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FOREWORD

I am pleased to put into the hands of readers Volume-5; Issue-6: Nov-Dec 2020 of **"International Journal of Environment, Agriculture and Biotechnology (IJEAB) (ISSN: 2456-1878)",** an international journal which publishes peer reviewed quality research papers on a wide variety of topics related to **Environment, Agriculture and Biotechnology**. Looking to the keen interest shown by the authors and readers, the editorial board has decided to release issue with DOI (Digital Object Identifier) from CrossRef also, now using DOI paper of the author is available to the many libraries. This will motivate authors for quick publication of their research papers. Even with these changes our objective remains the same, that is, to encourage young researchers and academicians to think innovatively and share their research findings with others for the betterment of mankind.

I thank all the authors of the research papers for contributing their scholarly articles. Despite many challenges, the entire editorial board has worked tirelessly and helped me to bring out this issue of the journal well in time. They all deserve my heartfelt thanks.

Finally, I hope the readers will make good use of this valuable research material and continue to contribute their research finding for publication in this journal. Constructive comments and suggestions from our readers are welcome for further improvement of the quality and usefulness of the journal.

With warm regards.

Editor-in-Chief
Date: Jan, 2021

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Ovarian function and pregnancy of dairy goats supplemented with pequi oil

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Abstract *— The objective of the work in question was to evaluate the ovarian function of Saanen goats supplemented with pequi oil. The experiment was carried out at the IFCE Ovine Caprinoculture vivarium, campus Crato. Eight pure Saanen goats were used, weighing an average of 1025,15 pounds, distributed in a completely randomized design with two treatments. The first treatment constituted soy oil as the lipid source and the second treatment with pequi oil. Two estrous cycles were evaluated to analyze the reproductive parameters of the females. In the first cycle, a non-castrated sheep was used as a ruffian to identify estrus, and the ovarian function was evaluated for the presence and number of follicles by ultrasound. In the second cycle, a Saanen was used in order to mate with the females. Ultrasound was performed to assess conception, fertility and prolificacy rates. The computer software SAEG 9.1 was used to analyze the values obtained. It was found that females supplemented with soybean oil stood out in comparison to goats treated with pequi oil, except for body weight, follicular diameter and body condition score, concluding that the use of pequi oil for Saanen goats it presents itself as an economically unfeasible alternative, as it has a higher acquisition value.*

Keywords *—* ***Goat, estral cycle, flushing, energetic, reproduction animal.***

I. INTRODUCTION

The Northeast region stands out from other Brazilian states in relation to the predominance of the practice of goat farming, with more than nine million goats, representing more than 90% of the national herd [8]. Dairy goat farming has been standing out in the semi-arid region of Brazil, being characterized as a profitable and viable practice mainly for small producers [6].

However, the precariousness of technical investments results in extensive or semi-intensive production as being the majority of the production systems found in the country [31], demanding from the animal good rusticity and adaptability to the prevailing climate in the region.

Among the main breeds used in dairy production, Saanen stands out. These animals are exotic in Brazil and need more intensive food handling when compared to other breeds, due to their clear coat and pink skin, which is less conducive to acclimatization in hot regions such as the semiarid [20]. Even with these characteristics, they are widely used due to the excellent milk production. However, several factors can influence this type of production, including reproductive and nutritional efficiency [15].

Nutrition plays an extremely important role in the demand for energy, protein and other vital nutrients for the productive and reproductive process of animals [32].

Supplementation is commonly performed aiming at increasing reproductive performance, which consists of external protein and / or energy supplementation. In goat females, lipid supplementation is found to be directly related to the ovulation rate, having been recommended at different times before the breeding season, during the season, during pregnancy, in pre and postpartum [18] and can be strategically effective in increasing the energy density of the diet of ruminant females.

The supply of a diet rich in lipids abundant in linolenic and linoleic unsaturated fatty acids increases the process of gluconeogenesis by raising propionate in the fermentation chamber, thereby stimulating insulin secretion, modulating follicle growth, oocyte maturation and embryo development [10].

With insulin secretion and through its action, gonadotropin-releasing hormone (GnRH) is released and secretes follicle stimulating hormone (FSH), luteinizing hormone (LH) and progesterone which in turn regulate ovarian activity and pregnancy, respectively [13].

For [18] supplementation rich in vegetable oils that has linoleic and linolenic acids in its composition, propels the process of gluconeogenesis by the influence of the increase of propionate produced, stimulating insulin and this in turn, modulates the growth of follicles, as it acts on regulation of the synthesis of gonadotropin-releasing hormone (GnRH) neurotransmitters; oocyte maturation in the sense that it controls the release of luteinizing hormone by the pituitary gland; and embryonic development.

In Brazil, due to its great biodiversity, there is a huge variety of vegetable oils extracted from fruits of endemic and regional species, such as pequi oil. The pequizeiro (Caryocar coriaceum) occurs in the semi-arid Northeast, commonly in the cariri from Ceará and Piauí. The pequi pulp is abundant in lipids, with more than 30% in its composition [14]. There are several studies on the use of pequi oil in the manufacture of natural medicines for animals and humans [24; 21; 23]. However, its use in animal supplementation is scarce, with few studies as a lipid source [25] and offspring of rats [19].

Pequi oil is a product rich in unsaturated fatty acids. Palmitic and oleic fatty acids are found in abundance in the pequi pulp [9; 14], with 41.1% and 54%, respectively. It is also composed of myristic (0.2%), palmitoleic (0.5%), stearic (1.9%), linoleic (1%) and linolenic (0.3%) acids in smaller quantities [7]. Studies show that these acids can interfere in the synthesis of steroids [33], serving as important precursors for the transition from the stage of cows luteogenesis [4].

In addition, in pequi oil there are phenolic compounds, being found in greater amounts in the peel (pericarp) that guarantee the fruit the role they play as antioxidants [35], together with carotenoids. Antioxidants are extremely important in reproduction, because in their absence, there will be a greater amount of free radicals, consequently causing oxidative stress causing deleterious effects on the female reproductive system, affecting from the oocyte maturation process until pregnancy [29].

Thus, the general objective of this work was to evaluate the effect of pequi oil as a lipid supplementation inserted in the concentrated food, on the performance and reproductive parameters of goats.

II. METHOD

The experiment was carried out at the Federal Institute of Education, Science and Technology, Campus Crato-CE, in the Ovinocaprinoculture production sector between May and July 2018, located by geographic coordinates: latitude 7 ° 12 '43 ”S and longitude 39 ° 26 '35 ”W and an altitude of 542m. This research was submitted to evaluation and approved by the IFCE Animal Use Ethics Committee, under protocol CEUA No. 4828200418.

Eight adult Saanen goats were used, with an average age of three years, with an average weighing an average of 1025,15 pounds and empty, with fertility proven through previous births. The animals were kept on the premises for a period of 15 days for prior adaptation. The goats were kept throughout the day in paddock irrigated by sprinkling and cultivated with Tifton 85 grass (Cynodon dactylon). At the end of the day, the animals were collected and contained with access to individual troughs, where the concentrate with supplementation was provided, totaling 90 days of the experimental period.

To evaluate the effect of supplementation with pequi oil on performance and reproductive parameters, the goats were divided into two groups, each group containing four animals. The control group consisted of females supplemented with soy bran-based concentrate (3%), ground corn grain (87%), urea (1%), mineral (2%) soybean oil (7%) and Caprinophos (Tortuga®). For the test group, the goats received the concentrate similar to the control group, differing from the vegetable oil used, which in this group was pequi oil (7%). During the supplementation period, the goats received a daily supply of 400 g of concentrate formulated based on the recommendations of nutritional requirements of the [17] for empty goats in the breeding season. The goats were identified by means of numbers attached to the ear and for better distance

observation, ribbons with different colors were placed around the neck.

The females were weighed weekly and the body condition score was assessed through visual assessment and palpation of the transverse and spinal apophyses. Fifteen days after the start of supplying the supplements, the goats were accompanied by an adult male sheep, not castrated, for the purpose of breeding. After acceptance of the mount by all females, ultrasound was performed on them on the day or the next day, to observe the number of follicles present and their diameter, implying knowing the influence that lipid supplementation was having on these animals.

At the beginning of the second estrous cycle, the females continued to be accompanied by an adult goat breeder of the Saanen breed, in order to allow the breeding, monitoring of the ovarian follicles as described for the first cycle. After thirty days of mating, a gestational diagnosis and evaluation of the functional corpus luteum was performed for all females. The reproductive efficiency of the goats was evaluated based on the pregnancy rate, fertility and prolificacy, where the pregnancy rate was obtained through the ratio of the number of positive diagnoses and the number of diagnoses performed.

The evaluation by means of ultrasonography was performed with a linear multifrequency probe, on the white line, in front of and close to the mammary gland, after a previous fast of at least 24 hours. The device used for ultrasound was the Mindray 3.300 Vet. The frequency used was 7.5 MhZ (megahetz), as shown in figure 1.

Fig.1: Ultrasonography with linear multifrequency probe to observe the number and diameter of follicles and to evaluate the corpus luteum

Source: Geo Brasil (2018)

The experiment was conducted in a completely randomized design with two treatments and four replications and the results obtained were subjected to analysis of variance and those that showed significance were subjected to the Tuckey test, with 5% significance, to compare the means using the package computational System for Statistical Analysis - SAEG 9.X.

III. RESULTS AND DISCUSSIONS

The goats that received pequi oil supplementation had a larger follicular diameter compared to the control treatment. Table 1 shows that the body weight of goats treated with the lipid supplementation of pequi oil stood out from the control treatment goats, which influences their body condition score index, that is, these goats had a greater amount of fat and muscles in bone structures.

Table 1. Zootechnical indexes of the goats according to their respective treatments.

	Body weight (kg)	Body condition score	Follicular Diameter (mm)
Pequi Oil	41,48	2,4	14,47
Soy oil	36,28	2,3	11,07

Source: Author (2018)

The increase in ovulation rate and prolificacy are dependent on an agile increase in the animal's body condition score. Supplementation provides this increase, since the moment before the breeding it is necessary to provide the animal with a supplementation of nutrients that improve its reproductive capacity, as well as its body conditions, which is one of the aspects that causes a benefit in reproduction [22].

The increase in the body condition score refers to the consumption of energy diets, causing a reduction in the concentration of insulin that minimizes the entry of glucose into the cell, causing a decrease in the steroid hormones that modify the quality of the oocyte, namely progesterone and estradiol. , also changing follicle growth and embryo viability [36].

In a study by [16] goats receiving high energy concentrated supplementation had a positive increase in body weight during pregnancy and reproductive performance, concluding that low weight and consequently low body condition score can affect fertility. For [1] the body condition score is an important practical methodology in the management of high production dairy animals, as they need adequate body reserve for energy and protein requirements during the beginning of lactation.

It was observed that for the treatment with pequi oil the follicular diameter was larger. Follicular diameter is directly related to the diameter of the oocyte and its competence in embryonic development [26]. The diameter is also related to the estradiol concentration rates and its increase is linked to the probability of ovulation and conception rate, being directly related to the corpus luteum in the final formation process. The corpus luteum of reduced volume is due to the formation of smaller follicles, strongly influencing less development of the embryo and as a consequence, low fertility.

For [27], linoleic and linolenic polyunsaturated fatty acids are important for fertility, as they affect follicle size, oocyte quality and corpus luteum size. This is because they have an effect on the concentration of steroid hormones and prostaglandins.

In a study with beef heifers supplemented with soybean oil it was observed that the quantity and diameter of the follicles increased [34]. These results are similar to those found in this study, according to the ovarian indices.

In studies by [12] with goats supplemented with soybean oil and barley, obtained as a result that these dietary lipid compounds increased the levels of progesterone in the blood during the period of ovulation and post-ovulation. According to research by [11], soybean oil is composed of the mystic fatty acids (<0.5), palmitic (7.0-14.0), stearic (1.4-5.5), linolenic (4.0-11 , 0) oleic (19.0-30.0) and linoleic (44.0-62.0), the latter two being characterized as more abundant. However, soy is rich in phytoestrogen, similar to the hormone estrogen and that mimics its functions, binding to its receptors.

For the control treatment, a larger amount of follicles was observed in a higher number of animals compared to the treatment with pequi oil. Table 2 shows the treatments according to the supplementation of the goats in the two estrous cycles. For the evaluated parameters, it is possible to highlight the beneficial effect that the pequi oil caused on the ovarian function.

Table 2. Number of follicles and embryos in Saanen goats supplemented with concentrate containing pequi oil or soybean oil, according to the EC (estrous cycles).

Treatment	Parameter	Animal				
		A	B	C	D	Média
1° EC Pequi oil	Number of embryos	3	1	1	1	1,5b
1° EC Soy oil	Number of embryos	4	4	5	1	3,5a
2° EC equi oil	Number of embryos	0	1	1	1	0,75b
2° EC Soy oil	Number of embryos	3	2	1	1	1,75a

* Lowercase letters compare averages in the column. Means followed by different letters differ from each other by Tukey's test at 5% significance.

Source: Author (2018).

In the first estrous cycle, the number of follicles for each treatment differed statistically, as well as for the number of embryos in the second estrous cycle, being higher in goats that received soy oil as a lipid supplement in the concentrate.

For [28], the brief supplementation that contains in the high energy capacity diet, simultaneously with the estrus synchronization, provides an increase in estrous activity, increasing the performance in sheep reproduction. In a study by the same authors, the objective was to assess whether supplementation, that is, a short-term high-energy diet, has any overstimulating effect on ovarian function in sheep, with the conclusion that supplementation improved estrus expression and ovarian activity .

The positive results in the zootechnical indexes in the test treatment with pequi oil may be due to components present in the oil of this fruit, such as the antioxidants that cause the balance of free radicals, which can generate negative changes in the maturation and fertilization processes of the oocytes.

The role of fatty acids on the quality of the embryo and concentration of levels of steroid hormones and prostaglandins proved their influence on the ovarian function of bovine females. Soy oil is abundant in linoleic and linolenic acids.

They obtained as a result that for the variables of superovulation, embryonic recovery rate and embryonic quality there was no significant difference, but even with the increase in polyunsaturated fatty acids, the production of arachidonic acid, which is a precursor of prostaglandins, decreased - an increase in prostaglandin production is expected, making the environment more luteotrophic, with the exception of a reduced number of degenerate embryos after supplementation with n-3 (polyunsaturated FA).

In research by [3] using sources of vegetable oils, sunflower and flaxseed, it was observed that polyunsaturated fatty acids had beneficial effects on the size of the ovarian follicles, as well as on the size of the corpus luteum.

According to previous information, it is known that linoleic and linolenic polyunsaturated fatty acids have an unquestionable role in reproduction, since they serve as precursors for processes that increase the energy density of the ruminant, influencing its ovarian function. Soy oil has these fatty acids in greater abundance when compared to pequi oil, with much lower percentages for the latter, which may hypothetically have influenced that the treatment with soy oil has positively excelled in the parameters of fertility, prolificacy and conception.

The amount to be used in the concentrate is also a major factor in terms of its functionality, however, the excess (above 7%) of fat can decrease the digestion of fiber by the ruminant, since there is the covering of its particles, preventing it from being degraded by microorganisms, negatively influencing the proportions of short-chain fatty acids to be produced in the rumen [30]. According to these authors, there is a reduction in insulin concentration and stimulation of body fat mobilization

However, factors such as degree of saturation, form of inclusion of the lipid source in the diet and length of the chain are preponderant in the responses of the animals [18].

IV. CONCLUSIONS

Although in the zootechnical parameters of body condition score and ovarian follicular diameter the animals have benefited more with lipid supplementation in comparation with pequi oil, for the other indexes the treatment with soybean oil obtained a higher value in the ovarian function of Saanen dairy goats.

The use of Pequi oil can become an economically unviable alternative based on the results obtained in this work, with the current reality being the greater use of soy oil, in addition to being easily found and having a lower purchasing value.

Because research related to the use of pequi oil in the ovarian function of ruminants is non-existent and vegetable oils are scarce, other studies should be conducted with a larger number of animals, as well as with other breeds and / or crosses to consolidate the results here reported. As well as the type of management (confined or grazed) can interfere in the results.

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Evaluation of Heavy Metal Pollution from Vehicular Exhausts in Soils along a Highway, Southwestern Nigeria

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***Abstract*—** *The Lagos-Ibadan Expressway is the busiest inter-state route in Nigeria and one of the largest road networks in Africa. It handles more than 250,000 PCUs (Passenger Car Units) daily. Amount of metal emissions being released daily from vehicles plying this road into the environment (air, soil, plant and water) is great! This study evaluated heavy metal composition in soils resulting from exhausts pollution from vehicular movement along Lagos-Ibadan Expressway. A total of two hundred and seventy-six soil samples were collected at 5, 15 and 25 m away from the edge of the road. The soil samples were collected with hand auger from the surface to a depth of 10 cm during both dry and wet seasons. Heavy metal concentrations were determined using Atomic Absorption Spectrophotometer (AAS). The heavy metal concentrations found in sampled soil during dry season in mg.kg^{-1} along the study sites revealed the following, Zn (558.03, 21.98), Pb (130.96, 3.64), Cr (24.08, 1.09), Cu (97.43, 4.41) and during wet season Zn (532.51, 5.72), Pb (120.52, 1.85), Cu (79.90, 1.05), Cr (19.82, 0.22). The results indicated a general reduction in heavy metal concentrations in the soil collected during rainy season, when compared with soil collected during dry season. Index of geo-accumulation (I_{geo}) of sampled soils revealed that contamination ranged from moderate to extreme in all the study locations during dry and wet seasons. Potential ecological risk Index (PERI) of the individual location in the study area ranged from 4.38 to 146.84. However, the total PERI of study area was 1136, which indicated that Lagos – Ibadan Expressway is generally polluted with heavy metals. Fe, Mn, Zn, Pb, Cu and Cr are the major heavy metal pollutants found in the soils during dry and wet seasons. The source of these heavy metals being vehicular exhausts emissions along Lagos – Ibadan Expressway.*

***Keywords*— Contamination, heavy metals, Potential Ecological Risk Index, soil, vehicular exhausts.**

I. INTRODUCTION

In many parts of the world today, vehicular exhaust emissions from heavy-duty trucks and other vehicles constitute one of the sources of plant and soil pollution (Akbar *et al.,* 2006). Olukanni and Adebiyi, (2012); Ezemokwe *et al.,* (2017) and Fosu-Mensah *et al.,* (2017) observed that heavy metals affect the ecosystem especially along major highways through these emissions. Soils along roadsides often contain high concentrations of heavy metals and other contaminants. These metals are released from fuel burning (either gasoline or diesel), wearing out of tires, leakage of oils, and corrosion of car metal parts (Dolan *et. al.,* 2006; Olukanni and Adebiyi, 2012). The resultant effect has adversely affected plants, soil, water, air and animals including humans in many urban areas around the world.

Lagos-Ibadan Expressway is a major highway in southwestern Nigeria that links other parts of the country. Nigeria, like other developing countries and some developed countries, has a problem of roadside pollution.

As Africa's most populous nation, Nigeria has its share of smog-filled cities, congested roads, aging vehicles, leaded fuel usage among others, which invariably resulted in large scale pollution of our highways by heavy metals through emissions from vehicle exhausts.The road under study (Lagos – Ibadan Expressway) is one of the busiest highways in Africa. It regularly witness vehicular traffic congestion since it was commissioned in 1978 and vehicular pollution has not been checked by environmental regulatory authorities, leading to elevated levels of pollution year in year out. The pollution level is critical and might be attributed to the poor economic situation of Nigerians. Large importation of old and fairly-used cars and poor vehicle maintenance culture cause increase in the emissions of dangerous substances through the exhaust pipes of vehicles. Large number of irreparable and decomposing car parts litter the road surfaces and roadsides. These expose the residents to serious health risks.

Little or no attention has been placed on metal contamination of the major roads in the country despite its direct contact with a greater part of the population. Poszyler-Adamska & Czerniak (2007) considered vehicle exhausts as first line of source of heavy metal pollutants in any major highway. Simon *et al.* (2013) pointed to the role of traffic emissions in the pollution of Wien soil by Cu, Pb, and Zn. These are the most important metal pollutants from heavy traffic exhaust owing to their presence in fuel as antiknock agent (Suzuki *et al.*, 2009; Atayese *et al.*, 2010). Hence, this present work deals with the evaluation of heavy metals pollution in soils along Lagos-Ibadan Expressway, south western Nigeria, from vehicular exhausts emissions. The objectives of the study are to determine the: (i) concentrations of the prevalent heavy metals in soils along Lagos – Ibadan Expressway from vehicular exhausts emissions; (ii) seasonal effects of heavy metals on soil along the highway; and (iii) level of pollution along Lagos-Ibadan expressway using Pollution Assessment Indices.

II. MATERIALS AND METHODS

2.1 The study area

The study was carried – out along Lagos-Ibadan expressway. The highway stretches from Ibadan (07º19.647'N, 003º52.528'E, 170 m a.s.l) through Ajanla Farms (06º55.140'N, 003º38.174'E, 58 m a.s.l) to 7up Bus stop at the Lagos end (06º35.976'N, 003º22.710'E, 30 m a.s.l). It covers a distance of 115 km.

The topography from Ibadan end of the road is high hills ranging from 58m to 170 m above sea level (Fig. 1) and characterized by low lying lands with gentle slopes in the vicinity of Sagamu Interchange.

Geologically, Lagos-Ibadan expressway is partly underlain by the crystalline rocks of the Precambrian Basement Complex and partly by the sedimentary rocks of the Dahomey basin. The Dahomey basin is a West African Atlantic Margin basin which is made up of Tertiary to Recent and Creteceous sediments (Omotsola & Adegoke, 1981; Obaje, 2009). Lying unconformably above the basement rocks is Abeokuta formation. This formation is overlain by Ewekoro formation which is in turn overlain by the Benin formation (Coastal Plain Sands).

2.2 Sampling Design

The area sampled covered a distance of 115km from Lagos end to Old Tollgate in Ibadan. Sampling started from 7up Bus - stop in Lagos through Ajanla Farms to Tollgate in Ibadan (Table 1). Soil samples were collected at intervals of 5 km in a zigzag manner across the expressway (Fig. 2). The samples were collected using hand auger from the surface to a depth of 10 cm. At each location, three subsamples were taken at 5 m, 15 m and 25 m away from the edge of the road (Fig. 3). These three subsamples were then made into one representative sample. The collected soil samples were stored in polythene bags and labelled apropriately and the coordinates of the sampling locations taken with a Global Positioning System (GPS). This sampling was done during dry and wet seasons for two consecutive years – 2015 and 2016. In all, a total of two hundred and seventy-six (276) samples, consisting of sixty- nine (69) samples each in both dry and wet seasons, were collected throughout the duration of the sampling exercise. Spots with obvious signs of disturbance, such as animal burrowing, engine oil spillage and landfills, construction sites, accidental vehicle deposit sites, burnt materials and/or deposits of any industrial waste were avoided. The samples were then taken to the laboratory for analysis.

2.3 Soil preparation, digestion and analysis

The soil samples were air-dried at room temperature to remove moisture. Thereafter, they were disaggregated and sieved using 2mm sieve size prior to digestion. The sieved soil samples were, then, packed into bottles and labelled appropriately. 5 g of the sieved samples were digested by adding HNO_3/Perchloric acid (2:1) and the mixture was then placed inside a digestor until a colourless fume was observed. It was then removed, filtered and the required volume of 50 ml was made by adding ultra-pure water. The digested samples were kept in clean polyethylene bottles in a refrigerator prior to analysis. Three replicates of the digested samples were then analyzed for the

following heavy metals: Cr, Pb, Cu, Zn, Fe, Mn, Ni and Co using Atomic Absorption Spectrophotometer (Buck Scientific AAS, Model 210VGP, Chapman & Pratt 1961). All the concentrations were recorded in mg/kg. Accuracy and precision of the analytical procedures were ensured by the analyses of standard reference materials and replicate samples. Blanks were also analyzed, contents of all the elements in the blanks were below the detection limits.

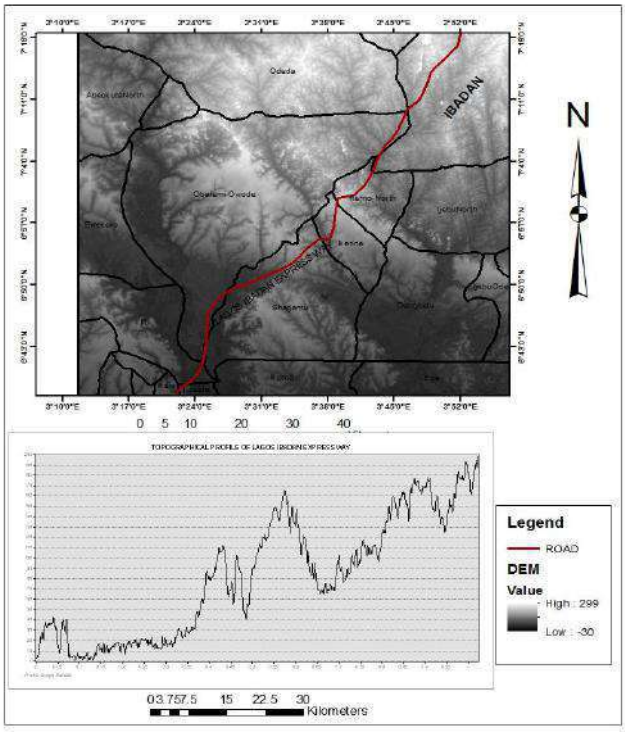

Fig. 1: Topographical Map and Digital Elevation Model (DEM) of Lagos-Ibadan Expressway Profile (Arcgis 10.3.1 Version).

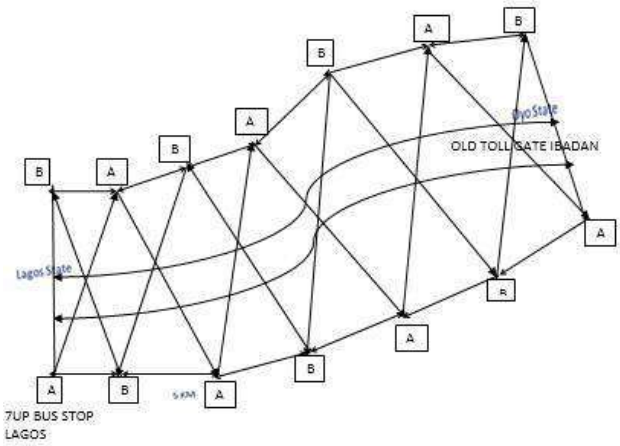

Fig. 2: Systematic zigzag sampling along Lagos-Ibadan Expressway in dry season (January 2015 and January 2016) and in Wet Season (July 2015 and July 2016)

Table 1. Coordinates, Elevations of Sampled Locations along Lagos-Ibadan Expressway

Location Name/Distance From Lagos (Km)	Coordinates N	Coordinates E	Elevation (M) A.S.L	Location Code
7up B/S Lagos (0)	06°35.976'	003°22.710'	30	L1
Secretariat (5)	06°37.494'	003°21.827'	18	L2
Opic (10)	06°39.194'	003°23.713'	11	L3
Arepo (15)	06°41.579'	003°24.910'	3	L4
Ile-Epo (20)	06°44.211'	003°25.175'	24	L5
Nasfat/Cntrl (25)	06°46.921'	003°25.451'	27	L6
Redeemed Area (30)	06°48.921'	003°27.103'	14	L7
Ileke Town (35)	06°49.998'	003°29.322'	15	L8
Mowe 1 (40)	06°51.147'	003°31.849'	34	L9
Mowe 2 (45)	06°52.483'	003°34.352'	91	L10
Mallo Fuel (50)	06°54.146'	003°35.881'	68	L11
Ajanla Farm (55)	06°55.140'	003°38.174'	58	L12
Trailer Park (60)	06°59.878'	003°39.106'	106	L13
General Park (65)	07°00.103'	003°40.653'	142	L14
Max Fuel (70)	07°01.958'	003°42.362'	85	L15
Quarry Jnctn (75)	07°04.321'	003°43.249'	61	L16
Stark Fuel (80)	07°06.450'	003°44.722'	92	L17
Namy Sch Area (85)	07°08.716'	003°45.884'	104	L18
Dominon (90)	07°10.770'	003°47.553'	126	L19
M/Mariana H (95)	07°13.950'	003°48.812'	153	L20
Aramed (100)	07°15.240'	003°50.076'	145	L21
Underb Guru (105)	07°17.135'	003°51.768'	132	L22
Toll Gate Ib (110)	07°19.647'	003°52.528'	170	L23

N = Northings, E = Eastings, a.s.l = above sea level

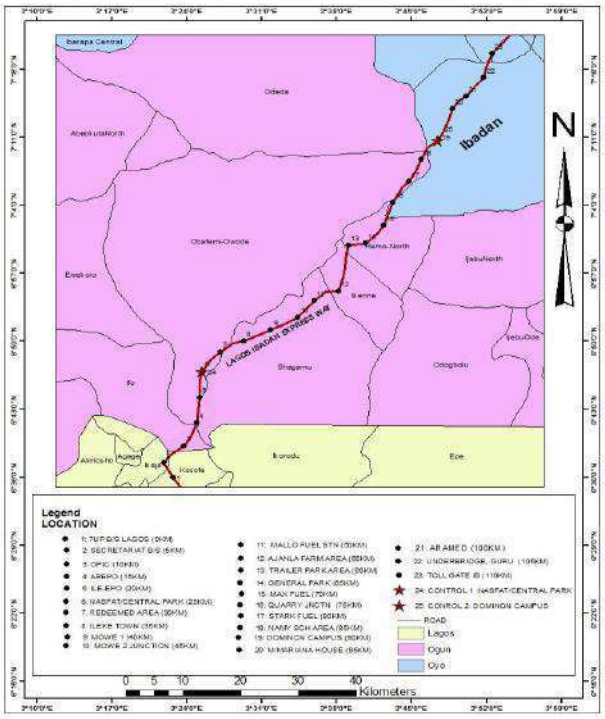

Fig. 3: Itinerary followed During Sample Collection along Lagos-Ibadan Expressway (ArcGIS 10.3.1 Version)

2.4 Data analysis and assessment of heavy metal pollution

The geochemical data from the laboratory were then subjected to statistical analyses using the SAS Statistical software 2014 version. Parameters evaluated are mean and standard deviation of the heavy metal concentrations at 5 m, 15 m and 25 m of each sampling point along the road from Lagos to Ibadan using Duncan's Multiple Range Test. In order to assess the pollution level of the soil, Index of geo-accumulation (I_{geo}), Single Ecological Risk Index (Ei) and Potential Ecological Risk Index (PERI) were computed using Excel^{TM} Software 2007 version

2.4.1 Index of geo-accumulation (I_{geo})

Index of geo-accumulation (I_{geo}) (Muller, 1981) was calculated using the formula below:

$I_{geo} = \text{Log}_2 (C_m/1.5*B_m)$ Equation 1

Where

C_m = Measured concentration of the examined metal in soil samples; and

B_m = Geochemical background reference value of the same metal.

The background reference adopted in this study was based on the world soil average abundance of metals by Levinson (1974). The constant 1.5 is the background matrix correction factor due to the lithogenic effects. The geo-accumulation values were interpreted using the I_{geo} classification shown in Table 2

Table 2. Classification of Geo-accumulation Index (I_{geo}) (After Muller, 1981)

Index	Value	Degree of Contamination
I_{geo}	$I_{geo} \leq 0$	Uncontaminated
	$0 < I_{geo} \leq 1$	Uncontaminated to moderately contaminated
	$1 < I_{geo} \leq 2$	Moderately contaminated
	$2 < I_{geo} \leq 3$	Moderately to strongly contaminated
	$3 < I_{geo} \leq 4$	Strongly contaminated
	$4 < I_{geo} \leq 5$	Strongly to extremely contaminated
	$I_{geo} \geq 5$	Extremely contaminated

2.4.2 Single Ecological Risk Index (Ei)

Single Ecological Risk Index (Ei), of a contaminant in an area, was calculated using this formula:

$E_i = T_i * CF_i$ Equation 2

Where

E_i = Single Ecological Risk Index, T_i=Toxic Response Factor for a given metal, CF_i = Contamination Factor (CF) for the same metal.

Prior to the computation of E_i, CF_i was first computed using the formula;

$CF_i = C_m/B_m$ Equation 3

Where

C_m = Measured concentration of the examined metal in soil samples; and

B_m = Geochemical background reference value of the same metal

The background reference in this study was based on the world soil average abundance of metals by Levinson (1974). The Ecological Risk Index values were interpreted using the classification by Hakanson (1980) (Table 3)

2.4.3 Potential Ecological Risk Index (PERI)

Potential Ecological Risk Index (PERI) was computed using this formula:

$PERI\ (RI) = \Sigma E_i$ Equation 4

Where

Σ = summation, E_i= Single Ecological Risk Index.

Both the Single Ecological Risk Index (E_i) and Potential Ecological Risk Index (PERI) values were interpreted using the classification in Table 3.

Table 3. Ecological Risk Index (Hakanson 1980)

E_i	Pollution Degree	RI	Risk Level	Risk Degree
$E_i < 30$	Low	RI < 40	A	Low
$30 \leq E_i < 60$	Medium	40 ≤ RI < 80	B	Medium
$60 \leq E_i < 120$	Strong	80 ≤RI < 160	C	Strong
$120 \leq E_i < 240$	Very strong	160≤RI < 320	D	Very strong
$E_i \geq 240$	Extremely strong	RI ≥320	-	-

III. RESULTS AND DISCUSSION

3.1 Heavy metal concentrations in sampled soil collected during dry and set seasons

The results of the mean heavy metal concentrations in soil samples collected during dry and wet seasons at 5m, 15 m and 25 m away from the edge of the road are presented in Tables 4a and 4b. The tables revealed that the mean concentrations of the analyzed heavy metals obtained at a distance of 5m away from the edge of the road, during both dry and wet seasons, were significantly higher (P>0.05) than all the other mean concentrations obtained at 15m and 25m away from the edge of the road. The mean concentrations of the heavy metals in the soil were in the order Fe > Mn > Zn > Pb > Cu > Cr > Co > Ni. Metals such as Fe, Mn, Pb and Zn showed considerable elevated concentrations in the soil.

When the results obtained from this study were compared with that of HSE – ENV (2004), the range of Cu values, for example, was lower than the range obtained from this study. This result is also true for Pb as well as Zn (Table 5). Similarly, the concentrations of the elements from this study were higher than those obtained from Yauri soil, Lagos – Badagry road as well as Osogbo area. For example, the range of concentration values from Yauri soil, Lagos-Badagry road and Osogbo area were 0.91 – 23.72(mg.kg^{-1}), 5.99 – 20.63(mg.kg^{-1}) and 27.69 – 21.19(mg.kg^{-1}) respectively for Cu. These values were lower than the 1.05 – 97.43(mg.kg^{-1}) range obtained from this study (Table 6). Table 7 revealed that the results obtained from this study were very similar to those obtained in India and Ethiopia.

3.2 Index of Geo-Accumulation (I_{geo}) of Sampled Soil during Dry and Wet Seasons

The Index of geo-accumulation (I_{geo}) for soil samples collected during the dry season is as shown in Fig. 4. A critical study of the table revealed that Fe had the highest I_{geo} values while Ni and Co had the lowest. The I_{geo} values for Fe obtained at distances 5m, 15m and 25m from the edge of the ranged from 28.71 – 28.88 with the highest value obtained at a distance of 5m away from the edge of the road. Co, on the other hand, had I_{geo} values of 1.71, 0.89 and 0.25 at 5m, 15m and 25m respectively away from the edge of the road. A similar trend was observed for the I_{geo} values for sampled soil in the raining season, Fe had the highest I_{geo} values, while Ni and Co had the lowest values (Fig. 5). The I_{geo} values for the elements are in the order Fe > Mn > Zn > Pb > Cr > Cu > Co > Ni. For all the analyzed elements, the I_{geo} values reduces with distance away from the edge of the road.

3.3 Single Ecological Risk Index (E_i) and Potential Ecological Risk Index (PERI) of Sampled Soil during dry and wet Seasons from the Edge of the Road

The Single Ecological Risk Index (E_i) for a single element and Potential Ecological Risk Index (PERI) of sampled soil during dry season for the study are shown on Table 8. These E_i values decrease as the distance from the edge of the road increases. The table revealed that elevated values of E_i were obtained for Cu while the E_i values for Cr, Mn, Ni and Co were very low. The range of *Ei of the heavy metals are* 0.11-0.34 for Cr, 4.59-13.54 for Pb, 28.32-45.71 for Cu, 1.42-1.99 for Zn, 0.21- 0.24 for Mn, 0.002-.007 for Ni and 0.009-0.023 for Co. In terms of the total Single Ecological Risk Indices (ΣEi) of the analyzed heavy metals, ΣEi is in the order Cu > Pb > Zn > Mn = Cr > Co > Ni. Therefore, Cu was the key influencing factor causing potential ecological risk.

Table 4a. Heavy Metal Mean Concentrations in Sampled Soil Collected during Dry and Wet Seasons from the Edge of the Road

DST	Cr		Pb		Cu		Zn	
	mg.kg^{-1}		mg.kg^{-1}		mg.kg^{-1}		mg.kg^{-1}	
	Dry Season	Wet Season	Dry Season	Wet Season	Dry Season	Wet Season	Dry Season	Wet Season
5m	15.24±10.67^{a}	10.27±8.48^{a}	54.17±51.06^{a}	84.76±47.00^{a}	41.14±30.20^{a}	31.72±26.40^{a}	189.48±166.88^{a}	158.65±157.52^{a}
15m	9.75±6.17^{b}	6.94±4.73^{b}	34.75±32.88^{b}	29.96±29.79^{b}	31.59±26.24^{b}	26.03±23.44^{b}	153.85±140.82^{b}	139.32±133.48^{b}
25m	4.92±2.64^{c}	3.42±2.09^{c}	18.34±19.53^{c}	15.72±18.11^{c}	25.49±23.73^{c}	21.99±22.44^{c}	134.58±130.65^{c}	129.44±128.13^{c}

*mean on the same column followed by the same letter are not significantly different at P<0.05. (Duncan's Multiple Range Test). DST = Distance

Table 4b. Heavy Metal Mean Concentrations in Sampled Soil Collected during Dry and Wet Seasons from the Edge of the Road

DST	Fe		Mn		Ni		Co	
	mg.kg^{-1}		mg.kg^{-1}		mg.kg^{-1}		mg.kg^{-1}	
	Dry Season	Wet Season	Dry Season	Wet Season	Dry Season	Wet Season	Dry Season	Wet Season
5m	15746.71±2780.15a	13212.61±2732.31^{a}	200.08±50.46a	175.03±42.48^{b}	0.09±0.13a	0.05±0.08^{a}	0.25±0.20^{a}	0.17±0.15^{a}
15m	14319.42±2238.08b	13020.96±2310.04^{ab}	187.82±48.34b	174.43±47.88^{ab}	0.04±0.07b	0.04±0.06^{b}	0.14±0.14^{b}	0.11±0.09^{b}
25m	13975.91±2622.84c	13129.91±2569.51^{b}	181.74±48.55c	176.79±48.02^{a}	0.02±0.07c	0.03±0.05^{c}	0.09±0.14^{c}	0.09±0.11^{c}

*mean on the same column followed by the same letter are not significantly different at P<0.05. (Duncan's Multiple Range Test). DST = Distance.

