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Effect of Distillery Spent-wash on Channel Bed and Groundwater Quality: Case Study of Unicol Distillery District Mirpurkhas

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***Abstract*— *The effect of treated spent wash on channel bed and groundwater qualities was investigated during 2014-15 at Unicol distillery as study area in district Mirpurkhas. For this purpose, samples of spent wash, soil/water were collected and determined for parameters related to physical and chemical properties. The impact of spent wash on channel bed and on the adjacent soils at different distances showed that highest soil EC (18.40 dS m-1) and TDS (11776 ppm) were determined for spent wash channel bed at surface soil and EC decreased with increasing distance from channel bed at sub-surface layers; while the highest overall soil pH (7.43) was observed at 450m distance at 30-45 cm soil depth. The highest total N (0.16%) and available K (0.53%) was observed at channel bed and at surface soil with highest available P (4.80%) at channel bed in sub-surface (15-30 cm) soil. The groundwater samples obtained from tube well and hand pump were also analysed for physical and chemical properties and compared with the spent wash from channel bed. The lower Na (1578.7 ppm) was determined in hand pump water samples than tube well water (2588.3 ppm); while highest (7050 ppm) in spent wash. The HCO_3 was lower in tube well water (247.00 ppm) than hand pump water (430 ppm); and highest (6166.70 ppm) in spent wash. The Chloride (Cl) content was lower (2117 ppm) in hand pump water samples than tube well water (5259 ppm); and highest in spent wash (14097 ppm). The groundwater EC was lower (11.077 dS m-1) hand pump water than tube well water (17.262 dS m-1) and highest (47.090 dS m-1) in spent wash. Similarly, the lower magnesium (465.3 ppm) was determined in hand pump water samples than tube well water (553.3 ppm) and exceptionally high (1300.7 ppm) in spent wash. The SAR of hand pump water samples was lower (11.583) than tube well water (20.390) and outstandingly high (35.693) for spent wash. In case of calcium content, it was lower in tube well water (359.33 ppm) than hand pump water (593.33 ppm) and exceptionally higher in spent wash (764.33 ppm). It was concluded that soil EC and TDS were lower at farther locations from spent wash channel bed at sub-soils. The surface soil contained higher organic matter; no effect of spent wash on soil organic matter was recorded. The soil pH was relatively higher at spent wash channel bed and its adverse effects were noted upto 300-meter distance. Total N was slightly (P>0.05) higher at channel bed than distant locations, while phosphorus was significantly higher at spent wash channel bed. The available potassium was also significantly influenced by the spent wash; and P was higher at channel bed, and decreased at the farther locations adjacent to the channel bed. The EC level, Na, HCO3, Cl, Mg and Ca contents as well as SAR for spent wash samples were manifold higher than the tube well and hand pump water samples.*

***Keywords*— spent-wash, hand pumps, tube wells, ground water quality, drainage channel bed quality.**

I. INTRODUCTION

One of the most important environmental problems faced by the world is management of wastes. Different industries create a variety of wastewater pollutants; which are difficult and costly to treat. Wastewater characteristics and levels of pollutants vary significantly from industry to industry [1~4]. The Effluent draining from distillery industries during the production of ethanol is considered as a major source of the environmental pollution. Distillery spent wash is considered as one of the big issues of pollution. This is because this effluent has extremely high values of chemical oxygen demand (COD), biological oxygen demand (BOD), inorganic solids, and low pH [5 & 6]. Basically, distillery spent wash industries are the agro-based industries and their waste effluent having high organic and inorganic compounds which are high strength based and difficult to disposed [7]. A typical cane molasses-based distillery generates 15 liters of spent wash per liter of ethanol produced [8]. This dark brown spent wash is being overloaded with high organic nitrogen, high organic and inorganic salts as a result having high electrical conductivity (EC) causes depletion of oxygen and produces bad smell [9~11]. Removal of distillery effluent on land is similarly harmful for the vegetative cover. It is described to reduce soil alkalinity and manganese availability, thus hindering seed germination defined by Kumar et al., [11].

Improper use of distillery effluent on soil without any suitable monitoring and checking, harmfully impacts the groundwater quality changing its physicochemical properties such as color, pH, electric conductivity (EC), through of leaching down of its organic and inorganic ions [13]. Due to high amount of salts in spent wash, soil can become sodic, saline, contaminated with a wide range of chemicals. Spent wash has high sodium content and when it is allowed to flow on land it causes negative impacts on soil properties [14 & 15]. This waste effluent may infiltrate into the sub-soil and put bad impacts on the ground water [16]. The discarding of huge quantities of biodegradable waste without systematic management, results in significant environmental pollution [17 & 18].

The spent wash contains high organic and inorganic contents which are high strength wastes and difficult to dispose [19] create a great destruction of natural and human resources [20]. Cane molasses also contains trace amount of dark brown pigment called melanoidins that impart color to the spent wash generated at the temperature range of 71-800C [21]. Distillery industry which produces a huge amount of wastewater is highly polluted and has very high chemical and biological oxygen demand (COD and BOD), heavy loaded of organic matter is dark brown reddish in color with unpleasant odour of indole, sketol and other sulpher compounds [22~24]. The research reports indicate that the spent wash contains heavy metals e.g. Hg, Cd, Cr which can accumulate and enter in food chain and biomagnifies to toxic level [25]. Germination percentage decreases with the concentration of effluent and put bad effect on livestock as well as Farmer's health and soil fertility; due to the effluent, groundwater qualities also deteriorated day by day [4, 26 ~28].

Ahmed et al. [29] assessed environmental impact of distilleries on soil quality. Research work is an experimental comparative study. Three locations were selected in Sindh Province of Pakistan among two were considered polluted and one was unpolluted or controlled site and controlled sample was taken from Sujawal City. Soil samples were taken as vertically and horizontally from and around distilleries. Spent wash samples were taken from the first two locations. Analytical assessment shown soil samples exceeded the limits those of the control soil samples, like pH of Mirpurkhas location surface soil 6.2 to 8.1, EC 308 µS/cm to 4.50 mS/cm, salinity 0.5%o to 2.4%o, T.N 24.8mg/l to 47.9mg/l, T.K 249.7mg/l to 291.5mg/l, T.P 0.29mg/l to 4.18mg/l, T.H 529mg/l to 1120mg/l, T.C 305mg/l to 690mg/l, S 71 mg/l to 101mg/l and Cl 27mg/l to 34.9mg/l and vertical soil of Mirpurkhas having pH 7.6 to 8.03, EC 221µS/cm to 725µS/cm, salinity 0.1%o to 0.4%o, T.N 32.7 mg/l to 49.8mg/l, T.P 0.26mg/l to 1.29mg/l, T.K 217.6 mg/l to 298.6mg/l, T.H 450mg/l to 1030mg/l, T.C 217mg/l to 630mg/l, S 65mg/l to 118mg/l and Cl 23.4mg/l to 39.3 mg/l, and deep soil having pH 7.9 to 8.30, EC 240µS/cm to 713µS/cm, salinity 0.2%o to 0.4%o, T.N 32.6 mg/l to 51.8mg/l, T.P 1.22 mg/l to 1.98 mg/l, T.K 264.2mg/l to 293.7mg/l, T.H 400 mg/l to 890mg/l, T.C 270mg/l to 450mg/l, S 67mg/l to 113mg/l and Cl 21mg/l to 35.2mg/l). Similarly, spent wash samples also exceeding the National Environmental Quality Standards limits.

II. MATERIALS AND METHODS

The study was carried out to evaluate the effect of treated spent wash on channel bed and groundwater qualities in district Mirpurkhas Unicol distillery. For this purpose, samples of spent wash, soil and water were collected.

Sample collection

Spent wash sample were collected in a clean glass container (the lid, seal and bottle were raised with boiling water before use) from outlets of Unicol distillery from MirpurKhas. The clean sterilized containers with stoppers were filled with the respective sample materials leaving one fourth of the containers empty. The samples were labeled properly and stored in ice box at 4°C and brought to the laboratory at the Centre of Excellence in Analytical Chemistry, University of Sindh Jamshoro. Standard procedures for chemical analysis of sample were used.

The Following parameters were determined for detailed analysis of the soil in surroundings of the spent wash channel bed.

EC (dS/m-1), TDS (%), Organic matter (%), pH

Nitrogen (%), Phosphorus (ppm), Potassium (%)

The water samples from different sources such as tube wells and hand pumps were also collected from the surroundings of the spent wash channel bed and their determinations were compared with spent wash. The collected water samples were labeled properly and brought to the laboratory at the Centre of Excellence in Analytical Chemistry, University of Sindh Jamshoro. Standard procedures for chemical analysis of sample were used.

The Following parameters were determined for detailed analysis of the water in comparison with spent wash.

S. #	Determinations	Method
1.	Na (ppm)	Flame Photometer (Jenway UK Model No. PFP-7)
2.	CO3 and HCO3 (ppm)	By titration with standard sulfuric acid (H2SO4) using phenolphthalein and methyl orange indicators respectively
3.	Cl (ppm)	titrating against standard silver nitrate (AgNO3) solution using potassium chromate (K2CrO4) indicator
4.	EC (dS/m-1)	portable conductivity meter (Hana Model-8733, Germany)
5.	Calcium and Magnesium (ppm)	titrating with std. versinate EDTA in the presence of NH4Cl+NH4OH buffer solution and Eriochrome Black T indicator
6.	Residual sodium carbonate (RSC)	RSC = (CO32- + HCO3-) - (Ca2+ + Mg2+) (Rowell, 1994)
7.	SAR [30]	$SAR = \frac{Na+}{[(Ca+2+Mg+2)/2]1/2}$
8.	Organic Matter	Walky and Black method [31]
9.	pH	portable pH meter (Orion-ISE Model-SA-720 USA)
10.	Total Nitrogen	Kjeldahl's apparatus [32]
11.	Available Phosphorus	Spectrophotometer (Model Specord-200 PC. Analytik Jen, Germany)
12.	Potassium	Flame Photometer (Jenway UK Model No. PFP-7)

III. RESULTS AND DISCUSSION

The study embodied in this thesis was carried out to investigate the effect of treated spent wash on channel bed and groundwater qualities in district Mirpurkhas Unicol distillery during the year 2015. For this purpose, samples of spent wash, soil/water were collected and determined for parameters related to physical and chemical properties.

The findings of the study showed that soil EC and TDS were lower at farther locations from spent wash channel bed at sub-soils. The surface soil contained higher organic matter; no effect of spent wash on soil organic matter was recorded. The soil pH was relatively higher at spent wash channel bed and its adverse effects were noted upto 300 meter distance. Total N was slightly ($P>0.05$) higher at channel bed than distant locations, while phosphorus was significantly higher at spent wash channel bed. The available potassium was also significantly influenced by the spent wash; and P was higher at channel bed, and decreased at the farther locations adjacent to the channel bed. The EC level, Na, HCO3, Cl, Mg and Ca contents as well as SAR for spent wash samples were manifold higher than the tube well and hand pump water samples. The present results are consolidately in agreement with many past researchers. Somawanshi and Yadav [33] indicated that additions of dilute spent wash would not add soluble salts to the soil provided there was sufficient leaching of soil solution. However, additions of concentrated spent wash would result in increased salinity of both soil and groundwater. Zalawadia et al. [34] observed increased P, K

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and S there by indicating the signs of improvement in soil properties. An increase in the soil available N, P and K by pre-sowing irrigation with distillery was noticed. The highest value of soil organic carbon (0.7%) was found in 87.5 m3/ha spent wash level. The available N, P and K content (216, 8.89 and 5488 kg/ha, respectively) of soil increased with an increase in spent wash levels. Baskar et al. [35] concluded that distillery effluent a waste water of distillery industry is of purely plant origin and contains large quantities of soluble organic matter and plant nutrients. Spentwash helped in better nutrition as it also supplemented micronutrients. It did not suppress biological activity and no significant changes were observed in them due to spentwash use. Diagan et al. [4] observed concentration of effluent spent wash depleted the groundwater quality. Kalaiselvi and Mahimairaja [8] concluded that the spent wash treated soil is enriched with the plant nutrients such as nitrogen, phosphorus and potassium. Chandraju et al. [36] concluded that diluted spentwash can be conveniently used for cultivation of leafy vegetables. Kanimozhi and Vasudeven [25] indicated that the spent wash contains heavy metals. Shenbagavalli et al. [37] reported that more than 50% of the samples were found unsuitable for irrigation purpose as they have shown greater potential for salinity hazards. Though no marked evidence was observed on the soil characteristics, the nutrients (N, P and K) and salt (Na, Ca, Mg, Cl and SO4) contents were relatively higher in these soils previously amended with the spentwash. Rakhi Chaudhary and Mahima Arora [27] reported soil and groundwater pollution due to spent wash channels. Mahar et al. [38] reported that spent wash has adversely affected the soil and groundwater parameters including pH, electrical conductivity, total dissolved solids (TDS), total hardness, chlorides, phosphates, alkalinity, nitrates, sulphates, chemical oxygen demand (COD), Na, K, Ca and Mg. Ajay et al. [28] argued that characteristics of spent wash do not allow its discharge into a water body; hence it requires treatment and dilution before discharge. Ansari et al. [11] conducted argued that spent wash requires treatment and dilution before discharge. Effluent originating from distilleries known as spent wash leads to extensive water pollution. Chandrakant and Kedar [39] considered spent wash as an ecotoxic effluent. Jha et al. [40] concluded that application of spent wash brings significant improvement in soil fertility and enhances the productivity of sugarcane in calcareous soil. Latha et al. [41] found that application of spent wash not only adds mineral N and carbon to soil, but also promotes the mineralization of soil organic C and N, thus resulting in large amounts of carbon, NH4 -N and NO3 -N in soil. Khandegar and Saroh [42] indicated that spent wash, leads to extensive soil and water pollution. Anoop and Renu [43] determined the impacts of sugar industry on ground water quality of area around the sugar industry. In villages, town and cities most of the population totally depend on the ground water for drinking purposes, domestic as well as for agriculture use, hence quality of ground water is extremely essential and must be analyzed. Ahmed et al. [29] took spent wash samples and shown soil samples exceeded the limits those of the control soil samples, like pH of Mirpurkhas location surface soil 6.2 to 8.1, EC 308 µS/cm to 4.50 mS/cm, salinity 0.5%o to 2.4%o, T.N 24.8mg/l to 47.9mg/l, T.K 249.7mg/l to 291.5mg/l, T.P 0.29mg/l to 4.18mg/l, T.H 529mg/l to 1120mg/l, T.C 305mg/l to 690mg/l, S 71 mg/l to 101mg/l and Cl 27mg/l to 34.9mg/l and vertical soil of Mirpurkhas having pH 7.6 to 8.03, EC 221µS/cm to 725µS/cm, salinity 0.1%o to 0.4%o, T.N 32.7 mg/l to 49.8mg/l, T.P 0.26mg/l to 1.29mg/l, T.K 217.6 mg/l to 298.6mg/l, T.H 450mg/l to 1030mg/l, T.C 217mg/l to 630mg/l, S 65mg/l to 118mg/l and Cl 23.4mg/l to 39.3 mg/l, and deep soil having pH 7.9 to 8.30, EC 240µS/cm to 713µS/cm, salinity 0.2%o to 0.4%o, T.N 32.6 mg/l to 51.8mg/l, T.P 1.22 mg/l to 1.98 mg/l, T.K 264.2mg/l to 293.7mg/l, T.H 400 mg/l to 890mg/l, T.C 270mg/l to 450mg/l, S 67mg/l to 113mg/l and Cl 21mg/l to 35.2mg/l). Similarly, spent wash samples also exceeding the National Environmental Quality Standards limits.

Electrical conductivity (EC dSm-1)

Soil samples were taken from the spent wash channel bed at varied distances (00, 150, 300, 450m) from outlet and determined for electrical conductivity at the soil depths 0-15, 15-30 and 30-45 cm. The data (Figure -1) showed that surface soil (0-15 cm) contained higher average EC level of 17.81 dSm-1, followed by EC levels of 14.89 dSm-1 and 12.91 dSm-1 at 15-30 and 30-45 cm soil depths, respectively. The effect of spent wash on EC level at different distance from spent wash channel bed indicated that at outlet, the EC level was highest (15.78 dS m-1); while the EC level of the soil significantly decreased to 15.35, 15.07 and 14.62 dS m^{-1} with increasing the distance from spent wash channel bed to 150, 300 and 450 meters, respectively.

The highest overall soil EC was determined for the soil samples obtained from spent wash channel bed at surface soil (18.40 dS m-1); while the lowest (12.43 dS m-1) in the samples obtained from the location at 450 meter distance from spent wash channel bed. There were significant ($P<0.05$) decrease in the EC level with increasing soil depth and increasing the distance from the spent wash channel bed.

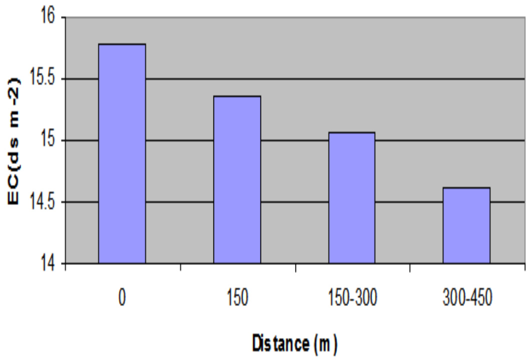


Fig.1: Soil EC (dS m^{-2}) at various distances from the spent wash channel bed

TDS (ppm)

The soil samples (0-15, 15-30 and 30-45 cm) were obtained from the spent wash channel bed and at varied distances (00, 150, 300, 450m) were examined for TDS. The data (Figure 2) indicated that the surface soil (0-15 cm) contained highest average TDS level of 10903 ppm, followed by 8863 and 8318 ppm TDS levels at 15-30 and 30-45 cm soil depths, respectively. The effect of spent wash on TDS level at different distance from spent wash channel bed showed that at channel bed, the TDS level was highest (10097 ppm); while the TDS level in soil markedly reduced to 9635, 9053 and 8660 ppm with increase in distance from outlet in spent wash channel bed to 00, 150, 300 and 450 meters, respectively. The highest overall TDS contents were noted in soil samples obtained from channel bed at surface soil (11776 ppm); while the lowest (7872 ppm) in the samples obtained from at location 450 meter distance from spent wash channel bed. There were significant ($P<0.05$) decrease in the TDS level with increasing soil depth and the TDS was higher in the soils closer to the spent wash channel bed.

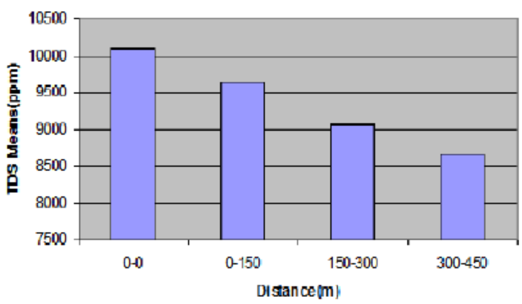


Fig.2: TDS in soil (ppm) at various distances from the spent wash outlet in the channel bed

Organic matter (%)

Soil organic matter is the most important element that influences the soil productivity; and soil amendments are mainly considered first to improve the soil organic matter. The soil samples at 0-15, 15-30 and 30-45 cm depths were collected from the channel bed as well as at varied distance from outlet of spent wash channel for examining the soil organic matter. The data (Figure 3) exhibited that the surface soil (0-15 cm) contained markedly highest organic matter (2.21%), while the soil organic matter declined to 0.81 percent and 0.65 percent at 15-30 and 30-45 cm soil depths, respectively. The effect of spent wash on soil organic matter at varied distance from spent wash channel bed showed that at channel bed, the soil organic matter was relatively higher (1.27%) as compared to 150, 300 and 450 meter distance from spent wash channel bed with average soil organic matter contents of 1.22, 1.21 and 1.19 percent, respectively. The highest overall soil organic matter (2.32%) was noted at channel bed near outlet; while the lowest soil organic matter (0.64%) was equally observed at 300 and 450 meter distance from spent wash channel bed in the 30-45 cm soil depth. The statistical analysis suggested significant effect of soil depth on soil organic matter ($P<0.05$); while the effect of spent wash at channel bed and at different distances from the outlet was statistically non-significant ($P>0.05$). This indicates that the surface soil always contained higher organic matter as compared to sub-surface layers; but the spent wash did not show a significant impact on the soil so far the soil organic matter is concerned.

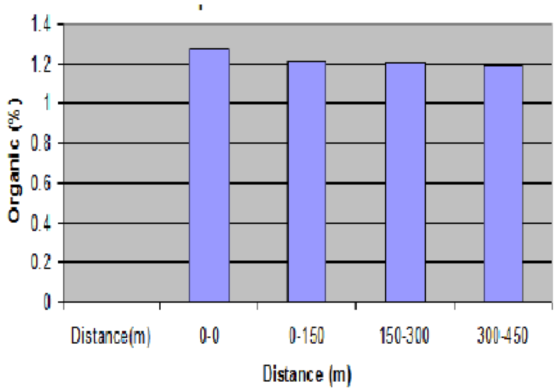


Fig.3: Organic matter in soil (%) at various distances from the spent wash channel bed

Soil pH

Soil pH is a key factor to influence the crop productivity and pH generally has close association with the soil EC. The soil samples at 0-15, 15-30 and 30-45 cm depths were achieved from the spent wash channel bed and at four distances from outlet for determining the soil pH. The data (Figure 4) described that the soil pH was highest at the surface soil (0-15 cm) with pH value of 7.39, and the soil pH decreased to 7.20 and 7.18 at 15-30 and 30-45 cm soil depths, respectively. The effect of spent wash on soil pH at varied distances from outlet of spent wash channel showed that near outlet the channel bed as well as at 150 meter distance, the soil pH was equally higher (7.32) as compared to 300 and 450 meter distance from outlet in spent wash channel bed with average soil pH of 7.27 and 7.10, respectively. The highest overall soil pH (7.43) was observed at 450 meter distance from channel bed at 30-45 cm soil layer; while the lowest soil pH (7.00) was equally observed at channel bed in surface (0-15 cm) and sub-surface (30-45 cm) soils. The statistical analysis suggested significant effect of soil depth and spent wash on soil pH (P<0.05). However, similarity in soil pH was observed for 0, 150 and 300 meter distances from channel bed (P>0.05); as well as for 15-30 and 30-45 cm soil depths (P>0.05). This indicates that spent wash channel has some adverse effects on the soil upto 300 meter distance.

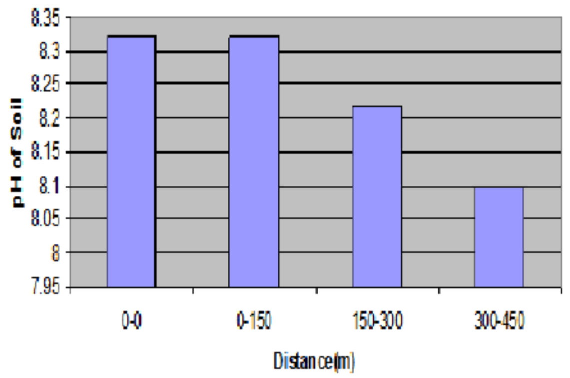


Fig.4: pH of soil at various distances from the spent wash channel bed

Total N in soil (%)

Nitrogen is the key element required by the plant for growth and without nitrogen crop production is rather impossible. The soil samples at 0-15, 15-30 and 30-45 cm depths at the experimental sit were obtained from the spent wash channel bed and at four distances from channel outlet for determining the total N. The data (Figure 5) showed that the total N was remarkably higher (0.148%) at the surface soil (0-15 cm), and N declined to 0.071 and 0.062 percent at 15-30 and 30-45 cm soil depths, respectively. The effect of spent wash on total N at progressive distances from outlet of channel showed that at zero and at 150 meter distance, the total N was higher i.e. 0.099 and 0.098% as compared to 300 and 450 meter distance from channel bed with average total N of 0.091 and 0.086 percent, respectively. The highest overall total N (0.16%) was observed at channel bed and 150 meter distance from channel bed at 0-15 cm soil layer; while the lowest total N (0.060%) was observed at 450 meters distance from channel bed at 30-45 cm soil layer. Statistically, the effect of spent wash on total N on the soil was non-significant (P>0.05); while significant (P<0.05) between the soil depths. The similarity (P>0.05) in total N was observed for 15-30 and 30-45 cm soil depths. Although, there was bit increase in total N at the channel bed over the distant locations, but the differences were negligible for spent wash effects; while sub-surface layers were lower in total N as compared to the surface soil at the studied location.

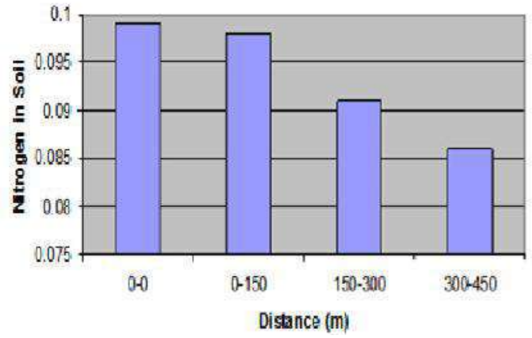


Fig.5: Soil Nitrogen (%) at various distances from the spent wash channel bed

Available P in soil (%)

Phosphorus is one of the essential elements required in the soil for plant growth and development. The effect of spent wash channel bed on the soil available P at different depths and distances was investigated. The data (Figure 6) indicated that the available P was remarkably higher (3.145%) at the surface soil (0-15 cm), and available P declined to 1.943 and 1.428 percent at 15-30 and 30-45 cm soil depths, respectively. The effect of spent wash on available P at progressive distances from channel bed indicated that at the channel bed, the available P was highest (3.60%) while available P simultaneously decreased to 2.23, 1.51 and 1.35% with increasing distance from channel bed to 150, 300 and 450 meters, respectively. The highest overall available P (4.80%) was noted at channel bed in 15-30 cm soil layer; while the lowest available P (1.01%) was recorded at 450 meters distance from channel bed in surface soil. Statistically, the differences in available P at various soil depths as well as the effect of spent wash channel bed at different distances was significant (P<0.05). However, similarity (P>0.05) in available P was recorded between 150, 300 and 450 meters distances as well as between 15-30 and 30-45 cm soil depths.

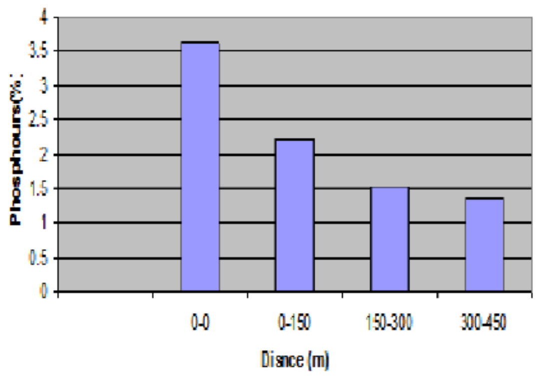


Fig.6: Available phosphorus in soil (%) at various distances from the spent wash channel bed

Available K in soil (%)

Potassium (K) is another element that required in the soil essentially for plant growth and development. The available K was determined at the experimental site at the 0-15, 15-30 and 30-45 cm soil depths to see the effect of spent wash channel bed on the soil available K at adjacent area. The data (Figure 7) showed that the available K was higher (0.49%) in the surface soil (0-15 cm), and declined to 0.22 and 0.14 percent at 15-30 and 30-45 cm soil depths, respectively. The effect of spent wash on soil available K indicated that at the channel bed, the available K was highest (0.31%), while available K showed a concurrent decrease to 0.28, 0.27 and 0.26% with increasing distance from channel bed to 150, 300 and 450 meters, respectively. The highest overall available K (0.53%) was noted at channel bed in surface soil; while the lowest available K (0.13%) was determined at 300 meters distance from channel bed in sub-surface soil (15-30 cm). Statistically, the differences in available K at various soil depths as well as the effect of spent wash channel bed at different distances were significant ($P<0.05$). However, similarity ($P>0.05$) in available K was observed between 300 and 450 meters distances from channel bed.

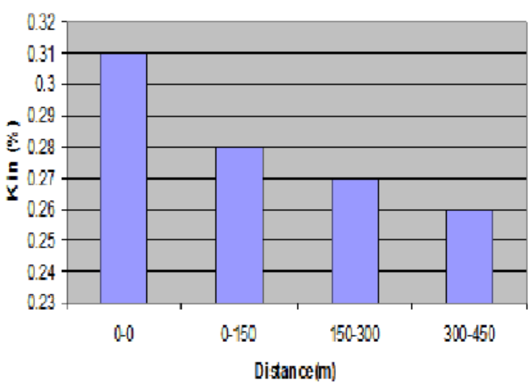


Fig.7: Available potassium in soil (%) at various distances from the spent wash channel bed

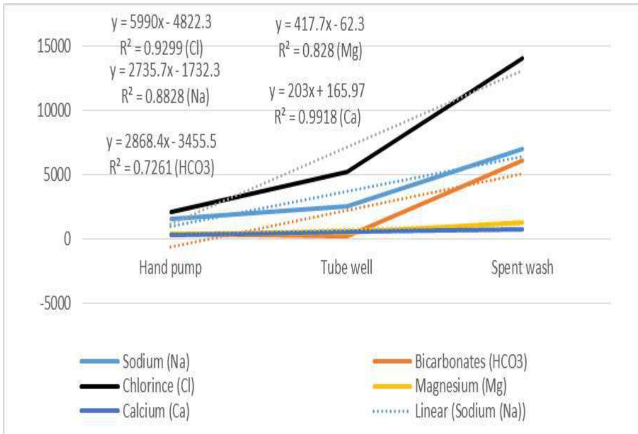
Comparative analysis of groundwater and spent wash

The groundwater samples were collected from two sources (tube well and hand pump) and their physical and chemical properties were compared with the spent wash samples collected from the channel bed.

Sodium (Na)

BOD, COD and pH removal

The regression analsyis for reedgrass plant height v/s effulent quality suggested that 56.63% variation in BOD (r^2 = 0.5663), 51.32% variation in COD (r^2 = 0.5132) and 67.49% change in pH (r^2 = 0.6749) was guided by the change in the reed grass plant height.



The sodium (Na) concentration in groundwater samples collected from different sources was determined and compared with spent wash. The data (Figure 8) showed that the lowest Na concentration (1578.7 ppm) was determined in groundwater samples collected from hand pumps; and the Na concentration in tube well water was 2588.3 ppm; while the highest Na concentration (7050 ppm) was determined in spent wash. The above figure for Na concentrations in analysed liquid showed a highly significant difference and clearly indicated that the Na concentration in spent wash samples is manifold higher than the tube well and hand pump water samples.

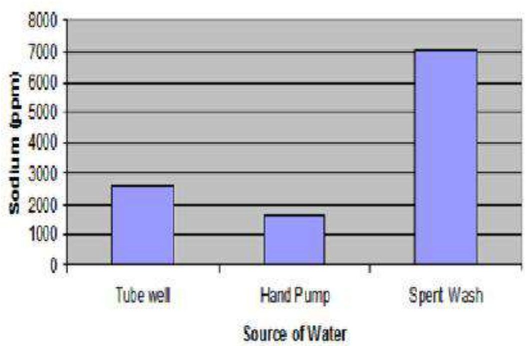


Fig.8: Groundwater analysis for Na (ppm) obtained from different water sources as compared to spent wash channel bed

Bicarbonate (HCO_3)

The bicarbonate (HCO3) concentration in groundwater samples collected from different sources was examined and compared with spent wash. The data (Figure 9) indicated that the lowest HCO3 concentration (247.00 ppm) was determined in groundwater samples collected from tube well; and the HCO3 concentration in hand pump water was 430.00 ppm; while the highest HCO3 concentration (6166.70 ppm) was determined in spent wash. The above data on HCO3 concentrations in analyzed water and spent wash samples showed a highly significant difference and concluded that the HCO3 concentration in spent wash samples was exceptionally higher than the water samples regardless the source of water. Moreover, differences in bicarbonate concentration between tube well and hand pump waters was also significant ($P<0.05$) showing that bicarbonate concentration in deep waters is lesser than the shallow waters.

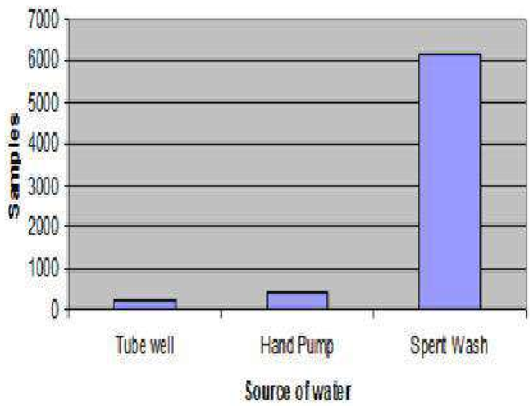


Fig.9: Groundwater analysis for HCO3 obtained from different water sources as compared to spent wash channel bed

Chloride (Cl)

The Chloride (Cl) content in groundwater samples collected from hand pumps and tube wells in surrounding areas of spent wash channel bed was determined and compared with chloride content of spent wash. The data (Figure 10) showed that the lowest chloride content (2117 ppm) was found in groundwater samples collected from hand pump; and the chloride content in tube well water was more than double to that of hand pump water (5259 ppm); while the highest chloride content (14097 ppm) was found in spent wash. This suggested that chloride quantities in spent wash are extraordinarily higher than the groundwater regardless the source of water. The differences in Chloride content between tube well and hand pump waters was also significant ($P<0.05$) indicated that the chloride content was higher in deep waters than the shallow waters.

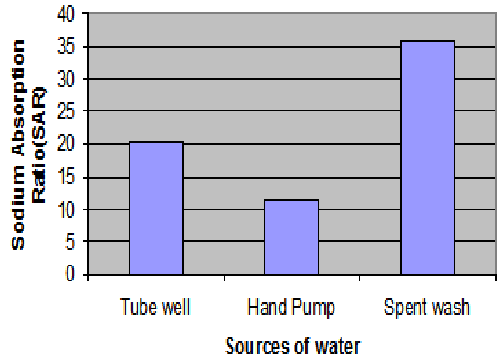


Fig.10: Groundwater analysis for Cl (ppm) obtained from different water sources as compared to spent wash channel bed

Electrical conductivity (EC)

The electrical conductivity (EC) level of groundwater samples collected from various sources such as tube well and hand pumps was determined and compared with spent wash. The data (Figure 11) indicated that the lowest EC level (11.077 dS m-1) was determined in groundwater samples collected from hand pumps; and the EC level in tube well water was relatively higher (17.262 dS m-1); while the alarmingly highest EC level (47.090 dS m-1) was determined in spent wash. This indicates that deep waters are of higher electrical conductivity as compared to that of shallow waters of hand pumps. However, spent wash was of huge level of electrical conductivity when compared with ground water irrespective of the source of water.

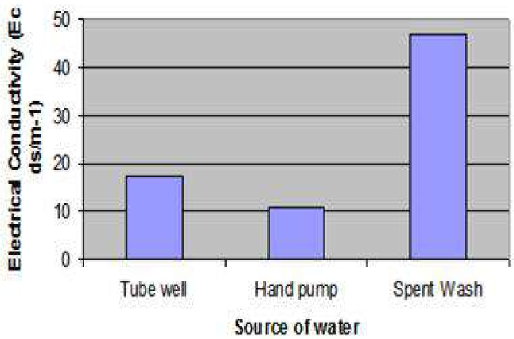


Fig.11: Groundwater analysis for EC (dS/m) obtained from different water sources as compared to spent wash channel bed

Magnesium (Mg)

The Magnesium content in groundwater samples collected from hand pumps and tube wells in surrounding areas of spent wash channel bed was examined and compared with magnesium content of spent wash. The data (Figure 12) exhibited that the lowest magnesium content (465.3 ppm) was determined in groundwater samples collected from hand pump; and the magnesium content in tube well water was significantly higher (553.3 ppm) than the water samples collected from hand pump water; while the exceptionally high magnesium content (1300.7 ppm) was recorded in spent wash. This suggested that magnesium quantities in spent wash were extremely higher than the groundwater samples apart from the source of water. The differences in Magnesium content between tube well and hand pump waters were also significant ($P<0.05$) indicated that the deeper water contained relatively higher magnesium contents as compared to shallow waters of hand pumps.

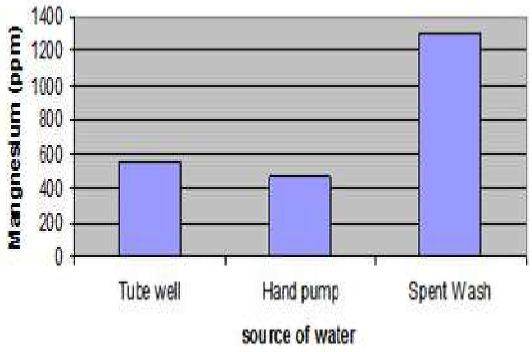


Fig.12: Groundwater analysis for Magnesium (ppm) obtained from different water sources as compared to spent wash channel bed

Sodium absorption ratio (SAR)

The sodium absorption ratio (SAR) of groundwater collected from hand pumps and tube wells in adjacent areas of spent wash channel bed was examined and compared with SAR of spent wash. The data (Figure 13) revealed that the lowest SAR (11.583) was analysed in groundwater samples collected from hand pump; and the SAR of tube well water was markedly higher (20.390) than the water samples collected from hand pump water; while the outstandingly high SAR (35.693) was analysed in spent wash. This suggested that SAR of spent wash was enormously higher than the groundwater samples and direct use of spent wash would not be useful. The differences in SAR for tube well and hand pump water were significant ($P<0.05$) indicated that the SAR was higher for deeper waters as compared to hand pumps.

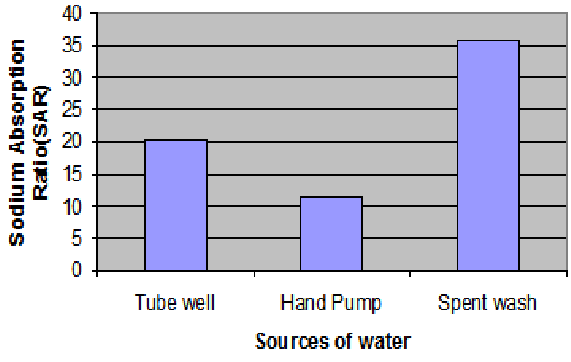


Fig.13: Groundwater analysis for SAR obtained from different water sources as compared to spent wash channel bed

Calcium (Ca)

The calcium content in groundwater of hand pumps and tube wells in the adjoining areas of spent wash channel bed was determined and compared with calcium content of spent wash. The data (Figure 14) indicated that the lowest calcium content (359.33 ppm) was observed in groundwater samples collected from tube well; and the calcium content in hand pump water was significantly higher (593.33 ppm) as compared to tube well water; while the spent wash contained superbly high (764.33 ppm). This indicated that calcium contents in spent wash were extremely higher than the groundwater samples apart from the source of water. The differences in calcium content between tube well and hand pump waters were also significant ($P<0.05$) indicated that the shallow water of hand pump contained significantly higher calcium as compared to deep waters of tube well. In view of the spent wash determination for calcium content, it is argued that without treatment of spent wash, its direct use would be harmful for the crops.

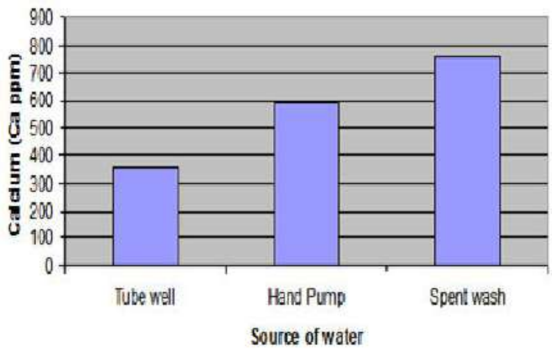


Fig.14: Groundwater analysis for Calcium (ppm) content obtained from different water sources as compared to spent wash channel bed

IV. CONCLUSIONS

The soil EC and TDS were lower at sub-surface soil layers and at farther locations from the spent wash channel bed. The surface soil contained higher organic matter as compared to sub-surface layers; but the spent wash did not show a significant impact on the soil organic matter. The soil pH was relatively higher at spent wash channel bed and spent wash adversely affected the soil pH up to 300-meter distance. There was a marginal increase in total N at the channel bed over the distant locations, but the differences were negligible for spent wash effects; while sub-surface layers were lower in total N as compared to the surface soil. The phosphorus was significantly higher at spent wash channel bed, which increased simultaneously with increase in the distance from channel bed. The available potassium was also significantly influenced by the spent wash; and P was higher at channel bed and decreased at the farther locations adjacent to the channel bed. The EC level, Na, HCO3, Cl, Mg and Ca contents as well as SAR for spent wash samples were manifold higher than the tube well and hand pump water samples.

REFERENCES

[1] Kuntal, M. H., A. K. Biswal, K. Bandyopdhayaya and K. Mishra. 2004. Effect of post methanation effluent on soil physical properties under a soyabean-Wheat system in a vertisol. Plant Nutri. & Soil Sci. 167(5): 584-590.

[2] Chakarbarty, R. N. 1980. Management of sugar and alcohol industry wastes-pollution control and conservation of energy and fertilizer. Proc. Symp. Disposal of sugar and distillery waste, U. P. Water pollution Control Board, Lucknow.

[3] Lin, Y. and S. Tanka. 2006. Ethonal fermentation from biomass resources: current state and prospects. App. Microbiol. Biotechnol, 69: 627-642.

[4] Diangan, J. M., T. Perez and R. Clveria. 2008. Analysis of land application as a method of disposal of distillery effluent. Int. J . Env. H. 2: 258-271.

[5] Shin, H. S.; Bae, B.U.; Lee, J. J.; Paik; B. C. (1992). Anaerobic digestion of distillery waste-water in a 2-phase UASB system. Water Science Technology; 25, 361-371.

[6] Saha, N. K.; Balakrishnan, M.; Batra, V. S.(2005). Improving Industrial Water Use: Case Study for an Indian Distillery. Res., Conserv. and Recyc.; 43, 163- 174. S

[7] Thakkar, A. (2013). Chemical Study on Distillery Effluent to Assess Pollution Load. Int. J. on Emerg. Tech.; 4, 121-123.

[8] Kalaiselvi, P and S. Mahimai Raja. 2009. Effect of bio-methanated spent wash on soil enzymatic activities. Bot. Res. Int.2 (4):267-272.

[9] Chauhan, A. (1991). Effect of distillery effluent on liver Wain Ganga, Indian J. Env. Hlth., 33, 203-207.

[10] Haroon, M. A. R.; Subash Chandra Bose, M. (2004). Use of distillery spent wash for alkali soil reclamation, treated distillery effluent for fertile irrigation of Crops. Indian Farm; March, 48-51. Oxford English Dictionary.

[11] Ansari, F., A.K. Awasthi and B.P. Srivastava. 2012. Physico-chemical characterization of distillery effluent and its dilution effect at different levels. Arch of App. Sci. Res, 4 (4): 1705-1715.

[12] Kumar, V.; Wati, L.; Fitz, F. G.; Nigam P.; Banat, I.M.; Singh, D.; Marchant, R. (1997a). "Bioremediation and de-colorization of an aerobically digested distillery spent wash", Biotech. Lett.;19, 311–313.

[13] Jain, R. K.; Kapur, M.; Labana, S.; Lal, B.; Sarma, P. M.; Bhattacharya, D.; Thakur, I. S. (2005). Microbial diversity: Application of microorganisms for biodegradation of xenobiotics. Current Science; 89, 101- 112.

[14] Christoph, M.; Theopisti, L.; Volker, J. D. (2001). Determination of Heavy Metals in Soils, Sediments and Geological Materials by ICP-AES and ICP-MS Mikrochim. Acta; 136, 123-128.

[15] Ramadura R., Gerard E. J. (1994). Distillery effluent and downstream products, SISSTA. Sugar Journal; 20,129-131.

[16] Akif, M.; Khan, A. R.; Sok, K. M.; Hussain, Z. M.; Abrar, K. M.; Muhammad, A. (2002). Textile Effluents and Their Contribution towards Aquatic Pollution in the Kabul River (Pakistan). Pak. J. Chem. 24, 106-111.

[17] Jain, N; Nanjundaswamy, C.; Minocha, A. K.; Verma, C. L. (2001). Indian journal of experimental biology; 39, 490-492.

[18] Pant, D.; Adholeya, A. (2007). Bio-resource technology; 98, 2321-2334.

[19] Billore, S.K., N. Singh, H.K. Ram, J.K. Sharma, V.P. Singh, R.M. Nelson and P. Dass. 2001. Treatment of molasses based distillery effluent in a constructed wetland in central India. Water Sci. and Tech. 44 (11-12): 441-448.

[20] Tao, F., J. Y. Miao, G. Y. Shi and K. C. Zhang. 2005. Ethanol fermentation by an acid-tolerant Zymomonas mobilis under non-sterlized condition. Process Biochemistry 40 (1): 183-187.

[21] Toma, M. M., U. Kalnenieks, A. Berzins, A. Vignts, M. Rikmnis and U. Viesturs. 2003. The effect of mixing on glcose fermentation by zymomonos mobilis continuous culture. Process Biochemistry, 38 (9) : 1347-1350.

[22] Nandy,T. S. Shasty and S. N. Kaul. 2002. Wastewater management in cane molasses distillery involving bioresources recovery. J. of Env. Management, 65 (1): 25-38.

[23] Uppal, J. 2004. Water utilization and effluent treatment in the indian alcohol industry: an overview. In: Tewari, P. K. (Ed), Liquid Asset, Proceedings of the Indo-EU workshop on promoting effluent water use in agro-based industries. TERI Press, New Delhi, India, Pp. 13-19.

[24] Yeoh, B. G. 1997. Two-phase anaerobic treatment of cane-molasses alcohol tillage. Water sci. and Technol. 36 (6-7) : 441-448.

[25] Kanimozhi, R. and Vasudevan, N. 2010. An over view of waste water treatment in distillery industry. Int. J. Env. Engi. 2: 159-184.

[26] Murugaragavan, R. 2002 Distillery spent wash on crop production in dry land soils. M. Sc (Environmental

Sciences) Thesis, Tamil Nadu Agricultural University, Coimbatore, India.
[27] Rakhi, C. and M. Arora. 2011. Study on distillery effluent: chemical analysis and impact on environment. Int. J. of Adv. Engg. Techno. II (II): 352-356.
[28] Ajay, F, A. F. Ansari and B.P. Srivastava. 2012 Physico-chemical characterization of distillery effluent and its dilution effect at different levels. Arch of App. Sci. Res, 1 (1/2): 29-37.
[29] Ahmed, M., J. Qureshi, Y. Nergis and M. Shareef. 2015. Environmental Impacts of Spent Wash on Soil Quality. Int. J. Econ. Environ. Geol. 6 (1) : 15-20.
[30] Rowell, D.L. 1994. Soil Science. Methods and Applications. Longman scientific & Technical, UK.
[31] Jackson, M. L. 1958a. *Soil Chemical Analysis*. 214-221.
[32] Jackson, M. L. 1958b. *Soil Chemical Analysis*. 498p. illus Englewood Cliffs, N.J.; Prentice-Hall, Inc. Kirk, P.L.
[33] Somawanshi, R. B. and A.M. Yadav. 1992. Effects of spent wash (distillery effluent) on soil chemical properties and composition of leachate. Proc. Ann. Con. of the Deccan Sug. Techn. Assoc. 1 : 101-108.
[34] Zalawadia, N.M., S. Raman and R.G. Patil, 1997. Influence of diluted spent wash of sugar Industries application on yield and nutrient uptake by sugarcane and changes in soil Properties. J. Indian Soc. Soil. Sci., 45: 767-769.
[35] Baskar, M., Kayalvizhi, C. and Bose, M.S.C. (2003) Ecofriendly utilization of distillery effluent in agriculture – A review. Agricultural Reviews 24, 16-30.
[36] Chandraju, S., R. Nagen draswamy, C. S. Chidan kumar and R. Venkat chalapathy. 2010. Nutritional additives of distillery Spent wash on the production of radish (Raphanous sativus), onion (Allium cepa) and garlic (Allium cepa) medicinal plants in normal and Spentwash Treated Soil. Bioresearch Bull 2: 59-65.
[37] Shenbagavalli. S., S. Mahimairaja and P. Kalaiselvi. 2011. Impact of biometha-nated distillery spentwash application on soil and water quality: A field appraisal. Int. J. Envir. Sci. 1 (7) : 1753-1758.
[38] Mahar, M.T., M.Y. Khuhawar, M.A. Baloch, T.M. Jahangir. 2012. Effects of spent wash of ethanol industry on groundwater: A case study of Rahimyar Khan District, Pakistan. Journal of Environmental Science and Water Resources, (4): 85 – 94.
[39] Chandrakant, M. and R. Kedar. 2013. Physico-Chemical Analysis and Microbial Degradation of Spent Wash from Sugar Industries. Research Journal of Chemical Sciences, 3(8): 53-56.
[40] Jha, C.K., S.K. Sinha and M. Alam. 2013. Utilization of bio-methanated distillery spent wash and bio-compost for sugarcane production and improving soil fertility. Environment and Ecology, 31 (4) : 1709-1713
[41] Latha, P., G. Thangavel, Rajannan and K. Arulmozhiselvan. 2014. Effect of Distillery Spent Wash on Carbon and Nitrogen Mineralization in Red Soil P. Nature Environment and Pollution Technology: An International Quarterly Scientific Journal, 12 (1) : 163-166.
[42] Khandegar, V. and A.K. Saroh. 2014. Treatment of distillery Spent wash by Electro coagulation. J. of Clean Ene Tech, 2 (3): 244-247.
[43] Anoop, Y. and D. Renu. 2014. Effect of sugar mill on physico-chemical characteristics of groundwater of surrounding area. Int. Res. J. Envir. Sci., 3(6): 62-66.

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Artificial Insemination Rate and Income of Farmers in Padang Pariaman District

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Abstract— *This study aims to determine the success rate of artificial insemination in beef cattle and the implications of artificial insemination in beef cattle on the income of farmers in Padang Pariaman Regency, West Sumatra. This study uses a survey method by interviewing 93 farmers and observing the cattle kept by the community in the research location. Sample selection was done by the accidental sampling method. The research data was obtained from artificial insemination reporting data in Padang Pariaman Regency which was taken as many as 957 acceptors, with 520 heads of Coastal cattle, 166 head of Bali cattle, 193 Simmental Peranakan cattle, 11 Brahman cattle, 35 PO cattle, and Limousin breed 32 tails. The variables observed were the characteristics of the breeder (age, education level, main occupation, experience in raising livestock, and the number of livestock kept). Inseminator Characteristics (age, education level, years of service, and activity of officers in the field), IB services, maintenance techniques (cattle that were maintained, feed given, maintenance management), IB success based on livestock reproduction (S/C, Conception rate, and Calving rate) and IB implications for farmer's income (revenues, costs incurred/profits). Based on the results of research on the success rate of Artificial Insemination in Beef Cattle in Padang Pariaman Regency, it can be concluded that Service per Conception (S/C) is 1.36 times, Conception Rate (CR) is 70.32%, Calving Rate (CVR) 45.98%. The implication of artificial insemination is that based on pregnancy and birth of livestock and the profits obtained from livestock that are born so that there is an added value and is reduced by the cost of feed, cage equipment, drug costs affect the income of farmers so that the net profits obtained by farmers are obtained.*

Keywords— ***Artificial Insemination, Calving Rate (CVR), Conception Rate (CR), IB Implications, Service per Conception (S/C).***

I. INTRODUCTION

The development of the livestock sub-sector in Indonesia needs to be increased, considering that the demand for livestock products tends to increase from year to year. On the other hand, the fulfillment of animal protein needs is still low, especially from ruminant meat, namely 5.5 g/capita/day or 2.02 kg/capita/year.

The increasing demand for beef is not matched by the population of beef cattle in Indonesia. The low population of beef cattle in Indonesia is due to the lack of successful livestock development in Indonesia (Talib et al. 2007). Therefore, it is necessary to increase the production and population of beef cattle.

The way to improve or increase beef cattle through seeds is by artificial insemination (Sudarmono and Sugeng, 2016). At the end of 2016, the government had launched the Upsus Siwab program (a special effort to accelerate the increase in the population of pregnant cows and buffalo) and now it has changed its name to Sikomandan (buffalo cattle, the mainstay of domestic commodities). The government claims the program can increase the population as well as improve the welfare of farmers.

Padang Pariaman Regency is one of the areas in West Sumatra that has intensively implemented an artificial insemination program since 1985. To optimize livestock reproduction, the government through the Sikomandan program has the principle of accelerating adult cows ready to become pregnant by conducting artificial insemination programs, pregnancy checks, and birth reporting. The Sikomandan program can be realized even better according to the birth targets set by each City/Regency. Pregnancy and birth are one of the main factors for the success of the Sikomandan program.

The total number of cattle in Padang Pariaman Regency in 2020 was recorded at 43,629 heads, but based on reports in the last 3 years there is a tendency to decrease the number of births. Where in 2017 the target for the birth of beef cattle was 4,717 heads and the realization exceeded the target of 5,908 heads. In 2018 the birth target increased to 5,244 tails with the realization of 5,900 birds. But in 2020 the target for beef cattle births decreased from 2019 to 5,076 heads with the realization decreasing from 2019 as many as 5,181 heads. It is not known for certain what causes births in Padang Pariaman Regency to decline in 2020.

According to the Ministry of Agriculture (2019), there are several problems in the implementation of artificial insemination, namely the knowledge of breeders is still low on AI, delays in the distribution of AI facilities and infrastructure, reproductive management, especially in semi-intensive and extensive areas, limited access to acceptors due to the difficult topography of the area and many conditions. livestock that is not good because of limited feed and reproductive disorders. Therefore, to optimize the results of successful artificial insemination through the Sikomandan program, these problems must be a serious concern to be resolved. The purpose of this study was to determine the success rate of artificial insemination based on reproductive performance in Padang Pariaman Regency and the implications of artificial insemination in beef cattle on the income of farmers in Padang Pariaman Regency.

II. MATERIAL AND METHOD

2.1. Materials Research

The materials used in this study were 520 coastal cattle, 166 Bali cattle, 193 Simental crossbreeds, 11 Brahman cattle, 35 PO cattle, and 32 Limousin crossbreed cattle which were IB acceptors and were recorded in Isikhnas.

2.2. Research Implementation

This study used a survey method with interviews with farmers and made observations of cattle kept by the community in the research location. The research samples taken are:

1. Data on the results of reporting on artificial insemination in beef cattle in Kabupaten Padang Pariaman were taken randomly. The data taken is the highest data on the implementation of artificial insemination in beef cattle in Padang Pariaman Regency, namely in June 2020, and data on livestock births from March to May 2021.
2. Farmers who have sold beef cattle resulting from artificial mating or artificial insemination. Determination of farmers who were interviewed as many as 93 farmers, using the accidental sampling method, which is a sampling technique based on chance, where anyone who coincidentally / incidentally meets the researcher can be used as a sample if it is seen that the person met is suitable as a data source (Sugiyono, 2005).

2.3. Observed variables

1. Characteristics of the breeder (age, education level, main occupation, experience in raising livestock, and the number of livestock kept).
2. Characteristics of Inseminators (age, education level, years of service, and activeness of officers in the field).
3. Maintenance techniques (seeds are maintained, feed given, maintenance management).
4. IB success based on livestock reproduction (S/C, Conception rate, Calving interval, Calving rate)
   a. Service per conception (S/C)

   Service per conception (S/C) is the number of inseminations needed for a pregnancy to occur. Data were obtained from inseminator records.

   b. Conception Rate

   The pregnancy rate or conception rate is the percentage of women who are pregnant in the first IB (Hafez, 2000).

   c. Calving Rate

   The calving rate is the percentage of the number of females giving birth to live calves of the total number of mated females.
5. Implications of IB on farmer's income (revenues, costs incurred)

The analysis used in this research is by looking at the comparison between revenue and total cost or R/C ratio so that from the comparison results can be seen the level of success and efficiency of farming. There are three possible conclusions from the analysis results from the above formulation, namely as follows:

- Revenue cost ratio (R/C) > 1 cattle business is feasible.
- Revenue costs ratio (R/C) = 1 cattle business reaches the break-even point, that is, neither gain nor loss.
- Revenue cost ratio (R/C) < 1 then cattle farming is not feasible.

2.3. Data Analysis

a. Service/Conception (S/C)

$$SC=\frac{number\ of\ inseminations\ that\ result\ in\ pregnancy}{Number\ of\ pregnant\ cows}$$

b. Conception Rate (CR)

$$CR=\frac{the\ number\ of\ pregnancy\ results\ of\ the\ 1st\ IB}{number\ of\ acceptors}\ x100\%$$

c. Calving Rate

$$Calving\ Rate=\frac{number\ of\ births}{number\ of\ acceptors}\ x\ 100\%$$

d. Income

π = TR –TC

Information:

π = net income / profit

TR = total gross income/receipt

TC = total cost of production

III. RESULT AND DISCUSSION

3.1. Characteristics of Breeders

Characteristics of breeders are one aspect that is quite important in a livestock business and has an influence on innovation. This is done to determine the identity of the farmers involved in this study. However, a farmer cannot be separated from the factors that influence his business in raising livestock, including age, gender, education level, main occupation, number of livestock kept, and experience of raising livestock as shown in Table 1.

Table 1. Characteristics of Beef Cattle Breeders in Kabupaten Padang Pariaman

No.	Characteristics	Amount (Person)	Percentage (%)
1.	Breeder's Age		
	< 30 Years	4	4,30
	30-50 Years	51	54,84
	> 50 Years	38	40,86
2.	Level of education		
	No school	4	4,30
	SD	39	41,94
	junior high school	27	29,03
	senior High School	20	21,50
	College	3	3,23
3.	The main job		
	Farmer	67	72,04
	Trader/Entrepreneur	22	23,66
	Employees/Retirees	4	4,30
4.	Number of Cattle Raised		
	< 5 Tails	47	50,54
	5-10 Tails	44	47,31
	> 10 Tails	2	2,15
5.	Farming Experience		
	1-5 Years	20	21,51
	6-10 Years	24	25,81
	> 10 Years	49	52,69

Source: Primary data that has been processed by researchers, 2021

3.2. Characteristics of Inseminator

Inseminator characteristics need to be known in addition to the characteristics of breeders because they can support the success of artificial insemination in the Padang Pariaman Regency, which can be seen in Table 2.

Table 2. Characteristics of Inseminators in Padang Pariaman Regency.

No.	Characteristics	Amount (Person)	Percentage (%)
1.	Age		
	20-60 Years	36	100
	> 60 Years	-	
2.	Level of education		
	Senior High School	19	52,78
	Diploma 4	1	2,78
	Bachelor Degree	16	44,44
3.	Years of service		
	0-3 Years	10	27,784
	4-10 Years	16	44,44

	11-20 Years	10	27,78
4.	IB Service		
	Active	33	91,67
	Passive	3	8,33

Source: Primary data that has been processed by researchers, 2021.

Based on Table 3 above, the characteristics of the age level of the inseminator officers in Padang Pariaman Regency are classified in the productive category with an average age of 20-60 years, namely 36 officers with a percentage of 100%.

The last education level of the inseminators was high school as much as 52.78% or 19 people, and D4 as many as 1 person (2.78%), and S1 as many as 16 officers (44.44%). This level of education shows that the inseminators in Padang Pariaman Regency have a good level of knowledge, and have sufficient experience with an average working period of 4-10 years, namely 44.44%. This shows that the implementation of IB is expected to run well because it has skilled and experienced inseminators.

In the artificial insemination service system, officers are quite active in serving farmers. Of the 36 officers as many as 33 active officers directly serve farmers to the field with a percentage of 91.67% and there are also passive officers as many as 3 people with a percentage of 8.33%. This is due to the dual work that is as an inseminator and as a civil servant.

3.3. Livestock Maintenance Techniques

The technical maintenance of livestock can be seen from the type of cattle kept, the feed provided, maintenance management, and disease prevention/treatment.

3.3.1. Types of livestock kept

The types of beef cattle kept by farmers in the Padang Pariaman Regency area can be presented in Table 3.

Table 3. Types of Seeds Raised by Breeders in Kabupaten Padang Pariaman

No	Breed Type	Amount (tail)	Percentage (%)
1.	Pesisir cow	520	54.38
2.	Bali Cow	166	17.33
3.	Simmental Crossbreed Cow	193	20.15
4.	Brahman Cow	11	1.15
5.	PO cows	35	3.65
6.	Limousine Breed Cow	32	3.34
		957	100.00

Source: Primary data that has been processed by researchers, 2021.

3.3.2. Feed

Types of feeding include forage, concentrate, and agricultural waste can be seen in Table 4.

Table 4. Types of Beef Cattle Feeding in Kabupaten Padang Pariaman.

No	Giving	Percentage (%)
1.	Forage only	65,59
2.	Forage and Concentrate	8,60
3.	Forage and Agricultural Waste Forage	21,51
4.	Concentrate and Agricultural Waste	4,30
		100.00

Source: Primary data that has been processed by researchers, 2021.

In Table 4, the provision of forage by breeders in Kabupaten Padang Pariaman is 65.59%. This is because farmers release their cows during the day and additional grass is given once in the afternoon or evening. From the results of interviews with farmers, they consider that the provision of forage is sufficient for the cows they raise and the provision of concentrate only adds to the cost. To produce good productivity, of course, it is necessary to provide good quality feed as well as support for cattle growth.

To spur increased productivity and reproducibility of livestock, it is necessary to support feed capacity both in quality and quantity. Leguminous plants are a type of forage that is prospectively developed because it has high protein content and is available in-situ on farmland. However, its potential has not been utilized by farmers as feed ingredients. The use of this forage as feed for ruminants can partially replace the need for concentrate (Hendri et al., 2010).

In addition, 8.60% of beef cattle breeders in Padang Pariaman Regency provide feed in the form of forage and concentrates. This breeder understands that it is also necessary to provide additional feed other than forage, usually farmers who provide this concentrate are for fattening cattle for beef cattle traders. The concentrate given is in the form of bran and tofu dregs. For the time of giving in the morning before giving forage, but some breeders give concentrate at night. For the provision of agricultural waste given in the form of banana stems, rice straw, and sago.

3.3.3. Maintenance Management

The management of beef cattle maintenance in Padang Pariaman Regency can be seen in Table 5.

Table 5. Management of Beef Cattle Maintenance in Padang Pariaman Regency.

No	Governance	Number of Breeders (person)	Percentage (%)
1.	Maintenance System:		
	Intensive	1	1,08
	Semi Intensive	92	98,92
	Extensive	-	-
2.	Cage Building:		
	Semi permanent concrete floor	2	2,15
	concrete floor wood	24	25,81
	Ground floor wood	67	72,04
3.	Cleaning the Cage:		
	Once a week	26	27,96
	Once a month	67	72,04
	Once a year	-	-

Source: Primary data that has been processed by researchers, 2021.

In Table 5, the most beef cattle rearing system is maintained with a semi-intensive system with 98.92%. Beef cattle are reared in a semi-intensive system, where the cattle are released during the day to look for food and at night are kept in cages. Livestock are released from 8.00 am to 5.00 pm. While the intensive rearing system is 1.08%, namely cattle are kept in cages without being released and full feeding is provided.

Beef cattle that are reared obtain forage in rice fields that are not planted with rice or that have been harvested, plantation areas, bushland, yards or roadsides. During the growing season, some cows are tied up by their owners somewhere to graze so as not to disturb the plants. In addition, farmers make fences in the planting area so as not to be disturbed by livestock that are allowed to roam. Besides getting forage from grazing, some farmers look for grass to give to livestock in the afternoon, especially during the rice planting season (Adrial, 2010). The cowshed building is generally made of wood that surrounds the entire cage ansd has 72.04% of the ground floor.

3.4. IB Success Rate Based on Livestock Reproduction

The success rate of artificial insemination is strongly influenced by four interrelated factors and cannot be separated from one another, namely the selection of acceptor cows, semen quality testing, the accuracy of estrus detection by breeders, and the inseminator skills. In this case, the inseminator and breeder have a very important role in the implementation of AI as well as the party responsible for the success or failure of the artificial insemination program in the field.

The success rate of the artificial insemination program in this study was also assessed from insemination per conception or Service per Conception (S/C), Conception Rate or conception rate (CR), and Calving Rate or birth rate (CVR).

Table 6. Value of Service per Conception (S/C), Conception Rate (CR), and Calving Rate (CVR) Beef Cattle in Padang Pariaman Regency.

No.	Reproductive Value	*n*	Results
1.	S/C	957	1.36 times
2.	CR	957	70.35 %
3.	CvR	957	45.98%

Source: Primary data that has been processed by researchers, 2021.

3.4.1. Value of Service per Conception (S/C)

Service per Conception is a number that shows the number of marriages until a pregnancy occurs. From the results of research that has been carried out the value of S/C on beef cattle in Padang Pariaman Regency is included in the good category. The value of S/C obtained is 1.36 times with the number of acceptor cattle as many as 957 heads. The lower the value, the higher the fertility value of the female livestock in the group, conversely the higher the S/C value, the lower the fertility value of the female group. The results of this study are almost the same as the results of research by Riyanto, et al (2015) S/C of beef cattle in Mojogedang District, Central Java is 1.33-1.71 times. The results of this study are close to Udin's (2012) statement that the perfect or ideal S/C value is one, which means that the animal is pregnant at one time IB, the optimum value is 1.6 or ranging from 1.4-1.8.

Nuryadi and Wahjuningsih (2011) stated that if the S/C is low, then the fertility value of the female cow is higher and if the S/C is high, the fertility rate of the female cow is lower. Following the opinion of Nebel (2002) which states that tilapia S/C is influenced by several factors including female fertility, semen quality, maintenance management, and inseminator skills.

The implementation of IB in Padang Pariaman Regency is carried out by experienced officers who have a permit to carry out Artificial Insemination (SIMI), have expertise in pregnancy inspection (PKB), ATR (Reproductive Technical Assistant), and semen handling. This is following the Decree of the Minister of Agriculture (2012) which states that the technical implementation of AI in the field requires officers who have special skills that are not easy for everyone to do.

The S/C value of beef cattle in Padang Pariaman Regency is said to be quite good, this situation is supported by most of the knowledge of farmers about signs of lust so that reporting to the inseminator officers is not too late. The S/C value indicates the level of livestock fertility. The greater the value of S/C, the lower the fertility rate. The high value of S/C is due to the delay of breeders and IB officers in detecting lust as well as inappropriate timing for IB, delays in IB causing pregnancy failure. Jalius (2011) explained that the high and low values of S/C were influenced by the accuracy of estrus detection, the timing of AI, and the reproductive condition of female cattle. Ihsan and Wahjuningsih (2011) added that the high S/C value is inseparable from the average nutrition content in the feed which greatly affects the reproductive condition of the female.

3.4.2. Conception Rate (CR) Value

Conception rate or conception rate is the main parameter in the assessment of artificial insemination results, namely the percentage of cows that are pregnant at the first insemination (Novita et al., 2019). The conception rate is determined based on the results of the diagnosis through a rectal examination (rectal palpation) by a veterinarian or special officer within 40 to 60 days after pregnancy (Feradis, 2010).

The results of the study can be seen that the success of AI in Padang Pariaman Regency is classified as very good because the average value of CR is 70.35%. This figure is almost the same as the research of Novita et al. (2019) in Juli District, Bireuen Regency, Aceh Province with a CR rate of 88.63%. This figure is higher than the statement by Hardjopranjoto (1995) that the ideal conception rate for a cattle population is 60-75%, the higher the CR, the more fertile the cows, and vice versa.

Fanani, et al. (2013) stated that the CR value was determined by male fertility, female fertility, and insemination techniques. Male fertility, one of which is the responsibility of the Artificial Insemination Center (BIB) which produces frozen semen in addition to storage management at the inseminator level. Female fertility is the responsibility of the breeder, assisted by a veterinarian in charge of monitoring the health of the mother cow. Meanwhile, the implementation of IB is the responsibility of the inseminator. The conception rate is the percentage of participants who are pregnant (Susilawati, 2011).

Rusumawati and Leondro (2014) that the physiological factors of female cattle also affect the conception rate, namely genetic factors, environmental factors, reproductive anatomy and hormonal conditions, body condition score, and eco parasites and endoparasites. In addition, the value of the conception rate is influenced by several factors including lust returning after giving birth and remarriage after giving birth (Dirgahayu et al., 2015).

3.4.3. Calving Rate (CVR)

The birth rate or Calving Rate is a number obtained by representing the number of children born from the result of one marriage (whether in the first marriage, second and so on) (Novita et al., 2019).

The Calving rate from the results of the research in Kabupaten Padang Pariaman is 45.98%. This result is lower than the research of Rosikh, et al. (2015) that the calving rate of Ongole Peranakan cattle in IB in Dukun District, Gresik Regency is 65%. According to Novita et al. (2019) that the value of the calving rate depends on the work efficiency of the inseminator, male fertility, female fertility during insemination, the health of female reproductive organs, and the ability to receive children in the womb until the time of birth. The absolute reproductive value of a female can only be determined after the birth of a live and normal cub.

Yulyanto et al. (2014) added that the most realistic assessment of IB is to calculate the number of offspring if the results of insemination have not produced children standing next to their mothers, then IB cannot be said to be successful. The high number for the Calving Rate is due to the inseminator skills that are already quite good, the ability of farmers to pay attention to their cattle during pregnancy and also the ability of the mother to take care of her child in the womb until the time of birth.

Generally, in the research area, many breeders do not separate their pregnant cattle and keep them in cages with other livestock. The treatment of farmers is also an influence on the birth rate of livestock. Susilo (2005) added that the factors that cause birth failure and can reduce CR are embryonic death, abortion, and fetal mummification during pregnancy. There were also several cases in the research area regarding the error of officers in giving insemination, namely the seeds given did not match the BCS (Body Condition Score) of the inseminated cows. According to the results of interviews with farmers, there was also an error by the officer in giving artificial insemination which turned out to be the acceptor was pregnant, causing the cow to abort or miscarry.

3.5. Livestock Reproduction Success Rate Based on Cattle Nation

Different breeds of cattle have different reproductive performances. The success rate of artificial insemination in Padang Pariaman Regency can be distinguished based on the breeds of beef cattle in Padang Pariaman Regency, namely Coastal cattle, Bali cattle, Simental crossbreeds, Brahman cattle, PO cattle, and Limousin crossbreeds (Table 7).

Table 7. Value of Service per Conception (S/C), Conception Rate (CR), and Calving Rate (CVR) of Cattle by Nation in Kabupaten Padang Pariaman.

No.	Breed Type	S/C	CR	CVR
1.	Pesisir cow	1.34	72.74%	48.46%
2.	Bali Cow	1.34	70.48%	40.96%
3.	Simmental	1.44	65.28%	45.08%
	Crossbreed Cow	1.54	63.63%	63.64%
4.	Brahman Cow	1.54	57.14%	40.00%
5.	PO cows	1.28	78.12%	37.50%
6.	Limousine Breed Cow			

Source: Primary data that has been processed by researchers, 2021.

Based on Table 8, the S/C value in Coastal cattle is the same as the S/C value in Bali cattle in Padang Pariaman Regency, which is 1.34 times. The S/C value of Bali cattle in this area is lower than the study by Nubatonis and Dethan (2021). The S/C value of Limousin crossbreed cattle was the lowest at 1.28 times. This indicates that the fertility of the Limousin cattle is quite good and the S/C value of Brahman cattle is the same as that of PO cattle, which is 1.54 times. This study is following the opinion of Ihsan and Wahjuningsih (2011) which states that Bos taurus (Limousin) cattle have high reproductive characteristics, large body size with medium to high growth rates, while the Bos indicus (PO) cattle have poor characteristics in terms of reproduction and speed of growth, but the nature of breastfeeding to their children (mothering ability) is very good.

The conception rate (pregnancy rate) is taken from the percentage of females who are pregnant in the first artificial insemination. The highest percentage of pregnant cows was Limousin crossbreed cattle, which was 78.12% and the lowest CR value was PO cattle with a percentage of 57.14%. The results of this study are following the research of Wiranto, et al (2020) who researched PO crossbreeds with Limousin crossbreed cattle with CR values of 58.97% and 74.03%, the conception rate value of Limousin Crossbreed cattle was better than Ongole Crossbreed cattle. . This is because farmers are more intensive in maintenance, especially in feeding.

Calving Rate is the percentage of the number of females that gave birth to live cows from the number of mated acceptors. The highest calving rate in beef cattle in Padang Pariaman Regency is Brahman cattle at 63.64%, while the lowest is Limousin crossbreeds at 37.50%. According to Yulyanto, et al. (2014) the physiological age status of cattle affects the percentage of CVR. Cows that have never given birth will be more sensitive to the risk of birth failure compared to cows that have given birth to calves. Ball and Peters (2004) stated that a large population of fertile cows inseminated with fertile semen could produce a CVR of 62%. The amount of CVR is influenced by the fertility of female cattle and male cattle or male semen. Susilo (2005) added that the factors that cause birth failure and can reduce CVR are embryonic death, abortion, and fetal mummification during pregnancy. CVR also depends on the work efficiency of the inseminator, male and female fertility, and the ability of the mother to raise children from the womb until the time of birth.

3.6. Implications of Artificial Insemination on farmer's income

The implications of the results of artificial insemination can be seen based on the results obtained and the costs incurred. From the results of the research, it was found that 45 farmers sold their livestock to 93 farmers who were used as respondents. With 45 artificially inseminated cattle that have been sold in the past year. Can be seen in Table 8.

Table 8. Beef Cattle Result of Artificial Insemination.

No.	Types of livestock produced by IB	Amount	%
1.	Female	9	20
2.	Male	36	80
		45	100%

Source: Primary data that has been processed by researchers, 2021.

3.6.1. Variable Cost

Variable costs are usually defined as costs whose size is influenced by the production obtained. For example, costs for production facilities, labor needs to be added, fertilizers also need to be added, and so on, so that these costs vary depending on the size of the desired production (Soekartawi, 1995). Variable costs can be seen in Table 9.

Table 9. Variable Costs of Beef Cattle Business.

No.	Variable Cost Component	Unit (Rp)
1.	Feed	2.379.096
2.	Drugs	20.537
3.	Farm labor	12.000.000
4.	Artificial Insemination Services	77.258
	Total Variable Cost	14.476.891

Source: Primary data that has been processed by researchers, 2021.

The cost component calculated for beef cattle farmers who use AI in the District of Padang Pariaman Regency is feed, where feed is the main support in the production process in addition to genetic and management factors (Parakkasi, 1999). Tumober (2014) also added that the cost of medicines/vaccines is the smallest of the total production costs. Variable costs incurred by farmers.

Table 9 shows the variable cost components for feed amounted to Rp. 14.476.891 from 93 respondents, while the cost of medicines from 45 respondents varies from Rp. 15,000 to Rp. 125,000 with a total cost of Rp. 20.537, which is only issued if the livestock is sick, giving vaccines and giving deworming medicine.

Labor costs amounted to Rp. 12,000,000. For labor, not all breeders use labor. There are only 5 breeders who use labor. Labor costs are calculated per day multiplied by the wages of farmworkers prevailing at the farmer's location. Furthermore, for the cost of artificial insemination services that are given free of charge because the cost of insemination itself is free but the farmers still provide it as a substitute for the transportation costs of officers, from 93 respondents amounting to Rp.14.476.891. artificial insemination amounted to Rp. 77.258.

3.6.2. Fixed Cost

Fixed costs are generally defined as costs that are relatively fixed in amount and continue to be incurred even though the product obtained is large or small. The amount of fixed costs does not depend on the size of the production costs obtained. For example, the costs for taxes will still be paid even if the results of the farming are large or fail (Soekartawi, 1995). Fixed costs can be seen in Table 10.

Table 10. Fixed Costs of Beef Cattle Business

No	Fixed Cost Component	Unit (Rp)
1.	Cowshed	289.010
2.	Cow barn equipment	41303
3.	Breeds	6.519.354
	Total Variable Cost	6.849.667

Source: Primary data that has been processed by researchers, 2021.

In Table 10, the cost components calculated for beef cattle farmers in Kabupaten Padang Pariaman are land rent, cages, and equipment. For land rent, generally, not all breeders rent land. Farmers who live in rural areas own their land and the livestock is only released on plantation land owned by farmers.

For housing depreciation costs around Rp. 125,000 to Rp. 1,000,000 depending on the construction of the cage made by the breeder, whether the cage is made of wood with a concrete floor or a wooden floor with a dirt floor. As for the equipment for the cages, farmers still use traditional equipment in the form of shovels, hoes, sickles, ropes to tie cattle and sacks. For seedlings amounting to Rp. 6.519.354, from the information, obtained the lowest cost of seeds is Rp. 3,000,000 and the highest 15,000,000.

Joesron and Fathorrozi (2003) stated that the total cost is the sum of total costs and variable costs in the production process. The variable cost of 93 respondents is Rp. 14.476.891 and fixed costs are Rp. 6.849.667 so the total cost is Rp. 21.326.558.

3.6.3. Reception

According to Soekartawi (1995), that farm income is the multiplication between the production obtained and the selling price. The revenue obtained is the selling value of the production of beef cattle farmers who use artificial insemination in the Padang Pariaman Regency (Table 11).

Table 11. Results of Analysis of Beef Cattle Business Revenues Using IB

No	Reception	Unit (Rp)
1.	IB Livestock Sales	10.333.334
2.	Temporary Livestock	13.638.709
3.	Price/Value IB Cattle Manure	47.825
	Total Reception	24.019.868

Source: Primary data that has been processed by researchers, 2021.

Based on the results of interviews with farmers for the sale of IB livestock, the price varies depending on the type of livestock sold. There are calves, non-productive or rejected females, and there are also males who are sold for slaughtering sacrificial animals. For the lowest price (<1 year) around Rp. 3,000,000 - Rp. 8,000,000 and the highest is for males with sales ranging from Rp. 13,000,000 - Rp. 22,000,000. rejected, sick, and barren female cattle at Rp. 8,000,000 - Rp. 11,000,000. Average sales are for cattle <1 year old and heifers.

The added value of livestock that is not sold from the 45 breeders, 48 farmers have not sold their livestock and will continue to increase in value if they continue to be maintained. The temporary livestock value added from this research is Rp. 24.019.868 of the 93 farmers.

3.6.4. Advantage

Profit or net income is obtained from the total gross income or revenue minus the total cost of production. The results of the profit analysis can be seen in Table 12.

Table 12. Results of Profit Analysis of Beef Cattle Breeders from IB

No.	Advantage	Unit (Rp)
1.	Reception	24.019.868
2.	Total Cost	21.326.558
	Advantage	2.693.310

Source: Primary data that has been processed by researchers, 2021.

Based on the results of the profit analysis in Table 18, farmers who use artificial insemination benefit from the difference between receipts or sales of IB livestock, sales of livestock manure and the value-added of livestock that is not sold (Rp. 24.019.868), and the total cost (Rp. 21.326.558). The average profit obtained from 93 farmers who have sold their beef cattle in the last 1 year of IB/head cattle in Padang Pariaman Regency is around Rp. 2.693.310/per year farmer who sells of cattle and breeder who has not sold his cattle. Where the average profit of this breeder is higher than the research of Motintja et al. (2015) where the average profit of IB breeders on PO cattle in West Tompaso District is around Rp. 733,475/head.

To see the level of success and efficiency of beef cattle breeders in Padang Pariaman Regency, it is necessary to calculate the Revenu Cost ratio (R/C). If R/C > 1 means that the beef cattle business is feasible to run, while R/C = 1 means that the cattle business has reached the break-even point, which is not making a profit (break-even revenue). Meanwhile, if the R/C < 1, the beef cattle business is not feasible. Where revenue is revenue divided by cost or production costs, as follows:

$$R/C = \frac{Rp.\,24.019.868}{Rp.\,21.326.558} = 1{,}12$$

From these results, the R/C > 1 means that the business is profitable and feasible to run. According to Sugiarti and Siregar (1998), AI treatment that has been carried out on cattle can have an impact on the income of farmers. With artificial insemination, it will increase the diversity of livestock and add superior seeds to livestock so that the selling value of livestock becomes higher. And with the presence of artificial insemination, can save farmers in the maintenance of males.

IV. CONCLUSION

Based on the results of research on the success rate of Artificial Insemination in Beef Cattle in Padang Pariaman Regency has been running very well, it can be concluded:

1. Service per Conception (S/C) 1.36 times, Conception Rate (CR) 70.32%, and Calving Rate (CVR) 45.98%.
2. The highest S/C value was in Brahman and PO cattle as much as 1.54 times and the lowest was in Limousin crossbreed cattle as much as 1.28 times. The highest CR value was in Limousin breeds of 78.12% and the lowest was in PO cattle of 57.14%. The highest CVR value was in Brahman cattle at 63.64% and the lowest was in Limousin crossbreeds at 37.50%.
3. The economic value or implication of artificial insemination is based on the pregnancy and the birth of the livestock and the profits obtained from the cattle that are born so that there is an added value and reduced by the cost of feed, cage equipment, drug costs affect the income of farmers so that the net profit obtained by the breeder. The average income of 1 farmer who performs artificial insemination is Rp.2.693.310 per year.

REFERENCES

[1] Adrial. 2010. Potensi Sapi Pesisir dan Upaya Pengembangannya di Sumatera Barat. Jurnal. Penelitian dan Pengembangan Pertanian 29(2): 66-72

[2] Badan Pusat Statistik Kabupaten Padang Pariaman. 2020. Jumlah Kelahiran Ternak Sapi Per Ekor Per Kecamatan Tahun 2018-2021. https://padangpariamankab.bps.go.id/indicator/24/149/1/jumlah-populasi-sapi-menurut-jumlah-per-kecamatan.html. Diakses pada tanggal 26 Januari 2020.

[3] Dirgayahu, F. F., M. Hartono, P.E. Santosa. 2015. Conception Rate pada Sapi Potong di Kecamatan Jati Agung

Kabupaten Lampung Selatan. Jurnal Ilmiah Peternakan Terpadu. Vol. 3 (1): 7-14.

[4] Fanani, S., Subagyo, Y. B. P dan Lutojo. 2013. Kinerja Reproduksi Sapi Perah Peranakan Friesian Holstein (PFH) di Kecamatan Pudak, Kabupaten Ponorogo. Tropical Animal Husbandry. Vol. 2 (1):21-27.

[5] Feradis. 2010. Reproduksi Ternak. CV. ALTABETA. Bandung.

[6] Hafez, E. S. E. 1993. Artificial insemination. In:HAFEZ, E.S.E. 1993. Reproduction inFarm Animals.

[7] Hendri, Z. Udin dan Jaswandi. 2004. Mata Kuliah Bioteknologi Reproduksi Ternak. Unand, Padang.

[8] Ichsan, Amirul. 2020. Penampilan Sifat Kualitatif dan Reproduksi Sapi Betina F1 Persilangan Sapi dan Sapi Pesisir di Kabupaten Padang Pariaman. Pascasarjana Universitas Andalas. Padang.

[9] Ihsan, M.N dan S. Wahjuningsih. 2011. Penampilan Reproduksi Sapi Potong di Kabupaten Bojonegoro. Jurnal Ternak Tropika. Vol 12(2) : 76-80

[10] Iswoyo dan P. Widyaningrum. 2008. Performans Reproduksi Sapi Peranakan Simmental (Psm) Hasil Inseminasi Buatan di Kabupaten Sukoharjo, Jawa Tengah. Jurnal Ilmiah Ilmu-ilmu Peternakan. 11(3): 125-133.

[11] Jalius. 2011. Hubungan Mortalitas Progresif dan Keutuhan Membran Sperma Dalam Semen Beku Sapi Bali Dengan Keberhasilan Inseminasi. Laboratorium Reproduksi Ternak Fakultas Peternakan Universitas Jambi. Jambi.

[12] Kusumawati, E.D dan H. Leondro. 2014. Inseminasi Buatan. Unikama, Malang.

[13] Luthfi, M., Anggraeny, Y.N., dan Purwanto. 2011. Perbedaan Performan Reproduksi Sapi PO dan Brahman Cross di Berbagai Lokasi di Jawa Tengah dan Jawa Timur. Seminar Nasional Teknologi Peternakan dan Veteriner Loka Penelitian Sapi Potong. Grati Pasuruan.

[14] Monintja, M.Y., F.S. Oley, B.F. Sondakh dan F.N.S. Oroh. 2015. Analisis Keuntungan Peternak Sapi Peranakan Ongole (PO) Yang Menggunakan Inseminasi Buatan (IB) di Kecamatan Tompaso Barat. Jurnal Zootek Vo.35 No.2: 201-209.

[15] Nuryadi dan S. Wahyuningsih. 2011. Penampilan Reproduksi Sapi Peranakan Ongole dan Sapi Peranakan Limousin di Kabupaten Malang. Jurnal Ternak Tropika. Vol 12(1) : 76-81.

[16] Novita, C. I., M. A. N. Abdullah, E.M. Sari, dan Zulfian. Evaluasi Program Inseminasi Buatan Pada Sapi Lokal Betina di Kecamatan Juli, Kabupaten Bireuen, Provinsi Aceh. Fakultas Pertanian Universitas Syah Kuala, Aceh. Jurnal Agripet Vol. 19.

[17] Parakkasi, A. 1999. Ilmu Nutrisi dan Makanan Ternak Ruminan. Universitas Indonesia. UI Press. Jakarta.

[18] Riyanto, J., Lutojo., Barcelona., D.M. 2015. Kinerja Reprodukti Induk Sapi Potong pada Usaha Peternakan Rakyat di Kecamatan Mojogedang. Jurnal Sains Peternakan. 3(2) : 73-39.

[19] Rusdi, B., Hartono, M., Suharyati, S., 2016. Calving Interval pada Sapi Bali di Kabupaten Pringsewu. Jurnal Ilmiah Peternakan Terpadu. Vol.4(4) : 277-283.

[20] Rosikh A, Aria AH, Qomaruddin M. 2015. Analisis perbandingan angka calving rate sapi potong antara kawin alami dengan inseminasi buatan di kecamatan dukun kabupaten gresik. Jurnal Ternak Vol.6(1):13-17.

[21] Soekartawi. 1995. Analisis Usaha Tani. Universitas Indonesia Press, Jakarta.

[22] Sudarmono, A. S. dan Sugeng Y. B.2016. Panduan Beternak Sapi Potong. Penebar Swadaya. Jakarta.

[23] Sugiyono. 2005. Metode Penelitian Kualitatif. Alfabeta, Bandung.

[24] Susilawati, T. dan Affandy, L. 2004. Tantangan dan Peluang Peningkatan Produktivitas Sapi Potong Melalui Teknologi Reproduksi Tantangan dan Peluang Peningkatan Produktivitas Sapi Potong Melalui Teknologi.

[25] Susilawati, T. 2011. Tingkat keberhasilan inseminasi buatan dengan kualitas dan deposisi semen yang berbeda pada sapi peranakan ongole. Ternak Tropika. Vol. 12 (2):15-24.

[26] Susilo, T. 2005. Efisiensi Produksi Program Inseminasi Buatan Terhadap Sapi Lokal Pada Daerah Lahan Basah dan Kering di Kabupaten Magelang, Provinsi Jawa Tengah. Tesis Master. Universitas Diponegoro, Semarang.

[27] Talib C, Inounu I, Bamualim A. 2007. Restrukturisasi Peternakan di Indonesia. Analisis Kebijakan Pertanian.1(5): 1-14.

[28] Tumober, J. 2014. Analisis Keuntungan Pemeliharaan Sapi di Kecamatan Suluun Tareran Kabupaten Minahasa Selatan. Jurnal Zootek Vol 24 No.2 : 18-26.

[29] Udin, Z. 2012. Teknologi Inseminasi Buatan dan Transfer Embrio Pada Sapi. Penerbit Sukabina Press, Padang

[30] Yulyanto, C. A., T. Susilawati dan M. N. Ihsan. 2014. Penampilan reproduksi sapi peternakan onggol (PO) dan sapi peranakan limousin di Kecamatan Sawo Kabupaten Ponorogo dan Kecamatan Tugu Kabupaten Trenggalek. Jurnal Ilmu-Ilmu Peternakan. Vol. 24 (2):49-57.

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Financial Analysis of Fishing Business using Jubi Catching Equipment in Bulutui Village, West Likupang District, North Minahasa Regency, North Sulawesi Province

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Abstract**—** *Traditional fishing gear jubi or arrows are still widely used by fishing communities in Bulutui Village, West Likpang District. The existence of this fishing gear continues to survive amid advances in fishing technology and this fishing gear is passed down from generation to generation. This research on the Financial Analysis of fishing gear business has never been carried out even though the results of this analysis will greatly assist fishermen in managing and developing their fishing business professionally.*
The purpose of this study in general, namely to examine the cost structure of the fishing business using jubi in Bulutui Village, while specifically this study aims to examine the financial analysis of the fishing business using jubi in Bulutui Village.

The method used in this research is a survey method. Data collection techniques by means of observation and filling out questionnaires. The sampling method is using the Simple Random method. In this study, 10 respondents were taken from a total population of 30 jubi fishermen in Bulutui Village.

The results of the financial analysis that have been carried out are obtained from the Total Cost obtained from Fixed Costs less Variable Costs amounting to Rp.115.913.900, Operating Profit obtained from Total Revenue minus Variable Costs amounting to Rp. 132,798,950, the total profit obtained from the total revenue is less, the variable cost is Rp. 112,486,100, the sales BEP is Rp. 18,511,638 and unit BEP of 3,161 individuals. This analysis shows that fishing business with jubi fishing gear in Bulutui Village is feasible to be developed. Based on the results of this analysis, it answers the problems in this study why jubi fishermen are able to survive in the midst of advances in fishing technology today because they are still profitable and feasible to be developed.

Keywords**— *Financial Analysis, Jubi, Bulutui Fisherman.***

I. INTRODUCTION

Fishermen are not only people who depend on catching fish in the sea, but also people who are involved in the process of catching fish at sea. Traditional fishermen are fishermen who use boats and simple equipment to go to sea such as boats and oars. In general, the so-called traditional fishermen are fishermen who utilize fishery resources with traditional fishing equipment, small business capital, and relatively simple fishing organizations.

Fishermen are very dependent on the season, during the fishing season the fishermen are very busy at sea and on the contrary during the lean season many are unemployed and what often happens is that when they return to fishing, they can buy expensive goods and when it is famine, their life is very bad. With such conditions, the decline of coastal communities/fishers in the abyss of poverty cannot be avoided. In this regard, it is necessary to have an effort to utilize natural resources towards a more optimal, self-supporting and community productivity in order to create a socio-economic life that has an impact on job creation and increasing income and standard of living (Nurfiarini, 2003).

Fisheries are all fishing businesses, fish cultivation and management activities to marketing the results (Mubiyarto, 1994 in Zubair and Yasin, 2011). According to Hanafiah and Saefuddin (2000) in Zubair and Yasin (2011) that fishing business is an activity to catch or collect animals or plants that live in the sea to earn income by making certain sacrifices. Capture fisheries is the business of catching fish and other aquatic organisms in the wild (seas, rivers, lakes, and other water bodies). The life of aquatic organisms in the wild and their factors (biotic and abiotic) are not intentionally controlled by humans.

The coastal area is defined as a land area bordering the sea, the land boundary includes areas that are waterlogged or not that are still affected by marine processes such as tides, sea breezes and salt intrusion. Boundaries in the sea are areas that are affected by natural processes on land such as sedimentation and the flow of fresh water into the sea, as well as marine areas that are affected by human activities on land (Bengen, 2001).

Coastal communities are a group of people living in coastal areas who live together and fulfill their needs from resources in coastal areas. People who live in cities or coastal settlements have socio-economic characteristics that are closely related to economic resources from the sea area (Prianto, 2005). Likewise, types of livelihoods that utilize natural resources or environmental services in coastal areas such as fishermen, fish farmers, and owners or workers of the maritime industry.

Traditional fishermen are referred to as people who are engaged in the marine sector by using sailboats without a motor (Mulyadi 2005). With simple fishing gear, the area of operation becomes limited, only around coastal waters. In addition to the limited catch, with the simplicity of the fishing gear owned, in certain seasons there is no catch that can be obtained. So that income is not in line with expectations and interferes with the welfare of fishermen, especially traditional fishermen. This has an impact on welfare which is reflected in the lifestyle and priority level of the livelihood needs of fishing communities in coastal areas.

Traditional fishing gear jubi or arrows are still widely used by fishing communities, including fishermen in Bulutui Village, West Likupang District. The existence of this fishing gear continues to survive amid advances in fishing technology and this fishing gear is passed down from generation to generation. This research on the Financial Analysis of fishing gear business has never been carried out even though the results of this analysis will greatly assist fishermen in managing and developing their fishing business professionally. Therefore, this research is deemed necessary to be carried out.

II. RESEARCH METHODS

2.1. Basic Method

The method that will be used in this research is the survey method. According to Sugiyono (2013), the survey method is a method used to obtain data from certain places that are natural and not artificial.

2.2. Data collection technique

The population in this study was the jubi fishing community in Bulutui Village, amounting to 30 people. Sampling using the sampling method, which is taking part of the population to be used as a sample of 10 respondents.

The data collected in this study are primary data and secondary data. Primary data in this study were taken by means of observation and interviews guided by questionnaires. Secondary data is data collected through a second party, usually obtained from agencies engaged in data collection such as statistical centers and others (Arikunto, 2013). Secondary data in this study were obtained from the Bulutui Village government and related agencies. Data collection techniques that will be used in this study are as follows:

1. Observation

Observation activities include systematic recording of events, behaviors, objects seen and other things needed to support the research being conducted (Sarwono, 2009). Observations were made by looking directly at the research location.

2. Questionnaire

Sugiyono (2008) states that a questionnaire or questionnaire is a data collection technique that is carried out by giving a set of questions or written statements to respondents to answer. The questionnaire in this study was used as a guide in interviewing respondents to find out the Financial Analysis of Fishing Business with jubi or arrow fishing gear.

2.3. Data analysis method

Data analysis will be carried out using the following financial analysis formula:

1. BEP

BEP is a situation where a business does not make a profit but also does not suffer a business loss. From the management point of view, BEP's situation does not mean financial loss, it's just that in terms of time they lose because of the time during production (business) they do not get more income as business profits.

2. R/C Ratio

R/C ratio is a value that shows the comparison between Business Revenue (Revenue = R) and Total Cost (Cost = C). Within the limits of the amount of R/C value, it can be known whether a business is profitable or not. Broadly speaking, it can be understood that a business will benefit if the revenue is greater than the cost of the business.

3. B/C Ratio

B/C ratio is a value that shows the comparison between Net Profit (Benefit = B) and Total Cost (Cost = C). Within the limits of the B/C value, it can be seen whether a business is profitable or not

4. Payback Period

Payback period is the ability of a company to return all invested capital/investment. Payback Period is expressed in units of time, for example months or years. The payback period is used as one of the complementary considerations in analyzing the feasibility of a business, because from the payback period, the payback period can be known for the payback period of all investment capital. The shorter the payback period, the more feasible a business is, this also means that the greater the net profit earned by the company.

III. RESULTS AND DISCUSSION

3.1 General Condition of Research Site

Bulutui Village is one of the villages located in West Likupang District, North Minahasa Regency and is one of the coastal villages of 20 villages in West Likupang District. The majority of the tribes in Bulutui Village are the Bajo tribe. Bulutui village before being inhabited by the community, was originally a coastal area of Likupang overgrown with water bamboo wilderness known as "bulutui". The name Bulutui Village itself is taken from the name of the bamboo.

In general, the topography of Bulutui Village is an area of hills / highlands, lowlands and coastal areas as well as tropical and rainy climates.

Most of the settlements are built on water, and most of the people in Bulutui Village work as fishermen, so that the largest income for the community is from the fishery sector.

Overall, the total population in Bulutui Village is 640 people, consisting of 190 families with the details, men amounting to 331 people and women totaling 309 people. Research on population can be seen from the following table1.

Table 1. Total Population in Bulutui Village, West Likupang District by Age Group

Age group(year)	Bulutui Village		Number of Souls	Percentage (%)
	Male	Female		
0-10	39	25	63	9,8
11-20	46	51	97	15,1
21-30	57	55	112	17,5
31-40	113	82	195	30,4
>41	76	97	173	27,1
Total	**331**	**309**	**640**	**100**

Source: Bulutui Village Office, 2021

The age group of Bulutui Village residents is 31-40 years old and the least is 0-10 years old. From the village office sources, no clear information was obtained regarding the composition of the population over 41 years of age. Age is one of the factors that quite influence a person's work productivity, but in the arrow business (Jubi) age cannot determine the level of income and gain profits, in this study the age of respondents with an age range of 31 years to 40 years amounted to 5 respondents. This age range with the largest number of respondents indicates that a mature age and in a healthy body condition determines the success of a business using arrow fishing gear (Jubi). While respondents aged over 41 years amounted to 2 people, indicating that the age of someone who is getting older means that the physical condition is somewhat declining so that fishermen who try to catch jubi gear are in small numbers. For details, the age range of respondents can be seen in the following table 2.

Table 2. Age Range of Respondents

No	Age Structure	Number of Souls	Percentage %
1.	**20-30**	**3**	**30**
2.	**31-40**	**5**	**50**
3.	**>41**	**2**	**20**
Total		**10**	**100**

Source: Primary Data, 2021

3.2 Education Level

The results showed that the education level of the respondents was mostly 5 people or 50% with elementary school education, while the lowest was respondents with high school education amounting to 2 people or 20%. The next level of education of respondents can be seen in the following table3.

Table 3. Respondents Education Level

No	Education Level	Number (people)	Percentage %
1.	SD	5	50
2.	SLTP	3	30
3.	SLTA	2	20
Total		6	100

Source; Processed Primary Data, 2021

3.3. Cost Structure of Jubi Capture Fishery Business

1 Investment Fee

Investment costs are funds that are not directly consumed but rotate to generate new revenues (Mantjoro, 1996). Investment costs in the arrow fishing business (Jubi) are in the form of costs incurred to obtain investment goods in the form of boats, outboard engines, compressors, coolbars, arrow fishing gear (Jubi) and flashlights. In the following table you can see all the investment costs in the Arrow Capture (Jubi) fishery.

Table 4. Total Investment Cost

No.	Description	Price (Rp)
1.	Boat	20.600.000
2.	Outboard Machine	21.900.000
3.	Compressor	4.650.000
4.	Coolbox	445.000
5.	Arrow (Jubi)	70.700
6.	Flashlight	1.085.000
Total Investment Cost		48.750.700

Source: Primary Data, 2021

Table 4 shows that the largest investment costs incurred for the purchase of outboard engines, amounting to Rp. 21,900,000 of the total investment costs and the smallest costs for making arrow fishing gear (Jubi) of Rp. 70,700.

2. Fixed Costs

Fixed costs consist of boat maintenance costs, outboard engines, compressors, colboxes, fishing gear and depreciation costs. All fixed costs are borne by the boat owner. Care and maintenance is carried out every three months when not carrying out fishing operations at sea. Here are the fixed costs in jubi fishing business

Table 5. Total Fixed Costs Per Year

NO	Description	Economic Life	Depreciation Cost	Maintenance Cost	Amount
1	Boat	10	2.060.000	500.000	2.560.000
2	Outboard Machine	5	4.380.000	700.000	5.080.000
3	Compressor	5	930.000	400.000	1.330.000
4	Coolbox	2	222.500		
5	Arrows (Jubi)	2	35.350		
6	Flashlights	1	1.085.000		
Total Fixed Cost					**10.312.850**

Source: Primary Data, 2021

3. Variable Costs

Variable costs are costs that are directly related to the fishing ground (fishing ground) and the length of time for fishing operations, namely the total operational costs of fishing. In the following table, it can be seen that the cost is not fixed once a trip.

Table 6. Total Variable Costs per Year

NO	Description	Amout	Price (Rp)
1.	Gasoline	15 Liter x @Rp.10.00 x 22 x 10	33.000.000
2.	Oil	1 Botol x @Rp.50.000 x 22 x 10	11.000.000
3.	Ice	190 Balok x @Rp.1.000 x 22 x 10	41.800.000

4.	Battery	1 Buah x @Rp.10.000 x 22 x 10	2.200.000
5.	Cigarettes	4 Bungkus x @Rp.20.000 x 22 x 10	17.600.000
6.	**Rubber**		**1.050**
Total Variable Cost			**105.601.050**

Source: Primary Data, 2021

4. Gross Revenue/Total Revenue

Gross income/total revenue is the total production result multiplied by the selling price of the production multiplied by the selling price of fish at the fish collector/buyer. The size of the gross income earned by fishermen is highly dependent on the number of catches. The following is a table of total revenue per year.

Table 7. Total Revenue

No	Description	Production (Kg)			Price (Rp)	Total Revenue (Rp)
	Catch	Per Trip	Per Month	Per Years		
1.	black goropa fish	5	80	800	30.000	24.000.000
2.	Sand Cooler Fish	5	80	800	17.000	13.600.000
3.	Lolosi Fish	7	112	1.120	20.000	22.400.000
4.	Octopus	2	32	320	15.000	4.800.000
5.	Lucky	5	80	800	40.000	32.000.000
6.	Parrotfish	5	80	800	20.000	16.000.000
7.	Red Snapper	10	160	1.600	60.000	96.000.000
8.	Blue Lobster	1	16	160	300.000	9.600.000
9.	Bobara Fish	5	80	800	25.000	20.000.000
Total		**45**	**720**	**7.200**		**238.400.000**

Source: Primary Data, 2021

5. Revenue (Operating Profit)

Operating profit is the profit of the arrow fishing business (Jubi) which is the difference between the total gross income and variable costs. The following is a table of revenue from jubi fishing business.

Table 8. Total Revenue

No	Description	Amount (Rp)
1.	Gross Income	238.400.000
2.	Variable Cost	105.601.050
***Operating Profit* (OP)**		**132.798.950**

Source: Primary Data, 2021

6. Total Profit ( )

The total profit is the total revenue minus the total cost, can be seen in the following table 9.

Table 9. Total Profit

No	Description	Amount (Rp)
1	**Total Receipt**	**238.400.000**
2	**Total Cost**	**115.913.900**
Total Profit (π)		**122.486.100**

Source: Primary Data, 2021

3.3. Financial Analysis

To find out the advantages of catching fish with jubi fishing gear in Bulutui Village, it is necessary to identify all the costs incurred and the income received. The amount of investment, fixed costs (FC), variable costs (VC), total costs (TC) and total revenues (TR) can be seen from the following table.

Table 10. Investment, FC, VC, TC, and TR

Description	Average
Investment	48.750.700
Fixed cost (FC)	10.312.850
Fixed cost (VC)	105.601.050
Total Cost (TC)	115.913.900
Total receipts (TR)	238.400.000

Source: Primary Data, 2021

The financial analysis carried out is as follows:

1. Total Cost (Total Cost)

Total Cost (TC) is the total number of fixed costs and variable costs incurred by the company to produce a number of products in a certain period.

Table 11. Total Cost (TC)

No	Description	Cost	
1	Fixed Cost	Rp	10.312.850
2	Variable Costs	Rp	105.601.050
Total Cost		**Rp**	**115.913.900**

Source: Primary Data, 2021

Based on the table 11, it is known that the variable cost that is issued per year is Rp. 105,601,050 and fixed costs (Fix Cost) incurred amounting to Rp. 10,312,850.

2. Revenue (Operating Profit/OP)

Operating Profit (OP) is the profit of fishing business with jubi fishing gear which is the difference between all gross income and variable costs.

Table 12. Revenue (OP)

No	Description	Total Cost
1	Admission Fee	Rp. 238.400.000
2	Variable Costs	Rp. 105.601.050
Operating Profit (OP)		**Rp. 132.798.950**

Source: Primary Data, 2021

Based on the table, Operating Profit Rp. 132.798.950,- is the profit earned and can be used for the next production cost.

3. Total Profit/Net Profit

The advantages of fishing with jubi fishing gear in Bulutui Village can be seen in table 13.

Table 13. Net Profit

No	Description	Amount
1	Total Revenue	Rp. 238.400.000
2	Total Cost	Rp 115.913.900
Total Profit		**Rp 112.486.100**

Source: Primary Data, 2021.

Net Profit or the total profit obtained in the fishing business with jubi in Bulutui Village is Rp. 112.486.100,-

4. Benefit Cost Ratio (BCR)

Benefit Cost Ratio (BCR) is an estimate of the expected benefits in the future or the ratio of revenues to all expenditures. If BCR> 1 then the business is feasible to run.

BCR=TR/TC

= Rp. 238.400.000,- / Rp. 115,913,900,- = 2,056

Based on the feasibility analysis of fishing with jubi in Bulutui Village, the B/C value is 2.056. This ratio indicates that the fishing business with jubi in Bulutui Village is feasible to run, because the benefits of fishing with jubi are greater than the costs.

5.Break Event Point

Sales BEP=FC/(1-VC/TR)

=(Rp.10.312.850)/(1-(Rp.105.601.050)/(Rp.238.400,000))

=Rp.18,511,667,6

Unit BEP=(Sales BEP)/(Unit Price)

=18,511,667.6/58,555

=Rp.3.161,3298 Tails

Based on the results of the analysis obtained from BEP sales, it shows the Break event point (Break event point) of the fishing business with this jubi is Rp. 18,511,667.6 and BEP units of Rp. 3,161.3298 tails.

IV. CONCLUSION

1. Based on the analysis of the feasibility of fishing with jubi in Bulutui Village, the Benefit cost ratio value is 2.056 > 1, indicating that the fishing business with jubi in Bulutui Village is feasible, because the benefits of fishing with jubi are greater than the costs expenditure.

2. Based on the results of the analysis of Net Profit or the total profit obtained in the fishing business with jubi in Bulutui Village of Rp. 112.486.100,- answering the problem in this study, why jubi fishermen are able to survive in the midst of advances in fishing technology today, apparently because it is profitable and feasible to be developed.

REFERENCES

[1] Bengen. D. G. 2010. Sinopsis Ekosistem dan Sumberdaya Alam Pesisir dan Laut.

[2] Pusat Kajian Sumberdaya Pesisir dan Lautan. Institut Pertanian Bogor.
[3] Faiza, 2011. Efektifitas dan Keberlanjutan Pengelolaan Daerah Perlindungan Laut Berbasis Masyarakat. Disertasi. Sekolah Pasca Sarjana. IPB. Bogor.
[4] Gabriella Selaindoong,2019. Analisis Finansial Usaha Budidaya Ikan Nila Pada Karamba Jaring Tancap Di Desa Eris Kabupaten Minahasa. Skripsi, FPIK.2019.
[5] Prianto, E. 2005. Proseding "Fenomena Ak tual Tema Dok toral Arsitektur dan Perkotaan". Semarang:Badan Penerbit Universitas Diponegoro.
[6] Riduwan. 2012. Metode & Teknik Menyusun Proposal Penelitian. Bandung: Alfabeta
[7] Roscoe, 2010, dikutip dari Uma Sekaran, 2006, Metode Penelitian Bisnis, Salemba Empat, Jakarta.
[8] Sugiyono. 2013. Metode penelitian Administrasi.Bandung.ALFABETA. Sugiyono, (2008). Metode Penelitian Kunatitatif Kualitatif dan R&D. Bandung Alfabeta
[9] Sugiyono. 2013. Metode Penelitian Kuantitatif, Kualitatif, dan R&D. Bandung: CV Alfabeta
[10] Subejo dan Supriyanto. 2005. Kerangka Pemberdayaan Masyarakat Pedesaan Menuju Pembangunan Yang Berkelanjutan. Jurnal Ilmu-Ilmu Pertanian.Vol. 1, No. 1. Juli 2005 Hal 17-32.

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Mitigating an Airport's Carbon Footprint Through the Use of "Green" Technologies: The Case of Brisbane and Melbourne Airports, Australia

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***Abstract*—*Like many other airports located around the world, Australia's major airports have installed and are operating new green energy systems, such as, photovoltaic (PV) solar and trigeneration systems. Using an instrumental case study research approach, this study has examined how Brisbane and Melbourne Airports have mitigated their carbon footprint by using green, renewable energy systems. The extensive use of these green, renewable energy has enabled both Brisbane and Melbourne Airports to reduce their annual carbon dioxide (CO_2) emissions, and thus, these measures have helped to mitigate the environmental impact of these airports' operations. The study revealed that the photovoltaic (PV) solar system at Brisbane Airport, will enable the airport to reduce its annual carbon dioxide (CO_2) emissions by an estimated 8,000 tonnes per year. The trigeneration system installed and operated by Melbourne Airport will deliver an estimated reduction of 920,000 tonnes carbon dioxide (CO_2) emissions over a 15 period. Melbourne Airport's photovoltaic (PV) solar is also delivering a reduction in the airport's annual carbon dioxide (CO_2) emission.*

Keywords*— Airports, Brisbane Airport, Melbourne Airport; Photovoltaic (PV) systems, Solar power, Trigeneration

I. INTRODUCTION

In the global aviation industry, passenger and air cargo services are provided within a value chain with the key stakeholders comprising aircraft manufacturers, leasing firms, airlines, airports, air traffic control service providers, aircraft maintenance organizations, flight catering providers, aircraft refuellers, ground handling agents, global distribution systems (GDS), travel agents, tour operators, and air freight forwarders (Jarach, 2017; Tretheway & Markhvida, 2013). Airports play a fundamental role in the air transport value by providing the critical infrastructure, such as, passenger terminals and runways that are necessary to facilitate the movement of passengers and air cargo. Accordingly, airports act as the critical interface point between the surface-based and air transport modes (Baxter et al., 2018a). Despite the significant social and economic benefits of air transport services, such services have an adverse impact on the environment. Noise, air and water pollution, and natural resources consumption present at an airport all have an adverse impact on the environment (Budd, 2017; Daley, 2016; Janić, 2011). In addressing their environmental impact concerns, airports are increasingly trying to become "green" or more environmentally friendly (Comendador et al., 2019; Janić, 2011). A "green airport" is an airport which has a minimal impact on the environment and is one that endeavors to become a carbon neutral facility in terms of carbon emissions, with the goal of producing zero greenhouse gas emissions (González-Ruiz et al., 2017).

In providing the necessary critical infrastructure, such as, runways, taxiways and airport facilities, airports consume large amounts of energy and, as such, are regarded as being very energy intensive (Akyuz et al., 2019; Baxter et al., 2018b; Ortego Alba & Manana, 2017). Considering this, airports around the world are increasingly embracing green energy technologies, such as photovoltaic solar (PV) systems, as a way of mitigating their carbon footprint. Adelaide Airport, Cochin Airport, Osaka's Kansai Airport, and Raja Bhoj International Airport, for example, have all introduced photovoltaic solar (PV) systems.

The objective of this study is to examine the solar photovoltaic (PV) systems installed at Brisbane and Melbourne Airports, Australia and to identify the environmental benefits that these two airports have obtained from the use of these systems. A secondary objective was to examine the trigeneration system installed at Melbourne Airport and to identify how this system has enabled the airport to mitigate its carbon footprint. The Brisbane and Melbourne Airports photovoltaic (PV) systems formed the focus for this case study as these are the largest solar photovoltaic (PV) systems installed at any Australian airport. A further factor in selecting Brisbane and Melbourne Airports as the case airports was the readily available case documentation which allowed for the in-depth analysis of these airports adoption of green energy systems and technologies.

The remainder of the paper is organized as follows: the literature review presented in Section 2 sets the context for the in-depth case study. The research method used in the study is described in Section 3. The Brisbane and Melbourne Airports case study is presented in Section 4. The key findings of the study are presented in Section 5.

II. BACKGROUND

2.1 Airport Energy Sources

Airports are comprised of the landside and airside precincts (Janić, 2011, 2017). The airside precinct includes the aircraft movement area, and the adjacent terrain and buildings/infrastructure. The landside precinct includes those parts of an airport together with the adjacent terrain and buildings that are not located in the airside precinct (Rossi Dal Pozzo, 2015). The actors operating within the airport's airside and landside precincts require a reliable and highly efficient supply of energy. Historically, the two primary energy sources have been electricity and fuel, for example, diesel, natural gas, and propane (Ortega Alba & Manana, 2016). Electrical energy is normally supplied directly to the airport through dedicated sub-stations (Janić, 2011). Typically, airports purchase electricity from the commercial grid and this electricity is supplied by a power company (Ortega Alba & Manana, 2016). As previously noted, in recent times airports have increasingly adopted the use of renewable energy sources. These include solar photovoltaic, concentrating solar power, wind power, oil and natural gas extraction, steam-generated power production and electricity transmission (Barrett et al., 2014). The use of renewable energy resources has provided airports with several favorable environmental related advantages. Green energy produces no greenhouse gas emissions from the combustion of fossil fuels. As a result, this reduces some forms of harmful air pollution (International Renewable Energy Agency, 2021; United States Environmental Protection Agency, 2021). Furthermore, renewable energy systems provide the airport with an alternative clean source of power (Kramer, 2010). Another important advantage is that solar power photovoltaic (PV) systems lower the airport's ground emissions (Sukumaran & Sudhakar, 2017). The airport's carbon footprint (carbon dioxide CO_2 emissions) can also be reduced by substituting solar PV based power generation for traditional, more heavily polluting, fossil-fuel based energy sources (Sukumaran & Sudhakar, 2017; Wybo, 2013). In addition, the use of green or renewable energy sources provides a firm or user with an important opportunity to optimize energy efficiency (Arman et al., 2013). Another advantage is that renewable energy sources normally have very little waste (Yerel Kandemir & Yayli, 2016).

According to Sukumaran and Sudhakar (2017), "the amount of power that a solar PV system can produce at an airport is dependent upon the available area". The amount of power is also dependent upon the type of photovoltaic (PV) solar system used at the airport, the PV system's orientation, and the available solar resource (Kandt & Romero, 2014). Many airports often have large tracts of open space that could be potentially used for the installation and operation of a PV system (Baek et al., 2016; Curran, 2016). Accordingly, many airports who meet the spare land use requirement are installing or plan to install large surfaces of PV panels (Figure 1). These PV systems are often capable of producing 20MWh or even higher amounts of sustainable energy (Wybo, 2013).

Fig.1: Solar photovoltaic system installed at Denver International Airport. Photograph provided courtesy of Denver International Airport.

2.2 Energy Usage at Airports

The primary areas of energy consumption at an airport are heating, cooling, lighting, and the energy required for operating the airport's facilities and systems (Janić, 2011; Radomska et al., 2018). At many airports, crude oil is often used for producing the fuel used to power the ground service equipment (GSE) and vehicles that are used in an airport's airside and landside areas, especially in the aircraft ground handling process (Janić, 2011). Fuel is also used for airport's heating boiler systems and emergency generators (Ortega Alba & Manana, 2016). The airport terminal's heating, ventilation, and air conditioning (HVAC) systems use the largest amount of energy (Akyüz et al., 2017).

2.3 A Brief Overview of Photovoltaic (PV) Solar Systems and the Key Issues for Airports

The solar photovoltaic (PV) systems being installed at airports are normally customized so that they optimize the use of the selected site (Baxter et al., 2019). Importantly, there are different environmental factors that will be applicable for each site. Consequently, these factors will influence the type of photovoltaic (PV) system that is required, and they will also impact its level of performance. Photovoltaic (PV) systems are comprised of the solar resource, photovoltaic cells, panel or module, array, battery, inverter, charge controller, electrical load – this includes the appliances and other devices that use the energy generated by the PV system, wiring and the surge protector – this is a device that safeguards against electrical shock from short circuits and damaging power fluctuations. The photovoltaic (PV) system wiring includes the wires that are known as conductors that connect the system components to complete circuits (Balfour et al., 2013, pp. 4-5). Quite often photovoltaic solar systems are collective in nature, that is, they are centralized systems that provide electricity to a group of users. These users include commercial customers (Bhattacharyya, 2015).

As previously noted, there is a growing use of solar power at airports located right around the world (Sreenath et al., 2020, 2021a, 2021b). However, there are several key issues that airport's need to be cognizant of when considering the installation and use of a photovoltaic (PV) solar system (Baxter et al., 2019). Solar photovoltaic (PV) systems are required to be installed at a sufficient distance from the airport's runway(s) and these systems should adhere to all relevant safety and fire measures applicable at the airport (Kandt & Romero, 2014). If inappropriately located at the airport, then there is a risk that the solar photovoltaic (PV) systems at airports can impact pilots, air-traffic controllers, aircraft, and air navigation systems due to the glare reflection (Mostafa et al., 2016). The solar system could cause either glint or glare, or possibly a combination both. This could potentially result in a brief loss vision, which would be an important safety concern for aircraft pilots (Anurag et al., 2017). Consequently, glare due to the reflection of sunlight from the metal parts of a solar PV panel could potentially provide a risk that may result in an adverse impact on aviation safety (Mostafa et al., 2016). There are several measures that an airport can use to mitigate the glare from their solar system. The first measure involves the application of anti-reflective coatings (Solanki & Singh, 2017). The second measure involves the surface texturing of the systems panels (Ahmed et al., 2017). Neither of these measures should have a noticeable impact on the solar PV system performance but will greatly assist in minimizing reflection from the PV system (Kandt & Romero, 2014).

III. RESEARCH METHODOLOGY

3.1 Research Method

This study used a qualitative instrumental case study research approach (Owens et al., 2021; Sorenson, 2021; West et al., 2021). An instrumental case study is the study of a case. An instrumental case study can study a firm(s). This research approach provides insights into a specific issue, enables researchers to redraw generalizations, or builds theory (Stake, 1995, 2005), whilst also facilitating the understanding of a specific phenomenon. An instrumental case study is designed around established theory (Grandy, 2010). The present study was designed around the established theory of green energy (Aswathanarayana, 2010; Bhowmik et al., 2017; Kalyani et al., 2015), and the use of solar power by airports (Baxter et al., 2019; Sreenath et al., 2020, 2021a, 2021b; Sukumaran & Sudhakar, 2017).

3.2 Data Collection

Data for the study was obtained from a variety of documents, airport industry-related journals, annual reports, press releases, company materials available on the internet and records as sources of case evidence. Documents included Brisbane and Melbourne Airports environmental policies, Brisbane Airport's masterplan, industry publications, and the airport's websites. Thus, this study used secondary data. The study followed data collection guidance of Yin (2018), that is, multiple sources of case evidence were used, a database on the subject was created, and there was of a chain of case evidence.

3.3 Data Analysis

Document analysis was used to analyze the documents gathered for the study. Document analysis focuses on the information and data from formal documents and company records that are gathered by the researcher(s) when conducting their case study (Andrew et al., 2011; Oates, 2006; Yin, 2018). The study paid particular attention to four criteria that need to be carefully considered when assessing the quality of historical documents: authenticity, credibility, representativeness and meaning (Scott, 2014; Scott & Marshall, 2009).

The document analysis process was undertaken in six discrete stages. Firstly, the types and required documentation and their availability were ascertained. In the second stage, the pertinent documents were collected and a system for managing them was developed. In the next stage, the documents were reviewed to assess their authenticity, credibility. It was also necessary to ascertain if any potential bias existed in the documents. The fourth stage involved the interrogation of the documents at which time the key themes, data and issues were identified. This was followed by a period of reflection and refinement at which time any difficulties with the documents were identified. Also, in this stage, a thorough review of the sources and the documents content was undertaken. The analysis of the data was finalized in the sixth stage of the document analysis process (O'Leary, 2004).

All the documents collected for the study were stored in a case study database (Yin, 2018). All the study's documents were in English. Each document was carefully read, and key themes were recorded in the case study (Baxter, 2021).

IV. RESULTS

4.1 Brisbane Airport Photovoltaic (PV) Solar System

4.1.1 A Brief Overview of Brisbane Airport

Brisbane Airport is bounded by the Brisbane River to the east, the Kedron Brook Floodway to the west, Moreton Bay to the north and the Gateway Motorway to the south of the airport precinct. The airport is located less than 20 kilometres from the Brisbane central business district (CBD) (Brisbane Airport, 2020). Brisbane Airport, IATA airport code BNE, is Australia's largest airport by land area (Brisbane Airport, 2021). The Brisbane Airport site is 2,700 hectares in size (Brisbane Airport, 2020). The airport has two separate terminals for domestic and international flights. The airport terminals are 4 kilometers apart. The international terminal has twelve gates with air bridges, two of which can handle the Airbus A380 aircraft. The domestic terminal has three satellites housing additional boarding gates and lounge facilities. There are twenty-eight gates with air bridges (Brisbane Airport, 2021). Brisbane Airport has two runways.

In July 1997, the Commonwealth Government entered into a 50-year lease agreement with the new owners and operators of Brisbane Airport as part of the government's airport privatization policy (Graham, 2018; Paul & De Groot, 2010; Solomon, 2009). Brisbane Airport Corporation Pty Limited, the operator of Brisbane Airport, is a private, unlisted Queensland-based business. Brisbane Airport Corporation acquired Brisbane Airport from the Australian Federal Government in 1997 under a 50-year lease agreement with an option to renew for a further 49 years (Brisbane Airport, 2020, p. 29).

Brisbane Airport is a key hub for Qantas Airways, Jetstar Airways and Virgin Australia. The airport is also linked to major international air travel markets and is well served by international airlines, such as, Air New Zealand, Qantas, and Singapore Airlines amongst others.

4.1.2 Brisbane Airport Environment and Sustainability Policy

The Brisbane Airport Corporation (BAC) is firmly committed to reducing the impact on the environment and has implemented programs to manage and minimize the long-term impacts of climate change and adverse environmental impacts from aviation and property-related development activities at the airport (Brisbane Airport, 2020).

The airport operator has the overall environmental responsibility for all the activities and operations undertaken at the airport. These include all airport operations and security, asset management, tenancy management as well as development projects. All other airport users are also responsible for the environmental management of their activities (Brisbane Airport, 2020).

In accordance with their Environment and Sustainability Policy, Brisbane Airport Corporation is committed to:

- Operating, managing, and developing Brisbane Airport in an environmentally responsible manner.
- Ensuring compliance with the applicable environmental laws, policies and other legal requirements which pertain to its operation. The airport aims to striving to meet and/or exceed these requirements wherever possible.
- Fostering an environmentally responsible culture amongst the company's employees.
- Minimizing adverse impacts on the environment that are caused by the company's operations.
- Continually striving to reduce natural resource consumption, waste generation, and prevent pollution.
- Working closely with government departments, agencies, and airlines to manage impacts of aircraft noise and the impacts of aviation on the community.

- Constantly striving to achieve continual improvement in environmental and sustainability performance through the implementation of an Environmental Management System (EMS) consistent with the international standard ISO14001:2015 and sustainability benchmarking evaluations (Brisbane Airport, 2020, p. 387).

To achieve this commitment, Brisbane Airport Corporation (BAC) will:

Take action to address potentially adverse environmental impacts.

- Communicate the Brisbane Airport Environment Strategy, policies and performance to employees, regulators, tenants, and the wider community.
- Establish, implement, and maintain an Environmental Management System (EMS) which includes the setting and reviewing of environmental objectives and targets.
- Periodically review the effectiveness of the Environmental Management System, and identify opportunities for environmental, social, economic, and operational sustainability performance improvements.
- Maximize energy, water, and waste efficiencies.
- Manage noise impacts, pollutant emissions and the impacts of climate change on the airport.
- Identify and seek to conserve objects and matters at the airport that have natural, indigenous, or historic heritage value.
- Achieve best practice in sustainable property development.
- Provide appropriate environmental training to the company's employees and encourage its tenants and contractors to do likewise.
- Build strong and active relationships with the wider community through engagement and sponsorship programs; and
- Provide the company's staff and resources necessary to meet these policy objectives (Brisbane Airport, 2020, p. 387).

4.1.3 Brisbane Airport Environmental Management System (EMS)

The Brisbane Airport Corporation Environmental Management System (EMS) has been developed in line with the internationally recognized EMS ISO 14001:2015 standard (Brisbane Airport, 2020). ISO 14001 is a worldwide meta-standard for implementing Environmental Management Systems (EMS) (Dentch, 2016; Grover & Grover, 2017; Heras-Saizarbitoria et al., 2011). The ISO 14001 Environmental Management System (EMS) has become one of the most widely used systems for managing corporate environmental aspects (Oliveira et al., 2011).

Brisbane Airport's EMS ensures that there is a systematic approach to manage environmental issues across the airport. Procedures and guidelines have been developed that are in accordance with specific aspects of the standard. These include:

- Risk and opportunities assessment.
- Compliance obligations.
- Training.
- Internal and external communications.
- Environmental auditing and document control.
- Incident and emergency preparedness.
- Sustainable procurement (Brisbane Airport, 2020, p. 389).

4.1.4 An Overview of Brisbane Airport's Photovoltaic (PV) Solar System

In 2017, Brisbane Airport made the decision to install a large photovoltaic (PV) solar system at the airport. The new 6MW photovoltaic (PV) solar system was to be installed across six sites at the airfield (Australian Aviation, 2019a; Shakra Energy, 2019). The system consists of five roof-mounted solar arrays which can generate a total of 5MW, and one ground-mounted solar array, generating about 1MW (Ecogeneration, 2017). The system collectively covers 36,000 square meters (Australian Aviation, 2019a; Maisch, 2019, Trina Solar, 2019).

In Stage one, which ran from early April 2018 to mid-May 2018, 700 solar roof panels, generating 0.2MWp, were installed at the Skygate facility. The Skygate facility is in the airport's "The Circuit" precinct. In Stage two, which also ran from early April 2018 to mid-May 2018, 700 solar roof panels generating 0.2MWp were installed at the Department of Home Affairs building, which is also located in the airport's "The Circuit" precinct. During Stage 3, which ran from mid-July 2018 to September 2018, 3,500 solar roof panels, generating 1.03MWp, were installed on the roof of the airport's domestic passenger terminal P1 multi-level car park. In Stage 4, which ran from mid-July 2018 to mid-October 2018, 3,400 solar panels generating 1.51MWp, were installed on the roof of the airport's domestic terminal P2 multi-level car park. During Stage 5, which covered the period mid-May2018 to mid-October 2018, 6,545 solar roof panels generating 1.8MWp were installed on the roof of the airport's international passenger terminal building. In the final stage, which ran from early June 2018 to December 2018, 4,000 solar ground panels generating 1.01MWp were installed in Brisbane Airport's Pandanus Avenue precinct (Brisbane Airport, 2018).

At the time of the installation of the photovoltaic (PV) solar system, the airport's international terminal roof installation was the largest single roof photovoltaic (PV) solar power installation in Australia. The photovoltaic (PV) solar power system installed on the roof of the airport's international passenger terminal consists of 7,133 solar panels that measured 11,675 square metres in area (Australian Aviation, 2017, 2019a; Brisbane Development, 2017; Halcol Energy, 2019).

The installation of the complete photovoltaic (PV) solar power system took one year to complete, and the solar panels became operational at the end of 2018 (Halcol Energy, 2019).

Brisbane Airport did not rely upon any grants or subsidies apart from "Large-scale Generation Certificates" (Ecogeneration, 2017). In Australia, large-scale renewable energy projects, such as solar farms, are entitled to create large-scale generation certificates (LGCs). One LGC is equivalent to 1 MWh of renewable electricity generated above the power station baseline (Clean Energy Regulator, 2021b). In Australia, it is permissible for Registered LGCs to be sold or transferred to entities with liabilities under Australia's Renewable Energy Target or to other companies who are seeking to voluntarily surrender LGCs. Liable entities are businesses (principally electricity retailers) that are required to purchase and surrender LGCs to Australia's Clean Energy Regulator each year in fulfilment of their obligations under the *Renewable Energy (Electricity) Act* 2000 (Clean Energy Regulator, 2021a).

4.1.5 The Environmental Related Benefits of Brisbane Airport Photovoltaic (PV) Solar System

The photovoltaic (PV) solar system has provided Brisbane Airport with some important environmental related benefits. It has been estimated that around 18% of the airport's electricity will be supplied from the solar PV system and this will meet approximately 6% of Brisbane Airport Corporation's overall load (Australian Aviation, 2019a; Brisbane Airport, 2018; Lenaghan, 2017). In addition, the photovoltaic (PV) solar system has reduced carbon dioxide (CO_2) emissions at the airport by an estimated 8,000 tonnes per year (Brisbane Airport, 2018).

4.2 Melbourne Airport Photovoltaic (PV) Solar and Trigeneration Energy Systems

4.2.1 A Brief Overview of Melbourne Airport

Melbourne Airport was officially opened on 1 July 1970 (Melbourne Airport, 2017a). The airport is located approximately 25 kilometres north-west of the Melbourne city central business district (Melbourne Airport, 2017c). Melbourne Airport is the State of Victoria's primary gateway for air travel, tourism, and freight (Melbourne Airport, 2017a). Melbourne Airport is Australia's second busiest airport. In July 1997, a long-term lease for the management and operation of Melbourne Airport was awarded to Australian Pacific Airports Corporation (Senguttuvan, 2007). At the time of the present study, Melbourne Airport was managed and operated by Australia Pacific Airports Corporation Limited (APAC) which is a privately held corporation that is owned by institutional investors, who are predominantly superannuation/pension funds (Australia Pacific Airports Corporation, 2019).

Melbourne Airport has four terminals, with Terminal 1 used for Qantas Airways domestic flights, Terminal 2 handles international flights, and Terminal 3 is used for Virgin Australia domestic services. Melbourne Airport officially opened its new Terminal 4 facility on 09 December 2015. The opening ceremony followed the gradual opening of the terminal to passengers in two stages. Tigerair Australia and Jetstar Airways commenced services from the terminal in the last few months of 2015 and Regional Express Airlines began services in early 2016. Melbourne Airport's Terminal 4 is located to the south of Terminal 3 and comprises 20,000 square metres of space over three levels. The facility has the capacity to accommodate up to 10 million passengers a year (Sadler, 2015).

The Melbourne Airport site is approximately 2,740 hectares in size. There is urban development to the east and south of the airport. This mainly consists of a mix of residential and industrial uses. Melbourne Airport has two runways. The longer Runway 16/34 measures 3.7 kilometres in length, while the shorter Runway 09/27 is 2.3 kilometres long (Australian Aviation, 2019b). Melbourne Airport has eighty aircraft stands that are used for the parking of aircraft (Australia Pacific Airports Corporation, 2019).

4.2.2 Melbourne Airport Environmental Policy

In 2018, Melbourne Airport defined and implemented a comprehensive environmental policy in which the airport committed to reducing its environmental impact. A key aspect of the policy is its desire to be an environmental leader for transport and logistics sites in the Asia Pacific region (Australia Pacific Airports Corporation, 2018).

In accordance with the environmental policy, Melbourne Airport is to be responsible for, and to protect the environment directly and indirectly by the airport's operations. The airport is also committed to the continuous improvement in its environmental performance using the certified Environmental Management System (EMS). The environmental policy also aims to make a material reduction in the energy consumption and the associated emissions by adopting measures to conserve natural resources and by adapting to climate change. Melbourne Airport's environmental policy also aims to minimize waste through the implementation of the waste management hierarchy and by adopting a life cycle approach to procurement. The airport also aims to reduce prevent, limit, and reduce pollution wherever possible. In accordance with its environmental policy, Melbourne Airport manages its land holdings to protect and enhance biodiversity and cultural heritage, whilst at the same time maintaining aircraft safety, which is a key priority. Melbourne Airport also ensures that it complies with all environmental and energy laws, policies, procedures, and other compliance obligations and, where appropriate, exceeds those requirements. A key aspect of the environmental policy is the airport's goal to make the best use of its facilities and design, and where needed, to construct and operate new facilities in support of the airport's environmental and sustainability goals (Australia Pacific Airports Corporation, 2018).

To achieve its environmental-related objectives, the airport authority works closely with its staff, tenants, business partners, regulatory agencies, and local and regional communities to develop new strategies to improve environmental performance whilst also protecting the environment (Australia Pacific Airports Corporation, 2018).

4.2.3 Melbourne Airport Environmental Management System (EMS)

In June 2004, Melbourne Airport became the first Australian airport to receive international certification of its Environmental Management System (EMS). At the time of the present study, Melbourne Airport's Environmental Management System (EMS) was being certified in accordance with the international standard ISO 14001:2015 (Melbourne Airport, 2017b).

4.2.4 Melbourne Airport Photovoltaic (PV) Solar Power System

During the period 2018 to 2021, Melbourne Airport installed three photovoltaic (PV) solar systems. The first system was a 12MW solar array that provides approximately 25% of the airport's energy needs (based on pre-COVID-19 levels), whilst the second system was comprised of a 1.8MW roof array on a warehouse located in the airport's Business Park. The final system was comprised of a 100KW array and 140KW battery solar array that is used to power an on-site water treatment plant (Melbourne Airport, 2017b).

The planning for these projects commenced in in 2018 (Thorn, 2020). Melbourne Airport's solar farm is one of Australia's largest behind the meter solar installations (Bates, 2020; International Airport Review, 2021; Matich 2020). The solar foam occupies an area of around 192,000m² (International Airport Review, 2021). The new solar farm at Melbourne Airport became operational in January 2021 and has the capability to produce enough renewable energy to power all four passenger terminals. The photovoltaic (PV) solar system can generate 17 GW hours of electricity per annum. This is equal to around 15 per cent of the airport's total annual electricity consumption (Air Transport Action Group, 2020; Bates, 2020; Matich 2020).

The solar panels were installed at a site located off Sunbury Road. This site was selected as the location due to the available land, proximity to the airport and the maximum direct sunlight without obstructions (International Airport Review, 2021). The solar farm used Canadian-made solar mono-facial panels that were designed to avoid glare that could otherwise have caused problems around the airport's runways (Jamieson, 2020). To alleviate the possibility of dazzling pilots from the solar system, Melbourne Airport installed fixed ground racking systems (Youd, 2021).

Melbourne Airport has also installed a 2MWdc capacity array on the roof of the Agility Logistics' facility. This system is approximately 30,000m2 in size and was the airport's first rooftop solar structure. The system will connect into the airport's embedded network (International Airport Review, 2021).

A key issue for Melbourne Airport was the surrounding wildlife and birds. To mitigate this problem, the shrubbery planted around the solar farm was kept simple, and does not produce any food for birds, such as seeds and berries (Youd, 2021).

4.2.5 Melbourne Airport Trigeneration System

In December 2016, Melbourne Airport completed the installation of its 8MW trigeneration plant (Australia Pacific Airports Corporation, 2017). The tri-generation power facility became operational in 2017. This system produces power, gas, and cooling. A key benefit of this system is that it turns excess heat into chilled and hot water (Melbourne Airport, 2017b). The airport's tri-generation facility has become a significant contributor to the airport's goal of reducing its carbon footprint (Australia Pacific Airports Corporation, 2017).

The tri-generation plant provides power into the airport's high voltage network. Importantly, both the solar farm and the tri-generation plant can operate in unison. This because the airport's load is more than the sum of what the solar farm and tri-generation plant can produce (International Airport Review, 2021).

4.2.6. The Environmental Benefits of the Solar Power (PV) and Trigeneration Systems at Melbourne Airport

The use of the photovoltaic (PV) solar power and trigeneration systems are helping Melbourne Airport to reduces its energy consumption. The reduction in energy consumption allows the airport to reduce its operational greenhouse gas (GHG) emissions and will help the airport to move to towards carbon neutrality (Melbourne Airport, 2017b).

The trigeneration system is delivering significant environmental-related benefits for Melbourne Airport. The airport has estimated that the system will reduce the airport's carbon dioxide (CO_2) emissions by 920,000 tonnes over a 15 period (Melbourne Airport, 2017b).

In the long-term, Melbourne Airport plans to maintain its commitment and investment in renewable energy projects, thereby minimizing its carbon footprint and maximizing energy efficiency. The airport's solar farm is expected to provide a sustained amount of electricity, thus reducing the dependency for grid electricity. The solar farm will reduce peak electrical demand on the network with Melbourne Airport's peak electrical demand, typically experienced during sunshine hours (International Airport Review, 2021). Like the trigeneration system, the airport's solar systems will also reduce carbon dioxide (CO_2) emissions. This is because photovoltaic (PV) solar power systems do not produce air pollution or greenhouse gases (GHG) (United States Energy Information Agency, 2020).

V. CONCLUSION

Airports are extremely energy intensive and where energy is produced from fossil fuels then there are emissions, such as, carbon dioxide (CO_2), that are harmful to the environment. Considering the environmental impact of their operations, airports around the world are increasingly adopting the use of new "green" technologies to reduce their carbon footprint. This study has examined the approaches taken by Brisbane and Melbourne Airports in Australia to mitigate their carbon footprint using photovoltaic (PV) solar systems. Melbourne Airport is now operating a trigeneration system and this system is also underpinning the airport's goal to reduce to its carbon footprint.

The case study revealed that Brisbane Airport built a large scale 5MWh system on six sites that are located throughout the airport precinct. The system has incorporated both rooftop and ground-based solar panels. The photovoltaic (PV) solar system has delivered Brisbane Airport important environmental related benefits. The photovoltaic (PV) solar system provides around 18% of the airport's electricity and the system will meet approximately 6% of Brisbane Airport Corporation's overall load. In addition, the photovoltaic (PV) solar system has enabled the airport to reduce it carbon footprint, as the system has reduced the airport's carbon dioxide (CO_2) emissions by an estimated 8,000 tonnes per annum.

Like Brisbane Airport, Melbourne Airport has also taken steps to "green' its operations. The case study found that the airport has taken a multi-faceted approach to its energy policy. The trigeneration system works in parallel with the airport's new photovoltaic (PV) solar system, and this system is predicted to reduce the airport's carbon footprint by an estimated 920,000 tonnes over a 15 period. The first system photovoltaic (PV) solar system is a 12MW solar array that will provide approximately 25% of the airport's energy needs (based on pre-COVID-19 levels), whilst the second system is a 1.8MW roof array on a warehouse located in the airport's Business Park and the final system is a 100KW array and 140KW battery solar. the airport's solar systems will reduce the airport's annual carbon dioxide (CO_2) emissions. At the time of the present study, the annual carbon dioxide (CO_2) emissions data was not publicly available.

REFERENCES

[1] Ahmed, S.S.U., Salehin, S., Rahman, M.M., & Sadral Islam, A.K.M. (2017). Harvesting solar energy: Fundamentals and applications. In P. Mondal & A.K. Dalai (Eds.), *Sustainable utilization of natural resources* (pp. 381-416). Boca Raton, FL: CRC Press.

[2] Air Transport Action Group. (2020). Melbourne Airport's sustainable solar approach. Retrieved from https://aviationbenefits.org/newswire/2020/08/melbourne-airport-s-sustainable-solar-approach.

[3] Akyüz, M.K., Altuntaş, O., & Söğüt, M.Z. (2017). Economic and environmental optimization of an airport terminal building's wall and roof insulation. *Sustainability*, 9(10), 1849. https://doi.org/10.3390/su9101849

[4] Akyuz, M.K., Altuntas O., Sogut M.Z., & Karakoc T.H. (2019). Energy management at the airports. In T. Karakoc, C. Colpan, O. Altuntas., & Y. Sohret (Eds.), *Sustainable aviation* (pp. 9-36). Cham: Springer

[5] Andrew, D.P.S., Pedersen, P.M., & McEvoy, C.D. (2011). *Research methods and design in sport management*. Champaign, IL: Human Kinetics.

[6] Anurag, A., Zhang, J., Gwamuri, J., & Pearce, J.M. (2017). General design procedures for airport-based solar photovoltaic systems. *Energies*, 10(8), 1194. https://doi.org/10.3390/en10081194
[7] Arman, H., & Yuksel, I. (2013). Preface. In H. Arman & I Yuksel (Eds.), *New developments in renewable energy* (pp. xi-xii). Rijeka: InTech.
[8] Aswathanarayana, U. (2010). Introduction. In U. Aswathanarayana., T. Harikrishan., & K.M Thayyib Sahini. (Eds.), *Green energy technology, economics, and policy* (pp. 1-4). Boca Raton, FL: CRC Press.
[9] Australia Pacific Airports Corporation. (2017). APAC Annual Report 2016/17. Retrieved from http://www.melbourneairport.com.au/getattachment/Corporate/About-us/Corporate-Information/Annual-reports/2017-Annual-report-(1).pdf.
[10] Australia Pacific Airports Corporation. (2018). Environmental policy. Retrieved from https://www.melbourneairport.com.au/getattachment/Corporate/About-us/Environment/Environment-at-Melbourne-Airport/Melbourne-Airport-Environment-Policy_August-2018.pdf.
[11] Australia Pacific Airports Corporation. (2019). APAC Annual Report 2018/19. Retrieved fromhttp://www.melbourneairport.com.au/getattachment/Corporate/About-us/Corporate-Information/Annual-reports/AP003ia-4-APAC-AR19¬_AW.pdf.
[12] Australian Aviation. (2017). Brisbane Airport unveils solar project. Retrieved from https://australianaviation.com.au/2017/09/brisbane-airport-unveils-solar-project/.
[13] Australian Aviation. (2019a). Brisbane Airport solar panels project reaches practical completion. Retrieved from https://australianaviation.com.au/2019/05/brisbane-airport-solar-panels-project-reaches-practical-completion/.
[14] Australian Aviation. (2019b). Melbourne Airport proposes north-south orientation for third runway. Retrieved from https://australianaviation.com.au/2019/11/melbourne-airport-proposes-north-south-orientation-for-third-runway.
[15] Baek, S., Kim, H., & Joon Chang, H. (2016). Optimal hybrid renewable airport power system: Empirical study on Incheon International Airport, South Korea. *Sustainability*, 8(6), 562. https://doi.org/10.3390/su8060562
[16] Balfour, J.R., Shaw, M., & Nash, N. (2013). *Introduction to photovoltaic system design*. Burlington, MA: Jones & Bartlett Learning,
[17] Barrett, S., Devita, P., Ho. C., & Miller, B. (2014). Energy technologies' compatibility with airports and airspace: Guidance for aviation and energy planners. *Journal of Airport Management*, 8(4), 318-326.
[18] Bates, J. 2020. New solar power plant being built at Melbourne Airport. Retrieved from https://airport-world.com/new-solar-power-plant-being-built-at-melbournes-tullamarine-airport/.
[19] Baxter, G. (2021). Achieving carbon neutral airport operations by 2025: The case of Sydney Airport, Australia. *Transport and Telecommunication*, 22(1), 1-14. https://doi.org/10.2478/ttj-2021-0001
[20] Baxter, G., Srisaeng, P., & Wild, G. (2018a). Sustainable airport waste management: The case of Kansai International Airport. *Recycling*, 3(1), 6. https://doi.org/10.3390/recycling3010006
[21] Baxter, G., Srisaeng, P., & Wild, G. (2018b). An assessment of airport sustainability, Part 2—Energy management at Copenhagen Airport. *Resources*, 7(2), 32. https://doi.org/10.3390/resources7020032
[22] Baxter, G., Srisaeng, P., Wild, G. (2019). Environmentally sustainable airport energy management using solar power technology: The case of Adelaide Airport, Australia. *International Journal for Traffic and Transport Engineering*, 9(1), 81 – 100.
[23] Bhattacharyya, S. 2015. Introduction. In P. Mohanty, T. Muneer, & M. Kolhe (Eds.), *Solar photovoltaic system applications: A guide for off-grid electrification* (pp. 1-6). Cham, Switzerland: Springer Nature Switzerland.
[24] Bhowmik, C., Bhowmik, S.,Ray, A., & Murari Pandey, K. (2017). Optimal green energy planning for sustainable development: A review. *Renewable and Sustainable Energy Reviews*, 71, 796-813. https://doi.org/10.1016/j.rser.2016.12.105
[25] Brisbane Airport. (2018). Brisbane Airport solar upgrade. Retrieved from https://www.bne.com.au/sites/default/files/docs/BAC3687-Solar-Fact-Sheet-v4.pdf.
[26] Brisbane Airport. (2020). Brisbane Airport 2020 master plan. Retrieved from https://www.bne.com.au/sites/default/files/docs/Brisbane-Airport-2020-Master-Plan_0.pdf.
[27] Brisbane Airport. (2021). Guide to Brisbane International Airport. Retrieved from https://www.brisbane-airport.com/.
[28] Brisbane Development. (2017). New solar system set to slash BNE's energy needs. Retrieved fromhttps://brisbanedevelopment.com/new-solar-system-set-to-slash-bnes-energy-needs/.
[29] Budd T. (2017). Environmental impacts and mitigation. In L. Budd & S. Ison (Eds.), *Air transport management: An international perspective* (pp. 283-306). Abingdon, UK: Routledge.
[30] Clean Energy Regulator. (2021a). Large-scale generation certificates. Retrieved from http://cleanenergyregulator.gov.au/RET/Scheme-participants-and-industry/Power-stations/Large-scale-generation-certificates.
[31] Clean Energy Regulator. (2021b). What is a renewable energy certificate (REC)? Retrieved from https://rec-registry.gov.au/rec-registry/app/public/what-is-a-rec.
[32] Comendador, V.F.G., Valdés, R.M.A., & Lisker, B. (2019). A holistic approach to the environmental certification of green airports. *Sustainability*,11(5), 4043. https://doi.org/10.3390/su11154043
[33] Curran, S. (2016). Economic viability of using grid-connected photovoltaic energy in an airport in Jamaica. *The Journal of the Association of Professional Engineers of Trinidad and Tobago*, 44(1), 16-21.
[34] Daley, B. (2016). *Air transport and the environment*. Abingdon, UK: Routledge.

[35] Dentch, M.P. (2016). *The ISO 14001:2015 handbook: Using the process approach to building an environmental management system*. Milwaukee, WI: Quality Press

[36] González-Ruiz,.D., Duque, E., Restrepo, J. 2017a. Green airport infrastructure in Colombia: Opportunities for public-private partnerships schemes. *Pertanika Journal of Science & Technology*, 25, 37 – 46.

[37] Graham, A. (2018). *Managing airports: An international perspective* (5th ed.). Abingdon, UK: Routledge.

[38] Grandy, G. (2010). Instrumental case study. In A.J. Mills., G. Durepos., & E. Wiebe (Eds.), *Encyclopedia of case study research*, Volume 1 (pp. 473-475). Thousand Oaks, CA: SAGE Publications.

[39] Grover, R.C., & Grover, S. (2017). *Winning the environmental challenge with ISO 14001: 2015: Implementation of an Environmental Management System*. Chennai, India: Notion Press.

[40] Halcol Energy. (2019). Brisbane Airport gets solar upgrade. Retrieved from https://halcolenergy.com.au/solar-news/brisbane-airport-gets-solar-upgrade/.

[41] Heras-Saizarbitoria, I., Landín, G.A., & Molina-Azorín, J.F. (2011). Do drivers matter for the benefits of ISO 14001? *International Journal of Operations and Production Management*, 31(2), 192-216. https://doi.org/10.1108/01443571111104764

[42] International Airport Review. (2021). The sun shines on Melbourne Airport's new solar farm. Retrieved from https://www.internationalairportreview.com/article/146676/ melbourne-airport-solar-farm/.

[43] International Renewable Energy Agency. (2021). Benefits. Retrieved from https://www.irena.org/benefits.

[44] Jamieson, E. (2020). Melbourne Airport using down time to open solar farm in 2021. Retrieved from https://globetrender.com/2020/10/13/melbourne-airport-solar-farm/.

[45] Janić, M. (2011). *Greening airports: Advanced technology and operations*. London, UK: Springer-Verlag.

[46] Janić. M. (2017). *The sustainability of air transportation: A quantitative analysis and assessment*. Aldershot, UK: Ashgate Publishing.

[47] Jarach, D. 2017. *Airport marketing: Strategies to cope with the new millenium*. Abingdon, UK: Routledge.

[48] Kandt, A., & Romero, R. (2014). Siting solar photovoltaics at airports. Retrieved from https://www.nrel.gov/docs/fy14osti/62304.pdf.

[49] Kalyani, V.L., Dudy, M.K., Pareek, S. (2015). Green energy: The need of the world. *Journal of Management Engineering and Information Technology*, 2(5), 18-26.

[50] Kramer, L.S. (2010). *Airport revenue diversification: A synthesis of practice*. Airport Cooperative Research Program Synthesis 19. Washington, DC: Transportation Research Board.

[51] Lenaghan, N. (2017). Brisbane Airport to save $1m a year from massive solar array. Retrieved from https://www.afr.com/property/brisbane-airport-to-save-1m-a-year-from-massive-solar-array-20170915-gyikfc.

[52] Maisch, M. (2019). Brisbane welcomes Australia's largest airport PV installation. Retrieved from https://www.pv-magazine-australia.com/2019/05/10/brisbane-welcomes-australias-largest-airport-pv-installation/.

[53] Matich, B. (2020). Fly me to the sun, Melbourne Airport's record solar farm. Retrieved from https://www.pv-magazine-australia.com/2020/08/07/fly-me-to-the-sun-melbourne-airports-record-solar-farm/.

[54] Melbourne Airport. (2017a). Connecting Victoria to the world since 1 July 1970. Retrieved from https://www.melbourneairport.com.au/Corporate/About-us/50th-anniversary#.

[55] Melbourne Airport. (2017b). Environment at Melbourne Airport. Retrieved from https://www.melbourneairport.com.au/Corporate/About-us/Environment/Environment-at-Melbourne-Airport.

[56] Melbourne Airport. (2017c). Passenger information: Maps. Retrieved from https://www.melbourneairport.com.au/Passengers/Passenger-information/Maps.

[57] Mostafa, M.F.A., Abdel Aleem, S.H.E., & Ibrahim, A.M. (2016). Using solar photovoltaic at Egyptian airports: Opportunities and challenges. In *Proceedings of the Power Systems Conference (MEPCON), 2016 Eighteenth International Middle East, Cairo, Egypt, 27-29 December*, 73-80.

[58] Mostafa, M.F.A., Abdel Aleem, S.H.E., & Zobaa, A.F. (2016). *Risk assessment and possible mitigation solutions for using solar photovoltaic at airports. In Proceedings of the Power Systems Conference (MEPCON), 2016 Eighteenth International Middle East, Cairo, Egypt, 27-29 December*, 81-88.

[59] Oates, B.J. (2006). *Researching information systems and computing*. London, UK: SAGE Publications.

[60] O'Leary, Z. (2004). *The essential guide to doing research*. London, UK: SAGE Publications.

[61] Oliveira, J.A., Oliveira, O.J., Ometto, A.R., Ferraudo, A.S., & Salgado, M.H. (2011). Environmental management system ISO 14001 factors for promoting the adoption of cleaner production practices. *Journal of Cleaner Production*, 133, 1384-1394. https://doi.org/10.1016/j.jclepro.2016.06.013

[62] Ortega Alba, S., & Manana, M. (2016). Energy research in airports: A review. *Energies*, 9(5), 349. https://doi.org/10.3390/en9050349

[63] Ortego Alba, S.O., & Manana, M. (2017). Characterization and analysis of energy demand patterns in airports. *Energies*, 10(1), 119. https://doi.org/10.3390/en10010119

[64] Owens, D.C., Sadler, T.D., & Friedrichsen, P. (2021). Teaching practices for enactment of socio-scientific issues instruction: An instrumental case study of an experienced biology teacher. *Research in Science Education*, 51, 375–398. https://doi.org/10.1007/s11165-018-9799-3

[65] Paul, J., & De Groot, E. (2010). Strategic planning: Study of Brisbane Airport. *Asian Case Research Journal*, 14(2), 1-29. https://doi.org/10.1142/S0218927510001416

[66] Radomska, M., Chernyak, L., & Samsoniuk, O. (2018). The improvement of energy-saving performance at Ukrainian airports. In T.H. Karakoç, C. Ozgur Colpan,, & Y. Şöhret

(Eds.), *Advances in sustainable aviation* (pp.189-203). Cham, Switzerland: Springer International Publishing.

[67] Rossi Dal Pozzo, F. (2015). *EU legal framework for safeguarding air passenger rights*. Cham, Switzerland: Springer International Publishing.

[68] Sadler, K. (2015). Melbourne Airport Terminal 4 officially opens. Retrieved from https://www.internationalairportreview.com/news/21446/melbourne-airport-terminal-4-officially-opens.

[69] Scott, J. (2014). *A dictionary of sociology* (4th ed.). Oxford, UK: Oxford University Press.

[70] Scott, J., & Marshall, G. (2009). *A dictionary of sociology* (3rd ed.). Oxford, UK: Oxford University Press.

[71] Senguttuvan, P.S. (2007). *Principles of airport economics*. New Delhi, India: Excel Books.

[72] Shakra Energy. (2019). Construction underway of massive solar installation at Brisbane Airport. Retrieved from https://shakraenergy.com/brisbane-airport-corporation/.

[73] Solanki, C.S., & Singh, H.K. (2017). *Anti-reflection and light trapping in c-Si solar cells*. Singapore: Springer Nature Singapore.

[74] Solomon, L.D. (2009). *The promises and perils of infrastructure privatization: The Macquarie Model*. New York, NY: Palgrave Macmillian.

[75] Sorenson, R. (2021). "How can I get better?": An instrumental case study of three experienced music teachers and their journey to expertise. *Contributions to Music Education*, 46, 225-244.

[76] Sreenath, S., Sudhakar, K., & Yusop, A.F. (2020). Technical assessment of captive solar power plant: A case study of Senai Airport, Malaysia. *Renewable Energy*, 152, 849-866. https://doi.org/10.1016/j.renene.2020.01.111

[77] Sreenath, S., Sudhakar, K., & Yusop, A.F. (2021a). Effect of PV material, texturing, orientation and tracking on glare impact: A simulation study from an Indian Airport. *Cleaner Engineering and Technology*, 4, 100236. https://doi.org/10.1016/j.clet.2021.100236

[78] Sreenath, S., Sudhakar, K., & Yusop, A.F. (2021b). Energy-exergy-economic-environmental-energo-exergo-enviroecono (7E) analysis of solar photovoltaic power plant: A case study of 7 airport sites in India. *Sustainable Energy Technologies and Assessments*, 47, 101352. https://doi.org/10.1016/j.seta.2021.101352

[79] Stake, R.E. (1995). *The art of case study research*. Thousand Oaks, CA: SAGE Publications.

[80] Stake, R.E. (2005). Qualitative case studies. In N.K. Denzin & Y.S. Lincoln (Eds.), *The SAGE handbook of qualitative research* (pp. 443-466). Thousand Oaks, CA: SAGE Publications.

[81] Sukumaran, S., & Sudhakar, K. (2017). Fully solar powered Raja Bhoj International Airport: A feasibility study. *Resource Efficient Technologies*, 3(3), 309-316. https://doi.org/10.1016/j.reffit.2017.02.001

[82] Thorn, A. (2020). Melbourne's gigantic solar farm to power on in January. Retrieved from https://australianaviation.com.au/2020/08/melbournes-gigantic-solar-farm-to-power-on-in-january.

[83] Tretheway, M., & Markhvida, K. (2013). Airports in the aviation value chain: Financing, returns, risk and investment. Retrieved from https://www.itf-oecd.org/sites/default/files/docs/dp201315.pdf.

[84] Trina Solar. (2019). Australia's largest airport solar installation achieves practical completion. Retrieved from https://www.trinasolar.com/au/resources/newsroom/auaustralia%E2%80%99s-largest-airport-solar-installation-achieves-practical-completion.

[85] United States Energy Information Agency. (2020). Solar explained: Solar energy and the environment. Retrieved from https://www.eia.gov/energyexplained/solar/solar-energy-and-the-environment.php.

[86] United States Environmental Protection Agency. (2021). Local renewable energy benefits and resources. Retrieved from https://www.epa.gov/statelocalenergy/local-renewable-energy-benefits-and-resources.

[87] West, J.J., Stanley, A.M., Bowers, J.P., & Isbell, D.S. (2021). Attrition, (de)motivation, and "effective" music teacher professional development: An instrumental case study. *Bulletin of the Council for Research in Music Education*, 229, 7-28.

[88] Wybo, J.L. (2013). Large-scale photovoltaic systems in airport areas: Safety concerns. *Renewable and Sustainable Energy Reviews*, 21, 402-410. https://doi.org/10.1016/j.rser.2013.01.009

[89] Yerel Kandemir S., & Yayli, M. Ö. (2016). Investigation of renewable energy sources for airports. In T. Hikmet, M. Baris Ozerdem, C.O. Colpan, O. Altuntas, E. Acikkalp (Eds.), *Sustainable aviation: Energy and environmental issues* (pp. 11-16). Cham, Switzerland: Springer International Publishing.

[90] Yin, R.K. (2018). *Case study research and applications* 6th ed.). Thousand Oaks: SAGE Publications.

[91] Youd, F. (2021). Sustainable energy: The airports harnessing green energy. Retrieved from https://www.airport-technology.com/features/sustainable-energy-the-airports-harnessing-green-energy/.

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Impact of silver nanoparticles on enhancing*in vitro* proliferation of embryogenic callus and somatic embryos regeneration of Date palm cv. Hayani

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***Abstract*—*The growth and development of in vitro plants are aided by silver nanoparticles. The Phoenix dactylifera is one in all the economically important fruit crops in many Arab countries. During this study, the various concentrations of Ag NPs (0, 0.1, 0.5, 1.0, 2.0, and 4.0 ml/l) were added to MS basal medium to judge their effects on the embryogenic callus proliferation, differentiation, and development, regeneration of somatic embryos of feather palm Hayani cv. to check the consequences of Ag NPs, research with two separate experiments was conducted. Within the first experiment, MS basal salt medium containing 3.0 mg/l 2,4-D, 1.0 mg/l 2ip (mg/l), and different concentrations of Ag NPs were wont to determinate the embryogenic callus proliferation under in vitro conditions. While within the second experiment, the effect of MS basal medium supplemented with 0.05 NAA, 0.1 BA (mg/l), and different concentrations of Ag NPs were examined on regeneration of somatic embryos. Results of the primary experiment indicated that various concentrations of Ag NPs had a significantly affected on the embryogenic callus proliferation and substantially increased somatic embryos formation on the callus when added Ag NPs at 1.0 ml/l in MS basal medium. Within the second experiment, the appropriate medium for regeneration of somatic embryos was added 1.0 ml/l Ag NPs intothe medium which had a positive effect on the number of somatic embryos and registered to the utmost number of somatic embryos 35.30 embryo/jar with the best length of embryos 1.80 cm, the best number of leaves 43.72 leaf/jar and also the highest length 3.87 cm. During this treatment, the full chlorophyll content was 2.584 mg/g. Further, higher Ag NPs concentrations had negative effects. There was genetic stability between shoots sample which exposure with 1.0 Ag NPs and therefore the mother plant.*

Keywords*— Date palm, Ag NPs, In vitro, Embryogenic callus, Somatic embryos, Chlorophyll content and RAPD (PCR) Molecular Marker.

I. INTRODUCTION

Date palm (*Phoenix dactylifera* L.) could be a monocotyledonous and dioecious species that belongs to the Arecaceae family and is recognized because the most vital fruit tree in many Arab countries; like Saudi Arabia and Iraq.

A feather palm tree is one among the traditional fruit crops cultivated in North Africa and therefore the Middle East **(Masmoudi-Allouche*et al*., 2011)**. In addition, it's a multi-purpose tree that's utilized in ornamental and landscape designs; Also, different parts of the tree such as; seeds, leaves, and trunk even have other uses that bring extra profit to this point palm growers **(Al-Khalifah and Shanavaskhan, 2012)**. The date palm tree has a necessary role within the improvement of sustainable agriculture in several drought and salinity damaged regions**(Khierallah and Hussein, 2013)**.

Commonly, a feather palm tree is propagated via seeds or offshoots, but plantlets produced from seeds aren't identical and have less quality than the mother tree. While propagation using offshoots is that the best method, the number of offshoots produced by the mother tree is proscribed especially in rare cultivars. The survival rate of offshoots is low and a high chance of infection attributed to the abundance of pests happens. Thus to beat these problems and produce a high number of plants freed from disease, it's necessary to develop another method of propagation, like the employment of plant structure culture technique**(Eshraghi*et al*., 2005)**.

One of these options is micro-propagation, which **is that the** true-to-type propagation of a genotype using *in vitro* culture procedures **(Al-Khalifah and Shanavaskhan, 2012)**. The tissue culture technique has numerous advantages, and plants have proven to be popular among farmers. These benefits include the ability to supply disease and pest-free cultivars of plants with desired qualities, large-scale multiplication of an outsized number of plantlets at any time of year, the ability to propagate elite cultivars that lack offshoots and produce seed only derived plants. Additionally to, avoiding the extent of plant quarantine regulations by facilitation of the exchange of plant materials between laboratories for research purposes without the chance of disease or pest transmission**(Johnson, 2011)**.*In vitro* date palm plantlets are produced by either somatic embryogenesis or organogenesis **(Mazri and Meziani, 2015)**. The *in vitro* pathway of somatic embryogenesis involved the induction of embryogenic callus which differentiation to somatic embryogenesis **(Al-Samir, 2015)**. Somatic embryogenesis is that the best date palm micro-propagation regeneration process. **(Fki*et al*., 2003)**. It's noted to be a swift and economical way for extensive propagation of *Phoenix dactylifera* and will even be extremely suitable for breeding programs **(El-Hadrami*et al*., 1998)**. Lots of studies are conducted to enhance the somatic embryogenesis of date palm by altering substance components and physical conditions **(Al-Khairy and Al-Bahrany, 2012; Baharan*et al*., 2015)**. Several previous studies of *Phoenix dactylifera* micro-propagation using the callus and somatic embryo pathways are published. **(McCubbin*et al*., 2000; Fki*et al*., 2011)**. On the opposite hand, Apical meristem tissues from axillary and lateral offshoots are now used because the widely accepted source of explants for date palm tissue culture after showing promising results **(Al-Khalifah*et al*., 2013; Hoffmann *et al*., 2013)**. In general, the development of cultured cells *in vitro* is especially dependent on the nutritional components and plant growth regulators **(Lima *et al.*, 2012)**.

Nanotechnology may be a technology applied at the nano-scale, involved the study of too small materials and its application among many other science fields, like chemistry, physics, biology, engineering, and agriculture. The worth of agricultural products is increased through the utilization of Nanotechnology and helps reduce environmental problems. Nanoparticles and powders have big reactivity because of extended specific surface area; these characteristics make the absorption of fertilizers that produced in nano-scale easier**(Mousavi and Rezaei, 2011)**.Present day, nanotechnology has introduced new valuable components and substances that help progress in ecological and environmental researches **(Miller and Senjan, 2006)**.Tiny metal-based nanoparticles (NPs) of 1–100 nm in size and is extremely low quantity has been tested as substitute plant mineral nutrients and stimulants due to the fast development of nanotechnology **(Saxena*et al*., 2016)**.

Several experiments have examined the influence of different nanoparticles on a number of commercially valuable plant species **(Monica and Cremonini, 2009; Krishnaraj*et al*., 2012)**.The main advantage of using nanoparticles in the field of biotechnology is focused on antimicrobial and nutrient properties and improvement of plant growth with different pathways. As date palm is an important traditional crop, many efforts have been made to provide higher yields and quality. In-plant tissue culture, there are promising indications of the utility of nano-materials to improve plant nutrition, plant growth, and development, seed germination, enhance plant growth and yield; enable plant genetic modification, improve bioactive compound production and achieve plant protection and tolerance to diseases**(Wang *et al*., 2016)**.

Silver Nanoparticles (Ag NPs) are a non-toxic substance with strong antimicrobial properties against fungi, bacteria, and viruses **(Abdi *et al*., 2008)**. The use of Ag NPs has grown in recent years as nanotechnology has advanced **(Luoma, 2008)**. *Brassica juncea*, common bean and corn plants increased their growth processes (shoot length, root length and leaf area) as well as biochemical parameters (chlorophyll, starch, protein content and antioxidant enzymes) **(Salama, 2012; Sharma *et al*., 2012)**. Moreover, the inclusion of Ag NPs in the plant tissue culture medium for the treatment of microbial contamination did not adversely affect shoot multiplication and subsequent rooting **(Gouran*et al.* 2014)**, on the contrary it proved its efficacy in different plant species **(Kumar *et al*., 2007)**.

The chlorophyll content may be a physiological characteristic that may affect plantlet quality, survival, growth, and development after transplantation. Chlorophyll could be a photoreceptor, an indicator of the photosynthetic potential of plants, and a catalyst for the conversion of sunlight into energy. It plays a significant role within the photochemical synthesis of carbohydrates **(Oliveira *et al*., 2016)**.

The aim of this study was to see how Ag NPs affected the growth and proliferation of embryogenic callus. Also,study the added impact of Ag NPs to medium on improvement and development of somatic embryos of date palm.

II. MATERIALS AND METHODS

This study was carried out in the Department of Breeding, Horticulture Research Institute, Agricultural Research Center, Giza, Egypt during 2018-2020.

2.1Plant material

The healthy offshoots of 5–7 kg in weight and 50–70 cm in length from date palm cv. Hayani were selected and detached from healthy, disease-free mother plants. The offshoots were carefully removed; the leaves were cut using a sharp knife until the shoot tip zone was exposed (apical meristems with leaf primordia). To avoid browning, the explants were washed in flowing tap water for 1 hour before being soaked in an antioxidant solution containing 150 mg/l ascorbic acid and 100 mg/l citric acid for 30 minutes.

2.2 Surface sterilization the explants

Surface sterilization was carried out to eliminate contamination agents. For sterilization under a sterile hood, the explants were removed from the antioxidant solution, then dipped in 70% ethanol for 1 minute and then surface sterilized with 0.1 mg/l $HgCl_2$ containing few drops of Tween-20 with continuous stirring for 75 minutes followed by washing with sterile distilled water 3 times and remove 1–2 leaves. After sterilization, the date palm shoot tips were divided longitudinally into eight segments and cultured on callus induction medium.

2.3 Culture medium

The culture medium used for *in vitro* cultures was the basal salts MS **(Murashige and Skooge, 1962)** supplemented with $Na_2H_2PO_4$ (170.0 mg/l), myo-inositol (100.0 mg/l), glutamine (200.0 mg/l), nicotinic acid (0.5 mg/l), pyridoxine-HCl (0.5 mg/l), thiamine (1.0 mg/l), 40 mg/l adenine sulphate, sucrose (40 g/l), agar (6 g/l), 0.5 g/l of activated charcoal. The pH of the medium was adjusted to 5.7, after that the media was dispensed into small jars (150 ml) in aliquots of 40 ml per jar and capped with polypropylene closures. Subsequently the media were autoclaved at 121 °C and 1.04 kg/cm^2 for 15 min.

2.4 Callus initiation

For callus culture initiation, the pieces of shoot tips were cultured into callus induction medium composed of basal salts MS with additional 10.0 mg/l Di-chlorophenoxy acetic acid (2,4-D) and 3.0 mg/l Isopentenyl adenine (2ip) **(Bekheet*et al.*, 2007)**. All cultures were incubated in a culture room under darkness at 27 ± 2 °C until initiation of white soft callus. The shoot tip explants were subcultures on the same medium and growth conditions every 4 weeks for 4 subcultures.

The primary callus was transferred on the same basal medium with added 5.0 mg/l 2,4-D and 2.0 mg/l 2ip, more callus growth occurs that can be easily separated from the original explants, after three subcultures with four weeks interval induced the embryogenic callus.

Isolate callus growth from original explants and transfer to callus multiplication medium which containing 3.0 mg/l 2,4-D and 1.0 mg/l 2ip and maintain in darkness until desired amount of callus.

2.5 Explant material and treatments used in this experiment:

2.5-1 Ag NPs characterization

Spherical silver nano-powder was purchased from Sigma-Aldrich Corporation. The particle size of Ag NPs was 10 nm, 0.02 mg/ml in aqueous buffer, and sodium citrate was used as a stabilizer, according to the manufacturer. Molecular weight at 107.87 and the pack size at 25 ml **(Fig. 1)**.

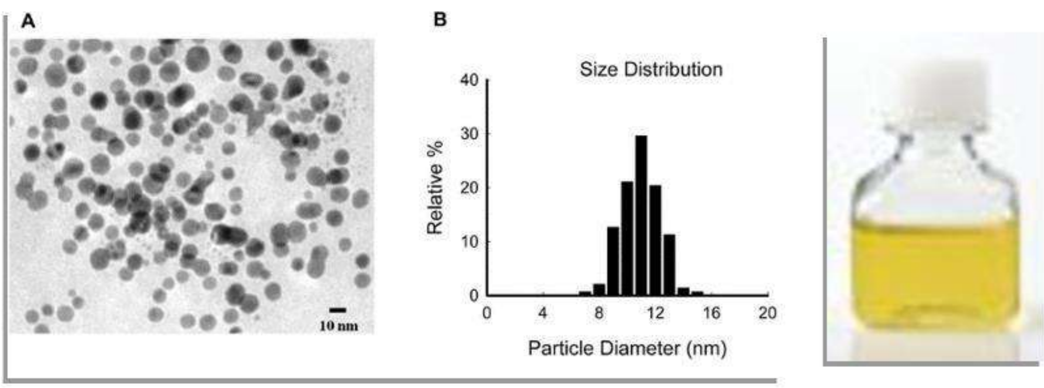


Fig. 1. Characterization of silver nanoparticles. Particles are mostly circular in shape with the average size of 10 nm by using scanning electron microscope

2.5-2 Effect of Ag NPs concentrations embryogenic callus growth and proliferation

The embryogenic callus at 0.5–1.0 g were used as the explant during this experiment to study the effect of Ag NPson the differentiation of embryogenic callus and improvement the growth of somatic embryos during two subcultures and each culture interval 4 weeks. Six treatments were used, five treatments from different concentrations of the Ag NPs at (0.1, 0.5, 1.0, 2.0 and 4.0 ml/l) were addition to MS basal medium supplemented with 2,4-D at 3.0 mg/l + 2ip at 1.0 mg/l and the treatment without Ag NPs was control.The each treatment contained 6 replicates.After the second subculture, the fresh weight of embryogenic callus was measured and also the number of somatic embryos induction on callus was counted.

2.5-3Effect of Ag NPs concentrations on growth and regeneration of somatic embryos

Transfer the small cluster consist of 6-10 somatic embryos of date palm cv. Hayani to differentiation medium which consist of MS basal medium supplemented with 0.05 mg/l Benzyl adenine (BA) + 0.1 mg/l Naphthalen acetic acid (NAA) **(Omar, 1988)**and added the different concentrations of Ag NPs, the cultures were incubated under light condition with 1500 lux for 16 hrs and 8 hrs dark at 27 ±2 °C. The somatic embryos were re-cultured on the fresh medium during two subcultures, each culture interval 4 weeks. At the end of experiment, the number of embryos/jar, length of embryos (cm), number of leaves/jar and length of leaves (cm) were measured.

2.6 Biochemical analyses

The chlorophyll content were determination leaves,Chlorophyll A, B and Carotenoids content (mg/g) as described by **(Lichtenthaler and Buschmann, 2001)** using Thermo Scientific, Orion Aqua Mate 8000, UV-Visible Spectrophotometer at wave lengths 660, 640 and 440 nm.

2.7 Detection of genetic stability at DNA Level Using RAPD (PCR) Molecular Marker

2.7-1 DNA extraction

Total DNA was extracted from mother plant of date palm Hayani cv. and*in vitro* shoots were exposure to 1.0 ml/l of Ag NPs. This work was carried out in the Horticulture Research Institute, Agricultural Research Center. DNA extraction was performed according to a specific procedure detailed by **Marzachì*et al*. (1999)**.Green fresh tissues were collected from healthy shoots *in vitro*. The genomic DNA was re-suspended in sterile distilled water in a volume of 100 liters.

2.7-2 PCR amplification

In the PCR reactions, the isolated DNA was used. Amplification was conducted using twelve primers PCRamplification, as shown in **Table (1).** was performed by adding 10 mg of template DNA to a solution consisting of 10 µM Tris-HCl, pH 9.0, 50 µM KCl, 0.1% Triton X-100, 1.5 µM $MgCl_2$, 200 µM DNTPs each, 0.4 µM primers and 2 units of Taq polymerase. The reaction volume was 25 µl. Thermal cycling parameters consisted of 45 cycles and annealing temperatures of 36 °C), 1 min denaturation at 95 °C (except for the first cycle: 5 min), 1 min annealing and 2 min extension at 72 °C (except for the last cycle: 5 min).

2.7-3 Gel Analysis

Amplified products were analyzed by electrophoresis in 1% agarose gel, stained with ethidium bromide and visualized with a UV transilluminator.

Table 1. The sequencing of Random Amplified Polymorphic DNA (RAPD) marker of mother plant of date palm Hayani cv. and sample of shoots exposure to 1.0 ml/l of Ag NPs

Numbe	Primer	Sequencing
1	OPA-1	CAGGCCCTTC
2	OPA-2	TGCCGAGCTG
3	OPA-3	CTCAGTCGCA
4	OPA-4	GTGAGGCGTC
5	OPA-5	GGACCCAACC
6	OPA-6	AGGGGTCTTG
7	OPA-7	TTGGCACGGG
8	OPA-8	GTGACGTAGG
9	OPA-9	GTGATCGCAG
10	OPA-10	AGTCAGCCAC
11	OPA-11	GAAACGGGTG
12	OPA-12	GGACCCAACC

2.8 Statistical analysis

With six replicates in each treatment, the experimental design was completely randomized. The best three results from each treatment were statistically analyzed using MSTAT Computer Program. To verify differences among means of various treatments, means were compared using Ducan's Multiple Range Test as described by **Ducan (1955).**

III. RESULTS AND DISCUSSION

3.1 Effect of Ag NPs concentrations embryogenic callus growth and proliferation

Many physiological and developmental changes during plant cellular growth are caused by the scale, concentration, and association of nano-materials with plant cells **(Khodakovskaya*et al.*, 2012)**.The results as demonstrated in **Table (2) and Fig. (2)** showed that the applications of Ag NPs significantly affected on callus growth and proliferation. The different doses of Ag NPs were employed *in vitro* on MS basal medium supplemented with 3.0 mg/l 2,4-D and 1.0 mg/l 2ip. Results indicated that a significant increase occurred in the fresh weight of embryogenic callus at 1.0 ml/l Ag NPs which recorded the maximum value 4.60 g/jar compared with the control treatment 2.52 g/jar, while a significant decrease in the fresh weight of callus obtained in the treatments 2.0 and 4.0 ml/l Ag NPs which resulted the lowest value 2.15 and 1.26 g/jar, respectively.

Added Ag NPs to medium were resulted increase in callus differentiation and produced somatic embryos, these results dependent on a dose, the concentration of Ag NPs at 0.5 ml/l was an average response which recorded 13.60 embryo/jar. The greatest results were found with MS medium supplemented with 3.0 mg/l 2,4-D, 1.0 mg/l 2ip and 1.0 ml/l Ag NPs, where the number of somatic embryos initiated on callus increased to 19.39 embryo/jar compared with control MS medium which recorded 6.13 embryo/jar. However, further increases in Ag NP concentration decreased the somatic embryos initiated. Addition the concentrations of Ag NPs at 2.0 and 4.0 ml/l, the induction response decreased further to 7.20 and 2.33 embryo/jar, respectively.

The auxin to cytokinin ratio has been shown to assess calli regeneration, and increasing the augmented auxin to cytokinin ratio suppresses calli plant regeneration frequency **(Din *et al.*, 2016)**. The cell culture which exposure to metallic NPs has been promote positive effects on callus induction, shoot regeneration and *in vitro* growth **(Kim*et al.*, 2017)**. Ag NPs can enhance the plant cell's nutrient and water uptake from the culture media by mutilating the cell wall, according to the mechanism of action of Ag NPs in plant cell development **(Ali *et al.*, 2018)**.

Table 2. Effect of Ag NPs concentrations on growth and proliferation of embryogenic callus of in vitro date palm after second subculture

Concentration of Ag NPs (ml/l)	F.W. of embryogenic callus (g)	Number of somatic embryos (embryo/jar)
0.0	2.52 d	6.13 e
0.1	3.24 bc	8.54 c
0.5	3.89 b	13.60 b
1.0	4.60 a	19.39 a
2.0	2.15 e	7.20 cd
4.0	1.26 f	2.33 f

Mean values followed by the same letter(s) within a column are not significantly different (P <0.05)

Low concentrations of NPs had an inductive effect on callus development and subsequent shoot regeneration in the current sample. Authors,it's conceivable that tiny NPs can join explants and affect certain genetic reprogramming features **(Shirazi and Ramezanpour, 2016)**. At a concentration of 20 mg/l, Ag NP induced a significant rise in ethylene and ABA levels, which was detrimental to callus regeneration. Increased ABA levels in plants are a symptom of stress and are dangerous to their growth and production **(Rai*et al.*, 2011)**.

The addition of Ag NPs to the culture media had an important effect on narrow-leaved lavender growth and production *in vitro*. The results differed depending on the metal concentration and the form of NP used. The lavender plants that were exposed to the lowest levels of NPs (1–5 mg dm3 Ag NPs) developed shoots **(Jadczak*et al.*, 2019)**. Determining the optimum concentration of any nano particles at which a plant cell can attain full and stable growth is very important for preventing toxicity concerns related to nanomaterial use on plants. When bean plant explants were treated with various amounts of Ag NPs, the most growth parameters during callus growth, like callus induction percent and biomass, were observed at the optimum concentration (50 mg/ml)**(Mustafa *et al.*, 2017)**. The effects of Ag NPs on the growth and anatomy of *solanumnigrum* callus *in vitro* culture were investigated, furthermore as changes in *S. nigrum*callus morphology, anatomy, including biomass (weight), and deforming cell shape and color, similarly as genetic instability in callus exposed to Ag NPs. They stated that exposure to Ag NPs increased callus fresh weight**(Emad*et al.*, 2015)**.

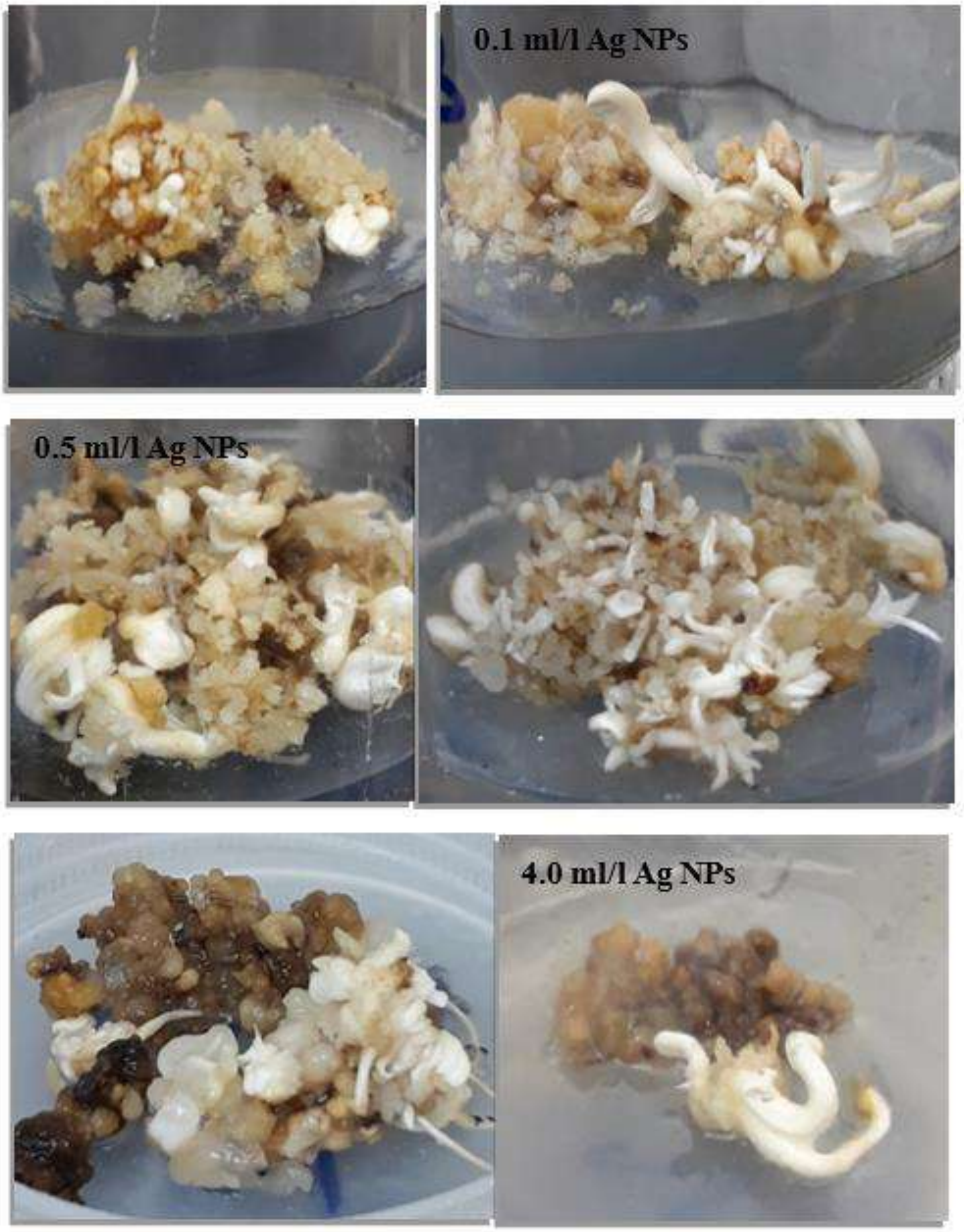


Fig. 2. Effect of different concentrations of Ag NPs on development and proliferation of callus embryogenic date palm Hayani cv. after the second subculture

The results of 8.0 mg/l Ag NPs together with 5.0 mg/l BA and 3.0 mg/l NAA on the event of callus in MS culture media were detected. There is the positive role of Ag NPs on callus induction and growth in the cultures of *Lycopersicumesculentum* supplemented with Ag NPs**(Alia *et al.*, 2019)**. When compared to the control medium, which had a callus induction frequency of 62%, the callus induction response was highest with Ag NPs concentrations of 10 mg/l, with callus induction frequency of 82%, followed by 69%% with 5 mg/l Ag NPs, 37% with 15 mg/l Ag NPs and 16% with 20 mg/l Ag NPs. The calli had a friable texture and a creamy appearance at lower concentrations of Ag NPs, but because the concentrations of Ag NPs rose, the calli became brown. The results of Ag NPs varied after the mediated calli were transferred to regeneration medium. Greening and organogenesis, on the opposite hand, decreased significantly because the concentration of NPs within the culture medium increased **(Manickavasagam *et al.*, 2019)**.

3.2 Effect of Ag NPs concentrations on growth and regeneration of somatic embryos

Results revealed that when adding Ag NPs into the cultivation medium, the response positively resulted on the number of somatic embryos initiated on callus, length of embryos, differentiation of embryos to leaves and length of leaves which supports the findings of **Salama (2012)** and **Sharma *et al.* (2012)** who found that Ag NPs increased root, shoot and leaf development as well as biochemical parameters.

The results as demonstrated in **Table (3) and Fig. (3)** showed that added Ag NPs into the culture medium had a positive effect on the number of somatic embryos. The different concentrations of Ag NPs in proliferation medium were caused produce the highest number of somatic embryos. The treatment with Ag NPs at 0.5 ml/l was resulted in high number of embryos 26.12 embryo/jar, by increasing the concentration of Ag NPs to 1.0 ml/l recorded an increase the number of somatic embryos and registered the maximum value as 35.30 embryo/jar.The other treatments of Ag NPs at 2.0 ml/l and 4.0 ml/l were recorded the lowest number of somatic embryos 17.83 and 10.66 embryo/jar, respectively compared with the control treatment 15.0 embryo/jar. These results were in good agreement with **Mahendran*et al*. (2018)** who reported the highest percentage of somatic embryo production of *Gloriosasuperba*was achieved on the MS medium containing 0.4 mg/l Ag NPs.

Moreover, there was a significant difference in the length of embryos with different concentrations of Ag NPs as shown in **Table (3) and Fig. (3)**. The highest length of embryos 1.80 cm was observed with the treatment 1.0 ml/l of Ag NPs. The other concentrations of Ag NPs (0.1, 0.5, 2.0 and 4.0 ml/l) were recorded a decrease in the embryos length as 0.50, 1.22, 0.65 and 0.33 cm, respectively.

There was a significant difference in the number of leaves by using the different concentrations of Ag NPs. There was an increasing gradually with different treatments of Ag NPs, the treatment 0.1 ml/l Ag NPs was resulted the number of leaves 25.60 leaf/jar which increased to 31.00 leaf/jar with increasing the concentration of Ag NPs to 0.5 ml/l. The highest number of leaves 43.72 leaf/jar were recorded with a treatment 1.0 ml/l Ag NPs. However, increasing Ag NPs to 2.0 ml/l caused the decrease in the number of leaves to 20.14 leaf/jar and also a treatment of Ag NPs at 4.0 ml/l recorded 10.35 leaf/jar which was lower than that in the control treatment 18.32 leaf/jar.

On the other hand, there was a significant difference in the length of leaves with the highest length 3.87 cm recorded with the treatment 1.0 ml/l of Ag NPs. The length of leaves decreased to 1.20 cm by increasing Ag NPs concentration to 2.0 ml/l. The lowest (0.1 ml/l) and highest (4.0 ml/l) Ag NPs concentrations recorded the least leaf lengths 0.58 and 0.65 cm respectively, compared with the control treatment 0.76 cm.

Table 3. Effect of different concentrations of Ag NPs on the development of somatic embryogenesis and shoots proliferation of in vitro date palm after the second subculture

Concentration of Ag NPs (ml/l)	No. of embryos (embryo/jar)	Length of embryos (cm)	No. of leaves (leaf/jar)	Length of leaves (cm)
0.0	15.00 e	0.76c	18.32 d	0.76 d
0.1	19.58 c	0.50 d	25.60 c	0.58e
0.5	26.12 b	1.22 b	31.00 b	1.92 b
1.0	35.30 a	1.80 a	43.72 a	3.87 a
2.0	17.83 cd	0.65 cd	20.14 d	1.20 c
4.0	10.66 f	0.33 e	10.35 e	0.65de

Mean values followed by the same letter(s) within a column are not significantly different (P <0.05)

In brief and after two subcultures, the results showed that Ag NPs with concentration 1.0 ml/l have positive response on the number of embryos and leaves. This result is in agreement with the result achieved by **Do *et al.* (2018)**who reported that adding 1.0 ppm Ag NPs to the banana shoot multiplication medium was found to be the optimum concentration to induce maximum shoot growth, the maximum number of leaves, and maximum total chlorophyll content.

Increasing the concentration of Ag NPs to higher than 1.0 ml/l decreased the number of embryos and length which was lower than the control treatment, which means that low Ag NPs concentrations stimulates proliferation and development while higher concentrations have an inhibitory effect similar to that reported by **Salama (2012)** for the increase in shoot and root lengths as well as leaf surface area and chlorophyll content of the two tested crop plants and on the contrary with the findings of **Caroline de Oliveira Timoteo*et al.* (2019)**who found a 90% reduction within the number of recent shoots of *C. rufa* nodal segments with 15.4 mg/l Ag NPs. However, the amount and length of leaves decreased by increasing Ag NPs concentration to 0.5 ml/l but re-increased to achieve its maximum with increasing the concentration to 1.0 ml/l.

Fig. 3. Effect of different concentrations of Ag NPs on growth and regeneration of somatic embryos date palm Hayani cv. after the second subculture

These results in agreement with, Ag NPs have a large surface area and interact with other particles in the medium to increase efficiency **(Ingle *et al.*, 2008)**.The Plant organisms, age, tissue types, and physiological status are among the parameters influenced by NPs **(Vannini*et al.*, 2013)**. More recently, silver nanoparticles have also been used due to their physical and chemical properties and their easy uptake and mobility into plant cells **(Sarmast and Salehi, 2016)**.

Different concentrations of Ag NPs were found to have a substantial positive effect on the growth of shoot and root in Zea mays in another study **(Salama, 2012)**. Shoot multiplication and length were greatly influenced when Ag NPs were added to the culture medium. These results were obtained by **Razzaq*et al.* (2016)** in seedlings of wheat (*Triticumaestuvum*) grown *in vitro* in MS medium with different concentrations of Ag NPs, finding better development in 25 mg/l of Ag NPs. **Spinoso-Castillo *et al.* (2017)** reported that in vanilla (*Vanilla planifolia*), using Ag NPs observed increased shoot production and length at a concentration of 25 mg/l of Ag NPs, whereas the lowest number of shoots were observed on MS medium supplemented with 200 mg/l.

3.3 Determination of chlorophyll content

The chlorophyll contents as represented in **Table (4)** decreased with the lowest Ag NPs concentration (0.1 ml/l) compared to the control then it gradually increased with increasing the Ag NPs concentration from 0.5 to 1.0 ml/l then decreased again with increasing Ag NPs to 2.0 and 4.0 ml/l.

Table 4. Effect of different concentrations of Ag NPs on the chlorophyll contents after the second subculture

Concentration of Ag NPs (ml/l)	**Chlorophyll (mg/g)**		
	A (660 nm)	**B (640 nm)**	**C (440 nm)**
0.0	1.125 d	0.583 c	1.425 d
0.1	1.106 d	0.471 d	1.151 e
0.5	1.461 b	0.743 b	1.937 b
1.0	1.925 a	0.815 a	2.584 a
2.0	1.233 c	0.420 d	1.683 c
4.0	0.519 e	0.251 e	0.572 f

Mean values followed by the same letter(s) within a column are not significantly different (P <0.05)

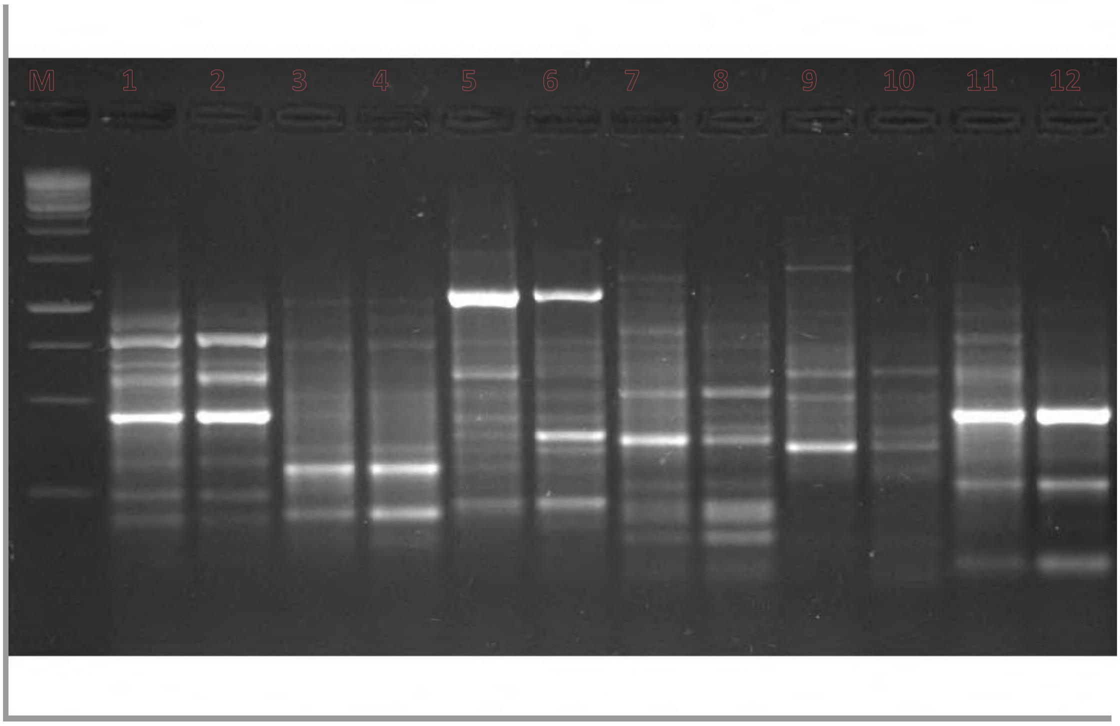
Chlorophyll results conform to **Castro *et al.* (2019)** who reported that chlorophyll A content was greater in Ag NPs than the control treatment. *In vitro* grown wheat, gross chlorophyll increased significantly at concentrations of25,50, and 100 mg/l of Ag NPs, according to**Razzaq*et al*. (2016)**.**Salama (2012)**, on the opposite hand, found that applying 60 mg/l of Ag NPs to beans and corn increased growth and chlorophyll content. An increase in chlorophyll content was found in the bean at concentrations of 50 mg/l of Ag NPs**(Saeideh and Rashid, 2014)**. The exposure of rice to 0.5 mg/l of Ag NPs has increased the content of chlorophyll and carotenoids **(Nair and Chung, 2014)**. Increased content of photosynthetic pigments in vanilla and sugarcane shoots treated with Ag NPs; this result was possibly caused by increased N, Mg and Fe concentrations in plant tissues exposed to Ag NPs, as these elements are linked to chlorophyll biosynthesis**(Spinoso-Castillo *et al.*, 2017; Bello-Bello *et al.*, 2017)**. The content of chlorophyll a, chlorophyll b, and overall chlorophyll in *A. thaliana* was decreased at 0.2, 0.5 and 3 mg dm^3 of Ag NPs **(Qian *et al.*, 2013)**.

3.4 Detection of genetic stability at DNA Level Using RAPD (PCR) Molecular Marker

The results in the present study revealed that the average polymorphism detected by the RAPD assay, indicating that there were no differences in the amplified DNA fragments among *in vitro* shoots sample of Hayani cv. which treated with 1.0 ml/l of Ag NPs and mother plant (control).Two date palm samples, as shown in **Fig. 4.**were fingerprinted using twelve random primers (RAPD) to detect the DNA polymorphism. Only two primers, namely OPA-5 and OPA-7 showed a difference of 5%, while other primers named (OPA-1, OPA-2, OPA-3, OPA-4, OPA-6, OPA-8, OPA-9, OPA-10, OPA-11 and OPA-12) showed that there were no differences of genetic variation (95%) between the sample treatment and the mother plant.This proves that using 1.0 ml/l of Ag NPs helps to produce true to type shoots for the mother plant.

Molecular diversity in date palm by using molecular marker tool was also documented by many workers **(Mohktar*et al.*, 1999)**. In this analysis, RAPD primers were used to amplify date palm sample and mother plant shoots in a PCR master cycler. RAPD markers produced a banding pattern that specifically identified clusters with genetic similarities.

In present study, RAPD markers have been successfully amplified for cultivar identification and genetic diversity analysis in the date palm Hayani cv. and the RAPD marker has given a good polymorphic data and hence they can be used for genetic diversity analysis. These finding were found similar as given by **(Ravi *et al.*, 2003; Kibria*et al.*, 2009; Hossain *et al.*, 2012)**.



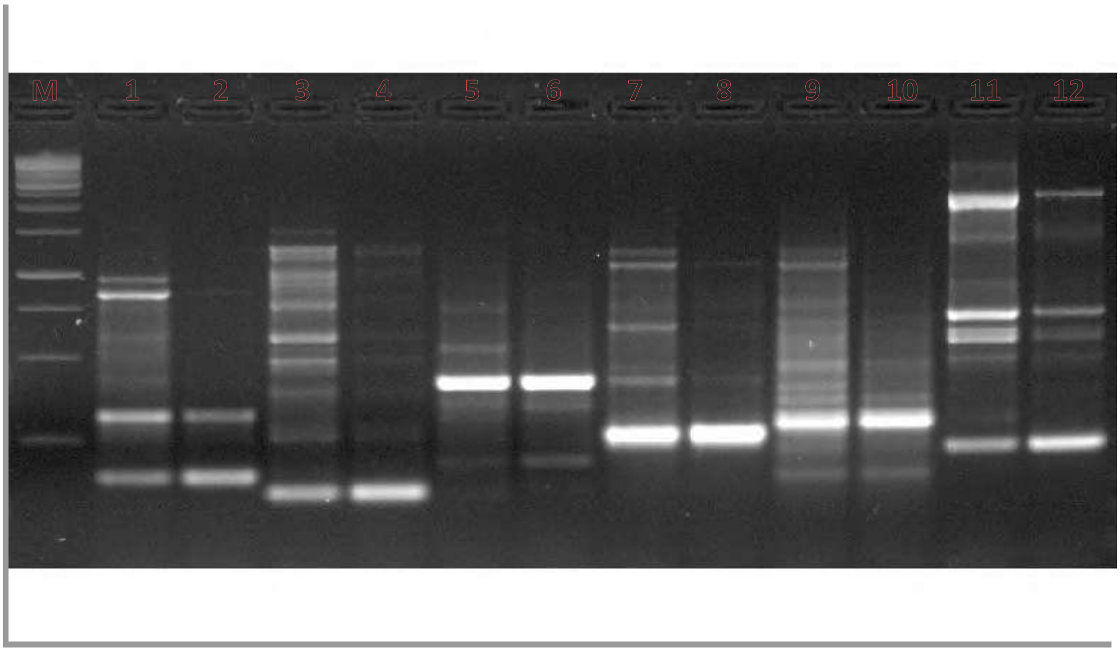


Fig. 4. RAPD's product of primers OPA-1, OPA-2, OPA-3, OPA-4, OPA-6, OPA-8, OPA-9, OPA-10, OPA-11 and OPA-12 showed similarity between the shoots sample which treated with 1.0 ml/l of Ag NPs and mother plant, while primer OPA-5 and OPA-7 showed the differences 5% between sample and mother plant

IV. CONCLUSION

Nano-materials are considered to be one of the most important inventions of modern science **(Wang *et al.*, 2011)**. Date palm organogenesis has gained much interest because of its high multiplication potential and production of true to type plantlets. It can be concluded that the right amount of Ag NPs can help with embryogenic callus growth and proliferation, as well as the development and regeneration of somatic embryos in date palm cv. Hayani*in vitro*.

Culture medium supplemented with 1.0 ml/l of Ag NPs was the best one for embryogenic callus differentiation and produced the highest number of somatic embryos. The suitable medium for regeneration of somatic embryos was added 1.0 ml/l Ag NPs. In this treatment, the number of somatic embryos, length of embryo, number of leaves, length of leaves and total chlorophyll content were 35.30 embryo/jar, 1.80 cm, 43.72 leaf/jar, 3.87 cm per explant and 2.584 mg/g, respectively. It was clear that there was significant effects in embryogenic callus and somatic embryos since exposure to low concentrations of AgNPs which stimulated growth, while increasing the concentration of AgNPs inhibits development.

The result inthis study indicated that the efficiency and ease of using RAPD markers for investigating genetic relationship and identification of varieties is good tool. Only two primers, namely OPA-5 and OPA-7 showed a difference of 5%, while other primers showed there were no differences of genetic variation (95%) between the samples were treated with 1.0 ml/l Ag NPs and the mother plant.

REFERENCES

[1] **Abdi, G.; Salehi, H. and Khosh-khuri, M. (2008).**Nano silver: A novel nanomaterial for removal of Bacterial contamination in Valerian (*V. officinalis*) tissue culture. Acta Physiol. Plant, 30: 709-714.

[2] **Alia, A. B.; Mohammadb, S.; Khanc, M. A.; Rajaa, N. I.; Arifc, M.; Kamilc, A. and Mashwani, Z. R. (2019).** Silver nanoparticles elicited *in vitro* callus cultures for accumulation of biomass and secondary metabolites in *Carallumatuberculata*. Artificial Cells, Nanomedicine, and Biotechnology, 47 (1): 715–724.

[3] **Ali, H.; Khan, M. A.; Ullah, N. and Khan, R. S. (2018).**Impacts of hormonal elicitors and photoperiod regimes on elicitation of bioactive secondary volatiles in cell cultures of*Ajugabracteosa*.Journal of Photochemistry and Photobiology B: Biology, 183: 242–250.

[4] **Al-Khairy, J. and Al-Bahrany, A. M. (2012).**Effect of abscisic acid and polyethylene glycol on the synchronization of somatic embryo development in date palm (*Phoenix dactylifera* L). Biotechnology, 11: 318-325.

[5] **Al-Khalifah, N. S. and Shanavaskhan, A. E. (2012).**Micro-propagation of Date Palms.Asia-Pacific Consortium on Agricultural Biotechnology (APCoAB) and Association of Agricultural Research Institutions in the Near East and North Africa (AARINENA). Riyadh, Saudi Arabia.

[6] **Al-Khalifah, N. S.; Askari, E. and Shanavaskhan, A. E. (2013).**Date Palm Tissue Culture and Genetical Identification of Cultivars Grown in Saudi Arabia.KACST Press. Riyadh, Saudi Arabia.

[7] **Al-Samir, E.; Al-Utbi, S. and Abass, M. (2015).**Phytotoxic effect of 2,4-D and dicamba on date palm (*Phoenix dactylifera* L.) tissue cultures at initiation stage. Advances in Agriculture and Botanics, 7: 96-108.

[8] **Baharan, E.; Pour, P.; Mohammadi, E.; Shahbaziand, S. and Hosseini, Z. (2015).**Effects of some plant growth regulators and light on callus induction and explants browning in date palm (*Phoenix dactylifera* L.) *in-vitro* leaves culture. Iranian Journal of Plant Physiology, 5: 1473-1481.

[9] **Bekheet, S. A.; Taha, H. S.; Saker, M. M. and Solliman, M. E. (2007).**Application of Cryopreservation Technique for *In vitro* Grown Date Palm (*Phoenix dactylifera* L.)Cultures. Journal of Applied Sciences Research, 3 (9): 859-866.

[10] **Bello-Bello, J. J.; Chavez-Santoscoy, R. A.; Lecona-Guzmán, C. A.; Bogdanchikova, N.; Salinas-Ruíz, J.; Gómez-Merino, F. C. and Pestryakov, A. (2017).**Hormetic Response by Silver Nanoparticles on *In Vitro* Multiplication of Sugarcane (*Saccharum spp.* Cv. Mex 69-290) Using a Temporary Immersion System. Dose-Response, 15 (4): 1559325817744945.

[11] **Din, A. R. J. M.; Ahmad, F. I.; Wagiran, A.; Samad, A. A.; Rahmat, Z. and Sarmidi, M. R. (2016).** Improvement of efficient *in vitro* regeneration potential of mature callus induced from Malaysian upland rice seed (*Oryza sativa* cv. Panderas). Saudi Journal of Biological Sciences, 23: S69–S77.

[12] **Do, D. G.; Dang, T. K. T.; Nguyen, T. H. T.; Nguyen, T. D.; Tran, T. T. and Hieu, D.D. (2018).** Effects of nano silver on the growth of banana (*Musa spp*.) cultured *in vitro*. Journal of Vietnamese Environment, 10 (2): 92-98.

[13] **Ducan, D. B. (1955)**. Multiple range and multiple F test. Journal of Biometrics, 11: 1-42.

[14] **El-Hadrami, I.; El-Bellaj, M.; El-Idrissi, A.; J'Aiti, F.; El-Jaafari, S. and Daaf, F. (1998).**Plant biotechnology and improvement of the date palm (*Phoenix dactylifera* L.) Pivot of Moroccan oasis agriculture.Cah. Agric., 7: 463-468.

[15] **Emad, A. E.; Said, A. D. and Ezzat, H. E. (2015)**.Evaluation of Callus Responses of *Solanumnigrum* L. Exposed to Biologically Synthesized Silver Nanoparticles.Nanoscience and Nanotechnology, 5 (3): 45-56.

[16] **Eshraghi, P.; Zarghami, R. and Mirabdulbghi, M. (2005).**Somatic embryogenesis in two Iranian palm cultivars. Afr. J. Biotechnol., 4: 1309–1312.

[17] **Fki, L.; Masmoudi, R.; Drira, N. and Rival, A. (2003).**An optimized protocol for plant regeneration from embryogenic

suspension cultures of date palm, *Phoenix dactylifera* L., cv. DegletNour. Plant Cell Reports, 21 (6): 517–524.

[18] **Fki, L.; Masmoudi, R.; Kriaa, W.; Mahjoub, A.; Sghaier, A. B.; Mzid R.; Mliki, A.; Rival, A. and Drira, N. (2011).**Date palm micro-propagation via somatic embryogenesis. In: Jain, S.M., Al-Khayri, J.M., Johnson, D.V. (Eds.). Date Palm Biotechnology.Springer. The Netherlands, pp. 47–68.

[19] **Gouran, A.; Jirani, M.; Mozafari, A. A.; Saba, M. K.; Ghaderi, N. and Zaheri**, **S. (2014).** Effect of silver nanoparticles on grapevine leaf explants sterilization at *in vitro* conditions, 2nd National Conference on Nanotechnology from Theory to Application, Isfahan, Iran, pp. 1–6.

[20] **Hoffmann, F.; Fortier, T. and Hoffmann-Tsay, S. S. (2013).**Micro-propagation of Date Palm (*Phoenix dactylifera*).Phoenix Institute of Desert Agriculture, San Diego, California.

[21] **Hossain, M. M.; Islam, M. M.; Hossain, H.; Ali, M. S. and da Silva, T. (2012).**Genetic diversity analysis of aromatic landraces of rice (*Oryza sativa* L.) by microsatellite markers. Genes, Genomes and Genomics, 6 (SI1): 42-47.

[22] **Ingle, A.; Gade, A.; Pierrat, S.; Sonnichsen.C and Rai, M. (2008).**Mycosynthesis of silver nanoparticles using the fungus *Fusariumacuminatum*and its activity against Some Human Pathogenic Bacteria.Current Nanoscience,4 (2):141 – 144.

[23] **Jadczak, P.; Kulpa, D.; Bihun, M. and Przewodowski, W. (2019).**Positive effect of Ag NPs and Au NPs in *in vitro* cultures of *Lavandulaangustifolia* Mill. Plant Cell, Tissue and Organ Culture, 139: 191–197.

[24] **Johnson, D. V. (2011).**Date palm biotechnology from theory to practice. In: Jain, S.M., Al-Khayri, J.M., Johnson, D.V. (Eds.), Date Palm Biotechnology. Springer Netherlands. The Netherlands, pp. 1–11.

[25] **Khierallah, H. S. M. and Hussein, N. H. (2013).**The Role of coconut water and casein hydrolysate in somatic embryogenesis of Date Palm and genetic stability detection using RAPD markers.Date Palm Research Unit, College of Agriculture, University of Baghdad, Iraq.

[26] **Khodakovskaya, M. V.; De Silva, K.; Biris, A. S.; Dervishi, E. and Villagarcia, H. (2012).** Carbon nanotubes induce growth enhancement of tobacco cells. ACS Nano, 6 (3): 2128–2135.

[27] **Kibria, K.; Nur, F.; Begum, S. N.; Islam, M. M.; Paul, S. K.; Rahman, K. S. and Azam, S. M. M. (2009).** Molecular Marker based Genetic Diversity Analysis in Aromatic Rice Genotypes Using SSR and RAPD Markers. Int. J. Sustain. Crop Prod. 4 (1): 23-34.

[28] **Kim, D. H.; Gopal, J. and Sivanesan, I. (2017).**Nanomaterials in plant tissue culture: the disclosed and undisclosed. The Royal Society of Chemistry Advances, 7: 36492–36505.

[29] **Krishnaraj, C.; Jagan, E. G.; Ramachandran, R.; Abirami, S. M.; Mohan, N. and Kalaichelvan, P. T. (2012).**Effect of biologically synthesized silver nanoparticles on *Bacopamonnieri* (Linn.)Wettst.Plant growth metabolism. Process Biochem., 47 (4): 51–658.

[30] **Kumar, V.; Ramakrishna, A. and Ravishankar, G. A. (2007)**. Influence of different ethylene inhibitors on somatic embryogenesis and secondary embryogenesis from *Coffeacanephora* P ex Fr. *In vitro* Cellular & Developmental Biology-Plant, 43: 602-607.

[31] **Lichtenthaler, H. K. and Buschmann, C. (2001).**Chlorophylls and carotenoids: Measurement and Characterization by UV-VIS. Current Protocols in Food Analytical Chemistry (CPFA), (John Wiley and Sons, M. New York): pp. F4.3.1-F4.3.8. [Nr. 107].

[32] **Lima, G. P.; Campos, R. S.; Willadino, L. G.; Terezinha, J. R. and Vianello, F. (2012).**Polyamines, gelling agents in tissue culture, micro-propagation of medicinal plants and bioreactors. In: Leva A, Rinaldi L., Eds. Recent advances in plant *in-vitro* culture. In Tech, pp. 165-185.

[33] **Luoma, N. S. (2008).** Silver nanotechnologies and the environment: Old problems or new challenges? The Project on Emerging Nanotechnologies.Project on Emerging Nanotechnologies of the Woodrow Wilson International Center for Scholars, 15: 66 pages.

[34] **Manickavasagam, M.; Pavan, G. and Vasudevan, V. (2019).**A comprehensive study of the hormetic influence of biosynthesized Ag NPs on regenerating rice calli of indica cv. IR64. Scientific Reports, 9 (8821): 1-11.

[35] **Marzachì, C.; Veratti, F.; D'Aquilio, M.; Vischi, A.; Conti, M. and Boccardo, G. (1999).**Molecular Hybridization and PCR Amplification of Non-Ribosomal DNA to Detect and Differentiate StolburPhytoplasma Isolates from Italy.Journal of Plant Pathology, 82 (3): 201-212.

[36] **Masmoudi-Allouche, F.; Gargouri-Bouzid, R.; Kriaâ1, W.; Saïdi, M. N.; Jamoussi, H.; Abdelkefi, Y. and Drira (2011)**. Investigation of genetic variability related to the *in vitro* floral hermaphrodism induction in Date palm (*Phoenix dactylifera* L.). Afr. J. Biotechnol., 10: 10567–10574.

[37] **McCubbin, M. J.; Van Staden, J. and Zaid, A. (2000).** A southern African survey conducted for off-types on date palm production using somatic embryogenesis. In: Proceedings of the date palm international symposium. Windhoek, Namibia, pp. 68–72.

[38] **Miller, G. and Senjan, R. (2006).**The disruptive social impacts of nanotechnology.

[39] **Mohktar, T.; Abdelmajid, R.; Ali, O.; Hela, S. and Mohamed, M. (1999).**Differention of Tunisian date-palm varieties by RAPDs technology. The International Conference of Date Palm, Nov. 9-11.

[40] **Monica, R. C. and Cremonini, R. (2009).**Nanoparticles and higher plants.Caryologia., 62 (2): 161–165.

[41] **Mousavi, S. R. and Rezaei, M. (2011).**Nanotechnology in agriculture and food production. Journal of Applied Environmental and Biological Sciences, 1: 414-419.

[42] **Murashige, T. and Skoog, F. (1962).** A revised medium for rapid growth and bio assays with Tobacco tissue cultures. PhysiologiaPlantarum, 15: 473-480.

[43] **Mustafa, H. S.; Oraibi, A. G.; Ibrahim, K. M. and Ibrahim, N. K. (2017).** Influence of silver and copper

nanoparticles on physiological characteristics of *Phaseolus vulgaris* L. *in vitro* and *in vivo*. International Journal of Current Microbiology and Applied Sciences, 6 (1): 834-843.

[44] **Nair, P. M. G. and Chung, I. M. (2014).**Physiological and molecular level effects of silver nanoparticles exposure in rice (*Oryza sativa* L.) seedlings. Chemosphere, 112: 105–113.

[45] **Oliveira, R. S.; Ma, Y.; Rocha, I.; Carvalho, M. F.; Vosátka, M. and Freitas, H. (2016).***Arbuscularmycorrhizal*fungi are an alternative to the application of chemical fertilizer in the production of the medicinal and aromatic plant *Coriandrumsativum* L. Journal of Toxicology and Environmental Health A, 79 (7): 320-328.

[46] **Omar, M. S. (1988).***In vitro* response of various date palm explants. Date Palm J., 6 (2): 371-388.

[47] **Qian, H.; Peng, X.; Han, X.; Ren, J.; Sun, L. and Fu, Z. (2013).**Comparison of the toxicity of silver nanoparticles and silver ions on growth of terrestrial plant model Arabidopsis thaliana. J. Environ. Sci., 25 (9): 1947–1956.

[48] **Rai, M. K.; Shekhawat, N. S.; Harish; Gupta, A. K.; Phulwaria, M.; Ram, K. and Jaiswal, U. (2011)**.The role of abscisic acid in plant tissue culture—a review of recent progress. Plant Cell Tissue and Organ Culture, 106: 179–190.

[49] **Ravi, M.; Geethanjali, S.; Sameeyafarheen, F. and Maheswaran, M. (2003).** Molecular Marker based Genetic Diversity Analysis in Rice (*Oryza sativa* L.) using RAPD. Euphytica, 133: 243–252.

[50] **Razzaq, A.; Ammara, R.; Jhanzab, H. M.; Mahmood, T.; Hafeez, A. and Hussain, S. (2016).**A novel nanomaterial to enhance growth and yield of wheat. Journal of Nano-science and Technology, 2 (1): 55–58.

[51] **Saeideh, N. and Rashid, J.(2014).**Effect of silver nanoparticles and Pb $(NO_3)_2$ on the yield and chemical composition of mung bean (*Vignaradiata*). Journal of Stress Physiology & Biochemistry, 10 (1): 316-325.

[52] **Salama, H. M. H. (2012)**. Effects of silver nanoparticles in some crop plants, Common bean (*Phaseolus vulgaris* L.) and corn (*Zea mays* L.). International Research Journal of Biotechnology, 3 (10): 190-197.

[53] **Sarmast, M. K. and Salehi, H. (2016).** Silver nanoparticles: an influential element in plant nano-biotechnology. Mol. Biotechnol., 58: 441–449.

[54] **Saxena, R.; Tomar, R. S. and Kumar, M. (2016).** "Exploring nano-biotechnology to mitigate abiotic stress in crop plants," Journal of Pharmaceutical Sciences and Research, 8 (9): pp. 974–980.

[55] **Sharma, P.; Bhatt, D.; Zaidi, M. G.; Saradhi, P. P.; Khanna, P. K. and Arora, S. (2012).** Silver nanoparticle mediated enhancement in growth and antioxidant status of *Brassica juncea*. Appl. Biochem. Biotechnol., 167: 2225–2233.

[56] **Shirazi, A. M. S. and Ramezanpour, Z. (2016).** "Toxicity effects of SiO_2 nanoparticles on green micro-algae *Dunaliella Salina*", International Journal ofNano-science andNanotechnology, 12 (4): 269-275.

[57] **Smith, F.; Gilles, M. A.; Hamilton, J. K. and Godess, P. A. (1956).**Colorimetric method for determination of sugar and related substances.Anal. Chem., 28: 350-358.

[58] **Spinoso-Castillo, J. L.; Chavez-Santoscoy, R. A.; Bogdanchikova, N.; Pérez-Sato, J. A.; Morales-Ramos, V. and Bello-Bello, J. J. (2017).** Antimicrobial and hormetic effects of silver nanoparticles on *in vitro* regeneration of vanilla (*Vanilla planifolia* Jacks. ex Andrews) using a temporary immersion system. Plant Cell, Tissue and Organ Culture, 129: 195–207.