Table 5. Comparison of Acceptable Range of few Heavy Metals in Some Studies with the Heavy Metals in Sampled Soil in the Present Study

[a]HSE-ENV (2004)

Metals (mg kg^{-1})	Acceptable range[a]	Observed range from this study
Cu	5 – 50	1.05 – 97.43
Pb	5 – 50	1.85 – 130.96
Zn	10 – 120	5.72 – 558.03

Table 6. Comparison of a few Heavy Metals Concentration in Sampled Soil in the Present Study with Similar Studies within Nigeria

Heavy metals ($mg.kg^{-1}$)	This study	[a]Yauri soil	[b]Lagos-Badagry road	[c]Osogbo area
Cu	1.05 – 97.43	0.91 - 23.72	5.99 - 20.63	27.69 - 21.19
Pb	1.85 – 130.96	35.9 - 484.9	0.25 - 4.24	92.07 - 68.74
Zn	5.72 – 558.03	79.6 - 202.4	NA	56.27 - 42.45

[a]Yahaya *et al*., (2010); [b]Adeniyi & Owoade (2010); [c]Fakayode & Olu-Owolabi (2003); NA: Not applicable

Table 7. Comparison of a few Heavy Metals concentration in Sampled Soil in the Present Study with Similar Studies in the World

Metals mg kg^{-1}	This Study	[a]Study in U.S.A	[b]Study in China	[c]Study in Poland	[d]Study in India	[e]Study in Ethiopia	[f]EU Reg Standard
Cu	2.67 – 97.43	2.86 – 101	7.26 – 55.1	2.0 – 18.0	5.34 – 198.23	23.7 – 93.0	50 – 114
Pb	3.64 – 130.96	4.62 – 55.4	9.95 – 56.0	7.1 – 50.1	ND – 623.95	20.3 – 325.4	90 – 300
Mn	113 – 271.62	43 – 2532	134 – 1740	83 – 1122	NA	NA	1500
Ni	0.003 – 0.24	2.44 – 69.4	7.73 – 70.9	2.0 – 27.0	343 – 1409	47.3 – 200.6	50

Source; [a]Yahaya *et al.*, (2010); [b]Shacklette & Boerngen (1984); [c]Bradford *et al*., (1996); [d]Dudka (1992); [e]Abida *et al.*, (2009) and [f]Melaku *et al*., (2005). ND= Not Detected; NA =Not Available

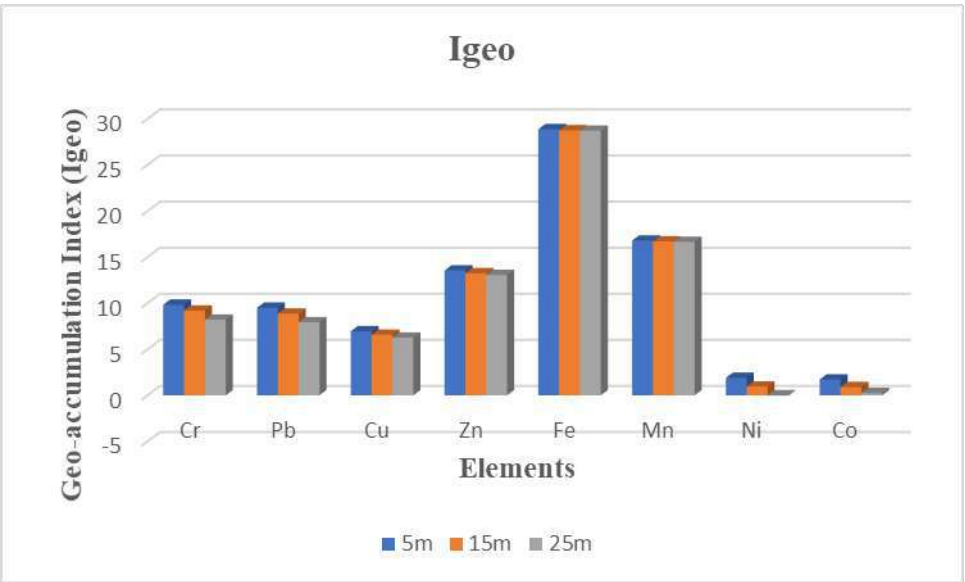

Fig. 4: Index of Geo-accumulation for sampled soil during dry season from the edge of the road inwards

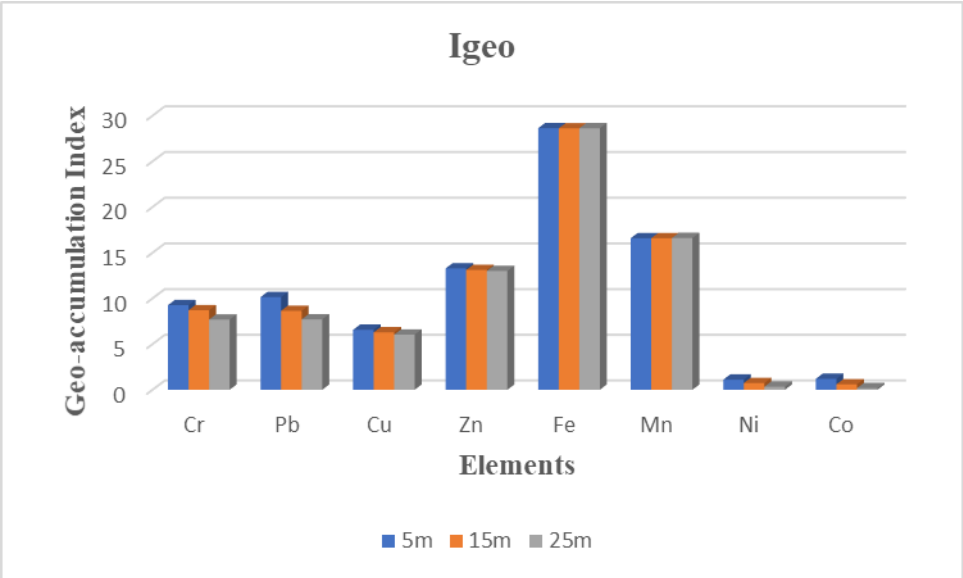

Fig. 5: Index of Geo-accumulation for sampled soil during wet season from the edge of the road inwards

Similar trends were observed for both *Ei* and PERI values for the sampled soil during wet season. Both the *Ei* and PERI values for the analyzed elements reduce with increasing distance away from the edge of the road. For example, while the PERI values was 58.77 at 5m, it reduced to 38.45 and 30.15 at 15m and 25m respectively. The range of *Ei of the heavy metals are* 0.08-0.23(Cr), 3.93-21.19(Pb), 24.44-35.25(Cu), 1.36-1.67(Zn), 0.21(Mn), 0.002-.004(Ni) and 0.0085-0.0173(Co). In terms of the total Single Ecological Risk Indices (ΣEi) of the analyzed heavy metals, ΣEi is in the order Cu > Pb > Zn > Mn > Cr > Co > Ni (Table 9). Therefore, Cu was the key influencing factor causing potential ecological risk.

The RI for the soil ranged between 34.86 - 62.05 and 30.15 – 58.77 for the soils in dry and wet seasons respectively.

Table 8. Single Ecological Risk Index (E_i) and Potential Ecological Risk Index (PERI) for Sampled Soil during Dry Season from edge of the Road

DIST	*Ei*							PERI
	Cr	Pb	Cu	Zn	Mn	Ni	Co	
5 m	0.34	13.54	45.71	1.99	0.24	0.007	0.023	62.05
15 m	0.22	8.69	35.10	1.62	0.22	0.003	0.014	46.07
25 m	0.11	4.59	28.32	1.42	0.21	0.002	0.009	34.86
PERI=Σ*Ei*	0.67	26.81	109.14	5.03	0.67	0.011	0.047	142.98

Table 9. Single Ecological Risk Index (E_i) and Potential Ecological Risk Index (PERI) for Sampled Soil during Wet Season from edge of the Road

DIST	E_i							PERI
	Cr	Pb	Cu	Zn	Mn	Ni	Co	
5m	0.23	21.19	35.25	1.67	0.21	0.004	0.0173	58.77
15m	0.15	7.49	28.92	1.47	0.21	0.003	0.0114	38.45
25m	0.08	3.93	24.44	1.36	0.21	0.002	0.0085	30.15
PERI=ΣE_i	0.46	32.61	88.61	4.50	0.62	0.008	0.0371	127.37

IV. DISCUSSION

The results of the heavy metal concentrations for sampled soil during dry and wet seasons at 5m, 15m, and 25m from the edge of the road revealed that the mean concentrations of heavy metals reduce as the distance increases away from the edge of the road. This is also in agreement with Mohammed & Folorunsho (2015) that the closer the soil is to the road, the more likely it will be polluted or contaminated by vehicular/human activities. Concentrations of heavy metals (Cr, Pb, Cu, Zn, Fe, Mn, Ni, Co) in soil were higher in dry season than in wet season at the same distance away from the road. These could probably be due to the fact the elements which were enriched at the top may be depleted/leached at the surface of the soil by rains (Ndiokwere, 1984). Furthermore, erosion, topography, vegetation cover, temperature, soil type and other human activities may be other factors that could add to or reduce the concentrations of these metals in soil in some of the locations along the study area and in general.

However, Pb concentration of 84.76 mg/kg was found to be highest at 5m during wet season when compared with Pb concentration of 54.17 mg/kg recorded during dry season at the same distance. This may be as a result of other anthropogenic activities apart from pollution from vehicular emissions.

The reasons for extremity in concentrations of some of these heavy metals (e.g. Fe, Mn and Zn) in the study area may be as a result of the closeness of some locations to parks especially those around Trailer Park, General Park, Toll Gate, and Ibadan. Other places like 7up Bus stop, OPIC and Redeemed Church which are prone to heavy traffic may likely acquire more deposits of some of these heavy metals.

The main pollutant found in sampled soil during dry and wet seasons are Zn, Fe, Cu, Pb and Mn. All the heavy metals mentioned above are actually associated with lead fuel, fuel burning (either gasoline or diesel), wear out of tires, leakage of oils, corrosion of car metal parts, traffic and other human activities. These are the daily activities experienced in the study area, Lagos – Ibadan expressway. According to Huntzicher *et al*., (1975); Ndiokwere, (1984) about 75% of Pb is emitted from the exhaust of motor vehicles in particulate form and it is also an important component of anti-knock fluid in petrol. Zn could also be present in tyres of motor vehicles.

Also the research work compared heavy metals obtained in the sampled soil with other previous studies in Nigeria. The result from other parts of the country indicated that Cu range is higher in Lagos-Ibadan Expressway than that of Yauri soil, Lagos – Badagry road and Osogbo area. This should be expected because the volume of traffic experienced in the study area, Lagos-Ibadan expressway,

is far more than in any other part of the country. This is also applicable to Zn while Pb was within the range of what was obtained at Yauri soil (Adeniyi & Owoade, 2010; Fakayode & Olu-Owolabi, 2003; Yahaya *et al*., 2010). Furthermore, the heavy metals concentrations obtained in this study are very similar to those of India and Ethiopia. This may be so because heavy metal emissions from vehicular emissions and other similar human activities are not regulated in most of the developing countries, India and Ethiopia inclusive.

The geo-accumulation index (I_{geo}) values for the soil samples were interpreted using Muller (1981) classification. The average I_{geo} values for the heavy metals (at 5m, 15m and 25m) in the soil samples in both dry and wet seasons are generally higher than 6 especially for Cr, Pb, Cu, Zn, Mn and Fe. This is an indication that the soil along Lagos – Ibadan Expressway are extremely contaminated with respect to these elements. The average I_{geo} values for both Co and Ni ranged between 0 – 2 (i.e. $0< I_{geo} \leq 2$) for both wet and dry seasons, indicating that the soils are uncontaminated to moderately contaminated. It also showed that pollution, in most of the study area, is not as a result of geological activities but as a result of vehicular movements and other anthropogenic activities. However, the average I_{geo} for Ni showed a negative value, at a distance of 25m away from the road, which indicates that the metal is not a pollutant to that environment at that location. Therefore, the source of the metal at this location could probably be from the rocks underlying the area. Furthermore, the I_{geo} values for soil sampled during dry season were higher than I_{geo} values for soils collected during wet season in the study area over the same period of time.

Single Potential Ecological Risk Index of metals in the soil was also assessed. During dry season, only Cu showed the highest risk index of 45.71 at 5m from the edge of the road, which reduced to 28.32 at 25m from the edge of the road. The total Single Ecological Risk Indices (ΣEi) of the analyzed heavy metals, ΣEi, is in the order Cu > Pb > Zn > Mn = Cr > Co > Ni. Therefore, Cu was the key influencing factor causing potential ecological risk. The RI at 5m (62.05) and 15m (46.07) away from the road indicated that the soil potential ecological risk index was level B corresponding to moderate ecological risk. A similar result was obtained for the *Ei* of the elements during wet season. The total ΣEi being in the order Cu > Pb > Zn > Mn = Cr > Co > Ni while the RI values indicated that the soil ecological risk index was medium at a distance of 5m away from the road.

V. CONCLUSION

There was a general reduction in heavy metal concentrations in the soil samples collected in rainy season when compared with sampled soil in dry season and when moved 5 m, 15 m and 25 m away from the edge of the road.

Index of geo-accumulation (I_{geo}) of sampled soil revealed that contamination ranged from uncontaminated to extreme contamination in the study locations during dry and wet seasons. The I_{geo} for Ni showed negative values in many sites which is an indication that the metal is not a pollutant from exhaust and the source is probably geogenic. The sampling points 5m away from the road exhibited a medium potential ecological risk for Cu.

The research results showed that Fe, Mn, Zn, Pb, Cu and Cr are the major heavy metal pollutants in the soil during dry and wet seasons, while Ni and Co are the least prevalent. A critical factor that could have contributed significantly to deposition and accumulation of these heavy metals in the soil for both seasons is the volume of traffic along Lagos-Ibadan Expressway which varied from time to time. Constructions, refuse dumps, effluent or release of industrial waste, accident on the highway resulting into spillage of dangerous chemicals into the environment may also have contributed to the deposition of these heavy metals in soil.

The study area, Lagos – Ibadan Expressway, is highly polluted and the major source of pollution is through anthropogenic activities, which are mainly from exhaust emissions from vehicular movement along the expressway over time. The study has also provided some relevant baseline information for accessing the public health risks, which could arise from living, farming, grazing or fish ponds along Lagos-Ibadan Expressway. For the people that have been living along the highway for years it is advisable for such people to go for comprehensive medical test to know their health status as regards the amount of heavy metals present in their body before it is too late.

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Impacts of COVID-19 on food production, environment and the economy: Review

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Abstract*— *The world is hit by another pandemic that has created a global threat. The Coronavirus disease of 2019 (COVID-19) has affected the entire human existence. The virus has taken over 1 million lives within 10 months. Governments are scrambling with different laws and regulations such as shelter-in-place orders, curfew, social distancing, mask-wearing, cancellation of public events, and traveling bans. These guidelines have helped reduce the spread and transmission of the virus in many countries, including some states in America. However, the virus has not only impacted lives, it has severally touched the agriculture, economy, and environment as well. This paper reviews the historical, origin, and mode of transmission of the COVID-19 and its impacts on human activities. On food production, it has created massive food insecurity around the world, especially in developing economies, resulting to collapse of small businesses and rising unemployment. Similarly, the environment has been largely impacted by improving ambient air quality and contributing to global waste management problems. Overall, the rate of increase in both atmospheric carbon dioxide (CO_2) and nitrogen dioxide (NO_2) dropped. On the other hand, the planet has seen over 200 metric tons of medical waste per day since the outbreak of the virus. Finally, doctors, scientists and farmers need to work together to reduce the impact of COVID-19 on human survival in all aspects. Therefore, this paper provides review of the COVID-19 pandemic, its effect on humans, environment, economy, agriculture, employment, and the potential mitigations to address the challenges of the pandemic in the near future.

Keyword*— *Coronavirus pandemic, COVID-19, impact, agriculture, economy and environment.

I. INTRODUCTION

Coronavirus disease 2019 (COVID-19) is an infectious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a novel member of the coronavirus family which was first reported by the World Health Organization (WHO) on December 31st, 2019 (WHO, 2020a). The mode of transmission was initially unknown, it has since been established that the SARS-CoV-2 virus is well-adapted for human-to-human transmission, and it is suspected that airborne transmission is the primary mode of transmission (Burke, 2020; Chen, 2020; Ghinai, 2020; Huang, 2020a; Li, 2020; Liu, 2020 Phan, 2020). The virus has so far taken the lives of over 1,000,000 people. The COVID-19 pandemic has negatively affected our planet and human activities. For instance, the poor agricultural productivity recorded across all continents, low economic activities – from the operation of major factories and businesses, and the halt in movement worldwide is debilitating. Several studies have been conducted on the COVID-19 infection, including its types, origin, mode of transmission, and health impact on humans. However, information on its impact on the environment, agricultural activities, economy, and unemployment is limited. Therefore, the objective of this review is to provide information on the impact of COVID-19 on global food production, economic activities, and environment.

1.1 Origin and history of the COVID-19 pandemic

Given that the COVID-19 is an emerging disease, there are a number of questions regarding its origin and

history. The SARS-CoV-2 virus is of natural origin and is thought to have originated from bats prior to having evolved the capability to infect humans (Andersen, 2020; Boni, 2020; Wan, 2020). The first cases of the disease were reported in Wuhan, China, and was linked with the Wuhan Huanan Seafood Market (Huang, 2020a; Wu, 2020; Zhou, 2020). From these observations, it shows that SARS-CoV-2, the causative agent of the current COVID-19 pandemic, is a novel, naturally-occurring virus likely of zoonotic origin with epidemiological data indicating Wuhan, China as the source of the current outbreak.

The first case to be reported outside mainland China occurred on January 13th, 2020, infecting a Chinese tourist in Thailand (Hui, 2020). The first case of the COVID-19 in the United States (U.S.) was reported on January 20th, 2020 (Holshue, 2020). In sub-Saharan Africa (SSA) countries, the first case was reported on January 28th, 2020 (Adepoju, 2020). The appearance of the disease was shortly followed by an explosive increase in confirmed cases reported from 21 different countries, including China, the U.S. and SSA countries. These events ultimately led to a declaration of the outbreak as a Public Health Emergency of International Concern (PHEIC) by the WHO on January 30th, 2020 (Bassetti, 2020; WHO, 2020b), and the declaration of COVID-19 as a pandemic by the WHO on March 11th, 2020 (Cucinotta, 2020). Cases in China are thought to have peaked in late January or early February 2020 (Callaway, 2020; Cyranoski, 2020), while cases in both the U.S. and SSA countries continue to rise. As at the time of this preparing this manuscript (26 October 2020), China was reported to have 62 cumulative cases per one million population, the U.S. was reported to have 24,367 cumulative cases per one million population, and SSA countries combined was reported to have11,891 cumulative cases per one million population (WHO, 2020c).

1.2 Transmission and containment of the virus

The symptoms of a SARS-CoV-2 infection appears after an incubation period of approximately 5 days (Li, 2020), and several recent reports have found strong evidence of asymptomatic transmission of the virus, although it is not yet clear whether asymptomatic transmission is a major factor in the spread of the disease (Huang, 2020b). Overall, these observations indicate that individuals can be infectious prior to being diagnosed with the disease, and that the virus easily moves from one individual to another individual.

1.3 Current and Future Considerations

The designation of COVID-19 as a pandemic has been in place for approximately six months at the time of this manuscript preparation. Currently, epidemiologists around the world are working on models to better prepare and prevent the spread of SARS-SoV-2 in both short-term and long-term projections (Scudellari, 2020). While these predictions do not always align, the models do agree that the COVID-19 is not going away in the foreseeable future. Though there remain many unknowns and the future remains uncertain even with the most accurate models, we do have a better understanding of the virus than we did in December 2019.

For example, we know that the spread of the disease differs depending on countries and locations. Recent reports have indicated that asymptomatic carriers could potentially pass the virus to other individuals, without knowing they have done so. This ‘silent transmission’ of SARS-CoV-2 indicates a need for rapid, reliable contact tracing methods and isolation of individual cases in order to suppress future outbreaks (Moghadas, 2020). We have also seen many countries implement drastic measures in an attempt to control the spread of the virus, including shutdowns/lockdowns, curfews, required quarantines, and travel restrictions, in response to the rapid rise in cases and the ease with which the virus has spread. These measures, along with physical distancing and self-isolation have led to a dramatic reduction in the spread of the virus, and therefore the number of cases in those parts of the world that have implemented them (Maier, 2020).

In addition to these, several efforts have been launched to develop a vaccine against SARS-CoV-2 infection. While the development of an efficacious vaccine against SARS-CoV-2 is critical in reducing the number of deaths and the spread of the disease, none have been approved for widespread use in the public as at the time of this manuscript preparation. There are several clinical trials designed to assess the efficacy and safety of potential vaccines currently in progress (Amanat, 2020). Despite worldwide efforts to accelerate the development of a safe and effective vaccine, current estimates maintain that successful administration of a SARS-CoV-2 vaccine is months, and perhaps years to come (Amanat, 2020; O’Callaghan, 2020). Therefore, the near-term course of the pandemic will largely depend on the response of affected communities. The long-term course of the pandemic will largely depend on the arrival of a successful vaccine that will be accessible and affordable by all countries.

II. IMPACT OF COVID-19 ON FOOD PRODUCTION

The COVID-19 pandemic has caused a lot of disruption in food production, distribution, and consumption across the world. The disruption caused by the pandemic has led to a significant impact on food prices and is a major concern to a world with such rapidly expanding human population (Torero, 2020). According to Global Food Crisis Report (2020), approximately 83 million to 132 million people may go to bed hungry due to the COVID-19 pandemic. These effects cut across the entire globe, but most significantly, countries with low economic GDP hit the hardest (Laborde et al., 2020a). It affects all sectors of agriculture, including poultry, livestock, and crop production. The pandemic has also affected the availability and access to food (Laborde et al., 2020a). This is partly due to lack of on-farm personnel, inability to import food, and the abrupt ending of school feeding programs that many school children rely on as a source of meal (Headey and Ruel, 2020).

On-farm production has seen an increase in unharvested or decomposing agricultural products due to labor shortage and closure of restaurants, and lack of transport within the borders of countries. The downside of this increase in food wastage extends from loss of income for the producers to global climate change through the emission of greenhouse gases (Aldaco et al., 2020; Goeb et al., 2020). However, it is also projected that many urban dwellers in developing countries could return to rural areas to take up farming as an alternative source of food and income (Laborde et al. 2020b). This is because of the loss of jobs associated with strict enforcement of COVID-19 lockdowns to curb down the spread of the virus.

Most of the agricultural industries worldwide have struggled to maintain personnel due to the COVID-19 outbreak. This has caused loopholes in the food supply chain, especially in Africa, where most of the farmers are subsistent with little or no farm equipment. These smallholder farmers rely on relative or family members, community youths, or religious societies to help with ploughing, planting, weeding, and harvesting along the production chain. The lockdowns, quarantine, isolation, and death have placed restrictions on labor, food processing, and transportation affected the lack of personnel. This effect extended to the animal and meat processing industries. These animals are forced to stay longer in the farms with limited feed resources leading to animal shrinkage in weight and loss of lives.

International travel restrictions between countries have posed a severe threat to food security, especially in the import-dependent countries (Cullen, 2020). These restrictions across different countries have limited agricultural food trade between countries because of the pandemic (Laborde, 2020). Limitations in food export and import causes a break in the production supply chain (Ivanov, 2020), which is threatening food security around the globe. The COVID-19 pandemic impact have severely affected the vulnerable people in the poorest nations (FAO, 2020). A widespread media coverage highlighted that the COVID-19 outbreak forced large scale farmers in the United States and Canada to dump their milk on the street. It was estimated that dairy farmers in the United States dumped about four million gallons of milk every day due to breakage in the milk supply chain, from the COVID-19 pandemic shutdown (Forstadt, 2020).

Similarly, Nepalese farmers had to dumped milk products worth $ 17 million and a lot more in deterioration (NepaliSansar, 2020). In Peru, producers are left with no option but to dump their white cocoa in landfills because hotels and restaurant that buys their product are closed. In India, farmers in rural areas are forced to feed strawberries to cows due to a lack of transportation to the city markets (Torero, 2020). In China, cattle are starving due to the unavailability of food and workforce labor due to the COVID-19 shutdown (Zhang, 2020). The United States, Canada, France, Germany, Italy, and Australia depend on seasonal foreign workers from North Africa and Eastern Europe to work on farms (Torero, 2020). Movement restriction disturbed food production, transportation, and distribution (figure 1) (Poudel et. al. 2020), while reducing labor availability; hence, food prices increase due to high demand and low supply.

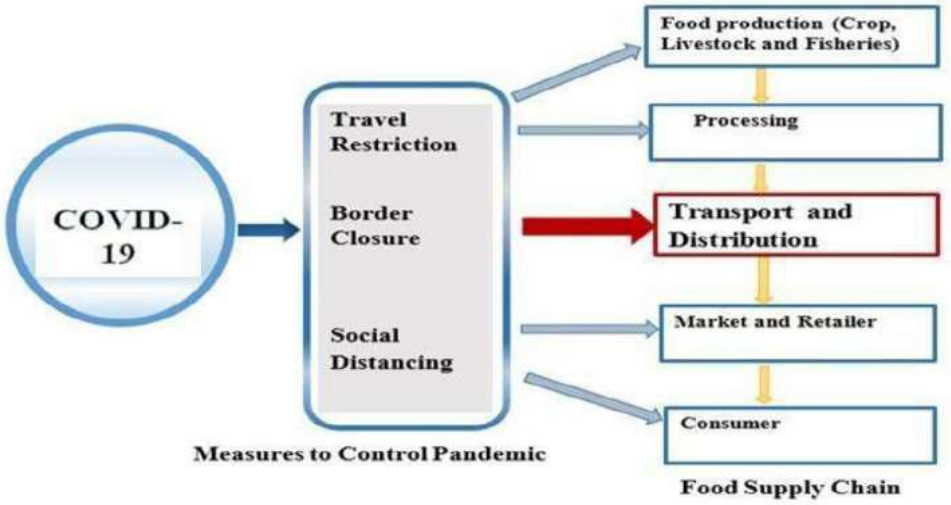

Fig.1: This figure shows COVID-19 affecting every stage of the food supply chain with a major impact on food transportation and distribution taken from Poudel et al., 2020.

III. POSITIVE IMPACT ON THE ENVIRONMENT

Due to bans on travel and orders to stay indoors to prevent the spread of COVID-19, researchers see a decrease in carbon emissions across the world in the U.S., China, and India. It is believed that environmental pollution dropped in these areas because of the pandemic (Henriques, 2020). Many small businesses shutdown or suspended production thereby reducing the negative impacts of the operations of these businesses on the environment. Depending on how long the pandemic lasts, it may contribute to an overall decrease in the yearly total of emissions, which will benefit the environment.

According to Jacinta Bowler, a scientific journalist at Science Alert, there is a clear positive impact on the environment (Bowler, 2020). Unfortunately, it is casually related to the lockdowns of different countries around the world. Bowler stated, "Resource economist Marshall Burke did some back-of-the-envelope calculations about recent air pollution drops over parts of China." (Bowler, 2020). Considering air pollution as a harm to the environment, physicist Jos Lelieveld states that "air pollution exceeds malaria as a global cause of premature death by a factor of 19" (Cassella, 2020). The pandemic has caused massive lockdowns, leading to a drop in CO_2 emissions, not just in China, but also around the world**.** It is worth noting that air pollution is a major contributor to global warming.

In Italy, canals have become apparent and dolphins are back in their waters. Researchers at Columbia University say that "CO_2 emissions are falling sharply," and "air pollution caused by vehicles in New York has been reduced by 50% from last year" (McGrath, 2020). Possibly, we can find ways to continue these positive impacts on the environment after the virus diminished.

Another positive effect that is more global in scale is the reduction of Carbon and Nitrogen emissions due to the halting of major transportation systems during the crisis. With fewer people traveling by car, and even fewer by airplane, global greenhouse gas emissions (GHG) has significantly dropped. According to the urbanism and city planning website Citylab, transportation usage is down from as low as 35% for San Francisco's Metropolitan Transport Authority (MTA), to as high as 90% with New York City's MTA (Bliss, 2020). These numbers suggest that many harmful emissions are curtailed for as long as the virus affects major metropolitan areas of the nation, benefitting the environment for the time being.

3.1 How COVID-19 affected the environment in China?

Greenhouse gas emissions were reduced by about 25% in the weeks following the Chinese New Year at the peak of the COVID-19 outbreak in China (Myllyvirta, 2020). Li

Shuo of Greenpeace China is concerned that the stimulus plan envisioned by the government will lead to industrial projects that might reverse these reductions (Network, 2020). Not only did greenhouse gas emissions lower, but air quality significantly improved. Nitrogen dioxide (NO_2) is a pollutant that primarily gets into the air from burning fuel and is linked to asthma and respiratory infections (EPA, 2020). This famous image gathered from satellite data indicates the reduction in NO_2 emissions before the COVID-19 outbreak and during the worst outbreak in China when many people in most major cities were asked to stay inside and not leave their homes (figure 2).

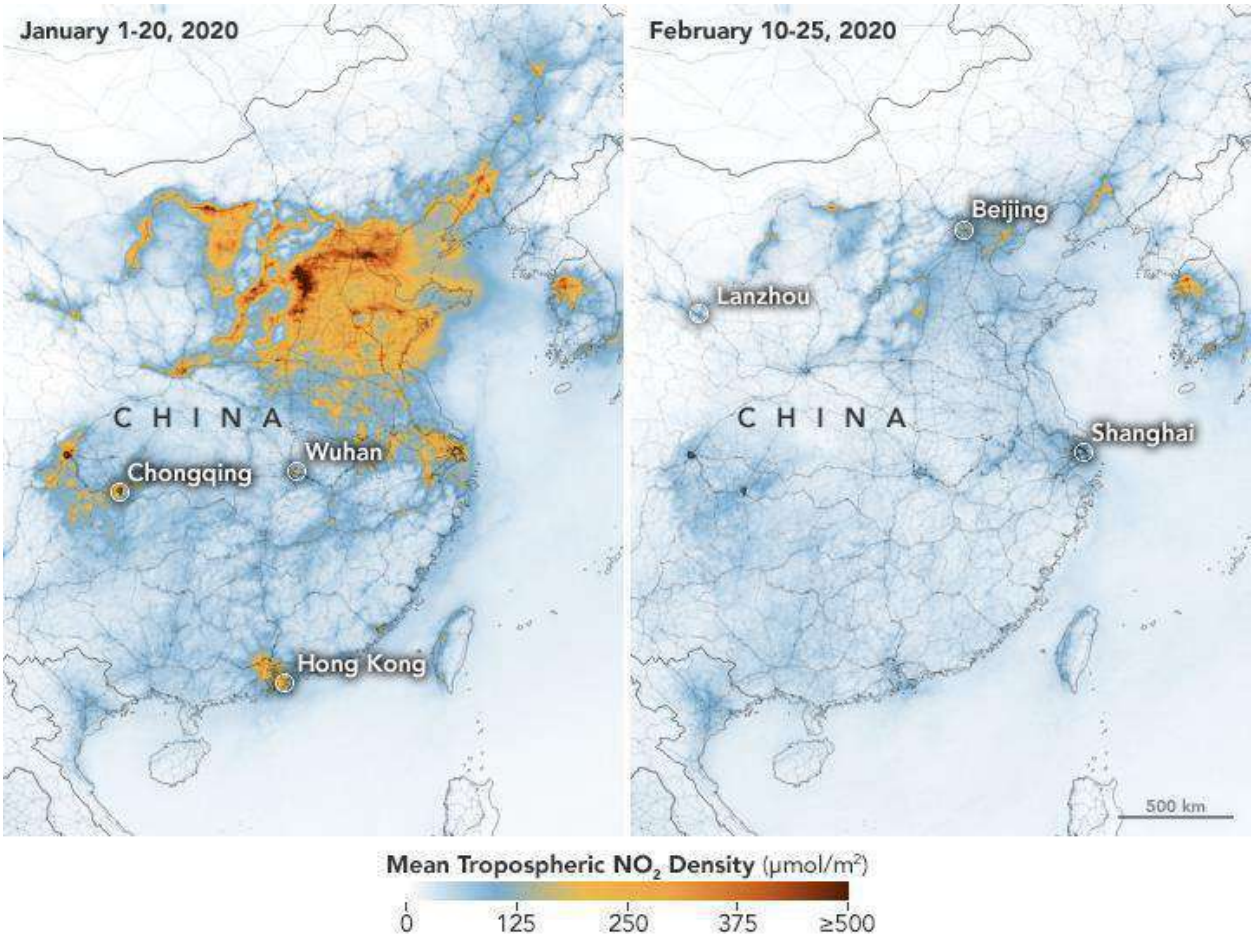

Fig.2: This figure shows the airborne nitrogen dioxide plummets over China during COVID-19 lockdown (Tan, Chou, Liang, Chou, & Shiu, 2009)

Despite improvements in air quality, notably in China and in Europe due to stay at home orders, the environmental side effects from the Coronavirus have not all been positive. For example, recycling programs and centers in both U.S. and Europe have been closed, disposable bag bans have been overturned, and the amount of medical waste produced is rising (Bloomberg News, 2020).

3.2 Air Pollution

Is air pollution a big deal? Air pollution is one of the world's biggest environmental problems. Five million deaths per year are attributable to air pollution (Figure 3) (Ritchie and Roser, 2019).

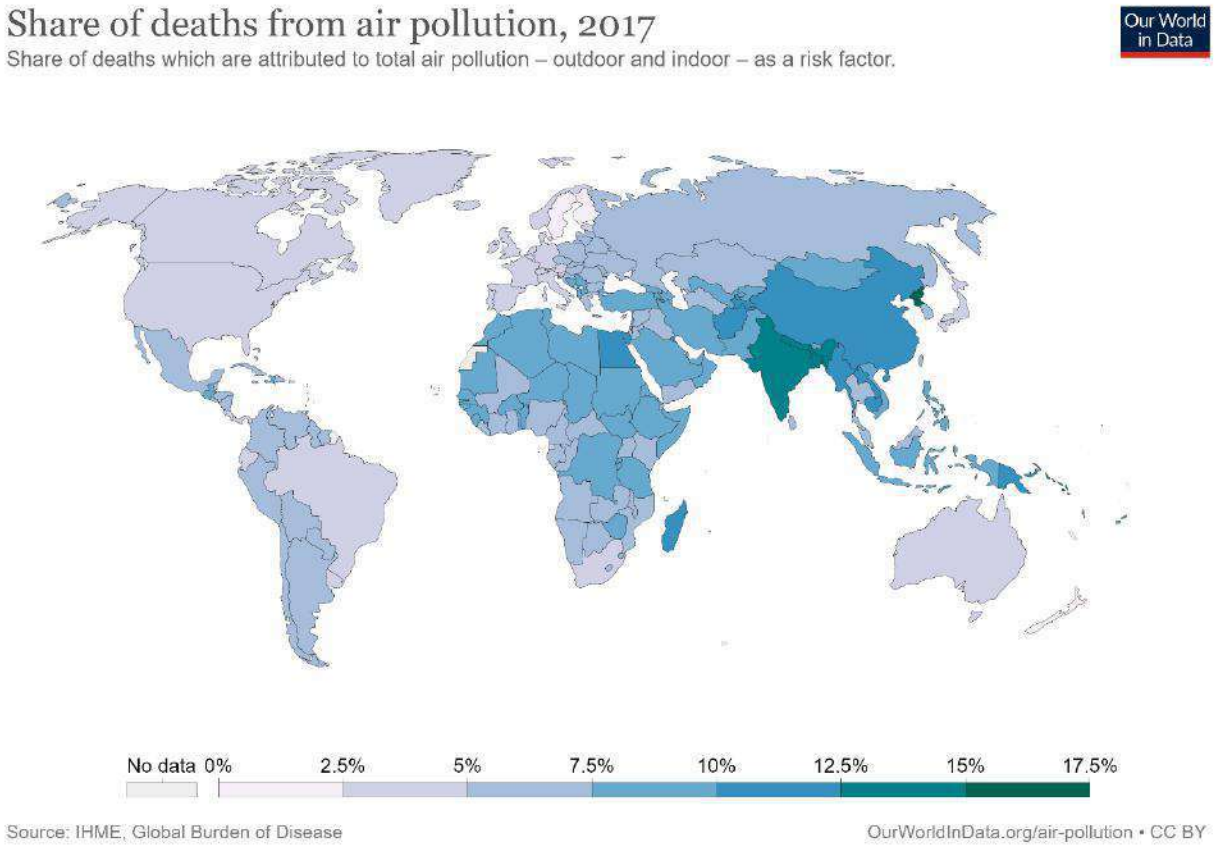

Fig.3: This figure shows the share of deaths from air pollution in 2017 (Ritchie & Roser, 2017)

While death rates in developed countries are low, air pollution exacerbates and makes other conditions worse. In the United States, for example, almost 4% of deaths can be attributable to air pollution. In China, this figure is much higher, at 11%. One researcher estimated that the coronavirus lockdown in China, which resulted in hundreds of millions of people staying home and thousands of factory closures, could have saved as many as 70,000 lives by curbing emissions from factories and vehicles (Burke, 2020).

3.3 Negative Impact on the Environment

A tremendous amount of medical wastes has been produced because of the COVID-19 pandemic. According the Verge Report, hospitals in Wuhan China are generating six times as much medical waste as compares to pre pandemic level. Wuhan is producing over 200 tons of medical waste per day and China has invested on building mobile medical waste disposable centers (theverge.com, 2020; chinadaily.com, 2020). As a planet, we already have tremendous trouble with waste management, often producing over three times more garbage than we can properly dispose of in just the United States. That waste usually ends up in the ocean – obstructing and harming wildlife and polluting the environments where the trash lays. We know, for instance, that hazardous waste is something that has the potential to harm human life (biohazardous needles, toxic sludge, etc.) and that this waste is not naturally present in the human environment, but rather is produced based on manufacturing efforts to advance our society (EPA, 2006).

Though air and water may be cleaner and clearer while pandemic protections are in place, it may be worse for the environment in the long run. People may simply be delaying trips, and when travel bans are lifted, the surge of travel could increase the level of pollution to pre-pandemic levels. Industrial pollution will also return to normal when businesses are back on track. There are also concerns that while people are changing their habits now with travel and work, environmental sustainability discussions could be pushed to the background (Henriques, 2020). Short-term

gains may be a reason to delay enacting more substantial, longer-lasting solutions.

One of the top ways Coronavirus can have negatively impacted the environment is by the abrupt increase in personal consumption of energy. Entire families at homes all day and night, in most cases means multiple television and computer monitors, lighted rooms, running inside and out to play, all of which cause an increase in energy usage. Another negative impact is the increase of single-use plastics. During this pandemic, if you walk through any store across the United States, all the shelves are cleared of paper and plastic plates, plastic cups, plastic silverware, and water bottles. The Plastic Industry Association is trying to get the single-use plastic bag ban reversed. They are saying reusable bags can spread the virus, so they are asking for these bans on bags to be reversed and to increase single-use plastics. According to NRDC.org, in "New York, 23 billion plastic bags are used by residents each year." Increase in single-use plastic can increase pollution in our waterways and land significantly (NRDC, 2020).

There are also some unfortunate side effects brought with all the positives. One negative effect is that companies and groups previously heavily involved in green initiatives have been forced to put on hold their environmental operations. For example, as Politico reports, the European Union has postponed climate law debates, Fridays for Future protests are forced online (Politico reports, 2020), and many industries that produce high amounts of carbon emissions are seeking government bailouts (theguardian.com, 2020). Of great concern to these activists is the fact that it is highly likely that post-recession, the world will see a large surge in emissions, possibly negating the positive reduction changes brought by COVID-19. Gernot Wagner, a clinical associate professor at New York University's Department of Environmental Studies, adds to the concerns over in addressing MIT's Technology Review: "Emissions in China are down because the economy has stopped and people are dying, and because poor people are not able to get medicine and food. This is not an analogy for how we want to decrease emissions from climate change" (Temple, 2020).

An article from Politico also notes a very real environmental threat that is increasing as the quarantine protocols continue - a heavy increase in waste production (Heath, 2020). Companies like Starbucks have switched from their reusable cups, deciding instead to serve their drinks in single-use cups that are not recyclable. Other restaurants that have transitioned to providing curbside or delivery options must provide packets of plastic utensils since their customers cannot enter the building to use metal silverware at their inside tables, such increase in the production and usage of plastic enormous and dangerous for the environment. Even worse than the restaurant plastic issues, medical facilities are producing exceedingly high amounts of waste (Jiangtao & Zheng, 2020). This "mountain of medical waste," as they label it, is mostly comprised of single-use items such as face masks and other personal protective equipment. Unfortunately, not only are these items piling up by the millions, but the incinerators used to dispose of them safely are quickly being overwhelmed – despite new construction in hundreds. These as well as medical waste facilities reaching the end of their viable lifespan, are causing extreme concern for the ability of many countries including the United States to contain the virus and prevent another outbreak. Will these problems also overwhelm other developing countries or will China's example spur proactive handling of such waste that will prevent the pandemic from becoming an even more significant threat to the environment? Only time will tell.

3.4 Positive Impact on the Economy

There may be significant economic growth amid the chaos and confusion created by the COVID-19 pandemic in the coming years. As we have seen, OPEC[1] has cut oil prices in the wake of COVID-19, and that has being reflected in reduced global oil prices. For instance, Oklahoma have seen gasoline price dropped to as low as $1.37 per gallon – far lower than it has been in years. This is due to OPEC ceasing their typical price gouging operations on oil barrels to support international economies during this challenging time (Forbes, 2020). This means that those interested in the stock market are more likely going to notice this trend and invest money into petroleum and refined gas markets, which will soar directly after the COVID-19 crisis is mitigated and oil prices return to new normal levels. It will more likely be a tight window to get money out of the market once OPEC does its price-fixing, but if pulled correctly, many investors may see a massive return on their investments.

Another positive impact is that we have learned that work could be more efficient and effective through virtual means than we might ever thought.

3.5 Negative Impact on the Economy

The extent of economic damage is dependent on the length of time that the damage-causing entity can flourish (CSIS, 2020). Those that are most likely to be affected are countries that are heavily hit by the Covid-19 pandemic. The most impacted industries are recognized as global air carriers and entertainment industries such as theatres, restaurants, casinos, and others, since social distancing prevents maximum utilization of these resources' customers (CSIS, 2020). Much of the world has experienced locked down or travel restrictions or quarantines measures to reduce the spread of the virus, but these restrictions may have come too little, too late to prevent intra-national spreading. Corporations involved in these two industries have suffered massive revenue loss, and there have already been massive layoffs and reduced spending in order to preserve the operation of corporations in the long run across other sectors such as manufacturing and mining.

In terms of the economy, COVID-19 has forced the closure of many small and large businesses in both developing and developed economies. For instance, in the US there exist a lot of uncertainty about the economy because closed businesses results to massive job loss culminating into lower incomes and decreased economic activity. On a larger scale, the government stimulus packages to heavily affected people in the United States are not enough to cushion the economic stress and frustrations caused by the Coronavirus pandemic. Medical care, health insurance, and paid sick leave should be the goals of businesses to help people survive the fallout (Waller, 2020). The severity of economic ramifications depends on how long the pandemic lasts in epic maleficence. Many taxpayers and voters are rethinking "Medicare for all" since they lost their jobs that provided their health insurance coverage. However, many who did not have company health insurance often saw their insurance premiums double and do not want the changes.

Gross Domestic Product (GDP) is an important measure of how well a country is doing since it represents all finished goods and services completed during a designated period. If we look at the following graph (figure 4), we note that COVID-19 ended the longest economic expansion on record.