[59] **Timoteo, C. O.; Paiva, R.; Reis, M. V.; Claro, P. I. C.; Silva, D. P. C.; Marconcini, J. M. and Oliveira, J. E. (2019).**Silver nanoparticles in the micro-propagation of *Campomanesiarufa* (*O. Berg*) Nied. Plant Cell, Tissue and Organ Culture,137: 359–368.

[60] **Vannini, C.; Domingo, G.; Onelli, E.; Prinsi, B.; Marsoni, M.; Espen, L. and Bracale, M. (2013).** Morphological and proteomic responses of *Eruca sativa* exposed to silver nanoparticles or silver nitrate. PLOS One, 8 (7): e68752.

[61] **Wang, J.; Asbach, C.; Fissan, H.; Hulser, T.; Kuhlbusch, T. A. J.; Thompson, D. and Pui, D. Y. H. (2011).** How can nano-biotechnology oversight advance science and industry: Examples from environmental, health and safety studies of nanoparticles (nano-EHS). J.Nanopart. Res., 13: 1373–1387.

[62] **Wang, P.; Lombi, E.; Zjao, F. J. and Kopittke, P. M. (2016).**Nanotechnology: A New Opportunity in Plant Sciences. Trends Plant Sci., 21 (8): 699-712.

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Factors Affecting Turmeric Production in Sunsari District, Nepal

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Abstract— *A study was conducted in Barahachhetra Municipality of Sunsari district from April to August, 2021 with the objectives to study factors affecting turmeric production. For the study, 100 respondents were selected through a simple random sampling method. A properly design questionnaire was administered for primary data after pretesting it. Secondary data was obtained from journals, research articles, publications, and reports. The obtained data were analyzed using computer software packages such as: MS excel (2016) and SPSS (V 26.0). The correlation coefficient tested the strength of the relationship between various dependent and independent variables. In the study, the majority of respondents were male about 68% with a literacy rate of 68% whereas, 63 respondents use their own production as seed sources. The study showed that educational status, seed treatment, Irrigation practices, cropping system, training, and product demand had a positive and significant relationship with the level of production. Similarly, seed quantity, manure quantity, labor cost, and land size under turmeric cultivation were found to have a positive and significant correlation with the level of production with a Pearson correlation coefficient of 0.525, 0.230, 0.511, and 0.791respectively. Quality planting materials, proper irrigation facilities, training related to cultivation, seed treatment should be provided to the farmers.*

Keyword— ***Turmeric, Production, Farmers.***

I. INTRODUCTION

Turmeric (Curcuma longa is a spice made from the dried rhizome of turmeric plants which is also referred to as the “Queen of spices”(Begum et al., 2019). Turmeric is an important spices crop(Karthik and Amarnath, 2014)which is one of the top five major spices grown for culinary and seasoning of foods in Nepal(HVAP, 2019). Turmeric is an annual crop belonging to the Zingiberaceae family which may be sown on sandy or clayed loam soil, and the best sowing time of April- May(Saeed et al., 2017).

Turmeric can be regarded as a good cash crop as well as main source of income for the peasants of tropical mid-hills in Nepal(Begum et al., 2019) as its production required less water, low technology, less capital investment and its can be grown with a comparatively lower application of fertilizers especially lower use or not any chemical fertilizers and low pest/disease infestation. Land topography of hilly region and nature of the soil is favorable for turmeric cultivation as it allows no accumulation of water in turmeric field which also aids in lower infestation of Diseases. Turmeric on sloppy land also be beneficial as it prevents soil erosion and the use of organic manure and mulching helps to retain the soil fertility(HVAP, 2019).

Turmeric cultivation areas are increasing day by day. Turmeric occupied about 11 percent of the area and about 15% of production among the top five spices crops in Nepal(MoALD, 2020; Acharya et al., 2021). In Nepal turmeric was cultivated in 9795 hectares and production of 99907 mt, while productivity was found to be 10.199mt/ha(Krishi Diary, 2078).

II. MATERIALS AND METHODS

Study site

We selected the Sunsari district of Nepal for study as there was a higher production potential of Turmeric. In Sunsari, we selected BarahachhetraMunicipality for our study, which lies in eastern Terai in Nepal and has remarkable area coverage by turmeric, a large number of Turmeric farmers, good production, and better access to market and transport facilities.

Selection of Turmeric farmers

The list of Turmeric growers was obtained from Prime Minister Agriculture Modernization Project-Ginger/Turmeric Zone, Sunsari, Nepal. A total of 100 respondents were selected from Barahachhetra Municipality by random sampling technique to draw a representative sample.

Data Collection and Analysis

The information was collected through a household survey using interview schedule, focus group discussion, and key informant interview.

Quantitative and qualitative data obtained from the survey were entered and analysed by using SPSS and Microsoft Excel. Different descriptive statistics such as means, frequency, percentage, and standard deviation were used to derive a conclusion from the data. Chi-square test ($\chi2$) was used to study the association of independent categorical variables; such as gender of the respondents, educational status, Seed treatment, Irrigation practices, cropping system, training received, and types of turmeric product with a dependent variable that is level of Turmeric production. Best of Average Turmeric production, production was categorized as high and low, and their relationships of individual production level to different socio-economic factors were analyzed using the Chi-square test ($\chi2$). Chi-square test ($\chi2$) was used to show the association between two categorized variables while the correlation was used to show the relationship between two continuous variables(Ghimire et al., 2019; Mahat et al., 2019).

III. RESULTS AND DISCUSSION

Socio-Economic Characteristics of the farmers

The Socio-economic variables such as Gender, Age, Ethnicity, Education, Seed source, Seed treatment, training, Irrigation practices, intercropping practices were studied. 68% of respondents were male while 32% were female as shown in Table-1.

Table-1: Socio-economic characteristics of the respondents

Gender	**Frequency**	**Percent**
Male	68	68
Female	32	32
Age		
Less than 30	12	12
30-50	66	66
Above 50	22	22
Ethnicity		
Janajati	72	72
Chhetri	17	17
Brahmin	9	9
Dalit	2	2
Education Level		
Illiterate	32	32
Primary	33	33
Secondary	20	20
Higher Secondary	10	10
Bachelor and above	5	5
Seed Source		
Self	63	63
PMAMP	12	12
Neighbor	14	14
Both Self and PMAMP	11	11
Seed Treatment		
Yes	6	6
No	94	94
Type of irrigation		
Rainfed	86	86
Seasonal Irrigation	14	14
Training		
Yes	23	23
No	77	77
Intercropping Practices		
Intercropping	25	25
Monocropping	75	75

The Majority of 66% of respondents farmers were from 30-50 year age groups while 22% were above 50 years and the remaining 12% were below 30 years. The findings revealed that 72 respondents were janajati and the remaining were followed by 17, 9, 2, by chhetri, brahmin and dalit respectively. From the study, it was found that the literacy rate was 68% which was higher than the national literacy rate 0f 66.22%(CBS, 2011). The percentage of the respondents who had primary, secondary, higher secondary, and bachelor and above was 33%, 20%, 10%, and 5% respectively. According to Battese & Coelli (1995) higher the education greater would be the efficiency of producers(Mahat et al., 2019).

The majority of the Turmeric growers stored their produced turmeric rhizome for seed sources for the next period of cultivation. From the analysis, it was found that 63% used their own rhizome as a seed source and other 12% used Turmeric seed provided by PMAMP, Ginger/Turmeric zone, Sunsari, 14% brought from the neighbour and remaining 11% used own and a half from PMAMP, zone Implementation unit as shown in Table-1. Generally, most of the farmers practiced cultivatingTurmeric without any chemical application but some do not have any idea of seed treatment yet. From the finding, only 6% practiced seed treatment for diseases/pests management.

The majority of the respondents i.e. 75% in the study site followed the sole cropping of Turmeric while only 25% of respondents followed the intercrop system with maize. Few respondents followed mixed cropping with maize only as they were utilizing space for additional income and we don't supply much irrigation to maize.The study revealed that, only 14% of the total respondents were found to follow the irrigated type of farming. Most of the households 84% followed a rain-fed type of farming which indicated that their farming system was entirely dependent on the mercy of the monsoon.

Production level of Turmeric

The average turmericproduction per household was calculated and it was found to be 366 kg per kattha (10.98mt per hectare). Based on this, turmeric was categorized into two production levels; low production (turmeric yield less than 366 kg) and high production level (turmeric yield more than 366 kg per kattha).

Table 2: Production level of turmeric in the study area

S. N	Production Level of turmeric	Frequency
1	High	62
2	Low	38

Note: 1 Kattha = 338.63 m^2(Source: Field survey, 2021)

Chi-Square analysis between factors and turmeric Production

Results of Chi-square test on the relationship between characteristics of ginger farmers and ginger production level revealed that gender hasno statistically significant relationship with the level of Turmericproduction at 0.05 probability level as shown in Table-3.

Table-3: Distribution of respondents according to different factors and level of production

Factors	Level of production			$\chi 2$ Cal	P-value
	High	Low	Total		
Gender				0.138 [ns]	0.711
Male	43	25	68		
Female	19	13	32		
Education status				8.619***	0.071
Illiterate	17	15	32		
Primary level	25	8	33		
Secondary level	14	6	20		
Higher Secondary	5	5	10		
Bachelor and above	1	4	5		
Seed treatment				3.912**	0.048
Yes	6	0	6		
No	56	38	94		
Irrigation practices				3.886**	0.049
Rainfed	50	36	86		

Seasonal Irrigation	12	2	14		
Cropping system				4.584**	0.032
Mono-cropping	51	24	75		
Intercropping	11	14	25		
Training				3.552***	0.067
Yes	18	5	23		
No	44	33	77		
Product demand				6.82**	0.033
Fresh Turmeric	16	12	28		
Dried Turmeric	35	12	47		
Processed Turmeric	11	14	25		

Note: * and ** indicate 10%, and 5% levels of significance, and ^{ns} indicate non-significant. Source: Field survey, 2021.

Results of the Chi-square test revealed that gender has no statistically significant relationship with the level of ginger production at 0.05 educational status probability level as shown in Table 4. However, educational status had a positive and strong relationship with the level of Turmeric production at a 10% level of significance. The higher the educational level, the higher will be the level of production. The level of production was also found to be positive and strongly dependent on seed treatment at a 5% level of significance. Seed treatment improved the level of production and quality of fresh Turmeric produced because it helps to prevent the spread of rhizome rot pathogens during the growth of Turmeric.

Similarly, Irrigation practices had also a positive and strong relationship with the level of production at a 5% level of significance. The cropping system and product demand had also a positive and strong relationship with the level of production at a 5% level of significance. Training received by the farmers was found to be positively significant at 10% with the level of production. Training received farmers used proper spacing for turmeric as well proper doses of manure, fertilizers with 2/3 times weeding and mulching with dried leaves help in improvingthe level of production.

Correlation

Correlation analysis revealed that manure quantity had a positive and significant relationship with the level of production at a 5% level of significance. However, family size showed a negative but not significant relationship with Turmeric production at a 0.05 level of significance. Area of Turmeric cultivation, seed quantity, labour cost, type of Irrigation practices hada positive and significant relationship with Turmeric production at a 1% level of significance. Similar, result was observed that seed quantity and labour cost show a positive and significant relationship with the production by Acharya et al., 2021.Begum et. al. (2019) also observed Land under turmeric, seed rhizome has significant relation with the production. However, family size shows non-significance with the level of production.

Table-4: Correlation analysis between factors and production.

Factors	Pearson correlation	Sig. (2 tailed)P	Sample size
Family size in number	-0.06 ^{ns}	.950	100
Land under turmeric cultivation	.791*	0.000	100
Seed Quantity	0.525*	0.00	100
Manure Quantity	.230**	0.021	100
Labour cost	.511*	.000	100
Type of irrigation	0.41 ^{ns}	.687	100

Note: ** and * indicates 5% and 1% level of significance and ^{ns} indicate non-significant.

IV. CONCLUSION

In the study area,a large number of farmers was found to be involved in Turmeric production and though they have idea or experience of turmeric cultivation and its importance, the majority were not satisfied with its production. The majority of 63% of the respondent use their own previous year's production as a seed source. However, 23 respondents have participated in training related to the cultivation of turmeric.

The results of the study revealed that practicing seed treatment, proper arrangement of irrigation, cropping system, availability of quality seed rhizomes, organic manure used, labour availability, and area under turmeric cultivation is the factor affecting turmeric production in Sunsari district of Nepal.

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REFERENCES

[1] *Acharya, S., Dhital, P. R., Bista, B., Ghimire, A. R., And, & Airee, S. (2021).* Resource Productivity Analysis of Organic Turmeric Production in Surkhet District, Nepal. Economic Affairs, *66(2), 189–194. https://doi.org/10.46852/0424-2513.2.2021.2*

[2] *Amarnath, J. S. and Kartik V.(2014).* An economic analysis of turmeric production in Tamil Nadu ,. *2(July), 66–76.*

[3] *Battese, G.; Coelli, T. (1995).* A Model for Technical Inefficiency Effects in a Stochastic Frontier Production Function for Panel Data. *Journal of Econometrics, 325–332.*

[4] *Begum, M., Miah, M. M., Rashid, M., Islam, M., & Hossain, M. (2019).* Economic analysis of turmeric cultivation: evidence from Khagrachari district. Bangladesh Journal of Agricultural Research, *44(1), 43–58. https://doi.org/10.3329/bjar.v44i1.40902*

[5] *CBS. (2011).* Statistical Year Book. Central Bureau of Statistics. Government of Nepal, National Planning Commission Secret*ariat*,Kathmandu, Nepal.

[6] Ghimire, S., Chalise, D. P., Neupane, J., & Devkota, K. (2019). Factors Affecting Ginger Production in Sunsari District, Nepal. *3(11), 181–186. https://doi.org/10.31080/ASAG.2019.03.0705*

[7] HVAP. (2019). Value Chain Analysis of Turmeric. vii–viii. https://doi.org/10.31826/9781463237745-toc

[8] Krishi Diary. (2078). Krishi Diary. Ministry of Agriculture and Livestock Development, 356.

[9] Mahat, S., Sapkota, S., Sapkota, S., & Katuwal, K. (2019). Factors Affecting Ginger Production in Surkhet District, Nepal. *International Journal of Applied Sciences and Biotechnology,* 7(2), 269–273. https://doi.org/10.3126/ijasbt.v7i2.24650

[10] MoALD. (2020). Statistical information Nepalese Agriculture.

[11] Saeed, R., Bashir, A., Khan, S. B., Bakhsh, K., & Qasim, M. (2017).An Economic Assessment of Turmeric Production in Punjab-Pakistan. 33(1), 85–99.

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Application of different concentrations of licorice and willow extracts as rooting stimulator in hardwood cuttings of olive (*Olea europaea L.*)

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***Abstract*—*Rooting in hardwood cuttings of olive was investigated at the College of Agricultural Engineering Sciences, University of Sulaimani, Kurdistan Region-Iraq under application of licorice and willow extracts with concentrations of (0, 3, 6 and 9 g.L^{-1}). The extracts were prepared from licorice root and willow shoots in 25% ethanol heated in a water bath at 40ºC for 3 hours, refrigerated for 24 hours and filtered through filter paper. The hardwood cuttings of olive cv. (Sorani) were taken from basal part of one-year-old suckers then soaked in the licorice and willow extract concentrations for 1 hour. The results revealed that the highest (66.66%) rooting was achieved in the cuttings soaked in 6 and 9 g.L^{-1} licorice extract for 1 hour, they were not different in comparison with control cuttings which gave (49.99%), but rooting percentage was reduced (38.8%) in the cuttings soaked in 6g.L^{-1} willow extract. The cuttings soaked in 9 g.L^{-1} willow extract and 6 g.L^{-1} licorice extract gave the best root number, root length, shoot length, shoot diameter and leaf number. Generally, depending on the obtained results in this study, 6 and 9 g.L^{-1} licorice extract with 9 g.L^{-1} willow extract have possibility to use as an alternative to induce root formation and improve root and shoot quality of the cuttings in olive.*

Keywords—**Licorice extract, willow extract, olive cuttings, root formation.**

I. INTRODUCTION

Olive propagation is desirably conducted through cuttings to provide guarantee for genetic uniformity and induce earlier bearing in comparison to seedlings from seeds (Awan *et al*., 2012). Rooting in olive cuttings is restricted by many internal and external factors which is more evident according to cultivar (Hechmi*et al*., 2013). Therefore, to overcome the difficulty in rooting, growth regulators are applied to the cuttings of olivein order to induce rooting, including auxins. It is observed that that 3000 ppm IBA was the best dosage for rooting and enhancing other traits in hardwood cuttings of olive (Rahman *et al*., 2002). However, using synthetic auxins in propagation are not permitted by European and North American regulations to obtain vegetative propagated materials in organic farming (Centeno and Gómez-del-Campo, 2008). Also, application of synthetic growth regulators is not recommended recently because of they are not friend of environment and may have toxic effect on plant, human and animals (El-Sherif, 2017); synthetic growth regulators are expensive and are not readily available in local markets as well (El-Shaima et al., 2018). Thereby, extract of some plants and natural products have been used as a substitute for synthetic growth regulators to promote rooting onto the cuttings. The more pronounced are extracts of vermin wash, Coconut water, willow leaf water, honey, humic acid, seaweed extract, Aloe vera, cinnamon powder, licorice and yeast extract (Gad and Ibrahim, 2018; Mohammed *et al*., 2020; Rajan and Singh, 2021).

Many researches have emphasized on root extract of licorice as a source of natural stimulant compounds which could be used instead of synthetic growth promoters because it contains phenolic compounds, mevalonic acid, amino acids, vitamins, biotin, folic acid, pantothenic acid, and many minerals (El-Dengawy*et al*., 2017). Licorice root extract is also counted as a source of phytohormones which have an important role in root formation onto the cuttings (Rady*et al*., 2019). Eid*et al*. (2018) reported that licorice extract is among the plant extracts could be considered as an alternative to growth regulators for rooting Picual olive cuttings. On the other hand, willow bark, shoot or leaf extracts contain growth promoting chemicals may effectively enhance rooting onto the cuttings. For example, some root-promoting and diffusate substances were observed in *Salix alba* which synergistically interact with IBA to augment rooting on mung bean cuttings (Kawase, 1970; Al-Amad and Qrunfleh, 2014). In addition, salicylic acid (SA) is a plant hormone prevalently was found in willow extract which stimulates root formation onto the cuttings (Sandoval-Yapiz, 2004; Hayat *et al*., 2010). Wise *et al*. (2020) summarized that 1.06 µL/L willow bark extract gave the best results with the cuttings of chrysanthemum and lavender. Thus, this study was carried out to determine the best concentration of licorice root extract and willow shoot extract for inducing rooting in hardwood cuttings of olive.

II. MATERIALS AND METHODS

The study was carried out at the College of Agricultural Engineering Sciences, University of Sulaimani, Kurdistan Region-Iraq to determinate the best concentration of willow shoot extract and licorice root extract for inducing root formation onto olive hardwood cuttings cv. (Sorani).

2.1 Preparation of the cuttings

The hardwood cuttings of olive cv. (Sorani) were taken on February 10, 2021 from basal part of the suckers of previous year with 20 cm long and about 4.5-6.5 mm diameter. After preparation, the bases of the cuttings were soaked in (0, 3, 6 and 9 g.L^{-1}) of licorice and willow extracts separately for 1 hour. Following treatment, the cuttings were planted in polyethylene bag with a size of 12×30 cm filled with sand medium. The experiment was laid out in RCBD design with three replications in a lath house, and in each bag six cuttings were planted. Initially, because of low temperature, the cuttings were covered with a polyethylene UV plastic sheet until April 14, 2021 then the plastic cover was removed till the time of taking the results. The average of maximum and minimum temperature inside the plastic cover was between 9-37 ºC and inside the lath house after removing the plastic sheet was between 17.2- 41.3 ºC.

2.2 Preparation of licorice and willow extracts

The dried root of licorice and dried one- and two-year-old shoots of willow were grinded and weighted in required amount, then the volume was completed with 25% of ethanol and placed in a water bath at 40ºC for 3 hours. After that, they were taken out from the water bath and refrigerated for 24 hours. In the next day, the extracts were filtered through filter paper and applied to the cuttings.

2.3 Statistical analysis

After 4 months and 20 days (on June 30, 2021) the cuttings were checked to calculate rooting percentage, root number, root length, shoot length and leaf number. XLSTAT software version 2019.2.2, one-way ANOVA-RCBD and Duncan’s multiple-range used for analyzing the data.

III. RESULTS AND DISCUSSION

The date in figure (1) showed that licorice and willow extracts did not significantly induce rooting percentage in hardwood cuttings of olivein comparison with control cuttings. However,rooting percentage was reduced in the cuttings soaked in 6 g.L^{-1} willow extract, and they were statistically different compared to the cuttings soaked in 6 and 9 g.L^{-1} licorice extract. The highest (66.66%) rooting was achieved in the cuttings soaked in 6 and 9 g.L^{-1} licorice extract, but the lowest (38.8%) rooting achieved in the cuttings soaked in 6 g.L^{-1} willow extract, while control cuttings and the cuttings soaked in 3 g.L^{-1}licorice and willow extracts gave (49.99%) rooting. These results could be attributed to that extract of licorice contains many chemicals that may induce root formation in cuttings. Also, licorice extract may synergistically elevate the metabolites in cuttings which have important role in rooting process. El-Shaima*et al*. (2018) found that licorice extracts increased rooting rate and root traits in the cuttings of grape similar to IBA and concomitantly resulted in the highest IAA content of the cuttings which is quite possible one of the factors augments rooting in the cuttings. Besides, Tahoori*et al*. (2019) found quercetin flavonoid in licorice extract, and quercetin was considered to be a substance stimulates root formation onto the cuttings (Tarragó*et al*., 2005). In that connection, do Prado *et al*. (2015) concluded that quercetin was a compound which enhanced performance of *Eucalyptus* cuttings owing to oxidative stress reduction and the encouragement of cell division. Also, phenols were found in licorice extract(Rao, 1993), and phenols were identified as rooting promoter in cuttings (Wilson and Staden, 1990).

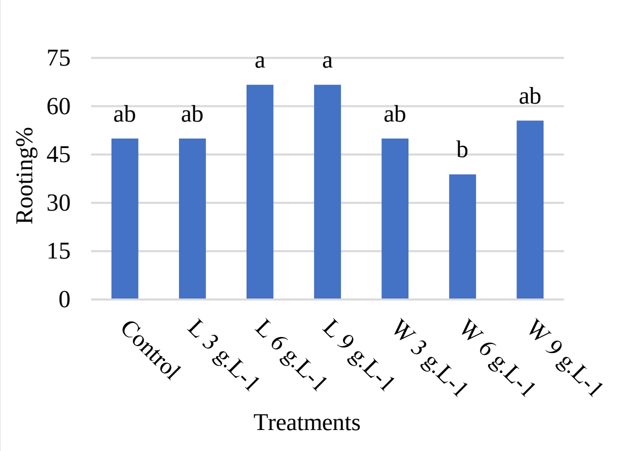

Fig. 1-Effect of different concentrations of licorice root extract (L) and willow shoot extract (W) on rooting percentage. Means sharing the same letter are not significantly different according to Duncan's multiple-range Test at $p \leq 0.05$.

Despite rooting percentage, extracts of licorice and willow remarkably increased root number (Figure 2). Numerous roots (11.23) found in the cuttings soaked in 9 $g.L^{-1}$ willow extract followed by (10.5) in the cuttings treated with 6 $g.L^{-1}$ licorice extract. Control cuttings and the cuttings were treated with 3 $g.L^{-1}$ willow extract gave the lowest (3.5 and 3.56, respectively) root number. It is noteworthy to mention that root number was increased by increasing concentration of willow shoot extract from 3 $g.L^{-1}$ to 9 $g.L^{-1}$. Similarly, the both 6 $g.L^{-1}$ licorice extract and 9 $g.L^{-1}$ willow extract significantly improved root length (Figure 3). The longest root (6.2 cm) was obtained in the cuttings soaked in 6 $g.L^{-1}$ licorice extract along with (5.98 cm) in the cuttings soaked in 9 $g.L^{-1}$ willow extract. The shortest roots (2.42 and 3.03 cm) were found in cuttings treated with 3 $g.L^{-1}$ willow extract and control cuttings, respectively. Willow contains phytohormones such as auxin and salicylic acid that may influence rooting and root traits of the cuttings as used in the form of "willow water" (Knapke*et al.*, 2018). Arena *et al.* (1997) reported greater root number in semi-hardwood cuttings of *Chionanthusretusus*when they were treated with willow diffusates with IBA after 75 days of the treatment. Al-Amad and Qrunfleh(2014) recognized babylon weeping willow extract to be a factor to raise root number in olive cuttings when they were soaked in it for 2 hours. Moreover, licorice extract 100% was found to be the best treatment to increase root number in grape cuttings near to the results of IBA (El-Shaima*et al.*, 2018). On the other hand, root elongation of the cuttings in the present study might be due to the stimulating agents exist in the two extracts. Rajan and Singh(2021) mentioned that natural decoction of many plants and natural substances have efficiency to elongate roots in cuttings, this is dueto occurrence of IAA and minerals in the extract of those substances. Wise *et al.* (2020) stated that a major component of willow bark extract is salicylic acid which is a phytohormone improved root growth and development. Additionally, rooting promoters accelerate earlier root formation onto cuttings as a result of breakdown and mobilization of carbohydrates and nitrogenous substances at the base of cuttings, particularly at high concentrations, thus roots are elongated excessively due to utilization of more nutrients (Babaie*et al.*, 2014).

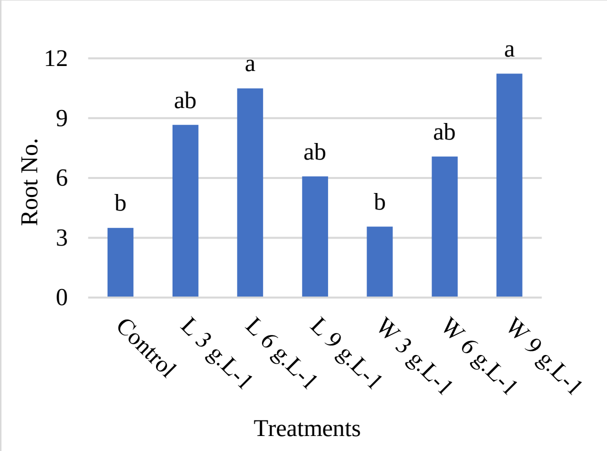

Fig. 2-Effect of different concentrations of licorice root extract (L) and willow shoot extract (W) on root number. Means sharing the same letter are not significantly different according to Duncan's multiple-range Test at $p \leq 0.05$.

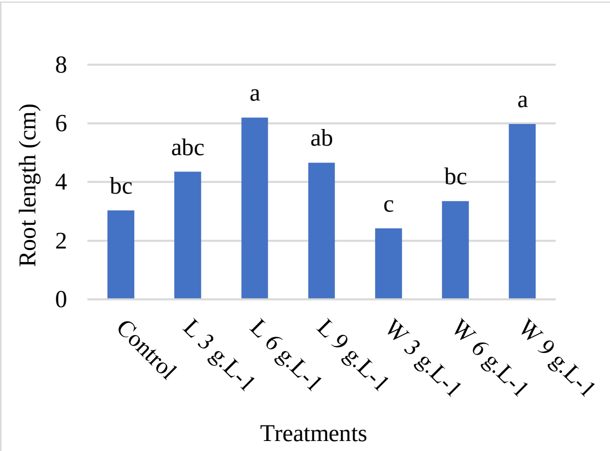

Fig. 3-Effect of different concentrations of licorice root extract (L) and willow shoot extract (W) on root length (cm). Means sharing the same letter are not significantly different according to Duncan's multiple-range Test at $p \leq 0.05$.

The results were shown in figure (4) confirmed that licorice and willow extracts gave rise to enhance shoot length in hardwood cuttings of olive compared to control cuttings. The longest shoot (2.18 cm) was found in cuttings treated with 9 g.L^{-1} willow extract, but control cuttings gave the shortest shoot (0.74 cm).

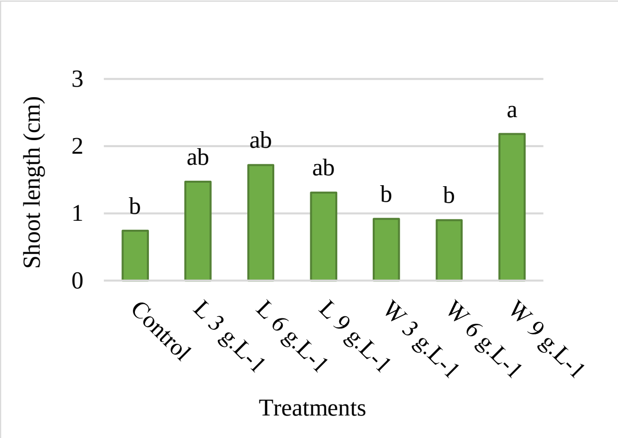

Fig. 4-Effect of different concentrations of licorice root extract (L) and willow shoot extract (W) on shoot length (cm). Means sharing the same letter are not significantly different according to Duncan's multiple-range Test at p ≤ 0.05.

Furthermore, shoot diameter was also significantly different according to concentration of the both extracts (Figure 5); 9 g.L^{-1} willow and licorice extracts increased shoot diameter significantly and reached in (l.41 and 1.38 mm, respectively), in contrast shoot diameter reduced to the lowest (0.91mm) in control cuttings.

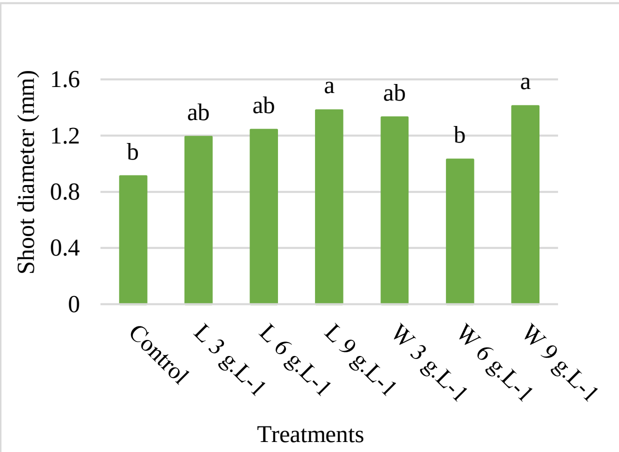

Fig. 5-Effect of different concentrations of licorice root extract (L) and willow shoot extract (W) on shoot diameter (mm). Means sharing the same letter are not significantly different according to Duncan's multiple-range Test at p ≤ 0.05.

Additionally, leaf number was notably elevated (17.02) in cuttings soaked in 6 g.L^{-1} licorice extract (Figure 6), contrarily leaf number diminished to (7.66) at 3 g.L^{-1} willow extract and control cuttings (8.13).

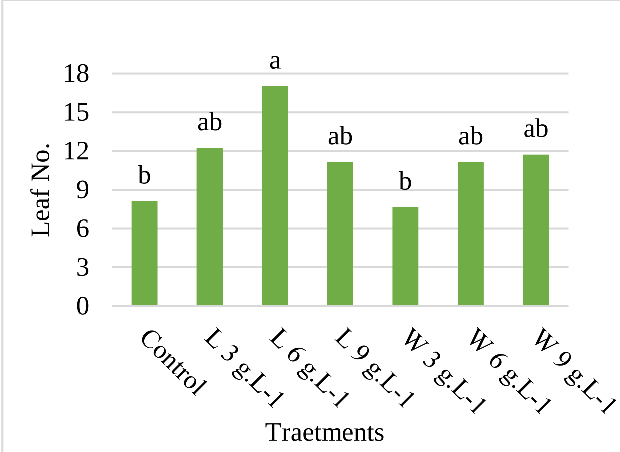

Fig. 6-Effect of different concentrations of licorice root extract (L) and willow shoot extract (W) on leaf number. Means sharing the same letter are not significantly different according to Duncan's multiple-range Test at p ≤ 0.05.

The above results revealed that the concentrations of the two extracts which encouraged better root number and length also gave the best shoot traits in terms of shoot length, shoot diameter and leaf number. Therefore, the analysis of correlation of the means in this study confirmed there was a positive association among root number and root length with shoot length and leaf number (Figure 7). According to the correlation analysis, root number and root length positively correlated with shoot length (r = 0.91, p-value = 0.005 and 0.004, respectively) and with leaf number (r = 0.82, p-value = 0.023 for root number) and (r = 0.84, p-value = 0.017 for root length). Perhaps these are because of better root characters lead to absorb more water and nutrients needed to superior shoot growth. Shukla *et al*. (2010) mentioned that growth of shoot in cuttings relies on favorable balance between root and shoot ratio crucial for uptake and translocation of water and nutrients. Branislav*et al*. (2009) summarized that vigorous shoot growth and development occurred in poplar cuttings subsequent earlier intensive root formation. Besides, it is possible that the extracts directly promoted shoot growth of the cuttings via inducing growth of buds as a result of metabolize reserved food and biostimulant effects on the growth of buds and formed shoots. Chandramouli (2001) showed that high concentration of auxin elongated shoot on the cuttings through elevating better utilization of nutrients such as carbohydrates and nitrogen. Ingle and Venugopal(2009) referred that elongation of shoot caused high node number which in turn means high leaf number. Apart from, a balance of auxin and cytokinin in cuttings is decisive to the best growth of vegetative parts, and cytokinin and auxin activate the genes involved in growth and regulate cell division in a harmonious manner (Bredmose*et al*., 2004). Auxin and cytokinin were found in licorice and willow extracts (Fujita *et al*., 2014; Rehman*et al*., 2018; Desoky*et al*., 2019).

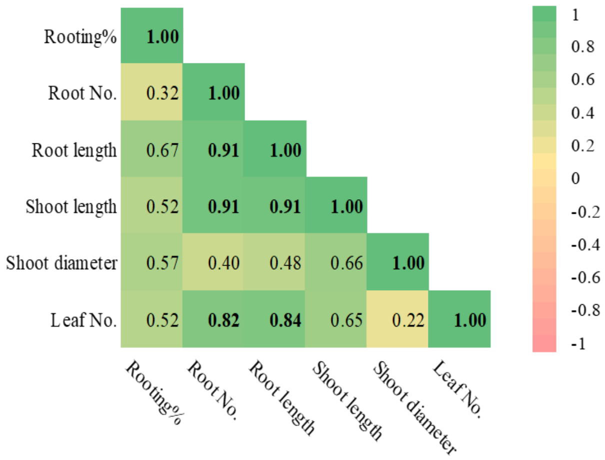

Fig. 7-Pearson correlation analysis of the six studied characteristics.

IV. CONCLUSION

The results of this study indicated that rooting percentage in hardwood cuttings of olive was increased by soaking them in 6 and 9 $g.L^{-1}$ licorice root extract, while 9 $g.L^{-1}$ willow shoot extract together with 6 $g.L^{-1}$ licorice extract improved most other shoot and root characteristics.

REFERENCES

[1] Al-Amad, I., &Qrunfleh, M. (2014). Effect of Babylon weeping willow (Salix babylonica L.) extracts on rooting of stem cuttings of olive (*Oleaeuropaea* L.)'Nabali'. In *XXIX International Horticultural Congress on Horticulture: Sustaining Lives, Livelihoods and Landscapes (IHC2014): 1130*, 391-396.

[2] Arena, M. J., Schwarz, O.J., & Witte, W.T. (1997). Experiments with locust and willow diffusates on rooting cuttings. *HortScience, 32*(4), 590D-590.

[3] Awan, A. A., Ullah, E., Abbas, S. J., Khan, O., &Masroor, S. (2012). Growth response of various olive cultivars to different cutting lengths. *Pak. J. Agri. Sci*, *49*(3), 283-287.

[4] Babaie, H., Zarei, H., Nikdel, K., &Firoozjai, M. N. (2014). Effect of different concentrations of IBA and time of taking cutting on rooting, growth and survival of *Ficusbinnendijkii*' Amstel Queen' cuttings. *NotulaeScientiaBiologicae*, *6*(2), 163-166.

[5] Branislav, K., Savo, R., Dragana, M., Petar, I., & Marina, K. (2009). Early shoot and root growth dynamics as indicators for the survival of black poplar cuttings. *New Forests*, *38*(2), 177-185.

[6] Bredmose, N., Kristiansen, K., & Nielsen, B. (2004). Propagation temperature, PPFD, auxin treatment, cutting size and cutting position affect root formation, axillary bud growth and shoot development in miniature rose (*Rosa hybrida* L.) plants and alter homogeneity. *The Journal of Horticultural Science and Biotechnology*, *79*(3), 458-465.

[7] Centeno, A., & Gomez-del-Campo, M. (2008). Effect of root-promoting products in the propagation of organic olive (*Oleaeuropaea* L. cv. Cornicabra) nursery plants. *HortScience*, *43*(7), 2066-2069.

[8] Chandramouli H (2001). Influence of growth regulators on the rooting of different types of cuttings in *Burserapenicillata*(DC) Engl. UniAgriSci Bangalore, MSc Thesis.

[9] Desoky, E. L., Elrys, A. S., &Rady, M. M. (2019). Licorice root extract boosts Capsicum annuum L. production and reduces fruit contamination on a heavy metals-contaminated saline soil. *International Letters of Natural Sciences*, *73*, 1-16.

[10] do Prado, D. Z., Dionizio, R. C., Vianello, F., Baratella, D., Costa, S. M., & Lima, G. P. P. (2015). Quercetin and indole 3-butyric acid (IBA) as rooting inducers in'Eucalyptusgrandis E. urophylla'. *Australian Journal of Crop Science*, *9*(11), 1057-1063.

[11] Eid, A. A. M., Nomier, S. A., Ibrahim, M. M., & Gad, M. M. (2018). Effect of some natural extracts, indolbutiric acid and naphthalene acetic acid on rooting of picual olive cuttings. *Zagazig Journal of Agricultural Research*, *45*(1), 119-136.

[12] El Sherif, F. (2017). *Aloe vera* leaf extract as a potential growth enhancer for Populus trees grown under in vitro conditions. *American Journal of Plant Biology*, *2*(3), 101-105.

[13] El-Dengawy, E. F. A., Wanas, A. L. E., &Farrag, M. H. (2017). Improvement of the Rooting Efficiency and Vegetative Growth in Date Palm Offshoots by Licorice Root Extract and Auxins Mixture Applications. *Journal of Plant Production*, *8*(7), 789-796.

[14] El-Shaima, M., El-Botaty,E. M., & Saleh, M.M.S. (2018). Effect of Some Natural Substances on Grape Cuttings Rooting. *Middle East Journal of Agriculture, 7*(4) 1702-1719.

[15] Fujita, K., Nomura, Y., Sawajiri, M., Mohapatra, P. K., El-Shemy, H. A., Nguyen, N. T., ... & Fujita, T. (2014). The extracts of Japanese willow tree species are effective forapoptotic desperation or differentiation of acute myeloid leukemia cells. *Pharmacognosy magazine*, *10*(38), 125-131.

[16] Gad, M. M., & Ibrahim, M. M. (2018). Effect of IBA and some natural extracts on rooting and vegetative growth of Picual olive sucker and shoot cuttings. *Curr. Sci. Int*, *7*(2), 191-203.

[17] Hayat, Q., Hayat, S., Irfan, M., & Ahmad, A. (2010). Effect of exogenous salicylic acid under changing environment: a review. *Environmental and experimental botany*, *68*(1), 14-25.

[18] Hechmi, M., Khaled, M., Abed, S., El-Hassen, A., Faiez, R., &M'hamed, A. (2013). Performance of olive cuttings (*Oleaeuropaea* L.) of different cultivars growing in the

agro-climatic conditions of Al-Jouf (Saudi Arabia). *American Journal of Plant Physiology*, *8*(1), 41-49.

[19] Ingle, M. R., &Venugopal, C. K. (2009). Effect of different growth regulators on rooting of stevia (*Stevia rebaudiana*Bertoni) cuttings. *Karnataka Journal of Agricultural Sciences*, *22*(2), 455-456.

[20] Kawase, M. (1970). Root promoting substances in Salix alba. Physiol. Plant. *23* (*1*), 159–170.

[21] Knapke, D., Readal, M., Stravinsky, D., Wells, L., & Wilson, M.J. (2018). The herb society of America's notable native herbal tree 2018 willow *Salix* L. species.The Herb Society of America. https://herbsocietyorg.presencehost.net/file_download/4f47ddcd-52f2-4b14-a959-698b475a7142.

[22] Mohammed, A. A., Ahmad, T. A., Noori, I. M., Aziz, R., & Ahmad, F. K. (2020). Application of baking yeast to induce rooting in hardwood cuttings of olive (*Oleaeuropaea* L.) cv. Sorani. *Euphrates Journal of Agriculture Science*, *12*(2).

[23] Rady, M. M., Desoky, E. S., Elrys, A. S., &Boghdady, M. S. (2019). Can licorice root extract be used as an effective natural biostimulant for salt-stressed common bean plants. *South African Journal of Botany*, *121*, 294-305.

[24] Rahman, N., Awan, A. A., Nabi, G., & Ali, Z. (2002). Root initiation in hard wood cuttings of olive cultivar coratina using different concentration of IBA. *Asian Journal of Plant Sciences*, *1*(5): 563-564.

[25] Rajan, R. P., & Singh, G. (2021). A review on the use of organic rooting substances for propagation of horticulture crops. *Plant Archives*, *21*(1), 685-692.

[26] Rao, K. V. S. (1993). A review on Licorice. *Ancient science of life*, *13*(1-2): 57-88.

[27] Rehman, A., Khan, M. S., Zakaria, S. A., &Malik, M. S. (2018). To Study the Effect of Willow Extract on Apple Cuttings for Different Time Duration. *Journal of Biology, Agriculture and Healthcare*, *8*(13), 21-24.

[28] Sandoval-Yapiz, M.R.(2004). Reguladores de crecimiento XXIII: efecto del acidosalicilicoen la biomasa del cempazuchitl (*Tageteserecta*). Tesis de Licenciatura. Instituto Tecnologico Agropecuario, Conkal, Yucatan, Mexico.

[29] Shukla, H. S., Tripathi, V. K., Awasthi, R. D., &Tripathi, A. K. (2010). Effect of IBA, PHB and Boron on rooting and shoot growth of hardwood stem cuttings of Peach. *Int J of Applied Agricultural Research*, *5*, 467.

[30] Tahoori, F., Ahmad, M. A. J. D., Nejadsattari, T., Ofoghi, H., &Iranbakhsh, A. (2019). Qualitative and Quantitative Study of Quercetin and Glycyrrhizin in In Vitro Culture of Liquorice (*Glycyrrhizaglabra* L.) and Elicitation with AgNO3. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, *47*(1): 143-151.

[31] Tarragó, J., Sansberro, P., Filip, R., López, P., González, A., Luna, C., &Mroginski, L. (2005). Effect of leaf retention and flavonoids on rooting of *Ilex paraguariensis* cuttings. *ScientiaHorticulturae*, *103*(4): 479-488.

[32] Wilson, P., &Staden, J. V. (1990). Rhizocaline, rooting co-factors, and the concept of promoters and inhibitors of adventitious rooting-a review. *Annals of Botany*, *66*(4), 479-490.

[33] Wise, K., Gill, H., & Selby-Pham, J. (2020). Willow bark extract and the biostimulant complex Root Nectar® increase propagation efficiency in chrysanthemum and lavender cuttings. *ScientiaHorticulturae*, *263*, 109108.

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A comparison of bacterial variability across biogeographic regions based on PGPR

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Abstract— *Plants are sessile organisms that experience abiotic stresses like sediment salinity, drought, and extreme temperatures. In light of our growing population and increasing demand for better nutrients and commercial quality foods, optimizing the use of our natural resources is essential to ensuring food security.Enhancing PGPR using modern tools and techniques of biotechnology can greatly contribute to achieving sustainable agriculture, by improving soil fertility, plant tolerance, crop productivity, and maintaining a balanced nutrient cycling. Plant rhizospheres could contribute to the development of robust plant growth-promoting (PGP) activities and stress tolerance capabilities under drought conditions. They enhance the adaptation of plants for different desertification environments because they are diverse and functionally redundant (PGPR). In addition to soil properties and plant species, there are a variety of biotic and abiotic factors that influence their composition and abundance. In this review article, we learn how Bacterial diversity is influenced by soil environment and geophysical conditions.*

Keywords— ***Endosphere, Halotolerant, Microniches, PGPR, Rhizosphere, Xerophytic Microflora.***

I. INTRODUCTION

Soil environment and geophysical conditions have a direct impact on bacterial diversity. The soil is composed of microinches with heterogeneous physical and chemical properties across a wide range of scales. Since bacteria live in small pockets within a niche, the properties of their immediate environment rather than the soil's mean properties influence the survival of their local community. Space heterogeneity has been shown to result in lower survival rates in local communities due to the heterogeneity of the bacterial communities on <1 cm **[1].** Abiotic factors such as soil physicochemical properties and geographical location are important factors that shape bacterial community abundance and diversity. The type and location of soil, as well as the plant species in an area, significantly change the structure of bacterial communities. The functional profiles of soil bacterial communities were influenced by both the location of the soil and the plants in the soil. Researchers have found that soil and plants play a major role in shaping rhizosphere microbiota's composition **[2]**. In rhizospheres, different soil properties such as pH, concentrations of phosphorus and potassium, as well as concentrations of other mineral nutrients, affect the composition of the bacterial communities differently **[3]**.

The PGPR bacteria are beneficial bacteria that colonize the roots of plants and enhance their growth through a variety of mechanisms **[4]**. These bacteria colonize the roots of plants and boost their growth in a variety of ways. Take steps to control insect and disease damage. Either directly or indirectly assist plants in growing. Reducing agricultural reliance on hazardous chemicals. Enhancing soil fertility by releasing nutrients. They affect plant growth in several ways, including direct mechanisms such as nutrition and growth regulation, as well as indirect mechanisms related to their ability to biocontrol **[5].** A key tool for boosting sustainable agriculture, PGPR has gained popularity over the last few decades. PGPRs can affect plant growth either indirectly or directly. Direct growth promotion by PGPR involves giving a plant a bacterium-produced compound, such as phytohormones, or encouraging the plant to take up nutrients from the environment **[6]**. By inhibiting the negative effects of phytopathogenic organisms, PGPR indirectly promotes plant growth. By doing so, antagonistic substances are produced or resistance is created against pathogens **[6].**

Rhizobacteria associated with plants are effective at raising plant tolerance to stress **[7][8][9]**named this phenotypic tolerance to abiotic stress "Induced General Tolerance" to describe plant responses to PGPR-induced abiotic stress. Based on their proximity and intimacy to roots, Rhizobacteria are divided into (i) soil-dwelling bacteria, which utilize roots' metabolites for carbon and nitrogen, (ii) rhizoplane- colonizing bacteria, (iii) bacteria that reside inside cortical cells, and (iv) bacteria living within specialized root structures. As the distance between the root and the plant changes, so does the association of plant growth-promoting bacteria. Extracellular PGPR (ePGPR) and intracellular PGPR (iPGPR) make up the vast majority of PGPR **[10].**PGPR (iPGPR) bacteria live inside plant cells and produce nodules. They are located in these specialized structural elements. PGPR (ePGPR) bacteria don't produce nodules but stimulate plant growth through the production of signal compounds. The species of *Rhizobium*, *Mesorhizobium*,*Bradyrhizobium*, *Allorhizobium*, and Frankia are examples of these. For higher plants, these fix atmospheric nitrogen **[11]**.Based on their level of association with roots, ePGPR can be classified into three types: bacteria living near but not in contact with roots; bacteria colonizing the surface of roots; and bacteria growing between the cells of root cortex **[12].** *Azotobacter*, *Caulobacter*, *Azospirillum*, *Bacillus*, *Erwinia*, *Chromobacterium*, *Agrobacterium*, *Erwinia*, *Flavobacterium*, *Arthrobacter*, *Micrococcus*, *Pseudomonas* and *Burkholderia* are some examples of ePGPRs. There is much greater diversity in host plant proximity, infection mode, and, most significantly, plant effect on ePGPR associations, compared to iPGPR**[13].**

Communication and environmental sensing are carried out by both beneficial and pathogenic bacteria using QS. It consists of a series of gene-regulated molecules known as autoinducers that act on bacteria at the gene level **[14]**. To maximize their population size and fitness, bacteria release AIs into the environment to assess their surroundings and adapt their gene expression accordingly. AHL, for example, is one of the many well-studied artificial ions produced by Gram-negative bacteria, whereas cyclic peptides are prevalent among Gram-positive bacteria **[15]**.Furthermore, QS is involved in several physiological behaviours such as symbiosis, virulence, antibiotic production, conjugation, competence, sporulation, and biofilm formation **[16]**. PGPRs also communicate with higher organisms, such as plants, using QS, whether they belong to the same species or not. As it turns out, plant-microbe communication is now evidently dependent on QS communication for microbial interactions at the rhizosphere **[17].**Evolutionary adaptations of plants have introduced an array of ways for them to respond to QS, such as sensing and responding to bacterial QS signals, and producing substances that mimic AHL and can impact QS in the plant-associated bacterial community. Numerous studies also reveal that the rhizosphere harbours more AHL producing bacteria than the bulk soil, and that these AHL molecules are capable of acting as inter-kingdom signalling molecules **[17][18].**When rhizobacteria form closer associations inside the roots of plants, they are called endophytes. The term is defined as "bacteria that can be isolated from surface-disinfected plant tissue, or that can be extracted from the plant itself without causing visible harm to the plant" **[19].**There are several types of endophytes, such as rhizobialnodulating bacteria and nitrogen fixers **[20].**

II. RHIZOSPERE

Life's terrain consists of the elements of an ecosystem that cannot be seen by the naked eye, such as soil or water. In soils, rhizosphere is likely the primary site of bacterial activity. "Rhizosphere" originates from the Greek words "rhiza" (root) and "soi" (ground). It can also be referred to as the root zone, soil zone, or hydrological region. PGPRS colonizes plant roots by inhabiting root tissues, around roots, and on the rhizoplane (root surface) **[6].**Rhizospheres were first described by German scientist Lorenz Hiltner (1904) as "areas of soil next to legume roots where bacteria thrive.". Therefore, it is a nutrient-rich area of soil directly surrounding roots of plants. Unlike other regions, this one has no distinct edges; it is influenced by compounds released by the roots and microbial life that feeds upon them. Its varied and dense flora makes it a highly dynamic region. By observing the composition and pattern of root exudates, we can gain insight into microbial activity and population proportions. Rhizosphere interactions affect root growth and function, ultimately resulting in changes to the plant's growth. Each plant species hosts a unique PGPR community**[21].**

III. PGPR IN DESSERTS: BACTERIAL COMMUNITY SHAPING FORESTS IN DESERT PLANTS

Deserts are dynamic, heterogeneous habitats that take up one third of the planet. A desert plant's adaptation to the desert environment is also determined by the microbial communities that colonize and inhabit its surrounding soils **[22].** It is extremely hard for microbes to thrive in deserts**[23].**Around the world, climates vary widely, but all are characterized by extreme temperatures, desiccation, soil salinity, minimal amounts of nutrients, and high levels of ultraviolet radiation during the summer. Xerophytic microflora flourishes in environments of constant water stress over an extended period of time. Due to the harshness of desert environments for so long, desert plants were forced to develop unique structures, including specialized leaves and stems, to adapt to the frequent stress conditions associated with deserts **[24].** It is observed that drought affects plants' water potential **[25]** as well as soil nutrient availability and distribution **[26]** also contributing to an increase in reactive oxygen species (ROS) **[27].** An excessive amount of ROS can cause lipid peroxidation, which harms plants in morphology and physiological functions **[28].**The plants use an array of antioxidant enzymes to keep them healthy, including superoxide dismutase (SOD), peroxidase (POD), catalase (CAT) and ascorbate peroxidase, and non-enzymatic antioxidants like glutathione and proline, which plants use to scavenge ROS and prevent membrane damage **[29][30][31].** Bacteria react differently to drought stress at different organizational levels depending on the intensity of the stress, period, species, and stage of growth. The PGPR could be beneficial for developing strategies to facilitate the conservation of plant water. The PGPR isolated from desert soils has evolved well to deal with extreme environmental factors like salinity and heat, enabling stress response genes to promote plant growth and enhance soil fertility **[32 [33][34][35][36].**

Although these pioneer desert plants were subjected to extreme conditions, their rhizospheres contained a rich diversity of bacteria. The composition of rhizosphere bacterial communities is influenced by abiotic factors such as soil type and composition, location, and soil properties. In similar geographical locations, the soil, rhizosphere, phyllosphere and associated bacterial communities are similar, whereas plants in distant geographic locations with distinct soil properties have a different soil bacterial community and have different associated bacterial communities. Furthermore, plant host genotyping has an effect on the community of bacteria in the Endosphere. There are a variety of bacterial communities in desert plants, but two major phyla are mainly dominant: Proteobacteria and Actinobacteria. Proteobacteria have a wide range of adaptation capabilities to a variety of environmental lifestyles, including nitrogen-fixing plant symbionts such as *Rhizobium*, *Mesorhizobium*, and *Sinorhizobium***[37].**The Actinobacteria have shown impressive versatility in their ability to grow under extreme conditions including salinity, low pH, low water availability, high radiation, and pressure, they include several diverse species (including alkalophiles, acidotolerants, thermotolerant, and halotolerant) **[38].**

There are certain features of desert plants that make them dependent on bacterial communities, such as high expression of genes related to dormancy and osmoregulation, and lower expression of genes related to nutrient cycling and catabolisms. Root exudates, plant species, genotypes, and plant compartments all determine the diversity of bacteria in the plant Endosphere **[21] [39][40][41] [42].**Plants mediated by PGPR are known to withstand drought stress in several ways, from producing exopolysaccharides (EPS) to 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase to reduce ethylene production **[43].** PGPR strains can reduce drought-induced oxidative damage to plants by increasing antioxidant enzyme activity **[44].** A PGPR strain *Mitsuaria* and a PGPR strain *Burkholderia* produced ACC deaminase and EPS that improved the root system of Arabidopsis by increasing proline content and antioxidant activity and decreasing malondialdehyde levels **[45].**The strains of *Variovorax paradoxus* RAA3, *Pseudomonas palleroniana* DPB16, and *Pseudomonas sp*. are capable of relieving wheat from drought stress. The UW4 strain **[46]**was isolated from pepper by *Bacillus licheniformis* K11 **[47].** *Glycyrrhiza uralensis* grew better under drought stress when *Bacillus pumilus* modified antioxidant levels **[30].** EPS is beneficial for plant survival during drought conditions **[48].** The EPS-producing strains of *Proteus penneri* (Ep1), *Pseudomonas aeruginosa* (Pa2), and *Alcaligenes faecalis* (AF3) have previously been isolated **[49].** There are abundant and diverse microbial communities in desert soils across the world, among which the four most universal phyla are *Actinobacteria*, *Proteobacteria, Bacteroidetes,* and*Cyanobacteria***[50].**

IV. IMPACT OF AQATIC PGPR

Due to the unique nature of the underwater life history, the competition for abiotic resources, like light availability, is more intense in the fresh water ecosystem. Additionally, freshwater ecosystems are typically biologically inaccessible. Rehabilitating macrophyte species and communities is more challenging than rehabilitating terrestrial plants. Aquatic plants, due to their adaptability to aquatic environments and rapid reproduction rate, offer good potential for restoring habitats and removing special pollutants, like pesticides and toxic metals **[51] [52].** A PGPR can increase aquatic plant growth by dissolving potassium, phosphorus, releasing hormones such as Cytokinin's and Indole-3-acetic acid (IAA), generating siderophores and 1-aminocyclopropane-1-carboxylate deaminases

[53], thereby enhancing plants' resistance to environmental stresses **[54].**

The PGPR strains can enhance the recovery of submerged plants in organically rich sediment **[55].** In many parts of the world, water ecosystems are experiencing deterioration in water quality and ecological structure **[56].**After a decrease in water nutrition level, macrophytes usually take decades to adjust to the new environment **[57].** The presence of organic sediment in lakes negatively impacts their ecological restoration because it acts as a stressor for macrophyte growth. It has been demonstrated that sediment anoxia inhibits the germination and sprouting of submerged macrophytes during restoration **[58],** and there are numerous issues associated with organic enrichment in water. An anoxic degradation pathway causes oxygen exhaustion and accumulation of potentially phytotoxic compounds, which causes benthic vegetation to decline **[59].**The high organic matter content of fertile sediments leads to decreases in aquatic plant biomass. Furthermore, excessive organic matter content can generate reductive sapropel, which threatens aquatic plants' survival **[60].**Phoridobacteria (PGPR) were screened from the rhizosphere of submerged macrophytes and selected for their ability to promote *Vallisneria natans* under the high preponderance of sediment organic matter **[55].**

V. IMPACT OF HALOPHYTES

Agricultural sustainability is seriously threatened by soil salinity. The salt content in soils is one of the major abiotic stressors known to affect arid and semi-arid regions, and this leads to significant losses in agriculture productivity.2009 determined that increases in salinity negatively affect the growth-promoting characteristics of PGPRs**[61]**. Therefore, using halotolerant PGPRs that are selected for high salt tolerance along with efficiency in expressing PGPR traits would allow crops to be grown successfully in environments with natural or induced salinity **[62].**

Salt-tolerant plants such as halophytes adapt to salt-contaminated environments and can survive at salinities as high as 1M NaCl **[63] [64].** Halophytic plants have a lot of salt-tolerant rhizobacteria in the rhizosphere, which is beneficial to crops at a low level of salinity stress **[65] [66] [67].** Rhizobacteria that live in high saline environments have evolved many strategies to survive, one of these strategies is the ability to accumulate compatible osmolytes, maintaining a balance between the forces of diffusion and growth **[68] [69].** These microorganisms exhibit multiple biochemical and physiological stress-related traits that facilitate plant immunity to salt stress under lower salinity levels **[69] [70] [71] [72].**

Halotolerant may grow in environments varying from one to thirty-third NaCl, as well as at intervals without NaCl **[73] [74].** Under low water potential owing to salt stress, they are thus found at intervals in the rhizosphere of halophytes **[61] [75].** The plant growth-promoting bacteria *Moneron pumilus, Mendocina spp., Arthrobacter spp., Halomonas spp., and Nitrinicolacisaponensis*have features such as phosphorus (P) solubilization, in addition to producing ACC deaminase, IAA, and siderophores. These traits are referred to as PGP traits because they allow plants to produce under limiting conditions, stimulate plant growth by serving as a phytohormone (IAA), provide metal to the plant through chelation (siderophores), and release a precursor to the plant stress hormone (ACC deaminase).

The endogenous phytohormone is regulated by PGPRs **[76][77][78]** and assists the signalling phytohormone (GA) in inhibiting the growth of plants under stress **[79][80]** that affect biological processes and elongation, hypocotyls, stem growth, leaf and root part size **[80][81][82].**

VI. TROPICAL MANGROVE ECOSYSTEM NEED TO BE MAINTAINED BY MAINTAINING MICROBIAL COMMUNITY DYNAMICS:

The mangrove forest is considered to be one of the most productive and biologically diverse wetlands on Earth, constituting an important natural reserve. There are currently fewer than 50% remaining, with half of it being degraded. Due to the deforestation of mangroves in many parts of the world, fish resources are dwindling, water supplies are being contaminated, and coastal erosion and salinization are rampant. Plants can use the nitrogen, phosphorus, and other nutrients being generated by the highly diverse and productive microbial community in tropical and subtropical mangrove ecosystems. Despite having a high organic matter content, mangrove ecosystems generally lack nutrient levels, especially nitrogen and phosphorus **[83][84][85].** Mangrove ecosystems are heavily dependent on microbial modification (bacteria and fungi) for nutrient cycling **[85 [86][87].** The world's mangrove ecosystems are important natural resources that require protection **[88].**A greater effort needs to be made to protect high-quality or primary mangrove sites. Mangrove rhizosphere bacteria can be used to enhance reforestation in Tamil Nadu's Pichavaram mangrove wetland, a habitat dominant in *Rhizophora* and *Avicennia* species.

There are some bacterial strains isolated from the root rhizosphere of mangrove plants that are useful as PGPBs and could be used to promote plant growth in programs of reforestation, or in the creation of mangrove wetlands in coastal lagoons. The inoculation of black mangrove plantlets with *M. chthonoplastes* caused roots to colonize rapidly, increasing nitrogen fixation **[89]** as well as nitrogen accumulation **[90].** When black mangrove seedlings were inoculated with a mixture of two bacteria, nitrogen was incorporated into the leaves twice as much and leaves developed more rapidly **[91].** Mangrove roots may contain bacteria that can be used as PGPB to improve the establishment and enhance the growth of coastal mangrove seedlings.

Nitrogen fixation occurs frequently in mangroves. Nitrogen was fixated at high rates in association with dead and decomposing leaves, pneumatophores (air roots), the root rhizosphere, tree bark, *Cyanobacterial* mats that covered surface sediments as well as the sediments themselves **[84][89][92][93][94][95][96][97][98][99].**Vari ous mangrove species were found to contain nitrogen-fixing bacteria grouped into the genera *Azospirillum, Azotobacter, Rhizobium, Clostridium,* and *Klebsiella* **[83][100].**

VII. BACTERIA ASSOCIATED WITH FOREST SOILS

Forest soil bacteria are an important part of the soil microbial community, though they are understudied.In forest ecosystems, bacteria perform diverse ecological functions such as organic matter decomposition, mycorrhizal symbiosis regulation, and participation in the N cycle. The major natural agents responsible for N fixation in forest ecosystems are bacteria **[101]** as well as other processes, such as mineral weathering, that cause inorganic nutrients to be released **[102].** A forest ecosystem provides many habitats for bacteria, including soils, plant tissues, streams, rocks, etc., however, soils and litter, which are particularly rich in bacteria, are predominant on the forest floor **[103].** According to most studies, Acidobacteria, Actinobacteria, Proteobacteria, Bacteroidetes, and Firmicutes are abundant in soil **[104].** Soil pH is one of the most important drivers in determining the composition of bacterial communities. in addition to ph. other components such as the organic matter content, nutrient availability, climate conditions, and biotic interactions (especially the influence of vegetation), influence bacterial community composition **[104].**

Two-thirds of the earth's C is stored in forest soils **[105].** A great deal of the global carbon balance is influenced by temperate and boreal floras as they will remain a large sink of anthropogenic carbon dioxide. Some soil bacteria are capable of fixing CO_2 in the soil. *Bradyrhizobium* (Betaproteobacteria) is a bacterium that inhabits forest soils in large numbers **[106].** For forest soils with aerobic conditions, methane represents a gaseous form of organic carbon **[107].** It has been reported that these methanotrophs are the primary consumers of atmospheric methane as well as methane from waterlogged, anaerobic soil horizons **[108].** Methane can be sequestered from the atmosphere by forest soils (especially those of boreal forests) because of the presence of methanotrophic bacteria. Most of the characterized methanotrophs belong to the *Alphaproteobacteria* and *Gammaproteobacteria***[109].**

In unmanaged environments, bacteria are estimated to contribute more than 95% of the N input **[101].**nifH is present in the *Alphaproteobacteria (Bradyrhizobium, Azospirillum, Hyphomicrobium, and Gluconacetobacter*species) and Deltaproteobacteria (*Geobacter*species). was observed in different temperate forest soils, demonstrating the ubiquity of some N-fixing bacteria, not only as symbiotic but also as free-living taxa **[106].**

VIII. CONCLUSION

The PGPR bacteria are beneficial bacteria that colonize the roots of plants and enhance their growth through a variety of mechanisms. In similar geographical locations, the soil, rhizosphere, phyllosphere and associated bacterial communities are similar, whereas plants in distant geographic locations with distinct soil properties have a different soil bacterial community and have different associated bacterial communities. Soil environment and geophysical conditions have a direct impact on bacterial diversity**.** The functional profiles of soil bacterial communities were influenced by both the location of the soil and the plants in the soil.

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REFERENCES

[1] O'Brien, Flora JM, et al. "Rhizosphere bacterial communities differ according to fertilizer regimes and

cabbage (Brassica oleracea var. capitata L.) harvest time, but not aphid herbivory." Frontiers in microbiology 9 (2018): 1620.

[2] Lundberg, Derek S., et al. "Defining the core Arabidopsis thaliana root microbiome." Nature 488.7409 (2012): 86-90.

[3] Edwards, Joseph, et al. "Structure, variation, and assembly of the root-associated microbiomes of rice." Proceedings of the National Academy of Sciences 112.8 (2015): E911-E920.

[4] Glick, Bernard R. "Plant growth-promoting bacteria: mechanisms and applications." Scientifica 2012 (2012)

[5] Boyetchko, Susan M., and Antonet M. Svircev. "A novel approach for developing microbial biopesticides." Biological Control Programmes in Canada 2001–2012 (2013): 37-43.

[6] Glick, Bernard R. "The enhancement of plant growth by free-living bacteria." Canadian journal of microbiology 41.2 (1995): 109-117.

[7] Etesami, Hassan, and Gwyn A. Beattie. "Plant-microbe interactions in adaptation of agricultural crops to abiotic stress conditions." Probiotics and Plant Health. Springer, Singapore, 2017. 163-200.

[8] Etesami, Hassan, and Dinesh K. Maheshwari. "Use of plant growth promoting rhizobacteria (PGPRs) with multiple plant growth promoting traits in stress agriculture: Action mechanisms and future prospects." Ecotoxicology and environmental safety 156 (2018): 225-246.

[9] Yang, Xiyan, and Xianlong Zhang. "Regulation of somatic embryogenesis in higher plants." Critical Reviews in Plant Science 29.1 (2010): 36-57.

[10] Martínez-Viveros, O., et al. "Mechanisms and practical considerations involved in plant growth promotion by rhizobacteria." Journal of soil science and plant nutrition 10.3 (2010): 293-319.

[11] Bhattacharyya, P. N., and Jha, D. K. (2012). Plant growth-promoting rhizobacteria (PGPR): emergence in agriculture. World J. Microbiol. Biotechnol. 28, 1327–1350. doi: 10.1007/s11274-011-0979-9

[12] Vessey, J. Kevin. "Plant growth promoting rhizobacteria as biofertilizers." Plant and soil 255.2 (2003): 571-586.

[13] Gray, E. J., and D. L. Smith. "Intracellular and extracellular PGPR: commonalities and distinctions in the plant–bacterium signaling processes." Soil biology and biochemistry 37.3 (2005): 395-412.

[14] Khan, N., Bano, A., Ali, S., and Babar, M. A. (2020). Crosstalk amongst phytohormones from planta and PGPR under biotic and abiotic stresses. Plant Growth Regul. 90, 189–203. doi: 10.1007/s10725-020-00571-x

[15] Hartmann, A., Rothballer, M., Hense, B. A., and Schröder, P. (2014). Bacterial quorum sensing compounds are important modulators of microbe-plant interactions. Front. Plant Sci. 5:131. doi: 10.3389/fpls.2014.00131

[16] Rutherford, S. T., and Bassler, B. L. (2012). Bacterial quorum sensing: its role in virulence and possibilities for its control. Cold Spring Harb. Perspect. Med. 2:a012427. doi: 10.1101/cshperspect.a012427

[17] Altaf, M. M., Khan, M. S. A., Abulreesh, H. H., and Ahmad, I. (2017). “Quorum sensing in plant growth-promoting Rhizobacteria and its impact on plant-microbe interaction,” in Plant-Microbe Interactions in Agro-Ecological Perspectives: Volume 1: Fundamental Mechanisms, Methods and Functions, eds D. P. Singh, H. B. Singh, and R. Prabha (Singapore: Springer), 311–331. doi: 10.1007/978-981-10-5813-4_16

[18] Elasri, Miena, et al. "Acyl-homoserine lactone production is more common among plant-associated Pseudomonas spp. than among soilborne Pseudomonas spp." Applied and environmental microbiology 67.3 (2001): 1198-1209.

[19] Quadt-Hallmann, A. N. D. R. E. A., J. W. Kloepper, and N. Benhamou. "Bacterial endophytes in cotton: mechanisms of entering the plant." Canadian journal of microbiology 43.6 (1997): 577-582.

[20] Lodewyckx, Cindy, et al. "Endophytic bacteria and their potential applications." Critical reviews in plant sciences 21.6 (2002): 583-606.

[21] Berg, G., and Smalla, K. (2009). Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere. FEMS Microbiol. Ecol. 68, 1–13. doi: 10.1111/j.1574-6941.2009.00654.x

[22] Vejan, Pravin, et al. "Role of plant growth promoting rhizobacteria in agricultural sustainability—a review." Molecules 21.5 (2016): 573.

[23] Marasco, R., Mosqueira, M. J., Fusi, M., Ramond, J.-B., Merlino, G., Booth, J. M., et al. (2018). Rhizosheath microbial community assembly of sympatric desert speargrasses is independent of the plant host. Microbiome 6:215. doi: 10.1186/s40168-018-0597-y

[24] Bechtold, Ulrike. "Plant life in extreme environments: how do you improve drought tolerance?." Frontiers in plant science 9 (2018): 543.

[25] Hsiao, Theodore C., and Liu-Kang Xu. "Sensitivity of growth of roots versus leaves to water stress: biophysical analysis and relation to water transport." Journal of experimental botany 51.350 (2000): 1595-1616.

[26] Vurukonda, Sai Shiva Krishna Prasad, et al. "Enhancement of drought stress tolerance in crops by plant growth promoting rhizobacteria." Microbiological research 184 (2016): 13-24.

[27] Gill, Sarvajeet Singh, and Narendra Tuteja. "Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants." Plant physiology and biochemistry 48.12 (2010): 909-930.

[28] Hou, Lin, et al. "Smart nanocomposite hydrogels based on azo crosslinked graphene oxide for oral colon-specific drug delivery." Nanotechnology 27.31 (2016): 315105.

[29] Mohammadi, Mohammad Hossein Sheikh, et al. "Molecular and physiological responses of Iranian Perennial ryegrass as affected by Trinexapac ethyl, Paclobutrazol and Abscisic acid under drought stress." Plant Physiology and Biochemistry 111 (2017): 129-143.

[30] Xie, Xiulan, et al. "The roles of environmental factors in regulation of oxidative stress in plant." BioMed research international 2019 (2019).

[31] Hinojosa, Leonardo, Janet B. Matanguihan, and Kevin M. Murphy. "Effect of high temperature on pollen morphology, plant growth and seed yield in quinoa (Chenopodium quinoa Willd.)." Journal of agronomy and crop science 205.1 (2019): 33-45.

[32] Paul, D., and Lade, H. (2014). Plant-growth-promoting rhizobacteria to improve crop growth in saline soils: a review. Agron. Sustain. Dev. 34, 737–752. doi: 10.1007/s13593-014-0233-6

[33] Makhalanyane, Thulani P., et al. "Microbial ecology of hot desert edaphic systems." FEMS microbiology reviews 39.2 (2015): 203-221.

[34] Eida, A. A., Ziegler, M., Lafi, F. F., Michell, C. T., Voolstra, C. R., Hirt, H., et al. (2018). Desert plant bacteria reveal host influence and beneficial plant growth properties. PLoS One 13:e0208223. doi: 10.1371/journal.pone.0208223

[35] Bokhari, A., Essack, M., Lafi, F. F., Andres-Barrao, C., Jalal, R., Alamoudi, S., et al. (2019). Bioprospecting desert plant Bacillus endophytic strains for their potential to enhance plant stress tolerance. Sci. Rep. 9:18154. doi: 10.1038/s41598-019-54685-y

[36] Eida, A. A., Alzubaidy, H. S., de Zélicourt, A., Synek, L., Alsharif, W., Lafi, F. F., et al. (2019). Phylogenetically diverse endophytic bacteria from desert plants induce transcriptional changes of tissue-specific ion transporters and salinity stress in Arabidopsis thaliana. Plant Sci. 280, 228–240. doi: 10.1016/j.plantsci.2018.12.002

[37] Pini, F., Galardini, M., Bazzicalupo, M., and Mengoni, A. (2011). Plant-bacteria association and symbiosis: are there common genomic traits in alphaproteobacteria? Genes 2, 1017–1032. doi: 10.3390/genes2041017

[38] Mohammadipanah, F., and Wink, J. (2016). Actinobacteria from arid and desert habitats: diversity and biological activity. Front. Microbiol. 6:1541. doi: 10.3389/fmicb.2015.01541

[39] Ramawat, K. G. (2010). Desert Plants Biology and Biotechnology. Berlin: Springer.

[40] Fierer, N., Leff, J. W., Adams, B. J., Nielsen, U. N., Bates, S. T., Lauber, C. L., et al. (2012). Cross-biome metagenomic analyses of soil microbial communities and their functional attributes. Proc. Natl. Acad. Sci. U.S.A. 109:21390. doi: 10.1073/pnas.1215210110

[41] Hacquard, S., Garrido-Oter, R., González, A., Spaepen, S., Ackermann, G., Lebeis, S., et al. (2015). Microbiota and host nutrition across plant and animal kingdoms. Cell Host Microbe 17, 603–616. doi: 10.1016/j.chom.2015.04.009

[42] Saad, Maged M., Abdul Aziz Eida, and Heribert Hirt. "Tailoring plant-associated microbial inoculants in agriculture: a roadmap for successful application." Journal of experimental botany 71.13 (2020): 3878-390.

[43] Belimov, Andrey A., et al. "Rhizosphere bacteria containing 1-aminocyclopropane-1-carboxylate deaminase increase yield of plants grown in drying soil via both local and systemic hormone signalling." New Phytologist 181.2 (2009): 413-423.

[44] Zhang, Min, et al. "Drought-tolerant plant growth-promoting rhizobacteria isolated from jujube (Ziziphus jujuba) and their potential to enhance drought tolerance." Plant and Soil 452.1 (2020): 423-440.

[45] Huang, Chao, et al. "Effect of Phanerochaetechrysosporium inoculation on bacterial community and metal stabilization in lead-contaminated agricultural waste composting." Bioresource technology 243 (2017): 294-303.

[46] Chandra, Rohan, et al. "Robusttp: End-to-end trajectory prediction for heterogeneous road-agents in dense traffic with noisy sensor inputs." ACM Computer Science in Cars Symposium. 2019.

[47] Kim, Jungmin, et al. "Methods of downstream processing for the production of biodiesel from microalgae." Biotechnology advances 31.6 (2013): 862-876.

[48] Sandhya, V. S. K. Z., et al. "Effect of plant growth promoting Pseudomonas spp. on compatible solutes, antioxidant status and plant growth of maize under drought stress." Plant Growth Regulation 62.1 (2010): 21-30.

[49] Naseem, Hafsa, and AsghariBano. "Role of plant growth-promoting rhizobacteria and their exopolysaccharide in drought tolerance of maize." Journal of Plant Interactions 9.1 (2014): 689-701.

[50] Alsharif, Mohammed H., et al. "Sixth generation (6G) wireless networks: Vision, research activities, challenges and potential solutions." Symmetry 12.4 (2020): 676.

[51] Ribeiro, ViníciusErlo, et al. "Enhanced essential oil and leaf anatomy of Schinusmolle plants under lead contamination." Industrial Crops and Products 132 (2019): 92-98.

[52] Saleh, Dina, et al. "Diversity, distribution and multi-functional attributes of bacterial communities associated with the rhizosphere and endosphere of timothy (Phleum pratense L.)." Journal of applied microbiology 127.3 (2019): 794-811.

[53] Saleem, Muhammad, et al. "Perspective of plant growth promoting rhizobacteria (PGPR) containing ACC deaminase in stress agriculture." Journal of Industrial Microbiology and Biotechnology 34.10 (2007): 635-648.

[54] Pérez-Montaño, F., et al. "Plant growth promotion in cereal and leguminous agricultural important plants: from microorganism capacities to crop production." Microbiological research 169.5-6 (2014): 325-336.

[55] Wang, Chuan, et al. "Plant growth-promoting rhizobacteria isolation from rhizosphere of submerged macrophytes and their growth-promoting effect on Vallisneria natans under high sediment organic matter load." Microbial Biotechnology 14.2 (2021): 726-736.

[56] Ho, PooiLeng, et al. "HEXIM1 peptide exhibits antimicrobial activity against antibiotic resistant bacteria through guidance of cell penetrating peptide." Frontiers in microbiology 10 (2019): 203.

[57] Sand-Jensen, Kaj, et al. "100 years of vegetation decline and recovery in Lake Fure, Denmark." Journal of Ecology 96.2 (2008): 260-271.

[58] Wu, Shan Shan. Enhanced phytoremediation of salt-impacted soils using plant growth-promoting rhizobacteria (PGPR). MS thesis. University of Waterloo, 2009.

[59] Soana, Elisa, et al. "Benthic nitrogen metabolism in a macrophyte meadow (Vallisneria spiralis L.) under increasing sedimentary organic matter loads." Biogeochemistry 124.1 (2015): 387-404.

[60] Phillips, M., Adrian Lee, and W. D. Leach. "The mucosa-associated microflora of the rat intestine: a study of normal distribution and magnesium sulphate induced diarrhoea." Australian Journal of Experimental Biology and Medical Science 56.6 (1978): 649-662.

[61] Upadhyay, Sudhir Kumar, et al. "Impact of PGPR inoculation on growth and antioxidant status of wheat under saline conditions." Plant Biology 14.4 (2012): 605-611.

[62] Zhu, Fengling, et al. "Isolation and characterization of a phosphate-solubilizing halophilic bacterium Kushneria sp. YCWA18 from Daqiao Saltern on the coast of Yellow Sea of China." Evidence-Based Complementary and Alternative Medicine 2011 (2011).

[63] Flowers, Timothy J., and Timothy D. Colmer. "Salinity tolerance in halophytes." New Phytologist 179.4 (2008): 945-963.

[64] Kumari, Baby, et al. "Plant growth promoting rhizobacteria (PGPR): modern prospects for sustainable agriculture." Plant health under biotic stress. Springer, Singapore, 2019. 109-127.

[65] Jha, Yachana, and R. B. Subramanian. "Paddy plants inoculated with PGPR show better growth physiology and nutrient content under saline condition." Chilean journal of agricultural research 73.3 (2013): 213-219

[66] Jha, Chaitanya Kumar, and Meenu Saraf. "Plant growth promoting rhizobacteria (PGPR)." JOURNAL OF AGRICULTURE RESEARCH AND DEVELOPMENT 5.2 (2015): 0108-0119.

[67] Shukla, Arvind K., et al. "Micronutrients and field crop production in India: a review." Indian Journal of Agronomy 57.3s (2012): 123-130.

[68] Nabti, Elhafid, Michael Schmid, and Anton Hartmann. "Application of halotolerant bacteria to restore plant growth under salt stress." Halophiles. Springer, Cham, 2015. 235-259.

[69] Sharma, Sandeep, Jayant Kulkarni, and Bhavanath Jha. "Halotolerant rhizobacteria promote growth and enhance salinity tolerance in peanut." Frontiers in microbiology 7 (2016): 1600.

[70] Rohban, R., Mohammad Ali Amoozegar, and A. Ventosa. "Screening and isolation of halophilic bacteria producing extracellular hydrolyses from HowzSoltan Lake, Iran." Journal of Industrial Microbiology and Biotechnology 36.3 (2009): 333-340.

[71] Siddikee, Md Ashaduzzaman, et al. "Isolation, characterization, and use for plant growth promotion under salt stress, of ACC deaminase-producing halotolerant bacteria derived from coastal soil." Journal of Microbiology and Biotechnology 20.11 (2010): 1577-1584.

[72] Bharti, Nidhi, et al. "Exiguobacteriumoxidotolerans, a halotolerant plant growth promoting rhizobacteria, improves yield and content of secondary metabolites in Bacopa monnieri (L.) Pennell under primary and secondary salt stress." World Journal of Microbiology and Biotechnology 29.2 (2013): 379-387.

[73] Larsen, Helge. "Halophilic and halotolerant microorganisms—an overview and historical perspective." (1986): 3-7.

[74] Khan, Naeem, and AsghariBano. "Modulation of phytoremediation and plant growth by the treatment with PGPR, Ag nanoparticle and untreated municipal wastewater." International journal of phytoremediation 18.12 (2016): 1258-1269.

[75] Ruppel, Silke, Philipp Franken, and Katja Witzel. "Properties of the halophyte microbiome and their implications for plant salt tolerance." Functional Plant Biology 40.9 (2013): 940-951.

[76] Bottini, Rubén, Fabricio Cassán, and Patricia Piccoli. "Gibberellin production by bacteria and its involvement in plant growth promotion and yield increase." Applied microbiology and biotechnology 65.5 (2004): 497-503.

[77] Kang, Sang-Mo, et al. "Plant growth-promoting rhizobacteria reduce adverse effects of salinity and osmotic stress by regulating phytohormones and antioxidants in Cucumis sativus." Journal of Plant Interactions 9.1 (2014): 673-682.

[78] Shahzad, Raheem, et al. "Plant growth-promoting endophytic bacteria versus pathogenic infections: an example of Bacillus amyloliquefaciens RWL-1 and Fusarium oxysporum f. sp. lycopersici in tomato." PeerJ 5 (2017): e3107.

[79] Magome, Hiroshi, and Yuji Kamiya. "Inactivation processes." Annu. Plant Rev 49 (2016): 73-94.

[80] Martínez, Oscar A., et al. "Serratia strains isolated from the rhizosphere of raulí (Nothofagusalpina) in volcanic soils harbour PGPR mechanisms and promote raulí plantlet growth." Journal of soil science and plant nutrition 18.3 (2018): 804-819.

[81] Guo, J. H., et al. "The plant healthy and safety guards plant growth promoting rhizo bacteria (PGPR)." Transcriptomics 3.109 (2015): 2.

[82] Wang, Xuefei, et al. "Biocontrol and plant growth-promoting activity of rhizobacteria from C hinese fields with contaminated soils." Microbial biotechnology 8.3 (2015): 404-418.

[83] Sengupta, Anjan, and Subhendu Chaudhuri. "Ecology of heterotrophic dinitrogen fixation in the rhizosphere of mangrove plant community at the Ganges river estuary in India." Oecologia 87.4 (1991): 560-564.

[84] Holguin, Gina, M. Antonia Guzman, and Yoav Bashan. "Two new nitrogen-fixing bacteria from the rhizosphere of mangrove trees: Their isolation, identification and in vitro interaction with rhizosphere Staphylococcus sp." FEMS Microbiology Letters 101.3 (1992): 207-216.

[85] Alongi DM, Christoffersen P, Tirendi F (1993) The influence of forest type on microbial-nutrient relationships in tropical mangrove sediments. J Exp Mar BiolEcol 171:201–223

[86] Holguin, G., C. L. Patten, and B. R. Glick. "Genetics and molecular biology of Azospirillum." Biology and fertility of soils 29.1 (1999): 10-23.

[87] Holguin, Gina, Patricia Vazquez, and Yoav Bashan. "The role of sediment microorganisms in the productivity, conservation, and rehabilitation of mangrove ecosystems: an overview." Biology and fertility of soils 33.4 (2001): 265-278.

[88] Kathiresan, Kandasamy, and Brian L. Bingham. "Biology of mangroves and mangrove ecosystems." (2001): 81-251.

[89] Toledo-Aral, Juan José, et al. "A single pulse of nerve growth factor triggers long-term neuronal excitability through sodium channel gene induction." Neuron 14.3 (1995): 607-611.

[90] Bashan, Yoav. "Inoculants of plant growth-promoting bacteria for use in agriculture." Biotechnology advances 16.4 (1998): 729-770.

[91] Rojas, Gabriela, et al. "Antimicrobial evaluation of certain plants used in Mexican traditional medicine for the treatment of respiratory diseases." Journal of Ethnopharmacology 74.1 (2001): 97-101.

[92] Gotto, John W., and Barrie F. Taylor. "N2 fixation associated with decaying leaves of the red mangrove (Rhizophora mangle)." Applied and Environmental Microbiology 31.5 (1976): 781-783.

[93] Zuberer, D_A, and WS242881 Silver. "Biological dinitrogen fixation (acetylene reduction) associated with Florida mangroves." Applied and environmental Microbiology 35.3 (1978): 567-575.

[94] Zuberer, D. A., and W. S. Silver. "N2-Fixation (acetylene reduction) and the microbial colonization of mangrove roots." New Phytologist 82.2 (1979): 467-471.

[95] Pots, Bernardus Franciscus Maria. "Turbulence and transport in a magnetized argon plasma." (1979)

[96] Uchino, Fuji, Gregori G. Hambali, and Michihiko Yatazawa. "Nitrogen-fixing bacteria from warty lenticellate bark of a mangrove tree, Bruguieragymnorrhiza (L.) Lamk." Applied and environmental microbiology 47.1 (1984): 44-48.

[97] Van der Valk, A. G., and P. M. Attiwill. "Acetylene reduction in an Avicennia marina community in Southern Australia." Australian journal of botany 32.2 (1984): 157-164.

[98] Hicks, Brendan J., and Warwick B. Silvester. "Nitrogen fixation associated with the New Zealand mangrove (Avicennia marina (Forsk.) Vierh. var. resinifera (Forst. f.) Bakh.)." Applied and environmental microbiology 49.4 (1985): 955-959.

[99] Mann, Fiona D., and T. D. Steinke. "Biological nitrogen fixation (acetylene reduction) associated with decomposing Avicennia marina leaves in the Beachwood Mangrove Nature Reserve." South African Journal of Botany 58.6 (1992): 533-536.

[100] Sengupta, A., and S. Chaudhuri. "Vesicular arbuscular mycorrhiza (VAM) in pioneer salt marsh plants of the Ganges river delta in West Bengal (India)." Plant and Soil 122.1 (1990): 111-113.

[101] Reed, Sasha C., Cory C. Cleveland, and Alan R. Townsend. "Functional ecology of free-living nitrogen fixation: a contemporary perspective." Annual review of ecology, evolution, and systematics 42 (2011): 489-512.

[102] Uroz, Stéphane, et al. "Bacterial weathering and its contribution to nutrient cycling in temperate forest ecosystems." Research in microbiology 162.9 (2011): 820-831.

[103] Hardoim, P. R. "Heading to the origins-Rice microbiome as functional extension of the host." Journal of Rice Research 3.2 (2015): 1-3.

[104] Lauber, Christian L., et al. "Temporal variability in soil microbial communities across land-use types." The ISME journal 7.8 (2013): 1641-1650.

[105] Xia, Ye, et al. "Multi-stress tolerant PGPR Bacillus xiamenensis PM14 activating sugarcane (Saccharum officinarum L.) red rot disease resistance." Plant Physiology and Biochemistry 151 (2020): 640-649.

[106] VanInsberghe, David, et al. "Non-symbiotic Bradyrhizobium ecotypes dominate North American forest soils." The ISME journal 9.11 (2015): 2435-2441.

[107] Lau, EeTiing, Choy Yuen Khew, and Siaw San Hwang. "Transcriptomic analysis of pepper plants provides insights into host responses to Fusarium solani infestation." Journal of biotechnology 314 (2020): 53-62.

[108] Savi, D.C., Aluizio, R., Galli-Terasawa, L. et al. 16S-gyrB-rpoB multilocus sequence analysis for species identification in the genus Microbispora .Antonie van Leeuwenhoek 109, 801–815 (2016). https://doi.org/10.1007/s10482-016-0680-y

[109] Esson, Kaitlin C., et al. "Alpha-and gammaproteobacterial methanotrophs codominate the active methane-oxidizing communities in an acidic boreal peat bog." Applied and environmental microbiology 82.8 (2016): 2363-2371.

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Influence of Inoculums Source and Pretreatment on Biogas Production from Cashew Nut Shells (*Anacardium occidentale*)

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***Abstract*— *Bioenergy recovery from cashew nut shells was investigated throughout using efficient inoculums source and appropriate pretreatment. Physicochemical characteristics of shells and inoculums including pH, volatile fatty acid (VFA, total solid, volatile solid, ash were determined using standard methods. Total anaerobes and methanogenic archaea from inocula were determined by MPP method. Wastewater (WW), sludge from bioreactor (SBR), bovine dung (BD) and mixed inoculums (MIX) were used to evaluate inoculums source in batch system. Biochemical methane potential of pretreated shells was evaluated. Biogas was measurement by liquid displacement. CH_4 and CO_2 were performed by GC. MIX and WW showed high concentration of methanogenic bacteria (2.3 10^2 CFU/mL). Best biomethane levels 70.38% with yields of 55.52 L biogas. $(Kg\ VS)^{-1}$ was observed with old shells inoculated by MIX. Low productivity was noted with thermal and biological treatment of old and fresh shells, 11.20 and 0.02 L CH_4. $(Kg\ VS)^{-1}$, respectively. Inoculums source has significant effect on biogas production. Mixed inoculums exhibited significantly high yields of biomethane. Thermo-biological pretreatment seems to be not appropriate for a better biomethane production. Combination of thermochemical and biological pretreatment could be necessary for best biomethane production yield.*

***Keywords*— Cashew nut shells, methanogenic bacteria, thermo-biological pretreatment, biomethane.**

I. INTRODUCTION

Worsening international food problems have created the need to develop agri-food sector in developing countries. Development of agro-food industries releases an important amount of organic waste or biomass which can be used as a feedstock source for bioenergy and or bioproduction. However, this technological way for biomass valorization remains still underused in large-scale condition in these countries. And their outdoor accumulation leads to environment and population health problems as highlighted by several studies (WHO, 2015, Franchitti *et al*., 2020). Nevertheless, some lab- and pilot-scale studies have been carried out for converting into bioenergy, biofuels and bio-based products (Mahdy *et al*., 2015,Na *et al*., 2021). Agricultural residues depend on locality. The third largest agricultural product for export, after cotton and sesame, is cashew nut which is still a poorly performing sector marked by low yields, processing and marketing. However, cashew production increased by 13% in 2018 (85,000 tons) and the processing rate from 40% (7,000 tons) (COMMODAFRICA, 2019). In Burkina Faso, cashew nut sector is experiencing increasingly significant development with production estimated at 81,000 tons in 2017 and expected to reach 200,000 tons in 2030 (Somé, 2014). Processing units generate a large amount of waste consisting of approximately 73% hulls and 6% skins (Tagutchou and Naquin, 2012). According to Lacroix (Lacroix, 2003), cashew shell contains a toxic acid, cashew nut shell liquid (CNSL) or balm, which makes production of almonds painful (during shelling). Improper drying procedures can create potentially hazardous situations for environment following release and infiltration of CNSL into soil. This would cause the death of trees in the area and infertility of the soil for several years. Cashew processing units are confronted with recurring energy problems. They use unsustainable energy sources such as wood and butane gas at excessive costs, leading them to resort to cashew shells as fuel in the processing chain, in particular for the embrittlement of nuts, cooking with steaming and drying almonds (Thiombiano *et al*., 2011). Godjo *et al.* (2015) showed cashew nut shells burning causes significant damage to environment and human health. Cashew nut consists of hard woody shell containing liquid (Cashew Nut Shell Liquid CNSL). CNSL is composed of 70 to 90% anacardic acid, 10 to 18% cardol and about 5% cardanol (Das, Sreelatha, & Ganesh, 2004; Patel, Bandyopadhyay, & Ganesh, 2006). Most cashew nut upgrading work is oriented towards CNSL extraction processes, some only talking about thermochemical treatment, in particular pyrolysis and gasification (Das *et al*., 2004, Singh *et al.* 2006, Tsamba *et al*. 2006). Faced to increasingly growing energy demand in processing units, and environmental problems linked to burning and release of shells into environment, a more ecological recovery of hulls is required. Several authors such as Saka *et al.* (2009) and Chandel and Singh (2011) showed difficulties during the bioconversion of plant species due to the structure and components of cell walls, which certainly influences digestibility. Indeed, Mahato *et al.* (2021) was reported possibility of citrus waste biotransformation, bio-waste which is antimicrobial in nature and inhibits fermentation process. The systematic process of bioproducts production from citrus biomass requires pretreatment steps including physical, chemical, physicochemical and biological pretreatment. Leitão *et al.* (2011) studied anaerobic digestion of cashew bagasse, but found no conclusive results given the complexity of this substrate. Nikiema *et al.* (2020) indicated the feasibility of biogas production from cashew nut shells and found experimental biochemical methane potential (BMP) was 46.84 CH_4 L. (Kg VS)$^{-1}$ and 1.98 CH_4 L. (Kg VS)$^{-1}$ for old and fresh shells, respectively. However, theoretical values could reach up to 526.206 CH_4 L. (Kg VS)$^{-1}$ and 666.937 CH_4 L. (Kg VS)$^{-1}$ for old and fresh shells, respectively. The presence of certain substances including anacardic acids, cardol and cardanol could explain lower yields observed in experimental study, since these substances constitute a limit to the bioconversion of cashew shells into biogas. Our study aims to contribute to ecological elimination of cashew nut for environment protection and at the same time to agro-resources valorization by bioconversion into bioenergy. Specifically, it aimed at to find out suitable source of inoculum and appropriate way of pretreatment of cashew nut shells for a better biogas production.

II. MATERIAL AND METHODS

2.1. Sampling of cashew nut shells and microbial inoculums

The samples required for biogas production have been collected at ANATRANS Company, a cashew scale transformation units, located in Bobo-Dioulasso, Burkina Faso, West Africa. Two types of waste samples were used: eight-year-old shells (OS) and fresh shells (FS) freshly produced. Four (04) types of inoculums were used: wastewater (WW), sludge from bioreactor (SBR), boving dung (BD), mixed (MIX) consisting of three (03) inoculums combination. Slaughterhouse effluents (wastewater and bovine dung) were sampled in anaerobic basin of Ouagadougou refrigerated slaughterhouse (12 ° 24'59 "N; 1 ° 28'29" W). Sludge from biodigester was sampled from biogas production unit of fecal sludge treatment center (CTBV) of Zagtouli in Ouagadougou. Sludges were sampled in 20 L flasks containing nitrogen gas (N_2) to maintain anaerobic conditions.

2.2. Mechanical pretreatment of cashew nut shells

Old hulls samples were crushed to obtain particles size ≤ 1 mm in diameter while new hulls were grounded to obtain cake (Figure 1).

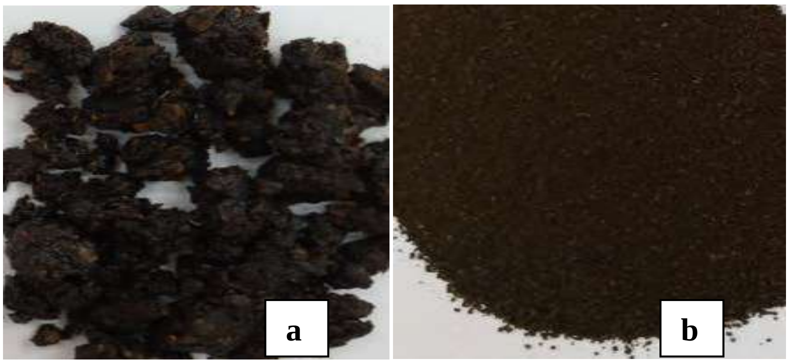

Fig. 1- Physical aspect of samples after mechanical treatment of cashew nut shells: (a) Grounded fresh shells; (b) old shells particles

2.3. Physichochemical characteristics of cashew nut shells

pH was determined using pH meter (WTW pH340) previously calibrated with buffer solutions at 25 °C. Five (5) gram of old shells particles and grounded fresh shells were homogenized in 45 mL of distilled water after measurement (Noutb *et al*., 1989). Total solids content (TS) was determined by drying 5 g sample in an oven at 105 °C until constant weight. Volatile solids (VS) and ash content were obtained by weight difference between dried waste and burnt waste at 550 °C for 4 hours (AFNOR, 1985).

2.4. Physicochemical characteristics of inoculums

pH was determined a described above. Total solid (TS), ash (As), volatile solid (VS) in sample were determined according to Sakaki (2014) methods. Titration assay method was used to determine volatile fatty acid (VFA) content. A volume of 25 mL of reactor supernatant was collected in a beaker and stirred. Initial pH was read. Using a 1/10 mL burette, a volume of sulfuric acid (0.1 mol. L^{-1} H_2SO_4) was poured until pH = 4. The volume poured was noted (V1) then the liquid was boiled 3 min. After cooling, Na_2CO_3 (0.05 mol. L^{-1}) was poured to pH = 7, and volume V2 poured in noted. Equation 1 shows formula using for determine VFA value.

$$VFA = 6.10^4 \times [(C2V2 / Ts] \text{ (mg (acetic acid). } L^{-1}) \quad (1)$$

V1: volume of sulfuric acid

V2: volume of sodium bicarbonate

Ts: Test sample (25 mL)

Viable total anaerobic and methanogenic bacteria were enumerated by the three-tube most probable number (MPN) technique during anaerobic digestion using modified medium of by Angelidaki et al. (2009). Cellulose 1g/L, glucose 1g/L, casein 1g/L, propionic acid 0.5g/L, n-butyric 0.5g/L, methanol 10 mM, sodium acetate 20 mM and sodium formate 20 mM were added as substrate. Tubes showing methane production at the GC were considered positive for methanogenic archaea. Tubes showing turbidity are considered positive for total anaerobes.

2.5. Thermal and biological pre-treatment of cashew nut shells

Thermal and thermobiological pretreatment was realized using method described by Fadil *et al.* (2003) and Aissam (2003) for biodegradation of effluents from olive oil production was adapted for the pretreatment of cashew shells. The fungus strains *Aspergillus niger* isolated from old shells matrices on sabouraud medium with chloramphenicol was used as inoculums for biological pre-treatment. Liquid culture medium (100 mL) with 2% (v/w) of shells placed in 250 mL Erlenmeyer flasks was used for improving aerobic fermentation. Medium was composed of yeast extract (0.1% w/v), $(NH_4)_2SO_4$ (0.5% w/v), KH_2PO_4 (0.4% w/v), $MgSO_4$ (0.05% w/v), NaCl (0.05% w/v). 25%, and pH was adjusted to 5. Erlenmeyer flasks were sterilized by autoclaving at 121 °C for 15 minutes. After fungi inoculation, cultures were incubated during 14 days at 30 °C with shaking at 150 rpm. Controls were uninoculated shells. The experiments were carried out in triplicate.

2.6. Assessment of biogas and gaseous metabolites production from cashew nut shells anaerobic digestion

Anaerobic digestion was realized on pretreated shells according to batch method described by Angelidaki *et al.* (2009). Estimation of biogas production was carried out using liquid displacement method. CH_4 and CO_2 were determined using a gas chromatograph (Girdel series 30 with catharometer equipped with thermal conductivity detector [TCD] and linked to the potentiometric recorder SERVOTRACE of type Sefram Paris 1mV). The chromatographical conditions for CH_4 and CO_2 measurement were as follows: injector temperature 90 °C, column temperature 60 °C, detector temperature 100 °C, filament current 150 mA, N_2 carrier gas pressure 1 bar, attenuation 32, paper speed 10 mm/min. Volume of gas phase 1 mL was injected into chromatograph using a sealed syringe graduated. CH_4 and CO_2 contents were determined using a standard curve established from CH_4 and CO_2 standards (Sawadogo et al., 2012). The yield of substrate-specific methane (YSM) was calculated from the Equation 2.

$$YSM = \frac{\text{Quantity of biomethane product (L)}}{\text{Quantity of volatile solide (Kg)}} \quad (2)$$

2.7. Data processing

Statistical analysis of data was realized using XLSAT 7.5.2 software. Analysis of variance (ANOVA) was performed to compare mean values of the different variables using Fisher's tests at probability p = 5%. Principal Component Analysis was carried out for distribution of biogas production variables from new and old hulls, CO_2 and CH_4 proportions depending on source of inoculums and pretreatment.

III. RESULTS AND DISCUSSION

3.1. Characteristics of feedstock and inoculums

Physicochemical of cashew nut shells feedstock and inoculums characteristics are pictured in Table 1. pH of fresh and old shells samples was respectively around 4.20 and 6.41. The acidic pH of dry hulls could be explained by high levels of acidic compounds such as anacardic acid (70 to 90%) and other phenolic compounds including 10 to 18% cardol and 5% cardanol (Das et al., 2004; Patel et al., 2006). Over time these compounds are degraded under the action of abiotic and biotic factors. Abiotic factors such as temperature, wind, rains as well as biotic factors (insects, microorganisms) can have an impact on the integrity of the shells. This explains the degraded condition of the old shells. Joutey *et al.* (2013) reported efficiency of microbial degradation depends on many factors, including chemical nature and the concentration of pollutants, their availability to microorganisms, and the physicochemical characteristics of the environment. High ash content of old shells could be explained by dilapidated condition of old hulls associated with presence of dust and dead insects. Volatile Solids content in fresh shells (88.74% VS) was significantly higher (P = 0.0001) than old shells (84.61% VS). High volatile solid indicated a preferred substrate for anaerobic digestion microorganisms according to Milaiti *et al.* (2003) and Nikiema *et al.* (2015). Significant difference as to parameters pH, VFA and methanogenic archaea concentration ($p < 0.0001$) was observed. The SBR, MIX, WW inoculums exhibited pH close to neutral (pH 7) with VFAs values of 1592, 1484 and 1756 mg (acetic acid). L^{-1}, respectively. Bovine dung (BD) inoculum had a slightly acidic pH (6.31) with high VFA concentrations of 2840 mg (acetic acid). L^{-1}, respectively. According to Vedrenne (2007) concentrations from 2000 to 3000 mg (acetic acid). L^{-1} of total VFA could inhibit anaerobic digestion process. Total anaerobic bacteria were higher than 10^7 CFU/mL for all inoculum. According to Wang *et al.* (2017) microbial community plays a role in process performance and stability. Mixed inoculums and wastewater exhibited high concentration of methanogenic archaea ($2.3 10^2$ CFU/mL) compared to SBR and BD inoculums. Low concentration of methanogenic archaea in bovine dung ($2.3\ 10^1$ CFU/mL) could be explained by unfavorable physicochemical conditions, namely low pH (6.3) and high VFA (2840 mg/L). It should be noted that number of bacteria still does not confirm effectiveness of inoculums.

Table 1. Characteristics of cashew nut shells feedstock and inoculums

	Feedstock			Inoculums				
Parameters	**Old Shells**	**Fresh Shells**	***p* value**	**SBR**	**MIX**	**WW**	**BD**	***p* value**
pH	6.41^{a}	4.29^{b}	0.0001	7.67^{a}	7.63^{a}	7.11^{b}	6.31^{c}	< 0.0001
TS g/L	90.66^{a}	$90,916^{a}$	0.067	13.44^{b}	13.90^{ab}	20.49^{a}	12.37^{b}	0.027
VS g/L	84.61^{b}	88.74^{a}	0.0001	90.76^{b}	90.60^{b}	92.90^{ab}	94.83^{a}	0.027
Ash g/L	$6,07^{a}$	$2,18^{b}$	> 0.0001	9.24^{a}	$9,40^{a}$	7.09^{a}	5.17^{b}	0.027
VFA (mg (acetic acid)/L)	--	--	--	1592^{b}	1484^{b}	1756^{b}	2840^{a}	< 0.0001
MA (CFU/mL)	--	--	--	$2.30\ 10^{2b}$	$4.30\ 10^{2a}$	$4.30\ 10^{2a}$	$2.30\ 10^{1c}$	< 0.0001
TA	--	--	--	$> 10^7$	$> 10^7$	$> 10^7$	$> 10^7$	---

(CFU/mL)

SBR: Sludge from Bioreactor; WW: Wastewater; BD: Bovine dung; MIX: Mixed of the three inoculums; TS: Total solid; VS: Volatile solid; VFA: Volatile Fatty Acid; MA: Methanogenic archaea; TA: Total Anaerobes

3.2. pH variation during the anaerobic digestion of cashew nut shells

Figure 2 shows pH variation during the anaerobic digestion process of shells with regard to inoculums source (SBR, WW, BD and MIX). From first days of anaerobic digestion, pH variation trended towards acidity (pH 6.5). Stability was obtained after 10th day for all inoculums. This stability depends on inoculums source, because with wastewater inoculum cultures continued to acidify after 20 days. This shows a strong activity of acidogenic bacteria of wastewater on old shells.

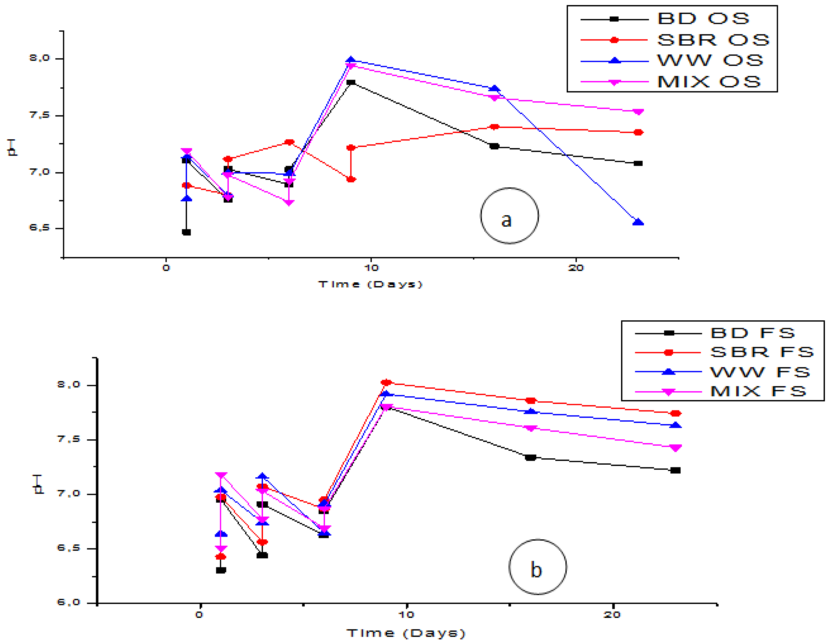

Fig. 2- pH evolution over the anaerobic digestion of cashew nut shells: (a) old shells, (b) fresh shells

3.3. Biogas production with regard to inoculums source

Biogas and biomethane production ranged according to inoculums source and type of shells as presented in Table 2. A significant difference of biogas and biomethane values was denoted between different inoculums ($p < 0.05$). Depending on type of shells, mixed inoculums and wastewater have best yields. SBR, BD and MIX inoculums gave best biogas yields for the old shells 64.18, 59.12 and 55.52 biogas L. (Kg VS)$^{-1}$, respectively, comparatively to values on fresh shells. The mixed inoculums showed a significantly high biomethane proportions with a value of 70.38%. There was no significant difference between biogas production with wastewater and mixed from fresh shells with 43.87 L biogas. (Kg VS)$^{-1}$ and 27.78 L biogas. (Kg VS)$^{-1}$, respectively. The high proportions of biomethane on fresh shells digestion were at the order of 10.30% and 9.81% CH_4 with wastewater and mixed inoculums, respectively. Similar results was obtained by our previous study (Nikiema *et al*., 2020) with yields of 46.840 CH_4 L. (Kg VS)$^{-1}$ and 1.982 CH_4 L. (Kg VS)$^{-1}$ upon old and fresh shells, respectively. Indeed, wastewater contains a microbial consortium very active in the degradation of complex substrates. The prediction in figure 3 shows that mixed inoculums is suitable in the production of biomethane from cashew nut shells compared to other types of inoculums. Nikiema *et al.* (2017) was showed possibility to realize a activated sludge for optimization of biomethane production by mixing several types of inoculums like wastewater and bovine dung. The low yields with old and fresh shells could be explained by low proportion of inoculum used which was 10%. Codigestion systems could increase biomethane production yields. Pouan (2011) found a production of 50 L biogas. (Kg ST)$^{-1}$ for *Jatropha curcus* cake, and showed that this production could reach 206 L biogas. (Kg ST)$^{-1}$ in codigestion with cattle manure. Singh et al. (2008) obtained a biomethane yield of 333 L. (Kg TS)$^{-1}$ from seed hulls of *Jatropha curcus* in co-digestion with bovine excreta. In addition to co-digestion, an appropriate pre-treatment could boost the anaerobic digestion process thus the production of biomethane (Forgacs *et al*., 2012, Wikandari *et al*., 2014, Mahato *et al*., 2021).

Table 2. Biomethane production from old and new shells according to the type of inoculums

Cultures	Mean values	
	Biogas L CH_4. (Kg SV)$^{-1}$	CH4 (%)
SBR OS	64.18^{a}	20.37^{b}
BD OS	59.12^{ab}	17.23^{b}
MIX OS	55.52^{ab}	70.38^{a}
WWFS	43.87^{abc}	10.30^{b}
WW OS	32.42^{abc}	29.19^{b}
MIXFS	27.78^{abc}	9.81^{b}
BDFS	18.67^{bc}	2.94^{b}
SBRFS	4.86^{c}	1.03^{b}

In a column the values which have a letter in common do not present a significant difference according to the Fisher LSD test at the probability threshold p = 0.05. OS: old shells; FS: fresh shells; WW: Wastewater; SBR: Sludge from Bioreactor; BD: Bovine dung; MIX: Mixed of inoculums

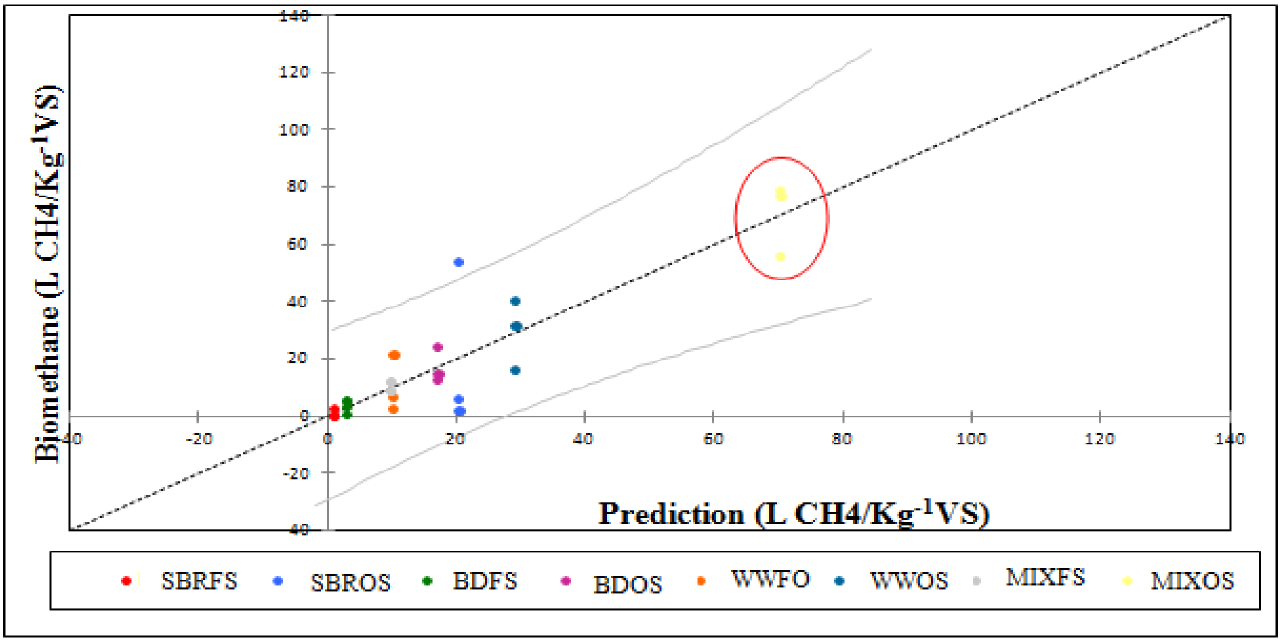

Fig. 3-Prediction of the biochemical methane potential of inoculums according to shell types

The typologies of the variables (Biogas, CH_4 and CO_2) and cultures according to the inoculums and the type of cashew shells on the factorial plans constituted by axes 1 and 2 are presented in Figure 4a and 4b. In this figure, only the variables close to the correlation circle need to be taken into account. In Figure 4a, there are clearly three groups of variables close to the circle (Biogas, CH_4 and CO_2, so that the projections on the axes F1 and F2 are 93.47%). Indeed, biogas is made up of CH_4 and CO_2. The representation of cultures as a function of inoculum and type of shell on the two factorial planes described by the axes F1xF2 (Figure 10a) allows them to be compared according to the production of biogas, CH_4 and CO_2. BD_{VC} crops have a significant production of biogas. MIXOS and WWOS cultures have high yields of CH_4. SBROS crops produce a high CO_2 production. The last batch formed by the cultures from the new hulls shows low production of biogas.

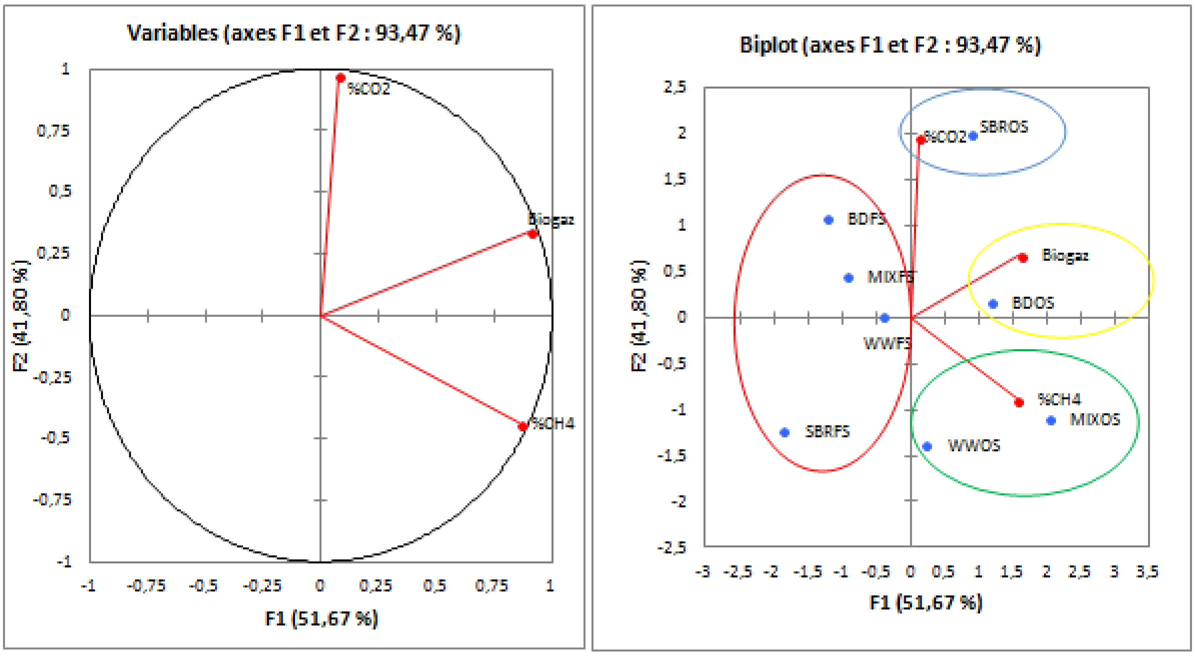

Fig. 4-Principal component analysis (a) plot of biogas production variables from old and fresh shells (OS and FS), CO_2 and CH_4 proportions (b) distribution of treatment types

3.4. Influence of pretreatment on anaerobic digestion of cashew nut shells

The results of pretreatment influence on shells anaerobic digestion are presented in Table 3. Thermo-biological treatment of cultures of old shells shows a significantly high production ($p < 0.0001$) of biogas (40.04 L. biogas (Kg VS)$^{-1}$) compared to control T-TTBOS (24.82 L. biogas (Kg VS) $^{-1}$) having only undergone heat treatment. This indicates that biological treatment improves yield after old hulls heat treatment. Same observation is made with new hulls. Yield was 16.55 L. biogas (Kg VS)$^{-1}$, a significant improvement ($p < 0.0001$) is remarkable at the end of biological treatment (27.63 16.55 L. biogas (Kg VS)$^{-1}$). The biomethane yield also underwent a significant improvement ($p = 0.001$) from 6.30 to 11.20 L. CH_4 (Kg VS)$^{-1}$. The opposite is remarkable for the new hulls with a significant decrease in biomethane productivity from 4.05 to 0.02 L. CH_4 (Kg VS)$^{-1}$. The biomethane yield values compared to work without thermobiological pre-treatment showed a decrease. Anaerobic digestion activities depending on inoculums source yielded 55.52 L. biogas (Kg VS)$^{-1}$ with 70.38% CH_4. Previous work had made it possible to find higher biogas yields than 70.38 L biogas. (Kg VS)$^{-1}$ with an also higher biomethane yield of 46.840 L CH_4. (Kg VS)$^{-1}$ with conditions similar to old untreated (Nikiema *et al*., 2020). Nikiema *et al*. (2020) had found yields of 1.982 L CH_4. (Kg VS)$^{-1}$ with the new untreated hulls. That indicates an improvement biomethane yield with thermal pretreatment only (4.05 L CH_4. (Kg VS)$^{-1}$), the biological treatment decreases value to 0.02 L CH_4. (Kg VS)$^{-1}$. Radziejewska-Kubzdela *et al.* (2020), enzymatic and thermal treatment of must made it possible to obtain highest content of phenolic compounds in *Berberis amurensis* Rupr juice, a plant whose total content of phenolic compounds is much higher high ranging from 261 to 1074 mg / 100 g (Hassanpour and Alizadeh, 2016). Thermal treatment weakens cell structuring and facilitates enzymatic activity through biological treatment. The differences in biogas production with untreated shells could be explained by the fact that treatment forms improve productivity of CO_2 contained in biogas. The low of biomethane yields obtained with old and new hulls as substrate could be explained by their physicochemical composition. In fact, cashew nut shells contain cashew nut shell liquid (CNSL) which is a liquid composed of 70% - 90% anacardic acid, 10% - 18% cardol and about 5% cardanol including new hulls contain abundantly. According to authors such as Liu *et al.* (2018), Zhang *et al*., (2019) and Liu *et al*. (2020), heat treatment degrades the cell structure and facilitates the extraction of soluble solids, polysaccharides, phenolic compounds from fruit and vegetables. These soluble sugars and other bioaccessible compounds will likely be used for microorganisms during the associated biological treatment. Heat treatment also results in the release of substances toxic to the microbial groups in anaerobic digestion. Bisaria and Ghose (1978) and Boulanger

(2011) showed thermo-chemical and physico-chemical treatments of organic matter could produce compounds such as hydroxyfulfurals and fulfural which can have a toxic effect for microorgansims. Liu *et al*. (2020) reported increase total phenols in finished product (wine) to 2.97–105.41% following heat treatment of *Crataegus* spp. fruits (Atanackovic *et al*., 2012). Lodish *et al.* (2000) showed with increasing temperature, thermal energy increases, thus facilitating cleavage of covalent bonds, resulting in the release of bound polyphenols. During the heat treatment, the ester and glucoside bonds can be broken, resulting in the release of the bound polyphenols. During the heat treatment, the ester and glucoside bonds can be broken, resulting in the release of the bound polyphenols. High temperatures can also lead to the formation of Maillard reaction products, altering the color of the material and contributing to the production of compounds such as hydroxyfulfurals and fulfural which can have a toxic effect for microorgansims (Bisaria and Ghose, 1978; Lavelli *et al*., 2009). Papoutsis *et al*. (2018) showed higher increase in total polyphenol contents (171.0 µM) compared to unheated control (71.8 µM) with treatment at 150 °C for 40 minutes of citrus peels extracts. At the same time, heat can lead to the transformation of polyphenols explaining why the content of some phenolic compounds increases while the content of others decreases (Buchner *et al*., 2006). Radziejewska-Kubzdela *et al.* (2020) showed with enzymatic and thermal treatment it possible to obtain highest content of phenolic compounds in *Berberis amurensis* Rupr juice, a plant with a much higher total content of phenolic compounds ranging from 261 to 1074 mg / 100 g (Hassanpour and Alizadeh, 2016). Decrease in our yields compared to previous work could be explained by inappropriate pretreatment which would contribute to production substances toxic to anaerobic bacteria. These pretreatment conditions cause an increase in the content of phenolic compounds and probable release of hydroxyfulfural and fulfural compounds. Saenab *et al.* (2017) worked on effects of anacardic acid isolated from cashew nut shells on production of methane and other products in rumen fermentation, found that the inhibition of methane production by l Anacardic acid was lower than that of biogas (crude extract of shells). However, as part of the bioactive compounds present inside the biogas, the contribution of anacardic acid to the total reduction of methane amounted to 77.36% while the contribution of other compounds amounted to 22, 64%. The other compounds besides anacardic acid are cardol, cardanol, dimethyl cardol according to Gandhi et al. (2013) and Njuku et al. (2014). Other forms of pretreatment could reduce the levels of these toxic compounds and thus promote the production of biomethane. Whether old or fresh shells, pretreatment is an essential step in increasing accessibility and biodegradation of macromolecules by anaerobic microorganisms. Indeed, Ghaderi-Ghahfarrokhi et al. (2017) investigated the effects of different treatment processes like boiling, autoclaving, roasting and soaking in solutions (water, acetic acid, NaOH and NaCl) on the removal of polyphenolic compounds from varieties of acorns, namely *Quercus brantii* var. persica and *Quercus castaneifolia* var. castaneifolia. According to these authors all the processes applied, with the exception of roasting, resulted in a significant decrease ($p < 0.05$) in the polyphenol content. Boiling reduced the polyphenol concentrations by approximately 52%. A considerable reduction in anti-microbial substances could lead to a significant improvement in biomethane production.

Table 3. Biogas, CH_4 and CO_2 production from pretreated shells

Culture	Mean values (L. Kg VS $^{-1}$)		
	Biogas	CH_4	CO_2
TTBOS	40.04^{a}	11.20^{a}	7.15^{a}
TTBFS	27.63^{b}	0.02^{c}	2.07^{b}
T-TTBFS	16.55^{c}	4.05^{b}	5.75^{a}
T-TTBOS	24.82^{b}	6.30^{b}	1.85^{b}
p value	< 0.0001	0.001	0.002

In a column, the values which have a letter in common do not present a significant difference according to the Fischer test (LSD) at the 5% threshold, TTBOS: Thermal and biological treatment of old shells, TBOS: Biological treatment of old hulls, T-TTBOS: Control-TTBOS, T-TTBFS: Control-TTBFS.

IV. CONCLUSION

The source of inoculums has a significant effect on biogas production from cashew shells. Mixed inoculums exhibited significantly high biomethane productions with old and new cashew shells. Thermobiological pretreatment of old and new shells performed was not effective in biogas production. It would be advisable either to experiment other forms of pretreatment and to find optimal time of biological pretreatment in order to increase yields or to examine agronomic possibility.

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REFERENCES

[1] AFNOR. (1985). *Matières fertilisantes et supports de cultures : échantillonnage, analyses chimiques et essais physico-chimiques. Amendements organiques et supports de culture - Détermination de la matière organique totale - Méthode par calcination*. Paris.

[2] Aissam, H. (2003). *Etude de la biodégradation des effluents des huileries (margines) et leur valorisation par production de l'enzyme tannase*. Thèse de doctorat, Université Idi Mohamed Ben Abdellah. p182.

[3] Angelidaki, I., Alves, M., Bolzonella, D., Borzacconi, L., Campos, J. L., Guwy, A. J., … Van Lier, J. B. (2009). Defining the biomethane potential (BMP) of solid organic wastes and energy crops: A proposed protocol for batch assays. *Water Science and Technology*, *59*(5), 927–934. https://doi.org/10.2166/wst.2009.040

[4] Atanackovic, M., Petrovic, A., Jovic, S., Gojkovic, L., & Bursac, M. (2012). Influence of winemaking techniques on the resveratrol content , total phenolic content and antioxidant potential of red wines, *131*, 513–518. https://doi.org/10.1016/j.foodchem.2011.09.015

[5] Bisaria, V.S., Ghose, T. K. (1978). Sorption characteristics of cellulases on cellulosic substances, in: Bioconversion of Cellulosic Substances into Energy, Chemicals and Microbial Protein. In *symposium proceedings, (Editor: Ghose, T.K.), IIT, New Delhi*, (pp. 155–164).

[6] Boulanger, A. (2011). *Préparation d ' un déchet ménager pour l ' optimisation du potentiel et de la cinétique méthanogène*. Institut des Sciences et Industries du Vivant et de l'Environnement AgroParisTech.

[7] Buchner, N., Krumbein, A., Rohn, S., & Kroh, L. W. (2006). Effect of thermal processing on the flavonols rutin and quercetin. *Rapid Commun. Mass Spectrom.*, *20*, 3229–3235. https://doi.org/10.1002/rcm

[8] Chandel, A. K., & Singh, O. V. (2011, March). Weedy lignocellulosic feedstock and microbial metabolic engineering: Advancing the generation of "Biofuel." *Applied Microbiology and Biotechnology*. https://doi.org/10.1007/s00253-010-3057-6

[9] COMMODAFRICA. (2019). Cajou : création du Conseil burkinabè de l'anacarde au Burkina Faso. Retrieved from http://www.commodafrica.com/21-03-2019-cajou-creation-du-conseil-burkinabe-de-lanacarde-au-burkina-faso

[10] Das, P., Sreelatha, T. D., & Ganesh, A. (2004). Bio oil from pyrolysis of cashew nut shell-characterisation and related properties. *Biomass and Bioenergy*, *27*(3), 265–275.

[11] Fadil, K., Chahlaoui, A., Ouahbi, A., Zaid, A., & Borja, R. (2003). Aerobic biodegradation and detoxification of wastewaters from the olive oil industry. *International Biodeterioration & Biodegradation*, *51*, 37–41.

[12] Forgacs, G., Pourbafrani, M., Niklasson, C., Taherzadeh, M. J., & Hovath, I. S. (2012). Methane production from citrus wastes :process development and cost estimation. *J Chem Technol Biotechno*, (June 2011), 250–255. https://doi.org/10.1002/jctb.2707

[13] Franchitti, E., Pascale, E., Fea, E., Anedda, E., & Traversi, D. (2020). Methods for Bioaerosol Characterization : Limits and Perspectives for Human Health Risk Assessment in Organic Waste Treatment. *Atmosphere*, *11*(452), 1–19. https://doi.org/doi:10.3390/atmos11050452

[14] Gandhi, T. S., Dholakiya, B. Z., & Patel, M. R. (2013). Extraction protocol for isolation of CNSL by using protic and aprotic solvents from cashew nut and study of their physico-chemical parameter. *Polish Journal of Chemical Technology*, *15*(4), 24–27.

[15] Ghaderi-Ghahfarrokhi, M., Sadeghi-Mahoonak, A. R., Alami, M., & Mousavi Khanegah, A. (2017). Effect of processing treatments on polyphenol removal from kernel of two Iranian acorns varieties. *International Food Research Journal*, *24*(1), 86–93.

[16] Godjo, T., Tagutchou, J., Naquin, P., & Gourdon, R. (2015). Valorisation des coques d ' anacarde par pyrolyse au Bénin. *Déchets Sciences et Techniques*, 11–18. https://doi.org/10.4267/dechets-sciences-techniques.3282

[17] Hassanpour, H., & Alizadeh, S. (2016). Scientia Horticulturae Evaluation of phenolic compound , antioxidant activities and antioxidant enzymes of barberry genotypes in Iran. *Scientia Horticulturae*, *200*, 125–130. https://doi.org/10.1016/j.scienta.2016.01.015

[18] Joutey, N. T., Bahafid, W., Sayel, H., & Ghachtouli, N. El. (2013). Biodegradation : Involved Microorganisms and Genetically Engineered Microorganisms. In R. Chamy & F. Rosenkranz (Eds.), *Biodegradation - Life of Science* (pp. 290–320). https://doi.org/http://dx.doi.org/10.5772/56194

[19] Lacroix, E. J. (2003). *Les anacardiers, les noix de cajou et la filière anacarde à Bassila et au Bénin*. Bénin.

[20] Lavelli, V., Pompei, C., & Aurelia, M. (2009). Quality of nectarine and peach nectars as affected by lye-peeling and storage. *Food Chemistry*, *115*(4), 1291–1298. https://doi.org/10.1016/j.foodchem.2009.01.047

[21] Leitão, R. C., Claudino, R. L., Freitas de Brito, C R, Alexandre, L. C., Cassales, A. R., & Saavedra, Pinto G A, Santaella, S. T. (2011). *Produção de biogás a partir do bagaço do caju*. https://doi.org/DOI: 10.13140/2.1.3771.7282

[22] Liu, J., Jiao, Z., Zhang, C., Yang, W., Liu, H., & Lv, Z. (2018). Effects of different drying methods on phenolic contents , antioxidant , and tyrosinase inhibitory activity of peach blossoms. *Journal of Food Measurement and Characterization*, *0*(0), 0. https://doi.org/10.1007/s11694-018-9850-0

[23] Liu, J., Yang, W., Lv, Z., Liu, H., Zhang, C., & Jiao, Z. (2020). Effects of different pretreatments on physicochemical properties and phenolic compounds of

hawthorn wine. *CYTA - Journal of Food*, *18*(1), 518–526. https://doi.org/10.1080/19476337.2020.1791962

[24] Lodish, H., Berk, A., Zipursky, S. L., Matsudaira, P., Baltimore, D., & Darnell, J. (2000). Molecular Cell Biology. In *Section 16.3, Photosynthetic Stages and Light-Absorbing Pigments* (4th editio). New York.

[25] Mahato, N., Sharma, K., Sinha, M., Dhyani, A., & Pathak, B. (2021). Biotransformation of Citrus Waste-I : Production of Biofuel and. *Processes*, *9*, 220. https://doi.org/https://doi.org/10.3390/pr9020220

[26] Mahdy, A., Mendez, L., Ballesteros, M., & González-fernández, C. (2015). Bioresource Technology Algaculture integration in conventional wastewater treatment plants : Anaerobic digestion comparison of primary and secondary sludge with microalgae biomass. *Bioresource Technology*, *184*, 236–244. https://doi.org/10.1016/j.biortech.2014.09.145

[27] Milaiti, M., Traoré, A. S., & Moletta, R. (2003). Essais de fermentation à partir de Calotropis procera production de CH4 en fonction de la charge en substrat et en fonction de la température. *Science et Médecine*, *02*(CAMES-Série A), 73–78.

[28] Na, R., Uchitani, K., Yoshida, K., & Shimizu, N. (2021). Stabilization of Anaerobic Co-Digestion Process via Constant the Digestate Solids Content. *Processes*, *9*(197), 1–11. https://doi.org/https://doi.org/10.3390/pr9020197

[29] Nikiema, M., Sawadogo, J. B., Somda, M. K., Traore, D., & Dianou, D. (2015). Optimisation de la production de biométhane à partir des déchets organiques municipaux Optimization of biomethane production from municipal solid organic wastes. *Internationnal Journal of Biologycal and Chemical Sciences*, *9*(5), 2743–2756.

[30] Nikiema, M., Somda, M. K., Adeoti, K., & Traore, D. (2017). Production of Efficient Microbial Complex for Organic Fraction of Municipal Organic Solid Waste Pretreatment Upstream Anaerobic Digestion. *International Journal of Environmental Bioremediation & Biodegradation*, *5*(3), 77–85. https://doi.org/10.12691/ijebb-5-3-1

[31] Nikiema, M., Somda, M. K., Sawadogo, J. B., Dianou, D., Traoré, A. S., & Ouattara, A. S. (2020). Valorization of Agricultural Waste: Theoretical Estimation and Experimental Biomethane Yield from Cashew Nut Hulls. *Journal of Sustainable Bioenergy Systems*, *10*(04), 113–130. https://doi.org/10.4236/jsbs.2020.104009

[32] Njuku, F. W., Mwangi, P. ., & Thiong'o, G. T. (2014). A Comparison of Cardanol and its Derivatives as Reactive Diluents in Alkyd. *Chemistry and Materials Research*, *6*(3), 50–61.

[33] Nout, M. J. R., Rombouts, F. M., & Havelarr, A. (1989). Effect of accelerated natural lactic fermentation of infant food ingredients on some pathogenic microorganisms. *Journal Food Microbiology*, *8*, 351–361.

[34] Papoutsis, K., Vuong, Q. V, Golding, J. B., Joaquín, H., Pristijono, P., Bowyer, M. C., … Stathopoulos, C. E. (2018). Pretreatment of citrus by-products affects polyphenol recovery : a review. *Food Reviews International*, *00*(00), 1–26. https://doi.org/10.1080/87559129.2018.1438471

[35] Patel, R. N., Bandyopadhyay, S., & Ganesh, A. (2006). Extraction of cashew (Anacardium occidentale) nut shell liquid using supercritical carbon dioxide. *Bioresource Technology*, *97*(6), 847–853.

[36] Pouan, F. (2011). *Valorisation Energétique des Tourteaux de Jatropha par Méthanisation*. 2IE.

[37] Radziejewska-Kubzdela, E., Szwengiel, A., Ratajkiewicz, H., & Nowak, K. (2020). Effect of ultrasound, heating and enzymatic pre-treatment on bioactive compounds in juice from Berberis amurensis Rupr. *Ultrasonics Sonochemistry*, *63*(October 2019). https://doi.org/10.1016/j.ultsonch.2020.104971

[38] Saenab, A., Wiryawan, K. G., Retnani, Y., & Wina, E. (2017). Anacardic Acid Isolated From Cashew Nut Shell (Anacardium occidentale) Affects Methane and Other Products in the Rumen Fermentation. *Media Peternakan*, *40*(August), 94–100. https://doi.org/https://doi.org/10.5398/medpet.2017.40.2.94

[39] Sakaki, S. (2014). *Etude de la production des acides gras volatils à partir de la fermentation acidogène des boues d 'épuration des effluents issus d'un e usine de pâtes et papiers*. Université du Québec.

[40] Sakar, S., Yetilmezsoy, K., & Kocak, E. (2009). Anaerobic digestion technology in poultry and livestock waste treatment – a literature review Suleyman. *Waste Management and Research*, *27*, 3–18. https://doi.org/10.1177/0734242X07079060

[41] Sawadogo, B. J., Dianou, D., & Alfred, S. (2012). Émissions de dioxyde de carbone et de méthane des termitières de Macrotermes bellicosus au Burkina Faso. *International Journal of Biologycal and Chemical Sciences*, *6*(3), 1223–1232.

[42] Singh, R. N. Ã., Vyas, D. K., Srivastava, N. S. L., & Narra, M. (2008). SPRERI experience on holistic approach to utilize all parts of Jatropha curcas fruit for energy. *Renewable Energy*, *33*, 1868–1873. https://doi.org/10.1016/j.renene.2007.10.007

[43] Singh, R. N., Jena, U., Patel, J. B., & Sharma, A. M. (2006). Feasibility study of cashew nut shells as an open core gasifier feedstock. *Renewable Energy*, *31*(4), 481–487. https://doi.org/10.1016/j.renene.2005.04.010

[44] Somé, L. F. M. C. (2014). *Analyse socio-conomiquedes systèmes de production d\'anacarde au Burkina Faso: cas des régions des Cascades et des Hauts-Bassins*. Master II, Université Polytechnique de Bobo Dioulasso.

[45] Tagutchou, J., & Naquin, P. (2012). Caracterisation et traitement thermochimique des coques d ' anacarde en vue de leur valorisation énergetique dans les procédés de transformation artisanale de noix de cajou. *Déchets Sciences et Techniques - Revues Francophone d'Ecologie Industrielle*, *N°62*, 28–35.

[46] Thiombiano, S. T., Weisman, N., & Blin, J. (2011). Adaptation de l ' outil exact et évaluation de l ' empreinte carbone de la filière anacarde au Burkina Faso. In *6ème Journées Scientifiques du 2iE du 4 - 8 Avril 2011* (pp. 1–4). Ouagadougou/Burkina Faso.

[47] Tsamba, A. J., Yang, W., & Blasiak, W. (2006). Pyrolysis characteristics and global kinetics of coconut and cashew nut shells. *Fuel Processing Technology*, *87*, 523–530. https://doi.org/10.1016/j.fuproc.2005.12.002

[48] Vedrenne, F. (2007). *Etude des processus de dégradation anaérobie et de production de méthane au cours du stockage des lisiers*. Ecole Nationale Supérieure d'Agronomie de Rennes.

[49] Wang, S., Hou, X., & Su, H. (2017). Exploration of the relationship between biogas production and microbial community under high salinity conditions. *Scientific Reports*, *7*(1), 1149. https://doi.org/10.1038/s41598-017-01298-y

[50] WHO. (2015). *Waste and human health : Evidence and needs*. Bonn, Germany.

[51] Wikandari, R., Millati, R., Cahyanto, M. N., & Taherzadeh, M. J. (2014). Biogas production from citrus waste by membrane bioreactor. *Membranes*, *4*(3). https://doi.org/10.3390/membranes4030596

[52] Zhang, L., Wu, G., Wang, W., Yue, J., & Yue, P. (2019). Anthocyanin profile , color and antioxidant activity of blueberry (Vaccinium ashei) juice as affected by thermal pretreatment. *International Journal of Food Properties*, *22*(1), 1036–1046. https://doi.org/10.1080/10942912.2019.1625366

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Plant formations of *Pterocarpus erinaceus* Poir. in Sudanian and Sahelian zones

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***Abstract*— *In the Sudano-Sahelian zones of West Africa, climate controls the distribution and composition of spontaneous vegetation. The study was conducted in Niger and Burkina Faso. It aims to (1) analyze the ecological and floristic characteristics in a heterogeneous environment of 8 forests: (2) determine the woody groups of plant formations in P. erinaceus along an agroecological gradient. A total of 206 plots of 1000 m^2 were established in 8 forests along an agroecological gradient in Niger and Burkina Faso. In each plot, dendrometric measurements such as DBH, Total height and two perpendicular diameters of the crown were performed on all woody individuals of all recorded species with DBH>5 cm. Our study identified 117 species belonging to 25 families. The four most represented families are Fabaceae (31.8%), Combretaceae (16.8%), Malvaceae (7.9%) and Rubiaceae (6.9%). Our results revealed that Micro-phanerophytes is the dominating biological type in all forests. The Shannon index shows that the species richness and diversity increase with increasing humidity from the Sahelian to the Sudano-Guinean zone. Sorensen's index shows that the similarity is greater between the forests of Tiogo, Cassou and Laba in Burkina Faso. The Ascending Hierarchical Classification and the Non-Multidimensional Scaling made it possible to determine six large groupings of P. erinaceus. We conclude that P. erinaceus has great ecological amplitude and its distribution zone extends from the Sahelian to the Guinean zones while associating with a diversity of plant species.*

Keywords— ***Plant formations, vegetation, Shannon index, agroecological gradient.***

I. INTRODUCTION

In Sudano-Sahelian zones of West Africa, the distribution, composition and structure of spontaneous vegetation depend on climate, soil, anthropogenic activities, herbivores and fire (Osborne et al., 2018; Coulibaly et al., 2019). The ecosystems have arguably been shaped by humans for thousands of years (Pennington et al., 2015). The interaction between anthropogenic, abiotic and biotic factors makes it difficult to identify and quantify the main determinants of plant association (Stevens et al., 2016). Tropical savannahs are habitat to many species and are among the richest ecosystems of the world (Loubota et al., 2018a; Ouédraogo et al., 2018). This characteristic is the most important challenge for understanding their functioning. The multiple interactions between species and with their environment make these forests extremely complex ecosystems (Loubota et al., 2018a). The heterogeneity of these environments, due to a pronounced rainfall gradient, influences the structure, composition and distribution of the characteristic species. The work of Osborne et al. (2018) indicated that the unique combinations of functional characteristics of plants characterizing the main associations of African savannahs make them differentially vulnerable and resilient to anthropogenic factors of ecosystem change.

Among the plants currently very exploited, we note *P. erinaceus* whose quality of wood, makes this species very sought after. This species is also exploited for various purposes such as fodder and various medicinal products etc. In addition, changes in land use patterns and their consequences on ecosystems have been recognized as a major component of global changes, of comparable significance to climatic and atmospheric variations (Mahamane et al., 2007). Given this complexity, one of the approaches adopted is the study of the dynamics of the species studied, chosen for their commercial and/or ecological interest. The set of inter-fertile individuals is considered a population, for the natural need of association, the populations of a given species cohabit with populations of other species evolving in the same natural environment. An association is a plant group characterized by a floristically determined and relatively constant composition within the limits of a given area (Solefacka, 2018). Any association represents a more or less stable stage of more or less long duration in a progressive or regressive series of association. The overexploitation of some species with great socio-economic values is one of the destabilizing factors of the plant association, especially in the Sahelian and Sudanian zones of West Africa.

Numerous studies have shown that woody species reflect better the variation of environmental factors (Chen et al., 2016; Mahamane et al., 2007; Loubota et al., 2018b) and represent the best indicators for assessing climatic and environmental variability (Thiombiano et al., 2006 ; Ligot et al., 2018; Gaisberger et al., 2017). According to Chen et al. (2016), the composition and structure of woody vegetation vary considerably from one agro-ecological zone to another. But African savannahs are considerably being modified by anthropogenic activities (Osborne et al., 2018). The present study seeks to 1) analyse the ecological and floristic characteristics in a heterogeneous environment of 8 forests; 2) determine the woody groups of *P. erinaceus* plant formations along agroecological gradient in Niger and Burkina Faso.

II. MATERIAL AND METHODS

Study sites

In this study we surveyed four (4) agroecological zones from North to South which are Sahelo-Sudanian and North Sudanian zones in Niger and South Sudanian and Sudanese-Guinean zones (Burkina Faso). In each area, representative forests were identified and investigated. The climatic characteristics and the geographical position of each site are summarized in Table 1.

In Niger three (3) forests were investigated (Park W Niger, Wildlife Reserve of Tamou and the Gorou Bassounga forest while in Burkina Faso, five (5) forests were surveyed (classified forests of Saponé, Tiogo, Cassou, Laba and a section of Wildlife Reserve of Comoé-Léraba) (Figure 1). In Niger, the dominant soil texture is lateritic imbricated with silty-clayey and gravelly soil beaches with vegetation dominated by Tiger bush, shrub savannahs and grassy savannahs in the Sahelo-Sudanian and North Sudanian zones (Saadou, 1990). While in Burkina Faso, the dominant soil texture is silty-clay to sandy-silty with vegetation dominated by more or less shrubby tree savannahs (Thiombiano et al., 2006; Gaisberger et al., 2017).

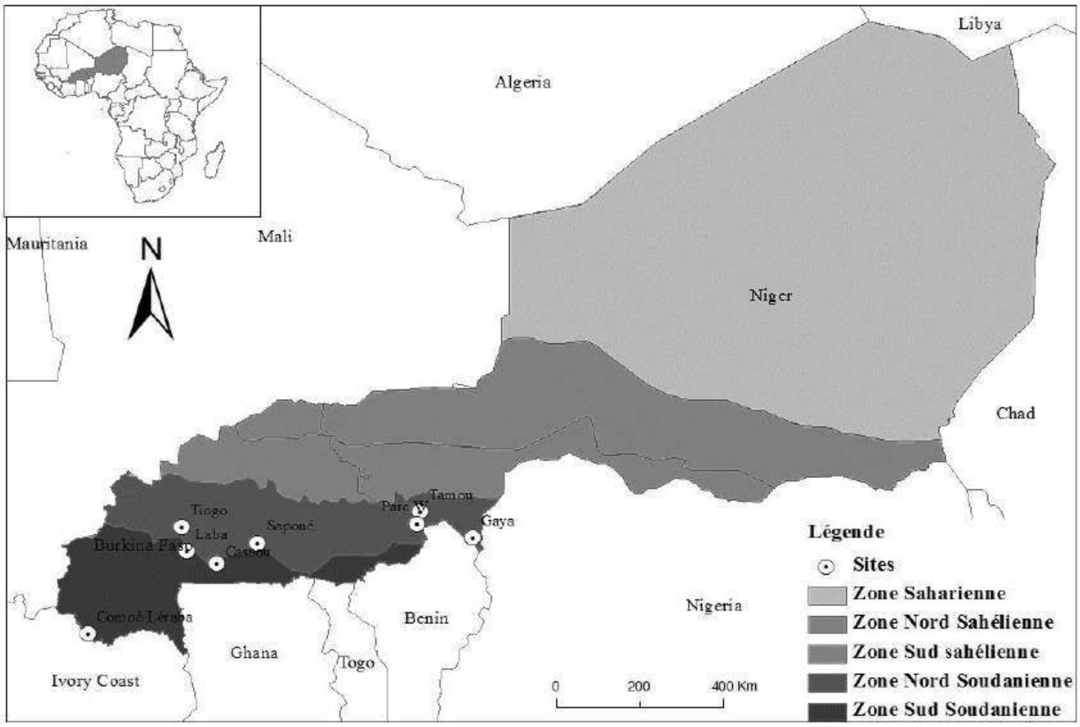

Fig.1. Location of study areas (Source: Rabiou et al., 2015)

Table 1. Characteristics of the surveyed sites

Country	***Sites***	***Agro-ecological zones***	***rainfall (mm/an)***	***Temperature (°C)***	***Area (ha)***	***Geographical coordinates***	
						Latitude	***Longitude***
Niger	Tamou	Sahelo-Sudanian	606 ± 99	36,7°C	76000	12°28’ and 12°50’	2°06’ and 2°24’
	Parc du W	Sahelo-Sudanian	704 ± 101	30° C	220000	11°00’ and 12°35’	2°00 and 3°50’
	GorouBassounga	North Sudanian	740±119,5	33° C	9970	11°52’ and 11°58’	3°20’ and 3°26’
Burkina Faso	Saponé	North Sudanian	806±102	26°C	100	12°07’ and 12°08’	1°33’ and 1°34’
	Tiogo	North Sudanian	827 ± 169	24°C	30389	12°11’ and t 12°24’	2°39’ and 2°52’
	Laba	South Sudanian	907 ± 157	24 °C	18501	11°48’ and 11°39’	2°44’ and 2°36’
	Cassou	South Sudanian	984 ± 103	29° C	29515	11°44’ and 11°21’	2°07’ and 1°44’
	Comoé-Léraba	Sudano-Guinean	1114,6±203	27,4°C	125000	9°39’ and 10°00’	4°25’ and 4°59’

Data collection and sampling

We collected the vegetation data through random sampling transects method (Gounot, 1969) in eight (8) plant formations in Niger and Burkina Faso. We surveyed a total of 206 plots of 1000 m^2 (20 m * 50 m) established at each 300 m along 41 transects. The number of plots and transects vary from 20 to 42 and from 4 to 8 respectively

(Table 2). In each plot, we measured all woody individuals with a diameter greater than or equal to 5 cm (Table 2).

Table 2. Number of transects and plots in all surveyed sites, ordered by decreasing latitude.

Agro-ecological zones	Sites	Number of transects	Number of plots
Sahelian	Tamou	8	39
	Parc W	8	42
North Sudanian	Gorou Bassounga	4	20
	Saponé	4	20
	Tiogo	5	25
South Sudanian	Laba	4	20
	Cassou	4	20
Sudano-Guinean	Comoé-Léraba	4	20
Total		41	206

Field measurements

In each plot, DBH, total height and two perpendicular crown diameters were measured for each recorded woody individual. These measurements were made using a forestry compass for large diameters, a calliper for small diameters, a tape measure, and a graduated pole for heights. For all individuals measured, a systematic count of young stems with a diameter less than 5 cm, resulting from regeneration (sowing, stump sprouts, layers or suckering) was carried out. To collect tree regeneration data, we set five subplots of 5m*5m (25 m^2) in each corner of the plot and a fifth in the center. In all surveyed sites, the dominant heights of young seedlings of all recorded species were systematically measured in accordance with the recommendations of SUN (2008). We recorded a total of 117 woody species in 207 plots carried out.

Characteristics of inventoried species

The biological type is a form of morpho-physiological adaptation of plants to unfavourable climatic conditions. The biological types of all recorded woody species were determined in accordance with the classification of Raunkiaer (1934) about woody phanerophyte vegetation. In fact, phanerophytes are woody plants whose buds are located more than 50 cm above the ground surface (Saadou, 1990). These include Nanophanerophytes (nPh) from 0.5 to 2 m; Microphanerophytes (mPh) from 2 to 8 m; Mesophanerophytes (MesoPh) from 8 to 30 m; Megaphanerophytes (MPh)> 30 m, Liana microphanerophytes (LmPh).

To determine the phytogeographic type of all recorded species we adopted the chorological subdivisions of White, (1983) that are being used (Mahamane et al., 2007; Osborne et al., 2018). Among these we have Cosmopolites (Cos) for species distributed in tropical and temperate regions of the world; African-American (AA) for species widespread in Africa and America; Pantropicals (Pan) concerning species widespread in Africa, America and tropical Asia; Paleotropical (Pal) that consists of species distributed in tropical Africa, tropical Asia, Madagascar and Australia; Afro-Malagasy tropical (AM) dedicated to species distributed in Africa and Madagascar; Afro-Tropicales (AT) for species widespread in tropical Africa; Pluri-regional (PA) that concern species whose range extends to several regional endemism centers; Soudano-Zambéziennes (SZ) that encompass species distributed in both Sudanian and Zambezian regional endemism centers; all species distributed in the Guinean region belong to Guinean-Congolese (GC); and . Sudanian (S): for widely distributed species in the regional center for Sudanian endemism.

Data analysis and processing

Alpha diversity indices

In each site we calculated several parameters such as the specific richness, the specific diversity of Shannon (H') and the fairness of Piélou (E). These last two parameters provide information on the distribution of individuals of each species. Indeed, the higher the index the greater the diversity.

$$H' = -\sum_{i=1}^{s} p_i \log_2 p_i \qquad E = \frac{H'}{\log_2 S}$$

with S = total number of species;

pi = (nj / N), relative frequency of the species;

nj = relative frequency of species j in the sampling unit;

N = sum of the specific relative frequencies;

Typology of plant groups

To identify a typology of plant groups, the species survey matrix was first subjected to an Ascending Hierarchical Classification (AHC) then to Non-Multidimensional Scaling analysis (NMDS) using PCord 5 software. These methods make it possible to summarize the information in the data table using dendrogram and factorial map.

The species with higher and statistically significant indicator values were considered to designate each group. However, since *P. erinaceus* was recorded in all relevés, therefore it will not be discriminating, and we removed it from the matrix before performing the analysis.

Sorensen index was calculated based on the number of common species between forest A and forest B, the number of species of forest A and the number of species of forest B. The index allows appreciating the level of similarity between two communities. It is calculated by the following formula:

$$IS = 2c / (a + b)$$

c = number of species common to A and B

a = number of species of A

b = number of species of B

The index varies from 0 to 1. When the index value is 1, the level of similarity is perfect and when the index value is o 0 the two communities have no common species.

The data table on the raw spectrum frequencies of the phytogeographic types of the 8 forests investigated was subjected to a Principal Component Analysis (PCA).

III. RESULTS

Floristic characteristics

Our results show that Fabaceae was the dominant family in all surveyed sites with 31.8% followed by Combretaceae with 16.8%. Malvaceae and Rubiaceae are represented by 7.9% and 6.9 respectively (Table 3). A total of 117 species were identified belong to 32 families. We observed the largest number of species (66 species) in the Comoé-Léraba forest (Sudano-Guinean zone) while the lowest number (42 species) was in Tamou forest located in Sahelo-Sudanian zone.

Table 3. Frequency of families (%) of surveyed sites

Familly	Parc W	Tamou	Gaya	Cassou	Comoé	Laba	Tiogo	Saponé	Global
Fabaceae	34.55	28.57	34	32.76	24.25	35.39	32.75	32.65	31.87
Combretaceae	20	19.05	14	17.24	16.67	13.85	17.24	16.33	16.80
Malvaceae	9.1	7.14	10	6.89	9.1	6.16	8.61	6.12	7.89
Rubiaceae	7.27	7.14	6	6.9	7.58	6.15	8.62	6.12	6.97
Anacardiaceae	3.64	9.52	8	5.17	3.03	7.69	6.9	8.16	6.51
Capparidaceae	7.27	9.52	4	0	0	4.62	3.45	2.04	3.86
Euphorbiaceae	1.82	2.38	2	3.45	3.03	4.62	3.45	2.04	2.85
Apocynaceae	0	0	4	3.45	1.52	3.08	3.45	2.04	2.19
Meliaceae	0	2.38	2	1.72	4.55	1.54	0	4.08	2.03
Sapotaceae	1.82	0	2	1.72	3.03	3.08	1.72	2.04	1.93
Bignoniaceae	3.64	2.38	2	1.72	1.52	0	1.72	2.04	1.88
Olacaceae	1.82	2.38	2	1.72	1.52	1.54	1.72	2.04	1.84
Loganiaceae	1.82	2.38	0	3.45	3.03	1.54	1.72	0	1.74
Balanitaceae	1.82	2.38	2	1.72	0	1.54	1.72	2.04	1.65
Celastraceae	1.82	0	0	1.72	1.52	1.54	1.72	2.04	1.30
Ebenaceae	1.82	0	0	1.72	1.52	1.54	1.72	2.04	1.30
Annonaceae	0	0	0	1.72	1.52	1.54	1.72	2.04	1.07
Hymenocardiaceae	0	0	2	1.72	1.52	0	0	2.04	0.91
Moraceae	0	0	2	0	3.03	0	0	2.04	0.88
Rhamnaceae	1.82	0	0	0	0	1.54	0	2.04	0.68
Verbenaceae	0	0	0	1.72	1.52	1.54	0	0	0.60

Familly	Parc W	Tamou	Gaya	Cassou	Comoé	Laba	Tiogo	Saponé	Global
Asclepiadaceae	0	4.76	0	0	0	0	0	0	0.60
Simaroubaceae	0	0	2	1.72	0	0	0	0	0.47
Flacourtiaceae	0	0	2	0	1.52	0	0	0	0.44
Chrysobalanaceae	0	0	0	0	3.03	0	0	0	0.38
Burseraceae	0	0	0	0	0	0	1.72	0	0.22
Polygalaceae	0	0	0	1.72	0	0	0	0	0.22
Sapindaceae	0	0	0	0	0	1.54	0	0	0.19
Araliaceae	0	0	0	0	1.52	0	0	0	0.19
Dipterocarpaceae	0	0	0	0	1.52	0	0	0	0.19
Myrtaceae	0	0	0	0	1.52	0	0	0	0.19
Opiliaceae	0	0	0	0	1.52	0	0	0	0.19
Total	100	100	100	100	100	100	100	100	100

Distribution of biological spectra of *P. erinaceus* formations

The biological type that dominates all of the investigated forests is the microphanerophyte followed by nanophanerophyte both in terms of raw and weighted spectrum. Mesophanerophytes and microphanerophyte lianas are poorly represented in both the Sahelian and Sudanian zones (Table 4). The frequency of the raw spectrum of microphanerophyte lianas decreases from the Sahelian to the Sudanian zone. These are mainly *Acacia erythrocalyx*, *Combretum aculeatum*, *Capparis tomentosa* and *Strophantus sarmentosus* in the Sahelo-Sudanian zone and *Opilia amentacea*, *Saba senegalensis*, *Baissea multiflora* and *Capparis sepiaria* in the Sudanian zone. Our findings show also that the frequency of microphanerophytes increases from Sahelian to Sudanian zones, while nanophanerophytes decreases (Table 3). However, the X^2 test shows that the distribution of phytogeographic types is not related to the agroecological zones (*Chi-Sq = 8.301; DF = 9; P-Value = 0.504*).

Table 4. Biological type. LmPh :Liane microphanerophytes ; mPh : Microphanerophytes ; nPh : Nanophanerophytes ; MesoPh : Mesophanerophytes.

TB	Sahelian		North Sudanian			South Soudanian		SudanoGuinean
	Tamou	Parc W	Gaya	Saponé	Tiogo	Laba	Cassou	Comoé-Léraba
Raw spectrum (%)								
LmPh	7.14	5.45	4	6.12	6.9	6.15	5.17	3.03
MesoPh	2.38	3.64	4	2.04	1.72	1.54	1.72	6.06
mPh	61.9	72.73	76	77.55	68.97	75.38	74.14	78.79
nPh	28.57	18.18	16	14.29	22.41	16.92	18.97	12.12
Total	100	100	100	100	100	100	100	100
Weighted spectrum (%)								
LmPh	1.85	2.88	0.43	1.25	0.56	1.01	0.29	3.9
MesoPh	0.03	0.06	0.18	1.17	0.14	0.11	0.25	1.28
mPh	53.28	81.22	78.57	87.73	86.09	74.56	89.23	78.64
nPh	44.84	15.83	20.81	9.84	13.21	24.31	10.23	16.17
Total	100	100	100	100	100	100	100	100

Distribution of phytogeographic types

The Principal Component Analysis PCA shows thatthe first two axes account respectively for 33.4 and 27.7% (both 61.1%) of the variance. PCA results highlights forest groups according to agroecological zones. For instance Park W is distinguished by chorological characteristics much closer to the Sudanian areas. The Sahelo-Sudanian zone is characterized by a predominance of the Paleotropical (Pal) and Sahelo-Saharan (SS) species. The Guinea Congolian (GC), Sudanian (S) and Pantropical (Pt) species dominate the North Sudanian zone. The Southern Sudanian zone is distinguished by a predominance of Sudano-Guinean (SG), Afro-Malagasy (AM) and Afro-tropical species (Figure 2).

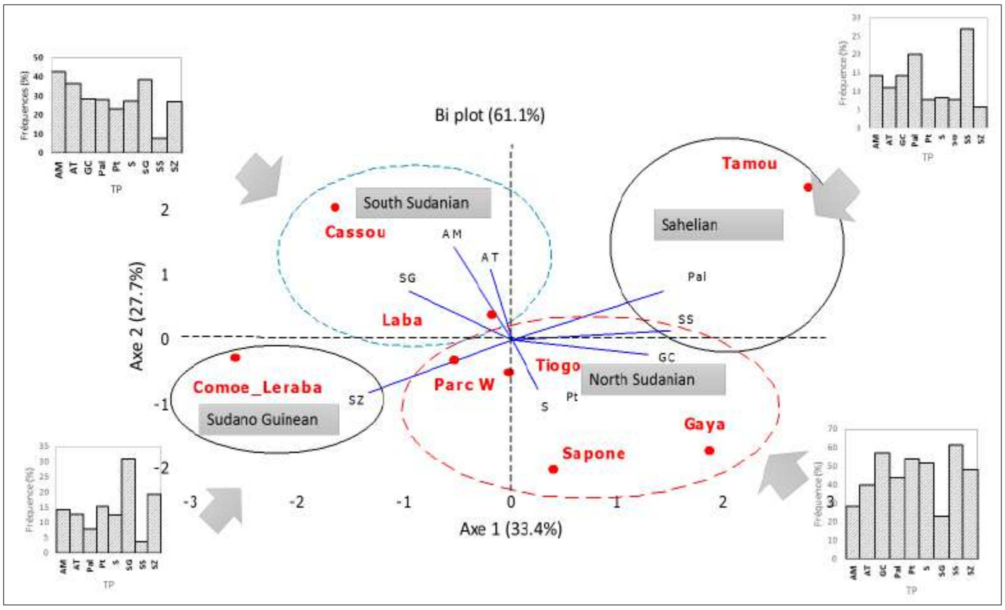

Fig.2. Distribution of biological types: AT: Afro-tropical; SG: Sudano-Guinean; AM: Afro-Malagasy; Pal: Paleotropical; SS: Sahelo-Saharan; GC: Guinean-Congolese; SZ: Sudano-Zambezian; S: Sudanian; Pt: Pantropical.

Analysis of woody species diversity

Analysis of the diversity indices reveals that the Tiogo and Comoé-Léraba forests are the most diversified as measured by Shannon diversity index which was 4.65 each one, although the species richness is greater in the Comoé-Léraba forest with 66 species, against 58 species in the Tiogo forest. Indeed, Piélou's fairness is of 0.7 for each forest. The dominant species in the Comoé-Léraba forest is *Terminalia mollis* representing 14.6% of the woody individuals, while *Combretum glutinosum* is the dominant species in the Tiogo forest and represents 12.9% of the woody individuals. The lowest diversity is observed at the Tamou wildlife reserve with a richness of 42 species and a low fairness of 0.54 (Table 5). In this reserve, the dominant species is *Guiera senegalensis* and represents 37.5% of the woody species. The analysis of the specific contribution index in the achievement of the Shannon index ($2^{H'}$) shows that in the Sahelo-Sudanian zone the dominance of certain species such as *Guiera senegalensis* and *Combretum micranthum* contributes largely to the weakness of the index. In Gaya and Tamou respectively only 9 and 8 woody species represent more than 90% of the stands. In the Sudanian zone where diversity is greater we observed up to 25 species representing 90% of the woody species.

Analysis of the diversity indices indicates that diversity is a function of humidity. The more watered sites recorded the large diversity of species due to the significant richness and fairness, suggesting a lack of pronounced dominance of certain species. In the Sahelian zone, the dominance of Combretaceae, in particular *Guiera senegalensis*, *Combretum nigricans* and *Combretum micranthum*, considerably reduces the Shannon diversity index, resulting in poor fairness. The Shannon diversity index and the species richness increase from the Sahelo-Sudanian to the Sudano-Guinean zone (Table 5).

Analysis of the species rarefaction curve shows that the accumulation of species richness is greater in the Sudanian and Guinean areas of Burkina Faso. Indeed, the high frequency of species observed in the Sahelo-Sudanian zone of Niger can be attributed to the high sampling effort. Figure 3 clearly indicates that for the same number of relevés, the cumulative variations in terms of species richness in the Sudanian and Guinean zones of Burkina Faso are clearly discriminated from those of the Sahelo-Sudanian zones of Niger. For the same number of relevés, the number of species is only 31 at Parc W, 29 at Tamou and 51 at Tiogo.

Table 5. Diversity indices: Wealth (S), diversity (H') and Piélou equitability (E)

Diversity indices	Sahelian		North Sudanian			South Sudanian		Sudano Guiean
	Tamou	Parc W	Gaya	Saponé	Tiogo	Laba	Cassou	Comoé-Léraba
H'	2.94	4.08	3.09	4.00	4.65	4.70	4.42	4.65
S	42	55	50	49	58	65	58	66
H max	5.39	5.78	5.64	5.61	5.86	6.02	5.86	6.04
E	0.54	0.71	0.55	0.71	0.79	0.78	0.75	0.77
$2^{H'}$	7.65	16.80	8.54	16.00	25.04	26.04	21.36	25.19

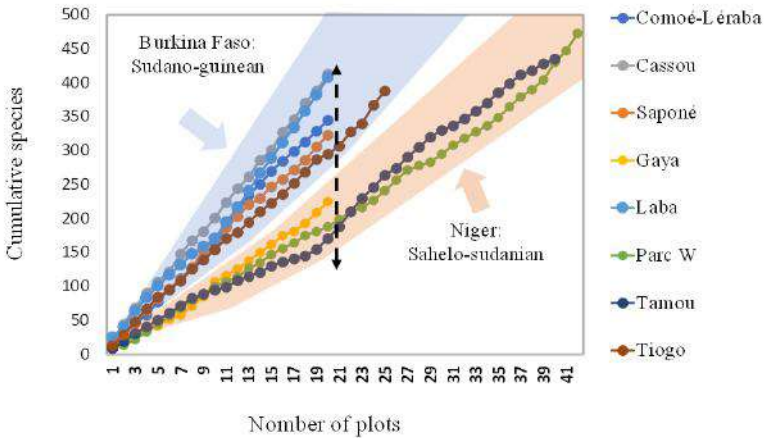

Fig.3. Species Curve of rarefaction and accumulation

Sorensen's similarity index (Is)

Analysis of the index shows that similarity is also a function of the rainfall gradient. Indeed, the sites located in the same agroecological zone have a higher level of similarity with higher Is indices. In the Sahelo-Sudanian zone, the level of similarity is of 0.66 between Park W and Tamou. In the northern Sudanian zone, the Is observed is 0.71 between Saponé and Tiogo. In the southern Sudanian zone, the index Is observed between Cassou and Laba is of 0.73. However, the greatest similarity index was observed between Tiogo and Cassou although these two forests are belonging to different agroecological zones. While, the lowest similarity index is of 0.27 and was observed between the Tamou wildlife reserve (Sahelo-Sudanian zone) and the Comoé-Léraba wildlife reserve (Sudano-Guinean zone) (Table 6).

Table 6. Values of the Sorensen Community index.

Sites	Tamou	Parc W	Gaya	Saponé	Tiogo	Laba	Cassou	Comoé-Léraba
Tamou	1							
Parc W	0,66	1						
Gaya	0,65	0,60	1					
Saponé	0,46	0,59	0,60	1				
Tiogo	0,56	0,69	0,59	0,71	1			
Laba	0,48	0,66	0,53	0,64	0,74	1		
Cassou	0,48	0,61	0,53	0,63	0,75	0,73	1	
Comoé-Léraba	0,27	0,49	0,43	0,52	0,53	0,61	0,62	1

Legend: Evolution of the similarity index; from the highest index to the lowest (from blue to red)

1	0,9	0,8	0,7	0,6	0,5	0,4	0,3	0,2	0,1	0

Individualization of plant groups

The CHA dendrogram made it possible to discriminate 6 plant communities with *P. erinaceus*. These communities vary according to agroecological zones at a threshold of proportion of the Similarity index (Figure 4) based on the Euclidean distance through the Ward method. Figure 4 shows by the position of the groups, the distance between them (if the distance between them is short, there is similarity and if it is long, it is dissimilarity). We found 35% of similarity that made it possible to determine 6 major groups distributed across the 4 different agroecological zones under study (Figure 5).

➢ **Sahelo-Sudanian zone**

The Sahelo-Sudanian zone is made up of the Regional Park W (Niger) and a section of the Tamou wildlife reserve. In this area two large groups were observed.

- Sahelo-Sudanian zone (Sah 1): *P. erinaceus* and *Combretum collinum*

The group was mainly observed in Park W where we recorded many species with the highest indicator value at the 5% threshold and statistically significant ($P < 0.05$) such as *Combrteum collinum* Fresen., *Anogeissus leiocarpa* (DC.) Guill. and Perr., *Lonchocarpus laxiflora* Guill and Perr., *Flueggea virosa* (Roxb. ex Willd.) Voigt., *Tamarindus indica* L., *Xeroderris stuhlamannii* (Taub.) Mendonça and Sousa.

- Sahelo-Sudanian zone (Sah 2): *P. erinaceus* and *Combretum nigricans*

This grouping was largely noticed in Tamou. The indicator species with the significant indicator values ($P < 0.05$) at the 5% level are: *Combretum nigricans* Lepr. exGuill. And Perr., *Acacia macrostachya* Reicheb. Ex DC., *Combretum micranthum* G. Don, *Guiera senegalensis* J. F. Gmel., *Feretia apodanthera* Del., *Acacia erhytrocalyx* Brenan, *Boscia senegalensis* (Pers.) Lam. ex Poir., *Boscia angustifolia* A. Rich., *Sclerocarya birrea* Hochst., *Combretum aculeatum* Vent., *Maerua angolensis* DC., *Dichrostachys cinerea* (L.) Wight and Am., *Strophanthus sarmentosus* DC. and *Cassia sieberiana* DC ..

➢ **Sudanian zone**

- North Sudanian zone (NS1): *P. erinaceus* and *Gardenia sokotensis*

This grouping is made up of surveys carried out in the North Sudanian zone particularly in (Gaya and the Wildlife Reserve of Tamou (Niger). The species with the highest indicator values are mainly, *Gardenia sokotensis* Hutch., *Grewia flavescens* Juss., *Acacia laeta* R. Br. Ex Benth., *Grewiabicolor*Juss., *Acacia nilotica* (L.) Wild.Ex Del.And *Gardenia erubescens*Stapf and Hutch.

- North Sudanian Zone (NS2): *P. erinaceus* and *Combretum glutinosum*

It is a group consisted mainly of surveys carried out in the North Sudanian Zone of Burkina Faso at Tiogo and Saponé sites. The species with large significant indicator values ($P < 0.05$) include *Entada africana* Guil. And Perr., *Lannea microcarpa* Engl. and K. Krause, *Lannea vellutina* A. Rich., *Combretum glutinosum* Perr. ex DC., *Piliostigma reticulatum* (DC.) Hochst., *Acacia gourmaensis* A. Chev., *Ximenia americana* L. and *Parkia biglobosa* (Jacq.) R. Br. ex G. Don f.

- South Sudanian zone (SS): *P. erinaceus* and *Lannea acida*

This group is made up of investigations carried out in the South Sudanian zone of Burkina Faso (Cassou and Laba). We recorded fifteen tree species with the largest indicator values. These include *Lannea acida* A. Rich., *Crossopteryx febrifuga* (G. Don) Benth., *Detarium microcarpum* Guill. et Perr., *Combretum molle* R. Br. ex G. Don, *Annona senegalensis* Pers., *Strychnos spinosa* Lam., *Burkea africana* Hook., *Grewia mollis* Juss., *Bridellia ferruginea* Benth., *Pteleopsis suberosa* Engl. and Diels., *Terminalia avicennoides* Guill. And Perr., *Acacia dudgeon* Craib. Ex Holl., *Balanites aegyptiaca* (L.) Del., *Terminalia macroptera* Guill. And Perr. And *Vitex doniana* Sweet.

- Sudano-Guinean Zone (SG): *P. erinaceus* and *Terminalia mollis*

In the Sudano-Guinean zone, only one plant community has been observed in the section of the Comoé-Léraba wildlife reserve. Nineteen significant indicator species ($P < 0.001$) were recorded by *Vitellria paradoxa* C. F. Gaertn., *Piliostigma thonningii* (Schumach.) Milne-Redhead, *Combretum fragrans* F. Hoffm., *Gardenia ternifolia* Schum. & Thonn., *Maytenus senegalensis* (Lam.) Exell, *Terminalia laxiflora* Engl. and Diels, *Pericopsis laxiflora* (Benth. ex Baker) Meeuwen, *Daniella oliveri* (Rolfe) Hutch. And Dalz., *Terminalia mollis* M. Laws., *Maranthes polyandra* (Benth.) Prance, *Cussonia arborea* Hochst. ex A. Rich, *Opilia amentacea* Roxb, *Parinari curatellifolia* Planch. Ex Benth, *Gardenia aqualla* Stapf and Hutch., *Saba senegalensis* (A.DC.) Pichon, *Diospyros mespiliformis* Hochst.ex A. DC., *Syzgium guineense* (Wild.) DC., *Khaya senegalensis* (Desr.) A. Juss. And *Isoberlinia doka* Craib and Stapf ..

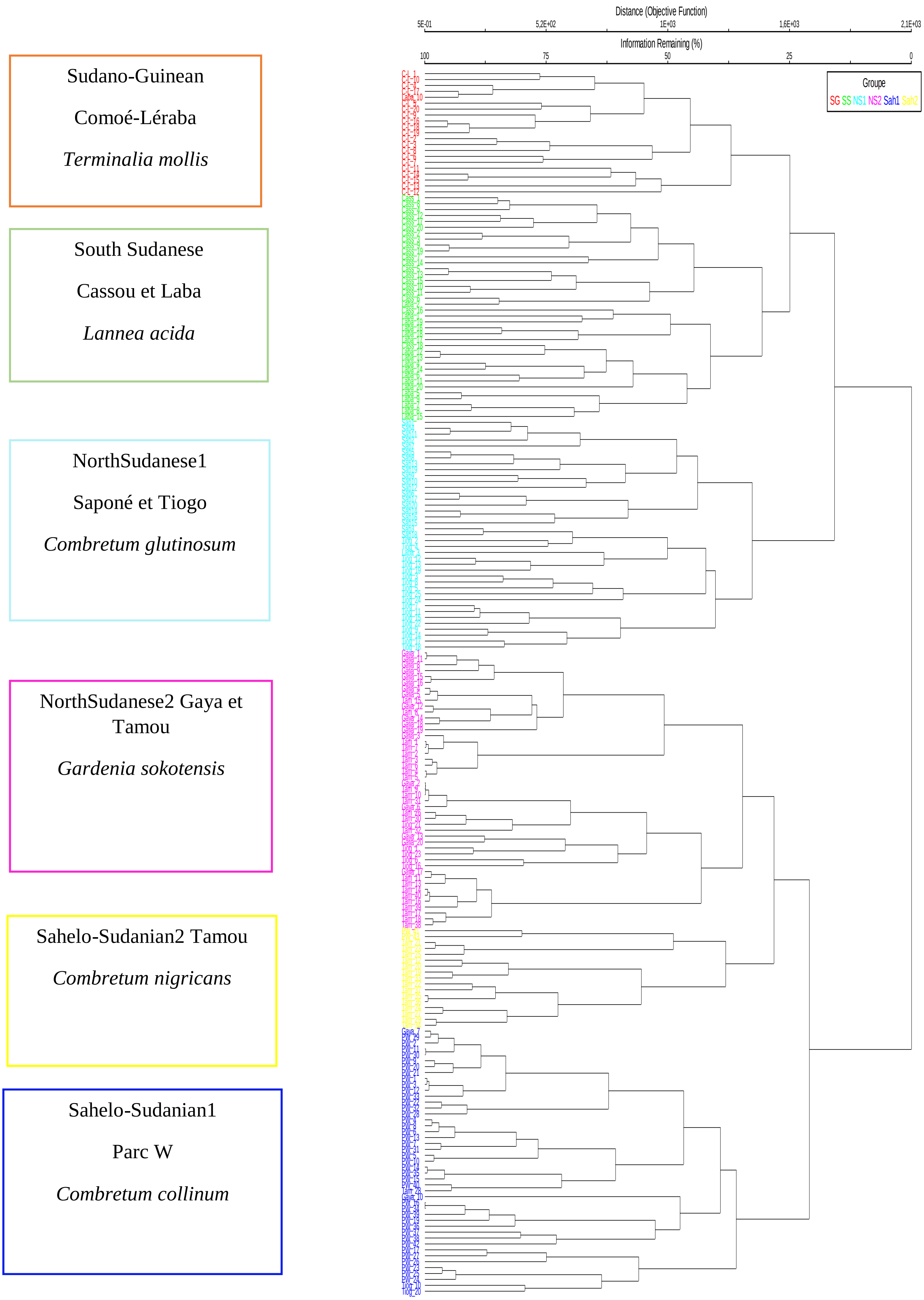

Fig.4. Distribution of relevés according to their similarity

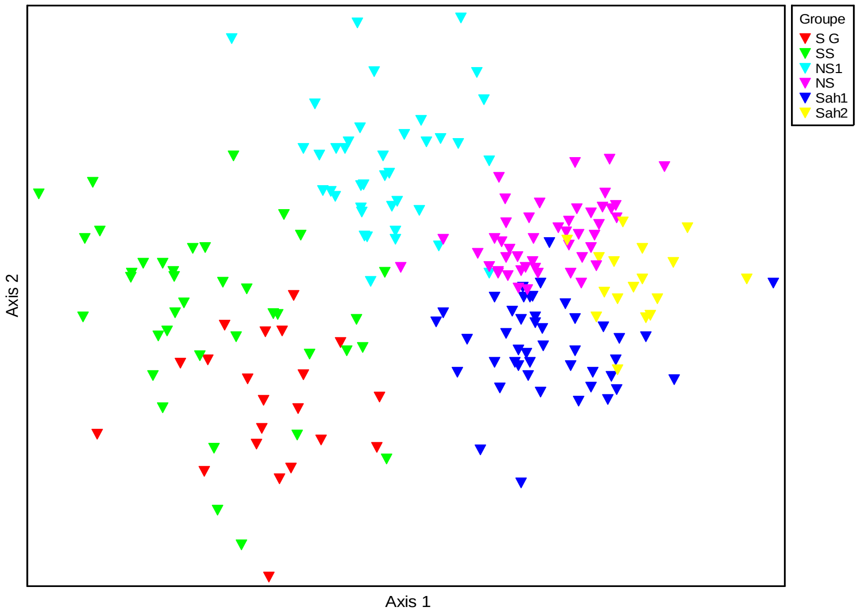

Fig.5. Representation of the distribution relevés using Non-Multidimensional Scaling (NMDS)

IV. DISCUSSION

The analysis of the floristic characteristics of all investigated agroecological zones showed a predominance of species belonging to 4 families classified according to their importance respectively Fabaceae, Combretaceae, Malvaceae and Rubiaceae. The distribution of these families varies according to agroecological zones. Ouédraogo et al. (2006) findings on the diversity of woody species in the eastern part of Burkina Faso showed the predominance of these families in the same agroecological zones. Moreover, the predominance of Combretaceae followed by Mimosaceae in the Sahelo-Sudanian zone has also been reported by several authors such as Diouf et al. 2010) in W park in Niger and Oborne et al. (2018) when studying the human impacts and the adaptation of the plant functional traits of African savannah. The decrease in frequencies of Combretaceae with increasing humidity could be explained by the decrease in xerophytic characteristics of the vegetation. According to Aubreville (1950), the predominance of the Combretaceae family is an indicator of a generally dry climate. On the other hand, the frequency of Caesalpiniaceae, which increases with increasing humidity, indicates a decrease in aridity from the Sahelian to the Sudanian zone. Numerous studies have shown that in the wetlands of West Africa the dominant taxa are gregarious Caesalpiniaceae (White, 1983; Osborne et al., 2018). The diversity analysis showed that the Shannon diversity, the Piélou fairness and species richness indices increase with the humidity gradient. Indeed, the dominance of one or two species is very marked in the driest areas and decreases with increasing humidity. Thus, in the Sahelian zones, notably at Tamou and Parc W, the dominant species are *Guiera senegalensis*, *Combretum micranthum* and *Combretum nigricans*. The regeneration of these species, according to Diouf et al. (2010) is mainly achieved through vegetative propagation. This type of regeneration, comprising several forms such as layering, suckering and stump sprouts (Bationo et al., 2005), is carried out using lying stems or felled tree stumpor burnt trees. In the section of Comoé-Léraba wildlife reserve (Sudano-Guinean zone) the dominance of *Terminalia mollis* is not very pronounced (14.6%) compared to the Tamou wildlife reserve where the dominant species (*Guiera senegalensis*) alone represents up to 37.5%. This is confirmed by the Piélou (E) fairness index with 0.54 and 0.77 respectively for Tamou and Comoé-Léraba. Our findings have shown that species richness is also a function of the humidity gradient, thus confirming the results of Ouédraogo et al. (2006) who showed that the floristic richness of woody people increases from the Sahelian to the Sudanian zones. This highlights the influence of climate, notably rainfall, in the distribution of woody taxa (Osborne et al., 2018).

The vegetation surveys carried out allowed the determination of *P. erinaceus* groups. The distribution of the groups observed shows that *P. erinaceus* is much more associated with Combretaceae. In fact, in the Sahelo-Sudanian zone two groups have been successfully discriminated. These are the *P. erinaceus* and *Combretum collinum* and *Combretum nigricans* groups in Parc W and Tamou respectively. These two contiguous plant formations recover the cuirass plateaus which appear in the form of large outcrops, resulting from the alteration of the sedimentary deposits of the Continental Terminal covering the bedrock (Diouf et al., 2010). The dominance of Combretumnigricans over these types of sedimentary soil has already been reported by Thiombiano et al. (2006). These authors have revealed that the species is abundant on clay-sandy or lateritic terrains in the Sahelo-Sudanian zone. In addition, the dominance of *Combretum nigricans* in these zones of cuirass plateaus can be explained by the particular characteristics of the vegetation. In fact, in these areas, the vegetation type is tiger bush and spotted bush characterised by alternating bare soil and strips of vegetation (thicket) (Diouf et al., 2010). The operating system of these arid zones is such that the runoff is concentrated in the center of the thicket. The microclimate thus created favors the installation of a dense vegetation network and even sensitive species such as *P. erinaceus*, *Gardenia sokotensis* and *Combretum nigricans*. The grouping of *P. erinaceus* and *Combretum glutinosum* observed in the forest of Tiogo, (northern Sudanian zone) has already been reported as a group dependent on slightly more humid and less hot zones (Thiombiano et al., 2006 ; Sanou et al., 2018). The frequency of *Combretum glutinosum* in the northern Sudanian zone is more than 70% (Ouédraodo et al., 2006). The grouping of *P. erinaceus* and *Terminalia mollis* was recorded in the wettest sites located in the Sudano-Guinean zone (the section of Comoé-Léraba). Gnoumou et al., (2015) findings indicated that in the Niangoloko forest located in the same zone, the grouping of *Terminalia mollis* of more than 100 ha in extent growing on low humus soils with pseudogley and weak surface acidity at the bottom of the valley. According to Thiombiano et al. (2006) and Schmidt et al. (2016) the latter species prefers the wettest sites in dry areas or areas with sufficient rainfall with an average rainfall of 1009±125 mm, an average relative humidity of 56±3.4% and an average annual temperature of 28±0, 6°C.

V. CONCLUSION

Our study demonstrates that the largest number of species is observed in the Comoé-Léraba forest in the Sudano-Guinean zone with 66 woody species and the lowest number is observed in the Sahelo-Sudanian zone in Tamou with 42 species. Overall the families mostly represented in all vegetation surveys carried out are Fabaceae, Combretaceae, Malvaceae and Rubiaceae. The diversity analysis shows that the Shannon index and the species richness increase with increasing rainfall. In fact, the Sahelo-Sudanian zone is dominated by Combretaceae, thus explaining the low recorded value of the Piélou fairness index. The study also made it possible to identify the *P. erinaceus* groupings in all the forests investigated, as well as the different phytogeographic and biological types which characterize these plant formations along the agroecological gradient. Our study reveals that *P. erinaceus* has great ecological amplitude and its distribution zone extends from the Sahelian to the Guinean zones while associating with a diversity of plant species. The high diversity of *P. erinaceus* habitat makes it complex the planning and management of the natural stands of this species.

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REFERENCES

[1] Bationo B. A., Karim S. et Dourma M., 2005. Argumentaire pour l'étude et l'utilisation des marcottes et drageons dans les pays à faible couvert ligneux. AUF, sécheresse, volume 1E, no3, 11 p.

[2] Chen Y., Yuan Z., Li P., Cao R., Jia H. and Ye Y., 2016.Effects of Environment and Space on Species Turnover of Woody Plants across Multiple Forest Dynamic Plots in East Asia. Front. Plant Sci. 7:1533. doi: 10.3389/fpls.2016.01533.

[3] Coulibaly A., Motelica-Heino M., Hien E., 2019.Determinants of Agroecological Practices Adoption in the Sudano-Sahelian Zone. Journal of Environmental Protection, 10, 900-918. https://doi.org/10.4236/jep.2019.107053

[4] Diouf A., Nicola B., Mahamane A., Lejoly J., Saadou M., Bogaert J., 2010. Caractérisation de la structure spatiale des individus ligneux dans une brousse tachetée au sud-ouest du Niger. CNRS. Rev. Can. Rach. For. Vol. 40 : 827-835.

[5] Gaisberger H., Kindt R., Loo J., Schmidt M., Bognounou F., Sylvestre D. S., Diallo O. B., Ganaba S., Gnoumo A., Lompo D., Lykke A. M., Mbayngone E., Nacoulma B. M. I., Ouedraogo M., Ouedraogo O., Parkouda C., Porembski S., Savadogo P., Thiombiano A., Zerbo G., Vinceti B., 2017. Spatially explicit multi-threat assessment of food tree species in Burkina Faso: A fine-scale approach. A fine-scale approach.PLoS ONE 12 (9): e0184457. **https://doi.org/10.1371/journal.pone.0184457**.

[6] Gnoumou A., Ouedraogo O., Schmidt M., Thiombiano A., 2015.Floristic diversity of classified forest and partial faunal reserve of Comoé-Léraba, southwest Burkina Faso. The journal of biodiversity data, Check List 11(1): 1557. doi:http://dx.doi.org/10.15560/11.1.1557.

[7] Gounot M., 1969. Méthodes d'étude quantitative de la végétation. Masson, Paris. 314 pp.

[8] Ligot G. Gourlet-Fleury S., Ou_Edraogo D.-Y., Morin X., Bauwens E. S, Baya F., Brostaux Y., Doucet J.-L., Fayolle A., 2018. The limited contribution of large trees to annual

biomass production in an old-growth tropical forest. Ecological Applications, 0(0), pp. 1–9.

[9] LoubotaPanzou G. J., Ligot G., GourletFleury S., Doucet J. L., Forni E., Loumeto J. J., Fayolle A., 2018. Architectural differences associated with functional traits among 45 coexisting tree species in Central Africa. Functional Ecology.1–11. DOI: 10.1111/1365-2435.13198.

[10] LoubotaPanzoua G. J., Fayolle A., Feldpausch T. R., Ligot G., Douceta J.-L., Fornid E., Zombo I., Mazengue M., Loumeto J.-J., Gourlet-Fleuryd S., 2018. What controls local-scale aboveground biomass variation in central Africa? Testing structural, composition and architectural attributes. Forest Ecology and Management 429: 570–578. https://doi.org/10.1016/j.foreco.2018.07.056.

[11] Mahamane A., Saadou M. et Lejoly J., 2007. Phénologie de quelques espèces ligneuses du Parc National du « w » (Niger), Sécheresse ; 18 (4) : 354- 358.

[12] Osborne C. P., Charles-Dominique T., Stevens N., John Bond W., Midgley G. F., Lehmann C., 2018. Human impacts in African savannahs are mediated by plant functional traits. New Phytologist, 220(1): 10–24. DOI:10.1111/nph.15236.

[13] Ouédraogo A., Adjima T., Hahn-Hadjali K., Guinko S. 2006. Diagnostic de l'état de dégradation des peuplements de quatre espèces ligneuses en zone soudanienne du Burkina Faso. Sécheresse, 17 (4): 485-491.

[14] Ouédraogo D.-Y., Doucet J.-L., Daïınou K., Baya F., Biwole A. B., Bourland N., Fétéké F., Gillet J.-F., Kouadio Y. L. Fayolle A., 2018. The size at reproduction of canopy tree species in central Africa. BIOTROPICA 50(3): 465–476. DOI: 10.1111/btp.12531.

[15] Pennington R. T., Hughes M., Moonlight P. W., 2015. The Origins of Tropical Rainforest Hyperdiversity. Trends In Plant Sciences, CellPress, 20(11) : 693-695. **http://dx.doi.org/10.1016/j.tplants.2015.10.005**

[16] Rabiou H., Diouf A., Bationo B. A., Segla K. N., Mahamane A., Adjonou K., Kokou K., Kokutse A. D., Saadou M., 2015. Structure des peuplements naturels de *Pterocarpuserinaceus* dans le domaine soudanien, au Niger et au Burkina Faso. Bois et forêts des tropiques, 325 (3) : 71-83. DOI: **https://doi.org/10.19182/bft2015.325.a31274**

[17] Raunkiaer C., 1934. The life forms of plants and statistical plant geography. Clarendon, Oxford.

[18] Saadou M., 1990. La végétation des milieux drainés nigériens à l'Est du fleuve Niger. Thèse de Docteur ès - Sciences Naturelles, Université de Niamey, 395 p. + annexes.

[19] Sanou L., Zida D., Savadogo P., Thiombiano A., 2018.Comparison of aboveground vegetation and soil seed bank composition at sites of different grazing intensity around a savannawoodland watering point in West Africa. Journal of Plant Research, Journal of Plant Research, 131, 773–788. https ://doi.org/10.1007/s1026 5-018-1048-3.

[20] Schmidt M., Mbayngone E., Bachmann Y., Hahn K., Zizka G., Thiombiano A., 2016. The impact of land use on species composition and habitat structure in Sudanian savannas – A modeling study in protected areas and agricultural lands of southeastern Burkina Faso. Candollea 71(2) : 265-274.

[21] Solefacka M. C. M., Fedoung E. F., Temgoua L. F., 2018. Factors determiningfloristic composition and functional diversity of plantcommunities of Mount Oku forests, Cameroon. Journal of Asia-Pacific Biodiversity, 11: 284-293. https://doi.org/10.1016/j.japb.2018.03.005.

[22] Stevens N., Lehmann C. E. R., Murphy B. P., Durigan G., 2016. Savanna woody encroachment is widespread across three continents. Global Change Biology, 23(1): 235-244. doi: 10.1111/gcb.13409.

[23] Thiombiano A., Schmidt M., Kreft H., Guinko S., 2006.Influence du gradient climatique sur la distribution des espèces de Combretaceae au Burkina Faso (Afrique de l'ouest). *Jour. Inter. Botaniq. Syst. Candollea* 61(1) : 189-213.

[24] White F., 1983. La végétation de l'Afrique. Mémoire accompagnant la carte de végétation de l'Afrique. L'Organisation des Nations Unies pour l'Education, la Science et la Culture (UNESCO).

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Effect of drinking saline water on performance, digestibility and nitrogen utilization of growing camels feed different quality roughages

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***Abstract*— *This study was conducted to evaluate the effect of three roughages that were fed ad lib. with two types of water on feed intake, digestibility, water utilization and performance of growing she-camels. Twenty-four healthy growing she-camels (30-36 months old and 448.50 ± 29.30 kg body weight) were housed individually in metabolic cages and randomly allotted to three treatments. The experiment lasted for 60 days. Three roughages were Egyptian clover hay to represent optimum grazing conditions, rice straw to represent dry season grazing and Atriplex halimus to represent arid rangelands dominated by halophytes. Roughages offered to camels ad lib. The concentrates used were corn grains and cottonseed meal. Concentrate intakes calculated, per unit metabolic body weight (kg0.73). Final body weight and ADG were affected by roughages. Nutrients intake was affected (P<0.05) by roughage type but not for drinking water and their interaction. Camels fed Atriplex had higher (P<0.05) in dry matter intake, roughage intake and roughage (% DMI) than hay and straw. Corn intake was greater (P<0.05) in camels fed Atriplex and straw whereas, was lower (P<0.05) in camels fed hay. Camels fed hay had higher (P<0.05) in dry matter digestibility followed by straw and then Atriplex. Organic matter, crude fiber and Nitrogen free extract digestibility were higher (P<0.05) in camels fed hay and straw as compared to Atriplex whereas, crude protein was higher (P<0.05) in Atriplex and hay as compared to straw. Free water intake, feed water, total water intake and fecal water were higher (P<0.05) in camels fed Atriplex as compared to hay and straw. Urinary water was higher (P<0.05) in camels fed Atriplex followed by hay and then straw. Free water intake, feed water, total water intake and fecal water were higher (P<0.05) in camels fed Atriplex as compared to hay and straw. Urinary water excretion was higher (P<0.05) in camels fed Atriplex followed by hay and then straw. In conclusion, camels fed on Atriplex showed a clear improvement in growth, digestibility, and nitrogen utilization in a similar way to camels fed on hay.*

***Keywords*— growing she-camels, roughages type, water type, intake, performance, water utilization.**

I. INTRODUCTION

Dromedary camel (*Camelus dromedarius*) is an important livestock species that is exceptionally well adapted to harsh environmental conditions. They are functionally and metabolically ruminant herbivores. Ruminant herbivores are distinguished by their multipart stomach. This anatomical structure and the microorganisms that inhabit the rumen network allow for longer retention of ingested feed and anaerobic microbial digestion of cellulosic materials, and consequently production of volatile fatty acids and microbial protein synthesis. Camels are bred because of their extraordinary strength to withstand hunger and thirst for a long time in the environmental conditions [1] In addition, its high ability to convert scarce desert resources into milk and meat makes it even more important to pastoralists [2,3].

Camels are non-selective grazers with a digestive system that has evolved - adapt to unfavorable conditions and has a greater activity of cellulolytic bacteria [6,7] Camels had a greater capacity to utilize low-quality roughages that are high in NDF and ADF and less digestible[6,7]. Camels are typically associated with a lower feed intake and greater efficiency of forage utilization, which may be due to their large body size and longer retention time, which gives more opportunity for microorganisms to digest non-structure carbohydrates [7]. On the other hand, camels prefer to consume salty bushes which are rich in moisture and salt. Salts present in such plants help to meet the physiological functions of camels [8,9] reported that the high moisture content of salt bushes ensures a good portion of the camel water requirement in areas where water is the most limiting factor for animals. Feeding halophytes especially for camels can be an appropriate method in arid regions to reduce the problem of forage shortage. Camels have adapted rumen microbial communities that enable them to take advantage of the non-protein nitrogen found in halophytes.

Camels have adapted mechanisms that allow it to withstand prolonged water deprivation especially in the absence of readily available water and survive when feed resources are scarce or of poor quality [10]. Camels are able to replenish in a relative short period of time the water lost. Whereas changes of water metabolism, body fluid and its regulation, body temperature, kidney function, appetite and hormonal aspects during dehydration have been studied in the past [11-14].

Lack of forage and water deprivation are important barriers to camel production in arid and semi-arid regions of the harsh climate. However, they are slowly being replaced by stable systems which should properly take into account the feeding of camels in these systems. Therefore, the present study was conducted to evaluate the effect of different forages and types of water on intake, nutrient digestibility, and performance of camels.

II. MATERIALS AND METHODS

The experiment was conducted at Maryout Research Station, Desert Research Center, Alexandria, Egypt.

2.1. Animals, diets, and experimental design

Twenty-four healthy growing she-camels (*Camelus dromedarius*) with an average initial body weight (BW) of 448.50 ± 29.30 kg and 30-36 months old were used and the experiment lasted for 60 days. Animals were housed individually in shaded floor pens for the duration of the experiment. The experiment was arranged as a 3 × 2 factorial experiment in a completely randomized design by using three forages and two types of water. Animal were weighed every two weeks after overnight fast and on two consecutive days and the average daily gain (ADG) was calculated.

The three roughages were used to represent the prevailing different grazing conditions in arid rangelands. Those were Egyptian clover hay to represent optimum grazing conditions, rice straw to represent dry season grazing and *Atriplex halimus* to represent arid rangelands dominated by halophytes. The concentrates used were corn grains and cottonseed meal selected as the commonly used energy and protein supplements, respectively. Roughages offered to camels *ad lib*. twice daily at 8:00 and 16:00 hours. Refusals were weighed at the following morning and daily intake was recorded on dry matter basis. Concentrate intakes calculated, per unit metabolic weight (kg 0.73), from a previous experiment [15], actual intake is presented below. in an attempt to control anticipated excessive soluble carbohydrates intake and possible adverse effects on rumen function and feed utilization [15]. The animals are drunk once every day, either tap water or salty 10,000 parts per million. The proximate composition of feed ingredients is presented in Table 1. Refusals were weighed daily, and feed intake was recorded. Samples of the roughages and concentrates were collected and analyzed for DM by drying to constant weight in a forced-air oven at 60°C for 48 h [16]. Samples were pooled for each camel.

Table 1. Proximate composition of feed ingredients, % DM basis.

Proximate Constituents	Corn grains	Cottonseed meal[1]	Egyptian clover hay	*Atriplex halimus*[2]	Rice straw
Dry matter	86.65	90.88	86.08	34.98	87.43
Ash	1.71	24.73	13.35	25.37	21.68
Organic matter	98.29	75.27	86.65	74.99	78.32
Crude protein	10.76	15.84	14.26	11.70	4.55
Crude fiber	3.77	19.30	34.23	28.62	28.86
Ether extract	3.92	10.86	4.40	2.94	2.52
N-free extract	79.84	29.27	33.76	31.37	42.39

[1]Un-decorticated, heat treated and mechanically pressed CSM, produced in a traditional oil mill,
[2]Leaves and succulent branches typically consumed by grazing animals.

2.2. Digestion trials

At the end of the experimental period, camels were placed in metabolic cages for 15 days, 8 days of adaptation to the metabolic cages and 7 days to collect faeces and urine. Total daily faecal output of each camel was collected thoroughly mixed and weighed. A 10% subsample of daily faecal output was analyzed for DM by drying to constant weight in a forced-air oven at 60℃ for 48 h [16]. Dried ingredients, orts and faecal samples were ground in a Wiley mill with a 1-mm screen. Samples were analyzed for ash, ether extract, crude fiber and crude protein according to [16]. Urine samples were collected in plastic containers containing 100 ml of H2SO4. Total daily urine output was weighed and recorded. A 10% subsample was collected and then analyzed for N [16].

2.3. Statistical analysis

Main effects and interactions were evaluated using the GLM repeated-measures analysis of variance procedures of the NCSS statistical package [17]. The type of roughage and concentrate levels were the independent variables, and type of water (water tap and saline water) levels were repeated within roughages. Newman-Keuls multiple comparison tests was applied to the means of the main effects, i.e. type of roughage, R-means, and level of concentrates, type of water (water tap and saline water) B-means. Statistical significance was declared at P≤0.05.

III. RESULTS

Chemical composition of the roughages is presented in Table 1. Three forages were selected differ in their crude protein content. Crude protein was higher for clover hay, intermediate for the *Atriplex halimus*, and lowest for rice straw. Two concentrates were selected differ in their chemical composition Table 1. Cottonseed meal was higher in their contents of crude protein, ash, crude fiber, and lower in nitrogen free extract compared with corn grain.

Effects of roughages type and drinking saline water on the performance of the camels are presented in Table 2. Initial body weight was not affected whereas, final body weight and ADG were affected by roughages type (P<0.05) but not for drinking water and their interaction.

Effects of roughages type and drinking saline water on the feed intake of the camels are presented in Table 3. Feed intake was affected (P<0.05) by roughage type but not for drinking water and their interaction. Camels fed Atriplex had higher (P<0.05) intake of dry matter, roughage intake and roughage (% DMI) than hay and straw. Corn intake was greater (P<0.05) in camels fed Atriplex and straw whereas, was lower (P<0.05) in camels fed hay.

Effects of roughages type and drinking saline water on the components of are presented in Table 4. Organic matter, crude protein, crude fiber, rumen degradable protein, rumen un-degradable protein intakes were affected (P<0.05) by roughage type but not for drinking water and their interaction. Camels fed Atriplex had higher values (P<0.05) of organic matter intake and crude fiber intake than hay and straw. Crude protein intake, rumen degradable protein intake and rumen un-degradable protein were greater (P<0.05) in camels fed Atriplex and hay whereas, were lower (P<0.05) in camels fed straw.

Effects of roughages type and drinking saline water on the apparent digestion coefficients of diets consumed by camels are presented in Table 5. Digestion coefficients of diets was affected (P<0.05) by roughage type but not for drinking water and their interaction. Camels fed hay had higher (P<0.05) in dry matter digestibility followed by straw and then Atriplex. Organic matter, crude fiber and nitrogen free extract digestibility were higher (P<0.05) in camels fed hay and straw as compared to Atriplex whereas, crude protein was higher (P<0.05) in Atriplex and hay as compared to straw.

Effects of roughages type and drinking saline water on the nitrogen utilization are presented in Table 6. Nitrogen utilization was affected (P<0.05) by roughage type but not for drinking water and their interaction. Nitrogen intake and digested nitrogen were higher (P<0.05) in camels fed hay and Atriplex as compared to straw whereas, fecal nitrogen, urinary nitrogen and nitrogen balance were not affected.

Effects of roughages type and drinking saline water on the water intake and execration are presented in Tables 7and8 Water intake and execration were affected (P<0.05) by roughage type but not for drinking water and their interaction. Free water intake, feed water, total water intake and fecal water were higher (P<0.05) in camels fed Atriplex as compared to hay and straw. Urinary water was higher (P<0.05) in camels fed Atriplex followed by hay and then straw.

IV. DISCUSSIONS

The present experiment showed that final body weight and ADG were affected by the type of roughages. Similar results were observed by [18] who reported that ADG g/day of camels fed hay and Atriplex were higher than those of their mates fed rice straw with limiting concentrate offered to 50%. In agreement with [19,15,7] the straw is characterized by its poor of digestion, a longer retention time in the rumen and its low nutritional value, which negatively affected the performance.

Effect of saline water on ADG camels fed straw and drinking salt water was lower final body weight and ADG Similar findings were reported by [20] who reported that female camels lost 1.9% of their initial live body weight when drinking saline water.

Our results indicate that camels consumed higher amounts of Atriplex compared to hay and straw. This is consistent with similar findings found by [21]. [22] reported that camels need salt more than other livestock in their diets, which they get from Atriplex, which contain salts that may reach 25% of DM [23].

Table 2. Effect of type roughages and drinking water and saline water on the performance of the camels.

Water type (B)	Roughage, *ad lib.* (R)			Water	*P*-value[1]		
	Atriplex	hay	Straw	Average	R	B	RxB
Initial body weight (kg)							
Fresh	448	446	446	447	0.35	0.61	0.33
Saline	446	447	446	446			
Roughage average	447	446	446	447			
± SEM	15.9						
Final body weight (kg)							
Fresh	480	483	468	477	<0.01	0.43	0.21
Saline	470	480	456	469			
Roughage average	475[a]	481[a]	462[b]	473			
± SEM	85.8						
Average daily gain (g/day)							
Fresh	531	608	360	500	<0.01	0.52	0.13
Saline	400	550	172	374			
Roughage average	465[a]	579[a]	266[b]	437			
± SEM	74.5						

[1] Probability values associated with roughage (R), water type (B), and roughage × water type interaction (R×B).

[a-e] Mean separation by Tukey MRT (P<0.05), valid comparison are between roughage average and between water watering an experiment.

Table 3. Average daily feed intake during the digestion trials, g/d/kg $^{0.75}$

Water type (B)	Roughage, *ad lib.* (R)			Water	*P*-value[1]		
	Atriplex	Hay	Straw	average	R	B	RxB
Dry matter intake(DMI)							
Fresh	85.5	63.5	56.0	68.3	<0.01	0.29	0.45
Saline	96.9	63.4	57.9	72.7			
Roughage average	91.2[a]	63.4[b]	56.9[b]	70.5			
± SEM	4.58						
Roughage (DMI)							
Fresh	56.3	34.7	27.1	39.4	<0.01	0.28	0.38
Saline	67.6	34.6	28.1	43.4			
Roughage average	61.9[a]	34.6[b]	27.6[b]	41.4			
± SEM	4.18						
Roughage (% in DMI)							
Fresh	65.5	54.6	48.3	56.1	<0.01	0.28	0.34
Saline	69.8	54.6	48.5	57.6			
Roughage average	67.6[a]	54.6[b]	48.4[c]	58.9			
± SEM	1.51						
Corn (DMI)							
Fresh	23.8	20.6	22.3	22.2	0.01	0.67	0.85
Saline	23.9	20.6	22.9	22.5			
Roughage average	23.8[a]	20.6[b]	22.6[a]	22.3			
± SEM	0.64						
Cotton seed meal (DMI)							
Fresh	5.37	8.24	6.70	6.77	<0.01	0.64	0.78
Saline	5.39	8.22	6.90	6.84			
Roughage average	5.38[c]	8.23[a]	6.80[b]	6.80			
± SEM	0.16						
Concentrate (DMI)							
Fresh	29.2	28.8	28.9	28.9	0.67	0.66	0..83
Saline	29.3	28.8	29.8	29.3			
Roughage average	29.2	28.8	29.4	29.1			
± SEM	0.79						

[1] Probability values associated with roughage (R), water type (B), and roughage × water type interaction (R×B).

[a-e] Mean separation by Tukey MRT (*P*<0.05), valid comparison are between roughage average and between water watering an experiment.

Table 4. Components of diets g/d/kg$^{0.73}$

Water type (B)	Roughage, *ad lib.* (R)			Water	*P*-value[1]		
	Atriplex	Hay	Straw	average	R	B	Rx B
Organic matter intake (OMI)							
Fresh	69.5	56.6	49.6	58.6	<0.01	0.29	0.47
Saline	77.9	56.5	51.2	61.9			
Roughage average	73.7^a	56.6^b	50.4^b	60.2			
± SEM	3.51						
Crude protein intake (CPI)							
Fresh	7.61	8.64	4.75	6.67	<0.01	0.29	0.46
Saline	8.47	8.61	4.89	7.33			
Roughage average	8.04^a	8.62^a	4.82^b	7.16			
± SEM	0.35						
Crude fiber intake (CFI)							
Fresh	17.9	13.8	10.3	14.0	<0.01	0.47	0.40
Saline	21.1	13.8	10.6	15.2			
Roughage average	19.5^a	13.8^b	10.4^b	14.6			
± SEM	1.20						
Rumen degradable protein intake (RDPI)							
Fresh	5.49	6.23	2.90	4.87	<0.01	0.30	0.44
Saline	6.13	6.21	2.99	5.11			
Roughage average	5.81^a	6.22^a	2.94^b	4.99			
± SEM	0.001						
Rumen un-degradable protein (RUPI)							
Fresh	2.12	2.41	1.85	2.83	0.01	0.28	0.51
Saline	2.34	2.40	1.91	2.22			
Roughage average	2.23^a	2.40^a	1.88^b	2.17			
± SEM	0.01						

[1] Probability values associated with roughage (R), water type (B), and roughage × water type interaction (R×B).
[a-e] Mean separation by Tukey MRT (P<0.05), valid comparison are between roughage average and between water watering an experiment.

Table 5. Apparent digestion coefficients of diets consumed by camels, %

Water type (B)	Roughage, *ad lib.* (R)			Water	*P*-value[1]		
	Atriplex	Hay	Straw	average	R	B	RxB
Dry matter							
Fresh	58.2	67.9	63.0	63.1	0.02	0.82	0.73
Saline	58.1	71.2	61.6	63.6			
Roughage average	58.1^c	69.6^a	62.3^b	63.3			
± SEM	2.97						
Organic matter							
Fresh	55.5	69.4	67.2	64.1	0.01	0.71	0.76
Saline	54.6	72.8	67.5	64.9			
Roughage average	55.1^b	71.1^a	67.3^a	64.5			
± SEM	2.94						
Crude protein							
Fresh	63.9	63.3	45.3	57.5	0.03	0.79	0.74
Saline	60.4	65.9	49.7	58.7			
Roughage average	62.1^a	64.6^a	47.5^b	58.1			
± SEM	5.22						
Crude fiber							
Fresh	28.8	57.5	58.4	48.2	0.01	0.42	0.51
Saline	37.6	66.1	54.1	52.6			
Roughage average	33.2^b	61.8^a	56.3^a	50.4			
± SEM	6.10						
Nitrogen free extract							
Fresh	63.5	75.5	72.6	70.5	0.03	0.71	0.59
Saline	59.6	76.6	73.1	69.8			
Roughage average	61.6^b	76.1^a	72.8^a	70.2			
± SEM	2.48						

[1] Probability values associated with roughage (R), water type (B), and roughage × water type interaction (R×B).
[a-e] Mean separation by Tukey MRT (P<0.05), valid comparison are between roughage average and between water watering an experiment.

Table 6. Nitrogen utilization, mg N/day/kg$^{0.73}$

Water type (B)	Roughage, *ad lib.* (R)			Water	*P*-value[1]		
	Atriplex	Hay	Straw	average	R	B	RxB
Nitrogen intake							
Fresh	1217	1382	760	1120	<0.01	0.29	0.46
Saline	1355	1378	783	1172			
Roughage average	1286[a]	1380[a]	772[b]	1145			
± SEM	56.1						
Fecal nitrogen							
Fresh	432	507	415	451	0.16	0.66	0.26
Saline	538	470	393	467			
Roughage average	485	488	404	459			
± SEM	42.4						
Digested nitrogen							
Fresh	785	875	345	668	<0.01	0.59	0.99
Saline	817	908	390	705			
Roughage average	801[a]	891[a]	368[b]	687			
± SEM	78.6						
Urinary nitrogen							
Fresh	457	734	240	477	0.11	0.74	0.34
Saline	763	521	285	523			
Roughage average	610	627	263	500			
± SEM	159						
Nitrogen balance							
Fresh	328	141	105	191	0.66	0.95	0.38
Saline	54.6	387	105	183			
Roughage average	191	264	105	187			
± SEM	172						

[1] Probability values associated with roughage (R), water type (B), and roughage × water type interaction (R×B).
[a-e] Mean separation by Tukey MRT (P<0.05), valid comparison are between roughage average and between water watering an experiment.

Table 7. Water intake and execration, mg N/day/kg$^{0.82}$

Water type (B)	Roughage, *ad lib.* (R)			Water	*P*-value[1]		
	Atriplex	Hay	Straw	average	R	B	Rx B
Free water intake							
Fresh	148	111	125	128	0.01	0.23	0.63
Saline	143	108	108	119			
Roughage average	146[a]	109[b]	116[b]	124			
± SEM	7.69						
Feed water							
Fresh	73.5	20.2	13.5	35.7	<0.01	0.87	0.58
Saline	75.3	16.8	14.1	35.4			
Roughage average	74.4[a]	18.5[b]	13.8[b]	35.6			
± SEM	2.48						
Total water intake							
Fresh	234	144	149	176	<0.01	0.34	0.73
Saline	233	138	133	168			
Roughage average	233[a]	141[b]	141[b]	172			
± SEM	9.21						
Fecal water							
Fresh	29.8	17.4	12.7	19.9	<0.01	0.46	0.27
Saline	36.6	13.5	15.6	21.9			
Roughage average	33.2[a]	15.4[b]	14.1[b]	20.9			
± SEM	3.02						
Urinary water							
Fresh	45.1	56.9	29.3	43.8	0.02	0.11	0.04
Saline	110	44.7	32.4	62.4			
Roughage average	77.6[a]	50.8[b]	30.8[c]	53.1			
± SEM	11.9						

[1] Probability values associated with roughage (R), water type (B), and roughage × water type interaction (R×B).
[a-e] Mean separation by Tukey MRT (P<0.05), valid comparison are between roughage average and between water watering an experiment.

Table 8. water intake, ml/day/kg$^{0.82}$

Water type (B)	Roughage, *ad lib*. (R)			Water	*P*-value[1]		
	Atriplex	hay	Straw	average	R	B	Rx B
Free water intake (FWI)							
Fresh	147.74	110.97	124.74	127.82	0.007	0.233	0.628
Saline	143.32	107.65	107.55	119.51			
Roughage average	145.53^{a}	109.31^{b}	116.14^{b}	123.66			
± SEM	7.687						
Feed water (FDWI)							
Fresh	73.48	20.18	13.54	35.73	0.0000	0.875	0.577
Saline	75.32	16.78	14.11	35.40			
Roughage average	74.40^{a}	18.48^{b}	13.82^{b}	35.57			
± SEM	2.482						
Metabolic water (MWI)							
Fresh	12.73	12.95	11.02	12.23	0.254	0.457	0.937
Saline	14.07	13.50	11.57	13.05			
Roughage average	13.40	13.22	11.29	12.64			
± SEM	1.255						
Total water intake (TWI)							
Fresh	233.96	144.10	149.30	175.79	0.0007	0.339	0.727
Saline	232.71	137.93	133.23	167.96			
Roughage average	233.34^{a}	141.02^{b}	141.27^{b}	171.87			
± SEM	9.208						
TWI/GE							
Fresh	1.256	0.915	1.112	1.096^{a}	0.001	0.013	0.272
Saline	1.108	0.885	0.961	0.984^{b}			
Roughage average	1.182^{a}	0.899^{c}	1.039^{b}	1.040			
± SEM	0.0392						
TWI/DE							
Fresh	2.229	1.313	1.669	1.737	0.002	0.130	0.836
Saline	1.991	1.212	1.415	1.539			
Roughage average	2.110^{a}	1.262^{b}	1.542^{b}	1.638			
± SEM	0.1381						

[1] Probability values associated with roughage (R), water type (B), and roughage × water type interaction (R×B).
[a-e] Mean separation by Tukey MRT (*P*<0.05), valid comparison are between roughage average and between water watering an experiment.

This was confirmed by [24] who explained that camels need 6 to 8 times the amount of salt than other livestock requirement, and also indicated that camels that do not regularly receive salty feed need about 140 grams of salt per dat. Moreover, Atriplex is a green plant more palatable and preferred for camels than alfalfa hay and rice straw as indicated by [22].

Our results indicate that the camels fed on clover hay had higher digestibility values, as clover hay is considered a good-quality roughage that is high in its available content of nutrients, which explains the high digestion parameters. These results are consistent with [18]

Camels can efficiently digest low quality roughage that is low in nutritional value, which explains the high fiber digestibility coefficient of camels fed straw.The more efficient utilization of low quality roughage by camels is mainly the result of a higher cellulolytic activity of the microorganisms [5] and to a longer retention time of solid particles [6,7]. Camels fed Atriplex showed improved protein digestibility, which may be due to their content of non-protein nitrogen (NPN). [7] reported that Atriplex differs from conventional forage in terms of its high NPN content and About 44.5% of the protein content is NPN. The NPN compounds are degraded and used as a source of N for the synthesis of microbial protein. The effective uptake of non-protein nitrogen by rumen microbes and subsequent conversion to microbial protein, diets should contain suitable and adequate source of energy [25-28]. This also explains the increase in corn intake in camels group fed Atriplex.

Our results indicated that camels fed on Atriplex and hay had higher values of nitrogen intake and digested nitrogen more than straw. These could mainly be due to the type of forage and its content of [29,23]

The roughage type significantly affected the water intake and excretion. Atriplex camels group recorded the highest free water intake. Because Atriplex had high salt content [30]. Camels can be fed on a high tolerance saline fodder than any other animal [31]. Camels fed this diet drink a lot of water in order to eliminate the salts ingested.

Atriplex camels group recorded significantly higher feed water intake than those hay and straw groups due to that Atriplex had higher moisture content about 65% similar findings were recorded by [21]. Camels consumed Atriplex recorded higher water execration values than those fed on straw or hay. These results indicated that camels' kidneys seem to be better adapted to handling salt load especially when they fed on halophytic plants [32,33] pointed out that increasing water excretion through the urinary pathway is believed to be an adaptive mechanism assisting the animal in getting rid of excess salts and maintain osmolality of food and other body fluids. In addition, [34,35] reported that increasing salts increased water excretion in urine and faeces.

V. CONCLUSION

In conclusion, the results indicate that drinking saline water did not affect feed intake, digestibility and nitrogen utilization. whereas, roughages type had effect intake, digestibility and nitrogen utilization. Camels fed on Atriplex showed a clear improvement in growth, digestibility, and nitrogen utilization in a similar way to camels fed on hay. As for the camels fed on straw, they had the least effect on growth, digestibility, and nitrogen utilization.

VI. REFERENCES

[1] Al-Dahash S, Sassi M (2009). A preliminary study on management, breeding and reproductive performance of Camel in Libya. Iraq J Vet Sci 23: 276-281.

[2] Kadim, I. T., Mahgoub, O., Al-marzooqi, W., Khalaf, S. K., and Raiymbek, G. (2013). Composition, quality and health aspects of the dromedary (*Camelus dromedarius*) and bactrian (Camelus bacterianus) camel meats: a review. J. Agric. Mar. Sci. 18, 7–24.

[3] Faye, B. (2015). Role, distribution and perspective of camel breeding in the third millennium economies. Emirates J. Food Agric. 27, 318–327.

[4] Ghali, M.B., Scott, P.T., Alhadrami, G.A. and Al Jassim, R.A.M., 2011. Identification and characterisation of the predominant lactic acid-producing and lactic acid-utilising bacteria in the foregut of the feral camel (*Camelus dromedarius*) in Australia. Animal Production Science, 51(7), pp.597-604.