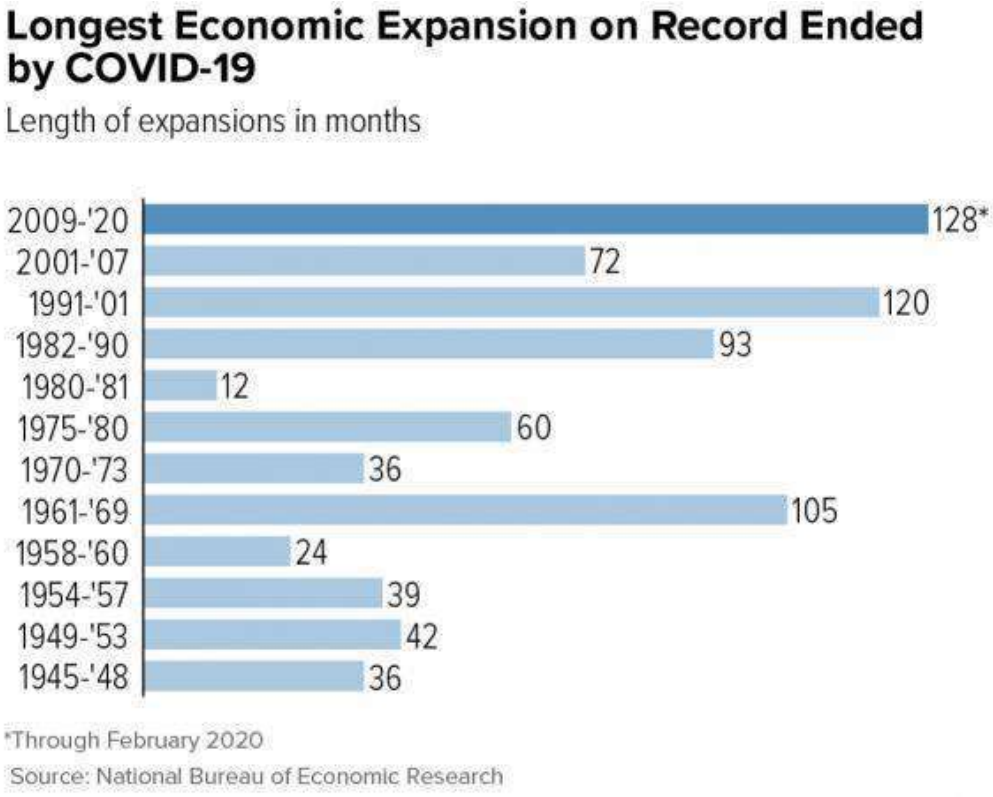

Fig.4: This figure shows the length of economic expansion on record ended by COVID-19 (Research, 2020)

In the United States, while the expansion was long, both the economy's average annual growth rate and the typical worker's earnings gains were relatively modest by the standards of earlier long expansions. President Donald Trump claimed that his policies would produce a substantial and sustained increase in economic growth, and his appointed Council of Economic Advisers believed that those policies would boost wages and employment substantially. By contrast, the Congressional Budget Office (CBO) and many other non-partisan analysts projected much slower economic growth and smaller increases in most workers' earnings.

The real GDP (adjusted for inflation) growth rate is shown in the following graph. Note that most quarters are positive, with two small negatives in 2011 and 2014. However, post-Covid-19 indicates that the United States is in a recession with strongly negative GDP growth for two quarters in a row after the start of the pandemic (Figure 5).

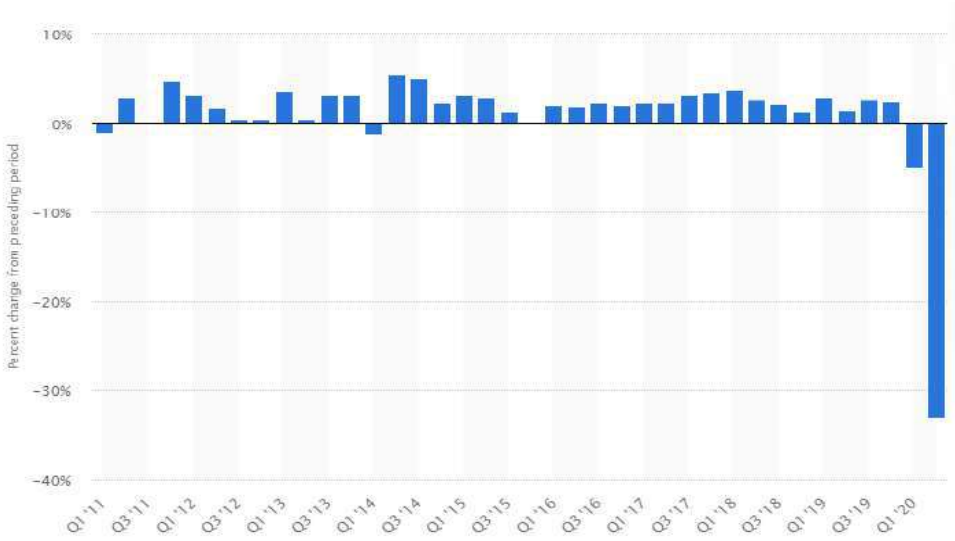

Fig.5: This figure show the quarterly growth of the real GDP in the United States from 2011 to 2020 (Duffin, 2020a)

Monthly unemployment is another important gauge of the economic health of a nation. The graph below indicates that after the shutdown of the economy that resulted from Covid-19 that unemployment rates skyrocketed (Figure 6).

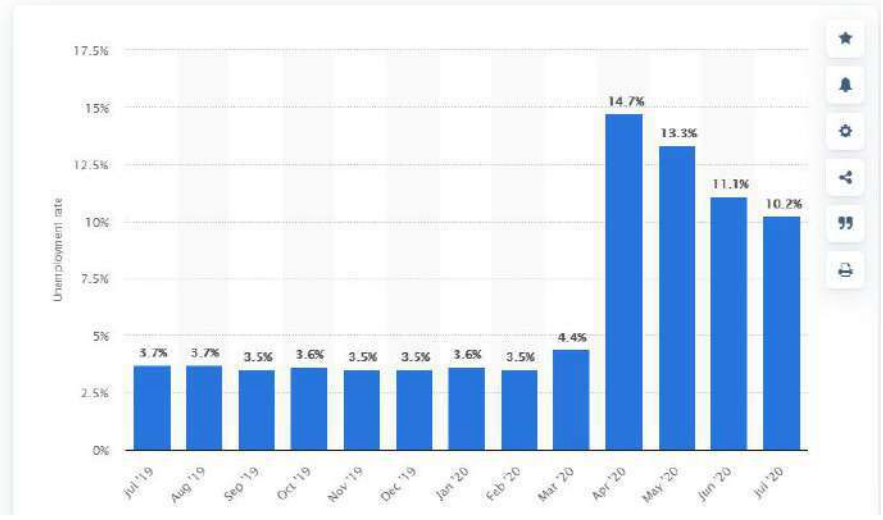

Fig.6: This figure shows the monthly unemployment rate in the United States from July 2019 to July 2020 (seasonally adjusted) (Duffin, 2020b)

Casual observers look at the stock market as the only barometer of the U.S. economy's health. They believe that wealthy investors know how well the economy is performing in all categories and that the stock market will rise and fall according to how well the economy is doing. However, investors are fickler than that and follow trends more readily than macroeconomists do. They often overlook factors such as workers losing health insurance, workers working either less than 40 hours or holding two or more jobs, and the impact of undocumented workers on our labor force. The following graph depicts what has happened to the U.S. Dow Jones Industrials stocks during the year 2020 after the introduction of the Covid-19 pandemic (figure 7). The index bottomed out in March when most of the country was placed in a lockdown, and deaths from the virus started increasing. However, as time goes on, the investors renewed their faith that their investments are sound. A large part of the large bounce back in the stock market is that the Federal government has pumped over three trillion dollars into the economy. It is noteworthy that many economists and significant investors believe that the market is overvalued and will decline regardless of the influence of COVID-19.

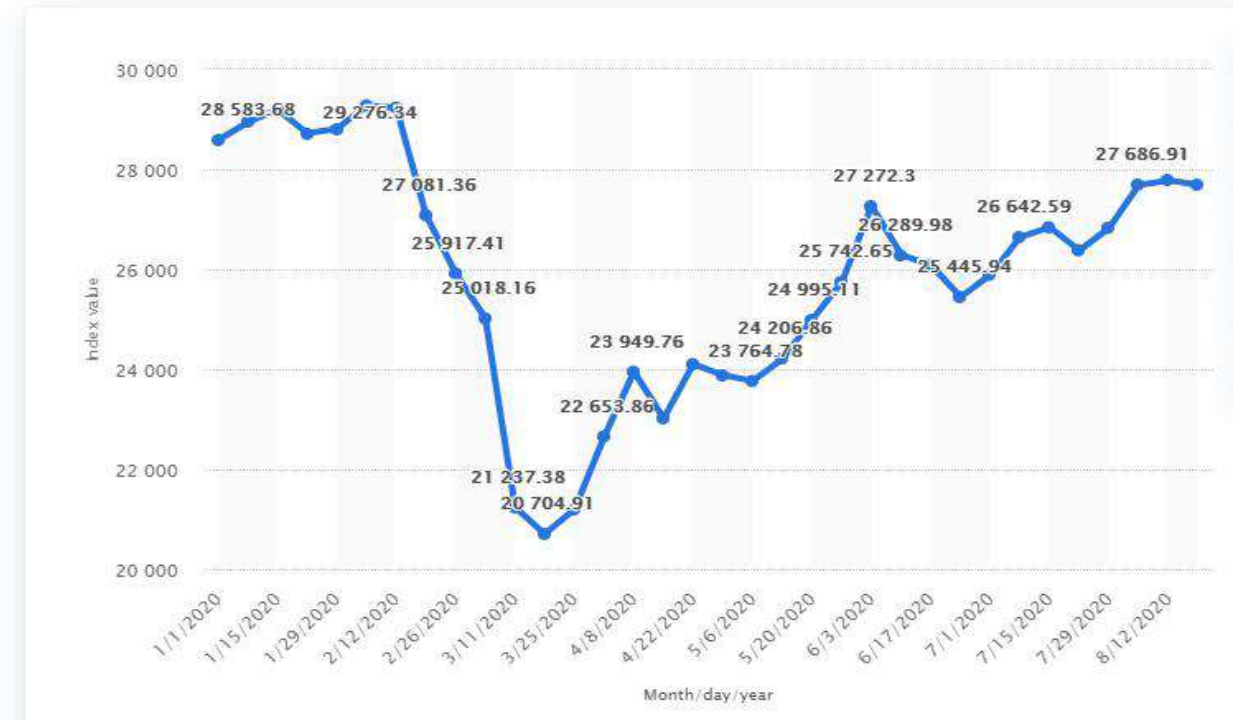

Fig.7: This figure show the weekly development of the Dow Jones Industrial Average index from January 2020 to August 2020 (Rudden, 2020)

Aside from the environmental impact the virus has had globally, it has also damaged many facets of the international economy; as it continues to spread to more and more countries, these effects are likely to increase exponentially as well. The infographic and data visualization website Visual Capitalist provide data on the economic impact China is facing, as well as predictions on the effects the U.S. and other countries may face next. As the data suggests, China's Economic Indicators – Investment in Fixed Assets, Retail Sales, Value of Exports, and Production – are all down in Year-over-Year change by at least 13%, with Investment hit the hardest at -24.5% (Ross, 2020). Although these numbers should increase as China continues its path to recovery, the country still faces heavy issues in product and service demand. International demand is negatively affected since an oversupply of goods is created due to other nations currently dealing with the outbreak, while local issues cause domestic demand to decrease sharply. A survey conducted in Beijing indicates, "up to 65% of respondents plan to 'restrain' their spending habits after the virus" (Ross, 2020).

Another problem for the economy is the companies that are switching their production and purpose of manufacturing medical and safety supplies for hospitals and other medical personnel (Ward, 2020). Among these companies are Ford, GE, General Motors, Dyson, and many more. While this is a great show of solidarity and support for the heroes that continue to sacrifice personal safety in fighting the virus, it could also have a negative effect in that products and services, mostly non-essential, are put at a halt. While the non-essentials are obviously a required sacrifice that will not significantly impact everyday life, it is still a factor that moves the economy further down into economic recession, as the new supplies are not producing as much revenue for these companies. This will more than likely drive prices up, as well as providing a hard-to-meet level of demand from consumers when production resumes post-crisis.

3.6 The Impact of Covid-19 in America

Many schools are no longer in session or have modified teaching formats across America and much of the developed world; stock markets are in free-fall; millions of Americans in the hospitality industry have already lost their jobs, leading to the largest number of unemployment claims in a single week since the United States (U.S.) has had unemployment insurance (figure 8) (Forbes.com, 2020a).

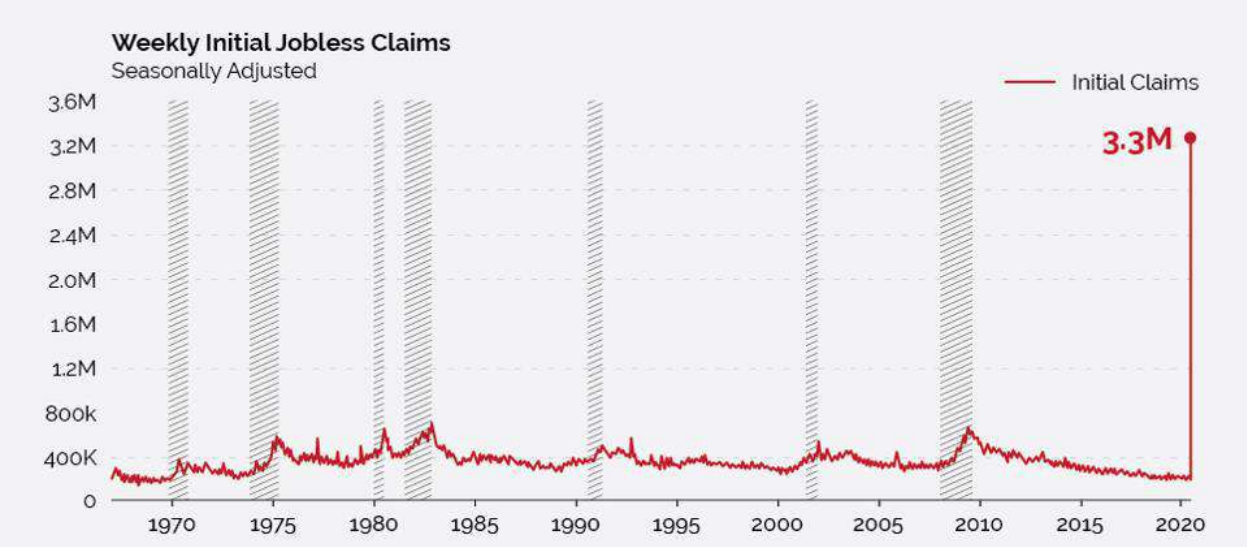

Fig.8: This figure shows the jobless claims over the years in the United States (Jones, 2020)

Leading economists suggest this is only the beginning of a recession[2]; many Americans believe a depression is likely (Jones, 2020). Questions may include: What has caused this economic catastrophe? Are there any side effects the people at Forbes are overlooking because these benefits and costs are not accounted for in the economy? What happens to the environment when certain segments of an economy shutdown?

Negative impact on the economy is coming in many different forms: from the stock market dropping, thousands of people losing their jobs, people not buying what and how they usually buy, and supply chains being disrupted. According to Forbes.com (2020), "Nearly 50% of companies say they are at least somewhat likely to conduct layoffs over the next three months due to coronavirus COVID-19, while more than one-third of firms (37%) say they already have instituted a hiring freeze.

IV. CONCLUSION

Businesses are closing, once-bustling streets are often nearly empty, and there is so much air of uncertainty encircling the globe while people obey quarantine restrictions meant to keep them safe. A global pandemic of the novel coronavirus COVID-19 has affected the world medically, socially, environmentally, and economically. As this pandemic continues, people must find new ways to cope with

the far-reaching effects and help slow its spread, which is vital in preventing more deaths.

The COVID-19 pandemic has negatively and positively affected our planet. Countries across the world are drastically changing their operations as a way of mitigating the impact of the virus on their population. Slow responders to the virus are being heavily hit. The most egregious example is the United States, where the sudden impact of the virus was met too late with action; the number of infected cases quickly grew to a massive peak of over 5.5 million, with the highest death toll of any country to date – more than 210, 000 people dead. According to public health experts, if the virus does continue this trend – or even worse, mutate and take on even more severe qualities - the human population worldwide could face a massive shock not seen since before the medical Revolution. On the hopeful side though, people from every corners of the world and every community are gaining an acute, experiential knowledge of what is and could be facing our global environment. It is worth noting the countries such as New Zealand, South Korea and Japan have done exemplary well in curtailing the devastating impact of the virus on their population.

COVID-19 may exacerbate global food shortage at a magnitude never seen before due to the economic recession hovering over many countries around the world. Currently, 690 million people may go to bed hungry, and this pandemic can cause this number to increase at a minimum of 773 million people (FAO, 2020). This year, farmers might not plant enough food to sustain the entire world population. Therefore, governments need to help farmers with input (seeds, fertilizers, pesticides, machinery, and animal feed), especially in low-income countries. Since the fertilizer industry in China has been affected by COVID-19 (Marlow, 2020), nations must start thinking about producing fertilizers such as organic fertilizers, which are cheap and affordable for smallholder farmers. Also, governments need to support farmers with storage facilities to preserve their produce until distributed. In addition, transportation should be provided so farmers can transport their goods to bigger cities for sale. Politicians need to strengthen international relationships to import and export food between countries. If not, COVID-19 will increase food insecurity by disrupting food production and distribution (Siche, 2020). If we do not help farmers plant enough food this year, the impact will be enormous next year. The starvation number will double whiles exposing children and adults to other diseases due to weak immune systems. The solutions to food shortages will have to be a concerted effort between politicians, scientists, and farmers. COVID-19 and many agricultural, economic, and environmental problems are too big to be dealt with on an individual level. They require the coordination of people, governments, and industry all working together towards a common goal.

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Effect of followed production procedures on the physicochemical properties of labneh anbaris

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Abstract—*. The aim of this study is to assess the different recorded processing attributes on physicochemical properties of anbaris and comparing them to commercially produced labneh done from pasteurized cow and goat milk. Comparing the industrially produced IP-anbaris with labneh industrially produced using cows and goat milk showed that the cow-labneh possessed significantly lower total-solids, titratable acidity, salt, ash, fat and protein content while having significantly higher pH and moisture content. As for the house hold produced anbaris, it was categorized into two classes according to the behavior of the household producer. Produced but in-between partial consumption of anbaris from the earthen-vessel and refilling during the process; HHC-anbaris; and produced but consumed only towards the end; HH-anbaris. Interestingly, the IP-anbaris compared to HHC-anbaris values did not differ significantly in any physicochemical property except in water activity, being significantly lower, and in titratable acidity, being significantly higher. As for the HH-anbaris, it possessed the significantly lowest water activity and protein content and the significantly highest titratable acidity compared to those recorded for IP- and HHC-anbaris. Traditionally earthenware-vessel was used in anbaris production but glass-vessel was recently introduced. In this study, there was no significant difference in the physicochemical properties between earthenware-anbaris or glass-vessel-anbaris. Furthermore, studying the characteristics of anbaris within the vessel before it is mixed showed that the bottom part was the significantly highest in total solids, fat and ash content compared to anbaris taken from the middle and upper section whose physicochemical properties did not differ significantly from each other.*

Keywords— ***Anbaris, Labneh, Earthen vessel, Water activity, Industrial, House hold.***

I. INTRODUCTON

Through the history, fermentation was mainly used to preserve food, longer shelf life, while being oblivion to the beneficial effects on health. This lead to fermented food being an integral parts of diet in many cultures [1] [2] [3]. Recently, product resulting from food fermentation microorganisms has been associated with health benefits [4]. During fermentation Lactic acid bacteria, one of the most studied microorganisms, synthesize vitamins and minerals, produce biologically active peptides with enzymes such as proteinase and peptidase, and remove some non-nutrients [5] [4]. Examples of health benefits are anti-oxidant, anti-microbial, anti-fungal, anti-inflammatory, anti-diabetic and anti-atherosclerotic activity products [4].

Labneh Anbaris, a traditional cheese-like fermented dairy product, is usually prepared from cow's and goat's milk following natural fermentation of raw milk in earthen vessel at around 30 ºC for 7 - 15 days after which the whey is drained and raw milk plus salt is added repeatedly until the earthenware vessel is full [6] [7]. Since raw milk is traditionally used, no starter culture is added nor CaCl2 solution to assist curdling of milk [8] [9]. Traditionally, anbaris production is during spring till the end of the summer [7]. The end product is recovered in September as a very concentrated and highly acidic type of Labneh. It is consumed fresh or made into small balls and submerged in vegetable oil in jars to be consumed later [6].

Anbaris is a special type of full fat, soft, cheese like fermented cheese produced from raw milk with a pH lower than 4. It is special since it is fermented with no addition

of culture, whey strained, earthen vessel refilled with milk and salt added then left again [10] [6] [7]. It is relatively safe against bacterial contamination but not yeast and mold especially if high moisture content anbaris is produced [10]. It can be used to reduce wastage of extra milk or milk that have reached 6.4 in pH thus out of the normal range. [10]

While exploring labneh anbaris production and physicochemical properties in Lebanon [10]. Furthermore, some minor differences were recorded in production such as usage of pasteurized milk instead of unpasteurized one, usage of glass vessel for fermentation instead of earthen vessel. In addition to that, some people would partially consume some of the anbaris after 7-15 days and fill again while others would wait till the whole earthen vessel is full with anbaris. All of these factors were the incentive to conduct this research to compare anbaris to the commercially produced labneh made from cow and goat milk. Also to register if the physicochemical properties are influenced by the change of the input product (raw milk), fermentation container (Glass or earthen vessel), household behavior (consumption during production or not). Last but not least, the profile of anbaris produced in the vessel - top versus bottom- was studied.

II. MATERIALS AND METHODS

2.1. Sampling procedure

2.1.1 Industrial anbaris samples

The industrial anbaris samples were divided into two categories according to milk pretreatment, Industrial-pasteurized (9 samples) and industrial-non-pasteurized (12 samples). Since there was no significant difference the pasteurized and the non-pasteurized industrially produced anbaris samples were all called Industrially produced (IP) Anbaris (21 samples).

2.1.2 House Hold Anbaris Samples

The house hold anbaris samples were divided according to the behavior of the house hold producers. Produced but in-between partial consumption of anbaris from the earthen-vessel and refilling during the process; HHC-anbaris (36); and produced but consumed only towards the end; HH-anbaris (21).

2.1.3 Samples of anbaris from different levels of earthen vessel and anbaris done in glass vessel

Six earthen vessels were used to produce anbaris labneh. From each earthen vessel samples were taken from three positions being the top 15 cm-, the middle 15 cm and the bottom 15 cm (Fig. 1) having around 20 liters volume. Top being the farthest from the drainage hole and bottom being at the level of the drainage hole.

Six glass vessels 20 liter each, similar to that of the earthen vessel (Fig. 1) were used to produce anbaris labneh.

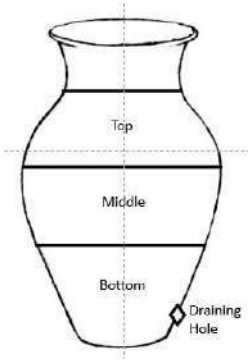

Fig.1 Earthen Vessel Sketch

2.1.4 Samples of anbaris compared to labneh produced from cow and goat milk

To compare anbaris to labneh from cows and goat milk, two companies, out of the 7 anbaris producing companies ((Dimassi, 2020 #555), agreed to provide us with 18 samples, from different lots, of cow, goat and anbaris labneh each.

2.2. Physico-chemical properties

Moisture content: Drying Oven and Balance method was used for moisture content determination. The oven used was Contherm designer series (Contherm Scientific LTD) following the ISO 5537:2004 [11].

Fat Determination: The fat content was determined using Soxhlet method as described by AOAC 922.06. [12]

Protein Determination: Protein content was determinedusing the Kjeldahl method according to AOAC 991.20. [12]

Ash Determination: Ash was determined using the AOAC 942.05 method. [12]

Weight determination: Weight was measured using Portable electronic balance Model 727 was used to measure the weight with an accuracy of ±1 gr (Jata Hogar).

pH: Microcomputer based pH /conductivity /TDS /salinity and temperature pocket meter Model pH/EC80 was used to measure the pH (Jenco VisionP).

Titratable Acidity: TA is expressed as percent lactic acid and is determined by titration of a known amount of reconstituted milk with 0.1 N NaOH using phenolphthalein as indicator [13].

Water activity: It was determined using Lab Start-aw water activity meter from novasina. Samples were flattened to cover the bottom of the cup and then water activity was measured at room temperature [14].

Salt: It was determined using Chloride QuanTab® Test Strips, 30-600 mg/L, Hach Company, Loveland, Colorado USA [15].

2.3. *Statistical analysis*

All tests and analysis were run in triplicates. General linear model performed via SPSS (statistical Package for the Social Sciences, version 17.0) was used to study the difference between the physicochemical properties and the score of the sensory attributes of the four different products. To study the difference between the physicochemical properties of the anbaris based on sample origion. Tamhane test was used for mean separation of the physicochemical properties.

III. RESULTS

3.1 Physicochemical values of the Industrially produced, house hold produced and house hold produced but with consumption anbaris

3.1.1 Water Activity (aw), moisture and total solids

The aw of anbaris did differ significantly where the water activity of the lowest for the HH-anbaris and significantly the highest for the IP-anbaris with the HHC-anbaris being in the middle (Table 1).

All for the moisture and the total solids content there was no significant differences between the values recorded for IP-, and HHC- anbaris, while the values of the HH-anbaris was significantly the highest (Table 1).

Table-1 Water activity (aw), moisture and total solids (TS) of anbaris from different origins

	Aw	Moisture (g/100gr)	TS (g/100gr)
	Mean ± SE	Mean ± SE	Mean ± SE
IP-anbaris	0.969a ±0.002	62.462a ±1.993	37.537a ±1.992
HH-anbaris	0.954b ±0.002	66.370b ±1.845	33.620b ±1.854
HHC-anbaris	0.962c ±0.001	61.457a ±1.495	38.457a ±1.500

- Within Columns, means with different alphabets are significantly different.
- IP: Industrial Produced, HH: House Hold, C: With consumption;

3.1.2 pH, titratable acidity and salt

The pH values all were lower than 3.8 thus they are in the high acid product category (pH<4.6) and there was no significant difference between the pH values of IP-, HH- and HHC- anbaris (Table 2).

As for the titratable acidity (TA) HH-anbaris possessed the significantly highest value followed by that of HHC-anbaris with the TA values of the IP-anbaris being the significantly lowest. (Table 2).

Concerning the salt concentration, the IP-anbaris had the significantly lowest salt value while the values of the HH-anbaris and those of the HHC-anbaris did not differ significantly (Table 2).

Table-2 pH, titratable acidity(TA) and salt content of anbaris from different origins

	pH	TA (g/100gr)	Salt (g/100gr)
	Mean ± SE	Mean ± SE	Mean ± SE
IP-anbaris	3.709a ±0.051	4.025a ±0.208	1.949a ±0.212
HH-anbaris	3.646a ±0.047	5.739b ±0.192	2.729b ±0.196
HHC-anbaris	3.607a ±0.038	4.736c ±0.156	2.636b ±0.159

- Within Columns, means with different alphabets are significantly different.
- IP: Industrial Produced, HH: House Hold, C: With consumption;

3.1.1 Fat, protein and ash content

The Fat content and the ash content of the IP-, HH- and HHC-anbaris, did not differ significantly from each other (Table 3).

As for the protein content the HH-anbaris had the significantly lowest value while their was no significant difference between the protein content values of IP- and HHC-anbaris (Table 3).

Table-3 Fat, protein and ash content of anbaris from different origins

	Fat (g/100gr)	Protein (g/100gr)	Ash (g/100gr)
	Mean ± SE	Mean ± SE	Mean ± SE
IP-anbaris	19.756a ±0.051	18.557a ±0.208	5.786a ±2.049
HH-anbaris	17.486a ±0.047	13.118b ±0.192	6.009a ±1.897
HHC-anbaris	20.226a ±0.038	19.007a ±0.156	8.096a ±1.536

- Within Columns, means with different alphabets are significantly different.
- IP: Industrial Produced, HH: House Hold, C: With consumption;

3.2 Physicochemical properties of Anbaris at different levels of Earthen Vessel

When the earthen vessel was full with anbaris samples were taken from 6 earthen vessels with 3 samples from each level (Fig. 1).

3.2.1 Water activity, moisture content and total solids

There was no significant difference in the water activity values of anbaris taken from the three different level. This was, however, different when the moisture content of the anbaris samples taken at the three levels. The samples of anbaris taken at the bottom showed the significantly lowest moisture content and consequently the significantly highest total solids (Table 4).

Table-4 Water activity (aw), moisture and total solids (TS) of anbaris from different earthen vessel levels

Position in Earthen-Vessel	Aw	Moisture (g/100gr)	TS (g/100gr)
	Mean ± SE	Mean ± SE	Mean ± SE
Top	0.931a ±0.001	77.111a ±1.531	22.879a ±1.490
Middle	0.931a ±0.001	74.501a ±1.531	25.488a ±1.541
Bottom	0.927a ±0.001	67.787b ±1.531	32.214b ±1.500

- Within Columns, means with different alphabets are significantly different.

3.2.2 pH, titratable acidity (TA) and salt content

There was no significant difference in the ph, titratable acidity and the salt content of the anbaris sample taken from the top, middle and bottom levels of the earthen vessels (Table 5).

Table-5 pH, titratable acidity(TA) and salt content of anbaris from different earthen vessel levels

Position in Earthen-Vessel	pH	TA (g/100gr)	Salt (g/100gr)
	Mean ± SE	Mean ± SE	Mean ± SE
Top	4.075a ±0.052	1.350a ±0.098	0.923a ±0.221
Middle	3.944a ±0.052	1.596a ±0.098	0.932a ±0.221
Bottom	3.976a ±0.052	1.536a ±0.098	0.977a ±0.221

- Within Columns, means with different alphabets are significantly different.

3.2.3 Fat, protein and ash content

Concerning fat, protein and ash content of the anbaris samples from the bottom level of the earthen vessel, were significantly the highest with those values from the middle and top position did not differ significantly (Table 6).

Table-6 Fat, protein and ash content of anbaris from different earthen vessel levels

Position in Earthen-Vessel	Fat (g/100gr)	Protein (g/100gr)	Ash (g/100gr)
	Mean ± SE	Mean ± SE	Mean ± SE
Top	7.818a ±1.011	10.316a ±1.713	1.755a ±0.123
Middle	6.667a ±1.011	13.927a ±1.713	1.907a ±0.123
Bottom	11.058b ±1.011	15.742b ±1.713	2.413b ±0.123

- Within Columns, means with different alphabets are significantly different.

3.3 Physicochemical properties of anbaris fermented and strained in glass vessel compared to that fermented and strained in earthen vessel

3.3.1 Water activity, moisture content and total solids

There was no significant difference between the water water activity value of anbaris originated from glass vessel compared to that originating from earthen vessel (Table 7).

However, when comparing the moisture content and total solids values of both, anbaris done in glass vessel had a significantly higher moisture content and consequently a significantly lower total solids when compared to that done in earthen vessel (Table 7).

Table-7 Water activity (aw), moisture and total solids (TS) of anbaris from fermented and strained in glass and in earthen vessel

Vessel	Aw	Moisture (g/100gr)	TS (g/100gr)
	Mean ± SE	Mean ± SE	Mean ± SE
Glass	0.968a ±0.003	70.963a ±2.165	29.038a ±2.165
Earth/clay	0.964a ±0.002	63.138b ±1.531	36.862b ±1.531

- Within Columns, means with different alphabets are significantly different.

3.3.2 pH, titratable acidity (TA) and salt content

The pH and titratable acidity of anbaris done using glass and earthen vessels did not differ significantly from each other. Salt, however, was significantly higher in anbaris samples done using glass vessel than those done using earthen vessel (Table 8).

Table-8 pH, titratable acidity (TA) and salt content of anbaris from fermented and strained in glass and in earthen vessel

Vessel	pH	TA (g/100gr)	Salt (g/100gr)
	Mean ± SE	Mean ± SE	Mean ± SE
Glass	3.897a ±0.146	1.644a ±0.201	3.751a ±0.381
Earth/clay	3.693a ±0.037	1.737a ±0.142	2.028b ±0.270

- Within Columns, means with different alphabets are significantly different.

3.3.3 Fat, protein and ash content

The fat and ash content of anbaris samples done using glass vessels did not differ significantly from those values of anbaris processed using earthen vessels.

Table-9 Fat, protein and ash content of anbaris from fermented and strained in glass and in earthen vessel

Vessel	Fat (g/100gr)	Protein (g/100gr)	Ash (g/100gr)
	Mean ± SE	Mean ± SE	Mean ± SE
Glass	20.997a ±0.146	11.841a ±0.201	5.522a ±0.381
Earth/clay	19.117a ±0.037	18.666a ±0.142	5.240a ±0.270

- Within Columns, means with different alphabets are significantly different.

Protein content, however, did differ significantly, where samples of anbaris manufactured utilizing earthen vessel had significantly higher protein values compare to those manufactured using glass vessels (Table 9).

3.4 Comparing physicochemical properties of anbaris to labneh (strained yogurt) done from cows and goat milk

3.4.1 Water activity, moisture content and total solids

There was no recorded significant difference when comparing the water activity of anbaris with labneh, strained yogurt [16], produced using cow's and goat's milk (Table 10).

The picture is different, however, when we compare moisture and total solids content, where anbaris samples did not differ significantly from labneh done from goat milk, both being significantly different from labneh values done from cow's milk, whose moisture content was significantly the highest and consequently the total solid content was significantly the lowest (Table 10).

Table-10 Water activity (aw), moisture and total solids (TS) of anbaris and labneh from cow and goat milk

	Aw	Moisture (g/100gr)	TS (g/100gr)
	Mean ± SE	Mean ± SE	Mean ± SE
Anbaris	0.965a ±0.001	56.907a ±2.375	43.093a ±2.365
G-labneh	0.963a ±0.001	56.807a ±2.375	43.193a ±2.365
C-labneh	0.958a ±0.001	72.499b ±2.375	27.551b ±2.365

- Within Columns, means with different alphabets are significantly different.
- C: Cow, G: Goat

3.4.2 pH, titratable acidity (TA) and salt content

The pH of anbaris did not differ significantly from the pH of labneh done from goat milk, both of which were significantly lower than pH of labneh done with cow's milk (Table 11).

Table-11 pH, titratable acidity (TA) and salt content of anbaris and labneh from cow and goat milk

	pH	TA (g/100gr)	Salt (g/100gr)
	Mean ± SE	Mean ± SE	Mean ± SE
Anbaris	3.825a ±0.025	1.566a ±0.062	2.116a ±0.039
G-labneh	3.868a ±0.025	1.602a ±0.062	2.290a ±0.039
C-labneh	4.093b ±0.025	1.395b ±0.062	1.670b ±0.039

- Within Columns, means with different alphabets are significantly different.
- C: Cow, G: Goat

As for the titratable acidity, the one recorded for anbaris was comparable to that of goat's milk labneh. Both being significantly higher than the titratable acidity value recorded for labneh produced from cow's milk.

The salt value of labneh from cow's milk had the significantly lowest salt content compared to anbaris and goat's milk labneh, while those values of both did not differ significantly.

Table-12 Fat, protein and ash content of anbaris and labneh from cow and goat milk

	Fat (g/100gr)	Protein (g/100gr)	Ash (g/100gr)
	Mean ± SE	Mean ± SE	Mean ± SE
Anbaris	20.259a ±1.94	21.538a ±0.062	6.81a ±0.099
G-labneh	22.634a ±1.94	24.458a ±0.062	5.03a ±0.099
C-labneh	14.983b ±1.94	10.892b ±0.062	4.659b ±0.099

- Within Columns, means with different alphabets are significantly different.
- C: Cow, G: Goat

3.4.3 Fat, protein and ash content

Concerning the fat protein and ash content values, they were significantly the lowest for labneh produced using cow's milk compared to anbaris and goat's milk labneh, while the values recorded for both did not differ significantly.

IV. DISCUSSION

Anbaris is a peculiar type of fermented cheese which is strained to get the peculiar gritty acidic taste [7] which is seemingly safe despite being done from raw milk [10] due to the low pH (<4) making it in the high acid food category [17]. Furthermore, there was no difference in the physicochemical properties of anbaris produced from pasteurized milk when compared to those produced from un-pasteurized milk. Nonetheless, sensory analysis should be done in another study since pasteurization lead to activation or inhibition of the plasmin/plasminogen complex, cathepsin D, lipoprotein lipase and alkaline phosphatase [18]. In addition to that, enzymes from psychrotrophic bacteria, acid phosphatase and xanthine oxidase, can survive pasteurization and may be active during ripening [18] while another array of enzymes will not survive. Also, it was noted that Lacto (a comparable fermented milk product in Zimbabwe) was less acceptable to the panelists when pasteurized milk was used compared to that produced from unpasteurized milk [19].

There is a difference between the industrially produced anbaris and that of the house hold produced anbaris. Interestingly the values of the house hold produced anbaris which is partially consumed during production, was very comparable to those from industrially produced anbaris. This might be explained by the fact that during production consumption is done from the top part (Fig. 1) of the earthen vessel before it is filled again with salt and milk were nearer to the industrially produced anbaris [10] [20]. Thus the significantly highest in moisture content and lowest in total solids will be consumed leading to more filling and draining times and thus higher total solid anbaris. In addition to that, the bottom part of anbaris is nearer to the draining hole and thus the higher protein and total solid content.

Comparing the physicochemical properties of anbaris produced using glass vessel and those produced using earthen vessel gave weight to the assumption of the house hold anbaris producers that earthen vessel produced anbaris which is different than anbaris produced utilizing neutral containers. Simply, earthen vessels are manufactured using clay, thus it allows the diffusion of water and salt and thus leads to lower moisture content and

higher protein content. This is in accordance with the results of Amasi, a fermented milk product in Zimbabwe, whose properties were found to be better when earthen vessels were used in comparison to amasi produced using glass jars [19] [21].

When comparing the labneh from cow's milk it had lower fat, protein ash, total solids and higher pH and moisture content. This is due to the fact that labneh from cows milk is strained for less than 7 day, 24 hours, in a cloth thus not as it is described usually in the production of labneh [22] which is not always practiced in the production of commercial cow's milk labneh. Since the 7 days straining period is practiced in goat and anbaris production the physicochemical properties were very comparable and in turn they were in accordance with values of labneh produced from cow's milk when 7 day straining was used [22].

V. CONCLUSION

Pasteurization did not affect the measured physicochemical properties of anbaris in this study, but a sensory analysis should be conducted to establish the profile for anbaris and other labneh products. The usage of earthen vessel is proven to lead to different anbaris product even at this level of testing. Comparing labneh from goat's, cow's milk origion and anbaris showed very similar physicochemical properties when straining time is applied. Thus, further tests should be conducted such as characterizing the different volatile compounds in the different labneh products.

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Effect of Addition of Mimba Leaf Extract (Azadirachta Indica A. Juss) to Corn with Different Types of Packaging on Water Content, Percentage of Moldy Seeds, and Aflatoxin Levels

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Abstract *— This study was conducted to determine the effect of mimba leaf extract and the type of packaging on corn stored at 4 weeks. This study used an experimental method with Completely Randomized Design (CRD) consisting of 4 treatments, and each treatment was replicated with 4 times. The treatment was the types of packaging, consist of cartoon without cover, plastic sacks, paperboard, and vacuum plastic. The measured variables were water content, moldy seed percentage, and aflatoxin content in corn after stored. Results of this study showed that the effect of adding mimba leaf extract on all types of packaging significantly different (P<0.05) on moisture content, the percentage of moldy seeds, and the aflatoxin content. The conclusion was the adding of mimba leaf extract on all types of packaging can maintain moisture content, percentage of moldy seeds, and reduce the aflatoxin content.*

Keywords*— *Aflatoxin, Corn, Mimba leaf, Type of packaging.

I. INTRODUCTION

Corn is the main raw material for poultry feed. Corn storage and processing greatly affect the quality of the corn. Corn is very easily contaminated if it is not stored properly. To reduce damage to corn, the storage method must be considered and the humidity temperature is considered so that people are competent in producing good quality corn.

Corn is a source of energy, with a carbohydrate / starch content of 64%. Domestic maize production has not been able to meet domestic needs, so it must import. In the period 1990 - 2001, the use of imported maize as raw material for the feed industry increased sharply at a rate of around 11.81% per year. Starting in 1994, the dependence of feed factories on imported maize was very high, around 40.29% and in 2000 it reached 47.04%, while the remaining 52.96% came from domestically produced corn (Deptan 2005).

Corn is one of the plants at risk of contamination by Aspergillus sp. especially Aspergillus flavus and Aspergillus parasiticus which produce aflatoxins that are carcinogenic and harmful to humans and animals. Aspergillus sp. can contaminate corn plants while they are still in the garden or during storage. These molds are naturally occurring molds that can grow in the soil so that parts of the corn plant are often affected by Aspergillus sp. These are the roots, then the stems, leaves, fruit of the corn, and then propagate to the deeper parts (Somantri 2005). According to Rahayu (2012), Aspergillus flavus is a type of fungus that often contaminates food, this type of mold can cause Aspergillosis infection and is also the mold that produces the most aflatoxins. If someone consumes food that is contaminated with low concentrations of aflatoxin continuously, it can damage the liver and reduce the immune system.