[5] Rabee, A.E., Forster, R.J., Elekwachi, C.O., Kewan, K.Z., Sabra, E.A., Shawket, S.M., Mahrous, H.A. and Khamiss, O.A., 2019. Community structure and fibrolytic activities of anaerobic rumen fungi in dromedary camels. Journal of basic microbiology, 59(1), pp.101-110.

[6] Kayouli, C., Jouany, J.P., Demeyer, D.I., Taoueb, H. and Dardillat, C., 1993. Comparative studies on the degradation and mean retention time of solid and liquid phases in the forestomachs of dromedaries and sheep fed on low-quality roughages from Tunisia. Animal feed science and technology, 40(4), pp.343-355.

[7] Khattab, I.M., Abdel-Wahed, A.M., Anele, U.Y., Sallam, S.M. and El-Zaiat, H.M., 2021. Comparative digestibility and rumen fermentation of camels and sheep fed different forage sources. Animal Biotechnology, pp.1-10.

[8] Payne, W.J.A., 1990. An introduction to animal husbandry in the tropics (No. Edn 4). Longman scientific and technical.

[9] Gauthier-Pilters, H. and Dagg, A.I., 1981. The camel. Its evolution, ecology, behavior, and relationship to man. The University of Chicago Press.

[10] Gaughan JB (2011) Which physiological adaptation allows camels to tolerate high heat load and what more can we learn? J Camelid Sci 4: 85-88.

[11] MacFarlane, W.V., Morris, R.J.H., Howard, B., 1963. Turnover and distribution of water in desert camels, sheep, cattle and kangaroos. Nature 197, 270–271.

[12] Schmidt-Nielsen, K., 1964. Desert Animals — Physiological Problems of Heat and Water. Oxford University Press, Oxford, pp. 33–70.

[13] Wilson, R.T., 1989. Ecophysiology of the Camelidae and Desert Ruminants. Springer Verlag, Berlin.

[14] Robertshaw, D., Zine-Filali, R., 1995. Thermoregulation and water balance in the camel: a comparison with other ruminant species. In: von Engelhardt, W., Leonhard-Marek, S., Breves, G., Giesecke, D. (Eds.), Ruminant Physiology: Digestion, Metabolism, Growth and Reproduction. Ferdinand Enke Verlag, Stuttgart, pp. 565–778.

[15] Farid, M.F.A., A.M. Abdel-Wahed, Safinaz M. Shawket and N.I, Hassan. (2010). Diet Selection, feed Intake capacity and performance of growing female camels: Effects of type of roughage and level of concentrates offered. J. American Sci., 6: 317-326

[16] AOAC – Association of Official Agricultural Chemists. (1990). Official Methods of Analysis, 15th edition. AOAC, Washington DC

[17] Hintze, (2007). NCSS. Kaysville, Utah, U.S.A.

[18] Abdel-Wahed, A. M. (2014). Effect of type of roughage of concentrate supplements on feed digestion and utilization in growing female dromedary camels. Journal of American Science 2014;10 (6): 198-206.

[19] Cianci, D., L. Goio, A.M. Hashi, S. Pastorelli, M. Kamoun, G.B. Liponi and M. Orlandi. (2004). Feed intake and digestibility in camels fed wheat straw and Meadow hay. J. Camel Science. 1: 52-56.

[20] Assad, F., El-Sherif, M.M.A., 2002. Effect of drinking saline water and feed shortage on adaptive responses of sheep and camels. Small Ruminant Research, 45, 279-290

[21] Abdel-Wahed, A.M., 2020. Water intake and excretion of growing she-camels in relation to the type of roughage fed and concentrate. International Journal of Environment, Agriculture and Biotechnology, 5,(4) Pages 953-964

[22] Shawket, S. M., K.M. Youssef and M. H. Ahmed. (2010). Comparative evaluation of Egyptian clover and *Atriplex halimus* diets for growth and milk production in camel. Animal Science Reporter, vol. 4: 9-21.
[23] Shawket, S.M., Khattab, I.M., Borhami, B.E. and El-Shazly, K.A., 2001. Performance of growing goats fed halophytic pasture with different energy sources. Egyptian J. Nutrition and feeds, 4, 251-264.
[24] Chamberlain, A. (1989). Milk production in the tropics. Intermediate Tropical Agricultural Series 13, Camels, 202-210.
[25] Cherdthong, A., Wanapat, M. and Wachirapakorn, C., 2011. Influence of urea–calcium mixtures as rumen slow-release feed on in vitro fermentation using a gas production technique. Archives of animal nutrition, 65(3), pp.242-254.
[26] Khattab, I.M., Salem, A.Z.M., Abdel-Wahed, A.M. and Kewan, K.Z., 2013. Effects of urea supplementation on nutrient digestibility, nitrogen utilisation and rumen fermentation in sheep fed diets containing dates. Livestock Science, 155(2-3), pp.223-229.
[27] El-Zaiat, H.M., Kholif, A.E., Khattab, I.M. and Sallam, S.M., 2021. Slow-release urea partially replace soybean in the diet of Holstein dairy cows: intake, blood parameters, nutrients digestibility, energy utilization, and milk production. Annals of Animal Science.
[28] Khattab, I.M. and Anele, U.Y., 2021. Dry matter intake, digestibility, nitrogen utilization and fermentation characteristics of sheep fed Atriplex hay-based diet supplemented with discarded dates as a replacement for barley grain. Journal of Animal Physiology and Animal Nutrition
[29] EI-Shaer, H.M. and H.M. Kandil. (1990). Comparative study on the nutritional value of wild and cultivated *Atriplex halimus* by sheep and goats in Sinai. Com. Sci. and Dev. Res., 29: 81-90.
[30] Khattab, I.M., 2007. Studies on halophytic forages as sheep fodder under arid and semi arid conditions in Egypt, Ph. D. Thesis. Alexandria University.
[31] Squires, V.R., 2015. Halophyte and Salt Tolerant Plants Feeding Potential to Dromedary Camels. In Halophytic and Salt-Tolerant Feedstuffs (pp. 273-284). CRC Press.
[32] Abou El-Nasr, H.M., M. Shawket, Safinaz, M.F. Farid and H.S. Khamis. (1988). Studies on saline drinking water for camels and sheep: 1- Water intake and excretion. Alex. J. Com. Sci. Dev. Res., 23: 131–139
[33] Ahmed, M.H. (1984). Studies on nitrogen metabolism in ruminants: effects of drinking saline water on nitrogen metabolism and rumen kidney function in sheep. Ph. D. Thesis, Alex. Univ., Egypt.
[34] El-Faramawi, A.A. (1984). Effect of drinking bring water on energy and water metabolism in sheep. M. Sc. Thesis, Ain Shams Univ
[35] Kandil, H.M., A.O. Sooud, M.FA. Farid, H.M. El-Shaer, and M.A. El-Ashry. (1985). Effect of drinking saline water on feed utilization and nitrogen and mineral balance in camels. First Inter. Congress on Animal Production in Arid zones 9 ZICAPAZ, Sep., 7 – 14, 1985, ACSAD, Damascus, Syria.

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Characterization and evaluation of the antibacterial potential of bacterial microbiota of cultivated soils of Cassava (*Manihot esculenta*) and Black pepper (*Piper nigrum*) in the city of Igarapé Açu – Pará, Brazil.

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Abstract— *Actinobacteria form a heterogeneous group of Gram-positive bacteria that have a great morphological and metabolic variety, are microorganisms with great biotechnological potential for the production of several substances of industrial and pharmaceutical interest, being considered the main source of antibiotics. Due to the increase in the occurrence of bacteria that are multiresistant to antibiotics used in clinical and hospital routine, the need to discover new substances with antibiotic capacity becomes evident. Thus, studies that assess the diversity and variety, composition and properties of metabolites produced by bacterial species present in cultivated soils are important and necessary. This work aimed to investigate the occurrence of actinobacteria in rhizosphere soils of areas cultivated with cassava (Manihot esculenta) and black pepper (Piper nigrum) in the city of Igarapé Açu - Pará, Brazil, to assess their potential as producers of antimicrobial substances. This is an analytical descriptive, experimental study that performs a microbiological analysis of soil samples. With great morphological diversity, 21 different bacteria were isolated, all with characteristics of actinobacteria. As a result, strains of actinobacteria with variable colonial characteristics were isolated, microscopically characterized as isolated and chained Gram-positive bacilli. Among the isolates, 8 strains were able to inhibit the growth of Escherichia coli and Klebsiella pneumoniae compared to in vitro. It emphasizes the need for more research aimed at the microbiology of Amazonian soils, due to the diversity of bacteria of biotechnological importance that can significantly contribute to the production of new antibiotics, thus generating a positive expectation in the fight against infections.*

Keywords— ***Actinobacteria, rhizosphere, Manihot esculenta, Piper nigrum, Amazon.***

I. INTRODUCTION

Soil is considered an important source of chemically diverse and biologically active substances; these compounds have been of great importance for industry and especially for pharmaceuticals. The microbiota in this ecosystem is extremely rich and the bioprospecting of several microorganisms and the analysis of their metabolic activities allow the discovery of potential antibacterial agents and other substances of industrial interest [1, 2].

Actinobacteria produce secondary metabolites with different chemical structures and biological activities, and many of these substances have been used to produce drugs for the treatment of various harm to human health and other organisms [3].

The Actinobacteria class is divided into 4 subclasses, 5 orders, 14 suborders, 50 families, 197 genera and 1936 species [4], they are mostly Gram-positive, aerobic bacteria [5], with high content of G+C in their DNA [6], similar to some fungi, these bacteria reproduce mainly by sporulation, in which each spore produced can give rise to a new organism [7].

Soils used for agriculture have different characteristics from uncultivated areas, due to anthropic action, type of fertilization and soil management can change the composition of the bacterial community in quantity and quality of microorganisms found in their rhizospheres [8,9].

The microorganisms present in the rhizosphere community of cultivated soils are of great importance for plants, as they influence their development and protect the roots against pathogens [10].

The search for new species of actinobacteria in these places is an essential component for the discovery of new substances and, based on the properties presented by actinobacteria, the study of microorganisms present in rhizosphere environments is of great importance, which can provide the discovery of new compounds and products of biological origin, with biotechnological potential for the production of metabolites with antibacterial action or other pharmaceutical and industrial applications.

The Amazon population still practices on a large-scale subsistence culture in small areas of land. Cassava (Manihot esculenta Crantz) and black pepper (Piper nigrum), are plants adapted to the tropical region, resistant to heat, demanding moisture and rainfall [11], both highly cultivated in regions of the state of Pará. The objective of this work is to characterize and evaluate the antibacterial potential of bacterial microbiota of cultivated soils of cassava (Manihot esculenta) and black pepper (Piper nigrum) in the city of Igarapé-ace – Pará.

II. MATERIAL AND METHODS

2.1 - Sample collection:

To carry out this work, 24 soil samples were used, collected in groups of 4 samples at each point. The collection will take place through the use of sterile spatulas and the samples will be packed in disposable and sterile bags. Each sample will have 100 g of soil from each collection point, which will be georeferenced to meet the need for subsequent collections if necessary.

2.2 - Isolation of microorganisms

For bacterial isolation, samples from the rhizosphere, before seeding for bacterial isolation, underwent a pre-treatment consisting of a 10g dilution of the sample in 90mL of sterile saline solution, followed by vertexing for 10 to 20 minutes. After shaking, each sample was heated in a 50°C water bath for 10 minutes, aiming to eliminate contaminating bacteria that spread quickly and are not the target of isolation. The resulting supernatant was seeded using the surface streak method, using 0.05 mL sterile and disposable loops, in 3 Petri dishes containing the culture media: modified Czapeck Agar, modified Hickey-Tresner Agar and the culture medium Vitamin Arginine. In all culture media, Amphotericin B (100µg/mL) was added to inhibit fungal growth. Petri dishes were incubated in a humid chamber bacteriological incubator for 24 hours up to 30 days at a temperature of 37°C to 45°C with daily visual observation of bacterial colony growth. The plates with bacterial growth were transferred to other plates and after purification they were kept in test tubes with slanted enrichment culture medium for their maintenance.

2.3 - Bacteria characterization

The isolated strains were characterized morphologically and physiologically through morphological characteristics of the colonies, microscopic characteristics of microorganisms and their metabolism through tests 8 biochemical tests. The macroscopic characterization was carried out by visual observation of color, shape and moisture characteristics, the evaluation of the microscopic aspect was carried out after making smears made of each characterized colony that were stained by the Gram stain method. The methodologies used in the biochemical tests were adapted from the manual of the National Health Surveillance Agency [12], the tests used were: Oxidase test, catalase test, Simmons Citrate test, Hemolysis test, TSI test (Triple Sugar Iron).

2.4 - Antimicrobial Sensitivity Test (TSA)

The TSA was performed using the diffusion test on plates containing Muller Hinton Agar, and the potential for inhibiting the growth of *Klebsiella pneumoniae, Staphylococcus aureus* and *Escherichia coli* strains was evaluated, bacterial suspensions were prepared with an approximate concentration of 0.5 cfu. /mL and the suspensions were used to seed plates containing Muller Hinton agar medium, using sterile disposable swabs, after 30 minutes at room temperature, the isolated and characterized strains from the soil were inoculated and pricked at specific points in the previously seeded plates, the formation of bacterial growth inhibition halos around the bite was considered a potential inhibiting power.

III. RESULTS

Despite the simplified methodology, this work clearly demonstrates the possibility of the existence of bacteria with biotechnological potential in the Amazon region. The antibacterial action of the isolates against Gram-positive bacteria such as *S. aureus* was not found in this research, but on Gram-negative bacteria such as *Escherichia coli* and *Klebsiella pneumoniae* there was inhibition of bacterial growth in vitro, this action was also described by Silva [7], in which only Gram-negative bacteria were affected.

The Amazonian soil microbiota is characterized by abundance and diversity, our findings were characterized by the isolation of bacteria with various macro and microscopic morphologies, similar to the results [13,10]. The organisms found in this research showed a wide morphological variety, but the presence of isolated Gram-positive bacilli or in chains predominated, similar findings are described in the literature, reinforcing the assertion of the vast amount of these microorganisms in the soil [3].

Ecological studies of actinomycetes populations are extremely important, as these microorganisms form spores and conidia, allowing their survival in adverse conditions [14]. In addition, actinomycetes stand out for the possibility of synthesizing vitamins, substances that inhibit enzymatic activity [15], antibiotics and other biologically active compounds [16].

There was bacterial growth at all collection points, with no growth restriction in relation to the soil depth in the places. Isolated colonies were macroscopically characterized based on their color and appearance presented in the media used. (Table 1).

The microscopic analysis of bacterial colonies, performed using the Gram stain technique, showed a great diversity of bacterial morphologies (Figure 1), described isolated and paired Gram-positive bacilli (BGPi), Gram positive coccobacillus (CBGP), Gram bacilli chain positives (BGPc) and grouped and isolated Gram-positive cocci (CGP), distributed according to table 2.

The biochemical characterization of the isolated colonies was performed using biochemical tests according to table 3.

In rhizosphere soils of black pepper, 13 plates with bacterial growth were analyzed, which presented colony characteristics similar to those of cassava. (Table 4).

Considering the morphology of the bacteria found through the Gram staining technique, they were identified in isolated and paired Gram-positive bacilli (BGPi), Gram positive coccobacillus (CBGP), Gram positive bacilli in chain (BGPc) and Gram-positive cocci grouped and isolated (CGP), distributed according to table 5.

The biochemical characteristics of colonies isolated from rhizosphere soil samples of black pepper did not show any difference from the characteristics isolated from rhizosphere soils of cassava. For the evaluation of the sensitivity test and antimicrobial activity of the strains, all 33 bacterial strains isolated were submitted to the methodology (Figure 2), of which only 8 strains showed inhibitory activity on the tested bacteria (Table 6).

IV. FIGURES AND TABLES

Table 1: Macroscopic characteristics of bacterial colonies isolated in cassava rhizosphere:

Colonial type	**N**	**%**
Mucoid white	8	38
Dry white	6	28,5
White cotton	4	19,2
Mucoid yellows	3	3
Total	**21**	**100,0**

Source: Authors

Table 2: Microscopic characteristics of bacterial colonies isolated in cassava rhizosphere:

Colonial type	**Microscopy**
Mucoid white	CGP e BGPc
Dry white	BGPi e BGPc
White cotton	BGPi e BGPc
Mucoid yellows	CBGP

Source: Authors

CGP: Gram-positive cocci; BGPc: Gram-positive bacilli in chain; BGPi: Isolated Gram-positive bacilli; CBGP: Gram-positive coccobacilli.

Table 3 – Biochemical characterization of colonies isolated from cassava rhizosphere.

Colonial type	**TSI**	**L**	**S**	**M**	**C**	**H**	**O**	**C**
Mucoid white	Ac/Alc	v	v	-	-	+	+	+
Dry white	Ac/Alc	+	+	-	-	+	+	+
White cotton	Ac/Alc	+	v	-	-	+	+	+
Mucoid yellows	Ac/Alc	+	+	-	-	+	-	-

Source: Authors

Captions: TSI: Triple sugar with iron, L: Lactose, S: Sucrose, M: Motility, C: Simmons citrate, H: Hemolysis test, O: Oxidase, C: Catalase.

+ Positive proof, - Negative proof, v: variable result.

Table 4: Macroscopic characteristics of bacterial colonies isolated in black pepper rhizosphere:

Colonial type	N	%
Mucoid white	3	23,05
Dry white	3	23,05
White cotton	2	15,4
Mucoid yellows	5	38,5
Total	**13**	**100,0**

Source: Authors

Table 5: Microscopic characteristics of bacterial colonies isolated in black pepper rhizosphere:

Colonial type	**Microscopy**
Mucoid white	CGP e BGPc
Dry white	BGPi e BGPc
White cotton	BGPi e BGPc
Mucoid yellows	CBGP

Source: Authors

CGP: Gram-positive cocci; BGPc: Gram-positive bacilli in chain; BGPi: Isolated Gram-positive bacilli; CBGP: Gram-positive coccobacilli

Table 6 – Biochemical characterization of colonies isolated from cassava rhizosphere.

Colonial type	**TSI**	**L**	**S**	**M**	**C**	**H**	**O**	**C**
Mucoid white	Ac/Alc	v	v	-	-	+	+	+
Dry white	Ac/Alc	+	+	-	-	+	+	+
White cotton	Ac/Alc	+	v	-	-	+	+	+
Mucoid yellows	Ac/Alc	+	+	-	-	+	-	-

Source: Authors

Captions: TSI: Triple sugar with iron, L: Lactose, S: Sucrose, M: Motility, C: Simmons citrate, H: Hemolysis test, O: Oxidase, C: Catalase.

+ Positive proof, - Negative proof, v: variable result.

Table 7 – Sensitivity test of colonies isolated in cassava rhizosphere.

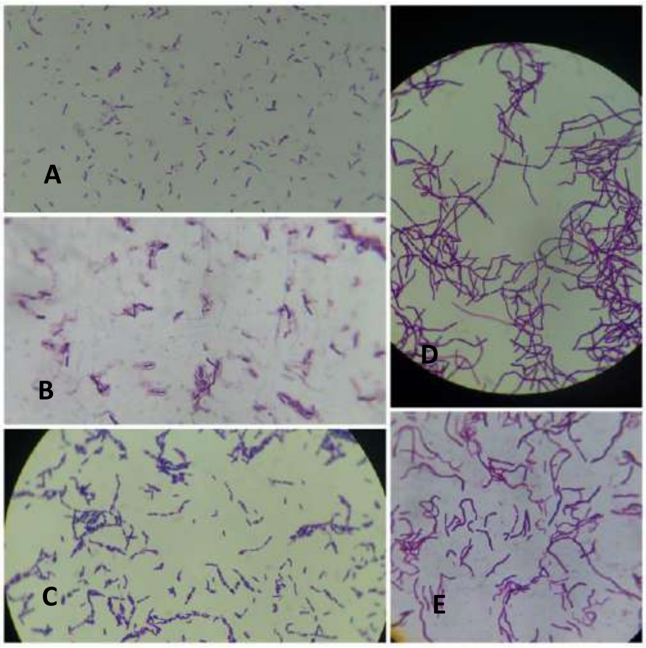
Colonial type	***E.coli***	***K.pneumoniae***	***S.aureus***
Cottony white	+	+	-
Dry white	-	-	-
Mucoid white	+	-	-
Mucoid yellow	+	+	-

Source: Authors

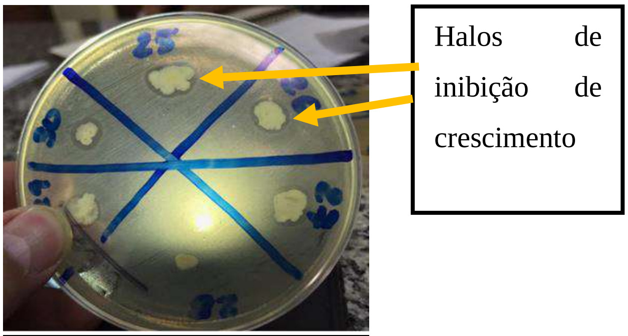
(+) Positive inhibition test

(-) Negative inhibition test

Figure 1 – A and B – Isolated and paired Gram-positive bacilli (BGPi), C, D and E Gram positive bacilli in chains (BGPc) (filamentous).



Source: Authors



Source: Authors

Fig.2: Sensitivity test demonstrating inhibition of Gram-negative bacteria growth by actinobacteria from isolated soils.

V. CONCLUSION

The Amazonian soil is still little explored in its biotechnological potential, the findings in this research demonstrate the possibility of discovering new bacterial strains with high potential for the production of new antibacterial drugs. Bacteria isolated from these soils proved to be organisms with relevant pharmaceutical potential. These organisms act as growth inhibitors of other microorganisms and are key agents in the biocontrol of diseases of human pathogens, and can significantly contribute to the production of antibiotics, thus generating a positive expectation in the fight against infections. The expected scientific benefits of greater knowledge about microbial diversity include, among others, better understanding of the roles played by microbial communities in terrestrial environments and knowledge of their interactions with other components of biodiversity.

Despite being relevant in several aspects, soil bacteria are still poorly studied and disseminated. It is proposed to carry out more in-depth research on the applications of actinobacteria in these processes, using more accurate tests and techniques to achieve these findings in order to highlight the economic importance of soil biota, and support the review and synthesis of the direct value and indirect soil biological species.

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REFERENCES

[1] CORRÊA, G.G.; SILVA, L.L.; LINS, M.R.C.R.; SILVA, W.O.; LOPES, W.; ARAUJO, E.A.; VASCONCELOS, N.B.; SOURCES VIEIRA, J.M.; SILVA, D.T.F.; LIMA, G.M.S.; ARAUJO, J. M. Isolation and antimicrobial activity of actinobacteria from the Caatinga rhizosphere. Federal University of Pernambuco, Recife PE, 2014.

[2] ESCHER, S K S. Bioprospection of actinobacteria isolated from the rhizosphere of *Aniba parviflora* Syn fragans (Macacaporanga) from Amazonia and evaluation of antimicrobial activity. Thesis (Doctorate). Federal University of Pernambuco. Graduate Program in Pharmaceutical Sciences. Recife, 2016.

[3] FRANCO, C.M.M. & COUTINHO, L.E.L. Coutinho, Detection of Novel Secondary Metabolites, Critical Reviews in Biotechnology, 11:3, 193-276, 1991.

[4] BORA, N.M.; WARD, A.C. The actinobacteria. In: GOLDMAN, E.; GREEN, L.H. (Ed). Practical handbook of microbiology. 2. ed. Boca Raton: CRC Press, 2009, p. 373-443.

[5] SANTOS, J.D. Analysis of the dominant microbiota of three sampling points in the Amazon savanna soil with potential for the production of bioactives. Federal University of Amapá. Dean of research and postgraduate research department and postgraduate master's degree in health sciences. 2012.

[6] VENTURA, M., CANCHAIA, C., TAUCH, A, CHANDRA, G., FITZGERAL, GF, CHATER, KF, van SINDERAM, D., Genomics of Actinobacteria: Tracing the Evolutionary History of an Ancient Phylum, Microbiology and Molecular Biology Reviews 71(3):495-548 · October 2007.

[7] SILVA, G. R. Bioprospection of actinobacteria isolated from the rhizosphere of *Caesalpinia pyramidalis* Tul. of the Caatinga biome. Federal University of Pernambuco, 2013.

[8] BRITO, P.H.; & NASCIMENTO, R. P. Evaluation of the enzymatic potential of actinobacteria isolated from coastal environments. XII Brazilian Seminar on Enzymatic Technology ENZITEC 2016. Federal University of Rio de Janeiro. Chemistry school. 2016.

[9] CANOVA, S.P. Diversity and Bioprospecting of Actinobacteria Isolated from Mangroves. Dissertation (Masters in Biotechnology). Institute of Biomedical Sciences, University of São Paulo, São Paulo, 66 f. 2009.

[10] SÁ, F.O., Biological control of sisal red rot (Agave sisalana Perrine) with *Trichoderma* ssp. And Actinobacteria. 2014. 133f. Thesis (Doctorate in Agricultural Sciences) - Federal University of Reconcavo da Bahia, Cruz das Almas, BA, 2014.

[11] RAMOS, B.C.N., Review of the literature of the microbiological study of pepper spice (*Piper nigrum*). Dissertation (Masters in Agricultural Sciences) - Federal University of Maranhão, São Luiz, MA, 2018.

[12] BRAZIL. National Health Surveillance Agency Clinical Microbiology for the Control of Infection Related to Health Care. Module 6: Detection and identification of medically important bacteria / National Health Surveillance Agency. – Brasília: Anvisa, 2013. 150p..: il.9 volumes.

[13] SOUSA, V.Y.K. Bioprospection of antimicrobial agents among environmental bacteria and medicinal plants from the Amazon. Faculty of Agronomy and Veterinary Medicine, University of Brasília, 69p. Masters dissertation. Brasilia, 2012.

[14] VOBIS, G. Morphology of *Actinomycetes*. In: MIYADOH, S. Atlas of *Actinomycetes*. Japan: Asakura, p. 180-191, 1997.

[15] TAGUCHI, S. et al. *Streptomyces subtilisin* inhibitor-like proteins are widely distributed in *Streptomycetes*. Applied and Environmental Microbiology, Washington, v. 59, no. 1, p. 4338-4341, 1993.

[16] PEREIRA, J.C.; NEVES, M.C.P.; DROZDOWICZ, A. Quantifications of bacterial populations in general, antibiotic resistant bacteria and *Actinomycetes* in soils. Seropédica: EMBRAPA-CNPAB, 1996. 21 p.

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Socio-economic impact of cassava wastewater on some communities in Benue State, Nigeria

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Abstract— *The socio-economic impact of cassava wastewater on some communities in Okpokwu and Ohimini Local Government Areas of Benue State, Nigeria were investigated. These communities are known for high rate of garri production in the State.Twenty structural questionnaires were administered on 293 residents within the study area. A large number (>80%) of the respondents in the community affirmed that garri wastewater had an offensive odour, changed soil colour, attracted mosquitoes and inhibited plant growth. The study revealed that a large number of the processor may not possess an in-depth knowledge on the effect of the cassava wastewater on the human health and environment at large. Environmental sanitation agencies may carry out routine inspection of processing areas as well as educate the processors of the hazards associated cassava wastewater.*

Keywords— ***socio-economic, cassava wastewater, environment, practises, benue.***

I. INTRODUCTION

1.1 Background to the Study

Nigeria is currently the world's largest producer of cassava accounting for about a fifth of the world's cassava production. It is followed by Thailand (the largest in Asia), Indonesia, Brazil, Ghana, Congo and others [10]; [18]. Consequently, out of the thirty-six (36) states in Nigeria, 24 states produce cassava and Benue State which is acronym the "food basket of the nation", is one of the states with the highest production of cassava [20];[3]. Cassava processing as an industry caters for 30% of the nation's informal sector in terms of employment and revenue [14]. In developing countries such as Nigeria, about 70% of harvested cassava roots are processed into garri. This is mostly done by small-scale processors with the aid of simple equipment for cassava processing. However, the traditional method of processing cassava into garri produces a large amount of waste [14]. Till date, several areas in Benue state that are known for the high rate of production of garri, carry out the traditional method of processing garri which incurs a lot of waste. Most individuals in the rural areas use part of their residence or a designated area for the cassava processing and are most times self-employed .Therefore, they produce and move their produce to the available market for sale. Cassava effluents are derived from the dewatering stage as shown in Plate 1 and accounts for about 16% of total cassava weight [7]. [3] reported an increase in the quantity of cassava produced in Nigeria in recent times due to many initiatives and support programmes. Therefore, an increase in waste production is also expected which may result in environmental pollution when it is not properly managed [16].Also, the establishment of cassava processing centres is an on-going process of the government in Nigeria [2]. Such centres have been sighted in Okpokwu Local Government area of Benue State. However, policies or guidelines for the disposal or treatment of cassava wastewater is lacking [13];[16] and [19]. This study is aimed at assessing the socio-economic impact of cassava wastewater on communities in the study area.

1.2 Cassava wastewater (Effluent)

Methods used for processing cassava produce solid and liquid wastes that contaminate the environment [12]. When processing cassava, the peel and liquid effluent squeezed out of the fermented parenchyma mash are the two important biological wastes that may cause damage to the environment. Most times the peels are discarded on the soil or water as waste and allowed to rot in the area as well as the liquid effluent which contains a lot of microorganisms [12]. At times homes around an area may discharge fermented cassava wastewater in small volumes. However, for several community individuals undergoing the same practice of fermentation could lead to a massive pollution on the receiving waters [15]. Cassava waste water have in suspended solids about 15,000 mg/L which are most times not treated but disposed freely into the environment, contaminating nearby water sources in the process. These effluents possess serious environmental impacts causing acidification due to the hydrolysis of cassava cyanogenic glucoside, linamarin and lotaustralin (Methyl linamarin) producing hydrogen cyanide which is also toxic to household animals, fisheries and other organisms [14]. [17] reported that Adult female catfish, Clarias gariepinus showed signs of gill and liver damage as a result of exposure to cassava effluent. Also stated was the histopathological examination carried out on the gill, kidney and liver of fingerlings of the Nile Tilapia (Oreochromis noliticus) that was treated with cassava effluent indicated damage as well. In Allium cepa root meristem, cassava wastewater causes anomalies in cell division process and chromosome aberration induction that could be as a result of heavy metal-cyanide interaction in cassava waste water [17]. Also, the study by [8] concluded that cassava wastewater alters the physicochemical characteristics of soils.

Plate 1: The dewatering stage of cassava processing that produces cassava effluent.

II. METHODOLOGY

Study area and sampling procedure

Benue State of Nigeria is located between latitude 7°43′50″N and longitude 8°32′10″E . Its vegetation cover consists of the Southern guinea savannah, with rainfall averages of 1,200 - 1,500mm, high relative humidity and fertile soil [11]. It comprises of twenty-three Local government Areas and farming is the common practice of the people with major crops such as yam, Soy beans, sesame, cassava, oil palm, mangoes, oranges, plantain and sweet potatoes cultivated seasonally. These elements contribute immensely as to why the state is termed the Food Basket of the Nation.

Judgmental/selective sampling was used to pick two local government areas namely; Okpokwu and Ohimini based on the high production rate of garri in these areas of Benue State. Collection of information on the socio-economic impact of cassava wastewater on inhabitants in the study area was done using structured questionnaire adopted from Godson-Ibeji and Chikaire (2016). 293 questionnaires were used for the final analysis.

III. RESULTS

Estimation method

The selected variables for this study comprised the socio-demographic data, Attitude and Practices of respondents, Environmental factors and Health factors affecting respondents. A complete description of these variables are given in Table 1, 2 and 3 respectively.

Table 1: Socio-demographic data, attitudes and practices of respondents (n=293).

Socio-demographic data	Percentage (%)
Gender	
Male	69
Female	31
Age	
15-29	52
30-54	38
55+	10
Education	
No formal education	10
Primary	19
Secondary	47
Tertiary	24
ıtion	
Farming	28
Garri processor	56
Civil servant	10
Others	6
Attitude and Practices	
Quantity of bags Pressed weekly	
1-5 bags	34
5-10 bags	18
11 bags and above	48
Nature of press	
Wooden	37
Hydraulic jack	63
Number of workers	
1-5	87
6 and above	13
Use of cassava waste	
Animal feed	48
Starch	3
Fertilizer	15
No usage of peel	14
No usage of wastewater	20

Table 2: Environmental factors affecting respondents (n=293).

Environmental factors	Percentage (%)
Method used for Garri frying	
Firewood	100
Kerosene	0
Gas	0
Others	0
Age of garri factory	
1-4 years	31
5-10 years	39
10+ years	30
Offensive odour of surroundings Present	
Yes	88
No	12
Colour change of soil where cassava is pressed	
Yes	96
No	4
Insects present in the surrounding of the factory	
Mosquito	87
Tsetse fly	5
Bug	8
Disposal of cassava peel	
Burn	9
Drop in refuse dump	86
Do not throw away	3
Others	2
Disposal of cassava wastewater	
Allow to flow on the ground	44
Collect in a container	13
Others	43
Do plants grow on contaminated soils	
Yes	11
No	89

Table 3: Health factors affecting respondents (n=293)

Health factors	Percentage (%)
How often do you treat malaria	
Weekly	3.8
Monthly	31.7
Quarterly	35.5
Yearly	20.1
None of the above	8.9
Dizziness when processing	
Yes	46
No	54
Nausea	
Yes	27
No	73
Difficulty in breathing	
Yes	41
No	59
Headaches	
Yes	60
No	40
Ever consumed the wastewater	
Yes	4
No	96

The socio-demographic data, attitude and practice of respondents in the study area are shown in Table 1. The proportion of male to female that carryout garri processing was 69% to 31% . The age range 15 – 29years which was the youngest had 52% while 30- 54 years had 38% and 55 years and above had 10%. 47% of garri processors had a secondary school education, 24% had a tertiary education and 19% had a primary school education. 10% had no formal education as shown in fig. 1. Most individuals combined other jobs with garri processing. Occupations of respondents in the study include Garri processing (56%), Farming (28%), Civil servant (10%) and others like students and traders (6%). Quantity of bags pressed weekly include, 11 bags and above (48%), 1-5bags (34%) and 6 – 10 bags (18%). Two nature of jack were used to squeeze out water from cassava mash. Hydraulic jack (63%) and wooden jack (37%). 1-5 number of workers had 87% while 6 and above number of workers had 13% indicating that it is a small scale business. 48% of respondents used cassava waste as animal feed, 3% used them as starch and 15% used them as fertilizers. 14% had no use for the peel while 20% had no use for the wastewater as shown in Fig. 2. Environmental factors affecting respondents are shown in Table 2. 100% of respondents used firewood to fry garri. The age of garri factory were as follows, 31% of respondent's factory were 1-4 years old, 39% were 5 – 10 years old and 30% were 10 years an above years old. This shows the length of time soils around such areas have been recieving the cassava wastewater. 88% of respondents agreed that cassava wastewater emits offensive odour while 12% disagreed. 96% of respondents agreed that there was a colour change in soils receiving cassava wastewater while 4% disagreed. 11% of respondents agreed that plants grow in soils contaminated with cassava wastewater while 89% disagreed as shown in Fig. 3. Insects present around the factory include mosquitoes (87%), flies (5%) and bugs (8%). 86% of respondents disposal their cassava peel by dropping them in the refuse dump, 9% burn them , 3% do not throw them away and 2% had other use for them like using it in their farmlands to cover the roots of trees. 44% of respondents dispose of their wastewater by allowing it to flow on the ground, 13% collect in containers and 43% had other ways of disposing it like digging trenches for the water to flow away from the area. Health factors affecting respondents are shown in Table 3. 35.5% of respondent agreed that they treat malaria quarterly, 31.7% agreed to monthly treatment, 20.1 % agreed to yearly treatment, 3.8 agreed to weekly treatment and 8.9% stated none of the above. Respondents agreed and disagreed to the following health conditions during processing as shown in Fig. 4. Dizziness, 46% agreed while 54% disagreed. 27% agreed to feel nauseous while 73% disagreed. 41% agreed to have difficulty in breathing while 59% disagreed. 60% agreed to have headaches while 40% disagreed.

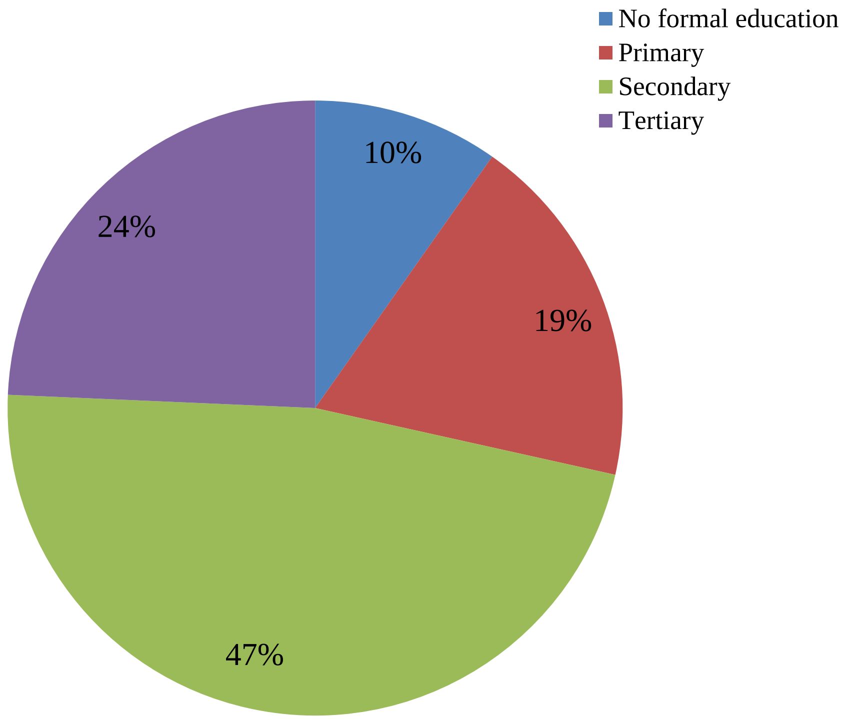

Fig.1: Education of Respondents in the Study Area.

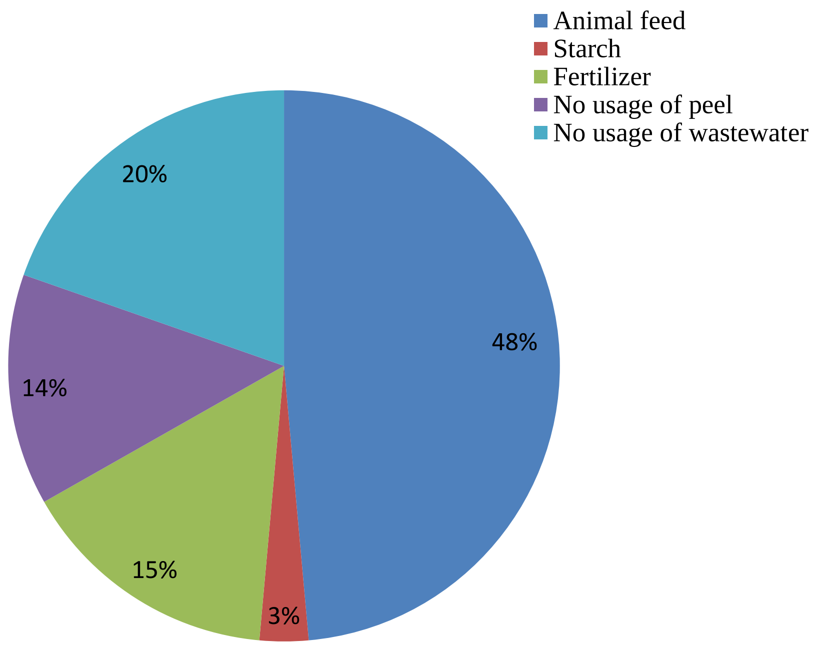

Fig.3: Use of Cassava Waste by Respondents in the Study Area.

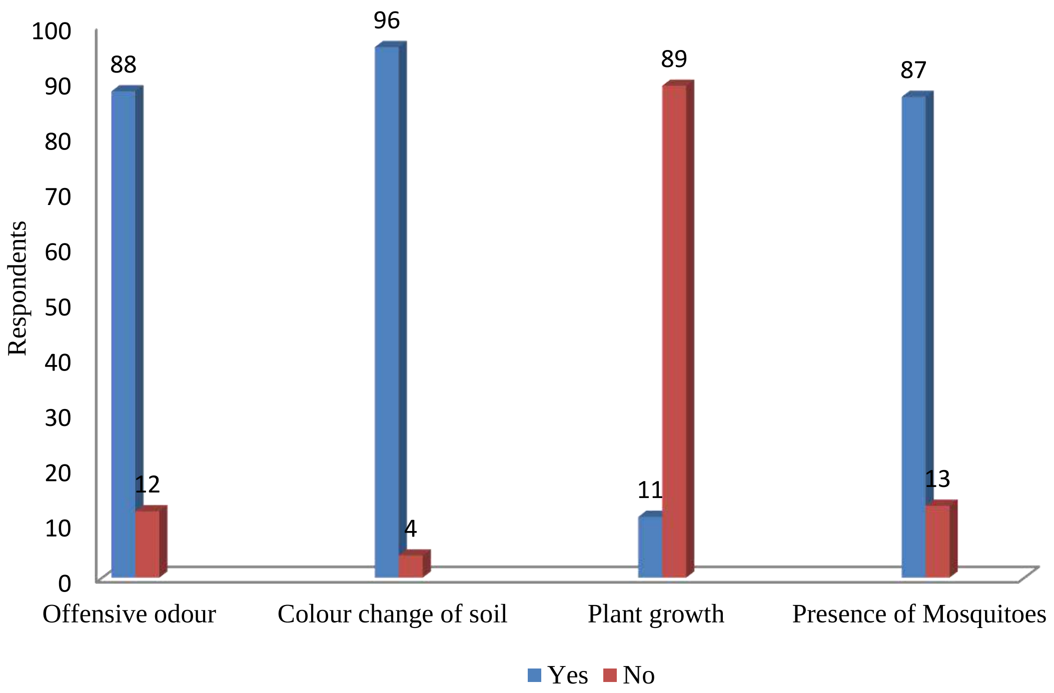

Fig. 3: Responses on the Effect of Cassava Wastes on Communities

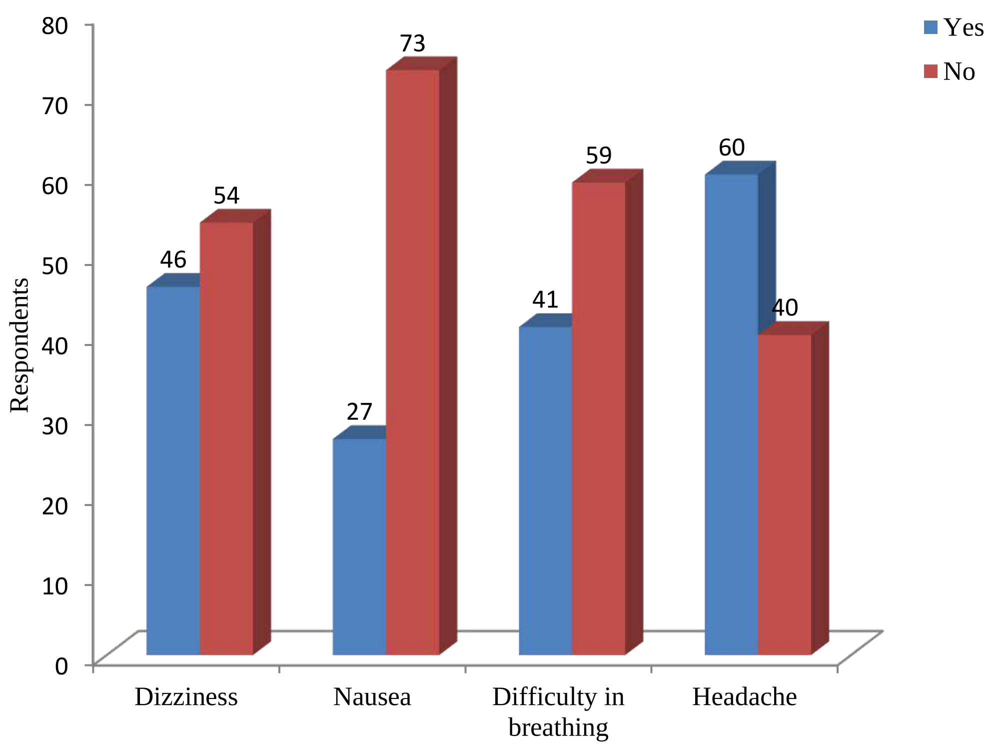

Fig. 4: Some Health Challenges Experienced by Garri Processor

IV. DISCUSSION

The socio-economic impact of the cassava wastewater on communities in the study area is shown in the pie chart diagrams. The gender pie chart reveals the male individuals dominating the females in cassava processing business. This could be attributed to the amount of strength needed to jack (dewater) the cassava flasks prior to frying. The hydraulic press in most of the sites need manual labour to work and this action is carried out by the male individuals. The age 15 – 29 showed the highest respondent. This indicates that the younger individuals participate more in this business due to the strenuous activities involved. Also, Respondents with secondary school education had the highest percentage (47%) and is consistent with work done by [16]. Lack of funds restricts most of them from getting a higher qualification therefore they engage themselves in the business for survival and creation of wealth. However, Individuals at this stage of education may not have an in-depth knowledge on the effect of the wastewater on the environment. A lot of respondent indicated garri processor as their occupation showing a high level of participation of individuals in the profession at the study area. Others who engage in other occupations also process garri at the side. 11 bags and above of cassava was pressed weekly which had the highest respondents indicates that most processors press large quantities of cassava weekly thereby leading to an increase in the amount of wastewater generated that eventually flows on the soil which contaminates it. The hydraulic press had the highest percentage and most times operated by the male individuals since it involves the use of strength. It is safer than the wooden press which collapses when the wood begin to rot or not tied properly. All respondents agreed to the use of firewood for the frying of garri. [4] also revealed that firewood was the main heating source for cassava processing communities in Ghana. Firewood is gotten from fell down trees which are chopped into smaller pieces. Thus the survey exposes the dependency of the rural dwellers on firewood as well as the concern of climate change that comes with this activity. The 10+ year old factory had the least percentage. This is due to a shift in the position of the jack or presses when the soil can longer retain water or begins to cake. The press is then shifted to a new area on the soil. 84% of respondents indicated that their surrounding had an offensive smell. This agrees with the research by[1] which states that the discharge of the cassava mill waste results in offensive odour originating from the biodegraded products of the waste. Also,[9] reports that its degradation is fast and spreads offensive odour in the environment. Majority (>90%) of respondents indicated a colour change in the soil where the cassava is pressed. Thus indicating that cassava wastewater alters the colour of the soil during processing. A lot of the respondents also indicated that they had never consumed the cassava wastewater. Majority attributed their decision to its offensive odour as well as the knowledge of it being poisonous or injurious to health. On the method of cassava waste disposal, this work agrees with the work by [16] where 90.5% of respondent dump their cassava waste in waste dump site. Also Majortiy (>80%) of respondent allow their wastewater to flow on the soil thereby contaminating the area. Dizziness, nausea, difficulty in breathing and headaches are associated with the inhalation of hydrogen cyanide gas [6]. However, only few respondents agreed that they experience these symptoms. Residents of the study area also faced the following challenges. Complaint from neighbours of seepage of wastewater into their well-water, poor road network and transportation of their goods out of the area, frequent malfunctioning of the jack and difficulty in squeezing out water from cassava especially when it contains a lot of starch, all jobs are done manually and it is labour intensive.

V. CONCLUSION

Majority of the processor were secondary school certificate holders who may not be have an in-depth knowledge on the effect of the cassava wastewater on the human health and environment at large.

A central area for cassava processing should be built in communities that majorly cultivate as well as process cassava products to avoid indiscriminate cassava waste disposal.

Environmental sanitation agencies may carry out routine inspection of processing areas as well as educate the processors of the hazards associated with the discharge of the wastewater directly into the environment.

Processing areas should be subject to public health regulations whereby such areas must comply with the necessary regulations to avoid termination.

Routine spraying of insecticides in the area should be encouraged to destroy breeding areas for disease causes insects

Also, the wastewater generated during processing should be collected in ponds and treated before disposal or reuse

REFERENCES

[1] Agwaranze, D.I, Nwugo, V.O, Ogodo, A.C., Onudibia, M.E., Nwaneri, C.B. and Aliba N.V. (2018). Effects of cassava mill effluent (CME) on bacteria diversity of soil and aquatic environments in South- South Nigeria. *MedCrave Open Acess Journal of Science, 2(4): 238 – 242.*

[2] Akpan, J.F., Eyong, M.O. and Isong, I.A. (2017). The Impact of Long-term Cassava Mill Effluent Discharge on Soil pH and Microbial Characteristics in Cross River State. *Asian Journal of Soil Science and Plant Nutrition, 2(1): 1-9.*

[3] Bankole, E. (2018, December 28). Discover the Leaders of Cassava Production In Nigeria. *Legit*. Retrieved April 25, 2019, from https://www.legit.ng/1208794-cassava-production-nigeria-by-state.html

[4] Francis, K., Ahmad, A. and Lawrence, D. (2015). Technical and Socioeconomic Potential of Biogas from Cassava Waste in Ghana. *Biotechnology Research International, Vol. 2015, Article ID 828576, 10pp.* Retrieved on April 25, 2019, from https://doi.org/10.1155/2015/828576.

[5] Godson-ibeji, C. C. and Chikaire, U. J. (2016). Consequences of Environmental Pollution on Agricultural Productivity in Developing Countries: A Case of Nigeria. *International Journal of Agricultural and Food Research, 5(3):1-12.*

[6] Goetz, C.G. (2010). Cyanides. In: Encyclopedia of Movement Disorders. *Science Direct*. 269-271

[7] Izah, S.C., Bassey, S. E. and Ohimain, E. I. (2017). Assessment of Heavy Metals in Cassava Mill Effluents Contaminated Soil in a Rural Community in the Niger Delta Region of Nigeria". *EC Pharmacology and Toxicology, 4(5): 186-201.*

[8] Izonfuo,W., Bariweni, P., and George, D.M.C. (2013). Soil Contamination from Cassava Wastewater Discharges in a Rural Community in the Niger Delta, Nigeria. *Journal of Applied Science and Environmental Management, 17 (1): 105 – 110.*

[9] Jideofor, M.I.(2015). Chemical Adjustment of Effluent from Cassava Processing Plant prior to Safe Disposal. *Nigerian Journal of Technology (NIJOTECH), 34 (4):883 – 889.*

[10] Mara, S. M. D., Mário, M. R., Anamaria de Sousa, D., Ênio Farias de França de Silva, Elvira, M. R. P., & Daniel da Costa, D. (2014). Chemical Attributes of Soil Fertilized with Cassava Mill Wastewater and Cultivated with Sunflower.*The Scientific World Journal, 2014:1-10.*

[11] Nnamonu, L.A and Eli, A.E. (2013). Perception of Agrochemical Use and Organic Farming in Makurdi, Benue State. *International Journal of Environmental Protection, 3(8):48-52.*

[12] Obueh, H.O. and Odesiri-Eruteyan, E. (2016). A Study on the Effects of Cassava Processing Wastes on the Soil Environment of a Local Cassava Mill. *Journal of Pollution Effects and Control, 4(4): 177.*

[13] Okunade, D.A. and Adekalu, K.O. (2014). Characterization of Cassava-waste Contaminated Soils in Ile-Ife, Nigeria. *European International Journal of Science and Technology, 3(4): 173 - 182.*

[14] Oladele, P.K. (2014). Cassava Processing and the Environmental Effect. The 4th World sustainability Forum 2014. Conference Proceedings Paper.

[15] Olukanni, D.O., Agunwamba, J.C. and Abalogu, R.U. (2013). Interaction between Suspended and Settled Solid Particles in Cassava Wastewater. *Academic Journals: Scientific Research and Essays, 8(10):414-424*.

[16] Olukanmi, D.O. and Olatunji, T.O. (2018). Cassava Waste Management and Biogas Generation Potential in Selected Local Government Areas in Ogun State, Nigeria. *Recycling, 3(58):1-12.*

[17] Omotosho, O. and Amori, A. (2015). Caustic Hydrogen Peroxide Treatment of Effluent from Cassava Processing Industry: Prospects and Limitations. *International Journal of Engineering and Technology Innovation, 5: 75-85.*

[18] Research and Markets (2019, February 21). Global Cassava Processing Market Report 2019: Industry Trends, Shares, Size, Growth, Opportunity and Forecasts 2011-2018 & 2019-2024. *GlobeNewswire*. Retrieved on April 25, 2019, from www.globenewswire.com

[19] Ukaegbu-Obi, K.M., Appeh, O.G., Itaman, O.V. and Nwosu, P.C (2018). Microbial and Physicochemical analyses of Soil Receiving Cassava Mill Wastewater in Umudike,Abia State, Nigeria. *Nigerian Journal of Biotechnology, 35 (2):99 – 107.*

[20] Wossen,T., Tessema, G., Abdoulaye, T., Rabbi, I., Olanrewaju, A.,Alene, A.,Feleke,S., Kulakow, P.,Asumugha, G., Abass, A. and Manyong, V. (2017). The Cassava Monitoring Survey in Nigeria Final Report. *International Institute of Tropical Agriculture (IITA), Ibadan , Nigeria*.

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Impact of climate change on the water balance of the Sankarani river basin in West Africa

Impact du changement climatique sur le bilan hydrique du bassin fluvial de Sankarani en Afrique de l'Ouest

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***Abstract*— *The Sankarani River is a tributary and powerful regulator of the hydrological regime of the Niger River. It plays a big role in the socio-economic development of Mali through the supply of electricity through the hydroelectric power station of Sélingué, its contribution in rice cultivation and fish farming. However, the inflow of water from this river over the past few decades has been very low, due to climate change and the degradation of its watershed. So this study set itself the objective of analyzing the evolution over different periods of the components of Sankarani's water balance. The data used for this are Landsat / OLI and SRTM satellite images, rainfall and temperatures from the NOAA database, and measured stream flows from 1980 to 2013. All these data have been aggregated at the watershed scale before being used to estimate the components of the water balance over two different periods, according to hydrometeorological standards. The Pettitt Test was used on the annual rainfall series to detect 1994 as the year of failure. Thus, the study revealed a strong degradation of the Sankarani watershed following its continuous anthropization, having led to a very remarkable decrease in the runoff coefficient because, in fact, the decrease in rainfall of 8.7% on the period 1995-2009 compared to 1980-1994 resulted in a decrease of 32.6% of the flow rate, of 26.2% of the variation of water reserve and only of 3.9% of evaporation. On the other hand, in terms of the volume of water compared to the average quantity of rain for each period, evaporation was more intensified by 4.3%, unlike the flow and the variation in reserve which, for their part, decreased by 3.5% and 0.9% respectively. The rainfall deficit in the Sankarani watershed was more negative for runoff.*

Keywords*— *Sankarani, Water balance, Flow rate, Watershed degradation.

I. INTRODUCTION

La rivière Sankarani est un affluent majeur du fleuve Niger. Grace au puissant barrage hydroélectrique de Sélingué à son embouchure, cette rivière joue un très grand rôle dans le développement socio-économique du Mali. En effet, ce barrage à vocation énergétique, agricole et halieutique à l'origine (Hathie et *al.*, 2017) a fourni 175,54 GWh d'électricité au pays en moyenne par an de 1981 à 2013, pour une productivité annuelle attendue d'environ 230 GWh (Bangneres, 2015). Il permet aussi l'irrigation de plus de 1000 ha de terre depuis 2001. Par ailleurs, depuis la mise en service de cet ouvrage en 1981, globalement l'apport en eau du fleuve Niger à fortement diminué, suscitant l'hypothèse de son probable effet sur le régime du Niger (Hassane et *al.*, 2000; Diawara, 2018). Pourtant, des études ont montré que les barrages hydroélectriques n'ont pas d'impacts significatifs à long terme sur les écoulements des cours d'eau (Olivry et *al.*, 1995 ; Assani et *al.*, 2002).

Plusieurs études menées sur le fleuve Niger et/ou sur ses affluents, avec ou sans aménagements, ont montrée aussi une diminution considérable des écoulements depuis les grandes sècheresses de la décennie 1970-1980, qui est causée plutôt par la variation de la pluviométrie. Bamba et *al.* (1996) ont trouvé que la diminution des écoulements du fleuve Niger et de ses différents affluents est plus importante que celle des pluies. Aussi, l'étude du bilan hydrologique du fleuve Niger à Koulikoro réalisée par Mahé et *al.* (1997) concluait qu'il y'a eu durant les années 1980, une diminution des écoulements de l'ordre de 55% suite à un déficit pluviométrique de 21%. Enfin durant la même période, selon Paturel et *al.* (2010), les débits moyens annuels de la rivière Bani, un autre affluent du Niger, ont baissé de 65% contre environ de 15% à 25% de diminution de la pluviométrie.

La diminution du coefficient d'écoulement dans le bassin versant du fleuve Niger et ses sous-bassins, démontrée par Mahé et *al.* (1995), et Olivry (2002), explique l'importance de la baisse des écoulements par rapport à celle de la pluie. En effet, cette caractéristique a passé de 19,0% à 15,3% entre les périodes 1961-1970 et 1981-1990 respectivement (Mahé et *al.*, 1995). Cela montre clairement une augmentation du taux des pertes d'eau de pluie dans ces bassins, notamment par l'évaporation et/ou l'infiltration, à l'inverse des écoulements. Les principales causes de cette réalité sont sans doute la dégradation du bassin versant et le changement climatique qui, se caractérisait par la persistance de la sècheresse (Paturel et *al.*, 1998 ; Diawara, 2019) en Afrique de l'Ouest en général, et dans le bassin versant du Sankarani en particulier (Konate, 2003), jusqu'à la dernière décennie du 20e siècle.

Le retour confirmé depuis quelques années d'une période humide en Afrique de l'Ouest sahélienne (Descroix et *al.*, 2015; Nouaceur, 2020) nous amène à se demander quelles sont la situation et les tendances actuelles des ressources en eau de la rivière Sankarani, très utile pour le pays aussi bien sur le plan énergétique que pour son apport important dans la régulation du régime du fleuve Niger, notamment dans le soutien de ses étiages (Olivry et *al.*, 1995; Kuper et *al.*, 2002). Par conséquent, cette étude vise à analyser l'impact du changement climatique sur les éléments du bilan hydrique de la rivière Sankarani.

II. PRESÉNTATION DU BASSIN VERSANT DE LA RIVIÈRE SANKARANI

La rivière Sankarani est un affluent majeur en rive droite du fleuve Niger en Afrique de l'Ouest, se situant dans le sous bassin du cours supérieur de ce dernier (Fig. 1). Elle prend sa source dans les hauts-plateaux guinéens d'où elle reçoit l'essentiel des eaux qui l'alimentent. En effet, long de 670 km, le Sankarani (le cours d'eau) naît au sud-est de la Guinée par la confluence des rivières Gbanhala et Kourou Kellé, traverse ensuite les territoires de la Côte d'Ivoire et du Mali, où il rejoint le cours principal du fleuve Niger au 160^{e} km depuis la source de celui-ci (ORSTOM, 1970). Ainsi, son bassin versant couvre 33 500 km^2, soit environ 29,6% du bassin supérieur du fleuve Niger jusqu'à Koulikoro au Mali (ORSTOM, 1970 ; UNESCO/IRD, 2011), et s'étend partiellement sur la Guinée Conakry (65,9%), le Mali (24,2%) et la Côte d'Ivoire (9,9%).

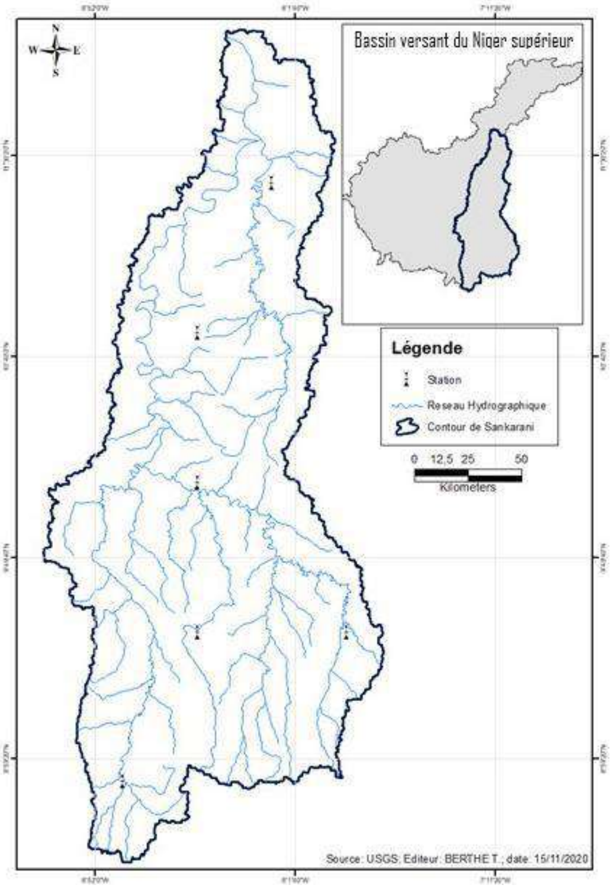


Fig.1: Carte de localisation du bassin versant de la rivière Sankarani

Le bassin versant du Sankarani se caractérise par un climat tropical avec l'alternance d'une saison sèche et d'une saison humide pendant laquelle on enregistre en moyenne une lame de pluie de 1100 à 2000 mm/an (Ferry et *al.*, 2018). Cela se reflète aussi sur le régime hydrologique avec des hautes et des basses eaux. En année moyenne, le bassin de Sankarani reçoit 45 km^3 d'eau de pluies dont

80,0% de la Guinée, 11,1% du Mali et 8,9% de la Côte d'Ivoire (Andersen et *al.*, 2006).

Le barrage de Sélingué construit de 1980 à 1982, avec un bassin de drainage vaste de 32 140 km^2 (soit plus de 90% de la superficie totale du bassin versant de Sankarani), possède une retenue d'eau de 450 km^2 de superficie et d'une capacité de stockage de 2,0 à 2,2 km^3 selon les sources (Kuper et *al.*, 2002 ; Ferry et *al.*, 2011). Bien que le volume d'eau stocké à Sélingué ne représente que 7,6% des écoulements moyens annuels du fleuve Niger à la station de référence de Koulikoro au Mali, ses débits sortants représentent plus de 50%, voire proches de 100% des débits du Niger à cette station en saison sèche (Kuper et *al.*, 2002). Cela montre la plus grande importance de ce cours d'eau dans la régulation du régime hydrologique du fleuve Niger en général. Ce barrage est multifonctionnel car il assure la production d'électricité grâce à une centrale hydroélectrique installée de 44 MW de puissance, la riziculture sur plus de 1000 ha et la production piscicole d'eau douce (Maïga et *al.*, 2011).

III. MATÉTIELS ET METHODS D'ANALYSE

3.1. Données et outils de traitement

Les données utilisées dans cette étude sont de plusieurs types. Il s'agit :

- des images satellitaires Landsat MSS et OLI des années 1974 et 2019 du mois de décembre, un peu après la fin de la saison des pluies, et SRTM 30 collectées à partir de la base de données de United States Geological Survey (USGS) ;
- des données climatiques journalières maillées (températures max et min, et précipitations) du bassin versant de Sankarani de 1980 à 2013, quant à elles, sont de source National Oceanic and Atmospheric Administration (NOAA) ;
- et enfin de la série des débits moyens annuels de la rivière Sankarani au niveau de Sélingué sur la période de 1980 à 2013, obtenue grâce aux archives des jaugeages effectués sur le cours d'eau.

Les principaux outils de traitement et d'analyse des données satellitaires utilisées sont GRASS GIS 7.6, ENVI 5.1 et ArcGIS 10.5. Le premier nous a permis de délimiter et d'analyser le bassin versant à partir de SRTM 30. Ensuite, ENVI 5.1 a servi pour le traitement des images Landsat MSS et OLI afin d'analyser les unités d'occupation et d'utilisation du sol du bassin versant. Enfin, ArcGIS10.5 a été utilisé pour la production des cartes.

Pour la détection de l'année de rupture dans la série des précipitations annuelles, l'outil statistique Khronostat a été utilisé.

3.2. Méthodes

3.2.1. Traitement des images satellitaires

L'image SRTM 30 a été prétraitée dans ArcGIS 10.5 afin de la restaurer à travers la correction des pixels qui manquaient d'informations ou qui contenaient des erreurs. Sur la base de ce Modèle numérique de terrain (MNT), le bassin versant de la rivière a été délimité et son réseau hydrographique extrait. Et par la suite, nous avons évalué les caractéristiques morphométriques du bassin versant et de son réseau hydrographique.

Afin d'évaluer le degré de dégradation du bassin versant de Sankarani, l'évolution des différentes unités d'occupation du sol a été analysée. Pour les identifier, la méthode de classification supervisée a été privilégiée (Dembélé, 2017), combinant la technique d'interprétation visuelle pour définir les thèmes basiques et le maximum de vraisemblance.

3.2.2. Agrégation des données hydroclimatiques

Les séries de données climatiques journalières (Tmax, Tmin et P) étant sous forme de mailles, nous les avons dans un premier temps générées pour six (06) nœuds, ici nommées stations, répartis de façon homogène en fonction des sous-zones climatiques du bassin versant (Fig.1). Ensuite pour chaque station, elles ont été agrégées à l'échelle mensuelle, puis annuelle selon les normes de traitement de l'OMM (OMM, 2011). La température moyenne annuelle est obtenue en faisant la moyenne de Tmax et Tmin.

A l'échelle du bassin versant, la méthode de la moyenne arithmétique simple nous a permis de calculer la température moyenne annuelle, alors que pour la pluie moyenne annuelle, la méthode des polygones ou méthode de Thiessen (Ouatiki, 2014) a été privilégiée. Ensuite, la rupture dans la série chronologique des précipitations annuelles a été détectée par le Test de Pettitt (Paturel et *al.*, 1995).

3.2.3. Evaluation des composantes du bilan hydrique et analyses

Les différentes composantes du bilan hydrique annuelle du bassin versant analysées dans cette étude sont présentées dans l'équation classique suivante :

$$\Delta R = P - E - h_Q \tag{1}$$

d'où ΔR - variation de réserve en eau du sol et/ou du sous-sol du bassin versant, en mm ; P – pluie reçue au cours d'une année, en mm ; E – lame d'eau évaporée du bassin versant en une année, en mm ; h_Q – lame d'eau écoulée (volume d'eau écoulée par unité de surface du bassin versant), en mm.

L'évaporation réelle (E, en mm) pour chaque année a été obtenue par la formule de Turc (Turc, 1955) ci-après présentée, en fonction de la température (T, en °C) et des précipitations (P, en mm) annuelles :

$$E = \frac{P}{\left(0{,}9 + \frac{P^2}{(0{,}05T^3 + 25T + 300)^2}\right)^{\frac{1}{2}}} \quad (2)$$

Enfin la lame d'écoulement par l'exutoire du bassin versant (h_Q, en mm) a été évaluée par la formule 3 (Roche, 1963).

$$h_Q = 31{,}54.10^3 \frac{Q_{an}}{A} \quad (3)$$

d'où Qan – le débit moyen annuel à l'exutoire du bassin versant, en m^3s^{-1} ; A – l'aire (superficie) du bassin versant, en km^2.

Les composantes du bilan hydrique du bassin versant ainsi calculées pour chaque année, ont été utilisées pour le bilan moyen pour chacune des périodes définies avant et après la rupture observée dans les précipitations. Et pour déterminer les périodes afin de faire une évaluation comparative du degré de variation des différentes composantes du bilan hydrique d'une période à l'autre, l'indice pluviométrique de Nicholson (Nicholson et *al.*, 1988) ci-après a été utilisé.

$$I_N = \frac{(x_i - \bar{x})}{\sigma} \quad (3)$$

d'où I_N – l'indice pluviométrique de Nicholson ; x_i – le cumul de pluie de l'année i ; $\bar{x}$ – la pluie moyenne sur la période totale de l'étude.

IV. RÉSULTATS ET DISCUSSION

4.1. Caractéristiques physiographiques du bassin versant

Le bassin versant a été délimité à partir de son exutoire situé au niveau de Sélingué, un peu en amont de sa jonction d'avec le fleuve Niger. Les principales caractéristiques morphométriques pour la compréhension de sa configuration sont récapitulées dans le Tableau 1.

Tableau 1: Caractéristiques morphométriques du bassin versant de Sankarani

Caractéristiques	Valeurs
Superficie (A, en km^2)	33 282
Périmètre (P, en km)	1 550
Indice de compacité (I_C)	2,38
Altitude max (Hmax, en m)	1 479
Altitude min (Hmin, en m)	323
Altitude moyenne (Hmoy, en m)	470
Ordre du réseau hydrographique	6
Longueur du cours d'eau principal (L, en km)	679
Densité de drainage (Dd, en km/km^2)	0,37
Densité hydrographique (Dh, en $1/km^2$)	0,08

Avec un indice de compacité supérieur à 2,38, ce bassin versant est de forme très allongée (Laabidi, 2016). La faible valeur de la densité de drainage indique une forte perméabilité du sol du bassin versant (Aoulmit, 2016), donc un taux d'infiltration à la recharge de la nappe d'eau souterraine plus élevé que celui de l'écoulement. Le cours d'eau principal dont la longueur fait 679 km est d'ordre six (6), comme on peut constater sur la Fig. 2, avec deux (2) principaux affluents, quant à eux d'ordre cinq (5).

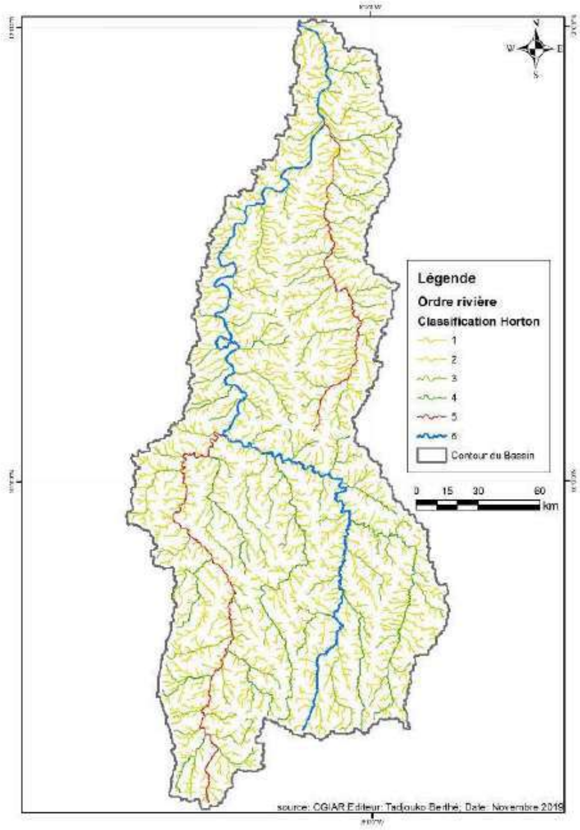
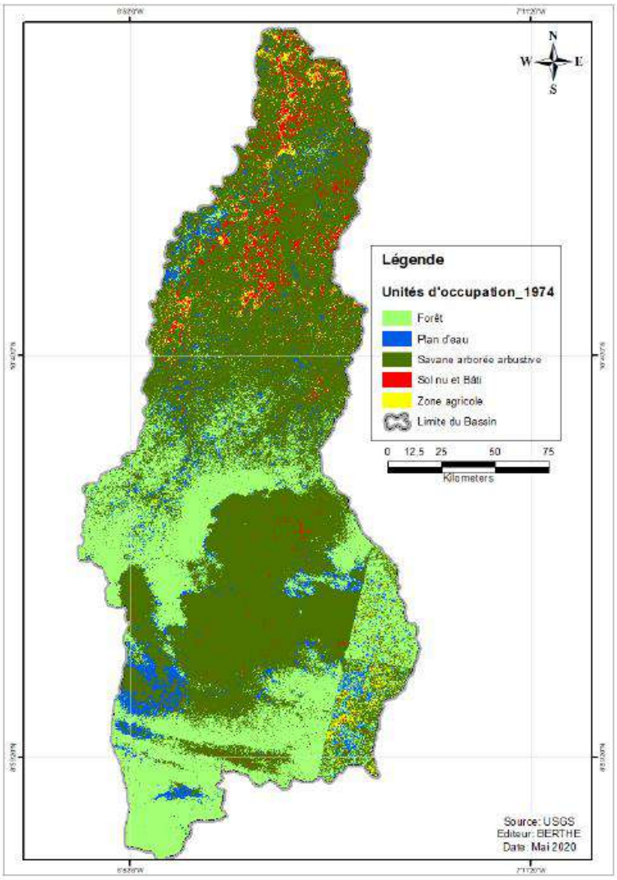


Fig.2: Réseau hydrographique du bassin versant de Sankarani.

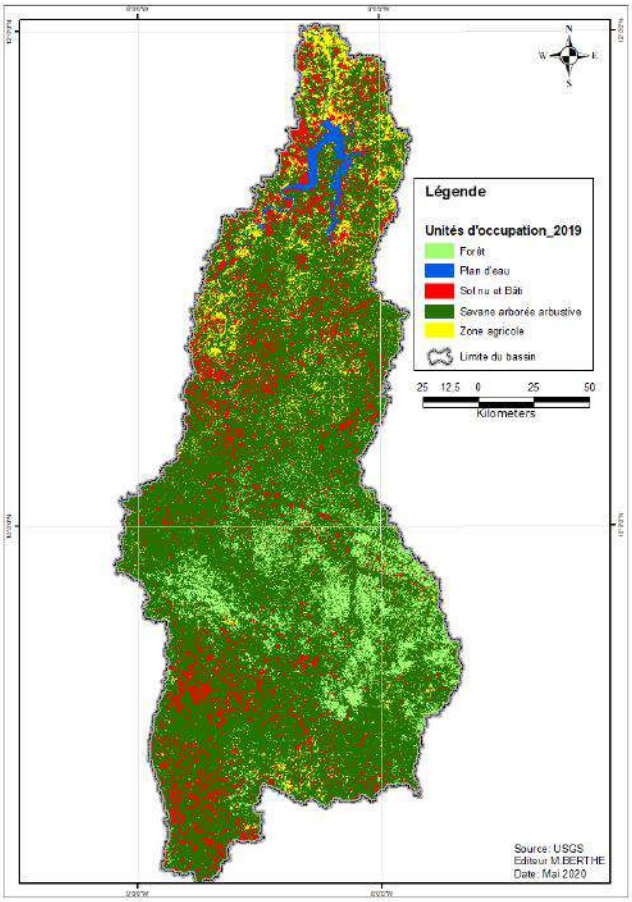
4.2. Analyse de l'occupation du sol du bassin versant de Sankarani

Les résultats des traitements d'images Landsat et OLI de décembre 1974 et 2019 sont présentés sous forme de cartes d'occupation du sol sur la Fig. 3. La comparaison de ces cartes nous informe sur la dynamique des unités d'occupation du sol, qui sont la forêt, le plan d'eau, la savane arborée/arbustive, la zone agricole et le sol nu/bâti.

Ces cartes nous permettent de constater que malgré le maintien des mêmes unités, l'occupation du bassin versant de Sankarani a beaucoup évolué entre 1974 et 2019. Par exemple, plusieurs plans d'eau visibles sur la carte de 1974 (a) dans la partie sud du bassin versant ont disparu sur celle de 2019 (b). Cela s'expliquerait par les effets du changement climatique en Afrique de l'Ouest, plus précisément par la baisse de la pluviométrie (Diawara, 2019). Par contre, nous observons apparaitre un lac proche de l'exutoire du bassin versant qui fait suite à la construction du barrage de Sélingué au début des années 1980. Par ailleurs, les sols nus/bâtis ont fortement augmenté, notamment dans la partie sud du bassin où ils étaient jadis inexistants.



(*a*)



(*b*)

Fig. 3: Cartes d'occupation du sol au mois de décembre 1974 (a) et 2019 (b) du bassin versant de Sankarani.

Les statistiques sur l'évolution des différentes unités d'occupation du bassin versant de Sankarani sont présentées sous forme de graphique sur la Fig. 4.

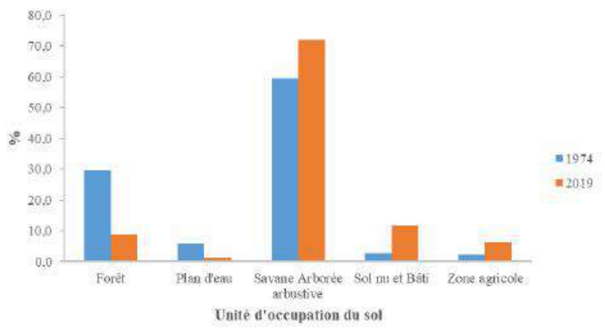


Fig. 4: Evolution des unités d'occupation du sol du bassin versant de Sankarani entre 1974 et 2019.

Au regard de cette Figure, nous notons une augmentation de la savane, du sol nu/bâti et de la zone agricole au détriment de la forêt et du plan d'eau. En effet, ces derniers ont diminué d'espace de 70,3% et 77,4% respectivement. Quant aux autres unités, elles ont augmenté de 21,1% pour la savane, de 308,3% pour sol nu/bâti et de 174,6% pour la zone agricole.

4.3. Analyse de la pluviométrie

Les données de pluie annuelle, après avoir été agrégées à l'échelle du bassin versant par la méthode de Thiessen, ont été soumises au Test de Pettitt afin de détecter les années de rupture dans la série. Sur la Fig. 5 se présente l'évolution de la variable U du Test de Pettitt.

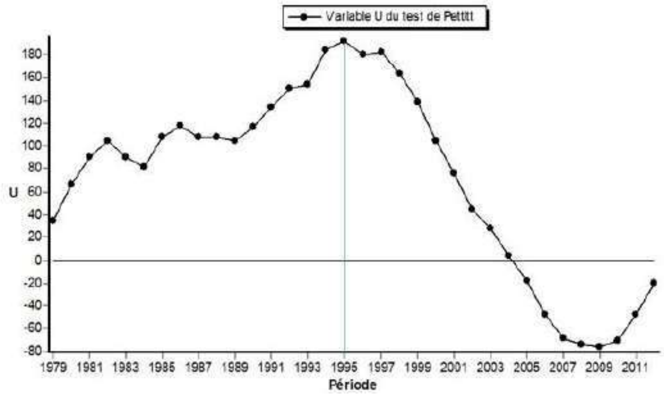


Fig. 5: Evolution de la variable U du Test de rupture de Pettitt pour la série de pluies annuelles dans le bassin de Sankarani.

Le Test de Pettitt a permis d'identifier 1995 comme l'année de rupture de la pluviométrie sur le bassin versant au seuil de confiance de 95%. Ce résultat concorde avec ceux d'autres études. En effet, Diawara (2019) a trouvé que depuis 1921, les décennies 1980-1990 et 1990-2000 ont été celles où il y'a eu les plus importantes diminutions de la pluie au Mali.

Ainsi, la distribution de l'indice de Nicholson pour la pluie sur la Fig. 6 nous montre clairement deux (2) périodes distinctes: 1980-1994 et 1995-2009. Pendant que pour la première période nous observons une situation de la pluviométrie annuelle de normale à excédentaire avec quelques années de sècheresse, pour la deuxième, pratiquement toutes les années s'avèrent déficitaires de modérées à fortes.

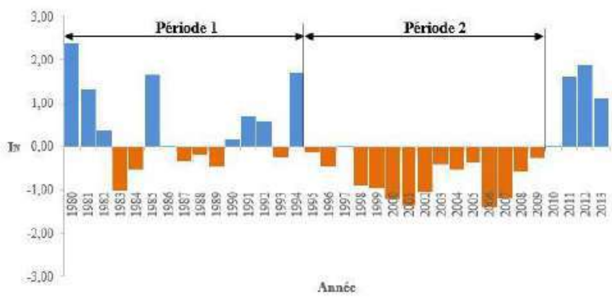


Fig. 6: Distribution de l'Indice de Nicholson pour la pluviométrie annuelle dans le bassin de Sankarani.

Cette figure semble aussi confirmer les études de Taylor et *al.* (2017), et Nouaceur (2020) par rapport à la reprise de la pluviométrie en Afrique de l'Ouest sahélienne car, en effet, à partir de 2010 nous observons le début d'une période plus humide dans le bassin versant de Sankarani.

4.4. Variation du bilan hydrique du bassin de Sankarani

Les résultats des évaluations des composantes du bilan hydrique du bassin versant de Sankarani pour les deux (2) périodes identifiées (1980-1994 et 1995-2009) se présentement dans le Tableau 2.

Tableau 2 : Composantes du bilan hydrique du bassin versant de Sankarani

Périodes	Nb. d'années	P (mm)	E		h_Q		ΔR	
			mm	%	mm	%	mm	%
Période 1 : 1980-1994	15	1455	1197	82,3	193	13,3	65	4,5
Période 2 : 1995-2009	15	1328	1150	86,6	130	9,8	48	3,6
Δ, en %	-	-8,7	-3,9	-	-32,6	-	-26,2	-

Il ressort de ce tableau une diminution de tous les éléments du bilan de la période 1 à la période 2. Par contre, les degrés de variation sont très différents d'un élément à un autre. En effet, une réduction de 8,7% de la pluviométrie a entrainé respectivement des réductions de 3,9% de l'évaporation, jusqu'à 32,6% de l'écoulement et de 26,2% de la variation de réserve. Il ressort ainsi que l'écoulement est l'élément le plus sensible à la variation de la pluviométrie sur le bassin versant de Sankarani. Ce constat a déjà été fait par d'autres auteurs, notamment Bamba et *al.* (1996), Olivry (2002) et Paturel et *al.* (2010), pour d'autres sous-bassins versants du fleuve Niger.

Par ailleurs, par rapport à la quantité moyenne de pluies tombées, le taux d'évaporation a passé de 82,3% à 86,6% (variation de +4,3%) pendant que ceux de l'écoulement et de la variation de réserve ont passé respectivement de 13,3% à 9,8% (variation de -3,5%), et de 4,5% à 3,6% (variation de -0,9%). Ainsi, on constate qu'avec la diminution de la pluie, le phénomène d'évaporation s'est intensifié contrairement à l'écoulement et à la variation de la réserve.

V. CONCLUSION

L'étude nous a permis d'évaluer le degré de dégradation du bassin versant de Sankarani due principalement aux activités anthropiques car, en effet, les espaces occupés par les sols nus/bâtis et les zones agricoles ont plus que doublé au détriment de la forêt et du plan d'eau, malgré la construction de la retenue d'eau du barrage de Sélingué. Et sur le plan du climat, elle a aussi permis de détecter deux périodes distinctes, de 15 ans chacune, dans la série des précipitations annuelles sur le bassin versant de Sankarani, par rapport à la rupture de 1994. Si pour la première période (1980-1994) la pluviométrie annuelle s'avère être de normale à excédentaire avec quelques années de sècheresse, pour la deuxième (1995-2009), pratiquement toutes les années s'avèrent déficitaires de modérées à fortes, par rapport à la normale de 1980 à 2013. Cette situation déficitaire de la pluie a eu certes comme effet la diminution de toutes les autres composantes du bilan hydrique du bassin versant de Sankarani, mais a été particulièrement négative pour l'écoulement qui a perdu 1/3 de son volume annuelle moyen.

REFERENCES

[1] Andersen, I., Dione, O., Jarosewich-Holder, M., Olivry J.-C. (2006). Le Bassin du fleuve Niger : Vers une vision de développement durable, La Banque mondiale, 150 p.

[2] Assani, A.A., Buffin-Belanger, T., Roy, A.G. (2002). Analyse d'impacts d'un barrage sur le régime hydrologique de la rivière Matawin (Québec, Canada). Revue des sciences de l'eau. *Journal of Water Science*, 15(2), 557–574. https://doi.org/10.7202/705469ar

[3] Bamba, F., Mahé, G., Briquet, J.-P., Olivry, J.-C. (1996). Changements climatiques et variabilité des ressources en eau des bassins du haut Niger et de la cuvette lacustre. XIIèmes Journées hydrologiques de l'ORSTOM du 10 au 11 octobre, Montpellier, 27 p.

[4] Bangneres, J. (2015). L'aménagement de Sélingué au Mali. Encyclopédie de l'énergie, 11 p.

[5] Descroix, Luc, Diongue Niang, A., Panthou G., Bodian, A., Sane, Y., Dacosta, H., Malam Abdou, M., Vandervaere, J.-P., Quantin, G. (2015). Evolution récente de la pluviométrie en Afrique de l'ouest à travers deux régions : la Sénégambie et le bassin du Niger moyen. *Climatologie*, vol. 12, p. 25-43.

[6] Diawara, H. (2018). Analyse spatio-temporelle de l'écoulement fluvial du fleuve Niger au Mali sur le siècle 1911-2010. *Actes de conférence SustainEng*, 2iE, p. 29-35.

[7] Diawara, H. (2019). Analyse de l'évolution Contemporaine de la Température de l'air et des Précipitations sur le territoire du Mali. *European Scientific Journal*, ESJ, 15(2), 154. https://doi.org/10.19044/esj.2019.v15n2p154

[8] Ferry, L., Mietton, M., Fujiki, K., Laval, M.; Coulibaly, N., Braquet, N., Martin, D. (2018). Faiblesse de la sédimentation dans le barrage-réservoir de Sélingué (Mali-Guinée). Témoin de la stabilité des savanes sud-soudaniennes à l'échelle d'un grand bassin versant très peu anthropisé. G-Eau Working Papers No.5a. Montpellier, France.

[9] Ferry, L., Muther, N., Martin, D., Mietton, M. (2011). Les principaux bassins versants du Niger au Mali. In : Niger supérieur : quelques résultats de recherche sur les ressources et usages de l'eau. Paris (FRA), Marseille : UNESCO ; IRD, 2 p.

[10] Hathie, I., Doucoure, O., Bagayoko, A., Syllan, A., Bocoum, N., Macalou, B. (2017). Étude comparative de la valeur actuelle du barrage de Sélingué et de la situation du bilan financier de l'état, GWI, 46 p.
[11] Hassane, A., Kuper, M., Orange, D. (2000). Influence des aménagements hydrauliques et hydro-agricoles du Niger supérieur sur l'onde de la crue du delta intérieur du Niger au Mali. *Sud Sciences & Technologies*, № 5, p. 16-31.
[12] Konate, S. (2003). Vulnérabilité et adaptation des ressources en eau aux effets des changements climatiques dans les bassins du Sankarani et du Baoulé, CNRST, 126 p.
[13] Kuper, M., Hassane, A., Orange, D., Chohin-Kuper, A., Sow, M. (2002). Régulation, utilisation et partage des eaux du fleuve Niger : Impact de la gestion des aménagements hydrauliques. In : Gestion intégrée des ressources naturelles en zones inondables tropicales. Orange Didier (ed.), Arfi Robert (ed.), Kuper Marcel (ed.), Morand Pierre (ed.), Poncet Yveline (ed.). Paris : IRD, 411-430. (Colloques et séminaires) ISBN 2-7099-1480-8 Séminaire international sur la gestion intégrée des ressources naturelles en zones inondables tropicales, Bamako, Mali, 20 Juin 2000/23 Juin 2000.
[14] Maiga, H., Marie, J., Morand, P., N'Djim, H., Orange, D. (2007). Présentation du fleuve Niger. *In : Avenir du fleuve Niger*, IRD éditions, p. 3-105.
[15] Mahé, G., Olivry, J.-C. (1995). Variations des précipitations et des écoulements en Afrique de l'Ouest et Centrale de 1951 à 1989. *Sécheresse*, n°1, vol 6, 109-117.
[16] Nicholson, S. E., Kim, J., Hoopingarner, J. (1988). Atlas of African rainfall and its interannual variability. Dept Meteorology, Florida State University, Tallahassee, Florida, USA.
[17] Nouaceur, Z. (2020). La reprise des pluies et la recrudescence des inondations en Afrique de l'Ouest sahélienne/Rain resumption and floods multiplication in western Sahelian Africa. *Physio-Géo*, Volume 15, p. 89-109.
[18] Olivry, J.-C. (2002). Synthèse des connaissances hydrologiques et potentielles en ressources en eau du fleuve Niger, 166 p.
[19] Olivry, J.-C., Bricquet, J.-P., Bamba, F., Diarra, M. (1995). Le régime hydrologique du Niger supérieur et le déficit des deux dernières décennies. In : Boulègue J. (ed.), Olivry Jean-Claude (ed.). *Colloques et Séminaires : Grands bassins fluviaux périatlantiques : Congo, Niger, Amazone*. Paris (FRA) : ORSTOM, 1993/11/22-24. ISBN 2-7099-1245-7. ISSN 0767-2896, p. 251-266.
[20] OMM. (2011). Guide des pratiques climatologiques, no 100.
[21] ORSTOM. (1970). Monographie hydrologique du bassin du Niger : 1ère partie, le Niger supérieur et le Bani.
[22] Ouatiki, H. (2014). Spatialisation des pluies et étude physiographique du sous bassin versant de Tillouguite à l'aide des outils de modélisation géospatiale. Mémoire de fin d'études, USMS, Maroc, 57 p.
[23] Paturel, J.-E., Servat, E., Kouame, B., Boyer, J. F., Lubes-Niel, H., Masson, J.M. (1995). Procédures d'identification de ruptures dans des séries chronologiques - modification du régime pluviométrique en Afrique de l'Ouest non sahélienne. *In : L'hydrologie tropicale : géoscience et outil pour le développement (Actes de la conférence de Paris, mai 1995)*. IAHS Publ. no. 238, p. 99-110.
[24] Paturel, J.-E., Diawara, A., A. Siou, L.K., Talin E., Ferry, L., Mahé, G., Dezetter, A., Muther, N., Martin, D., Rouche, N., L'Aour-Cres, A., Seguis, L., Coulibaly, N., Bahire-Kone, S., Koite, M. (2010). Caractérisation de la sécheresse hydropluviométrique du Bani, principal affluent du fleuve Niger au Mali. Global Change : Facing Risks and Threats to Water Resources (Proc. of the Sixth World FRIEND Conference, Fez, Morocco, October 2010). IAHS Publ. 340.
[25] Paturel, J.-E., Servat, E., Delattre, M.O., Lubes-Niel, H. (1998). Analysis of rainfall long series in non-Sahelian West and Central Africa within a context of climate variability. *Hydrological Sciences Journal*, 43:6, p. 937-946.
[26] Roche, M. (1963). Hydrologie de Surface, Gauthier-Villars, Paris, 430 p.
[27] UNESCO/IRD. (2011). Niger supérieur : quelques résultats de recherche sur les ressources et usages de l'eau, Paris (FRA)/Marseille, 12 p.
[28] Taylor, C.M., Belušić, D., Guichard, F., Parker, D.J., Vischel, T., Bock, O., Harris, P.P., Janicot, S., Klein, C., Panthou, G. (2017). Frequency of extreme Sahelian storms tripled since 1982 in satellite observations. *Nature*, 544, p. 475-478.
[29] Turc, L. (1955). Le bilan d'eau des sols : relations entre les précipitations, l'évaporation et l'écoulement. In : Pluie, évaporation, filtration et écoulement. *Compte rendu des troisièmes journées de l'hydraulique*, Alger, 12-14 avril 1954. Tome 1, p. 36-44.

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Butterfly (Lepidoptera) Fauna of Krishnarajanagar Town, Mysore District, Karnataka

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***Abstract*— A study was conducted to record the diversity of butterflies at Krishnarajanagara town of Mysore District over a period of six months from September 2019 to March 2020. Present survey was carried out in selected natural and man-made (parks) habitats of Krishnarajanagara town. A total of 1,147 individuals were recorded, photographed and identified which included 46 genera and 60 species belonging to five families. The relative abundance of butterflies of different families such as the Nymphalidae family was 43.33%, followed by families Lycaenidae, Hesperidae, Pieridae and Papilionidae representing 18.33%, 15%, 13.33% and 10% in the study area respectively. Results indicated that Eurema hecabe was the most dominant species followed by Ypthima huebneri, Catopsilia ponoma, and Junonia lemonias in the study area. Dominance of these species can be explained by the presence of their larval and host plants in the study area.*

***Keywords*— Butterfly, Man-made ecosystems, Pollard walk method.**

I. INTRODUCTION

The butterflies are the most beautiful and colourful creatures on the earth and have high ecological significance as they are very good pollinators apart from honey bees. They are considered as good bio-indicators as they are sensitive to slightest variation in environment such as temperature, wind speed, rainfall, humidity and solar radiation (Murphy and Weiss, 1998; Sparrow *et al.*, 1994; Spitzer *et al*., 1997; Brereton *et al*., 2011). Their distribution and abundance depends on different requirements for different habitat types for mating, breeding, and nectaring (Sprih Harsh, 2014). The present study aims to examine the distribution and abundance of butterflies across habitats studied. A checklist of butterfly species is also provided.

II. MATERIALS AND METHODS

Study area

Present study was carried out in selected Natural and Man-made ecosystems (parks) of Krishnarajanagara town, a taluk headquarters of Mysore district in the state of Karnataka, Southern India. It is situated at an altitude of 12°26'21.8"N 76°22'52.1"E. Climate of the study area is tropical landscape. This implies that the winter and the early part of the summer are typically dry periods. The rainy season falls in between June and early October.

Observation and identification of Butterflies

Field observations were conducted twice a month from September 2019 to March 2020 for a period of seven months. Distribution and abundance of butterfly species were recorded in the study area by selecting Natural and Man-made ecosystems (parks) (Table.1). Observations were made through Pollard walk method (PWM) by counting all the butterflies found in 10 meter, beside the observer and and Direct Visual Count Method (DVCM) (Kunte, 1997; Gupta *et al*., 2012; Kunte *et al*., 2012). Butterflies were observed, captured, identified, photographed and released immediately to their natural habitat carefully. Care was taken not to damage physical parts of the butterflies. Photography was made by using Nikon D5600 (55-300mm 24.1MP) DSLR camera.

The key characters used for identification were color pattern, wing span and mode of flight. Identifications were carried out with help of Evans (1932), Talbot (1947), Photographic guides of Smith (2006), Van der Poel & Wangchuk (2007) and also using Photography and guidelines of The Book of Indian Butterflies (Isaac Kehimkar).

Table.1 Study sites with GPS location.

Sites	Site name	GPS location
S1	H.B.C.S Layout	12°25'53.2"N 76°23'59.4"E
S2	Hale yadathore Cauvery river	12°28'03.0"N 76°23'31.3"E
S3	Mahatma Gandhi park	12°26'19.4"N 76°23'00.8"E
S4	Horticulture Department garden	12°25'19.1"N 76°23'49.5"E

III. RESULT

In the present study a total of 1,147 individual butterflies were recorded belonging to five families such as Nymphalidae, Pieridae, Lycaenidae, Papilionidae and Hesperidae with 46 genera and 60 species. Out of the five families recorded the relative abundance of Nymphalidae family is highest, representing 43.33% followed by families Lycaenidae, Hesperidae, Pieridae and Papilionidae representing 18.33%, 15%, 13.33% and 10% respectively (Fig.1). A checklist of species belonging to the five different families along with their status of occurrence in the study area is enlisted in Table 2.

Fig.2 depicts the relative abundance of butterfly species with respect to the total number of species recorded during the study period in each family. The relative abundance of Nymphalidae family was found to be 53.85% in site-4, 46.15% in site-1 and 3 whereas in site-2 it was 34.62%. On the contrary, out of the total 8 species recorded in Pieridae family 7 species (87.5%) were found in site-2 (Natural ecosystem) and 3 species (11.54%) in site-4 (Manmade ecosystem) whereas the members of Hesperidae and Papilionidae families preferred site-3 and site-4 (manmade ecosystems) as their relative abundance was high as compared to site-1 and site-2. Of the 11 species recorded, the relative abundance of Lycaenid butterflies in site-3 was found to be 63.64% (7 species) followed by site-1 with 36.36% (4 Species) and site-2 and site-3 recorded 27.27% (3 species) each.

Monthly variations in the number of butterfly species of different families are shown in Fig.3. The graph clearly indicates that all the families encountered in the study area are available throughout the study period (September-2019 to March-2020). Nymphalidae family dominated in all the months with more than 40% of the total recorded. Members of Nymphalidae family were abundant during December when compared to other months. On the contrary, percent occurrence of Pieridae, Lycaenidae, Papilionidae and Hesperidae was found to be high during October, February, March and November respectively.

Based on the status of occurrence in the study area and availability during the study period, the butterfly species were categorized into very common (11 species), Common (17 species) and rare (32 species) (Table.1).

IV. DISCUSSION

The study area may be favorable for better existence of butterfly community of Nymphalidae family as indicated by the results of the present study whereas the occurrence of Papilionidae in the study sites is comparatively less. The most plausible explanation would be habitat destruction due to urbanization which will be a threat to butterfly diversity. As a matter of fact, absence of food plants and nectar yielding plants in an area drives the butterfly population away due to the lack of feeding and breeding grounds. Anthropological interferences have an undeniably strong influence on the biodiversity of all existing species (Ricketts and Imhoff, 2003).

The study sites selected for the present study show variation in the abundance of butterfly species due to various factors, such as landscape, availability of host plants and most importantly anthropological disturbances. In the present study it may be noted that, Site-3 and site-4 were found to be rich in species diversity which had 31 and 28 species respectively with respect to total number of species recorded in the study area. Both the sites are manmade parks with large number of flowering plants. It is imperative that the richness in butterfly species diversity may depend on the type and variety of flowers and number of plants in a particular area and moreover the abundance, species richness and occurrence vary among different ecosystems. These results also indicate that, conservation of endemic species of butterflies may be possible by providing suitable environment to support the butterfly population and their survival (Myers *et al.*, 2000).

Among the species recorded two species *Pachliopta hector* L and *Hypolymnas misippus* L have a protected status under the schedule I part IV of Indian Wildlife Protection act, 1972 (Aurora, 2003) and *Lampides* *boeticus* under Schedule IV (Gupta *et. al*., 2005). In the present study it can be observed that *H.misippus* and *L.boeticus* were found only in site-3 and 4 (Manmade ecosystem) which implies that conditions are suitable for their conservation in these sites, whereas *P. hector* was recorded in all the four sites. These observations throw light on the fact that the study area has favorable habitat and climate which influence distribution and abundance of butterflies (Wynter-Blyth, 1957). From the results of this study it may also be noted that the diversity and abundance of Lycaenidae family members is affected due to the absence of grass fields (Harisha and Hosetti, 2013).

The diversity, distribution and the abundance of butterflies recorded may vary according to season of the year which obviously depend on diversity and distribution of floral species and their blooming season. It may be mentioned that conservation of native flora and reduced human interference may have positive effect on the butterfly population and their survival (Myers *et al*., 2000).

Table:2 Checklist of the Butterflies and their occurrence in study area

Sl. No	Family	Common Name	Scientific Name	Site 1	Site 2	Site 3	Site 4	Status
1	Nymphalidae	Common Four ring	***Ypthima huebneri* (Kirby,1871)**	+	+	+	+	VC
2		Lemon pansy	***Junonia lemonias* (Linnaeus, 1758)**	+	+	+	+	VC
3		Common crow	***Euploea core* (Cramer, 1780)**	+	+	+		VC
4		Tawny Castor	***Acraea terpsicore* (Linnaeus, 1758)**	+	+	+		VC
5		Blue tiger	***Tirumala limniace* (Cramer, 1775)**	+				R
6		Yellow pansy	***Junonia hierta* (Fabricius, 1798)**	+				R
7		Blue pansy	***Junonia orithya* (Linnaeus, 1764)**	+		+		C
8		Dark blue tiger	***Tirumala septentrionis* (Butler,1874)**	+				R
9		Plain tiger	***Danaus chrysippus* (Linnaeus, 1758)**	+	+	+	+	VC
10		Angled Castor	***Ariadne ariadne* (Linnaeus, 1763)**	+				R
11		Grey Pansy	***Junonia atlites* (Linnaeus, 1763)**			+	+	C
12		Peacock Pansy	***Junonia almania* (Linnaeus, 1758)**		+			R
13		Chocolate Pansy	***Junonia iphita* (Cramer, 1779)**			+	+	C
14		Painted lady	***Vanessa cardui* (Linnaeus, 1758)**			+		R
15		Great Egg fly	***Hypolimnas bolina* (Linnaeus, 1758)**			+	+	C
16		Danaid Egg fly	***Hypolimnas misippus* (Linnaeus,1758)**			+	+	C
17		Dark Evening Brown	***Melanitis phedima* (Cramer, 1780)**				+	R
18		Common Castor	***Ariadne merione* (Cramer, 1777)**		+		+	C
19		Common Baron	***Euthalia aconthea* (Cramer, 1777)**			+	+	C
20		Common Evening Brown	***Melanitis leda* (Linnaeus, 1758)**				+	R
21		Striped Tiger	***Danaus genutia* (Cramer, 1779)**	+			+	C
22		Common Bush Brown	***Mycalesis perseus* (Fabricius, 1775)**	+				R
23		Joker	***Byblia ilithyia* (Drury, 1773)**		+			R

24		Medus Brown	***Orsotriaena medus* (Moore, 1858)**		+			R
25		Tailed Palm Fly	***Elymnias caudata* (Butler, 1871)**				+	R
26		Common Sailor	***Neptis hylas* (Linnaeus, 1758)**				+	R
27	**Pieridae**	Common emigrant	***Catopsilia pomona* (Fabricius, 1775)**	+	+	+	+	VC
28		Common grass yellow	***Eurema hecabe* (Linnaeus, 1758)**	+	+	+	+	VC
29		Plain Orange Tip	***Colotis aurora* (Cramer, 1780)**	+				R
30		Mottled emigrant	***Catopsilia pyranthe* (Linnaeus, 1758)**	+	+	+		VC
31		Crimson tip	***Colotis danae* (Linnaeus, 1787)**		+			R
32		Common jezebel	***Delias eucharis* (Drury, 1773)**		+	+		C
33		Small Grass Yellow	***Eurema brigitta* (Cramer, 1780)**		+			R
34		Psyche	***Leptosia nina* (Fabricius, 1793)**				+	R

35	**Lycaenidae**	Gran blue	*Euchrysops cnejus* **(Fabricius, 1798)**	+				R
36		Common Silver Line	*Cigaritis vulcanus* **(Fabricius, 1775)**	+				R
37		Common Cerulean	*Jamides celeno* (**Cramer, 1779)**	+		+		C
38		Pea Blue	*Lampides boeticus* **(Linnaeus, 1767)**	+	+	+	+	VC
39		Plains Cupid	*Luthrodes pandava* **(Horsfield, 1829)**			+		R
40		Dark grass blue	*Zizeeria karsandra* **(Moore, 1865)**			+		R
41		Pale grass blue	*Pseudozizeeria maha* **(Kollar, 1844)**				+	R
42		Lesser Grass Blue	*Zizina otis* **(Fabricius, 1787)**		+	+		C
43		Common hedge blue	*Acytolepis puspa* **(Horsfield, 1828)**		+		+	C
44		Common Line blue	*Prosotas nora* **(R. Felder,1860)**			+		R
45		Zebra Blue	*Leptotes plinius* **(Fabricius, 1793)**			+		R
46	**Papilionidae**	Common Mormon	*Papilio polytes* **(Linnaeus, 1758)**	+	+	+	+	VC
47		Common Rose	*Pachliopta aristolochiae* **(Fabricius, 1775)**	+	+	+	+	VC
48		Lime Butterfly	*Papilio demoleus* **(Linnaeus, 1758)**		+	+		C
49		Crimson Rose	*Pachliopta hector* **(Fabricius, 1758)**			+	+	C
50		Blue Mormon	*Papilio polymnestor* (**Cramer, 1775)**			+	+	C
51		Tailed Jay	*Graphium Agamemnon* **(Fabricius, 1864)**				+	R
52	**Hes peri idae**	Dark Palm Dart	*Telicota bambusae* **(Moore, 1878)**	+	+			C
53		Asian Grizzled Skipper	*Spialia galba* **(Fabricius, 1793)**		+		+	C
54		Marbled Skipper	*Gomalia elma* **(Trimen, 1862)**		+			R
55		Rounded Palm-Red Eye	*Erionota torus* **(Evans, 1941)**			+		R

56		Common Banded Awl	*Hasora chromus* **(Cramer 1780)**			+		R
57		Grass Dart	*Taractrocera maevius* **(Fabricius, 1793)**			+		R
58		Grass Demon	*Udaspes folus* **(Cramer, 1775)**				+	R
59		Rice Swift	*Borbo cinnara* **(Wallace, 1866)**	+				R
60		Chestnut Bob	*Iambrix salsala* **(Moore, 1866)**				+	R

Status: VC- Very common, C- Common, R- Rare

Fig. 1 Relative abundance of Butterfly families in the study area

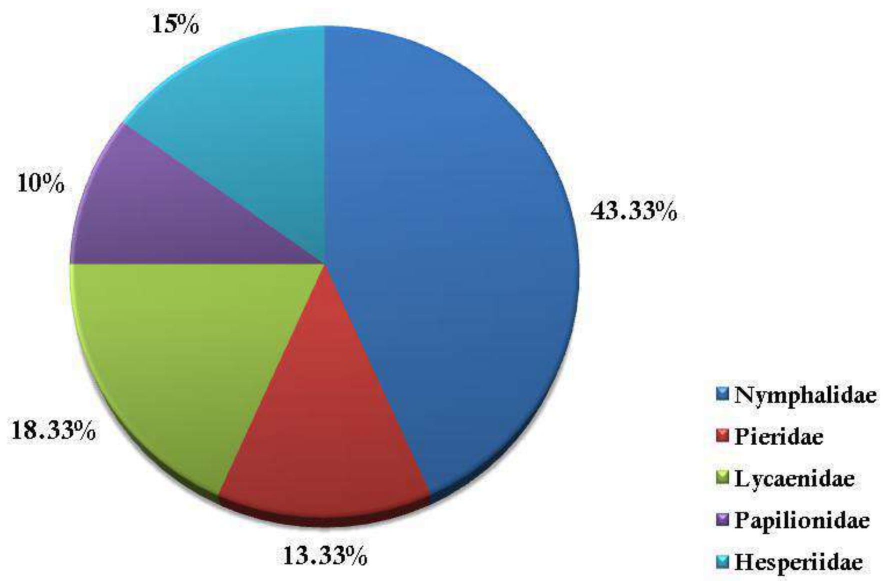


Fig. 2 Percent occurence of butterfly species in different study sites

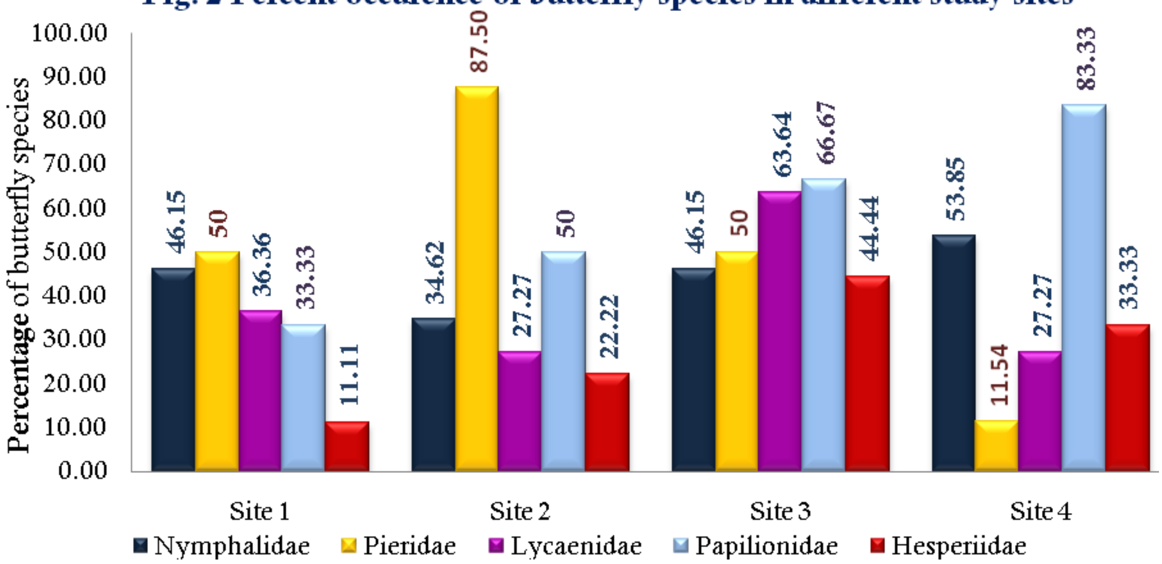


Fig.3 Monthly variation in the percent availabilty of butterfly families in the study area

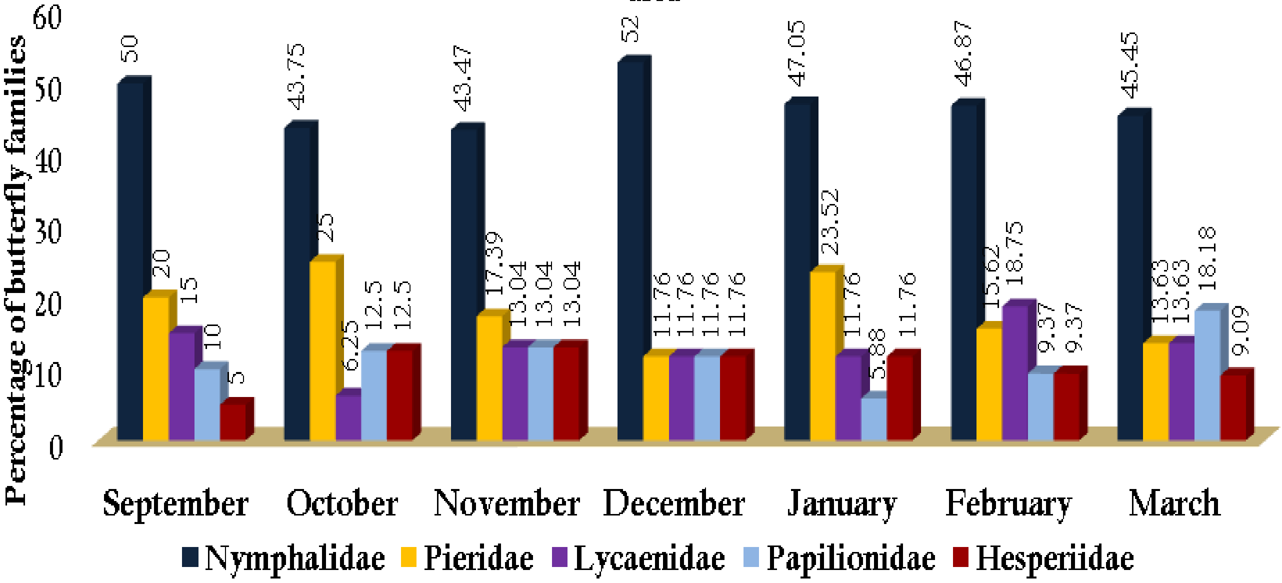
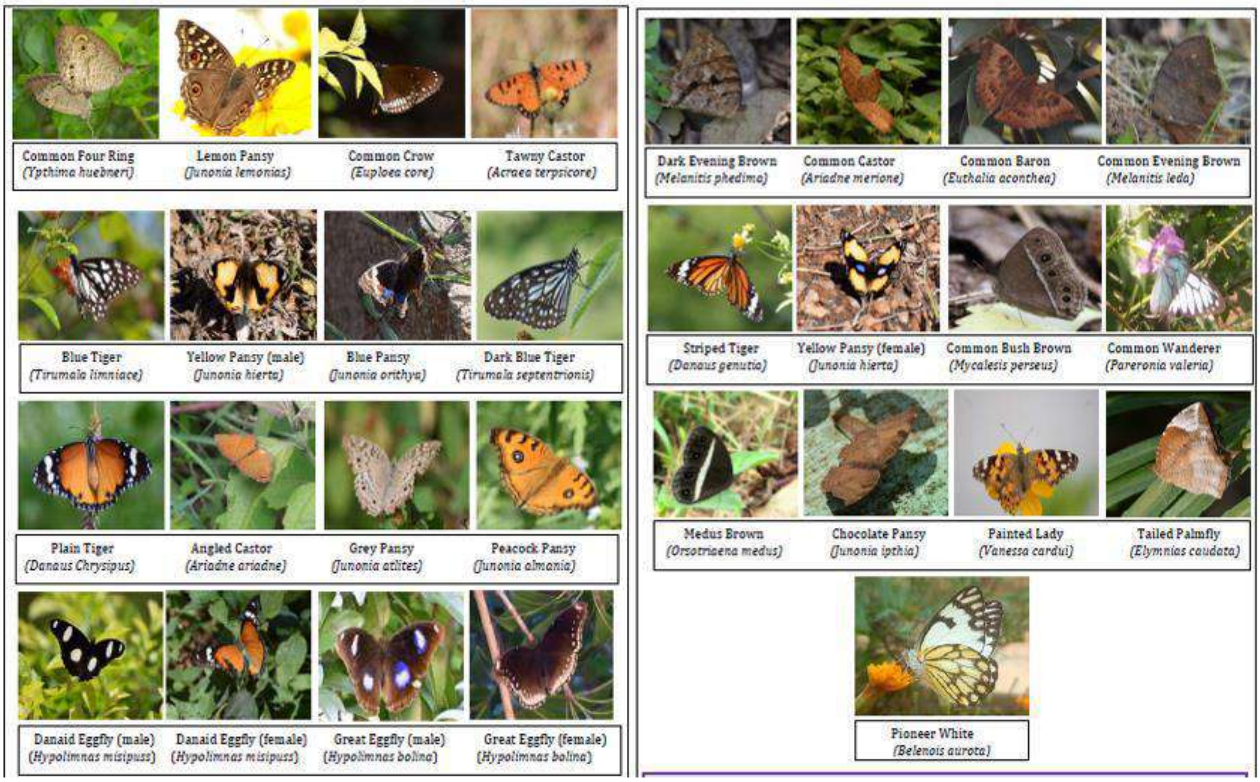
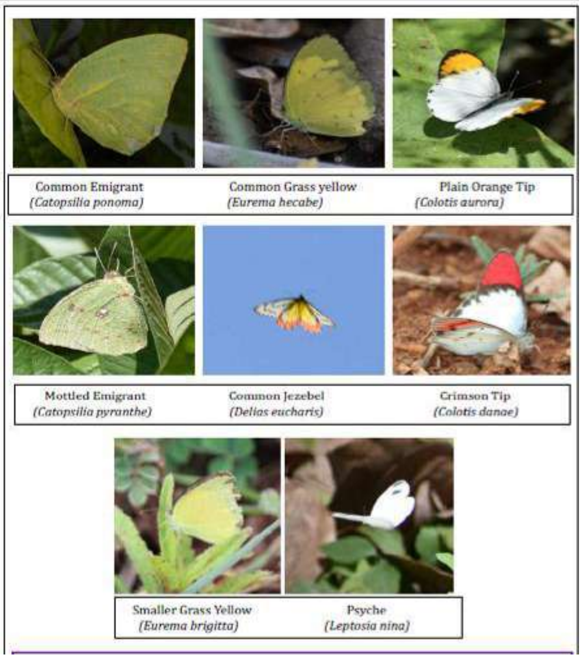


Plate.1 Butterflies of Nymphalidae family recorded in the study area

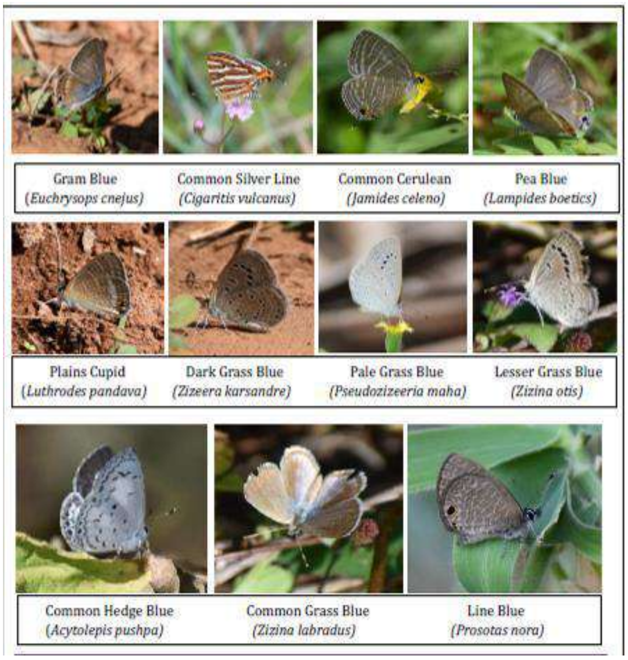


A **B**

Plate-2 Butterflies of Pieridae (C) and Lycaenidae (D) families recorded in the study area



C



D

Plate.3 Butterflies of Papilionidae (E) and Hesperiidae (F) families recorded in the study area

E

F

REFERENCES

[1] Aurora, K. (2003). Forest Laws. The Wildlife Protection Act, 1972 as amended by the Wild (Protection) Amendment Act, 2002. Professional Book Publishers, New Delhi, 85pp.

[2] Brereton T., D. B. Roy, I. Middlebrook, M. Botham, and M. Warren, (2011) "The development of butterfly indicators in the United Kingdom and assessments in 2010," Journal of Insect Conservation, vol. 15, no. 1, pp. 139–151.

[3] Evans, W.H. (1932). The identification of Indian Butterflies, 2nd Edition, Natural History Society, Bombay, India.

[4] Gupta, M.B., Rao, P.V.S., Reddy, D.S., Maddala, S.R.S.C.S. and Babu, P.M. (2012). A Preliminary Observations on Butterflies of Seshachalam Biosphere reservoir, Eastern Ghats, Andhra Pradesh, India, World Journal of Zoology, 7(1): 83-89.

[5] Harisha M.N., B.B. Hosetti (2013) Butterfly fauna of Daroji Sloth Bear Sanctuary, Hospet, Bellary District, Karnataka Journal of Research in Biology 3 (2), 840-846

[6] Kunte, K. (1997). Seasonal patterns in butterfly abundance and species diversity in four tropical habitats in Northern Western Ghats, Journal of Biosciences, 22: 593-603.

[7] Kunte, K., Sondhi, S., Sangam, B.M., Lovalekar, R., Tokekar, K. and Agarvekar, G. (2012). Butterflies of Garo Hills of Meghalaya, Northeastern India: their diversity and conservation, Journal of Threatened Taxa, 4(10): 2933-2992.

[8] Murphy D.D.and S.B.Weiss, (1988) "A long-term monitoring plan for a threatened butterfly," Conservation Biology, vol. 2, pp. 367–374,.

[9] Myers, N., Russell, A., Mittermelert, C., Mittermelert, G., Gustavo, A. B. and Fonseca, K. J. (2000), Biodiversity hotspots for conservation priorities. Nature, 403, 853- 858.

[10] Smith, C. (2006). Illustrated Checklist of Nepal's Butterflies. Craftsman Press, Bangkok.

[11] Sparrow H.R, T.D.Sisk, P.R.Ehrlich, and D.D.Murphy, (1994) "Techniques and guidelines for monitoring neotropical butterflies," Conservation Biology, vol. 8, no. 3, pp. 800–809.

[12] Spitzer, K.. J. Jaroˇs, J. Havelka, and J. Lepˇs, (1997) "Effect of smallscale disturbance on butterfly communities of an Indochinese montane rainforest," Biological Conservation, vol. 80, no. 1, pp.9–15.

[13] Sprih Harsh, (2014) "Butterfly Diversity of Indian Institute of Forest Management, Bhopal, Madhya Pradesh, India" Journal of Insects, Vol 2014, Art. ID 254972.

[14] Talbot, G.(1947). The Fauna of British India including Ceylon and Burma butterflies, 2nd Edition, Volume-II, Taylor and Francis Ltd., London, UK.

[15] Van der Poel, P. and T. Wangchuk. (2007). Butterflies of Bhutan Mountains, hills and valleys between 800 and 3000m. Royal Society for Protection of Nature (RSPN), Thimphy, Bhutan.

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Selected formulations of *Bacillus cereus* strain SLBE3.1AP with different storage durations for control *Fusarium oxysporum* f. sp. *capsici* Chili Plants

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***Abstract*— *The main disease in chili is fusarium wilt caused by Fusarium oxysporum f. sp. capsici that can cause losses up to 100%. The aim of this study was to obtain a solid formula for the endophytic bacterium Bacillus cereus strain SLBE3.1AP with a carrier that can be stored longer and is effective for controlling Fusarium oxysporum f. sp. capsici. This research was an experimental study to see the ability of the solid formula of Bacillus cereus bacteria strain SLBE3.1AP in controlling Fusarium oxysporum f. sp. capsici using Completely Randomized Design consisting of 15 treatments and 3 replications. The treatments consisted of carrier material for the formula bagasse, rice straw, bran, fungicide and control. Each solid formula of Bacillus cereus strain SLBE3.1.AP was introduced to chili seeds and seedlings. The results showed that the best formula for controlling Fusarium oxysporum f.sp. capsici and increasing the growth of chili plants was a solid formula of B. cereus strain SLBE3.1AP with 6 weeks storage of bagasse, 4 weeks of storage of rice straw, and 6 weeks of storage of rice bran.*

Keywords*— Bacillus cereus, chili, solid formula, Fusariumoxysporum f. sp. capsici, fusarium wilt.

I. INTRODUCTION

Red chili (*Capsicum annuum* L) is one of the horticultural crop commodities that have high economic value, so it is widely cultivated by farmers (Saptana *et al*., 2010). The higher demand for chili is sometimes not matched by the results of chili production. Chili productivity in Indonesia was relatively stable from 2014 to 2018, namely 8.35; 8.65; 8.47; 8.46; and 8.82 tons/ha (Central Bureau of Statistics, 2019). However, the productivity is stillclassified as low from the potential productivity of chili which can reach 12-15 tons/ha (Hadiyanti, 2016). One of the causes of the low productivity of chili is the attack of plant pathogens (Vivaldy *et al*., 2017).

One of the main diseases of chili is Fusarium wilt caused by *Fusarium oxysporum* f. sp. *capsici* (Yanti *et al*., 2020), Fusarium wilt disease results in losses and crop failure up to 50% if not controlled optimally (Rostini, 2011). *F. oxysporum*f. sp. *capsici* which is a pathogen of Fusarium wilt disease is a soil borne pathogen that is a soil inhabitant and can survive in extreme conditions with a chlamydiospore survival structure even in the absence of a host. *F. oxysporum* f. sp. *capsici* infects plants through wounds on the roots and inhibits the flow of water in the xylem tissue causing the plants to wilt (Chehri *et al*. 2010).

Efforts to control *F. oxysporum* f. sp. *capsici* that have been recommended include mechanically removing diseased plants, resistant varieties, crop rotation (Sila and Sopilena, 2016), and synthetic fungicides active ingredient Mancozeb (Sari, 2020) which can have a negative impact on the environment. Based on this, an alternative control is needed, namely: by utilizing microorganisms as biological control agents (Natalia *et al*., 2014). One of the biological agents that has been tested to control plant diseases and is widely used is endophytic bacteria (Sahu *et al*., 2019). Endophytic bacteria are bacteria that live in plant tissues and do not cause disease or significant morphological changes in plants (Wang *et al*., 2019).

One type of endophytic bacteria that has been widely used as a biological agent is Bacillus spp. because of its ability to sporulate and easily biodegradable by the environment. Biocontrol agents from *Bacillus* spp. including *B. pseudomycoides*, *B. mycoides*, *B. thuringiensis*, and *B. cereus* (Yanti *et al*., 2019). The successful use of *Bacillus* spp. which can inhibit the growth of the *Fusarium oxysporum* (Diarta *et al*., 2016). The use of *Bacillus* spp. singly causes *Bacillus* spp. cannot last long and is less than optimal both as a biocontrol agent and as a bioactivator, this is because bacteria need nutrients so that bacterial formulations need to be made (Oktrisna *et al*., 2017; Yanti *et al*., 2017).

The aim of the study was to obtain a solid formula of *B. cereus* strain SLBE3.1AP which was effective in controlling *Fusarium oxysporum*f. sp. *capsici* on chili plants.

II. METHODS OF RESEARCH

The research was carried out on March-September 2021 at the Microbiology and Phytopathology Laboratory, Department of Plant Diseases Pests and Experimental Gardens, Faculty of Agriculture, Andalas University, Padang. Studyis experimental using Completely Randomized Design (CRD) with 15 treatments and 3 replications. The treatment consisted of formula *B. cereus* strain SLBE3.1AP with various carrier materials from organic waste, namely: bagasse (AT), bran (D) and rice straw (JP) storage 0, 2, 4, and 6 weeks with a ratio of 1 :1 (v/v), fungicide treatment with the active ingredient Mancozeb, treatment without formulation and without inoculation of *F. oxysporum* f. sp. *capsici* (positive control), treatment without formulation and inoculation with *F. oxysporum* f. sp. *capsici* (negative control).

Propagation of B. cereus strain SLBE3.1AP

Propagation of *B. cereus* strain SLBE3.1AP was carried out in liquid culture by means of pure cultures of *B. cereus* strain SLBE3.1AP aged 2x24 hours were taken, put into 25 ml of NB medium in a culture bottle (volume 50 ml) and incubated on a rotary shaker for 24 hours. Next, 1 ml of preculture results were transferred to 49 ml of sterile coconut water in a culture bottle (100 ml volume) for mainculture and incubated in the same way for 2x24 hours at 150 rpm. Population density was determined by comparing the turbidity of the bacterial suspension with a *McFarland* scale 8 solution (population density estimated at 10^8 cells/ml) (Klement *et al*., 1990).

Preparation of solid formula carrier *B. cereus* strain SLBE3.1AP

The soft part of the bagasse is taken and cut into small pieces and then blended, the tofu pulp is put into aluminum foil and then baked, the rice straw is cut into small pieces then blended and the bran is filtered to get a smooth part. 9.5 g of each carrier was taken and put into a 100 ml Schott bottle and added 0.5 g of sucrose, then sterilized using an autoclave at 1 atm pressure at 121^{o}C for 15 minutes. The formula is cooled and 5 ml of suspension is added *B. cereus* strain SLBE3.1AP from mainculture 10^8 cells/ml. Each formula was stored at room temperature and incubated for 0, 2, 4, and 6 weeks (Yanti *et al*., 2017).

Pathogenicity Test

Foc inoculated by injuring the roots of the chili plants with scissors, then the 10 g *Foc* rice substrate was immersed into the soil 3 cm around the roots of the chili plants that had been injured. If the plant shows symptoms of wilting, the inoculum is classified as a pathogen (Chamzurni *et al*., 2010).

Propagation *Foc*

Propagation of Foc using rice media, as much as 2.5 kg of rice divided by 10 g for each treatment. The rice is washed and then the rice is dried, then put into a plastic 10x20 cm and sterilized in an autoclave at a temperature of 1210 C for 30 minutes. After the cold rice, the *Foc* cultures were cut 1x1 cm to be inoculated into the rice substrate and incubated for 21 days.

Introduction of formula *B. cereus* strain SLBE3.1AP

The introduction of the *B. cereus* formula SLBE3.1AP strain was carried out 2 times, namely at seeding and planting for 15 minutes.

Inoculation *Foc*

Before planting, the planting medium was inoculated with *Foc* 1 g. The *Foc* rice substrate was transferred to a test tube containing 10 ml of sterile distilled water, then homogenized with a vortex, the suspension was taken with a dropper and the number of conidia was counted using a haemocytometer under a microscope with a magnification of 40x10. The population used for inoculation into chili plants was 10^6 conidia/ml. Foc was inoculated by immersing 9 g of Foc rice substrate into the soil to a depth of approximately 3 cm to maintain soil moisture, the inoculated planting medium was covered with transparent plastic for 3 days, so that *F. oxysporum* f. sp. *capsici* grows well (Chamzurni *et al*., 2010).

Observation

Observations were made on disease development, seedling growth and chili plant growth. The data were analyzed by means of variance, if significantly different then continued with Least Significance Differences (LSD) at the 5% level.

III. RESULTS AND DISCUSSION

The introduction of a solid formula of *B. cereus* strain SLBE3.1AP with different storage times in chili plants, the results showed that all introduced formulas were able to suppress the development of Fusarium wilt disease. All formulas of *B. cereus* strain SLBE3.1AP were stable in suppressing incubation period, disease incidence and severity of Fusarium wilt disease in chili plants. This is presumably due to the type of formula, the nutritional content and the shelf life of the formula, the formula comes from endophytic bacteria which has many enzymes and carriers contain nutrients needed by bacteria to thrive. The best formula has a shelf life of 6 weeks during which time *B. cereus* can produce several resistance compounds. This is in accordance with the opinion of Taghavi *et al*., (2005) in Yuniawati *et al*., (2019) endophytic bacteria are able to produce enzymes, salicylic acid, ethylene and secondary metabolite compounds that play a role in inducing plant resistance. According to Hallman *et al*., (2009) in Munif (2003) before pathogens attack plants, endophytic bacteria that have been associated with plants can act as biological control agents, this is in accordance with the method in this study, namely the introduction of solid formula *B. cereus* strain SLBE3 .1AP before sowing and planting chili seeds,It is hoped that the antagonist bacteria will be able to suppress the growth and development of the fungus *F. oxysporum* which in turn can reduce the attack rate. The results of this study are also in accordance with the research of Yanti *et al.* (2017), which states that there are5 isolates Indigenous endophytic bacteria are one of them *B. cereus* strain SLBE3.1AP capable reduce the incidence of Fusarium wilt up to 100%.

The solid formula of *B. cereus* strain SLBE3.1AP introduced into chili seeds was able to increase chili growth in the seedling phase. The formula was able to increase seedling field emergence, seedling height, number of seedling leaves, wet weight and dry weight of seedlings compared to control (without treatment). From the research results, the stable formula in increasing seedling growth with 100% effectiveness was the rice straw formula which was stored for 4 weeks and the bagasse formula which was stored for 6 weeks. This is presumably because the best formula is a combination of carriers, which means that the more carriers there are, the more nutrients are contained in the formula, thereby increasing root fertility and plant growth.

Research result This is in accordance with the research of Resti, *et al*. (2018) which reported that endophytic bacteria can increase seedling growth and emergence of chili seedlings by 95% compared to controls. According to Lisnawita *et al* (2016), the increase in plant growth, both plant height and number of leaves, was positively correlated with the contribution of hormones produced by endophytic bacterial isolates. This is in line with the results of research by Marum *et al*. (2012), who reported that the application of bagasse can increase leaf area, dry weight and fresh weight of plants and provide effective yield growth of radish plants. This is becauseThe organic content of bagasse can reach 50% and has great potential as a source of organic matter that is useful for soil fertility (Ayu, 2018). Furthermore, Aldi *et al*. (2016) reported that the PGPR formulation in chilican accelerate the emergence of chili flowers at the age of 54 DAP with an average flower appearance of 84% of the total experimental plants and increase the number and weight of chilies.

IV. CONCLUSION

The best formula for controlling *Fusarium oxysporum* f.sp. *capsici* and increasing the growth of chili plants was a solid formula of *B. cereus* strain SLBE3.1AP with 6 weeks storage of bagasse, 4 weeks of storage of rice straw, and 6 weeks of storage of rice bran.

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REFERENCES

[1] Aldi, SES, Wuryandari, Y., Radiyanto, I. 2016. Growth and Production Response of Chili Plants Due to Administration of Formulas with Active Pseudomonas Fluorescent Isolate 122 in Various Forms and Doses. *Journal of Plumula* 5(2): 1-13.

[2] Ayu, DPS 2018. The Effect of Combination of Sugarcane Bagasse and Tea Bagasse as a Planting Media on the Growth of Mustard Pakchoy (Brassica rapa) and Its Contribution to High School Biology Learning. [Thesis] Faculty of Teacher Training and Education, Sriwijaya University.

[3] Central Bureau of Statistics. 2019. Center for Agricultural Data and Information, Ministry of Agriculture of the Republic of Indonesia. Jakarta.

[4] Chamzurni, T., Abduh, MU, Edi, D. 2010. Resistance Test of Several Tomato Varieties Against Wilting Disease. *Journal of Agista* 14(2): 62-67.

[5] Chehri, K., Saeed, TJ, Kasa, RNR, Saeed, A., Baharuddin, S. 2010. Occurrence of *Fusarium* spp. and Fumonisins in Stored Wheat Gains Marketed in Iran. *Toxins* 2(12): 2816-2.

[6] Diarta, IM, Javandira, C., and Widnyana, IK 2016. Antagonistic Bacteria *Pseudomonas* spp. and *Bacillus* spp. Against *Fusarium oxysporum* Fungus Causes Plant Wilt Disease. *Journal of Bakti Saraswati* (JBS), 5(1).

[7] Hadiyanti, D. 2016. Variety Improvement Can Increase Red Chili Productivity and Farmers' Income on Dry Land (Case Study in Lubuk Saung Village, Banyuasin III District, Banyuasin Regency). Proceedings of the 2016 National Seminar on Suboptimal Land, Palembang 20-21 October: 649-655.

[8] Hallman, JA., Quadt, HA., Mahafee, WF. and Kloeper, JW. 1997. Bacterial Endophytes in Agricultural Crops. Canadian Journal of Microbiology. 43:895-914.

[9] Klement, Z., Rudolph, K. and Sand, DC 1990. Methods in Phytopathology. Hungary: Kiado Academy.

[10] Lisnawita., Tantawi, AR, Safni, I., Khairunnisa. 2016. Utilization of Agricultural Waste and Local Antagonist Microorganisms to Control Root Chole Nematodes (*Meloidogyne* spp.) on Potato Plants. North Sumatra University Research Institute.

[11] Marum, J., Zulfita, D., Maulidi. 2012. Effect of Sugarcane Bagasse Compost on Radish Plant Growth and Yield on Red Yellow Podsolic Soil. *Agricultural Student Science Journal* 2(1): 1- 14.

[12] Munif, A. 2003. The Role of Endophytic Microbes as Biological Agents in Supporting Sustainable Agriculture. National Seminar and Product Title in the Field of Life Sciences. Management and Utilization of Biodiversity in the Framework of Sustainable Development. Bogor.

[13] Natalia, AG, Aeny, TN, and Prasetyo, J. 2014. Effectiveness Test of Trichoderma Spp. with Different Mixed Materials in Inhibiting the Growth of *Sclerotium rolfsii* Causes Sprout Sprout Disease in Peanuts. *Journal of Tropical Agotek*, 2(3)

[14] Oktrisna, D., Puspita, F., Zuhry, E. 2017. Bacterial Test of *Bacillus* sp. formulated with Some Waste against Rice Field Plants (*Oryza sativa* L.). Let's Faperta. 4(1): 1-12.

[15] Resti, Z., Sulyanti, E., Reflin. 2018. Consortium of Endophytic Bacteria as Biological Control of *Rastolnia solanacearum* and Stimulator of Chili Plant Growth. Prom Semnas Masy Biodiv Indonesia. 4(2): 208-214.

[16] Rostini, N. 2011. How to Grow Chilies Free of Pests and Diseases. Jakarta: PT Ago Media Pustaka.

[17] Sahu, PK, Singh, S., Gupta, A., Singh, UB, Brahmaprakash, GP, and Saxena, AK 2019. Antagonistic Potential of Bacterial Endophytes and Induction of Systemic Resistance Against Collar Rot Pathogen *Sclerotium rolfsii* in Tomato. Biological Control, 137, 104014.

[18] Saptana., Daryanto, A., Daryanto, HK, Kuntjoro. 2010. Analysis of Technical Efficiency of Big Red Chili Farming Production and Farmer Behavior in Facing Risk. Journal of Ago Economics. 28(2): 153-188.

[19] Sari, AL 2020. The Effectiveness of Contact Fungicide with Active Mankozeb in Controlling Potato Leaf Blight (Phytophthora infestans) in Karo Regency on Wet Months and in the Laboratory. [Thesis] University of North Sumatra. Medan.

[20] Schaad NW, Jones JB, And Chun W. 2001. Laboratory Guide for Identification of Plant Pathogenic Bacteria. St Paul: The American Phytopathological Society. 44(4): 546-546.

[21] Sila, S., and Sopialena. 2016. The Effectiveness of Several Fungicides on Disease Development and Production of Chili Plants (Capsicum frutescens). Agifor Journal. 15(1): 117-130.

[22] Taghavi, S., Barac, T., Geenberg, B., Borremans, B., Vangonsveld, J., and Van Der Lelie, D. 2005. Horizontal Gene Transfer to Endogenous Endophytic Bacteria from Poplar Improves Phytoremediation of Toluene. Applied and environmental microbiology, 71(12), 8500-8505.

[23] Vivaldy, LA, Max, R., Guntur, M. 2017. Incidence of Virus Disease in Chili Plants (Capsicum anuum) in Kakaskasen II Village, North Tomohon District, Tomohon City. Journal of In cocos. 1(6): 1-9.

[24] Wang, SS, Liu, JM, Sun, J., Sun, YF, Liu, JN, Jia, N., and Dai, XF 2019. Diversity of Culture-Independent Bacteria and Antimicrobial Activity of Culturable Endophytic Bacteria Isolated from Different Dendro bium stems. Scientific reports, 9(1), 1-12.

[25] Yanti, Y., Habazar, T., Resti, Z. 2017a. Solid Formulation of Rhizobacteria Indigenous Bacillus thuringiensis Ts2 and Storage Time to Control Pustule Disease of *Xhantomonas axonopodis* vp. *Glycine*. *Journal of Tropical Plant Pests and Diseases*. 17(1):9-18.

[26] Yanti, Y., Warnita., Reflin., Busniah, M. 2017b. Identificatiion and Characterizations of Potential Indigenos Endophytic Bacteria which Had Ability to Promote Gowth Rate of Tomato and Biocontrol Agents of *Ralstonia solanacearum* and *Fusarium oxysporum* f. sp. *solani*. *Indonesian Microbiology*. 11(4): 117-122.

[27] Yanti, Y., Hamid, H., and Habazar, T. 2020. The Ability of Indigenous Bacillus spp. Consortia to Control the Anthracnose Disease (*Colletrotricum capsici)* and Increase the Gowth of Chili Plants. *Biodiversity*. 21(1): 179–186.

[28] Yuniawati, R., Fatimah, S., Indrayati. R., Manzila, I., Priyatno, T., Susilowati, D. 2019. Improving Growth and Quality of Big Red Chili Fruit with Growth Hormone from Endophytic Bacteria. *Journal of Ago Biogen* 15(2): 75–82.

FIGURES AND TABLES

Table 1. The development of Fusarium wilt disease in chili plants introduced by each treatment

Treatment		Disease Progress						
Carrier Material	Storage Time	Incubation Period	Effectiveness (%)	Disease Incidence (%)	Effectiveness (%)	Disease Severity (%)	Effectiveness (%)	Attack Criteria
Bagasse	0	21.00 cde	117.16	100.00 a	0.00	41.67 ab	16.53	Heavy
Bagasse	2	29.67 abc	206.82	33.33 ab	66.67	16.00 bc	63.90	Light
Bagasse	4	30.67 ab	217.16	33.33 ab	66.67	14.00 bc	68.41	Light
Bagasse	6	34.00 a *	251.60	0.00 b*	100.00	0.00 c*	100.00	Healthy
Rice straw	0	19.00 de	96.48	100.00 a	0.00	37.67 ab	15.02	Heavy
Rice straw	2	27.33 abcd	182.62	66.67 ab	33.33	24.67 abc	44.34	Currently
Rice straw	4	29.67 abc	206.82	33.33 ab	66.67	15.00 bc	66.16	Light
Rice straw	6	30.67 ab	217.16	33.33 ab	66.67	16.33 bc	63.16	Light
Bran	0	18.33 ef	89.55	100.00 a	0.00	39.33 ab	15.02	Heavy
Bran	2	30.67 ab	217.16	33.33 ab	66.67	16.33 bc	63.16	Light
Bran	4	30.67 ab	217.16	33.33 ab	66.67	15.67 bc	64.65	Light
Bran	6	30.67 ab	217.16	33.33 ab	66.67	11.67 bc	73.67	Light
Mancozeb	-	24.33 bcde	151.60	66.67 ab	33.33	30.33 abc	42.09	Currently
+ control	-	34.00 a *	251.60	0.00 b*	100.00	0.00 c*	100.00	Healthy
Control -	-	9,667 f	0.00	100.00 a	0.00	54.00 a	0.00	Very heavy

*plants did not show symptoms until the end of observation (34 DAI)

*Numbers followed by the same lowercase letter in the same column are not significantly different according to LSD at the 5% level.

Table 2. Growth of chili seedlings introduced for each treatment

Treatment		Seed growth										
Carrier Material	Storage Time	Power appears field	Effectiveness (%)	Seedling height (cm)	Effectiveness (%)	Number of leaves (strands)	Effectiveness (%)	Effectiveness (%)	Wet weight (gam)	Effectiveness (%)	Dry weight (gam)	Effectiveness (%)
Bagasse	0	96.00	27.78	11.00 ij	26.87	5.66 bcde	62.00	26.08	0.12 jkl	33.33	0.02 ghij	00.00
Bagasse	2	96.00	27.78	11.50 gh	32.64	5.33 de	52.28	78.26	0.23 cd	155.55	0.05bcde	150.00
Bagasse	4	96.00	27.78	10.33 k	23.06	5.99 ef	42.85	78.26	0.14 ghijk	55.55	0.04 efgh	100.00
Bagasse	6	100.00	33.33	12.33 de	42.21	6.33 ab	80.85	147.82	0.26 bc	188.88	0.06 abc	200.00
Rice straw	0	96.00	27.78	11.67 fg	34.60	5.33 de	52.28	43.47	0.12 cl	33.33	0.03 fghi	50.00
Rice straw	2	96.00	27.78	11.00 ij	26.87	6.00	71.42	113.04	0.19def	111.11	0.04 def	100.00

						bcd						
Rice straw	4	100.00	33.33	12.83 bc	47.98	5.66 bcde	62.00	113.04	0.22 cd	144.44	0.05 cde	150.00
Rice straw	6	100.00	33.33	12.00 ef	38.40	5.66 bcde	62.00	104.34	0.15 ghijk	66.67	0.05 cde	150.00
Bran	0	92.00	22.22	11.33 ghi	30.68	5.33 de	52.28	39.13	0.08 l	- 4.44	0.01 j	- 50.00
Bran	2	100.00	33.33	12.33 de	42.21	5.33 de	52.28	69.56	0.15 ghijk	66.67	0.02 ghij	00.00
Bran	4	96.00	27.78	10.66 jk	19.14	5.66 bcde	62.00	95.65	0.13 hijk	44.44	0.07 ab	250.00
Bran	6	96.00	27.78	12.50 cd	44.17	6.00 bcd	71.42	56.52	0.20 de	122.22	0.04 efgh	100.00
Mancozeb	-	92.00	22.22	11.16 hi	28.83	5.16 ef	47.71	21.73	0.17 efgh	88.89	0.02 hij	00.00
Control	-	75.00	00.00	8.67 l	00.00	3.50 g	00.00	00.00	0.09 l	00.00	0.02 ij	00.00

*Numbers followed by the same lowercase letter in the same column are not significantly different according to LSD at the 5% level.

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FTIR (Fourier transform infrared spectroscopy) spectroscopic analysis of dried leaf and fruit peel extract of *Capparis divaricata lam.*

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***Abstract*— *Capparis divaricata lam commonly known as caper bush, belonging to genus Capparidaceae, found throughout India. In this study, we determine the phytocomponents in dried leaf and fruit peel extract of Capparis divaricata lam (cappardaecea).The FTIR (Fourier transform infrared spectroscopy) spectroscopy is an essential tool for determining the composition and structure of organic compounds. The FTIR spectroscopy is an essential tool for profiling biochemical compounds that exist in herbal extraction, FTIR method was selected because it is a very rapid and economic method for the characterizing of a functional group. The dried sample has been taken for the identification of chemical bonds which are present in the plant sample. The FTIR peaks analyzed in leaf shows the OH, CH_2, C=C, C-OH, CH_3 and CH, bonds while in fruit peel it shows OH, CH_2, C=C, C-OH, CH_3, CH and C=O bonds. The presence of C=O bond tells us that they are useful in organic synthesis catalysis and as catalyst precursors in homogenous catalysis. The CH_3 bonds suggest that the Methyl containing Amino Acid is present. The C-O-H group indicates the presence of Fatty Acids. Silicones and Sulfones the presence of various biological activities and are therapeutic targets. All the identified phytocomponents are having pharmacological activity and absorbance bond shows strong, stretching, symmetric and asymmetric bonds. So Capparis divaricata lam can be considered as a plant of phytopharmaceutical importance.*

***Keywords*— Capparis divaricata lam, fruit peel, leaves, FTIR.**

I. INTRODUCTION

Medicinal plants are a significant part of natural wealth. They have a large no of bioactive constituents therefore these plants are used to cure many infectious diseases. As per the reports of the world health organization (WHO), almost 80% of the global population depends on traditional medicine for the treatment of various disease and economic advantages. The various bioactive phytochemical constituents available in plants include alkaloids, saponins, glycosides, flavonoids, phenol, terpenes and carboxylic acid. Identification of the chemical present in the medicinal plants will provide some information on the different functional group responsible for their medicinal properties. Fourier Transform Infrared (FTIR) spectroscopy is an essential tool for determining the composition and structure of organic compounds. It is a very rapid and economical method for characterization of functional groups and creates an analytical data which is considered as fingerprinting of that particular sample. The Infrared spectrum which is obtained from the plants may show some small changes in the metabolites. According to Ramamoorthi and Kannan (2007) screened the bioactive group of chemicals in the dry leaf powder of *Calotropis gigantean* by FTIR analysis.

Kareru *et al*. (2008) detected saponins in a crude dry powder of 11 plants using FTIR spectroscopy. Muruganantham *et al.* (2009) carried out the FTIR spectroscopic analysis in the powder samples leaf, stem and root of *Ecliptaalba* and *Ecliptaprostrata.* The FTIR analysis of *Bauhinia racemosa* leaf extract in an aqueous methanolic solution for phytochemical compounds was done by Gauravkumar *et al.* (2010). Ragavendran *et al.* (2011) detected the functional groups in a various extract of *Aervalanata* using the spectroscopic method. Thangarajanstarlin *et al.* (2012) identified the elements and functional groups in the ethanol extract of the whole plant of *Ichnocarpusfrutescens* using FTIR spectroscopic method. Paraj.A.Pednekar and Bhanu Raman (2013) analyzed the methanolic leaf extract of *Ampelocissuslatifolia* through FTIR spectroscopy for an antimicrobial compound. So far, an FTIR analysis of the leaf and fruit peel extract of *Capparis divaricata* has not been done. Thus, we have attempted to analyse the functional groups of phytoactive compounds present in the leaf and fruit peel of *Capparis divaricata* by FTIR spectroscopic analysis.

II. MATERIALS AND METHODS

2.1 Collection of plant

Leaf and fruit sample of *Capparis divaricata* species were collected from Shingadgaon, Solapur, Maharashtra (India) in July. The specific plant species were identified with the help of Dr.Gore, Assistant professor of Walchand College of arts commerce and science Solapur.

2.2 Plant material

The leaf and fruit peel were washed thoroughly with running water and then with distilled water. The plant material was dried in shade dried for a couple of days and then dried in an incubator at 37^0C for 2-3 days. The dried leaves were then crushed in a mechanical grinder till it becomes a fine powder and then it was stored in an airtight container at room temperature.

2.3 Fourier transform infrared spectroscopy

A dry leaf and fruit peel powder of *Capparis divaricata* was taken. The dried leaf and fruit peel powder subjected to Fourier transform infrared (FTIR, IRA finite- university of Solapur, Solapur) spectroscopy measurement using the potassium bromide (KBr) pellet technique diffuse reflection mode at a resolution of 4cm^{-1}. The powder was mixed with KBr and exposed to an infrared source of 500 to 4000 cm^{-1}. A similar process was used for the FTIR studies of *Capparis divaricata* extract before and after bio-reduction.

III. FTIR ANALYSIS

Characterization of the biochemical molecules extracted from *Capparis divaricata* leaves and fruit peel depending on FTIR spectrum analysis is represented in fig. 1 and 2.

FTIR result revealed presence of hydroxyl group (OH) by peak at 3276.48cm^{-1},3292.24cm^{-1} 1417.74cm^{-}1,while frequency peak at 2918.24cm^{-1} ,2920.35 cm^{-1} , 2851.42 cm^{-1} refers to stretching of C-H aliphatic group, vibration peak (C=C aromatic) at,1621.48 cm^{-1} , 1615.67 cm^{-1} structure stretching frequency peak recorded at 1737.48 cm^{-1} assigned to presence (C=O), while (C-H and C-O) seems at 1019.22cm^{-1},1153.35 cm^{-1}, 1119.36 cm^{-1}, 1392.39cm^{-}1,1239.33 cm^{-}1. The 1579.82 cm^{-}1 shows (N-H bond). , 1007.00 cm^{-1}, 1330.59cm^{-}1, seems(S=O) bond. 1243.99cm^{-1}, 1320.99cm^{-1} seems (C-N) bond is present.

IV. RESULT AND DISCUSSION

The frequency of vibrational peak (ν) depends on two factors i.e., force constant and reduced mass, which can be explained by following equation.

$$\boxed{\nu = 1/2\pi c \sqrt{(k/\mu)}}$$

Here, c is the speed of light, k is force constant and μ is reduced mass.

If the reduced mass is constant, then the frequency is directly proportional to the force constant; therefore, increase in the frequency of any bond suggested a possible enhancement in force constant of the respective bond.

Table No. 1

Leaf extract of *Capparis divaricate*					
Sr no.	Peek	Bonds	Bond strength	Bond vibrations	Functional groups
1.	3276.48	O-H	Strong	Stretching	Alcohol
2.	2918.24	C-H	Medium	Stretching	Alkene
3.	1621.48	C=C	Strong	Stretching	α,β unsaturated ketone
4.	1579.82	N-H	Medium	Bending	Amine
5.	1417.74	O-H	Medium	Bending	Alcohol

6.	1320.99	C-N	Strong	Stretching	Aromatic amine
7.	1243.99	C-N	Medium	Stretching	Amine
8.	1153.35	C-O	Strong	Stretching	Aliphatic ether
9.	1119.36	C-O	Strong	Stretching	Secondary alcohol
10.	1007.66	S=O	Strong	Stretching	Sulfoxide

Table No. 2

Fruit peel extract *Capparis divaricata*					
Sr no.	Peek	Bonds	Bond strength	Bond vibration	Functional groups
1.	3292.24	O-H	Medium	Stretching	Alcohol
2.	2920.35	$C-H_2$	Strong	Stretching	Mainly lipids
3.	2851.42	$C-H_2$	Medium	Stretching	Mainly lipids
4.	1737.48	C=O	Strong	Stretching	δ- lactone
5.	1615.67	C=C	Strong	Stretching	α,β- unsaturated ketone
6.	1392.39	$C-H_3$	Medium	stretching	Phenol
7.	1330.59	S=O	Strong	Stretching	Sulfone
8.	1239.33	C-O	Strong	Stretching	Aliphatic ether
9.	1019.22	C-C, C-OH, CH ring and side group	Strong	Vibration	Anhydride

V. FTIR ANALYSIS

Presented data of FTIR strongly indicated the existence of phenolic compounds in *Capparis divaricata* leaves and presence of O-H group along with aromatic ring which consisting the basic unit of phenolic active components.

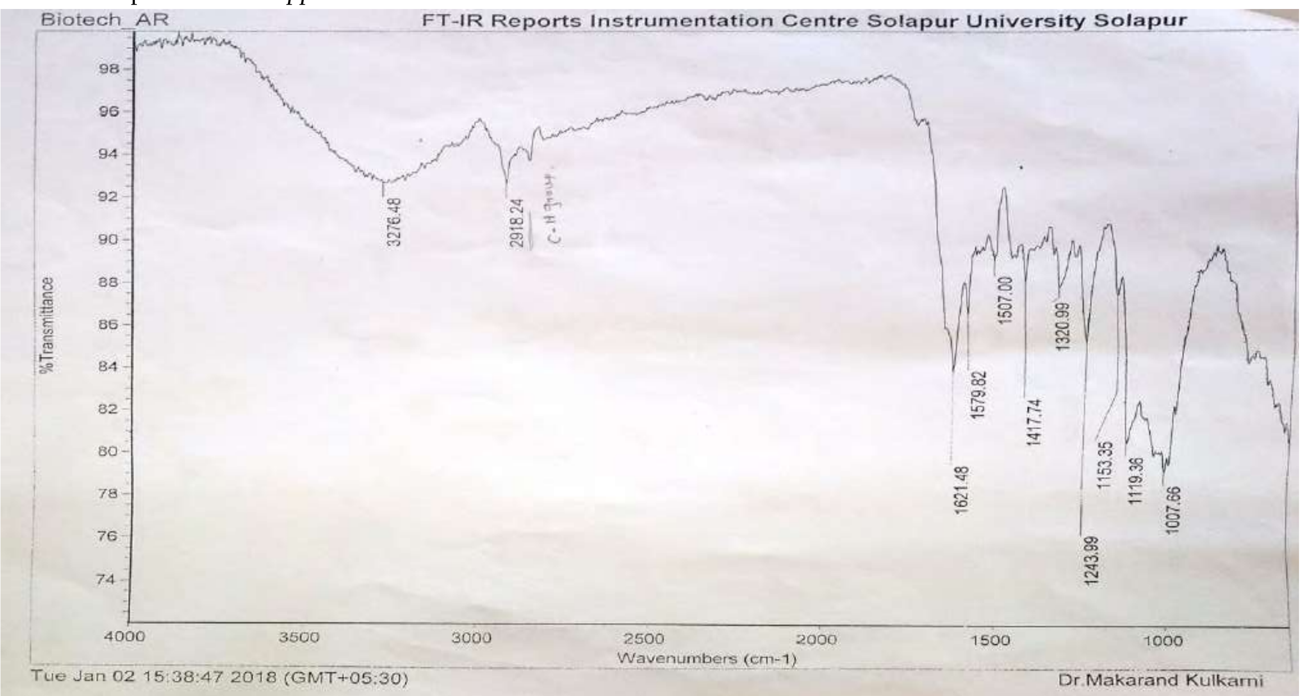

Fig.1: Leaf Extract (FTIR report of leaf extract of Capparis divaricata)

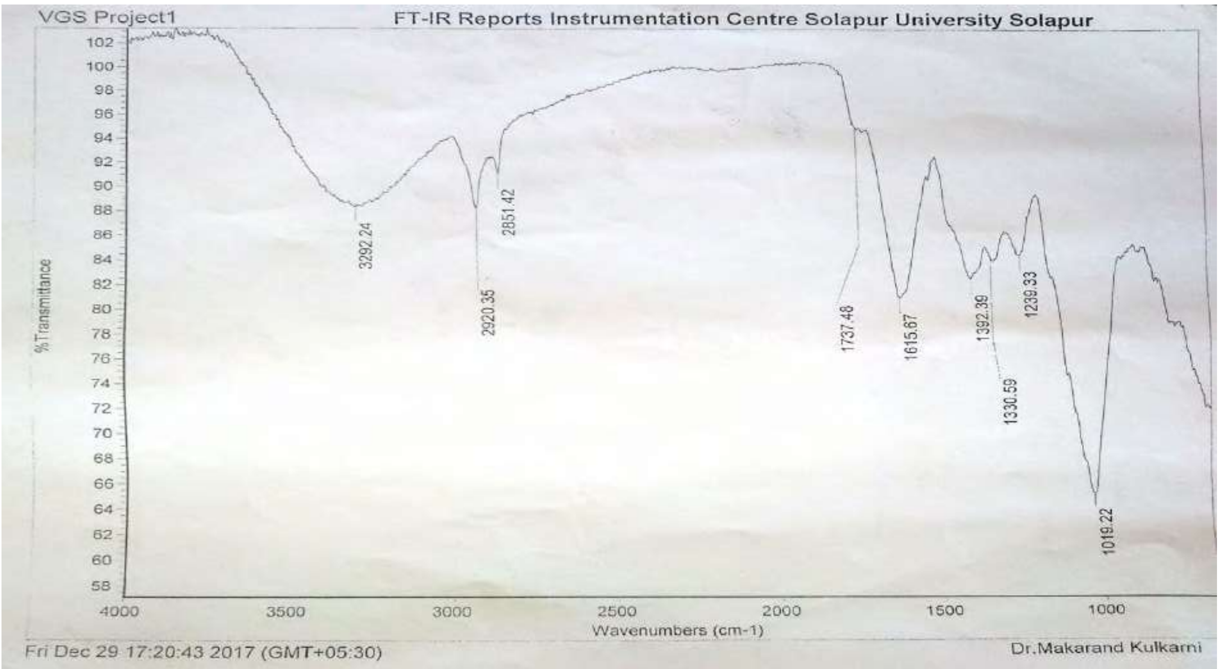

Fig.2: Fruit Peel (FTIR report of fruit peel extract of Capparis divaricata)

VI. CONCLUSION

The FT-IR data of *Capparis divaricate* plant extract i.e., fruit peel and leaves shows us the various active pharmaceutical ingredients. Characterization of biochemical molecule extracted from *Capparis divaricata* leaf and fruit peel depending on FT-IR spectrum analysis reveals the presence of hydroxyl group, absorption bond stretching peaks and vibrational aromatic ring. Present data of FT-IR strongly indicated the existence of phenolic compounds in *Capparis divaricata* leaves by the presence of OH group along with aromatic ring which consisting the basic unit of phenolic acetone components.

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REFERENCES

[1] Blessy M, Patel RD, Prajapati PN, Agrawal YK (2014) Development of forced degradation and stability indicating studies of drugs-A review. J Pharm Anal 4: 159-165.
[2] Cooper MA, Andrews JM, Ashby JP, Matthews RS, Wise R (1990) In-vitro activity of sparfloxacin, a new quinolone antimicrobial agent. J Antimicrob Chemother 26: 667-676.
[3] Davies M, Marks R (1976) Studies on the effect of salicylic acid on normal skin. Br J Dermatol 95: 187-192.
[4] Einstein A (1905) Does the inertia of a body depend upon its energy content. Ann Phys 18: 639-641.
[5] Garland SN, Valentine D, Desai K, Langer C, Evans T, Susan Li, Jun J mao. (2013) Complementary and alternative medicine use and benefit finding among cancer patients. J Altern Complement Med 19: 876-881.
[6] Guan XH, Chen GH, Shang C (2007) ATR-FTIR and XPS study on the structure of complexes formed upon the adsorption of simple organic acids on aluminium hydroxide. J Environ Sci (China) 19: 438-443.
[7] Gupta H, Aqil M, Khar RK, Ali A, Bhatnagar A, Mittal G. (2010) Sparfloxacin-loaded PLGA nanoparticles for sustained ocular drug delivery. Nanomedicine 6: 324-333.
[8] Hooper DC (2000) Mechanisms of action and resistance of older and newer fluoroquinolones. Clin Infect Dis 31: S24-S28.
[9] Hok J, Tishelman C, Polner A, Foess A, Falkenberg T (2008) Mapping patterns of complementary and alternative medicine use in cancer: an explorative cross-sectional study of individuals with reported positive “exceptional” experiences. BMC Complement Altern Med 8:48.
[10] Johnson JH, Cooper MA, Andrews JM, Wise R (1992) Pharmacokinetics and inflammatory fluid penetration of sparfloxacin. Antimicrob Agents Chemother 36: 2444-2446.
[11] Lenssen AW (2013) Biofield and fungicide seed treatment influences on soybean productivity, seed quality and weed community. Agricultural Journal 8: 138-143.
[12] Madan RK, Levitt J (2014) A review of toxicity from topical salicylic acid preparations.J Am Acad Dermatol70: 788-792.
[13] Mahendra Kumar Trivedi[1], Alice Branton[1*]Fourier Transform Infrared and Ultraviolet-Visible Spectroscopic

Characterization of Biofield Treated Salicylic Acid and Sparfloxacin. 10624 S Eastern Avenue Suite A-969, Henderson, NV 89052, USA

[14] Miner J, Hoffhines A (2007) The discovery of aspirin's antithrombotic effects. Tex Heart Inst J 34: 179-186.

[15] Pavia DL, Lampman GM, Kriz GS (2001). Introduction to spectroscopy. (3rdedn), Thomson learning,Singapor.

[16] Patil SA, Nayak GB, Barve SS, Tembe RP, Khan RR (2012) Impact of biofield treatment on growth and anatomical characteristics of*Pogostemoncablin* (Benth.). Biotechnology 11: 154-162.

[17] Planck M (1903) Treatise on Thermodynamics, (3rdedn) translated by Alexander OGG, Longmans, Green, London (UK).

[18] Raskin I (1992) Salicylate, A new plant hormone. Plant Physiol 99: 799-803.

[19] Salgado HRN, Moreno, PRH, Braga AL, Schapoval EES (2005) Photo degradation of sparfloxacin and isolation of its degradation products by preparative HPLC. J Basic ApplPharmSci 26: 47-54.

[20] Sances F, Flora E, Patil S, Spence A, Shinde V (2013) Impact of biofield treatment on ginseng and organic blueberry yield. Agrivita J AgricSci 35.

[21] Smith BC (1999) Infrared Spectral Interpretation: A systematic approach. CRC Press 1-288.

[22] Stein GE, Havlichek DH (1997) Sparfloxacin: Potential clinical and economic impact in the treatment of respiratory infections. Pharmacotherapy 17: 1139-1147.

[23] Stuart BH (2004) Infrared Spectroscopy: Fundamentals and applications (analytical techniques in the sciences (AnTs). John Wiley & Sons Ltd; Chichester, UK.

[24] Trivedi MK, Bhardwaj Y, Patil S, Shettigar H, Bulbule, A (2009) Impact of external energy on *Enterococcusfaecalis* [ATCC-51299] about antibiotic susceptibility and biochemical reactions-an experimental study. J Accord Integr Med 5: 119-130.

[25] Trivedi MK, Nayak G, Tallapragada RM, Latiyal O (2015) studies of the atomic and crystalline characteristics of ceramic oxide nano powders after biofield treatment, IndEng MANAFE 4:1000161.

[26] Trivedi MK, Patil S (2008) Impact of external energ on *Yersiniaenterocolitica* [ATCC-23715] about antibiotic susceptibility and biochemical reactions: an experimental study. Internet J Alternat Med 6-13.

[27] Trivedi MK, Patil S, Tallapragada RM (2013) Effect of biofield treatment on the physical and thermal characteristics of vanadium pentoxide powders. J Material SciEng S11, 001.

[28] Trivedi MK, Patil S, Tallapragada RM (2013) Effect of biofield treatment on the physical and thermal characteristics of silicon, tin and lead powders. J Material SciEng 2: 125.

[29] Trivedi MK, Patil S, Tallapragada RMR (2015) Effect of biofield treatment on the physical and thermal characteristics of aluminium powders. IndEng Manage 4: 151.

[30] Vane JR (1971) Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. Nat New Biol 231: 232-235.

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Growth of Lettuce (*Lactucasativa* L.) Plant Under Red-Blue-White Light and Grow Light LEDs in Plant Factory System

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***Abstract*— *Indoor culture requires a variety of inputs to get maximum biomass. These inputs are the nutrients, temperature, humidity, and light which plants needed to photosynthesize. Different types of light have been studied and it is known that the same spectrum will give different responses by different plants. The purpose of this study was to find out the effect of red-blue-white light LED on lettuce growth compared to grow light LED as a control which commonly used in plant factory rooms. The red-blue-white light is arranged on a 100 cm long aluminum rod, mounted along the plant in a gully DFT hydroponic fed by 1000-2000 ppm nutrients of ABmix plus with a pH of 5.5-6.5. LED grow light provided the plant a significantly higher height of 16.30% compared to red-blue-white light, but was no different to the length of lettuce root. The number and the area of leaves in red-blue-white light were markedly higher at 16.67% and 33.78% respectively than grow light. In addition, the red-blue-white light increased the chlorophyll content, fresh weight, and dry weight of lettuce plants, by 25.00%, 101.49% and 58.13% consecutively. Therefore, these results suggested that the red-blue-white LED light provided a significant higher biomass than the grow light LED.*

***Keywords*— plant factory, grow light LED, red-blue-white light LED, lettuce.**

I. INTRODUCTION

Foodstuffs, especially vegetables such as lettuce, is very useful for the health of the body. The vegetable should be produced close to consumers (in the city) to reduce transportation costs, but because of land limitation in urban areas, a new system is needed to grow indoor crops by utilizing technology so that products are cleaner and healthier, which we call such system is plant factory.

Plant factory requires various inputs such as nutrient solutions and other growing factors such as temperature, humidity, and light energy. Light bulbs are needed for plants to photosynthesize to produce biomasa. There has been a lot of research about the type of light spectrum with its combination to provide optimal intensity in spurring plant growth. According to Senger (1982), the same type of light will give a different mophogenesis response depending on plant species. Yorio *et al*. (2001) found that fluorescent cool-white light (CWF) is best light at spurring lettuce growth compared to red, blue, and green rays and their combinations.In contrast, Kim *et al*. (2004) showed different data, namely that red-blue LED lights with a 24% green supplement at wavelengths of 500-600 nm was most spur the growth of lettuce plants compared to all other types of combinations including CWF light. CWF is a light commonly used as a control of lights that are broad spectrum approaching to solar rays. Furthermore, Shimizu *et al*. (2011) showed that red monochromatic rays was best and markedly increased the fresh weight and dry weight of lettuce.Another study revealed that red-blue rays at a 4:1 ratio had the best effect on plant growth and chlorophyll leaf content compared to red-green-blue rays (Nguyen *et al*., 2021).Kobayashi *et al*. (2013) found that blue LED light rays stimulate vegetative growth and blue light spurs flowering.

In general, plants absorb spectrum rays at visible light wavelengths of 400-700 nm with concentrations in red and blue light. Plant chlorophyll absorbs unequal amounts of light energy to photosynthesize. Chlorophyll-a absorbs only blue-violet light (400-500 nm) and red light (650-700 nm) while chlorophyll-b requires only blue and orange spectrum (600-650 nm). Even a recent study conducted by Bugbee (2019) found that far red light was a light that can spur cell enlargement. Various types of rays and their combinations both in units of intensity, quality, energy, and wavelength in spurring plant growth are not yet known exactly. Therefore, research is needed on the use of red-blue-white LEDs light to know the comparison with grow light white LED. White grow light LEDs are lamps commonly used in plant factories that have been extensively researched.

II. MATERIALS AND METHODS

Materials and equipments

The materials used were the seedlings of the lettuce plant "Jonction RZ" in rock wool media, AB mix plus nutrient solution, pH lowering solution (nitric acid and phosphoric acid) and raw water. The equipments used were plant factory room, hydroponics system DFT (Deep Flow Technique), IoT System (which is able to show air humidity, room temperature, solution concentration, nutrient solution temperature, and able to regulate the pH of nutrient solution), white light LED polychromatic grow light (four spectrum peaks: λ = 450 nm, λ = 527-542 nm, λ = 608 nm, 54 watts), and red-blue-white combination LED lights with characteristic red light (λ=618 nm, 3 watts), blue (λ=452 nm, 2 watts), and white (λ=450 nm, λ=527-542 nm, λ=608 nm, 1 watt) (Figure 1).

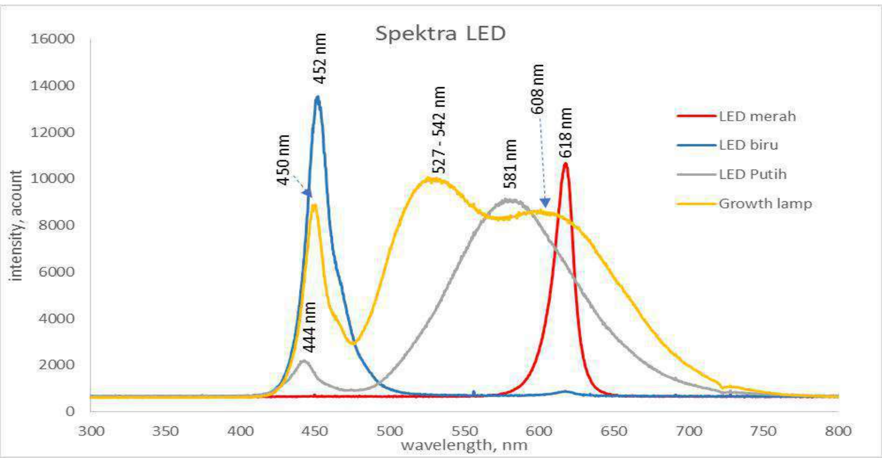

Fig.1: The spectrum of grow light LED and red-blue-white light LEDs were used in the study.

Seedling and planting

The seeds of *Lactuca sativa* L. cultivar "Jonction RZ" were immersed in rock wool with a size of 2 cm x 2 cm x 2 cm in a tray, then watered until saturated, covered with black plastic one night. Growing seedlings were then maintained and subjected to sunlight and watered every 2 days with a nutrient solution AB mix plus 500 ppm until two weeks old and transferred (transplanting) to gully in the DFT hydroponic system. The seedlingswere put into a net pot (basal diameter 3.5 cm, upper diameter 4.4 cm, height 5 cm) then placed in a gully hole with a distance of 20 cm.

LED light setup

The lights used in this experiment were grow light LED (Light Emitting Diode) lamp and a combined of red-blue-white light LED. Grow light LED lamp is a series of LED bulb with a total of 54 watts. Red-blue-white light LED lamp is a series of repeated 3 red bulb, 1 blue bulb, and 1 white bulb. The lampswere set along a hydroponic gully that was a total length of 3.6 meters. The lamp was turned on for 18 hours and then turned off for 6 hours intermittently as a photoperiodic for the process of photosynthesis and respiration of plants. The hydroponic system used was DFT (Deep Flow Technique) which has the advantage of water pooling as deep as 2 cm in gully that provides guarantees to plants still got nutrients even if the solution in gully stops flowing due to the pump damaged or the electricity went out. The DFT system scheme used in this study as Figure 2.

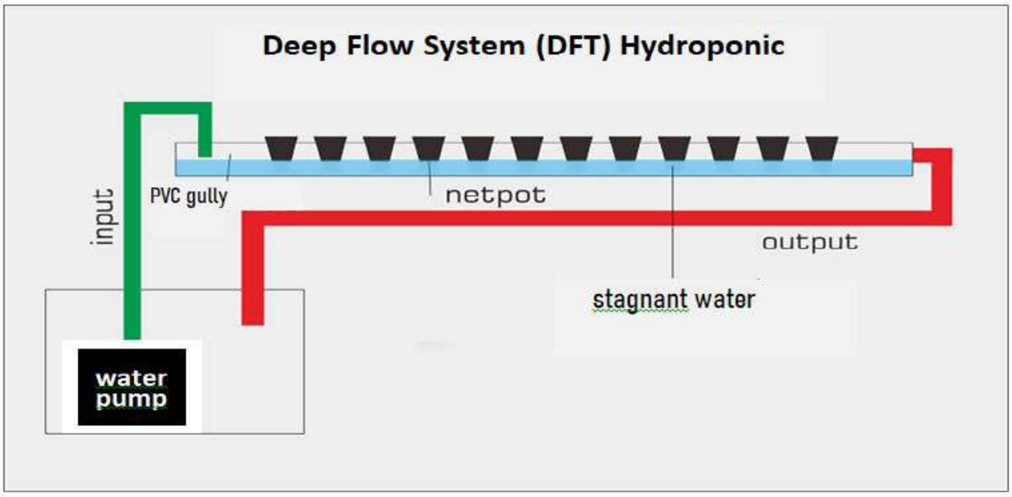

Fig.2: Scheme of deep flow system (DFT) hydroponic used in the plant factory. (Modified from: https://www.bing.com/images/search?view.)

Plant nutrient solution

The solution used to nourish plants was AB mix general vegetable containing macro and micro nutrients (Table 1) with the addition of Calcium nitrate (Ca(NO3)2) as much as 90 grams per literbased on method of Suryantini*et al*. (2019).

Table 1. Chemical contained onAB mix solution for vegetable general in onelitre

Description	Chemicals structure	Weight (g)	Concentration (ppm)	Group A/B
Calcium nitrate	$Ca(NO_3)_2$	138	235.4	A
Potasium nitrate	KNO_3	130.4	334.9	A
Monopothasiumphosphate	KH_2PO_4	34.5	87.1	B
Magnesium sulphate	$MgSO_4.7H_2O$	78.9	89.4	B
Amonium sulphate	$(NH_4)_2SO_4$	21.8	49.1	B
FeEDTA	$C_{10}H_{12}N_2O_8$ FeNa.3H_2O	3.08	2.03	A
FeEDDHA	$C_{18}H_{16}N_2O_6FeNa$	1.54	0.54	A
Boric acid	H_3BO_3	0.735	0.64	B
ZnEDTA	$C_{10}H_{14}MnNa_2O_8Zn$	0.186	0.13	B
MnEDTA	$C_{10}H_{14}MnN_2O_8$	0.988	0.64	B
CuEDTA	$C_{10}H_{14}CuN_2O_8$	0.096	0.07	B
Sodium molibdat	$Na_2MoO_4.2H_2O$	0.033	0.10	B

Observation variables

The observed plant variables were 1) plant height, measured from the base of the stem to the highest leaf end after being straightened up, 2) the length of the root, measured from the base of the stem down to the end of the root, 3) the number of leaves, calculated all the number of leaves present on the plant to the smallest leaves that can be counted, 4) fresh weight of the plant, measured by weighing the weight of the plant header using digital weighing equipment, 5) dry weight of the plant, measured the dry weight of the plant after drying in the oven with a temperature of 80°C until its weight was fixed, 5) the area of the leaves, measured by measuring the entire leaves of the observed plant,6) The amount of chlorophyll, done with the SPAD Units (Special Products Analysis Division Units). Each plant was measured three leaves and each leaf was measured in three parts, namely at the tip, middle, and the basal of the leaf, then averaged. All data was analyzed with a t-test of 5% as the indipenden group.

III. RESULTS AND DISCUSSIONS

Plant growth was observed weekly since the plant was transplanted (0 mst, weeks after transplanting) to 4 mst. Other variables namely fresh weight, dry weight, leaf area, and amount of chlorophyll were observed during harvest at 4 mst.

Plant height and plant root length

Lettuce plants in plant factorywere treated led grow light and red-blue-white LEDs with photoperiodic 18 hours of on lights and 6 hours off alternately. Lettuce plants that were exposed to a LED grow light exhibited the plant height every week higher than plants on red-blue-white LED lights (Table 2).The average height of plants in LED grow light was 15.7 cm,was markedly higher by 19.30% compared to plants illuminated by red-blue-white LED lights of13.16 cm. It was likely that plants experience mild ethiolation that occureddue to the intensity of grow light was low, allowing IAA growth hormone to be more active than the influence of red-blue-white light.Husen (2001) and Pacholczak*et al*. (2005) revealed that the presence of ethiolation will activate the IAA hormone that spured plant growth so that plants were higher, even the condition can also increase the number and length of roots on plant cuttings.

Although the LED grow light spectrum provided a more complete spectrum of between 420-750 nm with three peaks namely λ=450 nm, λ=527-542 nm, and λ=608 nm (Figure 1) but its intensity was low at about 9000 lux for blue light and 8500 lux for the red spectrum compared to the red-blue-white light with 13,500 lux for the blue spectrum and 10,000 lux for the red spectrum.

Table 2. The height of the lettuce plant on two different types of light bulbs

Light sources/colors	Weeks after transplanting (WAT)					
	0	1	2	3	4	Averages
LED grow light lamp (white)	5.25	8.41	17.40	21.10	26.35	15.70 a (19.30%)^z
LED red-blue-white lamp	5.23	6.47	14.00	17.50	22.60	13.16 b
Significance by t-test 5%,t_{count}=2.2798, t_{Table}=1.7341, ^{z}percentage of increase.						

The appearance of the lettuce plant at the age of 4 mst (weeks after transplanting) was presented in Figure 3 below.

Fig.3: Growth of lettuce plants. A) given a LED grow light: a. appears up, b. appears side; B) given a LED red-blue-white light: c. appears above, and d. appears side.

Number and areas of leaves

Lettuce leaves on red-blue-white LED lights every week were more leaves number than that exposed to led grow light (Table 3). The average number of leaves on a red-blue-white light (13.16 sheets) was 16.67% higher than in LED grow light (11.28 sheets). The plants at the red-blue-white light appear more lushes (Figure 3) and were supported by a much wider average leaf area of 2284.4

cm^2 on red-blue-and-white LEDs and 1707.6 cm^2 on grow light LED (Table 4). Much higher growth in these red-blue-white LEDs than lettuce affected by grow light was due to the role of chlorophyll-a which absorbs blue light and chlorophyll-b absorbs red light which was of higher spectrum quality than the same spectrum in grow light rays (Figure 1). This was supported by Saebo*et al*. (1995) which states that red light can increase carbohydrate accumulation in the leaves by inhibiting the translocation of photosintat out of the leaves.

Blue and red light can increase biomass productivity, seed germination, plant stem growth (Parks *et al*., 2001), chlorophyll content when given green light supplements (Bian*et al*., 2018) in lettuce plants. Furthermore, Brown *et al*. (1995), found that red light (660 nm) requires a blue light supplement for chili plants to grow normally.

Table 3. Number of lettuce plant leaves on two different types of light lamps

Light sources/colors	Weeks after transplanting (WAT)					
	0	1	2	3	4	Averages
LED grow light lamp (white)	5.20	7.00	10.80	14.50	18.90	11.28 a
LED red-blue-white lamp	5.33	7.60	11.90	16.80	25.60	13.16 b (16.67%)^z

Significance byt-test 5%, t_{count}=2.7138,t_{Table}=1.7341, ^{z}percentage of increase.

Table 4. The area of plant leaves on two different types of light lamps

Light sources/colors	Plant-1 (cm^2)	Plant-2 (cm^2)	Plant-3 (cm^2)	Average (cm^2)
LED grow light lamp (white)	2099.09	1707.79	1315.79	1707.6 a
LED red-blue-white lamp	2931.32	2456.74	1465.12	2284.4 b (33.78%)^z

Significance by t-test 5%, t_{count}=2.7855,t_{table}=1.7341, ^{z}percentage of increase

Chlorophyll content, fresh weight, and dry weight of plant

Plant chlorophyll was measured by SPAD Units (Special Products Analysis Division Units). The amount of chlorophyll contained in grow light LED and red-blue-white LED differ markedly which were 18.2 SPAD and 22.8 SPAD, respectively. The amount of chlorophyll in leaves on red-blue-white LED lights was 25% higher than plants that exposed to grow light LED (Table 5). More chlorophyll in this treatment will support higher photosynthetic activity,thereafter the fresh weight and dry weight of the plant became greater. The fresh weight of the plantand the dry weight of the plant on the red-blue-white light treatment were greater significantly than the plants irradiated by grow light, respectively increased by 101.49% and58.13% (Tables 6 and 7). Thesewere supported by research conducted by Senger (1982) which stated that blue light spurs the formation of chlorophyll, the opening of stomata, and the formation of various enzymes involved in the process of photosynthesis. Blue light encourages the development of chloroplasts and increased in the amount of chlorophyll (Akoyunoglou and Anni, 1984) and when combined with red light (1:4) can markedly increased the amount of chlorophyll, fresh weight, and dry weight of spinach plants (Nguyen *et al*., 2021).

Table 5. The content of plant chlorophyll in two different types of lamps

Light sources/colors	Plant-1 (SPAD)	Plant-2 (SPAD)	Plant-3 (SPAD)	Averages (SPAD)
LED grow light lamp (white)	19.5	16.7	18.5	18.2 a
LED red-blue-white lamp	21.4	19.0	28.0	22.8 b (25%)^z

Significance by t-test 5%,t_{count}=2.2962; t_{table}=1.7341, ^{z}percentage of increase.

Table 6. Total plant fresh weight in two different types of lamps

Light sources/colors	Plant-1 (g)	Plant-2 (g)	Plant-3 (g)	Plant-4 (g)	Average (g)
LED grow light lamp (white)	77.01	83.03	54.02	57.01	67.77 a
LED red-blue-white lamp	187.20	182.00	77.00	100.01	136.55 b (101.49%)^z

Significance by t-test 5%, t_{count}=2.3668, t_{table}=0.7176, ^{z}percentage of increase.

Table 7. Total plant dry weightin two different types of lamps

Light sources/colors	Plant-1 (g)	Plant-2 (g)	Plant-3 (g)	Plant-4 (g)	Average (g)
LED grow light lamp (white)	4.12	2.23	3.11	3.34	3.20 a
LED red-blue-white lamp	8.23	3.33	3.43	5.24	5.06 b (58.13%)^z

Significance by t-test 5%, t_{count}=1.5362, t_{table}=0.7176, ^{z}percentage of increase.

IV. CONCLUSION AND SUGGESTION

Conclusion

LED grow light exhibited the lettuce plant a significantly higher height of 16.30% compared to red-blue-white LED light. On the other hand, the number of leaf and leaf area in red-blue-white light LEDweremuch higher, which were 16.67% and 33.78% respectively compared to grow light LED. In addition, the red-blue-white light LED increased the content of chlorophyll, fresh weight, and dry weight of lettuce plants by 25.00%, 101.49%, and 58.13%, consecutively. Red-blue-and-white LED light provided a higher biomass than grow light LED.

Suggestion

More detailed studies should be done on the effect of red-blue-white lights LED on the nutritional and phytochemical content of lettuce plants in plant factory.

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REFERENCES

[1] Akoyunoglou, G. and H. Anni. 1984. Blue light effect on chloroplast development in higher plant. In Blue light effects in biological systems. Springer, Berlin, Heidelberg.

[2] Bian, Z.,Q.Yang, T. Li, R. Cheng, Y. Barnett, C. Lu. 2018. Study of the beneficial effects of green light on lettuce grown under short-term continuous red and blue light-emitting diodes. Physiol. Plant. 2018, 164, 226-240. [CrossRef].

[3] Brown, C.S., A.C. Schuerger, J.C. Sager. 1995. Growth and photomorphogenesis of pepper plants under red LEDs with supplemental blue or far-red lighting. Journal of the American Society for Horticultural Science 120(5):808-813. DOI:10.21273/JASHS.120.5.808.

[4] Bugbee, B. 2019. Far red: The forgotten photons. https://advanced growlights.com/ far-red-the-forgotten-photons/. [20 October 2021].

[5] Husen, A., M. Pal. 2001. Interactive effect of auxin and etiolation on adventitious root formation in cuttings of *Tectona grandis* Linn. f. Ind. For. 127(5):526-532.

[6] Kim,Hyeon-Hye, Gregory D. Goins, Raymond M. Wheeler, John C. Sager. 2004. Green-light supplementation for enhanced lettuce growth under red- and blue-light-emitting diodes. HortScience39(7):1617-1622.

[7] Kobayashi, K., T. Amore, and M. Lazaro. 2013. Light-Emitting Diodes (LEDs) for miniature hydroponic lettuce. Honolulu, USA 3, 7477.

[8] Nguyen, Thi-Phuong-Dung, Dong-Cheol Jang, Thi-Thanh-Huyen Tran, Quang-Thach Nguyen. 2021. Influence of green light added with red and blue LEDs on the growth, leaf microstructure and quality of spinach (*Spinacia oleracea* L.). Agronomy 11(9):1724 DOI:10.3390/Agronomy11091724.

[9] Pacholczak, A., W. Szydło, A. Tukaszewska. 2005. Effectiveness of foliar auxin application to stock plants in rooting of stem cuttings of ornamentalshrubs. Propagation of Ornamental Plants 5(2):100-106.

[10] Parks, B.M., K.M. Folta, and E.P. Spalding. 2001. Photocontrol of stem growth.Current Opinion in Plant Biology 4:436-444.

[11] Saebo, A., T. Krekling, and M. Appelgren. 1995. Light quality affects photosynthesis and leaf anatomy of birch

plantlets in vitro. Plant Cell Tissue Organ Culture 41:177-185.
[12] Senger, H. 1982. The effect of blue light on plants andmicroorganisms. Photochemistry and Photobiology35:911-920.
[13] Shimizu, H., Yuta Saito, Hiroshi Nakashima, Juro Miyasaka, Katsuaki Ohdoi. 2011. Light environment optimization for lettuce growth in plant factory. Preprints of the 18th IFAC World Congress Milano (Italy) August 28-September 2, 2011.
[14] Suryantini, N.N., G. Wijana, R. Dwiyani. 2021. Pengaruhpenambahan $Ca(NO_3)_2$terhadaphasiltanamanseladakriting (*Lactuca sativa* L.) pada hidroponiksistem Deep Flow Technique (DFT). Agrotrop 10(2):190-200.
[15] Yorio, N. C., G. D. Goins, H. R. Kagie, R. M. Wheeler, J. C. Sager. 2001. Improving spinach, radish, and lettuce growth under red light-emitting diodes (LEDs) with blue light supplementation. Hort Science36(2):380-383.

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Response of Cabbage (*Brassica oleracea var capitata*) to Organic and Inorganic Fertilizers on Growth and Yield Parameters and Incidence of Insect Pest

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Abstract— *Two field experiments were conducted for two cropping seasons at the Multipurpose Crop Nursery of the Akenten Appiah-Menka University of Skills Training and Entrepreneurial Development Mampong-Ashanti, from August to November, 2015and June to September, 2016 to determine the response of cabbage (Brassica oleracea var capitata) to organic and inorganic fertilizers on growth, yield and incidence of insect pest. The experimental design used for the field experiment was a randomized complete block design (RCBD) with three replications. The treatments were: poultry manure (20 t ha^{-1}) + Cypermetrine (30 l ha^{-1}), cow dung (20 t ha^{-1}) + Cypermetrine (30 l ha^{-1}), N.P.K (15:15:15) (300 kg) + Cypermetrine (30 lha^{-1}), foliar + Cypermetrine (30 l ha^{-1}), poultry manure (20 t ha^{-1}) + neem leaf extract, cow dung (20 t ha^{-1}) + neem leaf extract, N.P.K (15:15:15)(300 kg) + neem leaf extract, foliar + neem leaf extract and the control (no fertilizer and no insecticide). The result revealed that the application of organic manure (poultry manure and cow dung) and inorganic fertilization is a better option for soil fertility enhancement in cabbage production.High percentage crop establishment (>95%) was achieved across treatments with NPK + Cypermetrine, poultry manure + neem, N.P.K (15:15:15) + Neem or Foliar fertilizer + neem achieving 100% crop establishment. The application of Cow dung+ NLE produced the highest number of open leaves in both seasons. Application of poultry manure and N.P.K combined with Cypermetrine significantly improved head weight and cabbage yield.Application of poultry manure combined with Cypermetrine or neem leaf extract produced earliest head initiation and widest head diameter.In conclusion cabbage growers are encouraged to use poultry manure at 20 t ha^{-1} and N.P.K (15:15:15) (300 kg) combined with Cypermetrine at 30 l ha $^{-1}$ and neem leaf extract for the improvement of cabbage growth, yield and incidence of insect pest.*

Keywords—***foliar, cabbage, poultry manure, cow dung, cypermetrine, neem leaf extract.***

I. INTRODUCTION

Cabbage demand is high on the Ghanaian domestic market, serving as a source of vitamin and mineral requirements as well as a major source of income to the youth and women in urban and peri-urban areas (Timbilla and Nyarko, 2004). The crop is high in water content, fibre, protein, calcium, iron, and vitamins A and C (Adeniji *et al*., 2010; Meena *et al*., 2010). The rise in the consumption of cabbage has necessitated the increase production of the crop in Ghana. However, the productivity of cabbage per unit area is rather low compared to other developed countries of the world. This is as a result of numerous production challenges faced by farmers. Notable among them are soil nutrient and pest management which are required for better growth and yield of cabbage. Nutrient supply is an important input for realizing higher cabbage yield and its nutrient content

(Hasan *et al*., 2018). There is high cost of inorganic fertilizers (Snr *et al*., 2018). This makes it difficult for the local farmer to boost production and to increase yield, thereby reducing yield and their income levels. The habit of applying synthetic insecticides to control cabbage pest has been the traditional practice among most farmers in Ghana. The heightening consciousness of the danger associated with the use of synthetic insecticides as well as their high costs of control has necessitated for a less dangerous form of pest control. Organic pesticides have long been an alternative to synthetic chemical insecticides for pest control and management. This is because they pose little threat to the environment and to human health (Appiah*et al*., 2014).

Cabbage has high requirements for all nutrients, especially nitrogen and it demands for achieving high yields range from 130-310 kg/ha (Milas and Vincent, 2017). Nitrogen over-use in modern agriculture is of importance with respect to both environmental concerns and the quality of plant products. Cow dung is a potential source of nutrients and also a potential benefit to soil amelioration, especially for communal farmers who cannot afford fertilizers. However, getting maximum value out of the manure requires applying it at proper rates and frequency in conjunction to a particular soil (Pahlar*et al*., 2013).

The suitability and usefulness of cow dung has been attributed to high availability of N.P.K content and increased availability of organic matter content to degraded soil which may lead to the increasing activity of beneficial microorganisms and improves the physical properties of the soil (Snr *et al*., 2020). The plant has good responsiveness on animal manure application in quantity of 40 t/ha. Organic manuring enhances soil biological activity, improves nutrient mobilization, soil structure and increases soil water retention (Roy and Kashem, 2014). Crop production with integrated use of mineral and organic manure has proved to be highly beneficial by increasing yield as well as keep the environment sound. In the past, agricultural production was focused on maximizing the quantity of vegetables produced for commercial markets (Pavla and Pokluda, 2008); while in the last few decades the organic management of crops has gained popularity because of increased consumer's awareness of the health problems that come from food grown under conventional farming. Differences between organic manure and inorganic fertilizers, especially in soil fertility management may affect the nutritive composition of plants (Hassan and Solaiman, 2012). Shapla (2013) reported that manure applied in correct proportion, does not just improve soil porosity but it also contributes to good plant growth, development and yield, chlorophyll and N.P.K content.

In view of the benefits to the diet and the livelihood of the Ghanaian populace and to determine the effectiveness of fertilizers for soil improvement and maintenance on cabbage, it is of much importance to find out the response of cabbage (*Brassica oleracea var capitata*) to organic and inorganic fertilizers on growth and yield parameters and incidence of insect pest.

I. MATERIALS AND METHODS

2.1 Description of study area

Two field experiments were conducted on two different plots at the Multipurpose Crop Nursery at the Akenten Appiah-Menka University of Skills Training and Entrepreneurial Development (AAM- USTED) Mampong-Ashanti from August to November, 2015 and June to September, 2016. Mampong-Ashanti is located within the transitional agro-ecological zone of Ghana, lying between the semi deciduous forest to the south and the Guinea savannah region to the north. It is located at latitude 07^0 and 08^0 north of the Equator and longitude 01^0 and 24^o west. It is 457.5 m above sea level.

The climatic conditions at the experimental sites were determined during 2015 and 2016 cropping seasons. The climatic conditions during the field research periods show that differences in environmental factors (rainfall, temperature and relative humidity) were shown in both cropping seasons. The total monthly rainfall for 2015 cropping season was 287.5 mm and it occurred from August to November, 2015 with the peak in September and October (Ghana Meteorological Agency – Mampong Ashanti, 2015). The mean monthly temperature for the area for the 2015 cropping season ranged between 22.8 ^{o}C to 30.8 ^{o}C with the highest daily temperature of 32.6^{o}C occurring in November, 2015.The mean monthly relative humidity ranged from 60.3 to 92.2 % with the peak occurring between September and November.

In the 2016 cropping season, for experiment two (2), the total monthly rainfall was 647.8 mm and it occurred from June to September, with the peak in June, July and September (Ghana Meteorological Agency – Mampong Ashanti, 2016). The mean monthly temperature of the area for the 2016 cropping season ranged between 22.4 ^{o}C to 29.4 ^{o}C with the highest daily of 30.1 ^{o}C occurring in June. The mean monthly relative humidity ranged from 71 to 96 % with the peak occurring between June and September. The soil at the experimental site has been categorized as Chronic Luvisol and locally as the Bediesi series with a pH range of 4.0 - 6.5 suitable for root, cereal, vegetable and legume crops production legend (Asiamah *et al*., 2000).

2.2 Experimental design and treatments

The field was laid in a randomized complete block design (RCBD). There were nine (9) treatments with three replications. The nine treatments were made up of eight organic manure and fertilizer rates and the control (without amendment) and chemical insecticide (Cypermetrine) and neem leaf extract were assigned to each block. The treatments were: poultry manure (20 t ha^{-1}) + Cypermetrine (30 l ha^{-1}), cow dung (20 t ha^{-1}) + Cypermetrine (30 lha^{-1}), N.P.K (15:15:15) (300 kg) + Cypermetrine (30 l ha^{-1}), foliar + Cypermetrine (30 lha^{-1}), poultry manure (20 tha^{-1}) + neem leaf extract, cow dung (20 t ha^{-1}) + neem leaf extract, N.P.K (300 kg) + neem leaf extract, foliar + neem leaf extract and the control (no fertilizer and no insecticide).

2.3 Soil and manure application

Land clearing was by slashing and removing of stumps and this was immediately followed by lining and pegging. The experimental area was demarcated into plots and before transplanting, soil, poultry manure and cow dung were mixed thoroughly. Samples of no – manure soil (control) and organic manure (poultry manure and cow dung) was randomly taken prior to inorganic fertilizer application at a uniform depth of 0 – 20 cm for chemical analysis. Soil and organic manure samples analysis were carried out at the Soil Research Institute of CSIR laboratory in Kumasi, Ghana. The characteristics analyzed for included pH in (1:1 soil: distilled water ratio) and measured by the potentiometric method on a pH meter manufactured by VebPracitron in Dresden, Germany. Organic matter was determined by the Walkey and Black methodand total nitrogen was determined by the micro Kjeldahl method. Exchangeable cations were determined by flame emission photometry. Extraction was carried out by filtration or centrifugation. Ca and Mg were determined using an atomic absorption or spectrometry (AAS) after the removal of ammonium acetate and organic matter at pH 7.0. The result on nutrient level of organic manure and soil chemical properties are shown in Tables 1 and 2.

2.4 Soil and manure chemical analysis

Samples of soil, organic manure and a mixture of soil and manure from the various replicates and treatments with the exception of DI’GROW (Foliar fertilizer) plot were taken for analysis at the Soil Research Institute, Kwadaso in Kumasi. The characteristics analyzed for included; soil pH, organic matter, organic carbon, total nitrogen, exchangeable calcium, magnesium, potassium and sodium, effective cation exchange capacity, total exchangeable bases and available phosphorus and potassium.

2.5 Land preparation, fertilization and planting

The land was cleared by slashing and removal of stumps, ploughed and harrowed and then lined and pegged for planting. Cow dung and the poultry manure were applied depending upon the treatment at the rate of 20 t ha^{-1} and worked into the soil two weeks before transplanting of cabbage seedlings. The inorganic fertilizer (N.P.K15:15:15) at the rate of 300 kg ha^{-1} was applied to the designated plots on the various replicates two weeks after transplanting. The cabbage variety used for the study was Oxylus. The seedlings were transplanted four weeks after nursing at a spacing of 50 cm x 30 cm and at a depth of 1.0 cm. Transplanting of seedlings for each season was done early in the morning. Each experimental plot contained four (4) rows and ten (10) plants within each row. There were sixteen (16) plants within the harvest area (two central rows per plot).Each experimental plot measured 2.0 m x 3.0 m with 1.0 m between plots and 2.0 m between blocks. The total field size for each cropping season was 27.0 m x13.0 m (358.8 m^2).

2.6 Data collection and analysis

The vegetative data collected were percentage plant establishment and number of open leaves. The percentage plant establishment was measured at 21 days after planting (DAT).This was achieved by counting the number of plants in the two middle rows per plot and the percentage crop establishment estimated. The number of open leaves was counted from the two middle rows per plot at two weeks interval from 21 DAT to 63 DAT. Days to head initiation, head weight per plant and head diameter were estimated from the two central rows. Days to head initiation was counted from transplanting date to first harvest day when heads were firm. Head diameter was measured from the middle portion of the head using the vernier caliper. Cabbage head from the two middle rows per plant at harvest were weighed for the determination of head weight (kg) per plant using electronic weighing scale. Harvest index was estimated by dividing the fresh weight of head by the above ground fresh biomass of the plant. The data collected were analyzed using Analysis of Variance (ANOVA).The data obtained were analyzed using GenStat Release 11 statistical package and the Least Significant Difference (LSD) was used to separate the means at 5 % level of probability.

II. RESULTS AND DISCUSSION

3.1 Nutrient levels of organic amendments

Generally, the nutrient levels of the poultry manure applied in the 2015 cropping season was comparatively higher than the cow dung (Table 1). In 2016, both organic amendments had lower nutrient level compared to that of 2015. The level of potassium was 0.1 and 0.3 % for poultry manure and cow dung respectively in 2016.The pH for 2015 for both amendments was almost neutral and that of the 2016 was moderately acidic.

Table 1: Nutrient levels of organic amendments for 2015 and2016 cropping seasons

	Cropping seasons	pH	Ca (%)	Mg (%)	P (%)	K (%)	N (%)
re	2015	6.18	3.40	1.92	0.63	0.86	3.54
	2015	6.87	1.00	1.05	0.23	0.84	2.01
re	2016	5.97	2.11	0.48	0.70	0.10	0.86
	2016	4.89	0.14	1.10	0.25	0.34	0.76

3.1 Soil Chemical properties before and after treatment application for 2015 and 2016 cropping seasons

In 2015, soil analysis before application of treatments indicated that the soil was slightly acidic with a pH of 6.13, whereas that of 2016 was moderately acidic with a pH of 5.70 (Table 2). The Nitrogen, Potassium and Organic matter contents for both seasons were$\leq 0.06\ \%, 0.27\ \%$ and $\leq 1.16\%$ respectively (Table 2) (Soil analytical data guide of CSIR – SRI, 2007). Cations levels were low in the range of 0.1 – 4.8 meq/100g; calcium had an average of 4.5 meq/100g and the total exchangeable bases (TEB) were 6.13 and 5.45 for both cropping seasons. Effective cation exchange capacity for both cropping seasons was low, ranging between 6.00 meq/100g and 6.23 meq/100g respectively (Table 2). The pH of the fertilized soils remained slightly acidic or neutral (6.18 – 6.87) compared to the untreated soil which remained slightly acidic (6.13) after the 2015 season (Tables 2). After the 2016 cropping season, soils of the untreated, poultry manure or N.P.K (15:15:15) remained moderately acidic (5.70 -5.97) while that treated with cow dung became very acidic (Tables 2). The cow dung treated plots recorded higher levels of organic carbon than poultry manure, N.P.K (15:15)15), or the untreated (control) for both cropping seasons. After the 2015 cropping season, soil amendments slightly improved percentage total nitrogen, however, they still remained within the low range (Tables 2).Soil amendments improved percentage total N from the initial low levels to moderately high levels after the 2016 cropping season. While cow dung or poultry manure slightly improved organic matter, though still in the low category after the 2015 season, they significantly improved organic matter to the moderate or high levels after the 2016 cropping season. The application of cow dung left more organic matter in the soil in both years than the other amendments. Low levels of exchangeable cations, total exchangeable bases and effective cation exchange capacity were recorded for all treatments after both seasons, though slightly higher than the untreated (control). All the fertilized plots recorded moderate to high levels of available P or available K after both cropping seasons (Tables 2).

The differences in pH and nutrient levels of the organic manure in 2015 and 2016 could be due to differences in organic matter. It has been suggested that the growth of plants is optimal when soil pH is between 5.8 and 6.5 and sometimes to a maximum of 7.5 depending on the plant species (Ontario Ministry of Agriculture, 2009). The application of the poultry manure and cow dung allowed the soil pH fall within a range needed for maximum plant growth except for cow dung in 2016. The pH of the soil became acidic after the season because pH of the cow dung was acidic. This could be that the parent material for soil formation has a role to play in the overall pH of the soil formed. It has been established that soils respond differently to changes in pH depending on the soil's buffering ability (CEC) (Page-Dumroese *et al.* 2006). The initial CEC of the soil in 2016 before the application of the cow dung was low and this does not allow the holding of cations to the soil surfaces to aid in neutralization. Such soils are unable to control nutrient losses through leaching too.

The application of organic manure provides benefits of improved fertility, water holding capacity, structure, increased organic matter and organic carbon (Adebayo *et al.* 2011). Much organic matter and carbon was left on the cow dung treated plots principally because cow dung needs much time to decompose than poultry manure and therefore has a longer residual effect than poultry manure. Cow dung may have a longer stay to decomposition than poultry, hence the result. According to Zaman (2017), cow dung has been documented long as perhaps the best desired animal manures due to its high nutrient and organic matter content. The application of cow dung raises the organic carbon of degraded soils which may result in improving activity of beneficial soil microorganisms and the fertility of the soil by increasing availability of nutrients for plants from soil.

Table 2. Soil properties before and after treatment application for 2015 cropping season

Treatment	Year	pH 1:1	Org C (%)	Total N (%)	Org Matter (%)	Exchangeable Cations (meq/100g)				TEB	ECEC (meq/100 g)	Base Sat (%)	Avail P (ppm)	Avail K(ppm)
						Ca^{2+}	Mg^{2+}	K^{+}	Na^{+}					
Initial soil properties														
Cropping season	2015	6.13	0.64	0.05	1.10	4.81	1.07	0.25	0.10	6.13	6.23	98.39	24.32	47.99
Cropping season	2016	5.70	0.67	0.06	1.16	4.27	0.80	0.27	0.11	5.45	6.00	90.83	7.64	11.00
Soil properties after soil amendment														
Cow dung	2015	6.87	0.67	0.07	1.16	5.87	0.53	0.69	0.24	7.09	7.19	98.61	27.11	168.51
Poultry manure	2015	6.18	0.33	0.08	1.15	5.34	0.53	0.38	0.16	0.25	6.35	98.43	49.43	79.39
N.P.K	2015	6.29	0.30	0.06	1.02	5.34	0.27	0.27	0.13	5.88	5.98	98.33	33.56	51.96
Untreated soil	2015	6.13	0.64	0.05	1.10	4.81	0.25	0.25	0.10	6.13	6.23	98.39	24.32	47.99
Cow dung	2016	4.89	1.99	0.18	3.44	2.94	0.52	0.72	0.25	4.43	5.63	78.69	10.84	168.51
Poultry manure	2016	5.97	0.86	0.17	2.78	5.07	0.93	0.24	0.16	6.56	6.86	95.63	19.21	79.39
N.P.K	2016	5.87	1.61	0.14	1.48	3.34	0.67	0.28	0.15	4.44	4.84	91..74	10.34	51.96
Untreated soil	2016	5.70	0.67	0.06	1.16	4.27	0.80	0.27	0.11	5.45	6.00	90.83	7.64	11.00

3.2 Effect of soil amendment on vegetative growth performance

The percentage crop establishment was not significantly affected by any of the fertilizers combined with insecticides and was similar to the control in 2015 (Tables 3). Generally, higher percentage crop establishment (>95 %) was achieved across treatments with NPK + Cypermetrine, Poultry manure + neem, N.P.K (15:15:15) + Neem or Foliar fertilizer + neem achieving 100 % crop establishment. Similarly, in 2016, fertilizer application combined with insect control did not negatively affect crop establishment. The percentage established crop population ranged between 96 and 100 (Table 3). Generally, number of open leaves was not significantly affected by fertilizer and insecticide combinations from 21 DAT to 63 DAT in 2015 (Table 5). All the treatment effect increased between 21 and 35 DAT in number of open leaves, after which some begun declining. The number of open leaves increased from 21DAT to 35 DAT, peaked at 49DAT and then declined at 63 DAT in 2016 (Table 3). There were no significant differences between treatments in number of open leaves at 21DAT. However, at 35 and 45 DAT, cabbage treated withcow dung combined with Cypermetrine or neem produced significantly more open leaves than the control or foliar fertilizer combined with Cypermetrine (Table 3).

The high percentage crop stand establishment achieved with the application of the treatments is very important to cabbage production since crop stand at harvest is a very important determinant of yield at the end of the cropping season. A look at the number of open leaves dynamics is an indication that head initiation started from the point when the number of open leaves decline. Number of open leaves started declining at 49 DAT for the control which was 2 weeks later than for Poultry manure, cow dung or NPK (15:15:15) combined with any of the insecticides and was confirmed by data on days to head initiation. It is clear that plant nutrients play a major role in whether head initiation would happen at the right time or it would be delayed. According to the report of Radiovich *et al*., (2005), cabbage head development is quick and efficient when adequate nutrients are supplied to the plants and there are enough functional outer leaves for photosynthesis with N being the most needed nutrient.

According to the report of John *et al*., (2004), poultry manure contained essential nutrient elements associated with photosynthetic activities and thus promote roots and vegetative growth. Roy and Kashem (2014) also reported that cow dung resulted in significant increase in soil nitrogen and other soil properties necessary for crop yield and productivity. According to the report of Roy and Kashem (2014), adequate amounts of nitrogen may be obtained from reasonable amounts of organic matter applied to the soil and is directly responsible for vegetative growth of plants. Nitrogen functions in plants by being part of chlorophyll which is important in photosynthesis, and improves the quality of leaf (Roy and Kashem, 2014). According to the report of Patrick *et al.* (2012), yield of cabbage increased with increasing levels of nitrogen up to 390 kg/ha. Casely *et al.* (2006) observed that increasing rate of nitrogen (150-250 kg/ha) with basal P and K application increased yield of cabbage. The increase in water holding capacity in poultry manure and cow dung treatments also provided additional advantage for growth and yield to cabbage grown on such plots (Frempong *et al.*2006; Agyarko *et al.*2006; Ewulo, 2005).

Table 3: Effect of Treatments on Percentage Crop Establishment (%), and number of open leaves, (2015)

Treatment	Percentage Crop Establishment (%)		Number of open leaves (Days after transplanting)							
	Cropping season		Cropping season (2015)				Cropping season (2016)			
	2015	2016	21	35	49	63	21	35	49	63
PoultryMan+Cyper	95.83	100.00	19	16	11	16	13	19	23	19
Cow dung+ Cyper	95.83	100.00	19	17	14	17	13	21	27	19
N.P.K+ Cyper	100.0	97.92	18	18	13	18	11	18	23	17
Foliar + Cyper	95.83	97.92	17	18	16	18	11	19	22	18
PoultryMan+NLE	100.0	95.83	19	17	14	17	12	20	23	17
Cow dung+ NLE	95.83	100.00	19	22	20	22	13	20	28	18
N.P.K+ NLE	100.0	100.00	18	21	20	21	13	22	27	21
Foliar + NLE	100.0	100.00	14	14	15	14	12	20	26	23
Control	95.83	100.00	15	16	19	16	11	17	24	23
SED(0.05)	2.06	1.78	1.28	2.10	2.20	4.15	2.55	2.05	3.12	2.55

cyper = cypermetrine, man = manure

3.2 Effect of soil amendment on yield components

The days to head initiation was significantly influenced by fertilizer and insecticide treatments in the 2015 experiment (Table 4). Head initiation for all treatments started 2 – 3 weeks earlier than the control with poultry manure combined with neem or Cypermetrine producing the least days to head initiation. In 2016, days to cabbage head initiation ranged between 78 -97 days (Table 4). However, the production of cabbage without any form of fertilizer and insecticide application increased the number of days to head initiation by 10 to 19 days. The use of Poultry manure combined with neem or Cypermetrine required significantly less days (6-8days) to head initiation compared with NPK combined with Neem or Cypermetrine, cow dung combined with neem or foliar fertilizer combined with neem (Table 4).

Head diameter was also significantly influenced by the fertilizer and insecticide treatments (Tables 4). Poultry manure or cow dung regardless of the insecticide applied produced bigger cabbage heads which were 7 – 7.4 cm bigger than the control.

Weight of head per plant produced was significantly affected by fertilizer and insecticide application with poultry manure combined with Cypermetrine significantly producing heavier head per plant than the control, or foliar combined with neem or Cypermetrine (Table 4). The head per plant produced from the poultry manure combined with Cypermetrine were 0.80kg heavier than the control, foliar combined with Cypermetrine or neem (Table 4). The control, foliar fertilizer combined with neem or Cypermetrine, N.P.K combined with Cypermetrine, or cow dung combined with neem produced significantly lighter cabbage heads per plant compared with poultry manure combined with neem or Cypermetrine, cow dung combined with Cypermetrine, or N.P.K combined with neem (Table 4). The use of Poultry manure combined with neem or Cypermetrine produced cabbage heads per plant that were 350 – 450 % heavier than the cabbage heads of the control (0.20 kg). N.P.K combined with neem or Cypermetrine also produced heads that were 150 – 315 % heavier than the control (Table 4).

Cabbage yield (tonnes per hectare) was significantly influenced by fertilizer and insecticide treatment in 2015 (Table 4). The application of poultry manure and Cypermetrine or neem significantly produced 32.50 – 37.23 tonnes (468 – 537 %) more cabbage than the control. Foliar fertilizer regardless of the insecticide combined produced yields similar to the control (Table 4). In 2016, significantly higher yields of 278, 266, 289 and 131 % over the control (9 tonnes/ha) were recorded for poultry manure combined with neem, N.P.K (15:15:15) combined with neem, poultry manure combined with Cypermetrine, or cow dung combined with neem respectively over the control (Table 4). The control or foliar combined with Cypermetrine recorded least yields of 10 and 9 tonnes/ha respectively (Table 4). Harvest index was however, not influenced by the treatments (Tables 4). Poultry manure combined with neem leaf extract and N.P.K (15:15:15) combined with neem leaf extract had similar harvest index in 2016 (Table 4).

Table 4: Effect of fertilizer + insecticides on cabbage yield and yield components for 2015cropping season

Treatment	**Days to Head Initiation**		**Head Diameter (cm)**		**Head Weight (kg)**		**Yield (ton/ha)**		**Harvest Index**	
	2015	**2016**	**2015**	**2016**	**2015**	**2016**	**2015**	**2016**	**2015**	**2016**
Poultry Man+Cyper	80.00	79.00	10.03	10.03	1.03	1.10	39.44	32.56	0.83	0.79
Cow dung+ Cyper	82.00	82.00	10.00	10.00	0.80	0.73	24.58	19.11	0.85	0.82
N.P.K+ Cyper	86.00	85.00	8.67	8.67	0.53	0.50	22.78	18.11	0.79	0.75
Foliar + Cyper	82.00	82.00	5.07	5.07	0.17	0.17	9.86	10.00	0.67	0.77
Poultry Man+NLE	78.00	78.00	9.67	9.65	0.73	0.90	44.17	33.56	0.86	0.86
Cow dung+ NLE	85.00	85.00	9.67	9.67	0.73	0.50	23.33	20.56	0.75	0.79
N.P.K+ NLE	87.00	85.00	8.33	8.33	0.53	0.83	26.81	34.56	0.89	0.86
Foliar + NLE	87.00	87.00	5.33	5.34	0.20	0.40	18.47	17.10	0.54	0.83
Control	101.00	97.00	2.67	2.66	0.20	0.20	6.94	8.89	0.50	0.81
SED (0.05)	4.04	4.17	2.96	2.94	0.24	0.28	10.83	6.81	0.18	0.16

The head weight and yield of cabbage treated to poultry manure, cow dung or NPK combined with Cypermetrine or neem are indicative of the fact that nutrient supply was better on with those treatments than the foliar application. It also raises the question whether nutrient absorption for plant use may be effectively done by the roots than the leaves. Fageria *et al*., (2009) confirmed that while soil uptake is more common and most effective, especially when nutrients are required in higher amounts and that in such situations foliar supply alone may not be enough to supply the needed amount. The control or foliar combined with Cypermetrine recording least yields of 10 and 9 tonnes/ha respectively could be that the foliar application of fertilizer had no effect on the yield of cabbage. This disagrees with Narayan *et al*., (2016) who reported of maximum seed yield of cabbage (10.37 q/h) with the foliar application of $NP_2O_5K_2O$ (15-15-30) against the minimum seed yield (5.50 q/h) in untreated plots. The authors further attested that the spraying of Water Soluble Fertilizers (WSF) leads to proper development of flower buds and seed setting of cabbage which ultimately improves the seed yield. However, this did not reflect in this study.

II. CONCLUSION

Soil amendments both organic and inorganic fertilizers remarkably improved the soil physical and chemical properties. High percentage crop establishment (>95 %) was achieved across treatments with NPK + Cypermetrine, Poultry manure + neem, N.P.K (15:15:15) + Neem or

Foliar fertilizer + neem achieving 100% crop establishment. The number of open leaves increased from 21DAT to 35 DAT, peaked at 49 DAT and then declined at 63 DAT. However, application of Cow dung+ NLE produced the highest number of open leaves in both seasons. Application of poultry manure and N.P.K combined with Cypermetrine significantly improved head weight and cabbage yield.

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DECLARATION OF INTEREST

The authors declare that they have no competing interests.

REFERENCES

[1] Adeniji, O. T., Tanyongana, R. and Aloyce, A. (2010). Evaluation of head yield and participatory selection of horticultural characters in cabbage (Brassica oleraceae var capitata L.). *Journal of Plant Breeding and Crop Science*, 2 (8), 243 – 250

[2] Adebayo, A. G., Akintoye, H., A., Olufolaji, A. O., Aina, O. O., Olatunji, M. T., Shokalu, A. O. (2011). Assessment of organic amendments on vegetative development and nutrient uptake of Moringaoleifera Lam in the Nursery. *Asian J Plant Sci.*, 10:74-79.

[3] Agyarko, K., Kwakye, P.K., Bonsu, B.A., Asare – Donkor, N and Amanor, E. (2006). Agronomic evaluation of cattle manure for cabbage production: animal gender and physiological conditions. *Nepalese Journal of Agricultural Science*, 9: 76 – 89.

[4] Appiah, D. O., Osman, B., and Boafo, J. (2014). Land use and misuse. Human appropriation of land eco system services in Ghana. *International Journal Ecosystems*, 4 (1), 24-33.

[5] Asiamah, R. D., Adjei- Gyapong, T., Yeboah, E., Fening, J. O., Amponsah, E. O. and Gaisie, E. (2000). Soil characterization and evaluation of four primary cassava multiplication sites (Mampong, Wenchi, Asuansi and Kpeve) in Ghana. SRI Technical Report No.200, Kumasi.

[6] Casely, K. D., Bicudo, J. R., Schmidt, D. R., Singh, A., Gay, S. W., Gates, R. S., Jacobsen, L. D. and Hoff, S. J. (2006). Air quality and emissions from livestock and poultry production/ waste management systems, in: Rice, J.M., Caldwell, D.F. and Humenik, F.J. (Eds).

[7] Ewulo, B. S. (2005). Effect of poultry manure and cattle manure on sandy clay loam soil. *Journal of Animal and Veterinary Sciences*, 4:839 – 841.

[8] Fageria, N. K., Filho, M. B., Moreira, A., &Guimarães, C. M. (2009). Foliar fertilization of crop plants. *Journal of plant nutrition*, 32(6), 1044-1064.

[9] Frempong, M. E., Ofosu- Anim, J. and Blay, E. T. (2006). Nutrient supply strategies on growth and yield in okro (Abelmoschusesculentus. L) Moench. *Journal of Horticulture*, 5:73-81.

[10] Ghana Meteorological Agency – Mampong – Ashanti (2015).

[11] Ghana Meteorological Agency – Mampong – Ashanti (2016).

[12] Gudugi, I. A. S (2013). Effect of cow dung and variety on the growth and yield of okra (Abelmoschus esculentus L). *Eur.J. Exp. Biol.*, 3:495- 498.

[13] Hassan, A., Timerman, Y., Hamdan, R., Sela, N., Avetisyan, A., Hatachmi, N., Salzberg, N. (2018). An RNAi Screen identifies New Genes Required for Normal Morphogenesis of Larval Chordotonal Organs. G3 (Bethseda), 8(6): 1871-1884.

[14] Hassan, M. R. and Solaiman, A. H. M. (2012). Efficacy of organic fertilizer on growth of Brassica oleraceae var capitata L. (Cabbage). *International Journal of Agriculture and Crop Sciences*, 4 (3), 128 – 138.

[15] John, L. W., Jamer, D. B., Samuel, L. T. and Warner, L. W. (2004). Soil fertility and fertilizers. An introduction to Nutrient Management, Pearson Education, India pp: 106 – 153. *Journal of Plant Nutrition*, 31 (10): 1832 – 1843.

[16] Meena, M. L., Ram, R. B., Lata, R. and Sharma, S. R. (2010). Determining yield components in cabbage (Brassica oleraceae var capitata L.) through correlation and path analysis. *International Journal of Science and Nature*, 1(1): 27 – 30.

[17] Milas A. S and Vincent, R. K (2017). Monitoring Landside vegetation indices for different crop treatment and soil.

[18] Narayan, S., Ahmed, N., Mufti, S., Chattoo, M., Singh, P. and Khan, F. (2016). Response of Golden Acre Cabbage to Foliar Application of Water Soluble Fertilizers on plant growth and seed yield. *IRA-International Journal of Applied Sciences*, 4(2):226-229.

[19] Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA) (2009). Agronomy Guide for Field Crops. Publication 811- Queens Printer for Ontario, Toronto.

[20] Page–Dumroese, D., Jurgensen, M., Tiarks, A., Sanchez, F., Fleming, R., Krangbetter, M., Power, R., Ponder, F., Stone, D., Elioff, J. and Scott, A. (2006). Soil physical property changes on the North American Long Term Soil Productivity (LTSP) study sites: 1 and 5 years after treatment. *Can. J. for Res.*, 36:551 – 564.

[21] Pahlar, I., Tagwira, F., Muzemu, S., Chitamba, J. (2013). Effects of soil type and manure level on the establishment

and growth of Moringaoleifera. Int. Jour. Agric. and Forestry,

[22] Patrick, A., Lesaca A, Anabella P.T (2012). Increasing Cabbage Yield through SSNM. Bar Digest research and development

[23] Pavla, B. and Pokluda, R. (2008). Influence of Alternative Organic Fertilizers on the Antioxidant Capacity in Head Cabbage and Cucumber. Notuale Botanicae Horti Agrobotanici Cluj, 36(1) 63 – 67. PhD Thesis, Ahmadu Bello University, Zaira, p. 330.

[24] Radiovich, T. J. K., Kleinhene, M. D. and Streetter, J. G. (2005). Irrigation timing relative to head development influences yield components, sugar levels and glucosinolate concentrations in cabbage. Journal of the American Society for Horticultural Science, 130: 943- 949.

[25] Sajal, R. and Md. Abdul, K. (2014). Effects of organic manure in changes of some soil properties at different incubation periods. *Open Journal of Soil Science*, 4, 81-86.

[26] Shapla, A. S (2013). Effect of planting time and organic manure on growth and yield of broccoli Department of Horticulture, Sher-e-Bengla Agricultural University, Tongi, Bangladesh, MS Thesis

[27] Snr, P.A.P., Kyere, C.G., Jnr, P.A.P., Oppong, E. and Twumasi, G. (2020). Effects of Poultry Manure, N. P. K Fertilizer and Their Combination on the Growth and Yield of Sweet Pepper. *Asian Journal of Agricultural and Horticultural Research*, 5 (1), 14-22.

[28] Timbilla, J. A, Nyarko, K. O. (2004). A Survey of cabbage production and constraints in Ghana. Ghana Journal of Agricultural Science Vol.37 2004.93-101.

[29] Zaman, M. M. (2017). Effect of cow dung as organic manure on the growth, leaf biomass yield of Stevia rebaudiana and post-harvest soil fertility.

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Biofortification: Effect of Zn and Fe application on wheat genotypes in Bangladesh

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***Abstract*— *Biofortification of zinc (Zn) and iron (Fe) will be an important effort for the combat of malnutrition in Bangladesh. The experimental site was Bangladesh Agricultural University (BAU) farm to increase the Zn and Fe content in wheat grain. The design of the experiment was split-plot and replicated thrice. Ten varieties and seven advanced lines were tested under 3 treatments: control, Zn and Zn + Fe; for the study. For control plots, the grain Zn concentration varied from 20.3 – 30.5 µg g^{-1}, across the genotypes, with the highest performance by advanced line BAW 917 and the lowest performance by variety Sufi. The average grain Zn concentration over the 17 genotypes was noted as 26.3 µg g^{-1}. When Zn was applied to soil, the grain Zn concentration ranged from 29.1 - 40.9 µg g^{-1} with a mean of 34.2 µg g^{-1}. The Fe content ranged from 20-35 µg g^{-1} with a mean of 30.5 µg g^{-1}. The protein content also increases due to the Zn application. The Zn application increase the Zn content in grain as well as increase the yield with protein content. Among the genotype, there are some potential varieties for biofortification.*

***Keyword*— Zinc, iron, grain yield and wheat.**

I. INTRODUCTION

One of the very important micronutrient is zinc (Zn) for both human as well as plant and insufficient availability become a global health issue now by covering half of total population in earth (Hotz and Brown 2004; Stein 2010). The zinc deficiency in human and soil is very near that overlap geographically (Alloway 2008; Cakmak 2008) and show that there are a very close relationship between soil, food crops and human (Welch 2008). The people with cereal based food habitant are mainly suffering from Zn deficiency (Cakmak 2008; Gibson 2006) as bioavailability of Zn is low in cereal (Cakmak et al. 2010a).

As reported in Bangladesh by Islam *et al.* (2013), around 60% Zn and 55% Fe is provided from cereals in daily consumption. Anemia is widespread in Bangladesh especially to children and women due to inadequate Fe uptake. Increasing cropping intensity from 143% in 1971-72 to 194% in 2015-16 (BBS, 2018) declining soil fertility resulted micronutrient deficiency in Bangladesh. Among the micronutrient, Zn deficit is the top complication for crop growth. This element deficiency in the country was identified in late 1970s (Jahiruddin *et al.*, 1981) and with advancement of time its extent has increased. In Bangladesh, about 70% of the arable land is found Zn deficit (Jahiruddin and Islam, 2014).

Biofortification means to prepend micronutrients to food crops by improving breeding lines as well as fertilization methods that will create a opportunity for the rural people to get food intake with Zn as they could not afford fortified foods (Bouis, 2013). In Bangladesh, “baby zinc” tablet developed by icddr’b (Brooks, 2005) reduced child mortality from diarrhoea.

Iron (Fe) is an essential plant nutrient and its deficiency causes chlorosis, nutritional disorder and reduces crop yield. It will be very important if Fe could be increased in main food crops which can reduce common deficiency among the general people (Cakmak, 2002). Micronutrient deficiency is now a big challenges for the world population specially Zn and Fe (WHO, 2007). The grain yield and grain Zn concentration generally found have inverse relationship between them (McDonald et al. 2008). This inverse relationship problem can be address by breeding, transgenic technology or agronomic approaches. To increase the micronutrients in food grain an combined approaches needs to undertaken by both breeding (Potential genotype) and fertilizer management approaches for mitigation of Zn insufficiency among the general people (Cakmak *et al.,* 2004). This experiment was undertaken to increase the Zn and Fe content in wheat grain by fertilizer application and variety selection.

II. MATERIALS AND METHODS

Experimental Site

The experiment site was Bangladesh Agricultural University farm (BAU), Mymensingh, Bangladesh (location: 24^0 42´ 56.04´´ N and 90^0 25´ 31.01´´ E) and the agro-ecological zones (AEZs-9) is namely Old Brahmaputra Floodplain (FRG, 2018). The physical and chemical properties of soil at experiment sites are present at Table 1.

Table 1 Soil physical and chemical characteristics of the experimental fields

Textural Class	OC (%)	pH	Total N (%)	Avail. P (mg kg^{-1})	Exch. K (cmol kg^{-1})	Avail. S (mg kg^{-1})	Avail. Zn (mg kg-^{1})	Avail. Fe (mg kg^{-1})
Silt loam	1.14	6.5	0.11	7.5	0.12	14.0	0.78	55.4

Treatments and design

In these experiments there were three treatments of zinc and iron viz. Zn_0Fe_0, Zn_3Fe_0 and Zn_3Fe_4; subscripts represent the dose nutrients in kg ha^{-1}. All other fertilizers like N, P, K, S and B were applied at $N_{120}P_{30}K_{50}S_{12}B_{1.5}$ kg ha^{-1} to the all plots. The split plot design was used and replicated thrice.

Crop and Soil Management

There were 10 varieties and 7 advanced lines of wheat were tested for grain Zn & Fe concentrations as well as grain yield. The wheat varieties and advanced line were: Shatabdi (V_1), Sufi (V_2), Bijoy (V_3), Prodip (V_4), BARI Gom 25 (V_5), BARI Gom 26 (V_6), BARI Gom 27 (V_7), BARI Gom 28 (V_8), BARI Gom 29 (V_9) and BARI Gom 30 (V_{10}), and Rawal 87 (L_1), Vijay (L_2), BAW 917 (L_3), Fery 60 (L_4), BL 1040 (L_5), KRLI-4 (L_6), BL 1883 (L_7). Wheat seeds were sown on 16 November 2016 and the crop was harvested on 12 March 2017. The mature harvested crops were threshed, cleaned and processed for chemical analysis.

Chemical analysis

The soil samples of the experimental site were collected following standard procedure and processed by air-drying, ground and sieving in a 2-mm sieve. The soil texture, soil pH, organic matter, total nitrogen, exchangeable potassium, available phosphorus, sulphur, zinc and iron were measured following standard methods.

Analysis of plant sample

The harvested grain sample was collected from each plot and were analysed for N, Zn and Fe concentrations. The collected samples were dried in an oven at 65°C for about 48 hours and then ground by grinding machine to pass through a 20-mesh sieve to obtain homogenous powder. The prepared plant samples were kept in paper bags into desiccators for further analysis for the determination of N, Zn and Fe content.

Statistical analysis

The statistical analysis of the different plant parameters as well as soil and plant analysis data was done through computer based program (Statistics 10) and was followed the basic principles, as outlined by Gomez and Gomez (1984). For the determination of analysis of variance (ANOVA) of the significant effects of treatments, genotypes and their interaction were compared at 5% level of significance by Duncan's Multiple Range Test (DMRT).

III. RESULTS

Biofortification of Zn in wheat grain

Zinc fortification in wheat grain differed due to the treatments as well as to the different genetic makeup. The Zn concentration of wheat grain varied significantly with the genotypes (varieties and breeding lines) and with the Zn & Fe fertilization.

Genotypic effects

Different Zn content are found among the genotype used in the experiment. The treatment T_1 (control plots) presents a wide range of Zn concentration where the grain Zn concentration varied from 20.3 - 30.5 µg g^{-1}, across the genotypes. The highest Zn concentration was found in two advanced lines BAW 917 and Vijoy (30.5 µg g^{-1}) whereas in variety Sufi obtained lowest (20.3 µg g^{-1}) zinc concentration. The average grain Zn concentration over the 17 genotypes was noted as 26.3 µg g^{-1} (Table 2)

Fertilizer effect

The Zn concentration of the different genotype varied significantly due to the zinc fertilizer application. In treatment T_2, the grain Zn concentration ranged from 29.1 - 40.9 µg g^{-1} and the highest concentration was found in advanced lines BAW 917 (40.9 µg g^{-1}) and the lowest Zn concentration was found in variety Shatabdi (29.1 µg g^{-1}). The average Zn concentration was found 34.2 µg g^{-1} (Table 3) and the highest increase in Zn concentration in advanced line BL1883 is 11.7 µg g^{-1} where as the lowest increase is 3.5 µg g^{-1} is found in variety BARI Gom 30. The mean increase in Zn concentration in treatment T_2 is 7.99 µg g^{-1} which is very noticeable.

Regarding treatment T_3 where both Zn and Fe was applied, the maximum increased of Zn concentration was obtained from variety Sufi is 12.2 µg g^{-1} and the lowest increase in Zn concentration was found in variety BARI Gom 29 that is 2.8 µg g^{-1} ,. The mean increase in Zn concentration in treatment T_2 is 7.54 µg g^{-1} which is also very noticeable.

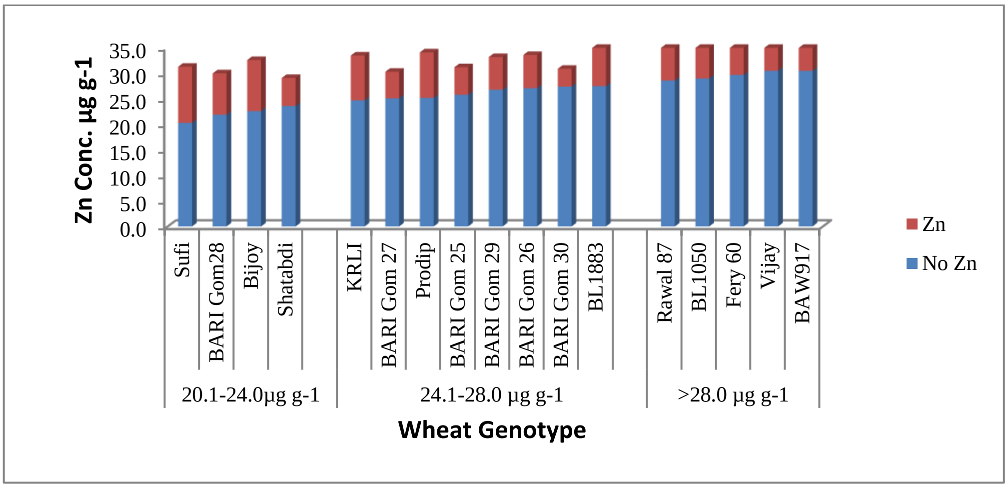

Fig. 1 Amount of zinc content of different wheat genotype

Table 2 Effects of Zn and Fe application on grain Zn concentrations (µg g^{-1}) of different genotypes of wheat

Genotypes	**T_1 (Control)**	**T_2 (Zn)**	**T_3 (Zn + Fe)**	**T_2-T_1**	**T_3-T_1**
V_1: Shatabdi	23.6 f-i	29.1 g	30.8 d	5.50	7.20
V_2: Sufi	20.3 i	31.3 d-g	32.5 cd	11.00	12.20
V_3: Bijoy	22.6 g-i	32.6 d-g	29.7 d	10.00	7.10
V_4: Prodip	25.2 d-h	34.1 cd	31.3cd	8.90	6.10
V_5: BARI Gom 25	25.8 c-g	31.2 d-g	31.1 cd	5.40	5.30
V_6: BARI Gom 26	27.1a-f	33.6 c-e	34.6 bc	6.50	7.50
V_7: BARI Gom 27	25.1 d-h	30.3 e-g	29.2 d	5.20	4.10
V_8: BARI Gom 28	21.9 hi	30.0 fg	31.7 cd	8.10	9.80
V_9: BARI Gom 29	26.8 b-f	33.2 c-f	29.6 d	6.40	2.80
V_{10}: BARI Gom 30	27.4 a-e	30.9 d-g	31.6 cd	3.50	4.20
L_1: Rawal 87	28.6 a-d	39.1 ab	37.8 ab	10.50	9.20
L_2: Vijay	30.5 a	37.6 ab	37.1 ab	7.10	6.60
L_3: BAW 917	30.5 a	40.9 a	39.3 a	10.40	8.80
L_4: Fery 60	29.7 ab	38.9 ab	36.9 ab	9.20	7.20
L_5: BL 1040	29.0 a-c	36.6 bc	38.2 a	7.60	9.20
L_6: KRLI-4	24.7 e-h	33.5 c-e	34.5 bc	8.80	9.80
L_7: BL 1883	27.5 a-e	39.2 ab	38.6 a	11.70	11.10
Max	**30.5**	**40.9**	**39.3**	**11.70**	**12.20**

Min	**20.3**	**29.1**	**29.2**	**3.50**	**2.80**
Mean	**26.3**	**34.2**	**33.8**	**7.99**	**7.54**

Genotypes V_1 - V_{10} represent varieties and L_1 - L_7 represent advanced breeding lines. Means followed by same letter in a column are not significantly different at 5 % level by DMRT.

Table 3 Effects of Zn and Fe application on grain Fe concentrations (μg g^{-1}) of different genotypes of wheat

Genotypes	T_1 (Control)	T_2 (Zn)	T_3 (Zn + Fe)	T_3-T_1
V_1: Shatabdi	26.5	27.9	33.2	6.70
V_2: Sufi	23.8	25.8	33.0	9.20
V_3: Bijoy	26.5	28.4	33.9	7.40
V_4: Prodip	24.0	23.4	30.5	6.50
V_5: BARI Gom 25	29.7	28.7	36.6	6.90
V_6: BARI Gom 26	24.7	26.3	31.9	7.20
V_7: BARI Gom 27	28.8	27.3	34.8	6.00
V_8: BARI Gom 28	23.9	26.5	33.4	9.50
V_9: BARI Gom 29	26.6	23.9	32.1	5.50
V_{10}: BARI Gom 30	23.7	26.5	32.7	9.00
L_1: Rawal 87	27.4	28.1	36.3	8.90
L_2: Vijay	29.4	28.9	37.5	8.10
L_3: BAW 917	25.7	28.0	36.1	10.40
L_4: Fery 60	27.5	29.8	36.6	9.10
L_5: BL 1040	28.3	31.8	39.7	11.40
L_6: KRLI-4	28.6	27.1	36.0	7.40
L_7: BL 1883	29.4	32.4	36.7	7.30
Max	**29.7**	**32.4**	**39.7**	11.40
Min	**23.7**	**23.4**	**30.5**	5.50
Mean	**26.7**	**27.7**	**34.8**	8.03

Genotypes V_1 - V_{17} represent varieties and L_1 - L_7 represent advanced breeding lines. Lettering was not done since the treatment effects were not significant.

Zinc efficiency of wheat genotypes

The increase in grain Zn concentrations of different wheat varieties and genotype differed in their response to Zn and Zn + Fe fertilization. Based on the % Zn efficiency [(Control Zn concentration / Treatment Zn concentration) x 100], the wheat genotypes could be classified into four groups (Fig. 2): inefficient (responsive to Zn application), moderately inefficient (moderately responsive), moderately efficient (moderately unresponsive) and efficient (unresponsive).

It is appearing that 2 genotype (variety Sufi and Bijoy) were found to be Zn inefficient (<70% Zn efficient), 8 genotype (2 varieties and 6 lines) moderately Zn inefficient (71-80% Zn efficient), 7 genotype (6 varieties and 1 line) moderately Zn efficient (81-90% Zn efficient) and no genotype are found Zn efficient (>90% Zn efficient). Varieties Shatabdi, BARI Gom 25, 26, 27, 29, and 30 are found moderately Zn efficiency with a advanced line Vijoy.

Thus, the results of two locations reveal that variety BARI Gom 26, breeding lines BAW, BL 1883 & BL 1040 were observed as commonly Zn efficient or moderately Zn efficient in which plots, Zn supplement was not done.

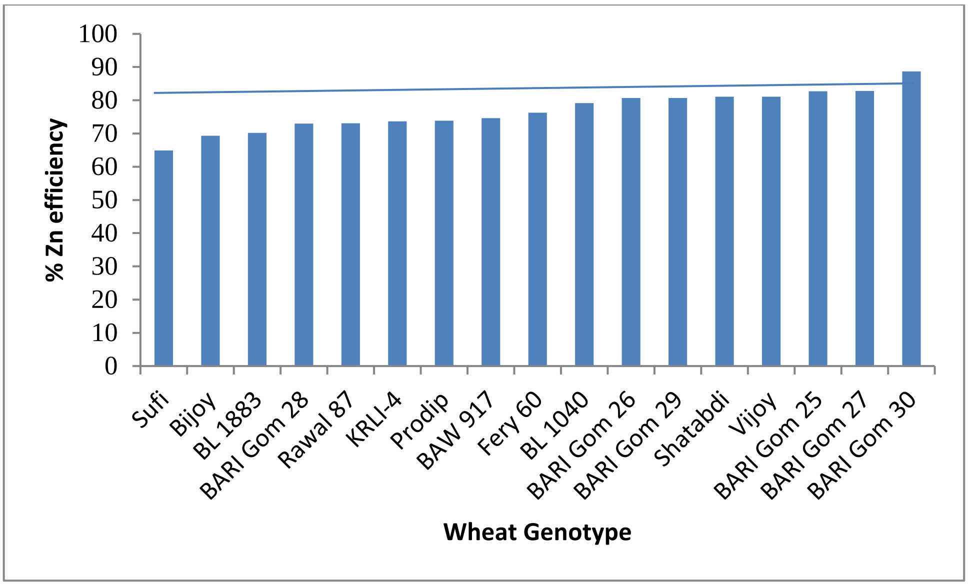

Fig. 2 Per cent Zn efficiency of different varieties and breeding lines of wheat genotype

Biofortification of Iron

The iron (Fe) was applied in treatment T_3 with the Zn fertilizer and result showed that the grain Fe concentration of 17 genotypes ranged from 23.7 - 29.7 µg g^{-1}, with the mean value of 26.7 µg g^{-1}. This result was obtained when Zn fertilizer was not used. The highest Fe concentration was found from variety BARI Gom 25 and variety BARI Gom 30 did the lowest. The grain Fe concentration increased by about 2 µg g^{-1} over Zn or Fe fertilized plots. In the Zn fertilized plots, the grain Fe concentration was found as 23.4 - 32.4 µg g^{-1}, mean 27.7 µg g^{-1} (Table 3). Among the Fe treated plots, the Fe concentration increased in all the genotypes and it ranged from 30.5 to 39.7 µg g^{-1} and the mean is 34.8 µg g^{-1}. The Fe concentration increased from 5.50 to 11.40 µg g^{-1} with a mean of 8.03 µg g^{-1} which is noticeable increment of Fe in wheat grain. The highest increase of Fe concentration was found from BL 1040 and BARI Gom 29 accumulated the lowest.

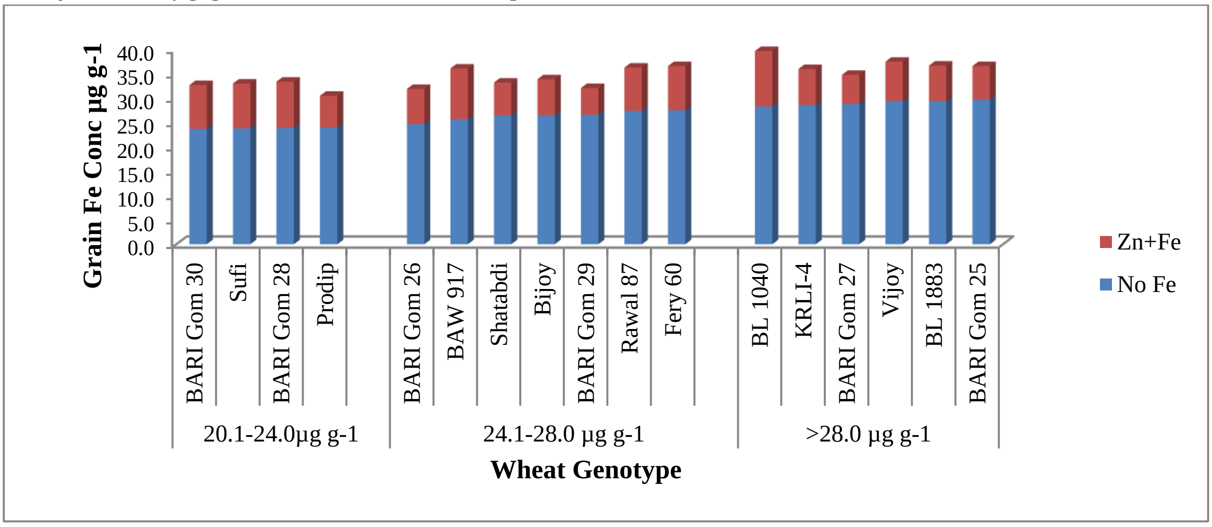

Fig. 3 Variation of iron concentration in grain of different wheat genotype

Protein content

The protein content of different varieties and advanced line are presented in Table 4.3. Obviously, the N concentration is considerably differed due to the genotype and has positively responded to the Zn fertilization. The effect of genotypes and Zn or Fe fertilization was the same for grain protein % since protein% was calculated as a multiple of 5.85 over grain N %.

The grain protein content of wheat, varied from 7.68 - 8.23%, the mean value being 7.91% (Table 4) when Zn or Fe fertilizer was not applied (control). Advanced line BL

1883 demonstrated the highest grain protein% and BAW 917 did the lowest.

The grain protein concentration of wheat, whether varieties or lines, markedly increased due to Zn fertilization; however, no effect was observed for Fe application. In Zn fertilized plots, the protein% varied from 8.67 - 9.87% with a mean of 9.14% and the Zn and Fe treatment plot lied between 8.78 - 9.69% with a mean of 9.02% (Table 4). This result indicates that Zn has influence on protein synthesis and plants take up these two elements (N and Zn) at a proportionate amount. The average increase in protein percentage is 1.23% when Zn fertilization was done.

Table 4 Effects of Zn and Fe application on protein content on grains of wheat genotypes

Genotypes	**T_1 (Control)**	**T_2 (Zn)**	**T_3 (Zn+Fe)**	**T_2 -T_1**	**T_3 –T_1**
V_1: Shatabdi	7.80	9.59	9.52	1.79	1.72
V_2: Sufi	7.74	9.01	9.20	1.27	1.46
V_3: Bijoy	7.98	9.32	9.03	1.35	1.05
V_4: Prodip	7.82	9.13	8.99	1.31	1.17
V_5: BARI Gom 25	8.17	9.87	9.69	1.70	1.52
V_6: BARI Gom 26	8.00	9.48	9.24	1.48	1.25
V_7: BARI Gom 27	8.11	9.56	9.52	1.44	1.40
V_8: BARI Gom 28	7.92	9.79	9.46	1.87	1.54
V_9: BARI Gom 29	8.00	9.48	9.17	1.48	1.17
V_{10}: BARI Gom 30	8.21	9.77	8.97	1.56	0.76
L_1: Rawal 87	7.76	8.93	8.85	1.17	1.09
L_2: Vijay	8.23	9.42	9.46	1.19	1.23
L_3: BAW 917	7.68	8.76	8.89	1.07	1.21
L_4: Fery 60	8.15	8.89	8.78	0.74	0.62
L_5: BL 1040	8.11	9.32	8.93	1.21	0.82
L_6: KRLI-4	8.60	9.48	9.63	0.88	1.03
L_7: BL 1883	8.23	9.77	9.38	1.54	1.15
Max	**8.23**	**9.87**	**9.69**	**1.87**	**1.72**
Min	**7.68**	**8.67**	**8.78**	**0.74**	**0.62**
Mean	**7.91**	**9.14**	**9.02**	**1.23**	**1.10**

Grain yield

Different varieties has different grain yield normally due to its difference of genetic potential. The varieties and advanced lines of wheat used in this study have produced different yield and there are variation of grain yield of wheat genotypes varied with varieties and breeding lines as well as fertilization.

Genotype effect

The grain yield of wheat generally varied with varieties and breeding lines which can be attributed to differences in genetic make-up. The grain yield does not have wide variation and ranged from 3.37 - 3.90 t ha^{-1}, the mean yield being 3.59 t ha^{-1} (Table 5). The highest grain yield (3.90 t ha^{-1}) was obtained from variety BARI Gom 30 and very close yield was given by BARI Gom 26, Fery 60 and BL1883. Among the tested genotypes, BARI Gom 28 performed the lowest yield (3.37 t ha^{-1}) and similar yield was demonstrated by Prodip and Sufi. The average yield over the 17 genotypes was found 3.59 t ha^{-1}.

Fertilizer effect

The grain yield positively responded to Zn fertilization over the varieties (Table 5). The BARI Gom 30 obtained the maximum yield (4.65 t ha^{-1}) followed by BARI Gom 26 (4.57 t ha^{-1}). Regarding at the % yield increase, it ranged from 9.22 - 27.40% having the best response by advanced line Bijoy and least response by KRLI-4. The BARI Gom 30 exhibited the highest yield (4.65 t ha^{-1}) due to Zn fertilization. The mean yield across the genotypes was 4.19 t ha^{-1} showing 0.6 t ha^{-1} higher over control yield. Virtually the yield remained unaffected by Fe fertilization.

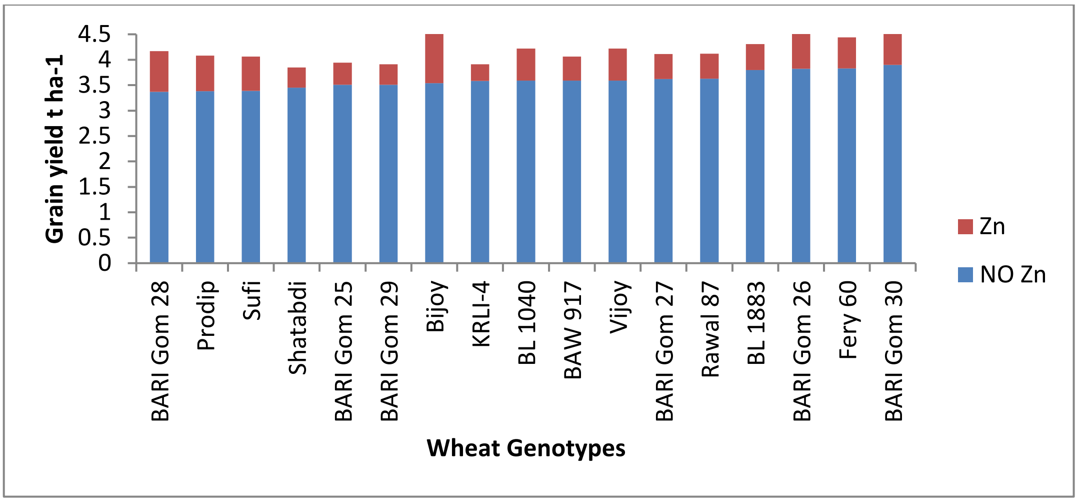

Fig. 4 Variation in grain yield of different genotypes of wheat

Table 5 Effects of Zn and Fe application on grain yield (t ha^{-1}) of wheat genotypes

Genotypes	T_1 (Control)	T_2 (Zn)	T_3 (Zn+Fe)	% Increase of T_2	% Increase of T_3
V_1: Shatabdi	3.45	3.85	3.90	11.59	13.04
V_2: Sufi	3.39	4.06	3.85	19.76	13.57
V_3: Bijoy	3.54	4.51	4.17	27.40	17.80
V_4: Prodip	3.38	4.08	3.99	20.71	18.05
V_5: BARI Gom 25	3.51	3.94	3.66	12.25	4.27
V_6: BARI Gom 26	3.82	4.57	4.40	19.63	15.18
V_7: BARI Gom 27	3.62	4.11	3.98	13.54	9.94
V_8: BARI Gom 28	3.37	4.17	3.71	23.74	10.09
V_9: BARI Gom 29	3.51	3.91	4.03	11.40	14.81
V_{10}: BARI Gom 30	3.90	4.65	4.68	19.23	20.00
L_1: Rawal 87	3.63	4.12	4.22	13.50	16.25
L_2: Vijay	3.59	4.22	4.35	17.55	21.17
L_3: BAW 917	3.59	4.06	4.12	13.09	14.76
L_4: Fery 60	3.83	4.44	3.92	15.93	2.35
L_5: BL 1040	3.59	4.27	4.22	18.94	17.55
L_6: KRLI-4	3.58	3.91	4.11	9.22	14.80
L_7: BL 1883	3.80	4.31	4.40	13.42	15.79
Max	**3.90**	**4.65**	**4.68**	**27.40**	**21.17**
Min	**3.37**	**3.85**	**3.66**	**9.22**	**2.35**
Mean	**3.59**	**4.19**	**4.10**	**16.52**	**14.08**

Genotypes V_1 - V_{10} represent varieties and L_1 - L_7 represent advanced breeding lines. Lettering was not done since the treatment effects were not significant.

IV. DISCUSSIONS

The existing wheat cultivars are not able to fulfill the Zn requirement for people in Bangladesh. Agronomic biofortification can solve this problem immediately which will be sustainable and easy adoptable to decipher Zn insufficiency in cereals (Qamar *et al*. 2017). The Zn concentration of wheat grain markedly increased due to Zn fertilization showing an increment of 3.5 - 11.7 µg g^{-1} Zn across the 17 genotypes used. Similarly, the grain Fe concentration had increased (5.5 - 11.4 µg g^{-1}) for the use of Fe fertilizer despite the fact that crop yield did not increase and further the experimental fields were not Fe deficient.

Some of the wheat varieties and breeding lines had potential of higher Zn accumulation and the Zn fertilization had an additive effect. The EDTA extractable Zn (0.78 mg kg^{-1}) was found low in the study location. The critical limit of Zn in Bangladesh soil is 0.60 mg kg-1 (FRG-2018). Yilmaz (1997) observed that Zn fertilization produced higher grain yield and increase the Zn concentration in grain as well in wheat and very crucial in soil where Zn is deficient (Cakmak, 2010). Duxbury *et al.* (2005) reported an elevated level of Zn, Cu and Mo in rice and wheat grains from their supplementary application.

The present study has screened out several varieties of wheat which have greater ability to uptake and accumulate Zn and Fe in grain, with a further possibility to enhance grain Zn concentration of wheat through Zn and Fe fertilization. Rawal 87, BL 1040, Vijoy & BAW 917 have been identified as Zn enriched breeding lines (>28.1 µg g^{-1} grain Zn. Virtually Fe fertilization did not influenced on Zn concentration in grain. Increment of grain Zn concentration due to Zn application is similar between varieties and lines tested. Findings from Cakmak (2010) showed that Zn concentration increased 11.7 (control) to 26.9 µg g^{-1} by Zn application.

Zinc fertilization depending on the varieties increased wheat yield by 9.2 - 27.4%. The grain yield differed with genotypes which can be attributed to differences in genetic make-up. Response of wheat yield to Zn application is much evidenced in Bangladesh and India (Khan *et al.*, 2009, Prasad *et al.*, 2010; Singh *et al*., 2012). Numerous studies have shown pronounced increase in grain yield (9–256%) and grain Zn concentration (9–912%) of wheat with Zn application to Zn deficient soils (Rafique *et al.*, 2006; IZA, 2009).

The results showed that Fe concentration of wheat grain generally increased for Fe fertilization, the increment being on an average 6-12 µg g^{-1}. The grain Fe concentrations of different varieties have been divided into four groups with an interval of 4 µg g^{-1} Fe: <20 µg g^{-1} Fe, 20.1 - 24 µg g^{-1} Fe, 24.1 - 28 µg g^{-1} Fe and >28 µg g^{-1} Fe. Similar to classification of grain Zn concentration, not all the grain Fe concentration of the same genotypes fell into the same class between two locations. This variation could be due to varied soil and climatic conditions.

Genetic biofortification together with agronomic approach extends a good possibility of development of new cultivars more efficient in accumulating minerals in the edible part (Mingotte *et al.*, 2018). Agronomic biofortifcation with Zn can provide a practical and cost-effective option to tackle the global Zn malnutrition problem (Cakmak and Kutman, 2018).

V. CONCLUSION

Some varieties and breeding lines of wheat have genetically greater ability to uptake and accumulate Zn and Fe in grain. It is possible to enhance further Zn and Fe level by their fertilization. Varieties BARI GOM 25, 27, 28 & 29 are identified as Zn enriched varieties, having 24 - 30 µg g^{-1} Zn in grain. Concerning Fe biofortification, Shatabdi, Prodip, BARI GOM 25 & 28 and Sufi are identified as Fe enriched varieties (24 - 30 µg g^{-1}). These genotypes would serve as breeding materials for biofortification of Zn & Fe in wheat, without compromising crop yield. Genetic biofortification coupled with agronomic approach (fertilization) would help develop of new cultivars of wheat that would have ability to accumulate Zn and Fe in grain.

REFERENCES

[1] Alloway BJ 2008: *Micronutrient Deficiencies and Global Crop Production.* Springer, Netherlands, 353 pp.

[2] BBS 2018: Year Book of Agricultural Statistics of Bangladesh. Bangladesh Bureau of Statistics. Ministry of Planning, Government of the Peoples Republic of Bangladesh, Dhaka.

[3] Bouis HE 2013: Biofortification: A new tool to reduce micronutrient malnutrition. In Proc.: XVII. *International Plant Nutrition Colloquium*, pp. 67-68.

[4] Brooks WA, Santosham M, Naheed A, Goswami D, Wahed MA, Diener-West M, Faruque ASG, Black RE 2005: Effect of weekly zinc supplements on incidence of pneumonia and diarrhoea in children younger than 2 years in an urban, low-income population in Bangladesh: randomised controlled trial. *The Lancet* **366** 999-1004

[5] Cakmak I, Kutman UB, 2018: Agronomic Biofortification of Cereals with Zinc: A Review. *European Journal of Soil Science* **69** 172-180.

[6] Cakmak I 2010: Biofortification of cereals with zinc and iron through fertilization strategy. 19th World Congress of Soil Science, 1-6 August, Brisbane, Australia, Proceeding: pp. 4-6.

[7] Cakmak I, Kalayci M, Kaya Y, Torun AA, Aydin N, Wang Y, Arisoy Z, Erdem H, Yazici A, Gokmen O, Ozturk L, Horst WJ 2010: Biofortification and localization of zinc in wheat grain. *Journal of Agricultural and Food Chemistry* **58** 9092–9102.

[8] Cakmak I 2008: Enrichment of cereal grains with zinc: Agronomic or genetic biofortification? *Plant and Soil* **302** 1–17.

[9] Cakmak I 2004: Identification and correction of widespread zinc deficiency in Turkey, A success story. *IFS Proceedings No. 552, International Fertiliser Society, York. UK*, pp 1–28.

[10] Cakmak I 2002: Plant nutrition research: Priorities to meet human needs for food in sustainable ways. *Plant and Soil* **247** 3–24.

[11] Duxbury JM, Bodruzzaman MD, Johnson S, Mayer ABM, Lauren J G, Meisner CA, Welch RM 2005: Impacts of increased mineral micronutrient content of rice and wheat seed/grain on crop productivity and human nutrition in Bangladesh. Li CJ, Zhang FS, Dobermann A, Hinsinger P, Lambers Marschner L, Maene L, Mcgrath S, Oenema O, Peng SB, Rengel Z, Shen QR, Welch R, Von Wiren N, Yan XL, Zhu YZ (Editor), *Plant Nutrition for Food Security, Human Health and Environment Protection* pp. 30-31.

[12] FRG (2018). Fertilizer Recommendation Guide-2018. Bangladesh Agricultural Research council (BARC), Farmgate, Dhaka, Bangladesh

[13] Gibson RS, Bailey KB, Gibbs M 2010: A review of phytate, iron, zinc, and calcium concentrations in plant-based complementary foods used in low-income countries and implications for bioavailability. *Food and Nutrition Bulletin* **31**: 34– 46.

[14] Gomez KA, Gomez AA 1984: *Statistical procedures for agricultural research (second edition)*. An International Rice Research Institute Book. John Wiley & Sons, Inc., USA. pp. 139-240.

[15] Hotz C, Brown KH (2004) Assessment of the risk of zinc deficiency in populations and options for its control. Food Nutr Bull 25:S91–S204

[16] Islam MR, Jahiruddin M, Islam MR, Alim MA, Akhtaruzzaman M 2013: Consumption of unsafe foods: evidence from heavy metal, mineral and trace element contamination. FAO Project Completion Report.

[17] IZA 2009: *Zinc in Fertilizers: Essential for Crops, Essential for Life!* International Zinc Association, Brussels, Belgium.

[18] Jahiruddin M, Bhuiya ZH, Hoque MS, Rahman L 1981: Effect of rates and methods of zinc application on rice. *Madras Agricultural Journal* **68** 211–216.

[19] Jahiruddin M, Islam MR 2014: Project Report (2011-2014). Requirement of Micronutrients for Crops and Cropping patterns. PIU-BARC (NATP Phase-1) project. Project ID No. 339. Bangladesh Agricultural Research Council, Dhaka.

[20] Khan A, Gurmani AR, Khan MS, Gurmani MH 2009: Residual, direct and cumulative effect of zinc application on wheat and rice yield under rice-wheat system. *Soil and Environment* **28(1)** 24-28.

[21] McDonald GK, Genc Y, Graham RD 2008: A simple method to evaluate genetic variation in zinc grain concentration by correcting for differences in grain yield. *Plant and Soil* **306** 49–55.

[22] Mingotte FLC, Revolti LTM, Unêda-Trevisoli SH, Lemos LB, Filho DF 2018: Rice (*Oryza sativa*) breeding strategies for grain biofortification. *African Journal of Biotechnology* **17(14)** 466-477,

[23] Prasad RK, Kumar V, Prasad B, Singh AP 2010: Long term effect of crop residue and zinc fertilizer on crop yield, nutrient uptake and fertility built-up under rice wheat cropping system in calcareous soil. *Journal of Indian Society of Soil Science* **58(2)** 205-211

[24] Qamar uz Zaman, Aslam Z, Yaseen M, Zahid Ihsan M, Khaliq A, Fahad S, Bashir S, P. M. A. Ramzani, Naeem M 2017: Zinc biofortification in rice: leveraging agriculture to moderate hidden hunger in developing countries. *Archives of Agronomy and Soil Science* **64:2** 147-161.

[25] Rafique E, Rashid A, Ryan J, Bhatti AU 2006: Zinc deficiency in rainfed wheat in Pakistan: magnitude, spatial variability, management, and plant analysis diagnostic norms. *Communications in Soil Science and Plant Analysis* **37** 181-197.

[26] Singh O, Kumar S, Awanish 2012: Productivity and profitability of rice as influence by high fertility levels and their residual effect on wheat. *Indian Journal of Agronomy*. **57(2)** 143-147.

[27] Stein AJ 2010: Global impacts of human mineral malnutrition. *Plant and Soil* **335** 133–154.

[28] Welch RM (2008) Linkages between trace elements in food crops and human health. In: Alloway BJ (ed) Micronutrient deficiencies in global crop production. Springer, Netherlands, pp 287–309

[29] WHO (World Health Organization) 2007: *World Health Report 2007*: *Reducing Risks, Promoting Healthy Life*, Geneva.

[30] Yilmaz A, Ekiz H, Torun B, Gultekin I, Karanlik S, Bagci SA, Cakmak I 1997: Effect of different zinc application methods on grain yield and zinc concentration in wheat grown on zinc-deficient calcareous soils in Central Anatolia. *Journal of Plant Nutrition* **20** 461–471.

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Molecular Characterization of Bacterial that degrades herbicides isolated from soil environment in Abuja.

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Abstract— *The study was aimed to determine the molecular characterization of bacterial that degrades herbicides isolated from soil environment in Abuja. The systemic chemical herbicide was applied on an experimental plot of land with weeds and its effects on soil bacteria and the physicochemical properties of the soil was examined for a period of seven weeks. The chemical herbicide, glyphosate, reduced the plate count of bacteria from 120 x 10^5 cfu/g/dwt to 48 x 10^5 cfu/g/dwt some hours after application and the reduction continued till the end of the sampling period. The isolated bacterial species were Simulium tani, Bacillus firmus, Pseudomonas tolaasii, Acinetobacter beijerinckii, Entrobacter sp, Citrobacter freundii , Pseudomonas poae. Organisms that were eliminated following glyphosate application were Bacillus magaterium, Pseudomonas tolaasii, Proteus sp, and Simulium tani while those that persisted throughout the experiment were Staphylococcus aureus, Pseudomonas poae, Bacillus firmus, and Entrobacter sp. It was concluded that glyphosate altered the microbial counts and had a temporary inhibitory effect on the type of bacteria present in the soil.*

***Keywords*—degrades, herbicide, soil, isolated, microbial count.**

I. Introduction

Herbicides are valuable tools for the selective control of un-desirable plants in crop production. However, various herbicides at recommended rates, whether applied to the foliage or soil, often persist in the soil for extended periods of time. These residues may cause serious damage to sensitive plant species grown the season(s) following application of the herbicides. The climatic and edaphic factors, e.g., temperature, moisture, pH, soil composition, and cation exchange capacity which affect the residual life of herbicides, are numerous and complex.

Herbicides cause a range of health effects ranging from skin rashes to death. The pathway of attack can arise from intentional or unintentional direct consumption, improper Agricultural application resulting in the herbicide coming into direct contact with people or wildlife, inhalation of aerial sprays, or food consumption prior to the labeled pre-harvest interval.Pesticides can enter the human body through inhalation of aerosols, dust and vapour that contain pesticides, through oral exposure by consuming food and water, and through dermal exposure by direct contact of pesticides with the skin (Cooper and Dobson 2007).

Herbicide are often applied directly to soil. They may also reach the soil through application to foliage via spray drift, run-off, or wash-off vectors. Once released to the environment, chemicals undergo various dissipation pathways, and the persistence of chemicals in the environment varies widely. Among factors affecting the local concentration of a compound are the amount of compound released, the rate of compound released, its persistence in the environment under various conditions, the extent of its dilution, its mobility, and the rate of biological or non-biological degradation (Ellis 2000 and Janssen *et al.* 2001).

Herbicide biodegradation involves a wide variety of microorganisms including bacteria and fungi operating under dynamic anaerobic and aerobic conditions. It is suggested that biodegradation of pesticides in soil ecosystems can only take place through the synergistic interactions of a microbial consortium, the activity of which is affected by many soil physical and chemical properties, as well as the nature and extent of the pesticide contamination.

Soil microbes make valuable contribution to soil fertility. Pesticides can exhibit, stimulate and neutral effect on soil microbes, depending on the nature and concentrations as well as strain or types of microbes (Busse*et al.*, 2001).

Herbicides will remain toxic in soil when conditions are not favorable for microbes. Degradation of the herbicide follows the population growth of the microbes. During the lag phase the microbial population increases in response to food source and rapid decomposition occurs. (Busse *et al.*, 2001).

It drastically reduces the microbial population when applied to any soil sample. Synthetic herbicides have the potential to influence plant disease by several mechanisms. They can enhance disease or protect plants from pathogens due to direct effects on the microbe, to effects on the plant, or to effects on both organisms.

II. MATERIALS AND METHOD

Collection of soil samples

Soil samples were collected from the research farm of National Root Crop Research Institute, Nyanya Out Station, Abuja, Nigeria (Latitude 9.0765N and Longtitude 7.3986E).

Soil samples were taken randomly with soil anger from each of the experimental plots and control plot, top soils of 0-15cm depth were used. Cassava and Maize arethecrops grown in the farm. These crops have been sprayed with glyphosate organophosphorusherbicide for the last 4-5years. Plots of land measuring 3x3m with four replicates arranged in randomized form was used for the experiment.Samples were collected before and after application of herbicide on from week one to the seventh week. Soil samples were sieved with 2.0mm mesh to remove stones and plant debris in soil. Samples were taken immediately to the laboratory into sampling bags for immediate analysis. (Makut and Ifeanyi 2017).

The herbicide, Glyphosate, was dissolved in distilled water at recommended rate of 50ml to five liter of water was used in this study, the mixture was then applied to the experimental plots and distil water was added to the control plots for comparison.

Isolation of Bacteria from soil contaminated with herbicide.

One (1.0) gram of the soil sample was weighed using weighing balance suspended in 9ml of sterile water. It was properly mixed and a 10-fold serial dilution was carried out into seven dilutions.

The identification and characterization of bacterial isolates were based on cultural, morphological and biochemical characteristic using standing method. (Mendes *et al.*, 2017).

Molecular Identification of Bacteria isolated from herbicide contaminated soil.

The molecular identification of bacteria isolated from herbicide contaminated soil where carried out using Bacterial genomic DNA extraction, DNA quantification, 16SrRNA Amplification and Sequencing.

Determining the effects of Temperature, pH and Days on biodegradation of herbicides

Biodegradation experiment was carried out at two different temperatures pH and weeks (herbicides 3.0mg/ml) using the methods of Thavasi *et al*., (2007).

Experiment to determine the effect of temperature on herbicides biodegradation by bacteria was carried out at various temperature for 15 days.

The effect of pH on biodegrading potential of bacteria was determined by adjusting the pH between pH4.5 and pH8.5 and were incubated for 15days.

Effect of Days on herbicides biodegradation was carried out by incubating for different weeks ranging from 1- 7 weeks.(Jurado, et al, 2011).

Quantification of Pesticide Residue

This was carried out using Gas chromatography spectrophotometer on the biodegraded sample. The aqueous samples were analyzed by directly derivatizing an aliquot and the derivatizing reagent mixture was prepared fresh by mixing one volume of Heptafluoro-butanol to two volumes of Trifluroacetic Anhydride.

III. RESULT AND DISCUSSION

The herbicide treatment used was observed to have negative effect on the microbial load. In glyphosate treated soil, there was a gradual decrease in bacterial population, that is, from 120 x 10^6cfu first day after application to 101 x 10^6cfu after one week of application. But by the third week of application, there was a sharp decrease of 77 x 10^6cfu to 48 x 10^6cfu by the sixth week of application. The bacteria count from contaminated and non-contaminated soil is as given in Table 1.

Table 1: Total Bacteria Counts for Bacteria10^6 (cfu) /g/ dwt.

SAMPLE	TIME OF SAMPLING	TBC
ES1	Immediately after herbicide application	120
CS1		132
ES2	A week after herbicide application	101
CS2		115
ES3	Two weeks after application	70
CS3		110
ES4	Three weeks after application	75
CS4		118
ES5	Four weeks after application	55
CS5		
ES6	Five weeks after application	59
CS6		
ES7	Six weeks after application	48
CS7		97

KEYS:

ES: Experimental Sample

CS: Control of Experimental Sample

TBC: Total Bacteria Count

Pseudomonas poae isolated from contaminated soil had percentage occurrence of 40.0% from Plot C and *Pseudomonas tolaasii* had 20.0% from plot A. *Proteus*sp had 60.0% occurrence from plot B, *Priestiaflexa* had 20.0% occurrence from plot B, *Bacillus firmus* had 20.0% occurrence from plot C, similarly *Bacillusmagaterium* had 20.0% occurrence from plot A and B respectively, *Simuliumtani* had 20.0% occurrence from plot B, *Acinetobacter beijerinckii*and *Citrobacter freundii* had 20.0% occurrence from plot C respectively, Table 2.

Table 2: Percentage Occurrence of different Bacteria from Contaminated Soil with Herbicide

Bacteria	No. sampled	Plot A No. (%)	Plot B No. (%)	Plot C No. (%)
Pseudomonas poae	5	0(0.0)	0(0.0)	2(40.0)
Pseudomonas tolaasii	5	1(20.0)	0(0.0)	0(0.0)
Proteus sp	5	0(40.0)	1(60.0)	0(0.0)
Priestia flexa	5	0(0.0)	1(20.0)	0(0.0)
Bacillus firmus	5	0(0.0)	0(0.0)	1(20.0)
Bacillus magaterium	5	1(20.0)	1(20.0)	0(0.0)
Simulium tani	5	0(0.0)	1(20.0)	0(0.0)
Acinetobacter beijerinckii	5	0(0.0)	0(0.0)	1(20.0)
Citrobacter freundii	5	0(0.0)	0(.0)	1(20.0)

The screening for survival of different bacteria in herbicides broth is as given in Table 3. The ability of the bacteria isolated from contaminated soil with herbicides showed that *Pseudomonas tolaasii*, *Pseudomonas poae*, *Proteus* sp, *Priestia flexa*, *Bacillus magaterium*, *Bacillus firmus*, *Simulium tani*, *Acinetobacter beijerinckii* and *Citrobacter freundii* were able to survival in herbicide concentration broth.

Table 3: Screening for Survival in Herbicides Broth

Bacteria	Lab code	Utilization
Pseudomonas tolaasii	Plot A 1a	+
Pseudomonas poae	Plot C 2b	+
Pseudomonas sp	Plot C 4a	-
Proteus sp	Plot A 1b	-
Proteus sp	Plot A 2a	-
Proteus sp	Plot B 5a	+
Priestia flexa	Plot A 3c	+
Bacillus magaterium	Plot B 4a	+
Bacillus sp	Plot B 5a	-
Bacillus firmus	Plot C 3c	+
Simulium tani	Plot B 2a	+
Acinetobacter beijerinckii	Plot C 4b	+
*Entrobacter*sp	Plot A 5c	-
Citrobacter freundii	Plot C 1c	+

The effect of temperature on utilization of herbicide is as shown in Table 4. *Pseudomonas tolaasii* had the highest utilization at 35℃ (2.19±0.26 mg/ml) followed by 30℃ (2.06±0.64mg/ml) and least was at 26℃ (1.23±01.1mg/ml).

Table 4: Effect of temperature on utilization of herbicides by different bacteria.

Isolates	**Herbicide Conc. (mg/ml)**	**Temperature (℃)** 26	30	35
Pseudomonas tolaasii	5	1.23±01.1	2.06±0.64	2.19±0.26
Pseudomonas poae	5	1.14±0.29	2.01±0.23	2.15±0.08
*Proteus*sp	5	0.53±0.86	1.97±0.05	1.92±0.16
Priestiaflexa	5	1.55±0.15	2.12±0.19	1.94±0.34
Bacillus magaterium	5	1.48±0.24	2.00±0.03	1.80±0.05
Bacillus firmus	5	1.02±0.86	2.02±0.57	1.62±0.08
Simuliumtani	5	1.12± 0.82	1.48±0.10	2.07±0.24
Acinetobacter beijerinckii	5	1.17±0.35	1.86±0.28	1.27±0.35
Citrobacter freundii	5	1.45±0.17	1.47±0.15	1.97±0.05

The effect of pH on herbicide utilization by bacteria isolates is as shown table 5. *Pseudomonas tolaasii* had the highest utilization at pH 7.0 (3.5±0.3mg/ml) followed by pH6.5 (3.1±0.3mg/ml), pH6.0 (2.1±0.1mg/ml) and the least was pH 5.5 (1.7±0.3mg/ml).

Table 5: Effect of pH on herbicide utilization by different bacteria isolates

Isolates	**Herbicide Conc. (mg/ml)**	**Degree of Acidity and Alkaline (pH)** 5.5	6.0	6.5	7.0
Pseudomonas tolaasii	5	1.7±0.3	2.1±0.1	3.1±0.3	3.5±0.3
Pseudomonas poae	5	2.0±0.1	1.9±0.8	2.1±0.3	2.8±0.8
Proteus sp	5	1.7±0.1	1.8±0.2	1.9±0.1	2.1±0.1
Priestia flexa	5	2.1±0.1	2.8±0.2	3.1±0.2	3.3±0.1
Bacillus magaterium	5	2.8±0.1	3.0±0.8	3.0±0.6	3.6±0.5
Bacillus firmus	5	2.3±0.2	2.6±0.1	2.8±0.2	2.8±0.3
Simulium tani	5	2.6±0.8	2.8±0.1	2.8±0.2	3.0±0.1
Acinetobacter beijerinckii	5	1.7±0.2	1.8±0.2	2.0±0.5	2.6±0.1
Citrobacter freundii	5	1.6±0.1	1.8±0.2	2.0±0.5	2.8±0.2

DNA sequence analysis/molecular identification of microbes

Blast analysis of the gene sequence of the pure bacteria culture identified three bacteria species of the genus Pseudomonas, Priestia and Bacillus, of which Pseudomonas dominated the samples, Table 6.

The relatively high abundance of Pseudomonas species in the samples might be due to their high ability to tolerate and degrade pesticides (Darsaet,al. 2014). Similar studies have been conducted by Asef 2014 and have documented the isolation, molecular characterization and pesticide degradation by Aspergillus species. Therefore, the presence of these bacterial species in our study can suggest their biodegradation potential towards pesticide**.**

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Table 6: Molecular Characterization of Bacterial Isolates

Sample ID	Organism Identified by BLAST	Identity (%)	Sequence Length (Bp)
Plot A 1a	*Pseudomonas tolaasii* strain Pt11 *Pseudomonas sp.* bs2935 *Pseudomonas sp.* MYb193 *Pseudomonas libanensis*strain DMSP-1	88	6475196
Plot C 2b	*Pseudomonas poae* strain PMA22 *Pseudomonas antarctica* strain BS2772 *Pseudomonas sp.* ADAK22 *Pseudomonas lurida* strain MYb11	90	6530734
Plot B 4a	*Bacillus magaterium* strain PHB06 *Bacillus sp.* strain magaterium-M1 *Bacillus sp.* strain AM136 *Priestia magaterium* strain R2A90	100	1493
Plot A 3c	*Priestia flexa* strain QG-3 *Priestia flexa* strain TH25 *Priestia flexa* strain FYF01 *Priestia flexa* strain BUMD13	100	1515
Plot C 3c	*Bacillus firmus* *Bacillus sp* mixed culture X3-37 *Bacillus sp* Al-Dhabi-17 BAU *Bacillus sp* strain 35-Lb11/2	95	1359
Plot B 2a	*Simulium tani* Uncultured bacterium clone MSD18_A02 *Bacillus sp.* mixed culture X3-37 *Bacillus sp.* Al-Dhabi-17	100	1420
Plot C 4b	*Acinetobacter beijerinckii* strain LMA2 Uncultured *Priestia sp* clone RJCEP_01 *Priestia aryabhattai* strain MSAR20 Uncultured actinobacterium clone STJ C42	88	786
Plot C 1c	*Citrobacter freundii* strain RHBSTW *Enterobacter sp.* RHBSTW-00975 *Enterobacterasburiae* MRY18-106 *Enterobacter sp.* HP19	100	109959

Phylogenetic analysis

The Phylogenetic analysis revealed that the soil contained diverse bacterial clustering into three orthologous groups. Reason maybe the combination of selective factors, proximity and functional capacity of microbes. Functionally, phylogenetically distant lineages can share common functional features and functions (Ning and Beiko 2015). Ning and Beiko 2015 also opined that functional similarities exist between operational taxonomic units (OTUs) that belong to different high-level taxonomic groups. Most of microbial sequences analyzed in different taxonomic divisions could be related to representatives with known metabolic traits.

Correlation between different parameters

Some correlations were also calculated from the results, at the end of the experiment when the organisms were suggested to be highly metabolic active. The negative correlation observed between pesticide degradation and colony count suggests the negative impact pollutants may have on biodiversity. These relationships would be useful to biodegradation of glyphosate and other organic contaminants in the environment (Showunmiet,al. 2020).

IV. CONCLUSION

From the findings of this study, it suggests that the organisms isolated and identified have the potential to degrade glyphosate pollutants when applied in the environmentally friendly technology clean-up (bioremediation) of glyphosate contaminated environment. Therefore factors promoting their growth should be encouraged.

Herbicides are phytotoxic chemicals used for destroying various weeds or inhibiting their growth. It is important to also know that excess use of herbicides in agroecosystems may change composition of weed populations and diversity.

Excess use of herbicide should be minimize in wildlands, as herbicides may increase the diversity of native species. Threats to plant biodiversity caused by habitat loss and invasive species are far greater than threats by use of herbicides.

It is also important to properly managed lands that are spread with herbicide as spray runoff in sandy soils may cause tree injury if followed soon after with irrigation or rainfall.

To prevent contamination of water bodies, management plans should carefully consider the hydrology of the system that is being treated. Hypothesize potential runoff scenarios and take appropriate measures (such as buffer zones) to prevent them. Underground aquifers and streams should be considered as well.

REFERENCES

[1] Asef, I. M. Isolation of pesticide degrading microorganisms from soil. Advances in Bioresearch 2014: 5(4): 164-168.

[2] Busse, M.D., Ratcliff, A.W., Stestak, C.J., Powers, R.F. (2001). Glyphosate toxicity and the effects of long term vegetation control on soil microbial communities. Soil Biol. And Biochemistry J. Vol.33:1777-1789.

[3] Cooper, J. and Dobson, H. 2007. The benefits of pesticides to mankind and the environment. Crop Protection 26(9): 1337-1348.

[4] Darsa, K., Thatheyus, J. and Ramya, D. 2014. Biodegradation of petroleum compound using the bacterium bacillus subtilis. Science International. 2014: 2: 20-25.

[5] Ellis, F. (2000). The determinants of rural livelihood diversification in developing countries. J. Agricultural Economis. 51, 289-302.

[6] Janssen, M.A. (2007). An update on the scholarly networks on Resilence, Vulnerability and Adaptation within the human dimentions of global environmental change. J. Ecology and Society. 12(2):9-15.

[7] Jurado, A.S., Fernandes, M.A.S., Videira, R.A., Peixoto, F.P., Vicente, J.A.F. (2011).Herbicides: The Face and the Reverse of the Coin. An in vitro Approach to the Toxicity of Herbicides in Non-Target Organisms. In: KORTEKAMP, A. (Ed.) Herbicides and Environment. Kroatia, 3- 45.

[8] Makut M.D. and Ifeanyi U. E. (2017). Screening for citric acid producing fungi from the soil Environment of Jos North, Plateau State, Nigeria.FULafia Journal of Science & Technology. 3 (1):1-12

[9] Mendes, K.F., Freguglia, R.M.O., Martins, B.A.B, Dias R.C., PimpinatoR.FandTornisielo V.L (2017). Cow bonechar for pesticide removal from drinking water. Scholars Journal of Agriculture and Veterinary Sciences. 4:504-512.

[10] Ning, J. and Beiko, G. 2015. Phylogenetic approaches to microbial community classification. Microbiome 2015: 3(1): 47-49.

[11] Sowunmi, K. Shoga, S.M. Adewunmi, O.M. Oriyomi, A.F. Sowunmi, L. 2020. Isolation and Molecular Characterization of Pesticide Degrading Bacteria in Polluted Soil. doi: https://doi.org/10.1101/2020.07.21.213942.

[12] Thavasi, R., Jayalakshmi, S., Balasubramanian T., Banat, I.M. (2007). Effect of salinity, temperature, pH and crude oil concentration on biodegradation of crude oil by Pesudomonas aeruginosa. Retrieved from www.acikenisim.uludag.edu.tr on Jan 12, 2021.

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The Effect of *Trichoderma* Biourine Application on Growth, Occurrence of Fusarium Wilt Disease and Yield of Several Shallot Varieties

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***Abstract*— *Biourine is a liquid fertilizer that contains complete elements, namely nitrogen, phosphorus, and potassium in small amounts as well as zinc, iron, manganese, and copper. Biourine can provide an increase in plant yields that is almost the same as plant fertilizers, besides that it can control Fusarium wilt disease. This study aims to determine the effect of Trichoderma biourin application on plant growth, Fusarium wilt disease and onion yield. The research was conducted using an experimental method in Senteluk Village, Batu Layar District, West Lombok Regency, West Nusa Tenggara using a Split Plot Design consisting of 2 factors. As the main plot, the shallot varieties consist of three levels, namely Bali Karet, Ampenan and Keta Monca, while as a sub-plot, the Trichoderma biourin application method consists of four levels, namely: without biourine, spraying the soil surface, spraying seed tubers, and spraying on plants 21 days after planting. The treatment was a combination of shallot varieties and Trichoderma biourine application method, each of which was repeated three times, so there were 36 experimental units. The results showed that: (a) The application of liquid biourine by spraying the soil surface, spraying seed tubers and spraying on plants 21 days after planting could increase the growth and yield of shallot bulbs. (b) The Bali Karet shallots varieties are more resistant to Fusarium wilt disease when compared to Ampenan and Keta Monca varieties.*
***Keywords*— Biourine, Trichoderma, Fusarium wilt disease, shallot, Bali Karet.**

I. INTRODUCTION

Shallots (*Allium cepa* var. *ascalonicum*). is a tuber vegetable that is quite popular among the public, in addition to its high economic value, red onion also functions as a flavoring and can also be used as an ingredient in traditional medicine or other pharmaceutical raw materials [1].

The province of West Nusa Tenggara or NTB is one of the centers of shallot production after Central Java, East Java and West Java. Shallot production in NTB from 2015-2019 has increased. NTB shallot production in 2015 was 160,201 tons with a harvested area of 14,524 ha, in 2016 as many as 211,804 tons with a harvested area of 19,275 ha, in 2017 as many as 195,458 tons with a harvested area of 17,904 ha, in 2018 as many as 212,885 tons with a harvested area of 19,341 ha, and in 2019 as many as 188,255 tons with an area of 16,688 ha. In 2017 the productivity of shallots in NTB decreased to 10.92 tons/ha whereas previously it was 11.03 tons/ha [2]. The productivity of shallots is still relatively low compared to the results of the study, which reached 15 tons/ha [3].

One of the causes of a decrease in the productivity of shallots in NTB is Fusarium wilt disease caused by the fungus *Fusarium oxysporum* f. sp. *cepa*e, the use of shallot seeds that are susceptible to Fusarium and poor quality seeds, as well as Fusarium wilt disease control techniques that still rely on the use of fungicides [3].

The varieties of shallots grown by farmers in NTB vary depending on the area where they are planted, for example, in Sembalun Bumbung Village, East Lombok Regency, farmers mostly plant the Bali Karet variety, in Senteluk Village, West Lombok Regency, farmers plant Ampenan and Super Philip varieties, in Santong Village, Lombok Regency. In the north, farmers plant Ampenan and Super Philip varieties, in Rada Village, Bima district, farmers plant Keta Monca and Super Philip varieties. The results of field observations turned out that all shallot varieties planted by farmers were attacked by the fungus *F. oxysporum* which causes wilt disease [3].

Fungal attack F. oxysporum f. sp. cepae can cause shallots to wilt quickly, leaves turn yellow and twist and the base of the stem rots. Fusarium wilt disease has caused damage and reduced tuber yield by up to 50% [4]. Attacks on plants if symptoms like this are found, then the plants are removed and destroyed [5]. Fusarium wilt disease develops in shallot planting centers in NTB starting from West Lombok, East Lombok, Sumbawa, and Bima which causes damage and reduces the yield of tubers by more than 45% [6].

Fusarium wilt disease on shallots is very difficult to control, because this fungus has *chlamydospores* which are structures that can survive in the soil as a saprophyte for about three to four years even without a host plant [7]. Thus, it is necessary to find an alternative to control Fusarium wilt that is effective and environmentally friendly. One control technique that has good prospects is biological technology using biourine fermented with *Trichoderma* fungus and the use of shallots varieties that have induced resistance to Fusarium wilt disease.

Biourine is a liquid fertilizer that contains complete elements, namely nitrogen, phosphorus, and potassium in small amounts as well as zinc, iron, manganese, and copper. Biourin can provide an increase in plant yields that is almost the same as plant fertilizers [8]. One of the microbes used for biourin fermentation is *Trichoderma* spp. Biourine containing *Trichoderma* spp. able to stimulate the growth of mustard greens when compared to mustard plants without being given biourine, besides that biourine containing *Trichoderma* spp., has the potential to protect mustard plants from clubroot disease (*Plasmodiophora brassicae*), this disease is an important disease in mustard and cabbage plants [9].

Trichoderma fungi isolated from the *rhizosphere* of shallot plants were reported to be effective in controlling the fungus *F. oxysporum* f. sp. *cepae* in vitro with inhibition percentage of 45% [10]. The fungus *T. harzianum* in suppressing the growth of the fungus *F. oxysporum* f. sp. *cepae* through the mechanism of competition for space and nutrients, mycoparasites and antibiosis [11]. In a greenhouse experiment, it was reported that the fungus *T. harzianum* was able to inhibit the incidence of Fusarium wilt disease in shallots up to 75% [12].

Several reports explain that the *Trichoderma* fungi is not only used for biourine fermentation, but also for the fermentation of other materials. The fungus *T. harzianum* used to ferment liquid biocompost from cow dung applied to vanilla plants can control Fusarium wilt disease [10]. The fungus *T. harzianum* applied to soybeans could inhibit the development of Fusarium wilt disease [13]. The fungus *T. harzianum* can control Fusarium wilt disease on banana plants [10]. Fusarium wilt disease in maize can be inhibited by the fungus *T. harzianum* [14]. The fungus *T. harzianum* is effective in controlling Fusarium wilt disease in soybeans [15]. The fungus *T. harzianum* was able to suppress Fusarium wilt disease in shallots [16]. The use of the fungus *T. harzianum* in the form of a tablet bioactivator formulation of 15 g/pot effectively controlled the fungus *F. oxysporum* f.sp. *cepae* on shallots reached 42.26% [17] and was able to increase plant growth and yield of shallots [18].

Induced resistance is the resistance of plants to pathogen infection because plants have been infected by other microorganisms before, both of the same type or of other types. Induced resistance can also occur after plants are inoculated early with biotic elicitors (avirulent, non-pathogenic, saprophytic microorganisms) [19]. Fusarium wilt control using onion varieties that have induced resistance have good prospects. The results of the preliminary study showed that the varieties of Bali Karet, Ampenan varieties, Keta Monca varieties, Bima Brebes and Super Philip varieties induced with the fungus *T. harzianum* caused immunity to Fusarium wilt disease [12] and induced resistance to Fusarium wilt disease [16].

The effect of *Trichoderma* biourine application in increasing growth and yield of several varieties of shallots in the field has never been studied. Therefore, a research was conducted on "The Effect of *Trichoderma* Biourine Application on the Growth and Yield of Three Shallot Varieties".

II. METHOD

1. Experimental Design

The study used an experimental method in Senteluk Village, Batu Layar District, West Lombok Regency, West Nusa Tenggara using a Split Plot Design consisting of 2 factors. As the main plot, the shallot varieties consist of three levels, namely Bali Karet, Ampenan and Keta Monca, while as a sub-plot, the

Trichoderma biourine application method consists of four levels, namely: without biourine, soil surface spraying, seed tuber spraying, and spraying on plants 21 days after planting. The treatment was a combination of shallot varieties and *Trichoderma* biourine application method, each of which was repeated three times, so there were 36 experimental units.

2. Experiment Execution

The production of *Trichoderma* biourine is carried out as follows: cow urine is collected in a holding tank. Furthermore, the standard solution of *Trichoderma* was put into a urine reservoir, then closed the fermentation container, and incubated for 4 weeks. Open the lid of the container once a week and stir for 15 minutes. After 4 weeks, the circulation was carried out using a ladder for 24 hours to remove the ammonia element which is pathogenic for plants. *Trichoderma* fermented biourin is ready to be applied to shallot plants.

The shallot seeds used were the Bali Karet, Keta Monca and Ampenan varieties purchased from seed breeders. Shallot seeds that are good to use are healthy and quality seeds with a shelf life of 2 months and there are visible growing points on the roots. The day before planting the seeds are cut off about part.

Tillage was carried out using a hoe to level the soil and making experimental plots with a size of 2 m × 4 m for each treatment plot. After processing the soil, basic fertilization is carried out using Phonska fertilizer of 100 kg/ha (50% of the recommendation). Basic fertilizer application was carried out by immersing it next to the planting hole, then the experimental plot was covered with plastic mulch.

The application of biourine was carried out according to the treatment, namely: by spraying the soil surface before installing plastic mulch, spraying shallot bulbs for 30 minutes, and spraying shallot plants after 21 days. Planting is done by inserting shallot seed bulbs into a hole with a depth of 2 cm and the hole is covered again with soil. Planting is done with a spacing of 20 × 20 cm.

3. Variable Observation

Observation of disease incidence was carried out by counting the number of wilted plants, observations were made from the age of 7 days after planting (DAP) until the shallots plants were 35 DAP. Disease incidence (%) is calculated using the following formula:

$$I = \frac{a}{b} x\ 100\ \%$$

where :

I = Percentage of disease incidence

a = Number of plants showing disease symptoms

b = Total number of plants observed

Observations of growth components, namely plant height and number of leaves of shallot plants were carried out from the age of 7 DAP to 35 DAP. Observations of yield components were carried out at harvest, namely at the age of more than 70 DAP. Observation of harvested dry shallot bulb weight was carried out by weighing the weight of bulbs at harvest per plot and then converted to hectares. Observation of the weight of stored dry shallots was carried out by weighing all parts of the plant in a dry state or after being stored in a wind-dried state for one month.

4. Data Analysis

Observational data were analyzed using Diversity Analysis with a significance level of 5% and further tested using the Honest Significant Difference test or HSD at the same significant level.

III. RESULT AND DISCUSSION

The results of the analysis of variance showed that the treatment of *Trichoderma* biourine application and shallot varieties were significantly different, while the interactions did not show significant differences in the incidence of Fusarium wilt disease, plant height growth and the number of shallots and shallot yields, namely the number of tillers, dry shallot bulb weight. harvest and weight of dry shallot bulbs stored.

1. Occurrence of Fusarium Wilt Disease on Shallots

The results of further tests on the effect of *Trichoderma* biourine application on the incidence of Fusarium wilt disease at the age of 7 DAP to 35 DAP using the 5% BNJ test are presented in Table 1.

Table 1. The Effect of Trichoderma Biourine Application on the Occurrence of Fusarium Wilt Disease in Shallots at the age of 7 DAP to 35 DAP

No.	How to Apply *Trichoderma* Biourine	Occurrence of Fusarium Wilt Disease (%)				
		7 DAP	14 DAP	21 DAP	28 DAP	35 DAP
1.	Without biourine	18.40 a[1)]	41.40 a[1)]	46.00 a[1)]	49.00 a[1)]	50.00 a[1)]
2.	Ground surface spraying	0.00 b	3.80 b	7.20 b	9.60 b	10.00 b
3.	Spraying of seed tubers	0.00 b	3.80 b	8.30 b	9.20 b	9.50 b
4.	Spraying plants aged 21 days	0.00 b	3.90 b	7.60 b	9.10 b	9.40 b

Notes: [1)] The numbers in each column followed by the same letter are not significantly different.

In Table 1, it can be seen that the application of *Trichoderma* biourine significantly affected the incidence of disease in shallot plants at the age of 7 DAP to 35 DAP. *Trichoderma* biourine which was applied by spraying the soil surface, spraying seed tubers and spraying on plants after 21 days could reduce the incidence of Fusarium wilt disease, while in control or without *Trichoderma* biourine treatment the incidence of Fusarium wilt at 35 DAP reached 50%.

The low incidence of Fusarium wilt disease in shallots after the application of *Trichoderma* biourine was due to the population of *Trichoderma* spp. in the rhizosphere increased markedly. The results of observations of the population of *Trichoderma* fungi in the rhizosphere showed an increase in the population in all biourine treatments, namely an average of 43.00 x 10^3 propagules/g soil, while in the control there was no *Trichoderma* spp. [20]. The fungus *Trichoderma* in soil is able to inhibit the development of disease-causing pathogens by means of competition both in terms of space and nutrients. The fungus *Trichoderma* can use various nutrient sources for growth by destroying cellulose, starch, lignin, and other soluble compounds such as protein and sugar [21]. In addition, *Trichoderma* can also inhibit the growth of pathogenic spores and hyphae with its ability to produce furonan group antibiotics [22]. The use of biofungicides fermented with *Trichoderma* spp. a minimum of 5 ml/plant can control Fusarium wilt disease through the mechanism of space competition, mocoparasites and antibiosis. [16].

2. The Effect of Trichoderma Biourine Application on Shallot Plant Growth

The results of further tests on the effect of *Trichoderma* biourine application on plant height and number of shallots at the age of 7 DAP to 35 DAP using 5% BNJ are presented in Tables 2 and 3.

Table 2. The Effect of Trichoderma Biourine Application on Shallot Plant Height at the age of 7 DAP to 35 DAP

No.	How to Apply Trichoderma Biourine	Shallot Plant Height (cm)				
		7 DAP	14 DAP	21 DAP	28 DAP	35 DAP
1.	Without biourine	3.50 a[1)]	15.50 a[1)]	18.80 a[1)]	20.10 a[1)]	24.30 a[1)]
2.	Ground surface spraying	7.80 b	21.60 b	30.80 b	36.10 b	37.00 b
3.	Spraying of seed tubers	7.90 b	22.50 b	31.30 b	36.30 b	38.00 b
4.	Spraying plants aged 21 days	8.20 b	23.30 b	31.40 b	36.60 b	38.40 b

Table 3. The Effect of Trichoderma Biourine Application on the Number of Leaf Shallots at the age of 7 DAP to 35 DAP

No.	How to Apply *Trichoderma* Biourine	Number of Leaves (strands)				
		7 DAP	14 DAP	21 DAP	28 DAP	35 DAP
1.	Without biourine	4.30 a[1)]	13.60 a[1)]	18.80 a[1)]	20.50 a[1)]	23.23 a
2.	Ground surface spraying	7.80 b	20.70 b	28.70 b	33.70 b	35.00 b
3.	Spraying of seed tubers	7.90 b	20.80 b	28.80 b	33.90 b	35.40 b
4.	Spraying plants aged 21 days	7.90 b	20.90 b	29.10 b	34.40 b	35.60 b

From Tables 2 and 3 it is known that the method of application of *Trichoderma* biourine significantly affected plant height and number of shallots. The plant height and the highest number of scallions began to be seen in all *Trichoderma* biourine applications.

From the results of this study, it can be said that all methods of biourin application can increase plant height and the number of leaves of shallot plants. The growth of plant height and number of leeks after the application of *Trichoderma* biourine is suspected because the Trichoderma fungus contained in the biourin has a role in stimulating ethylene in plant tissues so as to stimulate plant vegetative growth [11]. The fungus *T. harzianum* can stimulate seed germination and plant growth [23]. Ethylene is a hormone produced by the fungus *Trichoderma* spp. can stimulate plant flowering [24]. Treatment of the fungus T. harzianum on soybean plants can stimulate plant growth so as to increase plant height and number of plant leaves [21]. *Trichoderma* spp. able to stimulate plants to form the hormones gibberellin acid (GA3), Indolasetic acid (IAA), and benzylaminopurine (BAP) so that plant growth such as plant height and number of leaves is more and is healthy, tough and affects plant resistance to disease. Furthermore, gibberellins and auxin hormones also play a role in root and stem elongation, tuber formation and increase plant development [22].

3. The Effect of *Trichoderma* Biourine Application on Shallot Yield

The results of further tests on the effect of the application of *Trichoderma* biourine on the number of tillers of shallots, the weight of harvested dried shallots and the weight of stored dried shallots using BNJ 5% are presented in Table 4.

Table 4. The Effect of Trichoderma Biourin Application on Number of Tillers, Weight of Harvested Dried Shallot Bulbs and Dried Shallot Bulbs Weight of Stored

No.	How to Apply *Trichoderma* Biourine	Number of tillers (tubers)	Weight of Harvested Dried Bulbs (tons/ha)	Dry Bulbs Weight Save (tons/ha)
1.	Without biourine	6.70 a[1)]	6.80 a[1)]	4.50 a[1)]
2.	Ground surface spraying	11.80 b	13.90 b	12.30 b
3.	Spraying of seed tubers	11.90 b	14.20 b	12.40 b
4.	Spraying plants aged 21 days	12.20 b	14.40 b	12.60 b

Table 4 shows the number of tillers, the weight of harvested dry shallots, and the lowest weight of harvested dried shallots in the control or without using biourine. This indicates that the application of *Trichoderma* biourine can increase the number of tillers of shallot, the weight of harvested dry shallots, and the weight of harvested dry shallots.

The increase in the number of shallot tillers, harvested dry shallot bulb weight and stored dry shallot tuber weight after application of *Trichoderma* biourine was thought to be due to the role of this fungus in stimulating growth and increasing yield. It was reported that the fungus *T. harzainum* in the rhizosphere or plant root areas secretes ethylene which is diffused into the plant body through xylem which plays a role in promoting generative growth [11]. Treatment with conidia of *T. viride* and *T. koningii* fungi for controlling blight on strawberry plants was able to stimulate early flowering [25]. Ethylene is a hormone produced by the fungus *Trichoderma* spp. can stimulate flowering in plants [24]. Treatment of the fungus *T.harzianum* on soybean plants can stimulate plant growth so as to increase yield components [21]. *Trichoderma* spp. can produce certain hormones to increase the weight and number of pods in soybean plants [26]. *Trichoderma* spp. can stimulate plants to produce hormones gibberellin acid (GA3), Indolasetic acid (IAA), and benzylaminopurine (BAP) so that plant growth becomes optimum, and affects plant resistance. Gibberellins and auxin hormones play a role in root and stem elongation, and fruit (tuber) growth and increase plant growth [27].

Several previous researchers reported the successful use of *Trichoderma* fungi in various formulations on various plants. The use of biaoactivators fermented with *Trichoderma* spp. can increase the growth and yield of shallots in dry land [28]. The use of bioactivators fermented with *Trichoderma* spp. can increase the induced resistance of soybean plants to plant diseases and increase the growth and yield of soybean plants [29]. Tablet and liquid bioactivator fermented with *Trichoderma* spp. can increase soybean plant-induced resistance to wilt disease and increase soybean growth and yield [30]. Bioactivator and biocompost fermented with *Trichoderma* spp. can increase the weight of shallot bulbs [17]. The use of *Trichoderma* spp. which is formulated in the form of a stimulator biocompost can increase the growth and yield of maize in dry land [31]. The use of *Trichoerma* spp. in the form of a liquid bioactivator formulation as much as 5 ml/plant can increase the growth and yield of shallots [32]. Furthermore, it was also reported that the fungus *Trichoderma* spp. which is formulated in the form of biocompost can increase the growth and yield of soybean plants [33]. The use of *Trichoderma* biochar can increase soybean yield [34]. The use of *Trichoderma* biocompost can increase the yield of shallots [35]. The use of *Trichoderma* biofungicide can control Fusarium wilt disease and increase shallots yield [36]. The application of *Trichoderma* liquid biofungicide can increase the weight of harvested dried shallots bulbs [37]. The use of Trichoderma fungus can increase the resistance induced by banana seedlings to Fusarium wilt disease. The use of the saprophytic fungus *Trichoderma* antagonist causes shallots plants to become resistant to Fusarium wilt disease.

4. The Effect of Shallot Varieties on the Occurrence of Fusarium Wilt

The results of further tests using BNJ 5% the effect of shallot varieties on the incidence of Fusarium wilt disease at the age of 7 DAP to 35 DAP are presented in Table 5.

Table 5. The Effect of Shallot Varieties on the Occurrence of Fusarium Wilt Disease in Shallots at the age of 7 DAP to 35 DAP

No.	Shallot Varieties	Occurrence of Fusarium Wilt Disease (%)				
		7 DAP	14 DAP	21 DAP	28 DAP	35 DAP
1.	Bali Karet	0.00	2.50 a[1)]	4.90 a[1)]	6.40 a[1)]	7.70 a[1)]
2.	Ampenan	0.00	3.50 b	5.60 b	9.50 b	10.70 b
3.	Keta Monca	0.00	3.70 b	5.90 b	9.70 b	10.90 b

Notes: [1)] The numbers in each column followed by the same letter are not significantly different.

In Table 5 it can be seen that shallot varieties showed different effects on Fusarium wilt disease on shallots from 14 DAP to 35 DAP. Of the three varieties of shallots tested, it turned out that the Bali Karet variety showed a lower incidence of Fusarium wilt disease than the Ampenan and Keta Monca varieties.

The difference in the incidence of Fusarium wilt in the three varieties of shallot is thought to be because genetically these three varieties have different resistance. In addition, environmental factors such as sunlight, irrigation and soil conditions also affect resistance to Fusarium wilt disease. The Bali Karet, Ampenan and Keta Monca varieties in environmental adaptation tests at different altitude locations showed different resistance reactions to Fusarium wilt disease. The Bali Karet varieties grown in the highlands of Sembalun, the medium plains of Santong and the lowlands of Senteluk are resistant to Fusarium wilt disease [3].

5. The Effect of Shallot Varieties on Plant Growth

The results of further tests on the effect of shallot varieties on plant height and number of shallots at the age of 7 DAP to 35 DAP using 5% BNJ are presented in Tables 6 and 7.

Table 6. Effect of Shallot Varieties on Shallot Plant Height at the age of 7 DAP to 35 DAP

No.	Shallot Varieties	Shallot Plant Height (cm)				
		7 DAP	14 DAP	21 DAP	28 DAP	35 DAP
1.	Bali Karet	7.50 a[1)]	28.40 a[1)]	35.70 a[1)]	38.80 a[1)]	40.80 a[1)]
2.	Ampenan	6.70 b	22.70 b	31.80 b	35.80 b	37.50 b
3.	Keta Monca	6.50 b	22.50 b	31.70 b	35.60 b	37.30 b

Table 7. The Effect of Shallot Varieties on the Number of Leaves of Shallots at the age of 7 DAP to 35 DAP

No.	Shallot Varieties	Number of Shallots Leaves (pieces)				
		7 DAP	14 DAP	21 DAP	28 DAP	35 DAP
1.	Bali Karet	17.50 a1)	30.50 a1)	35.80 a1)	45.20 a1)	47.20 a1)
2.	Ampenan	7.50 b	22.70 b	28.80 b	38.70 b	41.50 b
3.	Keta Monca	7.40 b	22.50 b	28.60 b	38.50 b	39.90 b

From Tables 6 and 7 it is known that shallot varieties show different effects on plant height and the number of shallots on plants from the age of 7 DAP to 35 DAP. The Bali Karet variety showed higher plants than the Ampenan and Keta Monca varieties, while the Ampenan and Keta Monca varieties did not show a significant difference.

The occurrence of differences in plant height and number of leaves of shallot plants is thought to be due to genetic factors of each variety used, in addition to the adaptability of the variety to the environment such as sunlight, irrigation, rain intensity and soil conditions. Differences in plant growth are morphological adaptability, which in turn will affect the growth and yield of a plant [40]. The occurrence of variations in a plant can be caused by environmental influences and genetic factors. Differences in environmental conditions cause variations that can determine the final appearance of a plant [41]. The average plant height of the Bali Karet variety was 50-60 cm higher than the Ampenan and Keta Monca varieties, namely 26-45 cm and 26-46 cm. The number of leaves of the Bali Karet variety is 50-55 more than the Ampenan variety, which is 45-50 and the Keta Monca variety is 17-47 strands [3]

6. Effect of Shallot Varieties on Yield

The results of further tests on the effect of shallot varieties on plant height and number of tillers, weight of harvested dried shallots and weight of stored dried shallots using HSD 5% are presented in Table 8.

Table 8. Effect of Shallot Varieties on Number of Tillers, Weight of Harvested Dried Shallot Bulbs and Weight of Dried Shallots Saved

No.	Shallot Varieties	Number of tillers (tubers)	Weight of Harvested Dried Bulbs (tons/ha)	Dry Bulbs Weight Save (tons/ha)
1.	Bali Karet	13.40 a1	14.90 a1)	12.90 a1
2.	Ampenan	10.20 b	10.40 b	8.50 b
3.	Keta Monca	9.90 b	10.10 b	8.30 b

In Table 8 it can be seen that the shallot varieties showed different effects on the number of tillers of shallots, the weight of harvested dry shallots, and the weight of stored dry shallots. The Bali Karet variety showed that the number of tillers, harvested dry onion bulbs, and stored dry shallot bulbs were higher than the Ampenan and Keta Monca varieties.

, the weight of harvested dry shallots and the weight of stored dry shallots. The difference in the effect of shallot varieties is thought to be due to genetic factors of each variety and the influence of environmental conditions of planting. Genetic factors are one of the factors that affect plant growth and yield [42]. The average number of tillers of the Bali Karet variety was 7 – 14 higher than the Ampenan variety, 7 – 12 and the Keta Monca variety, 5 -9. The average yield of harvested dry shallot bulbs for the Bali Karet variety was 14-16 tons/ha, higher than the Ampenan variety, 12 tons/ha and the Keta Monca variety, 11 tons/ha [3]

IV. CONCLUSION

The results showed that:

1. The application of *Trichoderma* biourine by spraying the soil surface, spraying seed tubers and spraying on plants 21 days after planting can increase the growth and yield of shallot bulbs.
2. The Bali Karet shallots variety is more resistant to Fusarium wilt disease when compared to the Ampenan and Keta Monca varieties.

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REFERENCES

[1] Anonim, 2014. Cara Menanam Bawang Merah yang Baik dan Benar. http://diperta.jabarprov.go.id/index.php/subMenu/1156.

[2] Badan Pusat Statistik dan Direktorat Jenderal Hortikultura. 2019. Produksi Bawang Merah, Luas Panen Bawang Merah, dan Produktivitas Bawang Merah Menurut Provinsi Tahun 2015-2019.

[3] Sudantha, I. M., Suwardji, I. G. P. M. Aryana, I. M. A. Pramadya dan I. Jayadi,. 2018. Peningkatan Mutu Benih G0/Bibit Bawang Merah Dengan Teknologi Hayati Untuk Menunjang NTB Sebagai Sentra Benih G0/Bibit Bawang Merah Bersertifikat. Laporan Penelitian Unggulan Strategis Nasional. Direktorat Riset Pengabdian Masyarakat dan Lembaga Penelitian dan Pengabdian Kepada Masyarakat Universitas Mataram, Mataram.

[4] Wiyatiningsih, S. 2003. Kajian asosiasi *phytophthora* sp. dan *Fusarium Oxysporum* f. sp. *capsici* penyebab penyakit moler pada bawang Merah. Maperta. 5:1-6.

[5] Dwi J.A. Setiadi G , 2013. Pengendalian Hama, Penyakit dan Virus Pada Tanaman Bawang Merah Published 09/04/2013. By naissalintang. http://www.kiospertanian.com/pengendalian-hama-penyakit-dan-virus-pada-tanaman-bawang-merah/. Di Unduh 14 Pebruari 2014.

[6] Sudantha, I. M. 2015. Pemanfaatan Bioaktivator dan Biokompos untuk Meningkatkan Kesehatan, Kuantitas dan Kualitas Hasil Bawang Merah. Laporan Penelitian Mandiri Program Magister Pengelolaan Sumberdaya Lahan Kering Program Pascasarjana Unram.

[7] Sudantha, I.M. 2009. Aplikasi Jamur Trichoderma spp (Isolat ENDO-02 dan 04 serta SAPRO-07 dan 09) Sebagai Biofungisida, Dekomposer, dan Bioaktivator Pertumbuhan dan Pembungaan Tanaman Vanili dan Pengembangannya pada Tanaman Hortikultura dan Pangan Lainnya *di NTB*. Laporan Penelitian Hibah Kompetensi DP2M DIKTI, Mataram.

[8] Perdana, S.N., W.S. Dwi, M. Santoso. 2015. Pengaruh aplikasi biourin dan pupuk terhadap pertumbuhan dan hasil tanaman bawang merah (*Allium ascalonicum* L.). J. Prod. Tan. 3(6): 457-463.

[9] Sudana, I N., G. N. A. S. Wirya, I G. N. Raka, dan P. Sudiarta.2013. Pemanfaatan Biourin Sebagai Biopestisida dan Pupuk Organik dalam Usaha Budidaya Tanaman Sawi Hijau (*Brassica rapa* var. *parachinensis* L) Organik. Laporan Penelitian Hibah Unggulan Perguruan Tinggi. Universitas Udayana, Bali. 31 hal. https://simdos.unud.ac.id/uploads/file_riwayat_penelitian_1_dir/c415692b2c81eaef61f28e831f5cc492.pdf. Diunduh pada tanggal 26 Februari 2019.

[10] Sudantha, I. M. 2018. Buku Teknologi Tepat Guna: Penerapan Biofungisida dan Biokompos pada Pertanian Organik. LPPM Unram Press, Mataram. ISBN 978-602-53669-9-4. 125 hal.

[11] Sudantha, I. M. 2007. Karakterisasi dan Potensi Jamur Endofit dan Saprofit Antagonistik Sebagai Agens Pengendali Hayati Jamur *Fusarium oxysporum* f. sp. *vanillae* Pada Tanaman Vanili di Nusa Tenggara Barat. Disertasi Program Pascasarjana Universitas Brawijaya, Malang. 337 hal.

[12] Sudantha, I. M., Suwardji, dan M. T. Fauzi. 2016. Pemanfaatan Bioaktivator dan Biokompos Hasil Fermentasi Jamur *Trichoderma* spp. serta Fungi Mikoriza Abuskular Untuk Meningkatkan Kesehatan, Pertumbuhan dan Hasil Bawang Merah. Laporan Penelitian Dana PNBP Unram, Mataram.

[13] Sudantha I M. 2011. Uji aplikasi beberapa jenis biokompos (hasil fermentasi jamur *T. koningii* isolat Endo-02 dan *T. harzianum* isolat Sapro-07) pada dua varietas kedelai terhadap penyakit layu fusarium dan hasil kedelai. Jurnal Ilmu Pertanian Agroteksos, Fakultas Pertanian Universitas Mataram, Mataram. Vol. 21 No. 1 April 2011.

[14] Sudantha, I. M. dan Suwardji. 2013. Pemanfaatan Biokompos, Bioaktivator dan BiocharUntuk Meningkatkan Hasil Jagung dan Brangkasan Segar Pada Lahan Kering Pasiran Dengan Sistem Irigasi Sprinkler Big Gun. Laporan Penelitian Strategis Nasional, Mataram.

[15] Sudantha, I. M. dan Suwardji, 2014. Pemanfaatan Bioaktivator dan Biokompos (Mengandung Jamur *Trichroderma* spp. dan Mikoriza) Untuk Meningkatkan Kesehatan, Pertumbuhan dan Hasil Tanaman Kedelai di Lahan Kering. Laporan Hibah Tim Pascasarjana DP2M Dikti, Mataram.

[16] Sudantha, I. M., S. Suwardji, I G.P.M. Aryana, I M. A. Pramadya, I. Jayadi. 2020. The Effect of Liquid Bio Fungicides Dosage *Trichoderma* spp. against Fusarium Wilt Diseases, Growth and Yield of Onion. Journal of Physics: Conference Series 1594 (2020) 012013. IOP Publishing. doi:10.1088/1742-6596/1594/1/012013. https://iopscience.iop.org/article/10.1088/1742-6596/1594/1/012013/meta

[17] Sudantha, IM. and Suwardji. 2016. Growth and Yield of Onion (*Allium cepa* var. ascalonicum) as CA Result of Addition of Biocompost and Boactivity Fermented with *Trichoderma* spp. In: The 1st International Conference on Science and Technology (ICST) 2016, 1-2 Desember 2016, Universitas Mataram. http://eprints.unram.ac.id/4603/

[18] Sudantha, I. M., M. Isnaini, W. Astiko, N.M.L. Ernawati. 2018. The Influence of Arbuscular Mycorrhizal Fungi and Bioactivator (containing *Trichoderma* spp. Fungi and Legundi Leaf Extract) on Fusarium Wilt Diseases and Yield of Onion. CROP AGRO, Jurnal Ilmiah Budidaya, [S.l.], v. 11, n. 2, p. 94-103, Aug. 2018. ISSN 2621-5748. https://www.cropagro.unram.ac.id/index.php/caj/article/view/205.

[19] Sudantha, I. M., 2013. Patogen Tumbuhan Tular Tanah dan Pengendaliannya. Agra Puji Press, Matram. ISBN: 978-979-1025-56-0. 250 hal.

[20] Sudantha, I. M., Suwardji, dan I. G. P. M. Aryana. 2019. Desiminasi Pembuatan Bioaktivator, Biokompos Dan Biourin Limbah Kotoran Sapi Dengan Teknologi Fermentasi *Trichoderma* dan Aplikasinya Untuk Meningkatkan Hasil Bawang Merah. Laporan Akhir Produk Teknologi yang di Desiminasikan ke Masyarakat Kementerian Riset, Teknologi dan Pendidikan Tinggi Tahun Anggaran 2019.

[21] Latifah, A. Kustantinah, Loekas Soesanto. 2011. Pemanfaatan Beberapa Isolat *Trichoderma harzianum* Sebagai Agensia Pengendali Hayati Penyakit Layu Fusarium Pada Bawang Merah in Planta. Eugenia Vol.17 No 2. Universitas Jenderal Soedirman.

[22] Alfizar, Marlina and Hasanah. 2011). Upaya Pengendalian Penyakit Layu Fusarium oxysporum Dengan Pemanfaatan Agen Hayati Cendawan FMA dan *Trichoderma harzianum.* Jurnal Floratek, 6: 8-17.

[23] Windham, M., Y. Elad and R. Baker. 1986. A Mechanism of Increased Plant Growth Induced by *Trichoderma* spp. Phytopathology 76: 518 - 521.

[24] Salisbury, F. B. and C. W. Ross, 1995. Fisiology Tumbuhan Jilid 3. Perkembangan tumbuhan dan fisiologi Tumbuhan (Terjemahan D. R. Lukman dan Sumaryono). Penerbit ITB Bandung.

[25] Cook and Baker, 1983. *The Nature and Practice of Biological Kontrol of Plant phatogens*. The American Phytopathol Society Paul MN. 539 P

[26] Arianci, R. 2014. Pengaruh Campuran Kompos Tandan Kosong Kelapa Sawit, Abu Boiler dan *Trichoderma* terhadap Pertanaman Kedelai pada Sela Tegakan Kelapa Sawit yang Telah Menghasilkan di Lahan Gambut. Jurnal Teknobiolog*i*, *5*(1), 21-29.

[27] Triyatno, B. Y., 2005. Potential Multiple Control Agents against Ginger Rhizome Disease. Essay. Faculty of Agriculture, Jenderal Sudirman University. Purwokerto. 48

[28] Yusrinawati, Sudantha dan Astiko. 2017. The Effort of Increasing Growth And Harvest of Local Variety Red Onion With Applications of Some Dose of Indigenous Mycorrhizal And Bioactivator *Trichoderma* spp. in Dry Land. *IOSR Journal of Agriculture and Veterinary Sciensce (IOSR-JAVS). Volume 10, Issue 9 Ver. I (September 2017), PP 42-49 .e-ISSN: 2319-2402,p- ISSN: 2319-2380.* http://www.iosrjournals.org/iosr-javs/pages/ 10(9)Version-1.html

[29] Sudantha, I M. 2017. Eksplorasi Sumberdaya Alam (Biokompos, Bioaktivator, Biochar dan FMA) Untuk Mengembangkan Tanaman Pangan Sistem Organik Di Lahan Kering. Seminar Nasional MIPA Universitas Nahdlatul Wathan. Mataram. 136 – 150.

[30] Yudhiarti, S., I. M. Sudantha and M. T. Fauzi. 2020. Effect of Giving Arbuscular Mycorrhizal Fungi (AMF) and Bioactivator Dosage of *Trichoderma* spp. on the Growth and Products of soybeans (Glycine max L. Merr.). Traektoriâ Nauki= Path of Science 6 (9)

[31] Apzani, W.; I. M. Sudantha, and M. T. Fauzi. 2015. Aplikasi biokompos stimulator *Trichoderma* spp. dan biochar tempurung kelapa untuk pertumbuhan dan hasil jagung (zea mays l.) di lahan kering. Jurnal Agroteknologi, [S.l.], v. 9, n. 01, p. 21-35, June 2015. ISSN 2502-4906. Available at: https://jurnal.unej.ac.id/index.php/JAGT/article/view/3067

[32] Ningsih, D. H., I. M. Sudantha and Suwardji. 2019. Application of Liquid Bioactivator Contains *Trichoderma* spp. and Elements of Boron (B) as Growth of Growth and Improvement of Red Onion (*Allium Cep*a L.) Results. *International Journal of Multicultural and Multireligious Understanding*, *6*(6), 509–525.

[33] Prayoba, U. E., & I.M. Sudantha. 2019. Giving of Biochar and Biocompost to Growth, Results and N Uptake Soybean Plants (*Glycyne Max* (L) Merr.). International Journal of Environment, Agriculture and Biotechnology, *4*(2). http://journal-repository.com/index.php/ijeab/article/view/462

[34] Sukartono and I. M. Sudantha. 2016. Agronomic Response of Soybeans and Soil Fertility Status under Application of Biocompost and Biochar on Entisols Lombok, Eastern Indonesia. IOSR Journal of Environmental Science, Toxicology and Food Technology.

[35] Sudantha, I M. and S. Suwardji. 2021. The effect of biocompost *Trichoderma* spp. tablet in stimulating shallot growth and yield for climate change adaptation. 6th International Conference on Climate Change 2021 IOP Conf. Series: Earth and Environmental Science 824 (2021) 012032 IOP Publishing doi:10.1088/1755-1315/824/1/012032. https://iopscience.iop.org/article/10.1088/1755-1315/824/1/012033/pdf

[36] Sudantha, I M. and S. Suwardji. 2021. *Trichoderma* biofungicides formulations on shallot growth, yield and fusarium wilt disease resistance. 6th International Conference on Climate Change 2021 IOP Conf. Series: Earth and Environmental Science 824 (2021) 012032 IOP Publishing doi:10.1088/1755-1315/824/1/012032. https://iopscience.iop.org/article/10.1088/1755-1315/824/1/012032/pdf

[37] [37] Sudantha, I. M., S. Suwardji, I G.P.M. Aryana, I M. A. Pramadya, I. Jayadi. 2020. The Effect of Liquid Bio Fungicides Dosage *Trichoderma* spp. against Fusarium Wilt Diseases, Growth and Yield of Onion. Journal of Physics: Conference Series 1594 (2020) 012013. IOP Publishing. doi:10.1088/1742-6596/1594/1/012013. https://iopscience.iop.org/article/10.1088/1742-6596/1594/1/012013/meta

[38] Sudantha, I. M. 2021. Characterization and virulence of *Fusarium oxysporum* f. sp. *cubense* cause wilt disease in banana plants and its biological control using endophytic fungi *Trichoderma* spp. at West Nusa Tenggara, Indonesia. IOP Conference Series: Earth and Environmental Science, Volume 886, 2nd Biennial Conference of Tropical Biodiversity 4-5 August 2021, Makassar, Indonesia. https://iopscience.iop.org/article/10.1088/1755-1315/886/1/012016/pdf

[39] Sudantha, I M. and S. Suwardji. 2021. Biodiversity of *Trichoderma* antagonist saprophytic fungi and its use for

biocontrol of Fusarium wilt disease on shallots at Lombok Island, West Nusa Tenggara, Indonesia. IOP Conference Series: Earth and Environmental Science, Volume 886, 2nd Biennial Conference of Tropical Biodiversity 4-5 August 2021, Makassar, Indonesia. https://iopscience.iop.org/article/10.1088/1755-1315/886/1/012123/pdf

[40] Harjadi, S.S. 1991. Pengantar Agronomi. PT. Gramedia. Jakarta.

[41] Lakitan, B. 2004. Dasar-Dasar Fisiologi Tumbuhan. PT Raja Grafindo Persada. Jakarta.

[42] Darjanto dan Satifah. 1990. Pengetahuan Dasar Biologi Bunga dan Teknik Penyerbukan Silang Buatan. PT Gramedia. Jakarta.

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Antibacterial Activity and Identification of Active Compounds of Seaweed Extract *Sargassum* sp., *Halimeda opuntia* and *Halymenia* sp. from Lae-Lae Island of South Sulawesi

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***Abstract*—*In addition to containing primary metabolites, seaweed also contains secondary metabolites in the form of active compounds that function as antimicrobial and anticancer. Some species of seaweed include brown seaweed from the genus of Sargassum, green seaweed from the genus of Halimeda and red seaweed from the genus of Halymenia. The waters around Makassar City found species of Sargassum sp., Halimeda opuntia and Halymenia sp., which is abundant especially on Lae-Lae Island, but studies on its potential bioactivity are still very limited. This research aims to determine the bioactivity and identification of the active compound groups of the extracts of Sargassum sp., Halimeda opuntia and Halymenia sp. The research was carried out in December – April 2021. Samples were extracted using the meseration method. Antibacterial activity test used agar diffusion method and phytochemical test used Thin Layer Chromatography (TLC). The yield of methanol extract and seaweed hexane of Sargassum sp. respectively 2.4 and 2.3% and Halimeda opuntia of 0.53 and 1.15% and Halymenia sp. of 2.42 and 0.89%. The six seaweed extracts had no activity against E. coli bacteria. Hexane extract of Sargassum sp. had the highest activity against the bacteria of S. typhi with an average diameter of 25.67 mm and the highest level against the bacteria of A. hydrophila with an average diameter of 18.2 mm with methanol as solvent. The same activity was shown by methanol extracts of Sargassum sp. and Halymenia sp. against the bacteria of V. harveyi with an average zone diameter of 18.04 mm. However, only the methanol extract of Halimeda opuntia had activity against the bacteria of P. aeruginosa with an average diameter of 8.63 mm. An important finding that was found from the results of this research was the extract of Sargassum sp. showed the highest activity against S. typhi, A. hydrophila and V. harveyi with a higher diameter inhibition zone than the commercial antibiotic activity of ciprofloxacin as a positive comparison. This shows that the extract of Sargassum sp. has a great potential as a source of new antibiotics, especially against S. typhi. The extracts of methanol and hexane of Halimeda opuntia and methanol of Sargassum sp. contains 5 active compounds namely, alkaloids, flavonoids, tannins, triterpenonids and saponins. While the seaweed hexane extracts of Sargassum sp., only contains 3 active compounds namely, alkaloids, tannins and saponins. The methanol extracts of Halimeda opuntia contains 4 active compounds namely, flavonoids, tannins, triterpenonids and saponins, while the hexane extracts contain alkalaoids, flavonoids, tannins and saponins.*

***Keywords*— Seaweed extract, anti-bacterial, Sargassum sp., Halimeda opuntia, Halymenia sp.**

I. INTRODUCTION

Aquaculture production increased by 3.26% from 2016 to 2017 [1]. However, the increase in production and demand for freshwater fish commodities, both consumption and non-consumption (ornamental fish) in Indonesia will carry the risk of being attacked by fish pests and diseases that have the potential to damage the sustainability of the biological resources of fisheries. Fish and shrimp disease is one of the serious problems faced by aquaculture farmers because it has the potential to cause huge losses due to increased fish and shrimp mortality. Seeing these problems, other alternatives are needed as a solution to prevent infection by pathogenic organisms generally using antibiotics. However, the usage of antibiotics causes microbial resistance. The emergence of resistance and infection of antibacterial pathogenicity *E. coli*, *P. aeruginosa*, S. *tyhpi*, *V. harveyi*, *A. hydrophila* makes scientists seek to find drugs as new antibacterials. The alternative way is the use of seaweed.

In addition to containing primary metabolites, seaweed also contains secondary metabolites in the form of bioactive compounds that have the potential as antibacterial compounds [2]. This is in line with the research of Kasmiati et al., 2018 [3] which reported that several active compounds in seaweed function as antimicrobials to inhibit the growth of other competitive microorganisms and are potential for new secondary metabolites. Seaweed has been widely studied to have potential as an antibacterial [4], one of which is brown seaweed from the genus of *Sargassum*. *Sargassum polycystum* is rich in secondary metabolites, such as phenols, flavonoids, tannins, sterols, terpenoids, saponins, alkaloids and glycosides [5]. Red seaweed of the genus of *Halymenia* apart from being a source of natural pigments, also contains active components that have been widely reported as antioxidants, antibacterials, antimalarials, and antivirals [6]; [7].

The waters around Makassar City found species of *Sargassum* sp., *Halimeda opuntia* and *Halymenia* sp., which is abundant especially on Lae-Lae Island, but studies on its potential bioactivity are still very limited. This research aims to determine the active compound content and antibacterial activity of *Sargassum* sp., *Halimeda opuntia* and *Halymenia* sp. found on Lae-Lae Island of South Sulawesi.

II. MATERIAL AND METHODS

2.1 Materials

The materials used were seaweed of *Sargassum* sp., *Halimeda opuntia* and *Halymenia* sp., methanol and hexane, eule solution (methanol-chloroform (2:1)), 4% of H2SO4 solution, aluminum plate of KLT Silica Gel 60 F254, DMSO and ciprofloxacin commercial antibiotics. Test bacteria of *Escherichia coli*, *Salmonella typhi*, and *Pseudomonas aeruginosa* were obtained from Faculty of Medicine of Hasanuddin University, while *Aeromonas hydrophila* and *Vibrio harveyi* were obtained from the Brackish Water Aquaculture Center (BPBAP) of Takalar. Growth media for bacteria are Nutrient Broth (NB), Nutrient Agar (NA) and Tryptone Soya Agar (TSA). Disc paper in diameter of 6 mm, filter paper of Whatman No. 01. The equipments used are a blender, autoclave, oven, rotary vacuum evaporator, laminar air flow, vacuum pump, analytical scale, chamber, micropipette, incubator, magnetic plate, stirrer, and glassware.

2.2 Collection and Preparation of Seaweed

Seaweed samples of *Sargassum* sp., *Halimeda opuntia* and *Halymenia* sp. collected from the waters of Lae-Lae Island using snorkeling equipment. The sampling location was reached by motorboat. Samples were taken by removing the sand substrate and then washing it with seawater to clean it from adhering dirt such as sand, shellfish, and mud. Each sample was put in a plastic sample and stored in a cold box containing ice as a cooling medium to maintain the freshness of the seaweed during the trip to the laboratory. After arriving at the laboratory, each seaweed sample was washed with fresh running water to clean it from seawater and remaining dirt, then drained and weighed to determine the wet weight of the sample. The samples were dried in the shade for 7 - 10 days to obtain dried seaweed which was then weighed to determine the weight of the dry sample. Each species of seaweed is mashed using a blender to produce seaweed flour and then stored in the refrigerator in an airtight container.

2.3 Seaweed Extraction

Sample extraction using methanol and hexane solvent with maceration method refers to El Shafay et al., 2016 [8] which has been modified. A total of 150 g of each species of seaweed was put into a baker and then added with each solvent of methanol and hexane of 600 ml (1:4, w/v) and allowed to stand at room temperature for 3 days with stirring using a magnetic stirrer. The homogenate was filtered using filter paper of Whatman No.1 in a vacuum to separate the dregs and the filtrate. The filtrate was evaporated using a rotary vacuum evaporator to evaporate the solvent to obtain a crude extract of methanol and hexane in the form of a concentrate from each species of seaweed, thereby obtaining 2 crude extracts for each species of seaweed so that the total extracts of the three species of seaweed were 6 extracts. The crude extract was then weighed to determine its weight. The extract was stored in a glass container with a lid and stored in a refrigerator before being used in the test. Before the testing, the instrument was sterilized. Instrument sterilization is carried out by wrapping glassware using paper and then placing it in the oven and sterilizing it at 180°C for 2 hours.

2.4 Antibacterial activity

2.4.1 Preparation of Media and Test Bacteria

NB and NA media were prepared by dissolving separately 13 g of NB and 23 g of NA in 1 L of distilled water and then heated until dissolved. Both media were sterilized in an autoclave at 121°C for 15 minutes. Similarly, TSA media were prepared in the same way as NA media. The agar medium was cooled to about 50°C before being poured into the petridis. The test bacteria were rejuvenated on sterile sloping media and incubated at 37°C for 24 hours. A total of 1 ose of bacteria on slanted agar was inoculated on NB media, homogenized using a vortex and then incubated at 37°C for 24 hours to achieve viability of 108 colonies/ml.

2.4.2 Test of Antibacterial Activity

The antibacterial activity test of crude extracts of methanol and hexane refers to Bauer at al. (1996) [9] and Christobel at al. (2011) [10] with the modified agar diffusion method. A total of 1 ml of each bacterial culture was inoculated on NA media (except *A. hydrophila* and *V. harveyi* on TSA media) in petri dishes. Each extract of methanol and hexane was taken as much as 1000 g and dissolved in 50 µl of the solvent until completely dissolved. 10 µl was taken and applied to paper disk in a diameter of 6 mm at a dose of 200 µg/disk. After the solvent evaporates, each disk is dripped with 5 µl of DMSO and then placed on agar media which already contains the test bacteria. As a positive control used ciprofloxacin commercial antibiotic of 5 µg [8] while the negative control used DMSO of 5 µl. Petri dishes containing the test samples were wrapped in plastic wrap and incubated at 37°C for 24 hours. The clear zone formed around the disc indicated the inhibition of bacterial activity by the extract which was expressed in millimeters as the average value of three replications.

2.5 Identification of Active Compounds

2.5.1 Phytochemical Test

Identification of the group of active compounds contained in the methanol and hexane extracts of *Sargassum* sp., *Halimeda opuntia* and *Halymenia* sp. carried out through a phytochemical test using the method of Thin Layer Chromatography (TLC) refers to Harbone (1984) [11] by observing the presence of spots on the extract that has been sprayed on the TLC plate. Aluminum Plate of TLC Silica Gel 60 F254 was cut to a size of 1 x 5 cm for each test carried out. Then each extract to be tested was spotted on the prepared plate. Furthermore, the eluent solution of Methanol - Chloroform (2:1) was prepared and put into the chamber. After that, the TLC plate that has been stained with extract is put into a chamber that already contains eluent for the elution process. After completion of the elution process, the TLC plate was sprayed with certain reagents to see the color changes that occurred.

Flavonoid compounds were identified by spraying reagent solution of AlCl3 10% on a TLC plate that had gone through the elution process. Positive results were seen from the change in the color of the stain to light yellow-green. Triterpenoids/steroids were identified by spraying reagent solution of H2SO4 10%. A positive result was seen from the change in the color of the stain to brownish pink. Tannins were identified by spraying reagent solution of FeCl3 5%. Positive results were seen from the change in the color of the stain to dark blue-black.

Alkaloid compounds were identified by means of ± 2 mg of extract put into a mortar then 10 ml of chloroform was added and dissolved. Added 5 ml of chloroform-ammonia 0.05 M, filtered into a test tube. To the filtrate, 10 - 20 drops of sulfuric acid 2 N were added and then gently shaken for 2 - 3 minutes and allowed to form 2 layers. The top layer was taken and put into 2 test tubes and tested with Mayer and Dragendorff reagents. The formation of a white precipitate against the reagent of Mayer and an orange-red precipitate with the reagent of Dragendorff's showed a positive result of the alkaloid test. Saponins were identified by means of 1 ml of water fraction inserted into a test tube. The tube is shaken for 1 - 2 minutes. The formation of a permanent foam (not disappear for 5 minutes) indicates the presence of saponins.

III. RESULT AND DISCUSSION

3.1 Yield

Sample extraction was carried out in stages which included homogenization and partitioning of each sample in methanol and hexane. Homogenization of the sample with each solvent methanol and hexane at room temperature for three consecutive days aims to maximize the uptake of the active compound components. The concentrated homogenate was partitioned in methanol and hexane, respectively, so that crude extracts of methanol and hexane were obtained. The extracted yield of *Sargassum* sp. obtained from the waters of Lae-Lae island and it can be seen in Table 1.

Table 1. Yield of sample extraction

Sample	Gross Weight (BB) (g)	Simple Weight (BS) (g)	Extract	Extract Weight (BE) (g)	% Yield = BE/BS x 100%
Sargassum sp.	5650	300	methanol	7,2	2,4
			hexane	6,9	2,3
Halimeda opuntia	5350	300	methanol	7,27	0,53
			hexane	2,68	1,15
Halymenia sp.	3560	300	methanol	1,6	2,42
			hexane	3,45	0,89

Yield can be an important parameter to determine the total components that can be extracted and the effectiveness of an extract that can be utilized. The results after the extraction process are complete, the yield sequentially obtained is *Sargassum* sp. (methanol), *Sargassum* sp. (hexane), *Halimeda opuntia* (methanol), *Halimeda opuntia* (hexane), *Halymenia* sp. (methanol) and *Halymenia* sp. (hexane) of 2.4; 2.3; 0.53; 1.15; 2.42 and 0.89%. The three highest yields obtained were the extracts of methanol and hexane of *Sargassum* sp. and methanol extract of *Halymenia* sp. by 2.3%: 2.4% and 2.42%.

The extracts of *Sargassum* sp. and *Halymenia* sp. which used methanol as a solvent resulted in a higher yield when compared to hexane solvent. This is due to the dissolved bioactive components in nonpolar solvents are relatively small. The difference in yield is influenced by the type of solvent used. The yield of the resulting extract is influenced by several factors, including the selection of the type of solvent, the ratio of the number of samples to the solvent, the extraction temperature, the particle size of the sample and the extraction time [12], [13] dan [14].

Methanol is a form of alcohol with the chemical formula CH3OH (Araya et al., 2020). The chemical structure of methanol consists of a hydroxyl group (polar) and a carbon group (nonpolar) so that methanol is polar. Extraction with methanol produces more extracts, because the highly polar nature of methanol is thought to be able to extract more bioactive components that are highly polar and slightly nonpolar [15].

3.3 Antibacterial Activity

The antibacterial activity test in this research used the bacteria of *E. coli*, *P. aeruginosa*, *S. thypi*, *A. hydrophila* and *V. harveyi*. The use of these five bacteria aims to see the activity of seaweed sample extracts that can inhibit bacterial growth. The method used in this research was the agar diffusion method with a dose of each extract was 200 μg/disk. The positive control used the ciprofloxacin antibiotic of 5 μg/disk and the negative control used DMSO of 5 μg/disk. The results showed that the antibacterial activity of each sample extract against five bacteria was indicated by the formation of a halo zone around the paper disc. The results of the measurement of the inhibition zone formed can be seen in (Fig.1).

The research results, the six seaweed extracts namely *Sargassum* sp. (Methanol and hexane), *Halimeda opuntia* (methanol and hexane) and *Halymenia* sp. (methanol and hexane) had no activity against the bacteria *E. coli*. The hexane extract of *Sargassum* sp. had the highest activity against the bacteria of *S. typhi* with an average diameter of the inhibition zone of 25.67 mm and the highest against the bacteria of *A. hydrophila* with an average diameter of 18.2 mm with methanol as solvent. The same activity was shown by the methanol extract of *Sargassum* sp. and *Halymenia* sp. against the bacteria of *V. harveyi* with an average diameter of the inhibition zone 18.04 mm. However, only the methanol extract of *Halimeda opuntia* had activity against the bacteria of *P. aeruginosa* with an average inhibition zone diameter of 8.63 mm. This shows a wider activity as an antibacterial because it has inhibitory activity against all test bacteria except for *E. coli* (Fig. 2).

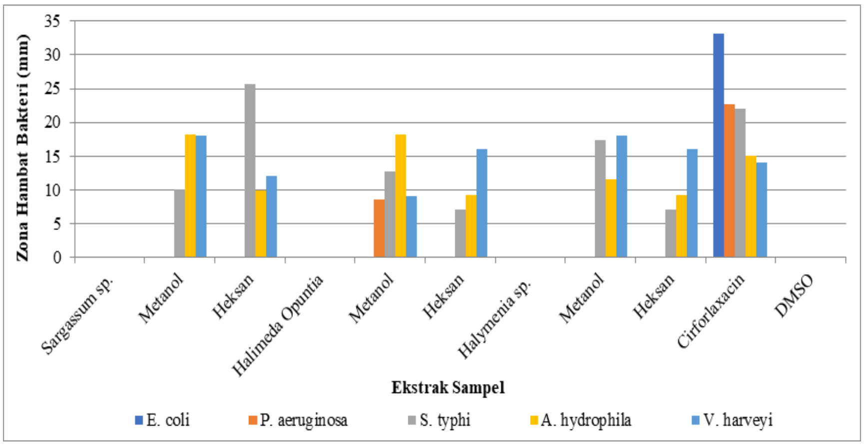

Fig.1. Diagram of antibacterial activity of seaweed extract samples of Sargassum sp., Halimeda opuntia and Halymenia sp. against the bacteria: E. coli, P. aeruginosa, S. thypi, A. hydrophila and V. harveyi, (+) Ciprofloxacin and (-) DMSO

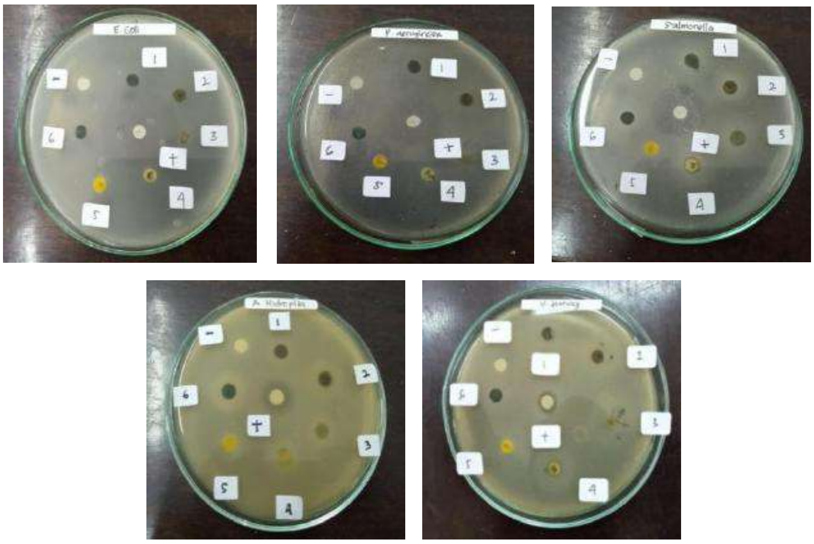

Fig.2. The results of antibacterial activities of the extracts of Sargassum sp., Halimeda opuntia and Halymenia sp. against the bacteria: E. coli, P. aeruginosa, S. thypi, A. hydrophila and V. harveyi, (+) Ciprofloxacin and (-) DMSO

The important finding that was found from the results of this research was the extract of *Sargassum* sp. showed the highest activity against *S. typhi*, *A. hydrophila* and *V. harveyi* with a larger inhibitory diameter than the commercial antibiotic of ciprofloxacin as a positive comparison (Figure 2). This indicates that the extract of *Sargassum* sp. has great potential as a source of new antibiotics, especially against the *S. typhi*. While the positive control of ciprofloxacin showed activity against all types of the test bacteria with an inhibition zone diameter range of 14.1 – 33.12 mm and the negative control of DMSO showed no activity against all the test bacteria.

The antibacterial ability of the six sample extracts was due to the active compounds contained in the seaweed extract. The inhibition mechanism of the bacteria by steroid compounds is thought to be damaging the bacterial cell membrane. Based on how it works, antibacterials are divided into bactericidal and bacteriostatic [16]. Bacteriostatic antibacterials are substances that work to inhibit bacterial growth [17], while bactericidal antibacterials are substances that work to kill bacteria [18].

The diameter of the inhibition zone in each bacterium showed differences, this could be due to several factors. The first factor at the time of 24-hours incubation of bacteria experienced a logarithmic phase where the growth of bacteria was twice that of the lag phase. The second factor is resistance by the bacteria by decreasing permeability so that it is difficult for antibacterials to enter cells, forming shortcuts to avoid the inhibited stages and increasing the production of enzymes that are inhibited by antibacterials.

The positive control of ciprofloxacin showed activity against all species of the test bacteria with a range of diameter of the inhibition zone from 14.1 to 33.12 mm. According to Mpila et al. (2012) [19] Ciprofloxacin is effective against bacteria that are resistant to other antibiotics such as penicillins, aminoglycosides, cephalosporins and tetracyclines and is effective against gram-negative and gram-positive bacteria. While the average diameter of the inhibition zone in the negative control treatment for the five bacteria was 0 mm. According to Handayani et al. (2009) [20] negative control treatment using dimethylsulfoxide (DMSO) is a solvent that dissolves almost all polar and non-polar compounds, besides that DMSO does not inhibit bacterial growth in the antibacterial activity test using the agar diffusion method. According to Bansemir (2006) [21], there are three categories of the ability of the test material to inhibit test bacteria which is characterized by the formation of a clear zone around the paper disc, namely the size of the inhibition zone >15 mm is classified as strong, from 8 to 15 mm is classified as moderate and from 1-8 mm is classified as weak activity, when the results of the research were compared with the positive control of ciprofloxacin, the antibacterial extracts of methanol and hexane of *Sargassum sp.* and methanol extract of *Halymenia sp.* against the bacteria of *A.hydropilla, S.typhi and V. harveyi* had a strong inhibition zone category. Methanol extract of *Halimeda opuntia* has a strong zone of inhibition against *A. hydropilla, S.typhi, P. aeruginosa and V. harveyi*. The hexane extract ability of *Halimeda opuntia* and *Halymenia sp.* categorized as weak against *S. typhi*, moderate against *A. hydrophila* and strong against *V. harveyi*.

3.2 Identification of Active Compounds

Phytochemical tests were carried out with the aim of looking at the class of active compounds contained in each sample extract which was marked by a color change that occurred after the reagent was added. This phytochemical test is a simple way to detect the presence of secondary metabolites in sample extracts.

Table 2. Results of screening for the bioactive compounds of seaweed extracts of Sargassum sp., Halimeda opuntia dan Halymenia sp.

No.	Sample	Test Types				
		Alkaloid	**Flavonoid**	**Tanin**	**Triterpenoid**	**Saponin**
1.	*Sargassum*sp. (methanol)	+	+	+	+	+
2.	*Sargassum*sp. (hexane)	+	-	+	-	+
3.	*Halimeda opuntia* (methanol)	-	+	+	+	+
4.	*Halimeda opuntia* (hexane)	+	+	+	-	+
5.	*Halymenia* sp. (methanol)	+	+	+	+	+
6.	*Halymenia* sp. (hexane)	+	+	+	+	+

Information:: (+) there are active compounds; (-) no active compounds

The data of research results (Table 2) shows the methanol extract of *Sargassum* sp. and *Halymenia* sp. with methanol and hexane as solvents containing 5 active compounds namely, alkaloids, flavonoids, tannins, triterpenonids and saponins. While the seaweed hexane extract of *Sargassum* sp. contains only 3 active compounds namely, alkaloids, tannins and saponins. The seaweed extract of *Halimeda opuntia* contains 4 active compounds namely, flavonoids, tannins, triterpenonides and saponins in the methanol extract and alkaloids, flavonoids, tannins and saponins in the hexane extract.

This is presumably due to the types of the solvent of methanol which is a universal solvent which has a polar group (-OH) and a nonpolar group (-CH3) so that it can attract polar analytes ([22]; [23]; [24]) and nonpolar [25] and [26]. Some secondary metabolites that can be dissolved by methanol such as flavonoids, alkaloids, saponins, tannins [27] and [28]. Meanwhile, hexane is a non-polar solvent [29]. Compounds that can be dissolved by this solvent are alkaloids, tannins and saponins [30] and [31].

The term of alkaloid comes from the Arabic, alkali meaning soda and from the Greek, eidos meaning appearance. This compound was introduced by W. Meisner in the early 19th century to denote a natural substance that reacts like a base. The biological activity of alkaloid compounds is due to the presence of a nitrogen-containing base group [32]. Its ability as an antibacterial is done by disrupting the peptidoglycan constituent components in bacterial cells, so that the bacterial cell layer is not fully formed and causes cell death in the bacteria.

Flavonoids are one of the secondary metabolites which are phenolic and play a role in pharmacology. Flavonoids have the ability to inhibit bacterial growth by several different mechanisms, including flavonoids causing damage to the permeability of bacterial cell walls [33], microsomes and lysosomes as a result of interactions between flavonoids and the DNA of bacteria [34], Different mechanisms were proposed by Di Carlo et al. (1999) and Estrela et al. (1995) in Sabir (2005) [35] which states that the hydroxyl group contained in the structure of flavonoid compounds causes changes in organic components and nutrient transport which will eventually lead to toxic effects on bacteria.

Saponin comes from the Latin word of Sapo which means foam producer [36]. The presence of saponins in the methanol extract is due to the presence of active glycoside bonds. Ganiswarna (1995) in Darsana et al. (2012) [37] stated that saponins work as antibacterial by inhibiting the synthesis of bacterial cell walls, interfering with the permeability of bacterial cells by binding to the outer membrane, resulting in damaged or destroyed cell walls (Arabsky et al., 2009), binding cholesterol in bacterial cells so that bacteria become lysed stability of bacterial cell membranes [38].

Tannins or commonly called tannic acid [39] are secondary compounds from abundant plants that are effective against bacteria. (The presence of tannins in methanol solvents is due to tannins are polyphenols which have an OH group [40] so that they are easily soluble in water, ethanol, acetone, or methanol [41]. Work mechanisms of tannins as an antimicrobial by shrinking the cell wall or cell membrane so that it interferes with the permeability of the bacterial cell wall itself.

Triterpenoid compounds from the extraction result are secondary metabolites that have the potential as antibacterial because they can lock the performance of enzymes that can bind DNA. In accordance with the opinion of Nurjanah et al. (2011) [42], triterpenoids can inhibit enzyme performance by binding to the active site of the enzyme which will bind to DNA and split it, so that the enzyme becomes locked and cannot bind DNA.

IV. CONCLUSION

Seaweed methanol extracts of *Sargassum* sp., *Halimeda opuntia* and *Halymenia* sp. had strong antibacterial activity against the test bacteria namely, *S. typhi*, *A. hydrophila* and *V. harveyi*, but was not active against the bacteria of *E. coli*. The methanol extract of *Halimeda opuntia* had wider antibacterial activity as indicated by the inhibition zone formed on four bacteria namely, *P. aeruginosa*, *S. typhi*, *A. hydrophila* and *V. harveyi.*

The extracts of methanol and hexane of *Halymenia* sp. and methanol of *Sargassum* sp. contains 5 active compounds namely, alkaloids, flavonoids, tannins, triterpenonids and saponins. Meanwhile, the hexane extract of *Sargassum sp.* only contains 3 active compounds namely, alkaloids, tannins and saponins. The methanol extract of *Halimeda opuntia* contains 4 active compounds namely, flavonoids, tannins, triterpenonids and saponins, while the hexane extract contains alkalaoids, flavonoids, tannins and saponins.

REFERENCES

[1] Badan Pusat Statistik. (2017). Rata-Rata Konsumsi per Kapita Seminggu Beberapa Macam Bahan Makanan Penting, 2007-2017. Diakses pada 1 Juni 2018, dari https://www.bps.go.id/statictable/2014/09/08/950/rata-ratakonsumsi-per-kapita-seminggu-beberapa-macam-bahan-makanan-penting2007-2017.html.

[2] Agbaje-Daniels, F., Babalola, A., Akintayo, I., Adeleye, A., & Beukes, D. (2017). Evaluation of Antibacterial Activity of Bryopsis pennata and Chaetomorpha antennina against Multidrug Resistant Morganella morganii and Salmonella Species Isolated from Healthy Individuals. Journal of Pharmaceutical Research International, 18(4), 1-7.Sfs

[3] Kasmiati, K., Yoshioka, Y., Okamoto, T., & Ojika M. (2018). New Crambescidin-Type Alkaloids from The Indonesian Marine Sponge Clathria bulbotoxa. Mar. Drugs, 6(3), 84.Dotulong, Jendra F.J, Margareth R. S, Grace D. Kandou. 2016. Hubungan Faktor Risiko Umur, Jenis Kelamin dan Kepadatan Hunian dengan Kejadian Penyakit TB Paru di Desa Wori Kecamatn Wori. Jurnal Kedokteran Komunitas dan Tropik. 3(2), pp.59-63.

[4] Dotulong, Jendra F.J, Margareth R. S, Grace D. Kandou. 2016. Hubungan Faktor Risiko Umur, Jenis Kelamin dan Kepadatan Hunian dengan Kejadian Penyakit TB Paru di Desa Wori Kecamatn Wori. Jurnal Kedokteran Komunitas dan Tropik. 3(2), pp.59-63.Dg

[5] Sedjati, S., Supriyantini, E., Ridlo, A., & Soenardjo, N. 2018. Kandungan Pigmen Total Fenolik Dan Aktivitas Antioksidan. Jurnal Kelautan Tropis. 21(2):137–144. doi : 10.14710/jkt.v21i2.3329.

[6] Deepak, P., Balasubramanian, B., Park, S., Sowmiya, R., Govindasamy, B., Aiswarya, D., Amutha, V., & Peruma, P. (2019). Phytochemical Profiling of Marine Red Alga, Halymenia Palmata and Its Bio-Control Effects Against Dengue Vector, Aedes Aegypti. South African Journal of Botany, 121.

[7] Malik, A. A. A., Bazire, A., Gamboa-Munoc, A., Bedoux, G., Robledo, D., García-Maldonado, J. Q., & Bourgougnona, N. (2020). Screening of Surface-Associated Bacteria from the Mexican Red Alga Halymenia floresii for Quorum Sensing Activity. Microbiology, 89(6), 778–788.

[8] El Shafay, S. M., Ali, S. S., & El-Sheekh, M. M. (2016). Antimicrobial Activity of Some Seaweed Species from Red Sea, against Multidrug Resistant Bacteria. The Egyptian Journal of Aquatic Research, 42(1), 65-74.

[9] Bauer, A.W., Kirby, W. M. M., Truck, H., & Shrecies, J. C. (1996). Antibiotic Susceptibility Testing by Standardized Single Disc Method. Am. J. Clin. Pathol., 45, 493-496.

[10] Christobel, G. J., Lipton, A. P., Aishwarya, M. S., Sarika, A. R., & Udayakumar, A. (2011). Antibacterial Activity of Aqueous Extract from Selected Macroalgae of Southwest Coast of India. Seaweed Res. Utiln., 33 (1&2), 67 – 75.Gf

[11] Harborne JB. 1987. Metode Fitokimia. Padmawinata K, penerjemah, Bandung (ID): Institut Teknologi Bandung. Terjemahan dari Phytochemical Methods. 345-354.Fgf

[12] Terblanche, Semakalu, Mtunzi, and Pillay (2017). Screening of Variables Influencing Extraction Yield of Cotyledon orbiculata: 2 Full Factorial Design. 9(3), 303-312.Fg

[13] Pagarra, H., Rahman, R. A., Muis, A., and Arifin, A. N. (2019). Screening of factors influencing Pectin Extraction of Pomelo peels using 2-Level Factorial Design IOP Conf. Series: Materials Science and Engineering 551, 1-7.Gf

[14] Tzanova, M., Atanasov, V., Yaneva, Z., Ivanova, D., and Dinev, T. (2020). Selectivity of Current Extraction Techniques for Flavonoids from Plant Materials. Processes 8(10).Fdg

[15] Edison, Diharmi, A., Ariani, N. M., and Ilza, M. (2020). Komponen Bioaktif dan Aktivitas Antioksidan Ekstrak Kasar Sargassum plagyophyllum. Jurnal Pengolahan Hasil Perikanan Indonesia 23(1), 58(66).

[16] Bertelloni, F., Cilia, G., and Fratini, F. (2020). Bacteriostatic and Bactericidal Effect of Tigecycline on Leptospira spp. Antibiotics 9(467), 1-7.

[17] Nemeth, h., Oesch, G., and Kuster, S. P. (2014). Bacteriostatic Versus Bactericidal Antibiotics for Patients with serious Bacterial Infections: Systematic Review and Meta-Analysis. Journal of Antimicrobial Chemotherapy 1-14.

[18] Rezaei-Zarchi, S., Imani, S., Z, A. m., Saadati, M., and Zaghari, Z. (2021). Study of Bactericidal Properties of Carbohydrate-stabilized Platinum Oxide Nanoparticles. International Nano Letters 2(21), 1-5.

[19] Mpila, D. A., Fatimawali, and Wiyono, W. I. (2012). Uji Aktivitas Antibakteri Ekstrak Etanol Daun Mayana (Coleus atropurpureus [L] Benth) terhadap Staphylococcus aureus, Escherichia coli dan Pseudomonas aeruginosa seacara In-Vitro 1-21.

[20] Handayani, S. 2013. Kandungan Flavonoid Kulit Batang Dan Daun Pohon Api-Api (Avicennia marina (Forks.) Vierh.) Sebagai Senyawa Aktif Antioksidan. Skripsi. Bogor: Institut Pertanian Bogor

[21] Bansemir, A., Blume, M., Schröder, S., and Lindequist, U. (2006). Screening of Cultivated Seaweeds for Antibacterial Activity Against Fish Pathogenic Bacteria. Aquaculture 252, 79-84.

[22] Meneses, N. G. T., Martins, S., Teixeira, J. A., and Mussatto, S. I. (2013). Influence of extraction solvents on the recovery of antioxidant phenolic compounds from brewer's spent grains. Separation and Purification Technology 108, 152-158.

[23] Altemimi, A., Lakhssassi, N., Baharlouei, A., Watson, D. G., and Lightfoot, D. A. (2017). Phytochemicals: Extraction, Isolation,and Identification of Bioactive Compoundsfrom Plant Extracts. Plants 6(42), 1-23.

[24] Sambou, M., Jean-François, J., Moutombi, F. J. N., Doiron, J. A., Hébert, M. P. A., Joy, A. P., Mai-Thi, N.-N., Barnett, D. A., Surette, M. E., Boudreau, L. H., and Touaibia, M. (2021). Extraction, Antioxidant Capacity, 5-Lipoxygenase Inhibition, and Phytochemical Composition of Propolis from Eastern Canada. Molecules 25(10).

[25] Astarina, Astuti, and Warditiani (2013). SKRINING FITOKIMIA EKSTRAK METANOL RIMPANG BANGLE (Zingiber purpureum Roxb.) Jurnal Farmasi Udayana, 1-6.

[26] Hakim, A., Jufri, A. W., Jamaluddin, Hadisaputra, S., and Adawiyah, S. R. (2020). Natural Product Chemistry Laboratory: Isolation Phenobarbitone from Mangrove Bark (Avicennia marina). Creative Education 11(9).

[27] Yadav, P., Kumar, A., Mahour, K., and Vihan, V. S. (2010). Phytochemical Analysis of Some Indigenous Plants Potent Against Endoparasite. Journal of Advanced Laboratory Research in Biology 1(1), 56-59.Dg

[28] Pertiwi, R. D., Suwaldi, Martien, R., and Setyowati, E. P. (2020). Radical Scavenging Activity and Quercetin Content of Muntingia calabura L. Leaves Extracted by Various Ethanol Concentration. Journal of Food and Pharmaceutical Sciences 8(2), 1-11.

[29] Ivanovic, S., Avramovic, N., Dojˇcinovic, B., Trifunovic, S., Novakovic, M., Teševic, V., and Mandic, B. (2020). Chemical Composition, Total Phenols and Flavonoids Contents and Antioxidant Activity as Nutritive Potential of Roasted Hazelnut Skins (Corylus avellana L.). Foods 9(430), 1-14.

[30] Putri, H. L., Retnowati, R., and Suratmo (2015). Fraksi n-heksana dari ekstrak Metanol daun Mangga Kasturi (Mangifera casturi Koesterm) dan Uji Fitokimia. Kimia Student Journal 1(1), 772-777.

[31] Simorangkir, D., Masfria, M., Harahap, U., and Satria, D. (2019). Activity Anticancer n-hexane Fraction of Cyperus Rotundus l. Rhizome to Breast Cancer MCF-7 Cell Line. Herbal Medicine in Pharmaceutical and Clinical Sciences 7(22), 3904-3906.

[32] Bribi, N. (2018). Herbal Medicine in Pharmaceutical and Clinical Sciences. Asian Journal of Botany.

[33] Xie, Y., Yang, W., Tang, F., Chen, X., and Ren, L. (2015). Antibacterial Activities of Flavonoids: Structure-Activity Relationship and Mechanism Current Medicinal Chemistry 22, 132-149.

[34] Cushnie, T. P. T., and Lamb, A. J. (2005). Antimicrobial activity of flavonoids. International Journal of Antimicrobial Agents.

[35] Sabir, A. (2005). Aktivitas Antibakteri Flavonoid Propolis Trigona sp Terhadap Bakteri Streptococcus mutans (in vitro). Maj. Ked. Gigi 38(3), 135-141.

[36] Juang, Y.-P., and Liang, P.-H. (2020). Biological and Pharmacological Effects of Synthetic Saponins. Molecules 25(4974).

[37] Darsana, I. G. O., Besung, I. N. K., and Mahatmi, H. (2012). Potensi Daun Binahong (Anredera Cordifolia (Tenore) Steenis) dalam Menghambat Pertumbuhan Bakteri

Escherichia Coli. Indonesia Medicus Veterinus 1(3), 337-351.

[38] Segal, R., Shatkovsky, P., and Ilana-Milo-Goldzwh (1974). On The Mechanism of Saponin Hemolysis-I Hydrolysis of the Glycosidic Bond. Biochemical Pharmacology 23, 973-981.

[39] Chung, K. T., Liu, Z., and Chou, M. W. (1998). Mechanism of Inhibition of Tannic Acid and Related Compounds on the Growth of Intestinal Bacteria. Food and Chemical Toxicology 36.

[40] Arbenz, A., and Avérous, L. (2015). Oxyalkylation of Gambier Tannin—Synthesis and Characterization of Ensuing Biobased Polyols. Industrial Crops and Products 67, 295-304.

[41] Fraga-Corral, M., García-Oliveira, P., Pereira, A. G., Lourenço-Lopes, C., Jimenez-Lopez, C., Prieto, M. A., and Simal-Gandara, J. (2020). Technological Application of Tannin-Based Extracts Molecules 25(614), 1-27.

[42] Nurjanah, Izzati, L., and Abdullah, A. (2011). Aktivitas Antioksidan dan Komponen Bioaktif Kerang Pisau (Sopen spp). Ilmu Kelautan UNDIP 16(3), 119-124.

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Development and validation of a bovine parainfluenza virus type 3 indirect ELISA

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Abstract— *Serological assays, including enzyme-linked immunosorbent assays (ELISA), provide an useful tool for screening animals for the presence of antibodies (Abs) against a wide range of infectious agents (including viruses that cause respiratory disease in cattle) and are mainly used in veterinary medicine to assist to the control and disease's monitoring. The aim of the present study was developing and validating one indirect enzyme-linked immunosorbent assay (ELISA) based on semi purified bovine parainfluenza virus type 3 (BPIV3).This test would allow to detect and quantify Abs against PI3 in serum sample from cattle and guinea pigs on both purposes diagnostic and typify/specify the quality of vaccines. The diagnostic sensitivity and specificity from the assay was 88% and 100% for bovine samples, using a threshold of corrected optical density, ODc =0.300, and 91% and 100% for guinea pig samples with a ODc =0.250.The intermediate precision expressed as the assays positive control coefficient of variation (CV) was 20% for bovines and 8.5% for guinea pigs. Both techniques reproducibility obtained in inter-laboratory assays was CV=17% for bovines and 15% for guinea pigs, which found the requirements of OIE (CV<30%). The efficacy of biological medicinal products, such as vaccines, relies an optimal model testing quality control. The validated ELISAs represents an important tool for testing vaccine quality, and quantifying and controling BPIV3 infections on cattle.*

Keywords— ***Bovine, Guinea pig, Indirect ELISA, Parainfluenza Virus Type 3.***

I. INTRODUCTION

Bovine parainfluenza virus type 3 (BPIV-3) is a Respirovirus, member of Paramyxoviridae family, antigenically related on human parainfluenza virus type 3 (hPIV-3)1. BPIV3 is an endemic infection of cattle, worldwide. Its presence is mostly documented in cattle, although there are investigations in other mammals, as a result of interspecies jumps, including rhinos, sheep, goats, guinea pigs and even humans 12. In Argentina, serological studies showed up a high incidence of antibodies against BPIV3 in cattle from the main livestock breeding regions [14,16,22].

In addition, positive serology was reported in domestic and wild South American camelids[16,3,17,21] and the virus was isolated from cattle, water buffaloes [15] and sheep [6]. BPIV3 infections are often asymptomatic, causing respiratory tissue damage and immunosuppression, predisposing animals to severe bacterial pneumonia. In some instances when animals are subjected to high stressful conditions, infection with BPIV3 can contribute to tissue damage, resulting in severe bronchopneumonia from secondary bacterial infections[5,8]. The resulting disease is part of the bovine respiratory disease complex (BRDC) and is considered as the most significant illness associated with feedlot cattle in the USA and possibly worldwide [24]. There is a demand for a fast detection and serological diagnosis of BPIV3 to monitor the presence of the virus and its antibodies in cattle, which is critical in designing suitable interventions and control.

Currently, the hemagglutination inhibition (HAI) is the gold standard technique to evaluate the immune status on a herd against BPIV3; however, this technique is laborious and has low sensitivity. Serological assays, including enzyme-linked immunosorbent assays (ELISA), provide a useful tool to screen animals to find antibodies's (Abs) against a wide range of infectious agents (including viruses that cause respiratory disease in cattle) and are highly used in veterinary medicine to assist on detecting disease in cattle and also in laboratory animal models, such as the guinea pig model validated in Argentina for vaccine potency testing [4,18,19].

The assay constitutes a critical method for final validation and transference to animal health authorities of the guinea pig model used to evaluate the skill to produce an effective level of immunization in the target species. This involves an alternative method for vaccine potency testing, which is in alignment with the 3R initiative of refining, reducing and replacing animal experimentation [11].

The aim of the present study was the development and statistical validation from an indirect enzyme linked immunosorbent assays (ELISAs) in order to diagnostic and typify / specify the quality of vaccines.

II. MATERIALS AND METHODS

2.1 Experimental design

A total of 85 bovine and 95 guinea pigs sera with known history have been used throughout every tests run in this work. The validation has included: diagnostic sensitivity and specificity; repeatability and intermediate precision within a laboratory along several years and the reproducibility in interlaboratory assays. To estimate the relevance of the assays, a concordance analysis between the results obtained by ELISA and by hemagglutination inhibition test was conducted.

2.2 Serum samples

A total of 85 bovine serum samples (53 positive and 32 negative for antibodies to BPIV3) and 95 guinea pig serum samples (49 positive and 46 negative for antibodies to BPIV3) were run to determine the cut-off of the assay and its associated sensitivity and specificity of the diagnostic. The serologically positive samples belong to naturally infected bovines and experimental hyperimunized guinea pigs. The negative serum samples were obtained from colostrum deprived calves –that were seronegative to BPIV3 for HAI in bovines. Guinea pig negative serum samples were from non-vaccinated or placebo animals.

The protocol was approved by the Institutional Ethical Committee-INTA (CICUAE-INTA N° 33/2012).

2.3 Internal bovine sample used as control sera

As there are no bovine reference controls for BPIV3 in order to have positive and negative controls, HAI-titrated sera positive and negative sera were chosen to make pools that were, titrated, aliquoted and stored at -20 ° C. Positive and negative control samples were included in each plate of each ELISA run, the positive control titer (HAI) was 5120 HAIU for bovine pool serum and 10240 HAIU for guinea pig serum pool. The assay was valid if the Ab titer of the positive control was the expected value ± standard deviation (2SD) and the negative control was below the cut off of the assay.

2.4 Hemagglutination inhibition assay

HAI assays were performed by incubating serial 2-fold dilutions of bovine or guinea pig serum at 25 °C for 30 min with 8 HA units per 25ul of BPIV-3. Subsequently, guinea pig red blood cells were added to each well, incubation was continued for 90 min and each well was then observed for hemagglutination. The sera were treated with kaolin to adsorb nonspecific hemagglutination inhibitors that were found there. It will be taken as the end-point of the serum activity (anti PI-3 HAI neutralizing Ab titer) the reverse of the maximum dilution in wichhaemagglutination phenomenon was inhibited. HAI titres were expressed as the reciprocal of the highest dilution of serum that was inhibited virus-mediated agglutination of erythrocytes. The final result is expressed in Hemagglutination Inhibitory Units (HAIU). The reciprocal of serum activity end-point dilution, multiplied by the virus hemagglutinating units (8 HAIU) will give as a result the number of serum haemagglutination inhibiting units per unit volume. The presence of HAI reactions at dilutions less than 1/10 (40-80 HAIU) are considered negative. Values greater than 1/80 (640 HAIU or more) represent infection response or to vaccination (Res. SENASA 598/12).

2.5 Enzyme linked immunosorbent assay

MDBK cells were infected with semipurified BPIV3 virus strain SF-4 in laminar flow at an m.o.i. of 0.1 TCID50 per cell and were incubated for 48 hours 37 ° C with 5% CO_2 in MEM-E, Gibco. After that time, the cytopathic effect of the culture was observed by a light microscope. Cells were lysed with a freeze / thaw process of -80 ° C for 10 minutes at 37 ° C for three cycles. The culture medium was clarified by low speed centrifugation and supernatant centrifuged at 13216x g for 1 h. The pellet was resuspended in pH= 7 NET buffer (Tris-HCl 500mM, Tris Base NaCl 100mM, NaCl EDTA 10mM) and titrated. ELISA plates (Inmulon IB; Dynatech, Laboratories) were coated, in alternating columns with positive antigen (BPIV3 amplified in MDBK cells) and negative antigen (mock: not infected cells). The incubation time was for 18 hours at 4°C. The blocking solution was about 50 ul of the phosphate buffer solution (pH 7.4) supplemented with 0.05% Tween20 (PBS-T) and ovalbumin 1% p/v (PBS-T OVA 1%) for thirty minutes at 37°C. The sera sample (1/40) were incubated for an hour. The following step was to add an anti-bovine conjugated with peroxidase (HRP, horseradish peroxidase - Kirkegaard& Perry Laboratories, KPL) in a (1:2000) dilution. Once the plate has been washed, it was been developed by adding distilled water, hydrogen peroxide (3% w / v H_2O_2) and the ortho-phenyldiamine(OPD), 0.06 mg/ml as substrate/chromogen system (Sigma), leaving it to act for about 5 minutes (developing solution) and it was made stopped using 7% sulfuric acid (H_2SO_4). The optical density was readspectrophotometrically at 490 nm.

The ELISA assay was adapted for detecting guinea pig antibodies by using the same procedure described on bovine samples. Peroxidase-labeled affinity purified goat anti guinea pig IgG(H+L) (Kirkegaard&PerryLaboratories,KPL) has been used as a conjugate in a 1:4000 working dilution.

2.6 Statistical methods for ELISA validation

2.6.1Feasibility studies, initial repeatability and intermediate precision

The repeatability was expressed as the optical density (OD) coefficient variation for positive control sera obtained in the different runs. The control sera were assayed at a 1/40 dilution duplicated on every ELISA run. The assay was valid if the Ab titer of the positive control was the expected value ± 2 standard deviation (2SD) and the negative control was below the cut off of the assay.

For full validation, the assay's intermediate precision was estimated by bovine and guinea pig positive control sera data, collected from ELISA runs conducted for 3 year in the our lab. For estimating the repeatability and intermediate precision from the ELISA on each species, an ANOVA for a nested model of variance has been carried out. The applied model allowed to quantify the relative contribution of different variation sources (intra assay: same plate and different plates; inter assay: different runs; and different samples: bovine or guinea pig serum) as the relative coefficient of variation (CV).

2.6.2Diagnostic sensitivity and specificity of the ELISA

The assay cut off of and its associated DSe and DSp were estimated basing the frequency (negative and positive sera) distribution of the ELISA values, obtained after analyzing the reference populations of 1/40 dilution. ELISA results were normalized by expressing the raw corrected optical density values (ODc; sample OD-mock OD)OD as the percentage of positivity (PP%) of the high-positive bovine and guinea pig reference control sera described in section “Internal bovine sample used as control sera”. The control sera were assigned a 100% PP% value and there were included in duplicate in each plate. An ELISA run was taken as valid if the positive control ODc fell on the established admissible working range (mean ODc ± 2 SD). In this study, the assay's sensitivity has been determinated like the probability of positive test results, when the animal was effectively positive for antibodies to BPIV3 (either due to infection or vaccination). Whereas the specificity has been defined like the probability of a negative test result when the animal was effectively negative for antibodies to BPIV3 (Greiner and Gardner, 2000) [7].

2.6.3 ROC analysis

The assay's cut-off selection for both species was also carried out with the aid of the receiver-operating (ROC) analysis, using the MedCalc Statistical Software version 18.11.6 (MedCalc Software bvba, Ostend, Belgium).

2.6.4 Detection limit and linearity of the assay

The relative analytical sensitivity or detection limit was defined as the least amount of antibody to BPIV3 detectable in an ELISA run positive sample with already known Ab titer (positive serum). The ELISA Ab titerwas estimated by the end-point, limiting dilution analysis from those reference sera. Briefly, dose response curves were constructed by using a total of 36 replicates of positive bovine serum and 16 replicates positive guinea pig serum, assayed in four-fold dilutions in several plates within different independent ELISA runs. To find out the analytic sensitivity linear regression curves has been determined by plotting the ELISA values of those sera expressed as ODc from positive control versus the log-transformed dilutions.

To verify the linearity of the system, the Mallows'sCpwere used to assess the fit of a regression model that has been estimated by using ordinary least squares.

2.6.5 Correlation between the antibody titer determined by ELISA and HAI

The correlation between the BPIV3 antibody titer determined by the HAI and ELISA have been tested in vaccinated animals. The vaccines tested included 2 experimental combined vaccines containing and 107.3 TCID50/dose of inactivated BPIV3 and one commercial vaccine (Biopoligen HS, Biogénesis-Bagó) applied to prevent bovine respiratory diseases. On this study, 90 bovine and 43 guinea pig serum samples were tested.

The relationship between the Ab titer obtained by ELISA and HAI in bovines and guinea pigs were studied. Specifically we have evaluated the Pearson´s correlation coefficient between HAI and ELISA Ab titer to BPIV3.

2.6.6 Reproducibility: inter-laboratory assay

The inter-laboratory assay included data collected in 3 laboratories for bovine and guinea pigs samples. The laboratories involved were Respiratory Virus and Animal Welfare division, Adventitious virus section, and INCUINTA from the Institute of Virology, INTA, Castelar. The laboratories received the kit and coded samples that included positive and negative controls and standard sera. The laboratories were requested to perform five ELISA assays following the supplied protocol, including 6 plates per run and testing the samples in 3 replicates per plate. The reproducibility of the assay was calculated on the coefficient of variation (relative standard deviation) using a nested ANOVA.

III. RESULTS

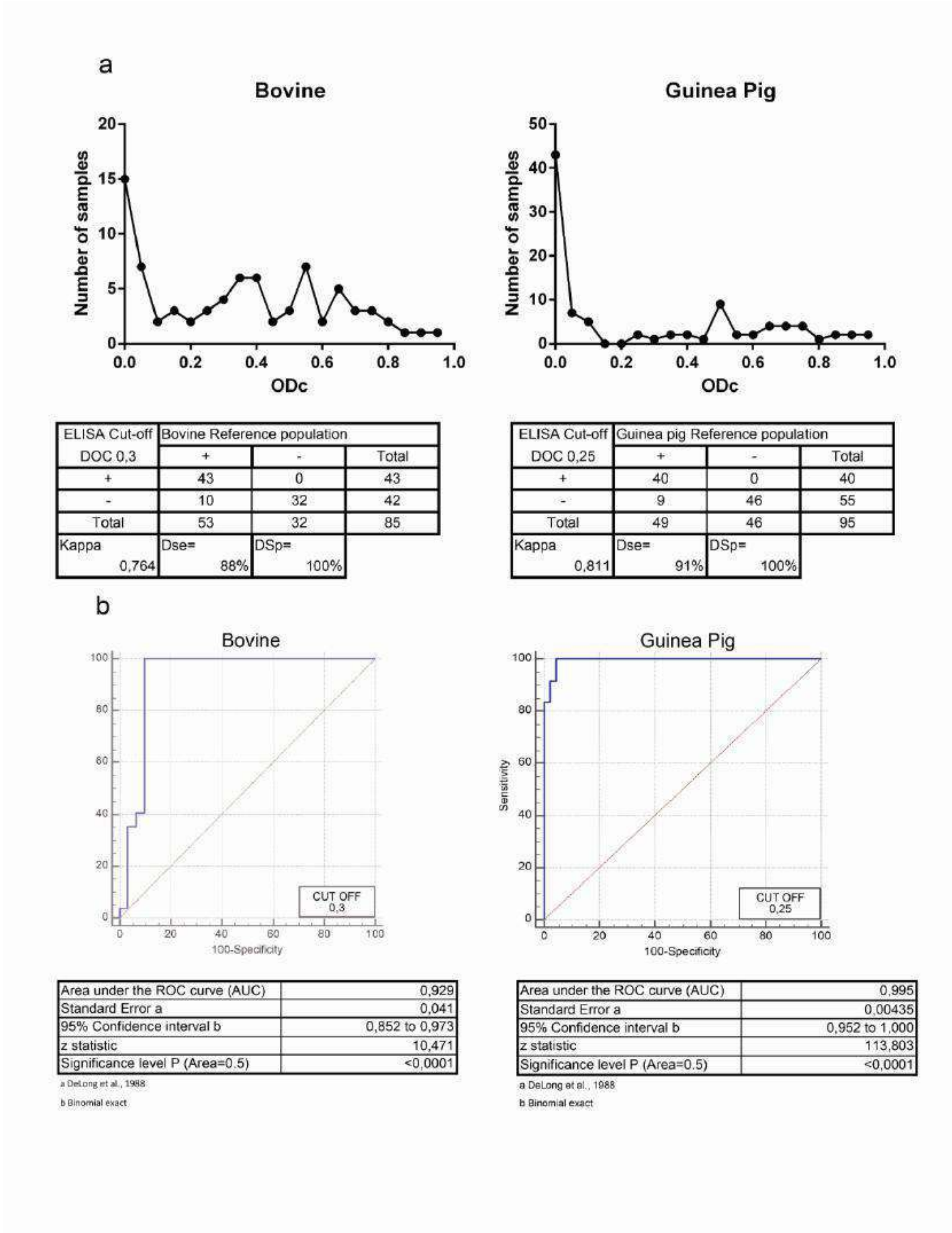
Table 1. Validation parameters

Validation parameters	Result		Acceptance criteria
	Bovine	Guinea Pig	
Cut-off (ODc)	0,3	0,25	
Diagnostic specificity	88%	91%	
Diagnostic sensitivity	100%	100%	
Positive control mean ODc	0,7	0,699	
Standard deviation (SD)	0,08	0,09	
N	85	95	
ODc admissible working range of the positive control (ȳ±1 SD)	0,62-0,78	0,61-0,79	
Repeatability	15,00%	5,60%	CV < 20%
Intermediate precision	20,70%	8,56%	CV < 25%
Linearity	Yes, R^2=0,9, p<0,0001	Yes, R^2=0,85, p<0,0001	
Inter-laboratory assay	(3 labs)	(3 labs)	
Reproducibility	17%	15%	CV < 30%

3.1 Cut-off, diagnostic sensitivity and specificity of the ELISA for bovine and guinea pigs: agreement with HAI

All bovine samples (n=85) were tested with HAI and ELISA. A total of 53 samples were positive for hemagglutination inhibition, while 32 were negative. For the ELISA, 43 out of 53 positives samples gave as a result higher ODc than 0.3 at the 1:40 dilution, and all of negative samples showed up values that were lower than 0.3. The frequency of positivity distribution percent obtained for positive and negative reference samples, run at 1:40 dilution is depicted in Fig.1a. Using a 0.3 ODc cut-off, the assay showed up a 88% diagnostic sensitivity and 100% diagnostic specificity and ~~a~~ high agreement with the HAI assay (kappa: 0.764) (Fig.1b); so it was considered suitable to use as a screening method for field surveys of infected animals. This cut-off was confirmed by ROC analysis conducted by MedCalc® software as the threshold that gives the highest accuracy (Table 1 and Fig. 1b). The area under the ROC curve value (AUC=0.929, p = 0.0001) indicated that the test has the ability to distinguish between the positive and negative samples with 95% confidence in the 93% of the times that a random simple is tested.

For guinea pig samples, the frequency analysis of positivity percent value distribution from reference sera, showed that a 0.25 ODc cut-off gave the highest levels of diagnostic sensitivity and specificity (Fig.1a). This value was also confirmed by ROC analysis (Table 1 and Fig.1b). Similarly to the ROC curve (AUC=0.995, p = 0.0001) obtained for bovine samples, the ELISA has the ability to score properly a guinea pig sample with 95% confidence in the 97% of the times that a random simple is tested. Using a 0.25 ODc cut-off the ELISA showed an almost perfect agreement with the HAI assay (kappa: 0.811) and a relative sensitivity and specificity of 91% and 100%, respectively.



ELISA Cut-off	Bovine Reference population		
DOC 0,3	+	-	Total
+	43	0	43
-	10	32	42
Total	53	32	85
Kappa 0,764	Dse= 88%	DSp= 100%	

ELISA Cut-off	Guinea pig Reference population		
DOC 0,25	+	-	Total
+	40	0	40
-	9	46	55
Total	49	46	95
Kappa 0,811	Dse= 91%	DSp= 100%	

Area under the ROC curve (AUC)	0,929
Standard Error a	0,041
95% Confidence interval b	0,852 to 0,973
z statistic	10,471
Significance level P (Area=0.5)	<0,0001

a DeLong et al., 1988
b Binomial exact

Area under the ROC curve (AUC)	0,995
Standard Error a	0,00435
95% Confidence interval b	0,952 to 1,000
z statistic	113,803
Significance level P (Area=0.5)	<0,0001

a DeLong et al., 1988
b Binomial exact

Fig. 1: Cut-offs of the assay for bovine and guinea pig samples and their associated diagnostic sensitivity (DSe) and specificity (DSp). (a) Frequency distribution of the ELISA values obtained, after analyzing the reference populations at a 1/40 dilution. ELISA values were expressed as corrected optical density (ODc). (b) ROC analysis, MedCalc® version 18.11.6 statistical software.

3.2 Feasibility studies, initial repeatability and intermediate precision

The coefficient of variation and the admissible working range of the ODc established for each bovine and guinea pig positive control are detailed in Table 1. The analysis of variance for the proposed nested model (5 assays, 6 plates per assay and 3 replicates per plate)has indicated that the assay had good values of repeatability. The coefficient of variation for bovine and guinea pig positive controls were 15% and 5.6%, respectively.

The intermediate precision of the assay given by the overall relative variation for both species was also acceptable (CVbovine = 20.7% and CVguinea pig = 8.56%) and met the OIE requirements (CV<25%) (Table1).

3.3 Detection limit and linearity of the assay

In the ELISA for bovine and guinea pig samples, the detection limit or absolute analytical sensitivity was estimated by the end-point limiting dilution analysis of positive control sera, assayed in four-fold dilutions on several plates within different independent ELISA runs. The detection limit of the assay was satisfactory and similar for both species (Fig.2a). In both cases the regression analysis from the obtained dose–response curves, has indicated that the assays showed up a range of linear behavior (Fig. 2a), R2= 0.9 and R2=0.85 for bovine and guinea pig respectively.

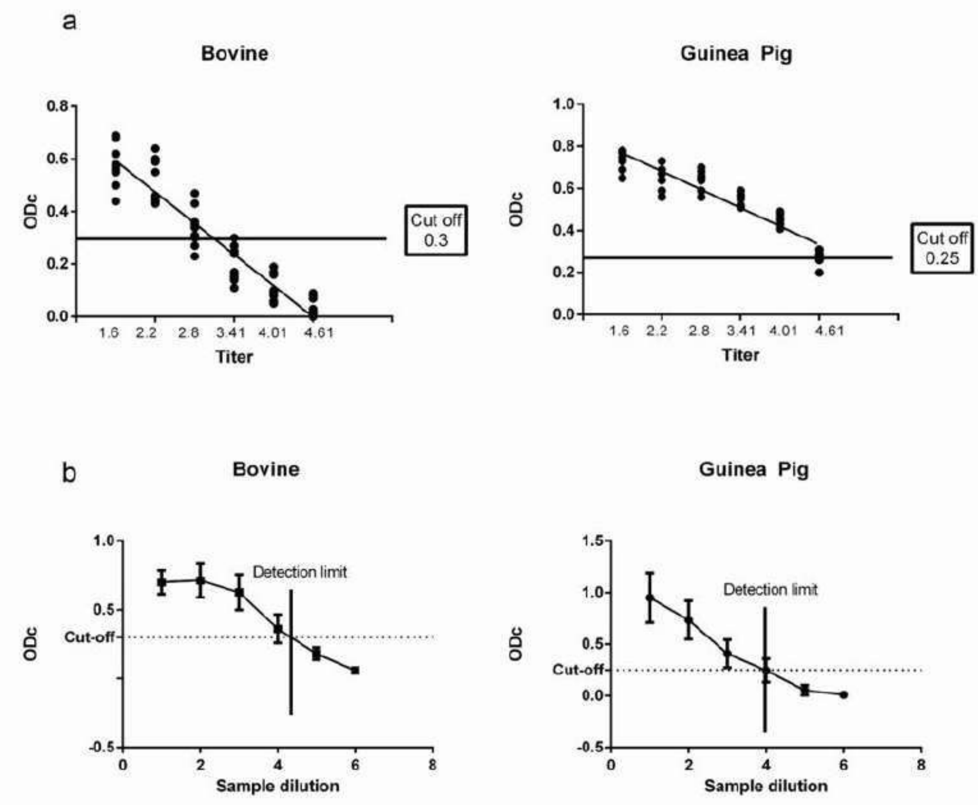


Fig. 2: Dose–response curves. Limit dilution analysis of bovine and guinea pig positive control serum, serial four-fold dilutions starting at 1:40. (a) Each point represents the value obtained for each replicate of the diluted sample in each plate from every independent ELISA run, and the curve is the linear regression; titerswere expressed as the log10. (b) The response detected by ELISA was expressed as the ODc versus the sample dilution. Each line represents the mean ODc value obtained for the different diluted sample replicates in every independent ELISA run.

3.4 Correlation between the antibody titer obtained by ELISA and HAI assay

There was a significant correlation between the ELISA and the hemagglutination inhibition antibodies titers to BPIV3. Excluding the negative animals, ~~the~~ Pearson correlation coefficients were lower, but still statistically significant ($p < 0.001$). The scatterplots, in which both coefficients are reported, are depicted in Fig. 3.

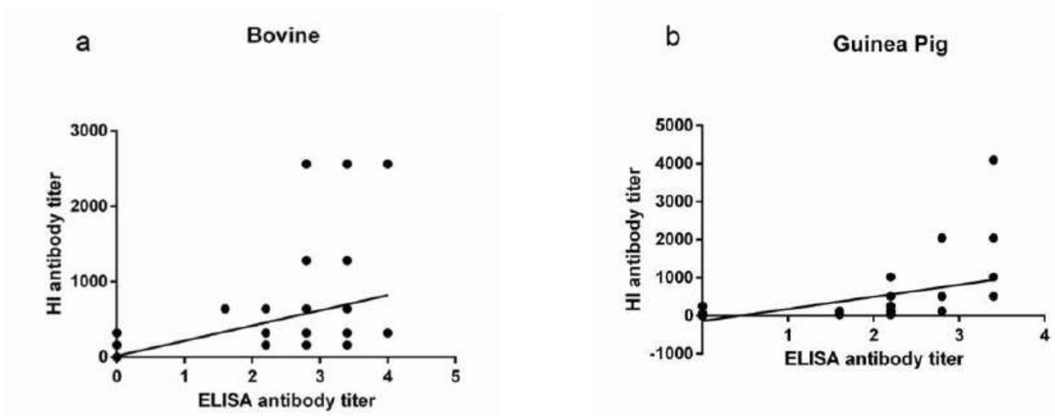


Fig. 3: ELISA relevance defined like the correlation between individual ELISA and neutralizing antibody titers induced by BPIV3 vaccines in groups of 5–10 (a) bovine calves, being sampled 60 days post-vaccination and (b) guinea pigs sampled 30 days post-vaccination. Bovine Pearson correlation coefficient: 0.52 (p=1.2E-6) (n=90) and Guinea Pig Pearson correlation coefficient: 0.51 (p=9.5E-4) (n=43).

3.5 Assay reproducibility: inter-laboratory assay

The inter-laboratory study data have been analyzed with an analysis of variance with 5 ELISA runs, 6 plates and 6 replicates and they were collected in three laboratories (defined in 2.6.6 section). The overall mean for bovine and guinea pig positive controls were 0.733 and 0.674 respectively, both falling within the range of acceptance (Table 1). The reproducibility of the assays expressed as the coefficient of variation was 17% for bovine and 15% for guinea pig positive control (Table 1).

IV. DISCUSSION

In this paper, we successfully validated an indirect ELISA assay by estimating the diagnostic sensitivity and specificity of the assays, the repeatability and precision within a laboratory over time, and also the precision and reproducibility of the assays inter-laboratory. This test will be used to evaluate and quantify antibodies against BPIV3 in bovine sera, thus improving the estimation of prevalence of this virus in herds of our country; and also in guinea pig sera, complementing the HAI tests in the guinea pig model.

Respiratory infections are one of the main causes of disease and death in calves during the first weeks of life and in the first stage of rearing. This is observable in both intensive dairy systems and in farmyard fattening systems, causing significant economic losses. Among the diseases that cause great economic losses of livestock industry there is the fever shipping associated to BPIV3.

Still, little information is available on the epidemiology of BPIV3 in Argentina: some studies demonstrate seropositivity in guanacos [17]. The presence of the three known genotypes of BPIV3 –a, b and c- has been documented in bovine and bubaline cattle in the north of the country, being the first cases in which the sequence is available in our country [15]. For the most part, the diagnosis of BPIV3 is by different serological techniques, including hemagglutination inhibition (HAI): reported seroprevalenceshave been made with this test, which is considered as the gold standard. The HAI principle is based on the ability of the virus, to agglutinate red blood cells, normally from guinea pigs, forming a red button on the cell plates where the assay is performed. However, there are several reports that reveal difficulties in sensitivity, due to low hemagglutination titers in inoculated animals[10] added to the fact that it requires several steps of molecules inactivation that unspecifically generate hemagglutination, resulting in false positives. The correlation coefficient obtained between both techniques is within acceptable values (Fig. 3).

A faster, cheaper, easier technic to repeat and execute is ELISA. Currently, there are two types of commercially BPI3 ELISA, one is an indirect format for the detection of antibodies in bovinesserum and milk, beeing similar to our assay, that is based on complete viral antigen. The second type is for detecting the virus directly (Biox, Belgium). In addition, the development of an indirect ELISA based on a truncated nucleocapsid protein obtained by recombination has been reported in the literature [25]. On the other hand, a multiplex ELISA was also reported to detect four complex respiratory bovine viruses in bovine serum [18]. In our country these kits are not available and, if they would be wanted to be imported, they would have a very high cost. Nowadays, polyvalent combined inactivated viral vaccines with the virus BPIV3, BoHV1, BDVD and BRSV and bacterial antigens are used as a control strategy for infectious diseases, aimed at facilitating the health calendar, and simultaneously, attacking a complex etiological problem, such as bovine respiratory syndrome. However, in many cases, they are not accompanied by objective data that supports their immunogenic quality and efficacy against each of the antigens that compose them. International control agencies (APHIS, USA; EMEA-CVMP, EU; OIE; VICH) require potency and efficacy tests in the species of application, for the approval of respiratory vaccines; this implies vaccination and infection to susceptible and seronegative cattle. Once the product is approved, the quality control for each serie must be carried out by means of a potency test that defines the immunogenicity of the product in cattle or in ~~an~~other laboratory animal model (*in vivo* test).

It must be statistically validated, possessing an acceptable degree of concordance with respect to the potency test in the target species, and to be functional as a vaccine efficacy predictive tool. The difficulty to obtain bovines with seronegatives bovine respiratory complex valences, and the high cost of immunogenicity tests in the host species, propose the need to develop a standardized test in laboratory animals. It would allow to compare potency evaluations of each batch of vaccine, in a harmonized way, thus guaranteeing the presence on the market of effective products. To date, the guinea-pig model validated for BPIV3 uses a range of antibody titers established by HAI to categorize the immunogenicity of vaccines [4]. In relation to animal welfare, this test responds to the guidelines of international organizations by replacing and reducing significantly the use of animals in experimental tests [11,2,9]. Not only due to the replacement of bovines by guinea pigs, but also because the change in technique (ELISA for HAI) implies not using red blood cells from animals.

The current situation, about the growth of the national market and the requirement of control organisms and private laboratories, makes it necessary to implement this type of quality control. Currently the technique for quantifying antibodies against BPIV3 validated for the guinea pig model is the HAI. However, due to the variability from each operator reading, the environmental conditions dependence and the fresh red blood cells availability to perform the assay, it was decided to develope these ELISAs. This production will allow us to have the necessary quantity of tests to evaluate the immunogenicity induced by commercial vaccines and to validate the guinea pig model to facilitate the vaccines quality evaluation.

An indirect ELISA for BPIV3 antibody detection in bovine and guinea pigs sera were validated. The statistical validation included the estimation of the assay reliability of the assays using certain cut-off values and their associated diagnostic sensitivity and specificity established in both of the species to make it fit on the assay purpose [7].

A 0.3 ODc cut-off, 88% sensitivity and 100% specificity has been considered suitable to use as a screening method for field surveys of infected animals. The BPIV3 ELISA showed similar performance as the hemagglutination inhibition and it has demonstrated to be more suitable for herd surveys. Similar values were obtained by the indirect biox ELISA; Relative sensitivity: 100% Relative specificity: 100%. On one hand, the agreement between the two tests is considered excellent (https://www.biox.com/en/bio-k-239-monoscreen-abelisa-bpi3-indirect-double-wells-p-255/). On the other hand, the NP-N elisa showed a sensitivity of 98.4% while the specificity was 100%[25]. None of these carried out reports were as exhaustive statistical studied as this work. In our work a significant correlation was obtained between the ELISA and HAI antibody titers in vaccinated animals analyzed at specific time point after vaccination (60 days for bovines and 30 days for guinea pigs), indicating the relevance of the ELISA's development to be applied on batch to batch vaccine potency testing in both species. To retain international trade markets, vaccination and serology surveys from herds will be mandatory in the near future. This test, the first in ELISA format, will allow us to evaluate the epidemiology of the virus in our region once it is commercially available. The used of this ELISA to detect antibodies in serum, in combination with BPIV3 RT PCR to detect virus genome represent a suitable method of high sensitivity and minimal risk of false negative results [15]. As well as this technique constitutes an important monitoring system for the implementation of eradication programs in the region.

In addition, it is well known that the sensitivity and specificity of an assay can varies either with the stage of infection or with immune host status[13]; thus, in positive reference population, we included naturally infected bovines from endemic farms, samples from vaccinated animals and samples from experimentally challenged animals [20,23].

This study reports the development of an alternative tool to detect antibodies against BPIV3. The additional advantage to the independence of the subjectivity of the operator reading and red blood cells disponibility, is the adjustment of the antibody titers range to assess the quality of vaccines with greater precision. It will be a valuable addition to improve the vaccines available in the market, and consequently, the population immunity to BPIV3 necessary to reduce the high circulation of the virus in the country.

In the near future this indirect ELISA could be offered as a commercial kit where the plates will be sensitized, dry and stable over time, making available a serological assay for BPIV3 in our region.

V. CONCLUSIONS

This assay will be useful to evaluate the seroprevalence in cattle and also for evaluation of the vaccines quality in a guinea pig model. The ELISA showed very good intermediate precision and reproducibility in both species.

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REFERENCES

[1] Almeida Vaucher R, Simonetti A, Roehe P. RT-PCR for detection of bovine parainfluenza virus type 3 (bPIV-3). *Acta Scientiae Veterinariae*. 2008; 36: 215-220.

[2] Akkermans A, Hendriksen C. Statistical evaluation of numbers of animals to be used in vaccine potency testing: a practical approach. *Dev Biol Stand.* 1999; 101:255-260.

[3] Barbieri S, Rodríguez D, Marin R, Setti W. Serological survey of antibodies against viral diseases of public health interest in llamas (Lama glama) from Jujuy province, Argentina. *Rev Argent Microbiol*. 2014; 46: 53-57.

[4] Camevet. Guía de prueba de potencia para vacunasbovinasinactivadas que contengan virus parainfluenza 3bovino (pi-3) [Potency Test Guide for Inactivated Bovine Vaccines Containing Bovine Parainfluenza 3 Virus (Pi-3)]. *Fundación PROSAIA*. 2012

[5] Ellis, J. Bovine parainfluenza- 3 virus. The Veterinary Clinics of North America. *Food Animal Practice*. 2010; 26(3):575-593

[6] Epstein B. Parainfluenza tipo III enbovinos y ovinos de Argentina. Patología, aislamiento y caracterización [Type III parainfluenza in cattle and sheep from Argentina. Pathology, isolation and characterization]. *Rev Argent Microbiol*. 1974; 55:397–406.

[7] Greiner M, Gardner I. Application of diagnostic tests in veterinary epidemiologic studies. *PrevVet Med*. 2000; 45:43-59.

[8] Haanes J, Guimond P, Wardley R. The bovine parainfluenza virus type-3 (BPIV-3) hemagglutinin/ neuraminidase glycoprotein expressed in baculovirus protects calves against experimental BPIV-3 challenge. *Vaccine*.1997; 15: 730-738.

[9] Halder M, Hendriksen C, Cussler K, Balls M. ECVAM's contributions to the implementation of the Three Rs in the production and quality control of biologicals. *Altern Lab Anim*. 2002; 30; 93-108.

[10] Haller A, Miller T, Mitiku M, Coelingh K. Expression of the Surface Glycoproteins of Human Parainfluenza Virus Type 3 by Bovine Parainfluenza Virus Type 3, a Novel Attenuated Virus Vaccine Vector. *J Virol.* 2000; 74:11626–11635.

[11] Hendriksen, C. Replacement, reduction and refinement alternatives to animal use in vaccine potency measurement. *Expert Rev Vaccines*. 2009; 8: 313-322.

[12] Horwood P, Gravel J, Mahony T. Identification of two distinct bovine parainfluenza virus type 3 genotypes. *J Gen Virol*. 2008; 89: 1643–1648.

[13] Kramps J, Banks M, Beer M, Kerkhofs P, Perrin M, Wellenberg G, Van Oirschot J. Evaluation of tests for antibodies against bovine herpes virus 1 performed in national reference laboratories in Europe .*Vet Microbiol*. 2004; 102: 169–181.

[14] Lager L, Sadir A, Schudel A. Enfermedadesrespiratoriasvirales de bovinos [Bovine viral respiratory diseases]. *J Información y DesarrolloenInvestigaciónAgropecuaria*. 1983; 1:55-58.

[15] Maidana S, Lomonaco M, Combessies G, Craig M, Diodati J, Rodriguez D, Parreño V, Zabal O, Konrad J, Crudelli G, Mauroy A, Thiry E, Romera S. Isolation and characterization of bovine parainfluenza virus type 3 from water buffaloes (Bubalusbubalis) in Argentina. *BMC Vet Res*. 2012; 8; 83.

[16] Marcoppido G, Parreño V, Vilá B. Antibodies to pathogenic livestock viruses in a wild vicuña (Vicugnavicugna) population in the Argentinean Andean altiplano. *J Wildl Dis*. 2010; 46:608–614.

[17] Marcoppido G, Olivera V, Bok M, Parreño V. Study of the kinetics of antibodies titres against viral pathogens and detection of Rotavirus and Parainfluenza 3 infections in captive crias of guanacos (Lama guanicoe).*TransboundEmerg Dis*. 2011; 58:37-43.

[18] Mayers J, Sawyer J. Development and evaluation of a multiplex enzyme-linked immunosorbent assay for the detection of antibodies to bovine respiratory diseases on the Meso Scale. *Journal of J Vet Diagn Invest*. 2012; 24:725–729.

[19] Parreño V, López M, Rodriguez D, Vena M, Izuel M, Filippi J, Romera S, Faverin C, Bellinzoni R, Fernandez F, Marangunich L. Development and statistical validation of a guinea pig model for vaccine potency testing against Infectious Bovine Rhinothracheitis (IBR) virus. *Vaccine*. 2010; 28:2539-2549.

[20] Parreño V, Romera SA, Makek L, Rodriguez D, Malacari D, Maidana S, Compaired D, Combessies G, Vena MM, Garaicoechea L, Wigdorovitz A, Marangunich A, Fernandez

F. Validation of an indirect ELISA to detect antibodies against BoHV-1 in bovine and guinea-pig serum samples using ISO/IEC 17025 standards. *J Virol Methods*. 2010; 169:143-153.

[21] Puntel M. Seroprevalencia de infeccionesviralesen llamas (Lama glama) en la república Argentina [Seroprevalence of viral infections in Lama Glama in Argentine]. *Rev Argent Microbiol.* 1997; 29: 38–46.

[22] Robles C. Relevamientosanitario e implementación de un plan para la prevención y control de enfermedadesenbovinos de productoresruralesminifundistascomunitarios de la provincia de Neuquén, Argentina [Sanitary survey and implementation of a plan for the prevention and control of diseases in cattle from rural small community farmers in the province of Neuquén, Argentina]. *Proinder-Inta.* 2008.

[23] Romera S, Hilgers L, Puntel M, Zamorano P, Alcon V, Dus Santos M, Blanco Viera J, Borca M, Sadir A. Adjuvant effects of sulfolipocyclodextrin in a squalane-in-water and water-in-mineral oil emulsions for BHV-1 vaccines in cattle. *Vaccine*. 2000; 19: 132–141.

[24] Snowder G, Van Vleck L, Cundiff L, Bennett G. Bovine respiratory disease in feedlot cattle: environmental, genetic, and economic factors. *J Anim Sci*. 2006; 84: 1999-2008.

[25] Yang Y, Wang, F, Sun N, Cao L, Zhang S, Zhu W, Wen, Y. Development and evaluation of two truncated recombinant NP antigen based indirect ELISAs for detection of bovine parainfluenza virus type 3 antibodies in cattle. *J Virol Methods.*2015; 15:47-50.

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Mass Propagation of Agarwood Producing Plant (*Aquilaria Malacensis* L.) with Application Auxin and Cytokinine Concentrations in Vitro Culture

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***Abstract*— *The objective of study to obtain: the best concentration of thidiazuron in encouraging explants to form shoots and the best combination of concentrations of NAA and BAP to regenerate callus and shoots to form plantlets. This experiment was carried out in Plant Tissue Culture Laboratory, Faculty of Agriculture Andalas University from May to October 2021. This study consisted of two experimental stages. where the first stage to callus induction with 6 levels of Thidiazuron concentration: 0.00, 0.125, 0.250, 0.375, 0.50 and 0.625 ppm. The second stage of the experiment was the shoot and callus regeneration stage with a combination treatment of NAA + BAP concentration, with 7 levels of treatment: 0.0 ppm NAA + BAP 0.0 ppm; 0.0 ppm NAA + 1.00 ppm BAP; 0.0 ppm NAA + 2.0 ppm BAP; 0.0 ppm NAA + 3.0 ppm BAP; 0.50 ppm NAA + 1.0 ppm BAP; 0.5 ppm NAA + 2.0 ppm BAP and 0.50 ppm NAA + 3.0 ppm BAP. The study was completely randomized design (CRD) with 3 replications in a The data were analyzed by using the F test and followed by the Least Significant Difference test (LSD). The experiment was conducted with completely randomized design with 3 replications. The result show the highest percentage of live explants was 100%, the fastest time callus induction was 15 days and the percentage of explants formed callus of was 70% at treatment with a concentration of 0.25 ppm Thiduazuron. The callus structure of all treatment levels was compact and the color of the callus was white, yellowish white and whitish yellow. callus forming the fastest shoots was 11.67 days, the percentage of callus forming shoots was 50% and the number of callus forming shoots was 5 pieces obtained at a concentration of 0.5 ppm NAA + 3.0 ppm BAP.*

Keywords— ***Auxin, Cytokines, In vitro culture, Mass propagation***

I. INTRODUCTION

Propagation of Agarwood-producing plants is usually carried out generatively using seeds but in its development in nature there are obstacles because Agarwood-producing plants only flower and bear fruit at the age of 7-10 years, while at the age of 5 years, farmers/loggers have started to harvest this plant and if there are trees that has been fruitful, then the ripe fruit is likely to be eaten by birds so that some are flown by birds to other places, and some fall under the tree, besides that the germination of agarwood seeds is relatively low, only about 47%; while vegetatively with cuttings and grafts it takes a long time to produce large numbers of seedlings, seedling growth is not uniform, depending on the season, is not free from systemic disease, and the percentage of growth is only about 55%.

The in vitro culture technique is the first step towards breeding Agarwood-producing plants that are faster, uniform, in large quantities, of better quality, and most importantly can be used as a source of germplasm for Agarwood-producing plants compared to conventional methods using seeds or cuttings or grafts. Furthermore, research on the propagation of Agarwood-producing plants, especially Aquilaria malaccensis species in vitro in Indonesia, has not yet developed, especially in the area of West Sumatra, research on this has not yet been reported.

The propagation of agarwood-producing plants in vitro is largely determined by the material from which the explants came from, the media, growth regulators, and the growing environment. Planting material (explants) taken from the parent tree must have criteria, including: healthy, exposed to sunlight, meristematic parts. The planting media made is adjusted to its purpose, if we want to form callus then we should use liquid media, and if for ordinary propagation we use solid media, then if we cultivate woody plants such as Agarwood-producing plants, we recommend using WPM media (Satria, Gustian Swasti and Kasim 2008).

Growth regulators are very influential in the success of tissue culture techniques. Growth regulators used for the formation of shoots are cytokines, while for the formation of roots or callus auxin is used as growth regulators (Untung and Nursandi, 2001 and Lestari, 2011). One type of cytokines that is widely used in the formation of shoots is thidiazuron. Thidiazuron belongs to the group of strong cytokines, thidiazuron with low concentrations is able to show a response to plants (Harahap, 2012). According to Khawar et al (2003) thidiazuron is able to induce shoot propagation faster than other types of cytokines. Administration of thidiazuron (TDZ) at a concentration of less than 1 M can induce shoots in woody plants (Huetteman and Preece, 1993).

Based on research by Fernando (2017), it was shown that the concentration of TDZ 0.25 mg/L in MS medium was the best concentration in inducing shoots of female Andalas plants. The use of TDZ can stimulate the growth of female Andalas plant explants to form shoots in vitro. Research by Swandra et al (2012) showed that shoot multiplication of Andalas (Morus macroura Miq. Var. macroura) using thidiazuron and different explant sources in vitro can produce shoots, both plants without colchicine induction and colchicine induction results. According to the results of Yunita's research (2004), it was shown that administration of thidiazuron on MS media resulted in higher melinjo shoot multiplication, both using in vitro explants and explants taken from the field. Warnita *et al.* (2021) also use MS media at potato culture.

Callus and shoot regeneration is largely determined by the balance of growth regulators Auxin and Cytokines, which will affect the growth, morphogenesis and regeneration of callus and shoots to form plant plantlets in vitro. The use of growth regulators is adjusted to the desired direction of plant tissue growth. Regeneration of callus and shoots to form plantlets in mangosteen plants at a combination of 0.50 ppm NAA + 1.75 ppm BAP was able to encourage shootlet and plantlet formation (Satria, Dwipa and Jamsari, 1999).

In order for the agarwood propagation technique in vitro to be used standardly to obtain quality, uniform, and uniform Agarwood plantlets in a relatively short time, and as a source of germplasm, the best composition of growth regulators must first be found. This study aims to obtain: 1. the best concentration of thidiazuron in encouraging explants to form shoots and 2. the best combination of concentrations of NAA and BAP to regenerate callus and shoots to form plantlets.

II. RESEACH METHODS

This experiment was carried out at the Tissue Culture Laboratory, Faculty of Agriculture, Andalas University, Padang, from May to October 2021. The materials used were: explants of agarwood axillary shoots of Aquilaria malacensis originating in the Mentawai district of West Sumatra, agar, sucrose, nutrients that make up the media MS, WPM and B5, vitamins, ZPT Thidiazuron, NAA, alcohol, spriritus, aquades, Benlate, insulating plastic, streptomycin, 10% bayclin, tween 80, NaOH, HCI. The tools used include: analytical balance, beaker, measuring cup, measuring flask, filter paper, electric heater, autoclave, oven, culture bottle, pH meter, spray bottle, suction pipette, tweezers, scalpel, scissors, petridis, laminar air flow cabinet, aluminum foil and others.

The experiment consisted of two stages, where the first stage was the shoot induction stage on MS media with Thidiazuron concentration treatment with 5 levels of treatment, namely: 0, 0.125 ,0.250 p, 0.375 ,0.50 and 0.625 ppm Thidiazuron. The second stage of the experiment was the shoot and callus regeneration stage, where the shoots and callus formed were regenerated on WPM media with a combination treatment of NAA + BAP concentration, with 7 levels of treatment, namely: NAA 0.0 ppm + BAP 0.0 ppm, NAA 0.0 ppm + BAP 1.0 ppm NAA 0.0 ppm + BAP 2.0 ppm, NAA 0.0 ppm + BAP 3.0 ppm, NAA 0,5 ppm + BAP 1,0 ppm, NAA 0,5 ppm + BAP 2,0 ppm and NAA 0,50 ppm + BAP 3,0 ppm. The experiment was arranged based on a Completely Randomized Design (CRD) with 3 replications, so that in each experimental stage there were 7 x 3 = 21 experimental units. Each experimental unit consisted of 10 culture bottles, so that 210 culture bottles were obtained at each stage of the experiment. The data were analyzed by using the F test and followed by the Least Significant Difference test (LSD) 5 %.

Prior to making stock solutions and culture media, sterilization of the equipment that will be used as a medium and culture bottles was carried out to grow the explants. The culture bottles were washed with detergent and rinsed thoroughly, then sterilized in an autoclave with a pressure of 15 psi at 121 ºC for 60 minutes. The sterilized bottles were stored in an oven at 80 ºC and the bottles were used as a medium. Plants such as petridish, scalpel, tweezers, scissors are also sterilized in an autoclave. Before putting these tools into the autoclave, they were first wrapped in parchment paper. Meanwhile, sterilizing the air flow cabinet is carried out with 70% alcohol and irradiating an ultra violet lamp for 30 minutes.

Preparation of media in stages 1 and 2 of the experiment: the nutritional ingredients were weighed (the nutritional composition of the MS (shoot induction) and WPM (shoot regeneration) then stock solution was made. After that the stock solutions were grouped into 6 groups (A, B.C, D, E, and F) and group I for vitamins (Myo inositol, Nyacin, Pyridoxin HO, and Thiamin HCI). Each nutrient group was placed in a container, namely a measuring flask with a size of 1000 ml, while the vitamins group was made into a separate container, namely a 100 ml volumetric flask. After the stock solution was prepared, it was put in each of the volumetric flasks, and stored in the refrigerator before use.

Preparation of culture media (according to treatment) is carried out by diluting the nutrient and vitamin stock solution according to the provisions. After the nutrient and vitamin solutions were well mixed, activated charcoal was given according to the dose, without PGR according to each treatment per liter of media. The volume of this mixed solution is made up to one liter by adding sterile distilled water. Furthermore, the pH was determined to be 5.8 by adding a solution of NaOH or HCL. Each mixture of media that had been made was labeled according to the treatment for both shoot induction and callus and shoot regeneration.

Furthermore, each treatment was heated with an electric heater while stirring continuously. Prior to reaching the boiling point, 7.0 grams of agar per liter of media was added in the third stage of the second series of experiments. After the solution became clear, the heating was stopped and immediately put into culture bottles as much as 15 ml per bottle with micro pinchers. Furthermore, the media in the culture bottle was sterilized in an autoclave for 20 minutes at a pressure of 15 psi with a temperature of 121 ºC. After sterilization, the vial containing the media was incubated for 1 (one) week in the transfer room before being used for explants.

The purpose of incubation is to determine whether the media in culture is completely sterile or uncontaminated. The media used for the third series of experiments in the second series (the stage of searching for sterile substances), the third series (the stage of finding the origin of explants), and the fourth series (the stage of finding the concentration of growth regulators) that were optimal were derived from the optimal culture media obtained from the first series of experiments. The procedure for making media for the second, third, and fourth series of experiments was the same as the first series of experiments.

The explants used in this experiment were shoots of Aquilaria macensis L.). The shoot explants were taken from the mother tree in the field, by cutting the shoots by 5-10 cm, then put into a 1 liter aqua bottle containing 0.05 grams of ascorbic acid solution and the bottle was closed. Furthermore, for the sterilization of explants, both explants measuring 0.50 cm were sterilized in a solution of: 0.05 grams of ascorbic acid per liter of aqua for 15 minutes; tween 80 2 drops per 300 ml of aqua for 1 minute; 70% alcohol for 1 minute; bayclin 20% for 5 minutes; and after each sterilization in the solution, the explants were rinsed three times with sterile distilled water, and all sterilization steps were carried out in a Laminar Air Flow Cabinet, but for the second series experiment the explants were also sterilized in various anti-fungal and bacterial solutions (according to the treatment). In the first stage of the experiment, the sterilized explants were immediately planted with 0.50 cm size explants in each culture bottle filled with 15 ml of MS media according to the treatment (Thiaduazuron concentration), then the weight of the culture was covered with plastic aluminum foil, and all the culture is done in Laminar Air Flow Cabinet. After that, the bottles containing the explants were stored in the culture room (incubation) at a temperature of 22 0C and the light intensity was regulated for approximately 12 hours. Furthermore, for the second stage of the experiment, explants in the form of callus and shoots formed in the first stage of the experiment were sub cultured (regenerated) on WPM media enriched with a combination of ZPT concentrations of NAA and BAP (according to treatment).

Culture space is always considered which includes; temperature of 22 0C, lighting of 40-watt TL lamp and humidity to prevent condensation in the culture bottle. If any planting material is contaminated by microorganisms (fungi and bacteria), it is immediately separated and removed from the culture room. The culture room was sprayed daily with 70% alcohol and once a month sterilized with formalin.

The variables observed in the shoot induction stage experiment, starting 1 (one) week to 10 weeks after explant culture, which include: Percentage of surviving explants, When explants form callus, Percentage of explants that form callus, Callus structure, Callus color, When callus explants form shootlets, Percentage of callus explants that form shootlets. The number of callus explants forming shoots.

III. RESULTS AND DISCUSSION

3.1. Percentage of live explants, when callus was formed and Percentage Explant forming callus

The concentration of Thiduazuron gave a significantly different effect on the percentage of live explants, when explants formed callus and Percentage Explant forming callus (Tabel 1).

Table 1. Percentage of live explants, when explant formed callus and Percentage Explant forming callus of Agarwood Producing Plant at the Concentration of Thidiazuron

Thiduazirone concentration (ppm)	Percentage callus %	time of callus formation (day)	Percentage Explant forming callus(%)
0	60,00c	25,00 c	20 d
0,125	80,00b	23,33 c	30 cd
0,250	100,00a	15,00 d	70 a
0,375	86,67 ab	30,00 b	50 b
0,500	80,00 b	32,00 b	40 bc
0,625	80,00 b	36,00 a	40 bc
KK(%)=	10,48	5,11	21,91

Note: The numbers in the same column, followed by the same lowercase letters according to the LSD test are significantly different at the 5% level.

Table 1 shows that the highest percentage of live explants was found at 100% and when explants formed the fastest callus were found in leaf petiole explants cultured on MS media enriched with 0.25 ppm Thidauzuron significantly different from other treatments (Figure 1). Response to changes in leaf petiole explants after being cultured on MS media it can be said to be quite fast. Initially, the explants changed from yellowish white to brown on the cut site and greenish on the uninjured area. On observation 1 week after culture, the explants swelled and the tips of the explants cracked, and 1 week later callus was formed. According to the research of Priyono et al. (2000), explants can form callus in a few weeks after sowing. Callus formation is caused by wound stimulation (Fowler, 1983). This stimulus causes the balance in the cell wall to change direction, some of the protoplasts flow outward so that callus begins to form.

For callus formation, depending on the type of explant used, the composition of the culture media, and the content of endogenous and exogenous auxin hormones, high auxin levels should be used (Suryowinoto, 1985 in Ambarwati, 1987). According to Priyono et al., (2000); Elliot (1982) and Widiastoety (1985) in tissue culture of ovule explants, where explants are able to regenerate without additional auxin from the outside, it is suspected that bananas contain sufficient endogenous auxin to mobilize cells to form new individuals.

The results of variance on the percentage of agarwood-producing explants formed callus due to the treatment of various culture media and types of plant explants Aquilaria malacensis L can be seen in Appendix 8c. The combination of explant types and culture media had a significantly different effect on the percentage of explants forming callus, after proceeding with the DMNRT test at a level of 5% which is presented in Table 1, and for more details on callus growth can be seen in Figure 1.

Table 1 shows that the highest percentage of explants forming callus was found in leaf petiole explants cultured on MS media enriched with Thiduazuron concentration and significantly different from petiole explants cultured on MS media, on leaf petiole explants. Leaf petiole explants cultured on MS media resulted in the highest percentage of explants forming callus, which was 50.00%. This shows that there is a strong growth response starting from day 2 of the leaf petiole in absorbing the nutrients present in the MS media and endogenous hormones found in the leaf petiole explants so as to stimulate tissue development which is characterized by elongation of the explants and callus begins to appear on average. on day 14. Callus formation started from the injured explant rim and then covered the explant surface. In cells damaged by injury, autolysis occurs, and from these damaged cells compounds are produced that stimulate cell division in the next layer to form callus.

In addition, the high callus formation in this type of explant is due to the fact that the petiole of the leaves has a lot of transport tissue that functions as a transport route for photosynthate so that it contains many nutrients and endogenous hormones. The cell wall against the protoplasm is reduced, this causes the protoplast to absorb water around the cell, so that the cell becomes long, especially the cells in the meristem. Besides, Auxin can also encourage the formation of a number of cells that are quite large but do not divide, this collection of cells is called a callus. Callus is formed due to the accumulation of cells that expand as a result of the entry of water, nutrients from the culture media and PGR into the cells, all of these materials cannot be spread throughout the plant body such as roots, stems and leaves, so they gather in one point. In accordance with the opinion of Wareing and Philips (1981); Wattimena (1988); Hendaryono and Wijayani, 1994 and; Suryowinoto, (1996) and Gunawan (1988) that only endogenous hormones present in explant tissue will affect plant physiological and morphological processes. Besides, according to George and Sherrington (1984) that MS media is the type of media most widely used in tissue culture where the specialty of MS media is the high content of nitrate, potassium and ammonium (Kyte, 1990).

The low percentage of callus formation was due to disruption of the balance of endogenous hormones above the optimum limit, so that the cell proliferation process eventually became disrupted and consequently the number of explants that formed callus decreased. In general, the low percentage of callus formation was also due to the large effect of phenol released by all types of explants. From the research that has been done, it was found that the ability of agarwood shoot explants to form callus was lower than that of petiole leaf explants.

According to Gunawan (1988) the callus formation ability of the tissue depends, among others; physiological age and tissue when isolated, plant parts used as a source of explants and plant species. Callus formation occurs when the ratio between the concentrations of Auxin and Cytokines is in a balanced state, both endogenous and exogenous (Rao, Sin, Kothagoda, and Hutchinson, 1981; and Widiastoety, 1985)., Wattimena, and Gunawan (1991) that the ability of explants to form callus and its growth rate may differ between parts of the explant tissue. This also happened because the different explants formed callus, caused by the difference in the toti potency of the explants. This is in accordance with the experimental results of Masyudi (1993) where there are differences in the ability of explants to form callus and callus regeneration power between parts of the explant tissue.

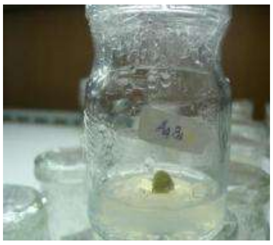

Fig.1 : . Placement of culture bottles containing explants in incubation room

3.2. Callus Structure and Callus Color

Observation of callus structure and callus color was done visually and using tweezers. The callus structure produced at various concentrations of Thiduazuron in Agarwood-producing plants is shown in Table 2. From Table 2 it can be seen that the responses of various types of explants cultured on various culture media showed differences in callus structure and callus color of Agarwood-producing plants. Generally, the callus structure formed in this experiment is compact and has a different color.

Based on these various results, the callus structure obtained in this experiment was influenced by the genotype of the explants used and the composition of the culture media, the endogenous regulators used and environmental conditions. Wattimena (1988) stated that the formation of callus or organs in in vitro culture is more influenced by genotype, initiation of culture, growing environment and tissue physiology used. The shape, texture, color and morphogenetic ability as well as cell differentiation depend on the age and purity of the tissue used as explants. The differences that occur will be greater if the explants are composed of more than one cell type (George and Sherrington, 1984).

Table 2. Callus structure and color callus of Agarwood Producing Plant at the Concentration of Thidiazuron.

Concentration Thiduaziron (ppm)	Callus structure	Color callus
0,00	Compact	White
0,125	Compact	Whitish Yellow
0,250	Compact	Yellowish White
0,375	Compact	White
0,500	Compact	Yellowish White
0,625	Compact	Yellowish White

Callus from various species can differ in texture, viability and color and callus formation is characterized by changes in the explant texture to become rough and the surface shiny when reflected by light (Wetherell, 1982). Based on the experimental results above, it is shown that generally the callus structure is compact, so this type of callus is suitable for organogenesis. Triatminingsih, Karsinah and Wahyuni (2000) stated that the shape and color of the callus will determine the direction of further morphogenesis. The crumb callus is suitable for embryogenesis while the compact callus is suitable for organogenesis.

Meanwhile, according to Darmawati (2002) reported that the callus type with a callus structure is easier to produce single cells in liquid media that is always shaken and will divide more quickly, compared to the type of callus with a compact or solid structure, as well as the opinion of Thomas and Davey (1975); Widiastoety (!987) that crumb callus is also called embryonic callus, where it is able to differentiate to form plants.

The compact callus structure obtained in this experiment showed that the opportunity for callus to be developed and grown further into direct plantlets was greater. This can be an added value for plant breeding in an effort to overcome the scarcity of Agarwood-producing plants in vitro. From Table 1 it can be seen that the response of various explants to form callus (%) to the concentration of Thiduazuron showed differences in callus structure and callus color of Agarwood-producing plants (Ficture 2). Generally, the callus structure formed in this experiment is in the form of crumbs and compact and has a different color.

Based on the various results, the callus structure obtained in this experiment was influenced by the genotype of the explants used and the culture media used. Wattimena et al (1992); Wetherell, (1982) stated that the formation of callus or organs in in vitro culture is more influenced by genotype, initiation of culture, growing environment and tissue physiology used. The shape, texture, color and morphogenetic ability as well as cell differentiation depend on the age and purity of the tissue used as explants. The differences that occur will be greater if the explants are composed of more than one cell type (George and Sherrington, 1984).

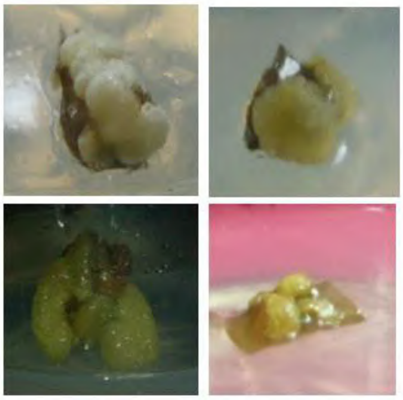

Fig.2: The forms of explants into plant callus agarwood producer

Triatminingsih *et al*, (2000) stated that the shape and color of the callus will determine the direction of further morphogenesis. The crumb callus is suitable for use for embryogenesis, while the compact callus is for organogenesis. The compact callus structure obtained in this study illustrates that the opportunity for callus to be developed and further grown into plantlets directly is greater. This can be an added value for plant breeding in an effort to overcome the scarcity of Agarwood-producing plants in vitro. Satria *et al (2017)* state that Discoloration occur can be caused by the pigmentation of the chlorophyll that undergoes degradation. The more the presence of chlorophyll, the greener the callus color. Wahyuni *et al. (2020)* report that colored callus yellow and compact struture has a chance used for organogenesis.

3.3. When callus form shootlet, Callus Percentage form Shootlet and Number of Callus form shootlet

The results of the variance on the time of shootlet formation and the percentage of callus regenerating to form shootlets (Figure 3), after the callus formed in the previous experiment was subcultured in the treatment of various combinations of NAA and BAP concentrations which gave significantly different effects in Appendix 1e and after being continued with the LSD test on level of 5% presented in the table. Table 5 shows that when callus formed shootlets the fastest was 11.67 days and the percentage of callus regenerated formed shootlets at a combined concentration of 050 ppm NAA + 3.0 ppm BAP. because various combinations of concentrations of 0.50 ppm NAA + 3.00 ppm BAP were able to encourage the fastest and highest growth and development of explants so that callus explants had the ability to live and have the ability to regenerate shootlets. Moore (1979); George and Sherrington (1984) reported that administration of growth regulators Auxins and Cytokines at low concentrations was able to stimulate the growth and development of explants and maintain the viability of explant tissues, but at high concentrations growth regulators could inhibit the development of morphogenesis explants.

Balance The concentration of Auxin and Cytokines growth regulators in explant tissue can increase the survival, growth and development of explant tissue (Satria, Dwipa, and Jamsari, 1999). Satria, Ferita, Dwipa and Jamsari, 1999b and Pierik, 1987 stated that due to endogenous PGR or exogenous Cytokines are able to stimulate cytokinesis, there is an increase in the number of cells. Cytokinesis is the process of cell division, in which cells that have absorbed more water, there is an addition of plasma and followed by these cells growing lengthwise, then the cells undergo differentiation which causes these cells to specialize in function.

Table 4. When Callus and Percentage of Explants Form Shootlet Agarwood-Producing Plants at Thidiazuron Concentration in Vitro at the age of 10 MST

Concentration NAA + BAP (ppm)	when explant callus form shootlet	percentage of callus forming shootlet	number of callus forming shootlet
0,0 + 0,0	33,00 a	10,00 c	1,0 c
0,0 + 1,0	29,67 ab	13,33 c	1,3 c
0,0 + 2,0	25,67 bc	16,33 c	1,6 c

0,0 + 3,0	22,67 c	33,33 b	3,3 b
0,5 + 1,0	20,33 cd	40,00 ab	4,0 **b**
0,5 + 2,0	16,33 d	43,33 ab	4,3 ab
0,5 + 3,0	11,67 e	50,00 a	5,0 a
KK(%) =	8,94	14,78	14,78

Fig.3 : . Callus explants regenerate to form shootlets

Furthermore, endogenous growth regulators are low in auxin and high in cytokines contained in explants, but in a balanced state encourage the growth of explants and the development of explants to form shootlets. Satria, Hervani and Gustian (2005) reported that high stockinins function in stimulating shoot formation, affecting cell metabolism and stimulating cells. Wiendi, et al (1991); and Wattimena 1988 reported that the growth and morphogenesis of plants forming shootlets and plantlets in vitro was controlled by the balance of growth regulators Auxins and Cytokines in explant tissues.

The addition of the number of shoots is one of the parameters that can be measured quantitatively, and is an indicator of the success of a tissue culture. Shoot growth is not only influenced by cytokines hormones and available nutrients, but each plant also has endogenous hormones that will affect shoot growth. The combination concentration of 0.50 ppm NAA + 3.00 ppm BAP, Cytokines was proven to be able to increase the number of shoots in agarwood-producing explants with an average value of 5.0 shoots (Figure 3).

From this research, it is known that the combination of auxin and cytokines growth regulators (NAA and BAP) has shown that with increasing concentrations of NAA and BAP in the media, the number of plant shoots tends to increase. This is in accordance with the statement of Bhojwani and Razdan (1983) that the higher the concentration of cytokines, the higher the number of shoots, but each of these shoots will be stunted. George and Sherrington (1984) also stated that BAP is best used to stimulate shoot formation. In contrast to research conducted by Rosdayanti (2007) research using a combination of cytokines (BAP and kinetin) at a level of 1 mg/L and 0.5 mg/L was able to produce 45 adventitious shoots in Aquilaria malaccensis. It is suspected that the combination of auxin and cytokines is effective in vertical multiplication (elongation) while the combination of cytokines is able to produce horizontal multiplication. Furthermore, according to Maulida (2005) stated that BAP stimulates shoot multiplication compared to kinetin. While kinetin has the effect of accelerating shoot induction. In addition, the suitability of the use of growth regulators is also a limiting factor for plant species (Wattimena 1992).

In the research conducted, the combination of growth regulators (NAA and BAP) on the number of shoots produced on average was only able to have 1 shoot in each explant (Figure 2). The small number of shoots formed was thought to be because the explants were less able to absorb the nutrients and hormones given to the media. In addition, during observations, it was seen that the explants that formed callus were getting bigger and then slowly covering all parts of the plantlet so that the plantlets did not experience the addition of new shoots at the nodes. The same thing was stated by Handayani (2003) that the addition of a growth regulator of NAA with a low dose followed by the administration of a high enough BAP could cause callus growth to be inhibited because the dose given was not balanced, as a result very little new callus was formed so that the shoots that grew relatively small or new callus did not produce a large number of shoots and did not even form buds at all until the end of the observation.

IV. CONCLUSIONS AND SUGGESTIONS

Based on the results of the research that has been carried out, it can be seen that the administration of growth regulator Thiaduazuron in the first stage of the study had an effect on the callus induction of Agarwood-producing plants (Aqulilaria malaccensis L.) in vitro. The callus formed in the first stage of the study was regenerated by giving a combination of NAA + BAP concentrations in the second stage of the study and showed an effect on callus regeneration to form shootlets.

Giving the concentration of ZPT Thiduazuron 0.250 ppm showed the highest percentage of live explant was 100%, when callus was formed the fastest (15 days) and the highest percentage of explants formed callus was 70%. Furthermore, the combination of 0.5 ppm NAA + 3.0 ppm BAP ZPT concentration showed that when callus explants formed the fastest shoots at 11.67 days, the highest percentage of callus explants formed shoots was 50% and the number of callus explants formed the most shoots was 5.0 shoots.

Based on the research activities that have been carried out, it is recommended to use explants with a larger size, and to update the sterilization technique used so that the percentage of explants that form callus can be greater. For the regeneration of callus explants, shoots should be carried out 14 days after the callus appears so that callus freshness is maintained.

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REFERENCES

[1] Zulkarnain. 2009. *Kultur Jaringan Tanaman : Solusi Perbanyakan Tanaman Budidaya*. Jakarta : Bumi Aksara. 185 hlm.

[2] Bhojwani SS, MK Razdan. 1983.*Plant TissueCulture: Theory andPractice*. Amsterdam: Elsevier Scientific Publishers.

[3] Dodds, J.H. and L.R. Roberts. 1982. *Experiments in Plants Tissue Culture*.Cambridge University Press. Cambridge.

[4] Fowler, M. W. 1983. *Commercial Application And Economic Aspects of Mass Plant Cell Culture. In : Plant Biotechnology*. Mantell Smith, H. London: Cambridge University Press.

[5] George, E.F. and P.D. Sherrington. 1984. Plant propagation by tissue culture.Handbook and directory of comercial laboratory. Exegeticts. Ltd. England. 709 p.

[6] Gunawan LW. 1995. Teknik Kultur In Vitro dalam Hortikultura. PT Penebar Swadaya. Jakarta.

[7] Gunawan, L.W. 1992. *Teknik Kultur Jaringan Tanaman*. Departemen Pendidikan dan Kebudayaan. Direktorat Jenderal Pendidikan Tinggi Pusat Antar Universitas Bioteknologi. Institut Pertanian Bogor.

[8] Handayani, 2003. Pengaruh Pemberian NAA dan BAP Terhadap Pertumbuhan Tunas *Eucalyptus urophylla* S.T. Blake dengan Sistem Kultur Jaringan, Skripsi Fakultas Pertanian Universitas Tanjungpura, Pontianak

[9] Harahap, F. 2012. *Fisiologi Tumbuhan: Suatu Pengantar*. Unimed Press. Medan.

[10] Hendaryono, Daisy. P.S dan A. Wijayani. 2004. *Kultur Jaringan : Pengenalan dan Petunjuk Perbanyakan Tanaman Secara Modern*. Karnisius. Yogyakarta

[11] Kosmiatin, M. A, Husni, dan I, Mariska. 2005. Perkecambahan dan Perbanyakan Gaharu secara *In Vitro*. Balai Besar Penelitian dan Pengembangan Bioteknologi dan Sumberdaya Genetik Pertanian, Bogor .

[12] Lestari, E. G. 2011. *Peranan Zat Pengatur Tumbuh dalam Perbanyakan Tanaman Melalui Kultur Jaringan.* Jurnal AgroBiogen. 7:63-68.

[13] Lizawati. 2012. Proliferasi Kalus Embriogenesis Somatik Jarak Pagar (*Jatropha Curcas* L.) Dengan Berbagai Kombinasi ZPT dan Asam Amino. 1(4): 65-72.

[14] Murashige, T. & Skoog, F., 1962. A Revised medium for rapid growth and bioassays with Tabaco tissue cultures. *Physiol Plant*, Volume 15, pp.473-497.

[15] Pierik RLM. 1987. *In Vitro Culture of Higher Plants*. Netherlands: Martinus Nijhoff Publishers.

[16] Priyono, D. Suhandi, dan Matsaleh. 2000. Pengaruh Zat Pengatur Tumbuh IAA dan 2-IP pada Kultur Jaringan Bakal Buah Pisang. *Jurnal Hortikultura*. 10 (3): 183 – 190.

[17] Saikia, M., K. Shrivastava and S.S. Singh. 2013. Effect of Culture Media and Growth Hormones on Callus Induction in *Aquilaria malaccensis* Lamk., a Medicinally and Comercially Important Tree Spedies of North East India. *Biological Science*, 6(2): 96-105.

[18] Santoso, U. Dan Nursadi, F. 2002. *Kultur Jaringan Tanaman*. Malang. UMM Press.

[19] Satria, B, D. Hervani,dan Gustian.2005. *Perbanyakan Vegetatif Tanaman Gaharu Pada Media WPM yang Diperkaya Dengan 2,4-D Secara In Vitro*. Laporan Penelitian dana SP4 jurusan BDP faperta Unand.24 hal.