Mimba plant is a medicinal plant that has various uses to be developed as a basic material for making vegetable pesticides. Mimba leaves contain active ingredients called azadirachtin and salanin (Balfas, 1994). Mimba can produce more than 20 different types of

secondary metabolites. The leaves and seeds contain several secondary metabolites which are active as vegetable pesticides including azadirachtin salanine, meliontriol, and nimbin. Azadirachtin is used as an active ingredient in botanical fungicides that can inhibit the growth of fungi that cause plant disease (Mirin, 1997). Syamsudin (2007) reports that the compounds contained in mimba leaves are azadirachtin salanin, nimbin and nimbidin, where these compounds function as a disruptor to cell growth which can lead to fungal cell death.

Mimba leaf extract contains the main active compound azadiractin. Azadirachtin acts as an ecdysone blocker or a substance that can inhibit the work of the ecdysone hormone, a hormone that functions in the metamorphosis process of insects. Insects will be disturbed in the molting process, or the process of changing from eggs to larvae, or from larvae to cocoons, or from cocoons to adults. Usually, failure in this process often results in death (Wiwin, 2008).

The packaging used to store feed ingredients can affect how long it can be stored. The packaging is one way to protect or preserve the product. The packaging is an important material in various industries. The damage caused by the environment can be controlled by packaging. The packaging must keep the product clean and provide protection against contamination and other contaminants. Packaging must provide protection for food against physical damage, water, air, and light and must function properly, efficiently, and economically in the packaging process, and must have size, shape, weight, and facilitate further processing and must provide information and appeal to consumers. (Syarief, et al., 1989). One of them is from the type of packaging in the form of a plastic sack.

Plastic bags have smaller pores than other types of packaging. Plastic bags are rapidly being used because they are strong, waterproof, inert, transparent, can be formed, filled, and sealed by machine. Plastic bags can be used as packaging materials because they protect products from light, air, heat transfer, contamination, and contact with chemicals. The flow of gas and water vapor through plastics is influenced by plastic pores, plastic thickness, and molecular size that diffuses the product (Syarief and Irawati, 1988).

II. MATERIAL AND METHOD

2.1. Materials Research

The materials used are mimba leaf flour, Aquades solvent, corn from PT Japfa comfeed with an aflatoxin content of 200 ppb with a moisture content of 15%, and the type of packaging (open container, plastic sack, paperboard, vacuum plastic). The tools used are evaporators, analytical scales, filter paper, and glassware.

2.2. Research Implementation

2.2.1. Experimental Design

This study was performed by using a completely randomized design with 4 types of packaging treatments and 4 replications. Treatment A (Plastic Sack), treatment B (Cardboard Paper), treatment C (Open Container), and treatment D (Vacuum Plastic). The mathematical model used is according to Steel and Torrie (1995). The data obtained were analyzed by ANOVA, if there is an influence on the measured variables, it will be followed by the DMRT test.

2.2.2. Mimba Leaf Extraction

Mimba leaves (Azadirachta Indica A. Juss) are dried first in the sun. After the ingredients become dry and then blended into flour, the extraction process begins by mixing 50 grams of mimba leaf flour (Azadirachta Indica A. Juss) with 250 ml of Aquades solvent then soaking for 24 hours so that the active substances contained in the mimba leaf flour dissolve in solvent. Furthermore, filtering is done with filter paper. The filtrate obtained is collected, and then evaporated with a vacuum evaporator at a temperature of 550C to obtain a concentration that resembles oil. Each extraction result is put into a basin of 20 ml or a dose of 2.5% of the weight of corn weighing 800 grams each. Because based on the research of Sonyarati (2006) the 2.5% dose has had an effect on the attack of warehouse pests sitophilus zeamir matsh on corn that has been given mimba leaf extract, then the corn that has been filled with extract is homogenized until evenly distributed, then the corn is dried and air dried for about 1-2 hours. or it can also be oven at 600C for 60 minutes so that the extract that has been mixed is absorbed by the corn. Furthermore, storage is carried out in different containers for 4 weeks using plastic sacks, paperboard, open containers, and vacuum plastic. Each treatment, a pile of corn is placed on a board to prevent evaporation. Samples were viewed weekly. Sampling was carried out after 4 weeks of age.

2.3. Observed variables

2.3.1. Percentage of Water Content of Corn

To calculate the moisture content in corn, it can be calculated using a tool commonly used by the company PT. Japfa Comfeed Indonesia Tbk, Padang branch, namely the PM 410 kett tester tool, can be seen in the picture below.

Fig.1: PM-410 Moisture Tester

Prepare the corn that has been stored for 4 weeks (100 grams per treatment), put it in a stainless glass measuring 100 grams of corn, then put it in the circle of this tool evenly, this tool works automatically and on the monitor screen the moisture content of the corn is read.

2.3.2. Determination Percentage of Visually Contaminated Corn with Fungus / Mold

This test is carried out visually with the sense of sight, corn that has been given a known weight treatment is initially separated then weighed and the percentage is calculated.

% of corn contaminated with fungus / mold = $\frac{weight\ of\ polluted\ corn}{weight\ of\ corn\ sample}\ x\ 100\%$

2.3.3. Determination of Percentage Qualitative Aflatoxin Contaminated Corn (UV Light)

Corn that we have observed in the previous stage and its weight has been known to be followed by visual observation under UV light, after being observed under UV light, then we calculate the percentage of corn contaminated by Aspergillus sp.

% Tainted Corn $= \frac{weight\ of\ aflatoxin-contaminated\ corn}{weight\ of\ corn\ contaminated\ with\ mold}$ x 100

III. RESULT AND DISCUSSION

3.1. Percentage of Water Content of Corn

Corn from PT Japfa Comfeed has a moisture content of 15%. The average percentage of mimba leaf extract on the packaging type to moisture content during storage for 4 weeks can be seen in Table 1.

Table 1. The effect of giving mimba leaf extract on the type of packaging on moisture content in maize with a storage time of 4 weeks.

Treatment	Percentage of Water Content of Corn (%)
Plastic Bag (PB)	14,63[a]
Paperboard (P)	14,58[a]
Open Container (OC)	14,65[a]
Vacuum Plastic (VP)	11,85[b]
SE	0,15

Note: Different superscripts in the same column show a very significant difference (P <0.01)

Table 1 shows that the average water content has a very significant effect (P <0.01) at 4 weeks of storage. Based on the DMRT further test, it showed that the average types of packaging in the form of plastic bags, paperboard, and open containers were not significantly different (P> 0.05) but significantly different (P <0.05) with vacuum plastic. In all types of packaging, the water content decreases after storage, this is due to the growth and metabolic activity of microorganisms requiring water to transport nutrients or waste materials into and out of cells, all these activities require water in liquid form. Water that crystallizes and forms ice or water that is chemically bound in a solution of sugar or salt cannot be used by microorganisms.

This is in accordance with the research of Widianingrum, et al. (2010) on storage of maize for the first four weeks, the moisture content decreased from the water content before storage. The type of packaging in the form of vacuum plastic (VP) has decreased drastically due to its low oxygen permeability and no permeability to carbon dioxide gas and low water vapor transmission rate.

In accordance with the opinion of Imdad and Nawangsih, (1999) packaging is a container or media used to wrap materials or commodities, as well as to provide protection for materials or commodities. Different packaging can affect the moisture content can be seen in Table 1. It shows that the moisture content value of corn in vacuum plastic packaging has the lowest value compared to other types of packaging up to 4 weeks of storage, this is because vacuum plastic packaging does not have pores. pores compared to other packaging types.

Susanto (2008) added that the ecological factors that influence the growth of fungi are water activity (wa), moisture content, temperature, O2 substrate, CO2,

microbial interactions, mechanical damage, insect infection, number of spores, and storage time. Syarief, et al (2003) added that the growth and metabolic activity of microorganisms require water to transport nutrients or waste materials into and out of cells. All of these activities require water in liquid form. According to (Syarief and Halid, 1993) Water content is the amount of bound water and free water contained in the material expressed in percent.

3.2. Percentage of Visually Contaminated Corn with Fungus / Mold

Table 2. Effect of giving mimba leaf extract in the form of packaging on moldy seeds in maize with a storage time of 4 weeks.

Treatment	Percentage of Moldy Seeds (%)
Plastic Bag (PB)	0,0[a]
Paperboard (P)	0,0[a]
Open Container (OC)	3,7[b]
Vacuum Plastic (VP)	0,0[a]
SE	0,15

Note: Different superscripts in the same column show a very significant difference (P <0.01)

The analysis of variance in Table 2 shows that the effect of the type of packaging given mimba leaf extract at 4 weeks of storage has a significantly different effect (P <0.05) on the percentage of moldy seeds. The DMRT further test results showed that the mean moldy seeds in the treatment of packaging types in the form of PB, P, and VP were not significantly different (P> 0.05), but significantly different (P <0.05) higher than OC.

The type of packaging in the form of an open container (OC) was significantly different (P <0.05) with a higher value than the type of packaging for PB, P, and VP. The high type of OC packaging (open container) due to temperature and relative humidity are the main factors in mold growth. This is in accordance with the opinion of Titik et al. (2001) stated that *Aspergillus flavus* was detected in corn storage at 2 weeks and the fungi *Aspergillus flavus* and *Aspergillus parasiticus* were able to grow at low water content. In contrast to the types of packaging PB, P, and VP, the absence of mold/mold fungi are stored for 4 weeks, this is because there is no or little oxygen circulation in the package, at a level where there is insufficient oxygen content, as well as other microorganisms very much determined by the oxygen level in a certain condition (Moreno-Martines et al. 2000) this proves that the three packages have almost the same ability to maintain the quality of shelled corn.

3.3. Percentage Qualitative Aflatoxin Contaminated Corn (UV Light)

The aflatoxin content of maize used in this study was 200 ppb, this corn is corn that is not used in feed factories because of its high aflatoxins. By giving mimba leaf extract and stored for 4 weeks in various packages, it is expected that a decrease in aflatoxin will occur. The average aflatoxin content and reduction of maize stored in various packages, storage for 4 weeks can be seen in Table 3.

Table 3. Effect of giving mimba leaf extract on the type of packaging on aflatoxins in maize with a storage time of 4 weeks.

Treatment	Aflatoxin content (ppb)	Decreased Aflatoxin (%)
Plastic Bag (PB)	91,50[a]	54,25%
Paperboard (P)	124,75[b]	37,62%
Open Container (OC)	173,00[c]	13,50%
Vacuum Plastic (VP)	129,00[b]	35,50%
SE	10,55	

Note: Different superscripts in the same column show a very significant difference (P <0.01)

The results of the analysis of diversity are in Table 3. It shows that giving mimba leaf extract to the type of packaging at 4 weeks of storage has a very significant effect (P <0.01) on aflatoxins. The DMRT further test results showed that the average aflatoxin in the 4 week storage period for the types of packaging in the form of cardboard (P) and plastic sacks (PB) was not significantly different (p> 0.05) higher than the type of cardboard packaging (P), and different. significantly (P <0.05) higher with open containers (OC) and significantly different (p <0.05) lower with plastic bags (PB).

The high reduction in aflatoxins occurred in the type of packaging in the form of plastic sacks (PB). This is presumably because plastic bags have a better ability to protect corn from moisture and oxygen so that the administration of mimba leaf extract (Azadirachta Indica A. Juss) is able to work well. During storage, there was a decrease in aflatoxins in corn kernels in all types of packaging PB, P, VP, and OC. This was because the mimba leaf extract was thought to contain very strong

phenolic compounds, triterpenoids, and coumarin, which are antioxidant compounds that will give up one or more electrons to radicals. free radicals so they can stop the damage caused by free radicals, as free radical scavengers and prevent chain reactions (Dyatmiko et al., 2000). This is thought to be the cause of the decrease in aflatoxins. Therefore, one of the purposes of giving mimba leaf extract in this study is to interfere with the metabolic process of Aspergillus sp in producing aflatoxins, wherein this extract there are phytochemical compounds that have an antifungal function.

This is supported by research (Lillehoj, 1989). The decrease in aflatoxin content is thought to be due to the absence of fungal growth in shelled corn so that there is also no aflatoxin production. Aflatoxins are toxins produced by fungi. In accordance with the opinion of Goldbalt (1969), which states that the formation of aflatoxins will continue to increase if the fungi increase in number and in a long storage time.

Of the 12 types of aflatoxins that have been identified according to Goto (1990), aflatoxins B1, B2, G1, G2 are commonly found in food and feed ingredients and aflatoxin M1 in milk, and it is known that aflatoxin B1 is produced mostly by Aspergillus flavus mushrooms in Indonesia (Dharmaputra 2002). Among these, aflatoxins B1 and M1 are the toxins that receive major attention because of their toxicity to animals and humans (Bhatnagar et al, 2006) and because they are the most dangerous, AFB1 is often used as the maximum threshold for aflatoxin in food and feed (Goto 1990).

According to Miskiyah, et al (2009) there are currently six types of aflatoxins, namely B1, B2, G1, G2, M1, and M2. Aflatoxins M1 and M2 are hydroxylated metabolites of aflatoxin B1 and B2 and can be found in milk and milk products obtained from animals consuming aflatoxin-contaminated feed. The order of toxicity levels based on the study of the effect of aflatoxins on liver cells in vitro is B1> G1> G2> B2. Aflatoxin B1 is the dominant type of aflatoxin in maize (cobs and shelled 23-367.4 ppb) and corn products (cornstarch, popcorn, and crackers 10-40 ppb) obtained from farmers, collectors, collectors, wholesalers, and markets. self-service (Dharmaputra et al. 1995; Dharmaputra and Putri 1997).

IV. CONCLUSION

The addition of 2.5% mimba leaf extract by using a type of packaging in the form of a plastic bag gave the best effect during 4 weeks of storage and could reduce the aflatoxin content by 54.25%.

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Anacardium Occidentale and Jatropha Curcas Seeds' Alkaloids: Their Feeding Inhibitory and Growth Retardatory Potentials on Rhyzopertha Dominica

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Abstract**—** *The damaging effect of Rhyzopertha dominica larvae and adult insects on the nutritive content and economic value of Zea mais seeds, and the cytotoxic nature of most phyto-alkaloids prompted this research work to be carried out. A. occidentale and J. curcas seeds' alkaloids were extracted and experimented for feeding inhibition and growth retardation on callow and mature adult R. dominica (Lesser grain borer) using maize seeds. Feeding inhibition for A. occidentale and J. curcas seeds' alkaloids on adult insects were 39.40%, 49.88%, 81.37%, 100%, 47.48%, 55.12%, 93.31%, 100% respectively at 50 µg/mL, 150 µg/mL, 300 µg/mL and 400 µg/mL concentrations. Alkaloids from Jatropha curcas seeds demonstrated higher feeding inhibitory potentials than alkaloids from Anacardium occidentale seeds. Relative Growth Rates (RGR) of callow and mature R. dominica were retarded by A. occidentale and J. curcas seeds' alkaloid treatments. The feeding inhibitory potentials of A. occidentale and J. curcas seeds' alkaloids at 400 µg/mL concentration on both the callow and mature adult insects were translated to growth retardation of the insects when compared to maize seeds without alkaloid treatment (controls) fed by the insects . Relative Consumption Rates (RCR) of maize by callow and mature R. dominica were also reduced for maize seeds treated with alkaloids, indicating growth is a function of consumption. Thus alkaloids from Anacardium occidentale and Jatropha curcas seeds demonstrated feeding inhibitory and growth retardatory potentials on callow and mature adult R. dominica.*

Keywords**— Alkaloids, Anacardium occidentale, Consumption, Extraction, Feeding Inhibition, Growth, Jatropha curcas, Rhyzopertha dominica.**

I. INTRODUCTION

1.1 *Rhyzopertha dominica* and its feeding behavior

Rhyzopertha dominica, also known as lesser or stored grain borer is in a monotypic genus of beetle, Rhyzopertha in the Bostrichidae family. It is a black-brown, slim, cylindrical, small insect of three millimeters length with shape similar to a bullet. Its head is attached to its thorax by a rounded neck shield covered with pits that get smaller toward the posterior end. A ten-segmented antenna is clubbed with last three segments forming a loose club. Each female lays up to five-hundred eggs either on kernels of grain or loosely in frass produced by the insects. Egg development takes five days at 36 ℃ and hatches into a larva which is white in color and c-shaped. Larval development often takes twenty seven to thirty one days at 28 ℃ and forty six days at 25 ℃ respectively. Pupation takes approximately five to six days at 28 ℃ and eight days at 25 ℃ respectively in an enlarged cell where the larval feeding tube ends, and the adult insect can live up to two hundred and forty days (Toews *et al*., 2006; Koehler *et al*., 2011; Mason, 2003).

Infestation of grains by *Rhyzopertha dominica* larvae and adults originate from either residual population inside storage structures or immigration from outside sources, and insects have been shown to enter grain silos and other storages through vents and poorly sealed silo bases (Jia *et al*., 2008). Both the larvae and mature adult insects are voracious feeders that cause severe damage to chick peas, dried potatoes, herbs, wood, wheat, maize, rice, millet, peanuts, cocoa beans as well as processed products such as macaroni, tobacco and dried spices (Toews *et al*., 2006; Doug, 2009). They feed on the endosperm of grains by chewing their way through the bran layer, reducing grains to shells of bran, leaving behind large amounts of flour, tunnels, irregular shaped holes, and a sweet odor in grains as signs of infestation. Interestingly, the sweet odor produced by male *Rhyzopertha dominica* contains pheromones which have shown effectiveness as lures for use in insect traps. On a contrary, the larvae and adult insects do not infest standing grain and can survive outside the grain environment on seeds and acorns of other plants (Koehler *et a*l., 2011; Doug, 2009).

1.2 *Anacardium occidentale*

Anacardium occidentale (Cashew) plant, a member of the Anacardiaceae family is a small tree having coriaceous leaves, polygamous flowers, with five petals and nine stamens. Fruits are fleshy, dark yellow or orange coloured (Ghodgate *et al*., 2013). Cashew nut shell, a honeycomb structure contains a natural resin, cashew nut shell liquid (CNSL) which is used as a raw material to develop drugs, antioxidants, fungicides etc., and for treatment of wood against termites. Medicinal properties of phytochemicals present in CNSL have been reported, and include cytotoxic activity against several tumor lines, anti-diabetic, anti-inflammatory and analgesic effects (Kanan *et al*., 2009). Cashew nut seeds are ground into paste to treat snake bites. Fruits, stem-bark and leaves have anti-fungal, antipyretic and antidiarrheal applications. They are used to treat sores and rashes. Leaf extracts have demonstrated growth inhibition against several species of bacteria and fungi (Azam-Ali and Judge, 2004; Akash *et al*., 2009).

1.3 *Jatropha curcas*

Jatropha curcas plant, a member of the Euphorbiaceae family is a small drought resistant tropical tree or shrub which exudes whitish coloured, watery latex when cut. Normally, it grows between three and five meters high, but can attain a height of up to eight or ten meters under favorable conditions. It has large green to pale-green leaves which are alternate with a spiral phyllotaxis. Petiole length ranges between 6-23 mm, and flowers are formed terminally and individually, with female flowers usually slightly larger and occur in the hot seasons. The plant is often used for fences or as hedges by locals especially farmers because it is not fed on by most animals and insects. Its latex contains an alkaloid known as "Jatrophine" which is believed to have anti-cancerous properties. It is applied externally as treatment for skin diseases, rheumatism, and sores on domestic livestock. Tender twigs of the plant are used for cleaning teeth, while juice of the leaves is externally applied against piles (Gupta *et al*., 2011). Its roots are used as an antidote for snake-bites. Seeds of this plant have been used traditionally to treat ailments like burns, convulsions, fever and inflammation. Seed oil is used externally for the treatment of sciatica, dropsy and paralysis. It has a strong purgative action, and has been reported to be a remedy for syphilis. Also, seed oil is known to be useful as an insecticide, for soap production and as a fuel substitute to produce biodiesel via trans-esterification.Explored chemical constituents of *Jatropha curcas* includes diterpenes, triterpenes, lignanes, coumarines, flavonoids, phytosterols (Demissie *et al*., 2013; Prasad *et a*l., 2012; Ikbal, 2009).

II. MATERIALS AND METHODS

2.1 Sample collection and authentication

Fruits of *Anacardium occidentale* and *Jatropha curcas* were randomly harvested from trees in Kintampo and Navrongo respectively in the Brong Ahafo and Upper East Regions of Ghana. Plants were authenticated at the Department of Applied Biology, University for Development Studies, Ghana.

2.2 Preparation of plants' seed samples

Fruits of the plants were dried under shade for about a week at 25 ^{0}C. Shells were removed and seed contents were dried under shade for 72 hours, and then ground into fine powder using a blender. The powders were stored in containers and later subjected to alkaloid extraction process.

2.3 Alkaloid extraction

Powdered seeds (50 g) of *Anacardium occidentale* and *Jatropha curcas* were separately soaked in 200 mL 95% ethanol solvent for three days and then filtered. Residues obtained were mixed with 1M HCl and allowed to stand for 1 hour and then filtered. Ethanol solvent was evaporated from crude extracts in a water bath at 45˚C leaving the concentrates. Concentrates were combined with HCl filtrates and mixed

with excess dilute 1M HCl. Acid mixtures were basified by adding equal amounts of dilute 5M Na_2CO_3 until swirls of white precipitates which were alkaloids clouded the solution. Next, an equal volume of n-hexane solvent was added to the mixture, poured in to a separating funnel and shaken. It was then allowed to stand overnight for the separation to take place. This step was repeated twice to get as much alkaloids as possible into the n-hexane solvent. When the separation was over, the n-hexane solvent was evaporated in a water bath leaving the concentrated alkaloids.

2.4 Test for crude alkaloids

Few drops of Wagner's reagent were added to 1mL solution of alkaloids, and brownish red precipitates indicated the presence of alkaloids.

2.5 Insect rearing

Ryzopertha. dominica insects (Callow adults of 21 days old and mature adults of 26 days old) reared in aerated containers at an average temperature of 32˚C and relative humidity of 75%, in light and dark conditions of 12:12 hours daily, and fed with maize grains at Savannah Agricultural Research Institute (SARI), Nyankpala in the Northern Region of Ghana were used for the experiment.

2.6 Feeding inhibition test

R. dominica insects were kept in aerated containers with each container having 6 insects. Containers with distilled water treated maize grains served as controls (three each for callow and mature adult insects), while those with alkaloids treated maize grains in distilled water solvent served as treatments. Alkaloids were tested in four different concentrations (50, 150, 300 and 400 µg/mL) on callow and mature adult insects, with each concentration in triplicate. Maize grains were soaked in these concentrations for 20 seconds and dried under shade for 24 hours. Insects in treatment containers fed on alkaloids treated maize while those in controls fed on distilled water treated maize. Weights of food consumed within 24 hours were recorded for six days, and the formula below was used to estimate feeding inhibition index of alkaloid extracts:

- Feeding Inhibition Index (FII) = $\frac{C-T}{C+T} \times 100$, where;

C = amount of distilled water treated maize grains eaten

T = amount of crude alkaloids treated maize grains eaten

Relative consumption rate was calculated as weight of food eaten divided by initial weight of insect.

- Relative Consumption Rate = $\frac{Wc}{W}$, where;

Wc = weight of maize grains eaten.

W = weight of insect at the beginning of the experiment.

Relative growth rate was calculated as the difference in weight of the insects between the last day and initial day divided by the initial weight of the insect *R. dominica.*

- Relative Growth Rate (RGR) = $\frac{A-B}{B}$, where;

A = weight of live insects on 6th day

B = initial weight of insects.

III. RESULTS

Table 1: Results for presence and % yield of alkaloids

Seed Alkaloids	Inferences	% yield
A. occidentale	+	10.34
J. curcas	+	13.23

Key: (+) = present: (−) = absent

Table 2: Feeding inhibitory indices of A. occidentale seed alkaloids on mature and callow adult R. dominica

Concentration (µg/mL)	% FII		Average % FII
	Mature adults	Callow adults	
50	7.83	70.97	39.40
150	16.99	82.76	49.88
300	62.73	100.00	81.37
400	100.00	100.00	100.00

Key: FII= Feeding Inhibitory Indices (Expresses feeding inhibition potential)

Table 3: Feeding inhibitory indices of J. curcas seed alkaloids on mature and callow adult R. dominica

Concentration (µg/mL)	% FII		Average % FII
	Mature adults	Callow adults	
50	37.88	56.98	47.43
150	47.60	62.63	55.12
300	86.62	100.00	93.31
400	100.00	100.00	100.00

Key: FII= Feeding Inhibitory Index (Expresses feeding inhibition potential)

Table 4: Effects of A. occidentale and J. cucas seed alkaloids on Relative Growth and Consumption Rates of R. dominica at 400 µg/mL

Parameter	*J. curcas*		*A. occidentale*	
	Mature adults	Callow adults	Mature adults	Callow adults
Control (RGR)	0.46	0.21	0.44	0.26
RGR	-0.28	-0.13	-0.18	-0.13
Control (RCR)	48.26	88.71	24.43	72.14
RCR	24.95	70.70	14.29	44.20

Key: RGR= Relative Growth Rate, RCG= Relative Consumption Rate

IV. DISCUSSION

Alkaloids were found present in the seeds of *Anacardium occidentale* and *Jatropha curcas* respectively (table 1). Feeding inhibitory potentials (% FII) for *A. occidentale* and *J. curcas* seeds' alkaloids on callow and mature adult *R. dominica* were concentration dependent, and increased as alkaloids' concentrations were increased. The alkaloids exhibited 100 % feeding inhibition on the callow and mature adult insects at 300µg/mL and 400 µg/mL respectively, implying the alkaloids were more effective feeding inhibitors on the callow adult insects than mature adults (tables 2 and 3). This is in line with documented reports that the mature adults are more voracious feeders and resistant to pesticides than the callow adults (Koehler et al., 2011; Doug, 2009). % FII of *A. occidentale* seed' alkaloids on the callow adult *R. dominica* (P-value = 0.0140914) was higher than that on the mature adult *R. dominica* (P-value = 0.024513). % FII of *J. curcas* seed' alkaloids on callow adult *R. dominica* (P-value = 0.002212) was also higher than that on the mature adult *R. dominica* (P-value = 0.007682). Thus the *J. curcas* seed' alkaloids had slightly higher feeding inhibitory indices compared to *A. occidentale* seed' alkaloids (tables 2 and 3). Growth rates of callow and mature adult insects that fed on maize treated with alkaloids were retarded, and consumption rates of maize reduced when compared with those of the controls (table 4). Hence the growth of callow and mature adult *R. dominica* is a function of consumption of maize. Prasad *et al*., 2012 reported that alkaloids in the seed, leaf and stem-bark extracts of *J. curcas* plant possessed antifeedant properties. Praveena *et al*., 2012 have also reported the antifeedant activity of *A. occidentale* seed oil to be concentration dependent.

V. CONCLUSION

Alkaloids from *Jatropha curcas* seeds exhibited slightly higher feeding inhibitory potentials than alkaloids from *Anacardium occidentale* seeds, which were later translated to slightly lower growth rates of both callow and mature adult insects at 400 µg/mL concentration. Therefore the *Anacardium occidentale* and *Jatropha curcas* seeds' alkaloids possess feeding inhibitory and growth retardatory potentials.

RECOMMENDATION

Further research works should be conducted to isolate specific alkaloidal compounds responsible for the feeding inhibitory and growth retardatory potentials of *Anacardium occidentale* and *Jatropha curcas* seeds' alkaloids on callow and mature adult *R. domiica*. Investigating these alkaloids to prove their non-toxic nature to mammalian cells will create an avenue for usage as protectants against Rhyzopertha dominica infestation of maize.

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Performance of Boer and their Crossbreed Goats in Nepal - A Review

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Abstract**—** *There are about 10.25 million goats in Nepal. Most of these are local and adaptive to different ecological zones of Nepal. Despite of big goat population, performance in terms of daily gains were very low as compared to the Boer crossbreeds. The crossbreed Boer goat is a remarkable small-stock ruminant that possesses distinctive qualities enabling it to excel as an efficient production performer. The Boer goats had faster growth rates and were heavier than the indigenous breeds of goats from Nepal. The daily gain of Boer is 80-140 gm/day and Boer crossbreeds is 100-200 gm/day varying with different blood level of Boer crossbreeds. It is early maturing, reaching a mean maximum weight ≈ 62 kg at 3.5 years of age on natural pasture under extensive grazing conditions. Twinning percentage is about 40-50% in the crossbreeds. The indigenous goats of Nepal are subjected to crossbreeding with Boer goats in order to utilize the Boer goat breed as an alternate breed for production and reproduction performances. Boer milk is rich in fat followed by lactose and protein. Dressing percentage is about 56-60 % at the age of full teeth placement. They are resistant to various disease due to better performance of adrenal cortex and high cortisol production. Mortality rate is more for quadruplet and triplet kids. They are the best browsers and used as a controller in bush encroachment. Flexibility in acclimatization and other vital characteristics signified better performance of Boer and their crossbreeds in diverse country like Nepal.*

Keywords**— Nepal, Boer, crossbreeds, performance.**

I. INTRODUCTION

Goat (*Capra hircus*), being the most common domestic animals reared, is the second most important meat animals after the buffalo meat in Nepal. Boer, Barberi, Jamunapari and Beetle are popular exotic breeds used for cross breeding to upgrade indigenous breeds like Khari, Terai, Sinhal etc.(Bhattarai et al., 2019).Low investment requirement attracts Nepalese farmer to get involved in goat farming, showing statistically 49.8% household rear goats with average holdings of 3.3/household (CBS, 2012).Sector of goat provides a strong sustenance in source of revenue of Nepalese farmers of hills and terai which constitute the higher share of land area and inhabitants of the country (Neupane et al., 2018). Figures regarding to goat population showed that there are more than 10.25 million heads of goats in the Nepal with the rise in goat population during 2001-2015 with 3.65% per year contributing about 20.1% to the total meat production in the country(Secretary & Division, 2015).Due to low producing ability of indigenous goats as compared to the exotic breeds, this production does not meet the growing demand and fulfillment is accomplished by importing from foreign country, especially from India and China. To overcome this situation, full blooded Boer goat are crossed with local breeds or developing Nepalese pure Boer goat by upgrading(Sciences, 2017).

Accomplishment of very productive goats within a short period of time and to obtain superior stock from elsewhere, crossbreeding appears to be an easy technique (Belay et al., 2014). The result will bring up the hybrid vigor or heterosis and it is expected that crossbreed give rise to improved performance compared to both parents for instance production or reproductive traits(Nugroho et al., 2019). Refining genotype within breed, compared to crossbreeding, selection via adaptation and genetic gain is sustainable but is a time-consuming method with genetic gain/generation as low as <5% (McDowell, 1972). Crossbreeding is taken as beneficial and decision of choice as crossbreeding goats can bring about their quick

enhancement by improving their genetic performance (Mustefa et al., 2019).

Originated in South Africa, Boer goats are meat category goats introduced in many countries counting Nepal as a result of good resistance capacity, quick growth rate, heavier body weight, high prolificacy with average litter size almost two and high carcass quality (Lu, 2001). Owing to these important qualities, crossbreeding of indigenous breeds with Boer has been common in Nepal since it is a better method to get productive goats in short period of time(Sapkota et al., 2016).Boer of origin Eastern Cape of South Africa ≈ 60 years ago, has been very adjacent to the perfect Boer to bred due to forethought of a no of Boer breeders, who stringently selected goats with a definitive breeding policy (Erasmus, 2000). Five phases of determination for performance testing of Boer started in 1970 under south African Mutton and Goat Performance and Progeny testing Scheme(N. H. Casey & Van Niekerk, 1988).Ability to convey its superior phenotypic physiognomies to other breeds when used for crossbreeding is supplementary in Boer(Barry & Godke, 1991).Boer was familiarized in Nepal from a private sector to advance growth performance of local goats subsidized by World bank, IFAD, AFSP etc. Goat research station; Bandipur, RARS (khajura), GDF (Budhitola) are the government-owned farms with nucleus herd of Boer goat in Nepal (Bhattarai et al., 2019).Indigenous goats are being collected at Lumle Agricultural Research Centre (LARC) since late 1980s from western hills aiming to evaluate their performance and explore the possibility of improvement of their genetic potential (Rasali & Khanal, 2002).Boer become a potential genetic resource in crossbreeding programs for improving Nepalese local goat productivity.

II. DISCUSSION

Boer as an exotic breed

Infusion of European, Angora and Indian goat, Boer is a horned breed with lop ears and varietal color pattern with most common white body with red head and muscular frame. Goat is full-bodied with decent shape and roman nose comprising short, well fleshed good thigs and hind legs for good carcass features (Campbell, 1984). Being popular for its browsing skill with limited impact on grass cover, mature buck masses 100-135 kg and does between 90-100 kg. With daily gains 200g in feedlot, ovulation rate ranges from 1-4eggs/doe with average of 1.7 and kidding rate of 200%. Male reaches puberty at about 6 months and females at 10-12 months and protracted breeding season making possible of 3 kids every 2 years (Bhattarai et al., 2017).Accountabilities for culling are a hollow forehead, slender mouth, folded ears, under-shot jaws, hollow back, weak pasterns, front x-shaped legs, small testes, hooves turning in or out, elongated coarse and wooly hair covering, thick, big teats, and less than 25% pigmentation (N. H. Casey & Van Niekerk, 1988). The Boer goats which are nowadays receiving quick acceptance in the industrialized (USA, New Zealand, Australia, Japan) and emerging (Sir Lanka, India) nations have reckless progress rate pre and post-weaning with an outstanding feed conversion ratio; 1:3.18-3.60(Feed & Council, 1997) and truncated cholesterol content are already familiarized in Nepal by a private farmer Mahesh Basnet in 1999(Panday, 2008).

Boer for crossbreeding

Boer goat producing decent crossbreeds having hybrid potency with indigenous breeds are more resilient to diseases compared to native breeds and its crosses correspondingly convey this resistance (Panday, 2008). In the last few decades(1990s), Khari goats were tremendously crossed with Indian Jamunapari and Barberi goats for growing production with postulation but is now substituted with Boer either naturally or through artificial insemination with frozen semen through the foremost private goat impresarios due to various research directed by NARC , ARSs, DLS etc.(Bhattarai et al., 2019).With the intention of conveying the superior phenotypic characteristics of a breed to the F1 progeny, Boer fits finest due to accessibility of these outstanding phenotypic characters which can be envisioned in its crossbreed offspring(Barry & Godke, 1991). The potentials of crossbreeding are adaptability, hardiness, better growth, early maturity, disease resistant, less kid mortality, better twinning percentage, improved milk production and lactation and many more.

Performances of Boer and their crossbreeds

Adaptability and hardiness:

Flexibility of farm animals in their capability to acclimatize to several climates and production schemes is an economically vital characteristics signified better by reproductive performance with reproductive life of 10 years(Campbell, 1984).Despite acclimatization being a slow procedure taking a year or extended, Boer characterizes itself as one of the robust amongst the small stock breeds having the capability to familiarize to almost any climate, from the hottest dry desert to the snow-covered mountains(Barry & Godke, 1991).

With strong and sturdy legs, Boer can travel long distances along dense shrubbery lands and over rugged mountains for food and water and survive droughts without supplementary feed being the best of all goat breeds.Small

dimensions, huge surface area to body weight, water conservation ability, restricted subcutaneous fat cover and the specific nature of their coats make Boer to adjust in hot than in cold environment (Shkolnik & Choshniak, 1985). The behavioural attribute of goat to feed selectively smooths their ability to endure under strict tropical and semi-arid surroundings(N. H. Casey & Van Niekerk, 1988). Ability to reside the latter environment with occasional management practices such as vaccination and medicating, the terms hardiness and adaptability are attached to this animal. Boer may finest be designated by the following quotation: "Certainly one of the most hardy of small-stock breeds on earth, with a great ability of adaptation; it is therefore found in such a wide variety of climates and grazing conditions"(Erasmus, 2000).

Growth Performances:

Heavier body weight and faster growing rate are the most notable among all superior traits.

Genetic parameters of development traits of kids are vital in enlightening their genetic makeup with respect to production and efficiency. In a research conducted in GRS Bandipur(NCRP, 2019), total no of 52 off-springs (23 male and 29 females) from 26 pure dams, the average weight at birth of pure Boer kid was 3.79 kg with average weaning weight 18.59 and eight month weight of 28.34 kg. The average yearling weight of the Boer was found 45.74 kg.

The average daily gain of the pure Boer kids in one year was found to be 114.93 gm/day which is comparatively more than that of Khari goats.

Table.1: Growth Performances of pure Boer, 2075/76(NCRP, 2019)

Age	**Male**		**Female**	
	Weight(kg)	Wt. gain(gm/day)	Weight(kg)	Wt. gain(gm/day)
Birth	4.02 ± 0.16		3.62±0.12	
Weaning	18.79±0.78	123.07	18.44±0.41	123.36
8 months	32.56±5.42	118.92	24.12±5.13	85.50
12 months	50.80±6.25	128.17	40.67±5.77	101.37

Boer goats are renowned having faster growing rate compared to others. Weaning methods, stress and compensatory growth affects the growth rate (Lu & Potchoiba, 1988).Illustration can be taken that growth rate of Boer kids can be abridged by adaptation in the internment alone (N. H. Casey & Van Niekerk, 1988). Incessant genetic selection improvement, feeding and supervision may subsidize to even a faster growing rate in Boer goats as well as their crosses (Lu, 2001).

Evaluation of different blood levels of the Boer goat in different ecological region of Nepal ongoing. 2.38 kg and 2.64 kg the average weight at birth, 15.87kg and 16.63 kg the average weaning weight, 26.88kg and 27.24kg the average eight months weight and 39.97kg and 41.01 kg the average annual weight was of 50% and 75% Boer goats found in the research of Boer X Khari goats.

Table.2: Growth Performances of Boer Crossbreed 50%, 2075/76(NCRP, 2019)

Age	**Male**		**Female**	
	Weight(kg)	Wt. gain(gm/day)	Weight(kg)	Wt. gain(gm/day)
Birth	2.72±0.62		2.06±0.72	
Weaning	17.42±2.94	122.50	14.34±2.18	102.37
8 months	28.28±2.33	106.55	25.49±2.90	97.66
12 months	41.08±1.93	106.61	38.88±1.65	102.32

Table.3: Growth Performances of Boer Crossbreed 75%,2075/76(NCRP, 2019)

Age	Male		Female	
	Weight(kg)	Wt. gain(gm/day)	Weight(kg)	Wt. gain(gm/day)
Birth	2.92±0.26		2.36±0.27	
Weaning	16.54±2.34	113.50	16.73±2.31	119.75
8 months	27.73±2.42	103.37	26.75±2.76	101.62
12 months	43.69±2.59	111.69	38.33±2.88	98.54

Average daily weight increase in 5th and 6th month of age was low which might be due to the weaning stress as weaning was done in the 4th month of age.

Rendering to the study in Sharabeshi of Bakrang, Gorkha, Boer crossbreeds (25% blood level) had achieved better performance in comparison to local Khari goat.

Table.4: Mean body weight and body weight gain Boer crossbreed (25% blood level) up to 12 months age at Sharabeshi, Gorkha in 2070/71, (NCRP, 2014)

Age	Male		Female	
	Weight(kg)	Wt. gain(gm/day)	Weight(kg)	Wt. gain(gm/day)
Birth	2.76		2.68	
4 months	13.65	80.00	12.50	66.67
8 months	22.20	76.67	20.30	68.33
12 months	31.50	76.67	28.00	63.33

Rendering to the study in Pekhuthana, Syangja, Boer crossbreeds had performed better performances in comparison to local Khari goat.

Table.5:Mean body weight and body weight gain Boer crossbreed (25% blood level) up to 12 months age at Pekhuthana, Syangja in 2073/74,(NCRP, 2017)

Age	Male		Female	
	Weight(kg)	Wt. gain(gm/day)	Weight(kg)	Wt. gain(gm/day)
Birth	2.76		2.67	
Weaning	13.42	88.83	12.68	83.41
8 months	21.89	79.71	19.12	68.54
12 months	30.47	75.91	29.22	72.74

Table.6: Mean body weight and weight gain of Boer crossbreed (50% and 75% blood level) up to five months of age at Pekhuthana, Syangja in 2075/76 (NCRP, 2019)

Age	Wt. of Boer (50%) kg		Wt. gain of Boer (50%) gm/day		Wt. of Boer (75%) kg		Wt. gain of Boer (75%) gm/day	
	Male	Female	Male	Female	Male	Female	Male	Female
Birth	2.93	2.75			3.26	2.50		
1st month	7.26	6.24	142.49	114.64	7.92	7.70	153.34	171.06
2nd month	12.42	10.87	202.52	152.25	16.65	17.96	287.15	337.41
3rd month	15.93	14.17	82.45	108.49	19.25	19.36	85.35	45.93
4th month	20.38	18.22	146.47	133.08	26.84	24.79	249.53	178.65
5th month	24.86	21.53	147.19	108.85	29.44	27.01	85.35	72.69

The birth weight of Boer X Khari cross kids is inferior than the birth weight of Boer kids but bear a resemblance to the birth weight of Khari kids (Rasali & Khanal, 2002). Weightier birth mass of male is reinforced by findings ofNeopane & Sainju, 1995 and Upreti & Mahato, 1995. The weaning weight of Boer X Khari cross kids differs by gender which is alike with Boer X Central highland cross kids (Deribe & Taye, 2013), though inferior than pure Boer weaning kids (Lu & Potchoiba, 1988).As per this study, weight gain was highest in Boer 50%. Kids born in monsoon were significantly weightier at birth due to availability of green forages and improved nutritional status, it did not affect their body weights up to 12 months of age as season of birth had zero significance on the body weight(Rasali & Khanal, 2002). Influences of genetics, nutrition, health and disease, breeding age and method of management system can vary the weight measurements (Lu, 2001).