[20] Satria, Dwipa dan Jamsari 1999. Regenerasi Kalus Tanaman Manggis (Garcinia mangostana L.) secara Kultur In Vitro. Jurnal Stigma. Fakultas Pertanian Unand, Padang. 41 hal

[21] Satria. B, Gustian, E.Swasti dan M.Kasim. 2008. Karakterisasi morfologi dan molekuler tanaman penghasil gaharu endemic Sumatera. Saintek (jurnal Akreditasi) FMIPA Biologi UNP. Padang. 45 hal.

[22] Satria, B., Gustian, S. Raesi, Nurbailis, M. Kasim. 2018. Pengaruh Pemberian Beberapa Konsentrasi 2,4-D pada Pembentukan Kalus Tanaman Gaharu Aquilaria malacensis Lamk) secara *In Vitro.* Prosiding Semnar Nasional Pembangunan Pertanian Indonesia 16 - 17 N0vember 2018. Fakultas Pertanian UPN Veteran Yogyakarta. Hal 1113 - 1122.

[23] Wattimena, G.A.. 1991. *Zat Pengatur Tumbuh Tanaman*. PAU IPB. Bogor. 145 hal.

[24] Wetherell, D. F. 1982. Pengantar *Propagasi Tanaman secara In Vitro* (diterjemahkan oleh Koensoemardiayah). IKIP Semarang Press : Semarang.

[25] Untung, S.dan F. Nursandi. 2001. *Kultur Jaringan Tanaman.* Universitas Muhammadiyah Malang. Malang.

[26] Wahyuni, A., B. Satria, A. Zainal. 2020. Induksi kalus Gaharu dengan NAA dan BAP secara *In Vitro*. Agrosains : Jurnal Penelitian Agronomi 22(1): 39-44,

[27] Warnita, W., R. Mayerni, N. Kristina, N. E. Nadila. 2021. Induction of Potatoes (*Solanum tuberosum* L.) Micro Tuber at Some Coumarin Consentratins and Incubation Temperatures. Plant Cell Biotechnology and Molecular Biology 22(33&34):1-12

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The use of Coastal Land for the Cultivation of Vegetable and Species on Small Islands in the Sub-District of Tatoareng Sangihe Regency

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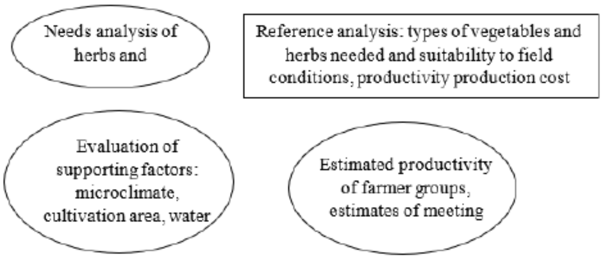
***Abstract*— *Communities on the small island of Tatoareng sub-district, Sangihe Regency, are faced with the problem of limited land ownership for vegetable cultivation, and low access to vegetable food. Vegetables purchased by the public have gone through four to five buying and selling processes starting from the production center, resulting of expensive vegetable prices. This chain of problems continues and has not yet found a way to solve it. Our team designed hydroponic vegetable and spice (herbs) cultivation on a small island in the District of Tatoareng. The initial stages of the study are analyzing the prospects for the cultivation of vegetables and spices that are the needs of the community, analyzing their suitability with ecological conditions especially the microclimate, and analyzing the economic benefits in this case income or reduction in expenditure versus production costs. Our team has conducted research on daily changes in the microclimate of residential, plantation and forest areas, as a reference for the choice of growing vegetables and herbs (spice). Our team has also conducted research on the economic condition of the family, on livelihoods, and average monthly family income. Initial research results indicate a monthly family income range between Rp. 1.000.000 to Rp. 2.000.000. Preliminary observations also indicate that each house has a yard measuring at least 4m x 8m which allows for the hydroponic cultivation of vegetables and herbs. This article describes the results of a prospective economic-ecological analysis of the cultivation of vegetables and herbs for low-income families. The results of the analysis present several choices of types of vegetables and herbs, prospects for economic benefits and their compatibility with microclimate conditions. Choice of vegetables and herbs that can be cultivated, has been confirmed to the public. The categories of choice of vegetables and herbs are: first priority is: tomatoes, second priority is mustard pakcoy, third priority is chilli. The results of this preliminary study became a reference for the follow up of the research on hydroponic vegetable and spice cultivation*

***Keywords*— Coastal Land, Cultivation, Vegetable, Small Islands**

I. INTRODUCTION

The area of Tatoareng Subdistrict, Sangihe Regency, consists of small islands that are close to each other, which can be reached by using a motorized boat belonging to the community in 20 – 40 minutes. People in this sub-district are faced with the problem of expensive vegetables and spices because they have to be bought from Tahuna. The main economic activity of the community is fishing, a small number of housewives make salted fish. Some people work as civil servants (teachers, local government employees, health/nursing personnel, TNI and Polri). This population condition indicates a high level of need for consumption of spices and vegetables. The free time that housewives have is potential to be directed to grow spices and vegetables.

The specific purpose of this study was to analyze the land conditions (soil, microclimate, availability of ground/surface water) for the cultivation of spices and vegetables designed individually (KK) or in groups based on land ownership and community agreement. The results of interviews with the community in 2017 revealed the desires and needs of the community for vegetable and spice cultivation but were constrained by several things: land ownership, procurement of seeds, cultivation and fertilization methods, long droughts, the influence of large waves that caused damage to crops in coastal areas. This research is a solution to the problem of food shortages and community empowerment innovations (especially housewives) in utilizing their spare time to cultivate spices and vegetables both for household needs and as a livelihood.



Analysis Model of Spice And Vegetable Cultivation To Fulfill Common Needs Tatoareng District is located in the southernmost position of the Sangihe District. Tatoareng District consists of nine small islands, six of which are permanent residents, while three islands are only a place for fishermen to rest. The total land area of Tatoareng District is 18.56 km2, with a population (in 2012): 4291 people, and a population density of 231 jima/km2 (Sangihe in figures, 2012). The settlement pattern is generally concentrated on the coast, so that the population density in the coastal area is relatively high. The area of land for agriculture is relatively small which is used for food crops (food ingredients for local people) such as cassava, sweet potatoes, vegetables, spices and fruits, and coconut plants. The main livelihood of the community is fishing (traditional), with low income due to limited marketing of catch products.

The small size of the catchment area, and the lack of vegetation cover raises the problem of water availability when there is a long dry season. Shah and Dulal (2015) [1], suggest that the strategy of the community in small islands in facing the problem of food availability, is significantly reduced due to climate change. Systemic analysis of resource management includes biophysical, socio-cultural and economic components as a whole and is a characteristic of small islands [2]. Small island agriculture requires a special approach to improve soil quality, such as organic content, aggregate stability, capacity to store groundwater, increase the ability of soil to bind carbon and minimize groundwater pollution [3].

The socio-ecological system approach should be a reference for natural resource management in small islands [4]. This system framework includes elements such as: existing cultivation practices, pressures from other sectors, such as tourism, land use habits, groundwater insecurity [3]. Leunufna and Evans (2014) [5] suggest that it is necessary to study sociological and technological aspects in the choice of utilization

land for the cultivation of spices and vegetables. To improve and strengthen the process of adaptation and integration of local knowledge with external knowledge, the community can form a forum for shared and collaborative use [6]. The process of adaptation and integration of knowledge has developed well through a participatory approach in terms of (1) planning processes [7], (2) geographic information systems [8], (3) scenario planning [9]

Based on the study of the characteristics of small islands: prone to climate change, problems of limited land related to food security, socio-economic (cost of living, local wisdom; plant cultivation habits, existing alternative economic activities, etc.), in the process of acceptance and adaptation, land use research Coastal areas for vegetable and spice cultivation are focused on:

1. Mapping of cultivation areas according to land characteristics (soil, availability of groundwater and surface runoff, microclimate), referring to the RTRW of Sangihe Regency as a development policy framework

2. Research on the physical condition of the land (microclimate, air temperature and humidity, irradiation and cycles/rainy and dry seasons).

3. Identification of community-owned cultivation patterns, strategies and mechanisms for integrating vegetable and spice cultivation technology.

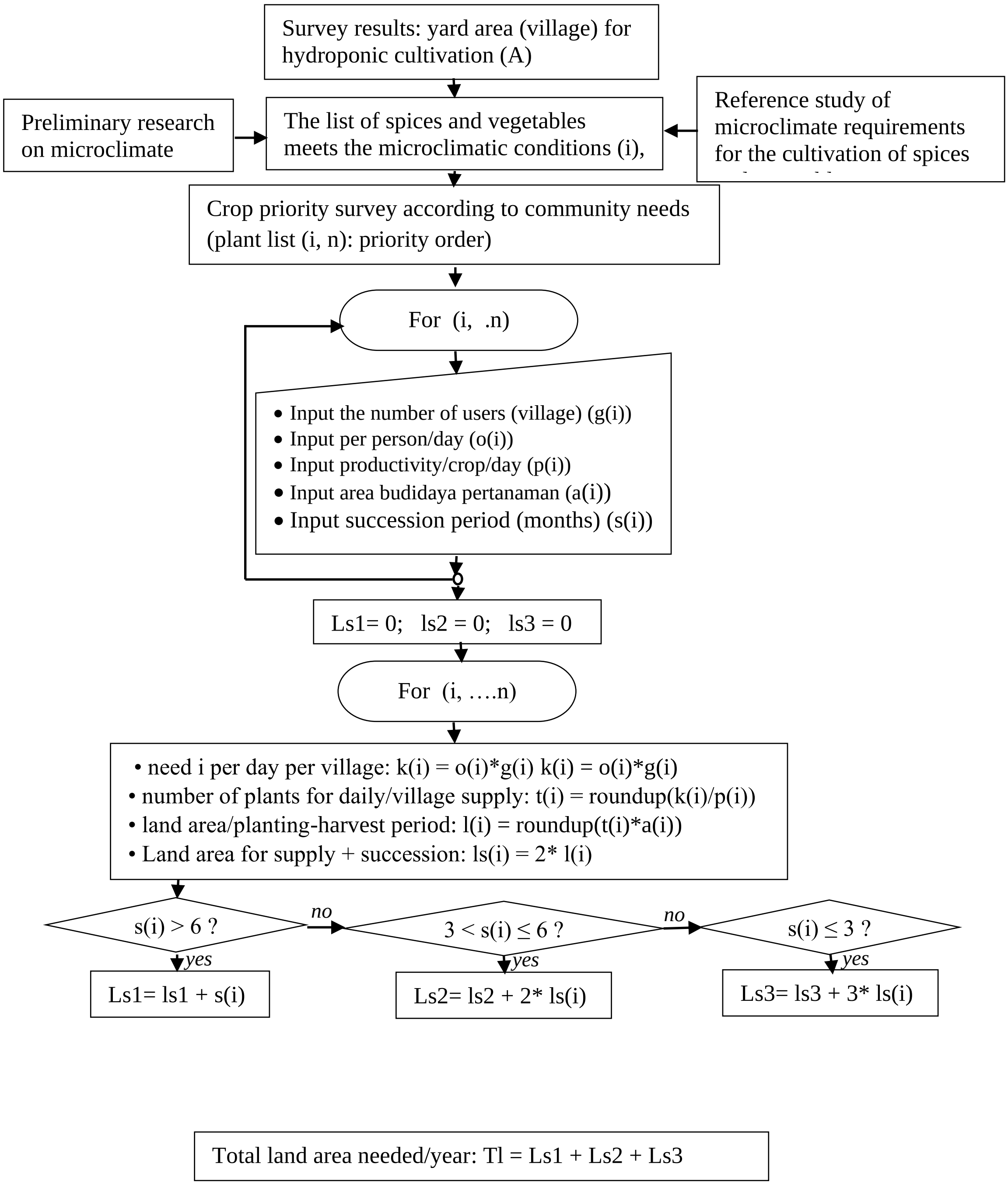
4. Socio-economic analysis of the community with inputs for land use and vegetable and spice cultivation activities.

5. The principles of land management and cultivation that ensure the sustainability of land use, availability of ground water.

6. Encouraging the role of the community in research (participatory model) so that the community directly carries out the technology adoption process and the integration of local customs/wisdom. This strategy is strengthened by coordination with local governments and organizations in the community.

II. RESEARCH METHODS

Flowchart of developing a spice and vegetable cultivation model to meet common needs on small islands.



III. RESEARCH RESULT

Prospect analysis of vegetable and spice cultivation

Types of spices/vegetables	Village needs /week (kg) (1)	Many plants needed (2)		Productive mass (3)	
		Total production /day (kg)	number of plants	harvest time	Month
Red onion	5 kg	0.71 Kg	3	1	3
Garlic	3.5 kg	0.5	2	1	4
Spring onion	3.25 kg	0.47	2	1	2,5
Beans	1.5 kg	0.16	2	1	1
Chilli	4.5 kg	0.64	3	1	3
Ginger	2 kg	0.29	1	1	4
Pumpkin	1.25 kg	0.18	1	1	2
Spinach	3 kg	0.43	5	1	1
Cabbage	4 kg	0.57	1	1	2
Potato	3 kg	0.43	2	1	3
Pakcoy	4.5 kg	0.64	4	1	1.5
Rggplant	1,7 kg	0.24	1	1	3
Tomatoes	18 kg	2.57	5	1	2
Carrot	2.5 kg	0.36	2	1	3

Information:

(1) results of survey/preliminary research

(2) a. determination of total production per day to meet needs (1) study of plant species productivity reference and analysis of data from demonstration gardens b. Number of plants needed: total production per week/plant productivity (productive mass) per week.

(3) study of plant productive mass reference, number of harvests and/or duration of production (months), and data analysis from the pilot farm

Most of the food needs for the islands in Tatoareng District are met from the surrounding islands or depend on other areas which can affect the prices of vegetables and spices. This condition causes vulnerability to food supply, due to very limited transportation infrastructure constraints and weather factors which greatly affect the smooth distribution of food ingredients. The table above shows various vegetables and spices that are the daily consumption needs of the surrounding community. In the district of Tatoareng itself, the community must import vegetables from outside the island such as cabbage,

potatoes, beans, pumpkins and eggplants to meet their daily consumption needs. Each type of imported vegetables that are needed the most per week are 4 kg of cabbage, 3 kg of potatoes and 2.5 kg of carrots.

In Tatoareng District, for local vegetables, only kale is available as well as spices such as chili, shallots, garlic, scallions and ginger, because the average household in the vicinity has their own garden. Even so, the existing production cannot meet the demands of the surrounding community. Spices and vegetables with the highest demand per week are tomatoes with a demand of 18 kg, onions 5 kg, and chili and pakcoy 4.5 kg

The characteristics of the islands in the Tatoareng sub-district, Sangihe Regency, have various soil conditions, microclimate, and availability of ground/surface water. Some of the land is not used optimally, only coconut is planted with grass cover plants. One of the efforts that can be done is to increase the ability to provide food independently by utilizing and optimizing local food sources according to the potential of each region. The results in the field show that the pH of the soil in the Tatoareng District ranges from 5.0 to 6.5 which can be categorized as normal to low. To increase the recommended vegetable and spice plants that can be cultivated.

IV. CONCLUSION

One of the efforts that can be done is to increase the ability to provide food independently by utilizing and optimizing local food sources in accordance with the potential of each region. The results of the analysis present several choices of types of vegetables and spices, prospects for economic benefits and their suitability for microclimate conditions. The choice of types of vegetables and herbs that can be cultivated has been confirmed to the community. The categories of choice or priority for vegetables and spices are: the first priority is tomatoes, the second priority is mustard pakcoy, the third priority is chili. Therefore, it is recommended that cultivating priority vegetables and remap can somewhat meet the needs of the community

REFERENCES

[1] Shah K, Dulal H (2015) Household capacity to adapt to climate change and implications for food security in Trinidad and Tobago. Reg Environ Change. doi:10.1007/s10113-015-0830- 1

[2] Mercer J., Dominey-Howes D., Kelman I., and Lloyd K. 2007. The potential for combining indigenous and western knowledge in reducing vulnerability to environmental hazards in small island developing states. Environmental Hazards. 7: 245–256

[3] Komnitsas K.A. and Doula M.K. 2017. Framework to improve sustainability of agriculture in small islands: The case of Pistacia vera L. cultivation in Aegina, Greece. Environmental Forensics . Volume 18, Issue 3. https://doi.org/10.1080/15275922.2017.1340370

[4] Glaser M, Glaeser B (2014) Towards a framework for cross-scale and multi-level analysis of coastal and marine social-ecological systems dynamics. Reg Environ Change. doi:10.1007/s10113-014-0637-5

[5] Leunufna S. and Evans M. 2014. Ensuring food security in the small islands of Maluku: A community genebank approach. Journal of Marine and Island Cultures.vol 3, 125–133

[6] Thornton, T. F., and Scheer A.M. 2012. Collaborative engagement of local and traditional knowledge and science in marine environments: a review. Ecology and Society 17(3): 8. http://dx.doi.org/10.5751/ES-04714-170308

[7] Daly, M.; Poutasi, N.; Nelson, F.; Kohlhase, J. 2010. Reducing the climate vulnerability of coastal communities in Samoa. J. Int. Dev., 22: 256–281.

[8] Corbett, J.; Rambaldi, G.; Kyem, P.; Weiner, D.; Olson, R.; Muchemi, J.; McCall, M.; Chambers, R. 2006. Mapping for Change: The Emergence of a New Practice. Participatory Learning and Action 54, 13–19

[9] Bohensky, E.L.; Butler, J.R.A.; Mitchell, D. 2011. Scenarios for knowledge integration: Exploring ecotourism futures in Milne Bay, Papua New Guinea. J. Marine Biology., doi:10.1155/ 2011/504651.

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An Assessment of Singapore Airlines Environmentally Sustainable Energy Management

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***Abstract*— *This study has examined how Singapore Airlines, a major global airline, manages its energy in an environmentally sustainable manner. The research used an in-depth qualitative longitudinal research design. The study period was from 2010/2011 to 2020/2021. Singapore Airlines four key energy sources are aircraft jet fuel, electricity, diesel, and petrol. The airline's passenger aircraft jet fuel consumption grew in line with its expansion of services and aircraft fleet. The airline's freighter aircraft fleet consumption exhibited a general downward trend. The annual electricity consumption displayed a general downward trend and benefited from the energy saving measures introduced by the airline over the study period. Diesel consumption grew in line with ground service equipment (GSE) energy requirements. The airline's annual petrol consumption fluctuated over the study period reflecting differing fuel requirements. Throughout the study period, Singapore Airlines implemented many energy efficiency measures. These measures include an aircraft weight reduction program, the installation of extensive light emitting diode (LED) lighting, the installation of more energy efficient plant and equipment, the installation of more energy efficient heat exchange system at its Silver Kris Lounge at Changi Airport, the upgrading of lifts with a more efficient model that had a Variable Voltage Variable Frequency (VVVF) motor, the installation of a large scale photovoltaic (PV) system, a computer system that optimizes the maximum zero fuel weight (MZFW) of its aircraft fleet, the use of lightweight catering items, the use of light weight aircraft containers, the use of sustainable aviation fuel, and the use of fixed electrical ground power and preconditioned air units at airports where its aircraft have night layovers or long transits.*

Keywords*— *Airlines, case study, energy, Singapore Airlines, sustainable airline energy management

I. INTRODUCTION

Airlines are one the most important actors in the global air transport industry value chain. The principal services provided by airlines are the transportation of passengers and air cargo. Other important activities undertaken by airlines include aircraft and ground service equipment (GSE) maintenance, aircraft ground handling and, in some instances, flight catering. Airlines are very energy intensive (Baxter et al., 2021). The largest energy requirement for an airline is typically jet fuel. The airline ground-based facilities and buildings also consume large amounts of electricity. In the case where an airline performs aircraft ground handling services then the ground service equipment (GSE) required for this function is also energy intensive. Ground service equipment (GSE) refers to vehicles and equipment that are used in the airport precinct to service whilst they are at the gate in between flights (Hazel et al., 2011).

In recent times, airlines have implemented a range of strategies and measures to sustainably manage their energy consumption. This sustainable energy management is playing a key role in mitigating airlines adverse impact on climate change and global warming (Baxter et al., 2021). To help reduce their energy jet fuel consumption, airlines are acquiring and deploying next generation, highly fuel-efficient aircraft such as the Airbus A350-900XWB and the Boeing 787 (Aircraft Commerce, 2016). The concerns related to climate change and energy supply have resulted in the production of more sustainable aviation fuels (Brooks et al., 2016). Accordingly, sustainable aviation fuels are increasingly being regarded as an ideal option for the airline industry to achieve large, near-term emissions reductions (Staples et al., 2014). Thus, as part of their sustainability policies, airlines are increasing their use of sustainable aviation fuels (Alam et al., 2021; Michaga et al., 2021; Rice et al., 2020).

In this study, Singapore Airlines, a major global full-service network airline, was selected as the case airline due to its long-term commitment to sustainable energy management. The sustainable management of its energy consumption is a key part of the airline's sustainability policy The objective of this paper is to analyze how Singapore Airlines manages its aircraft fuel and ground-based facility energy consumption. A second objective is to examine the role that renewable energy plays in the airline's energy policy. A final objective of the study is to examine the energy savings measures implemented by Singapore Airlines to mitigate its energy consumption. The study period was from 2010/2011 to 2020/2021.

The remainder of the paper is organized as follows: the literature review is presented in Section 2, and this sets the context for the Singapore Airlines case study. The research method used in the study is presented in Section 3. The case study is presented in Section 4. Section 5 presents the key findings of the study.

II. BACKGROUND

2.1 Airline Jet Fuel Consumption

As previously noted, the global airline industry is highly energy intensive (Baxter et al., 2021). Jet fuel accounts for the major share of an airline's energy consumption. There are various types of jet fuel used in the airline industry as well as for military aviation. During the 1960s, Jet-A fuel became the standard fuel used in the United States and by many commercial airlines (Brooks et al., 2016). This type of fuel was selected over the more highly flammable JP-4 for passenger safety reasons (Yildirim & Abanteriba, 2012). Jet A-I fuel is available globally, including in the United States (Brooks et al., 2016). Jet fuel typically represents the highest cost for an airline (Turner & Lim, 2015; Vasigh & Rowe, 2020).

2.2 Aircraft Fuel Efficiency

Increasing environmental concerns have drawn the significant attention of the air transport industry towards the requirement for judicious use of aviation fuel. Consequently, both economic and environmental sustainability concerns have led to dramatic progress in aviation fuel efficiency improvements over the past few decades (Singh et al., 2018).

In recent times, both airlines and the aircraft manufacturers have invested in new technologies and strategies to reduce aircraft fuel consumption and the concomitant aircraft emissions. Aircraft fuel has a close relationship with the emissions of carbon dioxide (CO_2) and other gases that result in climate change (Zou et al., 2016).

At a global level, the peak airline industry body – the International Air Transport Association (IATA) – have recognized the requirement to address the global challenge of climate change and has subsequently adopted a set of ambitious targets to mitigate carbon dioxide (CO_2) emissions from air transport. The association has targeted an average improvement in aircraft fuel efficiency of 1.5% per year from 2009 to 2020. IATA has implemented a multi-faceted approach: the four-pillar strategy to ensure that this objective is met (International Air Transport Association, 2021b). The strategy entails:

- Improved technology, including the deployment of sustainable low-carbon fuels.
- More efficient aircraft operations.
- Infrastructure improvements, including modernized air traffic management systems.
- A single global market-based measure, to fill the remaining emissions gap (International Air Transport Association, 2021b).

The term fuel efficiency for an airline refers to the consumption between the observed and least possible volume of fuel consumed in the production of a given level of output for the airline. Due to the complexity of airline operations, fuel efficiency is dependent upon a range of factors including aircraft size, market characteristics (short-haul versus long-haul services), service network structure (hub-and-spoke or point-to-point [P2P]), and so forth (Zou et al., 2016, p. 320). Fuel efficiency is also largely dependent upon aircraft fuel burn, the average aircraft speed, and other technical design factors. It is important to note that fuel efficiency can be controlled by an airline by the flying techniques that are employed, the distances flown, as well as other variables (Vasigh et al., 2012).

There are four methods available to assess airline fuel efficiencies. The method relevant to this study is ratio-based, which is the typically used metric in the airline industry, to determine airline fuel or environmental performance. When using this metric, fuel efficiency is measured as the ratio of fuel consumed to the output produced by the airline (Zou et al., 2016).

2.3 Airline Ground Service Equipment (GSE) Energy Consumption

To perform ground handling services of aircraft when they are being serviced on the ground in between flights, sophisticated technical equipment is required to perform the aircraft turnaround handling (Kazda & Caves, 2015; Roberts, 2018). The ground service equipment (GSE) used in servicing an aircraft includes push-back tugs, lower deck loaders, (main deck loaders for freighter aircraft), toilet and water truck, tugs (for towing cargo to and from the air cargo terminal and for towing baggage to and from the airport's baggage makeup area), aircraft container and pallet dollies, ground power unit, aircraft tail stand (for freighter aircraft), and aircraft bulk hold loaders. This ground service equipment is generally powered by diesel or petrol engines. Vehicles used by airlines are also often petrol-powered (Baxter et al., 2021).

It is important to note that during the aircraft ground handling function the ground service equipment (GSE) will have periods when their engine is in idle mode. Thus, to reduce fuel consumption, and hence, reduce harmful emissions, the idle rotation speed should be as low as possible (Mu & Tang, 2019).

2.4 Airline Property and Facilities Energy Consumption

To support their operations, airlines typically have extensive ground-based properties and facilities. These buildings include office buildings, aircraft and ground service equipment (GSE) maintenance facilities and hangars, air cargo terminals, and flight catering centres. Consequently, airlines require a reliable and highly efficient source of energy to power their airport and non-airport located buildings, facilities, and equipment. Electrical power is also required to run machinery, heating, ventilating, and air conditioning (HVAC) systems, building lighting, computers and so forth (Baxter et al., 2021). Aside from leasing airport terminal(s), airlines can potentially be one of several tenants in other airport-located multi-tenant buildings (Crider et al., 2011). Airports are very energy-intensive areas (Baxter et al., 2018; Ortega Alba & Manana, 2017; Sreenath et al., 2021). Thus, an airline's airport operations can be extremely energy intensive (Baxter et al., 2021).

As part of their sustainability measures, some airlines have installed photovoltaic (PV) solar systems. As will be noted in the case study, Singapore Airlines is one such airline that has installed a large photovoltaic (PV) solar system. The use of renewable energy resources has provided users with favorable environmental related advantages. Green energy produces no greenhouse gas (GHG) emissions from the combustion of fossil fuels. Consequently, this reduces some forms of harmful air pollution (International Renewable Energy Agency, 2021; United States Environmental Protection Agency, 2021). In addition, the use of green or renewable energy sources provides a firm or user with an important opportunity to optimize energy efficiency (Arman et al., 2013). Also, renewable energy sources normally have very little waste (Yerel Kandemir & Yayli, 2016).

III. RESEARCH METHODOLOGY

3.1 Research Method

This study used a qualitative longitudinal research design (Derrington, 2019; Hassett & Paavilainen-Mäntymäki, 2013; Neale, 2018). Qualitative longitudinal research aims to expand and develop theories (Derrington, 2019). The researcher's role when conducting case study research is to expand and generalize theories (analytical generalization). The researcher does not enumerate frequencies or makes any statistical generalizations (Rahim & Baksh, 2003).

3.2 Data Collection

The data used in the study was obtained from a range of documents, company materials available on the internet and records as sources of case evidence. Documents included the Singapore Airlines annual sustainability reports, and the airline's websites. An extensive search of the leading air transport journals and magazines was also conducted in the study.

The key words used in the database searches included "Singapore Airlines sustainability policy", "Singapore Airlines annual passenger aircraft fleet fuel consumption", "Singapore Airlines passenger aircraft fleet annual fuel productivity ratio", "Singapore Airlines annual freighter aircraft fleet fuel consumption", "Singapore Airlines Cargo freighter aircraft fleet annual fuel productivity ratio", "Singapore Airlines annual diesel consumption", "Singapore Airlines annual electricity consumption", "Singapore Airlines annual electricity intensity ratio", "Singapore Airlines annual petrol consumption", and "Singapore Airlines energy conservation measures".

This study used secondary data. The three principles of data collection as suggested by Yin (2018) were followed: the use of multiple sources of case evidence, creation of a database on the subject and the establishment of a chain of evidence.

3.3 Data Analysis

The data collected for the case study was examined using document analysis. Document analysis is quite commonly used in case studies. Document analysis focuses on the information and data from formal documents and a firm's records that are collected by a researcher(s) when conducting their case study (Andrew et al., 2011; Yin, 2018). Following the recommendations of Scott (2004, 2014) and Scott and Marshall (2009), the study's documents were examined according to four criteria: authenticity, credibility, representativeness and meaning.

The document analysis was undertaken in six distinct stages:

- Phase 1: The first phase involved planning the types and required documentation and their availability for the study.
- Phase 2: The data collection phase involved sourcing the documents and developing and implementing a scheme for the document management.
- Phase 3: The collected documents were examined to assess their authenticity, credibility and to identify any potential bias.
- Phase 4: The content of the collected documents was carefully examined, and the key themes and issues were identified.
- Phase 5: This phase involved the deliberation and refinement to identify any difficulties associated with the documents, reviewing sources, as well as exploring the documents content.
- Phase 6: In this phase the analysis of the data was completed (O'Leary, 2004, p. 179).

Following the guidance of Yin (2018), the study's documents were downloaded and stored in a case study database. All the documents gathered for the study were all written in English. Each document was carefully read, and key themes were coded and recorded in the case study research framework (Baxter, 2021).

IV. RESULTS

4.1 A Brief Overview of Singapore Airlines

The origins of Singapore Airlines date back to 28 January 1972. Singapore Airlines was established following the formation of Singapore as a Republic. Following Singapore's new republic status, Malaysia-Singapore Airlines (MSA) was divided into two individual airlines. These airlines subsequently became the national flag carriers of Singapore and Malaysia, respectively (Chant, 1997). Singapore Airlines began its commercial operations on 1 October 1972 (Green & Swanborough, 1975). Upon beginning its commercial operations, Singapore Airline served the same international destinations that had been previously served by Malaysia-Singapore Airlines (MSA). At this time, Singapore Airline operated a fleet of Boeing B707 and Boeing B737 aircraft (Chant, 1997).

A key milestone occurred on 2 April 1973, when Singapore Airlines began daily flights between Singapore and London. On 31 July 1973, the airline commenced a major expansion program following the delivery of its first wide body aircraft type, the four-turbofan powered Boeing B747-212B aircraft. The airline also commenced operating another wide body aircraft, the McDonnell-Douglas DC10-30. These aircraft were operated on the airline's medium-and-high density air routes (Chant, 1997). In the latter years of the 1970s, Singapore Airlines commenced services across the Pacific, terminating at San Francisco (Brimson, 1985).

On 20 December 1980, Singapore Airlines received its first Airbus A300B4-203 aircraft. Following its introduction into commercial service, this aircraft type complemented the Boeing B747-212B and McDonnell Douglas DC10-30 aircraft (Chant, 1997). Another key milestone occurred on the 15th of October 2007 when Singapore Airlines took delivery of its first Airbus A380 aircraft. Singapore Airlines was the first airline in the world to operate the Airbus A380 aircraft (Simons, 2014). The airline operated the first commercial Airbus A380 service from Singapore to Sydney and return in October 2007 (Simons, 2014; Sloan, 2019).

At the time of the present study, Singapore Airlines operated a modern passenger fleet of 113 aircraft and had outstanding orders for a further 87 aircraft. SIA Cargo operated a fleet of 7 Boeing B747-400 freighter aircraft, which had an average fleet age of 17 years and four months. The Singapore Airlines Group comprises the wholly owned subsidiaries SilkAir, Scoot Airways, and SIA Cargo (Singapore Airlines, 2021). On 25 July 2017, Scoot and Tigerair, the SIA Group low-cost carriers, were officially merged into a single entity that retained the Scoot brand (Gupta Kapoor, 2017; Ong, 2017; Singapore Airlines, 2019). Singapore Airlines became a member of the Star global passenger airline alliance in 2000 (Heracleous & Wirtz, 2012; Iatrou & Alamdari, 2005; Ramaswamy, 2002).

In February 2021, Singapore Airlines officially commenced the process of integrating its subsidiary SilkAir into its operations as part of merger plans between the two airlines (Centre for Aviation, 2021).

Figure 1 presents Singapore Airlines annual enplaned passengers and revenue passenger kilometres performed (RPKs) for the period covering the financial years 2010/2011 to 2020/2021. One passenger enplanement measures the embarkation of a revenue passenger, whether originating, stop-over, connecting or returning (Holloway,

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2016). One revenue passenger kilometre (RPK) is one passenger transported one kilometre (Belobaba, 2016; Gillen, 2017). Over the period FY2010/2011 to 2019/2020, Singapore Airlines enplaned passengers and RPKs showed quite consistent growth (Figure 1). Figure 1 shows, however, that there was a very steep decline in both the annual number of enplaned passengers and RPKs in 2020, which were both adversely impacted by the global Covid 19 pandemic. In 2020, the COVID-19 pandemic caused a decline in economic activity around the world. This decline in economic activity caused a major disruption in the air travel market supply and demand chain (Dube et al., 2021). As a result, the COVID 19 related restrictions had a very adverse impact on global airline passenger demand, and thus, on the total number of revenue passenger kilometres performed (RPKs) by the world's airlines in 2020 (International Air Transport Association, 2021a).

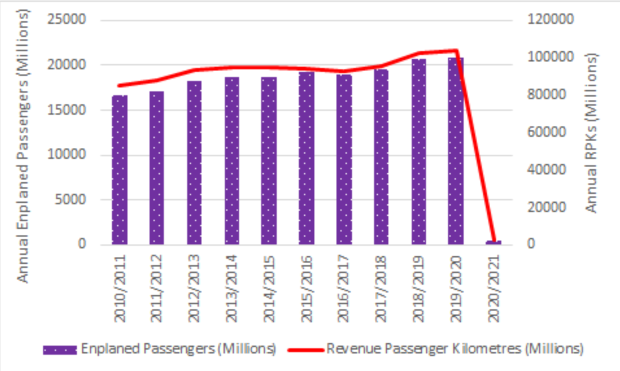

Fig.1: Singapore Airlines annual enplaned passengers and revenue passenger kilometres performed: 2010/11-2020/21. Note: Financial years from 1 April to 31 March. Source: Data derived from Singapore Airlines (2013, 2016, 2021).

4.2 Singapore Airlines Environmental Policy

Singapore Airlines has implemented a very comprehensive environment and sustainability related policy that is underpinned by four key pillars.

Pillar One: Improved technology: Singapore remains committed to its long-held policy of operating a modern and fuel-efficient fleet (Singapore Airlines, 2019). Singapore Airlines took delivery of its first Boeing 787-10 aircraft on March 25, 2018 (Field, 2018; Singapore Airlines, 2018). On September 22, 2018, Airbus delivered the first A350-900 Ultra Long Range (ULR) aircraft to Singapore Airlines (Airbus, 2018). As of 31 March 2021, Singapore Airlines operated a fleet of 113 aircraft, with an average age of five years and one month. In addition, the airline had 12 Airbus A350-900XWB, 31 Boeing 777-9s, 15 Boeing 787-10s, and 31 Boeing 737-8 MAX aircraft on firm order (Singapore Airlines, 2021). As part of its environmental policy, Singapore Airlines makes investments in engineering improvement packages for its aircraft airframes and engines. These improvements help to reduce drag and whilst at the same time improve engine efficiency, and thus, enhance fuel efficiency (Singapore Airlines, 2021).

Pillar Two: Operational measures: Importantly, as previously noted, Singapore Airlines maintains a modern and fuel-efficient fleet. As of 31 March 2021, the airline was operating 52 Airbus A350-900XWB aircraft. The Airbus A350-900XWB aircraft is acknowledged for its improved operating efficiency (Singapore Airlines, 2019). The Airbus A350-900XWB aircraft offers a 25% improvement in fuel efficiency and a 25% lower seat-mile (seat kilometre) cost when compared to its aluminum-based long-range competitors, such as the Boeing B777 aircraft (Otley, 2019). At the time of the present study, Singapore Airlines operated a fleet of fifteen Boeing 787-10 aircraft (Singapore Airlines, 2021). The Boeing 787-10 aircraft burns 20% less fuel (Boeing, 2021). A key focus of the airline's environmental policy is on improving aircraft fuel productivity through the implementation of "green" operations and by reducing fuel usage through highly efficient aircraft weight management together with the optimization of flight routes (Singapore Airlines, 2021).

Pillar Three: Improved infrastructure: Infrastructure improvements in the air transport industry present an opportunity for airlines to reduce both fuel use and carbon dioxide (CO_2) emissions. Such improvements can be achieved from the optimization of air routes through efficient air traffic control management. Singapore Airlines collaborates with Air Traffic Management stakeholders to investigate new ways to improve and enhance airspace incremental efficiency (Singapore Airlines, 2019, 2021).

Pillar Four: Global Market-Based Measure (MBM): Singapore Airlines recognizes that the International Civil Aviation Organization (ICAO) CORSIA scheme will play an essential role in achieving carbon neutral growth in a cost-efficient manner. As such, the company fully supports the ongoing efforts towards meeting the requirements in the ICAO CORSIA Monitoring, Reporting and Verification system of carbon emissions. As of 1 January 2019, Singapore Airlines commenced the monitoring and reporting of its carbon dioxide (CO_2) emissions on an annual basis. Furthermore, Singapore Airlines will voluntarily participate in the ICAO CORSIA program from 2021 to 2026, following which the scheme becomes mandatory (Singapore Airlines, 2019).

Singapore Airlines is dedicated to its long-term responsibility to protect the environment while delivering air transportation services that are of the highest quality. The airline has introduced a range of programs to enable the company to implement sustainable practices across its operations in a responsible manner. The airline actively manages issues such as carbon dioxide (CO_2) emissions, noise, waste, as well as energy and water consumption. Singapore Airlines continues to explore new sustainable practices across all areas of its operations. The company has adopted the International Air Transport Association (IATA) four-pillar strategy to address climate change, and, as a result, it seeks opportunities to reduce the carbon footprint of its operations. The airline also promotes eco-friendly habits among its employees and stakeholders. In addition, Singapore Airlines raises the awareness on the importance of taking action to reduce its impact on the environment (Singapore Airlines, 2020). Singapore Airlines became a signatory to the Ten Principles of the United Nations Global Compact in 2018. Singapore Airlines Engineering Division and Flight Operations Division have implemented an ISO 14001:2015 Environmental Management System (Singapore Airlines, 2021). ISO 14001 is a global meta-standard for implementing Environmental Management Systems (EMS) (Dentch, 2016; Grover & Grover, 2017; Heras-Saizarbitoria et al., 2011).

4.3. Annual Aircraft Fuel Consumption and Fuel Efficiency

Singapore Airlines passenger aircraft fleet annual fuel consumption (million/AG) and the year-on-year change for the period 2010/11 to 2020/21 is presented in Figure 2. As can be observed in Figure 2, Singapore Airlines annual passenger aircraft fleet fuel consumption (American gallons) has largely exhibited an upward trend reflecting the growth in services and in the aircraft fleet. This overall upward trend is demonstrated by the year-on-year percentage change line graph, which is more positive than negative, that is, more values are above the line than below. Figure 2 shows that there was a pronounced reduction in jet fuel consumption in 2020 (-78.22%), which was due to the lower level of operations because of the covid 19 pandemic. The other annual decrease in jet fuel consumption was recorded in the 2014/2015 financial year when the annual fuel consumption decreased by 1.96% on the previous year's levels (Figure 2).

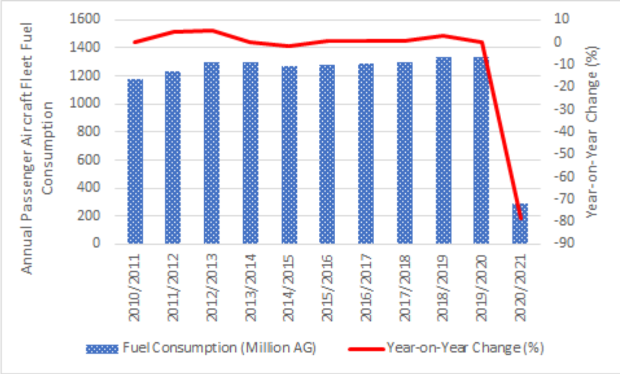

Fig.2: Singapore Airlines annual passenger aircraft fleet total annual fuel consumption (million/AG) and year-on-year change (%): 2010/11-2020/21. Source: Data derived from Singapore Airlines (2013, 2014, 2017, 2021).

Figure 3 presents Singapore Airlines passenger aircraft fleet annual fuel productivity ratio (LTK/AG) and the year-on-year change (%) for the period 2010/11 to 2020/21. According to CBS Netherlands (2021), a load tonne kilometre (LTK) is a "unit of measurement for transport capacity, representing the transport of a tonne (1000 kilograms) of load capacity over one kilometre". As can be observed in Figure 3, the airline's passenger fleet fuel productivity ratio (LTK/AG) remained relatively constant throughout the study period. Over the study period, Singapore Airlines expanded its passenger services, and thus, flew more load tonne kilometres. The airline has been able to achieve this growth whilst also maintaining its fuel productivity ratio at a relative constant value, which is a favorable outcome. In addition, the airline has added more next generation, fuel efficient aircraft, such as, the Airbus A350-900XWB and the Boeing 787-10, to its fleet in the latter years of the study; these aircraft are more fuel efficient when compared to the aircraft types that they have replaced. The lowest annual level was recorded in the 2020/2021 financial year (8.3 LTK/AG), whilst the highest level was recorded in the 2018/2019 financial year (10.74 LTK/AG). There were five years in the study period where this ratio declined on a year-on-year basis. These decreases occurred in the 2011/2012 (-0.98%), 2012/2013 (-0.59%), 2015/2016 (-0.29%), 2019/2020 (-1.58%), and 2020/2021 (-21.47%) financial years, respectively (Figure 3). The largest single annual decrease in this ratio was recorded in the 2017/2018 (+4.02%) financial year (Figure 3).

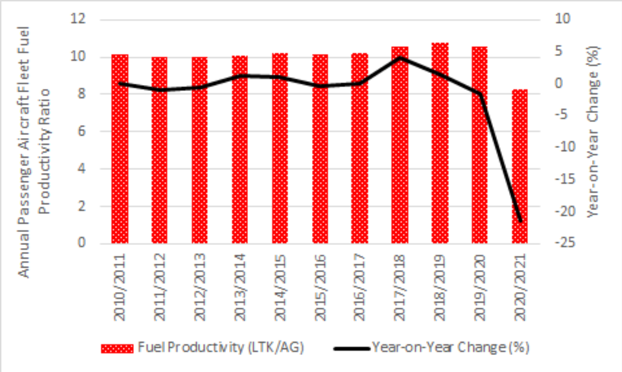

Fig.3: Singapore Airlines annual passenger aircraft fleet fuel productivity ratio and year-on-year change (%): 2010/11-2020/21. Source: Data derived from Singapore Airlines (2013, 2014, 2017, 2021).

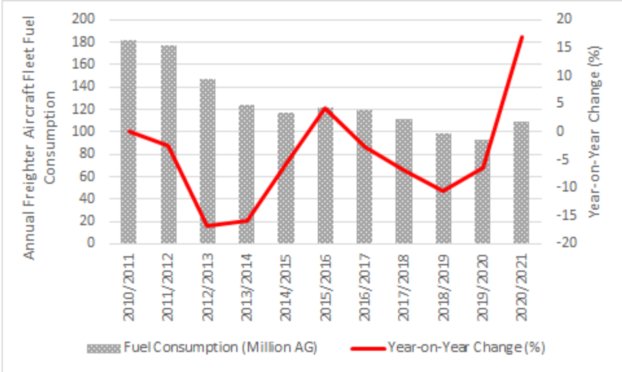

Fig.4: Singapore Airlines annual freighter aircraft fleet total annual fuel consumption (million/AG) and year-on-year change (%): 2010/11-2020/21. Source: Data derived from Singapore Airlines (2013, 2014, 2017, 2021).

The carriage of air cargo is also a core business activity of Singapore Airlines. The airlines air cargo division, SIA Cargo, operates a fleet of seven Boeing B747-400 freighter aircraft. A freighter aircraft is an aircraft that has been expressly designed or which has been converted to transport air cargo, express, and so forth, rather than passengers (Wensveen, 2016). Singapore Airlines Cargo freighter aircraft fleet annual fuel consumption (million/AG) and the year-on-year change (%) from 2010/11 to 2020/21 is presented in Figure 4. As can be observed in Figure 4, Singapore Airlines freighter aircraft fuel consumption has largely displayed a downward trend over the study period. This overall downward trend is demonstrated by the year-on-year percentage change line graph, which is more negative than positive, that is, more values are below the line than above. Figure 4 shows that Singapore Airlines freighter aircraft fuel consumption declined from a high of 182.02 million/AG in the 2010/2011 financial year to a low of 92.8 million/AG in the 2019/2020 financial years, respectively. There were two years in the study period where Singapore Airlines freighter aircraft fleet annual fuel consumption increased on a year-on-year basis. These increases occurred in the 2015/2016 (+4.17%) and 2020/2021 (+17.02%) financial years, respectively. The overall downward trend in freighter aircraft fleet fuel consumption is most favourable, particularly as Singapore Airlines Cargo annual freight tonnages remained relatively constant over the study period. This suggests Singapore Airlines Cargo has been able to accommodate its tendered air cargo traffic whilst at the same time reducing its freighter fleet fuel consumption.

Figure 5 presents Singapore Airlines Cargo freighter aircraft fleet annual fuel productivity ratio (LTK/AG) and the year-on-year change (%) for the period 2010/11 to 2020/21. As can be observed in Figure 5, Singapore Airlines Cargo freighter aircraft fleet annual fuel productivity ratio (LTK/AG) oscillated throughout the study period. This ratio exhibited a downward trend from 2010/2011 to 2015/2016, decreasing from 17.92 LTK/AG in the 2010/11 financial year to a low of 16.4 LTK/AG in the 2015/2016 financial year. This was followed by an upward trend with this ratio increasing from 16.4 LTK/AG in the 2015/2016 financial year to 17.68 LTK/AG in the 2017/2018 financial year. The ratio decreased on a year-on-year basis in the 2017/2018 (-4%), 2018/2019 (-2.43%), and 2019/2020 (-6.37%) before once again returning to positive growth in the 2020/2021 financial year (+3.4%) (Figure 5).

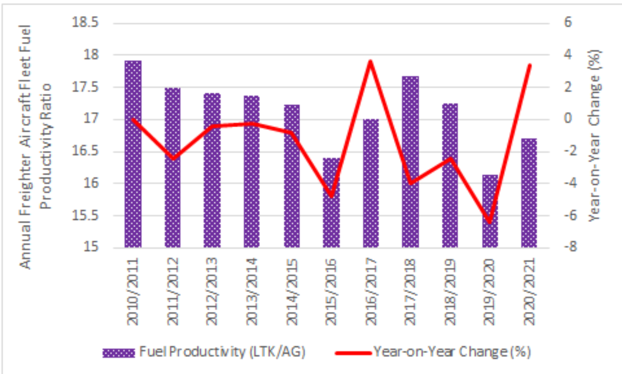

Fig.5: Singapore Airlines annual freighter aircraft fleet fuel productivity ratio and year-on-year change (%): 2010/11-2020/21. Source: Data derived from Singapore Airlines (2013, 2016, 2021).

4.4. **Singapore Airlines Facilities and Ground Service Equipment (GSE) Energy Consumption**

4.4.1. Annual Diesel Consumption

Singapore Airlines annual diesel consumption (litres) and the year-on-year change (%) for the period 2010/11 to 2020/21 is depicted in Figure 6. As can be observed in Figure 6, Singapore Airlines annual diesel consumption has displayed an upward trend, increasing from 3,376 litres in the 2010/2011 financial year to a high of 10,152 litres in the 2020/2021 financial year. This overall upward trend is demonstrated by the year-on-year percentage change line graph, which is more positive than negative, that is, more values are above the line than below. Figure 6 shows that there was a pronounced spike in the airline's diesel consumption in the 2018/2019 (+105.92%) and 2019/2020 (+91.98%) financial years. There was a significant decrease in diesel consumption in the 2017/2018 (-49.24%) financial year (Figure 6). There were also other smaller decreases in diesel consumption recorded in the 2011/2012 (-2.05%), 2012/2013 (-4.47%), and 2014/2015 (-9.57%) financial years, respectively. it is important to note that Singapore Airlines has grown its operations over the study period and the overall upward trend in diesel consumption reflect its ground service equipment (GSE) and vehicle consumption patterns.

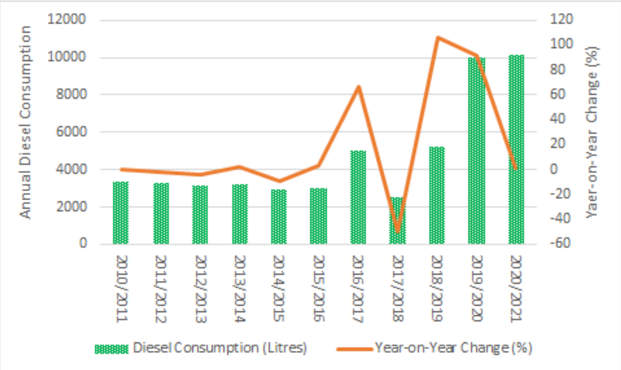

Fig.6: Singapore Airlines annual diesel consumption (litres) and year-on-year change (%): 2010/11-2020/21. Source: Data derived from Singapore Airlines (2013, 2014, 2017, 2021).

4.4.2. Annual Electricity Consumption

Singapore Airlines purchased electricity consumption is for four of the airline's properties – Airline House (ALH), SIA Training Centre (STC), TechSQ (TSQ), SIA Supplies Centre (SSC) and offices. Singapore Airlines annual electricity consumption (GWh) and the year-on-year change (%) from the 2010/11 financial year to the 2020/21 financial year is presented in Figure 7. As can be observed in Figure 7, Singapore Airlines annual electricity consumption has displayed a general downward trend, decreasing from 59.9 GWh in the 2021/2011 financial year to a low of 20.8 GWh in the 2020/2021 financial year. This overall downward trend is demonstrated by the year-on-year percentage change line graph, which is more negative than positive, that is, more values are below the line than above. Figure 7 shows that there were two years in the study period where Singapore Airlines electricity consumption increased on a year-on-year basis. These increases occurred in the 2011/2012 (+1.00%) and 2018/2019 (+1.07%) financial years, respectively. There was a very pronounced decrease in electricity consumption in the 2016/2017 financial year, when it decreased by 45.68% on the previous year's level. There was another significant decrease in the airline's electricity consumption in the 2020/2021 financial year, when it decreased by 25.44% on the 2019/2020 levels. Overall, this is a very favorable trend and suggests that the energy savings measures outlined below have had a very favorable impact on Singapore Airlines annual electricity consumption.

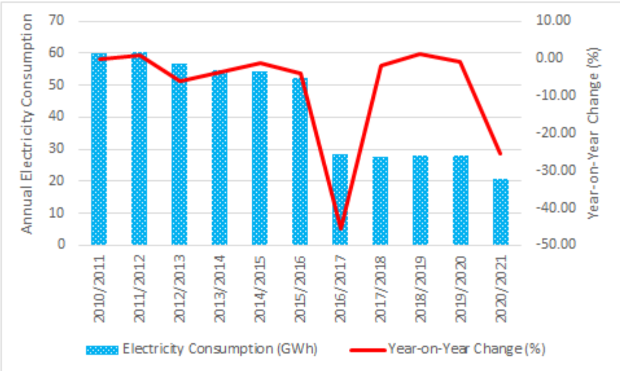

Fig.7: Singapore Airlines annual electricity consumption (GWh) and year-on-year change (%): 2010/11-2020/21. Source: Data derived from Singapore Airlines (2013, 2014, 2017, 2021).

Figure 8 presents Singapore Airlines annual electricity intensity ratio and the year-on-year change (%) from 2016/17 to 2020/21. Singapore Airlines annual electricity intensity ratio decreased from a high of 121.95 kWh/m^2 in the 2016/2017 financial year to a low of 98.1 kWh/m^2 in the 2020/2021 financial year (Figure 8). Figure 8 shows that there was just a single year in the study period when this ratio increased on a year-on-year basis. This increase was recorded in the 2018/2019 (+1.21%) financial year. There was a pronounced decrease in this ratio in the 2020/2021 financial year when it decreased by 18.48% on the 2019/2020 level (Figure 8). The general downward trend in this ratio is also a very favorable outcome and shows that Singapore Airlines is effectively managing its electricity consumption per square metre of building space.

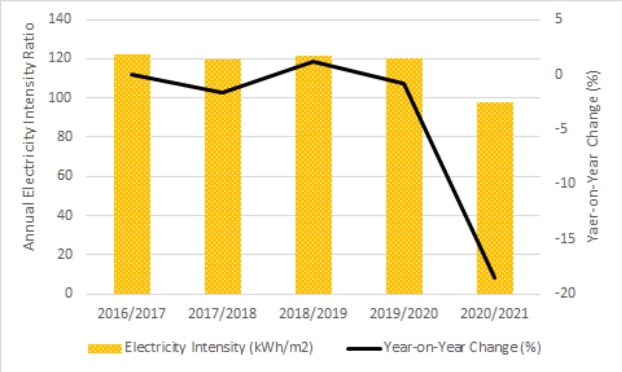

Fig.8: Singapore Airlines annual electricity intensity ratio and year-on-year change (%): 2016/17-2020/21.Note: data prior to 2016/17 not available. Source: Data derived from Singapore Airlines (2013, 2014, 2017, 2021).

4.4.3. Annual Petrol Consumption

Singapore Airlines annual petrol consumption (litres) and the year-on-year change (%) for the period 2010/11 to 2020/21 is depicted in Figure 9. Figure 9 shows that Singapore Airlines annual petrol consumption has fluctuated throughout the study period reflecting varying vehicle petrol consumption patterns. During the study period, there were four years where the annual petrol consumption decreased quite significantly on a year-on-year basis. These decreases occurred in the 2012/2013 (-20.83%), 2013/2014 (-13.59%), 2014/2015 (-24.87%), and 2020/2021 (-38.95%) financial years, respectively (Figure 9). As can be observed in Figure 9, there was a significant increase in the airline's petrol consumption in the 2017/2018 (+31.16%), and 2018/2019 (+20.26%) financial years, which reflected greater vehicle petrol consumption in those two financial years.

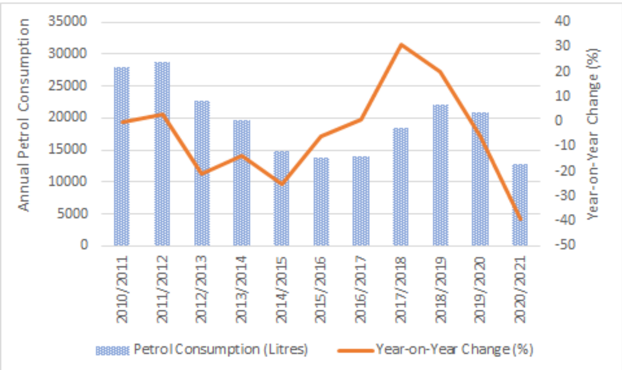

Fig.9: Singapore Airlines annual petrol consumption (litres) and year-on-year change (%): 2010/11-2020/21. Source: Data derived from Singapore Airlines (2013, 2014, 2017, 2021).

4.5 Singapore Airlines Energy Conservation Measures

4.5.1 Aircraft Weight Reduction Program

Airlines are increasingly introducing a range of measures that are designed to lower the weight of their aircraft, and hence, reduce aircraft fuel burn (Gilani & Körpe, 2019). Singapore Airlines has implemented aircraft weight saving measures initiatives both within its group airlines and with the Original Equipment Manufacturers (OEMs). Singapore Airlines has implemented an initiative that was designed to optimize the water uplift based on flight sector requirements through a tailored potable water program. Another initiative involved the removal of unutilized overhead storage compartments located in between the galleys of the airline's Boeing 777-300ER aircraft fleet. These two initiatives saved 2,400 tonnes of jet fuel per annum (Singapore Airlines, 2017).

4.5.2 Cleaner Energy Vehicles

The replacement of internal combustion engine powered airport ground support vehicles and equipment with cleaner energy powered vehicles could potentially reduce carbon dioxide (CO_2), carbon monoxide (CO), hydrocarbons (HC), nitrogen oxides (NO_x), and particulate matter (PM) (Gellings, 2011). Singapore Airlines has been cognizant of the environmental impact of fossil fuel vehicles and equipment and, as a result, in the 2020/21 financial year, Singapore Airlines installed six electric vehicles (EV) charging stations and promoted the use of electric vehicles (Singapore Airlines, 2021).

4.5.3 Ground-Based Facilities Energy Conservation Measures

Because of the growing problems of climate change and global warming, airlines are now actively promoting energy-savings (Tsai et al., 2014). Throughout the study period, Singapore Airlines introduced an extensive range of energy saving measures, which had a particular focus on air-conditioning, ventilation, lightings, and lifts within the company's buildings.

Singapore Airlines Office Management System (OMS) features innovative designs and best office practices that not only maximize space utilization but also reduce energy consumption (Singapore Airlines, 2015).

During the 2014/15 financial year, Singapore Airlines buildings and facilities were upgraded with more energy-efficient equipment and technology. These energy efficiency measures included the upgrading of lifts with a more efficient model that had a Variable Voltage Variable Frequency (VVVF) motor, the changing of high bay lights from metal halide lamps to more energy-efficient light emitting diode (LED) lighting, the replacement of neon signs on the company's main buildings with LED technology, the installation of a heat exchange system at the SilverKris Lounge at Changi Airport, which is used to produce hot water for the showers, and the replacement of an old chiller system with a higher energy efficiency model. These energy saving measures amounted to 80 MWh per month (Singapore Airlines, 2015).

In the 2016/17 financial year, Singapore Airlines energy saving measures focused on the management of air conditioning systems and lighting within its buildings. A key energy saving measure implemented by the airline was the replacement of high bay lighting for hangar operations (Singapore Airlines, 2017). These measures reduced both energy consumption. In the 2017/18 financial year, Singapore Airlines continued its energy saving measures program which included the completion of replacement of high bay lights project for the airline's Hangar 1 from 1,000W sodium metal halide lamps to 400W dimmable LED high bay lights. This measure provided energy efficiency savings. Other energy efficiency measures included the ongoing replacement of Fan Coil Units (FCUs) within the company's buildings with more efficient models as well as the replacement of Computer Air-Conditioning Units (CAUs) serving the aircraft simulator computers with higher efficiency units (Singapore Airlines, 2018). In the 2018/19 financial year, Singapore Airlines conducted a feasibility study that focused on the potential use of solar energy to support the company's use of green energy to meet its buildings' energy requirements. A tender was initiated for the installation of solar panels at the airline's Head Office building in Singapore. Other energy saving measures included the progressive upgrading of air-conditioning equipment to ensure that the most energy efficient equipment is used in the company's buildings and facilities, the airline also continued the replacement of Fan Coil Units (FCUs) within its buildings with more efficient models, and the company continued to replace old light fittings with LED lighting (Singapore Airlines, 2019).

In the 2019/20 financial year, Singapore Airlines continued to implement energy reduction initiatives, and these resulted in an estimated 800 MWh of energy savings principally through adjustments to the operating parameters of its chiller plants and air-handling units (AHU), and through the replacement of lighting with more energy efficient light-emitting diode (LED) light fittings. In addition to its energy reduction initiatives, the airline started adopting the use of renewable energy. As part of this strategy, Singapore Airlines entered into a partnership agreement with SembCorp Solar to install rooftop solar panels on three of its Singapore-based buildings: Airline House, SIA Training Centre and TechSQ. It was envisaged that the solar panels would come online in the second quarter of FY2020/21, and they would generate a projected 5,382 MWh of renewable energy annually, which is able to support up to 18 per cent of the company's buildings' electricity demand (Singapore Airlines, 2020). The installation of the solar panels on all its office buildings in Singapore was completed in the 2020/21 financial year (Singapore Airlines, 2021).

4.5.4 Improving Aircraft Maximum Zero Fuel Weights (MZFW) to Optimize Aircraft Fuel Uplift

An aircraft's maximum zero fuel weight (MZFW) is the maximum weight permitted on the aircraft before fuel is loaded (Jofré & Irrgang, 2000). By improving the accuracy of an aircraft's MZFW, the correct amount of fuel is uplifted on a flight. This reduces the overall weight of the aircraft. Singapore Airlines has implemented a computer system to improve the MZFW accuracy of its aircraft fleet. The airline has introduced a "ZFW Monitoring Dashboard". This system enables its Airport Operations department to monitor the MZFW of its aircraft fleet more closely. In the 2019/20 financial year, the optimization of aircraft MZFW weights resulted in an estimated 1,800 tonnes of fuel savings (Singapore Airlines, 2020).

4.5.5 Reduced Engine Aircraft Taxi in Procedure at Airports

As part of their sustainability policy, Singapore Airlines has implemented a "Reduced Engine Taxi In" procedure for their aircraft fleet to optimize fuel efficiency. Upon landing at their destination, pilots can use a single engine for twin-engine aircraft, or alternatively three engines for four-engine aircraft during taxiing process (Singapore Airlines, 2018). This measure therefore reduces the fuel consumption during the aircraft taxying phase of the flight at the destination airport.

4.5.6 Removal of Economy Class Footrests in Selected Aircraft

In 2019, Singapore Airlines removed some Economy Class footrests on selected aircraft. This measure delivered a weight saving of around 200kg to 300kg per aircraft (Singapore Airlines, 2019). The lower aircraft weight translates into lower fuel burn.

4.5.7 The use of aviation biofuels

Singapore Airlines has been an active member of the "Sustainable Aviation Fuel Users Group" (SAFUG) since 2011. Singapore Airlines views sustainable aviation fuels as a key long-term measure to support the air transport industry's carbon-neutral growth goal beyond 2020. Accordingly, the airline has pledged to advance and adopt aviation biofuels produced in a sustainable way. The sustainable aviation fuels should have minimal impact on biodiversity, such fuels should meet a sustainability standard, in relation to land, water and energy use, the production of sustainable aviation fuels should not displace or compete with food crops; and these fuels should also provide a positive socio-economic impact (Singapore Airlines, 2019).

In addition to the use of sustainable aviation fuels on a series of "Green Flights" in 2017, Singapore Airlines commenced working with Stockholm's Swedavia Airport in 2020 on the use of sustainable aviation fuels (Becken, 2021). In January 2020, Singapore Airlines began a year-long partnership with Swedish airport operator Swedavia. As part of Swedavia's sustainable aviation fuel (SAF) Incentive Scheme, the airline commenced using a blend of jet fuel and SAF on its flights between Stockholm and Moscow (Singapore Airlines, 2020). This arrangement between the two parties continued in the 2020/21 financial year (Singapore Airlines, 2021).

4.5.8 The use of Lightweight Aircraft Unit Load Devices

In recent times, airlines from all around the world have sought to minimize aircraft weight without compromising the business volume is using light weight aircraft unit load devices (ULDs) (Laniel et al., 2011). Aircraft unit load devices, or ULDs, are pallets and containers which are used to carry air cargo, mail and passenger baggage on wide-body passenger and freighter aircraft (Baxter et al., 2014; Lu & Chen, 2011). To achieve their fuel saving objectives, in recent times airlines have acquired new light-weight composite material aircraft unit load devices (ULDs) (Bandi & Lumia, 2013). Singapore Airlines is one such airline that has acquired light weight aircraft ULDs as a fuel saving measure (Singapore Airlines, 2021).

4.5.9 The Use of Light Weight Flight Catering Items on Regional Passenger Services

In 2020, Singapore Airlines introduced a new regional economy class menu which offered passengers additional meal choices. These were accompanied with bamboo cutlery and sustainable paper packaging. The new packaging was lighter, weighing half that of previously used plastics. This reduction in weight results in lower fuel consumption (Becken, 2021; Singapore Airlines, 2021).

4.5.10 The Use of Airport Mobile Ground Power Units

During the time an aircraft is on the ground between flights, electrical power is required on the airport apron to enable the ground handling of the aircraft prior to engine start-up. (Ashford et al., 2013; Horonjeff et al., 2010; Kazda & Caves, 2015). Singapore Airlines has introduced a policy where mobile ground power units and preconditioned air units are used during night layovers and long transits at airports. The use of fixed electrical ground power alleviates the reliance on its aircraft auxiliary power units (APUs) (Singapore Airlines, 2017, 2019). The use of fixed electrical ground power reduces fuel consumption as it is no longer necessary to use fuel to run the aircraft APU. In the 2019/20 financial year, this energy conservation measure delivered an estimated 910 tonnes in fuel savings (Singapore Airlines, 2020).

V. CONCLUSION

The global airline industry plays a vital role in the world economy by facilitating the movement of passengers and air cargo consignments. This transportation assists the global tourism industry as well as world trade. However, airlines are very energy intensive and, as a result, airlines are now seeking ways to manage their energy consumption on a more sustainable basis. Based on its commitment to sustainably manage its operations and its energy consumption, this study selected Singapore Airlines as the case airline. The study period was from 2010/2011 to 2020/2021. The secondary data collected for the study was examined by document analysis.

Singapore Airlines has four principal energy sources. The largest energy source is the jet fuel that is required to power its fleet of passenger and freighter aircraft. The airline's second largest energy source is the electricity that is required to power its Singapore-based facilities. The airline has a fleet of ground service equipment (GSE) and vehicles that underpin its ground operations. Thus, the third largest energy source for Singapore Airlines is the petrol used to power its fleet of vehicles. The final energy source is the diesel used to power its fleet of ground service equipment (GSE).

The case study found that Singapore Airlines jet fuel consumption has increased over the study period and is inline with its expansion of services and aircraft fleet. The annual fuel consumption for the passenger fleet declined very significantly in the 2020/2021 financial year due to the reduced scale of passenger operations because of the covid 19 pandemic. Air cargo is a core product of Singapore Airlines, and its freighter fleet fuel consumption exhibited a downward trend over the study period. This was a favourable outcome as the airline was able to satisfy its air cargo shippers' supply chain transportation requirements whilst at the same time reducing the annual consumption of jet fuel used to power its fleet of dedicated freighter aircraft. The case study revealed that the airline's diesel consumption had grown over the study period, increasing from 3,376 litres in the 2010/2011 financial year to a high of 10,152 litres in the 2020/2021, with the overall increase reflecting greater fuel consumption patterns. Singapore Airlines annual petrol consumption oscillated over the study period, with the highest annual consumption recorded in the 2011/2012 financial year (28,800 litres) and the lowest annual consumption in the 2020/2021 financial year (12,740 litres), respectively.

Singapore Airlines annual electricity consumption displayed a favourable downward trend, decreasing from 59.9 GWh in the 2010/2011 financial year to a low of 20.8 GWh in the 2020/2021 financial year.

A key energy saving strategy of Singapore Airlines has been the acquisition and deployment of the latest state of the art aircraft, such as the Airbus A350-900 XWB and Boeing 787-10 aircraft. These aircraft offer greater fuel efficiency than the aircraft models that they replaced. Like many other companies, Singapore Airlines has installed a photovoltaic (PV) system and this system will be capable of generating 5,382 MWh of renewable energy annually, which is able to support up to 18 per cent of the company's buildings' electricity demand.

As previously noted, throughout the study period, Singapore Airlines implemented various energy efficiency measures. These include an aircraft weight reduction program, the installation of extensive light emitting diode (LED) lighting, the installation of more energy efficient heat exchange system at its Silver Kris Lounge at Changi Airport, the installation of more energy efficient plant and equipment, the upgrading of lifts with a more efficient model that had a Variable Voltage Variable Frequency (VVVF) motor, the installation of a large scale photovoltaic (PV) system, a computer system that optimizes the maximum zero fuel weight (MZFW) of its aircraft fleet, the use of lightweight catering items, the use of light weight aircraft unit load devices, the use of sustainable aviation fuels, and the use of fixed electrical ground power and preconditioned air units at airports where its aircraft have night layovers or long transits.

REFERENCES

[1] Airbus. (2018). First ultra-long range A350 XWB delivered to Singapore Airlines. Retrieved from https://www.airbus.com/newsroom/press-releases/en/2018/09/first-ultra-long-range-a350-xwb-delivered-to-singapore-airlines.html.

[2] Aircraft Commerce. (2016). Fuel burn & operating performance of the 787-8, 787-9 and A350-900. *Aircraft Commerce*, 108, 16-27.

[3] Alam, A., Farhad Hossain Masum, M., & Dwivedi, P. (2021). Break-even price and carbon emissions of carinata-based sustainable aviation fuel production in the Southeastern United States. *GCB-Bioenergy*, 13(11), 1800-1813. https://doi.org/10.1111/gcbb.12888

[4] Andrew, D.P.S., Pedersen, P.M., & McEvoy CD. (2011). *Research methods and design in sport management*. Champaign: Human Kinetics.

[5] Arman, H., & Yuksel, I. (2013). Preface. In H. Arman & I Yuksel (Eds.), *New developments in renewable energy* (pp. xi-xii). Rijeka: InTech.

[6] Ashford, N. J., Martin Stanton, H.P., Moore, C.A., Coutu, P., & Beasley, J.R. (2013). *Airport operations* (3rd ed.). New York: McGraw-Hill.

[7] Bandi, S. & Lumia, R. (2013). Cycle time reduction of a composite panel manufacturing line. *International Journal of Mechanical Engineering and Robotics Research*, 2(2), 189-203.

[8] Baxter, G. (2021). Mitigating an airport's carbon footprint through the use of "green" technologies: The case of Brisbane and Melbourne Airports, Australia. *International Journal of Environment, Agriculture and Biotechnology*, 6(6), 29-39. https://dx.doi.org/10.22161/ijeab.66.4

[9] Baxter, G., Kourousis, K. & Wild, G. (2014). Fire resistant aircraft unit load devices and fire containment covers: A new development in the global air cargo industry. *Journal of Aerospace Technology and Management*, 6(2), 202-209. https://doi.org/10.5028/jatm.v6i2.327

[10] Baxter, G., Srisaeng, P, & Wild, G. (2018). Sustainable airport energy management: The case of Kansai International Airport. *International Journal for Traffic and Transport Engineering*, 8(3), 334 – 358.

[11] Baxter, G., Srisaeng, P., & Wild, G. (2021). A qualitative assessment of a full-service network airline sustainable energy management: The case of Finnair PLC. *WSEAS Transactions on Environment and Development*, 17, 167-180.

[12] Becken, B. (2021). Singapore Airlines targets net zero carbon emissions by 2050. Retrieved from https://www.businesstravelerusa.com/business-traveler-usa-story/singapore-airlines-targets-net-zero-carbon-emissions-by-2050/.

[13] Belobaba, P.P. (2016). Overview of airline economics, markets, and demand. In: P. Belobaba, A. Odoni & C. Barnhart (Eds.), *The global airline industry* (pp. 47-74) (2nd ed.). Chichester: John Wiley & Sons.

[14] Boeing Commercial Airplanes. (2021). Dreamliner advantages overview. Retrieved from https://www.boeing.com/commercial/787/by-design/#/dreamliner-advantages-overview.

[15] Brimson, S. (1985). *The airlines of the world*. Sydney: Dreamweaver Books.

[16] Brooks, K.P., Snowden-Swan, L.J., Jones, S.B., Butcher, M.G., Lee, G.S.J, Anderson, D.M., Frye, J.G., Holladay, J.G., Owen, J., Harmon, L., Burton, F., Palou-Rivera, L., Plaza, J., Handler, R., & Shonnard, D. (2016). Low-carbon aviation fuel through the alcohol to jet pathway. In C. Chuck (Ed.), *Biofuels for aviation: Feedstocks, technology, and implementation* (pp. 109-146). London: Academic Press.

[17] CBS Netherlands. (2021). Load capacity tonne-kilometre. Retrieved from https://www.cbs.nl/en-gb/onze-diensten/methods/definitions/load-capacity-tonne-kilometre#:~:text=Unit%20of%20measurement%20for%20transport,load%20capacity%20over%20one%20kilometre.

[18] Centre for Aviation. (2021). Singapore Airlines profile. Retrieved from https://centreforaviation.com/data/profiles/airlines/singapore-airlines-sq.

[19] Chant, C. (1997). *Airlines of the world*. London: Tiger Books International.

[20] Crider, R., Preisler, M., Autin, E., Roth, S., Armstrong, R.W., Fulton, S., Swartzlander, J., & Tharp, G. (2011). *Guidebook for developing and leasing airport property*. Airport Cooperative Research Program Report 47. Washington: Transportation Research Board.

[21] Dentch, M.P. (2016). *The ISO 14001:2015 handbook: Using the process approach to building an environmental management system*. Milwaukee, WI: Quality Press.

[22] Derrington, M.L. (2019). *Qualitative longitudinal methods: Researching implementation and change*. Thousand Oaks: SAGE Publications.

[23] Dube, K., Nhamo, G., & Chikodzi, D. (2021). COVID-19 pandemic and prospects for recovery of the global aviation industry. *Journal of Air Transport Management*, 92, 102022. https://doi.org/10.1016/j.jairtraman.2021.102022

[24] Field, J. (2018). Singapore Airlines receives first 787-10 Dreamliner. Retrieved from https://airwaysmag.com/airlines/singapore-airlines-receives-first-787-10-dreamliner/.

[25] Gellings, C.W. (2011). *Saving energy and reducing CO_2 emissions with electricity*. Lilburn: The Fairmont Press.

[26] Gilani, M., & Körpe, D.S. (2019). Airline weight reduction to minimize direct operating cost. In *Proceedings of the 4th International Aviation Management Conference (4th INTAVIC), Ankara, Turkey, November 18-19* (pp.1-12).

[27] Gillen, D. (2017). Aviation economics and forecasting. In: L. Budd & S. Ison (Eds.), *Air transport management: An international perspective* (pp. 27-45). Abingdon: Routledge.

[28] Green, W. & Swanborough, G. (1975). *The Observer's world airlines and airliners directory*. London: Frederick Warne & Co.

[29] Grover, R.C., & Grover, S. (2017). *Winning the environmental challenge with ISO 14001: 2015: Implementation of an Environmental Management System*. Chennai, India: Notion Press.

[30] Gupta Kapoor, N. (2017). Scoot and Tigerair merge. Retrieved from https://www.businesstraveller.com/business-travel/2017/07/26/scoot-tigerair-merge/

[31] Hassett, M., & Paavilainen-Mäntymäki, E. (2013). Longitudinal research in organizations: An introduction. In M. Hasset & E. Paavilainen-Mäntymäki (Eds.), *Handbook of longitudinal research methods in organisation and business studies* (pp. 1-12). Cheltenham: Edward Elgar Publishing.

[32] Hazel, R.A., Blais, J.D., Browne, T.J., & Benzon, D.M. (2011). *Resource guide to airport performance indicators*. Airport Cooperative Research Program Report 19A. Washington: Transportation Research Board.

[33] Heracleous, L. & Wirtz J. (2012). Strategy and organisation at Singapore Airlines: Achieving sustainable advantage through dual strategy. In: O. Inderwildi & S. King (Eds.), *Energy, transport, & the environment* (pp. 479-493). London: Springer.

[34] Heras-Saizarbitoria, I., Landín, G.A., & Molina-Azorín, J.F. (2011). Do drivers matter for the benefits of ISO 14001? *International Journal of Operations and Production Management*, 31(2), 192-216. https://doi.org/10.1108/01443571111104764

[35] Holloway, S. (2016). *Straight and level: Practical airline economics* (3rd ed.). Abingdon: Routledge.

[36] Horonjeff, R., McKelvey, F.X., Sproule, W.J., & Young, S.B. (2010). *Planning & design of airports* (5th ed.). New York: McGraw-Hill.

[37] Iatrou, K., & Alamdari, F. (2005). The empirical analysis of the impact of alliances on airline operations. *Journal of Air Transport Management*, 11(3), 127-134. https://doi.org/10.1016/j.jairtraman.2004.07.005

[38] International Air Transport Association. (2021a). Airline industry statistics confirm 2020 was worst year on record. Retrieved from https://www.iata.org/en/pressroom/pr/2021-08-03-01/.

[39] International Air Transport Association. (2021b). Fuel efficiency. Retrieved from https://www.iata.org/en/programs/ops-infra/fuel/fuel-efficiency/.

[40] International Renewable Energy Agency. (2021). Benefits. Retrieved from https://www.irena.org/benefits.

[41] Jofré, M., & Irrgang, M.E. (2000). Integration of cargo and passenger operations. In G.F. Butler & M.R. Keller (Eds.), *Handbook of airline operations* (pp. 259-271). New York: McGraw-Hill.

[42] Kazda, A., & Caves, R.E. (2015). *Airport design and operation* (3rd ed.). Bingley: Emerald Group Publishing.

[43] Laniel, M., Uysal, J. & Emond, J. P. (2011). Radio frequency interactions with air cargo container materials for real-time cold chain monitoring. *Applied Engineering in Agriculture*, 27(4), 647-652.

[44] Lu, H.A. & Chen, C.Y. (2011). A time-space network model for unit load device stock planning in international airline services. *Journal of Air Transport Management*, 17(2), 94-100. https://doi.org/10.1016/j.jairtraman.2010.09.004

[45] Michaga, M.F.R., Michailos, S., Hughes, K.J., Ingham, D., & Pourkashanian, M. (2021). 10 - Techno-economic and life cycle assessment review of sustainable aviation fuel produced via biomass gasification. *Sustainable Biofuels*, 269-303. https://doi.org/10.1016/B978-0-12-820297-5.00012-8

[46] Mu, H., & Tang, J. (2019). The composite control method for the GDI engine idle speed control. *International Journal of Circuits, Systems, and Signal Processing*, 13, 97-104.

[47] Neale, B. (2018). *What is qualitative longitudinal research?* London: Bloomsbury Publishing.

[48] O'Leary, Z. (2004). *The essential guide to doing research*. London: SAGE Publications.

[49] Ong, M. (2017). Scoot and Tigerair merger: What can we expect? Retrieved from https://www.asiaone.com/travel/scoot-and-tigerair-merger-what-can-we-expect.

[50] Ortega Alba, S., & Manana, M. (2017). Characterization and analysis of energy demand patterns in airports. *Energies*, 10(1), 119. https://doi.org/10.3390/en10010119

[51] Otley, T. (2019). Airbus A350: What you need to know. Retrieved from https://www.businesstraveller.com/features/a350-what-you-need-to-know/.

[52] Rahim, A. R., & Baksh, M. S. (2003). Case study method for new product development in engineer-to-order organisations, *Work Study*, 52(1), 25–36. https://doi.org/10.1108/00438020310458705

[53] Ramaswamy, K. (2002). Singapore International Airlines: Strategy with a smile. *Thunderbird International Business Review*, 44(4), 533-555. https://doi.org/10.1002/tie.10027

[54] Rice, C., Ragbir, N.K., Rice, S., & Barcia, G. (2020). Willingness to pay for sustainable aviation depends on ticket price, greenhouse gas reductions and gender. *Technology in Society*, 60, 101224. https://doi.org/10.1016/j.techsoc.2019.101224

[55] Roberts, A. (2018). Airside resource planning. In P.J. Bruce, Y. Gao & J.M.C. King (Eds.), *Airline operations: A practical guide* (pp. 152-161). Abingdon: Routledge

[56] Scott, J. 2004. Documents, types of. In: M. Lewis-Beck, A.E. Bryman. & T. Futing Liao (Eds.), *The SAGE encyclopedia of social science research methods* (pp. 281-284). Thousand Oaks: SAGE Publications.

[57] Scott, J. (2014). *A dictionary of sociology* (4th ed.). Oxford: Oxford University Press.

[58] Scott, J. & Marshall G. (2009). *A dictionary of sociology* (3rd ed.). Oxford; Oxford University Press.

[59] Simons, G.M. (2014). *The Airbus A380: A history*. Barnsley: Pen & Sword Aviation.

[60] Singapore Airlines. (2013). Sustainability report 2012/13. Retrieved from https://www.singaporeair.com/saar5/pdf/Investor-Relations/Annual-Report/sustainabilityreport1213.pdf.

[61] Singapore Airlines. (2014). Sustainability report 2013/14. Retrieved from https://www.singaporeair.com/saar5/pdf/Investor-Relations/Annual-Report/sustainabilityreport1314.pdf.

[62] Singapore Airlines. (2015). Sustainability report 2014/15. Retrieved from https://www.singaporeair.com/saar5/pdf/Investor-Relations/Annual-Report/sustainabilityreport1415.pdf.

[63] Singapore Airlines. (2016). Sustainability report 2015/16. Retrieved from https://www.singaporeair.com/saar5/pdf/Investor-Relations/Annual-Report/sustainabilityreport1516.pdf.

[64] Singapore Airlines. (2017). Sustainability report 2016/17. Retrieved from https://www.singaporeair.com/saar5/pdf/Investor-Relations/Annual-Report/sustainabilityreport1617.pdf.

[65] Singapore Airlines. (2018). Sustainability report 2017/18. Retrieved from https://www.singaporeair.com/saar5/pdf/Investor-Relations/Annual-Report/sustainabilityreport1718.pdf.

[66] Singapore Airlines. (2019). Sustainability report 2018/19. Retrieved from https://www.singaporeair.com/saar5/pdf/Investor-Relations/Annual-Report/sustainabilityreport1819.pdf.

[67] Singapore Airlines. (2020). Sustainability report 2019/20. Retrieved from https://www.singaporeair.com/saar5/pdf/Investor-Relations/Annual-Report/sustainabilityreport1920.pdf.

[68] Singapore Airlines. (2021). Sustainability report 2020/21. Retrieved from https://www.singaporeair.com/saar5/pdf/Investor-Relations/Annual-Report/sustainabilityreport2021.pdf.

[69] Singh, J., Sharma-Kumar, S., & Srivastava, R. (2018). Managing fuel efficiency in the aviation sector: challenges, accomplishments and opportunities. *FIIB Business Review*, 7(4), 244-251. https://doi.org/10.1177%2F2319714518814073

[70] Sloan, C. (2019). Flashback: Flying the inaugural 380 with Singapore Airlines (2007). Retrieved from https://airwaysmag.com/special-flights/onboard-a380-inaugural-flight-2007/.

[71] Sreenath, S., Sudhakar, K., & Yusop, A.F. (2021). Energy-exergy-economic-environmental-energo-exergo-enviroecono (7E) analysis of solar photovoltaic power plant: A case study of 7 airport sites in India. *Sustainable Energy Technologies and Assessments*, 47, 101352. https://doi.org/10.1016/j.seta.2021.101352

[72] Staples, M.D., Malina, R., Suresh, P., Hileman, J.I. & Barrett, S.R.H. (2014). Aviation CO_2 emissions reductions from the use of alternative jet fuels. *Energy Policy*, 114, 342-354. https://doi.org/10.1016/j.enpol.2017.12.007

[73] Tsai, W.H., Chang, W.C., Lin, S.J., Chen, H.C., & Chu, P.Y. (2014). A green approach to the weight reduction of aircraft cabins. *Journal of Air Transport Management*, 40, 65-77. https://doi.org/10.1016/j.jairtraman.2014.06.004

[74] Turner, P.A., & Lim, S.H. (2015). Hedging jet fuel price risk: The case of U.S. passenger airlines. *Journal of Air Transport Management*, 44-45, 54-64. https://doi.org/10.1016/j.jairtraman.2015.02.007

[75] United States Environmental Protection Agency. (2021). Local renewable energy benefits and resources. Retrieved from https://www.epa.gov/statelocalenergy/local-renewable-energy-benefits-and-resources.

[76] Vasigh, B., & Rowe, Z.C. (2020). *Foundations of airline finance: Methodology and practice* (3rd ed.). Abingdon: Routledge.

[77] Vasigh, B., Taleghani, R., & Jenkins, D. (2012). *Aircraft finance: Strategies for managing capital costs in a turbulent industry*. Fort Lauderdale.

[78] Wensveen, J.G. (2016). *Air transportation: A management perspective* (8th ed.). Abingdon: Routledge.

[79] Yerel Kandemir S., & Yayli, M. Ö. (2016). Investigation of renewable energy sources for airports. In T. Hikmet, M. Baris Ozerdem, C.O. Colpan, O. Altuntas, E. Acikkalp (Eds.), *Sustainable aviation: Energy and environmental issues* (pp. 11-16). Cham, Switzerland: Springer International Publishing.

[80] Yildirim, U., & Abanteriba, S. (2012). Manufacture, qualification and approval of new aviation turbine fuels and additives. *Procedia Engineering*, 49, 310-315. https://doi.org/10.1016/j.proeng.2012.10.142

[81] Yin, R.K. (2018). *Case study research and applications* (6th ed.). Thousand Oaks: SAGE Publications.

[82] Zou, B., Kwan, I., Hansen, M., Rutherford, D., & Kafle, N. (2016). Airline fuel efficiency: Assessment methodologies and applications in the U.S. domestic airline industry. In J.D. Bitzan., J. Peoples & W.W. Wilson (Eds.), *Airline efficiency* (pp. 317-352). Bingley: Emerald Group Publishing.

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The Performance and Haematological Indices of Broiler Chickens Fed Chromium Propionate, and Vitamin E Supplemented Diets

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Abstract*— *Aims: This study investigates the out-turn of Chromium Propionate (CrProp) and vitamin E dietary supplementation on broiler chickens' performance characteristics and haematological indices.

Study Design: The completely randomised design was used for this study.

Methodology: Six hundred- and forty-day-old Cobb 500 broiler chickens were randomly assigned to eight dietary treatments (10 birds/replicate). A basal diet was fractionated into eight equal parts and labelled diets 1 to 8. Diets 1 to 4 were supplemented with 0, 0.4, 0.8 and 1.2 mg/kg CrProp, respectively. The diets 5 to 8 were supplemented with 200 mg/kg vitamin E; 0.4 mg CrProp+200 mg vitamin C; 0.8 mg CrPro+200 mg vitamin E and 1.2 mg CrPro+200 mg Vitamin E, respectively.

Results: The final body weight (FBW) of the birds fed diets 2, 3, 4,7 and 8 were significantly (P<0.05) higher than those fed the control diet and diet 5 and 6, and total weight gain (TWG) of the birds fed diets 2,4,7and 8 were significantly (P<0.05) higher than those fed the control and diet 3,5,6. The CrProp supplementation at 0.4, 0.8 and 1.2 mg/kg levels improved (P<0.05) the FBW, and supplementation at 0.4 and 1.2mg/kg levels improved (P<0.05) the TWG of the birds, compared to the control. The vitamin E supplementation (200mg/kg) does not improve (P>0.05) the FBW and TWG of the birds. The haematological indices showed a significant difference (P<0.05) across the diets. However, supplementation of CrProp at 0.8mg/kg affects MCV, WBC, Heterocyte and lymphocyte count, whileat 1.2mg/kg, the MCHC value was significantly affected. Including vitamin E at 200mg/kg improves the MCV and MCH. The combination of CrProp at 0.4mg/kg and 200mg/kg vitamin E increased(P<0.05) heterocyte count, while supplementation at 0.8mg/kg and 200mg/kg vitamin E improves WBC and lymphocyte counts. The packed cell volume improved significantly by CrProp supplementation at 1.2mg/kg and 200mg/kg vitamin E.

Conclusion: The growth of the broiler chicken is enhanced by 1.2 mg/kg CrProp, 200 mg/kg vitamin E and a combination of CrPropand vitamin E dietary supplementations with significant changes in haematological indices of the birds.

Keywords*— *Avian, blood, chromium, growth, supplements, Vitamin E.

Authors' contributions

This work was carried out in collaboration among all authors. Authors OSO, OAA and CAC designed the study. Authors OAA and FBA performed the statistical analysis. Authors OSO, OAA and FBA wrote the protocol. Authors OSO and FBA wrote the first draft of the manuscript. All authors managed the analyses of the study. Authors OSO, OAA, FBA and SOA organised the literature searches. All authors read and approved the final manuscript

I. INTRODUCTION

Climate change can cause severe damage tofood production of animal origin for human consumption (Wang *et al*., 2017). Heat stress is considered one of the most critical environmental stressors that cause poor performance, imbalance of the oxidant/antioxidant system, and compromised immune and health statuses in egg-laying birds worldwide, namely, the poultry industry (Luo *et al.*, 2018; Sahin *et al.*, 2018). The tropical regions with high ambient temperature and humidity were more susceptible to high heat stress than the polar or temperate regions [Zhao *et al.* 2015]. Besides, meteorological factors such as temperature and humidity are significant factors that influencedomestic birds' production performance and haematological parameters [Ayo *et al*., 2011]. Notably, in Nigeria,the high environmental temperature may be responsible for reduced performance and increased mortality (Oguntunji and Alabi 2010; Yousaf *et a*l., 2019), and the provision of protection against heat which is usually in temporary light shades and radiation shield is usually inadequate [Ayo *et al.* 1996].

Feed consumption during heat stress is suppressed, leading to reduced nutrient intake (Khan *et al.* 2014). Increased mineral excretion from the body and decreases in the blood is significant consequence of heat stress, leading to deficiencies of these components in blood and tissues (Sahin *et al*., 2009). Heat stress may also increase the mortality rate, leading to economic losses (Khan *et al.*, 2011). However, the bodyweight of the broiler chickens raised under the heat-stressed environment is improved with dietary supplements and antioxidants [Zhao et al., 2015; Donkoh, 1989].

Presently, Chromium (Cr) is not yet generally considered an essential microelement for poultry. Still, it is thought that this trace element may play a beneficial nutritional and physiological role [Ognik*et al*., 2019]. Cr plays an essential role in the activation of certain enzymes and in stabilising the protein and nucleic acid [Ognik*et al.* 2019]. Khan et al. [2014] had earlier reported improved weight gain and reduced stress reactions in birds fed diets supplemented with Chromium. Despite these reported potentials of dietary Cr in poultry production, the National Research Council has not given its dietary inclusion recommended levels in poultry. Besides the beneficial effects of Cr, there is also a need for studies on the potentially toxic impact of wrong or inappropriate dosage in poultry. In addition, when combined with other antioxidants (e.g. vitamin E), Cr was reported to improve the stress influenced performance characteristics in laying chickens [Torki *et al*., 2017]. Vitamin E (VE) is a lipid-soluble antioxidant composed of eight compounds of similar structure, four tocopherols and four tocotrienols (TT) derivatives including α-, β-, γ- and δ-tocopherol and α-, β-, γ- and δ-tocotrienol. It is primarily bound to the hydrophobic interior of the cell membrane. It offers protection against injurious membrane oxidation by free radical scavenging [Birben*et al.* 2012] by donating an electron to lipid peroxidation products [Spiteller, 2006]. Vitamin E supplementation reduces the respiratory quotient in heat-stressed broiler chickens by supporting or enhancing increased fatty acid oxidation over the increase in protein-derived gluconeogenesis (Lin *et al.*, 2006)].

Therefore, the purpose of the present study was to evaluate the effects of dietary supplemental Chromium Propionate (CrProp) and vitamin E on the performance characteristics and haematological indices of broiler chickens.

II. MATERIALS AND METHODS

This feeding trial was carried out at the Avian Unit of The Federal University of Technology, Akure (FUTA) Teaching and Research Farm (TRF), during the peak of the dry season (i.e. between January and February 2020). The experimental pen's daily temperature-humidity index (THI) was 34.08°C±1.36. The THI was calculated (Tao and Xin, 2003) using the formula: THI= $0.85*T_{db}+0.15*T_{wb}$ Where T_{db} = dry bulb temperature (°C); T_{wb}= wet bulb temperature (°C).

2.1 Chromium Propionate and Vitamin E Source

The Chromium Propionate powder (purity level = 98%) was manufactured by Chemlock Nutrition Corporation (Cincinnati, OH, USA.), which provides 0.4% Cr. The L-alpha-tocopherol powder (purity level = 100% pure (USP/FCC grade) was manufactured by the Burgoyne Burbidges& Co (Supplies and Services) Limited, England.

2.2 Experimental Diets and Animals

A basal diet each was prepared for the starter (age 1-3 weeks) and the finisher (age 4-6 weeks) phases (Table 1) and analysed for proximate composition [AOAC.1995]. The basal diets were sundered equally into eight parts and labelled diets 1to 8 and supplemented as follows:

DESCRIPTION OF EXPERIMENTAL DIETS/TREATMENTS (T):

Treatment	Chromium source	Levels of Chromium	Level of Vitamin E
T1	Control	Basal diet+ Nil (Control)	Nil
T2	Chromium Propionate	Basal diet +0.4mg/kg	Nil
T3	Chromium Propionate	Basal diet + 0.8mg/kg	Nil
T4	Chromium Propionate	Basal diet + 1.2mg/kg	Nil
T5	Chromium Propionate	Basal + Nil	200mg
T6	Chromium Propionate	Basal diet +0.4mg/kg	200mg
T7	Chromium Propionate	Basal diet +0.8mg/kg	200mg
T8	Chromium Propionate	Basal diet +1.2mg/kg	200mg

Table 1. Composition of the experimental diets

Ingredients (%)	Starter feed	Finisher diet
Maize	52.35	59.35
Rice bran	0.00	6.00
Maize bran	7.00	0.00
Soybean meal	30.00	24.00
Soy oil	3.00	3.00
Fish meal	3.00	3.00
Limestone	0.50	0.50
Bone meal	3.00	3.00
Salt	0.30	0.30
Premix	3.00	3.00
Methionine	0.30	0.30
Lysine	0.25	0.25
Nutrient composition (%)		
*Crude protein	22.18	20.03
Metabolizable energy (Kcal/kg)	3018.89	3108.10
Methionine	0.68	0.66
Lysine	1.36	1.24
Available phosphorus	0.45	0.33
Calcium	1.01	0.99

2.3 Growth Performance

The body weights of the broiler chickens were measured on a weekly interval. The body weight gain was calculated by subtracting the birds' initial body weight from their final body weight and the initial body weight. The feed intake was also estimated by subtracting the quantity of feed given from the feed leftover. The total weight gain(TWG) was calculated by subtracting the initial weight from the final body weight.

2.4 Blood Sample Collection and Analysis

On day 42 of the experiment, three birds per replicate were randomly chosen, labelled, and phlebotomised with a syringe and needle via the wing vein.About 4 ml of blood was pass Out into Ethylenediaminetetraacetic acid bottles for haematological indices examination. The haematological studies were performed within 120 minutes post bleeding [Shastry.,1983];for red blood cells (RBC), packed cell volume (PVC), haemoglobin concentration (Hbc), White blood cells (WBC), granulocytes (GRA), lymphocytes (LYM) and monocytes (MON).

2.5 Data Analysis

All data were subjected to analysis of variance from the General Linear Model stratagem for complete randomised design with 4 CrProp levels x 2 Vitamin Elevels factorial setting of treatments. The data were checked for CrProp, Vitamin Eand interaction of CrProp with Vitamin E. When the treatment out-turn was significant (P<0.05), means were differentiated using Duncan's multiple range test using SPSS version 28.

III. RESULTS AND DISCUSSION

The final body weight (FBW) of the birds fed diets 2, 3, 4,7 and 8 were significantly (P<0.05) higher than those fed the control diet and diet 5 and 6, and total weight gain (TWG) of the birds fed diets 2,4,7and 8 were significantly (P<0.05) higher than those fed the control and diet 3,5,6. The improved final weight recorded in the broiler chickens fed diet 2 (0.4 mg/kg Cr Prop) , diet 3 (0.8 mg/kg Cr Prop), diet 4(1.2 mg/kg Cr Pro) diet 7 (0.8 mg/kg Cr Prop+200 mg/kg vitamin E) and diet 8 (1.2 mg/kg Cr Prop+200 mg/kg vitamin E), compared to those fed the control diet and diet 5 (200 mg/kg vitamin E) and diet 6 (0.4 mg/kg Cr Prop+200 mg/kg vitamin E) suggests Cr Prop supplementation at 1.2 mg/kg have growth performance-enhancing effects on the broiler chickens. Quite a few studies show that chromium Propionate dietary supplementation has a promoting effect on the growth performance of chickens [Kroliczewska*et al.*, 2005; Jackson *et al.*, 2008]. This result further unfolds another beneficial biological activity of Chromium when used as a dietary supplement [Khan *et al.* 2014]. CrProp improves growth by increasing insulin sensitivity, initiation of microRNA translation and consequently, the improvement in the stimulation of muscle protein synthesis [O'Connor *et al.* 2003). The vitamin E supplementation (200mg/kg) does not significantly (P>0.05) improve the final body weight, compared to the control. The feed intake of the broiler chickens fed diet 4 (1.2 mg/kg CrProp) increased (P<0.05) compared to those fed the rest diets. The 1.2 mg/kg CrPropsupplementation increased (P<0.05) the feed intake of the birds. In contrast,the interaction between CrProp and vitamin E significantly (P<0.05) affects the feed conversion ratio at 0.4 mg/kg Cr Prop and 0.8 mg/kg Cr Prop+200 mg/kg vitamin E.

Table 3 shows that the haematological indices of the birds, MCHC, WBC, Heterocyte, and lymphocyte counts were significantly (P<0.05) affected by CrProp. In contrast, vitamin E supplementationdoesaffect (P<0.05) the MCV, MCH values. The study showed that the CrProp in combination with vitamin E significantly (P<0.05) affects MCV, MCH, WBC and lymphocyte counts. The results of most blood indices across the various dietary treatments in this study show the supports of the CrPropand vitamin Eat the levels used in this study for normal blood formation. Granulocytes (neutrophils, eosinophils, and basophils) are phagocytes and possess granules of enzymes that digest the invading microbes.

Table 2. Effects of Chromium Propionate and vitamin Edietary supplementation on the performance characteristics of broiler chickens

CrProp (mg/kg	Vitamin E (mg/kg)	Initial weight (g/bird)	Final body weight (g/bird)	Total weight gain (g/bird)	Daily weight gain (g/bird)	Feed intake (g/bird)	Daily feed intake (g/bird)	FCR
Level of CrProp								
0		39.37±0.26	2601±58.61[b]	2562.60±58.63[b]	45.79±1.05[b]	5863.70±95.98[c]	104.71±1.71[c]	2.29±0.06
0.4		39.22±0.29	2679.17±106.51[b]	2639.95±106.26[b]	47.14±1.90[ab]	5976.47±82.30[b]	106.72±1.47[bc]	2.28±0.08
0.8		39.39±0.17	2880.97±67.55[ab]	2841.58±67.54[ab]	50.74±1.21[a]	6166.47±44.88[ab]	110.12±0.80[b]	2.17±0.04
1.2		40.29±0.51	2917.84±93.42[a]	2877.56±93.28[a]	51.38±1.67[a]	6403.90±121.41[a]	114.36±2.17[a]	2.23±0.06
Vitamin E								
	0	39.78±0.32	2817.20±49.80	2777.42±49.62	49.60±0.89	6075.97±91.97	108.50±1.64	2.19±0.02[b]
	200	39.36±0.15	2722.78±81.89	2683.42±81.81	47.92±1.46	6129.29±80.52	109.45±1.44	2.30±0.05[a]
CrProp x Vit E								
0	0	39.28±0.57	2667.43±94.15	2628.15±93.82[b]	46.93±1.68[b]	5734.99±152.69[b]	102.41±2.73[b]	2.18±0.06[a]
0.4	0	39.64±37.41	2873.26±37.41	2833.62±37.41[a]	50.60±0.67[a]	6032.07±23.64[ab]	107.72±0.42[ab]	2.13±0.02[a]
0.8	0	39.25±0.33	2832.94±113.37	2793.69±113.33[ab]	49.89±2.02[ab]	6153.27±88.70[ab]	109.88±1.58[ab]	2.21±0.06[b]
1.2	0	40.94±0.91	2895.15±124.49	2854.22±123.77[a]	50.97±2.21[a]	6383.57±211.99[a]	113.99±3.79[a]	2.24±0.04[b]
0	200	39.47±0.01	2536.51±63.47[ab]	2497.04±63.46[b]	44.59±1.13[b]	5992.40±78.63[b]	107.01±1.40[b]	2.40±0.03[c]
0.4	200	38.80±0.50	2485.07±132.84[b]	2446.27±132.46[b]	43.68±2.37[b]	5920.88±173.84[b]	105.73±3.10[b]	2.43±0.09[c]
0.8	200	39.53±0.14	2929.00±87.51[a]	2889.47±87.58[a]	51.60±1.56[a]	6179.67±45.05[ab]	110.35±0.80[ab]	2.14±0.05[a]
1.2	200	39.63±0.14	2940.53±166.22[a]	2900.90±166.26[a]	51.80±2.97[a]	6424.22±168.36[a]	144.72±3.01[a]	2.23±0.13[b]
Statistical significance								
CrProp		0.10	0.03	0.03	0.03	0.01	0.01	0.35
Vitamin E		0.20	0.24	0.24	0.24	0.58	0.58	0.04
CrProp x Vitamin E		0.24	0.04	0.03	0.02	0.04	0.03	0.05

Means with a different superscript in the same column are significantly (P<0.05) different; Cr Prop: Chromium Propionate

Table 3. Effects of Chromium Propionate and vitamin Edietary supplementation on the haematological indices of broiler chickens

CrProp (mg/kg	Vitamin E (mg/kg)	PCV (%)	RBC ($x10^6/1$)	HBc (g/d1)	MCHC (g/d1)	MCV (fl)	MCH (pg/cell)	WBC ($x10^9/1$)	HET (x109/1)	LYM ($x10^9/1$)	MON ($x10^9/1$)
Level of CrProp											
0		37.17±0.87	4.45±0.56	12.40±0.29	33.40±0.34	89.83±11.29^{a}	29.97± 3.77	4.47±0.94^{b}	2.08±4.19	2.30±0.42^{b}	0.08±0.02
0.4		38.17±1.64	4.90±0.43	12.73±0.55	33.42±0.34	81.52±8.73^{ab}	27.15±2.91	4.33±1.07^{b}	2.10±0.71	2.13± 0.39^{b}	0.08±0.05
0.8		40.67±1.17	4.95±0.65	13.57±0.39	33.83±0.36	87.67±8.70^{a}	29.25±2.89	8.88±1.47^{a}	3.20±0.52	5.60±1.05^{a}	0.11±0.04
1.2		39.67±1.09	4.98±0.09	13.22±0.37	34.45±0.15	79.78±2.87^{b}	26.58±0.96	5.87±1.41^{ab}	2.08±0.53	3.70±0.45^{ab}	0.08±0.04
Vitamin E											
	0	38.50±0.84	4.66±0.26	12.84±0.28	33.51±0.18	85.10±4.53	28.37±1.51	6.27±0.71	2.85±0.34	3.33±0.50	0.10±0.03
	200	39.33±0.98	4.98±0.38	13.12±0.33	34.04±0.28	84.30±6.93	28.11±2.31	5.51±1.22	1.88±0.43	3.53±0.80	0.08±0.03
CrProp x Vit E											
0	0	36.33±0.88	5.23±0.90	12.13±0.30	33.57±0.32	73.27±11.55^{b}	24.43±3.85^{b}	6.40±0.29^{ab}	3.17±0.26	3.13±0.29^{ab}	0.11±0.02
0.4	0	40.00±2.65	4.07±0.27	13.37±0.88	33.17±0.28	98.73±6.28^{a}	32.90±2.08^{a}	6.47±0.90^{ab}	3.47±0.78	2.87±0.20^{b}	0.13±0.10
0.8	0	40.00±1.53	4.30±0.21	13.33±0.52	33.10±0.26	93.33±4.60^{ab}	31.13±1.54^{ab}	8.67±1.79^{a}	3.17±0.79	5.43±1.33^{a}	0.09±0.05
1.2	0	37.67±0.67	5.03±0.20	12.53±0.23	34.20±0.20	75.07±3.29^{b}	25.00±1.10^{b}	3.53±0.52^{b}	1.60±0.50	1.90±0.46^{b}	0.05±0.02
0	200	38.00±2.65^{b}	3.67±0.59	12.67±0.91	33.23±1.16	106.40±26.26^{a}	35.50±8.75^{a}	2.53±1.33^{a}	1.00±0.72^{b}	1.47±0.57^{b}	0.06±0.02
0.4	200	36.33±1.76^{b}	5.73±0.39	12.10±0.59	33.67±0.66	64.30±6.75^{c}	21.40±2.25^{c}	2.20±0.58^{b}	0.73±0.18^{b}	1.40±0.40^{b}	0.02±0.01
0.8	200	41.33±2.03^{a}	5.60±1.27	13.80±0.67	34.57±0.19	82.00±18.03^{b}	27.37±5.99^{b}	9.10±2.76^{a}	3.23±0.84^{a}	5.77± 1.93^{a}	0.12±0.07
1.2	200	41.67±1.20^{a}	4.93±0.03	13.90±0.42	34.70±0.06	84.50±2.86^{b}	28.17±0.93^{b}	8.20±2.05^{a}	2.57±0.97^{a}	5.50±1.15^{a}	0.11±0.09
Statistical significance											
CrProp		0.19	0.79	0.20	0.05	0.04	0.71	0.02	0.05	0.01	0.96
Vitamin E		0.48	0.45	0.49	0.07	0.91	0.91	0.47	0.06	0.77	0.65
CrProp level*Vitamin E		0.05	0.06	0.15	0.19	0.02	0.02	0.02	0.46	0.05	0.45

Means with a different superscript in the same column are significantly (P<0.05) different; CrPic: Chromium Propionate; PCV: Packed cell volume; RBC: Red blood cell; HBc: Haemoglobin concentration; MCV: Mean cell volume; MCH: Mean cell haemoglobin; WBC: White blood cells; GRA: Granulocytes; LYM: Lymphocytes; MON: Monocytes; SEM: Standard error of the mean

IV. CONCLUSION

The 1.2mg/kg CrProp dietary supplementations improved the final body weight, total weight gain, daily weight gain and daily feed intake of the broiler chickens. Also, the 200 mg vitamin Esupplementation did not enhance the final body weight, total weight gain, daily weight gain and daily feed intake of the broiler chickens.

ETHICAL APPROVAL

This work was approved by the Research and Ethics Committee of the Animal Production and Health Department, The Federal University of Technology, Akure, Nigeria.

REFERENCES

[1] AOAC. (1995) Association of official analytical chemistry, official methods Of analysis(16th Ed.). Washington, DC; 1995.

[2] Ayo JO, Obidi JA, Rekwot PI. Effects of heat stress on the well-being, fertility and humidity of chickens in the Northern Guinea Savanah Zone of Nigeria: ISRN Veterinary Science. 2011;838606.DOI:10.5402/2011/838606.

[3] Ayo JO, Oladele SB, Fayomi A. Effect of heat stress on livestock production: a review. Nigerian Veterinary Journal. 1996; 1:58–68.

[4] Birben E, Sahiner UM, Sackesen C, Erzurum S, Kalayci O. (2012). Oxidative stress and antioxidant defense. World Allergy Organ J. 2012;5(1):9–19. https://doi.org/10.1097/WOX.0b013e3182439613.

[5] Donkoh A. Ambient temperature: a factor affecting performance and physiological response of broiler chickens. International Journal of Biometeorology. 1989.33(4): 259–265.

[6] Jackson, A.R., Powell, S., Johnston, S., Shelton, J.L., Bidner, T.D., Valdez, F.R., Southern, L.L.,2008. The effect of chromium propionate on growth performance and carcass traits in broiler. Research 17,476-483.

[7] Khan RU, Naz S, Dhama K, Saminathan M, Tiwari R, Jeon GJ, Laudadio V, Tufarelli V. Modes of action and beneficial application of Chromium in poultry nutrition, production and health: A review. International Journal of Pharmacology. 2014;10(7):357-367

[8] Khan RU, Naz S, Dhama K. 2014a. Chromium: pharmacological applications in heat-stressed poultry. Int J Pharm. 10(4):213–217. doi: 10.3923/ijp.2014.213.217

[9] Khan RU, Naz S, Nikousefat Z, Tufarelli V, Javdani M, Rana N, Laudadio V. 2011. Effect of vitamin E in heat-stressed poultry. Worlds Poult Sci J. 67(3):469–478. doi: 10.1017/S0043933911000511.

[10] KroliczewskaB., W. Zawadzki, T. Skiba, D. MiEta (2005): Effects of Chromium Supplementation on Chicken Broiler Growth and Carcass Characteristics. Acta Vet. Brno 2005,74: 543-549.

[11] Lin H, Jiao HC, Buyse J, Decuypere E. Strategies for preventing heat stress in poultry. World's Poultry Science Journal. 2006;62(1):71-85.

[12] Luo, X., Zheng, C., Xia, W., Ruan, D., Wang, S., Cui, Y., Yu, D., Wu, Q., Huang, D., Zhang, Y., Chen, W., 2018. Effects of constant or intermittent high temperature on egg production, feed intake, and hypothalamic expression of antioxidant and pro-oxidant enzymes genes in laying ducks. Journal of Animal Science 96, 5064–5074.

[13] O'Connor PM, Kimball SR, Suryawan A, Bush JA, Nguyen HV, Jefferson LS, Davis TA. Regulation of translation initiation by insulin and amino acids in skeletal muscle of neonatal pigs. American Journal of Physiology, Endocrinology and Metabolism. 2003;285(1):40-53

[14] Ognik K, Drazbo A, Stepniowska A, Kozlowski K, Listos P, Jankowski J. The effect of chromium nanoparticles and chromium picolinate in broiler chicken diet on the performance, redox status and tissues histology. Animal Feed Science and Technology. 2020;259:114326.DOI:10.1016/j.anifeedsci.2019.114326

[15] Oguntunji, O.M., Alabi, A.O. (2010). Influence of high environmental temperature on egg production and shell quality, a review. World's Poultry Science. J. 66, 739–774.

[16] Sahin K, Sahin N, Kucuk O, Hayirli A, Prasad AS. 2009. Role of dietary zinc in heat-stressed poultry: a review. Poult Sci. 88(10):2176–2183. doi: 10.3382/ps.2008-00560

[17] Sahin, N., Hayirli, A., Orhan, C., 2018. Effects of the supplemental chromium form on performance and metabolic profile in laying hens exposed to heat stress. Poultry Science 97, 1298–1305

[18] Spiteller G. (2006). Peroxyl radicals: inductors of neurodegenerative and other inflammatory diseases. Their origin and how they transform cholesterol, phospholipids, plasmalogens, polyunsaturated fatty acids, sugars, and proteins into deleterious products. Free RadicBiol Med.2006;41(3):36287. https://doi.org/10.1016/j.freeradbiomed.2006.03.013.

[19] Torki M., Karami, M and Mohammadi H. (2018). Effects of Dietary Supplemental Vitamin E and Chromium on Egg Production, Egg Quality and Blood Parameters of Laying Hens under Thermoneutral or Heat Stressed Conditions. Iranian Journal of Applied Animal Science (2018) 8(2), 295-303.

[20] Wang, R. H., Liang, R. R., Lin, H., Zhu, L. X., Zhang, Y. M., Mao, Y. W. and Luo, X. (2017). Effect of acute heat stress and slaughter processing on poultry meat quality and post mortem carbohydrate metabolism. Poultry Science 96(3), 738–746. [PubMed], [Web of Science ®], [Google Scholar]

[21] Yousaf, A., Jabbar, A., Rajput, N., Memon, A., Shahnawaz, R., Mukhtar, N., Farooq, F., Abbas, M., Khalil, R., 2019. Effect of environmental heat stress on performance and carcass yield of broiler chicks. World 9 (1), 26–30.

[22] Zhao Y, Ducharne A, Sultan B, Braconnot P, Vautard R. Estimating heat stress from climate-based indicators: present-day biases and future spreads in the CMIP5 global climate model ensemble. Environmental Research Letters. 2015; 10(8):084–013

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Profitability of blantic cattle traders as a supply chain institution in Minahasa Regency, Indonesia

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***Abstract*— *The blantic cattle traders as a supply chain institution in Indonesia play a role in the task of marketing cattle from farmer in the villages to buyers of cattle at the blantic cattle market. This research aims to study the profitability of blantic cattle traders in Minahasa Regency, Indonesia. The data were obtained from direct interviews with blantic cattle traders using questionnaires. The data were analyzed the factors that affect the profit with multiple linear regression. Blantic cattle traders profitability can be seen from the profit generated on profit and costs ratio (pcr) earned at 1.85% which is high. The number of cattle sold has a significant effect on the profits of blantic cattle traders, where every additional number of beef cattle sold by 1 head, the profit will increase by IDR 282,132.71, so that the more the number of cattle sold, the greater the amount of profit obtained. While age, education level, and work experience do not significantly affect the profits of blantic cattle traders. This is because the market demand for cattle tends to increase, therefore blantic cattle traders need to streamline their network with cattle farmers as a supply chain institution to maintain stock and total sales.*

Keywords*— *Blantic, cattle, profitability, supply chain.

I. INTRODUCTION

Distribution and marketing of cattle commodities is important to meet the increasing demand for food sources of animal protein in developing countries. This function is to facilitate the delivery of cattle from producers (farmers) to consumers. In distribution and marketing, supply chain has become an essential element (Al-Doori, 2019), due to cost reduction and improved customer service, suppliers are constantly looking for innovative ways in supply chain collaboration, involves collaborative work between buyers and suppliers, joint product development, common systems, and shared information (Alexander et al., 2014; Salam 2017; Saroha and Yadav, 2013).

Each supply chain has its own role and function in connecting the production sector to the consumption sector. However, in developing countries, the supply chain of agricultural commodities, including cattle, there is still a supply chain managed by traditional marketing institutions and practitioner participation in the market.

Market participation among beef cattle farmers is key to ensuring better income, food security, and sustainable beef supply. Farmers in the traditional beef cattle sector, nevertheless, are well known for their low market participation (Kibona and Yuejie, 2021), likewise, household beef cattle farmer in Minahasa Regency, Indonesia. Therefore, farmers in marketing their beef cattle need the help of intermediary cattle traders who market their cattle to global market or local traditional animal markets.

The beef cattle marketing system comprises numerous actors, including traders, brokers and butchers (Dahlanuddin et al., 2017). Cattle trading brokers in Minahasa Regency are known as "tukang blante" (blantic cattle trader), it is one of the supply chains institution that are still traditional, but location-specific and local wisdom, with location the "pasar blante" a traditional animal market. In addition, this blantic cattle market is accessed and used as a place to buy beef cattle by feedlot cattle breeders, cattle wholesale traders, inter-island traders and beef butchers from 12 regency and cities in North Sulawesi province.

The existence of blantic cattle trader and the traditional blantic cattle market has an impact on the advantages of 5 surrounding sub-districts so that it has the largest cattle production and population, and has become a center for cattle production in Minahasa Regency and even North Sulawesi Province. Even with transactions in traditional animal markets, it increases income for cattle farmers, blantic cattle traders and is proven to contribute to increasing regional income and driving economic activities in the region (Elly, 2009; Kimbal et al., 2012).

The blantic cattle traders play a role in the task of marketing cattle from farmer in the villages to buyers of cattle at the blantic traditional animal market, which has been going on for a long time, survived and continued until the modern marketing era. So it is necessary to study the profitability of blantic cattle traders in Minahasa Regency.

II. MATERIALS AND METHODS

1. Site, Time and Data Collection

This research was conducted in Kawangkoan, Minahasa Regency, North Sulawesi Province, Indonesia. The research was carried out from April to June 2021. The data used in this study were obtained from direct interviews with blantic cattle traders at the traditional blantic cattle market, using prepared questionnaires. Research respondents are blantic cattle traders in the traditional blantic cattle market, where cattle traders who can sell cattle in this market are only specifically for blantic cattle traders. The population of cattle traders in this blantic market is more than 100 traders, and about 30 to 65 traders (permanent and seasonal traders) every market day. The sample of respondin ents was selected based on the purposive sampling method with several criteria (Etikan et al., 2016), for this study the criteria were permanent (not seasonal) blantic cattle traders, blantic experience of more than 5 years and willing to be a respondent. Blantic cattle traders who met the criteria and were selected as respondents were 30 blantic cattle traders.

2. Data Analysis

The profitability of trading cattle blantic has been measured using profit analysis, with the formula according to Malope et al. (2007), where the notation is changed according to this study:

$$Y = TR - TC \;\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

Where: Y is the profit of the blantic cattle trader, TR is total revenue of the blantic cattle trader, and TC is total costs of the blantic cattle trader.

Furthermore, the analysis of factors that influence global profits is analyzed by multiple linear regression, according to the model from Gujarati (2003) :

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_4 + u \;\ldots\ldots \quad (2)$$

Where Y is the profit of the blantic cattle trader, X_1 is the number of cattle sold, X_2 is the respondent's age, X_3 is the respondent's education,

X4 is the experience of the blantic cattle trader, $\beta 0$ is a constant, $\beta 1$, $\beta 2$, $\beta 3$, $\beta 4$ is the regression coefficient for each variable X, and u is the stochastic error.

Furthermore, the model has been statistically analyzed using the F test to determine the simultaneous effect of the independent variables (Xi) on the dependent variable (Y). While the t test has been used to test the effect of each independent variable (Xi) partially on the dependent variable (Y). The data analysis has used SPSS Statistics software version 25.0.

III. RESULTS

1. Blantic cattle market system in Kawangkoan, Minahasa Regency

Blantic cattle market system in Kawangkoan, Minahasa Regency showed in figure 1, where only cattle blantic traders (cattle brokers) can sell cattles in the traditional blantic cattle market. Cattle blantic traders serve as intermediaries in the buy and sell of cattle between farmers and buyers. Traders serve to help cattle farmers who want to sell their cattle in the animal market, where between farmer and blantic trader deal on a minimum selling price for cattle in the animal market. Likewise, the amount of commission that farmers have to pay to blantic traders according to an agreement that depends market price to be sold at a minimum price from the farmer. If the cattle are sold at the same minimum on the animal unit, the blantic trader will only receive commission income from the farmer as agreed. Meanwhile, if the trader succeeds in selling the cattle above the agreed price with the farmer, the traders receive two kind revenues in the form of commissions from farmers and sales margins (the difference between the selling price and the price agreed with the farmers).

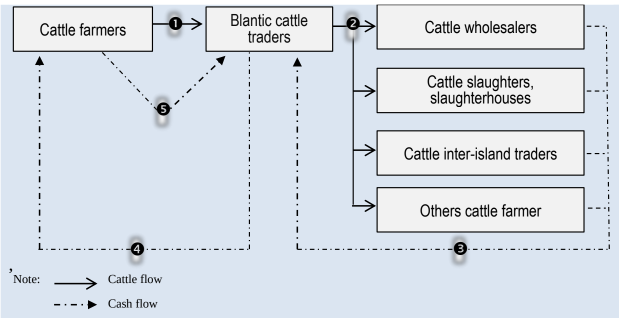

Figure 1 Blantic cattle market system in traditional cattle market in Minahasa

Based on Figure 1 shows the process of the cattle marketing system in the blantic cattle market, Minahasa Regency, as follows:

(1) Cattle farmers in rural area who want to sell their cattle, contact blantic cattle traders to market their cattle. Farmers and blantik will then carry out a dealing process regarding the price of cattle to be sold in the cattle market, with a commission that will be given by farmers to blantic traders. After an agreement is reached, then the blantic cattle trader will pick up the cattle owned by the farmers using an open ladbak car and bring and sell them to the blantic cattle market. Meanwhile, cattle in the rural around the market are only herded by blantic cattle traders on foot to the market.

(2) Blantic traders then bring and sell cattle to buyers (cattle wholesaler, cattle slaughter/slaughterhouses, inter-island traders and others cattle farmer) at the blantic cattle market.

(3) After the buy and sell transaction process takes place (deal), the buyer pays the cattle price to the blantic trader. It often happen, buyers also give commissions voluntarily to blantic cattle traders as has become the custom in this blantic cattle market.

(4) After successfully selling the cattle owned by the farmers in the blantic market, the blantic cattle traders will return to the cattle farmers, then pay or give some money from the sale of the cattle in the blantic market to the cattle farmers.

(5) The farmer then gives a commission to the blantic trader as a service for selling the cattle owned by the farmer according to the commission value they have agreed upon in advance.

2. Characteristics of Respondents

The number of cattle traders in this blantic market is is not much, but it can drive the development of cattle production in the area around the blantic cattle market and drive an increase in the regional economy. From the population, the number of research samples successfully obtained data totaling 30 respondents who met the renpondent criteria namely permanent (not seasonal) blantic cattle traders, blantic trader experience of more than 5 years and willing to be a respondent, with the characteristics consist of age, education level and experience of blantic cattle trader.

Factor age play an important role in human resource and management decisions (Garner and Campos, 2014), and productivity reductions at older ages are particularly strong for work tasks where problem solving, learning and speed are needed, while in jobs where experience and verbal abilities are important, older individuals' maintain a relatively high productivity level (Skirbekk, 2004; Pinto et al., 2014).

Table 1 Characteristics of Blantic Cattle Traders Respondents

No.	Description of characteristics	Proportion of number of respondents by characteristics (%)
1.	Age (years)	
	15 - 30	3.33
	31 - 45	26.67
	46 - 60	46.67
	> 60	23.33
	Total	100,00
2.	Education	
	Primary school	13.33
	Junior high school	26.67
	Senior High School	56.67
	College	3.33
	Total	100,00
3.	Blantic trader experience (years)	
	≤ 10	33.33
	11 - 20	33.33
	21 - 30	13.33
	31 – 40	6.67
	41 - 50	13.33
	Total	100,00

Table 1 shows the age of 73.74 % of respondents ranged from 31 to 64 years. This result is slightly different from the research of Montin et al. (2019) that the age of 64% of people ranged from 25 to 54 years, that represent the characterization of the age of the Brazilians people. The classification of respondents based on the age of blantic traders where most of the age of blantic traders are 46 to

60 years old (senior blantic traders) as much as 46.67%, while the fewest are young people aged 15 - 30 years in fact only 3.33% who are generally kids or former laborers by senior blantic traders. This corresponds to Keating and Little (1997) that parents want that from an early age, children receive messages from their parents about the advantages in their business.

Characteristics of respondents based on education level showed that most of the high school graduates were 17 people or 56.67%, while the least at the college education level was only 1 person with a percentage of 3.33%. High school education level is a good average education that can plan, implement and evaluate. This is because there is a positive relationship between work performance and education level (Doğuş, 2007).

The results showed that the experience of working blantik traders based on Table 1, the highest working experience of blantik traders is at the age of 3 - 10 years as much as 33.33%, and at the age of 11 - 20 years as much as 33.33%, while the lowest working experience is 31 - 40 years as much as 6.67%. In general, respondents have had sufficient experience in trading blantic cattle, so that with this experience, respondents are able to handle all jobs well, this is according to Putri (2020) that work experience has a positive effect on work performance, while job characteristics do not affect work performance.

3. Costs, Revenues and Profit of the Blantic Cattle Trader

Profit is obtained from the results calculation of total revenue less the results calculation of total costs, as showed in equation (1). Total costs for the blantic cattle traders are the costs incurred in the business activities of buy and sell cattle, starting from the costs to cattle farmers in rural, at the blantic cattle market, and returning to cattle farmers in rural, and so on, which consists of transportation, cattle feed and labor costs, and market administrative and fee costs in the blantic cattle market.

The revenue of blantic cattle traders comes from two sources. First, the revenue from the sales margin between the agreed price with the cattle farmer and the transaction price paid by the cattle buyer in the blantic cattle market; and second, the revenue earned by blantic cattle traders as blantic services as blantic services provided by cattle farmers when blantic cattle traders hand over the money from the sale of cattle to the cattle farmers.

Based on Table 2 shows that the total costs incurred by blantic cattle traders are IDR 755.647,500/year with an average of IDR 25,188,250/year per blantic cattle traders. Meanwhile, the income of blantic traders is IDR 2,150,945,500/year with an average of IDR 71,698,183/year per respondent. Thus the results of the study indicate that the profit which is the income of blantic cattle traders is IDR 1,395,298,000/year or an average of IDR 46,509,933/year per respondent. Business effectiveness and efficiency can be seen from the profit generated on profit and costs ratio (pcr). Profit and costs ratio = (profit/total costs) x 100% earned at 1.85% which is high, where the higher the ratio value, the better the business blantic cattle traders condition based on the profitability ratio.

Table 2 Costs, Revenues and Profitability of Blantic Cattle Traders

Description	Amount (IDR/year)	Average (IDR/year/ respondent)
(1) Cost		
▪ Transportation	499,200,000	16,640,000
▪ Cattle feed	14,565,000	485,500
▪ Labor	195,600,000	6,520,000
▪ Market fee	23,200,000	773,333
▪ Market administration	23,082,500	769,417
Total Cost (*TC*)	755,647,500	25,188,250
(2) Revenue		
▪ Margin of cattle sales by blantic traders	1,680,000,000	56,000,000
▪ Blantic service revenue (commissions) from cattle farmers	470,945,500	15,698,183
Total Revenue (*TR*)	2,150,945,500	71,698,183
(3) Profit ($TR - TC$)	1,395,298,000	46,509,933

4. Influence of factors on profitability of the blantic cattle trader

There are a number of factors that contribute to the success or failure of a business, for most businesses success is measured by profit. Moreover, while there are a number of factors that contribute to profitability (Zelles, 2015), where for this study the factors or variables related to profitability are the number of cattle sold, age, education and work experience. The results of multiple regression analysis of the effect of variables on the profitability of blantic cattle traders in the traditional blantic cattle market in Kawangkoan, Minahasa Regency can be seen in Table 3.

Based on the results of multiple regression analysis in Table 3, it is known that the R-Square value or the coefficient of determination of the regression results is 0.634. This means that the variable number of cattle sold, age of respondent, education of respondent, and blantic cattle trading experience affects the dependent variable on the income of blantic traders (Y) by 63.4%, while 36.6% is influenced by other variables that are not discussed in this study. The result of the calculation of the F-calculated value is 10.818 ($p<0.01$). This means that the independent variables of the number of cattle sold (X1), age (X2), education (X3), and work experience (X4) simultaneously have a significant effect on the income variable of blantic cattle traders in Minahasa Regency.

Table 3 Regression Analysis Result Of The Influence Of Factors On The Profitability Of Blantic Cattle Traders In The Traditional Blantic Cattle Market

Variables	Coefficients	t-calc	Sig.
Constant	12,269,641.670	1.204	0.240
Number of cattle sold (X_1)	282,132.716	6.284	0.000**
Age (X_2)	24,989.551	0.212	0.833
Education (X_3)	627,500.293	1.362	0.185
Blantic cattle trading experience (X_4)	4,970.531	0.059	0.954
R-square			0.634
F-calculated			10.818**

Note:

**) is very significant on the significance level $p <$ 0.01.

IV. DISCUSSION

The regression coefficient of the variable number of beef cattle sold (X_1) is 282,132.71, meaning that for every additional number of beef cattle sold by 1 head, the profit will increase by IDR 282,132.71, so that the more the number of cattle sold, the greater the amount of profit obtained. This is because the market demand for cattle tends to increase, so it is filled with imports. The main problem of cattle development in Minahasa Regency is the gap between demand (the needs) and supply (availability) of either cattle or feed (Osak et al., 2020). Based on the results of significant analysis obtained a significant value of 0.000 ($p < 0.01$) indicating that the variable number of cattle sold has a very significant effect on the profits of blantic cattle traders. The sale of beef cattle mainly depends on the number of cattle stock of the farmer (Kibona, 2021), therefore blantic cattle traders need to streamline their network with cattle farmers to maintain stock and total sales. Usually they get more profit than the farmer producers, and depend of beef cattle sold at a time (Dinku, 2019).

The regression coefficient of the age variable (X_2) is 24,989.55, meaning that for every 1 year addition to the age of the blantic trader, the profit will increase by IDR 24,989.55. However, based on the results of significant analysis, a probability value of 0.833 ($p > 0.05$) indicates that the age variable has no significant effect on the income of blantic cattle traders. This means that the profits of the older blantic traders are not significantly different from the earnings of the younger blantic traders. In contrast to Skirbekk (2004) that productivity reductions at older ages are particularly strong when problem solving, learning and speed are important, while older individuals maintain a relatively high productivity level in work tasks where experience and verbal abilities matter more. The oldest employees have a lower individual productivity potential than the middle-aged employees. It seems to be most demanding for the oldest workers to keep up with individual productivity potential of younger workers with high average skill loss for the oldest age group or high average skills level for all age groups or both (Børing and Grøgaard, 2021).

The regression coefficient of the education variable (X_3) is 627,500.29, meaning that for every additional 1 year of education for blantic traders, the profit will increase by IDR 627,500.29. Based on the results of significant analysis obtained a probability value of 0.185 ($p>0.05$) which indicates that the variable level or length of education has no significant effect on the profit of blantic traders. Entrepreneurs with higher levels of education should be able to generate greater income. However, in practice the level of education does not really affect the business of blantic traders, this is because of their tenacity and hard work that keeps them qualified. While other opinions that the level of education is an important factor that influences the mindset and performance of human resource. The significant positive effects of formal education and the low of effects of skills on the individual productivity potential (Børing and Grøgaard, 2021).

The regression coefficient for the blantic trader experience variable (X_4) is 4,970.53, meaning that for every additional 1 year of experience working as a blantic trader, the profit will increase by IDR 4,970.53. However, based on the results of significant analysis, a probability value of 0.954 ($p>0.05$) was obtained where this result showed that the experience variable had no significant effect on the profits of blantic traders. Working time, in its various dimensions, includes available empirical evidence influencing the types of flexible working time arrangements (Golden, 2012) and work experience was not äs important for successfull job performance, however, found a correlation between work experience and job performance (Ochonma et al., 2018; Hunter, 2017).

V. CONCLUSION

Blantic cattle traders profitability can be seen from the profit generated on profit and costs ratio (pcr) earned at 1.85% which is high. The number of cattle sold has a significant effect on the profits of blantic cattle traders, where every additional number of beef cattle sold by 1 head, the profit will increase by IDR 282,132.71, so that the more the number of cattle sold, the greater the amount of profit obtained. While age, education level, and work experience do not significantly affect the profits of blantic cattle traders. This is because the market demand for cattle tends to increase, therefore blantic cattle traders need to streamline their network with cattle farmers as a supply chain institution to maintain stock and total sales.

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REFERENCES

[1] Aldoori, J.A. 2019. The impact of supply chain collaboration on performance in automotive industry: Empirical evidence. Journal of Industrial Engineering and Management, 12(2), 241-253. doi: http://dx.doi.org/10.3926/jiem.2835

[2] Alexander, A., H. Walker, M. Naim. 2014. Decision theory in sustainable supply chain management: a literature review. Supply Chain Management: An International Journal, 19(5/6), 504-522. doi: https://www.emerald.com/insight/content/doi/10.1108/SCM-01-2014-0007/full/html

[3] Børing, P., J.B. Grøgaard. 2021. Do Older Employees Have a Lower Individual Productivity Potential than Younger Employees. Journal of Population Ageing. doi: https://doi.org/10.1007/s12062-020-09323-1.

[4] Dahlanuddin, B. Henderson, Dizyee, K., Hermansyah, A. Ash. 2017. Assessing the sustainable development and intensification potential of beef cattle production in Sumbawa, Indonesia, using a system dynamics approach. PLoS ONE, 12(8), e0183365. doi: https://doi.org/10.1371/journal.pone.0183365

[5] Dinku, A., B. Abebe, A. Lemma, M. Shako. 2019. Beef cattle value chain analysis: Evidence from West Hararghe Zone of Ethiopia. International Journal of Agricultural Science and Food Technology, 5(1), 077-087. doi: https://doi.org/10.17352/2455-815X.000046.

[6] Elly, F.H., B.M. Sinaga, S.K. Kuntjoro, N. Kusnadi. 2008. Development of smallholder cattle farming through integrated cattle-crops in North Sulawesi. Jurnal Litbang Pertanian, 27(2), 63-68. http://203.190.37.42/publikasi/p3272084.pdf

[7] Etikan, I., S.A. Musa, R.S. Alkassim. 2016. Comparison of convenience sampling and purposive sampling. American Journal of Theoretical and Applied Statistics, 5(1), 1-4. doi: https://doi.org/10.11648/j.ajtas.20160501.11

[8] Garner, E., A.P. O'Campos. 2014. Identifying the family farm: an informal discussion of the concepts and definitions. ESA Working Paper, 14-10. Rome, FAO.

[9] Golden, L. (2012). The effects of working time on productivity and firm performance: a research synthesis paper. International Labour Office. Conditions of Work and Employment Branch. Geneva: ILO.

[10] Gujarati, D.N. 2003. Basic Econometrics. 4th edition. New York: McGraw-Hill.

[11] Gürbüz, A. 2007. An assesment on the effect of education level on the job satisfaction from the tourism sector point of view. Doğuş Üniversitesi Dergisi, 8(1), 36-46. https://core.ac.uk/download/pdf/47254406.pdf

[12] Hunter, Jr.D.L. 2017. Using Work Experience to Predict Job Performance: Do More Years Matter? San Francisco, California: San Francisco State University.

[13] Keating, N.C, H.M. Little. 1997. Choosing the successor in New Zealand family farms. Family Business Review, 10(2), 157-171. doi: https://doi.org/10.1111/j.1741-6248.1997.00157.x

[14] Kibona, C.A., Z. Yuejie. 2021. Factors that influence market participation among traditional beef cattle farmers in the Meatu District of Simiyu Region, Tanzania. PLoS ONE 16(4): e0248576. doi: https://doi.org/10.1371/journal.pone.0248576

[15] Kimbal R.W., A. Suman, K. Azhar, A. Manzilati. 2012. Social capital in non-barter transaction chain in pasar blante Kawangkoan, North Sulawesi Province. Journal of Indonesian Economy and Business 27(3), 336-354.

[16] Malope, P., C.M. Tsopito, A.A. Aganga, O.R. Madibela. 2007. Profitability of dry season beef feed-lotting in grain deficit countries: the case of Botswana. Livestock Research for Rural Development, 19(6) #81. http://www.lrrd.org/lrrd19/6/malo19081.htm

[17] Mottin, C., C.E. Eiras, D.M. Chefer, V.da.C. Barcellos, T.R. Ramos, I.N.do. Prado. 2019. Influencing factors of consumer willingness to buy cattle meat: An analysis of survey data from three Brazilian cities. Acta Scientiarum. Animal Sciences, 41(1), e43871. doi: https://doi.org/10.4025/actascianimsci.v41i1.43871

[18] Ochonma, O.G., C.O. Nwodoh, G.J. Ani, E.O. Dyages, J.A. Ingwu, S.E. Igwe. 2018. Assessing the impact of years of work experience on managers' job performance: hospital managers' perspectives in a developing country. International Journal of Advanced Academic Research, 4(4), 47-64. https://www.ijaar.org/articles/Volume4-Number4/Sciences-Technology-Engineering/ijaar-ste-v4n2-feb18-p4.pdf

[19] Osak, R.E.M.F., M.L. Rundengan, T.F.D. Lumy. 2020. Impact of alternative feed technology utilization, between the increase in feed cost and revenue on rural cattle farming. IOP Conference Series: Earth and Environmental Science, 575 012077. doi: https://doi.org/10.1088/1755-1315/575/1/012077
[20] Pinto, A.M.G., R.S.C.M. da Silva, S.M.M.D. Nunes. 2014. Managing an aging workforce: What is the value of human resource management practices for different age groups of workers. Review of Applied Management Studies, 12, 58-68. doi: https://doi.org/10.1016/j.tekhne.2015.01.007
[21] Putri, I.A.J. 2020. The effect of work experience and characteristics of jobs on employee work achievement in shipping company. Social Sciences and Education Research Review, 7(1), 285-302. https://sserr.ro/wp-content/uploads/2020/07/SSERR_2020_7_1_285_302.pdf
[22] Skirbekk, V. 2004. Age and Individual Productivity: A Literature Survey. Vienna Yearbook of Population Research, 2(1), 133-153. https://www.jstor.org/stable/23025440
[23] Salam, M.A. 2017. The mediating role of supply chain collaboration on the relationship between technology, trust and operational performance An empirical investigation. Benchmarking: An International Journal, 24(2), 298-317. doi: https://doi.org/10.1108/BIJ-07-2015-0075
[24] Saroha, P., S.K.S. Yadav. 2013. Supply Chain Management: A Tool of Business Process Integration. International Multidisciplinary e-Journal, 2(11), 32-55.
[25] Zelles, R. 2015. Better profitability through higher employee engagement in the knowledge worker age. Journal of Human Resource Management, 11(8), 62-72. https://www.jhrm.eu/wp-content/uploads/2015/03/JournalOfHumanResourceMng2015vol18issue2-pages-62-72.pdf

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An Assessment of the Role of ISO 14001 Certified Environmental Management Systems (EMS) in Underpinning Environmentally Sustainable Airline Operations

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***Abstract*— *Based on an in-depth qualitative instrumental case study research approach, this study has examined the airlines that have implemented an ISO 14001 certified Environmental Management Systems (EMS). The study period was from 1990 to 2021. The qualitative data was analyzed by document analysis. The case study revealed that airlines located inBahrain, Canada, Ethiopia,Europe, Hong Kong, Japan, Singapore, South Korea, Taiwan, Thailand, Turkey,and the United States of America have implemented Environmental Management Systems (EMS) in accordance with the ISO 14001 based Environmental Management Systems (EMS) standard. The ISO 14001 Environmental Management System standard has been adopted by full-service network carriers, two leisure airlines, and by two major air cargo airlines. South Korea-based Asiana Airlines was the first airline to be ISO 14001 Environmental Management System (EMS) certified in 1996.Since the release of the ISO 14001:2015 Environmental Management System (EMS) standard, eight airlines have adopted this standard.The case study revealed that the use of an ISO 14001 certified Environmental Management Systems (EMS) underpins airlines environmentally sustainable operations. As part of their environmental management policies, airlines that have implemented an ISO 14001 Environmental Management System (EMS) have implemented a wide range of environmental conservation measures, which include the acquisition and deployment of the next generation, fuel efficient aircraft, the use of sustainable aviation fuel, energy efficient flight operations and air traffic management procedures optimization, aircraft weight reductions, aircraft engines washing, single engine aircraft taxying, sustainable waste management, electrification of ground service equipment (GSE) and vehicles, the use of photovoltaic (PV) solar systems, carbon offsetting programs, water conservation, and energy efficient offices and facilities.*

Keywords*—Airlines, Case study, ISO 14001 Environmental Management System (EMS), Environmental conservation measures.

I. INTRODUCTION

At a world-wide level, the environmental sustainability of air transport is receiving greater attention because of its critical impact on climate change(Teoh & Koo, 2016).In the air transport industry, passenger services are provided by full-service network carriers (FSNCs), low-cost carriers (LCCs), regional airlines, leisure airlines, andcharter airlines (Whyte & Lohmann, 2017). The carriage of air cargo is another important market with air cargo services being provided by full-service network carriers (FSNCs), dedicated all-cargo airlines, for example. Cargolux International Airlines and Nippon Cargo Airlines (NCA), and the integrated carriers, such as, DHL Express and FedEx (Baxter & Bardell, 2017; Baxter & Wild, 2021; Merkert & Alexander, 2018). As a byproduct of their passenger and air cargo services, air transport operations have a very substantial impact on the environment (Daley, 2016; Kumar et al., 2020; Schäfer & Waitz, 2014). These impacts include emissions, noise, wastes, and significant water consumption. To address their environmental impact, many airlines located around the world have recognized the importance of protecting the environment (Niu et al., 2016). Environmental issues in airline transport have grown in importance in recent times, and in response airlines have taken a proactive position by "greening" their operations (Hagmann et al., 2015; Mayer et al., 2012;Migdadi, 2020). Consequently, "greening" has become one of the most important emerging issues in the airline industry (Han et al., 2020).

The implementation of ISO 14001 certified Environmental Management Systems (EMS) has become one of the most important elements of corporate sustainability in recent times (Zobel, 2013). Environmental Management Systems (EMS) are now one of the principal tools used by firms to handle the environmental aspects and the impacts that their activities have on the environment (Campos et al., 2015; Ikram et al., 2019; MacDonald, 2005). Like many other industries, many airlines have now implemented ISO 14001 certified Environmental Management Systems (EMS)in recent times, and they use these systems as a tool to manage their environmental performance and to mitigate their environmental impact.

The key objective of this study is to empirically examine the airlines located around the world that have implemented an ISO 14001 certified Environmental Management System (EMS) as a tool to manage their operations in an environmentally sustainable manner. A second objective is to identify those airlines that have implemented an IS0 14001: 2015 Environmental Management System, which is the latest version of this ISO standard.A final objective is to examine the environmental mitigation measures implemented by the airlines that have adopted the use of an ISO 14001 certified Environmental Management System (EMS).

The remainder of the paper is organized as follows: The literature review is presented in Section 2. The research method that underpinned the study is outlined in Section 3. The case study is presented in Section 4. Section 5 presents the key findings of the study.

II. BACKGROUND

2.1 Environmental Impact of Airline Operations

Air transport has an environmental impact, particularly its impacts on climate change (Forsyth, 2011; Baumeister, 2020; Baumeister&Onkila, 2017). There is an environmental impact from its use of non-renewable resources (Forsyth, 2011).Air transport operations influence the environment at the local, regional, and global levels (Dileep, 2019; Marais et al., 2016).

A significant environmental impact of air transport operations are emissions (Graham, 2018). Indeed, the growth of commercial air transport has driven concerns over air quality around airports and their surrounding communities (Lobo et al., 2012). Aircraft operations are a growing source of greenhouse gas (GHG) emissions (Baer, 2020).Aircraft emissions produce air contaminants such as nitrogen oxides (NO_x), hydrocarbons (HC), and fine particulate matter (PM) (International Civil Aviation Organization, 2011). Aircraft emissions are contributing to climate change and to localized air pollution (Daley, 2016). Aircraft pollutant emissions may also directly or indirectly harmfully impact ecosystems and cultural heritage (Kurniawan &Khardi, 2011).Aircraft operating at subsonic speeds in flight have the following environmental impacts:

- Carbon dioxide (CO_2): CO_2 emissions are the most common and are acknowledged as a major contributor to climate change (Akpan & Akpan, 2012; Azarkamand et al., 2020;Sales, 2016).
- Oxides of nitrogen (NOx): at high altitudes NOx emissions help to form ozone in the upper troposphere.
- Water vapor (H2O): this is created through the burning of jet aircraft fuel. At altitude, condensation trails form, consisting of frozen ice crystals that deflect a small amount of sunlight away from the Earth's surface and reflect more radiation back towards the Earth. This results in an overall warming effect on the Earth's atmosphere (Sales, 2016, p.146).

Aircraft often travel long distances at a variety of altitudes, generating emissions that may potentially have an impact on air quality in not only local, but also regional and global environments (International Civil Aviation Organization, 2011).

Ground support equipment (GSE): for example, aircraft push-back tugs, aircraft loaders, and flight catering trucks,that are used to handle an aircraft whilst it is on the ground in between flightsare typically powered by diesel or petrol engines (Baxter et al., 2021), and hence,they also produce exhaust emissions. Ground service equipment (GSE) refers to vehicles and equipment that are used in the airport precinct to service whilst they are at the gate in between flights (Hazel et al., 2011).Thus, gaseous emissions of carbon dioxide (CO_2), as well as carbon monoxide (CO) and nitrous oxide (NO_x) and particulates (PMs) from aircraft, ground access transport, such as buses and taxis, on-airport ground transport vehicles, all negatively impact local air quality (Budd, 2017).

In recent years, aircraft noise disturbance and its impact on communities surrounding airports has become one of the most significant concerns affecting airport operations and developments (Gorji-Bandpy& Azimi, 2013; Reynolds, 2016; Young & Wells, 2011). Noise from airport operations may have a negative impact on the quality of life and the property values of members of a surrounding community (Luther, 2007; Suksmith&Nitivattananon, 2015). The major source of noise at airports is from aircraft, and this occurs during aircrafttake-off, landing and when aircraft are taxiing to and from the terminal and airportapron area (de Neufville &Odoni, 2013).An airport's apron area is the location where aircraft stands interface with airport terminal buildings, and they are the location where aircraft are handled whilst on the ground in between flights (Budd & Ison, 2017). Another source of noise at an airport is from the operation of diesel generators or other mobile ground power units that are used to provide power to aircraft parked and which are being serviced (handled) on the apron at airport terminals. A further source of noise is from the use of aircraft auxiliary power units (APU's) (Bennett & James, 1999).Aircraft auxiliary power units APU's are small gas turbine engines that are typically mounted in the rear of the aircraft fuselage and supply the essential requirements of the aircraft whilst it is on the ground at the airport and without the main engines operating, or when no external power source is available (Smith, 2004). However, this source of noise can be eliminated from the installation and use of fixed electrical ground power (FEGP) stations (Bennett & James, 1999) as such systems eliminate the requirement for airlines to use APU's whilst the aircraft is being serviced at the gate (Elmer & Leigland, 2014).

Airlines also produce large volumes of wastes from their in-flight services and ground-based operations (Baxter, 2020).Each year the airline industry generates a substantial amount of comingle waste (Blanca-Alcubilla et al., 2019).Consequently, in recent times. waste management and waste disposal have become one of the most significant issues in the environmental management in the world airline industry (Baxter, 2020; Li et al., 2003). As a result, airlines are now making substantial efforts to improve their waste management and reduce theirwaste generation (Blanca-Alcubilla et al., 2019; Moynihan &Walków, 2019).

Airlines are also energy intensive. The largest energy source is aircraft jet fuel which, as previously noted, produces harmful pollutants. Airlines also consume large amounts of electricity to power their ground-based facilities and buildings. Diesel and petrol are commonly used to power ground service equipment (GSE) and vehicles (Baxter et al., 2021).Environmental sustainability related concerns over the past few decades have resulted in the dramatic progress in aviation fuel efficiency improvements (Singh et al., 2018). The next generation aircraft, such as the Airbus A350-900XWB and the Boeing 787-9/10 being operated by the world's airlines are more fuel efficient, and thus, have lower emissions levels.The Airbus A350-900XWB aircraft, for example, offers a 25% improvement in fuel efficiency and a 25% lower seat-mile (seat kilometre) cost when compared to its aluminum-based long-range competitors, such as the Boeing B777 aircraft (Otley, 2019).

Airlines also consume large volumes of water. Water is carried on flights and is used on the ground inflight catering, aircraft, and ground service equipment (GSE) maintenance, in buildings and facilities and for maintain grounds and gardens.Importantly, water utilization can have a detrimental impact upon the environment (Payán-Sánchez et al., 2021). Water quality around an airport's precinct can be adversely impacted by runoff from aircraft and airport winter de-icing operations, as well as fuel leaks, and solid and liquid waste treatment and disposal (Marais et al., 2016).

2.2 ISO 14001 Environmental Management Systems (EMS)

Many firms operating around the world have implementedEnvironmental Management Systems(EMS), and firms have taken the additional step and had their Environmental Management Systems (EMS)certified in accordance with the international standard ISO 14001 (Jiang & Bansal, 2003). Thus, energymanagement system standards, also referred to as meta-standards, have now been widely implemented by an increasing number of businesses around the world (Heras-Saizarbitoria&Boiral, 2013). The International Organization for Standardization (ISO) has developed a series of voluntary standards and guidelines in the field of environmental management. These are referred to as the EN ISO 14000 series. These standards have been designed to provide an internationally recognized framework for environmental management, measurement, evaluation, and auditing (Škurla et al., 2002). The most important standard is ISO 14001 which was introduced in 1996 (Bansal & Bogner, 2002; Chin et al., 1999; Curkovic& Sroufe, 2011). The standard was slightly modified again in 2004 (de Vries et al., 2012). On September 15, 2015, the International Organization for Standardization (ISO) released the ISO 14001: 2015 standard, which had revised previous versions of the system (International Organization for Standardization, 2015).

According to Massoud et al. (2010), "the ISO 14000 series of international standards have been developed to integrate environmental aspects into processes and product standards". Accordingly, the ISO 14001 standard describes the requirements for a certifiable Environmental Management System (EMS) (Sartor et al., 2019). ISO 14001 is a global meta-standard for implementing Environmental Management Systems (EMS) (Heras-Saizarbitoria et al., 2011; Laskurain et al., 2017; Liu et al., 2020). The ISO 14001 Energy Management Systemstandard is based on the concept that more favorable environmental performance can be attained by a firm when environmental aspects are systematically identified and managed and are given a major contribution to sustainability, through pollution prevention, improved environmental performance, and from compliance with all applicable laws (Ciravegna& da Fonseca, 2015). The ISO standards are voluntary (Erauskin-Tolosa et al., 2020; Massoud et al., 2010).

Since its inception, the ISO 14001 Environmental Management System (EMS) standard has developed into one of the most widely used systems for managing corporate environmental aspects (Oliveira et al., 2011). Environmental Management Systems (EMSs) are intended to formalize procedures for managing and reducing environmental impacts of a firm's operations (Christini et al., 2004). Furthermore, the ISO 14001 Environmental Management System (EMS) standard has been designed to assist firms in the creation of structured mechanisms to enable the continuous improvement in their environmental performance (Kitazawa & Sarkis, 2000).

The basic elements of an environment management system (EMS) include the following:

- Reviewing the organization's environmental goals.
- Analyzing the firm's environmental impacts and compliance obligations (or legal and other requirements).
- Setting environmental objectives and targets to reduce environmental impacts and conform with compliance obligations.
- Establishing programs to meet these objectives and targets.
- Monitoring and measuring progress in achieving the objectives.
- Ensuring employees' environmental awareness and competence; and
- Reviewing progress of the EMS and achieving improvements (United States Environmental Protection Agency, 2021).

Firms can achieve very important environmental-related benefits from the use of an ISO 14001 EMS. These benefits include:

- Enhanced environmental awareness and accountability at all levels throughout the firm.
- Improved regulatory compliance.
- Enhanced operational procedures and controls.
- A reduced environmental footprint (lower emissions, discharges, and wastes).
- Continual system improvements resulting from the EMS objectives, goals, programs, periodic audits, and management reviews (Briggs, 2007, p. 67).
- Pollution prevention.
- Resource conservation
- New customers/markets
- Increased efficiency/reduced costs
- Enhanced employee morale
- Enhanced image with public, regulators, lenders, investors
- Employee awareness of environmental issues and responsibilities (United States Environmental Protection Agency, 2021)

The ISO 14001:2015 standard is applicable to any firm, regardless of type, size, and their nature of business. The ISO 14001 environmental management system (EMS) applies to the environmental aspects of its operations, products, and services that the firms sets and for which it can control and or influence (International Organization for Standardization, 2021; Shehabi, 2016).

III. RESEARCH METHODOLOGY

3.1 Research Approach

The present study used a qualitative instrumental case study research approach. An instrumental case study is the study of a case, for instance, a firm, that provides insights into a specific issue, redraws generalizations, or builds theory (Stake, 1995, 2005). The instrumental case study research approach provides researchers with a greater understanding of a specific phenomenon. An instrumental case study is designed around established theory of the phenomenon under study (Grandy, 2010). The present study was designed around the established theory of ISO 14001 Environmental Management Systems (EMS) (Dentch, 2016; Grover & Grover, 2017; Imtiaz Haider, 2010; Whitelaw, 2004).

3.2 Data Collection

The data used in the study was obtained from a range of documents, airlineannual sustainability reports, company materials available on the internet and records as sources of case evidence. An extensive search of the leading air transport and airline journals and magazines was also conducted in the study.

This study used secondary data. This study followed the guidance of Yin (2018) during the data collection phases, that is, the study used multiple sources of case evidence, database on the subject was created, and therewas a chain of evidence.

3.3 Data Analysis

The data collected for the case study was examined using document analysis. Document analysis is quite commonly used in case studies. Document analysis focuses on the information and data from formal documents and a firm's records that are collected by a researcher(s) when conducting their study (Andrew et al., 2011; Yin, 2018). Following the guidance of Scott (2004, 2014) and Scott and Marshall (2009), the documents gathered in the present study were examined according to four criteria: authenticity, credibility, representativeness and meaning.

The document analysis was undertaken in six discrete stages:

- Stage 1: The first stage involved planning the types and required documentation and their availability for the study.
- Stage 2: The data collection phase involved sourcing the documents and developing and implementing a scheme for the document management.
- Stage 3: The collected documents were examined to assess their authenticity, credibility and to identify any potential bias.
- Stage 4: The content of the collected documents was carefully examined, and the key themes and issues were identified.
- Stage 5: This stage of the document analysis process involved the deliberation and refinement to identify any difficulties associated with the documents, reviewing sources, as well as exploring the documents content.
- Stage 6: In this stage the analysis of the data was completed (O'Leary, 2004, p. 179).

Following the guidance of Yin (2018), the study's documents were downloaded and stored in a case study database. All the documents gathered for the study were in English. Each document was carefully read, and key themes were coded and recorded in the case study (Baxter, 2021).

IV. RESULTS

Aegean Airlines, which is based in Greece,was awarded the ISO 14001:2004 certification by TUV AUSTRIA HELLAS for the airline's implementation of an Environmental Management System for Passenger Services – Aircraft Services and Aircraft Maintenance at its principal hub at Athens' International Airport in September 2008. The Environmental Management System (EMS)certification underpins the airline's efforts to reduce the impact of its activities on the environment. The introduction of the electronic (e-ticket) in 2003, is one of the company's environmental practices. Other environmental practices include solid waste separation, collection of used paper, collection oflubricants and aircraft tyres for recycling, procedures for leakage management, and the saving of natural resources.In addition, the airline's staff and its partner organizations are kept well informed on ecological issues such as waste recycling, and the sensible use of water (Aegean Airlines, 2008).

Air Dolomiti,an Italian regional airline, was awarded with its ISO 14001: 2015 certification by DNV GL in December 2020. The implementation of the ISO 14001 certified Environmental Management System (EMS)involved the review of internal management processes to keep under control the environmental impact of its operations (Macca, 2020).In accordance with its sustainability policy, the airline has implemented a range of environmental-related measures in recent times. These measures include the elimination of plastic in on-board services, the packaging of snacks with compostablepackaging, use of glass or paper cups, the use of stirrer and food covers made from ecologicalmaterial, the elimination of plastic at company sites,the replacement of plastic cups and cutlery atfood courts with paper and bamboo cups and cutlery, and the airline encourages its staff touse their own cup and bottle instead of disposable cups and bottles. The airline is also focusing on its waste management and has aimed to separate and reduce waste. It has also implemented measures to integrate containers for glass collection withinthe company. Other environmental related practices adopted by the airline include aircraft flight plan optimization, the use of single engine taxi-in procedure, that is,closingdown of an engine after 2 minutes of cooldown following the landing of the aircraft at its destination airport, minimization of the use of aircraft auxiliary power units (APUs), the digitalization of manuals, navigation charts and other documents necessary forflights, and the use of electronic boarding passes, which saves printing and, therefore, paper consumption (Air Dolomiti, 2020).

Air France and KLM are ISO 14001 Environmental Management System (EMS)certified for all flights and for ground operations in France and The Netherlands. The two airlines social, societal, and environmental information is verified annually by an independent third party (Air France KLM Group, 2021).Air France KLMhave set a target to reduce their carbon dioxide (CO_2) emissions per passenger kilometreby50% by 2030 as compared to 2005 levels.To help achieve this target, the airlines have focused on their fleet renewal plans, with the order for 38 Airbus A350s and 60 Airbus A220s for Air France. These aircraft are quieter and more fuel efficient (Air France-KLM, 2020). Other environmental related measures implemented by the airlines are the use of sustainable aviation fuel (SAF)and achieving greater efficiency in the group's operations. These efficiency measures include designing more direct air routes, lightening the weight of aircraft, single-engine taxiing, and continuous descent air traffic management procedures (Poleri, 2021).

Air Transat, Canada's largest leisure airline, achieved ISO 14001:2015 Environmental Management System (EMS)certification for its Montreal-based head office in July 2019. The airline's ISO 14001 certified Environmental Management System (EMS) applies to its head office, comprising the administrative offices and maintenance centre, and includes building management as well as landscaping. The airline's environmental policy focuses on five primary areas: greenhouse gas (GHG) emissions, energy consumption, solid-waste production, hazardous materials, and wastewater. Mitigation targets have been defined for each of these areas, and all operations are standardized according to environmentally responsible practices supervised by the airline's Environmental Management System (EMS) team. The airline has implemented energy-optimization initiatives, which have included installation of a solar wall at the head office to lower energy consumption and greenhouse gas (GHG)emissions and banning of all single-use plastics in the cafeteria. Air Transat remains fully committed to continuing its many efforts to mitigate its environmental impact (Canadian Aviation News, 2019; Cision, 2019).

Asiana Airlines, which is based in South Korea,was the first airlines to receive ISO 14001 Environmental Management System (EMS)certification. The airline became ISO 14001 Environmental Management System (EMS)certified in 1996 (World Business Council for

Sustainable Development, 2009).To minimize environmental impactsfromits business activities, Asiana Airlines has selected four major environmental policies and related activities, from basic environmental pollution prevention activities such as air quality, water quality, and waste management to environment-friendly activities that it developsalong with its customers (Asiana Airlines, 2021).Asiana Airlines began its focus on its environmental efforts in 1994 when it developed and adopted a special emblem and the catchphrase "The one and only Earth, as precious as our customers".The airline has long focused on implementing environmental related protection measures. These measures include the introduction of environmentally friendly products and in-flight services. Asiana Airlineshas also focused on therecycling of waste so it can reduce consumption of resources.The airline has also made substantial efforts to reduce emissions by creating a roadmap and actively participating in global endeavors to prevent global warming. An example of this is the use ofground power units (GPU) at aircraft ramps instead of the aircraftauxiliary power unit(APU) power supply during ground maintenance and aircraft turnarounds.Jet fuel consumption and the associated gas emissions have also been reduced.Asiana Airlines has been carrying out various fuel reduction activities, which include improvements in flight procedures, flight plan optimization, and aircraft engine washing. In May 2008, Asiana Airlines started offsetting the carbon generated by all employees who travel on business trips on AsianaAirlines operated flights. The airline also introduced a passenger carbon offset scheme, where all funds raised from the carbon offset program are invested in offsets held by independent organizations (for example, government agencies) (World Business Council for

Sustainable Development, 2009).Other environmental related conservation measures implemented by the airline include the acquisition and deployment of the latest high fuel-efficient aircraft and engines, the continuous implementation of fuel saving policies, the implementation of environmentally friendly activities,

maintaining a green company, containing emissions within 50% of the legal standard, building voluntary reduction measures for emissions such as greenhouse gases (GHG), and a green campaign that is focused on its customers (Asiana Airlines, 2021). Announced in November 2020 and backed by the Korea Development Bank, Korean Air will formally acquire Asiana Airlines in 2022, with both airlines to fully merge and integrate their operations by 2024 (Flynn, 2021).

China Airlines, which is based in Taiwan, received its International Organization for Standardization's (ISO) Expansion of ISO 14001 Environment Management System (EMS) certification on 17 January 2014. The ISO 14001 Environmental Management System (EMS) certification was awarded by the British Standards Institution. The implementation of its Environmental Management System (EMS) underpins China Airlines' commitment to promote environmental sustainability as well as adhere to international standards of environmental management.In 2012, China Airlines' maintenance facility first passed the ISO 14001 Environment Management System (EMS)audit and was subsequently awarded with its certification. In 2013, China Airlines expanded its ISO 14001 Environmental Management System (EMS) certification to also include areas of its corporate headquarters, Songshan Airport, Taipei branch office, Kaohsiung branch office, as well as itsair cargo services. During 2011, China Airlines established a dedicated unit for enterprise-level environmental risk management, and an inter-departmental "Corporate Environmental Committee." These bodies were established for the management of environmental indicators to further prevent pollution, conserve energy, and reduce carbon emissions. Areas of environmental-related management include aircraft fuel savings, ground operations fuel savings, and savings in energy and resources. Other environmental related measuresimplemented by the airlineinclude China Airlines' maintenance facility, which covers air, water, waste, and noise pollution, as well as toxic chemicals, greenhouse gases, and energy management (China Airlines, 2014). As part of its corporate sustainability policy. China Airlines has introduced a "green" flight initiative, which aims to reduce the carbon footprint of its services around the world (China Airlines, 2012b). The airline's aircraft maintenance department also pays close attention to the environment and focuses on energy savings and eco-friendliness in the areas of wastewater and gas treatment, reuse of old parts, efficient lighting systems, and floor designs (China Airlines, 2012a). China Airlines also aims to reduce the carbon footprint of its offices, and, as a result, focuses on energy saving and carbon reduction initiatives (China Airlines, 2012c).

Croatia Airlines was awarded ISO 14001: 2015 Environmental Management System (EMS) certification in 2016 (Croatia Airlines, 2016). In line with its Environmental and Energy Policy, Croatia Airlines focuses on environmental awareness and energy efficiency improvements as part of goal and risk management (Croatia Airlines, 2021a). Croatia Airlines sustainable development hasfocusedon two principal areas, which are airline fleet requirements and the application of certain procedures that reduce fuel consumption and the associated carbon dioxide (CO_2) emissions and noise (Croatia Airlines, 2021b).

Czech Airlines has been awarded ISO 14001 certification for its Environmental Management System (EMS) (Airline Marketing Australia, 2021). The airline has implemented a comprehensive environmental policy and in line with this policy it aims to reduce the direct and indirect impact of its activities on the environment,as such aircraft and equipment are carefully deployed to mitigate their impact on the environment, the company is focusing on reducing its fuel consumption and the associated greenhouse gas (GHG) emissions, andnoise reduction. The airline is also using technologies and procedures to prevent or continuously reduce environmental pollution during aircraft and equipment maintenance as well as in ground operations (Czech Airlines, 2015).

Ethiopian Airlines, the largest aviation group in Africa was awarded with its ISO 14001:2015. Environmental Management System (EMS) certification on 30 August 2018. The airline's Environmental Management System(EMS) covers Ethiopian Cargo and Logistics Services, the airline's Head Office, the airline's Maintenance Repair and Overhaul, flight catering services, Aviation Academy, Flight Operations, flight simulators and equipment facility maintenance.Ethiopian Airlines aims to be an eco-friendly airline and to achieve this aim, the airline has made a large investment in the state of the art, next generation aircraft, serving its customers organic food without extra packaging, employing waste-reduction programs and the elimination of paper. The airline has implemented a carbon offsetting program titled "Fly Greener Program" to reduce carbon dioxide (CO_2) emissions (Ethiopian Airlines, 2018).

Gulf Air, which is based in Bahrain, received its ISO 14001 Environmental Management System (EMS) certification from RINA Services in January 2016 (ITP Media Group, 2016).With commercial operations across four continents, Gulf Air is actively addressing environmental challenges individually and collectively. An example of this is Gulf Air's membership of the Sustainable Aviation Fuel Users Group, which is focusing on the commercialization, certification, and provision of a viable market to accelerate the development of alternative aviation fuels (Gulf Air, 2021).Airlines now view the use of aviation biofuels as being a key environmental sustainability measure (Baxter et al., 2020; Bittner et al., 2015; Cortez et al., 2015). Accordingly alternative jet fuel (AJF) technologies have gained considerable interest and are now regarded as a way for the airline industry to achieve large, near-term emissions reductions (Staples et al., 2014).

Hong Kong-based Cathay Pacific Airways has been awarded ISO 14001:2015 Environmental Management System (EMS) certification. Positioned near Hong Kong International Airport, the airline's 134,000 square metres headquarters buildingscomprise Cathay Pacific City and CathayDragon House, airline stores, a hotel, and the flight training centre. The two premises are certified to the ISO 14001:2015 Environmental Management System (EMS) standard.To reduce its carbon footprint, Cathay Pacific Airways has invested in energy-saving measures such as low-impact lighting devices. Other reduction methods include sensors, chilled water system optimization andground fleet vehicle electrification (Cathay Pacific Airways, 2021). The flight catering division of Cathay Pacific Airways, Cathay Pacific Catering Services (H.K.) Ltd received its ISO 14001 Environmental Management System (EMS) certification in 1996 (Cathay Pacific Catering Services, 2007).

Jetairfly,a Belgian leisure and holiday airline, was awarded its ISO 14001 Environmental Management System (EMS) certification by the independent office Veritas in March 2015 (Orban, 2015). Jetairfly name has been changed to TUI fly, which is a brand name of TUI Airlines Belgium NV. TUI fly is a part of TUI Group(TUI fly, 2021a). The airline has implemented a wide range of environmental related measures, which include aircraft fleet modernization, the use of “winglets” on its Boeing 737 fleet, sustainable waste management practices, aircraft weight savings, optimal and dynamic flight planning, digitalization of flight manuals and other flight documentation, air traffic management optimization procedures, and single engine taxiing at the destination airport (TUI fly, 2021b).

Korean Airlines was awarded ISO 14001 Environmental Management System (EMS) certification on 25 November 2005. The airline subsequently obtained ISO 14001: 2015 Environmental Management System (EMS) certification on 10 December 2020 (Korean Airlines, 2020).Korean Air is optimizing fuel efficiency and reducing aircraft carbon emissions to achieve sustainable growth and minimize its environmental impact.The airline has also acquired and deploys the next generation environmentally friendly aircraft, such as, the Boeing 787-9 aircraft. The deployment of these next generation aircraft is enabling Korean Airlines to reduce its greenhouse gas (GHG) emissions. Korean Air has also taken steps to protect biodiversity and management of ozone-depleting substances to mitigate environmental pollution.Another environmental conservation measure is the planting of trees abroad, for instance, the “Korean Air Forest” in Baganuur, Mongolia, and the “Korea Air Green Garden” in Kubuqi Desert of Neimenggu, China (Korean Airlines, 2021).

LAN CARGO, an affiliate of LATAM Airlines Group, S.A., Environmental Management System (EMS) was awarded ISO 14001:2004 certification from Bureau Veritas for its air cargo ground operations and facilities at MiamiInternationalAirport on 5 January 2016. The ISO 14001:2004 Environmental Management System (EMS) certification covers both air cargo ground operations and the airline’s facilities at Miami InternationalAirport. By ensuring compliance with these standards the airline will be able to grow and develop its ground operation more efficiently, improve resource management and allocation, and reduce its operation’s environmental impact and carbon footprint.LAN CARGO ISO 14001:2004 Environmental Management System (EMS) certification coverscorporate and administrative activities, storage, warehousing and ground support for air cargo transportation and the repair station for maintenance of its aircraft in Miami. The certification was for three years and validated the impact of the development and implementation of policies by LAN CARGO to meet and comply with ISO 14001:2004 Environmental Management System (EMS)standards. The standards that govern LAN CARGO’s Environmental Management System (EMS), are based on a series of tools and initiatives that monitor key indicators to control and reduce environmental impact, whilst also promoting the continuous improvement of the airline’s operations.LAN CARGO’s Environmental Management System is designed to uphold a unified standard for all its ground operations. The objective of the EMS is to minimize environmental impact,comply with existing regulations and with the Company’s policy of security, quality, and environmental sustainability. The benefits of the Environmental Management System (EMS) include savings on costs and materials due to recycling, clear indicators to measure the development and progress of the different environmental protocols and programs, as well as the protection of natural resources (LAN CARGO, 2016).

Lufthansa Cargo AG was certified at its Frankfurt base to the international environmental management system standard ISO 14001 in February 2009 (Air Freight News, 2009).All of Lufthansa Cargo German stations have been ISO 14001 Environmental Management System (EMS)certified since 2010. This was extended to its subsidiary Jettainer GmbHin 2015 (Brice, 2018; Lufthansa Cargo, 2018).Lufthansa Cargo received its ISO 14001 environmental certification for its worldwide facilities in 2015 (Brice, 2018). The airline was once again recertified in accordance with the ISO 14001 environmental management systemstandard in February 2018. This certification also included for the first its subsidiary time:matters GmbH (Air Freight News, 2018; Lufthansa Cargo, 2018). The airline had set a target to reduce its specific carbon dioxide (CO_2) emissions by 25% by 2020 (Air Cargo News, 2016). Lufthansa Cargo operated its final McDonnell Douglas MD-11F flight on 17 October 2021. The airline had previously operated the McDonnell Douglas MD-11F flight for 23 years.The carrier first commenced operating the tri-jet freighter in 1998 with its fleet of MD-11Fs eventually numbering 19 of the model. The McDonnell Douglas MD-11F flight have now been replaced by a fleet of more efficient Boeing B777-2OO freighter aircraft (Brett, 2021a).Lufthansa Cargo and DB Schenker partnered together on November 29, 2020, to operate the first ever carbon dioxide (CO_2)-neutral freighter flights, powered by sustainable aviation fuel (SAF). The flight operated from Frankfurt to Shanghai utilizing a Lufthansa Cargo Boeing 777 freighter aircraft (Harry, 2020). In April 2021, DB Schenker and Lufthansa Cargo launched a regular carbon dioxide (CO_2)-neutral freighter route, operating between Frankfurt and Shanghai. The use of sustainable aviation fuel has reduced conventional kerosene usage of 174 tonnes per week on this route (Harry, 2021b). In 2021, as part of its sustainability policy, Lufthansa Cargo began to offer all its customers the option of carbon dioxide (CO_2) neutral transportation on segments that are operated by freighter aircraft. Lufthansa Cargo will achieve the carbon dioxide (CO_2) neutrality by using sustainable aviation fuel (SAF) or through certified carbon offsetting projects which will avoid or compensate for the emissions generated during air cargo transportation (Brett, 2021b).

Lufthansa CityLine was the world's first airline to launch a structured and officially certified environmental environment protection plan. In 2018, the airline wasrecertified in accordance with the international environmental standard ISO 14001: 2015 for its comprehensive Environmental Management System (EMS). The ISO 14001: 2015Environmental Management System (EMS) certificationis valid for each of the three company locations Munich, Frankfurt, and Cologne airports (Lufthansa CityLine, 2021a). Importantly, the protection of the environment is one of Lufthansa CityLine's most important goals. The airline aims to avoid environmental damage such as pollution, noise, waste, and wastewater whenever possible and keeps these to a minimum if they cannot be fully avoided (Lufthansa CityLine, 2021b).

Scandinavian Airlines (SAS) ISO 14001 certifiedEnvironmental Management System (EMS) encompasses all activities performed within SAS. SAS was certified according to ISO 14001 Environmental Management System (EMS) standard in 2010 (Scandinavian Airlines, 2021). Scandinavian Airlines (SAS) ISO 14001 certified Environmental Management System (EMS)was recertified in accordance with the ISO 14001: 2015 standard on 14 September 2018 (Scandinavian Airlines, 2018). The airline's system focuses on activities around administrative functions at the headquarter Frösundavik and at the airline's main bases at Stockholm-Arlanda Airport, Copenhagen-Kastrup Airport and Oslo-Gardermoen Airport.The system also includes other geographical areas through follow-up programs and contracted services. The airline's Environmental Management System(EMS) is based on shared environmental and sustainability policies, the Code of Conduct, the UN Global Compact, airline operational standards and ISO 14001. In addition, the system provides guidelines for a continuing cycle of planning, implementation, and evaluation, together with the improvement of processes and activities to meet operational and environmental targets (Scandinavian Airlines, 2021a).Scandinavian Airlines (SAS)has implemented a wide range of initiatives to mitigate its environmental impact. These measures include the acquisition and deployment of the latest start of the Airbus A350-900 XWB aircraft, Increased energy efficiency, sustainable products,and services, optimizingits flight schedule and aircraft size to optimally meet demand (particularly on regional routes with relatively low levels of demand), air space procedures optimization, collaboration with aircraft and enginemanufacturers and the airline is working with these key stakeholders on future low emission aircraft (Scandinavian Airlines, 2021b). Scandinavian Airlines (SAS) is regularly using sustainable fuel sources and has established a target to have all its Scandinavian domestic flights powered solely by sustainable aviation fuels by 2030 (Ahlgren, 2020).

Singapore Airlines has in placean Environmental

Management System (EMS) which is certified to the ISO 14001:2015 standard. The airline's EnvironmentalManagement System (EMS) covers the Engineering Division and FlightOperations Division, for the provision and management of aviation and engineering support services. Furthermore, the EnvironmentalManagement System (EMS) ensures that the airline's operations comply with the relevant local and international environmental regulations. Internal and external auditsare performed annually to ensure compliance with the requirements (Singapore Airlines, 2021). Singapore Airlines has implemented a wide range of environmental mitigation measures in recent times. These measures include an aircraft weight reduction program, the installation of extensive light emitting diode (LED) lighting, the installation of more energy efficient plant and equipment, the installation of more energy efficient heat exchange system at its Silver Kris Lounge at Changi Airport, the upgrading of lifts with a more efficient model that had a Variable Voltage Variable Frequency (VVVF) motor(Singapore Airlines, 2015), the installation of a large scale photovoltaic (PV) system, a computer system that optimizes the maximum zero fuel weight (MZFW) of its aircraft fleet, the use of lightweight catering items, and the use of fixed electrical ground power and preconditioned air units at airports where its aircraft have night layovers or long transits (Singapore Airlines, 2021).

Thai Airways International headquarters was awarded with its ISO 14001 Environmental Management System (EMS)certification by BVQI (Bureau Veritas QualityInternational) on 28 April 28, 2000. The airlines headquarters were recertified by BVQI (Bureau Veritas Quality International) on December 20, 2004. Thai Airways International Technical Department and the new Aircraft Maintenance Facility located at U-Tapao International Airport were awarded ISO 14001 Environmental Management System (EMS) certification from BVQI (Bureau Veritas Quality International) onMarch 16,2001. The airline's Customer Services Department has also been awarded ISO 14001 Environmental Management System (EMS) certificationfrom the Bureau Veritas Certification (Thai Airways International, 2021).Thai Airways International has established its environmental management policy in accordance with the Environmental Management System (EMS) ISO 14001:2015 standard, the airline's environmental policy comprises environmental protection, measures to prevent pollution,legal compliance, and constant improvement in its environmental performance. The airline has implemented a range of environmental related measures as part of its commitment to its environmental policy. These measures include carbon dioxide (CO_2) offsetting, the sustainable use of resources, energy efficient flight operations, flight route optimization, potable water uplift management, efficient fleet planning, energy, and water conservation its buildings and facilities, and sustainable waste management (Thai Airways International, 2020).

The Japan Airlines Group (JAL) has established Environmental Management Regulations based on the JAL Group Environmental Policy and is building and operating an Environmental Management System (EMS). Based on this Environmental Management System (EMS), the JAL Group aims to achieve its environmental goals, as well as complying with all applicable laws, regulations, and other rules related to the environmental activities of the company. A key goal of Japan Airlines is to reduce its environmental impact and prevent pollution. In addition, internal environmental audits are performed each year to confirm the conformity and legal compliance of the system and to verify the achievement status of the company's targets. Internal audits are conducted annually at each department in the airline's headquarters building, and over a three-year period at Group companies, and the results are linked to management reviews to ensure continuous improvement. In addition, JAL Royal Catering Co., Ltd. was awarded its ISO 14001 Environmental Management System certification in 2021 (Japan Airlines, 2021). As part of its sustainability policy, Japan Airlines has implemented environmental conservation measures that include a reduction in carbon dioxide (CO_2) emissions, operation of fuel-efficient aircraft, use of sustainable aviation fuels, reduction in the use of plastics, a reduction in the disposal of in-flight meal wastes, a reduction in industrial wastes, and the operation of low noise aircraft (Japan Airlines, 2020).

The Lufthansa Hub Munich received its ISO 14001 Environmental Management System (EMS) certification in May 2016 (Travel PR News, 2016). Munich is a key hub for Lufthansa and is also served by Lufthansa CityLine.

Turkish Airlines received its ISO 14001 Environmental Management System (EMS) certification in March 2013. The ISO 14001 Environmental Management System (EMS) certification coversallofTurkish Airline's activities and operations within the Republic of Turkey, and all of theactivities carried out onboard the airline's aircraft during flight operations even if they are not operated within the borders of theRepublic of Turkey. Within the airline's Environmental Management System (EMS), variousactivities are performedto increase environmental awareness of staff and to reduce the environmentalimpacts of its products, services, and activities. The airline focuses on its waste management within its offices and inflight activities. Turkish Airlines also promotes effective and efficient use of natural resources such as water,electricity, natural gas, and paper. As part of its sustainability policy, Turkish Airlines has implemented various environmental-related measures which include a fuel efficiency program, optimizing the use of APU (auxiliary power unit) while the aircraft is on ground, the introduction of anew optimized flight planning system, optimization of flight routes and aircraft speed, aircraft weight reduction, engine washing, investments in new technologies, and investments in infrastructure (Turkish Airlines, 2014).

V. CONCLUSION

Based on an in-depth qualitative instrumental case study research approach, this study has empirically examined the airlines that have implemented an ISO 14001 certified Environmental Management System (EMS) as a tool to manage their operations in an environmentally sustainable manner. The study period was from 1990 to 2021. The qualitative data was analyzed by document analysis. The case study revealed that airlines located Bahrain, Canada, Ethiopia,Europe, Hong Kong, Japan, Singapore, Thailand, South Korea,Taiwan, Turkey, and the United States of Americahave implemented the ISO 14001 based Environmental Management Systems (EMS). The ISO 14001 Environmental Management System standard has been adopted by full-service network carriers, two leisure airlines, and by two major air cargo airlines (LATAM Cargo and Lufthansa Cargo). South Korea-based Asiana Airlines was the first airline to receiveISO 14001 Environmental Management System(EMS) certification. The airline'sEnvironmental Management System was ISO 14001 certified in 1996.

The case study found that since the release of the ISO 14001:2015 Environmental Management System (EMS) standard, Air Transat, Cathay Pacific Airways, Croatia Airlines, Ethiopian Airlines, Korean Airlines, Scandinavian Airlines (SAS), Singapore Airlines, and Thai Airways International, have adopted this standard.

The case study revealed that the use of an ISO 14001 certified Environmental Management Systems(EMS) underpins airlines environmentally sustainable operations. The case study showed that as part of their environmental management policies, theairlines that have implemented anISO 14001 Environmental Management System(EMS) have defined and instigated a wide range of environmental conservation measures. Theseenvironmental conservation measures include the acquisition and deployment of the next generation, fuel efficient aircraft, such as, the Airbus A350 and Boeing 787, the use of sustainable aviation fuel, energy efficient flight operations and air traffic management procedures optimization, aircraft weight reductions, aircraft engines washing, single engine aircraft taxying, sustainable waste management, electrification of ground service equipment (GSE) and vehicle, the use of photovoltaic (PV) solar systems,carbon offsetting programs, water conservation, and energy efficient offices and facilities.

REFERENCES

[1] Aegean Airlines. (2008). Aegean Airlines receives ISO 14001 certification for environmental protection. Retrieved from https://en.about.aegeanair.com/media-center/press-releases/2008/aegean-airlines-receives-iso-14001-certification-for-environmental-protection/.

[2] Ahlgren, L. (2020). Sustainability: Which airlines are leading the way? Retrieved from https://simpleflying.com/sustainability-which-airlines-are-leading-the-way/.

[3] Air Cargo News. (2016). Lufthansa Cargo gains environmental certification. Retrieved from https://www.aircargonews.net/airlines/lufthansa-cargo-gains-environmental-certification/.

[4] Air Dolomiti. (2020). Environmental statement 2020-2023. Retrieved from https://www.airdolomiti.it/uploads/ckeditor/attachments/687/Dichiarazione_ambientale_ENG.pdf.

[5] Air France-KLM. (2020). Sustainability report 2019. Retrieved from https://sustainabilityreport2019.airfranceklm.com/.

[6] Air France KLM Group. (2021). Non-financial assessments. Retrieved from https://www.airfranceklm.com/en/non-financial-assessments.

[7] Air Freight News. (2009). Lufthansa Cargo certified to ISO 14001 environmental standard. Retrieved from https://airfreight.news/articles/full/lufthansa-cargo-certified-to-iso-14001-environmental-standard.

[8] Air Freight News. (2018). Lufthansa Cargo global environmental management system recognised again. Retrieved from https://airfreight.news/articles/full/lufthansa-cargo-global-environmental-management-system-recognised-again.

[9] Airline Marketing Australia. (2021). Czech Airlines. Retrieved from http://www.airlinemarketing.com.au/our-clients/czech-airlines/.

[10] Akpan, U.F., & Akpan, G.E. (2012). The contribution of energy consumption to climate change: A feasible policy direction. *International Journal of Energy Economics and Policy*, 2(1), 21-33.

[11] Andrew, D.P.S., Pedersen, P.M.,& McEvoy CD. (2011). *Research methods and design in sport management*. Champaign:Human Kinetics.

[12] Asiana Airlines.(2021). Sustainable management. Retrieved from https://flyasiana.com/C/US/EN/contents/environmental-management.

[13] Azarkamand, S., Wooldrige, C., &Darbra, A.M. (2020). Review of initiatives and methodologies to reduce CO_2 emissions and climate change effects in ports. *International Journal of Environmental Research and Public Health*, 17(11), 3858.https://doi.org/10.3390/ijerph17113858

[14] Baer, H.A. (2020).*Airplanes, the environment, and the human condition*. Abingdon, UK: Routledge.

[15] Bansal, P.,& Bogner, W.C. (2002). Deciding on ISO 14001: Economics, institutions, and context. *Long Range Planning*, 35(3): 269-290.https://doi.org/10.1016/S0024-6301(02)00046-8

[16] Baumeister, S. (2020). Mitigating the climate change impacts of aviation through behavioural change.

Transportation Research Procedia, 48, 2006-2017.https://doi.org/10.1016/j.trpro.2020.08.230

[17] Baumeister, S.,&Onkila, T. (2017). An eco-label for the airline industry?*Journal of Cleaner Production*, 142(4), 1368-1376.https://doi.org/10.1016/j.jclepro.2016.11.170

[18] Baxter, G. (2020). Sustainable airline waste management: A case study of Air New Zealand's waste management programs and strategies.*International Journal for Traffic and Transport Engineering*, 10(3), 351 - 370

[19] Baxter, G. 2021. Mitigating an airport's carbon footprint through the use of "green" technologies: The case of Brisbane and Melbourne Airports, Australia. *International Journal of Environment, Agriculture and Biotechnology*, 6(6), 29-39.https://dx.doi.org/10.22161/ijeab.66.4

[20] Baxter, G.S.,& Bardell, N.S. (2017). Can the renewed interest in ultra-long-range passenger flights be satisfied by the current generation of civil aircraft? *Aviation*, 21(2): 42-54.https://doi.org/10.3846/16487788.2017.1336485

[21] Baxter, G.,& Wild, G. (2021). Aviation safety, freight, and dangerous goods transport by air. In R. Vickerman (Ed.), *International encyclopedia of transportation* (pp. 98-107). Amsterdam, The Netherlands: Elsevier.

[22] Baxter, G., Srisaeng P., &Wild G. (2020). The use of aviation biofuels as an airport environmental sustainability measure: The case of Oslo Gardermoen Airport. *Magazine of Aviation Development*, 8(1), 6-17. https://doi.org/10.14311/MAD.2020.01.01

[23] Baxter, G., Srisaeng, P., & Wild, G. (2021). A qualitative assessment of a full-service network airline sustainable energy management: The case of Finnair PLC.*WSEAS Transactions on Environment and Development*, 17, 167-180.

[24] Bennett, M.,& James, P. (1999). ISO 14031 and the future of environmental performance evaluation. In M. Bennett., P. James & L.Klinkers (Eds.), *Sustainable measures: Evaluation and reporting of environmental and social performance* (pp. 76-97). Sheffield, UK: Greenleaf Publishing.

[25] Bittner, A., Tyner, W.E., & Zhao, X. (2015). Field to flight: A techno-economic analysis of the corn stover to aviation biofuels supply chain. *Bio Fuels, Bio Products & Biorefining*, 9(2), 201-210.https://doi.org/10.1002/bbb.1536

[26] Blanca-Alcubilla, G., Roca, M., Bala, A., Sanz, N., De Castro, N., &Fullana-i-Palmer, P.(2019). Airplane cabin waste characterization: Knowing the waste for sustainable management and future recommendations. *Waste Management*, 96, 57-64.https://doi.org/10.1016/j.wasman.2019.07.002

[27] Brett, D. (2021a). Lufthansa Cargo offers CO_2 'neutral' freight service. Retrieved from https://www.aircargonews.net/policy/environment/lufthansa-cargo-offers-co2-neutral-freight-service/.

[28] Brett, D. (2021b). High noon for Lufthansa Cargo's last MD-11F. Retrieved from https://www.aircargonews.net/airlines/freighter-operator/high-noon-for-lufthansa-cargos-last-md-11f/.

[29] Brice, A. (2018). Lufthansa subsidiary's ISO certification. Retrieved from http://magazine.groundhandling.com/news/timematters-gains-revised-iso-14001-certification/.

[30] Briggs, S.L.K. (2007). ISO 14001 hits 10-year mark. *Quality Progress*, 40(8), 67-68.

[31] Budd, L.,& Ison, S. (2017). Airfield design, configuration and management. In L. Budd& S. Ison (Eds.),*Air transport management: An international perspective* (pp. 41-60). Abingdon, UK: Routledge.

[32] Budd, T. (2017). Environmental impacts and mitigation. In L. Budd & S. Ison (Eds.),*Air transport management: An international perspective* (pp. 283-306). Abingdon, UK: Routledge.

[33] Campos, L.M.S., Aparecida de Melo Heizen, D., Verdinelli, M.A.,&Cauchick Miguel, P.A. (2015). Environmental performance indicators: A study on ISO 14001 certified companies. *Journal of Cleaner Production*, 99, 286-296.

[34] Canadian Aviation News. (2019). Air Transat obtains ISO 14001 certification for its head office. Retrieved from https://canadianaviationnews.wordpress.com/2019/07/16/air-transat-obtains-iso-14001-certification-for-its-head-office/.

[35] Cathay Pacific Airways. (2021). Sustainable development report 2020. Retrieved from https://sustainability.cathaypacific.com/wp-content/uploads/2021/05/Cathay-Pacific_SD-Report-2020_EN.pdf.

[36] Cathay Pacific Catering Services.(2007). Accreditation. Retrieved from https://www.cpcs.com.hk/eng/customers_e.html.

[37] China Airlines. (2012a). Aircraft maintenance. Retrieved from https://calec.china-airlines.com/csr/environment/en/action-maintain.html.

[38] China Airlines. (2012b). Eco flights: More eco, less CO_2. Retrieved from https://calec.china-airlines.com/csr/environment/en/action-flight.html.

[39] China Airlines. (2012c). Eco friendly offices. Retrieved from https://calec.china-airlines.com/csr/environment/en/action-office.html.

[40] China Airlines. 2014. China Airlines receives expansion of ISO 14001 Environment Management System Certification and ISO 50001 Energy Management System Certification. Retrieved from https://calec.china-airlines.com/csr/environment/en/charity-honor-11.html.

[41] Chin, K., Chiu, S.,& Rao Tummala, V.M. (1999). An evaluation of success factors using the AHP to implement ISO 14001-based EMS. *International Journal of Quality & Reliability Management*, 16(4), 341-362.https://doi.org/10.1108/02656719910248226

[42] Ciravegna, L.M.,& da Fonseca, M. (2015). ISO 14001:2015: An improved tool for sustainability. *Journal of Industrial Engineering and Management*, 8(1), 37-50.http://dx.doi.org/10.3926/jiem.1298

[43] Cision. (2019). Air Transat obtains ISO 14001 certification for its head office. Retrieved from https://www.newswire.ca/news-releases/air-transat-

obtains-iso-14001-certification-for-its-head-office-890655219.html.

[44] Cortez, L.A.B., Nigro, F.E.B, Nogueira, L.A.H., Nassar, A.M., Cantarella, H., Moraes, M.A.F.D., Leal, R.L.V, Franco, T.T., Schuchardt, U.F., &Baldassin, R. Jnr. (2015). Perspectives for sustainable aviation biofuels in Brazil. *International Journal of Aerospace Engineering*, 2015, 264898.https://doi.org/10.1155/2015/264898

[45] Croatia Airlines.(2016). Certifikat: ISO 14001 Environmental Management System. Retrieved from https://www.croatiaairlines.com/resources/dokumenti/iso-14001-valid-until-20dec22.pdf.pdf.

[46] Croatia Airlines.(2021a). Quality. Retrieved from https://www.croatiaairlines.com/company/social-responsibility/quality.

[47] Croatia Airlines.(2021b). Sustainable development. Retrieved from https://www.croatiaairlines.com/company/social-responsibility/sustainable-development.

[48] Curkovic, S.,& Sroufe, R. (2011). Using ISO 14001 to promote a sustainable supply chain strategy. *Business Strategy and the Environment*, 20(2), 71-93.https://doi.org/10.1002/bse.671

[49] Czech Airlines. (2015). Environmental policy. Retrieved from https://www.csa.cz/media/1423/environmental-policy-en.pdf.

[50] Daley, B. (2016). *Air transport and the environment*. Abingdon, UK: Routledge.

[51] de Neufville, R.,&Odoni, A. (2013).*Airport systems: Planning, design, and management*(2nd ed.).New York: McGraw-Hill.

[52] Dentch, M.P. (2016). *The ISO 14001: 2015 implementation handbook: Using the process approach to build an environmental management system*. Milwaukee, WI: ASQ Quality Press.

[53] de Vries, H.J, Bayramoglu, D.K.,& van der Wiele, A. (2012). Business and environmental impact of ISO 14001. *International Journal of Quality & Reliability Management*, 29(4), 425–435).https://doi.org/10.1108/02656711211224866

[54] Dileep, M.R. (2019). *Transport and travel management*. Abingdon, UK: Routledge.

[55] Elmer, V.,& Leigland, A. (2014).*Infrastructure planning and finance: A smart and sustainable guide*. Abingdon, UK: Routledge.

[56] Erauskin-Tolosa, A., Zubeltzu-Jaka, E., Heras-Saizarbitoria, I,.&Boiral, O. (2020). ISO 14001, EMAS and environmental performance: A meta-analysis. *Business Strategy and the Environment*, 29(3), 1145-1159.https://doi.org/10.1002/bse.2422

[57] Ethiopian Airlines. (2018). Ethiopian receives ISO Environment Management System certification August 30, 2018. Retrieved from https://corporate.ethiopianairlines.com/Press-release-open-page/ethiopian-receives-iso-environment-management-system-certification-august-30-2018.

[58] Flynn, D. (2021). Asiana Airlines brand to disappear after Korean Air take-over. Retrieved from https://www.executivetraveller.com/news/asiana-airlines-brand-to-disappear-after-korean-air-take-over.

[59] Forsyth, P. (2011). Environmental and financial sustainability of air transport: Are they incompatible? *Journal of Air Transport Management*, 17(1), 27-32. https://doi.org/10.1016/j.jairtraman.2010.10.006

[60] Gorji-Bandpy, M.,& Azimi, M. (2013). Passive techniques for fan noise reduction in new turbofan engines: Review.*Journal of Engineering Science and Technology Review*, 6(1), 59-61.

[61] Graham, A. (2018).*Managing airports: An international perspective* (5th ed.). Abingdon, UK: Routledge.

[62] Grandy, G. (2010). Instrumental case study. In A.J. Mills, G. Durepos& E. Wiebe (Eds.),*Encyclopedia of case study research*, Volume 1 (pp. 473-475). Thousand Oaks, CA: SAGE Publications.

[63] Grover, R.C.,& Grover, S. (2017). *Winning the environmental challenge with ISO 14001: 2015: Implementation of environmental management system*. Chennai, India: Notion Press.

[64] Gulf Air. (2021). Corporate social responsibility and sponsorship. Retrieved from https://www.gulfair.com/about-gulf-air/corporate-social-responsibility-and-sponsorship.

[65] Hagmann, C.,Semeijn, J., & Vellenga, D.B. (2015). Exploring the green image of airlines: Passenger perceptions and airline choice. *Journal of Air Transport Management*, 43, 37-45.https://doi.org/10.1016/j.jairtraman.2015.01.003

[66] Han et al., Chua, B.L., & Hyun, S.S. (2020). Consumers' intention to adopt eco-friendly electric airplanes: The moderating role of perceived uncertainty of outcomes and attachment to eco-friendly products. *International Journal of Sustainable Transportation*, 14(9), 671-685.https://doi.org/10.1080/15568318.2019.1607957

[67] Harry, R. (2020). Lufthansa Cargo and DB Schenker operate first CO_2-neutral freighter flights. Retrieved from https://www.aircargonews.net/policy/environment/lufthansa-cargo-and-db-schenker-partner-to-operate-co2-neutral-freighter-flights/.

[68] Hazel, R.A., Blais, J.D., Browne, T.J., & Benzon, D.M. (2011). *Resource guide to airport performance indicators*. Airport Cooperative Research Program Report 19A. Washington: Transportation Research Board.

[69] Heras-Saizarbitoria, I,.&Boiral, O. (2013). ISO 9001 and ISO 14001: Towards a research agenda on management system standards. *International Journal of Management Reviews*, 15(1), 47-65.https://doi.org/10.1111/j.1468-2370.2012.00334.x

[70] Heras-Saizarbitoria, I., Landín, G.A.,& Molina-Azorín, J.F. (2011). Do drivers matter for the benefits of ISO 14001? *International Journal of Operations and Production Management*, 31(2), 192-216.https://doi.org/10.1108/01443571111104764

[71] Ikram, M., Mahmoudi, A., Shah, S.Z.A.,& Mohsin, M. (2019). Forecasting number of ISO 14001 certifications of selected countries: application of even GM (1,1), DGM, and NDGM models. *Environmental Science and Pollution*

Research, 26, 12505–12521.https://doi.org/10.1007/s11356-019-04534-2

[72] Imtiaz Haider, S. (2010). *Environmental management system ISO 14001: 2004. Handbook of transition with CD Rom*. Boca Raton, FL: CRC Press.

[73] International Civil Aviation Organization. (2011).*Airport air quality manual*. Document No 9889. Montreal: ICAO.

[74] International Organization for Standardization. (2015). ISO 14001:2015 published. Retrieved from https://committee.iso.org/sites/tc207sc1/home/news/content-left-area/news-and-updates/iso-140012015-published.html.

[75] International Organization for Standardization. 2021. ISO 14001:2015 Environmental management systems — Requirements with guidance for use. Retrieved from https://www.iso.org/standard/60857.html.

[76] ITP Media Group. (2016). Gulf Air gains five IT ISOs. Retrieved from https://www.aviationbusinessme.com/airports/airport-business/content-403972.

[77] Japan Airlines. 2020. Sustainability. Retrieved from https://www.jal.com/en/sustainability/initiatives/pdf/sustainability.pdf.

[78] Japan Airlines. (2021). Environmental management. Retrieved from https://www.jal.com/en/sustainability/environment/environment-management/.

[79] Jiang, R.J.,& Bansal, P. (2003). Seeing the Need for ISO 14001. *Journal of Management Studies*, 40(4), 1047-1067.https://doi.org/10.1111/1467-6486.00370

[80] Kitazawa, S.,& Sarkis, J. (2000). The relationship between ISO 14001 and continuous source reduction programs. *International Journal of Operations & Production Management*, 20(2), 225-248.https://doi.org/10.1108/01443570010304279

[81] Korean Airlines. (2020). Certificate of registration. ISO 14001 Environmental Management System. Retrieved from https://www.koreanair.com/content/dam/koreanair/ko/footer/about-us/sustainable-management/green/ISO14001.pdf.

[82] Korean Airlines. (2021). Sustainability. Retrieved from https://www.koreanair.com/au/en/footer/about-us/sustainable-management/green.

[83] Kumar, A.; Aswin, A.; &Gupta, H. (2020). Evaluating green performance of the airports using hybrid BWM and VIKOR methodology. *Tourism Management*, 76, 103941.https://doi.org/10.1016/j.tourman.2019.06.016

[84] Kurniawan, J.S.,&Khardi, S. (2011). Comparison of methodologies estimating emissions of aircraft pollutants, environmental impact assessment around airports. *Environmental Impact Assessment Review*, 31(3), 240-252.https://doi.org/10.1016/j.eiar.2010.09.001

[85] LAN CARGO. (2016) LATAM Airlines Group cargo affiliate receives ISO 14001:2004 certification and inaugurates new maintenance hangar facility at Miami International Airport. Retrieved from http://incargonews.com/en/latam-airlines-group-cargo-affiliate-receives-iso-140012004-certification-and-inaugurates-new-maintenance-hangar-facility-at-miami-international-airport/#.YabjCNBhiUk.

[86] Laskurain, I., Ibarloza, A., Larrea, A.,&Allur, E. (2017). Contribution to energy management of the main standards for Environmental Management Systems: The case of ISO 14001 and EMAS. *Energies*, 10(11), 1758.https://doi.org/10.3390/en10111758

[87] Li, X.D., Poon, C.S., Lee, S.C., Chung, S.S., &Luk, F.(2003). Waste reduction and recycling strategies for the in-flight services in the airline industry. *Resources, Conservation & Recycling*, 37(3), 87-99.https://doi.org/10.1016/S0921-3449(02)00074-5

[88] Liu, J., Yuan, C., Hafeez, M.,& Li, X. (2020). ISO 14001 certification in developing countries: Motivations from trade and environment. *Journal of Environmental Planning and Management*, 63(7), 1241-1265.https://doi.org/10.1080/09640568.2019.1649642

[89] Lobo, P., Hagen, D. E.,& Whitefield, P.D. (2012). Measurement and analysis of aircraft engine PM emissions downwind of an active runway at the Oakland International Airport.*Atmospheric Environment*, 61, 114–123.https://doi.org/10.1016/j.atmosenv.2012.07.028

[90] Lufthansa Cargo.(2018). Lufthansa Cargo global environmental management system recognised again. Retrieved from https://lufthansa-cargo.com/-/lufthansa-cargo-global-environmental-management-system-recognised-again.

[91] Lufthansa CityLine.(2021a). Environmental certification. Retrieved from https://www.lufthansacityline.com/en/responsibility/environmental-certification.html.

[92] Lufthansa CityLine.(2021b). Environmental guidelines. Retrieved from https://www.lufthansacityline.com/en/responsibility/environmental-guidelines.html.

[93] Luther, L. (2007.) *Environmental impacts of airport operations, maintenance, and expansion*. CRS Report for Congress, Report RL33949. Washington, DC: Congressional Research Service, The Library of Congress.

[94] Macca, M. (2020) Air Dolomiti obtains ISO 14001 certification. Retrieved from https://airwaysmag.com/airlines/air-dolomiti-iso-14001/.

[95] MacDonald, J.P. (2005). Strategic sustainable development using the ISO 14001 Standard. *Journal of Cleaner Production*, 13(6), 631-643.https://doi.org/10.1016/j.jclepro.2003.06.001

[96] Marais, K., Wolfe, P.J., & Waitz, I.A. (2016). Air transport and the environment. In P. Belobaba, A. Odoni& C Barnhart (Eds.), *The global airline industry* (pp. 423-459) (2nd ed.). Chichester, UK: John Wiley & Sons.

[97] Massoud, M.A., Fayad, R., Kamleh, R.,& El-Fadel, M. (2010). Environmental Management System (ISO 14001) certification in developing countries: Challenges and implementation strategies. *Environmental Science and Technology*, 44(6), 1884–1887.

[98] Mayer, R.,Ryley, T., &Gillingwater, D. (2012). Passenger perceptions of the green image associated with airlines. *Journal of Transport Geography*, 22, 179-186.https://doi.org/10.1016/j.jtrangeo.2012.01.007

[99] Merkert, R.,& Alexander, D. (2018). The air cargo industry. In N. Halpern & A. Graham (Eds.), *The Routledge companion to air transport management* (pp. 29-47). Abingdon, UK: Routledge.

[100] Migdadi, Y.K.A.A. (2020). *Introduction to airline green operations strategy*. Hershey, PA: IGI Global.

[101] Moynihan, R., &Walków, M. (2019). Airlines are turning to cereal starch cutlery and sugarcane trays to fight 6.7 million tons of cabin waste every year. Retrieved fromhttps://www.insider.com/airlines-battle-cabin-trash-flights-plastic-alternatives-2019-8.

[102] Niu, S.Y., Liu, C.L., Chang, C.C., & Ye, K.D. (2016). What are passenger perspectives regarding airlines' environmental protection? An empirical investigation in Taiwan. *Journal of Air Transport Management*, 55, 84-91.https://doi.org/10.1016/j.jairtraman.2016.04.012

[103] O'Leary, Z. (2004). *The essential guide to doing research*. London, UK: SAGE Publications.

[104] Oliveira, J.A., Oliveira, O.J., Ometto, A.R., Ferraudo, A.S.,& Salgado, M.H. (2011). Environmental management system ISO 14001 factors for promoting the adoption of cleaner production practices. *Journal of Cleaner Production*, 133, 1384-1394.https://doi.org/10.1016/j.jclepro.2016.06.013

[105] Orban, A. (2015). Jetairfly, the first Belgian airline with ISO 14001 certification. Retrieved from https://www.aviation24.be/airlines/tui-aviation/tui-fly-belgium/jetairfly-the-first-belgian-airline-with-iso-14001-certification/.

[106] Otley, T. (2019). Airbus A350: What you need to know. Retrieved from https://www.businesstraveller.com/features/a350-what-you-need-to-know/.

[107] Payán-Sánchez, B., Pérez-Valls, M., Plaza-Úbeda, J.A., &Vázquez-Brust, D. (2021). Network ambidexterity and environmental performance: Code-sharing in the airline industry. *Business Strategy and the Environment*, 1-15.https://doi.org/10.1002/bse.2948

[108] Poleri, A. (2021). Air France-KLM to accelerate its environmental transition process to meet Paris Agreement objectives. Retrieved from https://www.aviacionline.com/2021/11/air-france-klm-to-accelerate-its-environmental-transition-process-to-meet-paris-agreement-objectives/.

[109] Reynolds, T.G. (2016). Green aircraft operations. In R. Agarwal, F. Collier, A. Schäfer&A. Seabridge (Eds.), *Green aviation* (pp. 369-380). Chichester, UK: John Wiley & Sons.

[110] Sales, M. (2016).*Aviation logistics: The dynamic partnership of air freight and supply chain*. London, UK: Kogan Pace.

[111] Sartor, M., Orzes, G.,& Moras, E. (2019). ISO 14001. In M. Sartor & G. Orzes (Eds.),*Quality management: Tools, methods, and standards* (pp. 199-216). Bingley: Emerald Publishing Limited.

[112] Scandinavian Airlines. (2018). Environmental Management System (ISO 14001) certificate. Retrieved from https://www.sasgroup.net/files/en/wp-content/uploads/sites/2/2014/09/scandinavian-airlines-system-iso-14001-2018.pdf.

[113] Scandinavian Airlines. (2021a). Environmental Management System (ISO 14001). Retrieved from https://www.sasgroup.net/sustainability/environmental-management-system-iso-14001/#:~:text=SAS%20is%20one%20of%20a%20handful%20airlines%20certified,Global%20Compact%2C%20airline%20operational%20standards%20and%20ISO%2014001.

[114] Scandinavian Airlines. (2021b).SAS annual and sustainability report fiscal year 2020:Travelers for the future. Retrieved from https://sas.prod.waas.site/files/documents/Corporate_governace/annual-reports/SAS_AST19-20_ENG2.pdf.

[115] Schäfer, A.W.; &Waitz, I.A. (2014). Air transportation and the environment. *Transport Policy*, 34, 1-4.https://doi.org/10.1016/j.tranpol.2014.02.012

[116] Scott, J. 2004. Documents, types of. In: M. Lewis-Beck, A.E. Bryman. & T. Futing Liao (Eds.),*The SAGE encyclopedia of social science research methods* (pp. 281-284). Thousand Oaks, CA: SAGE Publications.

[117] Scott, J. (2014). *A dictionary of sociology* (4th ed.).Oxford, UK: Oxford University Press.

[118] Scott, J.,& Marshall, G. (2009). *A dictionary of sociology* (3rd ed.).Oxford, UK: Oxford University Press.

[119] Shehabi, E. (2016). Application of the new version of the environmental management system, the impact on enterprise development. *Anglisticum Journal*, 5(5), 85–91.http://dx.doi.org/10.0001/(aj).v5i5.1081.g1313

[120] Singapore Airlines. (2015). Sustainability report 2014/15. Retrieved from https://www.singaporeair.com/saar5/pdf/Investor-Relations/Annual-Report/sustainabilityreport1415.pdf.

[121] Singapore Airlines. (2021). Sustainability report 2020/21. Retrieved from https://www.singaporeair.com/saar5/pdf/Investor-Relations/Annual-Report/sustainabilityreport2021.pdf.

[122] Singh, J., Sharma, S.K., &Srivastava, R. (2018).Managing fuel efficiency in the aviation sector: Challenges, accomplishments and opportunities. *FIIB Business Review*, 7(4), 244-251. https://doi.org/10.1177%2F2319714518814073

[123] Škurla, R., Kolar, V.,& Takač, A. (2002). Environmental management system: Current airline trends and practices in the EU with prospects for Croatia. Retrieved from https://www.bib.irb.hr/80746/download/80746.kurla_ISEP2002.doc.

[124] Smith, M.J.T. (2004).*Aircraft noise*. Cambridge, UK: Cambridge University Press.

[125] Stake, R.E. (1995). *The art of case study research*. Thousand Oaks, CA: SAGE Publications.

[126] Stake, R.E. (2005). Qualitative case studies. In N.K. Denzin & Y.S. Lincoln (Eds.),*The SAGE handbook of qualitative research* (pp. 443-466). Thousand Oaks, CA: SAGE Publications.

[127] Staples, M.D., Malina, R., Suresh, P., Hileman, J.I., &Barrett, S.R.H. (2014). Aviation CO_2 emissions reductions from the use of alternative jet fuels. *Energy Policy*, 114, 342-354.https://doi.org/10.1016/j.enpol.2017.12.007

[128] Suksmith, P.L.,&Nitivattananon, V. (2015). Aviation impacts on property values and management: The case of Suvarnabhumi International Airport.*IATSS Research*, 39(1), 58-71.https://doi.org/10.1016/j.iatssr.2014.07.001

[129] Teoh, L.E., &Khoo, H.L. (2016). Green air transport system: An overview of issues, strategies and challenges. *KSCE Journal of Civil Engineering*,20, 1040–1052 (2016).https://doi.org/10.1007/s12205-016-1670-3

[130] Thai Airways International. (2020). Sustainable development report 2019. Retrieved from https://thai.listedcompany.com/misc/sdreport/20200624-thai-sdreport2019-en.pdf.

[131] Thai Airways International. (2021). THAI with ISO. Retrieved from https://www.thaiairways.com/en/about_thai/public_information/general_information/thai_with_iso.page.

[132] Travel PR News.(2016). Lufthansa Hub Munich certified with the international standard ISO 14001 for environmental management systems. Retrieved from https://travelprnews.com/lufthansa-hub-munich-certified-with-the-international-standard-iso-14001-for-environmental-management-systems/travel-press-release/2018/05/16/.

[133] TUI fly. (2021a). Towards an even more sustainable TUI fly. Retrieved from https://www.tuifly.be/en/legal/company_information.

[134] TUI fly. (2021b). Company information. Retrieved from https://www.tuifly.be/en/more-sustainable.

[135] Turkish Airlines.(2014). Widen your world: Environmental and social responsibility report. Retrieved from https://investor.turkishairlines.com/documents/sustainability/environmental_and_social_responsiblty.pdf.

[136] United States Environmental Protection Agency. (2021). Learn about environmental management systems. Retrieved from https://www.epa.gov/ems/learn-about-environmental-management-systems.

[137] Whitelaw, K. (2004). *ISO 14001 environmental systems handbook*(2nd ed.). Oxford, UK: Elsevier Butterworth-Heinemann.

[138] Whyte, R., & Lohmann, G. (2017). Airline business models. In L. Budd & S. Ison (Eds.), *Air transport management: An international perspective* (pp. 107-121). Abingdon, UK: Routledge.

[139] World Business Council for Sustainable Development. (2009). Asiana Airlines: Environmentally friendly management and sustainability. Retrieved from http://docs.wbcsd.org/2009/12/Asiana_Airlines_Case.pdf.

[140] Yin, R.K. (2018). *Case study research and applications* (6th ed.). Thousand Oaks, CA: SAGE Publications.

[141] Young, S.B.,& Wells, A.T. (2011).*Airport planning and management*(6th ed.).New York, NY: McGraw-Hill.

[142] Zobel, T. (2013). ISO 14001 certification in manufacturing firms: A tool for those in need or an indication of greenness? *Journal of Cleaner Production*, 43, 37-44.https://doi.org/10.1016/j.jclepro.2012.12.014

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Decarbonizing International Air Cargo Transportation's Carbon Footprint: A Review of the World Air Cargo Carrying Airlines Current and Potential Environment Related Measures and Strategies

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Abstract— *Using an in-depth instrumental case study research design, this study has examined the current environmental related measures and strategies that have been defined and implemented to decarbonize air cargo operations by the world's air cargo carrying airlines. The study covered the period 2004 to 2021. The case study found that the world's air cargo carrying airlines are very cognizant of their environmental impact and, as a result, these airlines have defined and implemented an extensive range of measures and strategies to decarbonize their operations. These measures include the acquisition and deployment of fuel-efficient next generation aircraft, aircraft related carbon dioxide (CO_2) emissions offset programs, the use of fixed electrical ground power (FEGP) and pre-conditioned air at airports, the use of lightweight aircraft unit load devices (ULDs), use of sustainable aviation fuels, the use of renewable energy sources for ground based buildings and facilities, the electrification of air cargo carrying airlines ground service equipment (GSE), the optimization of air traffic management procedures, and the washing of aircraft engines. A key strategy adopted by the world's air cargo carrying airlines has been the use of sustainable aviation fuels (SAF), as this fuel source provides a very significant opportunity for the decarbonization of aircraft operations. Several key air cargo industry actors are planning in the future to use electric powered aircraft. Hydrogen as an aircraft energy source is also being considered. To mitigate the environmental impact of wastes, air cargo carrying airlines could consider adopting the circular economy waste management approach.*

Keywords— ***Air cargo carrying airlines, Air cargo carrying airlines decarbonization measures, Case study, Decarbonization, Sustainable aviation fuels.***

I. INTRODUCTION

Due to the adverse impact on the environment, the biggest issue confronting the global air transport industry today is sustainability (Turner, 2021g). Indeed, sustainability has assumed strategic importance in the air transport industry (Graham, 2021b). Consequently, all around the world, the environmental sustainability of air transport has been receiving greater focus in recent times due to its critical impact on climate change and on the environment (Budd, 2017; Chen, 2012; Teoh & Khoo, 2016). Environmental issues associated with the global air transport industry have grown in importance in recent years, and in response airlines have been proactive by demonstrating their "green" credentials (Mayer et al., 2012; Migdadi, 2018). In recent decades, greening (ensuring the sustainable development of the global air transport system) has been viewed as a highly significant part of the agenda by almost all the industry's involved stakeholders (Janić, 2011). These stakeholders include aviation organizations for international cooperation; international aviation organizations; air transport system operators, for example, airports, air traffic control (ATC) and airlines; aerospace manufacturers; non-governmental organizations; users, for example, passengers and air cargo shippers; and research, scientific and consultancy organizations (Janić, 2016). The aim of a "green airline" is to provide the green society with a transport system that reduces its carbon footprint, uses renewable energy, and produces less carbon dioxide (CO_2) emissions as well as other harmful pollutants (Abdullah et al., 2016). The concept of "greening" aviation firms, such as, airlines, can be best linked to their reduction of the level of emissions into the atmosphere, to the point where these airlines achieve near carbon neutrality (Sarkar, 2012).

The air transportation of goods/freight for commercial purposes plays a very significant role in the world economy (Alemán, 2010; Dewulf et al., 2019; Heng, 2016). Air cargo is defined as "anything carried in an aircraft except for mail or luggage carried under a passenger ticket and baggage check but including baggage shipped under an airway bill or shipment record" (Hui et al., 2004). Passenger baggage is associated with the carriage of passengers and is included as part of the individual passenger's air fare. Passenger baggage is therefore not a part of the air cargo service. (Dempsey & Gesell, 1997). Air cargo consignments are typically time sensitive and/or are high value-to-weight goods (Budd & Ison, 2017). The five key industry sectors that utilize the air cargo mode are equipment, consumer electronics, pharmaceuticals, healthcare, and retail products sectors. When combined they comprise around 90% of total world air cargo traffic. In addition, the air cargo mode is used by firms from varying market sectors and geographic region's locations to connect them to new customers and suppliers that may not be otherwise accessible by other transport modes (Turner, 2021f).

In the world air cargo industry, air cargo capacity is provided by three distinct types of airline operators. Combination passenger airlines are airlines that carry passengers on the main deck and transport air cargo in their passenger aircraft lower lobe belly-holds. Some combination airlines, for example, Cathay Pacific Airways, Qatar Airways, and Singapore Airlines, also operate freighter aircraft. Shippers may also decide to use dedicated all-cargo airlines, for example, Cargolux International Airlines or Nippon Cargo Airlines (NCA). The final type of air cargo carrying airline operator is the integrated carriers, for example, DHL Express, FedEx and United Parcel Service (UPS) (Baxter & Wild, 2021; Dresner & Zou, 2017; Merkert & Alexander, 2019). The integrated carriers operate very large fleets of freighter aircraft. A freighter aircraft is an aircraft that has been expressly designed or which has been converted to transport air cargo, express, and so forth, rather than passengers (Wensveen, 2016). All-cargo services are operated with freighter aircraft where all the available capacity is dedicated to air cargo transportation (Dresner & Zou, 2017; Tretheway & Andriulaitis, 2016). Dedicated all-cargo services are vital to the aviation industry, and to the global economy (Davies, 2013). Around 56 per cent of global air cargo revenue ton kilometres (RTKs) is carried in dedicated freighter aircraft (Boeing Commercial Airplanes, 2020). In providing air cargo services, airlines have various environmental impacts (Sales, 2013, 2016, 2017). These environmental impacts include the production of greenhouse gas (GHG) emissions both in-flight and from ground operations, the production of large volumes of waste, and the consumption of significant amounts of resources, such as, jet fuel, energy for ground-based facilities and ground handling equipment, and water.

Both climate change and carbon footprints are now frequently being regarded as two of the most urgent concerns confronting society and are now viewed as key issues of corporate responsibility (Hrasky, 2012). According to Wiedemann and Minx (2007, p. 5), "the carbon footprint is a measure of the exclusive total amount of carbon dioxide emissions that is directly and indirectly caused by an activity or is accumulated over the life stages of a product". Carbon footprints are now being extensively used as a measure of an organization's contribution to climate change (De Grosbois & Fennell, 2011). The measurement of its carbon footprint enables firms to estimate their own contributions to the changing climate (Gautam et al., 2012). Carbon foot printing is now one of the principal methods available to firms for quantifying their anthropogenic environmental impacts and for assisting them to tackle the threat of climate change (Williams et al., 2012).