Reproduction Performance:

Most important criterion relating to adaptation is reproductive performance. Maximum fertility appeared to attained at the relatively early age of 3.5 years. Crossbreed Boer is early maturing at the age of 1.5 years (Erasmus, 2000). Low post-natal mortality along with high rate of reproduction are the important requirements for meat producing animals; as it is best fit for goat than other domestic ruminants due to higher average litter sizes (Shelton, 1978).The reproductive performance of the Boer goat was documented to access the reproductive performance of pure Boer goat in GRS, Bandipur.

Table.7: Reproductive performance of pure Boer goat 2075/76 (NCRP, 2019)

Reproductive parameters	Days	No of Observations
Age at first service	270-400	21
Age at first kidding	410-510	15
Litter size at birth	1.73(1-3)	15
Post-Partum Estrus	110-170	15
Kidding interval	210-300	15

Boer X Khari goats are also subjected to access the reproductive performances (75% Boer cross doe and 50% Boer cross doe with Khari breeds).

Table.8: Reproductive performances of Boer X Khari crosses(F1) and Boer X Boer F1(F2) 2075/76(NCRP, 2019)

Reproductive parameters	F1(Boer 50% doe)	F1(Boer 75% doe)
Age at first service	220-270	210-290
Age at first kidding	360-380	300-400
Litter size at birth	1.54 (1-3)	1.55 (1-3)
Post-Partum Estrus	65-112	70-125
Kidding interval	240-360	220-380

Conception rate and kidding pattern of the Boer goats at 75% blood level and 50% blood level are similar but lesser in Boer cross 87.5% doe.

Table.9: Conception rate and kidding pattern of Boer crosses at GRS 2075/76(NCRP, 2019)

Parameters		Boer cross Doe blood Level		
		87.5%	**75%**	**50%**
Conception rate		66.66	93.60	96.08
Kidding type	**Single**	50.00	47.22	48.61
	Twin	50.00	50.00	48.61
	Triplet	0.00	2.78	2.78
Kidding rate/ doe		1.50	1.55	1.54

Performance testing scheme shows the following reproduction performances (Campbell, 1984).

Table.10: Reproduction Performance of Pure Boer Breed(Campbell, 1984) in South Africa

Females kidded	98
Singles Born	24
Twins Born	116
Triplets Born	45
Quadruplets Born	4
No of kids per parturition	1.93
Singles weaned*	26
Twins weaned	112
Triplets weaned	4

{* = Some kids were born as twins or triplets but reared as singles}

This suggests that with average litter size nearly equal to 2, Boer produced twinning kids and triplets up to 60% and 10-15% respectively. The birth type of Boer goat vary from 15-24.5% single, 59.2-67.5% twins and 15.3-16.3% triplets (Greyling, 2000).

Weak appearance of does leads to abortions which plagued Boer in some areas were described due to undernutrition. Experimental investigation byCoetzer & Van Niekerk, 1987,demonstrated the cause to be *Anaplasma ovies*(Barry & Van Niekerk, 1990). The organism is likely to cause abortion in prevalent areas by not only parasitizing the RBCs but also those of the aborted fetuses(N. H. Casey & Van Niekerk, 1988). Being well known for fecundity , a Boer goat ovulates 1.8(+ or – 0.9)ova per estrous cycle with a range of 1 to 4.3b (Greyling, 1990).

Table.11: Estrous cycle of Boer (Barry & Godke, 1991)

Length of estrus cycle	20.7 days
Does with cycles LT or EQ to 13 days	16.6%
Does with cycles GT or EQ to 25 days	10.2%
Mean length of Estrus	37.4 hours

Boer goat is a moderately seasonal breeder but complete anestrus does not occur in the breed(Van der Westhuysen, 1979), stirred by shortening daylight with ultimate sexual activity in April-May and least in October-January (Greyling & Van Niekerk, 1986).

Gestation length also varies with the birth type and postpartum anestrous period varies with season as represented:

Table.12: Gestation Period of Boer (Barry & Godke, 1991)

Mean Gestation Period	**148.2 days**
Does with singles	149.1 days
Does with twins	147.8 days
Does with triplets	146.8 days
Postpartum anestrous period	
Kidding in kidding season (sept-oct)	37.3 days
Kidding in off season (mar-apr)	59.9 days
First cyclic activity post-kidding	20 days
Kidding to pregnancy (no breeding season)	62 days

Estrus cycle are longer than 21 days during the period of least sexual activity.

Milk Production Performance:

Parental physiognomies of the goat doe are reliant on the figure of kids weaned per doe kidded. Milk production of the doe throughout the preweaning phase is one of the chief status to permit high growth rates of kids, particularly in does having multiple births as the fact that high fecundity is one of the Boer's sturdiest qualities(Erasmus, 2000). Foeto-placental units and placental weight determines the degree of mammary advance in milch goats (Hayden et al., 1979). Sequential lactation also intensifies the amount of milk produced (Prakash et al., 1971). Milk production in the doe regarding multiple born kids are strongly affected by seasonal influence. As Boer are not selected for milk producing trait, it is just an extension of reproduction under natural conditions and varies with litter size and lactation number (N. H. Casey & Van Niekerk, 1988).

Table.13: Mean milk production and milk composition during 12 weeks of lactation of Boer goat does (Raats et al., 1983)

Age of	Litter	Milk Yield	Milk composition			
Does(teeth)	Size	(kg/day)	% Protein	% Fat	% T. S	% Lactose
2	Single	1.5	4.5	7.5	17.3	4.7
2	Twins	1.9	4.4	7.0	16.8	4.7
2	Triplets	2.3	4.2	6.4	15.8	4.6
4	Singles	1.8	4.5	7.7	17.9	4.9
4	Twins	1.9	4.3	7.4	17.1	4.8
6	Singles	2.1	4.4	9.4	19.2	4.7
6	Twins	2.2	4.1	8.1	17.4	4.7
6	Triplets	2.5	3.9	7.6	17.0	4.7

In comparison with well intensive dairy goats, average yield of 1.5-2.5 kg/day may not seems well, capability of milk production of Boer can be raised by presence of milk ancestry with high nutritional regime(Raats et al., 1983). Milk composition and quality are the attributes which determines the nutritive value which are affected by stage of lactation, parity, breed and season.

Table.14: Milk analysis of Boer goats at GRS, Bandipur (NCRP, 2018)

FAT%	SNF%	Density	Lactose%	Solids%	Protein%	F. P	Conductivity
8.48	8.11	26.19	4.23	0.82	3.02	-0.54	4.29

The average daily gain (ADG) of kids is directly correlated to the milk production of dam with a preweaning ADG of 300gm, the doe most yield up to 2.5 litres of milk per day. 4th week of lactation must be the peak period of milk production. Though the doe produces sufficient milk to advance 2 kids, the supplementation of feedstuff to the doe with twin or triplets is decidedly prudent(Barry & Godke, 1991).

Meat production performance:

As the young Boer got tender and very tasty meat, it is raised for meat production. They must be marketed at a very young age to have better dressing percentage. The dressing percentage at specific ages is presented below:

Table.15: Dressed weight percentage of slaughtered Boer Goats (Barry & Godke, 1991)

Age	**Dressed Percentage**
8-10 months	48%
2 teeth	505
4 teeth	52%
6 teeth	54%
Full teeth placement	56-60%

Other mutton breed has no higher dressing percentage for young animals. 28-43 kg live-mass weight is best merchantable weight for young goats. There is no satisfactory fat casing in the carcass of young goats weighing < 18 kg and is often differentiated counter to kids implies the meat of goat with no perpetual incisors. This arrangement has superiority carcasses with a thin coating of fat. Too little or too abundant fat or a weak configuration causes aninferiorcategorizing of the carcass(Godke, 1977). The Boer goat used as crossbreeding increases growth and meat characteristics in local goats. Boer meat has a sturdier savour than mutton due to its habit of grazing bushes and shrubs comprehending aromatic compounds. Meat becomes tough due to less amount of subcutaneous fat during cold shortening(Norman Henry Casey, 1983).

Disease resistance performance:

Statistics about resistance of disease on the Boer is comparatively less. Regardless of this, Boer are believed to be relatively disease resistant due to their grazing habit (Skinner, 1972).Better management practices of natural grazing lowers the dose to goats against internal parasites (Greyling, 1990).Habit of tree climbing helps them to be less susceptible to parasite infection occurred in the grass cover(Erasmus, 2000). Being fairly healthy breed, they are resistant to blue tongue, prussic acid poisoning, enterotoxaemia (pulpy kidney *Clostridium perfringes* type-D) and gall-sickness(Hugo, 1968) but kids development is strappingly retarded by internal parasite infection (Erasmus, 2000)**.**During winter and spring Boer are subjected to bule tick infestations (*Linognathus africanus)* (Fourie et al., 1991). Due to being immune to tuberculosis benefit of utilizing goat milk is that this disease cannot be contracted by humans. It also cures erysipelas(rash) in small children and is prescribed to cure 'bawling babies'(Campbell, 1998). Higher cortisol production is maintained in Boer goat, a superior adrenal function. For the induction of several gluconeogenic enzymes that enables animals to survive stressful conditions, secretion of cortisol by the adrenal cortex is essential. CRF stimulates ACTH secretion form anterior pituitary as CRF is stimulated by the release of corticotropin-release factor (CRF) which is stimulated by stress. Glucocorticoids are secreted from the adrenal cortex promoted by ACTH and favour glucose production at the expense of glycolysis (Engelbrecht & Swart, 2000). Cortisol production is high in Boer goat in stress condition partially explain the superiority of disease resistant and adaptation. Being a good browser, in the unavailability of shrubs, it prefers not to graze in the early morning when there is higher chance of contamination due to dew on grass. This resistance ability of Boer makes its reproductive life cycle of up to 10years and more(Barry & Godke, 1991). Due to capability of drought resistance, tolerance of tannins, efficient fibre digestion, adaptation to various ambient temperature and lower water turnover rate, they become more disease resistance compared to other breeds and ruminants too(Lu, 2001).

Kid mortality:

Under extensive rearing the mortality seems relatively high. As kids born as single, twins, triplets and quadruplets, the mortality rate is 10.8%, 8.3%, 20.8% and 31.3% respectively (Erasmus et al., 1985). Kids born as triplets and quadruplets suffers the highest mortality (Els, 1995). Exposure to cold, milk shortage from doe, too small birth weight of kid are the important factors affecting kid mortality regardless of causes of mortalities in small stock (Olivier, 1980). Despite of these facts, cognisance should be taken of that other factors may influence eventual weaning rates. Predators like black-blacked jackal and lynx, stock thefts may contribute in kid losses(Erasmus, 2000). Out of 42 kids, 2 died (4.76%) in a research conducted in Boer 50% in GRS, Bandipur(NCRP, 2017).Higher survival rate of kids was found which are born in wet season (Hailu et al., 2006). Male kids had lower survival rate than female kids as similar impacts due to litter size and sex of kid on survival(Browning Jr & Leite-Browning, 2011).

Grazing performance:

Flexibility in harvesting forage and capability to survive under adversative scavenging conditions are the physiognomies of goat that set them apart from other livestock species (Lu & Potchoiba, 1988). Being reported as browsers, their diet consists of 82% browse and 18% grass. Study in Namibia indicated that 74% leaves and 26% grass are consumed by Boer goats(Viljoen, 1980).Acceptance of goats towards bitterness plays asignificantrole to take full advantage of grazing capacity and in biological control of weeds as they do not dig out roots under harsh grazing conditions(Lu, 2001). Bush and shrub encroachment are creating detrimental impact on animal production on global range. One direct consequence can be exampled the deterioration of natural vegetation which results in decreased carrying capacity ultimately limiting red meat production (Erasmus, 2000). Grazing of goat lies in a wider spectrum of plants than other type of small stock and are inclined to forage from the top downwards from approximately equal to 160 cm to 10 cm (Aucamp & Du Toit, 1980).*Portulacaria afra*, other shrubs and grasses are consumed in the ratio of 37:38:25 by Boer goats. This damages less to the extra sensitive

ground layer vegetation including seedlings, young plants and crown plants (Stuart-Hill, 1987). Despite of being heavy eaters, feed conversion ratio of Boer crossbreeds as well as purebred Boers about 2.5-3.5 kg dry matter per weight gain. Goat's adaptive ability enable it to perform equally on grass-only diet and survived where browse had been absent from their diet (Mahmoud, 1978). Apart from browser, it is also a good walker and is able to withstand the deteriorated natural pasture and is also mentioned as a controller of bush encroachment as they do not get tangled in thorn, bushes and shrubs and need not be protected from rain and cold (Campbell, 1998). Boer are also popular for maintenance of landscape in forest areas because regardless of their diets they are less choosy (Erasmus, 2000).

III. CONCULSION

The growth performances and growth rate of Boer crossbreed was highly significant different than local and other crossbreeds of goats found in Nepal. Boer goats are able to gain diverse gratitude for outstanding body configuration, fast growing rate and good carcass quality. Grouping of breed standards and performance testing is likely to be the well approach for the effective selection and enhancements of Boer goats. Heavier body weight and faster growing rate are the most notable traits among all superior traits for production of meat. Because of their desirable traits they have successfully improved productive performances, hardiness, adaptability, fertility and disease tolerant of indigenous breeds through cross breeding. Prominent improvements consist birth weight, growth weight, weaning weight, breeding weight, mature kids, kidding rate and carcass quality. These goats possess characteristics including versatility, harvesting forage and ability to survive under adverse foraging conditions that set them apart from other livestock species. Boer are reported to have superior adrenal function and able to maintain higher cortisol production. Kidding rate and twinning percentage of Boer cross was also better than other breeds and mortality rate was also very nominal as compared to Khari. It is concluded that Boer goat may be the best substitute breed for crossbreeding with indigenous breeds in order to upsurge performances from the goat farming in Nepal. Continuous upgrading in genetic assortment, feeding technique and supervision arrangement may subsidize to even faster growing rate in Boer crosses in future.

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Solid Waste Management in the Accra Metropolitan Area of Ghana

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Abstract**—** *Waste management is one of the key challenges that is facing the world today. This global threat of solid waste disposal has affected Africa in many ways especially causing diseases and increasing thepoverty rate. These problems emerge mainly as a lack of strategic planning for effective SWM, poor governance, resource constraint, poor coordination and lack of proper management. In most rapidly growing cities in developing countries, the major concern issue is the inefficient solid waste collection and disposal. This research focuses on how municipal solid waste is managed by the public administration system which is the AMA of Ghana. The study, therefore, focused on existing pieces of literature and came out with these results. It was revealed that the types and sources of solid waste in the study area include, domestic waste, commercial waste, agricultural waste, industrial stemming from food waste, rubber, paper, glass, metal, farm etc., The study also revealed the major policies guiding SWM in the study area which include the Environmental Sanitation Policy of 1999 Act (1994), Act 464. Another policy is the Environmental Assessment Regulation LI 1650 which had legal support from the Environmental Protection Agency Act 490 which spelt out the Environmental Assessment Regulation (EAR). Finally, the study revealed that there is a strong relationship between poor SWM and the impact on the health of people and the cases that were observed in the study area consist of malaria, typhoid fever, skin infections, and cholera.*

Keywords**— solid waste, solid waste management, AMA, sustainability, Ghana.**

I. INTRODUCTION

Waste management is one of the key challenges that is facing the world today. This problem is not only an issue for developing countries but also for developed worlds. Chazan (2002) added that SWM is a social problem in the perspective of the international construct. Thus, the problem of SWM has its root in both the developed and the developing nations as research has shown that some advanced nations are seriously facing this challenge.

According to Wilson et al. (2015), even this usage of 'waste' is still very broad, as it includes such unwanted outputs of human activity such as gases, liquids and solids as well as discharges to the three environmental receiving media of water, air and land. The UN Statistics Division uses the term' residuals' rather than 'waste' in this broad context, where they comprise one of six components of a comprehensive set of environmental statistics.

Many pieces of literature having their thought on the topic "SWM" attest that despite many efforts by various governments towards programmes designed to halt the problem of SWM, it has been noted that such programmes have failed to execute its intended goal (s). Although the developed countries have been able to design SWM technologies to curtail this bane, many pieces of research have come to the conclusion that the problems still exist. Kwawe (1995) supported this by attesting that even the invention of technology has not been able to effectively control waste generated in communities worldwide, however, it has rather degenerated the situation into a more uncontrollable mess.

According to Lyse (2003), SWM is one of the major problems facing Africa, nine out of ten cities in Africa face serious solid waste problems. MSW is not well managed in most low-income and developing countries because cities

and municipal councils cannot deal with the rate of solid waste production. This global threat of solid waste disposal has affected Africa in many ways especially causing diseases and increasing the poverty rate. These problems emerge mainly as a lack of strategic planning for effective SWM, poor governance, resource constraint, poor coordination and lack of proper management. In most rapidly growing cities in developing countries, the major concern issue is efficient solid waste collection and disposal.

In Ghana, the issue of inefficient solid waste collection and improper disposal resulting to heaps of solid waste materials involving organic waste and rubber bags scattered everywhere and disposal sites engulfed with filth which have a detrimental health effect like cholera, malaria and typhoid to residents who live close to the dumping site (Adu-Boahen et al., 2014). According to UNCHS (1996) report, it is estimated that approximately almost half of the waste generated within most cities in low and middle-income areas, (including Ghana) are not collected. Through the process wind and poor general attitudinal behaviour exhibited by many residents and other people, the wastes usually end up on illegal dumps, on street, roadsides, open spaces and drainage systems.

An environmental health issue reported by Heller & Catapreta (2003) demonstrates that the presence of sanitary or controlled landfill in urban areas and its implications for the health of the population that lives in its bordering area has been little investigated. Their findings continued to prove that the presence of these deposits, improper treatment of waste as a result of lack of financial constraint and logistics may draw many diverse insects and flies known as vectors to these places and facilitate the propagation of diseases from area to another with an acute health implications on the lives of many people living near and around the dumping sites.

There are rapid urbanization and high in-migration especially movement from the rural centres of Ghana to the city of Accra. Many pieces of research add that for the past four decades, the Accra metropolis has experienced overwhelming rapid urbanization thereby increasing the city's problems including SWM(GSS, 2014).

By estimate, the population of Accra in 1960 was 450000, in 1970, the population doubled and reached 1.3million in1984 (Boadi, K. O., & Kuitunen, M., 2003). Benneh *et al.*, (1993), also found that due to rapid urbanization as result of population growth and improvement in socio-economic development, the city of Accra is experiencing an uncontrollable expansion exceeding its boundaries to peripherical lands, however, the institutional capacity is limited in terms of budget allocation and logistics and this has resulted in the problem of inadequate SWM, posing health risks and burdens to many residents.

According to the EPA of Ghana's 2003 draft report on the environment, MSW is the "waste disposed of by households, hotels or motels, commercial, institutional and industrial sources. Statistics provided by the EPA shows that presently, only 55 per cent of waste in all forms generated within the area of Accra is properly collected and dumped by the AMA. In 1998, it was estimated that 765,000m of solid waste and 75,000m of liquid waste was generated in Accra per year.

1.2 Objectives of the Study

They study seeks to:

1. Examine the policies governing solid manage waste practices in the Accra Metropolitan Area of Ghana?
2. Identify the types and sources of waste as well as methods of waste disposal in the study area?
3. Examine the impacts of improper SWM on the health of people in the study area?

II. LITERATURE REVIEW

2.1 Solid Waste Management

According to Wilson et al. (2015), even this usage of 'waste' is still very broad, as it includes such unwanted outputs of human activity as gases, liquids and solids as well as discharges to the three environmental receiving media of air, water and land.

(Skenderovic et al., 2015) on the other hand, assess waste management as the process of gathering, transferring, organizing or categorizing, recycling, dumping, following, evaluating and monitoring of waste.

Chang, N. B. (2015) also defined MSWas comprisingof household waste, commercial waste, and institutional waste. Itincludes separatelycollected fractions from public service areas and private sectors,such as; garden and park waste (including cemetery waste),waste from markets.

2.1.1 Waste Generation

Waste generation is influenced by socio-economic development, level of industrial activities and climate. Generally, the level of economic prosperity and the size of the urban population of a particular country determines the amount of solid waste that will be generated (Bavel & Reher, 1999).

There is a variation from region to region and from season to season in terms of municipal solid waste generation and

also a strong link with the degree of socio-economic development and other activities. It is argued that high income countries generate more waste than low income countries. The UNESCAP(2012), added that high-income countries produce between 1.1 and 5.0 kilogram per capita per day; middle-income countries including Ghana generate between 0.52 and1.0 kilogram per capita per day, whilst low-income countries produce between 0.45 and 0.89 kilograms per capita per day.

Africa and particularly Ghana, are rich in terms of soil quality where the majority of food produced are consumed in its raw state, only a small proportion of these foods receive an added value through proper packaging. In Ghana, tuber content foods like cassava, yam, plantain etc account for the majority of solid waste generation in many households. It is estimated that the average quantity of solid waste generated in Accra per day is around One thousand five hundred tons (Anomanyo E.D. 2004). Anomanyo E.D also found that approximately two hundred tons of organic waste are directed into Accra's composting plant. Three hundred tons are left uncollected while the remaining waste accounting for about 1,000 tons are transferred to dumping sites. In the composition of waste production, there is an indication that the organic wastes hold the highest percentile of about 65% of the entire waste generated.

2.2 Types and Sources of Solid Waste Generation

The principal sources of solid waste include residential or households, commercial, institutional, construction and industrial wastes. However, a comprehensive example of the sources and types of solid waste presented by the UNESCAP (2012), is not different from the territorial boundary of the city Accra-Ghana. This is shown in table (2) below.

Table 1: Types and Sources of Solid Waste in the Study Area

Source	Waste generators	Example of solid wastes
Residential	Single and large family settings	Waste generated from food, polythene bags, electric cables, consumer electronic appliances, waste from back yards, paper, textiles, metals, glass etc
Commercial	Business buildings, guest houses, trade areas including stores and markets.	Paper, cardboard, plastics, wood, organic wastes, glass, metals, special wastes, hazardous wastes
Construction and demolition	Road construction and repairs, New building areas and renovation areas.	metal waste, waste from concreate mixture, waste from wood cutting, filth waste
Industrial	Light and heavy manufacturing, construction areas, power generation and chemical plants.	packaging, food wastes, construction and demolition materials, hazardous wastes, ashes, special wastes, scrap metals
Institutional	Schools, government centre, hospitals and Prisons.	Paper, cardboard, plastics, wood, food wastes, glass, metals, special wastes, hazardous wastes
Agriculture	Farming area, gardens, vineyards and crops	Spoilt food wastes and hazardous wastes like pesticides.
Process(manufacturing)	Heavy and light industrial manufacturing, petroleum and gas refineries, paint and chemical plants, power plants, mineral mining and processing	Industrial process wastes, scrap materials, slay, stakeouts etc.

Source: UNESCAP, (2012)

2.3 Methods of Solid Waste Collection and Disposal

The services of waste collection may be on a relatively small scale, providing primary collection to local neighbourhoods, or on a larger scale, providing either secondary collection or an integrated collection service across the city. In the collection of solid waste and disposal, the main types of vehicles currently used by the AMA are compaction and skip trucks. The wastes are taken from its source by road directly to the landfill sites(Anomanyo E.D, 2004).

According to Anomanyo E.D (2004), solid waste collection in the study area is both a franchise and contract basis. In his explanation, franchise involves a house to house collection (known as block collection) in high income residential areas while the contractors charge the households some fees based on a weekly frequency. However, at the initial stage, the contractors distribute recycling containers to these households at no charge while the latter is charged base on the services rendered to the customers. The contractors then pay a fee known as a tipping fee to the AMA for usingtheir dumping sites. Korfmacher, K. S. (1997) also share the same scenario by attesting that residents rely on the services their providers for disposing of their waste. In this system, a collection vehicle travels a scheduled route, stopping periodically for residents to bring their refuse.

Fig.1: Block Collection in High Income Area

Source: trendmap.com, retrieved on 24th May 2019

The contract aspect has to do with the AMA paying contractors to perform both block and communal container collection activities. As indicated above, the block collection takes place in high and middle-income areas with low population density and access to good roads. According to the study conducted by Anomanyo (2004), only 75 percent of the solid waste generated is collected and disposed of in these areas.

Fig.2: A Skip Truck Discharging Solid Waste Materials

Source: disposalknowhow.com, retrieved on 24th May 2019.

In high population density and low-income areas like Nima and James town, contractors use central communal containers for the collection of waste. Market areas are also part of this arrangement where people around the catchment area deposit their waste in communal containers and the frequency of collection is normally done by daily routine. However, waste generators do not pay any user fee. Also, in low-income areas, some of the waste generated by households are either buried or burnt.

Fig.3: A Communal Container filled with Solid Waste Materials

Source: ghananewsprime.com, retrieved on 24th May 2019.

2.4 Solid Waste Management Policy and Legal Framework in Ghana

As indicated by Saldeva, M. (2007), the Methodology for PAC gave their definition for policy as "game-plan or inaction embraced by the Government to address a given issue or interrelated arrangement of issues, or the manner by which the strategies for accomplishing the set objectives are resolved". So also, they defined the term "policy" as "intentional activity of Government that allows for a consequential adjustment or impacts the general public or economy outside the administration". It includes, but it is not limited to, taxation, regulation, expenditures, information, statements, legal requirements, and legal prohibitions.

The blueprint of policies for Ghana and that of the AMA appears very benign for the management of waste. However, in practical terms, these policies have failed to achieve the ultimate goal as a result of lack of clear definition of policies and implementation, lack of enforcement, lack of coordination and monitoring among others.

The Local Government Act (1994), Act 462 and the ESP of 1999 outline the policies and the legal framework guiding SWM in Ghana. However, implementation of the policy had many errors that consequently affected the goal of the policy for achieving sustainable SWM targets. In the year 2010, the policy was reviewed and amended by the policymakers to address and prevent future failures of the policies (Owusu-Sekyere, Bagah, & Quansah, 2015). The reformation of the policy enhanced the priorities for development in both the national and international context especially a reflection in the Ghana Poverty Reduction Strategy, the New Partnership for African Development (NEPAD) and the Millennium Development Goals.

Under the auspices of the Ministry of Environment and Science, Ghana, therefore, established the Environmental Protection Agency in 1994 and created the environmental legislation which basically made up of the Environmental Protection Act 490 and Environmental Assessment Regulation LI 1652, however, Environmental Assessment procedure is the main instrumental tool for SWM(Owusu-Sekyere, Bagah, & Quansah, 2015). Among all other things, the policy states that the disposal of solid waste must be consonant with standards and procedures directed by the EPA and any other regulatory agencies. It the overall responsibility of the Ministry of Local Government and Rural Development involving the Metropolitans, Municipalities and District Assemblies (MMDAs) to manage domestic solid waste although the regulatory power is invested in the EPA. Through the facilitation of the Waste Management Department (WMD) and Environmental Health and Sanitation Unit, the MMDAs, therefore, aid in the collection and final disposal of waste. The figure below therefore shows the institutional arrangements for SWM.

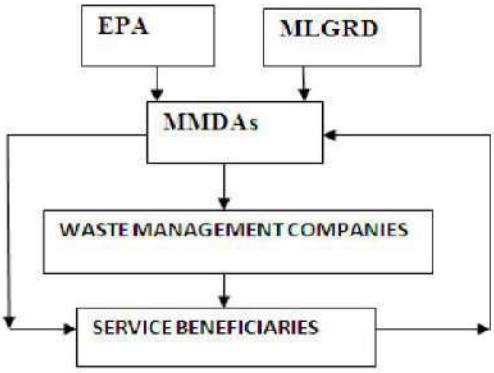

Fig.4: Institutional Arrangements for Solid Waste Management

Source: (Owusu-Sekyere, Bagah, & Quansah, 2015)

2.5 Impacts of Improper Solid Waste Management on Human Health, Animals and Aquatic Life

There are potential risks to the environment as well as the health of biotic organisms including humans, animals and aquatic species from improper handling of solid wastes. The result of all the types of pollution such as air, soil and water strongly correlate with improper SWM. In most populated urban areas, solid waste materials block drains, emanating from indiscriminate dumping of waste causing seasonal flooding's, stagnant water bodies for the breeding of insects like mosquitoes (Bavel & Reher, 1999).

Majority of solid wastes are disposed on the land in open dumps. Disposal of solid waste on the land without careful planning and management can bringmany dangers to the environment and human health. The environment should be catered for and less populated at all cost. This means that waste should be managed in such a way that the impacts on the environment will be minimal (US Environmental Protection Agency 2006).

Open dumpsites contribute to one of the major problems to the deterioration of the environment which in turn pollute the air that people inhale. Dumpsites emit unpleasant odours as well as smoke that may cause respiratory diseases to people living around (Marshal 1995). Issues on health and safety also arise from improper SWM. Organic waste in the form of human faecal matter is usually common in municipal solid waste, especially in low income areas. However, insects and rodents that set on this faecal matter can spread diseases like cholera and dengue fever (Bavel & Reher, 1999).

According to Wrensch et al., (1990), during active landfill site operation, dumpsites maybe a source of airborne chemical contamination through the expel of gases and particles as well as chemicals coming from dust. Lack of proper treatment of dumpsites may lead to soil and underground water contamination, especially organic solid waste materials containing filth saturated water content seeps through the soil and contaminates the water table. Heavy downpour also contaminates both the underground and running water (rivers, lagoons, lakes etc), especially through the process of washing away solid waste particles into these streams, some of which seeps the dirty particles into the underground water which have a negative repercussion on the health of people living in around the dumpsites. Another instance given by Wrensh (1990) shows that in some dumpsites, unstable (volatile) organic chemicals have been detected in odour air of nearby homes around the dumpsites. Bavel & Reher (1999) also found that using water contaminated by solid waste for bathing, irrigation purposes, and drinking can expose individuals to many diseases.

Songsore and McGranahan (1993) found that as a result of unsanitary conditions in many of the study area (Accra), the prevalence of parasites, cholera, diarrhoea and malaria has also increased extensively. The out-patient department (OPD) of many facilities in Accra also reported similar cases of infectious diseases especially among residents in and around slums areas, landfill sites including malaria, intestinal worms, and upper respiratory infections. The Environmental Protection Agency (2002) added that in most parts of the Accra, drains and gutters blocked by solid waste materials have created stagnated waters acting as habitat or breeding grounds for mosquitoes, which later transmits common diseases like malaria and fever to many residents.

3.0 Empirical Part: Solid Waste Management in Accra

Solid waste collection and disposal is in the hands of the Accra Metropolitan Assembly's Waste Management Department (WMD). Thus, the department is responsible for managing solid waste within the jurisdiction and

confines of the area including the management of the landfill sites in Mallam, Djanman and Oblogo.

3.1 Conceptual Framework of the Study

Figure 5 gives a summary representation of the SWM framework and its impacts on the livelihoods of people. The socio-economic activities and the livelihoods of people can be realized through the medium of commerce, domestic, industry and agriculture which eventually becomes the major ways and means of solid waste generation. Socio-economic activities are most influenced by the rate of population growth. Thus, the higher the rate of population growth, the higher the level of urbanization and growth of socio-economic activities which causes an uncontrolled generation of heaps of waste in many towns and cities. Low revenues for city councils, high SWM expenditures, inadequate logistics, poor general attitude for handling solid waste, inadequate public education, weak sanitation policies and laws, among others are the attributable factors for poor SWM (Ampofo, Soyelle, & Abanyie, 2016).

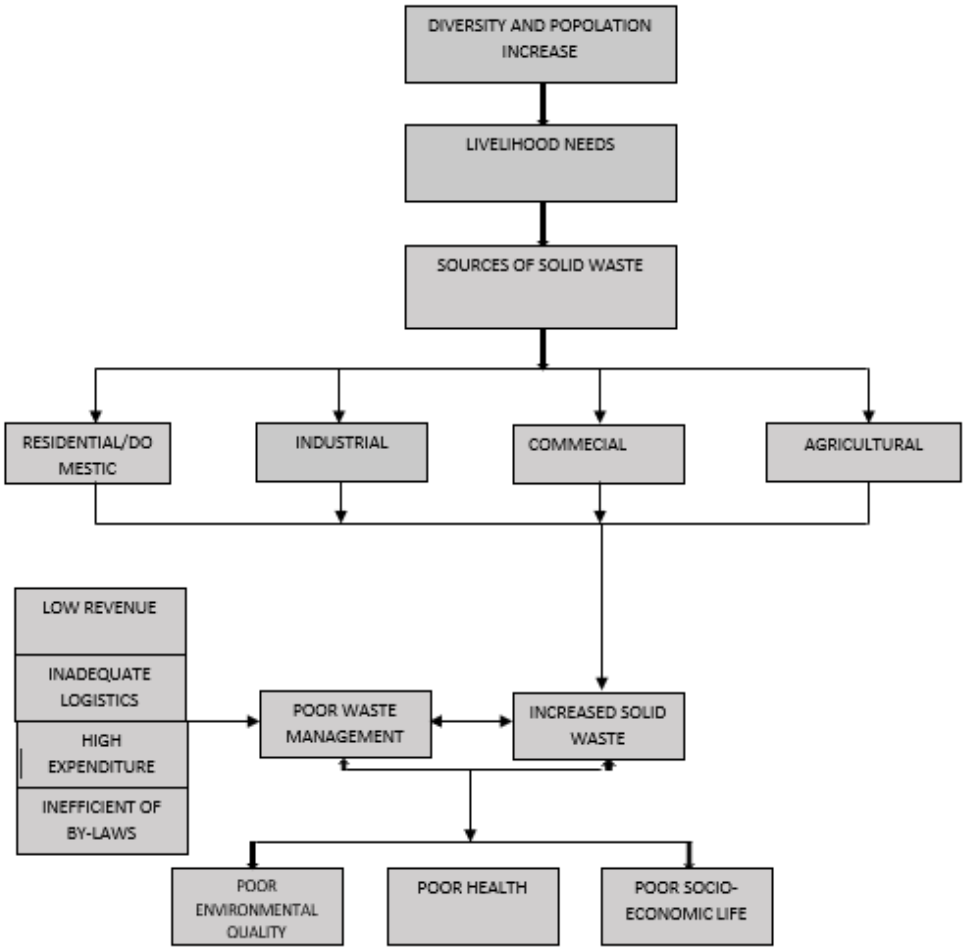

Fig.5: Conceptual Framework of Impact of Sustainable Solid Waste. Source: (Ampofo, Soyelle, & Abanyie, 2016)

3.2 Roles of Public Administration (AMA) in the Solid Waste Management

Good SWMplays an important role in the achievement of sustainable development.The entire sectors of the economy suffer when waste is poorly managed, as such, the impacts that may result from poor SWM include; poor health on the part of the citizens, land destruction, deterioration of the ecosystem involving the marine and coastal land, and most of all diminishes the gains from tourism and fisheries. Governance is also about responsibility, expressed through various legal and financial obligations, but also through a sense of 'ownership' of waste-related issues, which translates into involvement and care about the cleanliness of the open spaces in the community as well as protection of the broader environment and natural resources. The latter means that good waste governance goes beyond street cleaning and waste handling into the

realms of production and consumption (Wilson et al., 2015). Governmental institutions are established and given a specific mandate by law, as one of the instruments through which a nation's policy goals are defined and reached. For an effective waste solid management system, a strong, robust and transparent institutional framework is essential, within which institutions take various roles at different levels of government, from national to local. At the national level, the main role of the government and its institutions is that of policy maker and legislator, which includes strategic planning and preparation of policies, and their translation into legislation. Institutions deploy various participatory governance processes so as to benefit from the interaction with other societal actors in which they can contribute their knowledge, insights, queries and concerns on stakeholders.It has been documented that proper SWM can help promote sustainable development if critical attention is given, especially in the provision of finance, logistics, and effective management, however, due to some challenges like poor or lack of logistics, limited recycling plants, lack of funding for the management of waste, poor attitude and practices on the part of many citizens, lack enforcement and regulations and the obstacles of moving waste from one geographical area to another contribute largely to poor or insufficient solid waste management.

Dijk et al. (2017), noted that SWM in Ghana is the sole responsibility of the decentralized local government assemblies across the country. The assemblies represent the national government within their jurisdiction for the SWM. However, the local government assemblies across the country have strong backing from the Ghana Local Government Act 1993 (Act 462). This legal support gives them the mandatory power to enforce by-laws to guide and control SWM, sanitation, public cleaning and mitigate the act of indiscriminate disposal of waste in the city. The roles exercise by the public administration (national and local government) in Ghana, are discussed as follows;

National government; the central or the national government is responsible for the establishment of the institutional and legal framework. The national government provide support and ensure that local governments have the authority, power and capacity to effectively and efficiently manage solid waste. Schubeler, Wehrle and Christen (1996), added that the processes involved in the establishment of the institutional framework are usually done without the local government being given the needed support to build capacity.

Local government; generally, the local government are responsible for solid waste collection and disposal services. The obligation of upholding by-laws, controlling SWM activities and mobilizing funds and effective administration running for SWM as a responsibility of the local government is conferred by higher government authorities. Schubeler, Wehrle and Christen (1996) also made an elaborative relationship between the local government and the private firms by attesting that in a situation whereby a private company is contracted to provide solid waste services, the local government is mandated to regulate and control the activities such private firm or company.

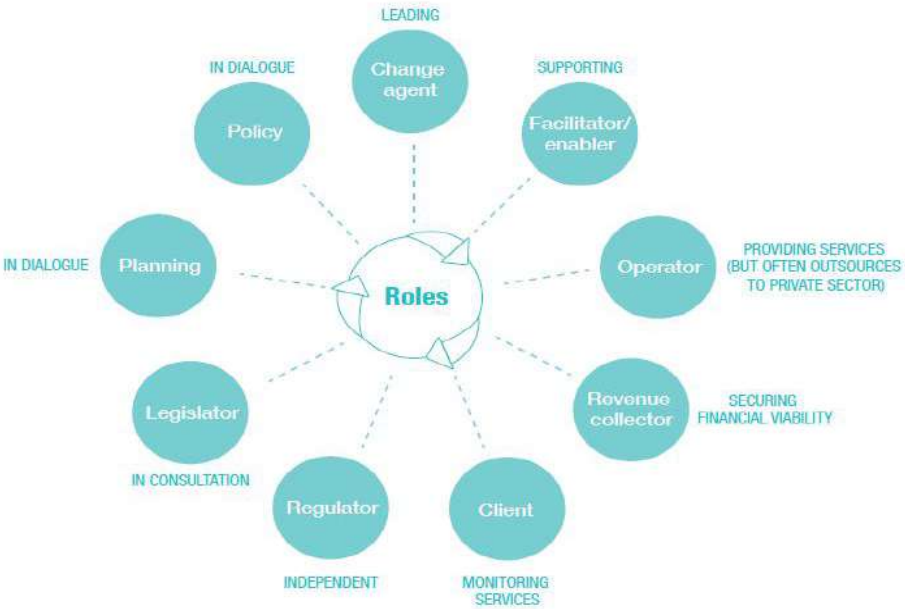

Fig.6: The Role of Public Administration (Government Institutions) in Waste Management

Source: United Nations Environment Programme, 2015

3.3 Management Process of Solid Waste

The Accra metropolitan through government intervention has commissioned a series of waste management plants including the recent Accra Waste Recovery Recycle plant and other waste transfer stations. However, coupled with some challenges like unstable power supply and limited capacities, majority of the waste generated end up at the landfill sites (retrieved from myjoyonline.com, on 25th May 2019). Ghana and for that matter, Accra has a complex management process in terms of SWM. However, due to the challenges mentioned above, ranging from the unstable power supply and lack of proper management and maintenance, the most commonly used management process is shown in figure (8) below.

*Hypothesis 1:*Inefficient enforcement of by-laws plays a significant role for poor solid waste management.

Hypothesis 2: There is no relationship between distance of solid waste dumpsites and spread of diseases among residents.

*Hypothesis 3:*Proper methods of solid waste disposal plays a major role for sustainable solid waste management,

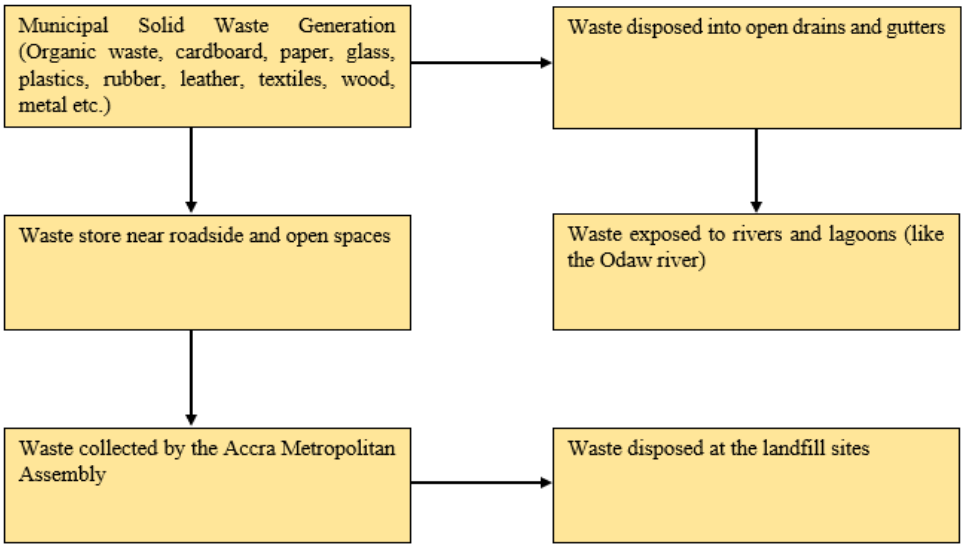

Fig.7: Common Management Process of Solid Waste

Source: Developed by the Author

III. DATA ANALYSIS AND DISCUSSION OF THE FINDINGS

The by-products of human activities and consumptions and its subsequent consequences become more concentrated following the movement of people into towns and cities globally. The impact of urbanization has also overrun spaces that could possibly be used to manage solid waste. However, it has become important for transporting waste to more distant areas and zones. It is, therefore, necessary for inter-municipal cooperation, under a different local administration to deal with solid waste which is far from its origin (Wilson et al. 2015). The profile of the study reveals an observed increase in the human population from time-time with the dangers of worsening the current situation of uncontrolled solid waste in the study area. However, there can be so many direct and indirect benefits if solid waste is properly managed by public administrators or government institutions (programmes designed to managed solid waste) to enhance sustainable waste management in the area. In the developing world, 'waste' is still largely an urban phenomenon. From this note, the questions and the discussions are structured as such;

1. What are the policies governing solid manage waste practices in the Accra Metropolitan Area of Ghana?

The findings revealed several policies governing SWM in Ghana which include the Environmental Sanitation Policy of 1999 which had the legal backing from the Local Government Act (1994), Act 464. However, the findings also revealed that this policy had many flaws in its implementation until it was reviewed and amended in 2010 which saw a significant reflection for structural change in the GPRS, the NEPAD and the MDGs at the national and international level. Another policy is the Environmental Assessment Regulation LI 1652 which had a legal support from the Environmental Protection Agency Act 490 which spelt out that the Environmental Assessment Regulation (EAR), is the main tool and also states that disposal of solid waste should be in relation to standards and

procedures for SWM prescribed by the EPA and other regulatory bodies. By and by, the findings revealed that there are inefficiencies in the policies. Owusu-Sekyere, Bagah, & Quansah (2015, pp. 53-54), observed that policies governing SWM are inadequate and even have become outdated. They are added that the obsolete nature of the SWM policies are scary to the extent that it appears to be protecting the environment and the people yet fooling them and which may rather expose them involuntary.

2. What are the types and sources of waste as well as methods of waste disposable in the study area?

The sources and types of solid waste generation primarily originate from domestic, commercial, institutional, constructional sites and industrial activities. These wastes include; plastics, metals, paper, organic wastes, glass, textiles, wood wastes, agricultural wastes and other forms of solid wastes. A study conducted by Kramer et al. (1994) cited by Boadi, K. O., & Kuitunen, M. (2003) shows that organic wastes constitute more than 70 per cent of all waste generated in the Accra metropolitan area, inert materials also accounted for 10.5 per cent, paper wastes constituted for 6.6 per cent, textiles and metals accounted for 2.2 and 2.1 respectively while glass and other forms of waste equated to 1.5 per cent and 0.7 per cent.

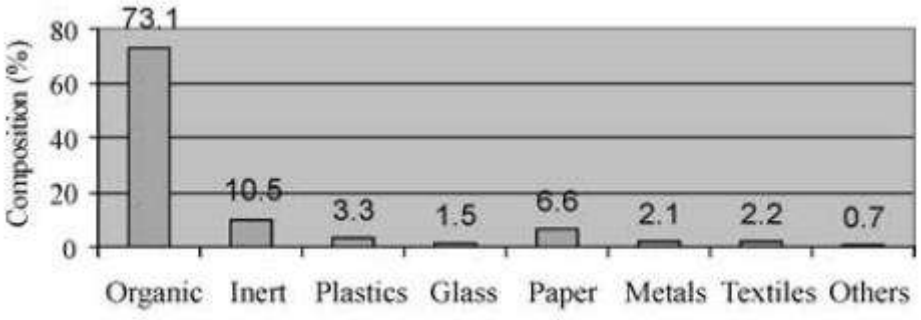

Fig.8: Components of Solid Waste Materials

Source: Kramer et al. (1994)

The Waste Management Department (WMD) of the AMA (2010 cited by Boamah A. Linda 2010) also revealed that on a daily basis, the city generates about 2200 tonnes of solid waste. By segregating these wastes into types, organic waste constitutes 65%, 6% for paper, 6% for plastic, 3% for glass, 2.5% accounts for metals while 1.7% and 15.8% accounts for both textiles and inert materials respectively.

The methods of solid waste collection and disposal vary from one geographical area to another. Generally, in high and middle-income areas, waste collection is done by block collection method. In this case, the solid waste service providers move from house to house to collect these wastes while in low income areas and public spaces like markets, central communal containers are used for solid waste collection and disposal. Again, in most low-income areas, solid waste burning is very common due to limited access to waste facilities. Areas isolated from the main town also dispose of their waste in open pits, however, in these areas, residents cover the pits with either sand or burn the waste from digging new pits especially it the pits are filled with waste (Boadi, K. O., & Kuitunen, M. 2003).

It is very important to note that despite all these waste collection methods, some proportion of the waste is left uncollected. According to the CCAMSWI (2014) reported that waste collection in the AMA is limited. It estimated that only 40 per cent of the households in the city have recycling or waste collections bins. However, the 60 per cent of the waste is indiscriminately dumped in informal areas like abandoned sites including quarry areas, dig holes, etc.

Fig.9: Landfill Site in Accra

Source: http://kevin-mcelvaney.com/portfolio/agbogbloshie/. Retrieved on 6th June 2019.

3. What are the impacts of improper waste management on the health of people in the study area?

To answer to this question, a hypothesis was tested as to whether improper solid waste management has a significant influence on the health of is tested. Ho: there is no relationship between distance of solid waste dumpsites and spread of diseases among residents. Ha: there is a strong relationship between distance of solid waste dumpsites and the spread of diseases among residents. Poor solid waste management has a negative effect on the three main media of nature namely, air, water and the environment (land). The damage to these important media consequently turns to affect the health of humans and living organisms including species in the water bodies, biodiversity and other species alike. The impact of environmental problems and its subsequent effects on human health, economic and other welfare losses can be inherently attributed to poor or improper SWM (Kolsch, F., & Ziehmann, G. 2004 cited by Bekoe-Nketia, 2015).

As reported by the Out-Patient-Department (OPD) unit of most facilities in Accra, common infectious diseases among people living in slum areas and close to landfill sites include malaria, intestinal worms, dengue fever and upper respiratory infections (Songsore & McGranahan 1993 cited by Anomanyo E.D 2004). The current situation in the area shows that proper SWM delivery is very limited, only 40 per cent of the entire households in the city have access to proper solid waste services (Climate and Clean Air Coalition Municipal Solid Waste Initiative 2014). This situation has therefore forced most residents to either bury or burn waste. In some cases, open places, bushes, drains, rivers become the only option for residents for disposing of waste, especially at night where people cannot see them. These poor attitude and practices raise several dangers to the health of many citizens as well as the spread of poverty. Bekoe-Nketia (2015) found that primary effect of improper solid waste management within most communities in Ghana including Accra is air pollution and unpleasant odour that many residents inhale from decomposed rotten materials and the smoke that comes out from dumpsites especially when burning solid waste materials. Kolsch, F., & Ziehmann, G. (2004), also confirms that the major cause of air pollution is due to the burning of solid waste materials at dumpsites, causing illness and reducing visibility, causing explosions of bottles and cans, making dumping sites dangerously insecure and probable spread of poverty. Alternatively, Al-Yaqout et al, (2002)and Vidanaarachchi et al. (2005) cited by Bekoe-Nketia (2015), confirm that one of the dangerous causes of air pollution is solid waste, therefore to enhance environmental quality through sustainable SWM it is very important to ameliorate the situation of improper solid waste management.

A study steered by Suleman (2016) reveals that there is a strong relationship between improper solid waste management and its subsequent consequences on the health of people. This was confirmed by a chi test results where the Pearson chi-value of 0.017 with a degree of freedom of 2 and a tested significance level of 0.05 in the table (3) below.

Table 2: Pearson Test for Improper Solid Waste Disposal and Impact on the Health of People

	Value	Df	Asymp. Sig. (2-sided)
Pearson Chi-Square	8.129[a]	2	0.017
Likelihood Ratio	8.719	2	0.013
Linear-by-Linear Association	5.684	1	0.017
N of Valid Cases	144		

Source: Suleman, Y. (2016),https://doi.org/10.4172/2165-784X.1000202, retrieved on June 6, 2019

However, from this result, the null hypothesis is accepted, since the Pearson correlation test confirms that there is a strong relationship between distance of solid waste dumpsite and the spread of diseases among residents. Further, the study of Suleman (2016) revealed that residents who stay closer to solid waste dumping sites stand the risk of contracting infectious diseases. However, the prevalence of disease acquisition reduces when the distance between residents and the dumping sites become wider. The study showed that 75 per cent of respondents who are close to the dumping sites had cases of malaria, while 2 per cent of the households relative far from the dumping sites had malaria cases. Alternatively, the findings revealed that 72 per cent and 75 per cent of the respondents whose houses are located close to the dumping sites had cases of skin infections and typhoid fever respectively while cases among residents far from the dumping sites were very minuet and this shown in table (4) below;

Table 3: Some Related Diseases contracted from Landfill Sites

Category	Frequency	Percent
Cholera	8	5%
Malaria	105	71%
Typhoid fever	15	10%
Skin infections	20	14%
Total	148	100%

Source: Suleman, Y. (2016),https://doi.org/10.4172/2165-784X.1000202, retrieved on June 6, 2019

IV. CONCLUSION AND RECOMMENDATION

According to Wilson et al, (2015) management of MSW is a key responsibility of the government to protect public health and theenvironment for all the citizens. SWM in the Accra metropolis not up to standard, as logistical equipment remains a key challenge in most part of the city especially low-income areas, hence needs immediate intervention by the government and other non-governmental bodies (Boadi and Kuitunen, 2003).

Challenges facing private sector involvement in the management of solid waste must be addressed by the government such as granting soft loans to procure equipment and also hire qualified personnel to deal with the problem of solid waste management. Private sector involvement and participation in SWM delivery must be done through a competitive arrangement and concerns for solid waste collection and disposal and dumping sites operations in order to improve environmental accountability(Bartone and Leitman, 1994).

There should also be the full involvement of informal waste collectors including waste carriers, waste recyclers, waste hunters (scavengers) and waste pickers in the SWM process to enhance the benefits of their contributions for sustainable city development. To enhance strategic solid waste management in Accra, there must be the involvement the active participation of those affected by solid waste problems, as well as those who regulate policies and implementation instruments, and those who possess relevant information and expertise (Bartone and Leitman, 1994). Decision making involving communities and especially the marginalized in society will augment self-confidence for mobilizing efforts and local resources for environmental management, particularly in low income areas. Also, more efforts must be directed at upgrading

local SWM infrastructure and services. This must include the provision of adequate facilities for waste management and requiring users to pay for the full cost of the service in order to enhance the efficiency and coverage of service delivery (Bartone and Leitman, 1994).Institutional capacity in the field of planning must be strengthened to effectively deal with the environmental and sanitation problems facing the city. For efficient waste management, every house in the city must be accessible by road, and this calls for proper layouts in the city (Boadi & Kuitunen, 2003). Capacity should also be strengthened in the area of poverty alleviation to enable the poor to afford the cost of environmental services. The metropolitan authorities must enact and improve the necessary policy measures to attract local and foreigndirect investments. These include institutional policies aimed at ensuring the safety of investments, removing bureaucratic bottlenecks to attract investors creating free trade zones and. City authorities must also encourage local investment for sustainable solid waste management through the provision of soft loans to small-scale businesses to expand their activities and also encourage local entrepreneurs to form co-operative organizations to mobilize funds for investment in sustainable SWM.

Lastly, attention must be geared towards local capacity in data collection and analysis in solid waste management. The availability of such data would be beneficial to determine and select the appropriate technology for the management of solid waste in Accra. Also, a periodic city-wide survey on SWM must be undertaken by Accra Waste Management Department (AWMD) that will provide complete and dependable information on the amount and the nature (different types of solid waste) that are generated on average basis so as to come out with a suitable and sustainable approach to treat and dispose such wastes.

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Effect of Plant Growth Promoting Rhizobacteria (PGPR) on Increasing the Activity of Defense Enzymes in Tomato Plants

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Abstract— *Growth-promoting rhizobacteria are non-pathogenic bacteria that can induce plant defense through induction of systemic resistance which can then activate defense enzymes such as Polyphenol Oxidase (PPO), Peroxidase (PO) and Penil Alanine Amoliase (PAL). This study aims to determine the activity of the PPO, PO and PAL defense enzymes with selected PGPR isolates. This research was carried out at the Microbiology and Greenhouse Laboratory of the Faculty of Agriculture, Andalas University, Padang, and then tested at the PAU IPB Laboratory, West Java Indonesia in March-July 2020. The results showed that tomato leaves that had been treated with PGPR showed that PGPR had the potential to increase the PO enzyme 0.072 µg · mL – 1, PPO 0.0009 µg · mL – 1 and PAL 14.15 µg · mL – 1. EAB 2.1 isolate is best isolate can increase PPO, PO and PAL.*

Keywords— ***PGPR, PPO, PO, PAL.***

I. INTRODUCTION

PGPR is a non-pathogenic microbe that can increase plant fertility and induce plant resistance from biotic and abiotic stresses through the ISR mechanism. Plant Growth Promoting Rhizobacteria (PGPR) is a group of soil-specific microorganisms that efficiently colonize rhizosphere and rhizoplan and can substantially improve plant health (Hatami and Ghorbanpour, 2016). Specific mechanisms between PGPR and plant pests and pathogens are by producing antibiotics, competition of substrate and ecological niches, siderophores, chitinase enzymes, β-1,3-glucanase, cyanide, parasitism, and inducing systemic plant resistance (ISR) in the host. (Khalimi and Wirya. 2009).

ISR is an effective defense mechanism manifested as a result of physiological changes in plants, such as modifications to the cell wall structure and the synthesis of antimicrobial compounds such as proteins associated with pathogenesis (PR) and phytoalexins, which wreak the spread of pathogens (Filippi *et al*., 2011). Antioxidant enzymes such as peroxidase (PO), phenylalanine amonialyase (PAL) and polyphenol oxidase (PPO) may be enzymes elicited by ISR (Yasmin *et al*., 2016). Induced systemic resistance (ISR) involves the production of oxidative enzymes such as peroxidase (PO) and polyphenol oxidase (PPO), which catalyzes the formation of lignin, and other oxidative phenols that contribute to the formation of defenses (Meziane *et al*., 2005; Jetiyanon, 2007). PGPR can stimulate systemic responses in tomatoes by inducing the activity of defense enzymes such as phenylalanine ammonialyase (PAL), peroxidase (PO), polyphenol oxidase (PPO) and chitinase as well as the level of phenolic accumulation which further reduces infection by biotic pressure (Ahmed et al., 2011) .

Several studies have shown the ability of PGPR to increase the activity of defense enzymes in plants, including cucumber roots treated with *Pseudomonas corrugata* 13 or *Pseudomonas aureofaciens* 63–28 can increase phenylalanine ammonia-lyase (PAL) activities, peroxidase (PPO) and polyphenol oxidase (PO) activity (Chen et al 2000). Turmeric plants introduced with *Bacillus amyloliquefaciens* BaTNAU5 and *Pseudomonas fluorescens* strain Pf3TNAU triggered an increase defense enzymes peroxidase (PO), polyphenol oxidase (PPO),

phenylalanine ammonia lyase (PAL), β-1,3-glucanase, chitinase, catalase and chemicals that trigger defense (total phenol) (Adhipati *et al*., 2014). In previous studies, there were 3 root bacteria and 4 endophytic bacteria which were able to suppress whitefly populations and tomato wilt disease (Yanti 2018 and Hamid 2020). This study aims to determine the activity of the PPO, PO and PAL defense enzymes with selected PGPR isolates.

II. MATERIALS AND METHODS

The research was carried out in the Microbiology laboratory and greenhouses of the Faculty of Agriculture, Andalas University, Padang. Subsequently, a defense enzyme analysis was carried out at the PAU IPB Bogor Laboratory, West Java, Indonesia from March to July 2020.

Rejuvenation and Propagation of Rhizobacterial Isolates

Rhizobacterial isolates were obtained from the Yanti collection (2017). Rhizobacterial isolates were rejuvenated by means of one ose of bacteria transferred to NA medium in a Petri dish by the scratch method and incubated at room temperature for 2x24 hours. Furthermore, rhizobacterial multiplication was carried out consisting of 2 stages: (1) pre-culture, 1 rhizobacterial colony from pure culture was transferred into 10 ml NB medium in culture bottles and incubated on a rotary shaker at a speed of 150 rpm for 24 hours at room temperature. (2) Main-culture, 1 ml of suspension from preculture was transferred into 25 ml of sterile coconut water in a culture bottle and incubated in the same manner for 2×24 hours (Habazar *et al*., 2007). Rhizobacterial suspension from main-culture was determined by population density based on comparison with McFarland scale 8 (BaCl 0,8 g + H_2SO_4 1% 9,2 g) (bacterial population density estimated at 10^8 cells/ml) (Klement *et al*., 1990).

Preparation of Planting Media

Tomato growing media is a mixture of soil and sterile manure with a ratio of 2: 1. The mixture of soil and manure is sterilized for 1 hour at 100ºC in a container measuring 45x40 cm2, then refrigerated for 24 hours. For the nursery, the soil is put into a pot-tray 22 grams / hole, while for planting chilies, the soil is put in a 4 kg /polybag

Introduction of rhizobacterial isolates

Rhizobacterial isolates was introduced twice, namely to seeds and seedlings.

Tomato seeds used are Warani Varieties. Tomato seeds are surface sterilized by soaking them in a 1% sodium hypochlorite solution for 3 minutes, after which they are drained and rinsed with distilled water 2 times and then dried. The seeds are soaked in rhizobacterial suspension for 15 minutes then planted in a pot-tray. Seedlings are maintained for 21 days.

Tomato seedlings that are 21 days old are transferred to polybags that have a mixture of sterilized soil and manure. Before planting, the roots are cleaned from the rest of the previous planting media and then immersed in rhizobacterial suspension for 15 minutes, then pla-nting

Measurement of Plant Defense Enzyme Activity

i. Peroxidase (PO)

The peroxidase enzyme activity test was performed by the Yanti method (2015). 1g roots and stems macerated, then added 2.5 mL Potassium phosphate 0.5% buffer pH 7 and 0.1g Polyvinyl pyrplidone (PVP). The suspension is homogenized and filtered using two layers of gauze, then centrifuged at a speed of 6,000 rpm (60 rpm = 1Hz) for 15 minutes at 4ºC. Supernatants are used to measure peroxidase activity.

Peroxidase activity measurements were carried out based on the Bateman method (1967). An enzyme extraction of 0.2 mL was added to a cup containing 5 mL pyrogallol (0.631 g pyrogallol in 0.005 M phosphate buffer pH 6, final volume 100 mL) and then shaken. The cup was placed on a spectrophotometer with 420 nm absorbance. 0.5 mL of 1% H_2O_2 is added to the cup, then shaken and immediately placed on a spectrophotometer. Changes in absorbance are observed every 5 seconds until there is no change. Peroxidase activity is expressed in µg/mL.

ii. Polyphenol oxidase (PPO)

1 g root and stem tissue samples were crushed and dissolved in 2 mL cold 50 mM phosphate buffer pH 6.5. The filtrate was then centrifuged at 16,000 g for 15 minutes at 4ºC. The resulting filtrate is used as a source of enzymes. A 2.6 mL phosphate buffer solution pH 6.5 of 2.6 mL, 0.1 mL L-3,4-dihydroxyphenylalanine (L-DOPA) 5 mM, 0.1 mL ascorbic acid 2.1 mM, and 1 mL EDTA 0.065 mM is mixed until homogeneous. A crude enzyme of 0.1 mL was added to the solution and incubated for 10 minutes at room temperature. The solution was then measured for absorbance at a wavelength of 265 nm with a spectrophotometer (Karthikeyan *et al*., 2006).

iii. Phenylalanine Ammonia Lyase (PAL)

1g of leaf tissue samples were crushed and dissolved in 2 ml of cold 0.1 M sodium borate buffer pH 7. The filtrate was then centrifuged at 16,000 rpm for 15 minutes at 4 ° C. The resulting filtrate is used as a source of

enzymes. 2.0 ml of 3 mM L-phenylalanine solution was added with 0.9 ml of deionized water and mixed until homogeneous (Karthikeyan *et al*., 2006).

PAL activity was measured by a modified method of Sainders and McClure (1975). The reaction was carried out for 60 minutes at 37ºC and an increase in absorbance at A290 nm were recorded at 15 min intervals. 200 mM Tris-HCl (pH 7.0) was used as a buffer solution and 20 mM L-phenylalanine as the enzyme test substrate. The rate curve of cinnamic acid formation was used as a measure of enzyme activity by using an absorbance increase of 0.01 at 290 nm as a cinnamic acid curve of 3.09 nmol. PAL activity was expressed in µ mol of cinnamic acid min-1 g-1. Protein calibration was measured according to the Bradford (1976) standard method.

III. INDENTATIONS AND EQUATIONS

Tomatoes treated with PGPR showed higher defense enzyme activity when compared to controls. Peroxidase (PO) enzyme activity in tomato leaves introduced by PGPR showed a 3-4 times higher increase compared to the untreated control. Tomatoes treated with EAB 2.1 isolate showed the highest peroxidase levels with a value of 0.0009 µg · mL^{-1} and the lowest was found in controls with a content of 0.0002 µg · mL^{-1} (Figure 1).

Apart from the PO enzyme, giving PGPR to tomatoes also increased the activity of the PPO enzyme. The PPO enzyme activity in tomatoes treated with PGPR also showed a 0.5-fold increase when compared to the control (Figure 2).. The highest PPO was found in plants introduced by EAB 2.1 isolate with a PPO content of 0.095 µg ·mL^{-1}, while the lowest was found in the control with a value of 0.045 ppm. All isolates used in this study showed enzyme activity above the control plants.

Furthermore, PGPR treatment on tomato plants can increase the activity of the enzyme Penylalanin Ammonia lyase. The activity of the PAL enzyme given PGPR can increase PAL by 0.17µg · µg · mL^{-1}. The highest PAL activity was found in EAB 2.1 isolates with a value of 15.98 µg · mL^{-1}, while the lowest was found in controls with a content of 13.98 µg · mL^{-1} (Figure 3). This is because PGPR can increase the level of peroxidase activity in plants.

Giving PGPR to tomato plants shows the induction mechanism of systemic resistance in tomatoes which is then expressed through the activation of defense enzymes such as PO, PPO and PAL. Ahmed *et al*., (2011) stated that PGPR is stimulating Systemic response in tomatoes by inducing high levels of enzymes activity of phenylalanine ammonialyase (PAL), peroxidase (PO), polyphenol oxidase (PPO) and chitinase as well as accumulation of high levels of phenolics. In the study, it was found that there was an increase in defense enzymes induced by PGPR in tomato plants with a 3-4-fold increase in PO, 0.5-fold PPO and 2-fold PAL. This was also found by Ahmed *et al.*, (2011) who reported that the administration of PGPR *P. puptida* and *P. flureccens* to tomato plants could increase PAL activity 3 times, PO 2 times and PPO 2 times.

Polyphenol oxidase is a plant defense enzyme from biotic and abiotic stress. Polyphenol oxidase is an enzyme in plants that regulate feeding, growth, development of insect pests, and play a major role in plant defense against biotic and abiotic stresses (Sharma *et al.*, 2009). PO is a group containing enzyme copper that catalyzes the oxidation of hydroxy phenols to derivative quinones, which have antimicrobial activity (Chunhua *et al*., 2001). In the research that has been done, the PO enzyme can increase 3-4 times and all isolates in the treatment showed enzyme activity above the control. This shows that all PGPR isolates used in this study have the potential to increase PO levels. Research conducted by Sharavankumar *et al*., (2006) states that giving PGPR to tea plants can increase the activity of the PPO enzyme. An increase in PO levels also occurred in rice plants treated with Baciluss spp (Rais *et al*., 2017).

Furthermore, there is the peroxidase enzyme which is one of the important enzymes in defense that plays a role in strengthening plant cell walls. Peroxidases produce reactions that regulate defense-related signal transduction pathways, initiate hypersensitivity reactions, and strengthen cell walls through increased lignification (Ralph et al., 2004). In the research, the peroxidase enzyme activity in untreated tomato plants was only around 0.0002 µg · mL^{-1} whereas those treated with PGPR could reach 0.0009 µg · mL^{-1}. This indicated that there was an increase in PO activity in plants treated with PGPR and all isolates showed more increases than the control. This is also obtainedby Sarvanan *et al*., (2004) that the increase in PPO activity in banana tubers introduced with *Psuedomonas fluorescence* and suppressed fusarium wilt disease. Rice plants induced by *Bacillus* spp can increase polyphenol oxidase enzymes as much as 3.0–3.8 fold and can suppress development *Pyricularia oryzae* (Rais *et al*., 2017)

PAL is one of the important enzymes that play a role in other phenolic synthesis related to plant defense (Daayf *et al*., 1997). PAL serves as a precursor file for the biosynthesis of lignin and other phenolic compounds that accumulate in response to infection (Klessing and Melany

1994). In studies that have been carried out PAL activity on treated tomatoes can increase up to 0.17 µg · mL^{-1} when compared to controls. Although there is only an increase 0.17 µg · mL^{-1} but all isolates used in this study had the potential to increase PAL activity in tomatoes. Yanti (2015) also reported thatOnions introduced with 8 rhizobacteria isolates showed an increase in peroxidase enzyme activity in the roots and leeks, PK2Rp3 was the best isolate with an increase in enzyme activity reaching (0.058 µg · mL^{-1}) in roots and leaves.

IV. FIGURES AND TABLES

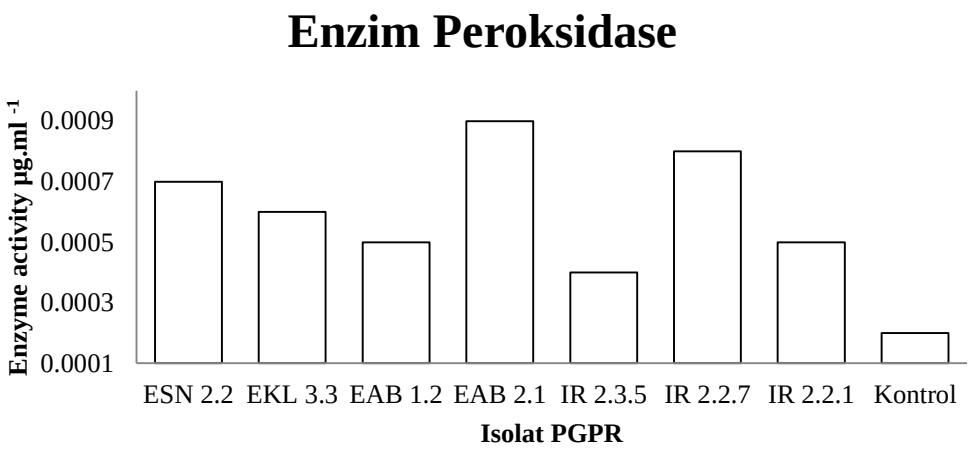

Fig.1: Production of Peroksidase in tomato plants introduced by PGPR

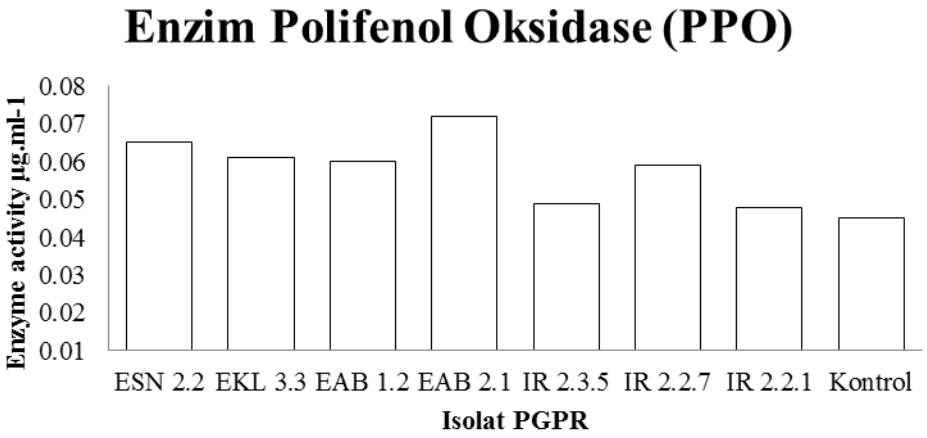

Fig.2: Production Polifenol Oksidase enzymes in tomato plants introduced by PGPR

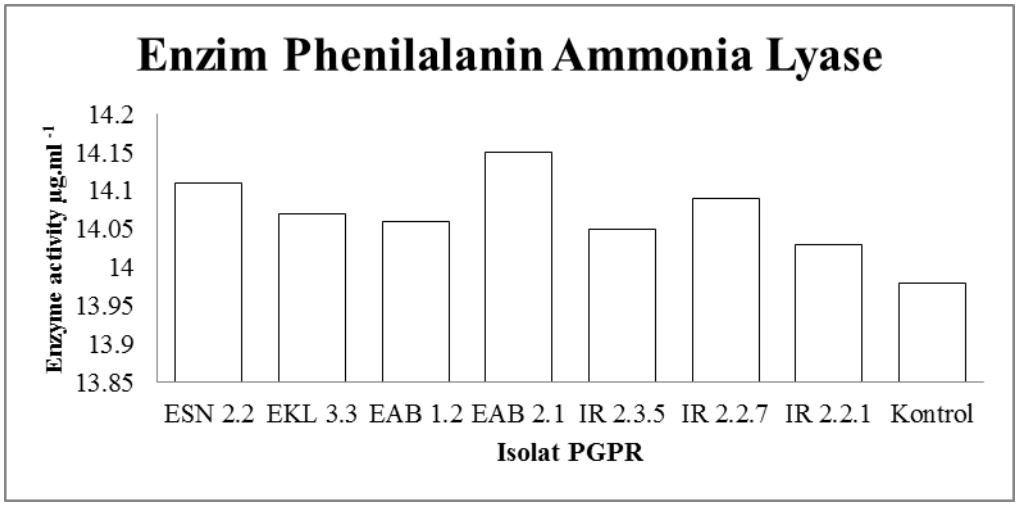

Fig.3: Production of Phenilalanan Ammonia Lyase enzymes in tomato plants introduced by PGPR

V. CONCLUSION

PGPR can induce plant resistance through ISR which further increases the activity of the PO, PPO and PAL enzymes. Seven isolates used in this study were able to increase the activity of the PO, PPO, and PAL enzymes, with Isolate EAB 2.1 being the best isolate in increasing enzyme activity.

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Mapping the Gravity Center of Fishing Ground on Skipjack Tuna Distribution in Bone Gulf-Flores Sea

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Abstract— *Bone Gulf and Flores Sea in the Fisheries Management Area 713 (WPP 713) are known as one of the best skipjack tuna fishing areas in Indonesia, where skipjack tuna is an export commodity and has high economic value. The potential of skipjack tuna is based on the spatial-temporal pattern of oceanographic conditions. The distribution of skipjack tuna can be predicted by the biophysical conditions of the environment. Sea surface temperature and chlorophyll-a are biophysical parameters that greatly affect the distribution of skipjack tuna and are often used to predict potential fishing grounds. The study used skipjack fisheries data from April to July in 2017 to 2019 and remote sensing satellite data on sea surface temperature and chlorophyll-a from Aqua / MODIS. This study aims to determine the gravity center of skipjack tuna fishing ground in Bone-Flores Sea using the Generalized Additive Model (GAM). The distribution of skipjack tuna was found to be significantly related ($p < 0.0001$) with preferred range of sea surface temperature at 29.02–31.03 °C and concentration of chlorophyll-a at 0.14–0.44 mg/m3. The gravity center of fishing ground from April shows that skipjack tuna shift to the north in May and June, then back to the south in July. This study dedicated on providing scientific information regarding the gravity center of skipjack tuna fishing ground in Gulf Bone-Flores Sea, as well as the applicability of remote sensing in contributing to optimalization and sustainable utilization of skipjack fisheries resource.*

Keywords— ***skipjack tuna, sea surface temperature, chlorophyll-a, gravity center of fishing grounds, remote sensing.***

I. INTRODUCTION

The Bone Gulf and Flores Sea in the Indonesia's Fisheries Management Area 713 are known as one of the best skipjack fishing areas in Indonesia [1], where skipjack is an export commodity with high economic value. This is evidenced by the various fishing gear fleets operating to exploit skipjack (*Katsuwonus pelamis*) such as purse seine, huhate (*pole and line*), hand line and trolling line.

The potential of skipjack tuna is influenced by spatial-temporal oceanographic conditions. The biophysical environment in Bone Gulf is certainly different from the biophysical environment in the Flores Sea. The availability of food both in quantity and quality affects the predation rate and is an important variable for the skipjack population. The distribution of fish can be predicted according to the biophysical conditions of the environment. Concentration of chlorophyll-a and sea surface temperature are biophysical parameters that greatly affect in fish distribution and are often used to predict potential fishing grounds [3, 6]

Several studies have shown that the distribution, migration and movement patterns of skipjack tuna are strongly influenced by oceanographic factors. Fish migrate due to changes in several environmental factors such as temperature, salinity and currents, efforts to find water areas where there is sufficient food and efforts to find spawning areas [5]. Sea surface temperature and chlorophyll-a have a significant effect on the catch per unit effort (CPUE) variation of skipjack tuna. The skipjack

tuna distribution pattern is associated with variables changes that reflect their preferred oceanographic. [8]

Thus from various studies show environmental biophysical conditions affect to distribution pattern of skipjack tuna, this study aim to determine the gravity center and distribution of skipjack fishing ground.

The contribution of this study is for the availability of scientific information regarding the gravity center of skipjack fishing ground in Bone Gulf-Flores Sea. As well as the results of this study can be used for optimalization and sustainable utilization of skipjack fishery resources.

II. MATERIALS AND METHODS

This research was conducted from April to July 2019, taking locations in two districts, Luwu Regency with fishing ground in Gulf Bone also Sinjai Regency with fishing ground in the Flores Sea (Fig. 1). Data processing was carried out at the Laboratory of Fisheries and Geospatial Information Systems, Faculty of Marine and Fisheries Sciences, Hasanuddin University.

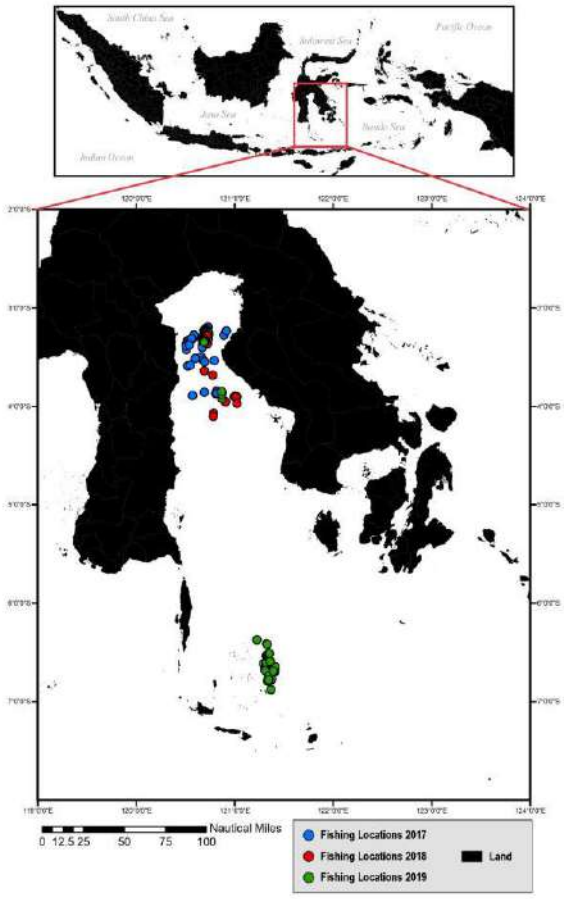

Fig.1: Map of study area in Bone Gulf and Flores Sea showing fishing location points from April to July 2017 to 2019.

a. Fishery data

Monthly catch data were collected from field surveys around the waters of Gulf Bone-Flores Sea from April to July 2019. As well as supporting data from previous studies, April to July in 2017 to 2018. The data obtained included catch per unit effort (CPUE) and fishing location (longitude and latitude).

b. Satellite remote sensing data

Sea surface temperature and chlorophyll-a subset data by Aqua/MODIS (Moderate Resolution Imaging Spectroradiometer) satellite measurements were used. The subset data were distributed by NASA Ocean Color on the website (http://oceancolor.gsfc.nasa.gov/). The data set is level 3 (4 km) monthly data from April to July 2017 to 2019 which coincides with the time of fish catch data to obtain sea surface temperature and chlorophyll-a values. Data sets are also used to obtain skipjack optimum oceanographic conditions and also to develop predictive models by Generalized Additive Model (GAM)

c. Generalized Additive Model (GAM) Analysis

Skipjack distribution and oceanographic variables have been shown to have a non-linear relationship optimal range of sea surface temperature and chlorophyll-a would be suitable for fish, whereas if the chlorophyll-a concentration and sea surface temperature were lower or higher than the optimal ranges, it would indicate a mismatch. [2]

The responses distribution in GAM is not limited to just the normal distribution, but the variables included in the exponential group can be analyzed using this model. The additive model itself is an extension of ordinary linear regression by replacing linear functions with additive functions that do not have a rigid shape, so that this model can be used even though the relationship between the response and predictor variables is not linear. Based on the characteristics of the catch data obtained, the GAM analysis was chosen as the most appropriate analysis to determine the relationship between fish distribution and sea surface temperature and chlorophyll-a concentration [2]

GAM analysis was carried out on CPUE, chlorophyll-a concentration, and sea surface temperature data at the fishing location using the following formula:

$$y = \alpha + s(x_i) + s(x_2) + \ldots + \varepsilon$$

where y is the CPUE value, α is the constant, s is the smooth spline function of the predictor variables (sea surface temperature and chlorophyll-a), x is the predictor variable, namely sea surface temperature (x_i) and chlorophyll-a (x_2), and ε is random error

d. Gravity center of fishing ground

To determine the gravity center of skipjack fishing ground in study area, the fishing location data were plotted on the average distribution of sea surface temperature and chlorophyll-a images from April to July. The satellite data were then extracted for each pixel corresponding to the fishing locations. The GAM results which are the relationship between oceanographic variables derived from satellites and skipjack tuna CPUE at all fishing positions produce CPUE predictive values. This predictive value along the fishing positions were used to determine the center gravity of fishing ground. The monthly center gravity fishing ground were mapped using ArcGIS 10.2 software. The center of gravity of the fishing area is obtained by the following formula [4,8]:

$$GC_{ij} = \frac{\sum L_{ij}\, CPUE_{ij}}{\sum CPUE_{ij}}$$

Where, GC_{ij} is the monthly gravity centre of fishing grounds at longitude and latitude position, L_{ij} is the latitude and longitude of fishing point, and $CPUE_{ij}$ is the catch per effort (fish/fishing set) at position ij.

III. RESULTS AND DISCUSSION

During April to July in 2017 to 2019, skipjack tuna were found in the study area at temperatures range from 29.02 - 31.03 ° C and chlorophyll-a concentrations 0.14 - 0.44 mg/m^3. The highest fishing frequency was at sea surface temperature range of 29.5 - 30 °C with a proportion of 50.21% (Fig. 3a) and chlorophyll-a concentration of 0.3-0.35 mg/m^3 with a proportion of 28.45% (Fig. 3b). The highest average catch was at a temperature of 30.24 ± 0.12 °C as many as 68 fishes (Fig. 3c) and a chlorophyll-a concentration 0.24 ± 0.02 mg/m^3 as many as 61 fishes (Fig.3d).

The variation of sea surface temperature in April is relatively higher than the following months, ranging from 29.38 °C to 32.60 °C and continues to decline every month. The sea surface temperature in Bone Gulf tends to be higher than the sea surface temperature in the Flores Sea (Fig. 3), this is due to the influence of cold water masses from the current circulation system and the occurrence of upwelling and downwelling. In contrast to the distribution of chlorophyll-a concentrations, the variation in April was relatively lower than in the following months, which ranged from 0.08 to 1.88 mg / m3 and continued to increase in concentration every month (Fig. 4).