The objective of this study is to examine the current environmental related measures and strategies that have been defined and implemented to decarbonize air cargo operations by the world's air cargo carrying airlines. The first objective is to identify the various environmental impacts that air cargo carrying airlines will have on the natural environment. The second objective is to identify and examine the various environmental related measures and strategies that have been implemented by air cargo carrying airlines, irrespective of their chosen business model, to decarbonize their air cargo operations. A further objective is to examine the benefits for air cargo carrying airlines of implementing sustainable waste management practices. This is because the disposal of wastes can also be a source of carbon dioxide (CO_2) emissions, especially if the wastes are disposed to landfill. The levels of emissions from waste are dependent upon how the waste is treated (Eurostat, 2020). The study covered the period 2004 to 2021. 'Decarbonization' often refers to the process of reducing 'carbon intensity'. This is achieved by a firm lowering the amount of greenhouse (GHG) gas emissions that is produced from the burning of fossil fuels (TWI Ltd, 2021).

The remainder of the paper is organized as follows: The literature review is presented in Section 2. Section 3 describes the study's research methodology that underpinned the study. The case study is presented in Section 4. Section 5 outlines the study's conclusions.

II. BACKGROUND

2.1 A Brief Overview of the Global Air Cargo Industry

Historically, the world air cargo industry has been a high growth industry (Albers, 2015). This trend can be observed in Figure 1 which shows the growth in total annual world domestic/international enplaned air cargo tonnage and freight tonne kilometres performed (FTKs) from 2000 to 2019. Over this period, the total annual enplaned tonnage and FTKs grew from 30 million tonnes and 118.0 billion FTKs in 2000 to 57.6 million tons and 225 billion FTKs in 2019, respectively. Freight tonne kilometres performed (FTKs) are calculated from the multiplication of freight tonnes carried by the distance of the flight. Most domestic cargo is transported in the USA and is principally carried by the integrated carriers, such as FedEx and United Parcel Service (UPS) (Doganis, 2019). The air cargo industry is cyclical in nature (Doganis, 2019; Morrell & Klein, 2018) and is sensitive to changes in any cyclical economic movements (Macário & Van de Voorde, 2011). Figure 1 shows that total world air cargo traffic (domestic and international enplaned tonnage and FTKs) showed marked declines in 2001 and 2008 following the tragic events of September 11 and the 2008-2009 global financial crisis (GFC).

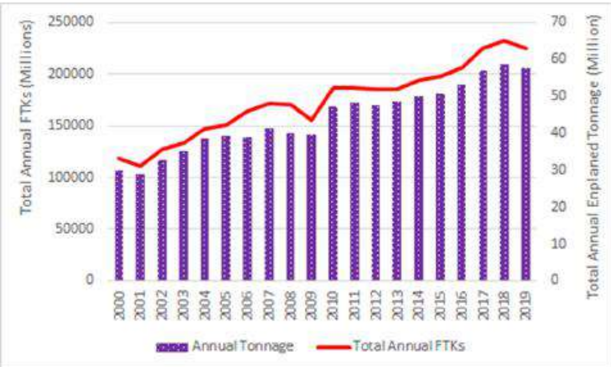

Fig.1: World air cargo industry total annual enplaned tonnage and freight tonne kilometres (FTKs) performed: 2000-2019. Source: International Civil Aviation Organization (2012, 2013, 2020).

Air cargo service providers are a heterogeneous group of actors. Together they deliver various logistics and supply chain services as well as their expertise. The air cargo industry is comprised of various commercial firms that provide shippers and/or consignees with their desired air cargo services. An airline provides transportation for air cargo consignments from the airport of origin to the destination airport. Combination airlines include airlines that provide both passenger and air cargo services. Most international carriers fall into this category. Combination airlines generally provide customers with airport-to-airport services and these airlines rely on air freight forwarders to perform the remaining transport logistics and supply chain services. Dedicated all-cargo airlines operate scheduled freighter flights on routes where there is regular heavy air cargo demand or where the potential passenger traffic is not large. The all-cargo airlines normally carry air cargo only on an airport-to-airport basis. The all-cargo airlines also provide charter services. The integrators are firms that integrate the air and ground transport services traditionally performed by separate firms and they provide a full door-to-door service. The integrators also provide supply chain and logistics solutions, which are underpinned by highly advanced IT systems (Baxter & Wild, 2021). International air freight forwarders are another very important actor in the global air cargo supply chain and these firms' contract with airlines for the physical carriage of goods. Freight forwarders often purchase block cargo space on airlines flights. Freight forwarders often work closely with complementary surface transportation service providers (Al Hajri, 1999).

2.2 The Environmental Impact of Air Transport Operations

2.2.1 Aircraft emissions

The growth of commercial air transport has driven concerns over air quality (Gössling & Upham, 2009) as air traffic growth has increased the air transport industry's annual carbon dioxide (CO_2) emissions (Fatihah & Abdul Rahim, 2017). The global aviation industry carbon dioxide (CO_2) emissions contribute to global warming (Chiambaretto et al., 2021). Thus, an environmental threat has emerged, that of aircraft emissions (Marais et al., 2016; Masiol & Harrison, 2014; Wey & Lee, 2017). By consuming fuel, aircraft produce emissions of carbon dioxide (CO_2), nitrogen oxides (NO_X), particles (principally soot) of sulphur oxides, carbon monoxide (CO), as well as various hydrocarbons. First, and generating the largest percentage share, are the emissions of carbon dioxide (CO_2), which are produced in direct proportion to the volume of jet fuel used to operate flights over any distance (Sales, 2017).

Aircraft operating at subsonic speeds in flight cause the following main effects to the environment:

- Carbon dioxide (CO_2): carbon dioxide (CO_2) emissions are the most common (Sales, 2016). Carbon dioxide (CO_2) emissions are acknowledged as a major contributor to climate change (Abd et al., 2021; Yadhav & Wang, 2017; Zhang et al., 2020).
- Oxides of nitrogen (NO_x): at high altitudes NOx emissions help to form ozone in the upper troposphere. They also reduce methane, which results in a cooling effect.
- Water vapor (H2O): Water vapor is created through the burning of jet fuel. At altitude, condensation trails form, consisting of frozen ice crystals that deflect a small amount of sunlight away from the Earth's surface and reflect more radiation back towards the Earth. This results in an overall warming effect on the Earth's atmosphere (Sales, 2016, p.146).

Pollutants are also produced during aircraft taxiing, at take-off, at landing, and often during the period when aircraft are sitting idle. Surface vehicles associated with the transportation of crews and their baggage are another source of pollutants (Prosperi, 2009).

2.2.2 Aircraft fuel efficiency

Increasing environmental concerns have drawn significant attention of the air transport industry towards the requirement for judicious use of aviation fuel. Consequently, both economic and environmental sustainability concerns have led to dramatic progress in aviation fuel efficiency improvements in the past decades (Singh et al., 2018).

In recent times, both airlines and the aircraft manufacturers have invested in new technologies and strategies to reduce aircraft fuel consumption and the concomitant emissions. Aircraft fuel has a close relationship with the emissions of carbon dioxide (CO_2) and other gases that result in climate change (Zou et al., 2016).

2.3 Carbon offsetting and reduction scheme for international aviation (CORSIA)

The 2015 Paris Climate Conference or COP 21 agreement provided a stimulus for the air transport industry to develop a global market-based measure (MBM) to reduce carbon dioxide (CO_2) emissions (Singapore Airlines, 2017). Effective in 2021, an increased share of the carbon emission growth in the international air transport industry will be subject to offsetting under the International Civil Aviation Organization (ICAO) "Carbon Offsetting and Reduction Scheme for International Aviation" (CORSIA) program (Kováčik et al., 2021; Maertens et al., 2019). Effective from 1 January 2021, all international flights will become subject to offsetting obligations under the CORSIA program (International Air Transport Association, 2021b). The objective of the Carbon Offsetting and Reduction Scheme for International Aviation, or CORSIA program, is the stabilization of carbon dioxide (CO_2) emissions at 2020 levels. Consequently, this will require airlines to offset the growth of their emissions from 2020 onwards. Airlines will also be required to monitor emissions on all their international services as well as offsetting emissions from air routes included in the scheme through the purchase of eligible emission units that are generated by projects that reduce emissions in other sectors (for example renewable energy) (European Commission, 2021).

The CORSIA program will be implemented in three discrete phases with the pilot phase operating from 2021-2023. The first phase will be from 2024 to 2026. Both the pilot and first phases are voluntary. The second phase of the program is targeted at the 2027 to 2035 time-period (Javed et al., 2019). Following the completion of the pilot and first phases, a second mandatory scheme will enter in effect for all ICAO member states, except for some least developed countries (Scott & Trimarchi, 2020). The global COVID-19 pandemic resulted in a very significant decline in the worldwide aviation industry traffic and, as a result, the International Civil Aviation Organization (ICAO) adjusted its CORSIA program by removing 2020 emissions from the baseline, which are now based on 2019 emission levels (Zhang et al., 2021). The voluntary pilot period for ICAO's CORSIA program became effective as of 2021 and will become mandatory for all airlines from 2027 onwards (Singapore Airlines, 2021).

2.4 The Reduction in Aircraft Weights as an Environmental Mitigation Measure

In recent times, airlines from around the world have implemented a range of measures that have been designed to lower the weight of their aircraft, and thus, reduce fuel burn and the associated harmful emissions. The weight saving initiatives include the correct stowage of items to avoid unnecessarily ordering catering supplies and other in-flight service equipment, the removal of rubbish, and the reduction in on-board company materials. In addition, airlines have implemented potable water strategies whereby they carefully optimize the water uplift on flights to satisfy passenger requirements whilst at the same time achieving fuel and emissions savings from the lower the aircraft weight (Baxter, 2016).

2.5 The Use of Sustainable Aviation Fuels as an Environmental Related Measure

Alternative fuels can help industry stakeholders to provide sustainable air transport since these fuels reduce the air transport industry climate-relevant emissions (Scheelhaase et al., 2019). Considering this one of the most important trends in the global airline industry in recent times has been the uptake in the use of sustainable aviation biofuel. Airlines now regard the use of aviation biofuel as being a key environmental sustainability measure (Baxter et al., 2020; Bittner et al., 2015; Cortez et al., 2015). As a result, alternative jet fuel (AJF) technologies have gained considerable interest and are now regarded as a way for the industry to achieve large, near-term emissions reductions (Staples et al., 2014). Currently, sustainable aviation fuel (SAF) is a drop-in solution. This means that current aircraft can use a 50 percent blend of sustainable aviation fuel (SAF) and common Jet A fuel without any modifications (Reichmann, 2021a).

Air cargo is in a strong position to lead the air transport industry in the adoption of green initiatives, with sustainable aviation fuel (SAF) proving to be the best way for the industry to reduce its carbon dioxide (CO_2) emissions (Brett, 2021a). Sustainable jet fuels now underpin the airline industry's strategy to reduce their carbon emissions (Gegg et al., 2014; Schäfer, 2016). According to Tavares Kennedy (2019), "depending upon the raw material used in its production, biofuels can reduce carbon dioxide (CO_2) emissions by 60-80%". The International Air Transport Association (2021a) have observed that sustainable aviation fuel can reduce emissions by up to 80% over its full lifecycle (International Air Transport Association, 2021a).

Aviation biofuels are therefore becoming an important substitute for fossil fuel in the airline industry. The use of aviation biofuels help with an airline's sustainability goals as they are environmentally friendly (Su et al., 2015). Furthermore, the replacement of fossil fuels by sustainable aviation biofuels is one of the principal strategies adopted in the global airlines to decrease their carbon dioxide (CO_2) emissions by 50% by 2050 (Bauen & Nattrass, 2018; Dodd et al., 2018). Hence, the airline industry now views the use of sustainable biofuels as being the key long-term technology for decarbonizing aviation (Fregnani & Andrade, 2017). Furthermore, airlines are counting on sustainable aviation fuels (SAF) to reduce their carbon dioxide (CO_2) emissions in the years prior to electric and hydrogen-based propulsion systems become widely available, which will most likely occur after 2035 (Air Cargo World, 2021).

III. RESEARCH METHODOLOGY

3.1. Research Approach

The research undertaken in the present study used an instrumental case study research approach (Beasley & Bernadowski, 2019; Honeycutt & Miller, 2021; Sadang et al., 2021). An instrumental case study is the study of a case so that it provides insights into a specific issue, redraws generalizations, or builds theory (Stake, 1995, 2005). An instrumental case study is designed around established theory of the phenomenon under study (Grandy, 2010). The present study was designed around the established theory of airline carbon footprints (Tuladhar et al., 2021), carbon footprints (Franchetti & Apul, 2013; Hunter, 2014; Ramachandra & Mahapatra, 2016), sustainable aviation (Budd et al., 2013; Palmer, 2020; Ydersbond et al., 2020), sustainable aviation biofuels (Cortez, 2014; Gegg et al., 2014; Schäfer, 2016), and the environmental impact of air cargo operations (Sales, 2013, 2016, 2017).

3.2. Data Collection

The documents collected and examined in the study included news items in the leading air cargo industry related journals and magazines, airline annual sustainability reports, press releases, and airline's websites. A comprehensive search of the air transport and air cargo industry related magazines was also conducted. The SCOPUS and Google Scholar databases were also examined in the study.

The study followed the three principles of data collection as recommended by Yin (2018), that is, the use of multiple sources of case evidence, creation of a database on the subject, and the establishment of a chain of evidence.

3.3 Data Analysis

The data collected for the case study was examined using document analysis. Document analysis focuses on the information and data that is obtained from the formal documents and company records gathered by the researcher(s) (Oates, 2006; Ramon Gil-Garcia, 2012; Yin, 2018). In this study, the documents were examined for their authenticity, credibility, representativeness, and meaning (Scott, 2014; Scott & Marshall, 2009).

The key words used in the database searches included "air cargo carrying airlines decarbonization strategies and measures", "air cargo carrying airlines acquisition and deployment of fuel-efficient next generation aircraft", "air cargo carrying airlines offsetting of aircraft related carbon dioxide (CO_2) emissions", "air cargo carrying airlines use of electric powered aircraft", " air cargo carrying airlines use of fixed electrical ground power at airports", "air cargo carrying airlines potential use of hydrogen as an aircraft energy source", "air carrying airlines use of lightweight aircraft unit load devices", "air cargo carrying airlines use of sustainable aviation fuels as an industry decarbonization related measure", "air cargo carrying airlines use of renewable energy sources for ground based buildings and facilities", "air cargo carrying airlines use of sustainable waste management principles", "electrification of air cargo carrying airlines ground service equipment (GSE)", the "Sustainable Air Freight Alliance", "the optimization of air traffic management procedures", and the "washing of aircraft engines by air cargo carrying airlines".

The study's document analysis was conducted in six distinct stages. The first stage involved planning the types and required documentation and ascertaining their availability for the study. In the second phase, the data collection involved sourcing the documents that were necessary to conduct the study. This stage of the document analysis process also involved the development and implementation of a scheme for managing the documents collected for the study. The documents were carefully examined to assess their authenticity, credibility and to identify any potential bias in the third stage of the document analysis process. In the fourth stage, the content of the collected documents was carefully examined, and the key themes and issues were identified and recorded. The fifth stage involved the deliberation and refinement to identify any difficulties associated with the documents, reviewing sources, as well as exploring the documents content. In the sixth and concluding stage, the analysis of the data was finalized (O'Leary, 2004).

Following the guidance of Yin (2018), the study's documents were stored in a case study database. All the documents collected for the study were in English. Each document was carefully read, and key themes were coded and recorded in the case study (Baxter, 2020).

IV. RESULTS

4.1 The Acquisition and Deployment of Fuel-Efficient Next Generation Aircraft

A key strategy adopted by the combination airlines, all-cargo airlines, and the integrated carriers in recent times has been the renewal of their aircraft fleets with more fuel efficient, and thus, lower-emissions aircraft. Over the past decade, next generation twin engine, long-haul aircraft have entered commercial service (Aircraft Commerce, 2016). The Airbus A350 XWB family was initially intended to have three variants: the Airbus A350-800, Airbus A350-900, and the Airbus A350-1000. The Boeing 787 Dreamliner family includes the 787-8, 787-9, and the 787-10. The Airbus A350-900XWB entered commercial service with Qatar Airways in 2014 (Aircraft Commerce, 2015). The first Airbus A350-1000 was delivered to Qatar Airways in February 2018 (Airbus, 2018). The Boeing 787-8 entered commercial service in 2011, whilst the 787-9 began commercial services in 2014 with Japan-based All Nippon Airways (Aircraft Commerce, 2015). Singapore Airlines took delivery of the first Boeing 787-10 on March 14th, 2018 (Boon, 2020). The Airbus A350 and Boeing 787 aircraft families are more fuel efficient (Salvatore, 2020). Airlines changing aircraft from the Boeing 767 and Airbus A340 to the newer Airbus A350, and Boeing 787 can reduce their aircraft emissions by 15-20% when flying on the same route (Szymczak, 2021). Thus, airlines operating Airbus A350 and Boeing 787 aircraft can reduce their carbon footprint. Furthermore, airlines have recently introduced new single-aisle aircraft (for example, the Airbus A220-100 and -300) and several derivative aircraft which have major propulsion and airframe technology upgrades (for example, the Airbus A320neo and A330neo, and the Boeing B737 MAX family). These new aircraft models have commenced commercial airline service in recent times and these aircraft provide substantial reductions in aircraft fuel burn (International Coordinating Council of Aerospace Industries Associations, 2019), and the concomitant aircraft emissions.

The 747-8 Intercontinental (the passenger variant of the Boeing 747-8 aircraft) improves fuel efficiency by 16% as compared to its predecessor the Boeing 747-400, Since introduction into commercial service in 2011, the Boeing 747-8's fuel efficiency has been further improved by an additional 3.5%. (Air Transport Action Group, 2021a)

As noted earlier, around half of world cargo traffic is carried in the lower lobe belly hold compartments of passenger aircraft with the remainder transported on dedicated freighter aircraft (Morrell, 2016). Since the late 2000s, the aircraft manufacturers, Airbus and Boeing Commercial Airplanes, have enhanced their freighter aircraft range and the improvement in new technologies has resulted in greater fuel efficiency, and thus, lower aircraft emissions. Small standard size and medium size freighter aircraft are operated by airlines because of their capability to operate high-frequency services. In recent times passenger to freighter (P2F) have assumed a key role in the global air cargo industry. The Airbus A321P2F, Boeing B737P2F, Boeing B737-400P2F, and Boeing 737-800P2F passenger aircraft have largely replaced the older Boeing B727 and McDonnell Douglas DC9F aircraft, with several companies offering Airbus A321 and Boeing B737 passenger-to-freighter (P2F) aircraft conversion services. Medium-capacity wide-body freighter aircraft such as the Airbus A330-200F and Boeing B767-300F, are operated extensively by the integrated carriers and by some non-integrated carriers.

The Airbus A330-200F was launched in January 2007, with commitments from Indian cargo start-up Flyington Freighters and lessor companies Intrepid Aviation Group and Guggenheim Aviation Partners for 32 aircraft (Kingsley-Jones, 2010). The A330-200F is a derivative of the very popular Airbus A330 Family. The A330-200F aircraft benefits from full operational commonality unique to Airbus due to the Airbus Fly-By-Wire technology which also enables faster pilot transitioning to and from other Airbus aircraft, both passenger and freighter aircraft. The Airbus A330-200 freighter is a mid-size, long-haul dedicated all-cargo aircraft. When operating in payload mode the aircraft can carry a 70 metric tons structural payload over a range of 4074.4 kilometres (2,200nm). In range mode, the aircraft can carry a 65 metric tons payload (Ierovante et al., 2015) over a range of 7,400km (4,000nm) (Kingsley-Jones, 2006).

Derived from the popular Boeing 767-300ER (extended range) passenger twinjet, the Boeing B767-300F benefits from the advanced avionics, aerodynamics, materials, and propulsion incorporated on Boeing 767 passenger aircraft. The Boeing B767-300 freighter aircraft can carry a payload of 52.5 tonnes over a range of 3,255 nautical miles/ 6,028 kilometres (Boeing Commercial Airplanes, 2021). Importantly, the Boeing B767-300 freighter aircraft is more fuel efficient than other aircraft offered in the medium sized freighter aircraft market segment (Boeing Commercial Airplanes, 2014)

Aimed at the large freighter market and launched in May 2005 on the back of the newly available Boeing 777-200LR airframe/engine combination, the Boeing 777-200LRF freighter aircraft offers a 103.9 tonne revenue payload capability (Norris 2006). The economics of the aircraft are extremely favourable with cargo density identical to the Boeing B747-400F (Conway, 2005), and 20 per cent lower fuel burn than the Boeing B747-400F, and thus, this aircraft has lower emissions levels. The Boeing 777 freighter aircraft can carry its full cargo load over a range of 9,000 kilometres (Ostrower, 2008). The Boeing B777-200LRF freighter entered service with Air France in 2009 (Conway, 2011).

The new-technology Boeing 747-8 Freighter was the latest version of the Boeing B747 freighter aircraft family. Boeing launched the 747-8 Family with orders for the freighter aircraft in November 2005 from Cargolux and Nippon Cargo Airlines (NCA), after considerable study of the market feasibility of a new 747 (Conway, 2012). By working together with customers and applying innovative new technologies from the 787 Dreamliner aircraft, Boeing was able to develop and offer the 747-8 freighter aircraft to airlines competing in the large aircraft size global air cargo market segment. The maximum payload of Boeing's next generation B747-8F is around 134 tonnes (Conway, 2005). The freighter aircraft has a range of 8,130km and has a 16% higher cargo volume than the 747-400. The Boeing 747-8 Freighter has a range of 4,475 nautical miles (8,275 km) and provides operators with 16 per cent more cargo volume than the Boeing 747-400 freighter aircraft (Airport Technology, 2021). In addition, the Boeing 747-8 freighter offered a 16% reduction in fuel usage (Benito & Alonso, 2018).

4.2 The Offsetting of Aircraft Related Carbon Dioxide (CO_2) Emissions

As previously noted, carbon offset programs are a mechanism available to the world's air cargo carrying airlines to mitigate their carbon footprint. Lufthansa Cargo is one such airline that offers such a program. At the time of the present study, Lufthansa Cargo offered two options for transporting shipments on a more sustainable basis. The first option is based on the use of sustainable aviation fuel (SAF), which is discussed below. The second option is a cargo offset program. Carbon dioxide (CO_2) emissions are produced during the production and transport of sustainable aviation fuels (SAF), and these emissions can be offset through compensation with environmentally friendly climate protection projects. As a result, the complete carbon dioxide (CO_2) neutrality of an air cargo consignment can be achieved either by combining both options or alternatively by 100 percent compensation in climate protection projects (Brett, 2021d).

4.2 The Electrification of Ground Service Equipment (GSE)

An important side effect of transportation is air pollution from the burning of fossil fuels. Electric powered vehicles can play an important role in improving air quality of transportation services (Longo et al., 2017). It is important to note that the ground service equipment (GSE) used at airports by airlines and ground handling agents to facilitate the turnaround of their aircraft produce harmful emissions, and hence, impact air quality at the airport (Budd, 2017). A solution to this issue is the use of electric powered vehicles and ground service equipment (GSE) (Gellings, 2011). In recent times, electricity powered aircraft loaders have started to replace diesel powered loaders (Ramsay, 2020). Electricity powered aircraft push-back tugs are also available, and there has been a trend among some operators to choose battery-powered aircraft tractors (Airside International, 2020). Electricity powered baggage tractors are also being used at airports, for example, Amsterdam's Schiphol Airport (Airside International, 2021). Air cargo carrying airlines also have the option to acquire and deploy electricity or battery powered air cargo tugs. Stuttgart Airport, for example, operates a fleet of electricity powered air cargo tractors. These tractors offer environmental related benefits, which include energy savings, emission reductions and noise reductions (Randall, 2019).

In March 2004, FedEx commenced using some hybrid electric trucks, which the company put into service in Sacramento, California. FedEx Express developed the truck in concert with Environmental Defense and Eaton Corporation, which produced the electric powertrain (Brown & Jackson, 2004). In 2010, FedEx added all-electric delivery vehicles in Paris and Los Angeles, building on its existing fleet of all-electric delivery vehicles in London. The integration of all-electric vehicles was a key part of the FedEx pledge to improve the fuel efficiency of its vehicle fleet by 20 percent through its Reduce, Replace and Revolutionize strategy (FedEx, 2010). In March 2021, FedEx announced that it would deploy deploying more zero-emission electric trucks for pick-ups and deliveries as part of its foal to achieve carbon neutrality by 2040 (Wolfsteller, 2021).

In the first quarter of 2021, the Deutsche Post DHL Group announced that it will invest €7bn to reduce its production of carbon dioxide (CO_2) emissions by 2030. As part of its roadmap to sustainability, the company will acquire and deploy 80,000 e-vehicles to be deployed for last-minute deliveries. This will result in a 60% electrification of the company's vehicle fleet **(**Grasso Macola, 2021**).**

4.3 The Optimization of Air Traffic Management Procedures

A further potential measure to reduce the carbon footprint of air carrying airlines is the optimization of air traffic management procedures. Emirates, Singapore Airlines and Cargolux Airlines International are examples of airlines that have worked closely with air traffic service providers to optimize aircraft fuel burn, and thus, the lower the associated emissions.

In 2018, Cargolux participated in the inaugural meeting to establish "continuous descent operations (CDO) at Luxembourg Airport Cargolux subsequently cooperated with the local air navigation service provider (ANSP) to develop CDOs arrivals into Luxembourg (Cargolux International Airlines, 2019). A continuous descent operation (CDO) is one in which the arriving aircraft descends from its cruise level to an airport with its engines at near-idle thrust (Itoh & Uejima, 2013). With the Cargolux CDO program a number of waypoints on the flight route were established that enable pilots to efficiently plan the descent of their aircraft. Both CDOs and CDAs (Continuous Descent Operations and Approach) methodologies offer the potential for significant fuel savings as well as substantial reduction in carbon dioxide (CO_2) emissions. For example, a CDO(F) arrival can provide fuel savings of approximately 250 kg per flight for a Boeing B747 freighter aircraft, as the engines remain at a near-idle thrust during the process (Cargolux Airlines International, 2019).

In 2010, Cargolux completed a program to equip all aircraft in its own aircraft fleet with the Iridium onboard satellite communications system. The Iridium system offers global coverage, including over the polar regions. As a result, in June 2010, Cargolux aircraft were able to operate on "Future Air Navigation System" (FANS) routes and thereby achieve significant savings in flying time (Cargolux International Airlines, 2011). The reduced flying time provided fuel savings and resulted in lower levels of aircraft emissions. The "Future Air Navigation System" (FANS) is an operational concept which relies upon satellite-based navigation and communication to provide the improvements required in communication, navigation, and surveillance (CNS) to efficiently handle the projected increase in traffic levels (Golmohammad & Mehdizadeh Dastjerdi, 2012).

Dubai-based Emirates Airline optimizes fuel efficiency in its operations. Once airborne, the airline's flight crew take advantage of opportunities to save aircraft fuel and thus lower emissions where practicable and supported by air traffic control. The airline fuel efficiency measures include continuous aircraft climbs and descents, and inflight rerouting on long-haul flights to consider more favorable shifting winds. Upon landing, the company's pilots use idle reverse thrust (instead of full reverse thrust) and switch off one engine while taxiing to the apron area where it is safe and practicable to do so (Emirates Airline, 2021).

A key focus for Singapore Airlines environmentally sustainable operations has been the adoption of several improved flight operation measures that not only reduce fuel consumption but also the associated carbon dioxide (CO_2) emissions. These measures include the use "continuous descent" operations, and to minimize fuel use (without impacting safety). In addition, operational procedures that reduce fuel burn for Singapore Airlines Airbus A380 operations have been implemented at London's Heathrow Airport. Singapore Airlines Airbus A380 aircraft departing from Heathrow Airport use less power when taking off, resulting in fuel savings and less carbon dioxide (CO_2) and nitrous oxide (NOx) emissions. Furthermore, Singapore Airlines optimization of flight operations and flight planning systems have led to more optimum flight route selection (Singapore Airlines, 2017, 2018), and thus, a reduction in carbon dioxide (CO_2) emissions.

4.4 The Use of Electric Powered Aircraft

United Parcel Service (UPS), as part of its environmental commitment, ordered vertical takeoff and landing electric (eVTOL) aircraft in April 2021. The aircraft that UPS are acquiring are the ALIA-250c, which is manufactured by Beta Technologies. With a flying range of 402 kilometres and cargo volume of 5.66 cubic metres (635 kilograms capacity), these aircraft will be limited to carrying smaller loads. These new aircraft will replace ground transport, which takes longer, and small feeder aircraft, which are more polluting and require airports for takeoff and landing. UPS has placed firm orders 10 eVTOL aircraft and UPS have the option to increase the purchase from Beta to 150 aircraft. These aircraft will require recharging, and UPS has also reserved Beta's recharging station allowing for a rapid recharge in under an hour, which is sufficient time for the aircraft to be unloaded and reloaded ready for the next flight (Humphries, 2021). UPS is expected to take delivery of the first ten aircraft in 2024 (Reichmann, 2021b).

In August 2021, DHL's air cargo subsidiary DHL Express, announced an order of 12 all-electric powered aircraft from Seattle-based manufacturer "Eviation". These new aircraft will be added to the company's fleet of powered vans and bikes. Once they enter service in 2024, the aircraft are scheduled to be operational on DHL Californian routes. According to DHL, the electrification of every transport mode plays a critical role and will significantly contribute to the company's overall sustainability goal of zero emissions. The aircraft will be flown by a single pilot and will only require 30 minutes or less to charge per hour of flight, carrying a maximum of 1,200kg over a range of 815km (DHL Group, 2021; Grasso Macola, 2021). DHL plans to operate its fleet of 12 electric planes for the middle-mile transportation of packages from major air hubs to smaller markets (Holland, 2021).

In September 2021, SAMAD Aerospace, a green technology start-up company based in the United Kingdom, announced that it has commenced the certification process for its Starling Cargo aircraft. The electrically powered aircraft will have a cruising speed of 95mph (152kh), a flight ceiling of 10,000 feet (3,048 metres) and a range of up to 135 miles (217.2 kilometres). The electric vertical take-off and landing aircraft will provide operators with the flexible point to point delivery of air cargo up to its 50-kilogram payload. The potential market segments for this aircraft are the air cargo industry that need to transport high value cargo. These include oil and gas firms, gemstone mining companies, medical logistics and emergency response companies. In addition, the new aircraft can be used for delivering humanitarian critical cargo of urgently required food, medicine, and equipment to often remote regions of the world which has underdeveloped or damaged local infrastructure (Holland, 2021; Turner, 2021e).

The substitution of jet fuel with electricity can influence the impact of aviation services on climate change, as electric powered aircraft do not produce any carbon dioxide (CO_2) emissions from fuel combustion. Importantly, however, these carbon dioxide (CO_2) benefits need to be considered on a life-cycle basis and will only occur if the electrical energy is sourced from lower carbon sources (Shah, 2021).

4.5 The Use of Fixed Electrical Ground Power at Airports

In addition to the carbon dioxide (CO_2) emissions generated by aircraft, aircraft ground operation carbon dioxide (CO_2) emissions can also be significant at airports (International Airport Review 2010). A variety of handling activities are undertaken at airports. The activities associated directly with the aircraft itself include the provision of power, cleaning, loading, or unloading of baggage/air cargo, lavatory services, aircraft marshalling, aircraft towing or pushback, and aircraft fuelling (Ashford et al., 2013; Kazda & Caves, 2015; Thompson, 2007). Consequently, electrical power is required on the airport apron to facilitate the aircraft turnaround process. External electrical power is also often used for aircraft engine start-up. Hence, to minimize aircraft auxiliary power unit (APU) usage, many airports provide aircraft electric power (and cooling capabilities) at the gate which are more efficient and cleaner than APUs powering generators and cooling packs (de Neufville & Odoni, 2013). An aircraft's auxiliary produces harmful emissions (Baxter, 2021). Thus, airports can help reduce aircraft emissions at airports through the provision of fixed electrical ground power (FEGP) and pre-conditioned air (PCA) to enable aircraft auxiliary power unit (APU) shutdown. In addition, to satisfy more stringent regulations on the supply of power at aircraft stand, operators and manufacturers are increasingly working towards smarter, more efficient, and more environmentally friendly usage and deployment of power units (Airside International, 2012). The use of fixed electrical ground power (FEGP) stations (and the supply of preconditioned air supply) eliminates the requirement for airlines to use APU's whilst the aircraft is at the gate (Bartsch, 2013; Elmer & Leigland, 2014; Yim et al. 2013). Many airports are now suppling electrical power from central power supplies that connect to the aircraft either by apron cable or by cable in the aerobridge structure (Ashford et al. 2013). Thus, the use of fixed electrical ground power (FEGP) and preconditioned air eliminates these harmful emissions from the environment (Baxter, 2021).

Nowadays, many air cargo carrying airlines are using fixed electrical ground power (FEGP) and pre-cooling) and this will help reduce their carbon footprint. Emirates Airline, for example, prioritizes the use of ground power and pre-conditioned air where it is available so that the aircraft's auxiliary power unit does not have to be used. This measure helps to save fuel and reduces their associated airborne emissions (Emirates Airline, 2021). Singapore Airlines also uses mobile ground power units and preconditioned air units during night layovers and long transits to reduce the reliance on its aircraft auxiliary power units (APUs) (Singapore Airlines, 2017, 2019).

4.6 The Potential Use of Hydrogen as an Aircraft Energy Source

At the time of the present study, hydrogen was being considered for future use in energy generation within the aviation industry due to its cleanness and abundance of supply (Baroutaji et al., 2019; Xu et al., 2015). Hydrogen powered aircraft can provide important environmental benefits as this energy source can substantially reduce or even eliminate aircraft pollutants, thus mitigating the impact on the environment (Schutte et al., 2016). Because of its environmental benefits, hydrogen is regarded as being a very likely energy source for the future of aviation, as it is a fuel source that has the potential of zero emissions (Khandelwal et al., 2013). However, the implementation of hydrogen technology has many technical challenges to overcome, including the development of sustainable production, storage and delivery systems that shall not diminish the environmental benefits of this fuel source (Rondinelli et al. 2017).

ASL Aviation Holdings in October 2021 announced its plan to purchase ten hydrogen-propulsion "conversion kits" for use on its ATR turboprop aircraft. Universal Energy is developing the hydrogen conversion kits as part of a broader effort to stimulate the use of hydrogen propulsion for aviation. ASL Aviation Holdings will be a global launch customer for the turboprop air cargo market and the company plans to purchase up to ten of Universal Hydrogen's ATR 72 conversion kits for installation into its existing or future turboprop aircraft fleet (Brett, 2021c).

4.7 The Use of Lightweight Aircraft Unit Load Devices

In addition to the aircraft unit load devices (ULDs) used to carry passenger baggage (Airline Ground Services 2013), airlines also use ULDs for the carriage of air cargo and mail, such as containers and pallets (Lu & Chen, 2012). Aircraft unit load devices (ULDs) are pallets and containers which are used to carry air cargo, mail and passengers' baggage on wide-body passenger and dedicated freighter aircraft (Baxter et al., 2014; Lu & Chen, 2011). Combination airlines who operate freighter aircraft, for example, Cathay Pacific Airways and Qatar Airways, dedicated all-cargo airlines, and the integrators, also use specially designed containers to fit the main deck of their freighter aircraft (Coyle et al. 2011; Morrell & Klein, 2018). In recent years airlines from all around the world have been seeking ways to become more fuel efficient so they can minimize their operational costs whilst at the same time mitigating their impact on the environment. One feasible way of achieving these objectives and minimizing weight without compromising the business volume is using light weight aircraft unit load devices (ULDs) (Laniel et al. 2011). Thus, the increasing use of lightweight aircraft unit load devices (ULDs) is being driven by airline requirements to save on fuel costs and reduce the carbon dioxide (CO_2) impact within the industry (Airline Ground Services, 2013).

In 2012, Etihad Airways, together with its Unit Load Device (ULD) partner Jettainer, embarked on a program to replace 3,000 aircraft containers from the airline's original aluminium ULD fleet with more environmentally friendly lightweight versions. The airline estimated that the use of the new lightweight aircraft containers would reduce annual carbon dioxide (CO_2) emissions by approximately 5,000 tons per year (Times Aerospace, 2012). In August 2012, Airberlin began modernizing its aircraft container fleet with lightweight aircraft containers. These containers are used to transport air cargo consignments and passengers' baggage. The use of the lightweight containers has reduced the actual flight weight by 200 kilograms, saving about 30,000 litres of fuel per year per aircraft. This enabled the airline to reduce its annual carbon dioxide (CO_2) emissions by 1,100 tonnes (Aviation Pros, 2012).

In 2013, FedEx finished retrofitting or replacing approximately 23,000 older-model aircraft ULDs, with more efficient lightweight containers. The use of new lightweight containers enabled FedEx to reduce its annual carbon dioxide (CO_2) emissions by around 35,289.4 tonnes per year (Lee, 2013). Swiss WorldCargo began replacing some of its unit load devices (ULDs) with state-of-the-art, lightweight containers during 2013. The airline's entire XKN container fleet, which are used for the transportation of valuables on long-haul routes, was replaced with AVA containers provided by outsourced ULD management company Jettainer. The new AVA containers are constructed of carbon fibre as well as partly recycled composite materials. These containers are around 40 kilograms lighter than the XKN containers previously used by the airline. This significant weight reduction resulted in an improved payload, increased fuel efficiency, as well as the reduction in the airline's annual carbon dioxide (CO_2) emissions (Air Cargo News, 2013b). Etihad Airways and Jettainer partnered together in 2013 to launch a new lightweight and double-width unit load device (ULD), known as the type ALF container. The new ALF container is almost 34 per cent lighter at 130 kilos, a saving of 66 kilos per unit, and offers a potential weight saving of 116 kilos per flight. Etihad predicted that the use of these new containers would offer an annual reduction in carbon dioxide emissions of 4,000 tonnes (Air Cargo News, 2013a).

On February 11, 2014, Nordisk Aviation Products completed the delivery of more than 4,300 light weight air cargo containers to Singapore Airlines. These new light weight containers helped the airline to save a minimum 77,600 kilograms on its ULD fleet (Nordisk Aviation Products, 2014). In May 2014, Japan Airlines acquired 480 new light-weight cargo containers for use on its international network. An important benefit for the airline was that the weight of new light weight containers was 40% lower, and this helped to reduce fuel consumption and the associated emissions (Japan Airlines, 2014). Since 2014, Finnair Cargo has acquired lighter-weight cargo pallets and the weight savings have delivered an environmental benefit to the airline (Salmi, 2019).

In 2015, Cargolux Airlines International became the first airline to carry cargo handling systems manufacturer Telair International's CAEROe ® advanced lightweight unit load devices (ULDs). The all-cargo airline began using CAEROe ®'s cookie sheet pallets, heavy gross weight pallets and fuel-saving containers on its fleet of Boeing B747 freighter aircraft. The pallets are up to 40 per cent lighter than standard aluminum ULDs. The use of the new light weight pallets and containers enabled Cargolux to reduce aircraft fuel burn and its annual carbon footprint (Air Cargo News, 2015a). Jettainer, a aircraft ULD leasing company, began the development of a new lightweight aircraft pallet in 2015. The new pallet weighs 70 kg, which is approximately 32 kg lighter than a traditional PMC aluminium unit. The new pallet is made of a composite material that was applied for the first time in the aviation sector. The new pallet offered airlines with an alternative to using thinner aluminium bases to reduce aircraft weight (Air Cargo News, 2015g), and thus, aircraft fuel burn. In 2015, Lufthansa Cargo reduced its fuel consumption by 2,000 tonnes per year and reduced carbon dioxide (CO_2) emissions by 7,000 tonnes from the use of light weight containers, that were made from lighter composite materials These new containers have a 14 kg weight saving over conventional AKE containers (Air Cargo News, 2015e). Portuguese flag carrier TAP Portugal began replacing its fleet of 3,500 Unit Load Devices with lightweight units as part of an outsourcing deal with CHEP Aerospace Solutions in 2015. As well as reducing aircraft fuel consumption, the significant reduction in container weight was anticipated to reduce TAP's carbon emissions by 11 000 tonnes over the four-year contract term (Air Cargo News, 2015d). On October 8, 2015, United Cargo announced a plan to purchase 8,500 Herculight S containers that were developed by Zodiac Aerospace, which will completely replace its current fleet of AKE baggage and cargo containers. United Airlines became the launch customer for the Herculight S, which weighs less than 55 kg, a reduction of 36 kg on the traditional AKE container. The switch to lighter containers was expected to reduce the airline's fuel consumption by two million gallons a year when completed, thus cutting the airline's annual carbon dioxide (CO_2) emissions by more than 19,400 tonnes (Air Cargo News, 2015f). As part of its goal to reduce its annual carbon footprint, Air France-KLM Air France-KLM have introduced new lightweight aluminum pallets that are 17kg lighter than standard pallets used by the two airlines. The new 83kg pallets gradually replaced all the current standard stock – each weighing 100kg – in the airline's fleet (Air Cargo News, 2015b). CHEP Aerospace Solutions signed a five-year agreement in 2015 to supply and manage Hong Kong-based Cathay Pacific Airways unit load device (ULD) fleet and pallet accessories. As part of this agreement CHEP Aerospace Solutions acquired Cathay Pacific's fleet of 25,000 ULDs, converting the majority to these to modern light weight composite units weighing 58 kg (Air Cargo News, 2015c).

In 2016, Fiji-based Fiji Airways signed a contract with CHEP Aerospace Solutions whereby CHEP introduced new lightweight containers from its 80,000 pool of assets to support Fiji Airways in reducing fuel costs and lowering the airline's carbon dioxide (CO_2) emissions. This initiative helped towards Fiji Airways sustainability targets (Air Cargo News, 2016b). Oslo-headquartered low-cost carrier (LCC) Norwegian Air Shuttle introduced new, lighter, and stronger baggage and cargo containers for use onboard its Boeing 787 'Dreamliner' aircraft in 2016. These new lighter weight containers were acquired to help improve the airline's fuel efficiency (Air Cargo News, 2016c). Scandinavian Airline (SAS) extended its contract with ULD service provider CHEP Aerospace Solutions in 2016, and as part of this contract, SAS began using CHEP's lightweight 65kg containers. These containers were over 20% lighter than the airline's existing units and as a result SAS saved over 13,000 tonnes of carbon dioxide (CO_2) over the five-year term of the new contractual agreement (Air Cargo News, 2016d). Also, in 2015, Jettainer replaced Italy-based Alitalia's entire fleet of 650 AKE containers, that were used for carrying belly hold freight and luggage on passenger aircraft, with the state-of-the-art, lightweight containers, as part of the contract between the two companies. The new replacement ULDs reduce each of the existing Alitalia containers' weight by 20 per cent – from 82 kilograms to a maximum of 66 kilograms. The weight reduction resulted in a significant reduction in fuel consumption and in turn lower carbon dioxide (CO_2) emissions (Air Cargo News, 2016a).

In 2017, Brussels Airlines converted its entire baggage and air cargo container fleet to new lightweight composite containers. This was done in partnership with CHEP Aerospace Solutions. The move by the airline to use lightweight containers was driven by the requirement to save on fuel costs and to reduce its carbon dioxide (CO_2) emissions (Airline Routes and Ground Services, 2018).

In 2019, aircraft ULD leasing firm Jettainer teamed up with Trilatec to offer a new lightweight pallet load distribution system that weighs approximately 80% less than traditional system. The trilatec squAIR-timber system, which is made of cardboard fibre, aims to replace the wooden planks that are frequently used for load distribution on airfreight pallets. The weight saving of approximately 80% leads to substantial reductions in fuel consumption and helps to reduce carbon dioxide (CO_2) emissions (Brett, 2019).

Virgin Atlantic Airways has acquired a fleet of light weight aircraft unit load devices (ULDs), which have enabled the airline to lower its carbon dioxide (CO_2) emissions by around 2,000 tonnes per year (Putzger, 2015).

In sum, the trend by airlines towards the use of new lightweight aircraft unit load devices (ULDs) has been underpinned by their objective to reduce aircraft fuel burn and the associated carbon dioxide (CO_2) emissions.

4.8 The Use of Sustainable Aviation Fuels as an Industry Decarbonization Related Measure

The use of sustainable aviation fuels (SAF) has been identified as one of the key elements in helping the world's airlines to achieve their goal of decarbonizing their operations. At the time of the present study, more than 45 airlines had trialed the use of sustainable aviation fuel. Since 2016, more than 370,000 flights have been operated with sustainable aviation fuel (International Air Transport Association, 2021a). The first step towards a carbon-free future for the air cargo industry is carbon-neutral growth, which implies that there should be no increase in carbon dioxide (CO_2) emissions despite the growth in air cargo traffic. Hence, making investments in sustainable aviation fuel (SAF) is viewed as a necessary step in this process and one of the primary instruments in reducing the industry's carbon dioxide (CO_2) emissions (Turner, 2021f). Considering the key role that sustainable aviation fuels will play in decarbonizing air cargo transportation, air cargo carrying airlines have developed sustainable aviation (SAF) programs, which are being offered to air freight forwarders, logistics service providers, and air cargo shippers.

Air France, KLM, and Martinair Cargo launched the world's first sustainable aviation fuel (SAF) program for the air cargo industry in December 2020. In June 2011, KLM operated the world's first commercial flight using sustainable aviation fuel (SAF), from Amsterdam Airport Schiphol to Paris Charles de Gaulle. A key objective of this program is to enable both shippers and air freight forwarders to stimulate and enlarge the market for sustainable aviation fuels (SAF) (Brett, 2020; Rimoczi, 2020; Turner, 2020). The sustainable aviation fuel program offered by Air France KLM Martinair Cargo (AFKLMP Cargo) enables shippers and air freight forwarders to select how much of their air cargo consignments will be flown on flights powered by sustainable aviation fuel (Air Cargo News, 2021c), which as noted earlier is a cleaner substitute for conventional jet fuel, thus reducing carbon dioxide (CO_2) emissions by up to 85% (Turner, 2021c). Bolloré Logistics joined the Sustainable Aviation Fuel (SAF) program offered by Air France KLM Martinair Cargo (AFKLMP Cargo) in January 2021. The agreement covered its shipments between Paris Charles de Gaulle and New York John F. Kennedy airports. It was anticipated that this agreement would reduce carbon dioxide emissions by 50% on this trade lane (Finn, 2021; Global Cargo Insight, 2021). Global transport and logistics services provider, Kuehne+Nagel partnered with Air France KLM Cargo in January 2021 to launch the first carbon neutral air freight lane between North America and Europe. In accordance with the agreement, 100% sustainable aviation fuel (SAF) will be used on Kuehne+Nagel cargo shipments carried on board Air France-KLM flights from Los Angeles to Amsterdam (Grover, 2021). On April 21, 2021, Koppert Biological Systems and Air France KLM Martinair Cargo (AFKLMP Cargo) entered into a partnership agreement covering the use of sustainable aviation fuel (SAF) on selected international cargo flights that transport Koppert's agricultural and horticultural products for biological pest control (Air Cargo Week, 2021; Koppert Biological Systems, 2021). In May 2021, Marinetrans (MT) joined the Sustainable Aviation Fuel (SAF) program of Air France KLM Martinair Cargo (AFKLMP Cargo) for its 2021 marine logistics shipments (Marinetrans, 2021). Best Global Logistics (BGL) and Dutch air freight forwarder Fast Forward Freight (FFF) also joined the program in May 2021 (Brett, 2021h). In June 2021, Airpharm Logistics SAU became the first Spanish-based air freight forwarder to join the Air France KLM Martinair Cargo (AFKLMP) Sustainable Aviation Fuel (SAF) program (Biofuels International, 2021a). Also, in June 2021, the Middle East South Asia (MESA) operation of Hellmann Worldwide become the first freight forwarder and logistics service provider in the region to join the Air France KLM Martinair Cargo (AFKLMP Cargo) Sustainable Aviation Fuel (SAF) program (Graham, 2021a; Times Aerospace, 2021). In July 2021, freight forwarders and logistics service providers AWOT Global Express, CTS International Logistics Corporation, Job-Mate International, Sinotrans e-Commerce Logistics and Xiamen Supertrans joined the Sustainable Aviation Fuel Program (SAF Program) offered by Air France KLM Martinair Cargo (AFKLMP Cargo). The use of sustainable aviation fuels enabled greener air cargo services on routes connecting China with Europe and South America (Harry 2021a; Saunders, 2021a; Turner, 2021d). In September 2021, Total Touch Cargo Holland BV (TTC) and Air France KLM Martinair Cargo (AFKLMP Cargo) ratified a partnership agreement as part of the airline's sustainable aviation fuel (SAF) program. Under the deal, AFKLMP Cargo will use sustainable aviation fuel (SAF) on selected cargo flights from Nairobi, Kenya, to Amsterdam Airport Schiphol. These flights carry TTC's fresh agricultural and horticultural products (Biofuels International, 2021b; Saunders, 2021b). Airflo and Tiger Freight joined the Air France, KLM and Martinair sustainable aviation fuel (SAF) program in November 2021. The sustainable aviation fuel (SAF) partnership agreement will make it possible to use sustainable aviation fuel on AFKLMP Cargo flights from Kenya and Zimbabwe carrying Airflo's and Tiger Freight's fresh agricultural and horticultural products. Thus, Airflo and Tiger Freight will be able to reduce the carbon dioxide (CO_2) emissions that they produce on their flowers and perishables air cargo consignments (Keen, 2021; Turner, 2021a).

All Nippon Airways (ANA) launched its "SAF Flight Initiative" on 24 October 2021. The aim of the program is to reduce carbon dioxide (CO_2) emissions. The new program was designed to promote Sustainable Aviation Fuel (SAF) through the collaboration with leading companies in this area. The program was the airline's latest effort to decrease its carbon footprint and adhere to the guidelines established by the United Nations Sustainable Development Goals (SDGs) as well as meet ANA Group's environmental commitments. Nippon Express Co., Ltd., Kintetsu World Express, Inc. and Yusen Logistics Co., Ltd. also announced that they would participate in the SAF Flight Initiative. ANA conducted a SAF-powered cargo flight in conjunction with these three major logistics and cargo companies on September 29. This was the first flight by a Japanese airline to conduct a joint operation using sustainable aviation fuel (SAF) (All Nippon Airways, 2021; Turner, 2021b).

Bolloré Logistics introduced a sustainable aviation fuel (SAF) program in July 2021. With this program, customers can reduce their carbon dioxide (CO_2) emissions by 80% (Brett, 2021d).

Cargolux Airlines International, one of the world's major dedicated all-cargo airlines, announced the launch of its sustainable aviation fuel (SAF) program on 25 October 2021. The introduction of the airline's sustainable aviation fuel (SAF) program is a key part of the airlines plan to decarbonizing its operations. The airline aims to be carbon neutral by 2050 (Cargolux Airlines International, 2021). The new program is designed to reduce the airline's carbon dioxide (CO_2) emissions and will help underpin its customer sustainability program, that will offer more sustainable options for customer transportation requirements (Saunders, 2021c).

CEVA Logistics became a member of United Airlines sustainability initiative, the "Eco-Skies Alliance" in April 2021. CEVA Logistics together with the other "Eco-Skies Alliance" companies are working with United Airlines to collectively purchase approximately 3.4 million gallons of sustainable aviation fuel (SAF) during 2021 (Harry 2021c).

DHL Express, one of the world's major integrated carriers, signed an agreement with energy firm Shell Aviation during December 2020 that will see it being supplied with sustainable aviation fuel (SAF) to power DHL Express flights from Amsterdam Schiphol Airport. This agreement formed part of DHL Express goal to achieve net zero emissions by 2050 (Harry, 2020b).

DHL Global Forwarding, the world's largest air freight forwarder, joined United Airlines sustainable aviation fuel (SAF) program in May 2021. As part of United Airlines "Eco-Skies Alliance program", DHL Global Forwarding will contribute towards the purchase of 3.4 million gallons of sustainable aviation fuel (SAF) during 2021. The reductions in DHL Global Forwarding carbon footprint are subsequently allocated by DHL to its customers (Brett, 2021e).

Logistics service provider Geodis began to offer its customers more sustainable transportation options for their air cargo shipments in September 2021. The company's customers can ship their air cargo consignments on flights that are partially powered by sustainable aviation fuels (SAF), thus enabling them to reduce their carbon footprint (Air Cargo News, 2021b; Harry, 2021d).

IAG Cargo partnered with Kuehne+Nagel (K+N) in June 2021 to complete its first net zero charter chain. This comprised 16 charter flights, that used sustainable aviation fuel (SAF). The charters operated between Stuttgart and Atlanta and were the first time that passenger-freighter flights were operated with net-zero carbon emissions (Harry, 2021e).

Kuehne+Nagel entered into an agreement with American Airlines in March 2021 to use 11 million litres of sustainable aviation fuel (SAF) for the transportation of its air cargo consignments. The agreement is part of K+N's "Net Zero Carbon program". Under the terms of the agreement, American Airlines will allocate a portion of the carbon reduction benefit generated through its use of sustainable aviation fuel (SAF) to Kuehne+Nagel (Harry, 2021f). Kuehne+Nagel and Lufthansa Cargo agreed on a partnership agreement in October 2021 for the promotion and use of power-to-liquid (PTL) synthetic sustainable aviation fuel (SAF). The two companies will purchase 25,000 litres of the fuel per year from what will become the world's first production site for synthetic crude oil in Werlte/Emsland in Germany (Brett, 2021f). Prior to this partnership agreement, Kuehne+Nagel and Lufthansa Cargo have used bio-based sustainable aviation fuels (SAF) to reduce the carbon footprint of air cargo consignments tendered for carriage by Lufthansa Cargo (Brett, 2021c). Kuehne+Nagel (K+N) began offering its customers with the option to purchase sustainable aviation fuel (SAF) for shipments so that customers can reduce the environmental impact of supply chains (Brett, 2021g). With the newly launched offering, all Kuehne+Nagel customers can easily request the use of sustainable aviation fuel (SAF) to be used instead of fossil fuel for air transport, and thus, they benefit from net zero carbon emissions air freight services. Sustainable aviation fuels (SAF) still produce some carbon dioxide (CO_2) emissions, thus under this program carbon neutrality is achieved by substituting each litre of jet fuel kerosene used with 1.33 litres of sustainable aviation fuel (SAF) (Turner, 2021c).

Lufthansa Cargo and DB Schenker teamed together on November 29, 2020, to operate the first ever carbon dioxide (CO_2)-neutral freighter flights, powered by sustainable aviation fuel (SAF). The flight operated from Frankfurt to Shanghai utilizing a Lufthansa Cargo Boeing 777 freighter aircraft (Harry, 2020a). In April 2021, DB Schenker and Lufthansa Cargo partnered together to launch a regular carbon dioxide (CO_2)-neutral freighter route, operating between Frankfurt and Shanghai (Air Cargo News, 2021a; Harry, 2021b). The use of sustainable aviation fuel has reduced conventional kerosene usage of 174 tonnes per week on this route (Harry, 2021b). In October 2021, Nokia Telecommunications joined the Lufthansa Cargo and DB Schenker sustainable aviation fuel (SAF) initiative. Each week, the global telecommunication network provider will avoid greenhouse gas (GHG) emissions by using the world's only freighter flight that is 100 percent powered by sustainable aviation fuel (SAF) (DB Schenker, 2021). In 2021, Lufthansa Cargo began to offer all its customers the option of carbon dioxide (CO_2) neutral transportation on segments that are operated by freighter aircraft. Lufthansa Cargo will achieve the carbon dioxide (CO_2) neutrality by using sustainable aviation fuel (SAF) or as noted earlier, through certified carbon offsetting projects which will avoid or compensate for the emissions generated during air cargo transportation (Brett, 2021g).

In September 2021, the United States government announced its goal of replacing all jet fuel with sustainable alternatives by 2050. The government's plan is to substantially boost the production of fuels made from waste or plants to reduce the environmental cost of aircraft emissions. The government's new goal targets the annual production of 3 billion gallons of sustainable fuels by 2030. This will provide a 20% reduction in aircraft carbon dioxide (CO_2) emissions. United States-based airlines have set a target of producing 2 billion gallons of alternative fuels by 2030 (Fried, 2021).

4.9 The Use of Renewable Energy Sources for Ground Based Buildings and Facilities

Airlines are extremely energy intensive. This is because airlines use very large amounts of power and heating and cooling for their buildings and facilities (Baxter et al., 2021a). The traditional power source used by airlines is electricity, which is typically sourced from the local grid. Air cargo carrying airlines could consider, where climatic conditions permit, the use of "green" or renewable energy sources. Renewable energy sources reduce air pollution and cut down carbon dioxide (CO_2) emissions (Spellman, 2015; United Nations, 2018; United States Energy Information Administration, 2021). Furthermore, the use of solar power helps a firm to mitigate its greenhouse gases (GHGs) and achieve it sustainability objectives (Sreeneth et al., 2021). The use of green or renewable energy sources provides a firm or user with an important opportunity to optimize their energy efficiency (Arman et al., 2013). A further advantage is that renewable energy sources generally have very little waste (Yerel Kandemir & Yayli, 2016).

FedEx Express, the world's largest air cargo carrying airline, has adopted the use of renewable energy as part of its environmental policy. On October 18, 2004, FedEx announced the plan to build a 904-kilowatt solar array that would provide about 80% of the peak load demand for FedEx Express' Oakland facility (Brown & Jackson, 2004). In August 2005, FedEx solar-electric system at its regional hub in Oakland, California became operational. The system can generate around 1 million kilowatt hours (KWh) of clean energy each year. The system can provide 20 percent of the airline's cargo facility's total electricity needs and meet 80 percent of its peak load demand. On October 20, 2008, FedEx commenced work on the installation of a 1.4-megawatt solar power system that would generate 1.3 gigawatt hours of electricity per year at its Cologne Hub in Germany (Jackson, 2008). On October 27, 2010, the photovoltaic (PV) solar system became operational, and the system can produce around 800,000 kWh per year (FedEx, 2010). FedEx announced plans to install its largest rooftop solar-electric system at its distribution hub in Newark, New Jersey on October 17, 2002. Upon completion, the system had the capacity to generate approximately 2.4 million kilowatt hours (kWh) and would provide over 15 percent of the hub's energy needs. In addition, the new photovoltaic (PV) enabled FedEx to reduce its carbon dioxide (CO_2) by around 1,807 tonnes per annum (FedEx, 2012). FedEx Freight has also completed installed solar electric systems at its California facilities in Whitter and Fontana. In Geneva, Switzerland, a FedEx cargo facility uses a system of pipes running deep into the ground to warm the building's air in winter and cool it in the summer. The system reduces the cargo terminal's reliance on gas for heating or freon for cooling, and thus, requires less energy to operate (Jackson, 2008).

Finnair's photovoltaic (PV) solar system, that had been installed on the Finnair's Cool Cargo terminal at Helsinki's Vantaa Airport, came online in 2018 generating electricity for the airline's air cargo terminal. During 2018, the solar panels produced 287 MWh of electricity (Finnair, 2019). In 2019, the system produced 297 MWh of power for the terminal's own use. This represented 8.7% of the total energy consumption of the air cargo terminal building (Finnair, 2020).

Singapore Airlines, a major air cargo carrying airline, has adopted the use of renewable energy. As part of this strategy, Singapore Airlines has entered a partnership with SembCorp Solar to install rooftop solar panels on three of its Singapore-based buildings: Airline House, SIA Training Centre and TechSQ. It was envisaged that the solar panels would come online in the second quarter of FY2020/21, and they would generate a projected 5,382 MWh of renewable energy annually, which is able to support up to 18 per cent of the company's buildings' electricity demand (Singapore Airlines, 2020). The installation of the solar panels on all its office buildings in Singapore was completed in the 2020/21 financial year. As well as delivering considerable energy savings, the new system has reduced the airline's carbon dioxide (CO_2) emissions by 4.3 million tonnes a year (Singapore Airlines, 2021).

4.10 The Use of Sustainable Waste Management Principles

In providing air transport services, airlines generate a substantial amount of comingle waste (Blanca-Alcubilla et al., 2019). Solid waste management and the disposal of wastes has therefore become one of the most significant issues in the environmental management of the world airline industry (Li et al., 2003). Accordingly, airlines are making substantial efforts to improve their waste management and reduce waste generation wherever possible (Blanca-Alcubilla et al., 2019). In providing air cargo services, airlines generate various types of waste which includes tyres, fluids from equipment, universal wastes (light bulbs, electronics, and batteries), wood and wooden pallets as well as plastic packing material. In addition to the general and food waste generated from offices, other significant sources of waste are plastic packing material and wood and wooden pallets (United States Federal Aviation Administration, 2013).

Waste avoidance refers to the measures that need to be implemented prior to a substance becoming waste (International Civil Aviation Organization, 2021). In an ideal situation, waste would be avoided. This means that in the waste management hierarchy, reducing or preventing waste should be the primary objective of the firm. A firm can reduce its wastes by implementing two key strategies. The first strategy involves the firm reducing the volume of waste generated and disposed to landfill. In following this strategy, the firm reduces the impact of wastes on the environment, it reduces the emissions generated from the wastes disposed in landfills and offers the firm energy and natural resources savings (Zhu et al., 2008). The second strategy involves the firm adopting an effective system to manage all unavoidable waste (Baxter et al., 2018).

There are four principal waste disposal methods available to airlines: composting, recycling, incineration (including waste to energy), or disposal of wastes to landfill (Baxter et al., 2021b). Composting waste is a process in which the organic portion of solid waste is converted into a humus-like product. The final product, which is inert in nature, can be utilized as a soil conditioner or used for landfill cover (Harper, 2004, p. 3). There are two key environmental related advantages associated with the composting of rubbish. First, there are lower levels of environmental pollution from the composting of wastes. Secondly, there is the beneficial use of the end products (Taiwo, 2011).

The incineration of solid waste has two particularly useful purposes in a waste management system. Primarily, it reduces the volume of waste to be disposed of by sanitary landfill (Rand et al., 2000). Secondly, incineration can also be used as a means of generating energy (Awasthi et al., 2019; Hettiarachchi & Kshourad, 2019). Energy recovery reduces the volume of waste that is disposed by landfill and produces useable energy, in terms of heat, electricity or fuel, through a variety of processes. These processes include combustion, gasification, pyrolysis, and anaerobic digestion (Rahman et al., 2017).

When recycling waste, the waste fraction is utilized again to produce consumer goods or other products. Recycling of wastes may also include the conversion of waste into energy through thermal treatment (processing). Disposal in landfill sites is regarded as the least desirable waste disposal option (Manahan, 2011; Pitt & Smith, 2003). Waste that is disposed of through landfilling and open dumping, is regarded as being environmentally unsafe due to emission of greenhouse gases (GHGs) (Ahmed et al., 2020; Trabold & Nair, 2019).

Air cargo carrying airlines could also adopt the circular economy waste management approach. The circular economy is an economic system that is based upon business models that replace the "end-of-life" concept with reducing or alternatively reusing, recycling, and recovering materials during the product/distribution and consumption processes of a firm (Ginga et al., 2020). The circular economy extends beyond recycling and is based upon a restorative industrial system that focuses on the treatment of waste as a resource (Ghosh, 2020). The circular economy is comprised of three primary activities: the reduction in the use of virgin raw materials, the re-use of already processed materials, and the recycling of waste. In some instances, there is a fourth circular economy activity, that of the redesign of products (Burneo et al., 2020; Kyriakopoulos et al., 2019). For a firm to achieve the benefits of a circular economy approach, the following steps need to be undertaken reuse, recycling, recovery, and waste prevention (Kyriakopoulos et al., 2019). A circular economy waste management approach respects the firm's environmental boundaries through an increase in the share of renewable or recyclable resources whilst at the same reducing the consumption of raw materials and energy. With this approach, emissions and loss of resources are thereby reduced (European Environment Agency, 2016).

Finnair, LATAM Airlines, and Singapore Airlines are examples of airlines that have adopted the circular economy approach to waste management.

4.11 Washing of Aircraft Engines

A further decarbonization measure available to air cargo carrying airlines is the washing the engines of their aircraft fleet. All aircraft engines become soiled during normal operations by airborne contaminants such as sand, dust, soot, salt, insects, and pollution. These contaminants can result in aircraft engines not operating efficiently, and, as a result, can lead to overall deterioration in engine health. Because of this an engine burns more fuel and operates at higher temperatures which can result in premature engine maintenance. Importantly, every kilogram of fuel saved by an airline result in the elimination of 3.15 kilograms of carbon dioxide (CO_2) emissions (Air Transport Action Group, 2021b).

Singapore Airlines, for example, have introduced an aircraft fleet-wide engine washing program, which has delivered an annual saving in fuel savings of 10,400 tonnes of fuel savings annum (Singapore Airlines, 2017). In addition to the greater fuel efficiency, this initiative has resulted in lower annual carbon dioxide (CO_2) emissions of around 32,670 tonnes.

4.12 Sustainable Air Freight Alliance

Delta Cargo joined the Sustainable Air Freight Alliance (SAFA) in September 2020. The Sustainable Air Freight Alliance (SAFA) is a business-led collaborative initiative whose goal is to reduce its members' environmental footprint. SAFA is a collaboration between shippers, freight forwarders, and airlines to track and reduce emissions from air cargo transportation. The organization promotes responsible freight transport (Air Cargo News, 2020; Brown, 2020; Finn, 2020). SEKO Logistics joined the Sustainable Air Freight Alliance (SAFA) in March 2021 (Brett, 2021b; Seko Logistics, 2021). The membership of this organization was part of Seko Logistics goal to accelerate its global decarbonization program and help the company's clients to achieve their own sustainability goals. AirBridgeCargo Airlines, American Airlines, Cargolux, Cathay Pacific, Delta Air Lines, LOT Polish Airlines, Lufthansa Cargo, Polar Air Cargo, Scandinavian Airlines (SAS), and United Airlines are members the Sustainable Air Freight Alliance (SAFA) (Seko Logistics, 2021). Ceva Logistics, a global logistics service provider, is also a member of the Sustainable Air Freight Alliance (SAFA) (Ceva Logistics, 2021). Unique Logistics International has also joined the Sustainable Air Freight Alliance (Unique Logistics International, 2021).

V. CONCLUSION

Based on an in-depth qualitative instrumental case study research design, this study has examined the strategies and measures being adopted by the world's air cargo carrying airlines to decarbonize their operations, and thus, mitigate their impact on the environment. The study also examined the potential strategies and measures that the key industry stakeholders are considering as part of their decarbonization goals. The study covered the period 2004 to 2021. The case study documentation was examined by document analysis.

The case study found that the world's air cargo carrying airlines are very cognizant of their environmental impact and, as a result, air cargo carrying airlines have defined and implemented an extensive range of measures and strategies to decarbonize their operations. These measures include the acquisition and deployment of fuel-efficient next generation aircraft, such as the Airbus A350, Boeing 787 passenger aircraft and the Boeing B747-8 freighter aircraft, aircraft related carbon dioxide (CO_2) emissions offset programs, the use of fixed electrical ground power (FEGP) and pre-conditioned air at airports, the use of lightweight aircraft unit load devices (ULDs), use of sustainable aviation fuels, the use of renewable energy sources for ground based buildings and facilities, the electrification of air cargo carrying airlines ground service equipment (GSE), the optimization of air traffic management procedures, and the washing of aircraft engines. A further decarbonization strategy has been the membership of key industry bodies, such as, the Sustainable Air Freight Alliance. A key strategy adopted by the world's air cargo carrying airlines has been the use of sustainable aviation fuels (SAF). Sustainable aviation fuels (SAF) provide a very significant opportunity for the decarbonization of aircraft operations as these fuels can reduce carbon dioxide (CO_2) emissions by up to 80% over its full lifecycle.

The case study also revealed that several key air cargo industry actors are planning in the future to use electric powered aircraft. The potential use of hydrogen as an aircraft energy source is also being considered.

A byproduct of air cargo transportation is the significant production of wastes. To mitigate the environmental impact of these wastes, air cargo carrying airlines could consider adopting the circular economy waste management approach.

REFERENCES

[1] Abd, A.A., Othman, M.R., & Kim, J. (2021). A review on application of activated carbons for carbon dioxide capture: present performance, preparation, and surface modification for further improvement. *Environmental Science and Pollution Research*, 28, 43329–43364. https://doi.org/10.1007/s11356-021-15121-9

[2] Abdullah, M.A., Chew, B.C., & Hamid, S.R. (2016). Benchmarking key success factors for the future green airline industry. *Procedia - Social and Behavioral Sciences*, 224, 246 – 253. https://doi.org/10.1016/j.sbspro.2016.05.456

[3] Ahmed, M., Ahmad, S., Tariq, M., Fatima, Z., Aslam, Z., Ali Raza, M., Iqbal, N., Akmal, M., Hassan, F.U., Akhtar Abbasi, N., & Hayat, R. (2020). Wastes to be the source of nutrients and energy to mitigate climate change and ensure future sustainability: options and strategies. *Journal of Plant Nutrition*, 43(6), 896-920. https://doi.org/10.1080/01904167.2020.1711944

[4] Airbus. (2018). Airbus delivers its first A350-1000 to launch customer Qatar Airways. Retrieved from https://www.airbus.com/en/newsroom/press-releases/2018-02-airbus-delivers-its-first-a350-1000-to-launch-customer-qatar.

[5] Air Cargo News. (2013a). Alf helps Etihad cut costs. Retrieved from https://www.aircargonews.net/uncategorized/alf-helps-etihad-cut-costs/.

[6] Air Cargo News. (2013b). Swiss WorldCargo opts for lightweight ULDs. Retrieved from https://www.aircargonews.net/uncategorized/swiss-worldcargo-opts-for-lightweight-ulds/.

[7] Air Cargo News. (2015a). Cargolux opts for Telair lightweight ULDs. Retrieved from https://www.aircargonews.net/business/cargolux-opts-for-telair-lightweight-ulds/.

[8] Air Cargo News. (2015b). Cargo pallets go on a diet. Retrieved from https://www.aircargonews.net/business/cargo-pallets-go-on-a-diet/.

[9] Air Cargo News. (2015c). Cathay and CHEP in ULD management deal. Retrieved from https://www.aircargonews.net/airlines/cathay-and-chep-in-uld-management-deal/.

[10] Air Cargo News. (2015d). Lightweight ULDs to cut TAP's fuel bill. Retrieved from https://www.aircargonews.net/airlines/lightweight-ulds-to-cut-taps-fuel-bill/.

[11] Air Cargo News. (2015e). Lufthansa launches flyweight LD3s. Retrieved from https://www.aircargonews.net/airlines/lufthansa-launches-flyweight-ld3s/.

[12] Air Cargo News. (2015f). United Cargo adopts lighter containers. Retrieved from https://www.aircargonews.net/technology/united-cargo-adopts-lighter-containers/.

[13] Air Cargo News. (2015g). WCS: Jettainer's lightweight pallet. Retrieved from https://www.aircargonews.net/airlines/bellyhold-airline/wcs-jettainers-lightweight-pallet/.

[14] Air Cargo News. (2016a). Alitalia opts for lightweight ULDs. Retrieved from https://www.aircargonews.net/airlines/alitalia-opts-for-lightweight-ulds/.

[15] Air Cargo News. (2016b). Fiji Airways renews ULD contract with CHEP. Retrieved from https://www.aircargonews.net/airlines/fiji-airways-renews-uld-contract-with-chep/.

[16] Air Cargo News. (2016c). Norwegian opts for Zodiac on B787s. Retrieved from https://www.aircargonews.net/technology/norwegian-opts-for-zodiac-on-b787s/.

[17] Air Cargo News. (2016d). SAS opts for lightweight ULDs. Retrieved from

https://www.aircargonews.net/region/europe/sas-opts-for-lightweight-ulds/.

[18] Air Cargo News. (2020). SAFA grows. *Air Cargo News*, 886, 16.

[19] Air Cargo News. (2021a). DB teams with Lufthansa to cut the carbon. *Air Cargo News*, 893, 9.

[20] Air Cargo News. (2021b). Geodis goes SAF green. *Air Cargo News*, 895, 8.

[21] Air Cargo News. (2021c). Salmon by sea puts focus on CO2. *Air Cargo News*, 889, 15.

[22] Air Cargo Week. (2021). AFKLMP Cargo welcomes Koppert to its sustainable aviation fuel programme. *Air Cargo Week*, 1(129), 8.

[23] Air Cargo World. (2021). Rolls-Royce, Shell deepens sustainable jet-fuel partnership. Retrieved from https://aircargoworld.com/news/technology/rolls-royce-shell-deepen-sustainable-jet-fuel-partnership/?utm_source=ACW+Main+List&utm_campaign=c8c4561b8b-ACW+Technology+Newsletter&utm_medium=email&utm_term=0_1711f92e66-c8c4561b8b-39015409.

[24] Aircraft Commerce. (2015). 787 & A350 XWB: How do they reduce maintenance costs? *Aircraft Commerce*, 102, 16-23.

[25] Aircraft Commerce. (2016). Fuel burn & operating performance of the 787-8, 787-9 and A350-900. *Aircraft Commerce*, 108, 16-27.

[26] Airline Ground Services. (2013). Containers can take the strain. *Airline Ground Services*, June, 20-23.

[27] Airline Routes and Ground Services. (2018). Containers can take the strain. Retrieved from https://airlinergs.com/issue-article/containers-can-take-the-strain/.

[28] Airport Technology. (2021). Boeing 747-8 Intercontinental airliner. Retrieved from https://www.aerospace-technology.com/projects/boeing-747-8/.

[29] Airside International. (2012). Getting it right: Aircraft power on the stand. *Airside International*, 19, 23-25.

[30] Airside International. (2020). Electric tow tractors: Evidence of a long-term trend? *Airside International*, 51, 6-10.

[31] Airside International. (2021). Aviaco GSE delivers electric baggage tractors into Schiphol. *Airside International*, 54, 26-27.

[32] Air Transport Action Group. (2021a). Boeing 747-8. Retrieved from https://aviationbenefits.org/case-studies/boeing-747-8/.

[33] Air Transport Action Group. (2021b). EcoPower engine wash. Retrieved from https://aviationbenefits.org/case-studies/ecopower-engine-wash.

[34] Albers, S. (2016). Cases on managing strategic alliances and networks. In J. Sydow, E. Schübler & G. Müller-Seitz (Eds.), *Managing interorganizational relationships: Debates and cases* (pp. 57-100). London, UK: Macmillan Publishers Ltd.

[35] Alemán, M.A. (2010) International aviation security practices relating to the global supply chain. In A.R. Thomas (Ed.), *International practices and innovations in moving goods safely and efficiently* (pp. 189-197). Santa Barbara, CA: ABC-CLIO, LLC.

[36] Al Hajri, G. (1999). *The impact of sea-air mode on air cargo transport*. Merstham, UK: A-Z Group Limited.

[37] All Nippon Airways. (2021). ANA launches SAF flight initiative to promote sustainability and reduced CO_2 emissions. Retrieved from https://www.anahd.co.jp/group/en/pr/202110/20211014.html.

[38] Arman, H., & Yuksel, I. (2013). Preface. In H. Arman & I. Yuksel (Eds.) *New developments in renewable energy* (pp. xi-xii). Rijeka, Croatia: InTech.

[39] Ashford, N.J., Stanton, H.P.M., Moore, C.A., Coutu, P., & Beasley, J.R. (2013). *Airport operations* (3rd ed.). New York, NY: McGraw-Hill.

[40] Aviation Pros. (2012). Airberlin modernises container fleet. Retrieved from https://www.aviationpros.com/gse/baggage-cargo/cargo-handling-equipment-accessories/uld-containers/news/10756191/lightweight-containers-save-fuel-and-cut-carbon-emissions.

[41] Awasthi, M.K., Sarsaiya, S., Chen, H., Wang, Q., Kumar Awasthi, S., Li, J., Liu, T., Pandey, A., & Zhang, Z. (2019). Global status of waste-to-energy technology. In S. Kumar, R. Kumar & A. Pandey (Eds), *Current developments in biotechnology and bioengineering: Waste treatment processes for energy generation* (pp. 31-52). Amsterdam, The Netherlands: Elsevier.

[42] Baroutaji, A., Wilberforce, T., Ramadan, M., & Ghani Olabi, A. (2019). Comprehensive investigation on hydrogen and fuel cell technology in the aviation and aerospace sectors. *Renewable and Sustainable Energy Reviews*, 106, 31-40. https://doi.org/10.1016/j.rser.2019.02.022

[43] Bartsch, R.I.C. (2013). *International aviation law: A practical guide*. Farnham, UK: Ashgate Publishing.

[44] Bauen, A., & Nattrass, L. (2018). Sustainable aviation biofuels: Scenarios for deployment. In M. Kaltschmitt & U. Neuling (Eds.), *Biokerosene: Status and prospects* (pp. 703-721). Berlin, Germany: Springer-Verlag.

[45] Baxter, G. (2016). *AERO2410 Airline operations: Topic 6 learning guide: Airline capacity management and passenger aircraft fleet planning*. Melbourne: RMIT University.

[46] Baxter, G. (2020). Sustainable airline waste management: A case study of Air New Zealand's waste management programs and strategies. *International Journal for Traffic and Transport Engineering*, 10(3), 351-370.

[47] Baxter, G. (2021). Achieving carbon neutral airport operations by 2025: The case of Sydney Airport, Australia. *Transport and Telecommunication*, 22(1), 1–14. https://doi.org/10.2478/ttj-2021-0001

[48] Baxter, G., & Wild, G. (2021). Aviation safety, freight, and dangerous goods transport by air. In R. Vickerman (Ed.), *International encyclopedia of transportation* (pp. 98-107). Amsterdam, The Netherlands: Elsevier.

[49] Baxter, G., Kourousis, K., & Wild, G. (2014). Fire resistant aircraft unit load devices and fire containment covers: A new development in the global air cargo industry. *Journal*

of Aerospace Technology and Management, 6(2), 202-209. https://doi.org/10.5028/jatm.v6i2.327
[50] Baxter, G., Srisaeng, P., & Wild, G. (2018). An assessment of airport sustainability, Part 1—Waste management at Copenhagen Airport. *Resources*, 7(1), 21. https://doi.org/10.3390/resources7010021
[51] Baxter, G., Srisaeng P., & Wild G. (2020). The use of aviation biofuels as an airport environmental sustainability measure: The case of Oslo Gardermoen Airport. *Magazine of Aviation Development*, 8(1), 6-17. https://doi.org/10.14311/MAD.2020.01.01
[52] Baxter, G., Srisaeng, P., & Wild, G. (2021a). A qualitative assessment of a full-service network airline sustainable energy management: The case of Finnair PLC. *WSEAS Transactions on Environment and Development*, 17, 167-180.
[53] Baxter, G., Srisaeng, P., & Wild, G. (2021b). Environmentally sustainable airline waste management: The case of Finnair PLC. *Environmental Research, Engineering and Management*, 77(4), 73–85. https://doi.org/10.5755/j01.erem.77.4.29574
[54] Benito, A., & Alonso, G. (2018). *Energy efficiency in air transportation*. Oxford, UK: Butterworth Heinemann.
[55] Beasley, K.T., & Bernadowski, C. (2019). An examination of reading specialist candidates' knowledge and self-efficacy in behavior and classroom management: An instrumental case study. *Education Sciences*, 9(2), 76. https://doi.org/10.3390/educsci9020076
[56] Biofuels International. (2021a). AFKLMP welcomes Airpharm Logistics to its SAF programme. Retrieved from https://biofuels-news.com/news/afklmp-cargo-welcomes-total-touch-cargo-to-its-saf-programme/.
[57] Biofuels International. (2021b). AFKLMP Cargo welcomes Total Touch Cargo to its SAF programme. Retrieved from https://biofuels-news.com/news/afklmp-welcomes-airpharm-logistics-to-its-saf-programme/.
[58] Bittner, A., Tyner, W.E., & Zhao, X. (2015). Field to flight: A techno-economic analysis of the corn stover to aviation biofuels supply chain. *Bio Fuels, Bio Products & Biorefining*, 9(2), 201-210. https://doi.org/10.1002/bbb.1536
[59] Blanca-Alcubilla, G., Roca, M., Bala, A., Sanz, N., De Castro, N., & Fullana-i-Palmer, P. (2019). Airplane cabin waste characterization: Knowing the waste for sustainable management and future recommendations. *Waste Management*, 96, 57-64. https://doi.org/10.1016/j.wasman.2019.07.002
[60] Boeing Commercial Airplanes. (2014). Boeing 767-300F designed to deliver. Retrieved from https://www.boeing.com/farnborough2014/pdf/BCA/bck-767_5_13_2014.pdf.
[61] Boeing Commercial Airplanes. (2020). World air cargo forecast: 2020-2039. Retrieved from https://www.boeing.com/resources/boeingdotcom/market/assets/downloads/2020_WACF_PDF_Download.pdf.
[62] Boeing Commercial Airplanes. (2021). Boeing freighter family. Retrieved from https://www.boeing.com/commercial/freighters/#/767f-highlight/767f/advantages/.
[63] Boon, T. (2020). Which airlines fly the Boeing 787-10? Retrieved from https://simpleflying.com/boeing-787-10-airlines/.
[64] Brett, D. (2019). Jettainer teams up with trilatec on weight-saving tech. Retrieved from https://www.aircargonews.net/services/uld/jettainer-teams-up-with-trilatec-on-weight-saving-tech/.
[65] Brett, D. (2020). Air France KLM Cargo launches sustainable fuel programme. Retrieved from https://www.aircargonews.net/airlines/air-france-klm-cargo-launches-sustainable-fuel-programme/.
[66] Brett, D. (2021a). Air cargo to lead aviation in carbon reduction. Retrieved from https://www.aircargonews.net/policy/environment/air-cargo-to-lead-aviation-in-carbon-reduction/.
[67] Brett, D. (2021b). Air cargo sector continues sustainability push. Retrieved from https://www.aircargonews.net/airlines/air-cargo-sector-continues-sustainability-push/.
[68] Brett, D. (2021c). ASL to buy hydrogen conversion kits for ATRs. Retrieved from https://www.aircargonews.net/policy/environment/asl-to-buy-hydrogen-conversion-kits-for-atrs/.
[69] Brett, D. (2021d). Bolloré Logistics launches SAF programme. Retrieved from https://www.aircargonews.net/freight-forwarder/bollore-logistics-launches-saf-programme/.
[70] Brett, D. (2021e). DHL Global Forwarding signs up for United's SAF programme. Retrieved from https://www.aircargonews.net/policy/environment/dhl-global-forwarding-signs-up-for-uniteds-saf-programme/.
[71] Brett, D. (2021f). K+N and Lufthansa Cargo partner on PTL aviation fuel. Retrieved from https://www.aircargonews.net/policy/environment/kn-and-lufthansa-cargo-partner-on-ptl-aviation-fuel/.
[72] Brett, D. (2021g). Lufthansa Cargo offers CO2 'neutral' freight service. Retrieved from https://www.aircargonews.net/policy/environment/lufthansa-cargo-offers-co2-neutral-freight-service/.
[73] Brett, D. (2021h). Two more forwarders sign up for Air France KLM Cargo's SAF programme. Retrieved from https://www.aircargonews.net/policy/environment/two-more-forwarders-sign-up-for-air-france-klm-cargos-saf-programme/.
[74] Brown, A. (2020). Delta Cargo commits to more sustainable future. Retrieved from https://www.aircargoweek.com/delta-cargo-commits-to-more-sustainable-future/.
[75] Brown, J., & Jackson, M. (2004). FedEx to build solar system at Oakland hub. Retrieved from https://www.bizjournals.com/memphis/stories/2004/10/18/daily5.html.
[76] Budd, L., & Ison, S. (2017). The role of dedicated freighter aircraft in the provision of global airfreight services. *Journal of Air Transport Management*, 61, 34-40. https://doi.org/10.1016/j.jairtraman.2016.06.003

[77] Budd, L., Griggs, S., & Howarth, D. (2013). Sustainable aviation futures: Crises, contested realities and prospects for change. In L. Budd, S. Griggs & D. Howarth (Eds.), *Sustainable aviation futures* (pp. 3-36). Bingley, UK: Emerald Publishing.

[78] Budd, T. (2017). Environmental impacts and mitigation. In L. Budd & S. Ison (Eds.), *Air transport management: An international perspective* (pp. 283-306). Abingdon, UK: Routledge.

[79] Burneo, D., Cansino, J.M, & Yñiguez, R. (2020). Environmental and socioeconomic impacts of urban waste recycling as part of circular economy. The case of Cuenca (Ecuador). *Sustainability*, 12(8), 3406. https://doi.org/10.3390/su12083406

[80] Cargolux International Airlines. (2011) *Annual report 2010*. Cargolux International Airlines, Luxembourg.

[81] Cargolux International Airlines. (2019) Sustainability report 2018. Retrieved from https://www.cargolux.com/media-library/files/csr/csr-report-20182.

[82] Cargolux International Airlines. (2021). Cargolux launches SAF program. Retrieved from https://cargolux.com/media-room/media-releases/media-releases/Cargolux-launches-SAF-program.

[83] Ceva Logistics. (2021). Our climate goal. Retrieved from https://www.cevalogistics.com/en/who-we-are/commitments/carbon-footprint.

[84] Chen, F.Y. (2012). Managers' views on environmental management: An examination of the Taiwanese airline industry. *Journal of Sustainable Development*, 6(1), 65-75.

[85] Chiambaretto, P., Mayenc, E., Chappert, H., Fernandez, A.S., & Le Roy, F. (2021). Where does flygskam come from? The role of citizens' lack of knowledge of the environmental impact of air transport in explaining the development of flight shame. *Journal of Air Transport Management*, 93, 102049. https://doi.org/10.1016/j.jairtraman.2021.102049

[86] Conway, P. (2005). Decline of the jumbo? *Airline Business*, 21(4), 62-63.

[87] Conway, P. (2011). Lifting the load. *Flight International*, 179(5291), 34-36.

[88] Conway, P. (2012) Volume switch. *Flight International*, 181(5344), 28-29.

[89] Cortez, L.A.B. (2014). *Roadmap for sustainable aviation biofuels for Brazil: A flightpath to aviation biofuels in Brazil*. Sao Paulo, Brazil: Blucher, 2014.

[90] Cortez, L.A.B., Nigro, F.E.B, Nogueira, L.A.H., Nassar, A.M., Cantarella, H., Moraes, M.A.F.D., Leal, R.L.V, Franco, T.T., Schuchardt, U.F., & Baldassin, R. Jnr. (2015). Perspectives for sustainable aviation biofuels in Brazil. *International Journal of Aerospace Engineering*, 2015, 264898. https://doi.org/10.1155/2015/264898

[91] Coyle, J.J., Novack, R.A., Gibson, B.J., & Bardi, E.J. (2011). *Transportation: A supply chain perspective* (7th ed.). Mason, OH: South-Western Cengage Publishing

[92] Davies, H. (2013). Freighter conversions. *Aircraft Technology*, 126, 50-54.

[93] DB Schenker. (2021). DB Schenker, Lufthansa Cargo and Nokia join forces on CO2-neutral air freight. Retrieved from https://www.dbschenker.com/global/about/press/db-schenker-lufthansa-cargo-and-nokia-join-forces-739578.

[94] De Grosbois, D., & Fennell, D. (2011). Carbon footprint of the global hotel companies: Comparison of methodologies and results. *Tourism Recreation Research*, 36(3), 231-245. https://doi.org/10.1080/02508281.2011.11081669

[95] Dempsey, P.S., & Gesell, L.E. (1997). *Air transportation: Foundations for the 21st century*. Chandler, AZ: Coast Aire Publications.

[96] de Neufville, R., & Odoni, A. (2013). *Airport systems: Planning, design, and management* (2nd ed.). New York, NY: McGraw-Hill.

[97] Dewulf, W., Meersman, H., & Van de Voorde, E. (2019). The strategy of air cargo operators: Carpet sellers and cargo stars. In K. Cullinane (Ed.). *Airline economics in Europe* (167-200). Bingley, UK: Emerald Publishing.

[98] DHL Group. (2021). DHL Express shapes future for sustainable aviation with the order of first-ever all-electric cargo planes from Eviation. Retrieved from https://www.dhl.com/global-en/home/press/press-archive/2021/dhl-express-shapes-future-for-sustainable-aviation-with-the-order-of-first-ever-all-electric-cargo-planes-from-eviation.html.

[99] Dodd, T., Orlitzky, M., & Nelson, T. (2018). What stalls a renewable energy industry? Industry outlook of the aviation biofuels industry in Australia, Germany, and the USA. *Energy Policy*, 123, 92-103. https://doi.org/10.1016/j.enpol.2018.08.048

[100] Doganis, R. (2019). *Flying off course: Airline economics and marketing* (4th ed.). Abingdon, UK: Routledge.

[101] Dresner, M., & Zou, L. (2017). Air cargo and logistics. In L. Budd & S. Ison (Eds), *Air transport management: An international perspective* (pp. 247-264). Abingdon, UK: Routledge.

[102] Elmer, V., Leigland, A. (2014). *Infrastructure planning and finance: A smart and sustainable guide*. London, UK: Routledge.

[103] Emirates Airline. (2021). Environment: Sustainability in operations. Retrieved from https://www.emirates.com/au/english/about-us/our-planet/sustainability-in-operations/.

[104] European Commission. (2021). Reducing emissions from aviation. Retrieved from https://ec.europa.eu/clima/policies/transport/aviation_en.

[105] European Environment Agency. (2016). Circular economy to have considerable benefits, but challenges remain. Retrieved from https://www.eea.europa.eu/highlights/circular-economy-to-have-considerable.

[106] Eurostat. (2020). Greenhouse gas emissions from waste. Retrieved from https://ec.europa.eu/eurostat/web/products-eurostat-news/-/DDN-20200123-1.

[107] Fatihah, S., & Abdul Rahim, A.S. (2017). The willingness to pay of air travel passengers to offset their carbon dioxide (CO_2) emissions: A Putrajaya resident case study. *Journal*

of Tourism, Hospitality and Environment Management, 2(5), 18-32.
[108] FedEx. (2010). FedEx inaugurates new solar-powered hub at Cologne Bonn Airport. Retrieved from https://newsroom.fedex.com/newsroom/fedex-inaugurates-new-solar-powered-hub-at-cologne-bonn-airport/.
[109] FedEx. (2012). FedEx Express breaks ground on new solar installation at Newark hub facility. Retrieved from https://newsroom.fedex.com/newsroom/fedex-express-breaks-ground-on-new-solar-installation-at-newark-hub-facility/.
[110] Finn, R. (2020). Delta Cargo commits to sustainable future. Retrieved from https://www.airfreight-logistics.com/delta-cargo-commits-to-more-sustainable-future/.
[111] Finn, R. (2021). AFKLMP Cargo and Bolloré Logistics team up under SAF program. Retrieved from https://www.airfreight-logistics.com/afklmp-cargo-and-bollore-logistics-team-up-under-saf-program/.
[112] Finnair. (2019). Sustainability report 2018. Retrieved from https.//company.finnair.com/~/media/Files/F/Finnair-IR/documents/en/…/Finnair-sustainability-report-2018.pdf,
[113] Finnair. (2020). Sustainability report 2019. Retrieved from https.//company.finnair.com/resource/blob/1994132/c493686a5af678b81ed6dbcd48eed150/fiinair-responsibility-report-2019-data.pdf
[114] Franchetti, M.J., & Apul, D. (2013). *Carbon footprint analysis: Concepts, methods, implementation, and case studies*. Boca Raton: CRC Press.
[115] Fregnani, A.T.G., & Andrade, O. (2017). Aviation initiatives and the relative impact of electric road vehicles on CO_2 emissions. Retrieved from https://www.boeing.com/resources/boeingdotcom/features/innovation-quarterly/aug2017/BTJ_CO2emissions_FULL.pdf.
[116] Fried, B. (2021). How air cargo is responding to climate change. Retrieved from https://www.aircargonews.net/freight-forwarder/how-air-cargo-is-responding-to-climate-change/.
[117] Gautam, M., Pandey, B. & Agrawal, M. (2012). Carbon footprint of aluminum production: Emissions and mitigation. In S. S. Muthu (Ed.), *Environmental carbon footprints: Industrial case studies* (pp. 197-228). Oxford, UK: Butterworth-Heinmann.
[118] Gegg, P., Budd, L., & Ison, S. (2014). The market development of aviation biofuel: Drivers and constraints. *Journal of Air Transport Management*, 39, 34-40. https://doi.org/10.1016/j.jairtraman.2014.03.003
[119] Gellings, C.W. (2011). *Saving energy and reducing CO_2 emissions with electricity*. Lilburn, GA: The Fairmont Press.
[120] Ghosh, S.K. (2020). Introduction to circular economy and summary analysis of chapters. In S.K. Ghosh (Ed.), *Circular economy: Global perspective* (pp. 1-23). Singapore: Springer Nature Singapore.
[121] Ginga, C.P., Ongpeng, M.C., & Klarissa Daly, M. (2020). Circular economy on construction and demolition waste: A literature review on material recovery and production. *Materials*, 13(13), 2970. https://doi.org/10.3390/ma13132970
[122] Global Cargo Insight. (2021). Bolloré joins AFKLMP's SAF programme. Retrieved from https://www.globalcargoinsight.com/bollore-joins-afklmps-saf-programme.
[123] Golmohammad, F., & Mehdizadeh Dastjerdi, A. (2012) The economic analysis of satellite-based CNS/ATM in Iranian air transportation. In R. Curran, L. Fischer, D. Pérez, K. Klein, J. Hoekstra, P. Roling & W.J.C. Verhagen (Eds.), *Air transport and operations: Proceedings of the Third International Air Transport and Operations Symposium 2012* (pp. 540-551). Amsterdam, The Netherlands: IOS Press BV.
[124] Gössling, S., & Upham, P. (2009). Introduction: Aviation and climate change in context. In S. Gössling & P. Upham (Eds.), *Climate change and aviation: Issues, challenges, and solutions* (pp. 1-23). London, UK: Earthscan.
[125] Graham, J. (2021a). First Asia operation joins AFKLMP Cargo SAF programme. Retrieved from https://www.aircargoweek.com/first-asia-operation-joins-afklmp-cargo-saf-programme/?mc_cid=959b1f51df.
[126] Graham, J. (2021b). ACL Airshop: Focused on growth. Retrieved from https://www.aircargoweek.com/acl-airshop-focused-on-growth/?mc_cid=5841c12e69.
[127] Grandy, G. (2010). Instrumental case study. In A.J. Mills., G. Durepos & E. Wiebe (Eds.), *Encyclopedia of case study research*, Volume 1 (pp. 473-475). Thousand Oaks, CA: SAGE Publications.
[128] Grasso Macola, I. (2021). DHL bets on electric cargo planes to deliver on zero emissions, Retrieved from https://www.airport-technology.com/features/dhl-bets-on-electric-cargo-planes-deliver-on-zero-emissions/.
[129] Grover, D. (2021). Kuehne+Nagel launch first carbon neutral air freight lane with Air France KLM Cargo. Retrieved from https://cargonewswire.com/kuehnenagel-launch-first-carbon-neutral-air-freight-lane-with-air-france-klm-cargo/.
[130] Harper, M. (2004). Introduction. In M. Ali (Ed.), *Sustainable composting: Case studies and guidelines for developing countries* (pp. 3-4). Loughborough, UK: Water, Engineering and Development Centre.
[131] Harry, R. (2020a). Lufthansa Cargo and DB Schenker operate first CO2-neutral freighter flights. Retrieved from https://www.aircargonews.net/policy/environment/lufthansa-cargo-and-db-schenker-partner-to-operate-co2-neutral-freighter-flights/.
[132] Harry, R. (2020b). Shell to supply SAF to DHL Express at Schiphol. Retrieved from https://www.aircargonews.net/sectors/energy-logistics/shell-to-supply-saf-to-dhl-express-at-schiphol/.
[133] Harry, R. (2021a). AFKLMP Cargo welcomes five East Asian companies to its SAF Programme. Retrieved from https://www.aircargonews.net/policy/environment/afklmp-cargo-welcomes-five-east-asian-companies-to-its-saf-programme/.
[134] Harry, R. (2021b). DB Schenker and Lufthansa launch Frankfurt-Shanghai CO_2-neutral freighter route. Retrieved

from https://www.aircargonews.net/policy/environment/db-schenker-and-lufthansa-launch-frankfurt-shanghai-co2-neutral-freighter-route/.

[135] Harry, R. (2021c). CEVA Logistics joins United's sustainability initiative. Retrieved from https://www.aircargonews.net/policy/environment/ceva-logistics-joins-uniteds-sustainability-initiative/.

[136] Harry, R. (2021d). Geodis the latest firm to offer SAF-powered flights for airfreight. Retrieved from https://www.aircargonews.net/policy/environment/geodis-the-latest-firm-to-offer-saf-powered-flights-for-airfreight/.

[137] Harry, R. (2021e). IAG Cargo and AFKLM partner with forwarders on SAF flights. Retrieved from https://www.aircargonews.net/airlines/iag-cargo-and-afklm-partner-with-forwarders-on-saf-flights/.

[138] Harry, R. (2021f). K+N and American partner to deploy 11m litres of SAF. Retrieved from https://www.aircargonews.net/airlines/kn-and-american-partner-to-deploy-11m-litres-of-saf/.

[139] Heng, Y.K. (2016). *Managing global risks in the urban age: Singapore and the making of a global city*. Abingdon, UK: Routledge.

[140] Hettiarachchi, H., & Kshourad, C. (2019). Promoting waste-to-energy: nexus thinking, policy instruments, and implications for the environment. In S. Kumar, R. Kumar & A. Pandey (Eds.), *Current developments in biotechnology and bioengineering: Waste treatment processes for energy generation* (pp. 163-184). Amsterdam, The Netherlands: Elsevier.

[141] Holland, F. (2021). DHL Express buys electric cargo planes for U.S. package delivery from start-up Eviation. Retrieved from https://www.cnbc.com/2021/08/03/dhl-express-buys-eviation-electric-planes-for-us-package-delivery.html.

[142] Holland, J. (2021). Samad starts certification process of fully electric VTOL Starling Cargo aircraft. Retrieved from https://www.aviationbusinessnews.com/cargo/samad-starts-certification-process-of-fully-electric-vtol-starling-cargo-aircraft/.

[143] Honeycutt, K.J., & Miller, D.R. (2021). Learner metacognitive insights from writing professional clinical practicum reflections: An instrumental case study of a University-Based MLS Program. *Journal of Allied Health,* 50(1), 67-72.

[144] Hrasky, S. (2012). Carbon footprints and legitimation strategies: Symbolism or action? *Accounting, Auditing & Accountability Journal*, 25(1), 174-198. https://doi.org/10.1108/09513571211191798

[145] Hui, G.W.L., Hui, Y.V., & Zhang, A. (2004). Analysing China's air cargo flows and data. *Journal of Air Transport Management*, 10(2),125-135. https://doi.org/10.1016/j.jairtraman.2003.08.003

[146] Humphries, M. (2021). UPS orders 10 electric aircraft for deliveries. Retrieved from https://au.pcmag.com/news/86509/ups-orders-10-electric-aircraft-for-deliveries.

[147] Hunter, N. (2014). *How carbon footprints work*. New York, NY: Gareth Stevens Publishing

[148] Ierovante, M., Somersalo, F., & Barth, A. (2015). ID for the freighter. Airbus Technical Magazine, October, 26-31.

[149] International Air Transport Association. (2021a). Developing sustainable aviation fuel (SAF). Retrieved from https://www.iata.org/en/programs/environment/sustainable-aviation-fuels/.

[150] International Air Transport Association. (2021b). Offsetting CO_2 emissions with CORSIA. Retrieved from https://www.iata.org/en/programs/environment/corsia/.

[151] International Airport Review. (2010). UK Airport operators launch new guidelines to reduce aircraft ground emissions. Retrieved from http://www.internationalairportreview.com/2717/airport-news/uk-airport-operators-launch-new-guidelines-to-reduce-aircraft-ground-emissions/.

[152] International Civil Aviation Organization. (2012). Annual Report of the Council – 2011, Document 9975. Retrieved from https://www.icao.int/publications/Documents/9975_en.pdf.

[153] International Civil Aviation Organization. (2013). Annual Report of the Council – 2012, Document 10001 Retrieved from https://www.icao.int/publications/Documents/10001_en.pdf.

[154] International Civil Aviation Organization. (2020). Presentation of 2019 air transport statistical results. Retrieved from https://www.icao.int/annual-report-2019/Documents/ARC_2019_Air%20Transport%20Statistics.pdf.

[155] International Civil Aviation Organization. (2021). Waste management at airports. Retrieved from https://www.icao.int/environmental-protection/documents/waste_management_at_airports_booklet.pdf.

[156] International Coordinating Council of Aerospace Industries Associations. (2019). Advancing technology opportunities to further reduce CO_2 emissions. Retrieved from https://www.icao.int/environmental-protection/Documents/EnvironmentalReports/2019/ENVReport2019_pg116-121.pdf.

[157] Itoh, E., & Uejima, K. (2013). Applying flight-deck interval management based continuous descent operation for arrival air traffic to Tokyo International Airport. In: *Proceedings of Tenth USA/Europe Air Traffic Management Research and Development Seminar (ATM 2013), June 10-13, 2013, Chicago* (pp. 1-10).

[158] Jackson, M. (2008). FedEx building German solar facility. Retrieved from https://www.icao.int/environmental-protection/Documents/EnvironmentalReports/2019/ENVReport2019_pg116-121.pdfhttps://www.bizjournals.com/memphis/stories/2008/10/20/daily1.html.

[159] Janić, M. (2011). *Greening airports: Advanced technology and operations*. London, UK: Springer-Verlag.

[160] Janić, M. (2016). *The sustainability of air transportation: A quantitative analysis and assessment*. Abingdon, UK: Routledge.

[161] Japan Airlines. (2014). JAL introduces new light-weight cargo container on its international routes. Retrieved from http://press.jal.co.jp/en/bw_uploads/20140507-JAL%20Introduces%20New%20light-weight%20Cargo%20Container%20on%20its%20International%20Routes.pdf.

[162] Javed, T., Ahmed, A., Raman, V., Alqualty, A.B.S., & Johansson, B. (2019). Combustion-based transportation in a carbon constrained world – A review. In R.A. Agarwal., A.K. Agarwal., T. Gupta & N. Sharma (Eds.), *Pollutants from Energy Sources: Characterization and Control* (pp. 7-34). Singapore: Springer Nature Singapore.

[163] Kazda, A., & Caves, R.E. (2015). *Airport design and operation* (3rd ed.). Bingley, UK: Emerald Group Publishing.

[164] Keen, G. (2021). Air France KLM Martinair Cargo welcomes Airflo and Tiger Freight to its Sustainable Aviation Fuel programme. Retrieved from https://www.caasint.com/air-france-klm-martinair-cargo-welcomes-airflo-and-tiger-freight-to-its-sustainable-aviation-fuel-programme/.

[165] Khandelwal, B., Karakurt, A., Sekaran, P.R., Sethi, V., & Singh, R. (2013). Hydrogen powered aircraft: The future of air transport. *Progress in Aerospace Sciences*, 60, 45-59. https://doi.org/10.1016/j.paerosci.2012.12.002

[166] Kingsley-Jones, M. (2006). Airbus closes in on A330F launch. *Flight International*, 169(5042), 11.

[167] Kingsley-Jones, M. (2010) Worth the weight. *Flight International*, 177(5241), 29-33.

[168] Koppert Biological Systems. (2021). Air France KLM Martinair welcomes Koppert Biological Systems to its Sustainable Aviation Fuel programme. Retrieved from https://www.koppert.com/news/2021/air-france-klm-martinair-welcomes-koppert-biological-systems-to-its-sustainable-aviation-fuel-programme/.

[169] Kováčik, L., Lusiak, L. & Novák, A. (2021). Reducing emissions from aviation and their impact on aviation work in agriculture. *Transportation Research Procedia*, 55, 220–227. https://doi.org/10.1016/j.trpro.2021.06.025

[170] Kyriakopoulos, G.L., Kapsalis, V.C, Aravossis, K.G., Zamparas, M., & Mitsikas, A. (2019). Evaluating circular economy under a multi-parametric approach: A technological review. *Sustainability*, 11(21), 6139. https://doi.org/10.3390/su11216139

[171] Laniel, M., Uysal, J., & Emond, J. P. (2011). Radio frequency interactions with air cargo container materials for real-time cold chain monitoring. *Applied Engineering in Agriculture*, 27(4), 647-652.

[172] Lee, D. (2013). Meet the new FedEx efficient containers. Lighter design. less fuel. Retrieved from https://www.3blmedia.com/news/meet-new-fedex-efficient-containers-lighter-design-less-fuel.

[173] Li, X.D., Poon, C.S., Lee, S.C., Chung, S.S., & Luk, F. (2003). Waste reduction and recycling strategies for the in-flight services in the airline industry. *Resources, Conservation and Recycling*, 37(3),87-99. https://doi.org/10.1016/S0921-3449(02)00074-5

[174] Longo, M., Lutz, N. M., Daniel, L. Zaninelli, D., & Pruckner, M. (2017). Towards an impact study of electric vehicles on the Italian electric power system using simulation techniques. In *Proceedings of the 2017 IEEE 3rd International Forum on Research and Technologies for Society and Industry (RTSI)*, 2017 (pp. 1-5). 10.1109/RTSI.2017.8065911.

[175] Lu, H.A., & Chen, C.Y. (2011). A time-space network model for unit load device stock planning in international airline services. *Journal of Air Transport Management*, 17(2), 94-100. https://doi.org/10.1016/j.jairtraman.2010.09.004

[176] Lu, H.A., & Chen, C.Y. (2012). Safety stock estimation of unit load devices for international airline operations. *Journal of Marine Science and Technology*, 20(4), 431-440.

[177] Macário, R., & Van de Voorde, E. (2011). Introduction. In R. Macário & E. Van de Voorde (Eds.), *Critical issues in air transport economics and business* (pp. 1-9). Abingdon, UK: Routledge.

[178] Maertens, S., Grimme, W., Scheelhaase, J., & Jung, M. (2019). Options to continue the EU ETS for aviation in a CORSIA-world. *Sustainability*, 11(20), 5703. https://doi.org/10.3390/su11205703

[179] Manahan, S.E. (20110. *Fundamentals of environmental chemistry* (3rd ed.). Boca Raton, FL: CRC Press.

[180] Marais, K., Wolfe, P.J., & Waitz, I.A. (2016). Air transport and the environment. In P. Belobaba, A. Odoni & C. Barnhart (Eds), *The global airline industry* (pp. 423-457) (2nd ed.). Chichester, UK; John Wiley & Sons.

[181] Marinetrans. (2021). Marinetrans (MT) has joined the Sustainable Aviation Fuel (SAF) programme of Air France KLM Martinair Cargo. Retrieved from https://marinetrans.com/newsroom/marinetrans-mt-has-joined-the-sustainable-aviation-fuel-saf-program-of-air-france-klm-martinair-cargo/.

[182] Masiol, M., & Harrison, R.M. (2014). Aircraft engine exhaust emissions and other airport-related contributions to ambient air pollution: A review. *Atmospheric Environment*, 95, 409-455. https://doi.org/10.1016/j.atmosenv.2014.05.070

[183] Mayer, R., Ryley, T., & Gillingwater, D. (2012). Passenger perceptions of the green image associated with airlines. *Journal of Transport Geography*, 22, 179-186. https://doi.org/10.1016/j.jtrangeo.2012.01.007

[184] Merkert, R., & Alexander, D. (2018). The air cargo industry. In N. Halpern & A. Graham (Eds.), *The Routledge companion to air transport management* (pp. 29-47). Abingdon, UK: Routledge.

[185] Migdadi, Y.K.A.A. (2018). Identifying the best practices of airlines' green operations strategy: A cross-regional worldwide survey. *Environmental Quality Management*, 28(1), 21-32. https://doi.org/10.1002/tqem.21575

[186] Morrell, P.S. (2016). The air cargo industry. In J.F. O'Connell & G. Williams (Eds.), *Air transport in the 21st century: Key strategic developments* (pp. 235-252). Abingdon, UK: Routledge.

[187] Morrell, P.S., & Klein, T. (2018). *Moving boxes by air: The economics of international air cargo* (2nd ed.). Abingdon, UK: Routledge.
[188] Nordisk Aviation Products. (2014). Nordisk completes delivery of 4,300 lightweight containers to Singapore Airlines. Retrieved from https://www.nordisk-aviation.com/en/news/nordisk-completes-delivery-of-4300-lightweight-containers-to-singapore-airlines/.
[189] Norris, G. (2006), Kings of cargo. *Flight International*, 169(5036), 36-38.
[190] Oates, B.J. (2006). *Researching information systems and computing*. London, UK: SAGE Publications.
[191] O'Leary, Z. (2004). *The essential guide to doing research*. London, UK: SAGE Publications.
[192] Ostrower, J. (2008). Green and versatile. *Flight International*, 174(5146), 46-50.
[193] Palmer, W. (2020). Sustaining flight: Comprehension, assessment, and certification of sustainability in aviation. In T. Walker, A.S. Bergantino, N. Sprung-Much & L. Loiacono (Eds.), *Sustainable aviation: Greening the flight path* (pp. 7-28). Cham, Switzerland: Springer Nature Switzerland.
[194] Pitt, M., & Smith, A. (2003). Waste management efficiency at UK airports. *Journal of Air Transport Management*, 9(2), 103–111. https://doi.org/10.1016/S0969-6997(02)00063-7
[195] Prosperi, D.C. (2009). Greening the world's airports. *World Transport Policy & Practice*, 14(4), 48-56.
[196] Putzger, I. (2015). Air cargo still waits for the breakthrough: Lightweight pallets that don't break easy. Retrieved from https://theloadstar.com/air-cargo-still-waits-for-the-breakthrough-lightweight-pallets-that-dont-break-easy/.
[197] Rahman, M., Pudasainee, P., & Gupta, R. (2017). Urban waste (municipal solid waste-MSW) to energy. In S. Devasahayam., K Dowling & M.K. Mahapatra (Eds.), *Sustainability in the mineral and energy sectors* (pp.499-530). Boca Raton: CRC Press.
[198] Ramachandra, T.V., & Mahapatra, D.M. (2016). The science of carbon footprint assessment. In S.S. Muthu (Ed.), *The carbon footprint handbook* (pp. 4-44). Boca Raton, FL: CRC Press.
[199] Ramon Gil-Garcia, J. (2012). *Enacting electronic government success: An integrative study of government-wide websites, organizational capabilities, and institutions*. New York, NY: Springer Science+Business Media.
[200] Ramsay, M. (2020). Loaders go electric. *Airside International*, 49, 8-11.
[201] Rand, T., Haukohl, J., & Marxen, U. (2000). *Municipal solid waste incineration: Requirements for a successful project*. Washington, DC: The World Bank.
[202] Randall, C. (2019). Stuttgart airport expands electric cargo tractor fleet. Retrieved from https://www.electrive.com/2019/10/30/stuttgart-airport-expands-electric-cargo-tractor-fleet/.
[203] Reichmann, K. (2021a). Sustainable aviation fuels aren't sustainable, not yet at least. Retrieved from https://www.aviationtoday.com/2021/08/05/sustainable-aviation-fuels-arent-sustainable-not-yet-least/.
[204] Reichmann, K. (2021b). UPS purchases Beta's eVTOL aircraft. Retrieved from https://www.aviationtoday.com/2021/04/09/ups-purchases-betas-evtol-aircraft/.
[205] Rimoczi, R. (2020). Air France KLM, Martinair introduce 'Cargo SAF' fuel program. Retrieved from https://airwaysmag.com/airlines/af-kl-ma-cargo-saf-fuel-prog/.
[206] Rondinelli, S., Gardi, A., Kapoor, R., & Sabatini, R. (2017). Benefits and challenges of liquid hydrogen fuels in commercial aviation. *International Journal of Sustainable Aviation*, 3(3), 200-216.
[207] Sadang, J.M., Palompon, D.R., & Suksatan, W. (2021). Older adults' experiences and adaptation strategies during the midst of COVID-19 crisis: A qualitative instrumental case study. *Annals of Geriatric Medicine and Research*, 25(2): 113–121. https://dx.doi.org/10.4235%2Fagmr.21.0051
[208] Sales, M. (2013). *The air logistics handbook: Air freight and the global supply chain*. Abingdon, UK: Routledge.
[209] Sales, M. (2016). *Aviation logistics: The dynamic partnership of air freight and supply chain*. London, UK: Kogan Page.
[210] Sales, M. (2017). *Air cargo management: Air freight and the global supply chain* (2nd. ed.). Abingdon, UK: Routledge.
[211] Salmi, H. (2019). Light-weight ULDs and other energy-saving gains by Finnair Cargo. Retrieved from https://cargo.finnair.com/en/cargo-news/light-weight-ulds-and-other-energy-saving-gains-by-finnair-cargo-1764834.
[212] Salvatore, D. (2020). *International economics* (13th ed). Hoboken, NJ: John Wiley & Sons.
[213] Sarkar, A. N. (2012). Evolving green aviation transport system: A Holistic approach to sustainable green market development. *American Journal of Climate Change*, 1, 164-180.
[214] Saunders, E. (2021a). AFKLMP Cargo adds five Chinese partners to its SAF Programme. Retrieved from https://www.caasint.com/afklmp-cargo-adds-five-chinese-partners-to-its-saf-programme/.
[215] Saunders, E. (2021b). AFKLMP Cargo welcomes Total Touch Cargo. Retrieved from https://www.caasint.com/afklmp-cargo-welcomes-total-touch-cargo/.
[216] Saunders, E. (2021c). Cargolux launches SAF program. Retrieved from https://www.caasint.com/cargolux-launches-saf-program/.
[217] Schäfer, A.W. (2016). The prospects for biofuels in aviation. In C. Chuck (Ed.), *Biofuels for aviation: Feedstocks, technology and implementation* (pp. 3-16). London, UK: Academic Press.
[218] Scheelhaase, J., Maertens, S., & Grimme, W. (2019). Synthetic fuels in aviation – Current barriers and potential political measures. *Transportation Research Procedia*, 43, 21-30. https://doi.org/10.1016/j.trpro.2019.12.015
[219] Schutte, J.S., Payan, AP., Briceno, S.I., & Mavris, D.N. (2016). Hydrogen-powered aircraft. In R. Agarwal., F.

Collier., A. Schäfer & A. Seabridge. *Green aviation* (pp. 165-176). Chichester: John Wiley & Sons.

[220] Scott, B.J. & Trimarchi, A. (2020). *Fundamentals of international aviation law and policy*. Abingdon, UK: Routledge.

[221] Scott, J. (2014). *A dictionary of sociology* (4th ed.). Oxford, UK: Oxford University Press.

[222] Scott, J., & Marshall, G. (2009). *A dictionary of sociology* (3rd ed.). Oxford, UK: Oxford University Press.

[223] Seko Logistics. (2021). Seko Logistics joins the Sustainable Air Freight Alliance. Retrieved from https://www.sekologistics.com/us/news/posts/2021/march/seko-logistics-joins-the-sustainable-air-freight-alliance/.

[224] Shah, Y.T. (2021). *Hybrid energy systems: Strategy for industrial decarbonization*. Boca Raton, FL: CRC Press.

[225] Singapore Airlines. (2017). Sustainability report 2016/17. Retrieved from https://www.singaporeair.com/saar5/pdf/Investor-Relations/Annual-Report/sustainabilityreport1617.pdf.

[226] Singapore Airlines. (2018). Sustainability report 2017/18. Retrieved from https://www.singaporeair.com/saar5/pdf/Investor-Relations/Annual-Report/sustainabilityreport1718.pdf.

[227] Singapore Airlines. (2020). Sustainability report 2019/20. Retrieved from https://www.singaporeair.com/saar5/pdf/Investor-Relations/Annual-Report/sustainabilityreport1920.pdf.

[228] Singapore Airlines. (2021). When it comes to carbon-neutrality, we're not sitting on the fence. Retrieved from https://www.singaporeair.com/en_UK/gb/flying-withus/our-story/giving-back/environmental-efforts/.

[229] Singh, J., Sharma-Kumar, S., & Srivastava R. (2018). Managing fuel efficiency in the aviation sector: challenges, accomplishments and opportunities. *FIIB Business Review*, 7(4), 244-251. https://doi.org/10.1177%2F2319714518814073

[230] Spellman, F.R. (2015). *Environmental impacts of renewable energy*. Boca Raton, FL: CRC Press.

[231] Sreeneth, S., Sudhakar, K., & Yusop, A.F. (2021). Suitability analysis of solar tracking PV system in the airport based on glare assessment. *International Journal of Automotive and Mechanical Engineering*, 18(1), 9061-9070.

[232] Stake, R.E. (1995). *The art of case study research*. Thousand Oaks, CA: SAGE Publications.

[233] Stake, R.E. (2005). Qualitative case studies. In N.K. Denzin & Y.S. Lincoln (Eds.), *The SAGE handbook of qualitative research* (pp. 443-466). Thousand Oaks, CA: SAGE Publications.

[234] Staples, M.D., Malina, R., Suresh, P., Hileman, J.I., & Barrett, S.R.H. (2014). Aviation CO_2 emissions reductions from the use of alternative jet fuels. *Energy Policy*, 114, 342-354. https://doi.org/10.1016/j.enpol.2017.12.007

[235] Su, Y., Zhang, P., & Su, Y. (2015). An overview of biofuels policies and industrialization in the major biofuel producing countries. *Renewable and Sustainable Energy Reviews*, 50, 991-1003. https://doi.org/10.1016/j.rser.2015.04.032

[236] Szymczak, R. (2021). Assessing the impact of a potential short-haul flights ban on European airports. In A. Kwasiborska, J. Skorupski & I. Yatskiv (Eds.), *Advances in air traffic engineering, selected papers from 6th international scientific conference on air traffic engineering, ATE 2020, October 2020, Warsaw, Poland* (pp. 146-163). Cham: Springer Nature Switzerland.

[237] Taiwo, A.M. (20110. Composting as a sustainable waste management technique in developing countries. *Journal of Environmental Science and Technology*, 4(2), 93-102.

[238] Tavares Kennedy, H. (2019). Finnair introduces CO_2 offsetting and biofuel service for customers. Retrieved from https://www.biofuelsdigest.com/bdigest/2019/01/19/finnair-introduces-co2-offsetting-and-biofuel-service-for-customers/.

[239] Teoh, L.E., & Khoo H.L (2016). Green air transport system: An overview of issues, strategies and challenges. *KSCE Journal of Civil Engineering*, 20(3), 1040-1052. https://doi.org/10.1007/s12205-016-1670-3

[240] Thompson, B. (2007). Ground handling opportunities for airports. *Journal of Airport Management*, 1(4), 393-397.

[241] Times Aerospace (2012). Etihad introduces lightweight cargo containers. Retrieved from https://www.timesaerospace.aero/news/air-transport/etihad-introduces-lightweight-cargo-containers.

[242] Times Aerospace. (2021). AFKLMP Cargo welcomes Hellmann Worldwide Logistics to its SAF programme. Retrieved from https://www.timesaerospace.aero/news/maintenance/afklmp-cargo-welcomes-hellmann-worldwide-logistics-to-its-saf-programme.

[243] Trabold, T.A., & Nair, V. (2019). Conventional food waste management methods. In T. Trabold & C.W. Babbitt (Eds.), *Sustainable food waste-to-energy systems* (pp. 29-45). London, UK: Academic Press.

[244] Tretheway, M.W., & Andriulaitis, R.J. (2016). Airport competition for freight. In P. Forsyth., D. Gillen., J. Müllen & H.M. Niemeier (Eds.), *Airport competition: The European experience* (pp. 137-150). Abingdon, UK: Routledge.

[245] Tuladhar, S., Ratna Bajracharyaa, T., & Raj Shakyaa, S. (2021). Evaluation and mitigation analysis of carbon footprint for an airline operator: Case of Nepal Airlines Corporation. *Journal of Innovation in Engineering Education*, 4(1), 18-33.

[246] Turner, Y. (2020). AFKLMP Cargo launch SAF programme for airfreight. Retrieved from https://www.aircargoweek.com/afklm-martinair-cargo-launch-saf-programme-for-airfreight/.

[247] Turner, Y. (2021a). Airflo and Tiger Freight join AFKLMP's SAF programme. Retrieved from https://www.aircargoweek.com/airflo-and-tiger-freight-join-afklmps-saf-programme/.

[248] Turner, Y. (2021b). ANA launches SAF Initiative. Retrieved from https://www.aircargoweek.com/ana-launches-saf-initiative/?mc_cid=edc4d6dfb8.

[249] Turner, C. (2021c). Kuehne+Nagel to offer SAF option for all shipments. Retrieved from

https://www.aircargoweek.com/kuehnenagel-to-offer-saf-option-for-all-shipments/.
[250] Turner, Y. (2021d). More join AFKLMP SAF programme. Retrieved from https://www.aircargoweek.com/samad-starts-to-certify-electric-cargo-aircraft/?mc_cid=2a1e293076.https://www.aircargoweek.com/more-join-afklmp-saf/?mc_cid=14644288a9.
[251] Turner, Y. (2021e). SAMAD starts to certify electric cargo aircraft. Retrieved from https://www.aircargoweek.com/samad-starts-to-certify-electric-cargo-aircraft/?mc_cid=2a1e293076.
[252] Turner, Y. (2021f). TIACA on sustainability: People, planet, prosperity. Retrieved from https://www.aircargoweek.com/tiaca-on-sustainability/?mc_cid=d8deab3dfc.
[253] Turner, Y. (2021g). The importance of air cargo to the global economy. Retrieved from https://www.aircargoweek.com/the-importance-of-air-cargo-to-the-global-economy/.
[254] TWI Limited. (2021). What is decarbonisation? Retrieved from https://www.twi-global.com/technical-knowledge/faqs/what-is-decarbonisation.
[255] Unique Logistics International. (2021). Unique Logistics International joins Sustainable Air Freight Alliance (SAFA). Retrieved from https://unique-logistics.com/news/unique-logistics-international-joins-sustainable-air-freight-alliance-safa/.
[256] United Nations. (2018). Renewable energy sources cut carbon emissions, efficiently increase electricity output worldwide, delegates say in Second Committee. Retrieved from https://www.un.org/press/en/2018/gaef3501.doc.htm.
[257] United States Energy Information Administration. (2021). Solar explained: Solar energy and the environment. Retrieved from https://www.eia.gov/energyexplained/solar/solar-energy-and-the-environment.php.
[258] United States Federal Aviation Administration. (2013). Recycling, reuse, and waste reduction at airports: A synthesis document. Retrieved from https://www.faa.gov/airports/resources/publications/reports/environmental/media/RecyclingSynthesis2013.pdf
[259] Wensveen, J.G. (2016). *Air transportation: A management perspective* (8th ed.). Abingdon, UK: Routledge.
[260] Wey, C., & Lee, C.M. (2017). Aircraft emissions: Gaseous and particulate. In E.S. Nelson & D.R. Reddy (Eds.), *Green aviation: Reduction of environmental impact through aircraft technology and alternative fuels* (p. 48). Leiden, The Netherlands: CRC Press/Balkema.
[261] Wiedemann, T.& Minx, J. (2007). A definition of 'carbon footprint'. In C.C. Pertsova (Ed.), *Ecological economics research trends* (pp. 1-11). New York, NY: Nova Science Publishers.
[262] Williams, I., Kemp, S., Coello, J., Turner, D.A., & Wright, L.A. (2012). A beginner's guide to carbon footprinting. *Carbon Management*, 3(1), 55-67. https://doi.org/10.4155/cmt.11.80
[263] Wolfsteller, P. (2021). FedEx invests $2bn in sustainable technologies. Retrieved from https://www.aircargonews.net/airlines/fedex-invests-2bn-in-sustainable-technologies/.
[264] Xu, W., Li, Q., & Huang, M. (2015). Design and analysis of liquid hydrogen storage tank for high-altitude long-endurance remotely-operated aircraft. *International Journal of Hydrogen Energy*, 40(46), 16578-16586. https://doi.org/10.1016/j.ijhydene.2015.09.028
[265] Yadhav, D., & Wang, J. (2017). Modelling carbon dioxide emissions from agricultural soils in Canada. *Environmental Pollution*, 230, 1040-1049. https://doi.org/10.1016/j.envpol.2017.07.066
[266] Ydersbond, I.M., Kristensen, N.B., & Thune-Larsen, H. (2020). *Nordic sustainable aviation*. Oslo, Norway: Nordic Energy Research.
[267] Yerel Kandemir S., & Yayli, M. Ö. (2016). Investigation of renewable energy sources for airports. In T. Hikmet, M. Baris Ozerdem, C.O. Colpan, O. Altuntas, E. Acikkalp (Eds.), *Sustainable aviation: Energy and environmental issues* (pp. 11-16). Cham, Switzerland: Springer International Publishing.
[268] Yim, S.H.L., Stettler, M. E.J., & Barrett, S.R.H. (2013). Air quality and public health impacts of UK airports. Part II: Impacts and policy assessment. *Atmospheric Environment*, 67, 184-192. https://doi.org/10.1016/j.atmosenv.2012.10.017
[269] Yin, R.K. (2018). *Case study research and applications* (6th ed.). Thousand Oaks, CA: SAGE Publications.
[270] Zhang, J., Zhang, S., Wu, R., Duan. M., Zhang, D., Wu, Y., & Hao, J. (2021). The new CORSIA baseline has limited motivation to promote the green recovery of global aviation. *Environmental Pollution*, 289, 117833. https://doi.org/10.1016/j.envpol.2021.117833
[271] Zhang, Z., Pan, S.Y., Li, H., Cai, J., Ghani Olabi, A., Anthony, E.J., & Manovic, V. (2020). Recent advances in carbon dioxide utilization. *Renewable and Sustainable Energy Reviews*, 125, 109799. https://doi.org/10.1016/j.rser.2020.109799
[272] Zhu, D., Asnani, P.U., Zurbrugg, C., Anapolsky, S., & Mani, S. (2008). *Improving municipal solid waste management in India: A sourcebook for policy makers and practitioners*. Washington, DC: The World Bank.
[273] Zou, B., Kwan, I., Hansen, M., Rutherford, D., & Kafle, N. (2016). Airline fuel efficiency: assessment methodologies and applications in the U.S. domestic airline industry. In J.D. Bitzan., J. Peoples & W.W. Wilson (Eds.), *Airline efficiency* (pp. 317-352). Bingley, UK: Emerald Group Publishing.

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Toxic Test of Lavender Leaf *(Lavandula angustifolia)* Ethanol Extract as Biolarvicide for Aedes aegypti Mosquitoes Vectors of Dengue Hemorrhagic Fever

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***Abstract*— *Toxic test of ethanolic extract of lavender (Lavandula angustifolia) leaf on mortality of Aedes aegypti mosquito larvae as vector of dengue hemorrhagic fever has been completed. The aim of the study was to determine the effective concentration of lavender (Lavandula angustifolia) leaf ethanol extract against the mortality of Aedes aegypti and LC_{50} mosquitoes for 24 hours. This study used a completely randomized design with 5 extract treatments, namely: 10ppm; 50ppm; 100ppm; 500ppm and 1000ppm and 1 control group with 3 replications. The results of the study were analyzed using one-way analysis of variance. and continued with the BNT test at a significance level of 0.05. The results showed that the ethanolic extract of lavender (Lavandula angustifolia) leaves was toxic to the larvae of the Aeders aegypti mosquito, which was indicated by the increasing number of larval mortality. Based on the test results, the concentration of 500 ppm lavender leaf ethanol extract was able to kill 100% of mosquitoes, and the effective concentration to kill 50% of test mosquito larvae was 87.0285 ppm. The ethanol extract of lavender (Lavandula angustifolia) leaves has the potential to be developed as a biolarvicide for the Aedes aegypti mosquito vector of dengue hemorrhagic fever.*

***Keywords*— Lavandula angustifolia leaves, biolarvicides, Aedes aegypti.**

I. INTRODUCTION

North Sulawesi in January 2015 was categorized as an Extraordinary Event (KLB), the five-year cycle of the Dengue Hemorrhagic Fever (DHF) outbreak that hit eight districts/cities in North Sulawesi, killing eight people who were positive for the virus transmitted by the Aides aegypti mosquito.

Until now, no vaccine has been found to kill the virus that causes dengue fever. One way to prevent the spread of dengue hemorrhagic fever (DHF) is to prevent dengue virus transmission, namely by controlling and eradicating vectors to cut off disease transmission. (WHO, 2005).

Fogging is one of the mechanical control methods. Unfortunately, smoking is considered less effective because it tends to repel mosquitoes from their nests, not kill them. The chemical method used is the spread of larvicides such as abate in mosquito breeding places. Indeed, the use of chemical larvicides has succeeded in controlling *Aedes aegypti* larvae, but the continuous use of chemical larvicides will actually cause resistance and various environmental problems. cause environmental pollution, poisoning, death of non-target organisms, and produce residues.

Due to the negative impact caused by chemical insecticides, it has encouraged experts to look for alternatives to vector eradication, namely by using natural insecticides that are safer, easier, cheaper, and do not have a toxic impact on humans.

Plants that have been isolated by researchers containing active compounds of vegetable insecticides in *Aedes aegypti* mosquito larvae are soursop seeds *(Annona muricata)* with LC_{50} = 117. 27 ppm (Komansilan *et al.* 2012), Hutun seeds *(Barringtonia asiatica Kurz)* with Lethal Concentration LC_{50} = 35.72 ppm (Komansilan and Suriani. 2016) and tuba root *(Derris elliptica)* with Lethal Concentration LC_{50} = 44.7526 ppm (Komansilan *et al.* 2017).

Lavender *(Lavandula angustifolia)* is one of the plants that can be used as a natural insecticide, because it is effective in controlling insects (mosquitoes). This is because lavender plants have kairomone as a chemical that causes an odor that mosquitoes don't like. Lavender plants also have active ingredients in the form of flavonoids; Rosmarinic acid, Chlorogenic acid, Caffeic acid 2-(3,4 dihydroxyphenyl) ethenyl ester (found in flowers), Flavonoids; Hypolaetin, Scutellarein, Salvigenin, Malvidin, Xanthomicrol, Delphinidine (found in leaves), and Terpenoi; Linalil acetate, Linalol, 1,8-

Cineole, Camphor, Ursolic acid, Oleanolic acid which acts as a repellent (insect repellent) by working as a contact poison and respiratory poison (Kherissat, 2009).

Based on research from Lekitoo (2009), it is known that the flowers and leaves of the lavender plant *(Lavandula angustifolia)* have no statistically different effect as a repellent against the *Aedes aegypti* mosquito.

Regarding the toxicity of lavender *(Lavandula angostifolia)* leaf extract, the results of research from Nindatu, *et al.* (2011} showed that lavender *(Lavandula angostifolia)* leaf extract was good and effective for controlling Culex sp mosquitoes, with an LC_{50} value of 0.259%

This study aims to determine the toxicity of the ethanolic extract of lavender *(Lavandula angustifolia)* leaves as a biolarvicide to the *Aedes aegypti* mosquito vector of dengue hemorrhagic fever.

II. RESEARCH METHODS

Location of Research Time

This research was conducted at the Integrated Science Laboratory, Faculty of Mathematics and Natural Sciences, Manado State University. This research was conducted from May to September 2021

Tools and Materials

The tools used are: vial, mosquito cage, blender, buchner, rotary evaporator, desiccator, digital scale, measuring cup and micro pipette. Research materials: Lavender leaves, ethanol, aquades, Aedes aegypti mosquito larvae, fish feed, filter paper .

Research design

The design used was a completely randomized design (CRD), with 6 treatments and 3 replications, namely: K1 = Control, K2 = .10ppm, K3 = .50ppm, K4 = 100ppm, K5 = 500ppm and K6 = 1000ppm

Observation

The parameter observed was the mortality percentage of *Aedes aegypti* mosquito larvae, which was calculated using the formula proposed by Kundra (1981):

$$\mathbf{M = a/b \times 100\%}$$

Where: M = percentage of mosquito mortality *Ae. aegypti*

a = number of mosquitoes *Ae. Aegypti* Dead

b = number of mosquitoes *Ae. aegypti* who used.

Work procedures

1. Reproduction of *Aedes aegypti* mosquito larvae

a) *Aedes aegypti* mosquito larvae media is made by filling a plastic container with water and the inner wall is lined with filter paper. Filter paper serves as a place for female mosquitoes to attach their eggs.

b) Eggs attached to filter paper are then dried at room temperature and stored in a closed container. For hatching eggs, filter paper is dipped in a plastic tray filled with water and after 24 hours the eggs will hatch and grow into first instar larvae.

c) First instar larvae will develop into second, third (4 days) and IV instar (2 days) instar larvae. Once every 2 days, the larvae were fed 1-2 grams of fish pellets. III/IV instar larvae used in the test.

2. Extract Making

The manufacture of lavender leaf extract is as follows:

a) Lavender leaves are separated from the stems, washed, and air-dried to dry indoors and away from sunlight.

b) The dried lavender leaves are mashed using a blender.

c) The mashed leaves were extracted by maceration using technical ethanol until all the components had been extracted.

d) The ethanol extract obtained was evaporated with a vacuum rotary evaporator until all solvent evaporated.

3. Toxicity Test.

a) Provide a solution of lavender leaf extract *(Lavandula angustifolia)* in a vial with concentrations of: 0ppm, 10ppm, 50ppm, 100ppm, 500ppm and 1000ppm.

b) In each vial, 10 larvae of *Aedes aegypti* mosquitoes were inserted with 3 replications

c) The calculation of mortality was carried out after 24 hours of treatment.

d) In the control tube using plain water.

Data analysis

The toxicity of lavender leaf extract to Aedes aegypti mosquito larvae was determined based on the LC_{50} value, namely the concentration where the test larvae died by 50%. Determination of LC_{50} was carried out using probit analysis. To distinguish the toxicity between treatments of several concentrations of lavender leaf extract against *Aedes aegypti* mosquito larvae, it was analyzed using one-way ANOVA analysis at a 95% confidence level (α = 0.05), followed by the BNT test.

III. RESULTS AND DISCUSSION

Based on mortality data of Aedes mosquito larvae that were tested for 24 hours with lavender leaf extract (Lavandula angustifolia), the average mortality obtained is presented in table 1.

Table 1. Average Mortality of Aedes aegypti Mosquito Larvae Testing With Lavender Leaf Extract (Lavandula angustifolia) For 24 Hours.

Test Concentration (ppm)	Average Mortality (%)
Control	0
10	3.33
50	23.33
100	60
500	100
1000	100

According to table 1, it can be seen that the average mosquito mortality at the lowest concentration, namely the 10ppm treatment was only able to kill 3.33% of the number of mosquito larvae tested and the 50ppm concentration treatment was able to kill 23.33% of the number of mosquito larvae tested. Furthermore, in the treatment with a concentration of 100 ppm the mortality of mortality was good, reaching 60% of the number of mosquitoes tested. While at concentrations of 500ppm and 1000ppm mortality mortality of Aedes aegypti mosquito larvae has reached 100%. %. The mortality data of *Aedes aegypti* mosquito larvae can be seen in the histogram in Figure 1.

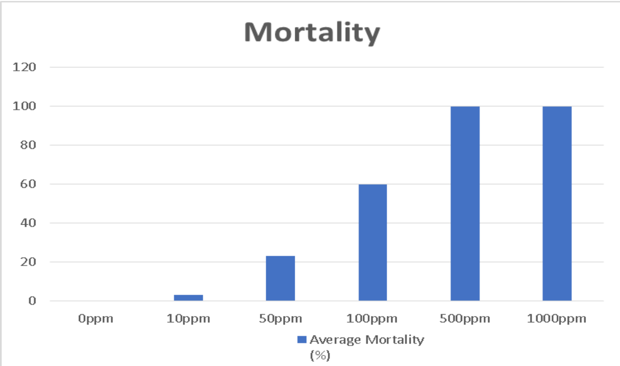

Fig.1. Histogram of Average Mortality of Larvae Ae. aegypti For 24 Hours Treatment with Lavender Leaf Extract (Lavandula angustifolia).

Furthermore, based on one-way analysis of variance of the toxicity of the ethanolic extract of lavender leaves on the mortality of *Ae. aegypti* data can be seen in table 2.

Table 2. Results of one-way analysis of variance on the toxicity of lavender leaf extract (Lavender angustifolia) on mortality of Ae. aegypti after 24 hours of treatment.

ANOVA

Death Rate

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	313.778	5	62.756	225.920	.000
Within Groups	3.333	12	.278		
Total	317.111	17			

From the results of one-way analysis of variance (ANOVA) in table 2, it shows that the calculated F value > table F (P < = 0.05). This means that the treatment given has a significant effect on the mortality of *Ae. aegypti*. Furthermore, to determine the toxicity of lavender *(Lavender angustifolia)* leaf extract which was significantly different to the mortality of *Ae. aegypti*, data analysis was continued with the Least Significant Difference (BNT) test using the SPSS for windows 15.0 program.

From the results of the BNT test shown in the table above, it shows that the control is not significantly different from a concentration of 10ppm, but significantly different from 50, 100,500 and 1000ppm. The concentration of 10ppm is not significantly different from 0ppm, but significantly different from 50, 100, 500 and 1000ppm. As for the concentrations of 50ppm 100ppm, 500ppm and 1000ppm, all of them looked significantly different both to the control and between treatments.

Based on the results of the calculation of the average mortality data and the BNT test, it can be seen that at all concentrations used, the higher the concentration of lavender leaf extract, the higher the mortality percentage of *Ae. Aegypti* as in Figure 2.

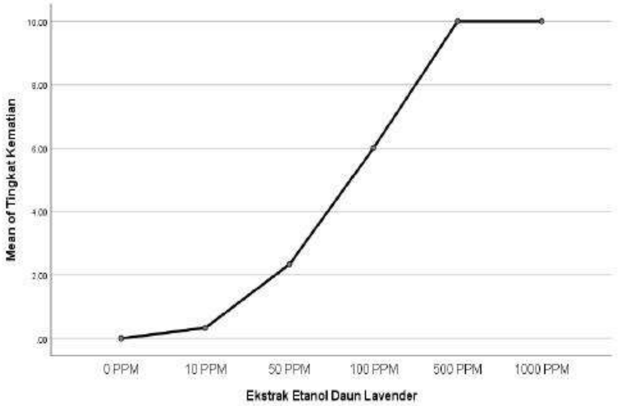

Fig.2. Graph of the relationship between the concentration of lavender leaf extract (Lavandula angustifolia) and mortality of Ae. Aegypti

Based on the results of the BNT test, probit analysis was carried out to determine the LC_{50} value of the effective concentration of lavender leaf extract *(Lavandula angustifolia).* kill 50% of *Ae. aegypti* which was tested for 24 hours, the data were analyzed using Probit Analysis (Finney Method) using Minitab 17 software.

The data used as a whole were obtained from 10 larvae of Ae. aegypti in each replication (there were 3 replications) so that 30 larvae of *Ae. aegypti* as a whole. Table 3. below presents the estimation parameters of the probit analysis model:

Table 3. Parameters of the estimated probit analysis model of clove leaf extract on Ae. aegypti larva larvae

Parameter Estimates Standard 95,0 % Normal Cl Parameter Estimate Error Lower Upper				
Location	87.0285	8.35754	70.6480	103.409
St Dev	45.3707	9.79414	29.7186	69.2666

Graphically, the probit analysis curve is presented as follows:

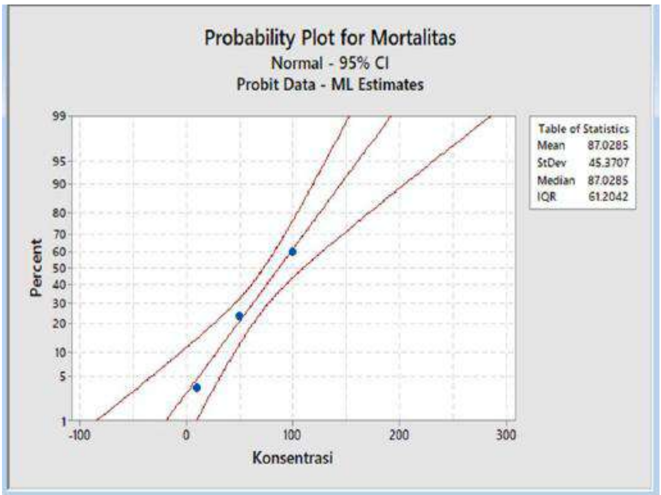

Fig.3. LC_{50} value of ethanol extract of lavender leaf samples on Ae. aegypti larvae after 24 hours of treatment.

Table 2 and Figure 3 present the LC_{50} or Mean Lethal Concentration values of the ethanol extract of lavender leaves based on the results of Probit Analysis. The test results show the value of the LC_{50} mortality concentration of *Ae. aegypti* is by giving a concentration of 245,802 ppm. Thus, the concentration figure of 245,802 ppm is the concentration of the lavender leaf ethanol extract which is the most effective for killing *Ae. aegypti* as much as 50% for 24 hours of treatment. According to the toxicity criteria based on the Australian Petroleum Energy Association (1994) the concentration of 245,802 from the ethanolic extract of lavender leaves or (LC_{50} = 245,802 ppm) at 24 hours of observation was included in the criteria for Toxic Poisoning.

IV. CONCLUSION

Conclusion

1. There is a significant difference between the mortality rate of *Ae. aegypti* at various concentrations of lavender leaf ethanol extract ranging from 0 ppm to 1000 ppm.

2. The results of the test of biolarvicide activity on the larvae of *Ae. aegypti* showed that lavender leaf ethanol extract was active as a larvicidal agent and effectively killed *Ae. aegypti* with a mortality concentration value of LC_{50} = 44.7526 ppm. included in the criteria for Toxic Poison.

Suggestion

1. It is necessary to separate and further identify the ethanolic extract of lavender leaves using chromatography and GC-MS spectrometer techniques.

2. The results of the separation, purification and identification of the compounds contained in the ethanol extract of lavender leaves were tested for their biolarvicidal activity on the larvae of *Ae. aegypti* to obtain the most toxic isolates as an ingredient in the formula for anti mosquito dengue fever.

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REFERENCES

[1] Alfrits Komansilan, Ni Wayan Suriani, Helen Lawalata. 2017. Test Toxic Tuba Root Extract as a Natural Insecticide on Larvae of Aedesaegypti Mosquito Vector of Dengue Fever. International Journal of ChemTech Research. Vol.10 No.4, pp 522-528,

[2] Khwerissat, 2009. Lavender. Http://www. Author stream.com/ presentation/ farawela235217-lavendereducation-ppt-powerpoin/. Diakses 14 januari 2017

[3] Komansilan A, Abdul L. A, Yanuwiadi B, and Kaligis D.A. 2012, Isolation and Identification of Biolarvicide from Soursop (Annonamuricata Linn) Seeds to Mosquito (Aedes aegypti) Larvae. International Journal of Engineering & Technology IJET-IJENS Vol: 12 No: 03 pp 28-32

[4] Komansilan A. and Suriani,N W. 2016. Effectiveness of Seed Extract Hutun (Barringtonia asiatica KURZ), on Larva Aedes aegypti Vector Disease Dengue Fever. International Journal of ChemTech Research Vol.9, No.04 pp 617-624

[5] Kundra. 1981. Dinamika Populasi. IPB, Bogor

[6] Lekitoo M, 2009. Respon nyamuk Aedes aegypti terhadap kairomon tanaman lavender (Lavandula angostifolia). Skripsi Fakultas Matematika dan Ilmu Pengetahuan Alam Universitas Pattimura. Ambon.

[7] Maria Nindatua, Novita L. Tuhumury, Martha Kaihena. 2011. PENGEMBANGAN EKSTRAK ETANOL DAUN LAVENDER *(Lavandula angustifolia)* SEBAGAI ANTI NYAMUK VEKTOR FILARIASIS *Culex sp*. Molucca Medica, Volume 4, Nomor 1, Oktober 2011, hlm. 19-27

[8] World Health Organization (2005). *Pencegahan dan pengendalian Dengue dan Demam Berdarah Dengue.* Penerbit buku kedokteran (EGC). Jakarta.

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Production and Marketing of Mandarin in Putalibazar Municipality of Syangja

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Abstract*— Mandarin is the most demanded citrus fruit and has been producing as the main source of income in the hilly region since mid-hills are likely to have a favorable climate. This study was conducted to analyze the production and marketing scenario of Putalibazar Municipality, Syangja. The field survey was carried out in 2021 to collect information from the mandarin growers and traders. Semi-structured questionnaires were used to collect the primary data from 75 producers,10 traders, 10 retailers, and 10 wholesalers by applying the simple random sampling method. The result shows that the majority of mandarin growers are commercial farmers. The overall mandarin producer has an average landholding of 1.05. The average area used for mandarin cultivation was 0.32. The average farm-gate price was NRS 48 and the average retail price is NRS 94. The average price spread was found 52% with producer share 51%. The market margin was NPR 46/Kg respectively. The overall average BC ratio was 2.3 indicating farmers are benefitted from their production. A Cobb-Douglas production model was run to find out the effect of different factors on the gross return of mandarin production and SWOT analysis to address strengths, weaknesses, opportunities, and threats.*

***Keywords*— Production, Marketing, Mandarin, Price spread, Market margin, SWOT.**

I. INTRODUCTION

Agriculture commenced independently in distinctive components of the globe and includes a diverse variety and Nepal has sixty six percentage of humans immediately engaged in farming (FAO , 2021). The rural zone contributes 27.6% to the country wide GDP, among which fruit contributes 7% to the total agricultural GDP (CBS, 2017). Citrus is one of the main fruit crops grown in the mid-hills of Nepal. Mandarin is an important species covering a major part of the citrus growing area globally. Mandarin occupies 65.3% and of the total citrus growing area and 67.2% of production in Nepal respectively (Pandey, et al., 2017). It covers a total growing area of 26,282 ha and has a production of 146,690 Mt (MoALD, 2017) Citrus is one of the most cultivated with higher production fruits in the large area in Nepal. In the category of citrus fruit, orange, lemon, and mandarin are the most demanded. Citrus has been grown in 62 districts of Nepal out of them Dhankuta, Terathum, Sindhuli, Ramechhap, Dhading, Kavre, Gorkha, Lamjung, Tanahun, Kaski, Syangja, Myagdi, Palpa, Salyan, Dailekh, Baitadi, Dadeldhura are the major citrus growing districts (Adhikari, 2014). Among citrus, mandarin orange is predominant which shares about 67 percent of the total citrus production in the country (FDD, 2009).

Syangja is one of the mid-hill districts of Nepal with a total mandarin cultivated area of 1,347H production of 14776 Mt and productivity of 10.97/Ha (MoALD, 2017). Despite the great potential of production in the mid-hill region of the country and continuous effort from the government, mandarin producers are facing problems such as poor marketing infrastructures like market information, physical facilities, marketing extension services, price uncertainty, and small scale of production. Furthermore, farmers are not organized (Pokhrel, 2011). This study conducts to explore production practices and market analysis of mandarin and the reasons for a high price for the consumer. This study can help farmers to understand different aspects of mandarin production and marketing scenarios, help the farmers in marketing research in the marketing management decision-making process.

II. MATERIALS AND METHODS

2.1 Study area, sampling techniques, and sample size

The study was conducted in Putalibazar Municipality. It lies in the Syangja district of Gandaki province of Nepal. Putalibazar Municipality has 14 wards in total and is considered as a Mandarin production Zone. 75 farmers were selected randomly from each group of each mandarin producing wards. 10 traders, retailers were selected from the Putalibazar Municipality, Syangja. Mandarin growers were divided into two categories i.e., farmers cultivating in the area less than 0.25ha (5 ropani) were small growers and the farmers cultivating above 0.25 ha (> 5 ropani) were commercial.

For the study, both primary and secondary data were collected. Primary data were collected through household survey and interview by using pre-tested semi-structured questionnaire survey among the farmers. Similarly, secondary data were collected from related publications from MoALD, annual reports of Putalibazar Municipality, books, various published and unpublished sources like journals, newspaper articles, etc.

2.2 Data analysis

. The data obtained during the study through house-hold survey and (KII) with traders, retailers was checked, arranged, revised, tabulated, and analyzed by using Microsoft Excel, different analytical tools and formulas, and SPSS.

Profitability analysis

The profitability of mandarin manufacturing from the viewpoint of an person farmer turned into measured in phrases of gross go back, gross margin, net income, and undiscounted benefit-cost ratio

Gross return: Total production x average market price

Gross margin: Gross Return – Total Variable Cost

Net profit: Gross Return – Total Costs

Undiscounted Benefit cost ratio: $\frac{\text{Gross return}}{\text{Total cost}}$

The Total cost includes sum of total fixed cost and total variable cost i.e., TC = TFC+TVC.

Market margin: Retail price- farmgate price

Price spread: $\frac{\textbf{consumer price} - \textbf{net price of producer}}{\textbf{consumer price}} * \mathbf{100}$

Producer share: $\frac{\textbf{farmgate price}}{\textbf{Retail price}} \times \mathbf{100}$

2.3 Factor affecting mandarin production

The Cobb-Douglas production function represents the relationship between two or more inputs - typically physical capital and labor and the range of outputs that may be produced. The regression coefficients constitute the pliancy of respective inputs, and its sum gives the return to scale cost. The form of CPDF used in this study is as follows:

$$Y = aX1^{b1}X2^{b2}X3^{b3}X4^{b4}Xn^{bn}e^{u}$$

Where Y is the total income from mandarin production (NRs. / ha), and X are the variable inputs, *e* error term and b are the coefficients to be determined.

2.4 SWOT analysis

Information related to mandarin production in Putalibazar Municipality was obtained from the KII with concerned personnel, through the study of Profile and Resource Maps of Putalibazar Municipality 2020; and as well as from the field survey. Then, an in depth evaluation was carried out to evaluate the internal factors (strengths and weakness) and verify the outside factors (possibilities and threats) of mandarin production in Putalibazar Municipality.

III. RESULT AND DISCUSSION

Production situation of mandarin

Table 1: Production situation per hectare

Particulars	Overall	Small Holders	Commercial producers
Total number of mandarin/ha	948	184	764
Average number of plants/ha	12.6	5.1	20.2
Average area under mandarin/ha	0.32	0.12	0.51
Production (in Tons)	3.45	1.64	5.12
Productivity (in Mt/ha)	9.56	10.2	8.9

The average area under mandarin cultivation in the study area was 0.32 ha. Among that, 0.12ha and 0.51 ha was inhibited by small-holder growers and commercial producers along with the 5.1 and 20.2 number of mandarin trees respectively. The overall production was 3.45 and productivity was 9.56. Gautam (2020) research found 2.163 Mt overall mandarin production in the study area, 0.987 Mt and 3.645 Mt on smallholder growers and commercial producers respectively in Gulmi.

Cost of production

Here the fixed cost is excluded and only variable cost incurred in a year is tabulated. The variable cost includes the cost of seedling, manure, irrigation, labor, transportation, Bordeaux mixture, pesticides, micro nutrients, and vermicompost. Where the variable cost incurred in the study area are seedling, manure, labor, Bordeaux mixture, transportation, vermicompost and mustard cake.

Table 2: Cost of production of mandarin

Particulars	Overall (NRS/Ha)	Small holders (NRS/Ha)	Commercial Producers (NRS/Ha)
Seedling	16,560	3990	12,570
Manure			
FYM	29465	8,220	21,245
Juto	4,585	3610	975
Poultry	13008.5	580	14,428.5
Mustard cake	4,749	2,074.5	2674.5
Chemical fertilizer	495	130.35	364.65
Bordeaux Mix	53,212	1,385	51827.5
Transportation	22,437.5	14,884	7553.85
Vermicompost	780	444	336
Total Variable cost (NRS/ha)	1,50,292	35,317.5	1,14,974.5

Economic analysis

Table 3: Economic indicators displaying productivity and profitability of mandarin farming.

Particulars	Overall	Small holder	Commercial producer
Total Variable cost (NRS/ha)	1,50,292	35,317.5	1,14,974.5
Average price/kg	48	44	52
Gross Revenue	7,09,125	1,32,062.5	5,77,062.5
Gross Margin	5,58,833	96,745	4,62,088
Net Income	5,49,658	93,148	4,56,510
Benefit Cost Ratio	2.3	1.9	2.7

The average total cost/ha was found to be NRs 1, 50,292 / ha and return was NRs 7, 09,125/ha. The average BC was found to be 2.3. Similarly, the gross margin was found to be 5, 58,833/ha. The total variable cost, gross revenue, gross margin, net income and BC ratio for commercial producers was NRs 1,14,974.5, NRs 5,77,062.5, NRs 4,62,088, NRS 4,56,510 and 2.7. Similarly, small holder mandarin producers' total variable cost, gross revenue, gross margin, net income and BC ratio was found NRs 35,317.5NRs 1,32,062.5, NRs 86,443.15, NRs 96,745, NRS 93,1478 and 1.9 respectively. This result was also consistent with another study of Kristhinachnechaur and Nirmalpokhari VDCs and BC ratios were 2.57 and 3.31 respectively (Kafle, 2018).

Price spread, market margin and producer share

Table 4: Price spread

Particulars	Overall average	Small holder	Commercial producer
Farm gate price (Rs/Kg)	48	44	52
Consumer price (Rs/Kg)	100	100	100
Price spread (%)	52%	56%	48%
Price spread (Rs/Kg)	52	56	48

Overall price spread of mandarin in the study area is 52% and small holder producer 56% and commercial producer is 48% respectively. This implies that the net price of an overall average producer is NRS 48. When mandarin is sold in the market at 100 NRS to a consumer, 52% of the price spread i.e., NRS 52 occurs which is due to higher marketing cost and margins obtained by intermediaries.

Table 5: Market margin and producer share

Particulars	Overall average
Retail price (Rs/kg)	94
Average farm gate price (Rs/kg)	48
Market margin (Rs/kg)	46
Producer share (%)	51%

The market margin of mandarin in the study area was 46 Rs/kg and producer share 51% which indicates higher marketing efficiency because a market is efficient when it provides the most consumer surplus and the most producer surplus possible. Kafle (2018) supports this study as he found market margin of Kristhinachnechaur and Nirmalpokhar were 11.43 and 10.89. Similarly, the producer share was 54.42.

Production function analysis

Table 6: Regression estimates for factors affecting gross income of mandarin growers

Variables	Coefficient	Standard error	t-value	P>|t|
Constant	2.827	0.219	12.938	0.000
Log_manure cost	0.084	0.025	0.943	0.349
Log_chemical fertilizer cost	0.073	0.050	0.470	0.640
Log_labor cost	0.739**	0.053	0.738	0.000
Log_Bordeaux Mixture Cost	0.083	0.027	0.996	0.323
Log_transportation cost	0.021	0.008	0.247	0.806
R square	0.610			
Adjusted R square	0.571			
F- value	26.640			
Return to scale	1			

** indicates significant at 5% level

The explanatory power of the estimated model for mandarin production was 0.610 F ratio being highly significant and the model was a good fit. All the explanatory variables had positive coefficients which suggest there is an opportunity to work on. It is evident that labor cost showed significance at a 5% level of significance on gross returns from mandarin production. Keeping all other factors constant. 100% increase in the labor cost will increase the return by 73%. Similarly, the regression analysis reveals that the coefficient of multiple

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determination was found to be 0.610. this implies that the estimated variables explained 61% of the variation in gross return of mandarin.

The sum of regression coefficients obtained from the cobb Douglas Production Function was 1 which indicated the constant return to scale in mandarin production. This implies that an increase in the cost of variable inputs would increase the amount of income from mandarin production.

Marketing channel

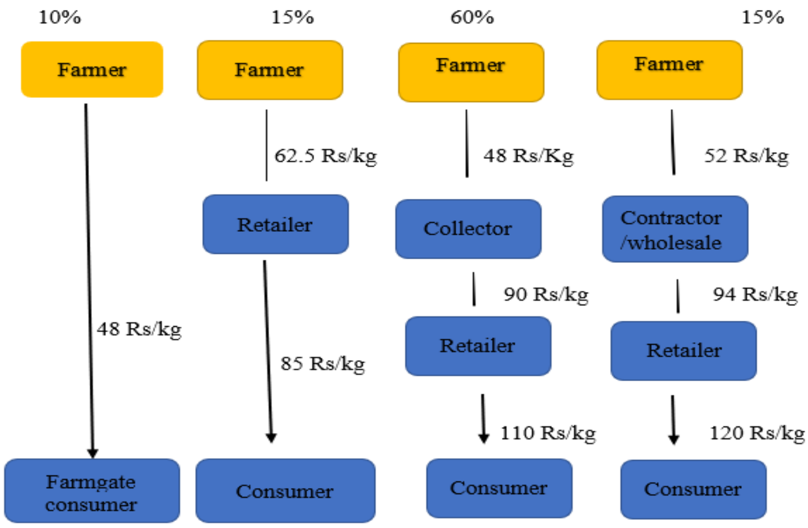
There had been 4 channels through which mandarin of Putalibazar flows from farmer to final clienti.e.,

Channel I- (farmer-farmgate consumer),

Channel II- (farmer-retailer- local consumer),

Channel III- (farmer-collector-Wholesaler/retailer-distant consumer),

Channel IV- (farmer-contractor/wholesaler-retailer-distant consumer).



SWOT analysis

Both internal factor (strength and weaknesses) and external factor (opportunities and threats) has a role in the production of mandarin and has been addressed accordingly.

Internal factors	**External Factors**
Strength	**Opportunities**
a. Availability of suitable climate for mandarin production, south-facing slopes with 1000- 1800 masl b. Better quality of mandarin with a juicer in nature. c. Adoption of organic farming practices to a greater extent, d. Availability of land for cultivation. e. The produce of Syangja has been recognized in the market as better quality, f. Availability of commercial experienced farmers who're able to increase gross revenue. Putalibazar is considered as a region for potential mandarin producing area	a. Scattered land and low production cause difficulty in commercial production. b. High transportation costs due to poor road facilities. c. Inadequate storage and processing facilities. d. High post-harvest loss and poor technical knowledge. e. Lack of coordination among different actors of mandarin. f. The limited capacity of farmers and their organization on marketing functions and decisions Poor functioning of collection centers due to limited facilities available.
Weakness	**Threats**

a. Increasing demand for mandarin in the national and international market. b. Better potential in Nepal as well as Japan in export due to good quality. c. Better access to market namely Pokhara, Butwal and Narayanghard d. Availability of collection center and postharvest storage facilities. e. High priority from government and non-government sectors to upgrade citrus value chain in the district.	a. Bad weather like heavy rainfall, hailstorm, and hailstones. b. Chinese and Indian mandarin with less per unit price c. Incidence of insect, pest and disease. d. The conflicting interest of market actors e. Lack of organized and assured market. f. Inadequate governmental policy for commercial mandarin producers. g. Incidence of the pandemic situation causing poor marketing.

IV. CONCLUSION AND SUGGESTIONS

Based on the study and findings it is concluded that mandarin production in Putalibazar Municipality, Syangja was a profitable business making a significant difference in the economic welfare of farmers. It was found that there is a high Benefit-Cost Ratio (B/C ratio) and Gross margin, which show that mandarin production is a profitable enterprise. BC ratio of commercial producers was high than smallholders which indicates mandarin commercialization is highly profitable. The return to scale was found 1 which represents the mandarin production scenario is a constant return to scale. The existing marketing channels were Channel 1 (farmer-farmgate consumer), channel 2 (farmer-retailer- local consumer), channel 3 (farmer-collector-retailer-distant consumer), channel 4 (farmer-contractor/wholesaler-retailer-distant consumer). The cobb-Douglas production model was run to find out the effect of different factors on the gross return of mandarin production in the study area. All the explanatory variables had positive coefficients. Production function analysis revealed that a one percent increase in the expenditure 100% increase in the labor cost will increase the return by 73%. and with estimated variables providing 61% of the variation in gross return. Putalibazar was facing marketing and production problems such as high monopoly of traders, price fluctuation, transportation, processing, and storage are major problems. Among the different marketing constraints, the low prices offered to the producers and the second major problem was lack of processing activities. To enhance production and marketing in the study area market-oriented policy and programs should be adopted.

Mandarin growers should improve their orchard management and post-harvest handling practices for higher productivity and quality of produce. Farmers should plant recommended number of mandarin sampling which is 20 trees/ ropani and 400 trees / hectare to increase the farm production and cost efficiency. The Collection centers and cooperatives should function properly to provide a direct link between producer groups and bulk end-buyers or processors, thereby cutting out middlemen (traders) and securing a higher share of the value-added for producers. The provision of output-based incentives can encourage the farmers to increase production and marketable volume.

REFERENCES

[1] Adhikari, G., 2014. In: *Causes of citrus tree dieback, citrus orchard decline and its management* . Kirtipur, Nepal: NCDP.

[2] CBS, 2017. In: *Statistical yearbook of Nepal 2017.* Kathmandu: Central Beaureau of Statistics, Government of Nepal.

[3] FAO , 2021. Nepal at a glance. *Food and Agriculture Organization of United Nations.*

[4] FDD, 2009. Annual Report 2008/2009. In: *Fruit Development Directorate* . kritipur, kathmandu,nepal: s.n.

[5] Gautam, A. et al., 2020. ECONOMICS OF PRODUCTION AND MARKETING OF MANDARIN IN GULMI, NEPAL. *Food and Agribusiness management.*

[6] Kafle, A., 2018. Comparative Analysis of Production and marketing of Mandarin Orange in Kristhinachnechaur and Nirmalpokhari Village Development Committees of Kaski,Nepal. *Neplease jornal of engineering* .

[7] MoALD, 2017. In: *Statistical Information on Neplease Agriculture.* Singha Durbar, Kathmandu: Ministry of Agricultural Development, Government of Nepal.

[8] Pandey, G., Basnet, S. & Pant, B., 2017. An Analysis of Vegetables and Fruits Production Scenario in Nepal. *Asian Research Journal of Agriculture.*

[9] Pokhrel, C. N., 2011. Analysis of market chain of mandarin in Nepal.

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Wetland Ecosystem – Interplay of Factors Influencing Microclimate and Seasonal Variation - a Review

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***Abstract*— *Water, is one of the necessities for the survival of human beings, flora, and fauna. Wetlands have been used as a source of water, and as dump grounds for discharging sewage and other human waste. Urbanization and industrialization have led to the overexploitation of water and water bodies making them disappear at an exponential rate. Seasonal variation and anthropogenic activities have a critical influence on the water quality, microclimate, and aquatic diversity. Researches reviewed in this study indicate that the various parameters are interdependent with each other and influence the productivity, biodiversity, and ultimately the micro-climate and seasonal variations of a wetland ecosystem. Physico-chemical parameters studied like pH, temperature, Chlorophyll content, light and phosphate content are known to directly affect productivity and biodiversity. Changes in productivity and biodiversity further affect the microclimate and seasonal variations of a wetland. Moreover, changes in physical parameters are influenced by changes in chemical parameters and vice versa. Also, changes in biodiversity and productivity are inter-dependent on physical and chemical parameters. This study establishes the interactions among various parameters that govern microclimate and seasonal variations. This review lays the foundation for similar studies on wetland ecosystems for better measures towards sustainability and maintenance.*

***Keywords*— Limnology, Microclimate, Seasonal variation, Wetland dynamics.**

Water is an important source of energy for all living systems on planet earth; it creates an ecosystem for flora and fauna. This vital elementary source of life runs through rivers into the seas and oceans. In urban communities, water is generally stored in catchment areas like small ponds, natural lakes, or even in man-made lakes. The water especially in wetlands like lakes creates an ecosystem, replenishes groundwater thereby regulating the survival of life forms. The dynamics of water quality in these inland water bodies are majorly affected by varied seasons influencing the microclimate of a wide region or a narrow locality. The parameters that influence the microclimate and seasonal variations can be categorized under physicochemical factors, biological factors, anthropogenic influences, etc… The nature and extent of influence can vary depending on the association of these factors to the above dynamics of a wetland. Few of these parameters have a direct influence while the others have an indirect influence. Micro-climate and seasonal variations in wetland dynamics is an important aspect as it is further known to directly or indirectly influence human activities, agriculture, underground water reservoir, and in the long run the local environment. More importantly, most of these factors are interconnected. Hence it is of great significance to carry out Limnological studies to understand the influence of these factors.

This paper tries to understand the influence of such factors on microclimate and seasonal variations of wetlands. The paper discusses the effect of each of these individual factors and their effect in totality. A relationship of inter-dependence of factors can be established viz that physicochemical factors influence each other and in-turn influences the microclimate and seasonal variations. Spatial variation of these factors also plays a key role in the overall changes observed in microclimate. The physicochemical factors studied in this review include temperature, light, pH, transparency, turbidity, pressure, humidity, wind speed, rainfall, and concentrations of chemical entities like phosphates, nitrates, chlorophyll, silica, and mercury. Most of these factors are known to either directly or indirectly influence the productivity and abundance of flora and fauna which in turn affects biodiversity and ill effects like eutrophication. Anthropogenic influences further are known to influence the increase and decrease of chemical factors leading to a drastic effect on productivity and eutrophication. These effects in turn have been shown to influence the micro-climate which further is known to influence seasonal variation. This can be predicted to be a cyclic reaction as per the relationship diagram (Fig 1) shown below.

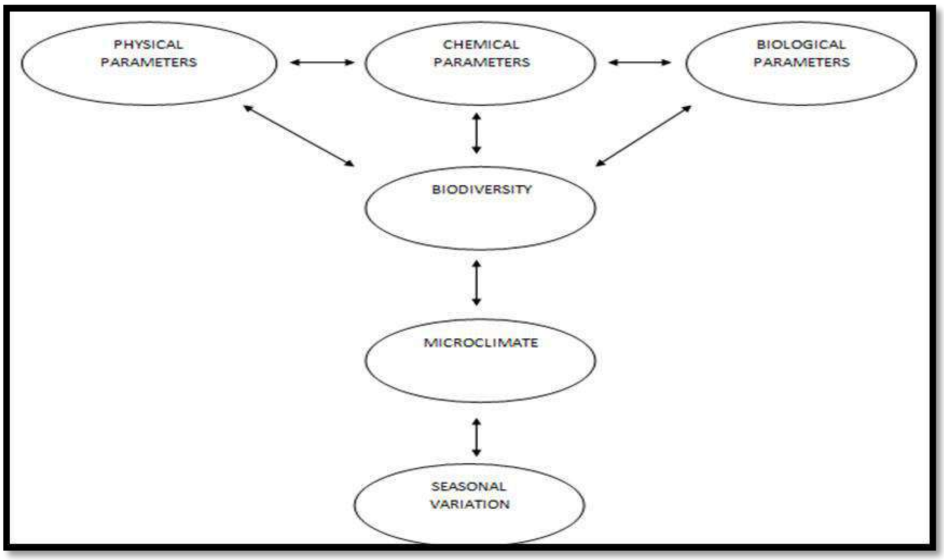

Fig 1: Representation of interdependence of various factors with microclimate and seasonal variation

Understanding such factors and their influence on the microclimate is an important component in the attempts towards sustainability and maintenance of natural resources. This paper helps in gathering an overall perspective on the changing dynamics of the above-mentioned parameters and useful insights that can be drawn towards prospects of sustainability and maintenance

A. Physico-chemical Parameters

pH

pH is the hydrogen ion concentration that categorizes any aqueous solution into acidic, neutral, and alkaline. Wetlands are also categorized into various types based on pH. pH governs the nutrients required for the growth and development of hydrophytes and wetland mesophytes. Temperature and dissolved oxygen have a positive correlation (Purohit & Singh 1980). It is also one of the primary factors influencing the growth of phytoplankton (Ersanli & Gonulol 2003). The primary productivity of a wetland ecosystem relies positively on water pH (Hajong & Ramnujam 2018). It is also a deciding factor concerning the usage of water for various domestic and industrial purposes (Ramanathan & Amsath 2018).

1. Temperature

Temperature is a physical parameter that controls the flow of energy in the universe. It regulates various biogeochemical cycles required for the survival of life forms. In a wetland ecosystem, temperature influences various parameters. The rise in temperature leads to a rise in temperature-dependent physical variables (Ramanathan & Amsath 2018). A study revealed that temperature has a positive dependence on pH and dissolved oxygen (Purahit & Singh 1980). Temperature is also known to impact the density of water (De 2002). It is known to negatively influence concentrations of salts such as phosphates, nitrates, and ammonia (Dar et al. 2013). Water quality and water levels in an aquatic ecosystem are further regulated by temperature (Sahoo et al. 2016). A lake is referred to as monomictic when it does not cool below the temperature of 4 degrees celsius (Purahit & Singh 1980). Concerning the influence of temperature on various biological systems, it was found that temperature directly influences zooplankton growth (Mecombie 1953; Das 1956; Bamforth 1958; Moitra & Bhattacharya 1965). Phytoplankton growth is also temperature-dependent (Ersanli & Gonulol 2003). It affects respiration and controls osmoregulation in aquatic animals (De 2002). Water temperature shows a positive correlation with total chlorophyll content (Dar et al. 2013; Giroldo 2014). It strongly influences activities like behavior, respiration, and metabolism in aquatic biota (Gayathri et al. 2013) and also has a direct effect on the growth of algae and aquatic weeds (Zafar 1964). Temperature plays a pivotal role in regulating a wetland ecosystem throughout various seasons; it controls the rate of evaporation, alters wind speed, wind direction, and thereby various attributes of the regional microclimate (Dorche et al. 2018).

2. Light

It is a physical parameter that is part of the electromagnetic spectrum which controls the flow of energy on planet earth through the sun being its primary source. Light again is an influencing factor for the well-being of wetlands and biota dependent on these wetlands. Studies indicate that the intensity of light varies according to seasons and changes the physicochemical parameters (Gayathri et al. 2013) high intensity of light generally observed during summers (Giroldo 2014) high light intensity has an ascending impact on the growth rate of members of Cyanophyceae (Araujo 2011), it contributes to the development pattern of benthic fauna (Kabir et al. 2011) and chlorophyll concentration (Giroldo 2014), the low light intensity, on the other hand, has an ascending effect on nitrogen-fixing bacteria (Araujo 2011). Light very evidently and greatly influences the algal growth and diversity in a lake (Hajong & Ramanujam 2018; Etisa et al. 2018). Thus the primary productivity of a wetland is dependent on light (Hajong & Ramanujam 2018).

3. Phosphate

Phosphate is a macronutrient present in water, soil, and food and it is required for the survival of life forms. In a wetland ecosystem, it regulates the growth of aquatic biota. Phosphate is temperature independent (Dar et al. 2013 and Gayathri et al. 2013) Phosphorous concentrations in lakes tend to rise along with other nutrients (Huszar et al. 2005). Increased phosphate concentration tends to increase chlorophyll concentration (Sahoo et al. 2016) but is known to restrict the growth of submerged aquatic plants (Poquet et al. 2008). An increase in phosphate concentration might be because of nutrient enrichment of the surface water or by the release of phosphate from dead algae and zooplanktons (Heron 1978). Phosphate composition varies with climate and increases during monsoons (Ramanathan & Amsath 2018). High phosphate inflow into the water body results in eutrophication of the water body (Araujo 2011) and thereby mesotrophic water bodies can turn out to be eutrophic (Garg et al. 2010).

4. Nitrate

Nitrate is a chemical nutrient required by phytoplanktons and it forms a major component of agricultural fertilizers. Anthropogenic activity leads to nitrate accumulation in a wetland ecosystem (Mishra et al. 2017). A study indicates the rise in other nutrient factors in association with the increasing concentration of nitrates (Huszar et al. 2005). Nitrates are also believed to vary negatively with temperature (Gayathri et al. 2013; Dar et al. 2013).

5. Transparency and turbidity

Turbidity is a measurement of murkiness in fluids. It indicates transparency and opaqueness of a water body which in turn determines the intensity of incident light reaching the benthic zone. Several studies have been carried out to understand the same. Transparency and turbidity are negatively interdependent with temperature (Gayathri et al. 2013; Hajong & Ramnujam 2018). Transparency is a prominent physical parameter that impacts and increases the productivity in a water body (Jayaweera & Asaeda 1996; Wei et al. 2004; Kuehl & Troelstrup 2013; Sukla et al. 2013; Balogun et al. 2014). Depth impacts the turbidity of a water body (Giroldo 2014) studies indicate a change in phytoplankton diversity with turbidity. Cyanophyceae members have been found in abundance in areas receiving sunlight as against nitrogen-fixing bacteria in low light regions (Araujo 2011; Giroldo 2014).

6. Pressure, humidity, wind speed, and rainfall

Atmospheric pressure over a water body is influenced by physical parameters like humidity, wind speed, and precipitation. The microclimate of a region is affected by wind speed and direction which in turn is dependent on temperature and dissolved oxygen (Dorche et al. 2018). Water levels and quality in an aquatic ecosystem are regulated by rainfall (Sahoo et al. 2016). The algal productivity varies concerning varying pressure (Agrawal 1999). The chlorophyll content of plants is also known to be influenced by the regional microclimate of a wetland ecosystem (Sahoo et al. 2016).

7. Chlorophyll

The total chlorophyll content of a plant determines its productivity. In an aquatic ecosystem, the total chlorophyll content is said to be dependent on physical parameters like water temperature and pH (Dar et al. 2013; Giroldo 2014). However, chlorophyll content was found to be independent of dissolved oxygen (Giroldo 2014). Chlorophyll content varies according to seasons (Araujo 2011, Hajong & Ramanujam 2018) Spectrophotometric analysis reported high chlorophyll pigments in submerged plants in comparison with floating hydrophytes (Dar et al. 2013).

8. Dissolved reactive silica

Silica is one of the elements found in soil that leaches out into the water and is known to influence phytoplankton. A study indicates high concentrations of silica during summer as compared to other seasons the study also reveals the concentration-dependent influence of silica on the growth rate of diatoms (Araujo 2011).

9. Mercury

Mercury is a liquid metal pollutant found in an aquatic ecosystem due to anthropogenic activity. It is found that aquatic flora and fauna accumulate mercury in the form of methylmercury, Methylation, and demethylation of mercury vary according to seasons and it was found to be increasing during summer seasons (Korthal & Winfrey 1987).

Biological Parameters

Wetland Biodiversity is the variety and variability of life in an aquatic ecosystem; it is inclusive of phytoplanktons, zooplanktons, hydrophytes, and terrestrial mesophytes. Phytoplanktons are autotrophic members which form the base for primary productivity of a freshwater ecosystem thereby regulating several aquatic food webs. Phytoplanktons play a decisive role in maintaining the fitness of a wetland ecosystem (Lopes et al. 2005). Zooplanktons are heterotrophic organisms that are majorly dependent on phytoplanktons. The growth, development, and productivity of phytoplankton are determined by several physical and chemical parameters. Physical parameters include pH, temperature, conductivity, light intensity, pressure, transparency, depth, and turbidity. Of these, phytoplankton productivity is negatively influenced by turbidity and depth (Giroldo 2014), while the rest of the parameters have a positive influence on the same. Dissolved oxygen, chlorides, phosphates, sodium, potassium, nitrates, silica, and mercury are a few of the chemical parameters that determine wetland diversity. Cyanophyceae, Bacillariophyceae, Dinophyceae, Chlorophyceae, Chrysophyceae, Zygnematophyceae, Trebouxiophyceae, and Xanthophyceae are the common phytoplankton members while Cladocerans and Rotifers are the common zooplanktons reported in aquatic ecosystems (Mecombie 1953; Das 1956; Bamforth 1958; Zafar 1964; Moitra & Bhattacharya 1965; Agrawal 1999; Ersanli & Gonulol 2003; Dixit et al.2005; Reynold et al. 2006; Araujo 2011; Etisa et al. 2018; Hajong & Ramanujam 2018; Dorche et al. 2018). Food production and nutrient cycle regulation in a wetland are controlled by phytoplanktons (Fathi et al. 2001; Khan 2003). Wetland biodiversity is subjective to seasonal variations in physical and chemical parameters. A study has reported high phytoplankton density during summer as compared to winter (Giroldo 2014). *Eichornnia crassipes*, *Lemna minor*, *Sagitaria latifolia*, *Hydro-cotyleranunculoids*, and *Cyprus* are the most abundant floating hydrophytes in the wetland ecosystem while the terrestrial mesophytes include *Hibiscus tiliaceus*, *Ipomoea pescaprae*, *Terminalia catapa*, and *Typha latifolia*(Mishra et al. 2017; Leidonald 2019).

Productivity

Primary productivity is defined as the amount of organic matter consumed during respiration and the rate of total photosynthesis. Productivity is dependent on water quality, diversity, and species abundance. Diversity of flora and fauna are further influenced by physicochemical parameters like transparency, alkalinity, temperature and dissolved oxygen (Jayaweera &Asaeda 1996; Wei et al. 2004; Kuehl & Troelstrup 2013; Sukla et al. 2013; Balogun et al. 2014; Hajong & Ramnujam 2018). Productivity was found to be high in shallow regions as compared to deeper zones in a water body due to nutrient regulation by sediments and macrophytic growth (Sontakke & Mokashe 2014).

Anthropogenic influences

Anthropogeny is the negative influence of human activities on the environment. It is responsible for the formation of recalcitrant entities. Deterioration of aquatic ecosystems can be traced to anthropogenic wastewater, agricultural run-offs, catchment area, medical waste, and industrial discharge (Kabir et al. 2011; Najar & Basheer 2012; Nyairo et al. 2015; Mishra et al. 2017; Sudarshan 2019). Human activities influence water quality in wetlands which in turn is responsible for seasonal variations (Kabir et al. 2011). Wetland ecosystems can be demarcated into clear-cut zones based on the extent of the negative influence by the anthropogenic activity which is reflected in the diversity and abundance of flora and fauna (Nikoloaidis et al. 1996; Kun li et al. 2017).

Microclimate

A microclimate is the prevalent atmospheric parameters over a locality that is contributed by temporal and vertical variation in humidity, temperature, and dissolved oxygen, rate of evaporation, wind speed, wind direction, and nutrient flow (Dorche et al. 2018). Wetlands are also known to regulate the microclimate of a region in its closed vicinity. Variations in microclimate over a wetland are governed by anthropogenic stress (Poquet et al. 2008; KunLi et al. 2017; Sudarshan 2019). Water quality and water levels are influenced by the rate of precipitation which in turn affects the wetland productivity. Unaltered aquatic ecosystems are lesser prone to eutrophication due to recharging and recycling of water which is not possible in the case of man-made aquatic bodies (Gorniak & Piekarski 2002; Sahoo et al. 2016). Seasonal variations in atmospheric gases result in demethylation, which further contributes to nutrient cycling, phytoplankton, and floral diversity implying the health of an aquatic ecosystem (Korthal & Winfrey 1987; Kennedy 1999; Lopes et al. 2005; Sudarshan 2019).

Spatial Variation

Spatial geometry is the geographical demarcation of a wetland ecosystem based on the variation in chemical nutrients and biodiversity. Anthropogenic activities around an aquatic ecosystem result in changes in water quality leading to spatial and seasonal variation, studies indicate variations in growth and development, diversity of phytoplanktons, and hydrophytes (Kun Li et al. 2017). The spatial variation of a water body is regulated by the hydrology and reed beds (Huszar 1996; Nikoloadis et al. 1996). Extensive overgrowth of hydrophytes as a result of human activities has led to ecological succession which further negatively influences the faunal and floral diversity (Mishra et al. 2017). Spatial variation and dimensions play a key role in strategizing the restoration and management of wetland ecosystems (Dorche et al. 2018).

RESULTS

This review paper has successfully gauged the interactions among the various parameters that influence the micro-climate and seasonal variations in a wetland ecosystem. Of all the parameters studied pH, chlorophyll content, temperature, light, and phosphate content of water are found to directly affect productivity by altering biodiversity of the wetland while parameters like pressure, humidity, wind speed, and rainfall are found to directly affect the microclimate of the given wetland. Factors like turbidity have been shown to negatively influence the growth of plants and hence the biodiversity and further productivity. Anthropogenic activities lead to a serious rise in chemical factors which further leads to a rapid increase in productivity. Studies have shown that an unchecked increase in productivity leads to eutrophication and in extreme scenarios loss of such wetland ecosystems due to ecological succession. Few studies reviewed reveal that productivity, a shift in biodiversity, eutrophication brings about a change in a microclimate which in turn results in rapid seasonal variations across the wetland ecosystem. Monitoring changes in the microclimate and seasonal variations of a wetland ecosystem is an important aspect of the maintenance and sustainable use of such wetlands in both urban and rural scenarios.

DISCUSSION AND CONCLUSION

Wetlands are water catchment areas; it includes freshwater bodies such as lakes, ponds, tanks, and rivers. These catchment areas support life forms that range from microscopic phytoplankton to diverse higher forms of flora and fauna. They serve as a source of water to humans and animals, they also function to replenish the groundwater table and act as natural sewage treatment zones. Wetlands contribute towards building an ecosystem and maintain homeostasis in the environment thereby making them a natural and a national asset. These aquatic ecosystems are known to alter atmospheric gases, humidity, and temperature thereby governing the wind speed and direction. These characters of a wetland contribute to regulating the microclimate of a locality and thus influence the seasons of a region. The review focuses on anthropogenic influence characterizing the quality of water. Various research studies indicate that there is a positive correlation established between pH, temperature, dissolved oxygen, phytoplankton diversity, and productivity. It governs respiration and osmoregulation in aquatic flora and fauna, while a few studies indicate chemical nutrients like nitrates, phosphates, alkaline earth metals, and heavy metals have a negative correlation with physical parameters. These physicochemical characters affect the functionality of the plant pigments and are said to have a direct impact on the productivity of an aquatic ecosystem and can infer the fitness of the same. Productivity varies concerning different zones in an aquatic ecosystem and is also influenced by varying seasons. Phytoplankton and zooplankton growth is said to vary in abundance with anthropogenic impact and seasonal dissimilarity. Phytoplankton diversity is high during the summer seasons in comparison with the other seasons. The growth and development of wetland-dependent plants and animals are regulated by these phytoplanktons since they are involved in controlling the biogeochemical cycles and production of food. An increase in physicochemical parameters and productivity shoots up the growth of aquatic weeds which blocks the entry of sunlight affecting underwater life forms leading to their death and altering the water quality. This is followed by the growth of terrestrial mesophytes dependent on these wetlands resulting in ecological succession over a water body resultant of which can lead to slow deterioration of overall water quality and death of a wetland in turn resulting in loss of an entire aquatic ecosystem. Various studies are indicative of the fact that anthropogenic stress-mediated variation in physical and chemical parameters leads to ecological succession. Ecological succession over a wetland is dependent on spatial geometry. This is due to spatial variation in chemical and physical qualities and anthropogenic activities across a water body. The above-discussed factors govern the microclimate over a wetland thereby having a critical influence on the seasons of a narrow region. Thus the above review suggests physicochemical, biological qualities and parameters of biodiversity that vary with human influence and varying seasons, converting the wetlands into cesspools which are reflected through changes in the microclimate and seasons over a narrow belt which in turn has a critical impact on global warming. The need of the hour is therefore to protect and conserve these aquatic ecosystems from further deterioration for maintenance of a homeostatic environment.

REFERENCES

[1] Agrawal S.C. 1999 Limnology, APH Publishing Corporation. Retrieved from https://www.biblio.com/

[2] Ahmed V., Parveen S., Khan A.A., Kabir H.A., Mola, H.R.A. & Ganai A.H. 2011 Zooplankton population in relation to physico-chemical factors of sewage fed pond of Aligarth (UP), India. Biology and Medicine, 3(2): 33634. Retrieved from https://www.longdom.org/

[3] Andrade R.D.R & Giroldo D. 2014 Limnological characterisation and phytoplankton seasonal variation in a subtropical shallow lake (Guaiba Lake, Brazil): a long-term study. Acta Limnologica Brasiliensia, vol. 26, no. 4, p. 442-456. https://doi.org/10.1590/s2179-975x2014000400011

[4] Bamforth S. S. 1958 Ecological studies on the planktonic Protozoa of a small artificial pond. Limnology and Oceanography, 3:398-412. https://doi.org/10.4319/lo.1958.3.4.0398

[5] Balogun K.J., Adedeji A.K., & Ladigbolu I.A. 2014 Primary production estimation in the euphotic zone of a tropical harbor ecosystem Nigeria. International Journal of Scientific and Research Publication, 4(8):1-8. Retrieved from http://www.ijsrp.org/

[6] De A.K. 1972 Environmental Chemistry. 4th edn, New Age International Publication, New Delhi, pp.231-232. Retrieved from http://files.stupidsid.com/

[7] Etisa D., Nega R. & Lule W. 2018 Seasonal Variation of Microalgae Diversity in Lake Abaya, Ethiopia. International Journal of Oceanography & Aquaculture, Volume 2, Issue 5, ISSN: 2577-4050. https://doi.org/10.23880/ijoac-16000150

[8] Das S. M., 1956 A Quantitative studies on qualitative and quantitative fluctuations of plankton in two Kumaon lakes, Nainital and Bhimtal (India). Acta Hydrobiolica. 2(1): 9-17. Retrieved from https://www.environmentaljournal.org/

[9] Dar N.A., Pandit A.K. & Ganai B.A. 2013 Seasonal Variation in the Pigment Content of Dominant Macrophytes from Wular lake, Kashmir Himalaya, India. Biochemistry and Pharmacology, volume 2 issue 4. https://doi.org/10.4172/2167-0501.1000124

[10] Dixit S., Gupta S. K. & Tiwari S. 2005 Nutrient overloading of fresh water lake of Bhopal, India. Electronic Green Journal, 1(21). 1-8. https://doi.org/10.5070/g312110588

[11] Eisa Ebrahimi Dorche., Mojgan Zare Shahraki., Omidvar Farhadian. & Yazdan Keivany. 2018 Seasonal Variations Of Plankton Structure As Bioindicators In Zayandehrud Dam Lake, Iran. Limnological Review, 18, 4: 157–165. https://doi.org/10.2478/limre-2018-0017

[12] Eric T. Korthalst & Michael R. Winfrey. 1987 Seasonal and Spatial Variations in Mercury Methylation and Demethylation in an Oligotrophic Lake. Applied and Environmental Microbiology, Volume 53, Pg. 2397-2404. https://doi.org/10.1128/aem.53.10.2397-2404.1987

[13] Elif Ersanl & Arif Gönülol. 2003 Study on the Phytoplankton and Seasonal Variation of Lake Simenit (Terme – Samsun, Turkey). Turkish Journal of Fisheries and Aquatic Sciences, 3: 29-39. https://doi.org/10.1080/02705060.2005.9664770

[14] Fathi A.A., Abdelzaher H.M.A., Flower R.J., Ramdani M. & Kraiem M.M. 2001 Phytoplankton communities of North African wetland lakes. The Cassarina Project, Aquatic Ecology, 35(3): 303–318. Retrieved from https://www.researchgate.net/

[15] Araujo F.G., Azevedo M.C.C.D. & Ferreira M.D.N.L. 2011 Seasonal changes and spatial variation in the water quality of a eutrophic tropical reservoir determined by the Inflowing River. Lake and Reservoir Management, pg 343 – 354 ISSN: 1040-2381. https://doi.org/10.1080/07438141.2011.627753

[16] Gajanan K. Sontakke & Satish S. Mokashe 2014 Seasonal variation in primary productivity of two freshwater lakes of Aurangabad district, Maharashtra, India. International Journal of Fauna and Biological Studies 1 (6): 07-10 ISSN 2347-2677. Retrieved from https://www.faunajournal.com/

[17] Garg R.K., Rao R.J., Uchchariya D., Shukla G. & Saksena D.N. 2010 Seasonal variations in water quality and major threats to Ramsagar reservoir, India. African Journal of Environmental Science and Technology, Vol. 4(2), pp. 061-076. Retrieved from https://www.ajol.info/

[18] Gorniak K. P. 2002 Seasonal and Multiannual Changes of Water Levels in Lakes of North eastern Poland. Polish Journal of Environmental Studies, Vol. 11, No. 4 (2002), 349-354. Retrieved from http://www.pjoes.com/

[19] Jeppesen E., Sondergaard M., Mazzeo N., Meerhoff M., Branco C., Huszar V. & Scasso F. 2005 Lake restoration and bio manipulation in temperate lakes: relevance for subtropical and tropical lakes. In: Restoration and management of tropical eutrophic lakes (Eds.: M.V. Reddy). Oxford and IBH Publishing Co. Pvt. Ltd., New Delhi. pp. 331-349. https://doi.org/10.1201/9781482294484

[20] Jayaweera M & Asaeda T. 1996 Modeling of biomanipulation in shallow, eutrophic lakes: an application to Lake Bleiswijkse Zoom. The Netherlands. Ecological Modelling, 85:113-127. https://doi.org/10.1016/0304-3800(94)00153-7

[21] Kennedy R.H. 1999 Reservoir design and operation: Limnological implications and management opportunities, Tundisi J.G, Straškraba M. (eds), Theoretical reservoir ecology and its applications, Backhuys, Leiden: 1–28. Retrieved from https://www.scielo.br/

[22] Khan T.A. 2003 Limnology of four saline lakes in western Victoria, Australia. Limnologica 33(4), 327–339. https://doi.org/10.1016/s0075-9511(03)80026-9

[23] Kuehl L.C. & Troelstrup N.H. 2013 Relationship between net primary production, water transparency, chlorophyll a and total phosphorus in Oak Lake, Brookings County, South Dakota. Proceedings –South Dakota Academy of Science, 92:67-78. Retrieved from https://scholar.google.com/

[24] Kun Li., Ling Wang., Zhaohua Li., Yujing Xie., Xiangrong Wang. & Qing Fang. 2017 Exploring the Spatial-Seasonal Dynamics of Water Quality, Submerged Aquatic Plants and their Influencing Factors in Different Areas of a Lake. Water, pg 1-17. https://doi.org/10.3390/w9090707

[25] Leidonald R., Lesmana I., Muhtadi A. & Desrita 2019 Biodiversity flora and fauna in tropical tidal lake. IOP Conference Series: Earth and Environmental Science, 260,012105. https://doi.org/10.1088/1755-1315/260/1/012105

[26] Lopes M.R.M., Bicudo C.E.M.& Ferragut M.C. 2005 Short term spatial and temporal variation of phytoplankton in a shallow tropical oligotrophic reservoir, southeast Brazil. Hydrobiologia, 542(1): 235–247. https://doi.org/10.1007/s10750-004-8332-z

[27] Mackereth F.Y.H., Heron J.G. & Talling J.J. 1978 Water analysis some revised methods for limnologist. Ambleside: Freshwater Biological Association. Oxford (UK): Blackwell Scientific Publication. https://doi.org/10.1002/iroh.19790640404

[28] Mecombie, A. M. 1953 Factors influencing the growth of phytoplankton. Journal of the Fisheries Research Board of Canada, 10 : 253-282. https://doi.org/10.1139/f53-019

[29] Mishra G.S., James A., Paliwal H.B. & Kumar H. 2017 Physico-Chemical, Biological Properties and Biodiversity of Aquatic Plant Species In Macferson Lake Allahabad, U.P. India, Current world environment, ISSN: 0973-4929, Vol. 12, No. (3) 2017, Pg. 630-634. https://doi.org/10.12944/cwe.12.3.14

[30] Moitra, S. K. & Bhattacharya B. K. 1965 Some hydrological factors affecting plankton production in a fresh pond in Kalyani, West Bengal, India. Ichthyologica.4: 8-12. Retrieved from https://scholar.google.lu/

[31] Najar A. & Basheer A. 2012 Assessment of seasonal variation in water quality of Dal Lake (Kashmir, India) using multivariate statistical techniques WIT. Transactions on Ecology and The Environment, Vol 164. https://doi.org/10.2495/wp120111

[32] Nikolaidis N.P., Koussouris T., Murray T.E., Bertahas I., Diapoulis A. & Gritzalis K. 1996 Seasonal Variation of Nutrients and Heavy Metals in Phragmites australis of Lake Trichonis, Greece. Lake and Reservoir Management, 12:3, 364-370. https://doi.org/10.1080/07438149609354277

[33] Nyairo W.N., Owuor P.O. & Kengara F.O. 2015 Effect of anthropogenic activities on the water quality of Amala and Nyangores tributaries of River Mara in Kenya. Environmental Monitoring and Assessment, 187, 691. https://doi.org/10.1007/s10661-015-4913-8

[34] Pranita Hajong. & Papiya Ramanujam. 2018 Seasonal variation in algal diversity and productivity in Dachi lake, Meghalaya Journal of Algal Biomass Utilization, 9(2), 9-24. Retrieved from http://www.jalgalbiomass.com/

[35] Poquet J.M., Mezquita F., Rueda J. & Miracle M.R. 2008 Loss of Ostracoda biodiversity in Western Mediterranean wetlands. Aquatic Conservation Marine and Freshwater Ecosystems, 18, 280–296. https://doi.org/10.1002/aqc.831

[36] Purohit R. & Singh S.P. 1981 Seasonal Variation in Physico-Chemical Limnology of shallow zones of Nainital Lake, Western Himalaya(India). Proceedings of Indian National Science Academy, B47, No. 2, pp 194- 203. Retrieved from https://insa.nic.in/

[37] Ramanathan S. & Amsath A. 2018 Seasonal Variations in Physico-Chemical Parameters of Puthukulam Pond, Pudukkottai, Tamilnadu. Research Journal of Life sciences, Bioinformatics, Pharmaceutical and Chemical sciences, 4(6), Pg.656. Retrieved from http://www.rjlbpcs.com/

[38] Reynolds C.S. 2006 The ecology of phytoplankton. Cambridge University Press, UK. https://doi.org/10.1017/cbo9780511542145.009

[39] Gayathri S., N. Latha N & Mohan M.R. 2013 Impact of Climate Change on Water Quality of Shoolkere Lake, Bangalore, Journal of Academia and Industrial Research (JAIR), Volume 2, Issue 6, pg 362- 368. Retrieved from https://www.semanticscholar.org/

[40] Sahoo P.K., Guimaraes J.T.F., Filho P.W.M., Silva M.S, Silva Junior R.O. & Pessim G. 2016 Influence of Seasonal Variation on the Hydro Biogeochemical characteristics of two upland lakes in the south eastern amazon, Brazil. Brazilian Academy of Sciences, 88, 2211-2227. https://doi.org/10.1590/0001-3765201620160354

[41] Shannon C.E. & Reid W.J. 2003 The mathematical theory of communication. University of Illinois Press, Illinois, USA.

[42] Sudarshan P., Mahesh M.K. & Ramachandra T.V. 2019 Assessment of Seasonal Variation in Water Quality and Water Quality Index (WQI) of Hebbal Lake, Bangalore, India. Environment and Ecology 37, pg: 309-317. Retrieved from https://www.sciencedirect.com/

[43] Sukla B., Patra A.K. & Panda R.P. 2013 Primary Productivity in River Birupa, India. Proceedings of the National Academy of Sciences India, Section B, Biological Science, 83(4), 593-602. https://doi.org/10.1007/s40011-013-0165-y

[44] Wei H., Sun J., Moll A., & Zhao L. 2004 Phytoplankton dynamics in the Bohai Sea observation and modelling. Journal of Marine System, 44:233-251. https://doi.org/10.1016/j.jmarsys.2003.09.012

[45] Zafar A.R. 1964. On the ecology of algae in certain fish ponds of Hyderabad, India. Distribution of Unicellular and colonial forms. Hydrobiologia, 24: 556 565. https://doi.org/10.1007/bf00142002

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Effect of use Probiotics and Organic Fertilizer on Yield of Peanut on Tra Vinh Province, Vietnam

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Abstract*— This study was to evaluate the effects, determine the appropriate, and assess the profitability of using locally produced organic foliar fertilizers on peanut production. The experiment was installed in CauNgang andTra Cu with two variety MD7, L14. Seven fertilizer treatments were designed.The treatments consisted of the application of T1:(Trichoderma sp + Bordeaux 1% + Probiotics 3M; T2: (organic fertilizer =10 ton/ha);T 3: (Trichoderma sp + Bordeaux 1% + Probiotics 3M + chemical fertilizers(35N-60P-60K + 150kg Ca + 40kg Mg) + organic fertilizer 100%) T4: (Trichoderma sp + Bordeaux 1% + Probiotics 3M + chemical fertilizers(35N-60P-60K + 150kg Ca + 40kg Mg) + organic fertilizer 75% ;T 5(Trichoderma sp + Bordeaux 1% + Probiotics 3M + chemical fertilizers + organic fertilizer50%) ;T6: chemical fertilizers(35N-60P-60K + 150kg Ca + 40kg Mg)+(Trichoderma sp + Bordeaux 1% + Probiotics 3M)and T7 control and treatment of farmers: (120 N-60 P-60 K + 200 Ca kg/ha). The experimental design adopted consisted of randomized complete blocks with three replications. Results showed that peanut plants flowered early when applied with T3 (Trichodermasp + Bordeaux 1% + CPVS 3M) + chemical fertilizer + 100% organic fertilizer) significantly increase plant height, number of branches/ plant, ability to absorb N. P. K. 100 seeds,weight of pods(g/ plant) and seeds of plant. The increase/decrease in fertilizer intake had a significant and statistically significant effect ($p \leq 0.05$) on peanut yield and quality in both treatments areas, with the exception of 100 grains. If only organic fertilizer is not combined with microbiological preparations, the manure of black spots and brown spots is higher than the experimental in combination with the treatment fertilizer combined with the balanced amount of fertilizer in the T5 treatments(Trichoderma sp + Bordeaux 1% + Probiotics 3M + chemical fertilizers + organic fertilizer50%).

***Keywords*— Peanut, Probiotics, nutritional status, N. P. K. yield, yield components.**

I. INTRODUCTION

Peanuts (*Arachishypogaea L.)* are also named groundnuts, belonging to the family Leguminosae that produces underground fruits known as shell beans (Aboelill et al.. 2012). Growing peanuts helps enrich the soil's nutrients due to its ability to fix nitrogen in the atmosphere. Peanuts require only a small amount of N because of their ability to fix nitrogen from the atmosphere (Jordan et al., 2017). Organic matter can be used as fertilizer because it contains essential nutrients such as nitrogen, phosphorus, potassium, calcium, iron, manganese, zinc, copper, magnesium and proteins that stimulate the metabolism of plants. Organic fertilizers are an excellent alternative to in innocuous fertilizers in crops that require fewer nutrients for their growth and development. The use of organic leaf fertilizer is beneficial. It contains microorganisms such as bacteria that accelerate the mineralization of organic materials and help plants absorb quickly.The impact of agrochemical pollution in air, water and soil profoundly affects human health through the accumulation of toxins from living in a toxic environment and consuming toxic foods (Katherine and Hendrik. 2010). This effect will increase the rates of asthma,Autism, physical disabilities, learning disabilities, reproductive disorders, diabetes, Parkinson's disease. Alzheimer's disease and cancer (Owens et al.,2010). In addition to directly affecting human health, chemicals from agricultural activities also affect the ecosystems of plants and animals. Finally, humans are affected by the consumption of these products and meats (Onder et al., 2011; Sharma and Singhvi, 2017).

The use of chemical fertilizers, pesticides and pesticides has continued for a long time in the agricultural production process and is widely found in farm systems (Aktar et al., 2009; Savci. 2012), where health effects are found both in consumers and farmers using chemicals for agricultural activities (Costa et al., 2014). Accordingly, organic products are a new trend and a great opportunity for manufacturers in the food industry. (Ferella et al., 2019). Biodegradable can significantly reduce soil N2O emissions, stabilize soil organic C, and the activity of microbial functional groups, especially debactericiologists (Yuan et al., 2017).

Chemical fertilizers have long been considered a necessary solution capable of replacing the natural fertility of the soil. Although they are effective, they are difficult to access, with many limitations such as being very expensive to buy, pollution and increasing the resistance of many pathogens to the commonly used dose of chemical fertilizers (Janny et al., 2003). For sustainable development, it is necessary to change behavior and innovate by proposing new ways to produce new cropping systems that are primarily based on natural processes to meet both food security needs and the need for more balanced management of natural resources. Many studies have focused on the biology of microorganisms that rapidly affect the rapid mineralization of organic matter (Higa, 1996). Native microorganisms (IMO) and effective microorganisms (EM) form a source of nutrient reserves.Their role as a mineralize increases soil fertility, while making them less compacted and eroded (Narasimha et al., 2012). IMO consists of microorganisms consisting mainly of bacteria,fungi and yeast. EM is a commercial solution of effective microorganisms consisting mainly of bacteria and yeast (Helen et al., 2006). The goal of this study is to assess the impact of native microbial-based organic fertilizers on peanut yields in the coastal region of TraVinh. In addition, the goal is to analyze the chemical physical properties of the soil before and after the application of fertilizer to determine soil fertility, and to assess the impact of vaccination of these microorganisms on the growth and productivity of peanuts at TraVinh.

II. MATERIAL AND METHODS

2.1. Varieties :MD7 and L14.

2.2. Experimental design and treatments

-Experiment was conducted at Tra Cu and CauNgangof TraVinh province, with sandy soil structures. Experimental soils have been growing peanuts for ten years and in recent years are managed in the conservation system for the peanut region. Prior to the experiment the soil layer was collected in each area in layers 0 to 30 cm deep to make up the composite sample, which was used to analyze chemical indicators according to the method of Raij et al., (2001) and particle size according to Camargo et al., (2009).

- The experiment was arranged on the farmer's field in a completely random mass (02 varieties, 7 treatments, 3 replications. at 02 locations. the area of plots is 25 m2).

Table 1: Treatments of fertilizers used experiments

no	Treatments	Contents	note
1	T1	(Trichoderma sp + Bordeaux 1% + Probiotics 3M)	
2	T2	organic fertilizer=10ton/ha	
3	T3	Trichoderma sp + Bordeaux 1% + Probiotics 3M) + chemical fertilizers(35-60-60 + 150kg Ca + 40kg Mg)+ organic fertilizer100%	(fermented cow manure)
4	T4	Probiotics (Trichoderma sp + Bordeaux 1% + Probiotics 3M) + chemical fertilizers(35-60-60 + 150kg Ca + 40kg Mg)+ organic fertilizer75%	
5	T5	(Trichoderma sp + Bordeaux 1% + Probiotics 3M) + chemical fertilizers(35-60-60 + 150kg Ca + 40kg Mg)+ organic fertilizer50%	
6	T6	Chemical fertilizers(35-60-60 + 150kg Ca + 40kg Mg)+(Trichoderma sp + Bordeaux 1% + Probiotics 3M)	
7	T7	fellowing farmers (120-60-60+ 200 Ca kg).(control)	

The process of planting and caring techniques (land preparation. planting density. care. harvesting) is carried out in accordance with Guidance No. 52/HD-SNN.

2.3. Data collection

Plant height: is determined by a ruler cm at the end of the harvest cycle (90 days) from the surface of the soil to the end of the main of 10 trees in each plot of experiment.

Number of leaves and number of branches: calculated at the end of the harvest period, using samples of 10 plants per experiment. Factors that constitute yield and yield components : The number of pods per plant , the number of seeds per plant is determined by counting the bark and seeds of the 10 plants selected from each experiment. 100 seeds weight (g): Weighs 100 seeds in each treatment weight of pods(g/ plant);Seed yield(gram) : The seeds obtained from each plot were weighed using weighing scale.Number of nodules : were counted.

Disease assessment

Table 2: Disease level assessment scale of iron and brown spots (Subrahmanyam et al. 1997)

Rust disease	**% infected**	**scale**	**Early leaf spot and late** lateleaf spot
No traces of disease.	0	1	No traces of disease.
There are a few small dots on the old leaves.	1-5	2	There are a few small spots on the old leaves.
A few small spots on the leaves are old and have the formation of spores like dust particles	6-10	3	Appears a few spots mainly on old leaves. there is weak spore formation
Many small spots are largely on young leaves and are located in the leaves themselves. the formation of spores	11-20	4	Many spots have small or large vows, most of which occur in the lower leaves low and the leaves in the middle, the stain appears clearly.
Small spots are easily visible in the lower leaves and the middle leaves of the plant have a mild spore formation.	21-30	5	Many pustules, mostly on lower and middle leaves yellowing and necrosis of some lower and middle leaves. moderately sporulating
It's like a 5 score -grade illness, but there are more spots.	31-40	6	The same with score but there are more spots
spotsappear most of the trees. there is necrosis in the low leaves and the middle leaves	41-60	7	Spots are easily visible at long distances, dots are present almost all over the leaves, there is deciduousness in the low leaves and the middle leaves.
The level of the disease is level 7, but the level of necrosis is more severe.	61-80	8	The level of the disease is level 7, but the level of necrosis is more severe
50-100% The leaves on the plant are fallen.	81-100	9	50-100% The leaves on the plant are fallen

2.4. Statistical analysis

All the data obtained was analyzed method by procedure in SAS (Version 9.4, SAS Institute Inc., 2017). The difference has the slightest meaning (LSD) at 5% to compare the differences between the tests.

III. RESULTS AND DISCUSSION

3.1. Experimental soil propertie

In the condition that the land of peanuts has been arranged to grow peanuts for 1 crop with innocuous fertilizer levels. Soil analysis results at Tra Cu and CauNgang locations showed that the soil protein parameters were 1.04% at Tra Cu and theCauNgangpoint was 0.95%. Organic C levels were not high (0.92% and 0.86%) (Tran et al., 2021) to 1.04(Tra Cu score to 0.95 (at CauNgang) at this experiment after planting a peanut experiment. (Table 3). Total protein also increased from 0.87- 0.93% for Tra Cu and CauNganginrespecity. Mild sour soil - neutral (pH,KCl 6.1-6.5). Peanuts grow best in slightly acidic soil with 6.0 to 6.5.

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Table 3: Some properties of the tested soil (0–30 cm depth) after harvested one season

Property	Tra Cu	CauNgang
% Nitrogen	0.87	0.93
% Potassium	150.2	128.4
% Sodium	71.6	83.5
% Magnesium	97.2	110.3
% Calcium	241	380.33
% Manganese	95.7	112.6
% Zinc	2.56	3.56
% Cooper	3.68	2.03
% Iron	79.5	108.8
% Organic	1.04	0.95
pH	6.1	6.5
% Sand	58.36	65.78
% Silt	31.5	40.20
% Clay	1.20	2.02

3.2. **Effects of fertilizers on peanut growth, development and productivity**

3.2.1. Analysis of the impact of fertilizers on tree growth .This analysis is based on three traits: plant height, number of branches on the plant and number of leaves on the plant in two different locations.

a) Experiment at Tra Cu

The height of fluctuating plant is statistically significant. The average height of the MD7 is 60.2cm in the T2 treatments (organic fertilizer) only. The tallest height in the T3 treatment is (65.8 cm). The average heighplant of L14 when not fertilized is 52.6 cm in the T1 treatment and the highest is also in the full fertilization treatment (T3) fully fertilized microbiological, organic and chemical fertilizer (58.3 cm). For the number of branches/ plant, the L14 and M D7 varieties both give the number of branches on the plant in the T3 treatment. The number of leaves is affected by varieties. The average number of leaves of the MD7 is lower than that of the L14. The MD7 has the highest number of leaves in the T3 treatment (79.4) and the lowest of 67.8 (T7). While the L14 has the lowest average value of 81.6 (T2 treatment) to 93.4 leaves in the T3 treatment.

b) Experiment **at CauNgang**

Similar to the experiment at Tra Cu , at CauNgang the average height plant of the MD7 is 60.2 cm in the T2 (organic fertilizationonly). The height is highest in the T3 treatment(65.8 cm). For the L14 the lowest height plant is also in the T1 treatment and the tallest in the F7 test is 52.6 cm, 59.6 cm respectively. For the number of branches/ plant, the L14 and MD7 varieties give a range of 9.2 to 11.7 for L14 and the number of branches on the plant the MD7 variety is 8.4 to 11.5 respectively. The number of leaves/ plant of the L14 variety is also higher than that of MD7,treatments are statistically different (table 4).

Table 4.Effects of fertilizers on peanut growth, development and productivity

Treatments(T)	MD7			L14		
	Height Plant (cm)	number of branches /plant	number of leaves /plant	Height Plant (cm)	number of branches /plant	number of leaves/ plant
Site 1: Tra Cu						
T1	62.3d	10.5b	79.3a	52.6e	10.3b	92.3b
T2	60.2e	10.2b	75.2c	56.4c	9.2c	81.6e
T3	65.8a	11.5a	79.4a	58.3a	11.7a	93.4a
T4	63.1c	10.7b	78.5b	57.2b	11.1a	90.8c
T5	61.4d	9.5c	78.4b	55.8d	10.8b	90.1c
T6	64.5b	9.7c	73.2d	55.3d	10b	89.6d
T7	61.7d	8.4e	67.8e	56.5c	10.4b	90.4c
Site 2: CauNgang						
T1	62.4d	9.0c	76.1b	56.3d	10.8b	93.3b
T2	57.2f	8.0d	68.2d	52.2e	8.3c	82.4f

T3	65.2a	11.0a	78.5a	59.5a	11.8a	94.8a
T4	64.3b	10.4b	76.3b	58.4b	11.2a	92.6c
T5	63.3c	10.0b	75.6c	57.3c	10.6b	91.6d
T6	61.7e	9.0c	78.2a	56.1d	10.6b	90.5e
T7	62.8d	10.0b	76.2b	59.6a	11.0a	91.4d

3.2.2. Effects of fertilizers on peanut yield and component yield

a) Experiment at Tra Cu

There were considerable differences in the mean number of pods per plant among the different plant of treatments. The treatment with a population of five plants per pot produced a mean number of 14.3-17.9 pods per plant for MD7. On average, the number of pods per plant affected by the variety and the proportion and dosage of adequate fertilizer according to the treatment (T3); the average number of seeds/ plant is significantly higher for both MD7 and L14 varieties at 17.8 and 18.6 inrespectively. This recorded the number of seeds on the T3 treatment for the higher number of particles on the MD7 and L14 varieties. Most of both the number of pod /plant and the number of seeds on plant are statistically significant. Except for the average volume of 100 seeds weight that are no different on fertilizer treatments.

b) Experiment at CauNgang

The experiment at CauNgangrecorded the number of pods perplant , the number of seeds on the plant of the two varieties MD7 and L14 are both statistically significant differences of the MD7 variety. The highest number of podsperplant is the T3 and T4 treatments, on the two varieties MD 7 and L14. The Hundred seed weight is also an important yield component which reflects the magnitude of seed development which ultimately reflects on the final yield of a crop. (Table 5)

Table.5. Effects of fertilizers on peanut component yield

Treatment(T)	MD7			L14		
	Number of pods per plant	seeds per plant	100 seed weight (g)	Number of pods per plant	seeds per plant	100 seed weight (g)
Site 1: Tra Cu						
T1	16.8b	30.7e	44.5a	17.2b	25.5a	35.3a
T2	14.3d	25.3f	44.1b	13.1d	19.8e	34.9a
T3	17.9a	35.8a	45.7a	18.6a	25.7a	35.3a
T4	17.5a	34.2b	45.2a	18.1a	25.1a	35.6a
T5	16.6b	33.3c	45.6a	17.6b	24.9b	35.4a
T6	15.4c	32.6d	45.5a	16.6c	20.2d	35.6a
T7	14.9d	30.6e	45.5a	16.7c	22.6c	35.3a
Site 2: CauNgang						
T1	19.2a	40.3a	44.9a	18.7b	30.3c	39.6a
T2	17.5c	30.2d	44.5a	15.8d	23.1d	38.3b
T3	19.7a	40.6a	44.8a	19.5a	32.9a	39.7 a
T4	18.5b	40.5a	44.2a	19.2a	32.7a	39.6a
T5	17.6c	37.9b	44.3a	18.9b	31.8b	39.1a
T6	17.4c	34.2c	44.7a	17.5c	30.4c	39.5a
T7	17.6c	37.5b	44.3a	17.7c	32.7a	39.2a

3.2.3. Effects of fertilizers on yield of peanut

a) Experiment at Tra Cu

Nodule number was significantly (P<0.01) influenced due to the main effects of peanut cultivars and application fertilizers.The average number of nodules is also affected by the variety and proportion, dosage of nitrogen fertilizers. In the Probiotics (Trichoderma sp + Bordeaux 1% + Probiotics 3M) + chemical fertilizers(35-60-60 + 150kg Ca + 40kg Mg)+ organic fertilizer 100%) (T3) the average number of nodules calculated for MD7 (46.5) is significantly different from the treatments(table 4). For the L14 in the T7,T3 and T4 tests the highest number of nodules is 43.9; 42.7;41.3 in respectively. Weight of pods(g/ plant)obtained on the plant on the MD7 variety is (29.3) highest in T3. Reducing the amount of fertilizer, the weight of pods(g/ plant)also decreased by 24.9 grams per plant on the T2 treatment. The yield of seeds on the MD7 variety is higher than L14 in the tests and is significant compared to the fertilization and non-fertilization test (table 4). At T1 treatment, the average weight of pods(g/ plant) is significantly different from the T2 and T7 treatment,seed yield is affected by the varieties, high productivity remains in T1, next to T4 and T5. The lowest particle yield in the non-fertilization treatment (T7) for the MD7 variety. (Table 6)

b) Experiment at CauNgang

The average number of nodules calculated at the ratios and dosage of nitrogen fertilizers varies significantly. T3 treatments has the highest nodules(46.2) , then T1(46.1) on the MD7. For the L14 variety, the highest number of nodules/ plant in thefollowed by T1, T3 and T5 treatments (fluctuations from 43.2. 43.1 and 43.1 in respectively). Weight of pods(g/ plant)was significantly (P<0.01) affected by the interaction effect .The yield of seeds on the MD7 variety is higher than L14 in both treatments and is significant compared to the fertilization and non-fertilization treatments (table 6). At T1 the highest weight of pods(g/ plant)(28.9 g/plant), then T3 (28.4 g / plant). While the weight of pods(g/ plant) is lowest in the non-fertilization treatments (20.7 g/ plant) at L14 variety. Seed yield is affected by the variety, with high yields remaining in T1 (16.4 g/plant), then T3 (15.4 g/plant) and T8 (15.2 g/ plant) for the MD7 variety. For the L14 variety the most yielding in T1 (17.6 g/ plant), then T6 (16.9 g / plant). On the L14 at CauNgangrecorded in the treatment (T2) the yield of the grain has decreased,this is the same with Tra Cu.