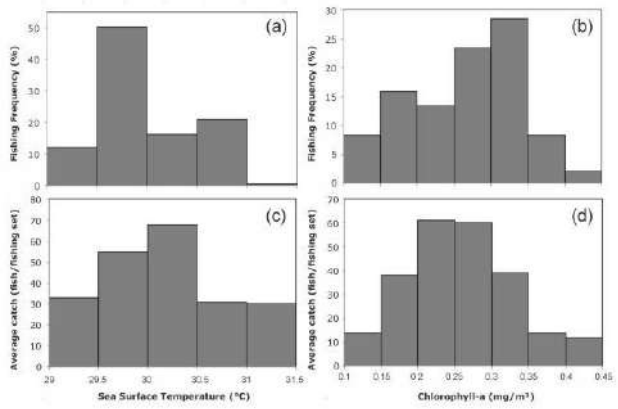

Fig.2: (a) The fishing frequency of skipjack tuna in sea surface temperature range; (b) The fishing frequency of skipjack tuna in chlorophyll-a range; (c) Average catch of skipjack tuna in sea surface temperature range; (d) Average catch of skipjack tuna in chlorophyll-a range in Gulf Bone-Flores Sea

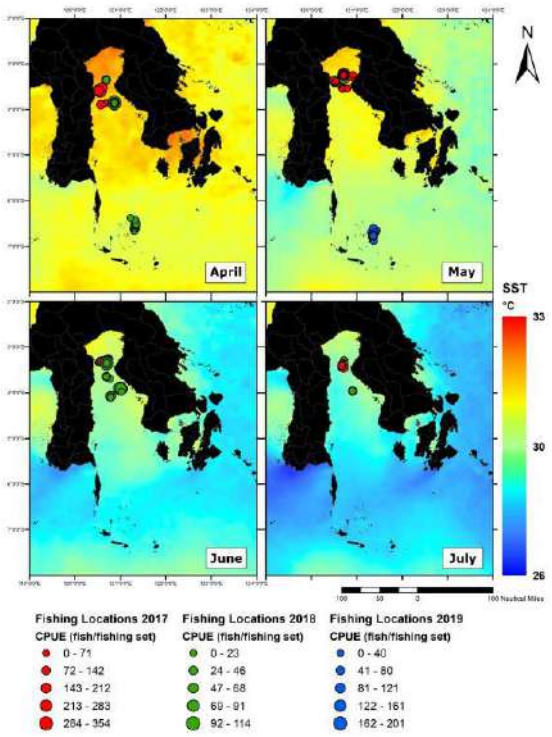

Fig.3: Map of average sea surface temperature distribution in Bone Gulf and Flores Sea showing fishing locations on April to July in 2017 to 2019

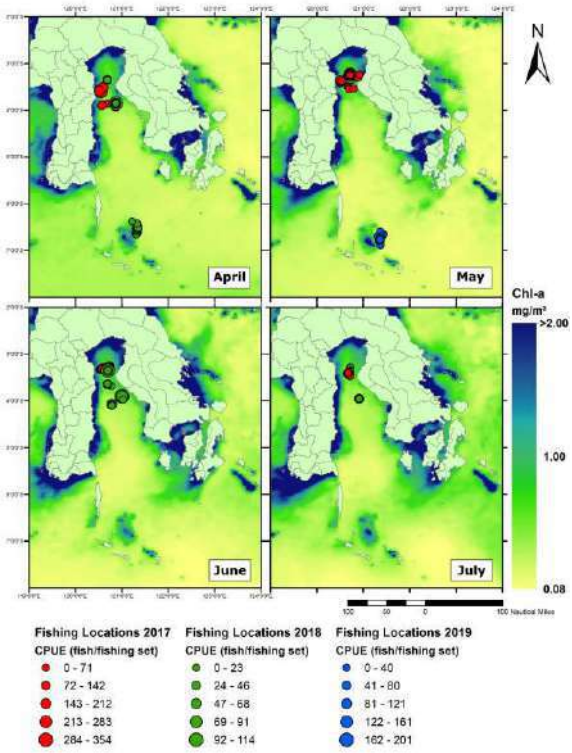

Fig.4: Map of average concentration of chlorophyll-a distribution in Bone Gulf and Flores Sea showing fishing locations on April to July in 2017 to 2019

The GAM plot can be interpreted as the individual effect of each predictor variable (sea surface temperature and chlorophyll-a) to CPUE value. The x-axis shows the predictor variables, sea surface temperature (Fig. 5a) and chlorophyll-a (Fig. 5b) and the y-axis shows the contribution of the smoother to responses value, CPUE. The percentage value is higher if the GAM function developed is above the zero axis which indicates the strong influence of a parameter and if it is below the 0 axis it indicates the weak effect of a parameter on skipjack tuna. The GAM plot on the horizontal axis represents the observed catch data points. The function indicated by the thick line and gray shade represents the 95% confidence interval (Fig. 5). Based on the results of GAM analysis in this study, it shows that sea surface temperature and chlorophyll-a concentration have a significant effect ($p < 0.0001$) on the CPUE value of skipjack tuna (Table 1).

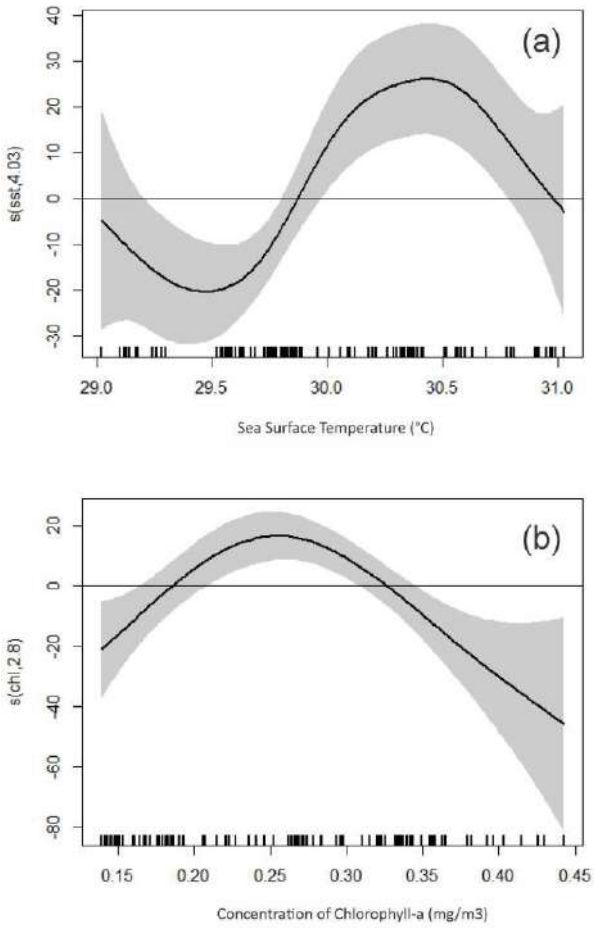

Fig.5: GAM analysis of skipjack tuna catch against: (a) sea surface temperature and (b) concentration of chlorophyll-a. Distribution of relative density data is shown on the x-axis.

Table 1. GAM analysis of sea surface temperature and chlorophyll-a on skipjack tuna CPUE

	edf	Ref.df	F	p-value
s(SPL)	4.029	4.943	6.407	0.0000167
s(Chl-a)	2.797	3.516	7.015	0.0000845
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1				

A strong relationship was observed in the sea surface temperature range of 29.7 - 30.9 °C (Fig. 5a) and chlorophyll-a concentrations of 0.17 - 0.33 mg/m^3 (Fig. 5b). This is evidenced by the distribution of the highest relative density of catch data (catch frequency) and the highest catch in this range. The results are also consistent

with the average catch and fishing frequency shown in Fig. 2.

The results obtained were the same as the results of research on skipjack tuna in the Coral Triangle area [7] that the distribution and dynamics of skipjack tuna habitat hotspots in Bone-Flores Sea is in the temperature range of 29.5-31.5 °C with a concentration of 0.15-0.35 mg/m^3.

The determining of gravity center of skipjack fishing ground shown that from April to July all of the gravity center were in Bone Gulf. The results of the GAM prediction also show that all the center points of gravity obtained are in the appropriate conditions for the skipjack tuna parameters (Fig. 6).

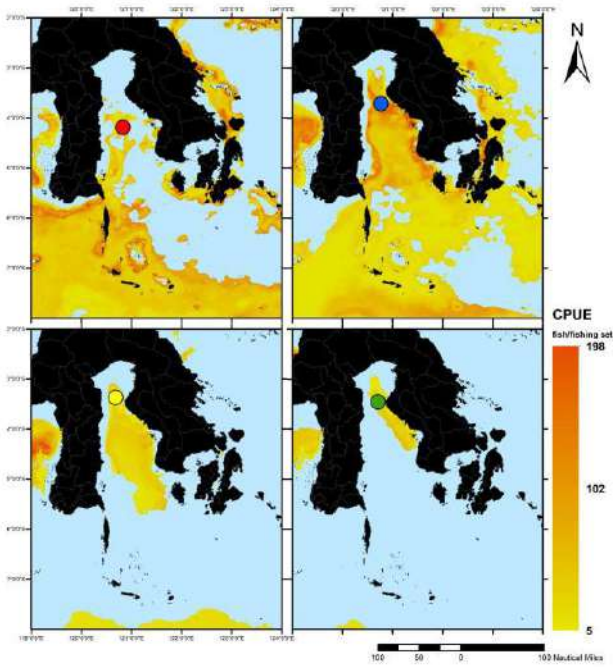

Fig.3: Map of skipjack tuna gravity center fishing ground overlay on GAM predictive raster from April to July

Figure 6 shows that in April, the gravity center point is 4°11'13.5"S, 120°49'50.6"E which shows that gravity center is in between the waters of Wajo Regency and Kolaka Regency. In May, the gravity center point is at 3°42'40.4"S, 120°46'18.5"E which indicates gravity center is in the waters of North Kolaka Regency. In June, the gravity center point is at 3°21'45.1"S, 120°41'07.0"E which indicates the gravity center is between the waters of Luwu Regency and North Kolaka Regency. In July, the gravity center point is at 3°27'08.9"S, 120°42'44.5"E which indicates gravity center is in North Kolaka Regency. By the results of determining the gravity center of fishing grounds, the distribution direction can be seen (Fig. 7) that from April, gravity center of skipjack tuna fishing ground shift to north in May and June, then returns to south in July.

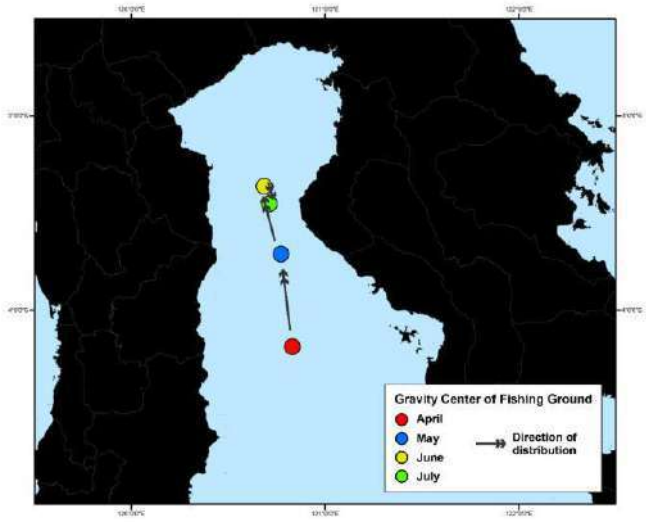

Fig.4: Direction of skipjack tuna gravity center movement estimated by GAM from April to July

Research which found that the movement pattern of skipjack tuna in the Makassar Strait during January-May depicts a shift in clockwise distribution. Skipjack tuna move from offshore to the Makassar Strait in January and then during February-March the movement continues towards the offshore area of Barru-Parepare-Pinrang Regency in the southeastern Makassar Strait. Then, during April-May the fish concentration moved westward to the offshore area and then began moving north to the waters off Mamuju Regency.[8]

These findings suggest that the pattern of skipjack distribution may be related to the dynamic movement of the preferred oceanographic conditions in terms of sea surface temperature and chlorophyll-a concentration.

IV. CONCLUSION

Based on the results and discussion of this research study, it can be concluded that the center of gravity of the fishing ground shows that the direction of distribution of skipjack tuna from April moves northward in May and June, then returns south in July.

V. SUGGESTIONS

Suggestions for the continuation of this research is further research is needed on the gravity center of skipjack fishing ground in the span of the year so that the movement pattern of skipjack tuna can be seen more obviously.

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Testing for the Environmental Fate and Safety of E-Waste using Nitrobacter and Mice Model

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Abstract**—** *The present study aimed to test for the environmental fate and safety of e-waste using Nitrobacter sp. and mice model assays. The Nitrobacter sp. toxicity test was designed in four treatments and control set-ups namely 6.25, 12.5, 25, 50 % and control separately in both fresh and marine water for 24 h at 25 °C. The Wister albino mice were separated into three set-ups of 8 animals each (4 females and 4 males) in which 1 ml/kg bw of normal saline which serves as control set-up was orally administered to Group 1 while 500 mg/kg bw doses of Products A and B preparations were administered to Groups 2 and 3, respectively once daily for 14 days at 25 °C. The result revealed higher values of heavy metals in products A and B. Products A and B had the highest and lowest EC_{50} values of - 111.19 % and - 922.26 % in fresh and marine water, respectively. The oral administration of 500 mg/kg bw of products A and B resulted in non-significant (P > 0.05) body/tissue weights decrease and increase in the levels of AST, ALT, ALP, urea and creatinine in the biochemical profile of the exposed mice. There were more serious but non-cancerous histopathological injuries to the kidney tissue structures than liver tissue structures. The study demonstrated the possible high toxicity of unregulated disposal of these e-wastes into the environments and animals and therefore recommends proper treatment or recycling of these wastes before disposal.*

Keywords**— E-waste toxicity, mice model, Nitrobacter sp., pollution, public health monitoring.**

I. INTRODUCTION

The growth and usage of electronic devices such as laptop of diverse brands especially in Nigeria has over the last two decades widely increased. The very possible reason for this increase could be due to high population density. As a result, there has numerous reports of unused and spoilt parts of these of devices containing poisonous heavy metals, metalloids, polyaromatic hydrocarbons, polychlorinated biphenyls scattered in major electronic waste dumps across significant cities and hence constitutes wastes (Manhart *et al*. 2011; Alabi *et al.* 2012; Uba *et al*. 2020a). These wastes commonly termed as E-wastes if not properly treated or managed could poised serious danger to public and animal health.

Evaluation of these wastes to determine their safeties when release either to aquatic or terrestrial ecosystems is usually carried out using chemical assays. This assay has been found to be limited in that it cannot detect the minutest concentration of these wastes and mostly not rapid. Bioassays using biological monitors has been found to overcome these demerits and as a result, deployed to complete the chemical toxicity assay (Uba *et al.* 2020b).

Previous studies by Nrior and Owhonda (2017); Nrior and Gboto (2017) and Uba *et al*. (2020a) reported significant lethal and inhibitory effects of spent phone and laptop battery wastes on *Nitrobacter* sp., *Phaseolus vulgaris* (common bean), *Sorghum bicolor* (guinea corn), *Allium cepa*, *Eisenia fetida* and *Selenastrum capricornutum* when these wastes are discharged into aquatic and terrestrial ecosystems. Alabi and Bakare (2014) reported a significant concentration-dependent induction of micronucleated polychromatic erythrocytes, sperm abnormalities and

decrease in sperm count across the treatment groups of mice exposed orally with e-waste contaminated underground water five weeks. In a study carried out by Andjelkovic *et al*. (2019), they reported that the acute exposure of adult Wistar rats to Cd and/or Pb induced toxic effects in their blood, liver and kidney tissues.

There is paucity of information on toxicity effects of spent laptop batteries on Nitrobacter sp. and mice model. To the best of our knowledge, the literatures available as at the period of this study, focused their scope of studies on toxicity effects of spent phone batteries only on *Nitrobacter* sp. and therefore demand the study. The current study was therefore aimed to test for the environmental fate and safety of E-waste using *Nitrobacter* and mice model assays.

II. MATERIALS AND METHODS

2.1 Collection of sample, processing and site description

The studied sites were Nigerian fresh water: River Niger (latitude 4°22'50"N - latitude 7°65'56.5"N and longitude 7°11'6.77"E - longitude 7°11'16.2"E) Anambra State and marine water: Onne Light Flows Sea water (latitude 6°7'50"N - latitude 6°9'30"N and longitude 6°45'47"E - longitude 6°46'20"E) Rivers State, respectively. The water samples were collected and processed as described in our previous study (Uba *et al.* 2020c). The spent laptop battery wastes (Dell brand was designated as product A and Lenovo brand designated as product B due to ethical issue) were bought at Onitsha Market, Nigeria and transported to Microbiology Laboratory, Chukwuemeka Odumegwu Ojukwu University, Nigeria. They were finally processed using by force rupturing of the battery lid and emptying of the contents into sterile plastic 1 L containers.

2.2 Specimen collection and adaptation

Healthy adult Wister albino mice (Equal sex, 23.2- 27.9 g, 16 – 17 weeks old) purchased from Green Stone Farm Okohio, Otolo Nnewi, Anambra State were placed, fed, cared and adapted in medium and spacious aluminum cages at 25 °C with 12 h light and 12 h darkness cycles for two weeks before carrying out the experiment according to the protocol of Organization for Economic Cooperation Development (OECD) (2009).

2.3 Heavy metal evaluation

The method of APHA (2012) and as previously described by Uba (2018) were employed for the determination of metal contents of products A and B using atomic absorption spectrophotometry.

2.4 Isolation of *Nitrobacter* sp.

The test organism *Nitrobacter* sp. was isolated from the fresh and marine water samples using Modified Winogradsky' Agar (MWA) as previously described by Odokuma and Nrior (2015). The organism was biochemically characterized and identified using Bergey's Manual for Determinative Bacteriology by Holt *et al.* (1994).

2.5 Toxicity evaluation

2.5.1 Toxicity evaluation using *Nitrobacter* sp.

The method of Uba *et al.* (2020c) was adopted in evaluating the toxicity of the laptop battery wastes on *Nitrobacter* sp. Pure culture of *Nitrobacter* sp. was prepared by inoculating a loopful into sterile Modified Winogradsky' Medium (MWM) until late log phase. After development of inoculum, 1 mL of the test organism was added to separate toxicant (Products A and B) concentrations (6.25, 12.5, 25, 50 and 0 %) in test tubes containing sterile fresh and marine water as separate diluents. The 0 % represents the control set-ups without the toxicant samples. After inoculation, zero-hour count plating was carried out on sterile Nutrient agar plates and incubated at 25 °C. Subsequently, 0.1 mL of each concentration of the toxicants was plated out and incubated after 4, 8, 12 and 24 h on sterile Nutrient agar plates for 48-72 h. The experiment was carried out in triplicates and the plates were later counted after emergence of colonies.

2.5.1 Toxicity evaluation using mice model

2.5.1.1 Acute toxicity testing

Acute toxicity testing of the laptop batteries wastes was carried out at oral dose administrations of 5, 50 and 500 mg/kg body weight (bw) for 24 h intervals in order to determine the safe dose in accordance with the guidelines of OECD (2009). Toxicity signs and mortality were monitored on each toxicant (Products A and B) set ups once daily for at least 14 days.

2.5.1.2 Sub-chronic toxicity testing

Repeated-dose of 500 mg/kg bw of the laptop batteries preparations was used to evaluate the sub-chronic toxicity testing as no mortality was recorded after the acute test. The Wister albino mice were separated into three set-ups of 8 animals each (4 females and 4 males). Then, 1 ml/kg bw of normal saline which serves as control set-up was orally administered to Group 1 while 500 mg/kg bw doses of Products A and B preparations were administered to Groups 2 and 3, respectively daily for 14 days. During the study, regular intake of water and food (TOP VITAL FEEDS, Kano State, Nigeria) were monitored. After the 14th day of the study, the mice were sacrificed after chloroform vapour sedation and whole blood was collected after dissection

through cardiac puncture and placed into labeled sample bottles.

2.5.1.3 Weight analysis

The body/tissue weight of mice in each set-up were determined at the beginning and end of the 14 days study.

2.5.1.4 Evaluation of biochemical indices

By adopting the method of Egurefa *et al*. (2020) and Uba *et al*. (2020b), the following biochemical profile were analyzed: alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), urea and creatinine.

2.5.1.5 Histopathological assay

As described by Egurefa *et al.* (2020) and Uba *et al*. (2020b), the liver and kidney tissues were fixed on slides, stained and counter-stained with hematoxylin and eosin (H and E stains) and finally viewed under a compound microscope (CX 23 Olympus, Japan). All the abnormalities from the normal architectural tissue structures were observed and noted.

2.6 Statistical Study

Mean of the triplicate values were determined at different concentrations and expressed in Tables and Figures. The mean values were further analyzed to determine the effects of products A and B on the growth survival of the *Nitrobacter* sp. using linear regression. Analysis of variance was used to compare the effects of products A and B on the weight and biochemical indices of the exposed Wister Albino mice using GraphPad Prism Software version 8.0.2.

III. RESULTS

3.1 Heavy metal profile

Table 1 showed the heavy metal profile of the products A and B. Product B had the highest values of mercury 1.532 ppm, nickel 22.802 ppm, lead 6.405 ppm while product A had the highest value of arsenic 0.179 ppm and cadmium 0.019 ppm, respectively.

Table 1: Heavy metal profile of the products A and B

Parameter	Concentration (ppm)		USEPA 2009/NESRA 2009 standards in water (ppm)
	Product A	Product B	
Mercury (Hg)	0.284	0.407	0.002
Nickel (Ni)	13.874	21.898	NA
Arsenic (As)	0.119	0.111	0.010
Cadmium (Cd)	0.191	0.000	0.005
Lead (Pb)	2.691	2.028	0.015 – 0.050

Key: Ppm = Part Per Million; NESRA = National Environmental Standards and Regulation Enforcement Agency (2009) permissible limits for drinking water; USEPA = US Environmental Protection Agency (2009); NA = Not available.

3.2 Nitrobacter toxicity profile

Figs. 1 and 2 showed the inhibitory response of *Nitrobacter* sp. to different concentrations of the toxicants (Products A and B). From the Figs., there were decrease in log count as the concentration and exposure period increased with fresh water having the highest log count of 5.48 logCFU/mL while marine water had the lowest log count of 5.11 logCFU/mL after 24 h, respectively. Similarly, Fig. 3 showed the 24 h toxic response of *Nitrobacter* sp. to products A and B. Products A and B had the highest and lowest EC_{50} values of - 111.19 % and - 922.26 %, respectively.

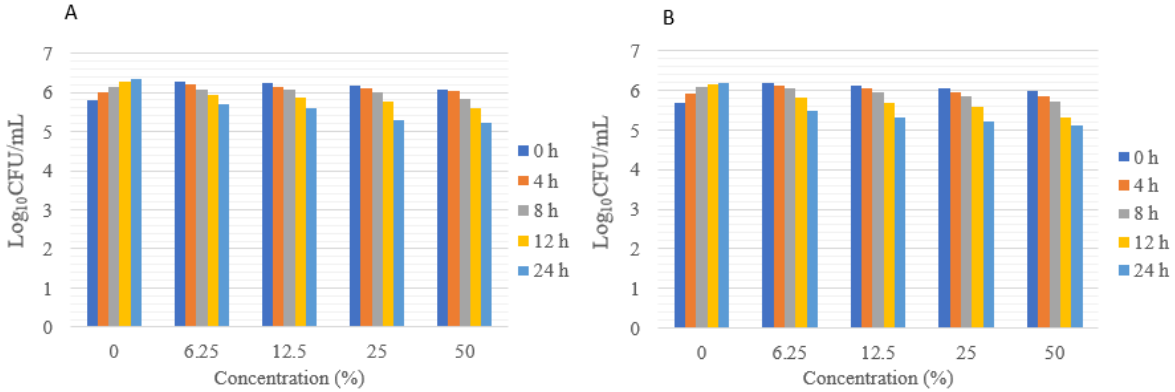


Fig. 1: Inhibitory response of Nitrobacter sp. to different concentrations of the Product A. A = Fresh water; B = Marine water; LogCFU/mL = Logarithmic colony forming unit per millilitre; h = Hour; % = Percent

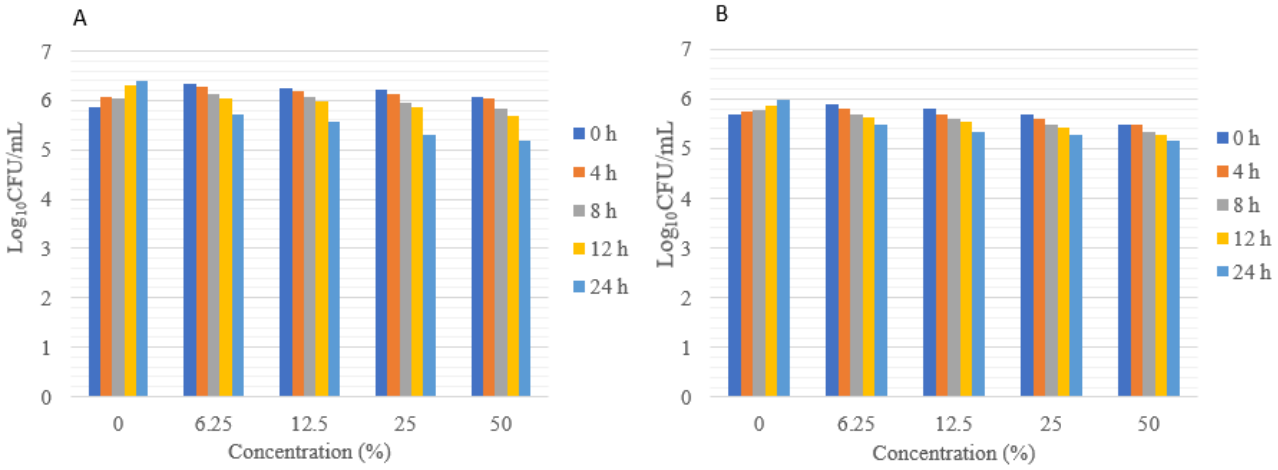


Fig. 2: Inhibitory response of Nitrobacter sp. to different concentrations of the Product B. A = Fresh water; B = Marine water; LogCFU/mL = Logarithmic colony forming unit per millilitre; h = Hour; % = Percent

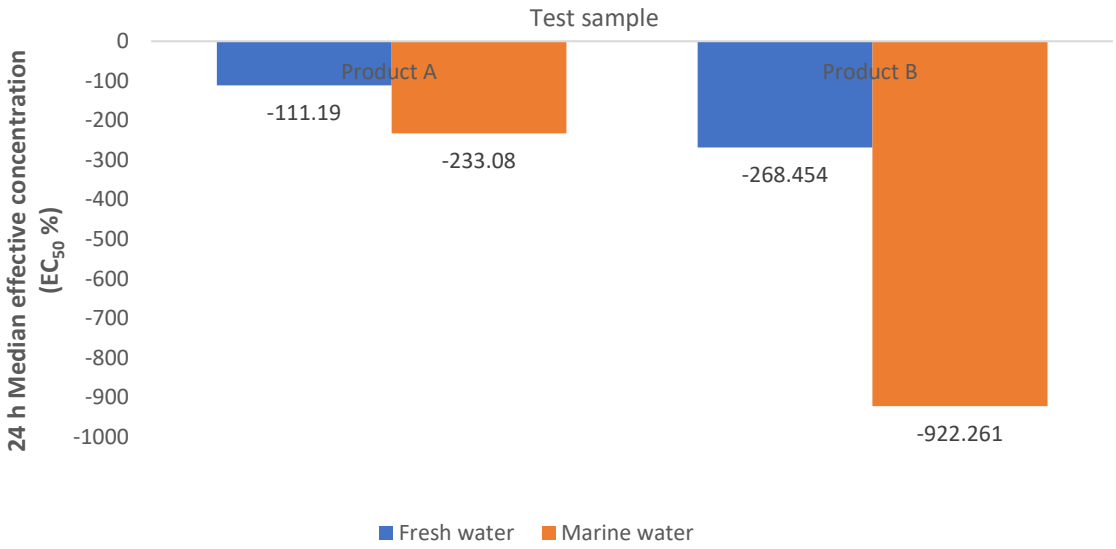
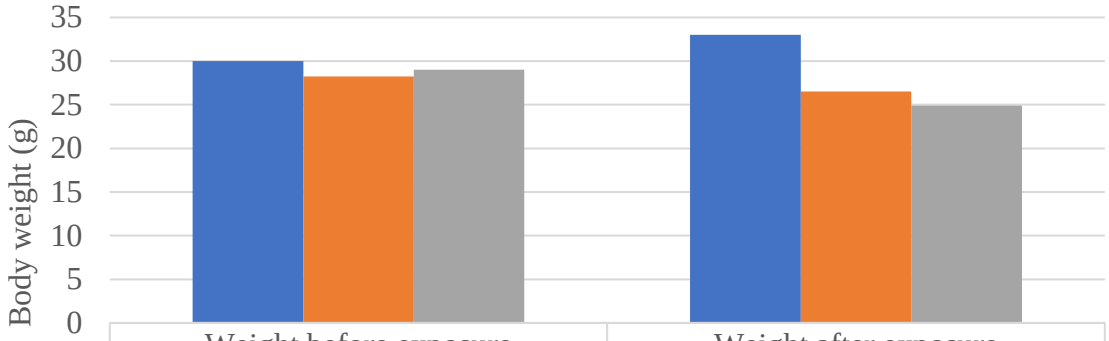


Fig. 3: 24 h toxic response of Nitrobacter sp. to products A and B. EC_{50} = 50 percentage median effective concentration; % = Percent; h = Hour

3.3 Mice toxicity profile

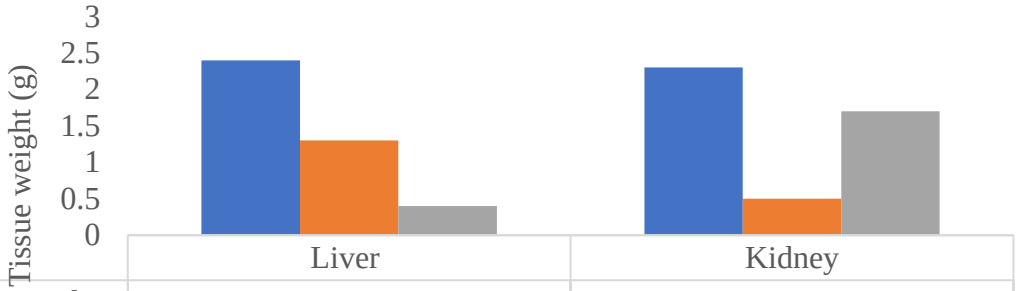
Figs. 4 and 5 displayed the body/tissue weights of the Wister albino mice before and after 14 days exposure to 5000 mg/kg bw of products A and B preparations and control set-up. The control set-up had the highest body weight values of 30.00 g before exposure while product B set-up had the lowest body weight values of 24.90 g after the 14 days exposure (Fig. 4). Similarly, control set-up had the highest liver and kidney weights of 2.40 g and 2.30 g; while product B and A set-ups had the lowest liver and kidney weights of 0.40 g and 0.50 g after the 14 days exposure (Fig. 5). Fig. 6 showed the biochemical indices of the Wister albino mice after 14 days exposure to 5000 mg/kg bw of products A and B preparations and control set-up. Product A set-up had the highest values of AST (20.00 U/L), ALT (28.00 U/L), ALP (84.00 U/L), urea (15.60 U/L) and creatine (240.00 U/L); while control set-up had the lowest values of AST (10.00 U/L), ALT (15.00 U/L), ALP (44.00 U/L), urea (7.80 U/L) and creatine (72.00 U/L) after 14 days exposure. Figs. 7a – c and 8a – c showed the micrograph of haematoxylin and eosin stained liver and kidney tissues of Wister albino mice after 14 days exposure to 5000 mg/kg bw of products A and B preparations and control set-up. Morphological aberrations of the product A liver tissues showed presence of inflammatory cells; while product B liver showed focal inflammatory and congestion of the central vein (Fig. 6a – c). The product A kidney tissue showed inflammatory cells emasculating the tubule while

product B kidney tissue showed vascular congestion in the medulla (Fig. 7a – c). The control mice set-ups revealed normal architecture of the liver and kidney structures.



	Weight before exposure	Weight after exposure
Control	30	33
Product A	28.23	26.5
Product B	29	24.9

Fig. 4: Body weight of the Wister albino mice before and after 14 days exposure to 5000 mg/kg bw of products A and B preparations and control set-up. g = Gram



	Liver	Kidney
Control	2.4	2.3
Product A	1.3	0.5
Product B	0.4	1.7

Fig. 5: Tissue weight of Wister albino mice after 14 days exposure to 5000 mg/kg bw of products A and B preparations and control set-up. g = Gram

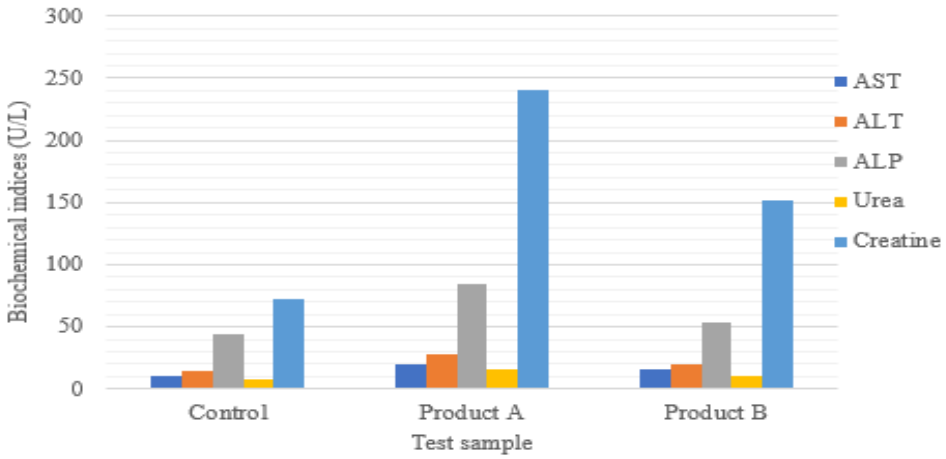


Fig. 6: Biochemical indices of the Wister albino mice after 14 days exposure to 5000 mg/kg bw of products A and B preparations and control set-up
Key: AST = Aspartate transaminase; ALT = Alanine transaminase; ALP = Alkaline phosphatase; BW = Body weight; mg/kg = milligram per kilogram; U/L = Unit per litre.

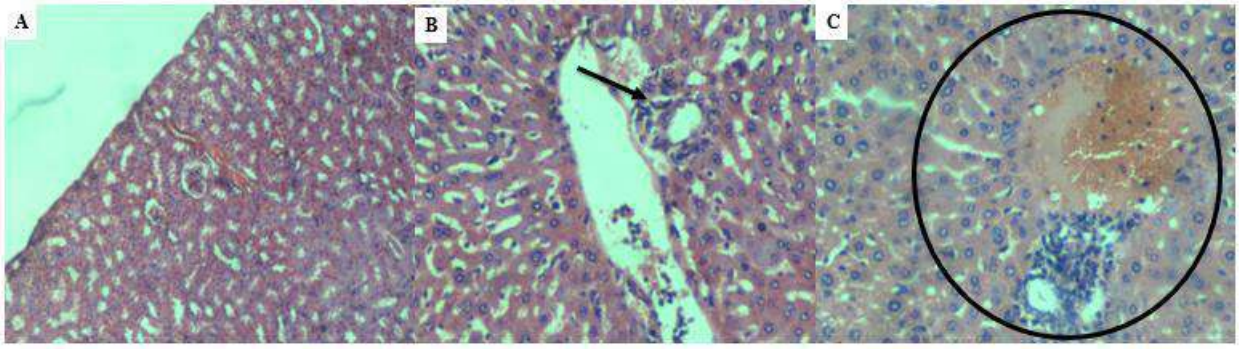


Fig. 7a – c: Micrograph of haematoxylin and eosin stained liver tissues of Wister albino mice after 14 days exposure to 5000 mg/kg bw of products A and B preparations and control set-up. A = Control; B = Product A; C = Product B.

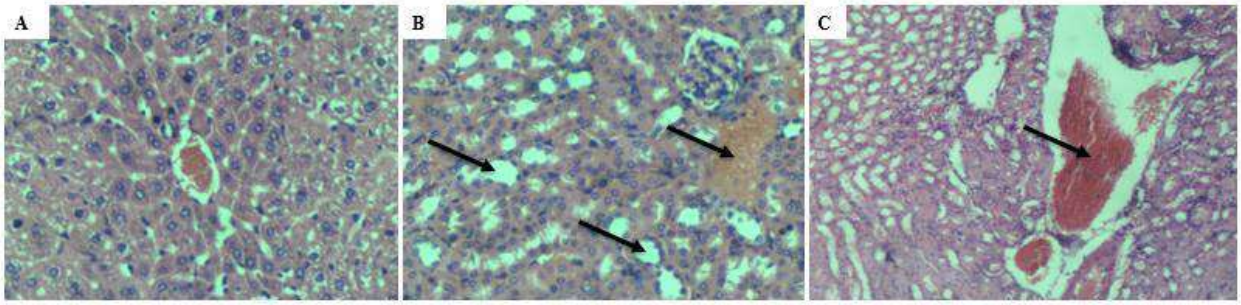


Fig. 8a – c: Micrograph of haematoxylin and eosin stained kidney tissues of Wister albino mice after 14 days exposure to 5000 mg/kg bw of products A and B preparations and control set-up. A = Control; B = Product A; C = Product B.

IV. DISCUSSION

The wide application of *Nitrobacter* sp. and mice model for assessing pollutant and chemical substances have been largely reported by numerous researchers (Odokuma and Nrior 2015; Nrior and Gboto 2017; Nrior and Owhonda 2017; Uba et al. 2020b; Alabi and Bakare, 2014; Andjelkovic *et al.* 2019). Earlier studies by Uba et al. 2020a and Alabi and Bakare, 2014 revealed higher levels of Ni, Pb and Cd heavy metals in their e-wastes preparations and leachates and similar findings were obtained in the heavy metal profile of products A and B e-wastes in this study.

In order to determine the fate of these wastes on the fresh and marine aquatic ecosystems, *Nitrobacter* sp. a key ecological test organism commonly found and isolated in both ecosystems was studied by exposing it to products A and B leachate preparations. The results in Figs. 1 and 2 revealed non - significant ($P > 0.05$) concentration-dependent reduction in the logarithm count of *Nitrobacter* sp. after 24 h exposure in all the set-ups except the control set-up with increase which could be as a result of the absence of e-waste preparations in them. Product B was found to be more toxic to *Nitrobacter* sp. than product A after 24 h exposure in both fresh and marine waters (Fig. 3) and the probable reason could be due to the higher profile of metals and metalloids present in product B than product A. Products A and B were classified as very acutely toxic according to Verma (2007) toxicity classification scheme because their EC_{50} values were < 1 %. Previous studies by Nrior and Gboto (2017); Nrior and Owhonda (2017) and Uba *et al.* (2020a) reported reduction in the percentage logarithmic survival of *Nitrobacter* sp. and bacteria in two aquatic ecosystems after exposure for 24 h and therefore upheld the findings in this study.

Furthermore, to widen our knowledge on the safety on these wastes on animals, mice model was studied by also exposing them to products A and B leachate preparations at 500 mg/kg bw. Figs. 4 and 5 demonstrated non-significant ($P > 0.05$) body/tissue weight reductions in the mice treated set ups in comparison to their controls. This revealed the

probable developmental health impacts of these wastes if disposed without treatment. There was non-significant ($P > 0.05$) elevated levels of ALT, AST, ALP, urea and creatine in the product A exposed groups than the product B exposed groups in comparison to the control unexposed groups (Fig. 6). These elevated features could suggest hepatic and renal functional impairment and injuries in both treated groups and the possible reasons could be linked to the presence of hazardous metal substances in the e-wastes. The results are similar to the findings of Yuan *et al*. (2014) and Cobbina *et al.* (2015) who reported increase in the hepatic and renal biomarkers in heavy metal exposed Wistar rats but contradict the findings of Zhu *et al*. (2014) and Andjelkovic *et al.* (2019) who reported decrease in these biomarkers. There were more serious injuries to the kidney structures than liver structures as depicted by the histopathological micrographs (Figs. 7a - c and 8a -c) which led to the secretions of more renal biomarkers than hepatic biomarkers. Andjelkovic *et al*. (2019) reported no serious disturbance on the liver structure exposed to single and combined heavy metal doses.

V. CONCLUSION

The study showed that product B was more toxic to *Nitrobacter* sp. than product A if discharged in the fresh and marine aquatic ecosystems. There was non-significant decrease in body/tissue weights of exposed Wistar mice. The hepatic and renal enzymes were found to be elevated with more serious but non-cancerous features in the kidney than the liver tissues. Hence, further study on reproductive, haematological and genetic fates of these wastes are recommended.

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Influence of Fertilizers on Incidence and Severity of Viral and Bacterial Potato (*Solanum tuberosum* L) Diseases under Field Condition

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***Abstract*— *The potato production in the Far North Region, Cameroon is confronted with rarities or unevenness of rainfall, diseases and pests. In order to improve the production of this plant, a study was conducted in two villages (Mouvou and Gouria) with the general objective of evaluating the impact of fertilizers on the development of viral, bacterial and pest diseases of this plant. The experimental design used was a completely randomized block S with 4 treatments repeated 3 times each. The fertilizer treatments were: Mycorrhizae (MYC), NPK (20-10-10) chemical fertilizers, chicken droppings (CD) and a control (T) that received no application. The plant material used was a local variety of potato (Dosa). Diseases were identified, incidence, severity and rainfall were evaluated. The viral diseases identified were: Virosis M, Rust Stain and PLRV. Bacterial diseases were Bacterial Wilt and Common Scab. The highest incidences of 18.91 % and 10.44 % were obtained with Virosis M and Rust Stain in MYC treatment at Mouvou and Gouria respectively. But, in CD treatment incidence was 2.22 % and 0 % at Gouria for Virosis M and Rust stain respectively. the average rainfall was 697.75 mm. Severity was low in CD treatment in all the sites (< 20 %). All diseases were present in Gouria. The average rainfall was higher in the Gouria site 716.5mm than in Mouvou site which received 679 mm of water. The CD treatment can be recommended to the farmers of Mogodé for the phytosanitary protection of potatoes.*

Keywords*— *Solanum tuberosum, fertilizers, diseases, incidence and severity.

I. INTRODUCTION

Among agricultural commodities, the potato (*Solanum tuberosum* L.) occupies a predominant place in the food supply of many countries because of the areas it occupies, the jobs it provides and the production volumes it generates. World production has been estimated at more than 368 million tons from 19.4 million hectares in 2013 (Issa et al., 2017), making it the fourth largest producer after wheat, rice and maize, making it the main non-cereal food in the world (FAO, 2013).