Table.6. Effects of fertilizers on yield of peanut

Treatment(T)	MD7			L14		
	number of nodules/ plant	weight of pods(g/ plant)	Seed yield (g/ plant)	number of nodules/ plant	weight of pods(g/ plant)	Seed yield (g/ plant)
site1: Tra Cu						
T1	40.1c	28.3b	18.8a	39.5e	23.2d	15.5d
T2	37.6 d	24.9d	10.7e	41.6c	20.7e	11.5f
T3	46.8a	29.3a	18.7a	42.7b	26.5a	18.4a
T4	45.3b	28.8b	18.1a	41.3c	25.2b	17.1b
T5	44.2b	27.1c	17.5b	40.8d	24.5c	16.6c
T6	44.1b	25.2d	16.3c	39.2e	23.8d	15.5d
T7	40.8c	25.6d	15.2d	43.9a	20.9e	14.4e
Site 2: CauNgang						
T1	46.2a	31.9a	18.3a	43.2a	23.5c	16.6b
T2	41.4e	25.2f	16.7c	40.3d	20.4f	10.3d
T3	46.3a	29.3b	18.8a	43.2a	25.3a	17.9a
T4	45.6b	28.5c	18.2a	43.1a	24.9b	17.5a
T5	44.3c	28.2c	17.5b	42.6b	23.1c	16.9b
T6	43.2d	26.7d	17.6b	41.7c	22.2e	15.8c
T7	43.8d	28.7c	16.5c	38.1f	23.7c	15.6c

3.3. Effects of fertilizers on rust disease

3.3.1. Rust disease

Rust disease is an economically important biotic stress that significantly reduces the pod and fodder yield and oil quality. It is caused by the *basidiomycete fungus*. Such as **:**Rust disease (*Pucciniaarachidis)*,Brown spots (*Cercosporaarachidicola)*, Black spots (*phaeoisariopsispersonala).*

Rust disease only occurs at the end of the growth and development of peanut plants. In the early stages, it is usually less common and the disease develops very strongly in the final stages. The varieties of peanut participating in the experiment suffered from rust disease from 1-3 score at this stage of 60 after sowing.

Brown spot disease and dark spot disease appear earlier than rust disease pediculosis from the time the peanut begin to flower causing severe harm at a 90-day period of 1-5 points for black spots on the T7 at CauNgang. Brown and black spots appear high in T2 on both CauNgangand Tra Cu.*Cercosporaarachidicola* is a fungal ascomycete plant pathogen that causes early leaf spot of peanut. All cultivars of peanuts are equally susceptible to peanut fungal pathogens; The results of this study indicated that genotypes MD 7 and L 14 were consistently tolerant under field conditions at TraCu and CauNgang with rust disease .

Table 7. Affected Rust disease.Earlyleaf spot (brown spot)of peanut and late leaf spot ((black spot)of peanut

Treatment(T)	MD7			L14		
	Rust disease	Early leaf spot	late leaf spot	Rust disease	Early leaf spot	late leaf spot
Site 1: Tra Cu						
T1	**0**	**2**	**2**	**0**	**2**	**2**
T2	**3**	**1**	**5**	**3**	**5**	**5**
T3	**0**	**1**	**1**	**0**	**1**	**1**
T4	**0**	**0**	**1**	**0**	**1**	**1**
T5	**0**	**0**	**0**	**0**	**0**	**0**
T6	**0**	**2**	**2**	**0**	**2**	**2**
T7	**0**	**2**	**3**	**0**	**3**	**1**
Site2: CauNgang						
T1	**0**	**2**	**2**	**0**	**2**	**2**
T2	**1**	**3**	**3**	**0**	**5**	**3**
T3	**0**	**1**	**1**	**0**	**1**	**1**
T4	**0**	**1**	**1**	**0**	**1**	**1**
T5	**0**	**3**	**3**	**0**	**1**	**3**
T6	**0**	**2**	**2**	**1**	**3**	**3**
T7	**0**	**3**	**5**	**0**	**3**	**3**

3.4. Discussion

These yields trend also to explain that liming alone cannot serve to achieve the maximum potential of an acid soil, thus suggesting that the soils are more depleted of N and K, which clearly influence crop performance as, was observed when these amendments (lime and P fertilizer) were applied in combination with manure (Farag and Zahran, 2014). Organic sources such as farm yard manure, ice husk ash, paper factory sludge along with chemical fertilizers improved the yield and quality of peanut kernels in a better and comparable way than lime (Basu et al., 2007). Table 4 shows that the agrocological properties of peanuts are affected by the application of different types of organic fertilizers. The methodical analysis shows that the heigh of plant, number of leaves varies significantly. Peanuts are treated with compost that flowers later than chemical fertilizers. According to (Jordan et al. (2017). Organic Fertilizers (Cow Manure.

Chicken) provide a higher amount of N nutrients that enhance the early flowering of peanut plants. Lalog, (2011) mentioned that a certified organic leaf fertilizer is rich in very good macronutrients.Yield and yield components indicators of peanuts are affected by the application of various organic fertilizers presented in Table 5. The methodological analysis shows that the weight (g) of 100 seeds, the weight of pods, and the seed yield (ton/ha-1) differ significantly between processing plants. Further explains that the power develops by increasing photosynthetic activity in the leaves, stimulating the need for water with leaves. Therefore, there is an increase in water absorption of the circuit system, which increases the quantity and quality of crop yields. On the other hand, the application of organic fertilizers as wet soil in combination with beneficial and effective microorganisms can be used instead of inorganic fertilizers. Likewise,tricoderma fungi improve the metabolism of plants and contribute to higher plant and yield production. It also enhances photosynthesis and increases the numbers of leaves of plants. Therefore, it increases the yield of crops (Mungkunkamchao. et al., 2013).

Incidence of Insect Pest and Diseases to Peanut

The rate of pests and diseases for peanuts. The rate of insect pests for peanut plants is presented in Table 7. The treatments shows high resistance to insect pests and resistance to levels 1-3 leaf spotting diseases. They produce reasonably higher productivity in all batches of experiments. Moreover, based on the reaction of the peanut plant (MD7 and L14 variety) to harmful insects and diseases, the variety is recommended for farmers because it is highly resistant to pests to medium. In fact, they can minimize the cost of pesticides. These results confirm the statement of (Brandenburg. et al., 2019).it is important to test the resistance of the varieties to pests before recommending it to farmers.

IV. CONCLUSION

Treatment (Trichodermasp + Bordeaux 1% + Probiotics 3M) + chemical fertilizer + 100% organic fertilizer) significantly increase plant height, number of branches/ plant, ability to absorb N. P. K. 100 seeds weight,weight of pods(g/ plant)and seeds yield. The increase/decrease in fertilizer intake had a significant and statistically significant effect ($p \leq 0.05$) on peanut weight of pods(g/ plant); seed of yield and quality in both treatmentsareas, with the exception of 100 grains. If only organic fertilizer is not combined with microbiological preparations, the manure of black spots and brown spots is higher than the experimental in combination with the treatment fertilizer combined with the balanced amount of fertilizer in the T5treatments(Trichodermasp + Bordeaux 1% + Probiotics 3M) + chemical fertilizer + 50% organic fertilizer).

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REFERENCES

[1] Aboelill A.A., Mehanna H.M., Kassab OM., &Abdallah EF. 2012. The Response of Peanut to Foliar Spraying with Potassium Under Water Stress Conditions. *Australian Journal of Basic and Applied Sciences,* 6(8): 626-634.

[2] Aktar, M.W., Sengupta, D., Chowdhury, A., 2009. Impact of pesticides use in agriculture: their benefits and hazards. Interdiscip. Toxicol. 2 (1), 1–12.

[3] Brandenburg R.L., Jordan D.L., Royals B.R., Mahoney D.J. & Johnson P.D. 2019. Utilization of Imidacloprid to Control Pests in Peanut in North Carolina. Peanut Science 46:1, 8-13. Online publication date: 24-Apr-2019.

[4] Basu M, Bhadoria PBS, Mahapatra SC. 2007. Comparative effectiveness of different organic and industrial wastes on peanut: Plant growth, yield, oil content, protein content, mineral composition and hydration coefficient of kernels, Archives of Agronomy and Soil Science 53(6), 645-658

[5] Camargo, O.A., Moniz, A.C., Jorge, J.A., Valadares, J.M. 2009. Métodos de análisequímica, mineralógica e física de solos do InstitutoAgronômico de Campinas. Rev. andupdated ed. InstitutoAgronômico de Campinas, Campinas, Boletimtécnico, 106. 77p.

[6] Costa, C., Garcia-Lestion, J., Costa, S., Coelho, P., Silva, S., Pingqarilho, M.,Valdiqlesias, V., Mattei, F., Dali Armi, V., Bonassi, S., Laffon, B., Snawder, J.,Teixeira, J.P., 2014. Is organic farming safer to farmers' health? A comparison between organic and traditional farming. Toxicol. Lett. 230, 166–176.

[7] Farag IAA, Zahran AA. 2014. Groundnut (Arachishypogaea L.) growth and yield responses to seed irradiation and mineral fertilization. Journal of Agriculture and Veterinary Science 7(15), p 63-70.

[8] Ferella, F., Cucchiella, F., D'Adamo, I., Gallucci, K., 2019. A techno-economic assessment of biogas upgrading in a developed market. J. Clean. Prod. 210, 945–957.

[9] Helen LG, Gerry G (2006). A handbook of preparations, techniques and organic amendments inspired by nature farming and adapted to locally available materials and needs in the western visayas region of the Philippines. Nature farming manure pp. 1-137.

[10] Higa T (1996). Effective microorganisms - Their role in Kyusei Nature Farming. Proceedings of the 3rd International Nature Farming Conference. USDA; Washington pp. 20-23
[11] Janny GM, Vos, Ritchie BJ, Flood J (2003). The discovery of cocoa, a guide for training facilitators. CABI Biosciences 115 p.
[12] Jordan D.L., Brandenburg R., Brown B., Bullen G., Roberson G. &Shew B., 2017. Peanut Information. *North Carolina Cooperative Extension Service Pub.* AG-331. 172 pages
[13] Katherine, K., Hendrik, W., 2010. Environmental Impacts of Agricultural Technologies.
[14] Evans School Policy Analysis & Research (EPAR). EPAR Brief No. 65.
[15] Lalog J.T. 2011. Dynapharm Company Incorporation, Quezon City, Philippines
[16] Mungkunkamchao T., Kesmala T., Pimratch S., Toomsan B. &Jothityangkoon D. 2013. Wood vinegar and fermented bioextracts: Natural products to enhance growth and yield of tomato. *Sci. Hortic*-Amsterdam. 154, 66-72.
[17] Narasimha G, Sumathi T, Janardhan A, Srilakhmi A, SaiGopal DVR (2012). Impact of indigenous microorganisms on soil microbial and enzyme activities. Archives of Applied Science Research 4(2):1065-1073.
[18] Onder, M., Ceyhan, E., Kahraman, A., 2011. Effects of agricultural practices on environment. international conference on biology. International Conference on Biology, Environment and Chemistry. IPCBEE 24, 28–32. Retrieved from. Http://ipcbee.com/vol24/6-ICBEC2011-C00015.
[19] Owens, K., Feldman, J., Kepner, J., 2010. Wide range of diseases linked to pesticides. Pestic. You 30, 13–21.
[20] Raij, Van B., Andrade, J.C., Cantarella, H., Quaggio, J.A. 2001. Análisequímica para avaliação da fertilidade de solos tropicais. InstitutoAgronômico, Fundação IAC, Campinas, 285 p
[21] Sharma, N., Singhvi, R., 2017. Effects of chemical fertilizers and pesticides on human health and environment: a review. Int. J. Agric. Environ. Biotechnol. 10 (6), 675–679.
[22] Savci, S., 2012. An agricultural pollutant: chemical fertilizer. Inter. J. Environ. Sci. Dev. 3(1), 73–80.
[23] Subrahmanyam, P. and Hildebrand, G.L. 1997. Responses of peanut genotypes to fungicidal control of early leaf spot in Malawi. Peanut Sci. 24:73-77.
[24] SAS Institute Inc. 2017. The MIXED procedure. SAS Institute Inc. Saxton, A. 1998. A macro for converting mean separation output to letter groupings in Proc Mixed. Proc. 23rd SAS Users Group Intl., SAS Institute, Cary, NC: 1243-1246
[25] Tran ThiKhanh Tran, Le Hoang Phuong, N. T. Phuoc. Nguyen thi Lang .2021. Effect of fertilizer on nutritional status and yield of peanut on TraVinh. Journal and Rural Development No. 17. Pages 45-53. 2021
[26] Yuan Y, Chen H, Yuan W, Williams D, Walker JT, Shib W. 2017. Is biochar-manure co-compost a better solution for soil health improvement and N2O emissions mitigation? Soil Biology and Biochemistry 113, p 14–25.

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Business Feasibility Analysis of Vaname Shrimp *(Litopenaeus vanname)* Cultivation Through Demonstration Farming in Barru Districk

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***Abstract*— *Shrimp vaname (Litopenaeus vanname) is one of the fishery commodities that become prima donna in the export market. Barru Regency is one of the areas that has the potential in developing vaname shrimp farming business with a land area owned by 2,500.11 ha. But the great potential is not accompanied by national shrimp productivity, one of the causes is the lack of awareness of cultivators in accepting technological innovation and lack of capital in applying cultivation technology. This research describes the Demonstration Farming program set by KKP with the aim of assisting cultivators in implementing new technologies through group cooperation. The study was conducted by qualitative method using descriptive analysis to describe the process of implementing demonstration farming program in Barru Regency. Quantitative methods use business feasibility analysis with Net Present Value (NPV), Net Benefit-Cost Ratio (Net B/C) and Internal Rate of Return (IRR) indicators. The results showed that (NPV) has a value of IDR. 418,514,544; (NetB/C) has a value of 2.24; and (IRR) has a value of 46.07%. The results of the analysis of vaname shrimp farming business through the Demonstration Farming program in Barru Regency are said to be feasible to run.*

***Keywords*— Business Feasibility, Demonstration farming, Vaname Shrimp Cultivation**

I. INTRODUCTION

The potential for shrimp cultivation development in Indonesia is very open because of the biophysical conditions of the waters that strongly support farm cultivation and the market is still very open, both abroad and nationally. Shrimp as the main commodity of Indonesian fishery exports that became prima donna by contributing a share of 34.83% of the total export value (KKP, 2021). The direction of aquaculture development policy by the government aims to increase production by policy transformation through sustainable management of aquaculture resources.

The use of advanced technology in the process of fisheries development is one of the absolute conditions in the development. The use of technology used in the process of fisheries development including aquaculture in farmland will not be separated from technological advances, especially if there is a desired change towards the progress of aquaculture itself. This can be seen one of them with the difference in the level of use of technology applied by shrimp farming communities in the field ranging from traditional to intensive, even super intensive (Yanti, 2015).

The difference in the level of this technology will further have implications for the financing needed in the implementation of shrimp farming activities. In this case it can be argued that traditional technology requires less financing than technology intensive. To increase the production and productivity of ponds today is not easy, because there is a reluctance to accept new technologies that have not been practiced and seen directly the accuracy in increasing productivity, as well as trauma experienced due to failure and material losses that are not a little invested in the pond due to disease attacks that cause crop failure.

In this case, the government took a role by rolling out the Demonstration Farming program which is a stimulant to stimulate the interest of shrimp farmers in increasing the knowledge and support of members of farming groups / fishermen and setting an example to apply new technologies through group cooperation. Demonstration farming can optimize ideal land, minimize failure and increase productivity and be environmentally friendly to maintain business continuity.

The program rolled out by the KKP established one of the regions in South Sulawesi province, Namely Barru Regency as a pilot in the Demonstration farming program. Barru Regency is one of the areas in South Sulawesi province that has the potential of vaname shrimp cultivation and has a cultivation area of 2,500.11 ha. Vaname shrimp cultivation system in Barru Regency there are 4 types namely (DKP, 2021): traditional pond (828.71 Ha), simple (1,484.67 Ha), semi intensive (130.05 Ha) and intensive (56.68 Ha). The largest number of farm land use with traditional and simple systems amounted to 92% of the total vaname shrimp cultivation land in Barru Regency.

The main problem in the vaname shrimp farming system is in the source of capital. The greater the capital owned, the use of technology in the cultivation system is growing. With the condition of pond cultivation carried out by individuals will always have difficulty to develop their business both from capital sources and human resources. Demonstation Farming program by means of cultivation application carried out in the form of groups will facilitate the development in receiving capital assistance and the cultivation system will be more well organized.

Based on the above problems, the government's efforts in dealing with these problems by optimizing production and produtivity require effective and efficient adaptive technology through demonstation farming pilots in vaname shrimp farming business. Financial business analysis shows the success rate in the vaname shrimp farming business that is being run. Financial business analysis includes total business costs, receipts, profits, Net Benefit-Cost Ratio (Net B/C) analysis, Net Present Value (NPV) analysis and Internal Rate of Return (IRR) analysis. Based on the above, the puIDRose of this study is to find out the amount of income and feasibility of the vaname shrimp farm cultivation business in the application of Demfarm in Barru Regency.

II. METHODOLOGY

2.1. Location and Time of Research

This research will be conducted from November 2020 to June 2021 in Barru Regency, South Sulawesi Province.. This location was chosen deliberately (puIDRosive) with the consideration that Barru Regency is one of the areas that implement demonstration farming program, with the aim to increase the produtivity of vaname shrimp in Barru Regency.

2.2. Sampling Methods

The population in the study was a vaname shrimp farmer who received assistance from the Demonstration Farming program. The total number of vaname shrimp farm farmers in Barru Regency who received demfarm program assistance as many as 37 people who were divided into 2 groups of cultivators or Pokdakan, among others (DKP, 2020): Pokdakan Rezky Flow (21 people) Balusu Subdistrict and Pokdakan Sipatangae (16 people) Tanete Rilau District.

The sampling technique in this study is puIDRosive sampling. PuIDRosive sampling is a sampling technique with certain considerations (Sugiyono, 2013) that this research sample is a group of recipients of the demonstration farming program specifically vaname shrimp in Barru Regency.

2.3. Types and Sources of Data

The type of research used is survey research. Survey research is a study that takes samples from one population and uses questionnaires as a basic data gathering tool (Singarimbun, 2008). To answer the puIDRose of the research, researchers use qualitative and quantitative analysis.

The data sources used in this study are primary data and secondary data. Primary data sources are obtained through documentation and interviews with pokdakan which includes the process of implementing the program, financial analysis, as well as the level of income and productivity of vaname shrimp during the Demfarm program. Secondary data is obtained from literature studies or literature studies that are relevant or related to the Demfarm program, data from the Fisheries Office and the Central Bureau of Statistics of Barru Regency, the results of reports, as well as previous research that can support research studies.

2.4. Data Analysis Methods

The data analysis method used is a mix method that is qualitative and quantitative methods. Qualitative methods are carried out descriptively according to analysis (Sugiyono, 2013) is a method that serves to describe or give an overview of the objects studied through data or samples that have been collected as is without conducting analysis and making conclusions that apply to the public.

Descriptive analysis is used to describe the implementation of demonstration farming program in Barru Regency which was implemented from 2019 to 2020.

Quantitative methods are used to determine the level of profit and feasibility of vaname shrimp farming business during the running of demonstration farming program. To find out the amount of production and profits received from the cultivation of vaname shrimp during the Demfarm program used the following equations (Bangun, 2010):

$$\Pi = TR - TC$$

Where :

π = Net Profit (IDR)

TR = *Total Revenue* (IDR)

TC = *Total Cost* (IDR)

To search for Total Acceptance can be used formula as follows (Wake, 2010):

$$TR = Q \times P$$

Where :

TR = *Total Revenue* (IDR)

P = Price (IDR)

Q = Quantity (Kg)

As for finding the Total Cost can be used formula (Bangun, 2010):

$$TC = FC + VC$$

Where:

TC = *Total Cost* (IDR)

VC = *Variable Cost* (IDR)

FC = *Fixed Cost* (IDR)

To calculate the feasibility of a business from the cultivation of vaname shrimp during the Demfarm program, use the calculations of NPV, Net B / C and IRR with the following calculation methods:

Net Present Value (NPV) can be formulated as follows (Pasaribu, Yusuf, & Amiluddin, 2004):

$$\text{NPV} = \Sigma \frac{B_t - C_t}{(1+i)^t}$$

Where:

Bt = annual gross income

Ct = Annual gross expense

(1+i) t = *discount factor(DF)*

T = interest rate

The decision-making criteria are based on:

NPV > 0, meaning that shrimp farming efforts are worth working on.

NPV = 0, meaning that shrimp farming business is as large as the value invested with the amount of value produced.

NPV < 0 means that the shrimp farm cultivation business is worth trying.

Net benefit-Cost ratio (Net B/C) can be formulated as follows (Primyastanto, 2011):

$$Net\ B/C = \frac{\sum_{t=0}^{t=n} NPV\ (+)}{\sum_{t=0}^{t=n} NPV\ (-)}$$

Where :

NPV (+) = NPV positif value

NPV (-) = NPV negative value

Internal Rate of Return (IRR) can be use formulated as follows (Pasaribu, Yusuf, & Amiluddin, 2004) :

$$IRR = i' + \left(\frac{NPV'}{NPV' - NPV''}\right)(i'' - i')$$

Where:

i' = Interest rates that generate positive NPV

i"= The interest rate that generates negative NPV

NPV ' = NPV at interest rate i'

NPV '' = NPV at interest rate i"

The decision-making criteria are based on:

IRR> i1 = shrimp farm cultivation efforts are considered feasible

IRR< i1 = shrimp farm cultivation efforts are considered unfit

III. RESULTS AND DISCUSSION

The implementation of a policy is basically a change or transformation that is multiorganization, where the changes implemented through the implementation strategy of this policy connect various layers of society. Demfarm program implemented in Kab. Barru involves various elements or institutions both government and private, demfarm program prioritizes a partnership system. Currently, the partnership system has been running well by involving several institutions, namely the Directorate General of Aquaculture through UPT Kab. Barru which monitors the production process, pest control and pond environment. Partners for production needs such as seedlings, feed, and medicines involve third parties who cooperate with PT. Esa Putli Barru and CV. Irawan. The extension team plays a role in accompanying pokdakan during the cultivation process ranging from land preparation to post-harvest.

Demonstation farming in Barru Regency through socialization activities carried out by the extension team under the supervision of the Marine and Fisheries Service. After that the extension team conducted a direct survey to demfarm location who will receive assistance and direction from the activity. Demfarm activities are a series of shrimp pond production processes in an integrated manner by applying technology, namely the use of watermills in traditional types of ponds whose puIDRose is to increase shrimp production. Demfarm's activities involve various elements such as government, private, partners, and prospective recipients of assistance (pokdakan).

The formation of a group of fish farmers (pokdakan) is one of the conditions in implementing Demfarm activities. Prospective recipients of demfarm program assistance must submit to the extension by attaching a budget proposal for activities. The group that had been formed under the supervision of the extension team was then submitted to the Barru Regency DKP office. The submission of the establishment of pokdakan must attach some requirements that have been set, such as a notary deed as proof of land ownership and not in dispute status, SKT, has a KUSUKA card (fisheries business card), a group of at least 10 people. Once all the requirements are met, the pokdakan already has the status as a prospective recipient of Demfarm program assistance.

The role of the private sector in the Demfarm program is to facilitate benur, feed and medicines to be used during the cultivation process. The partners participating in this program are PT. Irawan as a supplier of feed and medicine and PT. Benur Kita as a supplier of vaname shrimp benur. As for the needs of facilities and infrastructure such as ferris wheels, waterways, and fertilizers will be facilitated by the government. The Ministry of Marine Affairs and Fisheries through the Barru Regency Aquaculture Production Business Service Center provides technician assistance ranging from the application of technology, disease pest control and water quality. Technicians will give directions to pokdakan in each stage of cultivation and will conduct disease control and water quality every two weeks. Assistance by tekisi is carried out ranging from land preparation, water intake, benur spreading, shrimp maintenance, harvesting process to post-harvest.

Demfarm program has the main goal of changing the mindset of petambak from individually to communal (cluster system) and strengthen the entrepreneurial spirit among traditional farmers. The traditional way that shrimp farmers do, especially in Balusu and Tanete Rilau sub-districts in Barru Regency in the lau currently began to switch to the use of cultivation technology. Based on the results of research on intensive adoption of shrimp farming technology through the implementation of demonstration farming program in Karawang Regency shows that farmers who receive the program can adopt 91% of the overall technology recommended in intensive shrimp cultivation. The improvement of cultivation technology is inseparable from the institutional development of the cultivation group to be able to strive economically and profitably (Sukardi, 2002). Another thing that by increasing the productivity of farmland has a more positive impact compared to expanding cultivated land (Mu'tamar, et al 2013). Another advantage of this program is the establishment of good cooperation and synergy between farmers and governments, partners and stakeholders to advance and develop the national shrimp industry as had been achieved in the previous decade.

3.1 Financial Analysis of Vaname Shrimp Farm Cultivation Business

Feasibility study is a research study conducted on an institution on a particular project that is being or will be implemented. According to (Primyastanto, 2011) this study is used to provide direction on whether investments in certain projects are worth implementing or not, on the basis *of risk and uncertainty* in the future, multidisciplinary studies are needed before taking place.

Financial business analysis shows the success rate in the vaname shrimp farming business that is being run. Financial business analysis includes total business costs, receipts, profits, *Net Benefit-Cost Ratio* (Net B/C) analysis, *Net Present Value* (NPV) analysis and Internal Rate *of Return* (IRR) analysis. To find out the benefits of the vaname shrimp farm cultivation business in the application of Demfarm in Barru Regency, it is necessary to know the amount of investment, fixed costs, variable costs, total costs, receipts and the level of profit obtained in each production process of vaname shrimp ponds.

3.1.1. Vaname Shrimp Farming Business Investment

Investment is investment in an activity that has a relatively long period of time in various business fields (Amiluddin, et al 2020).

To see the amount of investment in vaname shrimp farming business can be seen in the following table.

Table 1. Average value of investment in vaname shrimp farming business

No.	Type of Investment	Average Investment Value (IDR)	Percentage (%)
1.	Farmland	238,648,649	82.5
2.	Generator set	7,658,000	2.6
3.	Water pump	4,917,189	1.7
4.	Ferris wheel	15,105,405	5.2
5.	Waring	21,628,378	7.5
6.	Anco	360,811	0.1
7.	Harvest mesh	913,514	0.3
8.	Spiral hose	173,020	0.1
	TOTAL	289,404,966	100

Source: Primary data after processing, 2021.

Based on the results of interviews and data analysis, it can be seen the components and value of investment in the business of shrimp farming vaname simple patterns in Barru Regency which includes farmland, generator sets, water pumps, ferris wheels, waring, anco and harvest roads. The average land area owned by demfarm program recipients is between 0.7 ha to 5 ha. Based on table 1 above it is known that the total average investment value is IDR. 289,404,966.

In the implementation of the Demfarm program, the government provides some assistance for investment value to pokdakan, namely in the form of water pumps, ferris wheels and generator sets. Demfarm program is designed to give stimulants to cultivators who still apply traditional pattern ponds to increase into simple or traditional plus patterns. The puIDRose of this program is to increase the productivity of vaname shrimp cultivated in Barru Regency.

3.1.2. Vaname Shrimp Farming Business Costs

3.1.2.1. Fixed Cost *(Fix cost)*)

A fixed cost is a cost that within a given period of amount is fixed, and does not depend on the amount of production. The fixed cost in question is the main capital and the result of the amount of depreciation costs in a year from investment of farmland, generator sets, water pumps, ferris wheels, waring, anco and harvest roads. The average amount of depreciation costs on the investment value of vaname shrimp farming business can be seen in the table below;

Table 2. Average depreciation cost / year on investment of vaname shrimp farming business

No.	Type of Investment	Average Depreciation Value (IDR)	Percentage (%)
1.	Farmland	23,864,865	72.4
2.	Generator set	1,914,500	5.8
3.	Water pump	983,438	3.0
4.	Ferris wheel	3,021,081	9.2
5.	Waring	2,703,547	8.2
6.	Anco	120,270	0.4
7.	Harvest mesh	182,703	0.6
8.	Spiral hose	173,020	0.5
	TOTAL	32,963,424	100

Source: Primary data after processing, 2021.

Based on table 2 above it is known that the average depreciation cost in the business of cultivating a simple pattern of vaname shrimp ponds with the highest value is pond land of IDR. 23,864,865 with a percentage of 72.4%, while the average lowest depreciation value is anco of 120,270. The total average depreciation value that must be incurred per year is IDR. 32,963,424 which is the total fixed cost (Fix cost).

3.1.2.2. Variable*Cost (Variable cost)*)

Veriabel cost is the amount of production costs that change according to the high amount of output to be produced. The greater the output or goods that will be produced, the greater the variable costs that will be incurred. The variable costs in question are benur, feed, lime, fertilizer, medicines, saponents, solar generators and labor.

Table 3. Average variable cost/year in vaname shrimp farming business

No.	Kind	Average Variable Cost (IDR)	Percentage (%)
1.	Benur	28,637,838	21
2.	Feed	40,199,351	29
3.	Lime	10,739,189	8
4.	Fertilizer	3,436,541	2
5.	Drugs	17,280,000	13
6.	Saponents	1,055,676	1
7.	Solar generator set	28,662,162	21
8.	Workforce	7,617,568	6
	TOTAL	137,628,324	100

Source: Primary data after processing, 2021.

Based on table 3 above it is known that the average variable cost incurred by the farmer of a simple pattern vaname shrimp pond with the highest value at feed cost of IDR. 40,199,351, while the average variable cost with the lowest value on saponent costs is IDR. 1,055,676. The total average variable cost to be incurred amounted to IDR. 137,628,324 per year.

3.1.2.3. Total *Cost (Total cost)*

The total cost is the overall amount of production costs incurred (Bangun, 2010) which is the total sum of fixed costs with variable costs incurred in the period of one year. The average total cost incurred by shrimp farm farmers vaname simple patterns in one year can be seen in the table below;

Table 4. Average total cost/year of vaname shrimp farming

Type of Cost	Average Value (IDR)	Percentage (%)
Fixed costs	32,963,424	19.3
Variable Cost	137,628,324	80.7
TOTAL	170,591,749	100

Source: Primary data after processing, 2021.

Based on table 4 above it is known that the average total cost incurred by the farmers of a simple pattern vaname shrimp pond during the one-year period is IDR. 170,591,749 which is divided from fixed costs of IDR. 32,963,424 and variable costs of IDR. 137,628,324.

3.1.3. Acceptance of Vaname Shrimp Farming Business

Business acceptance is the total income received by farmers from the activities of the sale of vaname shrimp that has not been reduced by the total cost. The average value of receipts in shrimp farming business vaname simple pattern for production for one year can be seen in the table below;

Table 5. Average value of receipts / year in vaname shrimp farming business

Price (IDR/Kg)	Average amount/year (kg)	Average receipt/year (IDR)
50,000	7,159	357,972,973

Source: Primary data after processing, 2021.

Based on table 5 above it is known that the average amount or yield in the business of shrimp farming vaname simple pattern in one year amounted to 7,159 Kg with an average total annual receipt of IDR. 357,972,973. The price that applies at that time is IDR. 50,000 for the sale of vaname shrimp with an average size of 50-60.

The large number of receipts from the cultivation of vaname shrimp is influenced by the level of use of cultivation technology applied through the Demfarm program. Based on the results of the interview to pokdakan which stated that before the implementation of the Demfarm program, the average harvest received was only 400-600 kg / Ha peaches and after receiving the Demfam program the average yield received was 700-1000 kg / Ha peaches. This is in accordance with the results of research conducted by (Rahman, 2015) in Blanakan Subdistrict which became one of the central areas of demfarm program in an effort to increase pond productivity. The results of the research conducted stated that the development of pond business with Demfarm system was able to increase the productivity of ponds by 7.00-7.50 tons / Ha or 7000-7500 kg / Ha, where such conditions provide support in the development of pond businesses.

3.1.4. Benefits of Vaname Shrimp Farming Business

Profit is the difference between the results of production sales and business costs incurred. The small amount of profit obtained by cultivators is influenced by the small cost of production incurred. Meanwhile, the small cost of production incurred affects the size of the land owned. As for the average profit received by shrimp farmers vaname simple patterns can be seen in the table below;

Table 6. Average profit/year in vaname shrimp farming business

Average receipt/year (IDR)	Average Cost/year (kg)	Average Profit/year (IDR)
357,972,973	170,591,749	187,381,224

Source: Primary data after processing, 2021.

Based on table 6 above it is known that the average profit received by shrimp farmers vaname simple pattern in one year amounted to IDR. 187,381,224. The amount of profit received by cultivators varies according to the area of land owned. The larger the land for the cultivation of vaname shrimp, the greater the production costs needed, the greater the crop and profits received from the shrimp farming business.

3.2. Business Faesibility Analysis

feasibility studies are used to provide direction whether investment in a particular project is feasible or not. On the basis of risk and uncertainty (risk and uncertainty) in the future, a multidisciplinary study is needed before making a decision (Primyastanto 2011). The total profit received in the vannamei shrimp farming business is not a measure of the success of the business to be maintained. After knowing the level of profit in vaname shrimp cultivation, it is necessary to analyze the feasibility of the business to see whether the business is feasible to run or will suffer losses in the next few years. The feasibility analysis used in this study uses indicators of Net Present Value (NPV), Net Benefit-Cost Ratio (Net B/C) and Internal Rate of Return (IRR), as follows:

Table 7. Financial and Feasibility Analysis in Shrimp Cultivation Business

No	Cash flow	Project Year					
		0	1	2	3	4	5
A	**Inflow**		357,972,973	369,821,878	382,062,983	394,709,267	407,774,144
B	**Outflow**						
1	DF 14%	1	0.877	0.769	0.675	0.592	0.519
2	Investment Fee						
a	Land	238,648,649					
b	generator	7,658,000					7,658,000
c	Water pump	4,917,189					
d	Ferris wheel	15,105,405					
e	Waring	21,628,378					
f	Anco	360,811				360,811	
g	Harvest Net	913,514					
h	Spiral hose	173,020					
	Total Investment	289,404,966				360,811	7,658,000
3	Variable Cost						
a	Seeds		28,637,838	29,030,176	29,427,890	29,831,052	30,239,737
b	feed		40,199,351	40,750,082	41,308,359	41,874,283	42,447,961
c	Chalk		10,739,189	10,886,316	11,035,459	11,186,644	11,339,901
d	Fertilizer		3,436,541	3,483,621	3,531,347	3,579,726	3,628,768
e	Drugs		17,280,000	17,516,736	17,756,715	17,999,982	18,246,582
f	Saponens		1,055,676	1,070,138	1,084,799	1,099,661	1,114,726
g	solar generator		28,662,162	29,054,834	29,452,885	29,856,390	30,265,422
h	Labor		7,617,568	7,721,928	7,827,719	7,934,958	8,043,667

	Total Variable Cost		137,628,324	139,513,832	141,425,172	143,362,697	145,326,766
4	Total Outfllow	289,404,966	137,628,324	139,513,832	141,425,172	287,447,015	305,969,531
5	Outflow 14%	289,404,966	120,726,600	107,351,364	95,457,963	170,191,709	158,910,987
C	**Net Cash Fllow**	(289,404,966)	220,344,649	230,308,046	240,637,811	107,262,252	101,804,613
	NCF 14%	(289,404,966)	193,284,780	177,214,563	162,423,668	63,507,864	52,874,126
D	**PV Positif**	649,305,000					
E	**PV negatif**	(289,404,966)					
F	**NPV**	418,514,544					
G	**IRR**	46.07%					
H	**NET B/C**	2.24					

Source: Primary data after processing,2021

3.2.1. Net Present Value (NPV)

Net Present Value (NPV) can be defined as the present value of cash flows generated by investments. Based on the results of the analysis on vaname shrimp cultivation in Barru Regency after the implementation of the Demfarm program, it is financially feasible to run. The results of the analysis of the data contained in table 7 are known that the feasibility value of the simple pattern vaname shrimp farming business with theindicator Net Present Value (NPV)has a value of IDR. 418,514,544. This can be seen from the Net Present Value (NPV) which has a positive number (greater than zero) at the applicable interest rate in 2020 of 14% which means the business will benefit with a positive residual value at the end of the year of activity, with 5 year project period.

3.2.2. Net Benefit-Cost Ratio (Net B/C)

Benefit and Cost Ratio (Net B/C Ratio) is a method of calculating the comparison between the present value of future net cash receipts and the present value of the investment. In the analysis of the Net Benefit-Cost Ratio (Net B/C) obtained from the comparison of positive net benefits and negative net benefits, it is the profit value that will be obtained from every one rupiah of costs (costs) incurred during the life of the business at an interest rate of 14% in Indonesia. 2020. The results of the analysis of the data contained in table 7 show that theindicator Benefit-Cost Ratio (Net B/C) has a value of 2.24 which means that every 1 rupiah spent will increase the benefit of IDR. 2.24. This is in accordance with (Nainggolan, 2018) which states that the profit-to-cost ratio (Net B/C) with a value > 0 indicates that the vannamei shrimp farming business is feasible to run. The greater the value of the profit-to-cost ratio, the greater the benefits that will be obtained from the business.

3.2.3. Internal Rate of Return (IRR)

Internal Rate Return is the interest rate that equates the present value of the expected cash outflows with the present value of the expected cash inflows, or also defined as the interest rate that causes the Net Present value (NPV) to be equal to zero. The value of internal business returns to show the level of business ability to pay interest on business loans during the life of the business is analyzed using IRR (Internal Rate of Return). The results of the analysis of the data contained in table 7 show that theindicator Internal Rate of Return (IRR) has a value of 46.07% which indicates that the vannamei shrimp farming business has the ability to pay loan interest if this business is developed using credit from formal financial institutions. This is consistent with the results of the study (Wafi.dkk, 2021) states that the value of the IRR greater than the interest rate in effect at this time, then the business 11 are eligible to run. By knowing the IRR value, the company can determine a productive business strategy during the current investment period.

IV. CONCLUSION

Implementation of the demonstration farming program on vaname shrimp cultivation in 2 sub-districts of Barru Regency succeeded in changing the mindset of farmers from the original farming individually to communal (cluster/group system) as well as strengthening the entrepreneurial spirit among the farmers. The traditional way carried out by shrimp farmers, especially in Balusu and Tanete Rilau sub-districts in Barru Regency in the past is now starting switch to the use of technology that they do on the basis of recommended technology applied to Demfarm. The implementation mechanism consists of: the whole series of cultivation processes starting from pre-production to post-harvest has been done well and can provide improvement of skills and income cultivation. Realization of program achievements carried out in accordance with the results expected, this is due to an increase the result of vaname shrimp cultivation previously only 500 kg/ha now to 1000 kg/ha.

Based on the results of the analysis on vaname shrimp cultivation business in implementation of demonstration farming program shows the level of profit with the value of the average received by cultivators in one year of Rp. 187,381,224 and the value of feasibility in the cultivation business simple pattern vaname shrimp with Net indicator Present Value (NPV) has a value of Rp. 418,514,544, the Benefit-Cost Ratio (Net B/C) indicator has a value of 2.24, and the Internal Rate of Return (IRR) indicator has a value of 46.07% and it can be concluded that the business is feasible to run.

REFERENCES

[1] Amiluddin, Baso, A., Najamuddin, & Hamzah. (2020). Financial Feasibility Analysis of Skipjack Tuna (Katsuwonus pelamis) Catching in Bone Bay, South Sulawesi, Indonesia. *In IOP Conference Series : Earth and Environmental Science* (p. 12164). Makassar: IOP Publishing.

[2] Bangun, W. (2010). *Microeconomic Theory.* Bandung: Publisher : Refika Aditama.

[3] Department of Marine Affairs and Fisheries (DKP). 2021. Fisheries and Cultivation Data Statistics of Barru Regency. Published annually.

[4] Maritime Affairs and Fisheries (KKP) (2021). Increasing the Competitiveness of Marine and Fishery Products.Retrieved from the Strategic Plan of the Directorate General of PDSPKP 2020-2024: https://kkp.go.id/djpdspkp/artikel/21730-draft-resntra-ditjen-pdspkp-tahun-2020-2024. diakses Selasa 30 November 2021

[5] Mu'tamar, Eriyanto, Machfud, & Soewardi. (2013). Dynamic Model Analysis of Raw Material Supply in Minapolitan Shrimp Agroindustry. *Industrial Engineering Latters*, ISSN 2224-6096. Vol 3. No.11.

[6] Nainggolan, A.,I.,S (2018). Study of Financial Feasibility of Vanamei Shrimp (Litopenaeus vannamei) Cultivation Business in Pantai Cermin District, Serdang Bedagai Regency, North Sumatra Province. Aquatic Resources Management study program. University of Northern Sumatra.

[7] Nasution, Z., & Yanti, B. I. (2015). Adoption of Intensive Shrimp Cultivation Technology in Pond Ponds. Journal of Social Policy *KP*, Vol. 5 No. 1. Hal 1-9.

[8] Pasaribu, A. M., Yusuf, D., & Amiluddin. (2004). *Fishery Project Planning and Evaluation. Makassar: Hasanuddin Press University (LEPHAS).*

[9] Primyastanto, M. (2011). *Feasibility Study Usaha Perikanan (Sebagai Aplikasi dari Teori Studi Kelayakan Usaha Perikanan).* Malang: Brawijaya *University* Press (UB Press).

[10] Rahman, S. (2015). *Study on the Effectiveness of the Implementation of the Shrimp Farm Demonstration Program in Subang Regency. Bogor: Bogor Agricultural University.*

[11] Singarimbun, M. d. (2008). *Survey Research Methods. Jakarta: LP3ES Library.*

[12] Sugiyono. (2013). *Research Methods Quantitative, Qualitative and R & D. Bandung: Alfabeta.*

[13] Sukardi. (2002). Improvement of Aquaculture Technology. Indonesian Journal of Ichtiology, Vol. 2. DKP.

[14] Wafi, A., Heri,A., Abdul,M., Benny, D.M.,(2021). Business Feasibility Of Intensive Vaname Shrimp (*Litopenaeus Vannamei*) With Non-Partial System. ECSOFiM: Journal of Economic and Social of Fisheries and Marine. 08(02):226-238.

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An Assessment of Sustainable energy management at a Major Scandinavian Hub Airport: The Case of Oslo Airport Gardermoen

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Abstract— *Using on an in-depth qualitative instrumental case study research approach, this study has examined Oslo Airport Gardermoen sustainable energy management. The study period was from 2005 to 2020. The qualitative data was analyzed by document analysis. The airport purchases electricity and heating and cooling energy from external vendors. The non-renewable energy sources include aircraft jet fuel, heating oil/diesel, fuel and biofuels for the airport's vehicles, and supplies of paraffin/Jet A1 fuel and propane, with the latter fuel sources being used for the fire drills conducted at the airport. The case study found that throughout the study period Oslo Airport Gardermoen has implemented extensive energy conservation measures and technologies that have enabled the airport to mitigate its impact on the environment. The energy conservation measures include the extensive use of LED lighting, the replacement of a ventilation unit with a free cooling unit, an upgrade to the airport's heat exchanger, the introduction of more energy efficient rotary heat recovery units, and the replacement of flood lighting around the airport's terminal building with new LED lighting. The airport also installed blanking pedestals on the existing lights in Hangar 8. Other energy saving measures included the installation of motion sensors and night lowering in Pir Syd, the night lowering of lighting in Pier Norda and SBV, and the automatic control of ground heating systems at the airport's apron. A very important environment-related energy measure has been the use of sustainable aviation fuels. Oslo Airport was the world's first airport to offer sustainable jet biofuel to all airlines serving the airport.*

Keywords—***Airports, Case study, Energy, Energy saving measures, Oslo Airport Gardermoen, Sustainable airport energy management.***

I. INTRODUCTION

Airports are quite unique entities that have substantial economic, social, and environmental impacts on local, regional, and often in many cases, national levels. However, there are both social and environmental costs associated with the construction and operation of airports (Culberson, 2011). The operations of airports have a variety of impacts on both local communities and the natural environment. These impacts include noise, local air quality issues, significant energy consumption, large water use, and the generation of wastes (Daley, 2016; Graham, 2018; Thomas & Hooper, 2013). Airports located throughout the world are increasingly becoming environmentally concerned, and, as a result, have increased their efforts to reduce air transport impacts on the environment by applying environmental management, certification systems, or other forms of ecological rating systems to their infrastructures and operation (Comendador et al., 2019). Indeed, in recent times, the air transport industry has initiated new sustainability efforts in response to society's greater requirements for living in healthier and more sustainable environments (Monsalud et al., 2015). Accordingly, many airports have implemented strategies and initiatives to "green" themselves, that is, to become more sustainable in the medium to long-term (Janić, 2011; Kumar et al., 2020; Prosperi, 2009).

Airports are extremely energy intensive (Baxter et al., 2018a, 2018b; de Rubeis et al., 2016). Consequently, many airports are increasing their energy efficiency as part of their efforts to reduce their impact on the environment and, more broadly, on climate change (Preston, 2015). Indeed, airports are now under growing pressure to use energy-efficient systems whilst also complying with increasingly stringent regulations. At the same time, airports are still required to provide occupant thermal comfort. Furthermore, energy efficiency enables airports to reduce their operating costs and reduce their carbon footprint (Taber & Steele, 2020).

In addition, airports are no longer simply based on aviation related functionalities (for example, passengers, air cargo, and aircraft handling facilities), rather they have changed, and many airports now have shopping and hotel complexes, conference facilities, industrial zones, logistic centres as well as inter-modal public transport hubs (Ferrulli, 2016). Airports have become multimodal/functional businesses and have established significant commercial development both inside and outside of their boundaries (Reiss, 2007). Many airports have become true "airport cities" (Appold & Kasarda, 2011; Efthymiou & Papatheodorou, 2018; Orth & Weidmann, 2014). These non-aviation facilities and their operations can increase the airport's energy requirements quite significantly.

The aim of this research is to empirically examine the sustainable aspects of airport energy management, in the context of Oslo Airport Gardermoen, Norway's principal air traffic hub. Environmental management forms an integral part of Oslo Airport Gardermoen's governance and management system. The airport's management system covers all regulatory requirements regarding internal control and is based on relevant requirements specified in international standards such as ISO 9001 Quality Management and 14001 Environmental Management Systems (EMS) (Avinor, 2021d). Oslo Airport Gardermoen Environmental Management System was certified as following the standard ISO 14001 standards in March 2014 (Vestvik-Lunde, 2014). Oslo Airport Gardermoen was selected as the single site in-depth exploratory case studied in this research. A further factor in selecting Oslo Airport Gardermoen as the case firm was the ready availability of the airport's annual environmental data for the period 2005 to 2020. A further key objective of the present study is to examine the airport's energy sources and the trends in the annual consumption of these energy sources, as well as examining the measures and technologies that have been implemented by Oslo Airport Gardermoen to mitigate the environmental impact of the energy consumed at the airport throughout the study period.

The remainder of the paper is organized as follows: Section 2 presents a review of the literature on sustainable airport energy management. The research method used to underpin the study is described in Section 3. The case study of Oslo Airport Gardermoen sustainable energy management is presented in Section 4. Section 5 presents the findings of the study.

II. BACKGROUND

As previously noted, airports are viewed as being extremely energy-intensive (Baxter et al., 2018a; Alba & Manana, 2017; Sukumaran & Sudhakar, 2017). Airport buildings are especially energy intensive (Kim et al., 2020; Yildiz et al., 2021). The significant energy consumption of an airport is due to the large buildings, which comprise the passenger terminals and non-passenger-related areas of the airport, that are equipped with heating and air-conditioning systems. Airports also have a high-power demand for lighting and electric equipment as well as the energy requirements from the many facilities located within the airport precinct (Cardona et al., 2006). Typically, an airport's heating, ventilation, and air conditioning (HVAC) system will represent the largest share of energy consumed in airport terminal buildings. It has been estimated that around 70% of the energy consumed in airport terminal buildings is used for heating, cooling, and air conditioning purposes. This energy consumption rate is higher in those countries that have a cold climate (Akyüz et al., 2017). Indeed, airport terminals have a high level of energy consumption for space heating in cold climate zones (Liu et al., 2021).

Airports need to provide electrical energy for the airfield aids that are used for air transport operations, for example, lighting and meteorological systems. Electrical energy is also required for airport buildings, aircraft hangers and other airport facilities (Kazda et al., 2015). Hence, energy management, which includes heating, ventilation, air conditioning, and lighting, is extremely important for airports (Graham, 2018). Furthermore, airports need to ensure that they have a guaranteed, appropriately priced, and secure energy supply. This because airports need to meet peak demand from their service partners and passengers thereby optimizing their operational capacity (Thomas & Hooper, 2013).

The operation of more efficient heating and cooling systems and the performance of the building envelope can result in significant reductions in energy consumption.

This can be achieved without compromising comfort conditions in the airport terminal buildings (Akyüz et al., 2018). To ensure that energy demand can be met when the needs arise, airports are increasingly focusing on energy-conservation measures in the design (and operations) of terminal buildings and infrastructure (Thomas & Hooper, 2013). Some airports have also developed and operate new power-generation systems that provide reliable and affordable sustainable energy whilst also lowering their energy costs (Budd & Budd, 2013). Importantly, airports around the world are shifting toward the utilization of clean energy technologies together with the implementation of practices that reduce local emissions. This environmental-related strategy includes replacing fossil fuel-based with electricity-based operations at the airport (Sajed Sadati et al., 2018). Accordingly, airports are increasing their use of renewable energy sources. These include solar photovoltaic, concentrating solar power, wind power, oil and natural gas extraction, steam-generated power production and electricity transmission (Barrett et al., 2014).

Energy consumed by airports can be broken down into the energy consumed by the airside activities undertaken at the airport as well as the energy consumed in the provision of the airport's landside area activities (Janić, 2011). The airside means the movement area at an airport, adjacent terrain and buildings/infrastructure, or portions, the access to which is restricted. Landside means those parts of an airport as well as the adjacent terrain and buildings or portions thereof that are not in the airside precinct (Rossi Dal Pozzo, 2015). In the airport's airside area, energy requirements include the fuel that is consumed by aircraft during the landing and take-off (LTO) cycles. Also, ground vehicles serving aircraft during the turnaround process at the apron/gate complex consume energy. In the airport landside area, the principal consumers of energy are the airport ground access systems/modes and passenger and air cargo terminals together with other administrative buildings serving the airport. In most cases, the primary energy sources are from non-renewable fossil fuels and to a smaller extent from renewable wind, water, and solar sources (Janić, 2011).

Electrical energy is normally sourced from different sources. The electrical energy is supplied directly to the airport through dedicated sub-stations. As noted earlier, this energy is primarily used for heating, cooling, lighting, and operating the airport's facilities, equipment, and other devices in the processes of servicing passengers and their baggage and air cargo consignments in passenger and cargo terminals, respectively. Electrical energy is also used for the provision of heating, cooling (air conditioning), and lighting other administrative buildings at airports (Janić, 2011).

III. RESEARCH METHODOLOGY

3.1 Research Method

This study used a qualitative longitudinal research design (Derrington, 2019; Hassett & Paavilainen-Mäntymäki, 2013; Neale, 2018). Qualitative longitudinal research aims to expand and develop theories (Derrington, 2019). A case study enables the exploration of complex phenomena (Remenyi et al., 2010; Yin, 2018), and thus, the collection of rich, explanatory information that provides in-depth insights into the phenomenon being investigated (Ang, 2014). Case studies also enable the researcher(s) to connect with real world practice (McCutchen & Meredith, 1993).

3.2 Data Collection

The qualitative data for this study was obtained from the annual Oslo Airport Gardermoen and Avinor AS environmental reports. Qualitative data was also gathered from the Avinor AS web sites. The study therefore used secondary data analysis to investigate the research problem.

A comprehensive examination of the leading airport-related journals and airport industry magazines was also conducted. The study also included a search of the SCOPUS and Google Scholar databases.

The key words used in the database searches included "Oslo Airport Gardermoen total annual electricity consumption", "Oslo Airport Gardermoen total annual electricity consumption for airport specific installations", "Oslo Airport Gardermoen total annual purchased heating and colling energy", "Oslo Airport Gardermoen total annual electricity consumption for electrode boiler", "Oslo Airport Gardermoen total annual electricity consumption for compressors and pumps", "Oslo Airport Gardermoen total annual purchased heating and cooling energy", "Oslo Airport Gardermoen total annual heating oil consumption", "Oslo Airport Gardermoen total annual recovered energy", "Oslo Airport Gardermoen total annual heating and cooling energy", the annual jet fuel consumption at Oslo Airport Gardermoen", "Oslo Airport Gardermoen total heating oil/diesel consumption", "Oslo Airport Gardermoen total annual consumption of bio-heating oil", "Oslo Airport Gardermoen total annual consumption of bio-fuel for airport vehicles", "Oslo Airport Gardermoen total annual consumption of fuel for airport vehicles", and "Oslo Airport Gardermoen total annual consumption of fuels for firefighting drills", The study followed the three principles of data collection as recommended by Yin (2018), that is, the use of multiple sources of case evidence, creation of a database on the subject, and the establishment of a chain of evidence.

3.3 Data Analysis

The data collected for the case study was examined using document analysis, which is a research technique that has been extensively used in case study research. Document analysis focuses on the information and data from formal documents and company records (Oates, 2006; Ramon Gil-Garcia, 2012). Following the recommendations of Scott (2004, 2014) and Scott and Marshall (2009), the documents that were gathered for analysis in the study were examined for their authenticity, credibility, representativeness, and meaning.

The study's document analysis was conducted in six distinct stages. The first stage involved planning the types and required documentation and ascertaining their availability for the study. In the second phase, the data collection involved sourcing the documents from Oslo Airport Gardermoen and Avinor AS. This stage of the document analysis process also involved the development and implementation of a scheme for managing the documents collected for the study. The documents were carefully examined to assess their authenticity, credibility and to identify any potential bias in the third stage of the document analysis process. In the fourth stage, the content of the collected documents was carefully examined, and the key themes and issues were identified and recorded. The fifth stage involved the deliberation and refinement to identify any difficulties associated with the documents, reviewing sources, as well as exploring the documents content. In the sixth and concluding stage, the analysis of the data was finalized (O'Leary, 2004).

Following the recommendation of Yin (2018), all the gathered documents were downloaded and stored in a case study database. All the documents collected for the study were in English. Each document was carefully read, and key themes were coded and recorded in the case study (Baxter, 2021).

IV. RESULTS

4.1 A Brief Overview of Oslo Airport Gardermoen

Oslo opened a new airport at Gardermoen, a redundant military airfield, in 1998. This new airport replaced Oslo Fornebu Airport (Feldman, 1998; Iatrou & Williams, 2008). The capacity at Oslo Airport Fornebu was a restraining factor on traffic growth (Lian, 2010). The new airport related high-speed train and improved road system to the airport cost almost NOK 20 billion ($USD 3 billion) (Feldman, 1998). Oslo Airport Gardermoen (IATA Airport Code OSL) is Norway's major airport and air traffic hub. The airport is operated by Oslo Lufthavn AS, which was incorporated in 1992. Oslo Lufthavn AS is a subsidiary of Avinor AS (Bråthen & Fuglum, 2016).

The airport is in the municipality of Ullensaker, approximately 47 kilometres north of Oslo (International Airport Review, 2010). Oslo Airport Gardermoen occupies an area of 13 square kilometres and has two runways 2,950 and 3,600 metres in length. The airport has a total of 72 gates (44 with aerobridges) and 7 gates (4 Code D and 3 Code F) located at the cargo terminals (Avinor, 2021a). A new passenger terminal, which opened in April 2017, has expanded the airport's annual capacity to 32 million passengers (Avinor, 2021e). The new passenger terminal was constructed west of the existing terminal building, and occupies an area of 117,000m². The new triangular-shaped pier termed 'Pier B' or 'North Pier' was built north of the existing terminal building and this pier has a floor area of 63,000m² (Airport Technology, 2017a).

The terminal building 265,000 square metres in size (Avinor, 2021a) and the airport's buildings are equipped with large glass walls. This requires large cooling demands in summer as well as large heating demands in during the winter period (Eggen & Vangsnes, 2006). In addition, the expansion of the airport terminal building has also meant that there has been an increase in the heating and cooling requirements at the airport (Mæx Moe, 2018).

Figure 1 presents the growth in passenger traffic (domestic and international) at the airport from 2005 to 2020. The global financial crisis had an adverse impact on passenger travel with the annual number of enplaned passengers at Oslo Airport Gardermoen declining from 2008 to 2009. Since 2010, passenger traffic has shown a steady increase, however, it declined significantly in 2020 (Figure 1). In 2020, the COVID-19 pandemic caused a decline in economic activity around the world, and this resulted in disruptions in the supply and demand chain for the air travel market (Dube et al., 2021). In addition, because of the global coronavirus crisis, most countries placed restrictive measures in order to confine the pandemia (Maria Iacus et al., 2020), and these restrictions had an adverse impact on airline passenger demand. International passengers comprise the largest share on enplaned passengers at Oslo Airport Gardermoen.

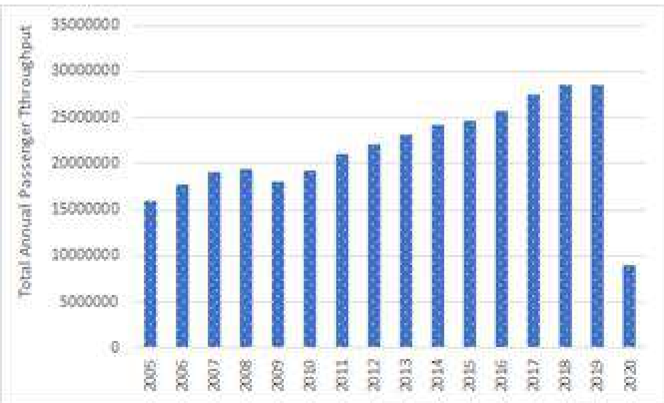

Fig.1: Total annual domestic and international enplaned passengers at Oslo Airport Gardermoen: 2005-2010. Note: From 2013 passengers comprise scheduled and charter and includes infants. Source: Data derived from Avinor AS (2021b).

Figure 2 shows the total annual domestic and international aircraft movements at the airport from 2005 to 2020. Like the annual enplaned passenger traffic, there was a decline in aircraft movements in 2009, however, since 2010, the annual aircraft movements showed a steady annual growth rate before declining significantly in 2020, with the decrease in aircraft movements reflecting the lower level of airline operations as a result of the Covid 19 pandemic.

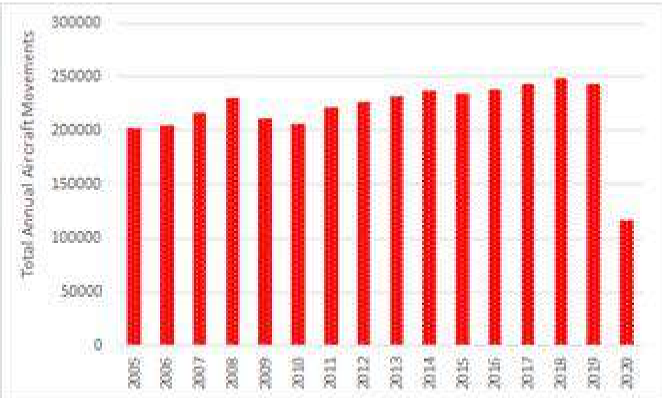

Fig.2: Total annual aircraft movements (domestic and international) at Oslo Airport Gardermoen: 2005-2010. Source: Data derived from Avinor AS (2021b).

4.2 Avinor AS Environmental and Corporate Social Responsibility Policy

Avinor AS is a fully owned state limited company under the Norwegian Ministry of Transport and Communications. Avinor AS is responsible for the management and operations of 44 state-owned airports located throughout Norway (The Avinor Group, 2021). Oslo Airport Gardermoen is managed and operated by Avinor AS. Avinor AS has defined and implemented a comprehensive policy which describes the general principles for environmental and social responsibility in the company. The purpose of this policy is to improve Avinor's own environmental performance, ensure the company is a driving force in the environmental work in the aviation industry, and for the company to be a leader in the work on corporate social responsibility in the Norwegian aviation industry (Oslo Lufthavn AS, 2021, p. 4).

The environmental principles prescribed in the policy are as follows:

- Avinor works to constantly improve its environmental performance and the company actively works to reduce the impact of the business on the environment.
- Avinor is required to comply with all regulatory requirements and its own requirements, and the company's environmental management must be in accordance with ISO14001, to ensure a systematic approach to coordination and follow-up of environmental work.
- Avinor must ensure there is a high degree of environmental awareness and expertise throughout the entire group.
- Employees and partners at the airport must be aware of the Avinor Group's significant environmental aspects.
- Avinor must emphasize and integrate environmental
- Considerations early in the planning and implementation of projects and when purchasing products and materials. Furthermore, the business
- Avinor must place a strong emphasis on the environment in expansion projects.
- Avinor aims to maintain open, constructive, and proactive communication with its partners, local communities, authorities, aviation organizations and other stakeholders in order to reduce environmental impact of its operations.
- Avinor seeks solutions to environmental challenges through cooperation with research and development communities, authorities, and other organizations both at a national and international level (Oslo Lufthavn AS, 2021, p. 3).

Environmental management is an integral part of Avinor's management system. As previously noted, in March 2014, Oslo Airport Gardermoen was certified according to EN-NS ISO14001: 2004. The airport is now a part of the common Avinor certificate, which is also in accordance with ISO standard 14001:2015 Environmental Management System (Oslo Lufthavn AS, 2021). ISO 14001 is a worldwide meta-standard for implementing Environmental Management Systems (EMS) (Dentch, 2016; Grover & Grover, 2017; Heras-Saizarbitoria et al., 2011).

The recognition of being a “green airport” is an important strategic objective for Oslo Airport Gardermoen (Oslo Airport, 2020). A "green airport" is an airport which has a minimal impact on the environment and is one that endeavors to become a carbon neutral facility in terms of carbon emissions, with the aim to ultimately produce zero greenhouse gas emissions (González-Ruiz et al., 2017). The concept underpinning a “green airport” is for the airport to create a centre of sustainable practices (Sumathi et al., 2018). Oslo Airport Gardermoen "Green Airport" strategic objective involves an improvement in the performance and understanding the mechanisms that influence the airport’s environmental reputation. In addition, the airport envisages that through the change of infrastructure and processes, it will reduce the airport’s environmental impact through continuous improvement, innovative solutions and through the focus on the most effective measures. Also, the airport envisages that open and active communication will further strengthen the airport’s environmental reputation (Oslo Lufthavn AS, 2020).

It is important to note that Oslo Airport Gardermoen Energy central has a high capacity and consequently is subject to regulations regarding greenhouse gas emission (GHG) allowance trading. Oslo Airport has been granted a quota-regulated emission permit from the Norwegian Environment Agency for emissions which are subject to quotas and are compensated annually for carbon dioxide (CO_2) emissions in the EU’s quota system (Oslo Lufthavn AS, 2019).

Oslo Airport has been working extremely proactively on energy efficiency measures in recent years, and the airport’s percentage of renewable energy now exceeds 90 percent annually. Furthermore, there has been considerable expansion at Oslo Airport Gardermoen in recent times, and energy consumption is predicted to increase in the future, even when energy efficiency measures are implemented. These energy saving initiatives are examined in the latter section of the paper. Oslo Airport Gardermoen aim was to use only renewable energy by 2020. This goal was anticipated to be achieved through the selection and implementation of energy efficient solutions when modernizing and replacing equipment as well as changing from fossil energy to bioenergy (Oslo Lufthavn AS, 2019).

4.3 Oslo Airport Gardermoen Energy System

The energy system that supplies Oslo Airport’s buildings, tenants, and road heating systems with energy for heating and cooling is comprised of a remote heating plant, a remote cooling plant, a snow cooling plant, a groundwater plant as well as a wastewater heat exchanger system (sewage) (Oslo Airport, 2020, p.11). Heating and cooling energy is distributed through a district heating and cooling network to the airport’s tenants, the Police, the Flyporten Business Centre, the airport terminal, the railway station, the Scandinavian Airlines (SAS) operations building, the SAS Radisson Hotel and the airport’s own buildings (Oslo Lufthavn AS, 2009)

One of the largest groundwater aquifers in Norway is located underneath the airport (Birhanu et al., 2015; French & Binley, 2004; Wejden & Øvstedal, 2006). This aquifer is used for both heating and cooling of Oslo Gardermoen Airport. During summer, ground water is pumped from cold wells and is then used for cooling before it is returned to the warm wells. In winter, this process is reversed, as ground water from the warm wells is used as heat source for the heat pump. The district cooling water is pre-cooled by the ground water, and post cooled by the combined heat pump/refrigeration plant. The airport’s base heat load is covered by the heat pump. Additional heat is supplied from a heat energy central with biofuels as well as oil heated and electrically heated boilers. (Eggen & Vangsnes, 2006). In 2018, the drilling of two deep water geothermal wells 1,500 metres in depth was completed. These wells are used to supply the ground heating system located in the airport’s aircraft engine test area. By keeping snow and ice free all year round, at the time of the present study, the wells were each supplying between 60-100 KWh of heat and did not require the use of a heat pump (Oslo Lufthavn AS, 2019).

Oslo has a continental type of climate, with the city typically experiencing cold winters (Climates to Travel, 2012; Larssen et al., 1994). During the winter period, the airport’s remote heating plant ensures that the buildings located within the airport precinct are kept sufficiently warm. This remote heating plant uses water-based heating. In addition, Oslo Airport also has its own remote heating plant which uses remote heating from Statkraft Varme AS. Woodchips are the source of the heating provided by Statkraft Varme AS (Oslo Lufthavn AS, 2020).

During summer, Oslo Airport Gardermoen remote cooling plant ensures that airport’s buildings are kept sufficiently cool. At Oslo Airport Gardermoen, snow is stored during the winter season in a large basin. During summer, the melting water is used for cooling the airport’s terminal on those days that require extra cooling needs (Oslo Lufthavn

AS, 2020). At Oslo Airport Gardermoen, the snow cooling infrastructure and system stores snow and ice from the runway in the winter and, as previously noted, uses the water from the melting snow for cooling in the summer. This is achieved through the exploitation of the characteristics of snow's thermal energy potential. At Oslo Airport Gardermoen the snow is stored in the ground and is thermally insulated with wood chips (Mæx Moe, 2018). At Oslo Airport Gardermoen, the gathered snow is divided into two categories, pure and impure, that is, whether it contains or does not contain de-icing chemicals from runway and taxiway de-icing. The pure snow is collected in a large snow stockpile shaped like a basin. When the basin is full it is covered with sawdust. Sawdust insulates very effectively, allowing the airport to utilize the cold temperatures in the snow and ice. The coldness of the meltwater is recovered in a heat exchanger and transferred to the airport's central cooling plant. The meltwater is subsequently returned to the stockpile to repeat the heat exchange with the snow and ice in the snow stockpile. This is followed by a new cycle of transmission of cold energy to central cooling systems. The energy in the snow and the cold meltwater is subsequently used to cool the airport's North Pier on hot days (Avinor, 2021f).

Oslo Airport Gardermoen also uses grey water and the ground water under the airport for its heating (Airport Technology, 2017b). Grey water is water that is slightly contaminated by human activities and may possibly be reused following suitable treatment (Liu et al., 2010). The airport's terminal building is also designed as a "passive house" which is a German concept; this means the terminal building is designed so it requires less energy. Furthermore, the airport's terminal building has good insulation throughout the whole building; the windows minimize energy loss, and the terminal building has a solar screen to keep the sun away during sunny periods (Airport Technology, 2017b).

In addition, the airport's groundwater plant provides an interim storage facility for surplus energy. Large heat pumps, groundwater wells, heat exchangers to the airport's sewerage system (from the municipality of Ullensaker's treatment plant) and surface water represent the principal contribution to Oslo Airport's high percentage of renewable energy. The fossil fuel boilers have been assigned a low priority. These boilers are only used for test operations and during the times that Statkraft Varme and the electric boiler are unable to supply sufficient energy (Oslo Lufthavn AS, 2020).

Oslo Airport Gardermoen's passenger terminal building was also the first airport building to be awarded an Excellent BREEAM rating (Building Research Establishment Environmental Assessment Method) (Business Traveller, 2017; Ros, 2017).

4.4 The Annual Electricity Consumption at Oslo Airport Gardermoen

Prior to examining the trends in Oslo Airport Gardermoen, it is important to note that in Norway hydropower accounts for the most of Norwegian power supplies. One unique characteristic of the Norwegian hydropower system is its high storage capacity. Norway has half of Europe's reservoir storage capacity. Moreover, more than 75 % of Norwegian production capacity is flexible. Production can thus be rapidly increased and decreased as needed, at low cost (Norwegian Ministry of Petroleum and Energy, 2021). Hydroelectric power (hydro) is regarded as a renewable energy source because it relies on the Earth's natural water cycle's kinetic energy to generate electricity (McEntee, 2021). Hydropower is a climate-friendly energy source, as it generates power without producing harmful air pollution or toxic by-products (National Hydropower Association, 2021). In addition, renewable power plants are typically sited in Norway where there is access to resources. At the start of 2021, there were 53 wind farms located in Norway. This corresponds to approximately 13.1 terra watts (TW) in a normal year. In addition, during the beginning of 2021, the total installed capacity for solar power throughout was 160 megawatts (MW) (Norwegian Ministry of Petroleum and Energy, 2021). Because it is renewable, wind power generates almost no emissions of greenhouses gases (GHGs) and other pollutants (Ledec et al., 2011; Wang & Prinn, 2010; Warren et al., 2005). Solar energy systems/power plants do not produce air pollution or greenhouse gases. Thus, the use of solar energy can have a positive, indirect impact on the environment when solar energy replaces or reduces the use of other energy sources that have larger environmental effects (United States Energy Information Agency, 2020).

The total annual electricity consumption at Oslo Airport Gardermoen and the year-on-year change (%) for the period 2005 to 2020 is presented in Figure 3. The highest single annual electricity consumption was recorded in 2018, when the airport consumed a total of 120 GWh of electricity (Figure 3). As can be observed in Figure 3, the airport's annual electricity consumption has largely displayed an upward trend. This is demonstrated by the year-on-year percentage change line graph, which is more positive than negative, that is, more values are above the line than below. The increase in Oslo Airport Gardermoen total annual electricity consumption throughout the study period has been due to a range of factors. During 2005, 2006, and 2007 there was an increase in electricity usage in the airport terminal building, the eastern area of the airport as well as in external areas. In addition, there was also an increase in the airport's runway electricity requirements (Oslo Lufthavn AS, 2008). Oslo Airport Gardermoen opened a new multi-level car park in 2008. The electricity consumption for this new facility was estimated to be around 2.8 GWh. However, because of the reduced energy demands in the airport's other facilities, there was only a small (+0.13%) increase in electricity consumption compared to the 2007 levels (Oslo Lufthavn AS, 2009). One of the key factors influencing the increase in electricity consumption in 2009 was the completion of the airport's terminal building eastern extension as well as the apron extension in the western area of the airport (Oslo Lufthavn AS, 2010). An airport apron is the defined area of land located within the airport precinct that accommodates, the parking, loading/unloading, refueling and maintenance activities of an aircraft (Budd & Ison, 2017). Also, there was an increase in the electricity consumption of a new indoor car parking facility (this facility became operational in the fall of 2008) (Oslo Lufthavn AS, 2010). In 2010, the total energy consumption of the airport's Energy Centre increased due to higher energy requirements. The opening of the new hotel Park Inn in 2010 also contributed to the higher electricity consumption recorded by the airport in 2010 (Oslo Lufthavn AS, 2011).

A key factor that influenced Oslo Airport Gardermoen's higher electricity consumption in 2011 was the major building projects at the airport, with the concomitant worker accommodation, and the offices and service facilities that were in the north area of the airport terminal building together with the building site facilities associated with the work on the southern terminal pier. In addition, the extension of technical installations and commercial areas of the airport terminal, and a 10.8% growth in passenger volumes contributed to the higher energy requirements, some of which were offset by various energy saving initiatives (as discussed below) (Oslo Lufthavn AS, 2012). In 2012, electrical consumption for electricity-specific installations increased by 5.7 GWh. The factors that contributed to this increase in electricity consumption were the infrastructure expansions, such as, the airport's runway system at the northern end, South Pier, and restaurants in the West Pier, together with increased building activities and associated barrack rigs. Another factor was the growth in passenger volumes at the airport. In addition, the consumption of thermal energy production, district heating and electrical energy reflected the weather conditions in 2012 and increased by approximately eight per cent as compared with 2011 (Figure 3) (Oslo Lufthavn AS, 2013). In 2013, the consumption of thermal energy production, district heating and electricity reflected the addition of new airport buildings the prevailing weather conditions (Oslo Lufthavn AS, 2014).

Figure 3 also shows that there were three years in the study period where the airport's total annual electricity consumption decreased on a year-on-year basis. These decreases were recorded in 2007 (-0.93%), 2019 (-2.5%), and 2020 (-15.38%) and reflected lower energy requirements during these years.

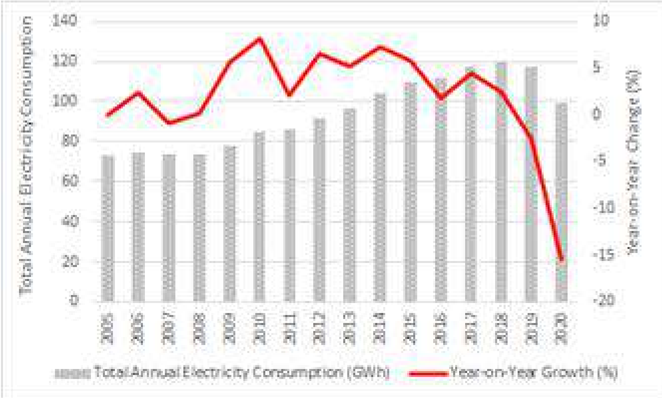

Fig.3: The annual electricity consumption at Oslo Airport Gardermoen and year-on-year change (%): 2005-2020. Source: Data derived from Oslo Airport AS (2008, 2011, 2016, 2021).

Through the airport's own high-voltage grid, Oslo Airport Gardermoen supplies electricity to its tenants, which include the police, the Flyporten Business Centre, the airport terminal, the airport's railway station and the airport's own buildings and installations. This energy is referred to as electric specific installations (Oslo Lufthavn AS, 2009). Oslo Airport Gardermoen's total annual electricity consumption for airport-specific installations (KWh) and the year-on-year change (%) for the period 2005 to 2020 is depicted in Figure 4. As can be observed in Figure 4, the airport's annual electricity consumption for airport-specific installations has largely displayed an upward trend. This is demonstrated by the year-on-year percentage change line graph, which is more positive than negative, that is, more values are above the line than below. As previously noted, the increases in airport-related infrastructure have resulted in an increased energy consumption. Figure 4 shows that there was a marked decrease in this metric, when electricity for airport specific installations decreased by 15.38% in 2020 on the 2019 levels. During the study period, there were two years where this metric declined. These decreases occurred in 2007 (-0.93%) and in 2020 (-15.38%).

Fig.4: The annual electricity consumption for airport specific installations at Oslo Airport Gardermoen and year-on-year change (%): 2005-2020. Source: Data derived from Oslo Airport AS (2008, 2011, 2016, 2021).

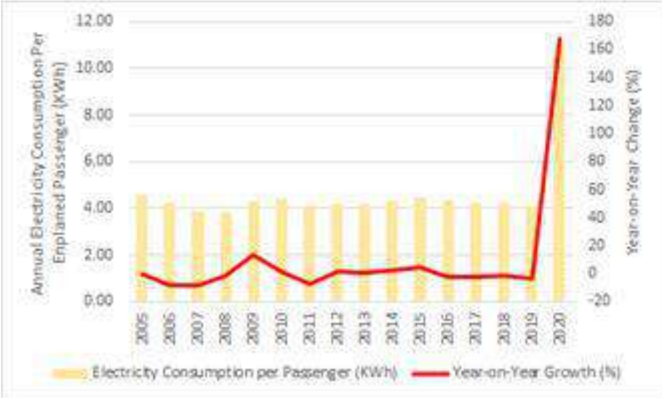

Fig.5: The annual electricity consumption per enplaned passenger at Oslo Airport Gardermoen and year-on-year change (%): 2005-2020. Source: Data derived from Oslo Airport AS (2008, 2011, 2016, 2021).

An important measure of an airport's energy efficiency is the energy consumption (electricity, gas, or fuel) per enplaned passenger (Graham, 2005). One passenger enplanement measures the embarkation of a revenue passenger, whether originating, stop-over, connecting or returning (Holloway, 2016). Figure 5 presents Oslo Airport Gardermoen's total annual electricity consumption per enplaned passenger (KWh) and the year-on-year change (%) for the period 2005 to 2020. As can be observed in Figure 5, the total annual electricity consumption per enplaned passenger fluctuated throughout the study period. The lowest annual electricity consumption per enplaned passenger was recorded in 2007 (3.43 KWh per passenger), whilst the highest level of annual electricity consumption per enplaned passenger occurred in 2016 (4.08 KWh per passenger). Figure 5 shows that there were sharp increases in the annual electricity consumption per passenger in 2009 (+8.93%), 2014 (+9.41%), and 2020 (+168.21%), respectively. Figure 5 also shows that the total annual electricity consumption per enplaned passenger decreased on a year-on-year basis in six years of the study period (2006 -8.24%, 2007 -3.65%, 2010 -0.52%, 2011 -6.38%, 2013 -0.82%, 2017 -0.98%, and 2018 -2.72%). In each of the years, there was an increase in both electricity consumption and enplaned passengers, thus the total annual consumption was spread over a greater volume of passenger traffic, which resulted in the decrease in electricity consumption per enplaned passengers.

Airport energy efficiency can also be measured by energy consumed per aircraft movement (Janić, 2017). Oslo Airport Gardermoen's total annual electricity consumption per aircraft movement (KWh) and the year-on-year change (%) for the period 2005 to 2020 is depicted in Figure 6. As can be seen in Figure 6, the total annual electricity per aircraft movement at Oslo Airport Gardermoen has exhibited an upward trend, increasing from 305.65 KWh per aircraft movement in 2005 to a high of 846.15KWh per aircraft movement in 2020. Figure 6 shows that there were three pronounced annual increases in the electricity per aircraft. These occurred in 2010 when the annual electricity per aircraft movement increased by 8.24% on the previous year's level and in 2014, when there was increase of 11.61% on the 2013 levels, and again in 2020, when the electricity consumption per aircraft movement increased by 76.46% on the 2019 levels. It is important to note that there was a 52.04% decrease in the number of aircraft movements in 2020, whilst the total annual electricity consumption in 2020 decreased by 15.38% on the 2019 level. Thus, the large increase in electricity per aircraft movement is due to the lower number of aircraft movements in 2020. Throughout the study period, there were three years when the total annual electricity consumption per aircraft decreased on a year-on-year basis. These decreases were recorded in 2008 (-3.43%), 2011 (-4.07%), and 2018 (-1.53%), respectively (Figure 6). It is important to note that throughout the study period, the size of commercial aircraft has increased. The Airbus A380 entered commercial service with Singapore Airlines in October 2007 (Jackson, 2021; Simons, 2014). The Boeing 747-8 Intercontinental first commercial flight took place on 1 June 2012 (Asian Aviation, 2012). The Boeing 787-8 first entered commercial service in 2011. The Airbus A350-900XWB first commercial flight was operated by

Qatar Airways in 2014 (Aircraft Commerce, 2015). Singapore Airlines took delivery of the first Boeing 787-10 on March 14th, 2018 (Boon, 2020). The Boeing 787-8 is around 20 seats larger than the Boeing 767-300ER, whilst the Boeing 787-9 has about 20 seats more capacity than the A330-200 (Aircraft Commerce, 2016).

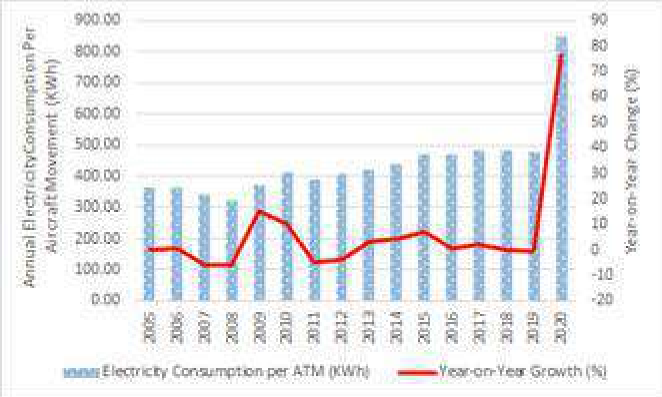

Fig.6: The annual electricity consumption per aircraft movement at Oslo Airport Gardermoen and year-on-year change (%): 2005-2020. Source: Data derived from Oslo Airport AS (2008, 2011, 2016, 2021).

4.5 The Annual Consumed Energy for Heating and Cooling at Oslo Airport Gardermoen

Like other airports around the world, Oslo Airport Gardermoen purchases both heating and cooling energy. The total annual purchased heating and cooling energy consumption and the year-on-year change (%) for the period 2005 to 2020 is depicted in Figure 7. As can be observed in Figure 7, the annual purchased heating and cooling energy consumption at the airport increased from 41.3 GWh in 2005 to 65 GWh in 2020. The highest annual consumption of purchased heating and cooling energy occurred in 2018, when the total annual consumption was 71 GWh. The largest single annual increase in the airport's purchased heating and cooling energy was recorded in 2016, when this energy source increased by 24.44% on the 2015 levels. Figure 7 also shows that the airport was able to decrease its consumption of heating and cooling energy in six years of the study period. These decreases reflected lower consumption levels and were recorded in 2007 (-3.87%), 2008 (-3.13%), 2011 (-12.9%), 2014 (-1.47%), 2015 (-5.46%), and in 2019 (-9.85%), respectively (Figure 7). The 2019 result is particularly favorable given the larger heating and cooling requirement of the airport's expanded terminal building, which as noted earlier, became operational in 2017.

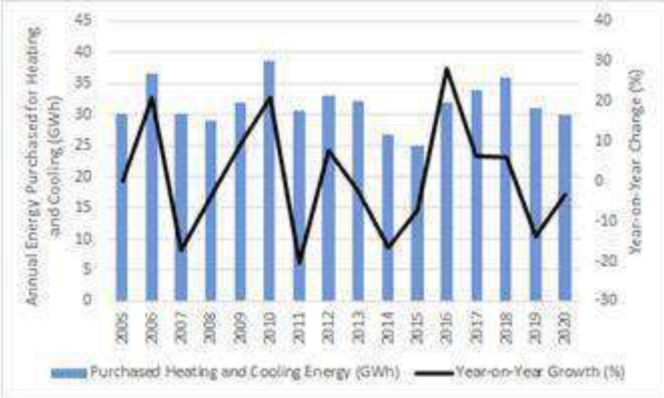

Fig.7: The annual purchased heating and cooling energy by Oslo Airport Gardermoen and year-on-year change (%): 2005-2020. Source: Data derived from Oslo Airport AS (2008, 2011, 2016, 2021).

4.6 The Annual Electricity Consumption for Compressors and Pumps at Oslo Airport Gardermoen

The total annual electricity consumption for the compressors and pumps used at Oslo Airport Gardermoen and the associated year-on-year change (%) from 2005 to 2020 are depicted in Figure 8. As can be observed in Figure 8, the annual electricity consumption for the compressors and pumps used at the airport has gone through discrete stages. In stage 1, that is, from 2005-2009, the annual energy consumption remained relatively stable at around 6.24 GWh per year. From 2010 to 2013, stage 2, there was a pronounced increase in this energy source, with the highest single annual consumption was recorded in 2010, when the total annual consumption increased by 315% on the 2009 levels. In the third stage, from 2014 to 2020, the consumption remained relatively consistent, averaging between 9 GWh and 11 GWh per annum (Figure 8).

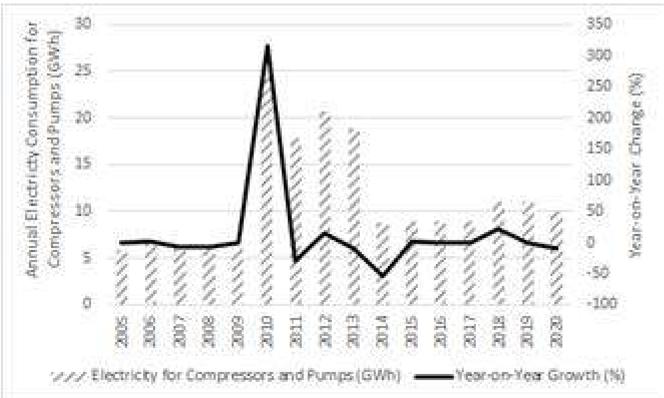

Fig.8: The annual consumption of electricity for compressors and pumps at Oslo Airport Gardermoen and year-on-year change (%): 2005-2020. Source: Data derived from Oslo Airport AS (2008, 2011, 2016, 2021).

4.7 The Annual Electricity Consumption for Electrode Boiler at Oslo Airport Gardermoen

The total annual electricity consumption for the Oslo Airport Gardermoen electrode boiler and the year-on-year change (%) from 2005 to 2020 are depicted in Figure 9. As can be observed in Figure 9, the annual electricity consumption for the airport's electrode boiler oscillated throughout the study period. Figure 9 shows that there was a pronounced spike in this metric in 2009, when the annual electricity consumption increased by 375% on the 2008 level. In 2010, the total annual electricity consumption rose by 50% on the 2009 level (Figure 9). There was a further spike recorded in 2014, when the boiler annual electricity consumption increased by 68.75% on the 2013 level (Figure 9). Figure 9 also shows that throughout the study period, there were ten years where the year-on-year total annual electricity consumption decreased on a year-on-year basis. The largest single annual decrease was recorded in 2007, when the boiler's annual electricity consumption decreased by 53.33% on the 2006 level. Figure 9 also shows that there were two significant decreases recorded in 2019 (-50%) and 2020 (-25%), respectively.

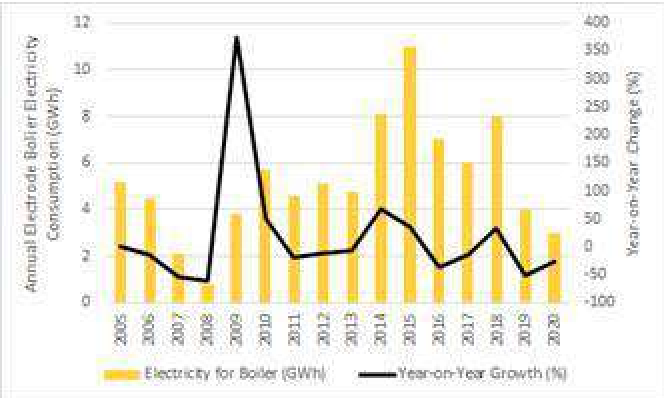

Fig.9: The annual consumption of electricity for electrode boiler at Oslo Airport Gardermoen and year-on-year change (%): 2005-2020. Source: Data derived from Oslo Airport AS (2008, 2011, 2016, 2021).

4.7 The Annual Consumption of Hafslund Fjernvarme AS Supplied District Heating Energy at Oslo Airport Gardermoen

From 2005 to 2014, Oslo Airport Gardermoen purchased district heating from Hafslund Fjernvarme AS. Hafslund Fjernvarme AS. used biofuels and oil in the production of district heating energy. In 2005, Hafslund Fjernvarme AS. installed an additional boiler, whereupon the airport signed an additional agreement for deliveries of district heating energy as this was a key part of the airport's heating and cooling system (Oslo Lufthavn AS, 2008). Figure 10 presents the total district heating energy purchased from Hafslund Fjernvarme AS and the year-on-year change (%) for the period 2005 to 2014. As can be observed in Figure 10, the annual consumption of district heating energy fluctuated throughout this period and reflected the airport's heating and cooling requirements. In 2006, there was a pronounced spike when this energy source consumption increased by 66.17% on the 2005 levels. The second most significant annual increase was recorded in 2010 (+17.45%). Figure 9 also shows that there were four years when the annual consumption of purchased district heating energy declined on a year-on-year basis. These decreases were recorded in 2007 (-22.12%), 2011 (-28.51%), 2013 (-8.69%), and in 2014 (-53.43%), respectively (Figure 10). In 2007, the number of degree days was 11% below the norm experienced at Oslo Airport Gardermoen, which resulted in a lower demand for thermal energy (Oslo Lufthavn AS, 2008).

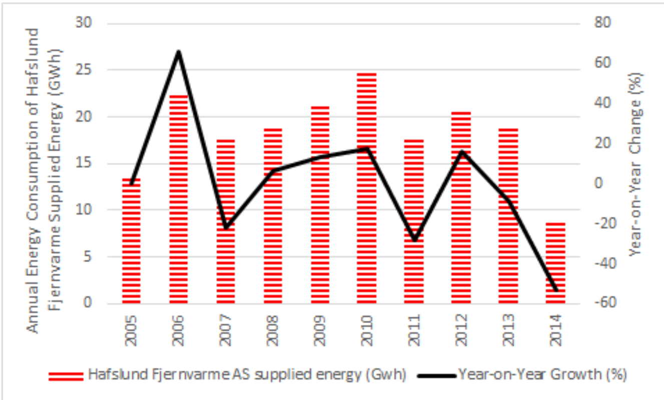

Fig.10: The annual purchased district heating from Hafslund Fjernvarme by Oslo Airport Gardermoen and year-on-year change (%): 2005-2014. Source: Data derived from Oslo Airport AS (2008, 2011, 2016).

4.8 The Annual Consumption of Statkraft Varme AS Supplied District Heating energy at Oslo Airport Gardermoen

From 2015 to 2020, Oslo Airport Gardermoen purchased remote district heating from Statkraft Varme AS. Statkraft Varme AS supplied district heating is sourced from woodchips (Oslo Lufthavn AS, 2020). The total annual purchased district heating energy from Statkraft Varme AS and the year-on-year change for the period 2005 to 2020 is presented in Figure 11. As can be observed in Figure 11, the annual consumption of district heating sourced from Statkraft Varme AS fluctuated over the study period due to differing heating requirements at the airport. Figure 11 shows that the highest annual consumption of district supplied heating from Statkraft Varme AS was recorded in

2019 (17 GWh), whilst the lowest level of consumption occurred in 2015 (5 GWh). The highest single annual increase in this district heating occurred in 2016, when the annual consumption increased by 200 % on the 2015 levels. Figure 11 shows that there were two years in the study period where the annual district heating consumption decreased on a year-on-year basis. The decreases were recorded in 2018 (-6.25%) and in 2020 (-5.88%), respectively, and reflected the lower heating requirements in these two years (Figure 11).

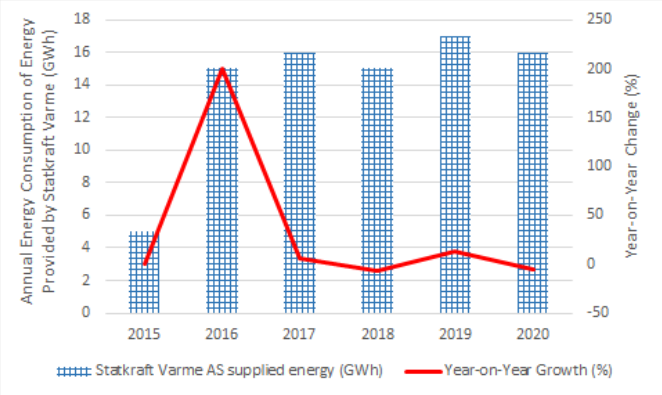

Fig.11: The annual purchased district heating from Statkraft Varme AS by Oslo Airport Gardermoen and year-on-year change (%): 2015-2020. Source: Data derived from Oslo Airport AS (2016, 2021).

4.9 The Annual Heating Oil Consumption for Energy Central at Oslo Airport Gardermoen

The total annual heating oil consumption for Oslo Airport Gardermoen's Energy Central and the year-on-year change (%) from 2005 to 2020 is depicted in Figure 12. As can be observed in Figure 12, the airport's annual heating oil consumption for Energy Central has principally displayed a downward trend. This is demonstrated by the year-on-year percentage change line graph, which is more negative than positive, that is, more values are below the line than above. However, as can be observed in Figure 12, there were four years during the study period where there were pronounced spikes in the annual consumption of heating oil for the airport's Energy Central. These spikes occurred in 2007 (+53.84%), 2010 (+66.66%), 2014 (+200%), and 2017 (+300%) with these increases being the result of greater heating requirements in these years. Figure 12 also shows that there were five years during the study period, where the airport's annual consumption of heating oil for its Energy Central declined quite significantly on a year-on-year basis. These decreases were recorded in 2006 (-52.72%), 2009 (-74.28%), 2014 (-71.42%), 2018 (-50%), and 2019 (-50%) (Figure 12). In recent times, heating oil has only been used at the airport to a limited extent. The reduction in the use of heating oil is a result of the airport's greenhouse gas (GHG) reduction measures. As noted below, the airport has been transitioning to biofuel oil to further reduce consumption. In addition, the oil boilers that are in the airport's thermal plant are only used for emergencies and consumption of fuel oil is limited to test runs (Avinor AS, 2021c).

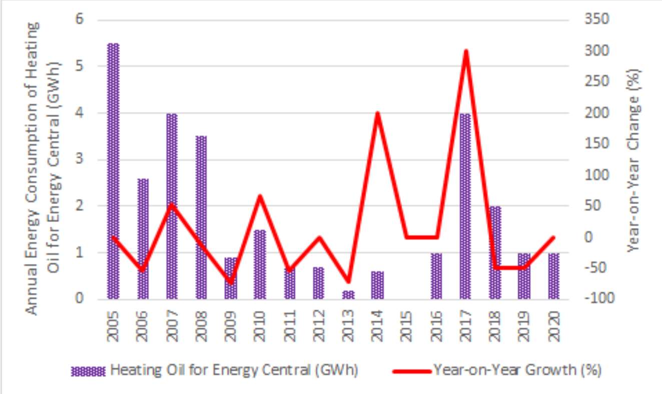

Fig.12: The annual heating oil consumption for Energy Central at Oslo Airport Gardermoen and year-on-year change (%): 2005-2020. Source: Data derived from Oslo Airport AS (2008, 2011, 2016, 2021).

4.10 The total annual recovered energy at Oslo Airport Gardermoen

The total annual recovered energy at Oslo Airport Gardermoen and the year-on-year change (%) for the period 2005 to 2020 is shown in Figure 13. Energy recovery is defined as the process of extracting useful energy from waste, for example, heat from the incineration of wastes (Allaby & Park, 2007). Figure 13 shows that the total annual recovered energy increased from 11.1 GWh in 2005 to a high of 35 GWh in 2020. Figure 13 also shows that there has been a general upward trend with pronounced spikes in 2007 + 47.95%, 2014 + 28.57%, and in 2017 + 29.16%, respectively (Figure 13). Throughout the study period, the total annual recovery at the airport decreased on a year-on-year basis in six years, that is, 2006, 2008, 2009, 2010, 2015 and 2019, respectively. the largest single annual decrease in the annual recovered energy occurred in 2006, when the total annual recovered energy decreased by 11.71% on the 2005 level (Figure 13).

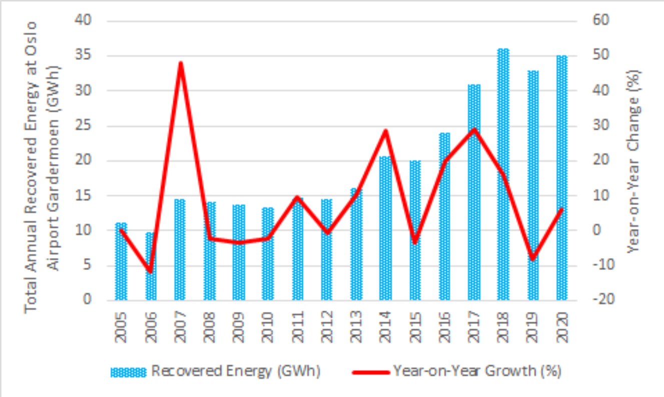

Fig.13: The annual recovered energy at Oslo Airport Gardermoen and year-on-year change (%): 2005-2020. Source: Data derived from Oslo Airport AS (2008, 2011, 2016, 2021).

From an environmental perspective, energy recovery enables firms to reduce their greenhouse gas emissions as well as minimizing waste to landfill. Indeed, energy recovery enables the generation of energy more sustainably (Energy Australia, 2021). As material recycling, energy recovery has a concomitant linkage with the prevention and minimization of waste. Energy recovery is one of the two primary means of valorizing the waste that does occur and hence reduces its environmental and economic impacts. When taking together, material recycling and energy recovery offer a firm alternative and complementary ways of gaining the greatest sustainable benefit from natural resources and their wastes, thus reducing the consumption of virgin resources (Shulman, 2019).

4.11 Non-renewable Energy Resources Usage at Oslo Airport Gardermoen

4.11.1 Annual Bio-heating Oil Consumption at Oslo Airport Gardermoen

The total annual bio-heating oil consumption at Oslo Airport Gardermoen and the year-on-year change (%) from 2014 to 2020 is depicted in Figure 14. Bio heating oil is a blend of biodiesel fuel that is blended with traditional heating oil (Apgar Oil, 2021). Figure 14 shows that there has been an upward trend in the airport's consumption of bio-heating oil. The overall increase in bio-heating oil consumption at the airport is demonstrated by the year-on-year percentage change line graph, which is more positive than negative, that is, more values are above the line than below. The largest single annual increase in the consumption of bio-heating oil occurred in 2017, when the consumption of this energy source increased by 185.71% on the 2016 level. This reflected the airport's heating requirement in 2017. Figure 14 shows that there was a single annual decrease in the consumption of bio-heating oil during the study period. This decrease was recorded in 2019, when the annual consumption of bio-heating oil decreased by -1.56% on the 2018 level, due to the lower heating requirement (Figure 14).

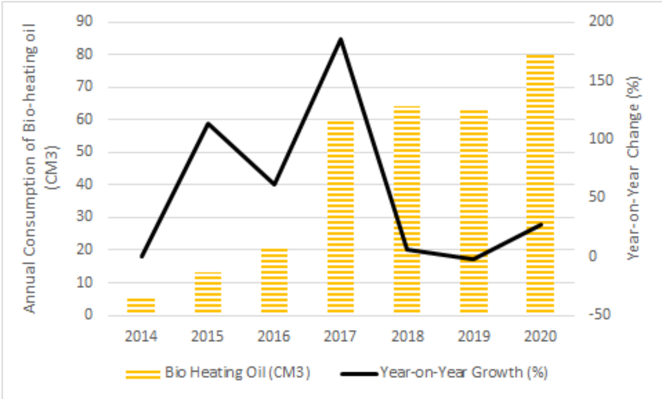

Fig.14: The annual bio-heating oil consumption at Oslo Airport Gardermoen and year-on-year change (%): 2014-2020. Source: Data derived from Oslo Airport AS (2016, 2021).

Bio-heating oil offers users several environmental-related advantages. Bio-heating oil is a clean burning alternative fuel, that is produced from both domestic and renewable resources. Biofuel contains no petroleum, and it can be blended at any level with petroleum diesel to create a biofuel blend. Bio heating oil is also considered safe and provides a cleaner, more complete, and energy-efficient burn. This fuel source is a renewable energy source that is environmentally friendly. A further advantage is that bio-heating oil reduces global warming gas emissions; it is also non-toxic, biodegradable, and suitable for sensitive environments. In addition. Bio-heating oil is essentially free of sulphur and aromatics. A final advantage is that bio heating oil extends equipment life and reduces periodic maintenance of the (Apgar Oil, 2021).

4.11.2 Annual Consumption of Biofuel for Oslo Airport Gardermoen Vehicles

An important metric in the airport industry is the airport vehicles and ground service vehicles that have been converted to energy efficient types and is an indicator of airport's objective to reduce energy consumption, and the use of fossil-based fuels. Consequently, many airports located throughout the world have decided to convert their airport vehicles to more energy efficient vehicles (Hazel et al, 2016).

Through a framework agreement, advanced biodiesel is purchased by Oslo Airport Gardermoen that satisfies the European Union's sustainability criteria and this biodiesel is also guaranteed without palm oil or palm oil products.

Advanced biodiesel is used at the airport in vehicles that cannot be easily electrified, for example, snow blowers and sweepers. At the end of 2020, the vehicle fleet of administrative vehicles at Oslo Airport Gardermoen was comprised of 23 zero-emission vehicles (Oslo Lufthaven, 2021).

Figure 15 shows that Oslo Airport Gardermoen began using biofuels for its vehicles in 2015 and continued to use these fuels over the remainder of the study period. As can be seen in Figure 15, there was a general upward trend in the annual consumption of biofuels with a pronounced spike recorded in 2016 (+822.22%). There was just one year when the annual biofuels decreased on a year-on-year basis, and this decrease occurred in 2020, when the annual consumption decreased by -47.67%, due to the reduced vehicle fuel requirement. From an environmental perspective, biofuels can reduce a firm's consumption of fossil fuels, and hence, reduce carbon dioxide (CO_2) emissions. This is because biofuels are carbon neutral (Hanaki & Portugal-Pereira, 2018). In 2020, for example, the use of zero emission vehicles at the airport resulted in a reduction in greenhouse gas emissions of 1,439 tons of carbon dioxide (CO_2) (Oslo Lufthaven, 2021).

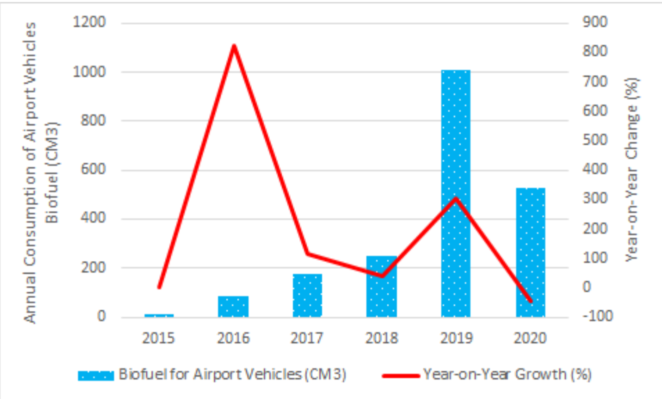

Fig.15: The annual bio-fuel consumption for Oslo Airport Gardermoen vehicles and year-on-year change (%): 2015-2020. Source: Data derived from Oslo Airport AS (2016, 2021).

4.11.3 Annual Consumption of Fuel for Fire Drills at Oslo Airport Gardermoen vehicles

In the air transport industry, the incidences of fires and emergencies occurring at an airport are often quite rare, however instances of fires and emergencies, particularly on aircraft, the fire fighting and rescue capabilities at an airport may mean the difference between life and death for pilots, passengers, and other associated airport personnel (Young & Wells, 2011). It is important to note that there is a finite probability that an accident may occur sooner or later. Many accidents have occurred during take-off and landing, that is, they are often in the vicinity of the airport. Consequently, the airport operator must be fully prepared for such an eventuality (Kazda & Caves, 2015). The degree of firefighting and rescue protection is dependent upon the size of the largest aircraft operating to the airport together with the frequency of operation (Ashford et al., 2013). Importantly, in accordance with the provisions of Annex 14 to the 1944 Chicago Convention on International Civil Aviation— Aerodromes, Volume I — Aerodrome Design and Operations, Member States of the International Civil Aviation Organization are required to provide rescue and firefighting equipment and services at an airport. All personnel (regular and/or auxiliary) provided for aircraft rescue and firefighting duties, should be fully trained in the performance of their duties, and operate under the direction of a designated chief of emergency crew (International Civil Aviation Organization, 2014).

The total annual consumption of paraffin and Jet A1 aircraft fuel for fire drills at Oslo Airport Gardermoen and the year-on-year change (%) from 2005 to 2020 are presented in Figure 16. Figure 16 shows that there has largely been a downward trend in the amount of paraffin/Jet A1 fuel used for fire drills at Oslo Airport Gardermoen. This is demonstrated by the year-on-year percentage change line graph, which is more negative than positive, that is, more values are below the line than above. Figure 16 shows that there was quite a large increase in this fuel source in 2006 when its consumption increased by 60.51% on the 2005 levels. The single largest annual decrease in paraffin/Jet A1 fuel for fire drills was recorded in 2014, when the amount of this fuel source decreased by 38.86% on the 2013 level. Figure 16 shows that there were no reported usage of paraffin and Jet A1 aircraft fuel for fire drills at Oslo Airport Gardermoen during 2019 and 2020.

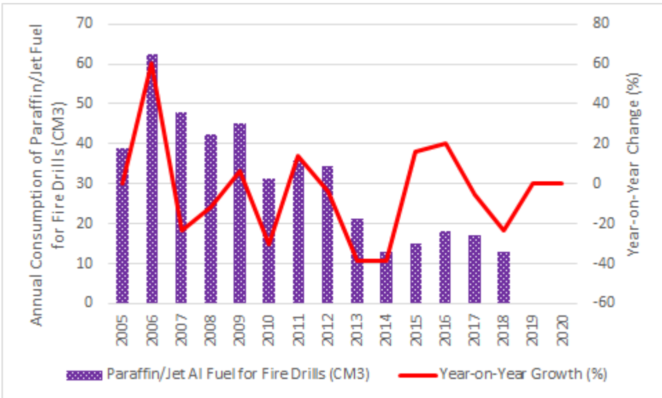

Fig.16: The annual consumption of fuel (paraffin/Jet A1) for fire drills at Oslo Airport Gardermoen and year-on-year change (%): 2005-2020. Source: Data derived from Oslo Airport AS (2008, 2011, 2016, 2021).

Figure 17 presents the total annual consumption of propane fuel that is used for fire drills at Oslo Airport Gardermoen and the year-on-year change (%) from 2005 to 2020. As can be observed in Figure 6, the annual consumption of propane for fire drills at Oslo Airport Gardermoen declined from a high of 2.6cm3 to zero in both 2019 and 2020. Figure 17 shows that there were two pronounced spikes in the consumption of propane, and these were recorded in 2010 (+46.15%) and in 2018 (+200%), respectively. Figure 17 also shows that there were significant decreases in the amount of propane used for fire drills at the airport. These decreases occurred in 2006 (-11.53%), 2007 (-39.13%), 2011 (-63.15%), 2013 (-14.28%), 2015 (-33.33%), and in 2016 (-25%) (Figure 17). As noted in Figure 17, the volume of paraffin and Jet A1 fuel have been the primary sources for fire drills at the airport.

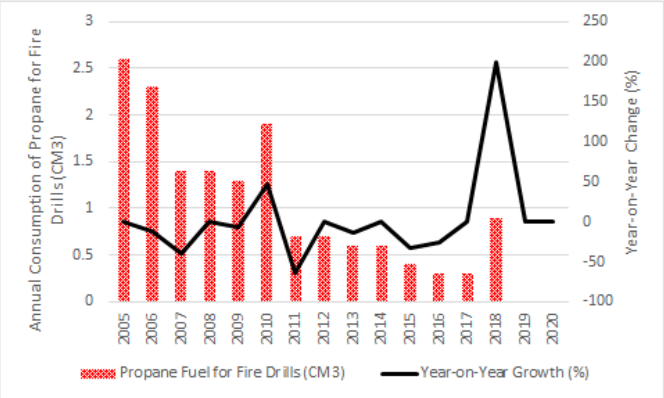

Fig.17: The annual consumption of fuel (propane) for fire drills at Oslo Airport Gardermoen and year-on-year change (%): 2005-2020. Source: Data derived from Oslo Airport AS (2008, 2011, 2016, 2021).

4.11.4 Annual Consumption of Fuel for Oslo Airport Gardermoen vehicles

The annual consumption of fuel for Oslo Airport Gardermoen vehicles and the year-on-year change (%) from 2005 to 2020 are presented in Figure 18. As can be observed in Figure 18, the airport's annual consumption of fuel for its fleet of vehicles has oscillated throughout the study period reflecting varying annual vehicle fuel requirements. The largest single annual increase was recorded in 2014, when the vehicle total fuel consumption increased by 35.02% on the previous year's level. Figure 18 shows that there was a significant decrease in this fuel source in 2019 (-74.87%) and 2020 (-79.13%), respectively. In these later years, the airport had transitioned to biofuel. The third most significant decrease in vehicle fuel consumption occurred in 2013, when the total vehicle fuel consumption decreased by 19.42% on the 2012 levels (Figure 18).

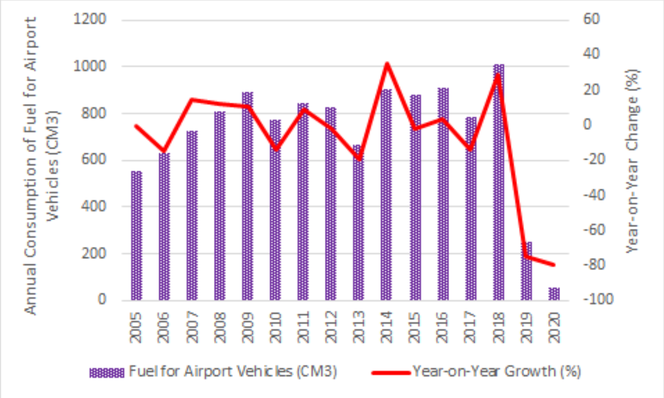

Fig.18: The annual fuel consumption for Oslo Airport Gardermoen vehicles and year-on-year change (%): 2005-2020. Source: Data derived from Oslo Airport AS (2008, 2011, 2016, 2021).

4.11.5 Annual Heating Oil/Diesel Consumption at Oslo Airport Gardermoen

The total annual heating oil/diesel consumption at Oslo Airport Gardermoen together with the associated year-on-year change (%) for the period 2005 to 2020 is presented in Figure 19. As can be observed in Figure 19, the airport's annual consumption has declined from a high of 682 cubic metres in 2005 to a low of 26 cubic metres in 2020. Figure 19 also shows that there were three years in the study period where there were pronounced increases in the year-on-year consumption. These increases occurred in 2007 (+44.35%), 2010 (+36.91%), and in 2017 (+155.49%), respectively (Figure 19). The higher annual consumption of heating oil/diesel is reflective of the climatic conditions experienced in Oslo during these years.

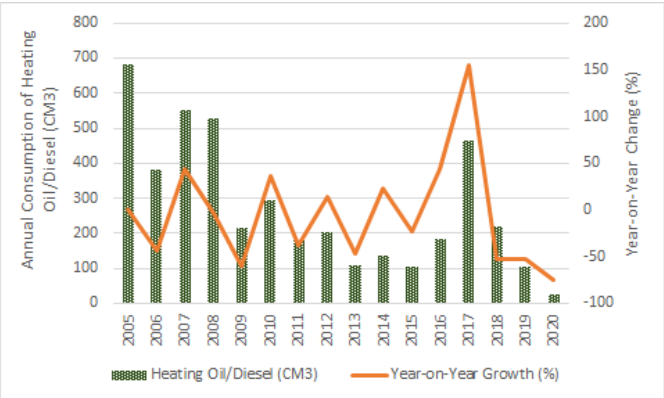

Fig.19: The annual heating oil/diesel consumption at Oslo Airport Gardermoen and year-on-year change (%): 2005-2020. Source: Data derived from Oslo Airport AS (2008, 2011, 2016, 2021).

4.11.6 Annual Aircraft Jet Fuel Consumption at Oslo Airport Gardermoen

The total annual aircraft jet fuel consumption at Oslo Airport Gardermoen from 2005 to 2020 and the year-on-year change is presented in Figure 20. As can be observed in Figure 20, there has been an overall upward trend in jet fuel consumption at the airport, which reflects the growth in the annual aircraft movements as well as the larger sizes of the aircraft using the airport. The overall increase in aircraft jet fuel consumption at the airport is demonstrated by the year-on-year percentage change line graph, which is more positive than negative, that is, more values are above the line than below. The highest annual single increase in aircraft jet fuel consumption was recorded in 2006, when the annual aircraft jet fuel consumption increased by 14.91% on the 2013 level. During the study period, there were three years where the annual aircraft jet fuel consumption declined on a year-on-year basis. These decreases were recorded in 2009 (-5.68%), 2015 (-3.1%), and in 2020 (-58.47%) (Figure 20). The pronounced drop in 2020 was due to the significant reduction in aircraft movements due to the downturn in traffic and flights because of the Corona virus pandemic.

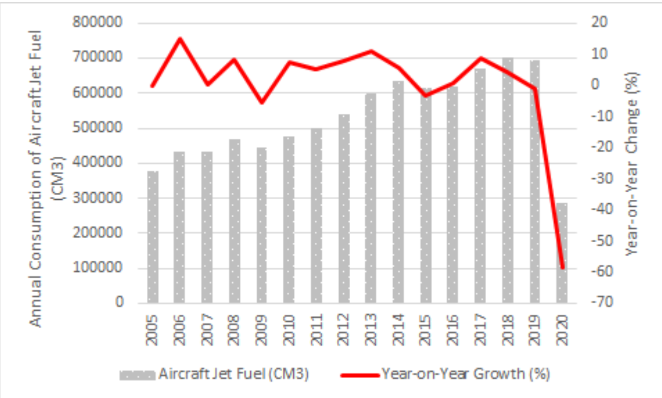

Fig.20: The annual aircraft jet fuel consumption at Oslo Airport Gardermoen and year-on-year change (%): 2005-2020. Source: Data derived from Oslo Airport AS (2008, 2011, 2016, 2021).

An important development in the air transport industry in recent trends has been increasing trend by airports and airlines to use aviation biofuel as a key environment sustainability measure (Baxter et al., 2020). Indeed, the growing concerns of climate change and energy supply have been driving the production of more sustainable aviation fuels (Brooks et al., 2016). As a result, alternative jet fuel (AJF) technologies have gained strong interest, and thus, are increasingly being viewed as a way for the airline industry to achieve large, near-term emissions reductions (Staples et al., 2014). Aviation biofuels are therefore becoming an important substitute for fossil fuel in the airline industry. Aviation biofuels offer several advantages including sustainability, they are environmentally friendly, and they offer good adaptability (Su et al., 2015). Considering these advantages, the aviation industry has acknowledged the importance of sustainable biofuels as being the key long-term technology for decarbonizing aviation (Fregnani & Andrade, 2017).

Oslo Airport became the world's first airport to offer sustainable jet biofuel to all airlines serving the airport in 2016. The jet biofuel is supplied by Air BP. Lufthansa Group, SAS and KLM have signed agreements to purchase the fuel. Norwegian airport operator Avinor AS played a vital role in the commercial offtake agreements by offering incentives for all flights at Oslo Airport that use sustainable jet fuel. The aviation biofuel is produced by Neste in accordance with the framework of the demonstration project ITAKA. The first batch of this sustainable jet fuel is made from camelina oil that is Roundtable on sustainable biomaterials (RSB) certified (Airport Technology, 2016). Airlines that participate in this initiative are rewarded with lower carbon dioxide (CO_2) taxes on domestic flights in Norway and the aviation biofuel is also exempted from the European Union (EU) quota system (Biofuels International, 2016). In addition, Avinor AS has set a goal that by 2030 goal, thirty per cent of aviation fuel consumed in Norway. This would equate to around 400 million litres (Mosvold Larsen, 2017).

4.12 Energy Conservation Measures at Oslo Airport Gardermoen

Oslo Airport Gardermoen has historically placed a high focus on identifying the opportunities to conserve energy, and thus, mitigate or minimize any environmentally harmful impacts associated with its energy consumption. Energy conservation is the saving of energy by a firm through the elimination of wasteful use, making more efficient use of energy, or reducing the firm's total use of energy (Allaby & Park, 2007).

In 2007, Oslo Airport Gardermoen implemented a fourth quarter trial set-up for the potential upgrading of light-fittings to long-life light sources. This initiative showed the potential annual savings of 150 MWh in the airport's terminal piers. An additional benefit was the reduced demand for cooling energy (Oslo Lufthaven AS, 2008). During 2008, Oslo Airport Gardermoen modified 142 light fittings in the airport terminal buildings with light fittings that used 10% less energy. This initiative resulted in an annual energy saving of 17 MWh, other benefits included reduced heat emission, improved technical properties and a six-fold increase in the lighting's service life (Oslo Lufthaven AS, 2009). In 2009, Oslo Airport Gardermoen installed new light fittings on the airport's railway platforms. The new light fittings delivered a 43 per cent saving in the lighting energy consumption. Also, during 2009, the airport terminals and other buildings lighting control systems were improved. In addition, the improved control of the street heating system located outside the Terminal Building reduced the annual thermal energy consumption by an estimated 600-800 MWh (Oslo Lufthaven AS, 2010).

Airports around the world are increasingly transitioning to the use of light emitting diodes (LED) lighting systems (Bullough, 2012; Freyssinier, 2014; Taylor, 2011). Oslo Airport Gardermoen is one such airport that has followed this trend. LED lighting can improve the quality of lighting and comfort of building occupants, whilst at the same time deliver energy savings that are beyond improved source efficiency (United States Department of Energy, 2019). In 2010, Oslo Airport Gardermoen's up-light fixtures consisting of 1000 watts lamps located in the airport terminal were modified and this resulted in annual saving of 552 MWh. During December 2010, a series of downlight fixtures were replaced with LED downlights and this new lighting technology was estimated to provide an annual energy saving of 33 MWh (Oslo Lufthaven AS, 2011). Oslo Airport Gardermoen continued its program of installing LED lighting during 2011, when the airport completed the modification of 1000 watt light fixtures in the airport terminal as well as the replacement of downlight fixtures with LED lamp in the arrival hall. These lighting measures enabled the airport to achieve a 552 MWh and 43 MWh energy savings, respectively (Oslo Lufthaven AS, 2012).

In 2012, Oslo Airport Gardermoen modified the heat exchangers in the airport's terminal building ventilation system. Other energy saving measures implemented in 2012 included the modification of 100 downlight fixtures in the international arrivals hall, which resulted in a forty percent energy saving. These two energies saving measures produced an annual saving of 1,452 MWh. The airport also installed LED flood lighting in the general aviation terminal, which is located to the west airport, and this measure resulted in annual energy savings of 21 MWh (Oslo Lufthaven AS, 2013). During 2013, Oslo Airport Gardermoen converted the remaining 100 uplights in the international arrival's hall to more energy efficient lighting, which delivered an annual energy saving of 213 MWh. Also, in 2013, the airport modified 280 uplights in Pier East and Pier West and this lighting modification resulted in around 70% of these lights becoming operational resulting in a reduction in consumption of approximately 50 MWh per annum. Fluorescent lights were also replaced in the airport's administration building in 2013 and this measure enabled the airport to reduce energy consumption by 25 MWh per annum (Oslo Lufthaven AS, 2014).

During 2014, Oslo Airport Gardermoen continued its program of implementing further energy saving measures that delivered an annual saving in energy consumption of 1,265 MWh. The energy saving measures implemented in 2014 included the modification of uplights in the airport's Pier East and Pier West as well as the upgrading of LED lights in the terminal and on the runways. In addition, heat exchangers in the airport's ventilation system were modified and the air conditioning unit was replaced with a free cooling unit (Oslo Lufthaven AS, 2015). The energy saving measures implemented by Oslo Airport Gardermoen in 2015 resulted in an annual energy reduction of 216 MWh. These measures included the replacement of halogen runway lights with LED lighting. Around 800 runway lights were replaced, and this initiative delivered an annual energy saving of 149 MWh. The airport also replaced a ventilation unit with a free cooling unit, saving up to 67 MWh per annum. In addition, the airport's heat exchange, which comprised a free exchanger and low-temperature heat pump, as well as treated wastewater from the municipality of Ullensaker, became operational in 2015. The treated wastewater acts as a heat source during the winter period and as a heat sink during cooling throughout the summer period (Oslo Lufthaven AS, 2016).

A range of energy savings measures were implemented at the airport in 2016. Around 867 halogen runway lights were replaced with LED lights providing a reduction in power consumption of 117 MWh per annum. In addition, some LED lights were also replaced in the passenger terminal and this initiative resulted in an annual energy saving of 68 MWh. The most significant energy saving measure in 2016, however, was the replacement of heat recovery units in two ventilation systems. The airport switched from battery recovery units to much more energy efficient rotary recovery units. The annual energy saving from this initiative was 710 MWh. Also, in 2016, the temperature in Hangar 8 was reduced to further save energy and power during the winter season. The annual saving for this measure was estimated to be 87 MWh (Oslo Lufthaven AS, 2017).

Oslo Airport Gardermoen continued its program of implementing further energy saving measures during 2017 and these measures delivered total annual energy savings of 2,398 MWh. The measures included the further replacement of halogen lights with LED lighting. In total, 1,100 lane lights were replaced as part of the lighting upgrade program, which resulted in the annual power consumption of around 198 MWh. Furthermore, some replacements were made to the airport terminal's LED lighting, which contributed to an annual energy saving of 250 MWh. The largest single annual energy saving measure, however, was the replacement of lighting system in the airport's parking lot P-11. This upgrade involved the entire lighting system of 1,250 luminaires being replaced with more efficient LED lighting. Each luminaire has a motion detector sensor that dims down the light if there is no nearby activity. This lighting project delivered annual energy savings of around 1,950 MWh (Oslo Lufthaven AS, 2018).

During 2018, the range of additional energy saving measures enabled Oslo Airport Gardermoen to reduce its energy consumption by 5,102 MWh. This significant annual saving was achieved through the better monitoring and controlling of its energy requirements. This was done by setting the time control and night reduction in the airport's ventilation system, and through the pulses of lighting. In 2018, the largest energy savings came from the replacement of the entire lighting system in the airport's parking garage P-10. This project involved the replacement of 6,224 luminaires with more efficient LED lighting with motion detection capability. This lighting upgrade resulted in an annual energy saving of 3,000 MWh per annum. Also, in 2018, two ventilation units in the Pier West terminal were replaced with a larger unit that had a much higher heat recovery and more efficient fans. In addition, a lot of old lighting was also changed in other buildings, and the replacement of lane lighting with LED lights continued in 2018. Overall, these measures resulted in a reduction of 1,300 MWh/year (Oslo Lufthaven AS, 2019).

Oslo Airport Gardermoen defined and implemented a range of new energy saving measures in 2019 that enabled the airport to further reduce its energy consumption by 1.4 GWh/year. During 2019, new LED lighting was installed in the airport's sweeper halls and in part of the Pier Makeup Zone (PMZ). Furthermore, a contract was signed for the replacement flood lighting around the airport terminal building with new LED lighting. This measure would not only deliver safety benefits, it will also save electricity, and reduce the airport's overall power consumption. In 2019, three new energy-efficient rotor recyclers in the passenger terminal were installed together with a new heat exchanger that helps the airport to recover around 3-4 GWh of heat energy from the plant located at Ullensaker. Other energy saving measures implemented in 2019 included the installation of blanking pedestals on the existing lights in Hangar 8, the installation of motion sensors and night lowering in Pir Syd, the night lowering of lighting in Pier Norda and SBV, together with the automatic control of ground heating systems at the airport's apron (Oslo Lufthaven AS, 2020). The airport apron comprises the individual aircraft stands that interface with the airport terminal building(s) and where aircraft are ground handled in between flights (Budd & Ison, 2017). When combined, these energy saving measures enabled Oslo Airport Gardermoen to reduce its energy consumption by around 2 GWh in 2019 (Oslo Lufthaven AS, 2020).

In August 2020, eight electric 18-meter buses were delivered to Oslo Airport Gardermoen. These new buses were a replacement for eight fossil fuel-powered buses. The electric buses are to be used to carry passengers between the airport terminal and remotely parked aircraft (Oslo Lufthaven AS, 2021).

V. CONCLUSION

Using an instrumental case study research approach this study has examined Oslo Airport Gardermoen, Norway's principal air traffic hub, environmentally sustainable energy management. The study covered the period 2005 to 2020. The documents gathered for the case study were analyzed using document analysis.

The case study revealed that Oslo Airport Gardermoen has multiple energy sources. The airport purchases electricity that is used for the airport's specific installations as well as for the airport's electrode boiler and compressors and pumps. The airport also purchases heating and cooling energy from external vendors, such as, Statkraft Varme AS. The non-renewable energy sources include aircraft jet fuel, heating oil/diesel, fuel and biofuels for the airport's vehicles, and supplies of paraffin/Jet A1 fuel and propane, with the latter fuel sources being used for the fire drills conducted at the airport.

Over the study period, the airport's total consumption increased from 72.8 GWh in 2005 to 99 GWh in 2020. The highest single annual consumption of electricity was recorded in 2018, when a total of 120 GWh of electricity was consumed at the airport. The annual electricity consumption per enplaned passenger fluctuated throughout the study period. The annual electricity consumption per aircraft movement exhibited an upward trend, which was in line with larger aircraft types using the airport plus the increased aircraft movements at the airport..

The heating and cooling energy purchased by the airport is influenced by weather patterns, and thus, the heating and cooling requirements reflected the necessity to heat or cool the airport's buildings. The case study revealed that Oslo Airport Gardermoen has been transitioning to biofuels as the energy source for its heating requirements. Biofuels are a cleaner, more complete, and energy efficient burning source. The use of biofuels enables users to reduce their greenhouse gas (GHG) emissions. The use of biofuels for the airport's vehicles will also result in a reduction in greenhouse gas (GHG) emissions at the airport.

The case study found that there has been an overall upward trend in jet fuel consumption at Oslo Airport Gardermoen. As noted in the case study, there has been a general increase in the number of aircraft movements at the airport, and these increases translate into higher jet fuel consumption.

The case study also shows that throughout the study period Oslo Airport Gardermoen has identified and implemented a wide range of energy conservation measures and technologies that have further enabled the airport to mitigate its impact on the environment. The energy conservation measures include the installation of LED lighting in the airport terminals and administration building, the installation of LED flood lighting in the airport's general aviation terminal, and the installation of LED lighting on the airport's runways and taxi lanes. LED lighting was also installed in the airport's car parking lots (P-1 and P-10). Other energy conservation measures implemented throughout the study period included the replacement of a ventilation unit with a free cooling unit, an upgrade to the airport's heat exchange which consisted of a free exchanger and low temperature pump that used treated wastewater from the municipality of Ullensaker, the introduction of more energy efficient rotary heat recovery units, and the replacement of flood lighting around the airport's terminal building with new LED lighting. The airport also installed blanking pedestals on the existing lights in Hangar 8, the installation of motion sensors and night lowering in Pir Syd, the night lowering of lighting in Pier Norda and SBV, as well as the automatic control of ground heating systems at the airport's apron.

Another important energy and environment-related measure has been the use of sustainable aviation fuels. As noted earlier, Oslo Airport became the world's first airport to offer sustainable jet biofuel to all airlines serving the airport. The case study also revealed that in 2018, the drilling of two deep water geothermal wells 1,500 metres in depth was completed. These wells are used to supply the ground heating system located in the airport's aircraft engine test area.

REFERENCES

[1] Aircraft Commerce. (2015). 787 & A350 XWB: How do they reduce maintenance costs? *Aircraft Commerce*, 102, 16-23.

[2] Aircraft Commerce. (2016). Fuel burn & operating performance of the 787-8, 787-9 and A350-900. *Aircraft Commerce*, 108, 16-27.

[3] Airport Technology. (2016). Oslo Airport becomes world's first airport to supply sustainable jet fuel to airlines. Retrieved from https://www.airport-technology.com/uncategorised/newsoslo-airport-becomes-worlds-first-airport-to-supply-sustainable-jet-fuel-to-airlines-4793272/.

[4] Airport Technology. (2017a). Oslo Airport Terminal 2. Retrieved from https://www.airport-technology.com/projects/oslo-airport-terminal-2/.

[5] Airport Technology. (2017b). Sustainable success: Oslo Airport opens 'world's greenest' terminal. Retrieved from https://www.airport-technology.com/features/featuresustainable-success-oslo-airport-opens-worlds-greenest-terminal-5885304/.

[6] Akyüz, M.K., Altuntaş, Ö., & Söğüt, M. Z. (2017). Economic and environmental optimization of an airport terminal building's wall and roof insulation. *Sustainability*, 9(10), 1849. https://doi.org/10.3390/su9101849

[7] Akyüz, M.K., Altuntas O., Sogut M.Z, Karakoc T.H., & Kurama, S. (2018). Determination of optimum insulation thickness for building's walls with respect to different insulation materials: A case study of International Hasan Polatkan Airport terminal. *International Journal of Sustainable Aviation*, 4(2), 147-161.

[8] Alba, S.O., & Manana, M. (2017). Characterization and analysis of energy demand patterns in airports. *Energies*, 10(1), 119. https://doi.org/10.3390/en10010119

[9] Allaby, C., & Park, M. (2007). *A dictionary of environment and conservation*. Oxford, UK: Oxford University Press.

[10] Ang, S.H. (2014). *Research design for business & management*. London, UK: SAGE Publications; 2014.

[11] Apgar Oil. (2021). What is bioheat? And is it better than heating oil? Retrieved from https://www.apgaroil.com/bioheat-better-heating-oil/.

[12] Appold, S.J., & Kasarda, J.D. (2011). Seeding growth at airports and airport cities: Insights from the two-sided market literature. *Research in Transportation Business & Management*, 1(1), 91-100. https://doi.org/10.1016/j.rtbm.2011.06.011

[13] Ashford, N.J., Martin Stanton, H.P., Moore, C.A., Coutu, P., & Beasley, J.R. (2013), *Airport operations* (3rd ed.). New York, NY: McGraw-Hill.

[14] Asian Aviation. (2012). 747-8I enters commercial service. Retrieved from https://asianaviation.com/747-8i-enters-commercial-service/.

[15] Avinor AS. (2021a). About Oslo Airport: Facts and figures. Retrieved from https://avinor.no/en/corporate/airport/oslo/about-us/about-oslo-airport/tall-og-fakta.

[16] Avinor AS. (2021b). About Oslo Airport: Traffic statistics. Retrieved from https://avinor.no/en/corporate/airport/oslo/about-us/traffic_statistics/trafikkstatistikk.

[17] Avinor AS. (2021c). Energy. Retrieved from https://avinor.no/en/corporate/airport/oslo/community-and-environment/energi/enok.

[18] Avinor AS. (2021d). Environmental management. Retrieved from: https://avinor.no/en/corporate/airport/oslo/community-and-environment/miljostyring/.

[19] Avinor AS. (2021e). The new Oslo Airport 2017. Retrieved from https://avinor.no/en/corporate/airport/oslo/development/this-is-new/this-is-new.

[20] Avinor AS. (2021f). Renewable energy. Retrieved from https://avinor.no/en/corporate/airport/oslo/community-and-environment/energi/fornybar-energi.

[21] Barrett, S., Devita, P., Ho. C., & Miller, B. (2014). Energy technologies' compatibility with airports and airspace: Guidance for aviation and energy planners. *Journal of Airport Management*, 8(4), 318-326.

[22] Baxter, G. (2021). Mitigating an airport's carbon footprint through the use of "green" technologies: The case of Brisbane and Melbourne Airports, Australia. *International Journal of Environment, Agriculture and Biotechnology*, 6(6), 29-39. https://dx.doi.org/10.22161/ijeab.66.4

[23] Baxter, G., Srisaeng, P., & Wild, G. (2018a). An assessment of airport sustainability, Part 2—Energy management at Copenhagen Airport. *Resources*, 7(2), 32. https://doi.org/10.3390/resources7020032

[24] Baxter, G., Srisaeng, P., & Wild, G. (2018b) Sustainable airport energy management: The case of Kansai International Airport. *International Journal for Traffic and Transport Engineering*, 8(3), 334-358.

[25] Baxter, G., Srisaeng, P., & Wild, G. (2020). The use of aviation biofuels as an airport environmental sustainability measure: The case of Oslo Gardermoen Airport. *Magazine of Aviation Development*, 8(1), 6-14. https://doi.org/10.14311/MAD.2020.01.01

[26] Biofuels International. (2016). Norway's Oslo Airport begins jet biofuel delivery for all airlines. Retrieved from https://biofuels-news.com/news/norways-oslo-airport-begins-jet-biofuel-delivery-for-all-airlines/.

[27] Birhanu, Z.K., Kitterød, N-O., Krogstad, H.E., & Kværnø, A. (2015). Temperature boundary conditions for ATES systems. *AIP Conference Proceedings*, 1648, 030032. https://doi.org/10.1063/1.4912349

[28] Boon, T. (2020). Which airlines fly the Boeing 787-10? Retrieved from https://simpleflying.com/boeing-787-10-airlines/.

[29] Bråthen S., & Fuglum K. (2016). Developing airports in a long-term perspective: The case of Tromsø, Norway. In G. Williams & S. Bråthen (Eds.), *Air transport provision in remoter regions* (pp. 221-230). Abingdon, UK: Routledge.

[30] Brooks, K.P., Snowden-Swan, l.J., Jones, S.B., Butcher, M.J., Lee, J.S.J., Anderson, D.M., Frye, J.G., Holladay, J.E., Owen, J., Harmon, L., Burton, F., Palou-Rivera, I., Plaza, J., Handler, R., & Shonnard, D. (2016). Low-carbon aviation fuel through the alcohol to jet pathway. In C. Chuck (Ed.), *Biofuels for aviation: Feedstocks, technology and implementation* (pp. 109-146). London, UK: Academic Press.

[31] Budd, L., & Budd, T. (2013). Environmental technology and the future of flight. In L. Budd, S. Griggs & D. Howarth (Eds.), *Sustainable aviation futures* (pp. 87-107). Bingley, UK: Emerald Group Publishing.

[32] Budd, L., & Ison, S. (2017). Airfield design, configuration, and management. In L. Budd & S. Ison, (Eds.), *Air transport management: An international perspective* (pp. 59-77). Abingdon, UK: Routledge.

[33] Bullough, J. D. (2012). Light-emitting diode airfield lighting systems. Retrieved from https://www.internationalairportreview.com/article/10414/light-emitting-diode-airfield-lighting-systems/.

[34] Business Traveller. (2017). Oslo airport opens terminal extension. Retrieved from https://www.businesstraveller.com/business-travel/2017/04/27/oslo-airport-opens-terminal-extension/.

[35] Cardona, E., Piacentino, A., & Cardona, F. (2006). Energy saving in airports by trigeneration. Part I: Assessing economic and technical potential. *Applied Thermal Engineering*, 26(14-15), 1427–1436. https://doi.org/10.1016/j.applthermaleng.2006.01.019

[36] Climates to Travel. (2012). Climate – Oslo, Norway. Retrieved from https://www.climatestotravel.com/climate/norway/oslo.

[37] Comendador, V.F.G., Valdés, R.M.A., & Lisker, B. (2019). A holistic approach to the environmental certification of green airports. *Sustainability*, 11(15), 4043. https://doi.org/10.3390/su11154043

[38] Culberson, S.D. (2011) Environmental impacts of airports. In N.J. Ashford, S.A. Mumayiz & P.H. Wright (Eds.), *Airport engineering: Planning, design, and development of 21st century airports* (pp. 704-738) (4th ed.). Hoboken, NJ: John Wiley & Sons.

[39] Daley, B. (2016). *Air transport and the environment*. Abingdon, UK: Routledge.

[40] Derrington, M.L. (2019). *Qualitative longitudinal methods: Researching implementation and change*. Thousand Oaks, CA: SAGE Publications.

[41] Dentch, M.P. (2016). *The ISO 14001:2015 handbook: Using the process approach to building an environmental management system*. Milwaukee, WI: Quality Press

[42] de Rubeis, T., Nardi, J., Paoletti, D., Di Leonardo, A., Ambrosini, D., Poli, R., & Sfarra, S. (2016). Multi-year

consumption analysis and innovative energy perspectives: The case study of Leonardo da Vinci International Airport of Rome. *Energy Conversion and Management*, 128, 261-272. https://doi.org/10.1016/j.enconman.2016.09.076

[43] Dube, K., Nhamo, G., & Chikodzi, D. (2021). COVID-19 pandemic and prospects for recovery of the global aviation industry. *Journal of Air Transport Management*, 92, 102022. https://doi.org/10.1016/j.jairtraman.2021.102022

[44] Eggen, G., & Vangsnes, G. (2006). Heat pump for district cooling and heating at Oslo Airport, Gardermoen [Aquifer thermal energy systems (ATES)]. Retrieved from https://www.osti.gov/etdeweb/biblio/20886519.

[45] Energy Australia. (2021). Energy recovery fact sheet. Retrieved from https://www.energyaustralia.com.au/sites/default/files/2020-11/Energy%20Recovery_Fact_%20Sheet.pdf.

[46] Efthymiou, M., & Papatheodorou, A. (2018). Evolving airline and airport business models. In N. Halpern & A. Graham (Eds.), *The Routledge companion to air transport management* (pp. 122-135). Abingdon, UK: Routledge.

[47] Feldman, J. (1998). 25 years to life. *Air Transport World*, 35(9), 91-96.

[48] Ferrulli, P. (2016). Green airport design evaluation (GrADE) – Methods and tools improving infrastructure planning. *Transportation Research Procedia*, 14, 3781-3790. https://doi.org/10.1016/j.trpro.2016.05.463

[49] Fregnani, A.T.G., & Andrade, O. (2017). Aviation initiatives and the relative impact of electric road vehicles on CO_2 emissions. Retrieved from https://www.boeing.com/features/innovation-quarterly/aug2017/feature-technical-co2.page.

[50] French, H., & Binley, A. (2004). Snowmelt infiltration: Monitoring temporal and spatial variability using time-lapse electrical resistivity. *Journal of Hydrology*, 297(1-4), 174-186. https://doi.org/10.1016/j.jhydrol.2004.04.005

[51] Freyssinier, J.P. (2014). The long-term performance of LEDs. *International Airport Review*, 18, 38–41.

[52] González-Ruiz, J.D., Duque, E., & Restrepo, J. (2017). Green airport infrastructure in Colombia: Opportunities for public-private partnerships schemes. *Pertanika Journal of Science & Technology*, 25, 37 – 46.

[53] Graham, A. (2005). Airport benchmarking: A review of the current situation. *Benchmarking: An International Journal*, 12(2), 99-111. https://doi.org/10.1108/14635770510593059

[54] Graham, A. (2018). *Managing airports: An international perspective* (5th ed.). Abingdon, UK: Routledge.

[55] Grover, R.C., & Grover, S. (2017). *Winning the environmental challenge with ISO 14001: 2015: Implementation of an Environmental Management System*. Chennai, India: Notion Press.

[56] Hanaki, K., & Portugal-Pereira, J. (2018) The effect of biofuel production on greenhouse gas emission reductions. In K. Takeuchi, H. Shiroyama, O. Saito & M. Matsuura (Eds.), *Biofuels and sustainability: Holistic perspectives for policymaking* (pp. 53-71) Tokyo, Japan: Springer Japan.

[57] Hassett, M., & Paavilainen-Mäntymäki, E. (2013). Longitudinal research in organizations: An introduction. In M. Hasset & E. Paavilainen-Mäntymäki (Eds.), *Handbook of longitudinal research methods in organisation and business studies* (pp. 1-12). Cheltenham, UK: Edward Elgar Publishing.

[58] Hazel, R.A., David Blais, I., Browne, T.J., & Benzom, D.M. (2016). *Resource guide to airport performance indicators*. Airport Cooperative Research Program Report 19A. Washington, DC: Transportation Research Board.

[59] Heras-Saizarbitoria, I., Landín, G.A., & Molina-Azorín, J.F. (2011). Do drivers matter for the benefits of ISO 14001? *International Journal of Operations and Production Management*, 31(2), 192-216. https://doi.org/10.1108/01443571111104764

[60] Holloway, S. (2016). *Straight and level: Practical airline economics* (3rd ed.). Abingdon, UK: Routledge.

[61] Iatrou, K., & Williams, G. (2008). New for old: An analysis of the impact of the new Athens and Oslo airports. *Journal of Airport Management*, 2(3), 235-248.

[62] International Airport Review. (2010). Oslo Airport – Europe's most efficient airport. Retrieved from https://www.internationalairportreview.com/article/3989/oslo-airport-europes-most-efficient-airport/.

[63] International Civil Aviation Organization. (2014). *Airport services manual Part 1: Rescue and firefighting*. (4th ed.). Document 9137- AN/898. Montreal, Canada: ICAO.

[64] Jackson, R. (2021). *Airbus A380*. Barnsley, UK: Pen & Sword Books.

[65] Janić, M. (2011). *Greening airports: Advanced technology and operations*. London, UK: Springer Verlag London Limited.

[66] Kazda, T., & Caves, B. (2015). Airport emergency services. In A Kazda & R.E Caves (Eds.), *Airport design and operation* (pp. 427-456) (3rd ed.). Bingley, UK: Emerald Group Publishing.

[67] Kazda, T., Caves, B., & Kamenický, M. (2015). Environmental control. In A. Kazda & R.E. Caves (Eds.), *Airport design and operation* (pp. 457-500) (3rd ed.). Bingley, UK: Emerald Group Publishing.

[68] Kim, S.C., Shin, H.I., & Ahn, J. (2020). Energy performance analysis of airport terminal buildings by use of architectural, operational information and benchmark metrics. *Journal of Air Transport Management*, 83, 101762. https://doi.org/10.1016/j.jairtraman.2020.101762

[69] Kumar, A., Aswin, A., & Gupta, H. (2020). Evaluating green performance of the airports using Hybrid BWM and VIKOR methodology. *Tourism Management*, 76, 103941. https://doi.org/10.1016/j.tourman.2019.06.016

[70] Larssen, S., Grønskei, K.E., Gram, F., Hagen, L.O., & Walker, S.E. (1994). Verification of urban scale time-dependent dispersion model with subgrid elements in Oslo, Norway. In S.E. Gryning & M.M. Millán (Eds.), *Air pollution monitoring and application X* (pp. 91-99). Boston, MA: Springer.

[71] Ledec, G.C., Rapp, K.W., & Aiello, R.G. (2011). *Greening the wind: Environmental and social considerations for wind power development*. Washington, DC: The World Bank.

[72] Lian, J. I. (2010). Network dependency and airline competition – Consequences for remote areas in Norway. *Journal of Air Transport Management*, 16(3), 137-143. https://doi.org/10.1016/j.jairtraman.2009.07.007

[73] Liu, S., Butler, D., Memon, F.A., Makropoulos, C., Avery, L., & Jefferson, B. (2010). Impacts of residence time during storage on potential of water saving for grey water recycling system. *Water Research*, 44(1), 267-277. https://doi.org/10.1016/j.watres.2009.09.023

[74] Liu, X., Zhang, T., Liu, X., Li, L., Lin, L., & Jiang, Y. (2021). Energy saving potential for space heating in Chinese airport terminals: The impact of air infiltration. *Energy*, 215(Part B), 119175. https://doi.org/10.1016/j.energy.2020.119175

[75] Mæx Moe, J. (2018). Using stored snow as cooling at Oslo airport, Norway. *Civil Engineering*, 171(5), 11-16. https://doi.org/10.1680/jcien.17.00041

[76] Maria Iacus, S., Natale, F., Santamaria, C., Spyratos, S., & Vespe, M. (2020). Estimating and projecting air passenger traffic during the COVID-19 coronavirus outbreak and its socio-economic impact. *Safety Science*, 129, 104791. https://doi.org/10.1016/j.ssci.2020.104791

[77] McEntee, F. (2021). How green is hydropower? Retrieved from https://www.sustaineurope.com/how-green-is-hydropower-20180229.html.

[78] McCutchen, D.M., & Meredith, J.R. (1993). Conducting case study research in operations management. *Journal of Operations Management*, 11(3), 239-256. https://doi.org/10.1016/0272-6963(93)90002-7

[79] Monsalud, A., Ho, D., & Rakas, J. (2015). Greenhouse gas emissions mitigation strategies within the airport sustainability evaluation process. *Sustainable Cities and Society*, 14, 414-424. https://doi.org/10.1016/j.scs.2014.08.003

[80] Mosvold Larsen, O. (2017). Aviation biofuels at Oslo Airport. Retrieved from https://www.icao.int/Meetings/altfuels17/Documents/Olav%20Mosvold%20Larsen%20-%20Avinor.pdf.

[81] National Hydropower Association. (2021). Clean & sustainable. Retrieved from: https://www.hydro.org/waterpower/why-hydro/clean-and-sustainable/#:~:text=Hydropower%20is%20a%20climate%2Dfriendly,over%2038%20million%20passenger%20cars.

[82] Neale, B. (2018). *What is qualitative longitudinal research?* London, UK: Bloomsbury Publishing.

[83] Oates, B.J. (2006). *Researching information systems and computing*. London, UK: SAGE Publications.

[84] O'Leary, Z. (2004). *The essential guide to doing research*. London, UK: SAGE Publications.

[85] Norwegian Ministry of Petroleum and Energy. (2021). Energy facts Norway. Retrieved from https://energifaktanorge.no/en/norsk-energiforsyning/kraftproduksjon/.

[86] Orth, H., & Weidmann, O. (2014). Airport cities and airport public transport access: Demand balancing or peak exacerbation? Case of Zurich Airport, Switzerland. *Transportation Research Record: Journal of the Transportation Research Board*, 2449(1), 24-33. https://doi.org/10.3141%2F2449-03

[87] Oslo Lufthavn AS. (2008). Environmental report 2007. Retrieved from https://avinor.no/globalassets/_oslo-lufthavn/om-oslo-lufthavn/om-oss/rapporter/en/environmental/Environmental-report-2007.

[88] Oslo Lufthavn AS. (2009). Environmental report 2008. Retrieved from https://avinor.no/globalassets/_oslo-lufthavn/om-oslo-lufthavn/om-oss/rapporter/en/environmental/Environmental-report_2008.pdf.

[89] Oslo Lufthavn AS. (2010). Environmental report 2009. Retrieved from https://avinor.no/globalassets/_oslo-lufthavn/om-oslo-lufthavn/om-oss/rapporter/en/environmental/Environmental-report_2009.pdf.

[90] Oslo Lufthavn AS. (2011). Environmental report 2010. Retrieved from https://avinor.no/globalassets/_oslo-lufthavn/om-oslo-lufthavn/om-oss/rapporter/en/environmental/Environmental-report-2010.pdf.

[91] Oslo Lufthavn AS. (2012). Environmental report 2011. Retrieved from https://avinor.no/globalassets/_oslo-lufthavn/om-oslo-lufthavn/om-oss/rapporter/en/environmental/Environmental-report-2012.pdf.

[92] Oslo Lufthavn AS. (2013). Environmental report 2012. Retrieved from https://avinor.no/globalassets/_oslo-lufthavn/om-oslo-lufthavn/om-oss/rapporter/en/environmental/Environmental-report-2012.pdf.

[93] Oslo Lufthavn AS. (2014). Environmental report 2013. Retrieved from https://avinor.no/globalassets/_oslo-lufthavn/om-oslo-lufthavn/om-oss/rapporter/en/environmental/environmental_report_2013.pdf.

[94] Oslo Lufthavn AS. (2015). Environmental report 2014. Retrieved from https://avinor.no/globalassets/_oslo-lufthavn/om-oslo-lufthavn/om-

oss/rapporter/en/environmental/environmental-report-2015.pdf.

[95] Oslo Lufthavn AS. (2016). Environmental report 2015. Retrieved from https://avinor.no/globalassets/_oslo-lufthavn/om-oslo-lufthavn/om-oss/rapporter/en/environmental/environmental-report-2015.pdf.

[96] Oslo Lufthavn AS. (2017). Environmental report 2016. Retrieved from https://avinor.no/globalassets/_oslo-lufthavn/om-oslo-lufthavn/om-oss/rapporter/en/environmental/environmental-report-2016.pdf.

[97] Oslo Lufthavn AS. (2018). Environmental report 2017. Retrieved from https://avinor.no/globalassets/_oslo-lufthavn/om-oslo-lufthavn/om-oss/rapporter/en/environmental/environmental-report-2017.pdf.

[98] Oslo Lufthavn AS. (2019). Environmental report 2018. Retrieved from https://avinor.no/globalassets/_oslo-lufthavn/om-oslo-lufthavn/om-oss/rapporter/en/environmental/environmental-report-2018.pdf.

[99] Oslo Lufthavn AS. (2020). Environmental report 2019. Retrieved from https://avinor.no/globalassets/_oslo-lufthavn/om-oslo-lufthavn/om-oss/rapporter/en/environmental/environmental-report-2019.pdf.

[100] Oslo Lufthavn AS. (2021). Environmental report 2020. Retrieved from https://avinor.no/globalassets/_oslo-lufthavn/om-oslo-lufthavn/miljo-og-lokalsamfunn/miljodokumenter/miljoarsrapport-2020-engelsk.pdf.

[101] Preston, K. (2015). Sustainability initiatives helping airports address climate change. *International Airport Review*, 19(5), 16-19.

[102] Prosperi, D. (2009). Greening the world's airports. *World Transport Policy & Practice*, 14(4), 48-56.

[103] Ramon Gil-Garcia, J. (2012). *Enacting electronic government success: An integrative study of government-wide websites, organizational capabilities, and institutions*. New York, NY: Springer Science-Business Media.

[104] Reiss, B. (2007). Maximising non-aviation revenue for airports: Developing airport cities to optimise real estate and capitalise on land development opportunities. *Journal of Airport Management*, 1(3), 284-293.

[105] Remenyi, D, Williams, B, Money, A., & Swartz, E A. (2010). *Doing research in business and management: An introduction to process and method*. London, UK: SAGE Publications.

[106] Ros, M. (2017). Why is Oslo Airport called the world's greenest? Retrieved from https://edition.cnn.com/travel/article/oslo-airport-worlds-greenest/index.html.

[107] Rossi Dal Pozzo, F. (2015). *EU legal framework for safeguarding air passenger rights*. Cham, Switzerland: Springer International Publishing.

[108] Sajed Sadati, S.M., Cetin, K., Ceylan. H., Sassani, A., & Kim, S. (2018). Energy and thermal performance evaluation of an automated snow and ice removal system at airports using numerical modeling and field measurements. *Sustainable Cities and Society*, 43, 238-250. https://doi.org/10.1016/j.scs.2018.08.021

[109] Scott, J. (2004). Documents, types of. In: M. Lewis-Beck, A.E. Bryman. & T. Futing Liao (Eds.), *The SAGE encyclopedia of social science research methods* (pp. 281-284). Thousand Oaks, CA: SAGE Publications.

[110] Scott, J. (2014). *A dictionary of sociology* (4th ed.). Oxford, UK: Oxford University Press.

[111] Scott, J. & Marshall G. (2009). *A dictionary of sociology* (3rd ed.). Oxford, UK; Oxford University Press.

[112] Shulman, V.L. (2019). Tire recycling. In T. M. Letcher & D. A. Vallero (Eds.), *Waste: A handbook for management* (pp. 489-515) (6th ed.). Amsterdam, The Netherlands: Elsevier.

[113] Simons, G.M. (2014). *Airbus A380: A history*. Barnsley, UK: Pen & Sword Books.

[114] Staples, M.D., Malina, R., Suresh, P., Hileman, J.I., & Barrett, S.R.H. (2014). Aviation CO_2 emissions reductions from the use of alternative jet fuels. *Energy Policy*, 114, 342-354. https://doi.org/10.1016/j.enpol.2017.12.007

[115] Su, Y., Zhang, P., & Su, Y. (2015). An overview of biofuels policies and industrialization in the major biofuel producing countries. *Renewable and Sustainable Energy Reviews*, 50, 991-1003. https://doi.org/10.1016/j.rser.2015.04.032

[116] Sukumaran, S., & Sudhakar, K. (2017). Fully solar powered airport: A case study of Cochin International airport. *Journal of Air Transport Management*, 62, 176-188. https://doi.org/10.1016/j.jairtraman.2017.04.004

[117] Sumathi, N., Phanendra, M.G.V.S., & Teja, K.G. (2018). Green airports- Solution to stop pollution! *International Journal of Latest Technology in Engineering, Management & Applied Science*, VII(IV), 78-85.

[118] Taber, C., & Steele, B.A. (2020). Impact of HVLS fans on airplane hangar air destratification. *ASHRAE Journal*, April, 26-32.

[119] Taylor, J. (2011). Technology innovation and advance LEDs for airfield lighting. Retrieved from https://www.internationalairportreview.com/article/7451/technology-innovation-and-advance-leds-for-airfield-lighting/.

[120] The Avinor Group. (2021). About the company. Retrieved from https://avinor.no/en/corporate/about-us/the-avinor-group/about-the-company.

[121] Thomas, C., & Hooper, P. (2013). Sustainable development and environmental capacity of airports. In

N.J. Ashford, H.P.M. Stanton, C.A. Moore, P. Coutu & J.R. Beasley (Eds.), *Airport operations* (pp. 553-578) (3rd ed.). New York, NY: McGraw-Hill.

[122] United States Department of Energy. (2019) DOE BTO lighting R&D program, 2019 Lighting R&D Opportunities. Retrieved from https://www.energy.gov/sites/prod/files/2020/01/f70/ssl-rd-opportunities2-jan2020.pdf.

[123] United States Energy Information Agency. (2020). Solar explained: Solar energy and the environment. Retrieved from https://www.eia.gov/energyexplained/solar/solar-energy-and-the-environment.php

[124] Vestvik-Lunde, J. (2014). Oslo Airport achieves environmental management certification. Retrieved from: https://www.dnvgl.com/news/oslo-airport-achieves-environmental-management-certification--7403.

[125] Wang, C., & Prinn, R.G. (2010). Potential climatic impacts and reliability of very large-scale wind farms. *Atmospheric Chemistry and Physics*, 10(4), 2053–2061.

[126] Warren, C.R., Lumsden, C., O'Dowd, S., & Birnie, R.V. (2005). 'Green On Green': Public perceptions of wind power in Scotland and Ireland. *Journal of Environmental Planning and Management*, 48(6), 853-875. https://doi.org/10.1080/09640560500294376

[127] Wejden B., & Øvstedal J. (2006). Contamination and degradation of de-icing chemicals in the unsaturated and saturated zones at Oslo Airport, Gardermoen, Norway. In J.H. Tellam, M.O. Rivett, R.G. Israfilov & L.G. Herringshaw (Eds.), *Urban groundwater management and sustainability* (pp. 205-218). NATO Science Series (IV: Earth and Environmental Sciences), Vol 74. Dordrecht, The Netherlands: Springer, Dordrecht.

[128] Yildiz, O.F., Yilmaz, M., & Çelik, A. (2021). Energy analysis of cold climate region airports: A case study for airport terminal in Erzurum, Turkey. *International Journal of Sustainable Aviation*, 7(1), 66-92

[129] Yin, R.K. (2018). *Case study research and applications* (6th ed.). Thousand Oaks: SAGE Publications.

[130] Young, S.B., & Wells, A.T. (2011). *Airport planning & management* (6th ed.). New York, NY: McGraw Hill.

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Towards Carbon Neutral Airline Operations by 2045: The Case of Finnair PLC

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Abstract—*In response to the growing concerns over their impact on the environment and climate change, a number of the world's airlines have announced plans to become fully "carbon neutral". Using an in-depth instrumental case study research approach, this study examines the strategies defined and implemented by Finnair to meet its goal of becoming a "carbon neutral" airline by 2045. The qualitative data, covering the period 2010 to 2019, was analyzed using document analysis. The study found that the operation of a modern, fuel-efficient aircraft fleet underpins Finnair's goal of becoming a carbon neutral airline. Other measures implemented by Finnair include a carbon offset program for its passengers and corporate customers, more sustainable waste management practices, the use of aviation biofuels where possible to power their aircraft operations, the use of biodiesel for ground service equipment (GSE), the electrification of ground vehicles, and measures to reduce aircraft weight. Finnair's annual Scope 1 carbon dioxide (CO_2) emissions from jet fuel usage have increased from 2010 to 2019 due to fleet and route network expansions. The airline's Scope 1 carbon dioxide (CO_2) emissions from ground vehicles fuel usage declined over the study period. The annual Scope 2 carbon dioxide (CO_2) emissions for electricity and heating oscillated over the study period reflecting changes in usage patterns, particularly for heating during the winter periods. Finnair's annual Scope 3 carbon dioxide (CO_2) emissions exhibited an upward trend due to the carbon dioxide (CO_2) emissions associated with the manufacture of the airline's new Airbus A350-900XWB aircraft, the greenhouse gas emissions (GHGs) associated with the production and transportation of jet fuel, emissions from leased vehicles, and emissions from cargo flights that were operated on behalf of Finnair Cargo.*

Keywords—***Aircraft, Airline, Airline carbon footprint, Carbon neutral airline operations, Carbon dioxide (CO_2) emissions, Case study, Finnair.***

I. INTRODUCTION

Many airlines around the world have recognized the importance of environmental protection (Niu et al., 2016), and considering this many airlines have taken strong environmental positions (Roza, 2009). Indeed, airlines have become increasingly committed to becoming more "green," or environmentally friendly (Hagmann et al., 2015; Jalalian et al., 2019; Migdadi, 2018, 2020c; Zhou & Zhang, 2020). A "green airline" is a relatively new concept – and represents initiatives by the airline to support sustainable social and economic development without impacting the local and global environment (Sarkar, 2012). The objective of a "green airline" is to provide the green society with a transport system that reduces its carbon footprint, uses renewable energy, and produces less carbon dioxide (CO_2) emissions as well as other harmful pollutants (Abdullah et al., 2016). The concept of "greening" aviation firms, such as, airlines, can be best linked to their reduction of emissions into the atmosphere, to the point where they achieve near carbon neutrality (Sarkar, 2012). Carbon neutrality means every ton of anthropogenic carbon dioxide (CO_2) emitted is compensated with an equivalent amount of carbon dioxide (CO_2) removed (Levin et al., 2015). Furthermore, the adoption of a green operations strategy by an airline is a combination of green operational actions that are undertaken to acknowledge green indicators (Migdadi, 2020b).

The world's peak global airline industry body – the International Air Transport Association (IATA) – have recognized the requirement to address the global challenge of climate change and in response has adopted a set of ambitious targets to mitigate carbon dioxide (CO_2) emissions arising from air transport operations. The association has targeted an average improvement in aircraft fuel efficiency of 1.5% per year from 2009 to 2020, a cap on net aviation carbon dioxide (CO_2) emissions from 2020, and a reduction in net aviation carbon dioxide (CO_2) emissions of 50% by 2050, relative to 2005 levels (International Air Transport Association, 2018). Importantly, the direct greenhouse gas emissions that are produced from aircraft operations and the use of ground service equipment (GSE) contribute to the world's total greenhouse gas emissions (Migdadi, 2020a). Aviation emissions are the source of around 2 to 3 per cent of global greenhouse gas emissions (Birchfield, 2015).

Considering the IATA policy to reduce carbon dioxide (CO_2) emissions, member airlines have introduced plans and strategies that are aimed to achieve this policy objective. Furthermore, several of the world's major airlines have announced plans to become fully "carbon neutral" (Becken, 2020; Cui & Li, 2021; Cui et al., 2020). The key aim of this study is to gain an insight into how airlines can achieve their goal of carbon neutral operations. To achieve this aim, Finland-based Finnair PLC. was selected as the case airline for this study. Finnair was the first airline in the world to announce their intention to cut their net carbon emissions by 50% from 2019 levels by the end of 2025, and for the airline to be fully "carbon neutral" by 2045 (Bailey, 2020; Finnair, 2021b; Taylor, 2020). The key objective of this study was to examine the strategies that the airline has defined and implemented to achieve its carbon neutral operations goal. A second objective was to examine Finnair's annual carbon footprint and to identify the impact that the carbon neutrality strategy has had on its carbon footprint. According to Wiedemann and Minx (2007, p. 5), "the carbon footprint is a measure of the exclusive total amount of carbon dioxide emissions that is directly and indirectly caused by an activity or is accumulated over the life stages of a product". An additional objective is to examine the role that the airline's aircraft fleet will pay as part of its carbon neutrality strategy. The study covers the period 2010 to 2019.

The remainder of the paper is organized as follows: the literature review is presented in Section 2, and this sets the context of the case study. The research method that underpinned the study is outlined in Section 3. The Finnair case study is presented in Section 4. Section 5 presents the findings of the study.

II. BACKGROUND

2.1 Aircraft and Ground-Based Equipment and Vehicle Emissions

Aviation emissions are a significant contributor to global climate change (Markham et al., 2018). By consuming fuel, aircraft produce emissions of carbon dioxide (CO_2), nitrogen oxides (NO_X), particles (principally soot) of sulphur oxides (SO_X), carbon monoxide (CO), as well as various hydrocarbons. First, and generating the largest percentage share, are the emissions of carbon dioxide (CO_2) which are produced in direct proportion to the volume of jet fuel used to operate flights over any distance (Sales, 2013, 2016). Water vapor is formed from the burning of jet fuels. At altitude, condensation trails from the aircraft. These comprise frozen ice crystals which deflect a small amount of sunlight away from the earth's surface and reflect more infrared radiation back toward earth. This produces an overall warming effect on the earth's atmosphere (Sales, 2013). After water vapor, carbon dioxide (CO_2) is regarded as the second most important of all the greenhouse gases (Drewer et al., 2018; Ngo & Natowitz, 2016).

Aircraft emissions present potential risks relating to public health (Barrett et al., 2010; Levy et al., 2012). Furthermore, aircraft often travel considerable distances at a variety of altitudes, generating emissions that may potentially have an impact on air quality in not only local, but also regional and global environments (International Civil Aviation Organization, 2011).

Growing environmental concerns in recent times have also drawn the attention of the airline industry towards the requirement for judicious use of aviation fuel. As a result, both economic and environmental sustainability concerns have resulted in significant progress in aviation fuel efficiency improvements in recent times (Singh et al., 2018). The airlines and the aircraft manufacturers have invested in new technologies and strategies to reduce fuel consumption and the related emissions (Zou et al., 2016). Aircraft fuel burn is highly correlated with emissions whilst also directly contributing to transport externalities (Park & O'Kelly, 2014).

Air pollution at an airport is also produced from the ground service equipment (GSE) used during aircraft turnaround and ground handling operations (Sameh & Scavussi dos Santos, 2018; Testa et al., 2014). Ground service equipment (GSE) is any piece of mobile equipment, whether powered or self-propelled, that is purpose designed, built, and used for the ground handling, servicing, or field maintenance of civil transport aircraft on the ramp or apron area of an airport (International Organization for Standardization, 2014). Accordingly, the ground service equipment (GSE) carbon dioxide (CO_2) emissions can also be significant at airports (International Airport Review, 2010).

2.2 Carbon Offsetting and Reduction Scheme for International Aviation (CORSIA)

In October 2016, the Member States of the International Civil Aviation Organization (ICAO) reached a decision to adopt a world-wide market-based measure for aviation emissions (Attanasio, 2018; Scheelhaase et al., 2018). In 2021, an increasing share of the carbon emission growth in international air transport will be subject to offsetting under the ICAO "Carbon Offsetting and Reduction Scheme for International Aviation" (CORSIA) program (Maertens et al., 2019). CORSIA is a worldwide based market-based measure that has been designed to offset international aviation carbon dioxide (CO_2) emissions to stabilize the levels of such emissions from 2020 onwards. The offsetting of carbon dioxide (CO_2) emissions in the air transport industry will be achieved through the acquisition and cancelation of emissions units from the global carbon market by aircraft operators (International Civil Aviation Organization, 2021). The CORSIA program will be rolled out in three phases with the pilot phase operating from 2021-2023. The first phase will be from 2024 to 2026. Both the pilot and first phases are voluntary. The second phase of the program is targeted from 2027 to 2035 (Javed et al., 2019). Following the pilot and first phase, a second mandatory scheme will enter in effect for all ICAO Member States, except for some least developed countries (Scott & Trimarchi, 2020). The COVID-19 pandemic resulted in a very significant decline in the global aviation industry traffic (Czerny et al., 2021; Li, 2021; Liu et al., 2020) and, as a result, the International Civil Aviation Organization (ICAO) adjusted its CORSIA program by removing 2020 emissions from the baseline, which are now based on 2019 emission levels (Zhang et al., 2021). The voluntary pilot period for ICAO's CORSIA program became effective as of 2021 and will become mandatory for all airlines from 2027 onwards (Singapore Airlines, 2021).

2.3 Offsetting of Carbon Emissions by Airline Passengers

Carbon offsetting has become an integral element of the airline industry strategy to reduce its carbon emissions (Becken & Mackey, 2017). Consequently, airlines are now offering carbon offset schemes for their passengers so that they can reduce their carbon footprint (Chen, 2013; Ritchie et al., 2020; Zhang et al., 2019). Voluntary carbon offsetting by airline passengers is a useful measure that could help reduce environmental damage caused by air travel (Babakhani et al., 2017). In the global airline industry, carbon offsetting is a means for individuals or firms, in this case airline passengers and corporate customers, to "neutralize" their proportion of an aircraft's carbon emissions on a particular journey through an investment in carbon reduction projects (International Air Transport Association, 2021b). The principle of carbon offsetting is that the emissions for each flight are allocated amongst the passengers. Each passenger can therefore pay to offset the emissions caused by their portion of the flight's emissions. Passengers can offset their emissions through an investment in carbon reduction projects that generate carbon credits (International Air Transport Association, 2016).

Passengers participating in carbon offset programs can purchase carbon credits generated by certified renewable energy and energy efficiency projects in developing countries. These projects have been verified that they will reduce greenhouse gas emissions. A carbon credit is a permit that represents one tonne of carbon dioxide (CO_2) that has either been removed from the atmosphere or alternatively saved from being emitted. Once used these carbon credits are subsequently "cancelled" on an official register to ensure that they cannot be sold or used again (International Air Transport Association, 2016).

Carbon credits establish a market for the reduction in greenhouse emissions by providing a monetary value to the cost of polluting the air (International Air Transport Association, 2016). There are two major types of carbon credits: certified emission reductions (CERs) and voluntary emission reductions (VERs) (Bayon et al., 2009; Harris, 2019).

2.4 The Greenhouse Gas Protocol

The Greenhouse Gas Protocol has established a comprehensive global standardized framework to measure and manage greenhouse gas (GHG) emissions from both the private and public sectors, through value chains, and mitigation actions (Greenhouse Gas Protocol, 2021). The Greenhouse Gas Protocol categorizes greenhouse gases into both direct and indirect emissions and further categorizes them into Scope 1, Scope 2, and Scope 3 emissions (Jones, 2009). Scope 1, direct emissions, includes those emissions from sources that are owned or controlled by the firm (Girella, 2018; Vásquez et al., 2015). Scope 2, indirect emissions, come from the purchase of electricity, heat, steam or cooling. Scope 3 emissions are all the other indirect emissions that arise from the consequences of the various activities undertaken by a firm but occur from sources that are not owned nor controlled by that firm (Mazhar et al., 2019).

Although there are variations in air quality regulations by country (Budd, 2017), airlines are now increasingly recording and reporting emissions in terms of Scope 1, Scope 2, or Scope 3 emissions. Finnair, the case airline in the present study, is one such airline that follows this practice.

2.5 Reduction in Aircraft Weight

In recent times, airlines from around the world have implemented a range of measures that have been designed to lower the weight of their aircraft, and thus, reduce fuel burn and the associated harmful emissions. The weight saving initiatives include the correct stowage of items to avoid unnecessarily ordering catering supplies and other in-flight service equipment, the removal of rubbish, and the reduction in on-board company materials. In addition, airlines have implemented potable water strategies whereby they carefully optimize the water uplift on flights to satisfy passenger requirements whilst at the same time achieving fuel and emissions savings from the lower the aircraft weight (Baxter, 2016).

2.6 Sustainable Airline Waste Management

Airlines produce substantial volumes of waste which typically includes food and drink containers, newspapers and magazines, food waste (from offices, lounges/cafeterias, and in-flight services), light bulbs, printer toner, paper, documents, and computer print outs (Baxter et al., 2018). Deplaned aircraft waste is waste that originates on an airline's flights. Cabin waste is comprised of two principal streams: cleaning waste and catering (galley) waste (International Air Transport Association, 2021a). The volume and characteristics of waste generated on an aircraft are normally dependent upon the length of the flight being operated (Chandrappa & Das, 2012).

Airlines often dispose of their wastes through recycling, incineration, composting, or by landfill (Baxter et al., 2021). When recycling waste, the waste fraction is utilized again to produce consumer goods or other products. Recycling of wastes may also include the conversion of waste into energy through thermal treatment (processing) (Fulekar, 2010; Skrifvars & Åkesson, 2016). Energy recovery reduces the volume of waste that is disposed by landfill and produces useable energy, in terms of heat, electricity or fuel, through a variety of processes. These processes include combustion, gasification, pyrolysis, and anaerobic digestion (Rahman et al., 2017). With incineration the waste fraction is incinerated. During waste incineration, there are substantial emissions of carbon dioxide (CO_2) (Reinhardt et al., 2008; Tarczay et al., 2011). There may also be smaller amounts of methane and nitrous oxide emissions (Tarczay et al., 2011). Composting waste is a process whereby the organic portion of solid waste is converted into a humus-like product. The final product, which is inert in nature, can be utilized as a soil conditioner or for landfill cover (Harper, 2004, p. 3). There are several advantages associated with the composting of rubbish: lower operational costs, lessened environmental pollution, as well as the beneficial use of the end products (Taiwo, 2011). Wastes disposed by landfill undergo biological, chemical, and physical transformations that result in changes in solid, liquid (leachate), and gas phases (Pawlowska, 2014). Disposal in landfill sites is regarded as the least desirable option (Barlow & Morgan, 2013; Manahan, 2011; Pitt and Smith, 2003).

2.7 The use of Sustainable Aviation Biofuels

In recent times, there has been a growing trend by airlines to use aviation biofuel as an environmental sustainability measure (Baxter et al., 2020; Dodd & Yengin, 2021; Neuling & Kaltschmitt, 2018). Concerns associated with climate change and energy supply have been driving the production of more sustainable aviation fuels (Brooks et al., 2016). Accordingly, alternative jet fuel (AJF) technologies have gained considerable interest as a way for the industry to achieve large, near-term emissions reductions (Staples et al., 2014). Sustainable jet fuels represent an especially important element in the airline industry's strategy to reduce their carbon emissions (Gegg et al., 2014; Schäfer, 2016). Depending upon the raw material used in its production, biofuels can reduce carbon dioxide (CO_2) emissions by 60-80% (Bioenergy International, 2019; Tavares Kennedy, 2019).

Aviation biofuels are therefore becoming an important substitute for fossil fuel in the airline industry as they offer several advantages including sustainability, they are environmentally friendly, and they offer good adaptability (Su et al., 2015). In addition, the replacement of fossil fuels by jet-biofuels is one of the primary strategies to decrease carbon dioxide (CO_2) emissions by 50% by 2050 (Bauen & Nattrass, 2018; Dodd et al., 2018). Thus, the aviation industry has recognized the importance of sustainable biofuels as being the key long-term technology for decarbonizing aviation (Fregnani & Andrade, 2017).

III. RESEARCH METHODOLOGY

3.1 Research Method

This study used an instrumental case study research approach. An instrumental case study is a research approach that facilitates the understanding of a phenomenon (Grandy, 2010). An instrumental case study is also the study of a specific case, for example, a firm, that provides insights into a specific issue, redraws generalizations, or builds theory (Stake, 1995, 2005). The goal of the case study approach is to expand and build theories rather than perform statistical analysis to test a study's specific hypothesis (Rahim & Baksh, 2003). The present study was designed around the established theory of sustainable (green) aviation management (Abdullah et al., 2016; Agarwal, 2009, 2012; Budd et al., 2020; Dryer, 2017; Palmer, 2020).

3.2 Data Collection

Data for the study was obtained from a range of documents: Finnair's annual sustainability reports, Finnair's annual reports, media releases, and the airline's websites. These documents provided the sources of the study's case evidence. A comprehensive search of the leading air transport journals and magazines was also conducted in the study. A search of the SCOPUS and Google Scholar databases was also conducted.

The key words used in the database searches included "Finnair environmental responsibility policy", "Finnair passenger carbon offset program", "Finnair's membership of CORSIA", "Finnair aircraft fleet fuel efficiency", "Finnair's aircraft fleet modernization", "Finnair annual Scope 1, 2 and 3 carbon dioxide emissions (CO_2)", "Finnair sustainable waste management" and "Finnair carbon neutrality measures", "Finnair's use of sustainable aviation biofuels".

This study used secondary data. The three principles of data collection as recommended by Yin (2018) were followed: the use of multiple sources of case evidence, creation of a database on the subject and the establishment of a chain of evidence.

3.3 Data Analysis

Document analysis was used to examine the data collected for the case study. Document analysis is extensively used in case studies (Grant, 2019; Monios, 2016) and focuses on the information and data from formal documents and company records collected for the case study (Baxter, 2021; Ramon Gil-Garcia, 2012). The effective use of documents gathered for a study is dependent on them being appraised in terms of four key criteria: authenticity, credibility, representativeness and meaning (Scott, 2004, 2014).

Prior to commencing the formal analysis of the gathered documents, the soundness and authorship was assessed (Scott, 2004). According to Scott and Marshall (2009, p.188), "soundness refers to whether the document is complete and whether it is an original and sound copy". Authorship of documents relates to such issues as collective or institutional authorship. In this study the source of the case study documents was Finnair. When conducting document analysis in a study, it is necessary to interpret the understanding and the context within which the document was produced. This enables the researcher(s) to interpret the meaning of the document. The evidence found in the documents collected and used in the study were all clear and comprehensible (Baxter, 2021; van Schoor, 2017).

The document analysis was conducted in six distinct stages. The first stage involved planning the types and required documentation and ascertaining their availability. The second stage in the document analysis process involved gathering the documents and developing and implementing a scheme for the document management. Following the conclusion of Stage 2, the documents were reviewed to assess their authenticity, credibility and to identify any potential bias. In the subsequent stage, the content of the collected documents was interrogated, and the key themes and issues were identified and were incorporated into the case study. Stage 5 involved reflection and refinement to identify any difficulties associated with the documents, reviewing sources, as well as exploring the documents content. The analysis of the data was completed in Stage 6 of the document analysis process (O'Leary, 2004).

All the gathered documents were downloaded and stored in a case study database (Yin, 2018). The documents gathered for the study were all in English. Each document was carefully read, and key themes were coded and recorded. Documents were collected from multiple sources. This approach helped verify the themes that were detected in the documents that were used in the study (Baxter, 2021; Kitamura, 2019).

IV. RESULTS

4.1 A Brief Overview of Finnair

Finnair was established by private interests as Aero O/Y on 1 November 1923. The airline commenced operations on 20 March 1924 with a service from Reval in Estonia, and shortly thereafter a Helsinki-Stockholm service via Turku was started in conjunction with ABA of Sweden (Chant, 1997; Green & Swanborough, 1975). The airline operated exclusively with seaplanes prior to the opening of Finland's first airports in 1936 (Taylor & Young, 1975). In the immediate post World War II years, the airline operated a fleet of ex-military Douglas DC3 aircraft. Services were expanded to other European countries. The Finnish Government commenced purchasing stock in the airline in the 1950s and 1960s, and today the airline is substantially government owned (Brimson, 1985).

In 1986, the airline changed its name to Finnair. At this point of time the company was seeking to establish a more distinctive, nationalistic image (Brimson, 1985). Finnair joined the major global airline alliance **one**world in September 1999 (Hayward, 2019).

Today, Finnair is a full-service network carrier (FSNC) that specializes in both passenger and air cargo transportation. According to Ehmer et al. (2008, p. 5), a "full-service network carrier is an airline that focuses on providing a wide range of pre-flight and onboard services, including different service classes, and connecting flights". Finnair also offers package tours under its Aurinkomatkat-Suntours (later Aurinkomatkat) and Finnair Holidays brands. The cornerstone of Finnair's sustainable, profitable growth strategy is the airline's competitive geographical advantage, which enables the quickest connections in the growing market of air traffic between Asia and Europe (Finnair, 2019a, 2020a). At the time of the present study, the Finnair aircraft fleet consisted of 80 aircraft, which included 16 state-of-the art Airbus A350-900XWB aircraft (Finnair, 2021c).

4.2 Finnair Environmental Responsibility Policy

Finnair's environmental management is predicated upon the principle of continuous and systematic improvement. The company has identified the key environmental aspects arising from its operations, their impacts, risks, and opportunities involved, and has a range of targets related to them. Finnair aims to be an engaged leader in the field of environmental responsibility. As noted earlier, the airline is committed to the common goal of the global airline industry to achieve carbon neutral growth from 2020 and reduce the emissions from its flight operations by half by 2050 from the 2005 level. Finnair aims to be a pioneer in evaluating, reducing, and reporting environmental impacts. The company is also fully committed to comply with existing environmental legislation; however, its environmental work aims at exceeding statutory requirements (Finnair, 2020b).

Finnair is an active participant in civil aviation environmental committees as well as in industry workgroups in Finland and the Nordic countries. The airline actively promotes the necessity for the reduction of the aviation sector's environmental load. The airline maintains an open dialogue with different stakeholders and aims to continuously develop its operations according to the latest available information. Where possible, Finnair implements new technologies as part of its environmental responsibility. Finnair regularly reports on the company's environmental impacts through its annual reports and as a part of the Carbon Disclosure Project (CDP) (Finnair, 2020b).

In 2016, Finnair became a part of the International Civil Aviation Organization's "Carbon Offsetting and Reduction Scheme for International Aviation" (CORSIA) (Finnair, 2018b). All the company's environmental goals, targets, impacts, and promotion are managed and continuously developed through Finnair's "Environmental Management System" (EMS). The EMS system complies with the International Air Transport Association (IATA), the peak global airline body, "Environmental Assessment Program (IEnvA) Stage 2" as well as the ISO 14001:2015 Environmental Management System standard. IEnvA is an environmental management program that was developed by IATA specifically for airlines. Finnair's use of this program enables them to make use of the best industry practices in the industry (Finnair, 2020b).

Finnair considers all environmental aspects and impacts in all its flight and ground operations. In addition to the energy solutions that help reduce the company's environmental load, Finnair's environmental strategy also includes the implementation of circular economy principals as well as the preservation and promotion of natural diversity. The latter is known as biodiversity thinking (Finnair, 2020b).

During the period 2020 to 2025, Finnair will be investing €60 million in sustainability related measures (Air Transport Action Group, 2020; Centre for Aviation, 2020). This investment is in addition to the mandatory European Union Emissions Trading Scheme (ETS) and other environment related payments (Centre for Aviation, 2020). Effective from 1st January 2012, the European Union decided to include the aviation industry in the European Union Emission Trading Scheme (EU-ETS). This decision was in accordance with the Directive 101/2008/EC (Li & Tang, 2017; Meleo et al., 2016; Nava et al., 2018).

4.3 Finnair Annual Carbon Footprint

Virtually all of Finnair Group's greenhouse gas (GHG) emissions arise from the airline's flight operations. In 2015, as part of the implementation of the company's new IEnvA Environmental Management System (EMS), Finnair updated its carbon dioxide (CO_2) reduction target. Finnair committed to reducing its carbon dioxide (CO_2) emissions by 20% per one hundred tonne kilometres flown from the 2009 level by 2017 (Finnair, 2015). Finnair's long-term efficiency target has subsequently been to reduce carbon emissions by 17% relative to the revenue tonne kilometres (RTKs) from 2013 levels by the end of 2020. At the end of the 2019, the airline's emission efficiency had decreased by 8.8% and Finnair predicts it can reach 12–13% reduction by 2020 leaving the performance 4–5% short from the original emissions reductions target. The principal reason for this is that Finnair has been growing faster than the market in general and the original aircraft fleet renewal schedule from some time ago has changed from the previously estimated aircraft fleet requirements (Finnair, 2020b).

Figure 1 presents Finnair's annual Scope 1 direct carbon dioxide (CO_2) emissions from aircraft jet fuel usage over the period 2010 to 2019. As can be observed in Figure 1, Finnair's annual Scope 1 direct carbon dioxide (CO_2) emissions from jet fuel usage have predominantly increased over the study period reflecting the growth in the aircraft fleet and the airline's route network expansion. The largest increases occurred in 2011 (13.55%), 2015 (13.52%), and 2018 (11.89%), respectively (Figure 1). Figure 1 also shows that there were several years where the annual Scope 1 direct carbon dioxide (CO_2) emissions from jet fuel usage declined, with the largest decline occurring in -5.51%. Small decreases were also recorded in 2012 (-1.9%) and 2014 (-0.90%) (Figure 1).

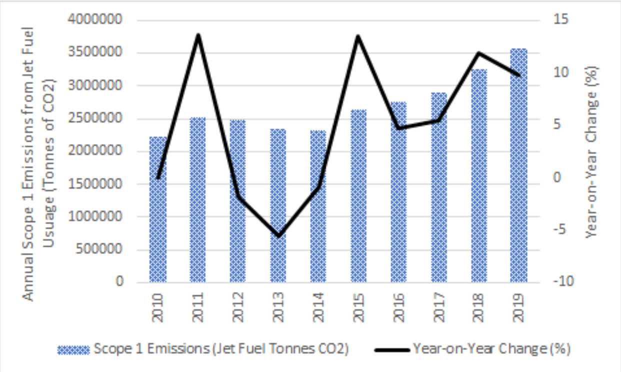

Fig.1: Finnair's total annual Scope 1 carbon dioxide (CO_2) emissions from jet fuel: 2010-2019. Data derived from Finnair (2013, 2015, 2019b, 2020b).

Figure 2 presents Finnair's annual Scope 1 carbon dioxide (CO_2) direct emissions from ground vehicle fuel usage over the period 2015 to 2019. As can be observed in Figure 2, there was a decline in the carbon dioxide (CO_2) levels from 2010 to 2014, with the largest annual fall in carbon dioxide (CO_2) emissions occurring in 2013 (a reduction of 83.65%). In 2015 and 2016, the annual carbon dioxide (CO_2) emission levels remained relatively constant. There was, however, an increase of 229% in carbon dioxide (CO_2) emissions in 2017, reflecting higher ground vehicle and equipment usage. During 2019, the level of carbon dioxide (CO_2) emissions once again declined (11.52%), reflecting the use of more environmentally friendly ground vehicles and equipment (Figure 2). During the study period, Finnair was transitioning from diesel powered vehicles and ground service equipment (GSE) to electric powered vehicles, which will result in lower levels of carbon dioxide (CO_2) emissions. The electrification of vehicles and ground service equipment used at airports results in lower carbon dioxide (CO_2) emissions (Gellings, 2011).

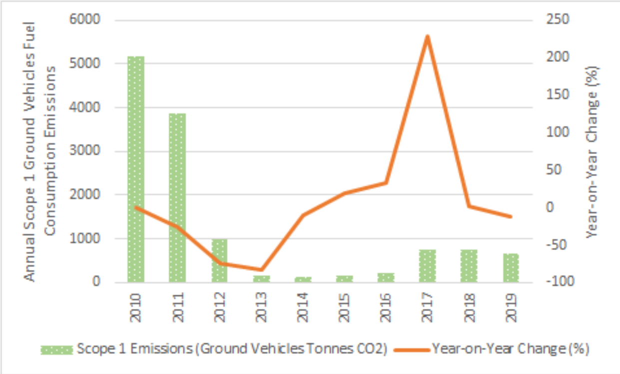

Fig.2: Finnair's total annual Scope 1 carbon dioxide (CO_2) emissions from fuel for ground vehicles and equipment: 2010-2019. Data derived from Finnair (2013, 2015, 2019b, 2020b).

In Finland, the energy consumption of buildings accounts for over a third of total greenhouse gas (GHG) emissions. Finnair uses various measures, for example, repairs, alterations, preventive maintenance together with user training, to ensure the energy efficiency of its business premises and to mitigate the greenhouse gas emissions arising from the energy consumption of its buildings (Finnair, 2020b). Figure 3 presents Finnair's annual Scope 2 carbon dioxide (CO_2) direct emissions from electricity usage from 2010 to 2019. As can be observed in Figure 3, there was a very large spike in Finnair's annual Scope 2 carbon dioxide (CO_2) emissions from electricity in 2011, which increased by 434.5% on 2010 levels. There was a smaller increase recorded in 2012 of 16.54% (Figure 3). Figure 3 also shows that from 2013 to 2015, Finnair's annual Scope 2 carbon dioxide (CO_2) direct emissions from electricity usage declined each year reflecting more favorable electricity usage. However, over the period 2016 to 2018, the annual Scope 2 direct carbon dioxide (CO_2) emissions from electricity increased each year which could be attributed to greater electricity requirements. However, in 2019, Finnair's annual Scope 2 direct carbon dioxide (CO_2) emissions from electricity usage decreased by 26.93%, the second largest decrease recorded in the study period. This could be contributed to the lower electricity requirement by the airline in 2019 (Figure 3).

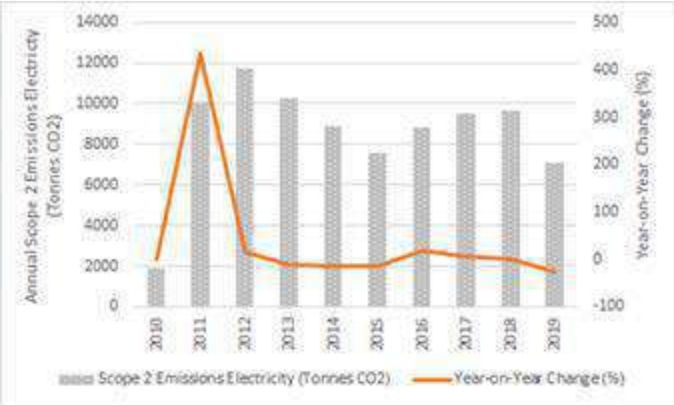

Fig.3: Finnair's total annual Scope 2 carbon dioxide (CO_2) emissions from electricity: 2010-2019. Data derived from Finnair (2013, 2015, 2019b, 2020b).

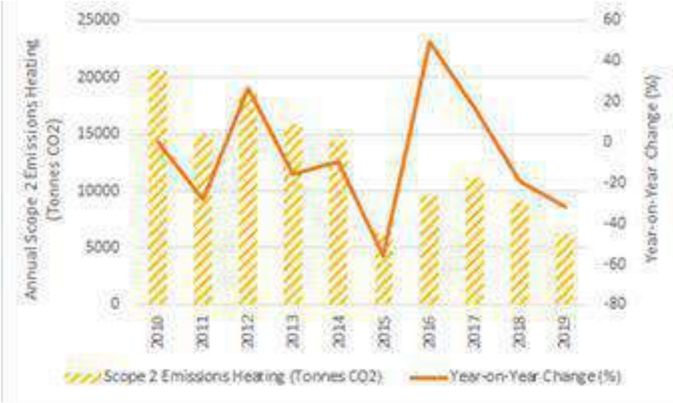

Fig.4: Finnair's total annual Scope 2 carbon dioxide (CO_2) emissions from heating: 2010-2019. Data derived from Finnair (2013, 2015, 2019b, 2020b).

Figure 4 presents Finnair's annual Scope 2 direct carbon dioxide (CO_2) emissions from heating facilities usage over the period 2010 to 2019. Finland's climate is characterized by long, cold winters (Climates to Travel, 2021). As can be seen in Figure 4, the largest level of emissions from heating occurred in 2010. Finland's 2009–2010 winter was the coldest experienced in the country since the 1986–1987 winter (Finnish Meteorological Institute, 2011), and thus, there was a greater requirement for the heating of facilities in 2010. The second highest level of carbon dioxide (CO_2) emissions from heating facilities occurred in 2012 with a 26.59% increase on the 2011 levels. During 2012, there was a greater requirement for Finnair to heat their facilities due to the cold weather experienced in Finland (Figure 4). The total annual carbon dioxide (CO_2) emissions from heating declined in 2013, 2014 and 2015, respectively. In 2015, carbon dioxide (CO_2) emissions from heating decreased by 55.53% on 2014 levels (Figure 4), which represented the largest single decline during the study period. However, the carbon dioxide (CO_2) emissions from heating rose again in 2016 and 2017 reflecting greater heating requirements due to weather conditions before declining again in 2018 and 2019 (Figure 4). The lowest level of Scope 2 direct emissions from heating occurred in 2019 (6,205 tonnes) (Figure 4).

Prior to examining Finnair's annual Scope 3 carbon dioxide (CO_2) emissions, it is important to note that Finnair (2020b) have observed that the greenhouse gas (GHG) emissions arising from the production and transport of jet fuel constitute a significant proportion of the airline's indirect greenhouse gas (GHG) emissions balance. In addition, any business travel made on another airline's services is also reported under the airline's Scope 3 indirect carbon dioxide (CO_2) emissions.

Figure 5 presents Finnair's annual Scope 3 indirect carbon dioxide (CO_2) emissions from 2015 to 2019. As can be observed in Figure 5, the annual Scope 3 indirect carbon dioxide (CO_2) emissions have increased on year-on-year basis. This is illustrated by the year-on-year percentage change line being all positive. During this period, the largest increase occurred in 2018 when the company's total annual Scope 3 emissions increased by 11.97%. However, in 2019 the total annual Scope 3 carbon dioxide (CO_2) emissions increased at a lower rate (9.77%) than that recorded in 2018 (Figure 5).

There have been a range of factors that influenced the annual Scope 3 emissions over the study period. The indirect greenhouse gas emissions arising from the manufacture of four Airbus A350-900XWB aircraft amounted to an estimated 8,484 tonnes of carbon dioxide (CO_2) that were included in Finnair's emissions balance in 2016. At the end of 2016, Finnair had a total fleet of 708 leased cars. Their combined emissions amounted to 1,663 tonnes of carbon dioxide (CO_2), which was 4.5% higher than in 2015. Finnair's indirect carbon dioxide (CO_2) balance also included the air cargo capacity that was purchased from other airlines by Finnair Cargo, Finnair's air cargo division. In 2016, this additional air cargo capacity produced around 8,943 tonnes of carbon dioxide (CO_2) emissions. The amount of these emissions includes cargo flights that were operated solely for Finnair Cargo (Finnair, 2017). The greenhouse gas emissions associated with the production and transportation of jet fuel amounted to an estimated 632,974 tonnes of carbon dioxide (CO_2) in 2017. The indirect greenhouse gas emissions arising from the manufacture of seven Airbus A321 and four Airbus A350-900XWB aircraft amounted to an estimated 13,077 tonnes of carbon dioxide (CO_2) in 2017 (Finnair, 2018a). The greenhouse gas (GHG) emissions associated with the production and transport of jet fuel amounted to around

701,701 tonnes of carbon dioxide (CO_2) in 2018 (Finnair, 2019b) (Figure 5).

Finnair Cargo purchases transport services from trucking firms, and the statistical practices of these firms at the time of the present study did not allow the actual emissions to be calculated. Finnair Cargo's main trucking partners use vehicles classified as EURO 4 as a minimum (Finnair, 2020b).

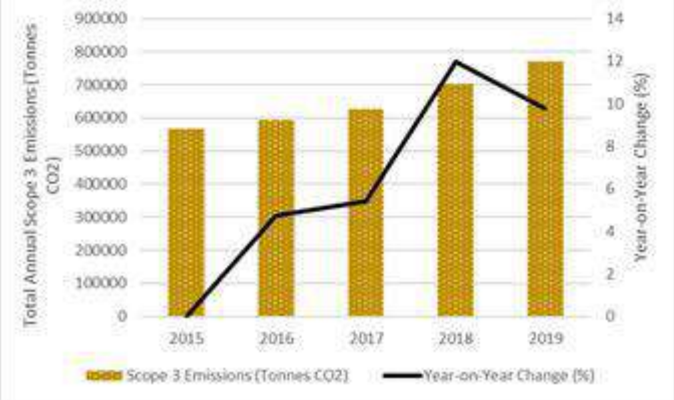

Fig.5: Finnair's total annual Scope 3 carbon dioxide (CO_2) emissions: 2010-2019. Note: data prior to 2015 is not available. Data derived from Finnair (2015, 2019b, 2020b).

Figure 6 shows Finnair's annual carbon dioxide (CO_2) emissions per revenue passenger kilometre (RPK) performed (RPK) from 2010-2019. Revenue passenger kilometres (RPKs) are a measure of airline output and can be obtained by multiplying the number of passengers by the distance (kilometres) flown (Dileep & Kurien, 2022). During the study period, the carbon dioxide (CO_2) emissions per revenue passenger kilometre performed (RPKs) largely exhibited a downward trend, which is illustrated by the year-on-year percentage change line being more negative than positive. As can be observed in Figure 6, the annual carbon dioxide (CO_2) emissions per revenue passenger kilometre performed (RPK) declined over the period 2011 to 2014. In 2015, the annual carbon dioxide (CO_2) emissions per revenue passenger kilometre performed (RPK) increased by 32.97%, the largest single annual increase recorded in the study period. Having peaked in 2015, the annual carbon dioxide (CO_2) emissions per revenue passenger kilometre performed (RPK) exhibited a downward trend from 2016 to 2019 (Figure 6). Figure 6 also shows that Finnair's carbon dioxide (CO_2) emissions per revenue passenger kilometre performed (RPK) have declined from a high of 125.91 grams in 2015 to 113.01 grams in 2019. This favorable trend could be attributed to the operation of the modern, state-of-the art aircraft, for example, the Airbus A350-900XWB, which offer lower carbon dioxide (CO_2) emissions when compared with previous generation aircraft. In addition, Finnair's annual RPKs have grown strongly reflecting greater passenger patronage. Also, during the study period, Finnair expanded its route network which provided the airline with the opportunity to grow its annual revenue passenger kilometres (RPKs) performed.

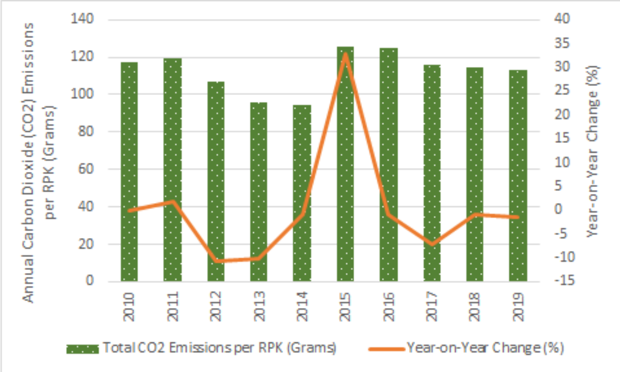

Fig.6: Finnair's total annual carbon dioxide (CO_2) emissions per revenue passenger kilometre (RPK): 2010-2019. Data derived from Finnair (2013, 2015, 2019a, 2019b, 2020b).

Figure 7 presents Finnair's annual carbon dioxide (CO2) emissions per available seat kilometre for the period 2010-2019. An available seat kilometre (ASK) is a measure of an airline's flight's passenger carrying capacity. ASKs are calculated by multiplying the number of seats on an aircraft by the distance that the aircraft has flown (Heshmati & Kim, 2016; Vasigh et al., 2015). Figure 7 shows that Finnair's annual carbon dioxide (CO_2) emissions per available seat kilometre declined during the period 2010 to 2014. In 2015, there was, however, a pronounced spike of 32.97% (Figure 7). This was the only increase recorded during the study period. Since 2015, there has been a consistent downward annual decrease in Finnair's annual carbon dioxide (CO_2) emissions per available seat kilometre from the high of 125.91 grams in 2015 to 113.01 grams in 2019 (Figure 7). Despite the growth in passenger capacity (ASKs) arising from Finnair's route network expansion, the operation of a modern, state-of-the art aircraft fleet over the enlarged route network has contributed to this favorable trend in lower carbon dioxide (CO_2) emissions per ASK since 2015.

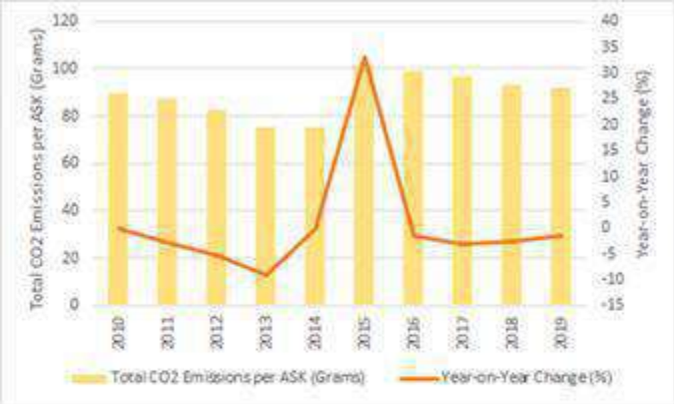

Fig.7: Finnair's total annual carbon dioxide (CO2) emissions per available seat kilometre (ASK): 2010-2019. Data derived from Finnair (2013, 2015, 2019a, 2019b, 2020b).

4.4 Acquisition of a Modern, Fuel-Efficient Aircraft Fleet

Modern aircraft are regarded as being more fuel-efficient and quieter than previous generation aircraft, and thus, Finnair's most significant environmental action has been its continuous, ongoing investments in a modern aircraft fleet (Finnair, 2020b). In addition, the next-generation aircraft, such as, the Airbus A350-900XWB, consume around 20–25 per cent less fuel than their predecessors, whilst the aircraft's carbon dioxide (CO_2) emissions decline by a corresponding amount (Finnair, 2019b).

In June 2010, Finnair announced its plans to replace four Boeing 757 aircraft with five Airbus A321 aircraft, fitted with sharklet wingtips, becoming the launch customer for the modified aircraft type. The "Sharklets" are designed to reduce the aircraft's fuel consumption by up to 4% (Kaminski-Morrow, 2010). The "Sharklet" equipped Airbus A321 aircraft has the lowest emissions in its class (Kainulainen, 2013). In September 2013, Finnair took delivery of the first "Sharklets" equipped Airbus A321 aircraft (Airbus, 2013).

Finnair was also the launch customer for the Airbus A350-900XWB aircraft, and in 2007 placed an order for 11 aircraft, with the option to acquire a for further eight (Airbus, 2014). The fleet investment, which was the most significant in the company's history, saw Finnair take delivery of a total of 19 Airbus A350-900 XWB aircraft. These aircraft support the company's target of reducing carbon dioxide (CO_2) emissions by 17% per cent per revenue tonne kilometre (RTK) flown by the end of 2020, using 2013 as the baseline year (Finnair, 2019b). Revenue tonne kilometres are the product of the revenue earning load as measured in tonnes and the distance over which the revenue load was carried (Cole, 2001). A revenue tonne kilometre (RTK) is one tonne carried one kilometre (Holloway, 2016).

In September 2013, Finnair made important progress toward the company's twin goals of fuel-efficiency and greener operations when it became the world's first commercial operator of Airbus A321 aircraft equipped with new, fuel-saving Sharklet wing tip devices. A total of three Airbus A321s with Sharklets entered the fleet in 2013 (Finnair, 2014). During 2014, Finnair retired the last of its Boeing B757 aircraft and replaced these with Airbus A321 equipped with the new, fuel saving Airbus A321 aircraft equipped with the "Sharklet" winglets (Finnair, 2015). During 2015, Finnair took delivery of its first Airbus A350-900XWB aircraft on 7 October 2015 (Finnair, 2016). Finnair took delivery of four new Airbus A350-900 XWB aircraft during 2016. The airline had a fleet of seven of these aircraft as at the end of 2016. During 2016, three Airbus A340-300 widebody aircraft and two Embraer E170 narrow body aircraft were retired from the airline's fleet (Finnair, 2017).

In 2017, Finnair completed the first phase of its long-haul aircraft fleet renewal, when four new Airbus 350-900XWB aircraft joined the fleet, increasing the number of Airbus 350-900XWB to eleven. Finnair also took delivery of seven new leased Airbus A321 aircraft (Finnair, 2018a). In addition to one new Airbus A350-900XWB aircraft, the airline also added one new Airbus A321 to its fleet in 2018 (Finnair, 2019b). Finnair added two new A350-900XWB aircraft in 2019, bringing its total fleet of A350s to 14 (Finnair, 2020b).

During the period 2020-2025, Finnair will be investing a further € 3.5-4 billion on aircraft fleet renewal and growth. Finnair envisages that its fleet renewal will reduce carbon dioxide (CO_2) emissions on its European services by 10-15% per annum (Finnair, 2020b; Otley, 2020). The new Airbus A350-900XWB aircraft will also enable Finnair to reduce its carbon dioxide (CO_2) emissions on the routes that the aircraft are operated on.

4.5 Finnair Fuel Efficiency Measures to Mitigate Greenhouse (GHG) Gas Emissions

Finnair monitors the fuel efficiency of its flights principally by the aircraft payload indicator revenue tonne kilometres (RTK), which considers the passenger load factor, the total enplaned air cargo consignments transported, as well as the distance between the origin and destination of the flight. A revenue tonne-kilometre (RTK) is an output measure in the airline industry and is defined as a tonne of payload flown over one kilometre (Shaw, 2016). Finnair's fuel efficiency is achieved through highly fuel-efficient flight planning, reductions in the weight of the aircraft operated, and operating each flight as fuel efficiently as possible. The airline's pilots play a key role in this as they have a considerable impact on fuel burn, and thus, on the carbon dioxide (CO_2) emissions produced during flights. In addition, the flexible deployment of the Finnair Airbus fleet, makes it possible for Finnair to allocate an optimal aircraft type to each route on any given day of the year (Finnair, 2020b).

As compared with the 2005 fuel efficiency rate, Finnair has improved its aircraft fleets' fuel efficiency by 27.2% over the period 2005 to 2019. This equates to a 2.3% annual reduction (Finnair, 2020b). Considering the relationship between fuel burn and greenhouse gas (GHG) emissions (Craggs & Gilbert, 2018), the fuel efficiency achieved by Finnair will not only result in lower fuel costs but will also lead to lower emissions of greenhouse gases (GHGs) and their associated impact on the environment. Indeed, a 2% increase in Finnair's fuel efficiency corresponds to around 15 million kilograms of annual fuel savings, which in turn equates to a reduction of nearly 50,000 tonnes of carbon dioxide (CO_2) emissions (Finnair, 2015).

4.6 Finnair Sustainable Waste Management Program

As part of its environmental management policy, Finnair has set a goal to include circular economy principles in all its business operations increasing waste recovery, cost efficiency and safety. The airline also plans to reduce the volume of waste generated. As a starting point the airline has prescribed long-term targets aiming for inflight catering sustainability. There have been ongoing actions directed at achieving this goal with some of the first definitive changes being a reduction in the use of single packaged milk, the introduction of cardboard packaged hot meals to replace "cPET" casseroles, the reduction in plastic amenity kits, and redesigning the packaging of the onboard sales selection (the Nordic Kitchen Brand). At the time of the current study, these changes had resulted in the annual reduction of 80.0 tonnes of wastes annually. Furthermore, in adopting and implementing the circular economy principles, Finnair aims to recycle at least 50% of the plastics returning to its Helsinki Airport hub. In addition, recycled materials as part of its service design, for example, salad containers and business class slippers, are being made from recycled polyethylene terephthalate (PET) (Finnair, 2020b).

As a result of contagious animal health concerns and regulations in place some parts of Finnair's waste flows are considered unsafe for material recycling or for biogas production. All in-flight wastes arriving at Helsinki Airport are reused either as energy, heat, biogas, manure, or material, importantly, most importantly, no waste is disposed to landfill (Finnair, 2020b). As previously noted, the disposal of waste to landfill is viewed as being the least preferable method in sustainable waste management (Barlow & Morgan, 2013; Bolton & Rousta, 2019).

The airline's sustainable waste management policy will also play an important role in the airline achieving its objective of being "carbon neutral" by 2045. In this regard, Finnair (2021b) aims to reduce at least 50% of single-use plastics out of the business by the end of 2022. This will enable the airline to reduce its annual plastic waste by 230 tons. Finnair also plans to reduce its food waste by 50% in the same period (Finnair, 2021b; Otley, 2020). To offset carbon dioxide (CO_2) emissions that are produced from meat production whilst also catering for changing tastes, more vegetarian options will be offered to passengers during 2020 (Green Air, 2020).

4.7 Finnair Use of Sustainable Aviation Biofuels

As previously noted, in recent times, there has been an increasing trend by airlines to use aviation biofuel as an environment sustainability measure (Baxter et al., 2020). Finnair is an active member of the "Nordic Initiative for Sustainable Aviation Working Group". This working group is comprised of Nordic-based airlines, airport operators and government ministries who are working together with aircraft manufacturers to rapidly develop biofuel in the aviation industry (Finnair, 2020b).

Finnair first operated flights using biofuel in 2011, On 23 September 2014, Finnair's flight from Helsinki to New York was operated using a more environmentally friendly biofuel mixture that was partly manufactured from used cooking oil (Finnair, 2015).

In December 2019, Finnair announced that it would be contributing funding, along with other companies, that include Neste, to a feasibility study on a potential synthetic fuels pilot production plant in Eastern Finland. The industrial-scale pilot facility is based on power-to-x technology and the plant will be used to produce carbon-neutral fuels for transportation. The main raw materials that will be used will be excess hydrogen produced by chemical company Kemira and carbon dioxide (CO_2) from the Finnsementii cement facility, which is in Lappeenranta, located in southeastern Finland. Hydrogen and carbon dioxide (CO_2) can be combined in a synthesis process to provide synthetic methanol. This methanol can subsequently be further processed into synthetic, emission-free transportation fuels (Green Air, 2020).

Also, during 2019, Finnair operated three biofuel powered flights, reducing carbon dioxide (CO_2) emissions by 81.8 tonnes (Finnair, 2020b, 2021a). As an element of its "Push for change" initiative launched in early 2019, Finnair used sustainable aviation fuels sourced from used cooking oil on two flights from San Francisco to Helsinki under a purchase agreement with Shell, World Energy and SkyNRG (Green Air, 2020).

At the time of the present study, Finnair was increasing its use of sustainable aviation fuels. By the end of 2025, the airline anticipates spending around €10 million annually on sustainable aviation fuels. Finnair's aviation biofuel partner is Finland-based Neste, the world's largest producer of sustainable aviation fuels that are refined from waste. In addition, Finnair in conjunction with Neste and Finavia, are developing a model which will enable corporate customers to decrease the carbon dioxide (CO_2) emissions of their travel using biofuel (Finnair, 2020b; Green Air, 2020).

4.8 Finnair Use of Biodiesel for Ground Vehicles and Ground Service Equipment (GSE)

Prior to examining Finnair's strategy to mitigate carbon dioxide (CO_2) emissions from its ground operations, it is important to note that a range of functions are performed during the time that that an aircraft spends on the ground and is being serviced in between flights. These functions include any combination or singular selection of the following activities: aircraft loading/unloading, cargo handling, lavatory services, aircraft marshalling, aircraft towing or pushback, aircraft fueling; and auxiliary ground power support (Thompson, 2007). To perform ground handling services, sophisticated technical equipment is required (Kazda & Caves, 2015; Roberts, 2018). This ground service equipment is typically powered by diesel engines. Vehicles used by airlines are also often petrol-powered.

Finnair's environmental policy has included replacing the use of fossil fuels in ground vehicles and ground service equipment (GSE) with biodiesel powered equipment and vehicles. During 2019, all Finnair's diesel driven ground service vehicles were running on biodiesel. This resulted in an annual reduction of 155 tonnes of carbon dioxide (CO_2) emissions (Finnair, 2020b). Biofuels can reduce a firm's consumption of fossil fuels, and hence, reduce carbon dioxide (CO_2) emissions. This is because biofuels are carbon neutral (Hanaki & Portugal-Pereira, 2018). Finnair is also using some electric powered ground vehicle and ground service equipment (GSE) fleet (Finnair, 2020b).

4.9 Passenger Carbon Dioxide (CO2) Emissions Offset Scheme

Finnair has introduced a carbon offsetting scheme for its passengers, whereby passengers can offset their carbon dioxide (CO_2) emissions through an emissions reduction project, and/or using sustainable aviation biofuels. Effective 1 September 2020, Finnair plans to offset carbon dioxide (CO_2) emissions from its corporate customers. Finnair will also introduce new types of passenger tickets that will permit passengers to support aviation biofuels or other offsetting measures during 2020 (Finnair, 2021d).

4.10 Reductions in Aircraft Weight

Finnair has implemented a program to reduce the weight of its fleet of aircraft, as this weight reduction has a direct impact on fuel burn, and thus, aircraft emissions (Finnair, 2021a). The use of new technology and high-quality lightweight materials has enabled Finnair to reduce the empty weight of its aircraft. For instance, in 2014, Finnair replaced all its baggage containers used on its narrow body aircraft with lightweight composite containers. In addition, weight is considered one of the key considerations in all procurement activities related to aircraft-related equipment (Finnair, 2015).

Finnair has developed a new plan to remove unnecessary weight from the airline's aircraft, with a target of reducing fuel consumption by 15,000 tonnes per year (Green Air, 2020). As of the end of April 2020, Finnair will remove travel retail sales from its short-haul European services and instead will focus on pre-orders (Preston, 2020). This policy is expected to reduce on average 50-100 kg per flight and will result in a 70,000 kg saving in fuel consumption and 220,000 kg of carbon dioxide (CO_2) emissions per year (Green Air, 2020).

4.11 Implementation of the United Nations Sustainable Development Goals (SDGs)

In 2015, all United Nations Member States adopted the "2030 Agenda for Sustainable Development" and its seventeen 17 Sustainable Development Goals (SDGs). Each SDG comprises a range of targets to be achieved by 2030 (Katila et al., 2019; United Nations, 2021). The United Nations Sustainable Development Goals (SDGs) provide a framework for business and government to solve global economic, social, and environmental challenges (Air New Zealand, 2018).

At the time of the present study, Finnair was contributing to all 17 Sustainable Development Goals (SDGs). The company has chosen to particularly focus on six SDGs which are the most relevant for its business: gender equality (SDG 5), industry innovation and infrastructure (SDG 9), responsible consumption and production (SDG 12), climate action (SDG 13), peace, justice, and strong institutions (SDG 16), and partnerships for the goals (SDG 17) (Finnair, 2021e). The SDG most pertinent to this study is SDG 13, that is, to take urgent action on climate change.

V. CONCLUSION

In conclusion, this study has examined the strategies defined and implemented by Finnair to meet its objective of becoming a "carbon neutral" airline by 2045. To achieve the objectives of the study, Finnair was selected as the case airline. The research was undertaken using an in-depth qualitative instrumental case study research approach. All the data collected for the study was examined using document analysis. The study was underpinned by a case study research framework that followed the recommendations of Yin (2018).

Finnair has defined and is implementing a range of strategies and environmental-related measures to meet its objective of becoming a "carbon neutral" airline by 2045. The airline has introduced a carbon offsetting scheme for its passengers, whereby passengers can offset their carbon dioxide (CO_2) emissions through an emissions reduction project, and/or using sustainable aviation biofuels. Sustainable waste management is another important element in Finnair's goal to become a carbon neutral airline by 2045. Wherever possible, Finnair is planning to recycle at least 50% of the plastics returning on its flights to its home base at Helsinki Vantaa Airport. Importantly, no waste is disposed by landfill; this practice eliminates the greenhouse gases associated with landfill waste, and thus helps alleviate climate change. Finnair is also planning to reduce its food waste by 50% and to reduce single use plastics by 50% as well by the end of 2022. Another important strategy implemented by Finnair is the increased use of sustainable aviation biofuels. Aviation biofuels are an important substitute for fossil-based fuel as they are more environmentally friendly and offer airlines the chance to decarbonize their operations. Finnair's environmental policy has included the replacement of fossil fuels in its ground service equipment (GSE) and ground vehicles with biodiesel. The use of biodiesel is producing annual reductions in the airline's ground service equipment (GSE) and ground vehicles carbon dioxide (CO_2) emissions. Another important measure adopted by Finnair has been to reduce the weight of its aircraft. In this regard, Finnair is using new technology and high-quality lightweight materials where possible.

The case study revealed that the acquisition and deployment of modern, fuel-efficient aircraft have underpinned Finnair's goal to become a carbon neutral airline. The operation of modern fuel-efficient aircraft is the airline's most significant environmental action and forms a key part of the airline's sustainability strategy. The airline's fleet of modern, state-of-the art, Airbus A350-900XWB aircraft consume around 20-25% less fuel than their predecessors, and very importantly, their carbon dioxide (CO_2) emissions decline by a corresponding amount. Also, as noted in the case study, Finnair took delivery of a fleet of Airbus A321 aircraft equipped with the "Sharklet" wing tips. These aircraft have the lowest emissions in their class.

The burning of aviation fuel results in the emissions of carbon dioxide (CO_2) and other harmful gases. Thus, fuel efficiency has a concomitant impact on aircraft emissions. Finnair places a very high focus on fuel efficiency, and this is achieved through optimized flight planning, reductions in aircraft weight, and the flexible deployment of its aircraft fleet.

As noted in the case study, virtually all of Finnair's greenhouse gas emissions come from its flight operations. Over the study period, Finnair expanded its route network and aircraft fleet, and, as a result, there was an associated increase in the airline's Scope 1 carbon dioxide (CO_2) emissions from jet fuel usage. These emissions grew from 2,220,388 tonnes of carbon dioxide (CO_2) in 2010 to 3,566,409 tonnes of carbon dioxide (CO_2) in 2019. Finnair's annual Scope 1 carbon dioxide (CO_2) emissions from fuel used for its ground service equipment (GSE) and ground vehicles declined from a high of 5,181 tonnes of carbon dioxide (CO_2) in 2010 to 668 tonnes of carbon dioxide (CO_2) in 2010. Finnair is transitioning from diesel powered vehicles to electric powered vehicles, and these will deliver more favorable environmental benefits. Finnair's annual Scope 2 carbon dioxide (CO_2) emissions from electricity oscillated over the study period with annual increases being recorded in 2011, 2012, 2016, 2017, and 2018. In 2019, Finnair's annual Scope 2 carbon dioxide (CO_2) emissions from electricity amounted to 7,068 tonnes of carbon dioxide (CO_2), this was the second lowest level of emissions recorded during the study period. Finnair's annual Scope 2 emissions from the heating of its facilities also oscillated during the study period. There were large spikes in emissions during the cold winters experienced in Finland. Conversely, during more mild winters there was a decline in carbon dioxide emissions (CO_2) due to the lower heating requirements. During the period 2015 to 2019, Finnair's annual Scope 3 carbon dioxide (CO_2) emissions exhibited an upward trend, increasing from 567,902 tonnes of carbon dioxide (CO_2) in 2015 to 770,802 tonnes of carbon dioxide (CO_2) in 2019. The airline's annual Scope 3 carbon dioxide (CO_2) emissions were impacted by a range of factors which included the estimated tonnage of carbon dioxide (CO_2) emissions associated with the manufacture of the airline's new Airbus A350-900XWB aircraft, the greenhouse gas (GHG) emissions associated with the production and transportation of jet fuel, emissions from leased vehicles, and emissions from cargo flights that were operated on behalf of Finnair Cargo.

REFERENCES

[1] Abdullah, M.A., Chew, B.C., & Hamid, S.R. (2016). Benchmarking key success factors for the future green airline industry. *Procedia - Social and Behavioral Sciences*, 224, 246 – 253. https://doi.org/10.1016/j.sbspro.2016.05.456

[2] Agarwal, R.K. (2009). Sustainable (green) aviation: Challenges and opportunities. *SAE International Journal of Aerospace*, 2(1), 1-20. https://doi.org/10.4271/2009-01-3085

[3] Agarwal, R.K. (2012). Review of technologies to achieve sustainable (green) aviation. In R.K. Agarwal (Ed.), *Recent advances in aircraft technology* (pp. 427-464). Rijeka, Croatia: InTech.

[4] Air New Zealand. (2018). Sustainability report 2018. Retrieved from https://www.airnewzealand.com.au/sustainability-reporting-and-communication.

[5] Airbus. (2013). Finnair receives world's first A321 with Sharklets. Retrieved from https://www.airbus.com/newsroom/press-releases/en/2013/09/finnair-receives-world-s-first-a321-with-sharklets.html.

[6] Airbus. (2014). Finnair orders eight additional A350 XWBs. Retrieved from https://www.airbus.com/newsroom/press-releases/en/2014/12/finnair-orders-eight-additional-a350-xwbs.html.

[7] Air Transport Action Group. (2020). Finnair's anti-carbon program lifts the ambition-level in the aviation industry. Retrieved from https://aviationbenefits.org/newswire/2020/03/finnairs-anti-carbon-program-lifts-the-ambition-level-in-the-aviation-industry/.

[8] Attanasio, G. (2018). Naples International Airport and Airport Carbon Accreditation (ACA). In J. Casares., J. Longhurst., G, Passerini., G. Perillo & J. Barnes (Eds), *WIT transactions on ecology and the environment: Air pollution XXVI* (pp. 465-474). Southampton, UK: WIT Press.

[9] Babakhani, N., Ritchie, BW., & Dolnicar, S. (2017). Improving carbon offsetting appeals in online airplane ticket purchasing: Testing new messages and using new test methods. *Journal of Sustainable Tourism*, 25(7), 955-969. https://doi.org/10.1080/09669582.2016.1257013

[10] Bailey, J. (2020). Finnair aims to cut emissions by 50% in just 5 years. Retrieved from https://simpleflying.com/finnair-aims-to-cut-emissions-by-50-in-just-5-years/.

[11] Barlow, C.Y., & Morgan, D.C. (2013). Polymer film packaging for food: An environmental assessment. *Resources, Conservation and Recycling*, 78, 74-80. https://doi.org/10.1016/j.resconrec.2013.07.003

[12] Barrett, S.R.H., Britter, R.E., & Waitz, I.A. (2010). Global mortality attributable to aircraft cruise emissions. *Environmental Science & Technology*, 44(19), 7736-7742. https://doi.org/10.1021/es101325r

[13] Bauen, A., & Nattrass, L. (2018). Sustainable aviation biofuels: Scenarios for deployment. In M. Kaltschmitt & U. Neuling (Eds.), *Biokerosene: Status and prospects* (pp. 703-721). Berlin, Germany: Springer-Verlag.

[14] Baxter, G. (2016). *AERO2410 Airline Operations: Topic 6 Learning Guide Airline Capacity Management and Passenger Aircraft Fleet Planning*. Melbourne, Australia: RMIT University.

[15] Baxter, G. (2021). Mitigating an airport's carbon footprint through the use of "green" technologies: The case of Brisbane and Melbourne Airports, Australia. *International Journal of Environment, Agriculture and Biotechnology*, 6(6), 29-39. https://dx.doi.org/10.22161/ijeab.66.4

[16] Baxter, G., Srisaeng, P., & Wild, G. (2018). Sustainable airport waste management: The case of Kansai International Airport. *Recycling*, 3(1), 6. https://doi.org/10.3390/recycling3010006

[17] Baxter, G., Srisaeng P, & Wild G. (2020). The use of aviation biofuels as an airport environmental sustainability measure: The case of Oslo Gardermoen Airport. *Magazine of Aviation Development*, 8(1), 6-17. https://doi.org/10.14311/MAD.2020.01.01

[18] Baxter, G., Srisaeng, P., & Wild, G. (2021). Environmentally sustainable airline waste management: The case of Finnair PLC. *Environmental Research, Engineering and Management*, 77(4), 73-85. https://doi.org/10.5755/j01.erem.77.4.29574

[19] Bayon, R., Hawn, A., & Hamilton, K. (2009). *Voluntary carbon markets: An international business guide to what they are and how they work*. London, UK: Earthscan.

[20] Becken, S. (2020). Major airlines say they're acting on climate change, but research reveals they're way off course. Retrieved from https://www.abc.net.au/news/2020-02-17/are-major-airlines-doing-enough-to-act-on-climate-change/11966892.

[21] Becken, S., & Mackey, B. (2017). What role for offsetting aviation greenhouse gas emissions in a deep-cut carbon world? *Journal of Air Transport Management*, 63, 71-84. https://doi.org/10.1016/j.jairtraman.2017.05.009

[22] Bioenergy International. (2019). Finnair introduces carbon offsetting and biofuel service for customers. Retrieved from https://bioenergyinternational.com/markets-finance/finnair-introduces-carbon-offsetting-and-biofuel-service-for-customers.

[23] Birchfield, V.L. (2015). Coercion with kid gloves? The European Union's role in shaping a global regulatory framework for aviation emissions. *Journal of European Public Policy*, 22(9), 1276-1294. https://doi.org/10.1080/13501763.2015.1046904

[24] Bolton, K., & Rousta, K. (2019). In M.J. Taherzadeh, K. Bolton, J. Wong & A. Pandey (Eds.), *Sustainable resource recovery and zero waste approaches* (pp. 53-63). Amsterdam, The Netherlands: Elsevier.

[25] Brimson, S. (1985). *The airlines of the world*. Sydney, Australia: Dreamweaver Books.

[26] Brooks, K.P., Snowden-Swan, l.J., Jones, S.B., Butcher, M.J., Lee, J.S.J., Anderson, D.M., Frye, J.G., Holladay, J.E., Owen, J., Harmon, L., Burton, F., Palou-Rivera, I., Plaza, J., Handler, R., & Shonnard, D. (2016). Low-carbon aviation fuel through the alcohol to jet pathway. In C. Chuck (Ed.), *Biofuels for aviation: Feedstocks, technology and implementation* (pp. 109-146). London, UK: Academic Press.

[27] Budd, T. (2017). Environmental impacts and mitigation. In L. Budd & S. Ison (Eds.), *Air transport management: An international perspective* (pp. 283-306). Abingdon, UK: Routledge.

[28] Budd, T., Intini, M., & Volta, N. (2020). Environmentally sustainable air transport: A focus on airline productivity. In T. Walker, A.S. Bergantino., N. Sprung-Much & L Loiacono (Eds.), *Sustainable aviation: Greening the flight path* (pp. 55-77). Cham, Switzerland: Palgrave Macmillan.

[29] Centre for Aviation. (2020). Finnair to reduce net emissions by 2025, achieve carbon neutrality by 2045. Retrieved from https://centreforaviation.com/analysis/reports/aviation-sustainability-and-the-environment-capa-09-mar-2020-516511.

[30] Chandrappa, R., & Das, D.B. (2012). *Solid waste management: Principles and practice*. Berlin, Germany: Springer Verlag.

[31] Chant, C. (1997). *Airlines of the world*. London, UK: Tiger Books International.

[32] Chen, F. Y. (2013). The intention and determining factors for airline passengers' participation in carbon offset schemes. *Journal of Air Transport Management*, 29, 17-22. https://doi.org/10.1016/j.jairtraman.2013.01.001

[33] Climates to Travel. (2021). Climate – Finland. Retrieved from https://www.climatestotravel.com/climate/finland.

[34] Cole, S. (2001). *Applied transport economics: Policy, management and decision making* (2nd ed.). London, UK: Kogan Page.

[35] Craggs, L., & Gilbert, P. (2018). Sustainable greenhouse gas reductions from bioenergy systems - Climate change: A bioenergy driver and constraint. In P. Thornley & P. Adams (Eds.), *Greenhouse gas balances of bioenergy systems* (pp. 1-10). London, UK: Academic Press.

[36] Cui, Q., & Li, X.Y. (2021). Which airline should undertake a large emission reduction allocation proportion under the "carbon neutral growth from 2020" strategy? An empirical study with 27 global airlines. *Journal of Cleaner Production,* 279, 123745. https://doi.org/10.1016/j.jclepro.2020.123745

[37] Cui, Q., Lin, J.L., & Jin, Z.Y. (2020). Evaluating airline efficiency under "Carbon Neutral Growth from 2020" strategy through a Network Interval Slack-Based Measure. *Energy*, 193, 116734. https://doi.org/10.1016/j.energy.2019.116734

[38] Czerny, A.I., Fu, X., Lei, Z., & Oum, T.H. (2021). Post pandemic aviation market recovery: Experience and lessons from China. *Journal of Air Transport Management*, 90, 101971. https://doi.org/10.1016/j.jairtraman.2020.101971

[39] Dileep, M.R., & Kurien, A. (2022). *Air transport and tourism: Interrelationship, operations and strategies*. Abingdon, UK: Routledge.

[40] Dodd, T., & Yengin, D. (2021). Deadlock in sustainable aviation fuels: A multi-case analysis of agency. *Transportation Research Part D: Transport and Environment*, 94, 102799. https://doi.org/10.1016/j.trd.2021.102799

[41] Dodd, T., Orlitzky, M., & Nelson, T. (2018). What stalls a renewable energy industry? Industry outlook of the aviation biofuels industry in Australia, Germany, and the USA. *Energy Policy*, 123, 92-103. https://doi.org/10.1016/j.enpol.2018.08.048

[42] Drewer, J., Howard, D., & McNamara, N. (2018). Greenhouse gas (GHG) and biogenic volatile organic compound (bVOC) fluxes associated with land-use change to bio-energy crops. In P. Thornley & P. Adams (Eds.), *Greenhouse gas balances of bioenergy systems* (pp. 77-96). London, UK: Academic Press.

[43] Dryer, J.E. (2017). Perspectives on the future of green aviation. In E.S. Nelson & D.R. Reddy (Eds.), *Green aviation: Reduction of environmental impact through aircraft technology and alternative fuels*. (pp. 335-346). London, UK: CRC Press/Balkema.

[44] Ehmer, H., Berster, P., Bischoff, G., Grimme, W., Grunewald, E. & Maertens, S. (2008). Analyses of the European air transport market: Airline business models. Retrieved from https://ec.europa.eu/transport/sites/transport/files/modes/air/doc/abm_report_2008.pdf.

[45] Finnair. (2013). Sustainability report 2012. Retrieved from https.//investors.finnair.com/~/media/Files/F/Finnair-IR/documents/en/reports-and-presenntation/2013/Finnair-sustainability-report-2012.pdf.

[46] Finnair. (2014). Annual Report 2013. Retrieved from https://investors.finnair.com/~/media/F/Files/Finnair-IR/documents/en/reports-and-presentation/2014/annual-report-2013.pdf.

[47] Finnair. (2015). Annual report 2014. Retrieved from https://investors.finnair.com/~/media/F/Files/Finnair-IR/dcouments/en/reports-and-presentation/2015/annual-report-2014.pdf.

[48] Finnair. (2016). Finnair Group interim report 1 January – 30 September 2015. Retrieved from https://investors.finnair.com/~/media/Files/F/Finnair-IR/documents/en/reports-and-presentation/2015/finnair-q3-2015-interim-report.pdf.

[49] Finnair. (2017). Annual report 2016. Retrieved from https://investors.finnair.com/~/media/F/Files/Finnair-IR/dcouments/en/reports-and-presentation/2017/annual-report-2016.pdf.

[50] Finnair. (2018a). Annual report 2017.Retrieved from https://investors.finnair.com/~/media/F/Files/Finnair-IR/dcouments/en/reports-and-presentation/2018/annual-report-2017.pdf.

[51] Finnair. (2018b). Finnair environmental history: Major green milestones. Retrieved from https://cargo.finnair.com/en/cargo-news/environment-sustainability-results.
[52] Finnair. (2019a). Financial information 2018. Retrieved from https://investors.finnair.com/~/media/F/Files/Finnair-IR/dcouments/en/financial/finnair-2018-en.pdf.
[53] Finnair. (2019b). Sustainability report 2018. Retrieved from https://investors.finnair.com/~/media/Files/F/Finnair-IR/documents/en/reports-and-presentation/2019/Finnair-sustainability-report-2018.pdf.
[54] Finnair. (2020a). Annual report 2019. Retrieved from https://investors.finnair.com/~/media/F/Files/Finnair-IR/dcouments/en/reports-and-presentation/2020/annual-report-2019.pdf.
[55] Finnair. (2020b). Sustainability report 2019. Retrieved from https://company.finnair.com/resource/blob/1994132/c493686a5af678b81ed6dbcd48eed150/fiinair-responsibility-report-2019-data.pdf.
[56] Finnair. (2021a). CO_2 reduction. Retrieved from: https://company.finnair.com/en/sustainability/co2-reduction.
[57] Finnair. (2021b). Finnair's anti-carbon program lifts the ambition-level in the aviation industry, Media Release March 06. Retrieved from https://company.finnair.com/en/media/all-releases/news?id=3593081.
[58] Finnair. (2021c) Finnair fleet. Retrieved from https://www.finnair.com/au/gb/flights/fleet.
[59] Finnair. (2021d). How Finnair is putting sustainability at its core. Retrieved from https://www.finnair.com/au-en/bluewings/sustainability/how-finnair-is-putting-sustainability-at-its-core-2053792.
[60] Finnair. (2021e). Sustainability. Retrieved from: https://company.finnair.com/en/sustainability.
[61] Finnish Meteorological Institute. (2011). 2010 – a year of weather extremes. Retrieved from https://en.ilmatieteenlaitos.fi/press-release/125205.
[62] Fregnani, A.T.G., & Andrade, O. (2017). Aviation initiatives and the relative impact of electric road vehicles on CO_2 emissions. Retrieved from https://www.boeing.com/features/innovation-quarterly/aug2017/feature-technical-co2.page.
[63] Fulekar, M.H. (2010). *Environmental biotechnology*. Boca Raton, FL: AK Peters/CRC Press.
[64] Gegg, P., Budd, L., & Ison, S. (2014). The market development of aviation biofuel: Drivers and constraints. *Journal of Air Transport Management*, 39, 34-40. https://doi.org/10.1016/j.jairtraman.2014.03.003
[65] Gellings, C.W. (2011). *Saving energy and reducing CO_2 emissions with electricity*. Lilburn, GA: The Fairmont Press.
[66] Girella, L. (2018). *The boundaries in financial and non-financial reporting: A comparative analysis of their constitutive role*. Abingdon, UK: Routledge.
[67] Grandy, G. (2010). Instrumental case study. In A.J. Mills., G. Durepos., & E. Wiebe (Eds.), *Encyclopedia of case study research*, Volume 1 (pp. 473-475). Thousand Oaks, CA: SAGE Publications.
[68] Grant, A. (2019). *Doing excellent social research with documents: Practical examples and guidance for qualitative researchers*. Abingdon, UK: Routledge.
[69] Green Air. (2020). Finnair signs SAF agreement with Neste as it targets a 50 per cent reduction of net emissions by 2025. Retrieved from https://www.greenaironline.com/news.php?viewStory=2625.
[70] Green, W., & Swanborough, G. (1975). *The Observer's world airlines and airliners directory*. London, UK: Frederick Warne & Company Limited.
[71] Greenhouse Gas Protocol. (2021). About us. Retrieved from https://ghgprotocol.org/about-us.
[72] Hagmann, C., Semeijn, J., & Vellenga, D.B. (2015). Exploring the green image of airlines: Passenger perceptions and airline choice. *Journal of Air Transport Management*, 43, 37-45. https://doi.org/10.1016/j.jairtraman.2015.01.003
[73] Hanaki, K., & Portugal-Pereira, J. (2018). The effect of biofuel production on greenhouse gas emission reductions. In K.Takeuchi., H. Shiroyama,, O. Saito & M. Matsuura (Eds.), *Biofuels and sustainability: Holistic perspectives for policymaking* (pp. 53-71). Tokyo, Japan: Springer Japan KK.
[74] Harper, M. (2004). Introduction. In M. Ali (Ed.), *Sustainable composting: Case studies and guidelines for developing countries* (pp. 3-4). Loughborough, UK: Water, Engineering and Development Centre.
[75] Harris, N. (2019). *Green chemistry*. Waltham, UK: ED-Tech-Press.
[76] Hayward, J. (2019). The story of the oneworld Alliance. Retrieved from https://simpleflying.com/oneworld-alliance-story/.
[77] Heshmati, A., & Kim, J. (2016). *Efficiency and competitiveness of international airlines*. Singapore: Springer Science+Business Media.
[78] Holloway, S. (2016). *Straight and level: Practical airline economics* (3rd ed.). Abingdon, UK: Routledge.
[79] International Air Transport Association. (2016). IATA carbon offset program: Frequently asked questions. Retrieved from http://www.iata.org/contentassets/922ebc4cbcd24c4d9fd55933e7070947/icop20faq20general20for20airline20particpants20jan202016.pdf.
[80] International Air Transport Association. (2018). Climate change & CORSIA. Retrieved from https://www.iata.org/contentassets/c4f9f0450212472b96dac114a06cc4fa/fact-sheet-climate-change.pdf.
[81] International Air Transport Association. (2021a). Airline cabin waste. Retrieved from https://www.iata.org/en/policy/environment/cabin-waste/.
[82] International Air Transport Association. (2021b). IATA Carbon Offset Program. Retrieved from

https://www.iata.org/en/programs/environment/carbon-offset/.

[83] International Airport Review. (2010). UK airport operators launch new guidelines to reduce aircraft ground emissions. Retrieved from http://www.internationalairportreview.com/2717/airport-news/uk-airport-operators-launch-new-guidelines-to-reduce-aircraft-ground-emissions/.

[84] International Civil Aviation Organization. (2011). *Airport air quality manual*. Document No 9889. Montreal, Canada: ICAO.

[85] International Civil Aviation Organization. (2021). What are the mechanisms for the CORSIA implementation? How will ICAO support States to implement the CORSIA? Retrieved from https://www.icao.int/environmental-protection/Pages/A39_CORSIA_FAQ4.aspx.

[86] International Organization for Standardization. (2014). ISO 6966-2:2014(en) Aircraft ground equipment — Basic requirements — Part 2: Safety requirements. Retrieved from https://www.iso.org/obp/ui/#iso:std:iso:6966:-2:ed-2:v1:en.

[87] Jalalian, M., Gholami, S., & Ramezanian, R. (2019). Analyzing the trade-off between CO_2 emissions and passenger service level in the airline industry: Mathematical modeling and constructive heuristic. *Journal of Cleaner Production*, 206, 251-266. https://doi.org/10.1016/j.jclepro.2018.09.139

[88] Javed, T., Ahmed, A., Raman, V., Alqualty, A.B.S., & Johansson, B. (2019). Combustion-based transportation in a carbon constrained world – A review. In R.A. Agarwal., A.K. Agarwal., T. Gupta & N. Sharma (Eds.), *Pollutants from energy sources: Characterization and control* (pp. 7-34). Singapore: Springer Nature Singapore.

[89] Jones, M. (2009). *Sustainable event management: A practical guide* (2nd ed.). Abingdon, UK: Routledge.

[90] Kainulainen, M. (2013). Finnair receives world's first Airbus A321 with Sharklets. Retrieved from https://www.helsinkitimes.fi/business/7600-finnair-receives-world-s-first-airbus-a321-with-sharklets.html.

[91] Kaminski-Morrow, D. (2010). Finnair scraps order for two A330s to launch sharklet A321. Retrieved from https://www.flightglobal.com/finnair-scraps-order-for-two-a330s-to-launch-sharklet-a321/94025.article.

[92] Katila, P., Pierce Colfer, C.J., de Jong, W., Galloway, G., Pacheco, P., & Winkel, G. (2019). Introduction. In P. Katila., C.J. Pierce., C.J. Colfer., W. de Jong., G. Galloway., P. Pacheco & G. Winkel (Eds.), *Sustainable development goals* (pp. 1-16). Cambridge, UK: Cambridge University Press.

[93] Kazda, A., & Caves, R.E. (2015). *Airport design and operation* (3rd ed.). Bingley, UK: Emerald Group Publishing.

[94] Kitamura, K. (2019). A conceptual framework for managerial analysis under economic nationalism and globalization: A study of Japanese automakers in the USA. In H. Chandan & B. Christiansen (Eds.), *International firms' economic nationalism and trade policies in the globalization era* (pp. 35-58). Hershey, PA: IGI Global.

[95] Levin, K., Song, J., & Morgan, J. (2015). COP21 Glossary of terms guiding the long-term emissions-reduction goal. Retrieved from https://www.wri.org/blog/2015/12/cop21-glossary-terms-guiding-long-term-emissions-reduction-goal.

[96] Levy, J.I., Woody, M., Haeng Baek, B., Shankar, U., & Arunachalam, S. (2012). Current and future particulate-matter-related mortality risks in the United States from aviation emissions during landing and takeoff. *Risk Analysis: An International Journal*, 32(2), 237-249. https://doi.org/10.1111/j.1539-6924.2011.01660.x

[97] Li, X.Y., & Tang, B.J. (2017). Incorporating the transport sector into carbon emission trading scheme: An overview and outlook. *Natural. Hazards*, 88, 683–698. https://doi.org/10.1007/s11069-017-2886-3

[98] Li, Z. (2021). Air emergency transport under COVID-19: Impact, measures, and future. *Journal of Advanced Transportation*, 2021, 5560994. https://doi.org/10.1155/2021/5560994

[99] Liu, J., Qiao, P., Ding, J., Hankinson, L., Harriman, E.H., Schiller, E.M., Ramanauskaite, L., & Zhang, H. (2020). Will the aviation industry have a bright future after the COVID-19 Outbreak? Evidence from Chinese airport shipping sector. *Journal of Risk and Financial Management*, 13(11), 276. https://doi.org/10.3390/jrfm13110276

[100] Maertens, S., Grimme, W., Scheelhaase, J., & Jung, M. (2019). Options to continue the EU ETS for aviation in a CORSIA-world. *Sustainability*, 11(20), 5703. https://doi.org/10.3390/su11205703

[101] Manahan, S. E. (2011). *Fundamentals of environmental chemistry* (3rd ed.). Boca Raton, FL: CRC Press.

[102] Markham, F., Young, M., Reis, A., & Higham, J. (2018). Does carbon pricing reduce air travel? Evidence from the Australian 'Clean Energy Future' policy, July 2012 to June 2014. *Journal of Transport Geography*, 70, 206-214. https://doi.org/10.1016/j.jtrangeo.2018.06.008

[103] Mazhar, M.U., Bull, R., Lemon, M., & Bin Saleem Ahmad, S. (2019). Carbon management planning in UK universities: A journey to low carbon-built environment. In W. Leal Filho & R. Leal-Arcas (Eds.), *University initiatives in climate change mitigation and adaptation* (pp. 33-56). Cham, Switzerland: Springer International Publishing.

[104] Meleo, L., Nava, C.R., & Pozzi, C. (2016). Aviation and the costs of the European Emission Trading Scheme: The case of Italy. *Energy Policy*, 88, 138-147. https://doi.org/10.1016/j.enpol.2015.10.008

[105] Migdadi, Y.K.A.A. (2018). Identifying the best practices of airlines' green operations strategy: A cross-regional worldwide survey. *Environmental Quality Management* 28(1), 21.32. https://doi.org/10.1002/tqem.21575

[106] Migdadi, Y.K.A.A. (2020a). *Airline green operational strategies: Emerging research and opportunities*. Hershey, PA: IGI Global.

[107] Migdadi, Y.K.A.A. (2020b). Green operational strategies for airlines: Content and regional analysis. In P. Yang (Ed.), *Cases on green energy and sustainable development* (pp. 193-229). Hershey, PA: IGI Global.

[108] Migdadi, Y.K.A.A. (2020c). Identifying the effective taxonomies of airline green operations strategy. *Management of Environmental Quality*, 31(1), 146-166. https://doi.org/10.1108/MEQ-03-2019-0067

[109] Monios, J. (2016). *Institutional challenges to intermodal transport and logistics: Governance in port regionalization and hinterland integration*. Abingdon, UK: Routledge.

[110] Nava, C.R., Meleo, L., Cassetta, E., & Morelli, G. (2018). The impact of the EU-ETS on the aviation sector: Competitive effects of abatement efforts by airlines. *Transportation Research Part A: Policy and Practice*, 113, 20-34. https://doi.org/10.1016/j.tra.2018.03.032

[111] Neuling, U., & Kaltschmitt, M. (2018). Techno-economic and environmental analysis of aviation biofuels. *Fuel Processing Technology*, 171, 54-69. https://doi.org/10.1016/j.fuproc.2017.09.022

[112] Ngo, C., & Natowitz, J. (2016). *Our energy future: resources, alternatives and the environment*. Hoboken, NJ: John Wiley & Sons.

[113] Niu, S.Y., Liu, C.L, Chang, C.C., & Ke, K.D. (2016). What are passenger perspectives regarding airlines' environmental protection? An empirical investigation in Taiwan. *Journal of Air Transport Management*, 55, 84-91. https://doi.org/10.1016/j.jairtraman.2016.04.012

[114] O'Leary, Z. (2004). *The essential guide to doing research*. London, UK: SAGE Publications.

[115] Otley, T. (2020). Finnair aims for carbon neutrality by 2045. Retrieved from https://www.businesstraveller.com/features/finnair-aims-for-carbon-neutrality-by-2045/.

[116] Palmer, W. (2020). Sustaining flight: Comprehension, assessment, and certification of sustainability in aviation. In T. Walker, A.S. Bergantino., N. Sprung-Much & L. Loiacono (Eds.), *Sustainable aviation: Greening the flight path* (pp. 7-28). Cham, Switzerland: Palgrave Macmillan.

[117] Park, Y., & O'Kelly, M.E. (2014). Fuel burn rates of commercial passenger aircraft: Variations by seat configuration and stage distance. *Journal of Transport Geography*, 41, 137-147. https://doi.org/10.1016/j.jtrangeo.2014.08.017

[118] Pawlowska, M. (2014). *Mitigation of landfill gas emissions*. Leiden, The Netherlands: CRC Press/Balkema.

[119] Pitt, M., & Smith, A. (2003). Waste management efficiency at UK airports. *Journal of Air Transport Management*, 9(2),103-111. https://doi.org/10.1016/S0969-6997(02)00063-7

[120] Preston, A. (2020). Finnair wants to banish its carbon footprint. Retrieved from https://www.inflight-online.com/finnair-wants-to-banish-its-carbon-footprint/.

[121] Rahim, A.R., & Baksh, M.S. (2003). Case study method for product development in engineer-to-order organisations. *Work Study*, 52(1), 25–36. https://doi.org/10.1108/00438020310458705

[122] Rahman, M., Pudasainee, P., & Gupta, R. (2017). Urban waste (municipal solid waste-MSW) to energy. In S. Devasahayam., K. Dowling, & M.K. Mahapatra (Eds.), *Sustainability in the mineral and energy sectors* (pp. 499-530). Boca Raton, FL: CRC Press.

[123] Ramon Gil-Garcia, J. (2012). *Enacting electronic government success: An integrative study of government-wide websites, organizational capabilities, and institutions*. New York, NY: Springer Science-Business Media.

[124] Reinhardt, T., Richers, U., & Suchomel, H. (2008). Hazardous waste incineration in context with carbon dioxide. *Waste Management Research*, 26(1), 88-95. https://doi.org/10.1177%2F0734242X07082339

[125] Ritchie, B.W., Sie, L., Gössling, S., & Dwyer, L. (2020). Effects of climate change policies on aviation carbon offsetting: a three-year panel study. *Journal of Sustainable Tourism*, 28(2), 337-360. https://doi.org/10.1080/09669582.2019.1624762

[126] Roberts, A. (2018). Airside resource planning. In P.J. Bruce., Y. Gao & J.M.C. King (Eds.), *Airline operations: A practical guide* (pp. 152-161.). Abingdon, UK: Routledge.

[127] Roza, G. (2009). *Reducing your carbon footprint on vacation*. New York, NY: The Rosen Publishing Group,

[128] Sales, M. (2013). *Air cargo management: Air freight and the global supply chain*. Abingdon, UK: Routledge.

[129] Sales, M. (2016). *Aviation logistics: The dynamic partnership of air freight and supply chain*. London, UK: Kogan Pace.

[130] Sameh, M.M., & Scavussi dos Santos, J. (2018). Environmental sustainability measures for airports. In A. de Mestral, P.P Fitzgerald & M. Tanveer (Eds.), *Sustainable development, international aviation, and treaty implementation* (pp. 62-80). Cambridge, UK: Cambridge University Press.

[131] Sarkar, A. N. (2012). Evolving green aviation transport system: A Holistic approach to sustainable green market development. *American Journal of Climate Change*, 1, 164-180.

[132] Schäfer, A.W. (2016). The prospects for biofuels in aviation. In C. Chuck (Ed.), *Biofuels for aviation: Feedstocks, technology and implementation* (pp. 3-16). London, UK: Academic Press.

[133] Scheelhaase, J., Maertens, S., Grimme, W., & Jung, M. (2018). EU ETS versus CORSIA – A critical assessment of two approaches to limit air transport's CO_2 emissions by market-based measures. *Journal of Air Transport Management*, 67, 55-62. https://doi.org/10.1016/j.jairtraman.2017.11.007

[134] Scott, B.J., & Trimarchi, A. (2020). *Fundamentals of international aviation law and policy*. Abingdon, UK: Routledge.

[135] Scott, J. (2004). Documents, types of. In: M. Lewis-Beck, A.E. Bryman. & T. Futing Liao (Eds.), *The SAGE encyclopedia of social science research methods* (pp. 281-284). Thousand Oaks, CA: SAGE Publications.

[136] Scott, J. (2014). *A dictionary of sociology* (4th ed.). Oxford, UK: Oxford University Press.
[137] Scott, J., & Marshall, G. (2009). *A dictionary of sociology* (3rd ed.). Oxford, UK: Oxford University Press.
[138] Shaw, S. (2016). *Airline marketing and management* (7th ed). Abingdon, UK: Routledge.
[139] Singapore Airlines. (2021). Sustainability Report 2020/21. Retrieved from https://www.singaporeair.com/saar5/pdf/Investor-Relations/Annual-Report/sustainabilityreport2021.pdf.
[140] Singh J, Sharma-Kumar, S., & Srivastava R. (2018). Managing fuel efficiency in the aviation sector: Challenges, accomplishments and opportunities. *FIIB Business Review*, 7(4), 244-251. https://doi.org/10.1177%2F2319714518814073Skrifvars, M., & Åkesson, D. (2016). Recycling of thermoset composites. In M.J. Taherzadeh & T. Richards (Eds.), *Resource recovery to approach zero municipal waste* (pp. 249-260). Boca Raton, FL: CRC Press.
[141] Stake, R.E. (1995). *The art of case study research*. Thousand Oaks, CA: SAGE Publications.
[142] Stake, R.E. (2005). Qualitative case studies. In N.K. Denzin & Y.S. Lincoln (Eds.), *The SAGE handbook of qualitative research* (pp. 443-466). Thousand Oaks, CA: SAGE Publications.
[143] Staples, M.D., Malina, R., Suresh, P., Hileman, J.I., & Barrett, S.R.H. (2014). Aviation CO_2 emissions reductions from the use of alternative jet fuels. *Energy Policy*, 114, 342-354. https://doi.org/10.1016/j.enpol.2017.12.007
[144] Su, Y., Zhang, P., & Su, Y. (2015). An overview of biofuels policies and industrialization in the major biofuel producing countries. *Renewable and Sustainable Energy Reviews*, 50, 991-1003. https://doi.org/10.1016/j.rser.2015.04.032
[145] Taiwo, A.M. (2011). Composting as a sustainable waste management technique in developing countries. *Journal of Environmental Science and Technology*, 4(2), 93-102.
[146] Tarczay, K., Nagy, E., & Kis-Kovács, G. (2011). Energy production, industrial processes, and waste management. In L. Haszpra (Ed.), *Atmospheric greenhouse gases: The Hungarian perspective* (pp. 367-386). Dordrecht, The Netherlands: Springer Science+Business Media.
[147] Tavares Kennedy, H. (2019). Finnair introduces CO_2 offsetting and biofuel service for customers. Retrieved from https://www.biofuelsdigest.com/bdigest/2019/01/19/finnair-introduces-co2-offsetting-and-biofuel-service-for-customers/.
[148] Taylor, I. (2020). Finnair to axe inflight sales amid pledge 'to banish' carbon. Retrieved from http://www.travelweekly.co.uk/articles/361270/finnair-to-axe-inflight-sales-amid-pledge-to-banish-carbon.
[149] Taylor, J., & Young, S. (1975). *Passenger aircraft and airlines*. London, UK: Marshall Cavendish Publications.
[150] Testa, E., Giammusso, C., Bruno, M., & Maggiore, P. (2014). Analysis of environmental benefits resulting from the use of hydrogen technology in handling operations at airports. *Clean Technologies and Environmental Policy*, 16(5), 875-890. https://doi.org/10.1007/s10098-013-0678-3
[151] Thompson, B. (2007). Ground handling opportunities for airports. *Journal of Airport Management*, 1(4), 393-397.
[152] United Nations. (2021). Sustainable development goals. Retrieved from https://sustainabledevelopment.un.org/?menu=1300.
[153] van Schoor, B. (2017). *Fighting corruption collectively: How successful are sector-specific coordinated governance initiatives in curbing corruption*. Wiesbaden, Germany: Springer.
[154] Vasigh, B., Fleming, K., & Humphreys, B. (2015). *Foundations of airline finance: Methodology and practice* (2nd ed.). Abingdon, UK: Routledge.
[155] Vásquez, L., Iriarte, A., Almeida, M., & Villalobos, P. (2015). Evaluation of greenhouse gas emissions and proposals for their reduction at a university campus in Chile. *Journal of Cleaner Production*, 108(Part A), 924-930. https://doi.org/10.1016/j.jclepro.2015.06.073
[156] Wiedemann, T., & Minx, J. (2007). A definition of 'carbon footprint'. In C.C. Pertsova (Ed.), *Ecological economics research trends* (pp. 1-11). New York, NY: Nova Science Publishers.
[157] Yin, R.K. (2018). *Case study research and applications* (6th ed.). Thousand Oaks: SAGE Publications.
[158] Zhang, B., Ritchie, B., & Mair, J. (2019). Is the airline trustworthy? The impact of source credibility on voluntary carbon offsetting. *Journal of Travel Research*, 58(5), 715-731. https://doi.org/10.1177%2F0047287518775781
[159] Zhang, J., Zhang, S., Wu, R., Duan. M., Zhang, D., Wu, Y., & Hao, J. (2021). The new CORSIA baseline has limited motivation to promote the green recovery of global aviation. *Environmental Pollution*, 289, 117833. https://doi.org/10.1016/j.envpol.2021.117833
[160] Zhou, T., & Zhang, J. (2020). Behavioural research on transport and energy in the context of aviation. In J. Zhang (Ed.), *Transport and energy research: A behavioral perspective* (pp. 279-294). Amsterdam, The Netherlands: Elsevier.
[161] Zou, B., Kwan, I., Hansen, M., Rutherford, D., & Kafle N. (2016). Airline fuel efficiency: Assessment methodologies and applications in the U.S. domestic airline industry. In J.D. Bitzan., J. Peoples & W.W. Wilson. (Eds.), *Airline efficiency* (pp. 317-352). Bingley, UK: Emerald Group Publishing.

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Assessment of Phenotypic Diversity in Breeding Lines of Barley at Rampur, Chitwan

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Abstract— *The experiment was conducted during winter at the research field of National Maize Research Program (NMRP), Rampur, Chitwan situated in the inner-terai region of Nepal. Field experiment was conducted in the Completely Randomized Block Design (CRBD) with three replications. Three blocks was made on the field as local control to obtain more accurate data. Each block contains 14 experimental units. The collected data was recorded, entered and tabulated in Microsoft Excel and then analyzed by using R-software. Analysis showed non-significant variation for number of heads per meter square and significant variation for all other remaining traits. CHZ-NP-108-OY (111DAS) showed highest days to maturity and B86019-1K-2K-0K3 showed lowest. Days to booting, heading and anthesis were found highest in variety Bonus (std. check) with 81 DAS, 88 DAS and 98 DAS, respectively. Likewise, total quantity of tillers and number of effective tillers were highest in variety B90K-090-0K (2.9 and 2.4 respectively) and lowest in B86019-1K-2K-0K3 (1.9) and B86099-50K (1.6) respectively. Plant height was highest in variety B90K-024-1-1-2-0K (107cm) and lowest in Xveola-15 (82cm). Xveola-38 showed highest result for seed length (11.2mm) and grain yield (1634 kg/ha). Correlation between most of the traits was significant. Plant height changed into vast and undoubtedly correlated with tiller range (0.3*), powerful tiller (0.44***), flag leaf width (0.41***) flag leaf period (0.35**). Grain yield in keeping with hectare changed into significantly and definitely correlated with days to maturity (0.07**), flag leaf width (0.41***) plant peak (0.45**) and grain yield according to spike (0.1**). B86019-1K-2K-0K3 showed better adaptability and results for phenotypic characters whereas Xveola-38 performed best for the yield and yield attributing traits. So these varieties have the possibilities and potential for further study in Rampur, Chitwan.*

Keywords— ***Significant, Correlated, Yield and Non-significant.***

I. INTRODUCTION

Barley is a short seasoned, early maturing annual grain crop with some degree of tolerance to drought and salinity, which allows its production in a wide range of climatic zones including both irrigated and dry land production areas (Baniya, 1989). The genetic system of the crop is simple; however the species are genetically diverse (Vitrakoti et al., 2016).Barley is cultivated in extensive variety of environments in Nepal(Baniya et al., 1997).It has the capability to evolve well in adverse climatic and stress conditions and moreover, can be managed with little input. The geographical diversification of Nepal including fertile plains, sub-alpine forest hills and the Himalayas serves for the disproportionately large bio-diversification, relative to its size. There are different varieties of the barley suiting different agro-climatic condition. The diversity assessment of barley helps to choose high-yielding, well adapted varieties to a particular region.

Barley occupies fourth position worldwide with the production of 141423028 tones and fifth in Nepal with production of 30510 tones (FAO, 2018). The total land under barley cultivation in Nepal is 24648ha (Krishi Diary, 2076). The significance of barley as a meals crop will increase with ascending altitude toward the North, wherein other cereals can't be cultivated correctly because of poor edaphic factors and excessive environmental stress, especially drought(OGTR, 2008). Although having so much potential, not many study and research have been conducted on barley as it's far taken as underutilized vegetation. although suggested barley manufacturing within the terai has declined in the past decade from 6000 to 3000 tons in keeping with year, manufacturing in the hills over the equal length has multiplied from 9000 to twelve,000 tons and from 8000 to 10,000 tons in the mountains(Baniya, 1989). This popularity in hills and mountains may have resulted due to its early maturing ability (1 month) before wheat favoring the summer crops. Also, the low adaptability of local varieties and fewer introductions of improved varieties may have caused the decreased production in terai. The research and studies made are limited. Barley has its growing popularity worldwide.

Huge topographical and agro-climatic diversification inside the country does not favor a single variety within all ecological niches. The cultivation and production of barley is low in the terai region due to the less knowledge on suitable lines in the tropical climate. Limited research and study works is major problem regarding the assessment of diversity of this crop. And hence, it calls for a need to study the performance of different varieties in different agro-climatic conditions and assigned the best breeding lines for particular climate type. This research is significant as assessing the high yielding breeding lines in accordance with the ecological diversity is the only way to improve the production and productivity of the marginalized farmers. Also, the availability of the scientific literature is limited because this crop has not been given much importance and is less researched. Moreover, the high yielding varieties of barley are assessed for temperate regions and less significant study has been made for the tropics. This research provides detailed estimates of agro-morphological and phenotypic diversity of available lines for pre-breeding purpose and functional diversity. despite its top notch capacity and possibilities, the crop has no longer been able to engross pleasant attention concerning studies and improvement in Nepal and remains underutilized and ignored (Yadav et al., 2018).

II. MATERIALS AND METHODS

Available breeding lines of barley at NARC were characterized and assessed for different traits. The experiment became conducted at some point of winter season at the studies subject of national Maize research program (NMRP), Rampur, Chitwan located within the inner-terai vicinity of Nepal from December, 2019 to April, 2020. Area test was conducted within the completely Randomized Block design (CRBD) with 3 replications, each containing 14 experimental units. 14 treatments were assigned randomly into each experimental units. The barley lines were allotted into treatments as below:

Table 1: Allotment of barley lines into treatments

S.N.	Treatments	Barley lines
1	1	B90k-014-1-1-2-0K
2	2	Xveola-53
3	3	B90K-090-0K
4	4	B86019-1K-2K-0K3
5	5	LG51/Xveola-5-77
6	6	Xveola-38
7	7	CHZ-NP-108-OY
8	8	B86099-5-0K
9	9	Xveola-15
10	10	B90k-024-1-1-2-0K
11	11	NB-1003-37/1034
12	12	Bonus(std check)
13	13	Local check
14	14	CO11#112-14

Two to three harrowing with a disc harrow mounted on tractor followed by leveling and uniform grading was done for proper plant establishment and management. The field was divided into three blocks containing 14 plots each of size 3*2metre square. The seeds of different germplasm were line sown in each experimental unit by maintaining the row-row distance of 25cm.Irrigation was provided at two critical stages tillering and flowering. Fertilizer dose was applied at the ratio of45:30:0 kg NPK/ha where the Nitrogen fertilizer was divided into 3 times among which half of the dose was applied as basal application and remaining half was divided equally again to be applied on tillering and flowering.Ten plants were sampled randomly for recording observation for each entry and 50 percentages completion out of the total plants was taken for days to heading, booting, anthesis and maturity. The different germplasm were harvested when most of the plants of particular germplasm turned yellow and dried up. When the spikes were well dried, threshing and cleaning was done followed by packing and storage. Data entry and processing was carried out using the Microsoft Office Excel 2010 software and means and standard deviation for all traits were compared. The testing of hypothesis and analysis of variance was calculated using R-software.

III. RESULTS AND DISCUSSION

Phenological and growth attributing traits

In our experiment there was high significant variation for days to booting, heading, anthesis and maturity among the tested barley germplasms.

A maximum day to booting was observed in the treatment Bonus (std. check) in 81 days and minimum days to booting was observed in the germplasm LG51/Xveola-5-77 in 68 days. CV value for days to booting was 2.81; LSD value among the tested germplasms was 3.39 and mean days to booting for the treatments was 72days.It was reported by(Vitrakoti et al., 2016),the days of booting for different barley genotypes ranged from 58-84 which is likely with our result. The mean days for booting were 69 days which is slightly lower than our result. The CV and LSD value were 3.47 and 4.77 respectively which were slightly higher than ours.

A maximum day to heading was observed in the treatment Bonus (std. check) in 81 days and minimum days to heading was observed in the germplasm LG51/Xveola-5-77 in 72 days. CV value for days to heading was 2.4087; LSD value among the tested germplasms was 3.157078 and mean days to heading for the treatments was 78 days. (Vitrakoti et al., 2016), reported the days of heading for barley ranged from 71-96 days whereas our barley varieties were already headed till 81 days. This might be the result of the different varieties use in these two researches. The genetic characteristics of the different varieties might have contributed in this difference for days to heading.

A maximum day to anthesis was observed in the treatment Bonus (std. check) in 98 days and minimum days to anthesis was observed in the germplasm B90K-014-1-1-2-0K in 79 days. CV value for days to anthesis was 1.694734; LSD value among the tested germplasms was 2.386527 and mean days to anthesis for the treatments was 84 days. Days of flowering ranged (92-117DAS) as reported by(Gupta et al., 2009) is slightly higher than this research which might have caused due to the difference in climate of Jumla and Chitwan or may be due to the use of naked barley germplasms in Mr. Gupta's research.

A maximum day to maturity was observed in the treatment CHZ-NP-108-OY in 111 days and minimum days to maturity was observed in the germplasm B86019-1K-2K-0K3 in 97 days. CV value for days to maturity was 2.848386; LSD value among the tested germplasms was 5.045745 and mean days to maturity for the treatments was 106 days. Days of maturity ranged to 139-149 as reported by in Khumaltar, Lalitpur(Gupta et al., 2009). This is very high than our result. This could be the result of temperature difference between the two cities or increased global warming over time.

Table 2: Phenological and growth attributing traits

Genotype	**Days to Booting (days)**	**Days to Heading (days)**	**Days to anthesis (days)**	**Days to maturity (days)**
B90k-014-1-1-2-0K	69.7c	73.7ef	79.0h	100.0de
Xveola-53	70.3c	77.7cd	80.3fgh	102.7cd
B90K-090-0K	71.0c	79.0c	84.3cd	106.7abc
B86019-1K-2K-0K3	67.7c	73.0ef	81.7efg	97.3e
LG51/Xveola-5-77	67.7c	72.3f	79.7gh	100.0de
Xveola-38	70.3c	75.7de	81.0efgh	107.3abc
CHZ-NP-108-OY	70.3c	80.3c	85.7c	111.0a
B86099-5-0K	69.7c	78.3cd	82.3def	104.0bcd
Xveola-15	69.0c	78.3cd	82.3def	108.0ab
B90k-024-1-1-2-0K	70.3c	75.7de	80.3fgh	109.3a

NB-1003-37/1034	71.0c	78.3cd	85.7c	109.3a
Bonus(std check)	81.0a	87.7a	97.7a	106.0abc
Local check	76.3b	79.0c	83.0de	107.3abc
CO11#112-14	79.7ab	84.3a	91.7b	108.7ab
CV	**2.8**	**2.4de**	**1.7**	**4.3**
LSD	**3.4**	**3.2b**	**2.4**	**0.2**
MEAN	**71.7**	**78.1**	**83.9**	**2.3**
F-test	***	***	***	***

Note: Mean separated by DMRT and columns represented with the same letter (s) are non-significant at 5% level of significance, CV= Coefficient of Variation, LSD= Least Significant Difference at 0.05 level of significance and SD= Standard Deviation

Growth attributing traits

In our experiment there was highly significant variation for tiller number among the tested barley germplasms. Maximum tiller number was exhibited by the treatment B90K-090-0K with 2.9 tillers and lowest tiller number was shown by the germplasm B86019-1K-2K-0K3 with 1.9 tillers. CV value for tiller number was 4.254319; LSD value among the tested germplasms was 0.1625237 and mean tiller number for the treatments was 2.3.(Gupta et al., 2009) reported the range for total tillers from 2.4-3.9 which is slightly higher than our result. This might be the result of better adaptability and thriving of barley in cool climatic condition of Jumla. The total tiller number affects the number of effective tillers which in higher number might favor the higher yield per plant.

In our experiment there was highly significant variation for effective tillers among the tested barley germplasms. Maximum effective tillers was exhibited by the treatment B90K-090-0K with 2.4 tillers and lowest effective tillers was shown by the germplasm B86099-5-0K with 1.6 tillers. CV value for effective tillers was 8.198947; LSD value among the tested germplasms was 0.2634164 and mean effective tillers for the treatments was 1.9. (Gupta et al., 2009), reported similar result for the effective tillers ranging from 1.9-7. The number of effective tillers is the ultimate characteristic causing variation in the yield.

In our experiment there was highly significant variation for flag leaf length among the tested barley germplasms. Maximum flag leaf length was shown by the treatment Xveola-38 with 12.9cm and lowest flag leaf length was shown by the germplasm Local check with 6.3cm. CV value for flag leaf length was 4.594492; LSD value among the tested germplasms was 0.7685383 and mean flag leaf length for the treatments was 9.9cm. The flag leaf length of barley germplasm was ranged from 9.78cm to13.6 cm in the research conducted by(Joshi et al., 2020).

In our experiment there was highly significant variation for flag leaf width among the tested barley germplasms. Maximum flag leaf width was shown by the treatment CHZ-NP-108-OY with 0.9cm and lowest flag leaf width was shown by the germplasm Bonus (std. check) with 0.4cm. CV value for flag leaf width was 4.515979; LSD value among the tested germplasms was 0.05383119 and mean flag leaf width for the treatments was 0.7cm. The flag leaf width of barley germplasm was ranged from 0.68 to 1.08 cm in the research conducted by(Joshi et al., 2020). It is slightly greater than our result. This might be due to the different germplasms used in the two studies.

Table 3: Growth attributing traits

Genotype	Total tiller number	Effective Tillers	Flag leaf length (cm)	Flag leaf width (cm)
B90k-014-1-1-2-0K	2.1ef	1.9cdef	10.4e	0.7de
Xveola-53	2.3cd	2.1bcd	10.6de	0.8bc
B90K-090-0K	2.9a	2.4a	10.7bcde	0.7e
B86019-1K-2K-0K3	1.9g	1.7fg	10.6cde	0.7cde
LG51/Xveola-5-77	2.0fg	1.8defg	10.4de	0.8ab
Xveola-38	2.2de	1.9cdef	12.9a	0.7de
CHZ-NP-108-OY	2.3cd	2.1bc	11.1bcd	0.9a
B86099-5-0K	2.1ef	1.6g	11.4b	0.8bcd

Xveola-15	2.1ef	1.8defg	8.7f	0.7e
B90k-024-1-1-2-0K	2.3cd	1.9bcde	9.3f	0.8b
NB-1003-37/1034	2.4bc	2.2ab	11.4bc	0.8ab
Bonus(std check)	2.6b	1.7efg	6.9g	0.4h
Local check	2.1ef	1.7fg	6.3g	0.5g
CO11#112-14	2.4bc	2.0bcd	8.7f	0.6f
CV	**4.3**	**8.2**	**4.6**	**4.5**
LSD	**0.2**	**0.3**	**0.8**	**0.1**
MEAN	**2.3**	**1.9**	**10.0**	**0.7**
F-test	***	***	***	***

Note: Mean separated by DMRT and columns represented with the same letter (s) are non-significant at 5% level of significance, CV= Coefficient of Variation, LSD= Least Significant Difference at 0.05 level of significance and SD= Standard Deviation

Growth and yield attributing traits

In our experiment there was moderate significant variation for flag leaf sheath length among the tested barley germplasms. Maximum flag leaf sheath length was shown by the treatment CHZ-NP-108-OY with 22.7cm and lowest flag leaf sheath length was shown by the germplasm Bonus (std. check) with 18.1cm. CV value for flag leaf sheath length was 5.529546; LSD value among the tested germplasms was 1.905999 and mean flag leaf sheath length for the treatments was 20.5cm.

The flag leaf sheath stores photosynthetic products and transport to grains after heading which contributes yield up to 10-20% in case of rice(Gupta et al., 2009), reported similar result for flag leaf range from 14.02cm to 21.78cm.

In our experiment there was high significant variation for plant height among the tested barley germplasms. Maximum plant height was shown by the treatment B90k-024-1-1-2-0K with 106.7cm and lowest plant height was shown by the germplasm Xveola-15 with 82.3cm. CV value for plant height was 7.230024; LSD value among the tested germplasms was 11.48829 and mean plant height for the treatments was 94.7cm. This result is almost similar to the reported by(Amgai et al., 2011). Also study reported the plant height for barley ranged from 85.8cm to 135.67cm. The variation in leaf appearance rate, leaf size and genetic makeup might have resulted in the variation of plant height.

In our experiment there was high significant variation for awn length among the tested barley germplasms. Maximum awn length was shown by the treatment B90K-090-0K with 10.8cm and lowest awn length was shown by the germplasm Xveola-38 with 8.9cm. CV value for awn length was 2.613249; LSD value among the tested germplasms was 0.4432893 and mean awn length for the treatments was 10.1cm.

Table 4: Growth and yield attributing traits

Genotype	**Sheath length (cm)**	**Plant height (cm)**	**Awn length (cm)**
B90k-014-1-1-2-0K	20.9abc	98.6abcd	10.6ab
Xveola-53	20.5bcd	92.4def	9.4d
B90K-090-0K	21.0abc	99.9abcd	10.8a
B86019-1K-2K-0K3	18.6de	90.6def	10.1c
LG51/Xveola-5-77	21.8ab	104.8ab	10.8a
Xveola-38	20.3bcd	94.4bcde	8.9d
CHZ-NP-108-OY	22.7a	104.4abc	10.8a
B86099-5-0K	19.9cde	82.4f	9.9c
Xveola-15	20.8bc	82.3f	10.1c
B90k-024-1-1-2-0K	20.9abc	106.8a	10.2bc

NB-1003-37/1034	20.6bc	96.2abcd	9.9c
Bonus(std check)	18.1e	83.1ef	10.7a
Local check	19.7cde	93.3cdef	10.0c
CO11#112-14	21.6abc	96.1abcd	9.1d
CV	**5.5**	**7.2**	**2.6**
LSD	**1.9**	**11.5**	**0.4**
MEAN	**20.5**	**94.7**	**10.1**
F-test	**	***	***

Note: Mean separated by DMRT and columns represented with the same letter (s) are non-significant at 5% level of significance, CV= Coefficient of Variation, LSD= Least Significant Difference at 0.05 level of significance and SD= Standard Deviation

Yield attributing traits

In our experiment there was high significant variation for spike length among the tested barley germplasms. Maximum spike length was shown by the treatment CHZ-NP-108-OY with 7.7cm and lowest spike length was shown by the germplasm Local check with 4.9cm. CV value for spike length was 3.798795; LSD value among the tested germplasms was 0.4019691 and mean spike length for the treatments was 6.3cm. Bista (2015) reported that spike length was ranged from 5.41cm to 7.03 cm which is almost similar with our result. Likewise, similar result was shown by (Eshghi & Akhundova, 2009).

In our experiment there was non-significant variation for number of head per meter square among the tested barley germplasms. Maximum head per meter square was shown by the treatment B90K-090-0K with 251.3 heads and lowest head per meter square was shown by the germplasm B86099-5-0K with 179.7 heads. CV value for head per meter square was 20.49503; LSD value among the tested germplasms was 71.88255 and mean head per meter square for the treatments was 209 heads.

In our experiment there was high significant variation for grain per spike among the tested barley germplasms. Maximum grain per spike was shown by the treatment B90K-014-1-1-2-0K with 56.4 and lowest grain per spike was shown by the germplasm Bonus (std. check) with 26.9 grains. CV value for grain per spike was 11.79152; LSD value among the tested germplasms was 9.41914 and mean grain per spike for the treatments was 47.6 grains per spike.(Eshghi & Akhundova, 2009) reported the number of grains per spike to be 17-59 and 26-58 respectively which is almost similar to our result.(Eshghi & Akhundova, 2009), recorded the mean number of grains per spike to be 17 which is way lower than our result. This difference in the mean data might have occurred due to the climatic difference between Nepal and Rajasthan and also the use of different germplasms.

In our experiment there was high significant variation for grain yield per spike among the tested barley germplasms. Maximum grain yield per spike was shown by the treatment B90K-090-0K with 1.65gm per spike and lowest grain yield per spike was shown by the germplasm Bonus (std. check) with 0.73 gm per spike. CV value for grain yield per spike was 23.38698; LSD value among the tested germplasms was 0.5099844 and mean grain yield per spike for the treatments was 1.3gm per spike. (Eshghi & Akhundova, 2009), had a little higher result (1.4cm to 2.5cm) than that of ours for the grain yield per spike.

In our experiment there was high significant variation for seed length among the tested barley germplasms. Maximum seed length was shown by the treatment Xveola-38 with 11.2mm and lowest seed length was shown by the germplasm B86099-5-0K with 9.8mm. CV value for seed length was 2.910947; LSD value among the tested germplasms was 0.508445 and mean seed length for the treatments was 10.4mm.This result is very higher than the seed length 6.9-9mm as reported by(Eshghi & Akhundova, 2009),which may have resulted due to the difference in number for grain per spike, genetic constitution, flag leaf area and chlorophyll content among different germplasms.

Table 5: Yield attributing traits

Genotype	Spike length (cm)	Number of head/m2	Grain per spike	Grain yield per spike (gm/spike)	Seed length(mm)
B90k-014-1-1-2-0K	6.2c	204.7a	56.4a	1.5ab	10.3cde
Xveola-53	6.2cd	191.0a	51.5abc	1.3abc	10.2cde
B90K-090-0K	5.5ef	251.3a	49.4abcd	1.7a	11.0ab
B86019-1K-2K-0K3	6.0cd	193.7a	44.5cd	0.9cd	10.0de
LG51/Xveola-5-77	6.9b	209.0a	49.9abcd	1.5ab	10.5cd
Xveola-38	5.4f	201.7a	51.5abc	1.7a	11.2a
CHZ-NP-108-OY	7.7a	187.7a	41.4d	1.2abc	10.6bc
B86099-5-0K	5.8de	179.7a	47.1abcd	1.1bcd	9.8e
Xveola-15	6.1cd	244.7a	51.5abc	1.4abc	10.6bc
B90k-024-1-1-2-0K	6.4c	237.7a	51.5abc	1.5abc	10.3cde
NB-1003-37/1034	6.1cd	187.0a	45.3bcd	1.3abc	10.2cde
Bonus(std check)	7.6a	248.3a	26.9e	0.7d	9.9e
Local check	4.9g	206.3a	45.1bcd	1.4abc	10.3cde
CO11#112-14	7.5a	183.0a	54.5ab	1.1bcd	10.6bc
CV	**3.8**	**20.5**	**11.8**	**23.4**	**2.9**
LSD	**0.4**	**71.9a**	**9.4**	**0.5**	**0.5**
MEAN	**6.3**	**209.0**	**47.6**	**1.3**	**10.4**
F-test	*******	**Ns**	*******	*****	*******

Note: Mean separated by DMRT and columns represented with the same letter (s) are non-significant at 5% level of significance, CV= Coefficient of Variation, LSD= Least Significant Difference at 0.05 level of significance and SD= Standard Deviation

Yield and yield attributing traits

In our experiment there was high significant variation for seed width among the tested barley germplasms. Maximum seed width was shown by the treatment CHZ-NP-108-OY with 3.4mm and lowest seed width was shown by the germplasm Xveola-53 with 2.7mm. CV value for seed width was 3.936254; LSD value among the tested germplasms was 0.2034439 and mean seed width for the treatments was 3mm. This result is almost similar to the result reported by (Bista, 2015), 2.17mm to 3.06mm and(Eshghi & Akhundova, 2009) .

In our experiment there was high significant variation for thousand seed weight among the tested barley germplasms. Maximum thousand seed weight was exhibited by the treatment B90k-024-1-1-2-0K with 36.9gm and lowest thousand seed weight was shown by the germplasm Co11#112-14 with 19.8gm. CV value for thousand seed weight was 2.263947; LSD value among the tested germplasms was 1.083716 and mean thousand seed weight for the treatments was 28.5gm. Gupta et al. (2009), reported the thousand seed weight to range from 36.4-46gram which is greater than our result.(KARKOUR et al., 2019), also recorded higher thousand seeds weight than ours which is 39 gm.

In our experiment there was high significant variation for grain yield per hectare among the tested barley germplasms. Maximum grain yield per hectare was exhibited by the treatment Xveola-38 with 1634.4kg/ha and lowest grain yield per hectare was shown by the germplasm Bonus (std. check) with 382.2kg/ha. CV value for grain yield per ha was 24.66688; LSD value among the tested germplasms was 454.1431 and mean grain yield per ha for the treatments was 1096.984 kg/ha. Study reported the grain yield per hectare range from 1666.67kg/ha to 5141.67kg/ha. This result is much greater than our result which might have resulted due to the differential performance and adaptability of the respective germplasms in the climatic condition of Khumaltar and Chitwan respectively.

Table 6: Yield and yield attributing traits

Genotype	Seed width(mm)	Thousand seed weight (gm)	Grain yield per plot (kg/plot)	Grain yield per hectare (kg/ha)
B90k-014-1-1-2-0K	2.9cd	31.3bc	0.7abc	1221.1abc
Xveola-53	2.7d	25.8e	0.5def	753.3def
B90K-090-0K	3.1bc	31.4bc	0.7bcd	1161.1bcd
B86019-1K-2K-0K3	2.9cd	21.5f	0.7bcd	1121.1bcd
LG51/Xveola-5-77	3.2ab	31.7b	0.7bcd	1115.6bcd
Xveola-38	3.1bc	36.2a	0.9a	1634.4a
CHZ-NP-108-OY	3.4a	30.6bc	0.8abc	1275.6abc
B86099-5-0K	3.0bc	24.7e	0.4ef	646.7ef
Xveola-15	3.1b	25.0e	0.8ab	1388.9ab
B90k-024-1-1-2-0K	3.2bc	36.9a	0.8abc	1352.2abc
NB-1003-37/1034	3.1bc	28.7d	0.8abc	1302.2abc
Bonus(std check)	3.1bc	25.3e	0.2f	382.2f
Local check	3.1bc	30.4c	0.6cde	933.3cde
CO11#112-14	3.1bc	19.8g	0.6bcde	1070.0bcde
CV	**3.9**	**2.3**	**24.7**	**24.7**
LSD	**0.2**	**1.1**	**0.3**	**454.1**
MEAN	**3.1**	**28.5**	**0.7**	**1097.0**
F-test	***	***	***	***

Note: Mean separated by DMRT and columns represented with the same letter (s) are non-significant at 5% level of significance, CV= Coefficient of Variation, LSD= Least Significant Difference at 0.05 level of significance and SD= Standard Deviation

Table 7: Correlation of quantitative traits

	DTB	DTH	DTA	DM	tn	Et	Fl	Fw	Sl	PH	Al	spl	GPS	GYS	sel	sw	tswt	GY
DTB		0.83 ***	0.84***	-0.09	-0.11	-0.17	-0.44 ***	-0.37**	0.13	-0.2	0.15	0.12	0.43 ***	-0.31 *	-0.01	-0.18	-0.1	-0.24
DTH			0.78***	-0.09	-0.06	-0.12	-0.3	-0.25	0.07	-0.3	0.05	0.06	0.42 ***	-0.34 **	0.05	-0.12	-0.17	-0.13
DTA				-0.1	-0.06	-0.12	-0.39 **	-0.44 ***	-0.09	-0.4***	0.01	0.25	0.46	-0.46	-0.1	-0.23	-0.24	-0.32
DTM					0.1	-0.02	0.03	-0.04	0.23	0.13	-0.18	0.32 **	0.06 ***	0.13 ***	0.03	0.24	0.24	0.07 **
tn						0.87 ***	0	0.1	0.02	0.3 *	0.08	0.06	0.17	0.16	-0.04	-0.11	0.13	-0.01
et							0.21	0.21	0.11	0.44***	0.11	0.1	0.25	0.27	0.02	-0.07	0.22	0.09
Fl								0.46 ***	0.26	0.35 **	-0.32*	0.08	0.2	0.33 **	0.2	0	0.22	0.23
Fw									0.44***	0.41 ***	-0.07	0.04	0.3 *	0.28	-0.08	-0.01	0.18	0.41 ***
Sl										0.5 ***	-0.06	0.22	0.31 *	0.39 ***	0.2	0.16	0.31*	0.18
PH											0.04	0.07	0.24	0.47 ***	0.14	-0.02	0.45 ***	0.35 **
Al												-0.04	0.19	-0.17	0	0.13	-0.14	0.1
spl													0.21	-0.26	0.04	0.04	-0.1	-0.28
GPS														0.73***	0.17	-0.07	0.26	0.23
GYS															0.32**	0.08	0.78 ***	0.39 **
Sel																0.2	0.24	0.1
Sw																	0.15	-0.15
Tswt																		0.19
GY																		

DTB- Day to booting, DTH- Days to heading, DTA- Days to anthesis, DTM- Days to maturity, tn- Tiller numbers, et- number of effective tillers, fl- Flag leaf length, fw- Flag leaf width, sl- Flag leaf sheath length, PH- Plant height, al- Awn length, spl- Spike length, GPS- Grains per spike, GYS- Grain yield per spike, sel- Seed length, sw- Seed width, tswt- Thousand seed wight and GY- Grain yield per hectare, ***p=0.001>, **p= 0.01-0.001, *p=0.04-0.01.

Correlation between all quantitative traits was analyzed. The days to booting was highly significant and positively correlated with days to heading (0.83***) and days to booting (0.84***). Days to maturity was non-significant and negatively correlated with days to booting (-0.09ns), days to heading (-0.09ns) and days to anthesis (-0.1ns).

Tiller number and effective tillers were highly significant and positively associated with each other (0.87***). Plant top became tremendous and negatively correlated with days to heading (-0.3*) while non-sizable and negatively correlated with days to booting (-zero.2ns) and days to maturity (-0.04ns). Plant top changed into significant and undoubtedly correlated with tiller wide variety (zero.three*), notably good sized and positively correlated with powerful tiller (0.44***), flag leaf width (zero.forty one***) while reasonably good sized and positively correlated with flag leaf duration (zero.35**).Flag leaf length and flag leaf width were highly significant and positively correlated (0.46***) with each other. Grains per spike and grain yield per spike were highly significant and strong positively associated (0.73***) with each other.

Thousand seed weight was highly significant and positively correlated with plant height (0.45***) and grain yield per spike (0.78***). Thousand seed weight and grain yield were non-significant and positively correlated (0.19ns) with each other.

Grain yield per hectare was moderately significant and positively correlated with days to maturity (0.07**), plant height (0.45**) and grain yield per spike (0.1**) whereas highly significant and positively correlated with flag leaf width (0.41***). Grain yield per hectare was non-significant and negatively correlated with days to booting (-0.24ns), days to heading (-0.13ns), days to anthesis (-0.32), tiller number (-0.01ns), spike length (-0.28ns) and seed width (-0.15ns).

Characters like flag leaf width and tiller numbers, seed length and awn length, seed width and flag leaf length are not associated (0.00) with each other.

In the article published by(Vitrakoti et al., 2016),grain yield in keeping with hectare had advantageous and noticeably massive end resultwith thousand seed weight (which was positive but non-significant in our result), positive and significant correlation with spike length (which was negative and non-significant in our result) whereas grain yield per hectare showed negative highly significant correlation with days to flowering. Although there exists positive relationship between yield and the other components, the negative correlation between some of them cannot make a useful choice for all as a factor in increasing the yield (Kannenberg 1976).There was non-significant positive correlation between plant height and thousand seed weight (Drikvand 2011), which was positive and highly significant in our result. Thousand grain weights had positive high significant association with grain yield per hectare (which was positive and non-significant in our result) followed by spike length (which was negative and non-significant in our result) and negative high significant association with days to flowering followed by days to booting and days to heading (similar to our result).

Highly significant favorable correlation among yield attributing tendencies suggests that, the unit increment in one of the trait will motive a unit increment inside the every other relatedtraits, which in turn will lead to increase in the grain yield. Hence, these traits could be pointed and noted for the improvement of grain yield by focusing on the associated traits (Vitrakoti 2016).

IV. CONCLUSION

Analysis showed non-significant for the number of heads per meter square and significant variation for all other traits. The analysis indicated that highly significant difference existed for most of the quantitative data which implies that there exists vast diversity among the breeding lines for different traits.

Plant height turned into considerable and undoubtedly correlated with tiller variety, incredibly significant and positively correlated with powerful tiller, flag leaf width while substantially and positively correlated with flag leaf length. Grains consistent with spike and grain yield in step with spike had been pretty widespread and sturdy undoubtedly associated with every different. Grain yield per hectare became drastically and undoubtedly correlated with days to adulthood, plant height and grain yield in line with spike whereas exceptionally giant and definitely correlated with flag leaf width. Xveola-38 performed better for yield attributing traits whereas B86019-1K-2K-0K3 showed better phenotypic characters and adaptability. These lines of barley have the possibilities and potentials for further study and research in Rampur, Chitwan. Early maturing lines like CHZ-NP-108-OY, B90k-024-1-1-2-0K, NB-1003-37/1034 and CO11#112-14 which have short crop period can be suggested for cultivation in drought prone areas as they can escape drought period. B86019-1K-2K-0K3 showed better adaptability and results for phenotypic characters which can contribute for the crop improvement and plant breeding program. Likewise, Xveola-38 performed best for the yield and yield attributing traits, so this variety could have the potentiality to be released for commercial cultivation in Rampur, Chitwan which can be justified after further study and research.

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AUTHORS DECLARATION

The authors declare that there is no any conflict of interest. Mr. Anup Adhikari designed the layout and work plan for this research while Pabitra Ale, Jigyasha Gautam, Babita Dhungana and Aakash Adhikari performed cultivation practices, recorded the data, performed statistical analysis of data, revised the first draft minutely and elaborated the final manuscript for publication. All the authors have read the manuscript and approve the final version.

REFERENCES

[1] Amgai, R. B., Pantha, S., Chhetri, T. B., & Mudwari, A. (2011). Nepalese barley: Variation in agro-morphology characteristics. *Proceedings of 28th National Winter Crops Workshop, Held On*, 9–10.

[2] Baniya, B. K. (1989). Present status of barley improvement in Nepal. *A Workshop/Training on Barley February*, *24*.

[3] Baniya, B. K., Dongol, D. M. S., & Riley, K. W. (1997). Characterization of Nepalese barley germplasm. *Barley and Wheat Newsletter*.

[4] Eshghi, R., & Akhundova, E. (2009). Genetic diversity of the monomeric prolamins and hordein in hulless barley genotypes and their relation with agronomical traits. *African Journal of Biotechnology*, *8*(9).

[5] Gupta, S. R., Upadhyay, M. P., & Shah, U. S. (2009). Agro-morphological variability study of barley (Hordeum vulgare L.) landraces in Jumla, Nepal. *Nepal Agriculture Research Journal*, *9*, 1–11.

[6] Joshi, B. K., Gorkhali, N. A., Pradhan, N., Ghimire, K. H., Gotame, T. P., KC, P., Mainali, R. P., Karkee, A., & Paneru, R. B. (2020). Agrobiodiversity and its Conservation in Nepal. *Journal of Nepal Agricultural Research Council*, *6*(March), 14–33. https://doi.org/10.3126/jnarc.v6i0.28111

[7] KARKOUR, L., FENNI, M., RAMLA, D., GAAD, D., & BENBELKACEM, A. (2019). Evaluation of agronomic performances of rainfed barley double-haploids (DHs) lines under semi-arid conditions. *Biodiversitas Journal of Biological Diversity*, *20*(5).

[8] Vitrakoti, D., Aryal, S., Rasaily, S., Ojha, B. R., Kharel, R., & Sapkota, M. (2016). Study on genotypic response and correlation analysis of the yield and yield attributing traits of different barley (Hordeum vulgare) genotypes. *International Journal of Applied Sciences and Biotechnology*, *4*(4), 529–536.

[9] Yadav, R. K., Gautam, S., Palikhey, E., Joshi, B. K., Ghimire, K. H., Gurung, R., Adhikari, A. R., Pudasaini, N., & Dhakal, R. (2018). Agro-morphological diversity of Nepalese naked barley landraces. *Agriculture & Food Security*, *7*(1), 1–12.

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Role of Trees in Farm Bunds – A Case study in Balaghat district, Madhya Pradesh, India

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Abstract**—** *Agroforestry systems are multifunctional systems that can provide a wide range of economic, sociocultural and environmental benefits. Agroforestry can be particularly important for smallholder farmers because it generates diverse products and services on a limited land area. The tree resource outside the forest (TOF) is a highly diverse and locally different natural renewable resource. In many regions they play a prominent role in securing rural livelihoods viz., provision of small timber, firewood, fodder, fruits, medicinal value, etc.The present study was conducted at Balaghat districct of Madhya Pradesh to study the types of tree species being maintained in farm bunds, potential benefits of trees, reason for maintaining trees in bunds, etc. The study reveled that, farmers' are maintaining around 25 different tree species for various outputs. Marginal farmers are maintaining trees mainly to meet out their daily needs and small and large farmers for future investment. In this paper, the reasons for opting bund planting, level of domestication and adoption and constraints facing by farmers are discussed in detail.*

Highlights:

1. *This article gives idea about trees are being maintained by farmers in Balaghat district of Madhya Pradesh.*
2. *Gives an idea about reasons for opting trees in farm bunds, level of domestication and adoption levels in different farming category.*
3. *Also, deals in details about constraints facing by farmers in TOF.*

Keywords**— *Trees Outside Forests, Domestication, Bund Planting, NTFPs.***

I. INTRODUCTION

Agroforestry involves raising trees in combination with other agricultural enterprises. providing fodder, fuel, wood, and other products, trees in agroforestry systems promote soil and water conservation, enhance soil fertility and act as windbreaks for nearby crops. Agroforestry is known to have the potential to mitigate the climate change effects through microclimate moderation, conservation of natural resources and creation of additional source of livelihood and income opportunities. Agroforestry is evolved over a period of time and intricately related to human life from generations to generations. The maintenance of trees either on field boundary or near habitat is traditional practices to fulfill day-by-day needs to secure livelihood and this practice scientifically known as agroforestry from last four to five decades.In recent past, we have witnessed that tree species are receiving more and rapid attention in India to achieve the national goal of 33% green cover, mostly in the form of Trees Outside Forests (TOF) category. TOF have potential to increase social, economic and environmental stability and overall, net farm income (Saravanan, 2017).

As of now about 25 Mha area is under Agroforestry land use in India and supports almost half of the demand of fuelwood, two third of small timber, 70-80% wood for plywood industry, 60% raw material for paper pulp and 9-

11% of green fodder requirement of livestock. Although, current average biomass productivity in India is less than 2tha^{-1}yr^{-1} but it can be enhanced to 10tha^{-1}yr^{-1}through careful selection of compatible tree crop combination not only to bridge the gap in demand and supply but also to make country surplus in plywood, paper pulp and small timber.Deep tree roots also help prevent soil erosion. Agroforestry systems can also be used as carbon sinks within an environment, and to an extent, help counter the effects of continued deforestation on the carbon cycle.

In India, agroforestry is practiced in various ways like tree on field boundary, block plantation; alley cropping, scattered trees in field, home gardens and homestead gardens etc. Among these, bund plantation is very common practice through-out the country.In such conditions, mostly multipurpose tree species is likely to be chosen to plant on field bunds to achieve the benefits like fruits, fibre, fuel wood, fertilizer, food and medicine. In systematic agroforestry, the industrial hub for plywood and pulp has developed Eucalyptus and Poplar based agroforestry system on 8-9 lakh ha area in Tamil Nadu, Andhra Pradesh, Maharashtra, Karnataka, Punjab, Haryana and western Uttar Pradesh. The income obtained from these system are two-three times higher than conventional cropping practice and this has forced farmer to spare fertile land for agroforestry. Poplar based Agroforestry is classical example of this system where in trees are grown alongside the crops/grasses to obtain diverse produce.Agroforestry not only provides environmental services, but also economic gains, as about 65% of the country's timber requirement is met from the trees grown outside forests (Dhillon *et al.*, 2018).

Demand and supply of industrial wood is elevated at higher pace after the outlawing the harvesting of green timber from the reserved as well as private lands of India through the implementation of National Forest Policy 1988. As per the FSI (2019) reports states that as being 21.67% of forest area contributes only 3 million cubic meter wood (approximately 6 %) and remaining 44 million cubic meter wood comes from agroforestry sector of the country. The demand for furniture, paper and small wood has been rising between 8-12% annually (Shrivastava, 2017).Planting and maintaining trees in farm bunds is a type of agroforestry system, in order to meet the demands of fuel wood, small timber, fodder and other tree products. These trees strengthen economic security of poor farmers in case of disasters and emergencies by selling trees for cash. The effect of trees grown at bunds of cultivated fields on grain yield of wheat, barley, paddy and potato were studied by many authors (Dhillon *et al.*, 1982, 1984; Grandstaffe *et al.*, 1986; Saxena *et al.*, 1990 and Sac-Lee *et al.*, 1992). Therefore promotion of agroforestry is becoming well proven solution in various parts of countries to cater the needs of farming community as well as environmental security.

The present study was conducted in Balaghat district of Madhya Pradesh to study the types of tree species being maintained in farm bunds, potential benefits of trees, reason for maintaining trees in bunds, etc. are discussed in detail.

II. MATERIALS AND METHOD

Study area: This study was conducted at Balaghat district of Madhya Pradesh falls under Chhattisgarh plain agroclimatic zone of Madhya Pradesh, with the population of 1.70 million people (Census, 2011). It is a very important cultural center and its geographical coordinates are parallels of East Longitude′00° to 81′00° and 80′30° to 22′30°latitude with an elevation of 288 m MSL. The normal annual rainfall of Balaghat district is 1294.5 mm. About 91.3% of the annual rainfall received during monsoon season i.e. June to September. The normal maximum temperature recorded during the month of May is 43 C° and minimum is 8 C° during the month of December. The district is generally covered with Black cotton soils, Sandy loam and lateritic soil.

Sampling procedure and Survey method: A questionnaire was prepared for collecting information from the study area of Balaghat district, Madhya Pradesh. Using this questionnaire relevant information was collected from 450 farmers, comprising each 150 of marginal, medium and large farmers' category which were randomly selected from the study area. Data were recorded from these selected farmers from May – June, 2021. In conformity with the set objectives of the study, a set of preliminary survey schedules has been designed for collection of data for the study. Thus, the final survey schedule has been prepared in a simple manner maintaining logical sequences and necessary adjustments.

Data analysis: The collected data was analysed by 'Garrett scoring Technique' (Garrett and Woodworth, 1969) and presented in the results and discussion part.

III. RESULTS AND DISCUSSION

1. Tree species planted and maintained in farm bunds

During the extensive survey it was noticed that, about 25 different types of tree species (Table-1) are being maintained in the farm bunds for eight main reasons*viz.*, fodder, small timber, fuel wood, fruits, timber, NTFP, medicine and green manure.Among the 25 species recorded in bund planting, nine tree species (36%) belongs to Fabaceae family followed by three tree species (12%) in Myrtaceae family. Among various uses, usage for fuel wood ranked first (60%) followed by small timber (50%) and least for medicinal use (12%) (Fig.1). Jharia *et al.*, (2013) also reported that, other than *Eucalyptus*, some other MPTs including *Terminalia arjuna*, *Terminalia tomentosa*, *Albizia procera*, *Mangifera indica*, *Butea monosperma*, *Zizyphus mauritiana*, *Azadirachtaindica* and *Gmelina arborea* are also plantedin the farm bunds. Farmers of Gujaratprefered and retains tree species of *Acacia nilotica, Acacia catechu, Dalbergia sissoo, Mangifera indica, Zizyphus mauritiana* and *Gmelina arborea* along with crops. In Bihar, *Dalbergia sissoo, Litchi chinensis* and mango are frequently grown on field, but for boundary plantation, *Sissoo* and *Wendlandia exserta* are most commonly used. Farmers of Sikkim, grow bamboo species (*Dendrocalamus strictus*, *Bambusa bambos*) all along the irrigation channels (Jhariya *et al.*, 2015). Pohjonen and Pukkala (1990) revealed that, *Eucalyptus globulus* trees are unpalatable to goats, sheep and cattle, thus they have a distinct advantage as boundary planting in Ethiopia.

2. Reasons for opting bund planting

The reasons were obtained from farmers and ranked based on 'Garrett scoring Technique' and presented in Table-2 and Fig.2.During survey, reasons were given to farmers and asked them to rank for opting bund planting. The farmers were classified in to marginal, medium and large farmer category and noted the reasons for maintaining trees in farm bunds. Marginal farmers expressed that, meeting their daily needs from trees ranked 1st followed by higher income and future investment with the mean score of 59.84, 56.24 and 54.81 respectively. Least score of 47.13 was goven for the last rank with the reason for efficient land utilization. Medium farmers are expressed that, higher income is the prime reason for maintaining trees in farm bunds with the mean score of 60.42 followed by future investment and promotion of TOF with the mean score of 58.36 and 54.68 respectively. Least score was given to efficient land utilization with the mean score of 49.81. With reference to large farmers' category, future investment recorded 1st reason for adoption of bund planting with the mean score of 62.56 followed by higher income (59.61) and promotion of TOF (55.71). Meeting day to day needs reason registered least score of 45.47 in large farmers' category. Bargali *et al.*, (2004) reported that, small landholders preferred only bamboo and Eucalyptus for bund and boundary plantations to meet their household requirements. Medium and large farmers preferred a number of species (see table) as they are into commercial production and they have better resources in the state of Chhattisgarh.Depommier *et al.*, (2002) articulated that the needs and strategies of small farmers usually correspond to subsistence agriculture with low inputs and, interestingly, a high level of diversification, which includes tree products and services. The multipurpose use of species partly satisfies the basic needs of poor farmers. Saravanan (2021) revealed that, nonavailability of agricultural labour (ranked first in four agroclimatic zones) and higher returns from tree components (ranked first in two agroclimatic zones) registered higher mean score and ranked among other reasons for adoption of agroforestry systems in different agroclimatic regions of Tamil Nadu.

3. Level of domestication and extend of adoption of bund planting

The level of domestication and extend of adoption was analysed and presented in Table-3 with reference to three farming category. The access to quality planting material (15.1%) and technical know how on tree cultivation is low in marginal farmers (15.1% & 30.0%) category compared to medium and large farmers' category (42.9 & 80.2 and 52.9% & 74.7%). From this study, it is showing that, marginal farmers' are not in the position to give proper maintenance including protection measures compared to marginal and large farming category. This level of domestication and extend of adoption of bund planting mostly depends on farmers' choice and self interests. Moreover, socioeconoic factors also plays important role in this aspect. Medium and large farmers are easily access to quality planting material and other related services in tree cultivation compared to marginal farmers. Economic status also plays important role in access to elite planting material and technical know how.

4. Constraints faced by the farmers in adoption of bund planting

The constraints faced by the farming communities are analysed and presented in Table-4 and revelead that, most of the farmers are facing constraints in getting quality planting materials (82.70%) and this leads to poor growth and form of trees in later stage. Moreover, there is no certified central nurseries in this region for distribution of quality planting material to stakeholders especially to farmers. In the case of technology, non availability of tree cultivation techniques (88.44%) and poor forestry extension strategies (83.33%) plays major constraints in promotion of TOF in this region. Unlike in agriculture, there is no proper extension mechanasim for forestry in promotion of TOF and also there is no or very limited resources are available in tree cultivation techniques, which are acceable to farmers'. In agroforestry, marketing (89.11%) and value chain models are playing major role. Unlike in agriculture, in forestry/agroforestry, there is no market intellegience/updated information for farming communities. Moreover, middleman plays a major role (81.11%) in procurring the agroforestry products with low cost compared to market rates. Presence of wood based industries near by area also plays a vital role in procurring the agroforestry produces. Unlike in agriculture, tree growers are not considered for bank loan, incentives, crop insurance and other benefits. These are also creating major constraints among tree growers and reduce the interests in TOF.Taimoor Hassan Farooq *et al.,* (2017) reported that lack of technical assistance, proper awareness and water shortage were the major constraints faced by the farmers for planting trees on their farmlands.Monica Mbatha Masibo *et al.,* (2018) revealed that, education, capacity building and training is vital to enhancing the success of agroforestry programmes.The resultas are in tune with Ibrahim *et al.,* (2019) reported that, the major constraints to agroforestry practices were limited use of machineries (75%), poor access to credit (70%), fast growing nature of trees (68.3%) land tenure (65.8%) and marketing channels (62.5%).

IV. CONCLUSION

From the above study, it showed that, all the farming category are showing interests in maintaing trees in farm bunds for various end uses. Further, the level of domestication and extend of adoption varies according to farmers category and their economic condition. Invariabely, farmers are facing constraints in getting quality planting materials and tree cultivation technologies. Also, there is no regulated market mechanism and value chain for agroforestry products. Farmers will come forward to grow more trees especially in farm bunds, if these constraints will be addressed for the benefit of farming community.

REFERENCES

[1] Bargali, S.S, Singh, S.P, and Pandya, K.S. 2004. Effects of Acacia nilotica on gram crop in a traditional agroforestry system of Chhattisgarh Plains. **Int. J. Ecol. Environ. Sci**. 30(4):363-368.

[2] Census of India, 2011. Registrar General and Census Commissioner of India.

[3] Depommier, D, Laurent, J, Cassou, J, Demenois, J, Heurtaux, A, and Grard, P. 2002. Multipurpose trees and agroforestry practices for sustainable development and conservation of environment in South India: linear plantations and living fences in the dry lands of Karnataka and Tamil Nadu. In: Proceedings of National Seminar on Conservation of Eastern Ghats. Tirupati. p: 161-168.

[4] Dhillon, G. S., S. Singh, M. S. Dhillon and Atwal, A. s. 1982. Developing agrisilvicultural practices: studies on the shading effect of *Eucalyptus* on the yield of adjoining crops. **Indian Journal of Ecology**, 9: 228-236.

[5] Dhillon, M. S., Singh, S., Atwal, A.S. and Dhillon, G. S. 1984. Developing agrisilvicultural practices: effect of *Dalbergia sissoo* and *Acacia nilotica* on the yield of adjoining crops. **Indian Journal of Ecology**, 11: 249-253.

[6] Dhillon, R. S., Chavan, S. B., Bangarwa, K. S., Bharadwaj, K. K. Sushil Kumari and Chhavi Sirohi. 2018. *Eucalyptus* based agroforestry system under semi-arid condieion in North-western India: An economic analysis. **Indian Journal of Ecology**, Vol. 45 (3): 470-474.

[7] Forest Survey of India. 2019. State of Forest Report, 2019 (Ministry of Environment, Forests and Climate Change, N. Delhi), Dehradun, India.

[8] Garrett, H. E and Woodworth, R. S. 1969. Statistics in Psychology and Education. Bombay, Vakils, Feffer and Simons Pvt. Ltd.

[9] Grandstaf, S. W., Grandstaff, T. B, Rathaketta, P., Thomas, D.E. and Thomas, J.K. 1986. Trees in paddy fields in northeast Thailand. In Mortan G. G (ed.). Traditional Agriculture in South Eastasia, Westview Press, 358 p.

[10] Ibrahim, A. O., Adedeji, A. S. and Meduna, P. N. 2019. Constraints facing agroforestry practices among farmers in New Bussa, Nigeria. **Journal of Research in Forestry, Wildlife & Environment,** Vol. 11(3).

[11] Jhariya, M. K. Raj, A. Sahu, K. P. and Paikra, P. R. 2013. Neem- A Tree for Solving Global Problem. **Indian Journal of Applied Research**. **3**(10): 66-68.

[12] Jhariya, M. K., Bargali, S. S, and Abhishek Raj. 2015. Possibilities and perspectives of agroforestry in Chhattisgarh. Open access peer-reviewed chapter. doi: 10.5772/60841.

[13] Monica Mbatha Masibo, Joseph Hitimana, Ann Sitienei Yegon and Yuda Odongo Owino. 2018. Challenges Affecting the Adoption of Agroforestry Practices around Chepalungu Forest in Bomet County, Kenya. **Asian Journal of Advanced Research and Reports**, Vol. 2(1): 1-10.

[14] Pohjonen, V. and Pukkala, T. 1990. *Eucalyptus globulus* in Ethiopian Forestry. **Forest Ecology and Management**. **36**:19–31.

[15] Sae-Lee, S, Vityakon, P. and Prachaiyo, B. 1992. Effects of trees on paddy bund on soil fertility and rice growth in northeast Thailand. **Agroforestry Systems**, 18, 213-223.

[16] Saravanan, S and Nanita Berry. 2021. Agroforestry practices in Tamil Nadu, India – a boon for farmers for livelihood security. **Current Science**, Vol. 120 (4): 644-653.

[17] Saravanan, S. 2017. Cultivation of *Melia dubia* cav. – a fast growing native tree species and constraints faced by the farmers in western region of Tamil Nadu. **Indian Journal of Agroforestry**, 19 (2): 56-60.

[18] Saxena, A. K. Singh, P. K and Singh, B.P. 1990. Effect of mango trees on growth and yield of wheat. In Singh, R.K, Saxena, A.K. and Singh, I. S. (Eds.). Agroforestry: Present status and scope for future development in the farming system, N. D. Uni. of Agri. And Tech. Kumargani, Faizabad, India, pp. 276-287.

[19] Shrivastava, S. 2017. Forest Productivity-Lumber slumber. Down to

Earth.https://www.downtoearth.org.in/news/forestproductivity-lumber-slumber-57323.

[20] Taimoor Hassan Farooq, Muhammad Farrakh Nawaz, Muhammad Waqqas Khan, Matoor Mohsin Gilani, Supaporn Buajan, Junaid Iftikhar, Norela Tunon and Pengfei Wu. 2017. Potentials of agroforestry and constraints faced by the farmers in its adoption in District Nankana Sahib, Pakistan. **International Journal of Development and Sustainability**, Vol. 6 (8): 586-593.

Table-1. List of tree species planted and maintained in farm bund in Balaghat district of Madhya Pradesh

Tree species	Family	Main uses							
		Fodder	Small timber	Fuel wood	Green manure	Medicine	Timber	Fruits	NTFP
Acacia catechu	Fabaceae	*	§	ō	--	--	--	--	--
Aegle marmelos	Rutaceae	--	--	--	--	⁂	--	ʆ	--
Albizia lebbeck	Fabaceae	--	§	ō	Ώ	--	◐	--	--
Albizia procera	Fabaceae	--	§	ō	Ώ	--	--	--	--
Annona squamosa	Annonaceae	--	--	--	--	--	--	ʆ	--
Artocarpus heterophyllus	Moraceae	*	--	--	--	--	◐	ʆ	--
Azadirachta indica	Meliaceae	--	§	ō	Ώ	⁂	◐	--	¥
Butea monosperma	Fabaceae	--	§	ō	--	--	--	--	¥
Cassia siamea	Fabaceae	--	§	ō	Ώ	--	--	--	--
Dalbergia sissoo	Fabaceae	*	§	ō	Ώ	--	◐	--	--
Dendroclamus strictus	Poaceae	--	§	ō	--	--	--	--	¥
Eucalyptusterticornis	Myrtaceae	--	§	ō	--	--	--	--	--
Gmelina arborea	Lamiaceae	*	§	--	Ώ	--	◐	--	--
Leucaena leucocephala	Fabaceae	*	--	ō	Ώ	--	--	--	--
Mangifera indica	Anacardiaceae	--	§	ō	--	--	--	ʆ	--
Melia azedarach	Meliaceae	--	--	ō	Ώ	--	--	--	--
Millettia pinnata	Fabaceae	--	--	--	Ώ	--	--	--	¥
Moringa oleifera	Moringaceae	--	--	--	--	--	--	ʆ	--
Phyllanthus emblica	Phyllantheaceae	--	--	--	--	--	--	ʆ	¥
Psidium guajava	Myrtaceae	--	--	--	--	--	--	--	¥
Syzygium cumini	Myrtaceae	*	--	ō	Ώ	--	--	ʆ	¥
Tectona grandis	Lamiaceae	--	--	--	--	--	◐	--	--
Terminalia arjuna	Combretaceae	--	--	ō	--	⁂	--	--	¥
Vachellia nilotica	Fabaceae	*	§	ō	--	--	--	--	--
Ziziphus mauritiana	Rhamnaceae	*	--	--	--	--	--	ʆ	¥

* Fodder; § small timber; ō Fuel wood; Ώ Green manure; ⁂ Medicine; ◐ Timber; ʆ Fruit; ¥ NTFP

Table-2: Reason for opting bund planting in Balaghat district, Madhya Pradesh

Reasons	Marginal farmers		Medium farmers		Large farmers	
	Mean score	Rank	Mean score	Rank	Mean score	Rank
Meeting day to day needs	59.84 (112)* 74.6%	I	52.83 (80) 53.3%	V	45.47 (68) 45.3%	VI
Higher income	56.24 (104) 69.3%	II	60.42 (119) 79.3%	I	59.61 (105) 70.0%	II
Future investment	54.81 (96) 64.0%	III	58.36 (112)	II	62.56 (119)	I

			74.7%		79.3%	
Less risk and inputs	52.56 (84) 56.0%	IV	57.45 (88) 58.7%	IV	48.25 (76) 50.7%	V
Promotion of TOF	49.63 (78) 52.0%	V	54.68 (97) 64.7%	III	55.71 (97) 64.7%	III
Efficient land utilization	47.13 (69) 46.0%	VI	49.81 (72) 48%	VI	52.37 (85) 56.7%	IV

* The value within the brackets are frequency (n=150), followed by percentage to the frequency.

Table-3. Level of domestication and extend of adoption of bund planting in Balaghat district, Madhya Pradesh

Reasons	Marginal farmers		Medium farmers		Large farmers	
	Frequency*	Percentage	Frequency	Percentage	Frequency	Percentage
Knowledge on tree cultivation	135	30.0	238	52.9	336	74.7
Quality planting material	68	15.1	193	42.9	361	80.2
Site selection	52	11.6	247	54.9	286	63.6
Type of planting material	74	16.4	200	44.4	301	66.9
Time of planting	361	80.2	395	87.8	406	90.2
Proper spacing	253	56.2	382	84.9	339	75.3
Digging pits in correct size	148	32.9	273	60.7	345	76.7
Soil mixture	82	18.2	225	50.0	224	49.8
Mulching	41	09.1	156	34.7	208	46.2
Irrigation	38	08.4	127	28.2	327	72.7
Application of fertilizer	35	07.8	159	35.3	184	40.9
Plant protection measures	167	37.1	259	57.6	355	78.9

* Frequency (n = 450)

Table-4. Constraints faced by the farmers in adoption of bund planting in Balaghat district of Madhya Pradesh

Constraints	Frequency (m = 450)	Percentage
Inputs		
Quality planting material	372	82.70
Cost of inputs like fertilizers	238	52.90
Cost of plant protection measures	168	37.30
Technology		
Non availability of tree cultivation techniques	398	88.44
Poor forestry extension strategies	375	83.33
Marketing		
Non availability of marketing intelligence	401	89.11
Middle man role	365	81.11
Low cost for forestry products at farm gate	336	74.67
Loan/Credit/Tree insurance facilities		
Non availability of tree loan	247	54.89
Non availability of incentives from government	412	91.56
Non availability of tree insurance or lesser information	351	78.00
Others		
Establishment and linking of tree growers association	365	81.11
Creation of value chain models	369	82.00

Easy in timber transport rules	400	88.89
Less support from SFDs towards TOF	383	85.11
Absence of certified tree nurseries for QPM	348	77.33

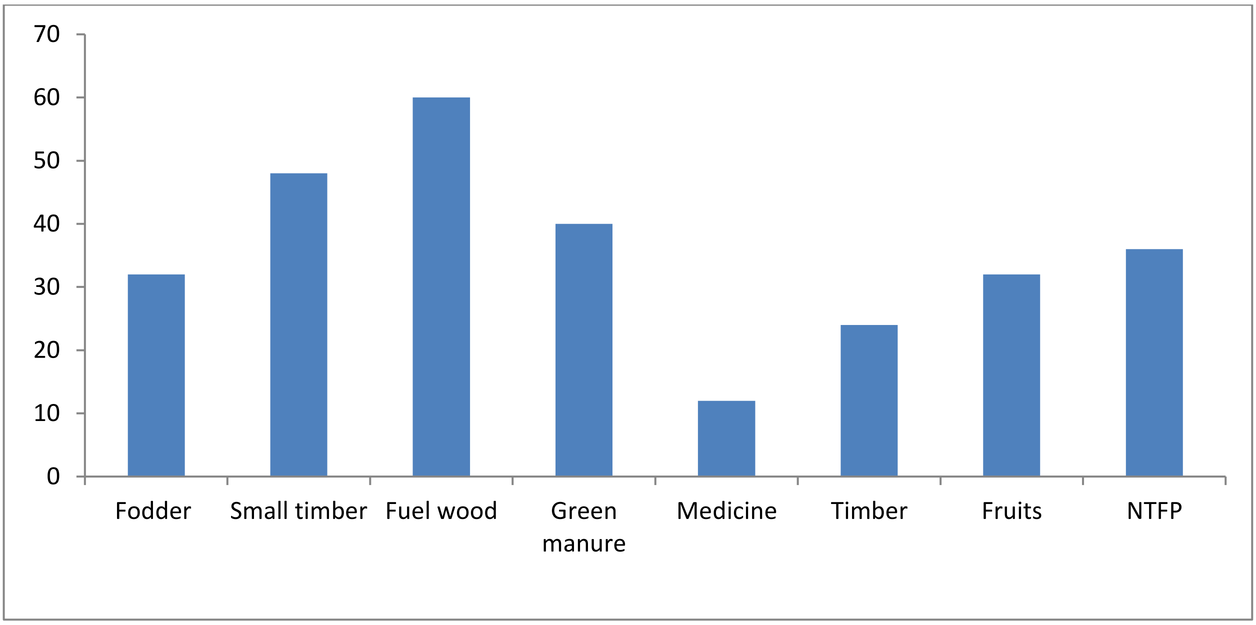

Fig-1: Usage of trees in farm bunds in Balaghat district, Madhya Pradesh

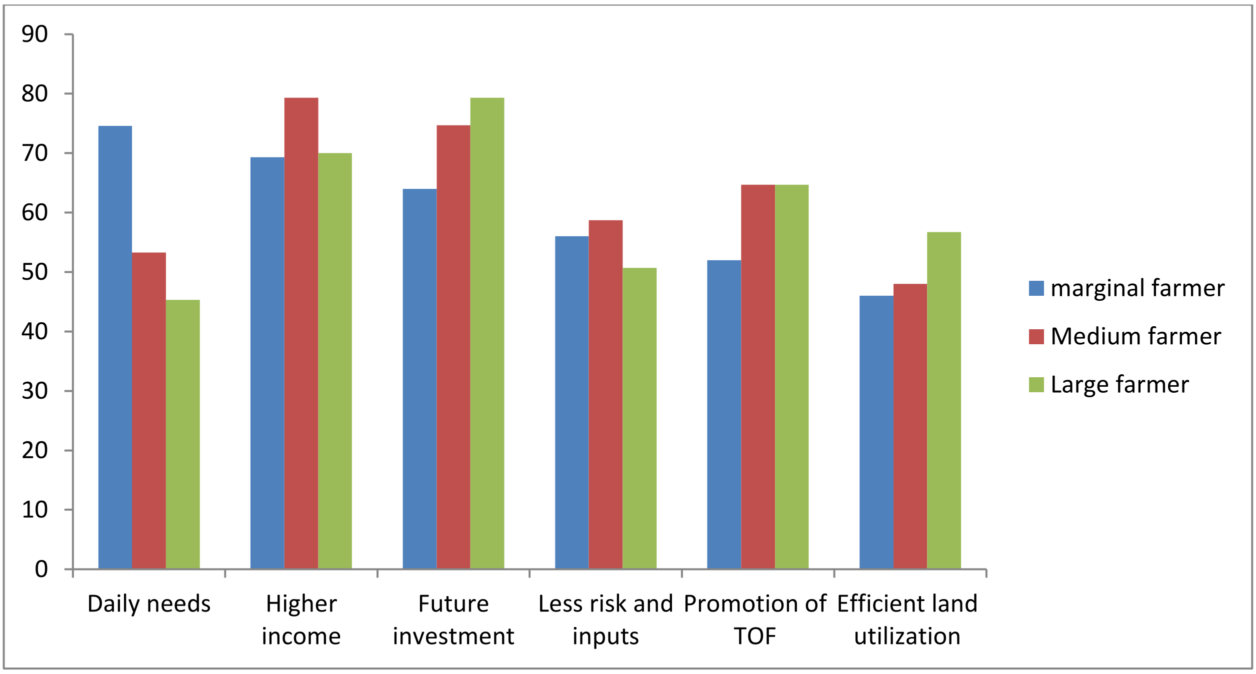

Fig-2: Reason for opting bund planting in Balaghat district, Madhya Pradesh

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Effect of *Alchornea cordifolia, Tithonia diversifolia* and *Mezoneuron benthamianum* treatment time on agromorphological parameters of tomato in Daloa (Côte d'Ivoire)

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***Abstract*— *The cultivation of tomato (Solanum lycopersicon L.) is confronted with many production problems related to the strong parasite pressure and especially to the fertility of soils. Thus, the effectiveness of powdery extracts of three plants (Alchornea cordifolia, Tithonia diversifolia and Mezoneuron benthamianum) was evaluated on the tomato crop and their ability to improve agromrphological parameters. Quantities of 5 g and 15 g of these extracts were used to treat the cultivation soil one month, one week and the day of transplanting of tomato plants. The results showed that A. cordifolia, Tithonia diversifolia and Mezoneuron benthamianum significantly improved the growth parameters and development of the plants at the amounts of 5 g and 15 g, on. The efficacy of the extracts varied depending on the plant used, the concentrations and the period of soil treatment. These plant extracts have biostimulatory properties and can be used as renewable resources for healthy and sustainable agriculture.*
***Keywords*— Alchornea cordifolia, Tithonia diversifolia, Mezoneuron benthamianum, extracts, symptoms.**

I. INTRODUCTION

Tomato (*Solanum lycopersicon*) is one of the most important vegetable crops in the world (Naika et al., 2005). According to the Food and Agriculture Organization of the United Nations (FAO), it is the second most important vegetable crop after potatoes. It is cultivated in more than 170 countries of the world in various climates, including relatively cold regions thanks to the development of crops under cover (FAO, 2007). Global tomato production has grown significantly since 1978, when it increased from 48 million tons to over 180 million tons in 2017 (FAOSTAT, 2018). Tomato, is grown for its fleshy, highly sought after fruit, rich in phenolic antioxidants and then mineral elements such as lycopene, carotenoids, vitamins A, C and E (Daniel et al., 2012; Ignace et al., 2015). Tomato is one of the most important fruit vegetables in the human diet as it contributes to a healthy and balanced human diet that can be consumed fresh or processed [6] (Willcox et al., 2003).

En Côte Ivoire, fresh tomato production was estimated by the FAO in 2014 at 32,364 tons per year and increased to 44078 tons in 2018.Tomatoes are one of the main revenue crops in vegetable farming because many actors (producers and traders) make a living from tomato production and marketing (Coulibaly et al., 2019). However, environmental degradation is occurring due to the use of chemicals for both soil fertilization and plant treatments to increase tomato production.

In an effort to significantly reduce the use of chemicals, several studies have been conducted on biological control with satisfactory results. A study focused on composting showed that it improves crop yields compared to chemical fertilizers (Outéndé, 2016). In addition, the cost of compost is low compared to the purchase of chemical fertilizers. Another study conducted on natural plant extracts showed that plants such as Moringa oleifera and Azadirachta indica can be used respectively in the control of aphids and caterpillars as well as for soil fertilization and (CORAF, 2010). It is in this context that our work aims to improve the cultivation of tomato through the use of plant extracts.

II. MATERIAL AND METHODS

Collection and treatment of plant material

The plant material consisted of leaves of Alchornea cordifolia, Tithonia diversifolia, and Mezoneuron benthamianum. These leaves were collected in the locality of Daloa during the month of May 2020 and were used for the treatment of the tomato crop soil.

Fresh harvested leaves were shade dried at room temperature in the laboratory (25 to 27°c±2) for three weeks, as exposure to sunlight often reduces their efficacy (Anjarwalla et al., 2016). Furthermore, the dried leaves were powdered separately using a grinding machine (RETSCH type: SK100/C Gusseinsen) to obtain the powdered ones**.**

Setting up the experimental plot

For the realization of the experiment, a plot of 68 m2 was weeded with a machete and then weeded with a daba. Then 6 ridges of 3 m by 3.5 m were made. On each ridge 7 lines separated by 40 cm were made and on each line 6 sowing points separated by 50 cm were also made. The experimental set-up consisted of two completely randomized blocks of 6 ridges. Each ridge constituted a treatment and each treatment consisted of 7 lines. Each treatment was repeated twice.

Treatments

Quantities of 5 and 15 g of *Alchornea cordifolia, Tithonia diversifolia,* and *Mezoneuron benthamianum* powders were weighed for the treatment of the crop soil at the level of the pits. These treatments were performed as follows:

- Treatment 1: cultivation soil treated with the powdery extracts on the day of transplanting
- Treatment 2: cultivation soil treated with powdery extracts one week before transplanting
- Treatment 3: cultivation soil treated with powdery extracts one month before transplanting.

Transplanting of plants

Tomato plants aged 21 days were transplanted at the level of the sowing points made with the different treatments. Watering was done twice a day for eight weeks and once a day until harvest. Regular weeding was done to maintain the plot. The staking of the plants was carried out four weeks after transplanting.

Data collection

Plant survival rate, growth and yield parameters were evaluated. Plant survival rate was recorded at three days, seven days and two weeks after transplanting. The survival rate was calculated according to formula (1).

$$\mathbf{Ts}= \frac{\text{nps}}{\text{np}} \times 100 \quad (1)$$

Ts: survival rate, nps: number of surviving plants, np: total number of plants

Growth parameters such as number of leaves per plant, plant height (cm), leaf area (cm^2) and number of branches were recorded from three weeks (21 days) after transplanting and the operation was repeated every two weeks until fruit ripening. The mean values were then calculated according to the formula (2) :

$$\mathbf{y_m} = \Sigma x_i / x_p \quad (2)$$

ym: average observed parameter; xi: observed parameter; xp: total number of plants observed.

Yield parameters were recorded from four weeks for the number of flowers and five weeks for the number of fruits.

Statistical Analysis

Data were subjected to a multi-factor analysis of variance (ANOVA) using STATISTCA 7.1 software. ANOVA was used to evaluate the effect of the extracts of the three plants on the agro-morphological parameters and the incidence and severity of diseases. When a significant difference was observed (P<0.05) between the different factors, multiple comparisons were performed using the LSD Fisher test at the 5% threshold.

III. RESULTS

Effect of *A. cordifolia, T. diversifolia* and *M. benthamianum* extracts on tomato plant survival rates

The survival rates of tomato plants ranged from 80.50 to 100 %. These rates were higher for the *A. cordifolia*, (94.44 to 100 %) and *T. diversifolia* (91.67 to 97.17 %) treatments one week and one month before transplanting. On the other hand, for *M. benthamianum*, it is rather the treatments on the day of transplanting and one week before transplanting at the quantity 5 g that gives the best survival rates. However, statistical analysis showed that there was no significant difference (P>0.05) between the survival rates with the different treatments of the extracts of the three plants (Table 1

Table 1 : Survival rates of tomato plants according to treatments, concentrations and extracts of A. cordifolia, T. diversifolia and M. benthamianum

Traitements	Quantity	*A. cordifolia*	*T. diversifolia*	*M. benthamianum*
	C0	93,28	93,28	93,28
1	C1	80,50	94,33	97,17
	C2	88,67	100	100
2	C1	97,17	91,67	100
	C2	100	97,17	88,67
3	C1	94,33	97,17	88,83
	C2	100	97,17	80,50
P		0,11	0,64	0,25

P: Probability; 1, 2, 3: soil treated on the day of transplanting, one week and one month before transplanting CO: Control; C1: 5 g dose; C2: 15 g dose.

Effects of *A. cordifolia, T. diversifolia* and *M. benthamianum* extracts on agromorphological parameters of tomato

The effectiveness of the plant extracts used varied according to the plant species and agromorphological parameters studied. Thus, statistical analysis showed a significant difference (P< 0.05) in the span, leaf area and number of flowers. However, plant size, number of leaves, crown circumference, number of branches and number of fruits were not influenced by the type of extract (P > 0.05) (Table 2). Also, *Alchornea* obtained the highest values in some parameters.

Table 2: Agromorphological parameters of tomato according to plant extracts

Plantes	TM	NFE	EM	SFM	DM	NRM	NFL	NFR
Achornea	25.70±1.10	21.60±1.08	51.76±1.87^{a}	590.37±30.04^{a}	5.60±0.19	4.75±0.31	9.90±0.64^{a}	6.513±0.54
Tithonia	24.72±1.11	20.10±1.02	48.60±1.79^{b}	527.72±28.23^{b}	5.37±0.17	4.16±0.26	9.03±0,59^{ab}	5.83±0.55
Mezoneuron	25.30±1.12	19.57±1.05	49.12±1.83b	544.75±29.50^{ab}	5.32±0.18	4.18±0.26	8.21±0.56^{b}	5.65±0.50
Témoin	23.89±3.24	19.19±2.5	47.20±5.14c	509.70±76.45c	5.15±0.46	4.12±0.61	8.88±1.43b	5.42±1.28
P	0.49	0.12	0.03	0.02	0.13	0.11	0.04	0.28

P: probability ; Values with the same letters in the same column are statistically equal. TM: mean plant size; NFE: mean number of leaves; EM: mean plant span; SFM: mean leaf area of plant; CM: mean crown circumference; NRM: mean number of branches; NFL: number of flowers; NFR:

Effects of *A. cordifolia* extracts on agromorphological parameters of tomato

Agromorphological parameters of tomato plants treated with *A. cordifolia* varied with concentration and soil treatment period. Thus, the concentration 15 g (C2) of the soil treated one week before transplanting (treatment 2) recorded the highest values for the agromorphological parameters, except for the number of branches, whose highest value was obtained at the concentration 5 g (C1) of the soil treated one month before transplanting (treatment 3). The smallest values were obtained at the concentration 15 g (C2) of the treated soil on the day of transplanting (treatment 1). Statistical analysis showed a highly significant effect (P<0.01) of the treatments performed on the different parameters studied (Table 3).

Effects of *T. diversifolia* extracts on agromorphological parameters of tomato

The effect of *T. diversifolia* also varied according to concentrations and treatments on agromorphological parameters. Statistical analysis showed a highly significant difference (P<0.01) among the studied parameters. The concentration 5 g (C1) of the soil treated one month before transplanting (treatment 3) recorded the highest values for the different parameters, except for the number of flowers, neck circumference and number of fruits, for which the highest values were obtained at the concentration 15 g (C2) of the same treatment (Table 4).

Table 3 : Agromorphological parameters of tomato plants treated with Alchornea cordifolia powder extracts according to treatments and concentrations

T	QT	TM	NFE	EM	SFM	DM	NRM	NFL	NFR
	C0	23.89±3.24ac	19.19±2.52ac	47.20±5.14ab	509.70±76.45c	5.15±0.46c	4.12±0.61ac	8.88±1.43bc	5.42±1.28ab
1	C1	25.45±3.48^{cd}	21.28±3.23^{ab}	55.47±6.11^{b}	643.0695.44^{bd}	5.37±0.54^{c}	4.46±0.77^{ac}	8.03±1.34^{ed}	6.34±1.52^{ab}
	C2	20.25±3.09^{d}	18.59±2.54^{ac}	45.03±5.88^{ac}	480.00±90.80^{ef}	4.44±0.45^{bc}	3.21±0.59^{ac}	4.91±1.13^{j}	3.19±0.99^{ac}
2	C1	26.42±3.76^{bc}	21.97±3.37^{ab}	53.56±5 .50^{bc}	572.09±81.31^{cd}	5.34±0.48c	3.83±0.63^{ac}	9.03±1.52^{bc}	7.09±1.66^{b}
	C2	30.58±3.59^{a}	32.44±4.35^{a}	65.56±6.07^{a}	903.47±115.93^{a}	7.26±0.66^{a}	5.92±0.78^{b}	13.92±2.00^{a}	12.97±2.28^{a}
3	C1	27.46±2.85^{ab}	24.72±4.26^{b}	52.53±5.95^{c}	574.69±81.26^{cd}	6.30±0.56b	7.75±1.62^{a}	14.78±3.34a	7.28±1.95^{b}
	C2	29.46±3.18^{b}	18.47±2.31^{ac}	52.06±4.55^{c}	610.62±81.76^{ab}	6.25±0.44b	5.21±0.92^{ab}	12.00±1.92c	5.50±1.19^{ab}
P		0 .001	0.001	0.001	0.001	0.001	0.001	0.001	0.001

P: Probability; Values with the same letters in the same column are statistically equal. TM: average size; NFE: average number of leaves; EM: average span; SFM: average leaf area; DM: average diameter at the crown; NRM: average number of branches; NFL: number of flowers; NFR: number of fruits. 1, 2, 3: soil treated on the day of transplanting, one week and one month before transplanting; CO: Control; C1: 5 g dose; C2: 15 g dose; QT: Quantity, T= Traitement

Table 4 : Agromorphological parameters of tomato plants treated with Tithonia diversifolia powder extracts according to treatments and concentrations

T	QT	TM	NFEM	EM	SFM	DM	NRM	NFL	NFR
	C0	23.89±3.24ac	19.19±2.52ac	47.20±5.14ab	509.70±76.45c	5.15±0.46c	4.12±0.61ac	8.88±1.43bc	5.42±1.28ab
	C1	20.42±3.06c	20.31±3.17ac	46.28±5.65bc	480.62±74.03bc	5.00±0.52ab	3.62±0.63ab	7.59±1.44ac	4.44±1.20ac
1	C2	20.29±3.51c	22.78±3.55b	54.81±6.34b	587.34±91.37ab	5.57±0.58ab	5.08±0.69b	9.50±1.50ab	5.59±1.34ab
	C1	27.29±3.02b	15.19±2.12c	42.84±4.23ac	414.90±73.02bc	4.81±0.44c	2.50±0.63c	4.97±1.08c	3.75±1.18ec
2	C2	27.42±3.27b	17.69±2.61c	45.87±4 .94bc	477.12±72.73bc	5.17±0.43ab	2.67±0.61j	5.47±1.03bc	4.22±1.29ac
	C1	28.67±4.15a	22.62±3.33b	55.75±6.07a	679.81±114.62a	5.97±0.53b	5.79±1.06a	14.59±2.76a	7.69±1.59b
3	C2	26.71±3.14b	25.37±4.16a	50.28±4.87ab	580.62±83.90ab	6.35±0.65a	5.08±0.92b	12.75±2.15b	10.50±3.15a
	P	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0 .001

P: Probability; Values with the same letters in the same column are statistically equal. TM: average size; NFE: average number of leaves; EM: average span; SFM: average leaf area; DM: average diameter at the crown; NRM: average number of branches; NFL: number of flowers; NFR: number of fruits. 1, 2, 3: soil treated on the day of transplanting, one week and one month before transplanting; CO: Control; C1: 5 g dose; C2: 15 g dose; QT: Quantity, T= Traitement

Effects of *M. benthamianum* extracts on agromorphological parameters of tomato

As for *M. benthamianum*, statistical analysis also showed a highly significant difference (P<0.01) between agromorphological parameters (Table 5). Thus, the soil treated one week before transplanting (treatment 2) recorded the highest values of the agromorphological parameters at concentration 5 g (C1), except for the number of flowers whose highest value was obtained at concentration C2 of the soil treated one month after transplanting (treatment 3). The smallest values were obtained at concentrations C1 and C2 of the treated soil on the day of transplanting (treatment 1).

Table 5 : Agromorphological parameters of tomato plants treated with Mezoneuron benthamianum concentrations powder extracts according to treatments and concentrations

T	QT	TM	NFE	EM	SFM	DM	NRM	NFL	NFR
	C0	23.89±3.24ab	19.19±2.52bc	47.20±5.14bc	509.70±7.,45e	5.15±0.46b	4.12±0.61ab	8.88±1.43bc	5.42±1.28ab
	C1	22.00±3.32bc	15.03±2.38ac	48.65±5.76bc	481.34±77.56c	5.14±0.54b	2.62±0.46ac	4.72±1.15bc	2.19±0.81c
1	C2	19.81±3.71c	16.82±4.32ac	37.61±5.44c	325.36±61.53bc	3.85±0.56c	2.28±0.90c	3.21±1.22c	3.14±1.56ac
	C1	32.71±3.28a	28.75±4.21a	65.09±6.13a	846.50±11.42a	6.60±0.62a	6.62±0.86a	10.94±2.05b	11.34±2.22a
2	C2	24.08±3.41ab	20.37±3.88ab	44.78±6.09bc	528.78±108.20ab	4.65±0.59ac	3.75±0.86ac	5.16±1.27bc	4.94±1.63ac
	C1	27.87±3.30b	16.66±1.86ac	52.34±4.81b	585.78±74.68ab	5.73±0.42b	4.67±0.58ab	11.00±1.83b	5.92±1.09ab
3	C2	28.87±3.24b	21.25±2.69b	50.56±4.31ab	578.50±77.65ab	6.27±0.52a	5,04±0,83b	11.81±1.87ab	6.72±1.27b
P		0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001

P: Probability; Values with the same letters in the same column are statistically equal. TM: average size; NFE: average number of leaves; EM: average span; SFM: average leaf area; DM: average diameter at the crown; NRM: average number of branches; NFL: number of flowers; NFR: number of fruits. 1, 2, 3: soil treated on the day of transplanting, one week and one month before transplanting; CO: Control; C1: 5 g dose; C2: 15 g dose; QT: Quantity , T= Traitement

IV. DISSCUSSION

Tomato is a very demanding crop in terms of water but also in terms of mineral elements, which makes the periodic replenishment of the soil reserves in these elements essential to maintain a good productivity. The powdery extracts of *T. diversifolia, A. cordifolia* and *M. benthamianum* were used to improve the fertility parameters of tomato growing soil.

The results show that the survival or recovery rates of the seedlings did not vary from one treatment to another, but the values remained high (80 to 100%). This result shows that the treatments performed influenced the recovery of the seedlings or that the vigor of the seedlings used favored their recovery. Indeed, the work of Ojetayo et al. (2011) and Musas (2012) showed that the recovery rates of vegetable plants from the nursery does not depend on the mineral elements contained in the soil but rather on the vigor of the plants. Also the work of Soro et al. 2008 showed that the age of transplanting of nursery tomato plants influences the mortality rate of the plants. These authors showed that plants aged 21 to 25 days are more resistant to attacks by *Pythium* sp. responsible for the death of young plants. Treatments performed on tomato plants showed that the powdery extracts of the three plants applied four weeks before transplanting of the plants was more effective on morphological and production parameters of tomato. This could be explained by the synchronization of nutrient release from the extracts during their decomposition and assimilation by the plant. Indeed, Cobo et al. (2002) showed that the rate of decomposition of organic matter and the increase in yields was closely related to the timing between the release of nutrients and their assimilation by the plant. The various extracts incorporated into the soil thus appear to have a suitable decomposition rate that allowed the plant to assimilate a large proportion of the nutrients released during the decomposition of the organic matter. Also, an increase in parameters such as, number of flowers, spread and leaf area was recorded on the plants whose soils were treated with Alchornea cordifolia. This would result from the importance and the quantity of nutrients such as phosphorus, nitrogen, potassium contained in this extract which contribute to the nutrition of the plant. Indeed, Alchornea cordifolia is a plant that is found in Ivorian fallow soils and contributes to increase the organic matter of the soil. The effectiveness of the extracts varied according to the plants and the concentrations. Thus, *A. cordifolia* was more effective at the 5g concentration (C2), while *T. diversifolia* and *M. benthamianum* were more effective at the 15g concentration (C1). This seems to be due to the fact that the plant extracts would have different effects on the growth parameters and development of the plants. This is in agreement with the results of NARC work on Garcinia kola in 2012 which found a variation in growth parameters depending on the substrate. The improvement of the growth parameters suggest that these extracts have inhibitory properties of soil pathogens. The anti-parasitic effects of plant extracts have been reported by different authors. Thus Kankam & Sowley (2006), demonstrated that the amendment of infested chilli plants by extracts of A. indica leaves allows a significant decrease of nematode populations. These results concur with those of Asare-Bediako et al. (2014) who highlighted the ability of extracts of *A. indica*, *C. papaya*, *Allium* sp., *Capsicum* sp. (Solanaceae), Anacardium sp. (Anacardiaceae) to minimize the severity of whitefly virosis and increase the yield of treated plots. This is explained by the fact that the extracts of these plants contain chemical substances such as terpenoids, steroidal heterosides, flavonoids, tannins, saponins, carbohydrates that act on the pest population and thus reduce their infections.

V. CONCLUSION

The results of this study showed that the powdery extracts of *A. cordifolia*, *T. diversifolia* and *M. benthamianum* had satisfactory effects on the improvement of growth and development parameters of tomato plants. However, only the Alchornea treatments had more significant effects on the studied parameters. For the different concentrations of plant extracts studied, C1 (5 g) gave the best results. It is therefore the optimal dose for treatment efficiency. The results also showed that the treatment of the soil one week before transplanting gave the best agromorphological parameters for *A. cordifolia* and *M. benthamianum* contrary to T. *diversifolia* which records the highest parameters when the treatments are done one month before transplanting. The work carried out showed the capacity of the extracts of these plants to be used to boost quantitatively and qualitatively the production of tomato

REFERENCES

[1] Anjarwalla P., Belmain S., Sola P., Jamnadass R. et Stevenson P. (2016). Optimisation des plantes pesticides : technologie, Innovation, sensibilisation réseaux. *Guide des plantes pesticides*, Kenya, 1 (4) : 63, 4-15.

[2] Asare-Bediako E., Addo-Quaye A. and Bi-Kusi. (2014). Comparative Efficacy of Phytopesticides in the Management of Podagrica spp and Mosaic Disease on Okra (*Abelmoschus esculentus* L.). American Journal of Experimental Agriculture 4(8): 879-889.

[3] Coulibaly N., Fondio L., N'gbesso M. et Doumbia B. (2019). Evaluation des performances agronomiques de quinze nouvelles lignées de tomate en station au centre de la Côte d'Ivoire. Int. J. Biol. Chem. Sci. 13(3): 1565-1581.

[4] Cobo J.G., Barrios E., Kaas D. and Thomas R.J. (2002). Nitrogen mineralization and crop uptake from surface-applied leaves of green manure species on a tropical volcanic-ash soil, biology and fertility of soils, 36: 87- 92.

[5] CORAF. (2010). Cultures maraîchères des extraits végétaux à la place des insecticides de synthèse. Producteurs et Utilisateurs au Centre de la Recherche Agricole ,16p

[6] Daniel C., Alphonse A., Jacques B., Romaric E., Ulrich G. etElvis J. (2012). Inventaire préliminaire de l'entomofaune des champs de tomates (*Lycopersicon esculentum* Mill) dans la Commune de Djakotomey au Bénin. Int. J. Biological. Chemical. Science, 6(4) : 1798-1804.

[7] FAO. 2007. FAO statistic. Côte Ivoire, www.faostat.fao.org/site/612/default.aspx#ancor (consulté le 11/ 11/2020).

[8] FAOstat. 2018. Record historique de la production mondiale de tomate. Côte Ivoire www.hortitecnews.com (consulté 10/05/2020).

[9] Ignace S., Moumouni K., Constantin D., Lamousssa P., Valérie B., Adama H., Charlemagne G., Eloi P. et Roger H. (2015). Etude de l'influence des modes de transformation sur les teneurs en lycopène de quatre variétés de tomates de la région du nord du Burkina Faso. Int. J. Biological. Chemical. Science, 9(1) : 362-370.

[10] Kankam F. and Sowley E. (2016). Evaluation of neem (*Azadirachta indica* L.) products for the control of root-knot nematode of chilli pepper (*Capsicum annum* L.). Arch. Phytopathol. Plant Protection, 49(5-6) : 111-119.

[11] Maisara M. (2015). Sources naturelles de la résistance contre les nématodes à galles *Meloidogyne javanica* chez la plante modèle *Arabidopsis thaliana*. Thèse de Doctorat : Biologie. Paris-Sud : Université Paris-Sud, 114p.

[12] Musas N.N., (2012). Valorisation agronomique des bio déchets et gestion de la fertilité des sols en agriculture urbaine et périurbaine : Effets des doses croissantes des engrais minéraux, des fèces humaines et de leur combinaison sur la production de l'oignon (*Allium cepa*) et de l'épinard (*Spinacia oleracea*). Mémoire de fin d'études, faculté des sciences agronomiques, Unilu, 43 p.

[13] Soro S., Doumbouya M., Koné D. et Kouadio Y.J., Potentiel infectieux des sols de cultures de tomate (*Lycopersicon esculentum* Mill.) sous abri et incidence de l'âge de repiquage sur la vigueur des plants vis-à-vis de *Pythium* sp. à Songon Dabou en Côte d'Ivoire. *Tropicultura*, 26(3) (2008) 173-178. French.

[14] Naika S., Jeude J. V., Goffau M., Hilmi M., Van dam B. et Florijn A. 2005. La culture de la tomate : production, transformation et commercialisation, Agrodo, 17, 9 p.

[15] Ojetayo A.E., Olaniyi J.O., Akanbi W.B. and Olabiyi T.I. (2011). Effect of fertilizer types on nutritional of two cabbage varieties before and after storage. *Journal of Applied Biosciences*, 48 3322 – 3330.

[16] Outéndé. T. 2016. Evaluation des caractéristiques chimiques et agronomiques de cinq composts de déchets et étude de leurs effets sur les propriétés chimiques du sol, la physiologie et le rendement du maïs (*Zea mays* L. Var. Ikenne) et de la tomate (*Lycopersicum esculentum* L. Var. Tropimech) sous deux régimes hydriques au Togo. Mémoire de thèse en Biologie Végétale Appliquée, Université de Lome en Cotutelle Avec L'université De Limoges, 195 p.

[17] Willcox J., Catignani G. and Lazarus S. 2003. Tomatoes and cardiovascular health. Critical Review in food Science and Nutrition, 43(1): 1-18.

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Quality Characteristics of Chicken Burger Processed from Broiler Chicken Fed on Different Levels of Quinoa Seeds

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***Abstract*— *The study was carried out to evaluate the effect of feeding broiler chickens on different levels of quinoa seeds on the quality characteristics of chicken burger during frozen storage at -20°C for 90 days. A total of 480 one-day-old chicks of (Ross 308) were used for this study. Treatments were: (T1) control group fed on corn–soybean basal diet; (T2) fed on basal diet with 5 % quinoa seeds; (T3) fed on basal diet with 10 % quinoa seeds; (T4) fed on basal diet with 15 % quinoa seeds. Results showed that feeding broiler chickens on different levels of quinoa seeds had significant effects on pH values, cooking loss %, color measurements and shear force values. No significant differences were found in shrinkage measurements. Supplemented quinoa seeds in broilers diets can be potentially used for improving color stability and controlling TBA values in processed chicken burger during frozen storage at -20°C for 90days.*

***Keywords*— Broiler feed, quinoa seeds, Chicken burger, frozen storage, Quality characteristics.**

I. INTRODUCTION

Lipid oxidation in foods specifically, meat and meat products is the major cause of quality deterioration. Chicken meat is subjected to quality deterioration caused by lipid oxidation because of its high content of polyunsaturated fatty acids and low natural antioxidants (Aziza et al., 2010). Synthetics antioxidants have been widely used in poultry diets to prevent the lipid oxidation and improved color stability in meat and its products (Avila-Ramos et al., 2013).

Many studies have revealed that using synthetics antioxidants have been found to exhibit adverse health effects because of their toxicity and carcinogenicity. This has led to growing interest in the use of natural antioxidants in meat and meat products because of their safety and consumer acceptability (Mokhtar et al., 2014).

Quinoa (*Chenopodium quinoa* Willd) belongs to Chenopodiaceae. Quinoa is unique seeds it has high ability to adapt different types of soil and climatic changes therefore, it could be cultivated in different environments. Quinoa is a grain with exceptional health benefits, nutritional and functional value (Gordillo-Bastidas et al., 2016). Quinoa seeds had large variety of bioactive compounds phenolic compounds include phenolic acids (rosmarinic and chlorogenic acids), flavonoids (quercetin and isoquercetin), and nitrogen-containing compounds (betacyanins, and betaxanthins). Most of the bioactive compounds in quinoa seeds are related to their antioxidant activity (Fernández-López et al., 2020).

Using quinoa seeds extract in broiler diet significantly affected on broilers performance and improved the meat quality. Quinoa extract had antioxidative properties which resulting in delaying the lipid oxidation of broiler meat during storage (Easssawy et al., 2016).

This study aimed to evaluate the effect of feeding broiler chicken on different levels of quinoa seeds on the processing and quality characteristics of chicken burger during frozen storage at -20° for 90 days.

II. MATERIAL AND METHODS

2.1. Preparation of quinoa seeds

Quinoa seeds (*Chenopodium quinoa* Willd) were supplied by the project of climatic smart agriculture entrepreneurship development of quinoa value chain in Egypt. The seeds were soaked in distilled water for 48 h thereafter the soaked seeds were washed with distilled water several times in a row, drained and dehulled, according to the method described by Udensi et al. (2008). Seeds were dried in a room with a temperature of 30 to 32ºC and a humidity of 15% with stirring until complete drying (about 8 days).

2.2. Experimental design

The experimental procedures were approved by the Animal Breeding Department, Animal and Poultry Production Division, Desert Research Center.

A total of 480 one-day-old chicks of (Ross 308) strain were used for this study, the chicks were randomly assigned to four treatment groups. Each group consisted of 6 replicates and each replicate was made up of 20 chicks. Treatments were: (T1) control group fed on corn–soybean basal diet; (T2) fed on basal diet with 5 % quinoa seeds; (T3) fed on basal diet with 10 % quinoa seeds; (T4) fed on basal diet with 15 % quinoa seeds. The basal diet was formulated to meet the nutrient requirements of broiler chicken following the National Research Council (NRC, 1994). Diets were offered in two feeding phase's starter: one-day-old till 21 days of age and grower: 22 days till 35 days. The composition and calculated analysis of basal diets are showed in Table 1. The chicks were raised at 33 ± 0.5 °C and then the temperature was gradually decreased until 28 ± 1 °C was reached by day 15 and then left with the case of natural temperature.

Table 1 Feed ingredients and chemical analyses of experimental diets

Ingredients (%)	Starter (1-21 d)				Grower (22-35 d)			
	Q0	Q5	Q10	Q15	Q0	Q5	Q10	Q15
Yellow corn	53.35	49.60	45.95	43.44	57.70	54.05	50.12	45.56
Soybean meal (44%)	33.14	32.14	31.00	29.50	28.65	28.26	27.34	26.8
Corn gluten meal (62%)	6.35	6.35	6.35	6.35	5.75	5.20	5.20	5.20
Quinoa	0.00	5.00	10.00	15.00	0.00	5.00	10.00	15.00
Soybean oil	3.00	2.70	2.39	1.40	3.95	3.50	3.28	3.30
Calcium carbonate	1.23	1.23	1.23	1.23	1.07	1.17	1.15	1.15
Di-calcium phosphate	1.93	1.93	2.03	2.03	1.98	1.85	1.88	1.90
Broiler premix*	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
Salt	0.45	0.45	0.45	0.45	0.40	0.40	0.40	0.40
DL-methionine	0.20	0.20	0.20	0.20	0.15	0.17	0.20	0.22
L-lysine	0.00	0.05	0.05	0.05	0.00	0.05	0.08	0.12
Total	100	100	100	100	100	100	100	100
Chemical analysis								
ME (kcal kgG1)	3050	3050	3050	3050	3150	3150	3150	3150
Crude protein	23.00	23.00	23.00	23.00	21.00	21.00	21.00	21.00
Calcium	1.00	1.00	1.00	1.00	0.95	0.95	0.95	0.95
Av. phosphorus	0.48	0.48	0.48	0.48	0.45	0.45	0.45	0.45

*Premix: (1%) provided the following (per Kilogram of complete diets). 1400 IU vitamin A, 3000 IU Vitamin D3, 50 mg vitamin E, 4 mg vitamin K, 3 mg Vitamin B6, 6 mg Vitamin B12, 60 mg Niacin, 20 mg Pantothenic acid, 0.20 mg folic acid, 150 mg Choline, 48 mg Ca, 3.18 mg P, 100 mg Mn, 50 mg Fe, 80 mg Zn, 10 mg Cu, 0.25 mg Co, 1.5 mg Iodine.

2.3. Slaughtering of birds

At the end of the experiment (42 days), 80 birds (20 birds from each group) were selected based on similar body weight for slaughtering. Slaughtered birds were scalded in hot water bath, plucked and eviscerated manually. Chicken meat from thigh and abdominal muscles were collected, packed and frozen at -20ºC until further analyses and processing of chicken burger were completed.

2.4. Preparation of chicken burger

Chicken meat of thigh and abdominal muscles were collected from each experimental diet and separately ground through a 3mm plat meat grinder (K-R-SU, Model: KMG1700. China). Meat of each dietary treatment was formulated with 1.5% salt, 0.5% black pepper, 0.5% spices and 7.5% onion as describe by Mikhail et al. (2014). The formula of each dietary treatment was handily mixed and formed by using manual burger press machine (Metaltex No.25.17.25 Made in PRC). Chicken burgers (1cm thickness, 10cm diameter and 70±2g weight) were placed in plastic foam trays packed in polyethylene bags and frozen at -20ºC±1until further analysis.

2.5. Physical analysis

2.5.1. pH value

Raw chicken burger was measured for pH value as described by Hood (1980). Ten grams of sample were homogenized with 100ml distilled water and measured using a digital pH-meter Jenway 3310 conductivity and pH meter. Values of pH were determined in triplicate for each dietary treatment at 0, 30, 60 and 90 days of storage at -20°C.

2.5.2. Cooking measurements

Chicken burger samples of each treatment were cooked in preheated grill (at110°C for 10 min each side) to an internal temperature 70°C±1. Three replicates per treatment were done for cooking loss measurement. Cooking loss was calculated by using the following equation as reported by Naveena et al. (2006).

Cooking loss (%)

$$= \frac{(\text{Uncooked sample weight}) - (\text{Cooked sample weight})}{(\text{Uncooked sample weight})} \times 100$$

2.5.3. Shrinkage measurements

The reduction in diameter and thickness of chicken burger were measured as described by Berry (1993) using the following equation:

Reduction in diameter (%) =

$$\frac{(\text{Uncooked sample diameter}) - (\text{Cooked sample diameter})}{(\text{Uncooked sample diameter})} \times 100$$

Reduction in thickness (%) =

$$\frac{(\text{Uncooked sample thickness}) - (\text{Cooked sample thickness})}{(\text{Uncooked sample thickness})} \times 100$$

Shrinkage was calculated by using the following equation as reported by Murphy *et al.* (1975).

Shrinkage (%) =

$$\frac{[(\text{Raw thickness - Cooked thickness}) + (\text{Raw diameter - Cooked diameter})]}{(\text{Raw thickness} + \text{Raw diameter})} \times 100$$

2.5.4. Shear force value

Cooked chicken burger samples were sheared for three times at different positions by using Instron Universal Testing Machine (Model 2519-105, USA). The average shear force was calculated from the three obtained results ($Kg/_f$).

2.5.5. Color measurements

Color of raw chicken burger samples was measured by Chroma meter (Konica Minolta, model CR 410, Japan) calibrated with a white plate and light trap supplied by the manufacturer (CIE, 1976). The color was expressed as lightness (L*), the redness (a*) and the yellowness (b*). The average of three spectral readings at different locations was obtained for burgers of each dietary treatment during storage periods 0, 30, 60 and 90 days of storage at -20°C.

2.6. T.B.A value

Measurement of lipid oxidation: The extent of lipid oxidation in raw chicken burger was assessed by measuring 2- thiobarbituric acid reactive substances (TBARS), as described by AOCS (1998). TBARS values were determined in triplicate for each sample at 0, 30, 60and 90 days of storage at -20°C.

2.7. Statistical analysis

All data generated from each treatment were analyzed using statistical analysis system (SAS, 2000). Two- way ANOVA was applied for pH, TBA and color measurements. In case of shrinkage measurements and physical analysis one – way ANOVA was applied.

III. RESULTS AND DISCUSSIONS

Physical properties of chicken burger

Physical properties (pH values, cooking loss, shear force values and color parameters) of chicken burger processed from broiler fed on different levels of quinoa seeds are shown in Table 2. The results of pH values of chicken burger indicated that feeding broilers on different levels of quinoa seeds had significant differences in pH values. Burger processed from control feeding group (T1) exhibited significantly higher in pH value followed by burger of T2 group. Burger from feeding groups of high levels of quinoa seeds (T3 and T4) showed lower pH values. In the same line, similar trends of pH values were found by Marino et al. (2018). Conversely, Shim et al. (2018) found that no significant difference were found in pH values of broiler meat fed on different levels of dried grains.

Table 2 Physical properties of chicken burger

Parameters	Treatments				
	T1	T2	T3	T4	SEM
pH	5.64^{a}	5.59^{b}	5.46^{d}	5.54^{c}	0.01
Cooking loss (%)	42.08^{b}	40.83^{b}	49.43^{a}	47.64^{a}	1.11
Shear force ($Kg/_{f}$)	2.08^{a}	1.59^{c}	1.62^{bc}	1.99^{ab}	0.11
Color parameters					
L	49.36^{b}	51.47^{a}	50.69^{ab}	50.95^{a}	0.79
a	4.71^{ab}	4.44^{b}	5.09^{a}	4.26^{b}	0.15
b	7.59^{b}	8.30^{ab}	8.73^{a}	8.99^{a}	0.33

$^{a-d}$ means within the same row with different superscripts letters are different ($p<0.05$).

T1: control diet, T2: diet contains 5 %, T3: diet contains 10 % and T4: diet contains 15 %.

SEM: standard error of means.

No significant differences were found in cooking loss between burger processed from control feeding groups (T1) and treated feeding group (T2). On the other hand, no significant differences were found in cooking loss between burger processed from (T3) and (T4) feeding groups. These results came in accordance with that obtained by Zaki et al. (2018) they found that no significant differences were observed in cooking loss of chicken burger processed from broiler fed on different types of diets and feed additives.

Results of shear force values of burger samples are revealed that feeding broilers on different levels of quinoa seeds had a significant effect on tenderness of processed chicken burgers. Burger of control group (T1) showed the higher shear force value (less tender) than the burger of quinoa seeds feeding groups. In this regard, our data reflect that the increasing of quinoa seeds level in broilers diet resulting in increasing in shear force values of processed chicken burgers. Burger of (T2) group which processed from broiler fed on the lowest level of quinoa seeds (5 %) showed the lowest shear force value (more tender) than the other quinoa feeding groups. Similar results were obtained by Marino et al. (2018) they found significant differences in WBSF values of meat fed on diets supplemented with quinoa. They found that control group showed the highest WBSF value (less tender) while; meat of quinoa feeding groups showed the lowest WBSF value (more ender).

Data of color measurements of chicken burger processed from different level of quinoa seeds showed that no significant differences were found in L* values of chicken burger samples of T2 and T4. Slight significant differences were found between burger of T1 andT3 and T4.

Supplemented broiler diet with different quinoa levels had significant effects on redness of processed burger (a* value). Burger of (T3) showed the highest *a value and no significant differences were found between T4 and T2. Chicken burger processed from broiler fed on different levels of quinoa seeds exhibited significantly higher in b* values than burger processed from control group (T1). View of the present data, it could be concluded that that the yellowness of chicken burger increased as feeding broiler on quinoa seeds levels increased. This finding came in accordance with the results obtained by Marino et al. (2018) they found that feeding on quinoa or/ and linseed showed a significant higher on color parameters (L* , a* and b* values) than meat feeding on control groups.

Shrinkage parameters of chicken burgers processed from broiler fed on different levels of quinoa seeds are shown in Table 3. Results of reduction in diameter % revealed that supplemented broiler diets with quinoa seeds had no significant effect on reduction in diameter % of burger, despite of burger of T2 had the lowest reduction in diameter % while, burger of T3 had the highest percentage but, the differences among burger groups were not significant . The same trends were found in data of reduction in thickness %.

View of the current results, it could be concluded that shrinkage measurements % of chicken burger did not affected by supplemented broiler diets with different quinoa seeds levels. These results are in line with that obtained by Zaki et al. (2018) they found that no significant differences were found in shrinkage measurements % of chicken burger processed from broilers fed on different feeding diets and feed additives. However, results of shrinkage measurements are consistency with data of cooking loss% and shear force values.

Table 3 Shrinkage parameters of chicken burgers

Treatments	Parameters		
	Reduction in diameter (%)	Reduction in thickness (%)	Shrinkage (%)
T1	21.23	19.44	21.69
T2	20.12	18.43	21.53
T3	22.37	22.22	22.73
T4	21.90	21.77	22.51
SEM	1.49	2.11	1.44
Sig.	NS	NS	NS

T1: control diet, T2: diet contains 5 %, T3: diet contains 10 % and T4: diet contains 15 %.

SEM: standard error of means. Sig : significant, NS: non significant.

Effect of frozen storage on the quality characteristics of chicken burger

Changes in pH values

Data in Table 4 showed the pH values of chicken burger processed from broiler fed on different levels of quinoa seeds during frozen storage at -20°C for 90 days. It can be noticed that a significant difference were found in pH values of burger treatments, the highest pH values found in burger of control feeding group (T1).While, slight significant differences were found among burgers of quinoa seeds feeding groups (T2, T3 and T4). Regarding frozen storage, during 30 days of storage no significant changes in pH values were found in both of burger of T1 (control feeding group) and burger of T2 (low level quinoa feeding group). Conversely, burger from higher quinoa levels feeding groups (T3 and T4) showed significantly decreased in pH values after 30 days of storage.

Table 4 Changes in pH values of chicken burger during frozen storage at -20°C for 90 days

Treatments	Storage periods (days)			
	0	30	60	90
	pH values			
T1	5.64^{Ac}	5.64^{Ac}	5.95^{Ab}	6.29^{Aa}
T2	5.59^{Bc}	5.57^{Bc}	5.84^{Bb}	6.16^{Ba}
T3	5.46^{Cc}	5.35^{Dd}	5.78^{Cb}	6.01^{Ca}
T4	5.54^{BCc}	5.44^{Cd}	5.85^{Bb}	6.03^{Ca}
SEM	0.02	0.02	0.02	0.02

$^{a-d}$ (→) means within the same row with different superscripts letters are different (p<0.05).

$^{A-D}$ (↓) means within the same column with different superscripts letters are different (p<0.05).

T1: control diet, T2: diet contains 5 %, T3: diet contains 10 % and T4: diet contains 15 %. SEM: standard error of means.

However, significant increased were found in pH values of all burger treatments during 60 and90 days of frozen storage. These discrepancies in pH values during frozen storage could be explained separately, the decreasing in pH values could be attributed to psychrophilic bacteria especially lactic acid bacteria which resulting in breakdown of glycogen during frozen storage; thereby increase in lactic acid which caused the reduction in pH values (Shelef, 1975). Conversely, the increasing in pH values may be due to the breakdown of protein in meat during frozen storage resulting in releasing of amino acids and accumulation of ammonia and consequently, increasing in pH values (Jin et al., 2007). These results are consonance with that obtained by Alabdulkarim et al. (2012) they found that pH values of chicken patties significantly decreased after 20 days of frozen storage and then increased during the rest of frozen storage period (60 days). The same results were found by Ozer and Sariçoban (2010) they indicated that during frozen storage, pH values of chicken patties samples tended to decrease after 2 months of storage and significantly increased as the time of frozen period increased (6 months).

Color parameters

Effect of frozen storage on the color measurements of chicken burger processed from broiler fed on different levels of quinoa are shown in Table 5. It can be noticed that fresh burger (at zero time) showed slight differences in L* values of all burger samples. After 30days of storage L* values significantly decreased, followed by significant increased throughout the storage period (90 days of storage). Fernandez-Lopez et al. (2003) indicated that pH values are the most factor affected on meat color because of its effect on chemical state of meat pigments. In this regard, data of pH values are consistency with results of L* values which could be explained the changes in L* values during frozen storage. Similar trend were obtained by Ozer and Sariçoban (2010) they found that L* values of chicken patties significantly decreased during 4 months of frozen storage and then increased at the end of frozen period.

Table 5 Changes in color parameters of chicken burger during frozen storage at -20°C for 90 days

Treatments	Storage periods (days)				
	0	30	60	90	SEM
			*L**		
T1	49.36^{Ba}	46.39^{Bb}	46.84^{Bb}	48.79^{Ba}	0.65
T2	51.47^{Aa}	48.22^{ABb}	50.22^{Aab}	52.49^{Aa}	0.65
T3	50.69^{ABab}	49.72^{Ab}	48.60^{ABb}	52.28^{Aa}	0.65
T4	50.95^{Aa}	46.44^{Bb}	47.21^{Bb}	48.36^{Bb}	0.65
			*a**		
T1	4.71^{ABa}	4.41^{ABa}	4.46^{Aa}	3.55^{Bb}	0.19
T2	4.44^{Ba}	4.13^{Bab}	3.86^{Bb}	3.88^{ABb}	0.19
T3	5.09^{Aa}	4.74^{Aa}	4.53^{Aab}	4.34^{Ab}	0.19
T4	4.26^{Ba}	4.10^{Ba}	3.68^{Bab}	3.61^{Bb}	0.19
			*b**		
T1	7.59^{Bb}	10.04^{Aa}	10.49^{Aa}	9.94^{Aa}	0.42
T2	8.30^{ABb}	10.25^{Aa}	9.91^{Aa}	8.94^{Aab}	0.42
T3	8.73^{Ab}	10.28^{Aa}	9.98^{Aa}	9.92^{Aa}	0.42
T4	8.99^{Aa}	9.79^{Aa}	9.88^{Aa}	9.10^{Aa}	0.42

$^{a-b}$ (→) means within the same row with different superscripts letters are different ($p<0.05$).

$^{A-B}$ (↓) means within the same column with different superscripts letters are different ($p<0.05$).

T1: control diet, T2: diet contains 5 %, T3: diet contains 10 % and T4: diet contains 15 %.

SEM: standard error of means.

Burger of T3 showed the highest a*value (more red), followed by burger of control group (T1). No significant differences were found between burger of T2 and T4. Regarding frozen storage, decreasing trends were observed in (a*) values for all burger samples as the time of frozen storage increased. This may be attributed to the oxidation of oxymyoglobin to metmyoglobin which resulting in dark color (Ozer and Sariçoban, 2010). In addition, at any time of frozen storage burger of T3 showed the highest a* value (more red) than other burger samples. These results are in line with the results of Vieira et al. (2009) they found significant decreased were observed in a* of all beef samples as the time of frozen storage increased. The same results were found by Fernandez-Lopez (2006) who found that a* values of burger decreased as the time of storage increased. Also, Gahruie et al. (2017) reported that significant decrease in a* values were found in all beef burger formulations during frozen storage.

The results revealed that a significant increased was found in b* values after 30days of frozen storage, after that b* values tended to decrease gradually with the time of storage increased up to 90days despite the fact that differences in b* values were not significant as the time of frozen storage increased. These results are consonance with Vieira et al. (2009) they found a significant decreased in b* values of meat after 90days of frozen storage. The same results were found by Ibrahim et al. (2011) they found that all chicken burger formulations tended to increased in b* values after 45 days of storage and slightly decreased after 90 days of frozen storage.

The results of the current study revealed that supplemented quinoa seeds in broilers diets resulting in increasing the antioxidant activity in chicken meat which can be potentially used as a natural antioxidant for controlling color parameters (L*, a* and b*) values in processed chicken burger during frozen storage.

Changes in TBA values

Table 6 showed the TBARS values of chicken burger during frozen storage at -20°C for 90 days. It can be noticed that at zero time burger of control group (T1) showed the lower TBA value and no significant differences were found between burger of T2 and T3 while, the highest TBA value were found in burger of T4. After 30 days of frozen storage significant decreased were found in all burger samples especially, in burger processed from chicken fed on high level of quinoa seeds (T4). On the other hand, the differences between burger treatments were not significant. After 60 days of storage TBA values increased for all burger treatments and such increase was continued as the time of frozen storage increased. These results are consonance with that obtained by Gahruie et al. (2017) they found that TBARS values of all burger treatments were significantly increased as the time of frozen storage increased.

Table 6 Changes in TBA values of chicken burger during frozen storage at -20°C for 90 days

Treatments	Storage periods (days) 0	30	60	90
	T.B.A value (mgMDA/kg)			
T1	0.209^{Ca}	0.029^{Bc}	0.105^{Bb}	0.111^{Bb}
T2	0.235^{Ba}	0.033^{ABd}	0.099^{Cc}	0.110^{Bb}
T3	0.239^{Ba}	0.034^{Ad}	0.109^{Bc}	0.116^{Bb}
T4	0.409^{Aa}	0.035^{Ad}	0.118^{Ac}	0.132^{Ab}
SEM	1.88	1.88	1.88	1.88

$^{a-d}$ (→) means within the same row with different superscripts letters are different ($p<0.05$).

$^{A-C}$ (↓) means within the same column with different superscripts letters are different ($p<0.05$).

T1: control diet, T2: diet contains 5 %, T3: diet contains 10 % and T4: diet contains 15 %.

SEM: standard error of means.

Also, Wei et al. (2017) found that TBA values of breast chicken meat were gradually increased during frozen storage period (0-5 months) but the significant increased was found during 7- 8 months of storage. Generally, it is clear that at any time of frozen storage, T.B.A. values of all burger samples remained lower than T.B.A. values at zero time. Based on the present data, it could be concluded that incorporation of quinoa seeds in broiler diets resulting in inhibited lipid oxidation of chicken burger during frozen storage. This is may be attributed to the higher antioxidant activity of quinoa seeds because of its remarkable content of phenolic and flavonoid compounds which play as a source of free radical scavenging agents. Thereby, addition of quinoa seeds in broilers diet resulted in increment of the antioxidative properties of chicken burger. This finding came in accordance with the results of Easssawy et al. (2016) they reported that addition of quinoa seeds extract in broiler diets can be successfully delayed the lipid oxidation of chicken meat during refrigerated storage for 7 days.

IV. CONCLUSION

The aim of the current study was to evaluate the quality characteristics of chicken burger processed from broiler chicken fed on different levels of quinoa seeds and stored under frozen storage. Addition of quinoa seeds in broiler diets has a positive effect on quality traits of chicken burger. Quinoa in broiler chicken diets would subsequently affect the oxidative stability during frozen storage and improving the color of burger during frozen storage.

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REFERENCES

[1] Alabdulkarim, B., Bakeet, Z. A. N., & Arzoo, S. (2012). Effect of frying oils on quality characteristics of frozen chicken patties incorporated with honey. *African Journal of Biotechnology, 11(12)*, 2985-2992. https:// doi: 10.5897/AJB11.3389.

[2] AOCS. (1998). American Oil Chemists' Society. Official method Cd 19-90. 2-Thiobarbituric acid value. Direct method. In: D, Firestone (Eds.), *Official Methods and Recommended Practices of the American Oil Chemists' Society*, 5th ed. Champaign, p.3.

[3] Avila-Ramos, F., Pro-Martínez, A., Sosa-Montes, E., Cuca-García, J. M., Becerril-Pérez, C., Figueroa-Velasco, J. L., Ruiz-Feria, C. A., Hernández-Cázares, A. S., & Narciso-Gaytán C. (2013). Dietary supplemented and meat-added antioxidants effect on the lipid oxidative stability of refrigerated and frozen cooked chicken meat. *Poultry Science, 92 (1)*, 243–249. https:// doi: 10.3382 /ps. 2012-02409.

[4] Aziza, A. E., Quezada, N., & Cherian, G. (2010). Antioxidative effect of dietary camelina meal in fresh, stored, or cooked broiler chicken meat. *Poultry Science, 89(12)*, 2711–18. doi:10.3382/ps.2009-00548.

[5] Berry, B.W. (1993). Fat level and freezing temperature affect sensory, shear cooking and composition properties of ground beef patties. *Journal of Food Science, 58*, 34-42. https:// doi: 10.1111/j.1365-2621.1993.tb03204.x.

[6] CIE (Commission International de l'E´ clairage). (1976). *Official recommendations on uniform colour spaces.* Colour difference equations and metric colour terms, Suppl. No. 2. CIE Publication No. 15 Colourimetry. Paris. https:// doi.org/10.1002/j.1520-6378.1977.tb00102.x.

[7] Easssawy, M. M. T., Abdel-Moneim, M. A., & El-Chaghaby, G. A. (2016). The use of quinoa seeds extract as a natural antioxidant in broilers' diets and its effect on chickens' performance and meat quality. *J. Animal and Poultry Production Mansoura Univ., 7(5)*, 173- 180. https:// doi: 10.21608/jappmu.2016.48696.

[8] Fernández-López , J., Viuda-Martos , M., Sayas-Barberá, M. E., Navarro-Rodríguez de Vera, C., Lucas-González, R., Roldán-Verdú, A. Botella-Martínez, C., & Pérez-Alvarez, J. A. (2020). Chia, quinoa and their coproducts as potential antioxidants for the meat industry. *Plants, 9*, 1359-1380. https:// doi: 10.3390/plants9101359.

[9] Fernandez-Lopez, J. (2006). Quality characteristics of ostrich (*Struthio camelus*) burgers. *Meat Science, 73*, 295–303. https://doi: 10.1016/j.meatsci.2005.12.011.

[10] Fernandez-Lopez, J., Sayas-Barbera, E., Perez-Alvarez, J. A., & Aranda-Catala, V. (2003). Effect of sodium chloride, sodium tripolyphosphate and pH on color properties of pork meat. *Color Research and Application, 29*, 67–74. https://doi.org/10.1002/col.10215.

[11] Gahruie, H. H., Hosseini, S. M. H., Taghavifard, M. H., Eskandari, M. H., Golmakani, M. T. &Shad, E. (2017). Lipid oxidation, color changes, and microbiological quality of frozen beef burgers incorporated with shirazi thyme, cinnamon, and rosemary extracts. *Journal of Food Quality*, Article ID 6350156, 9 pages. https://doi.org/10.1155/2017/6350156.

[12] Gordillo-Bastidas, E., Díaz-Rizzolo, D.A., Roura, E., Massanés, T., & Gomis, R. (2016).Quinoa (Chenopodium quinoa Willd), from Nutritional Value to Potential Health Benefits: An Integrative Review. *Journal of Nutrition & Food Sciences, 6(3)*, 497-507. doi:10.4172/2155-9600.1000497.

[13] Hood, D. E. (1980). Factors affecting the rate of metmyoglobin accumulation in prepackaged beef. *Meat Science, 4*, 47–50. doi: 10.1016/0309-1740(80)90026-1.

[14] Ibrahim, M. A., Salama, M. F., & Hussein, A. A. (2011). Production of Functional Low- Fat Chicken Burger. *Australian Journal of Basic and Applied Sciences, 5(12)*, 3149-3154.

[15] Jin, S. K., Kim, I. S., Jung, H.J., Kim, D.H., Choi, Y.J., & Hur, S. J. (2007). The development of sausage including meat from spent laying hen surimi. *Poultry Science, 86*, 2676-2684. https:// doi: 10.3382/ps.2006-00451.

[16] Marino, R. Caroprese, M., Annicchiarico , G., Ciampi, F., Ciliberti, M.G. , della Malva, A., Santillo, A., Sevi , A., & Albenzio , M. (2018). Effect of diet supplementation with quinoa seed and/or linseed on immune response, productivity and meat quality in merinos derived lambs. *Animals, 8*, 204-217. https:// doi: 10.3390/ani8110204.

[17] Mikhail, W.Z.A., Sobhy, H. M., Khallaf, M. F., Ali, H. M. Z., El-Askalany, S. A., & Ezz El-Din, M. M.,(2014) Suggested treatments for processing high nutritive value chicken burger. *Annals of Agricultural Science*, 59, 41–45.

[18] Mokhtar, S. M. , Youssef, K. M., Morsy, N. E. (2014). The effects of natural antioxidants on colour, lipid stability and sensory evaluation of fresh beef patties stored at 4ºC. *Journal of Agroalimentary Processes and Technologies, 20(3)*, 282-292.

[19] Murphy, E. W., Criner, P. E., & Grey B. C. (1975). Comparison of methods for calculating retentions of nutrients in cooked foods. *Journal of Agricultural Food*

Chemistry, 23, 1153–1157. https:// doi: 10.1021/jf60202a021.

[20] Naveena, B. M., Muthukumar, M., Sen, A. R., Babji, Y., & Murthy, T. R. K. (2006). Quality characteristics and storage stability of chicken patties formulated with finger millet flour (Eleusine coracana). *Journal of Muscle Foods, 17 (1),* 92–104. https://doi.org/10.1111/j.1745-4573.2006.00039.x.

[21] NRC (1994).National Research Council. Nutrient Requirements of Poultry 9th Ed. *Composition of poultry feedstuffs.* National Academy Press, Washington, DC, USA.

[22] Ozer, O., & Sariçoban, C. (2010). The Effects of Butylated Hydroxyanisole, Ascorbic Acid, and α-Tocopherol on Some Quality Characteristics of Mechanically Deboned Chicken Patty during Freeze Storage. *Czech Journal of Food Science, 28 (2),* 150–160. https: // doi. org /10. 17 221 /160/2009-CJFS.

[23] SAS. 2000. *User's Guide Statistics*. SAS Institute, Inc. Cary, N.C., USA.

[24] Shelef, L. A. (1975). Microbial spoilage of fresh refrigerated beef liver. *Journal of Applied Bacteriology, 39,* 273-280. https://doi.org/10.1111/j.1365-2672.1975.tb00572.x.

[25] Shim, Y. H., Kim, J. S., Hosseindoust, A., Choi, Y. H., Kim, M. J., OH, S.M., Ham, H.B., Kumar, A., Kim, K.Y., Jang, A., & Chae, B.J. (2018). Investigating meat quality of broiler chickens fed on heat processed diets containing corn distillers dried grains with solubles. Korean *Journal for Food Science of Animal Resources,* 38*(3),* 629-635. https: // doi. org /10. 58 51 /kosfa.2018.38.3.629.

[26] Udensi EA, Arisa NU, Maduka M (2008). Effect of processing method on the level of antinutritional factors in Mucuna flagellipes. *Nigerian Food Journal 26(2),* 53-59. https://doi: 10.4314/nifoj.v26i2.47437.

[27] Vieira, C., Diaz, M.T., Martinez, B., & Garcia-Cachan, M.D. (2009). Effect of frozen storage conditions (temperature and length of storage) on microbiological and sensory quality of rustic crossbred beef at different states of ageing. *Meat Science, 83,* 398–404. https://doi: 10.1016/j.meatsci.2009.06.013.

[28] Wei, R., Wang, P., Han, M., Chen, T., Xu, X. & Zhou, G. (2017). Effect of freezing on electrical properties and quality of thawed chicken breast meat. *Asian Australasian Journal of Animal Science, 30(4),*569-575 . https:// doi.org/10.5713/ajas.16.0435.

[29] Zaki, E. F., El Faham A. I., & Nematallah, G. M. (2018). Quality characteristics of chicken burger processed from broiler chicken fed on different types of vegetable oils and feed additives. *Int. J. of Health, Animal science and Food safety 5:* 1 – 11.

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Assessment on Motivation Factors of Agripreneurs in Nueva Ecija

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Abstract— *Stimulating entrepreneurial activity in agriculture, through targeted training in agricultural entrepreneurship, could possibly an answer to the emerging situation. Entrepreneurship shifts the focus from producing more of the same things to producing value-added goods and services through managed agricultural risks.The study described personal and business profile of the respondents. The study assessed the motivation factors of agripreneurs in terms of Job Characteristics, Critical Psychological State and Job Satisfaction. Further, this study determined the relationship between and among the profile of the respondents and motivation factors of agripreneurs.This study used descriptive-correlational design. This study determined the relationships among sociodemographic and business profile of agripreneurs, motivation factors and challenges that were gathered through an online questionnaire.The researcher used purposive quota sampling in selecting the respondents. A quota of 62 agripreneurs in Nueva Ecija was established. Based on the results of the study, agripreneurs in Nueva Ecija are motivated by job characteristics where they understood that their job requires a wide range of skills because it is not simple or repetitive. They believed their job has meaning for them because of its importance and usefulness, which meant a lot to them as agripreneurs. This contributed to how they impact the well-being of their clients. Thus, agripreneurswere satisfied with their jobs when they face personal work challenges. Then, the main motivating factor for agripreneurs was found to be job satisfaction.Future researchers may explore more on the demographic of the agripreneurs. They may include courses of the agripreneurs if these were related to their job, trainings, and seminars attended in relation to agribusiness.Profile of the agripreneurs have positive correlations to motivating factors such critical psychological state and job satisfaction but only limited to age, highest educational attainment, number of years in the agribusiness, and average monthly income. Further, job characteristics as a motivating factor were found no significant relationships with the profile of the agripreneurs.*

Keywords— ***Agripreneurs, Motivation Factors, Agribusiness, Agriculture.***

I. INTRODUCTION

Stimulating entrepreneurial activity in agriculture, through targeted training in agricultural entrepreneurship, could possibly an answer to the emerging situation. Entrepreneurship shifts the focus from producing more of the same things to producing value-added goods and services through managed agricultural risks. In order to encourage the development of opportunities and value creation in this sector, current farmers need to be trained to become more entrepreneurial and to educate future generations to become agricultural entrepreneurs (Santiago &Roxas, 2015).

In the context of aging farmers and rural-urban youth migration, attention has shifted to agricultural or agribusiness entrepreneurship as a strategy to modernize the agricultural sector (Yusoff, 2017). Bairwa, Lakra, Kushwaha, Meena and Kumar (2014) stated that applying the principles of entrepreneurship to the agricultural sector can provide a host of benefits, ranging from employment and income generation to the creation of innovative products and poverty reduction.

Based from the review report of the Philippine Statistics Authority in 2004, Nueva Ecija shared the largest number of farms in Central Luzon and also the biggest among the provinces. Palay was the major temporary crop in Nueva Ecija alongside with tubers, roots and bulbs, corn, sugarcane, and fruit bearing vegetables. It was reported a high proportion of livestock such as goats and hogs and also raising poultry like ducks, chicken,and quails came from this province. In addition to their review, thousand household members in Nueva Ecija were engaged in agricultural activities. While male operators dominated the agricultural operation, females engaged as helpers of their own farms or workers of other farms. In general, based on the report, farmers in Nueva Ecija are actually helpers or workers either on their own farms or from others and none was mentioned if these farmers were engaged into entrepreneurship.

With the scenarios presented, it is important to know how the agripreneurs are doing despite some challenges or problems encountered. There is a shortage of studies in the Philippines that examine motivation factors of these agripreneurs in relation to job satisfaction.

II. CONCEPTUAL FRAMEWORK

Using Oldham and Hackman's Job Characteristic Theory, the research employed variables that specifically anchored to the theory and also based on empirical studies. Research on job satisfaction and motivation are ones of the most addressed issues in organizational psychology (Judge &Kammeyer-Mueller, 2012).

There is still a lack of effective focus on building an entrepreneurial mindset among agriculturalists. Investing in physical structures or providing training for better farm productivity is the usual response of governments to improve output in the agriculture sector. These efforts are important but wasteful if not coupled with a shift in thinking (Audinet&Haralambous, 2005).

III. OBJECTIVES OF THE STUDY

The study described personal and business profile of the respondents. The study assessed the motivation factors of agripreneurs in terms of Job Characteristics, Critical Psychological State and Job Satisfaction. Further, this study determined the relationship between and among the profile of the respondents and motivation factors of agripreneurs.

IV. METHODOLOGY

This study used descriptive-correlational design. Correlational research data may be used to determine the prevalence of variables and their relationships, as well as to forecast occurrences based on available data and information (Curtis et al., 2016). This study determined the relationships among sociodemographic and business profile of agripreneurs, motivation factors and challenges that were gathered through an online questionnaire. The researcher used purposive quota sampling in selecting the respondents. A quota of 62 agripreneurs in Nueva Ecija was established.

V. RESULTS AND DISCUSSIONS

Table 1. Personal Profile of the Respondents

Age	Frequency	Percentage
30 and below	1	1.61
31 - 45	9	14.52
46 – 60	48	77.42
Above 60	4	6.45
Total	62	100
Sex	**Frequency**	**Percentage**
Male	38	61.29
Female	24	38.71
Total	62	100
Civil Status	**Frequency**	**Percentage**
Single	2	3.23
Married	54	87.10
Widowed/Separated	6	9.68
Total	62	100
Highest Educational Attainment	**Frequency**	**Percentage**
High School	14	22.58
College	46	74.19
Graduate Studies	2	3.23
Total	62	100

Table 1 presents the socio-demographic profile of the respondents in terms of their age, sex, civil status, and their highest educational attainment. Based on the data presented, the following results were drawn.

In terms of age, majority of respondents with a total of 48 (77.42%) were 46 to 60 years old. In terms of sex, there were 38 (61.29%) male respondents and there were 24

(38.71%) female respondents. In terms of civil status, most of the respondents with a total of 54 (87.10%) were married. It can also be derived that there were 46 (74.19%) respondents attained college level in terms of highest educational attainment.

Table 2. Business Profile of the Respondents

Nature of Agribusiness		Frequency	Percentage
Crops		28	45.16
Fisheries		4	6.45
Poultry		20	32.26
Other livestock		10	16.13
	Total	62	100
Number of years in Agribusiness		**Frequency**	**Percentage**
5 years and below		12	19.35
6 to 10 years		24	38.71
11 to 15 years		12	19.35
Above 15 years		14	22.58
	Total	62	100
Initial Investment for Agribusiness		**Frequency**	**Percentage**
P200,000 and below		14	22.58
P200,001 to P400,000		25	40.32
P400,001 to P600,000		6	9.68
Above P600,000		17	27.42
	Total	62	100
Source of Funding		**Frequency**	**Percentage**
Personal Savings		32	51.61
Loans		28	45.16
Others		2	3.23
	Total	62	100
Average Monthly Profit		**Frequency**	**Percentage**
P100,000 and below		33	53.23
P100,001 to P200,000		15	24.19
P200,001 to P300,000		1	1.61
Above P300,000		13	20.97
	Total	62	100

Table 2 presents the business profile of the respondents in terms of its nature of agribusiness, number of years in agribusiness, initial investment, source of funding and average monthly profit.

In terms of nature of agribusiness, there were 28 (45.16%) respondents with crops as their agribusiness, while there were 20 (32.26%) respondents with poultry as their agribusiness. Most of the respondents with a total of 24 (38.71%) were already on 6th to 10th year in their agribusiness. A total of 25 (40.32%) respondents initially invested P200,001-P400,000 to their respective agribusinesses. Most of the respondents with a total of 32 (51.61%) funded their agribusiness through their personal savings. On the average, it can be derived that a total of 33

(53.23%) respondents were earning P100,000 and below monthly profit.

Table 3. Assessment on Motivation Factors of Agripreneurs in terms of Job Characteristics

Statement/s	WM	Verbal Interpretation
Skills Variety		
1. My job provides a lot of variety.	3.27	Strongly Agree
2. My job is very demanding and requires many different skills.	2.61	Agree
3. Many aspects of my job are simple and repetitive.	2.44	Disagree
Task Identity		
4. I consider my job as holistic.	3.29	Strongly Agree
5. I can not only partially but rather comprehensively influence the development of my clients.	2.65	Agree
6. In my job, I do not have the ability to edit a complete a task from beginning to end.	2.66	Agree
Task Significance		
7. My job is important to the life and well-being of the clients.	3.26	Strongly Agree
8. The quality of my job directly impacts my clients.	3.03	Agree
9. All in all my job is not very important and significant.	2.45	Disagree
Autonomy		
10. I have full liberty in deciding how to structure my job.	3.23	Agree
11. I can independently plan and organize my job.	2.95	Agree
12. I have become so hampered by guidelines and specifications that I can hardly bring original ideas into my job.	2.53	Agree
Feedback		
13. In carrying out my job activities, it is easy for me to say, how well I perform my job.	2.87	Agree
14. My job provides little feedback on the actual quality of my work.	2.73	Agree
Average Weighted Mean	2.86	Agree

Legend	***Verbal Interpretation***
3.25 - 4.00	Strongly Agree
2.50 - 3.24	Agree
1.75 - 2.49	Disagree
1.00 - 1.74	Strongly Disagree

Table 3 presents the assessment on motivation factors of agripreneurs in Nueva Ecija in terms of job characteristics. Job characteristics can be divided into five aspects namely, skills variety, task identity, task significance, autonomy, and feedback.

The result shows that agripreneur-respondents had an average mean of 2.86 in overall job characteristics as a motivation factor which has a verbal interpretation of "Agree".

To highlight the statements with highest means for each aspect are as follows: Skills Variety - Item 1 *"My job provides a lot of variety."* (Mean = 3.27; Strongly Agree); Task Identity - Item 4 "." (Mean = 3.29; Strongly Agree); Task Significance - Item 7 *"My job is important to* *the life and well-being of the clients."* (Mean = 3.26; Strongly Agree), Autonomy – Item 10 "*I have full liberty in deciding how to structure my job."* (Mean = 3.23, Agree); and Feedback – Item 13 "*In carrying out my job activities, it is easy for me to say, how well I perform my job.*" (Mean = 2.87, Agree).

Agripreneurs in Nueva Ecija are responsible for more than just management and business management. They were also hands-on in their business, performing tasks that an owner would not normally perform because the majority of them did not have staff (farmers) or a large number of staff to assist them. Growing and harvesting crops, raising poultry, and preparing them for market are all jobs for agripreneurs.

The findings indicate that agripreneurs in Nueva Ecija are motivated by job characteristics. They understand that their job requires a wide range of skills because it is not simple or repetitive. In their business tasks, they identify their job holistically by influencing the development of their customers comprehensively, which entails the ability to complete tasks as soon as they begin it. They find meaning and significance in their job, which contributes to how they impact the well-being of their clients. Agripreneurs are self-sufficient in their job execution as well as in planning, organizing, and managing the structure of their job.

As a result, they can contribute their own ideas to their job. They are also motivated when they receive feedback from their customers, whether it is positive or negative. According to Meyerding (2018), insights into the attractiveness of job characteristics as well as reliable knowledge about the characteristics of applicants available to employers contribute to a more open situation in which better-informed decisions can be taken by candidates and employers, resulting in improved job satisfaction, success and longevity of careers. Further of Wolf &Qenani-Petrala (2004) stated that the influence of job characteristics was taken into account by including variables such as form of occupation, area of employment, position in the business and starting salary.

Table 4. Assessment on Motivation Factors of Agripreneurs in terms of Critical Psychological State

Statement/s	WM	Verbal Interpretation
Experience Meaningfulness		
1. The work I am doing means a lot to me.	3.26	Strongly Agree
2. I consider my work to be very important and useful.	3.34	Strongly Agree
3. Many of my tasks seem useless and insignificant.	2.39	Disagree
Experienced Responsibility		
4. I feel a great deal of personal responsibility for the work I am doing.	3.16	Agree
5. I am clearly responsible for whether my clients learn something or not.	2.77	Agree
6. It is often hard for me to worry much about whether my work is done well.	2.65	Agree
Knowledge of Results of the Work Activities		
7. I usually know if I have done my job satisfactorily.	3.13	Agree
8. I find it often easy to predict whether I have done my job well or poorly.	2.71	Agree
9. I can hardly determine whether I am doing my job well or not.	2.40	Disagree
Average Weighted Mean	2.87	Agree

Legend	***Verbal Interpretation***
3.25 - 4.00	Strongly Agree
2.50 - 3.24	Agree
1.75 - 2.49	Disagree
1.00 - 1.74	Strongly Disagree

Table 4 presents the assessment on motivation factors of agripreneurs in Nueva Ecija in terms of critical psychological state. Critical psychological states are divided into three aspects namely experience meaningfulness, experienced responsibility, and Knowledge of results of the work activities. The result shows that agripreneur-respondents had an average mean of 2.87 in critical psychological state as a motivation factor which has a verbal interpretation of "Agree".

To highlight the statements with highest means for each aspect are as follows: Experience Meaningfulness - Item 2*"I consider my work to be very important and useful."* (Mean = 3.34; Strongly Agree); Experienced Responsibility - Item 4 *"I feel a great deal of personal responsibility for the work I am doing."* (Mean = 3.16; Agree); and Knowledge of Results of the Work Activities - Item 7*"I usually know if I have done my job satisfactorily."* (Mean = 3.13; Agree).

These findings imply that agripreneurs in Nueva Ecija believe their work has meaning for them because of its importance and usefulness, which means a lot to them as agripreneurs. They also gain personal responsibility for the outcomes of their work by addressing their personal responsibilities and acknowledging how their work was completed. Furthermore, agripreneurs understand the success of their work because it is often easy to predict whether they have done their job well or poorly. This confirms the result of Ulianchenko et al. (2019) as labor motivation is an employee's attitude to the work carried out, which is based on the priorities of wants, desires and motivation. And, on the other hand, the company's management should conduct those actions directed at the staff's efforts to fully achieve the organization's goals. However, Bessell et al. (2002) found that there should be thinking about incentives and recognition, it is also a good idea to recognize career growth and where people are in their careers. Pay rewards, for example, may appeal to young employees, opportunities for professional advancement may interest mid-career associates, and being part of policy and strategic planning may interest long-term associates.

Table 5. Assessment on Motivation Factors of Agripreneurs in terms of Job Satisfaction

Statement/s	WM	Verbal Interpretation
1. All in all I am very satisfied with my job.	3.27	Strongly Agree
2. I often think about changing jobs.	2.69	Agree
3. In general, I am satisfied with the nature of my job.	3.08	Agree
4. I am very satisfied with my prospects with the current institution. I am very satisfied with the opportunities of my current situation.	2.90	Agree
5. I am very satisfied with the respect and fair treatment by management. I am very satisfied with the respect and fair treatment we have in this business.	2.94	Agree
6. I am very satisfied with the income of my business	2.85	Agree
7. I am very satisfied with the extent of support that I receive for my business.	3.10	Agree
8. I am very satisfied with the extent to which I can reward myself and family based on the performance of my business.	2.94	Agree
9. I am very satisfied with the quality of my management of my business.	2.89	Agree
10. I am very satisfied with the ability to develop myself personally through my job.	2.85	Agree
11. I am very satisfied with the feeling of achieving something valuable by my job	2.92	Agree
12. I am very satisfied with the security of my job.	2.89	Agree
13. I am very satisfied with the degree of independence regarding thinking and acting at my job.	2.97	Agree
14. I am very satisfied with the extent to which my job challenges me personally	3.23	Agree
Average Weighted Mean	2.97	Agree

Legend	*Verbal Interpretation*
3.25 - 4.00	Strongly Agree

2.50 - 3.24	Agree
1.75 - 2.49	Disagree
1.00 - 1.74	Strongly Disagree

Table 5 presents the assessment on motivation factors of agripreneurs in Nueva Ecija in terms of job satisfaction.

The result shows that agripreneur-respondents had an average mean of 2.97 in job satisfaction as a motivation factor which has a verbal interpretation of "Agree".

The statements with highest means are as follows: Item 1 *"All in all I am very satisfied with my job."* (Mean = 3.27; Strongly Agree), Item 14 *"I am very satisfied with the extent to which my job challenges me personally"* (Mean = 3.23; Agree), Item 7 *"I am very satisfied with the extent of support I receive for my business."* (Mean = 3.10; Agree), and Item 3 *"In general, I am satisfied with the nature of my job."* (Mean = 3.08; Agree).

The findings indicate that agripreneurs in Nueva Ecija, in general, are satisfied with their jobs, whether they like the job itself or specific aspects or facets of it. According to their responses, agripreneurs are satisfied even when they face personal work challenges. This can be attributed to the respect and fair treatment they they have in their business. Another factor influencing agripreneurs' job satisfaction is their level of independence in terms of thinking and acting at their job. Moreover, because they enjoy their job as agripreneurs, they regard it as valuable and significant. Thus, Hoque et al. (2016) found that a well-built organizational culture, empowerment and participatory method acknowledgement are established by the specific business. Recognition and career policies and congenial business policies help to meet corporate goals very efficiently and effectively. Further, this confirms the study of Marri et al. (2012) that employees in agriculture sector institutions have shown a clear commitment to a cooperative and team-oriented environment; Employees' efforts should be compensated reciprocally by the company in order to ensure that they have an equitable chance of advancement in life and equal opportunity for professional growth and career progression.

Table 6. Correlation between Profile of the respondents and Motivation Factors

	Age	Sex	Highest Educational Attainment	Number of years in the Agribusiness	Initial Investment for Agribusiness	Average Monthly Profit	JC Mean	CPS Mean	JS Mean
Age	1								
Sex	.085	1							
Highest Educational Attainment	-.160	-.307*	1						
Number of years in the Agribusiness	.347**	-.197	.062	1					
Initial Investment for Agribusiness	-.076	-.179	.129	-.481**	1				
Average Monthly Profit	.137	-.278*	.442**	-.009	.620**	1			

JC Mean	-.049	-.046	-.096	.179	-.197	-.052	1		
CPS Mean	.106	-.182	.335**	.354**	-.091	.257*	326**	1	
JS Mean	.282*	-.239	.098	.284*	.156	.480**	.305*	378**	1

Table 6 shows the results significant relationships of the personal profile of the agripreneur-respondents and their motivation factors using Pearson r correlation coefficient. The profile of the agripreneur-respondents include age, sex, highest educational attainment, number of years in the agribusiness, initial investment for agribusiness,and average monthly profit. The motivation factors include job characteristics, critical psychological state, and job satisfaction.

Results show that age (r = .282, p <.05), the number of years in agribusiness (r=.284, p<.05) and the average monthly profit (r=.326, p<.01) have weak positive correlations to job. This indicates that as the age of the agripreneurs go higher, and/or they have more number of years in the agribusiness, and/or there is an increase on their average monthly profit, the more they are motivated. They also have more contentment or satisfaction with their *job*, whether they like the *job* of individual aspects or facets of *jobs*. Further, results show that the highest educational attainment (r=.335, p <.01), the number of years in agribusiness (r=.354, p<.01), and the average monthly profit (r=.326, p<.01) have weak positive correlations to with the motivation factor which is critical psychological state. This implies that the higher the educational attainment, and / or higher number of years in the agribusiness, and/or higher average month profit of the agripreneurs, the more they feel the value and meaning of their job and the more they know how successful their work has been. It was also found out that the three aspects of motivation factors such job characteristics, critical psychological states, and job satisfaction have weak intercorrelations with one another. This means that when the score of motivation factor increases the score of other motivation factors also increases. However, the profile of the agripreneur-respondents was not found to be correlated to the job characteristics as a motivating factor.

VI. CONCLUSIONS AND RECOMMENDATIONS

Based on the results of the study, the following conclusions are made:The majority of Nueva Ecija'sagripreneurs were all middle aged adults. Because there were nearly equal numbers of male and female agripreneurs in Nueva Ecija, the job of agripreneur was not sex-determined. Majority of them were already married and attained college level in terms of highest educational attainment.Crops were the most of the products of agripreneurs. They have been agripreneurs for about a decade. They funded their initial investment for their respective agribusinesses through their personal savings. Most of the agripreneurs in Nueva Ecija are earning below their initial investment as reflected on their monthly profit.

Agripreneurs in Nueva Ecija are motivated by job characteristics where they understood that their job requires a wide range of skills because it is not simple or repetitive. They believed their job has meaning for them because of its importance and usefulness, which meant a lot to them as agripreneurs. This contributed to how they impact the well-being of their clients. Thus, agripreneurswere satisfied with their jobs when they face personal work challenges. Then, the main motivating factor for agripreneurs was found to be job satisfaction.

Profile of the agripreneurs have positive correlations to motivating factors such critical psychological state and job satisfaction but only limited to age, highest educational attainment, number of years in the agribusiness, and average monthly income. Further, job characteristics as a motivating factor were found no significant relationships with the profile of the agripreneurs.

Future researchers may explore more on the demographic of the agripreneurs. They may include courses of the agripreneurs if these were related to their job, trainings, and seminars attended in relation to agribusiness. Studies on agripreneurs should not only focus on related to crops, poultry, and aquaculture. Agripreneurs related to innovations such as farm machineries can be included. Business profile should also include partnerships with other entrepreneurs and business agencies.

REFERENCES

[1] Bairwa, S. L., Lakra, K., Kushwaha, S., Meena, L. K., & Kumar, P. (2014). Agripreneurship development as a tool to upliftment of agriculture. International

Journal of Scientific and Research Publications, 4(3), 1-4.

[2] Bessell, I., Dicks, B., Wysocki, A., &Kepner, K. (2002). Understanding motivation: an effective tool for managers. *Florida: University of Florida*, 1-4

[3] Hoque, F., Rabbany, G., Anny, S. A., &Akhter, A. (2016). Level of job satisfaction in agribusiness sector in Bangladesh: An application of Hertzberg two factors motivation theory. *International Journal of Economics, Commerce and Management*, *4*(6), 419-445.

[4] Judge, T. A., &Kammeyer-Mueller, J. D. (2012).Job attitudes. *Annual review of psychology*, *63*, 341-367.

[5] Marri, M. Y. K., Sadozai, A. M., Zaman, H. M. F., &Ramay, M. I. (2012). The impact of Islamic work ethics on job satisfaction and organizational commitment: a study of agriculture sector of Pakistan. *International Journal of Business and Behavioral Sciences*, *2*(12), 32-45.

[6] Meyerding, S. G. (2018). Job preferences of agricultural students in Germany–A choice-based conjoint analysis for both genders. *International Food and Agribusiness Management Review*, *21*(1030-2018-1788), 219-236..

[7] Philippine Statistics Authority (2004).A Review of the Agriculture Sector in Central Luzon.released on November 8, 2004 with Reference No. 124, retrieved from https://psa.gov.ph/content/review-agriculture-sector-central-luzon on June 3, 2021.

[8] Qenani-Petrela, E., & Wolf, M. M. (2007). Differential Earnings of the Agricultural Graduates: New Evidence from the Agribusiness Industry. *International Food and Agribusiness Management Review*, *10*(1030-2016-82447), 49-66.

[9] Santiago, A., & Roxas, F. (2015). Reviving farming interest in the Philippines through agricultural entrepreneurship education. Journal of Agriculture, Food Systems, and Community Development, 5(4), 15–27. http://dx.doi.org/10.5304/jafscd.2015.054.016

[10] Ulianchenko, O. V., Sheludko, R. M., Ponomarova, M. S., &Sheludko, L. V. (2019).Motivation management as the key factor of financial and economic growth of small agribusiness enterprises. *Financial and credit activity: problems of theory and practice*, *4*(31), 239-248.

[11] Yusoff, A. (2017). Antecedents of agropreneurship intention and behavior among Malaysia Gen Y: A longitudinal approach (Doctoral Dissertation). Retrieved from https://doi.org/10.13140/RG.2.2.10970.85448

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Influence of different Temperatures and Substrates on the Germination of Munguba (*Pseudobombax munguba* (Mart. & Zucc.) Dugand.)

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***Abstract*— *Due to the ecological and economic importance of the species Pseudobombax munguba (Mart. & Zucc.) Dugand. (Munguba), it is necessary to investigate the most appropriate conditions for conducting its germination process. Therefore, the objective of this work was to evaluate seed moisture, number of seeds/kg, and the influence of different temperatures and substrates on the process of seed germination. The initial seed moisture was evaluated with samples containing four replicates of 10, 20, 30, 50, 60, 70, 80, 90 and 100 seeds dried in an oven at 105±3ºC. Germination was tested in three different substrates: sand, vermiculite, and Germitest® paper rolls (three sheets/roll) at constant temperatures of 25, 30 and 35ºC in a germination chamber. The number of 50 seeds per replicate had more homogeneous representation with 7.2% moisture. Sand or vermiculite substrates at a temperature of 25°C can be indicated to conduct the germination of the species under controlled conditions. Germination rates in sand substrate reached 69% when assessed for root protrusion, and 53% for seedling formation. Germination rates in vermiculite were 74% for root protrusion and 56% for seedling formation. The initial development was homogeneous and there were higher rates of germination speed in both substrates when assessing seedling formation.*

***Keywords*— Central Amazon, normal seedlings, root protrusion, seed moisture.**

I. INTRODUCTION

Knowing the proper conditions for seed germination is of great importance, since species can present different responses to several intrinsic factors such as dormancy, and extrinsic factors such as water, light, temperature, oxygen and the action of pathogens associated with the substrate for germination (Ferreira and Borguetti, 2004; Brazil, 2009). These authors mention that among the external factors that directly affect the germination process, temperature and substrate exert significant influence, which can have an effect on the establishment of plant communities.

Pseudobombax munguba (Mart. & Zucc.) Dugand. is a tree species of the family Malvaceae, which is typical of the Amazonian floodplains (Gribel and Gibbs, 2002; Gribel, 2003). Its seeds are consumed by the local fauna and its wood is used in the production of paper, boats and housing (Maia, 2001), thus making this species relevant for the riverside communities in the Amazon, who mostly build their houses along the banks of rivers and streams, and mainly use boats and canoes as their main means of transportation.

In view of the ecological, economic and social importance of the species, it is necessary to further the knowledge of the most appropriate conditions for conducting its germination process and ensuring the production of seedlings for timber plantations and/or enrichment of areas along the watercourses in the Amazon. Thus, the objective of this work was to evaluate the influence of temperature and substrate on the germination process of *P. munguba* seeds.

II. MATERIALS AND METHODS

The fruits used in this study were collected directly from the canopy of trees, with the aid of trimmers, when they began to naturally fall during the third week of August in the lowland areas (03°03'40.0"S - 60°06'36.1"W). The collection period took place at the end of the rainy season. As this species is typical of the floodplains of the Amazon region, its fruiting occurs from June to September (Gribel, 1995; Gribel and Gibbs, 2002; Gribel, 2003).

The degree of moisture of the seeds was defined with samples containing four replicates of 10, 20, 30, 40 (eliminated from the experiment due to an accidental occurrence and damage during manipulation in the oven), 50, 60, 70, 80, 90 and 100 seeds, which were placed in aluminum cans (6 x 5 cm - diameter/height) after the evaluation of their initial mass before drying. The opened cans were taken to drying in an oven at 105±3ºC, according to Brasil (2009). Cooling was performed in a desiccator containing blue silica.

Analyses to determine the degree of moisture of the seeds were carried out in a completely randomized design, with the analysis of variance of treatments (ANOVA). The mean values were compared by Tukey's test at the level of 5% probability (Santana and Ranal, 2004).

The effects of different substrates and temperatures on seed germination were evaluated in Gerbox® boxes containing sand, vermiculite or Germitest® paper rolls (three sheets/roll), with four replications of 25 seeds per treatment. The boxes were wrapped in transparent plastic bags, closed, and placed in germinators, at constant temperatures of 25, 30 and 35ºC, with a photoperiod of 12 h of light/dark (10 µmol m^{-1} s^{-1} of radiation).

The criteria to consider a seed as germinated were primary root protrusion (approximately 2 mm in length) and seedling formation, according to Brasil (2009). Germination was evaluated by the percentage of germinated seeds, mean germination time (days), and Germination Speed Index - GSI, according to Maguire (1964), and Santana and Rana (2004). The experimental design used was completely randomized in a 3 x 3 factorial scheme (three temperatures and three substrates). Statistical analyses were performed using analysis of variance (ANOVA), and means were compared by Tukey's test at the level of 5% probability (Santana and Ranal, 2004).

III. RESULTS

The species *P. munguba* has an average seed weight of 0.01888 g, 1000 seed weight of 18,88475 g, and number of seeds/kg of 52,953.

Seed moisture ranged between 6.8 and 8.5%, with the highest value occurring in the treatment with 10 seeds. The lowest values were observed in the treatments with 100, 90 and 80 seeds, which showed no differences by Tukey's test. However, there were no significant differences between treatments with 20, 30, 50, 60 and 70 and all other treatments (Table 1).

Table 1 – Seed moisture (%) of Pseudobombax munguba (Mart. & Zucc.) Dugand., obtained with different numbers of seeds per sample.

Samples	Means (%)	Minimum (%)	Maximum (%)	Standard Deviation	Difference between replicates (%)
10	8.5 A	7.4	9.8	1.07	2.4
20	7.6 AB	7.4	7.9	0.26	0.5
30	7.3 AB	7.0	7.6	0.28	0.6
50	7.2 AB	7.0	7.3	0.13	0.3
60	7.4 AB	6.9	8.6	0.78	1.7
70	7.1 AB	6.9	7.3	0.17	0.4
80	7.0 B	6.9	7.1	0.08	0.2
90	7.0 B	6.9	7.2	0.13	0.3
100	6.8 B	5.8	7.1	0.65	1.3
Means	7.32	6.9	7.8	0.39	0.86

C. V.: 8.18%. Means followed by the same capital letter in the column do not differ statistically from each other by Tukey's test at 5% probability.

The moisture content of 7.2% in the 50-seed treatment showed the lowest standard deviation (0.13%) in the intermediate group of treatments, with no significant differences between them and all other treatments. Differences between replicates ranged from 0.2 to 2.4% between all treatments. The 50-seed treatment had a difference of 0.3% from the others. These results show that a treatment with 50 seeds is more representative for evaluating the moisture content of *P. munguba* seeds.

The germination rates for root protrusion in *P. munguba* seeds in sand substrate at temperatures of 25°C, 30°C, and 35°C were respectively 69, 66, and 71%; in paper rolls, 64, 71, and 65%; and in vermiculite, 74, 60, and 65%. In the sand substrate there was a reduction in germination at 30°C, however, it was similar at 25°C and 30°C. In the paper rolls, germination rates were 64, 71, and 65%, with an increase at 30°C and lower values at 25°C and 35°C. Germination rates in vermiculite substrate were 74% at 25°C, 60% at 30°C, and 65% at 35°C (Figure 1 A).

The highest germination rates in each substrate were 71% in sand at 35°C, 71% in germination paper rolls at 30°C, and 74% in vermiculite at 25°C (Figure 1 A).

The highest germination rates for seedling formation were obtained in sand (53%) and vermiculite (56%), both at 25°C.

Germination rates for seedling formation at 25, 30, and 35°C were respectively: 53, 34, and 20% in the sand substrate, 17, 16, and 4% in germination paper rolls, and 56, 15 and 8% in vermiculite (Figure 1 B).

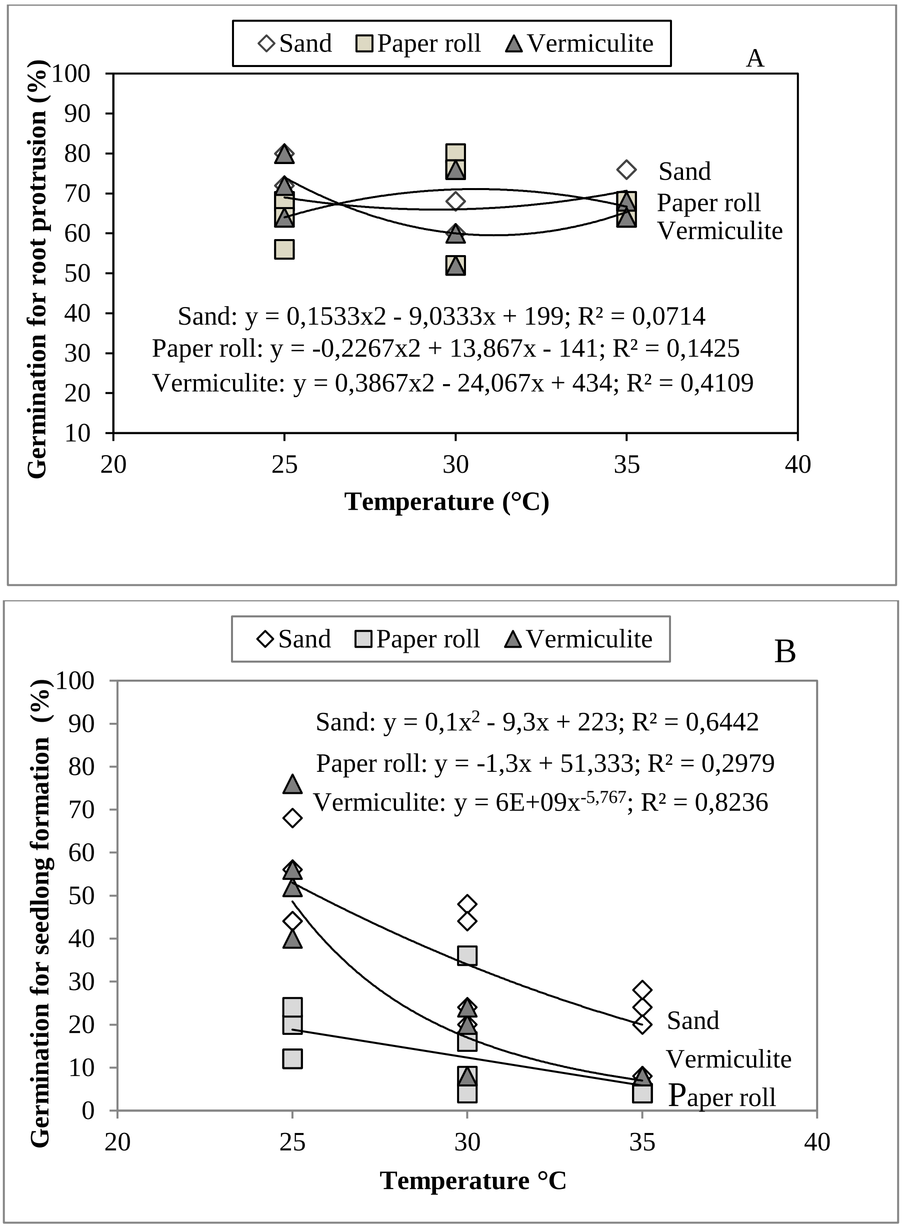
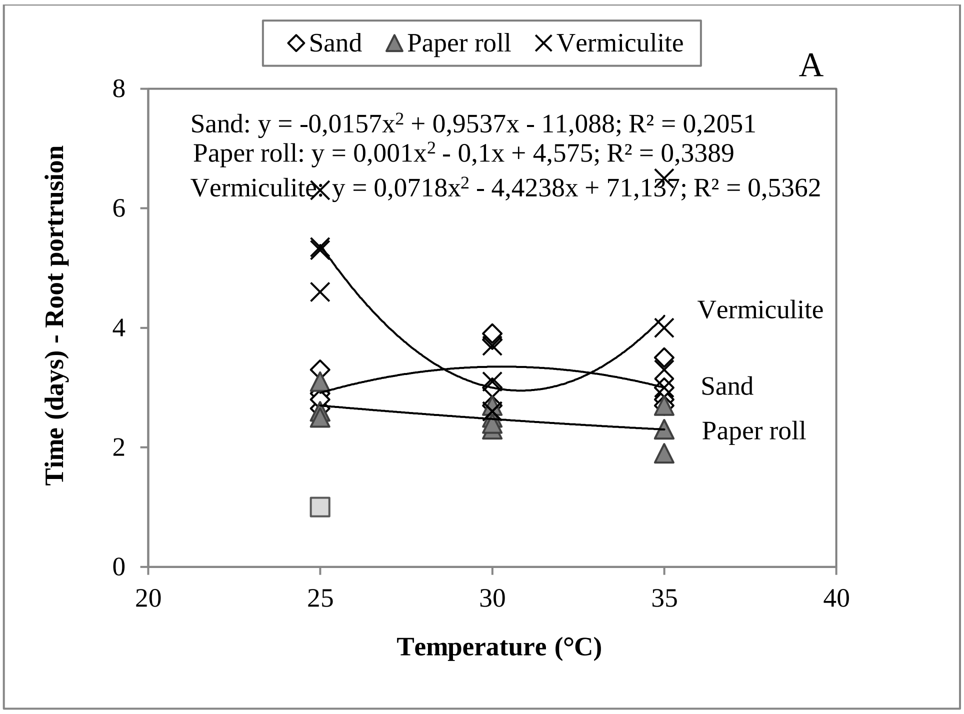


Fig.1 - Germination percentages of Pseudobombax munguba (Mart. & Zucc.) Dugand. seeds with different temperatures and substrates. A - Root protrusion; B - Seedling formation.

The highest germination rates for root protrusion show that the time between root protrusion and normal seedling formation at higher temperatures in interaction with substrates reduces the number of germinated plants. Thus, for a large-scale production of *P. munguba* seedlings, seed germination in sand or vermiculite at a temperature of 25°C is higher.

The average germination times for root protrusion at temperatures of 25, 30 and 35°C in the sand substrate were 2.9, 3.4 and 3.0 days, respectively. In paper rolls, these times were 2.7, 2.5 and 2.3 days, whereas in vermiculite, they were 5.4, 3.0 and 4.2 days.

In the sand substrate, the longest time occurred at a temperature of 30°C (3.4 days), while the shortest time occurred at the temperature of 25°C (2.9 days), which was close to the result found at 35°C (3.0 days). In germination paper rolls, these values had less variation (2.7 days at 25°C, 2.5 days at 30°C, and 2.3 days at 35°C). In vermiculite there was greater variation in germination time (5.4 days at 25°C, 3 days at 30°C, and 4.2 days at 35°C) (Figure 2 A).



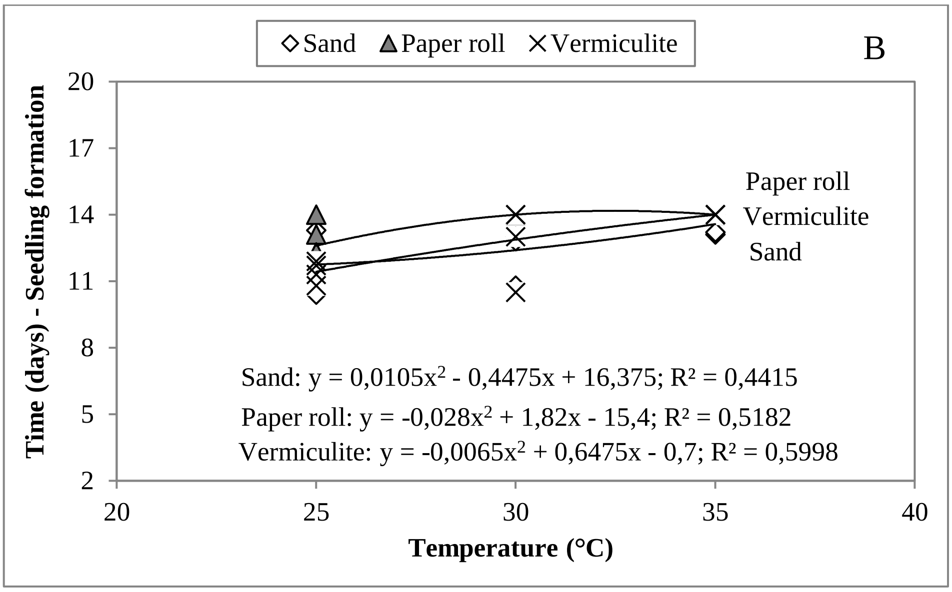


Fig.2 - Mean time (days) for seed germination of Pseudobombax munguba (Mart. & Zucc.) Dugand. as function of temperature and substrate. A - Root protrusion; B - Seedling formation.

The average times for seedling formation in the sand substrate were 11.8 days at 25°C, 12.4 days at 30°C, and 13.6 days at 35°C. In germination paper rolls the times were 12.6 days at 25°C, 14.0 days at 30°C, and 14.0 days at 35°C. In vermiculite, the times were 11.4 days at 25°C, 12.9 days at 30°C, and 14.0 days at 35°C (Figure 2B).

The shortest seedling formation times observed in this study were 11.8 days in the sand substrate, and 11.4 days in vermiculite, both at temperature of 25°C.

The time difference between root protrusion and seedling formation at 25°C was 6 days in vermiculite, and 8.9 days in sand. The longest intervals occurred in germination paper rolls at 30°C (11.5 days) and 35°C (11.7 days).

The germination speed index (GSI) values obtained for root protrusion at a temperature of 25°C were 4.9 in the vermiculite substrate, 6.1 in sand, and 6.4 in paper rolls. At 30°C, GSI values were 5.8 in sand and vermiculite, and 7.0 in paper rolls. At 35°C, GSI values were 6.6 in sand, 7.2 in paper rolls, and 5.0 in vermiculite (Figure 3A).

The GSI values obtained for seedling formation at 25°C were 1.2 in the sand and vermiculite substrates, and 0.4 in germination paper rolls. At the temperature of 30°C, GSI values were 0.7 in sand, and 0.3 in both paper rolls and vermiculite. At 35°C, GSI values were 0.4 in sand, and 0.1 in both paper rolls and vermiculite (Figure 3B).

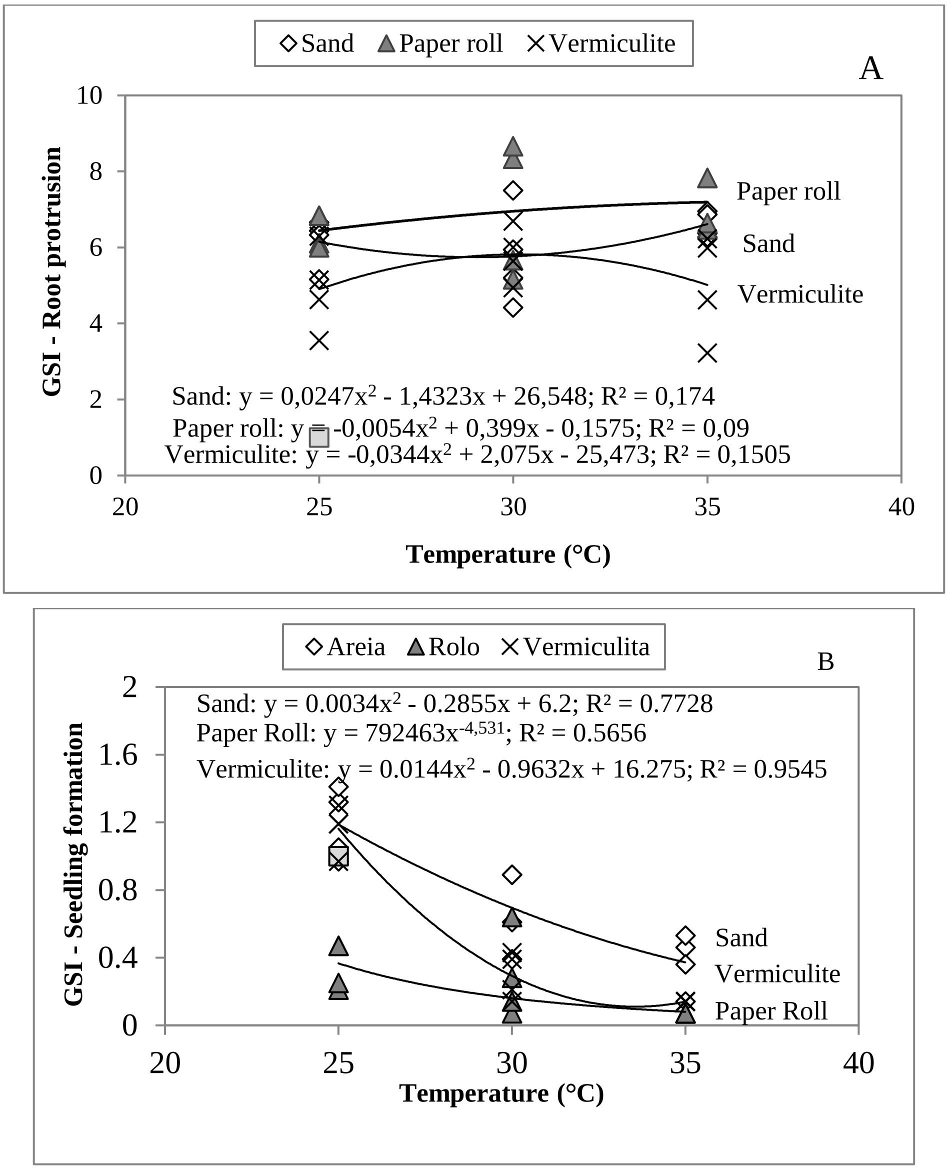


Fig.3 - Germination Speed Index (GSI) of seeds of Pseudobombax munguba (Mart. & Zucc.) Dugand., obtained with different temperatures and substrates. A - Root protrusion; B – Seedling formation.

The highest GSI values concerning to root protrusion occurred in the germination paper roll substrate (7.0 at 30°C, and 7.2 at 35°C). On the other hand, the lowest value (4.9) was observed in the vermiculite substrate at 25°C. The highest GSI value for seedling formation (1.2) was observed in sand and vermiculite substrates at 25°C, whereas the lowest value occurred in germination paper rolls (0.07).

The highest germination rates for seedling formation were obtained when using sand and vermiculite substrates at a temperature of 25°C, which proved to be more efficient than the temperatures of 30 and 35°C. There were smaller differences in the mean germination time between root protrusion and the formation of seedlings, thus showing greater homogeneity in the germination process of *P. munguba* seeds.

The lowest germination rates for seedling formation were obtained when using germination paper rolls. The results showed the greatest time differences between root protrusion and seedling formation, the highest GSI values in root protrusion, and also the lowest values in seedling formation at all temperatures of the experiment. Therefore, germination paper rolls are less suitable for *P. munguba* seed germination and seedling formation.

IV. DISCUSSION

The number of seeds/kg of the species *P. munguba*, calculated from its 1000 seed weight (18,88475 g) was 52,953. These results were obtained from the seeds of 5 trees, collected in the same location, on a beach in the city of Manaus.

However, the number of seeds/kg of 67,558, calculated with data from Meniccuci (2007), was obtained from 16 to 43 individuals of each population in 11 locations in the Amazon: Beruri – AM, Caracarai – RR, Catalão – AM, Cruzeiro do Sul – AC, Caxiuanã National Forest – PA, Japurá River – AM, Madeira River – AM, Paraná do Mapixi by Purus River – AM, Tefé – AM, Tabatinga – AM (right bank), and Tabatinga – AM (left bank). Analysis of molecular variance using all populations showed that most of the genetic variation found can be explained by the variation contained within populations (59%). When excluding monomorphic populations, this value rose to 66%. Analysis by the Mantel test, which correlates genetic and geographic distances between the populations analyzed in pairs, indicated that there is no isolation by distance between the populations of *P. munguba* in the Brazilian Amazon.

Given the above, these differences can be understood by the potential genetic variability that must exist in the species. Gribel and Gibbs (2002) reported that although *P. munguba* does not have a conventional mechanism for stigmatic or stylar self-incompatibility, the combination of exclusive pollination by a wide-ranging bat vector and the death of most self-fertilized eggs provide this species with an outcrossing breeding system.

The result of 7.2% moisture in the treatment with 50 seeds, which showed the smallest standard deviation (0.13%) and a 0.3% difference between replicates, made it possible to consider it as the most representative treatment for moisture evaluation with this number of *P. munguba* seeds. According to the Seed Analysis Rules (Brasil, 2009), when the number of seeds/kg is greater than 5,000, the difference between replicates must be lower than 0.6%, which happened in this treatment, since the difference was 0.3%. Germination rates for the formation of seedlings also showed the highest values in sand or vermiculite substrates at a temperature of 25°C, and inferior results at 30 and 35°C. There were smaller differences in the mean germination time between root protrusion and seedling formation, and there was greater homogeneity in the germination process of *P. munguba* seeds. Similar results were found by Ladeia *et al*. (2011) and Ladeia *et al*. (2012) in the germination of a species of the same genus as *P. munguba* (*Pseudobombax longiflorum*). The authors concluded that the most suitable treatment for the production of seedlings was by using sand substrate at a temperature of 30°C.

A treatment with sand at a temperature of 25°C also resulted in higher rates of *Acosmuim nitens* seed germination and seedling formation (Varela *et al*., 2005). Barbosa (1995) also found higher seed germination rates at 25°C with the species *Rheedia benthamiana* P er Tr. and *Couroupita guianansis* Aubl. for the production of seedlings used in revegetation in areas affected by a hydroelectric power plant. Those plants, similarly to *P. munguba* also grow naturally on the banks of watercourses in the Amazon.

V. CONCLUSIONS

The species *Pseudobombax munguba* has 52,953 seeds/kg and its seeds have water content between 6.4 and 8.5%. When germinated under controlled conditions, in sand or vermiculite substrates at a temperature of 25°C, they reached the highest germination percentages (53 to 56%), the most homogeneous initial development of seedlings, higher GSI levels, and a large number of seeds per kg. This species has become highly important for riverside populations, as well as for reforestation or the recovery of areas degraded by deforestation along watercourses in the Amazon.

REFERENCES

[1] Barbosa, A.P. 1995. Estudos de espécies florestais amazônicas visando a revegetação de áreas de depleção da usina hidrelétrica de Camargos, Itutinga, MG. **Tese de Doutorado**; Universidade Federal de Lavras, MG. 96 p.

[2] Brasil. 2009. ***Regras para Análise de Sementes***. Ministério da Agricultura e Reforma Agrária. SNDA/DNDV/CLAV, Brasília, 395pp.

[3] Ferreira, A. G.; Borghetti, F. ***Germinação: do básico ao aplicado***. Porto Alegre: Artmed, 2004. 323 pp.

[4] Gribel, R. 2003. Polinização por morcegos em Bombacaceae: Conseqüências para o sistema reprodutivo e estrutura genética das populações. In: Jardim, M.A.G.; Bastos, M.N.C.; Santos, J.U.M. (Eds.). ***54º Congresso Nacional de Botânica****, 2003, Belém. Desafios da Botânica no Novo Milênio: Inventário, Sistematização e Conservação da Diversidade Vegetal.* MPEG, UFRA, EMBRAPA, Belém, PA. p.108-110.

[5] Gribel, R. 2005. Reproductive biology of two bombacaceae trees in the Brazilian Central Amazon. **Doctor of Philosophy's Thesis.** University of St. Andrews, United Kingdom, 185 p.

[6] Gribel, R.; Gibbs, P.E. 2002. High outbreeding as a consequence of selfed ovule mortality and single vector bat pollination in the Amazonian tree *Pseudobombax munguba* (Bombacaceae). ***International Journal of Plant Sciences,*** 163(6):1035-1043.

[7] Ladeia, E.S.; Coelho, M.F.B.; Azevedo, R.A.B. 2011. Germinação de sementes de *Pseudobombax longiflorum* (Mart. &Zucc.) A. Robyns. (Malvaceae) de duas procedências em diferentes temperaturas. ***Revista de Ciências Agrárias***, 54(3): 290-298.

[8] Ladeia, E.S.; Coelho, M.F.B.; Azevedo, R.A.B.; Albuquerque, M.C.F. 2012. Procedência do fruto e substratos na germinação de sementes de *Pseudobombax longifl*orum (Mart. &Zucc.) A. Robyns. ***Pesquisa Agropecuária Tropical***, 42(2): 174-180.

[9] Maguire, J. O. 1964. Speed of germination and in selection and evaluation for seedling emergence and vigor. ***Crop. Science***, 2(2):176-177.

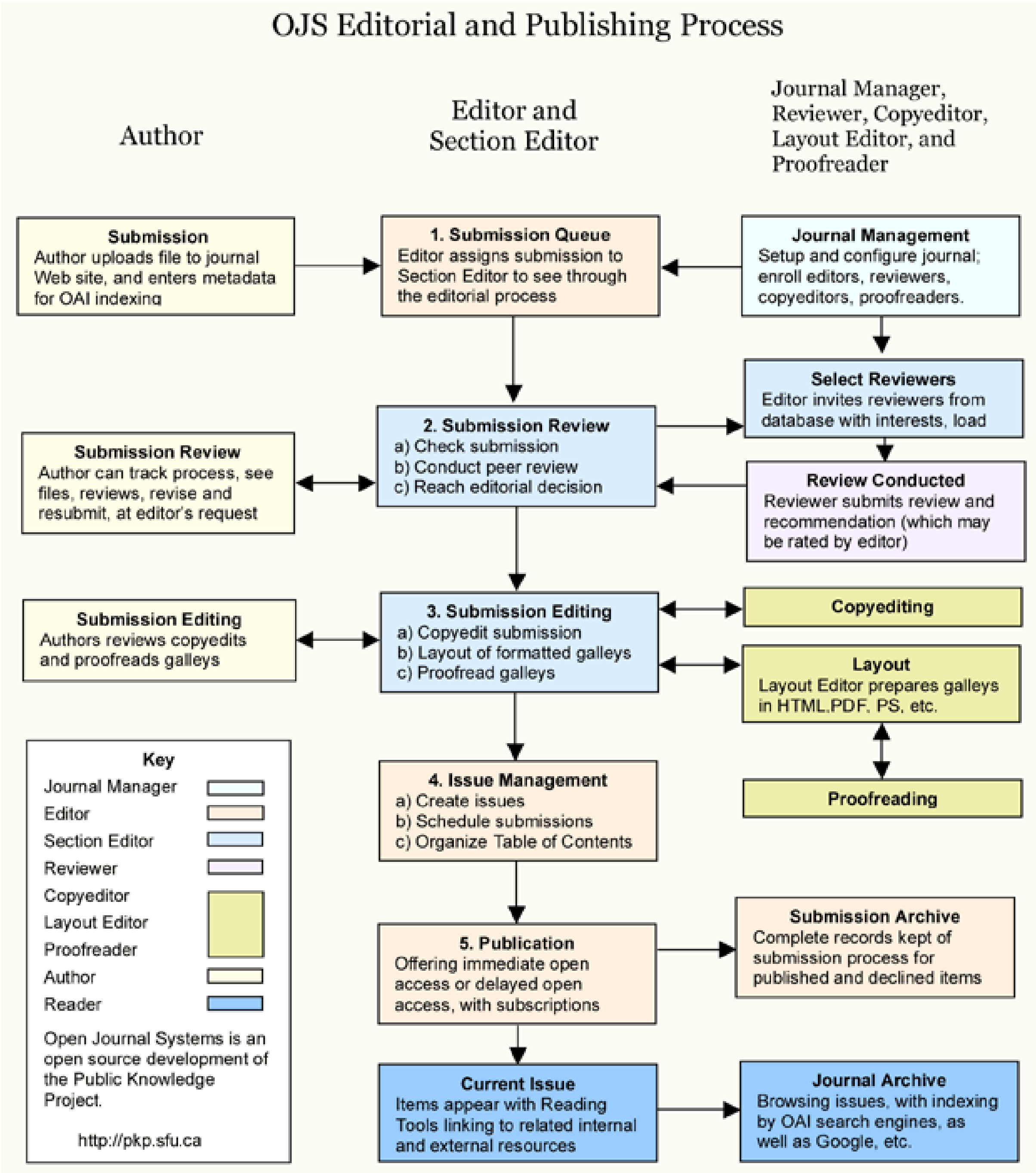
[10] Maia, L.M.A. 2001. **Frutos da Amazônia: fonte de alimentos para peixes.** *Programa de Desenvolvimento Empresarial e Tecnológico.* INPA, SEBRAE/AM. Manaus. 143pp.

[11] Menicucci, T.A. 2007. Fitogeografia e estrutura genética de populações de mungubeira (*Pseudobombax munguba* Mart & Zucc.) Dugand, Malvaceae – Bombacoideae) na Amazônia Brasileira. **Dissertação**. Mestrado em Genética, Conservação e Biologia Molecular. INPA/UFAM – Programa Integrado de Pós-Graduação em Biologia Tropical e Recursos Naturais. 66pp.

[12] Poulsen, K.M.; Parrat, M.J.; Gosling, P.G. (Eds.). **Topical and subtropical tree and shrub seed handbook**. Zurich: ISTA,1998. 204p.

[13] Santana, D.G.; Ranal, M.A. 2004. **Análise estatística.** In: Ferreira, A.G.; Borguetti, F. (Eds.). *Germinação: do básico ao aplicado*. Artmed, Porto Alegre. p.197-208.

[14] Varela, V.P.; Costa, S.S.; Ramos, M.B.P. 2005. Influência da temperatura e do substrato na germinação de sementes de itaubarana (*Acosmium nitens* (Vog.) Yakovlev) – Leguminosae, Caesalpinoideae. ***Acta Amazonica***, 35(1): 35-39.



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