Originally from the highlands of Peru (Spooner et al., 2005), it was introduced to Cameroon in 1940. It is cultivated in high altitude zones (1000 to 3000 m) (IRAD, 2012) and extensively in six of Cameroon's ten regions (North-West, South-West, West, Adamaoua, Littoral and Extreme-North), mainly by rural people and mainly women (Fontem et al., 2005). It constitutes a staple food for the populations of these regions

(annual consumption of 4 to 10 kg per inhabitant per year) an important source of income because production surpluses are either sold on the local market or exported to neighboring countries. National production was estimated at 229,000 tons on 23,500 hectares in 2009 (IRAD, 2012). Thus, despite the importance of potatoes in the national economy, total production remains below real potential, yields are generally low (Burton, 1989; Diop et al., 2019) and range between 3 and 11t/ha, while those of European countries average 25 t/ha (Sayed et al. 2015) and reach 60 t/ha.

In the Far North Cameroon Region, particularly in the Mogodé subdivision (Mayo-Tsanaga), the only and main production area, the low yields observed are associated with poor peasant farming practices (Ngoyi et al., 2020), the scarcity or inequality of the rains and especially to diseases and pests. Many diseases have been reported on potatoes in several countries (Masum et al., 2011; Habtamu et al., 2012 ; Alkher et al., 2015 ; Son et al., 2018) and in Cameroon (Fontem et al., 2003; Fontem et al., 2005 ; Lontsi et al., 2019).

Potato crop losses due to bacterial diseases could be direct or indirect. Brown rot and Bacterial wilt (*Ralstonia solanacearum*) of potato are the major bacterial diseases in potato production area (Prior et al. 2016 ; Kong et al. 2016 ; Charkowski, 2020). Whereas more than 50 different viruses and one viroid have been reported infecting potatoes worldwide only a handful of them cause major losses globally (Chiunga and Valkonen, 2013). PVY and PLRV (potoato leafroll virus) are now the most damaging viruses of potato world-wide, with PVY having overtaken PLRV as the most important. Tuber yield losses are caused by either of them in single infections and can reach more than 80% in combination with other viruses. PVX or PVM occurs commonly worldwide and causes losses of 10–40% in single infections and is particularly damaging in combination with PVY or PVA (Baldo et al., 2010).

Several solutions have been proposed to overcome these diseases production constraints: These include the use of varieties with high production potential, resistant to diseases and adapted to the agro-ecological zone (IRAD, 2012) and the use of chemical fertilizers by farmers. However, these fertilizers when there are within the reach of farmers, there is lack of control over their tis can leads to risks that can disrupt the environmental balance. In the other hand, diseases encountered in the fields are caused by some cultural practices that maintain the development, proliferation or reduction of the pathogens responsible for these diseases, as well as on the parameters that govern the development of epidemics (Thurston, 1992; Tompkins et al., 1992; Reid et al., 2004). Organic amendments and composting can affect the inoculum which is the primary source of disease infestation in the field (Thurston, 1992; Compaore et al., 2010).

On the other hand, the addition of certain mineral and organic fertilizers can lead to the rapid development of plants, making them more or less susceptible to attack by pathogens (Thresh, 1982; Nawal et al., 2014; Abiodum et al., 2015). Despite the knowledge about the relationship between fertilizer application and diseases expression, fertilizer application is adopted by farmers in Cameroon just to increase their crop yield. But, little information is available on the effect of this applied fertilizer on the incidence and severity of diseases of potato. Nevertheless, knowledge of host nutrition in relation to disease development provides a basis for modifying current agricultural practices to reduce disease incidence and severity.

It is necessary to implement strategies aimed at improving agricultural production that are based on the respect of ecological, economic and toxicological functionalities in the context of food security and environmental protection. Hence bio fertilization by the use of mycorrhizae, is more resistant to pathogenic bacterial and fungi attacks and exposure to soil toxins (Moser and Haselwandter, 1983; Ngonkeu, 2003; Gnamkoulamba et al., 2018) and organic fertilization by the use of chicken droppings that provide more mineral elements (potassium) improves plant resistance to pathogens and environmental balance. Therefore, a study was undertaken with the general objective of evaluating the impact of fertilizers (mycorrhizae and chicken droppings) on the development of potato viral, bacterial diseases in the district of Mogodé (Mayo-Tsanaga) Far-North, Cameroon.

II. MATERIALS AND METHODS

Plant material and Fertilizers

The plant material used for this trial was a local variety of potato (Dosa). Its development cycle is three (3) months. It has a round shape, white skin and flowers.

The mycorrhizal inoculum used for this work consisted of a mixture of spores from fungi of the genera *Glomus* and *Gigaspora*. It was provided by the Nkolbisson Biotechnology Centre of the University of Yaoundé I.

The organic manure used was chicken droppings from the Teufack Poultry Farm in Mokolo.

The chemical fertilizer used was NPK (20-10-10) as a maintenance and background fertilizer.

Experimental design

The experimental design was in completely randomized blocks. Each block consisted of 4 treatments constituting plot units repeated three times each. The fertilizer treatments consisted of: Mycorrhizae (MYC), chemical fertilizer (NPK); Chicken droppings (CD) and control (T) that received no application. Each site had a total of 60 plants per plot unit. That is 160 plants per block and 480 plants per site. A total of approximately 960 potato plants were used to set up the trial at the two sites. Each plot was 5m long and 5m wide. The plots were 1m long and 2m wide. Each site had an area of 525 m^2, 25 m long and 21m wide.

Seeding and fertilizer application

The sowing was direct seeding with 80 cm between rows and 25 cm between bunches at a rate of one pre-germinated tuber per bunch. The depth of sowing was about 2-3 cm.

For mycorrhizae (MYC), the method used was coating. Ten (10g) of inoculum was applied with the tubers and per pot at the time of sowing. For the treatment of chicken droppings (CD), 200 g of were taken and applied per bunch at sowing. Twenty (20 g) of NPK was applied per bunch 30 days after sowing. The control treatment (T) did not received any application during the entire study.

Evaluation of the effect of treatments on the development of diseases

Incidence and severity were measured to assess the development of the diseases identified in the treatments. Environmental parameters were quantified by monitoring rainfall at both sites.

Assessment of incidence

Incidence of diseases were evaluated using the following formula: $\boldsymbol{I}(\%) = \frac{\boldsymbol{np}}{\boldsymbol{N}} \times \mathbf{100}$ where, I is incidence; np number of plants showing symptoms per plot ; and N total number of plant in plot

Assessment of severity

The severity of the disease was assessed by estimating the leaf area occupied by the symptoms of the disease using the formula: $\mathbf{Sévérité} = \frac{\sum \boldsymbol{n} \ \mathbf{X} \ \boldsymbol{I}}{\boldsymbol{N}} \times \mathbf{100}$

Where Σ is the sum of the products between the number of diseased plants (a) and the number of plants with the index given in% (b). N is the total number of plants in the plot The severity index was used as an estimation scale: 0=no symptoms; 1/4 of the diseased leaf corresponds to 25%; 2/4=50% of the attacked leaf; 3/4=75% and 4/4=100% of the diseased leaves. The number of diseased leaves per plant was associated with this index.

Quantification of the environment

The evaluation of rainfall provides information on the degree of precipitation that can influence the state of crop planting and the evolution of the disease. The data were collected using two rain gauges installed in the middle of the fields in Mouvou and Gouria. Each rain gauge consisted of a conical tube, hung on a wooden support. Rainfall was measured after each rainfall.

Data Analysis

Data collected were subjected to analysis of variance (ANOVA) and means were separated using the Duncan's multiple range test (5%). SPSS 16.0 software was used to perform the statistical analyses.

III. RESULTS AND DISCUSSION

Diseases identified by site

Table 1 : Diseases identified in the Mouvou and Gouria sites (Ngoh Dooh et al. 2020 in Press)

	MOUVOU	GOURIA
Viral diseases	Virose M	PLRV
	Rust stain	Virose M
		Rust stain
Bacterial diseases		Bacterial wilt
		Common Scab

Impact of fertilizers on the incidence of diseases in the field

Incidence of Virosis M

The analysis carried out revealed a statistically significant difference (P=0.02) between treatments, with the highest incidences obtained with the MYC treatments, 18.91 % and 16.83% respectively in Mouvou and Gouria. But, in Gouria incidence was 18.91 %. In control, incidence was higher in Mouvou,15 %, than in Gouria, 5 % (Figure 1).

Impact of treatments on the incidence of Rust Stain

The analysis performed showed that the treatments significantly (P=0.013) influenced the incidence of the disease in both sites. The highest incidence was obtained in Gouria with the MYC treatment, 10.44 % in contrast to the CD treatment. In contrast, in Mouvou, the highest incidence was obtained with the NPK treatment, 8.88 % while in CD treatment incidence was low 2.22 % (Figure 1).

Impact of treatments on the incidence of PLRV

The incidence was highest with the NPK treatment 5.55 % than in control 3 %. On the contrary, in MYC and NPK treatments the disease was absent in Gouria. (Figure 1). This disease was absent in Mouvou site.

Impact of treatments on the incidence of bacterial wilt

The treatments applied significantly influenced (P= 0.03) the average incidence of the disease in Gouria. The highest incidence was obtained with the NPK treatment, 5.55 %. In control, incidence was about 2.3 %. In MYC and CD treatment in Gourai as well as all treatments in Mouvou, disease was absent (Figure 1).

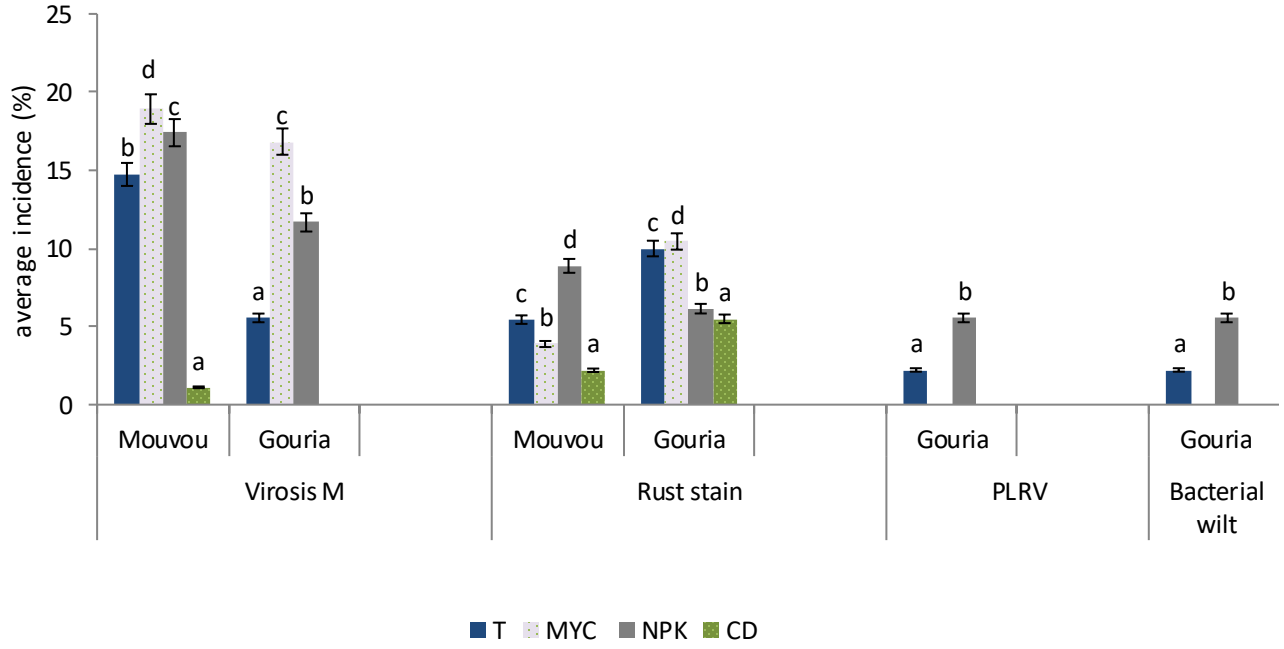

Fig.1: disease incidences at both sites.

T= control; MYC= mycorrhizae; NPK= chemical fertilizers; CD = chicken dropping
Values followed by the same letter in the same site are not significantly different at the 5% threshold according to Duncan's test.

Impact of treatments on severity of diseases in the field

Impact of treatments on the severity of Virosis M

The analysis performed showed a statistically significant difference (P=0.003) between the different treatments applied. This analysis showed that, in Mouvou, the highest severity was obtained with the control treatment and NPK, 27.94±5.08 and 27,45±5,01 % respectively, while in CD treatment it was 11.08±2.14%. In the Gouria site, the MYC treatment obtained the highest severity, 10.97±3.03 %. Disease was absent in CD treatment (Table 2).

Impact of treatments on the severity of Rust Stain

Statistical analyses show significant difference between treatments (P < 0.05 %) in the two sites. Highest severities, 21.11±10.58 % and 20.65±5.15 % were obtained with the

control and MYC treatments respectively in Gouria and Mouvou. In CD treatment in Mouvou, severity was low, 9.67±1.1 % (Table 2).

Impact of treatments on the severity of PLRV

The analysis carried out showed that there is no statistically significant difference (P=0.70) between the different treatments applied to Gouria. It emerged from this analysis that the highest severity was obtained with the NPK treatment, 10.33% in contrast to the MYC and FP treatment where the disease was absent. The control treatment obtained the average severity of 8 % (Table 2).

Effect of treatments on the severity of bacterial wilt

The treatments applied had a significant influence (P=0.03) on the severity of the disease in Gouria. This analysis shows that the highest severities were obtained with the control and NPK treatments, 10.33% and 8.65% respectively. The disease was absent in the MYC and CD treatments (Table 2).

Table 2: severity of different diseases in the two sites.

Sites	Diseases	Treatments			
		T	MYC	NPK	CD
Mouvou	Virosis M	27.94±2.08c	24.89±1.9[b]	27.45±2.01[c]	11.08±2.14[a]
	Rust stain	9.83±1.3[c]	20.65±1.5[d]	14.16±1.01[a]	9.67±2.51[b]
Gouria	Virosis M	8.33±1.15[a]	10.97±3.73[b]	10.71±2.22[b]	*
	Rust stain	21.11±2.58[c]	12.35±1.0[a]	18.33±1,9[b]	18±3.1[b]
	PLRV	8.0[a]	*	10,33[b]	*
	Bacterial wilt	8.65[a]	*	10,33[b]	*

T= control; MYC = mycorrhizae; NPK= chemical fertilizers; CD = chicken droppings.
Values followed by the same letter in the same disease are not significantly different at the 5% threshold according to Duncan's test.
*Means that the disease was absent in the treatment.

Evolution of rainfall in the two study sites

Rainfall was ranged from 0 mm (week 1 of June) to 1620 mm (week 2 of September) at the Mouvou site with a peak of 1620 mm observed in the second week of September and from 0 mm (week 1 of June) to 1800 mm (week 1 of September) with a peak of 1800 mm observed in the first week of September at the Gouria site. Average rainfall was higher at the Gouria site (716.5mm) than in Mouvou site, which received (679 mm) (Figure 2).

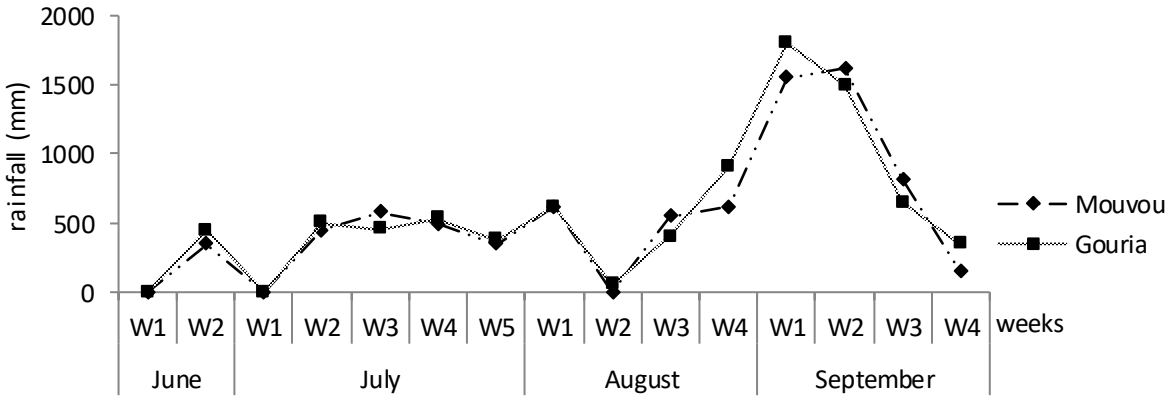

Fig.2: Evolution of rainfall in the two study sites.

IV. DISCUSSION

The present work was based on the evaluation of the impact of bio (mycorrhizae), organic (chicken droppings) and chemical (NPK) fertilizers on the development of viral, bacterial and pest diseases of potato crops in the district of Mogodé (Mayo-Tsanaga) Far North, Cameroon. Results obtained in the field showed that potato production is confronted with viral (Virosis M, Rust Stain and PLRV), bacterial (Bacterial Wilt and common scab), ringworm and pest (lepidopteran larvae) diseases. These attacks occurred after germination, and mainly during the phases of vegetative growth, flowering, fruiting-maturation and harvesting. These results corroborate those of Mulger and Turkensteen, (2005) and Chiunga and Valkonen (2013). According to these authors, the vegetative propagation mode per tuber favours a greater spread of pathogens than by seed multiplication and it is not rare for a tuber to harbour several pathogens.

Numerous viral diseases and pests such as Virosis M and rust stain were respectively observed during the vegetative growth and flowering phase. This can be explained by the fact that the presence of young leaves, fruiting organs and characteristic odours attract many insects that are vectors of viral diseases. N'gbesso et al, (2013), (Lepoivre (2003) and Chiunga and Valkonen (2013) have also showed that the flowers of many plants attract pollinating insects that are considered as vector agents of viral diseases.

The almost homogeneous distribution of viral diseases under all treatments in the two sites is explained by the fact that both sites are located in the same agro-ecological zone (Sudano-Sahelian zone), and therefore, there is the presence of similar vector agents (insects) responsible for the transmission of viral diseases disseminated in the zone. This result corroborates that of Traoré (1997) who has found that in the sahelian zone, insects are more abundant and therefore responsible for the transmission of many viral diseases. Ngoko, (1994) in his work, highlighted the presence of grey spot (viral disease) in all the agro-ecological zones of Cameroon.

The treatments applied influenced the development of the diseases identified in the two study sites. The incidence and severity of the viral diseases were low in the CD and MYC treatments in the control. In the CD treatment, the low rate of M virus and rust stain was explained by the effectiveness of the chicken droppings which provided more mineral elements (e.g. potassium) that improved plant resistance to pathogens and reduced both soil and leaf pathogens. These results are in agreement with those of Hachicha et al. (1992), Compaoré et al. (2010) who have shown that, many minerals like phosphorus and potassium can enhanced resistance of plant in field

On contrary, higher incidence obtained in NPK can be explain by solidity or well being of plant which attracked insects, vectors for pollinisation and viral diseases (Issa et al. 2017). In fact, a potash deficiency will have a direct impact on the maintenance of cell turgidity and thus the regulation of water in the plant. Potash will also be essential for the quality of tuberization. Finally, it will enable the plant to increase its natural resistance, particularly against frost, disease and drought (Reid et al., 2004). In the other hand, under- or over-fertilization of nitrogen is detrimental to crop productivity. In underdose, it does not allow the plant to have an optimal growth. In over-dose it will favour an over-abundant foliage which will be favourable to the development of diseases and will delay maturity and harvest. Nitrogen remains nevertheless essential to ensure good growth (Son et al., 2018).

V. CONCLUSION

The incidence and severity of diseases were almost 0 % in the CD treatment as compared to the control, NPK and MYC treatments. The CD treatment proved to be the most beneficial treatment in terms of potato phytosanitary protection.

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The dinamics of water quality on tiger shrimp (*Penaeus monodon*) cultivation using probiotic in semi intensive pond

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Abstract— *Tiger shrimp (Penaeus monodon) is one of the aquaculture commodities which is still be Indonesia leading export commodity. However, since the outbreak of white spot syndrome virus (WSSV), tiger shrimp cultivation began to leave by farmers and switch to vannameiwhich is more profitable and more resistant to disease. This condition should be avoided considering tiger shrimp are native shrimp of Indonesia whose market demand is still quite high. Therefore, some efforts to improve tiger shrimp cultivation is necessary. One of the efforts to improve tiger shrimp cultivation is by improving culture medium in pond using RICA probiotics. The study was conducted on experimental pond installation in Takalar regency. The treatments were: A) liquid RICA probiotics, B) powdered RICA probiotics. Data of water quality i.e: phosphate, nitrate, nitrite, organic matter, ammonia, TSS,alkalinity and temperature, salinity, DO, pH were analyzed descriptively. The results of the study showed that the use of liquid RICA probiotics had average of phosphate 0.3531 mg/L, nitrate 0.2164 mg/L, nitrite 0.0090 mg/L and pH 8.11 whereas by using powdered RICA probiotics the average phosphate was 0.1851 mg/L, nitrate 0.0573 mg/L, nitrite 0.0069 mg/L and pH 8.51. Application of liquid and powdered probiotics in semi-intensive tiger shrimp could effectively maintain water quality parameters like NH_3-N, NO_2-N, NO_3-N and total organic matter*

Keywords— ***probiotic, semi intensive, tiger, shrimp, water quality.***

I. INTRODUCTION

Since 2000, probiotics began to be used for overcoming crop failure. Many types of probiotics on the market, one of which functions to improve the pond bottom by decomposing organic material by the bacterium *Bacillus sp* (Moriarty, 1997; Poernomo, 2004). According to Hirota, *et. al*., 1995 in Maeda (1999), the presence of *B. subtillis* in an anaerobic sedimentary layers can cause sulfide concentrations to decrease, therefore the potential redox (*Eh*) increases which indicates an enhancement in the pond sediment condition quality.

The cause of the decline in the pond waters environment quality is the disposal of aquaculture wastewater during operations containing high N and P elements, discharged into the surrounding environment (Boyd, 1999; Horowitz & Horowitz 2000; Montoya & Velasco, 2000). According to Teicher Coddington, *et al*. (1996), states that the waste load increases along with the increasing of shrimp stocking densities and feed conversion ratio (FCR).

Various efforts can be done to increase the shrimp production from the shrimp aquaculture in ponds, and one of them is the fulfillment of disease-resistant superior seeds. A seed production technique, using environmental improvements by using probiotics, needs to be done to get the disease-resistant seeds. A seed production technology through the use of probiotics has been successfully carried out at Tiger Shrimp Hatchery Installation in Barru Regency, however, it is necessary to do enlargement on the pond to see further performance.

Probiotic bacteria are able to accelerate the breakdown of organic waste into minerals that are useful for phytoplankton in a pond so that the regeneration process of nutrients has been applied faster in Indonesia (Poernomo, 2004). Furthermore it is said that probiotics applied in a pond must be able to live in a pond, grow, breed, and work actively in their respective fields as expected (Muliani, *et al*. 2004; Muliani, *et al*. 2006), reported that several international researchers have isolated probiotics from the culture environment and from organisms that have been maintained, then examined the use of these probiotics to improve the cultivation environment (Muliani, *et al*., 2008), to increase survival of pet organisms (Aly, *et al*., 2008; Markidis, *et al*., 2008), to improve digestion of pet organisms (Kumar, *et al*., 2006), to stimulate growth and immune systems of pet organisms (Aly, *et al*., 2008) and to countermeasure shrimp larvae disease (Tjahyadi, *et al*., 1994: Haryanti et al., 2000). Cruz, *et al*. (2012), reported that the use of probiotics in a practical aquaculture system could increase disease resistance, increase the growth of aquatic organisms, and increase the feed efficiency. Matiasi, *et al*. (2000), reported that the use of certain commercial probiotics in Malaysia has the potential to improve water quality, and be able to increase shrimp production from aquaculture ponds. Furthermore Wang, *et al.* (2005), reported that the use of probiotics was able to increase the density of ammonification bacteria, *Bacillus sp*, and Protein Mineralizin Bacteria (PMB) significantly, therefore the concentration of nitrogen and phosphorus decreased, resulting in increased shrimp production. Furthermore Gunarto, *et al*. (2006), states that the use of probiotics can improve the pond environment such as improving the redox potential value of the pond sediment, reducing the concentration of ammonia, the total organic matter (TOM), and suppressing the growth of *Vibrio sp* population in pond water.

The RICA 1, 2, and 3 probiotics are the probiotics produced by the Brackishwater Aquaculture Research and Development Center in Maros Regency, which are isolated from pond, marine, and mangrove leaf sediments. These probiotics have been tested in several ponds in South Sulawesi, even in East Java on traditional plus tiger shrimp cultivation, and the results are proven to increase shrimp survival and production (Atmomarsono, *et al*., 2010: Susianingsih and Atmomarsono, 2014). However, to complete the information about the application of RICA probiotics, a semi-intensive study of tiger shrimp ponds was conducted, comparing the liquid form RICA probiotics with powder probiotics. This research aims to obtain data and information about the effect of using different probiotics on the dynamics of water quality in tiger shrimp aquaculture in ponds.

II. METHODS AND MATERIAL

This research was conducted in the Punaga experimental pond, the Brackish Aquaculture Fisheries Research Institute, in Takalar District, South Sulawesi, using four ponds with an area of 1000 m^2 each. The ponds construction was made of concrete with a drainage system using a central drainage system. Each plot was equipped with a two leaf mill, thus the water quality remained excellent. The water preparation used the main reservoir which functioned as sedimentation of seawater Sterilize the container by using a 40 ppm chlorine solution equally across the surfaces of the ponds, then washed with clean sea water. The water filling in the ponds was carried out in step by step until the depth reached 1 m.. The growth of natural feed was with urea fertilizer at 150 kg/ha, and SP-36 fertilizer at 75 kg/ha. This research used two treatments, namely: A = liquid RICA probiotics, and B = RICA powder probiotics; each of two replications. Application of probiotics at a dose 0.5-1 mg/L per week (Atmomarsono et al., 2014). The applied density was 20 shrimp/m^2. The test animals were black tiger shrimp seeds with PL 20 in size, free from *White Spot Syndrome Virus* (WSSV) based on *Polymerase Chain Reaction* (PCR) observation. During the ongoing maintenance, the shrimp was fed by 3-20% dosage of commercial feed, decreased along with the increasing of the shrimp biomass. The feeding frequency was 2-4 times a day evenly. The water replacement began after entering the 30^{th} day as much as 5-10%. The maintenance time was estimated at 120 days. The observed water quality was Total Organic Matter, ammonia, alkalinity, nitrite, nitrate, phosphate, and Total Suspended Solid. The samples were taken as much as 500 mL in each plot, analyzed in the laboratory. Specifically for temperature, salinity, pH, and oxygen were measured *in situ* on the ponds. The water quality data during the research were analyzed and discussed descriptively.

Table 1. Parameters and tools used for water quality analysis

Variable	Unit	Tools//Methods	Description
Temperature	^oC	DO meter	In situ
Dissolved Oxygen	mg/L	DO meter	In situ
pH		pH meter	In situ
Salinity	ppt	Handrefractometer	In situ
Ammonia (NH_3)	mg/L	Spektrofotometer/SNI	Laboratory
Nitrate ($NO3$)	mg/L	Spektrofotometer/SNI	Laboratory
Nitrite ($NO2$)	mg/L	Spektrofotometer/SNI	Laboratory
Phosfate (PO_4)	mg/L	Spektrofotometer/SNI	Laboratory
Total Organic Matter	mg/L	Gravimetri/ SNI	Laboratory
Alkalinity	mg/L	Gravimetri/ SNI	Laboratory

III. RESULTS AND DISCUSSION

Water quality is very influential on the growth and survival rate of shrimp (Fast & Lester, 1992; Prihutomo, 2013). In addition, quality parameter influences the life cycle of organisms, and are a limiting factor for the spread of species (Rochmady, 2011; Susiana, *et al*., 2014). In environmental parameter, water quality plays an important role in the metabolic processes of aquatic organisms (Susiana, 2011, 2015; Rochmady, *et al*., 2016).

Table 2. Range of water quality on tiger shrimp maintenance during the experiment

Variable	Treatment	Observation time	Range value	Standard deviation	Optimal Range/ References
Temperature (^oC)	A	Morning	27.00-31.77	0.00-0.20	26.6-30.0 (Atmomarsono *et al*., 2003)
	B		27.05-31.83	0.21-0.23	
	A	Afternoon	26.90-31.85	0.07-0.14	
	B		26.50-31.05	0.21-0.71	
pH	A	Morning	8.05-9.61	0.05-0.06	7.5-9.0 (Tharavathy,2014)
	B		7.96-9.45	0.17-0.47	
	A	Afternoon	6.12-8.99	0.25-1.06	
	B		6.44-9.00	0.08-0.18	
Dissolved Oxygen (mg/L)	A	Morning	3.35-5.47	0.18-0.38	4-7 Mangampa *et al* (2003)
	B		3.58-5.47	0.15-0.29	
	A	Afternoon	3.98-7.80	0.18-0.51	
	B		4.12-7.33	0.76-1.00	
Salinity (ppt)	A	Morning	28.64-36.68	0.11-2.53	10-35 Murdjani (2007)
	B		28.25-36.26	0.11-2.18	
	A	Afternoon	28.74-36.84	0.05-2.14	
	B		28.26-37.29	0.26-2.16	

Temperature is an energy or a limiting variable which has an important role for life growth, and accelerates the metabolic processes of aquatic animal organisms. In Table 2, the water temperature observation results in the morning range from 27.00 to 31.77°C in Treatment A, and 27.05 to 31.83°C in Treatment B; whereas in the afternoon it ranges from 26.90 to 31.85°C in Treatment A, and 26.50 to 31.05°C in Treatment B. The water quality temperature in the morning is higher than in the afternoon. The optimum water temperature for the fish and shrimp aquaculture in ponds is 28 to 32°C (Effendi, 2003). According to Chanratchakool, *et al.* (1995), water temperature influences the shrimp feeding response when the temperature is higher than 32°C, and the shrimp appetite will decrease by 30% to 50% if the water temperature is lower than 25°C; the optimal temperature for the shrimp growth ranges from 26 to 39°C (Atmomarsono, 2003). The metabolic rate from several aquaculture cultivation research is strongly influenced by water temperature factor, apart from the activity, size, and age of the species (Suther and Rissik, 2008). The optimal temperature range for shrimp growth is 25 to 32°C (Fast & Lester, 1992; Sutanti, 2009).

Oxygen plays an important role as a water quality indicator, since dissolved oxygen plays a role in the oxidation and reduction of organic and inorganic materials. In Table 2, the lowest oxygen measurement result in Treatment A ranges from 2.98 to 7.80 mg/L, however, in Treatment B it is higher, which ranges from 4.12 to 7.33 mg/L in the afternoon, and in the morning ranges from 3.35 to 5.47 mg/L in Treatment A, and in Treatment B ranges from 3.58 to 5.47 mg/L. The value of the oxygen range in the administration of liquid probiotics in the afternoon decreases, ranging from 2.98 - 7.80 mg/L, however, in Treatment B it arises, ranging from 4.12 to 7.33 mg/L. According to Pushparajan and Soundarapandian (2010), the minimum dissolved oxygen value is 3.9 mg/L, and the maximum value is 4.9 mg/L during shrimp rearing in a pond. Shailender, *et al.* (2010), reported that the minimum dissolved oxygen value is at 4.5 mg/L, and the maximum value is at 5.5 mg/L in tiger shrimp maintenance, plus for 140 days can support the growth of tiger shrimp to reach 40.2 g/shrimp.

The salinity during this research in each treatment was almost the same, which ranged from 28.64 to 36.66% in Treatment A, and 28.25 to 36.26 ppt in Treatment B in the morning, while in the afternoon was 28.74 to 36.84 ppt in Treatment A, and 28.26 to 37.29 ppt in Treatment B. For tiger shrimp aquaculture, the salinity ranges from 10 to 30 ppt (Chanratchakool, *et al.*, 1995), and 10 to 35 ppt for an intensive maintenance (Murdjadi, *et al*., 2007). In this research, the salinity was quite high. At high salinity, a lot of energy transformation is needed for the osmoregulation process rather than for flesh formation, thus it can affect the shrimp growth.

pH level of pond water during the study ranged from 8.05 to 9.61 in Treatment A, and 7.96 to 9.45 in Treatment B in the morning. and in the afternoon from 6.12 to 8.99 in Treatment A, and 6.44 to 9.00 in Treatment B. To increase the shrimp growth, a pH range of 6.8 to 8.7 is needed (Suwoyo and Sahabuddin, 2017). The water with a pH of 7.5 to 9.0 is considered a suitable value for shrimp production, a pH below 5.0 can inhibit the shrimp growth. Usually a low pH is followed by a high content of accumulated organic matter, and no perfect oxidation occurs (Anonymous, 1985). Low pH can result in the shrimp reduced appetite, unstable alkalinity, and easy stress. This can be anticipated by applying lime, both during the pond preparation period, and during cultivation. The pH value is very determining the suitability of a marine environment for shrimp (Ratnawati, 2008; Nengsih, 2015).

The phosphate content in Treatment A on the second observation was higher than Treatment B (Figure 1A). In Treatment A, the phosphate content increased to 0.8516 mg/L, whereas in Treatment B it decreased to 0.1621 mg/L. The phosphate content in the Treatment A and B in the third observation decreased until the end of the research. In a shrimp aquaculture activity, the suitable phosphate content ranges from 0.05 to 0.5 mg/L (Kasnir, *et al*., 2014). Phosphate is a form of phosphorus that can be utilized by high and low level plants (microalgae), thus affecting the level of aquatic productivity.

During the observation, the nitrite content (Figure 1B) fluctuated and tended to increase even though it was very small. The nitrite content in Treatment A and Treatment B in the first and fourth observation was smaller than 0.001 mg/L. Adiwijaya, *et al.* (2003), that the optimal range of nitrite for aquaculture is 0.01 to 0.05 mg/L. For shrimp aquaculture, nitrite content is < 0.25 mg/L (Kasnir, *et al*., 2014). Nitrites are formed in the process of nitrification, that is the oxidation of ammonia becomes nitrite by the bacterium Nitrosomonas (Wetzel, 1983). The detection result of water nitrite content in ponds must be less than 0.01 mg/L (Puryaningsih, 2003).

The nitrate content had a different pattern in Treatment A and Treatment B (Figure 1C). Treatment A in the third observation was much higher than Treatment B. Treatment A was 0.5427 mg/L, and Treatment B was 0.0901 mg/L. This is as a result of the performance of the bacterium *Bacillus sp* in A to convert NO_2 – N water into

NO_3 – N. Madigan, *et al.* (1997), in Mustafa and Mangampa (1990), stated that the bacterium *Bacillus sp* is one type of bacteria that can convert NO_2 – N to NO_3 – N. According to Clifford, (1994) that the optimal nitrate content for shrimp aquaculture ranges from 0.4 to 0.8 mg/L.

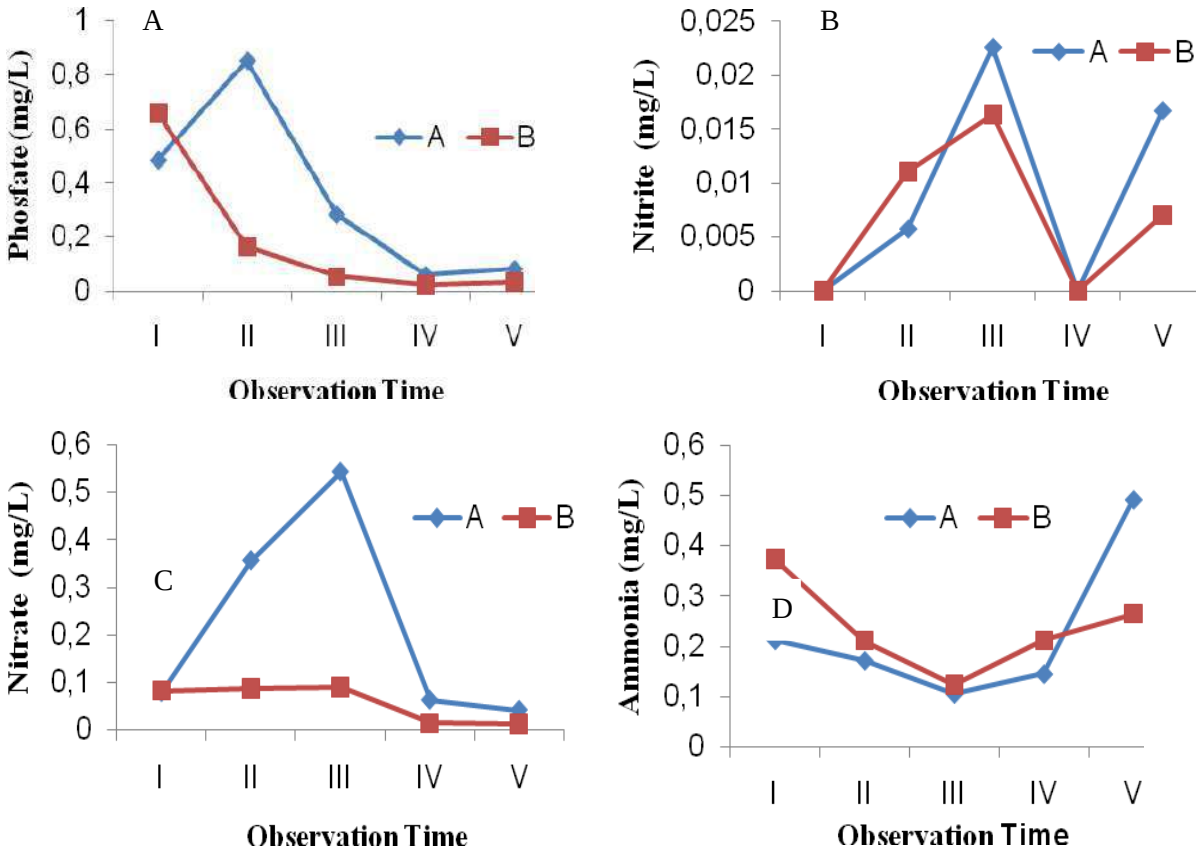

Fig.1: Fluctuation of Phosphate (A), nitrite (B), nitrate (C) and ammonia (D) in pond water during tiger shrimp (P.monodon) cultivation

The phosphate content in Treatment A on the second observation was higher than Treatment B (Figure 1.A). In Treatment A, the phosphate content increased to 0.8516 mg/L, whereas in Treatment B it decreased to 0.1621 mg/L. The phosphate content in the Treatment A and B in the third observation decreased until the end of the research. In a shrimp aquaculture activity, the suitable phosphate content ranges from 0.05 to 0.5 mg/L (Kasnir, *et al.*, 2014). Phosphate is a form of phosphorus that can be utilized by high and low level plants (microalgae), thus affecting the level of aquatic productivity.

During the observation, the nitrite content (Figure 1.B) fluctuated and tended to increase even though it was very small. The nitrite content in Treatment A and Treatment B in the first and fourth observation was smaller than 0.001 mg/L. Adiwijaya, *et al.* (2003), that the optimal range of nitrite for aquaculture is 0.01 to 0.05 mg/L. For shrimp aquaculture, nitrite content is < 0.25 mg/L (Kasnir, *et al.*, 2014). Nitrites are formed in the process of nitrification, that is the oxidation of ammonia becomes nitrite by the bacterium *Nitrosomonas* (Wetzel, 1983). The detection result of water nitrite content in ponds must be less than 0.01 mg/L (Puryaningsih, 2003). This shows that application of probiotics is quite effective in reducing nitrite levels. This shows that application of probiotics is quite effective in reducing nitrite levels. Both forms of probiotics could effectively maintain water quality parameters like total organic matter, NH_3-N, NO_2-N, and TBV/TPC ratio (Atmomarsono, 2020)

The nitrate content had a different pattern in Treatment A and Treatment B (Figure 1.C). Treatment A in the third observation was much higher than Treatment B. Treatment A was 0.5427 mg/L, and Treatment B was 0.0901 mg/L. This is as a result of the performance of the bacterium *Bacillus sp* in A to convert NO_2 – N water into NO_3 – N. Madigan, *et al.* (1997), in Mustafa and

Mangampa (1990), stated that the bacterium *Bacillus sp* is one type of bacteria that can convert $NO_2 - N$ to $NO_3 - N$. According to Clifford, (1994) that the optimal nitrate content for shrimp aquaculture ranges from 0.4 to 0.8 mg/L.

The ammonia content (Figure 1.D) in both treatments, from the initial shrimp rearing until the third observation, decreased from 0.3740 mg/L to 0.1241 mg/L in Treatment B, and decreased from 0.2129 mg/L to 0.1061 mg/L in Treatment A; after the fifth observation, the ammonia content rose to 0.4920 mg/L. Kumar, *et al*. (2016), that the value of ammonia nitrogen content in shrimp rearing, using probiotics and without probiotics, respectively are 0.32 to 0.71 mg/L and 2.1 to 2.7 mg/L; and further stated to maintain the culture media and stabilize ammonia, it is recommended to use probiotics. Mangampa (2010) states that the direct effect of high ammonia levels, but not deadly, will damage the gill tissue, then the gill sheet will swell (*hyperplasia*), therefore the function of the gills as a means of breathing will be disrupted in terms of the oxygen binding of water. High levels of ammonia can increase the concentration of ammonia in the blood, thereby reducing blood activity (*hemocyanin*) in oxygen binding. In addition, high levels of ammonia can also increase the susceptibility of shrimp to disease.

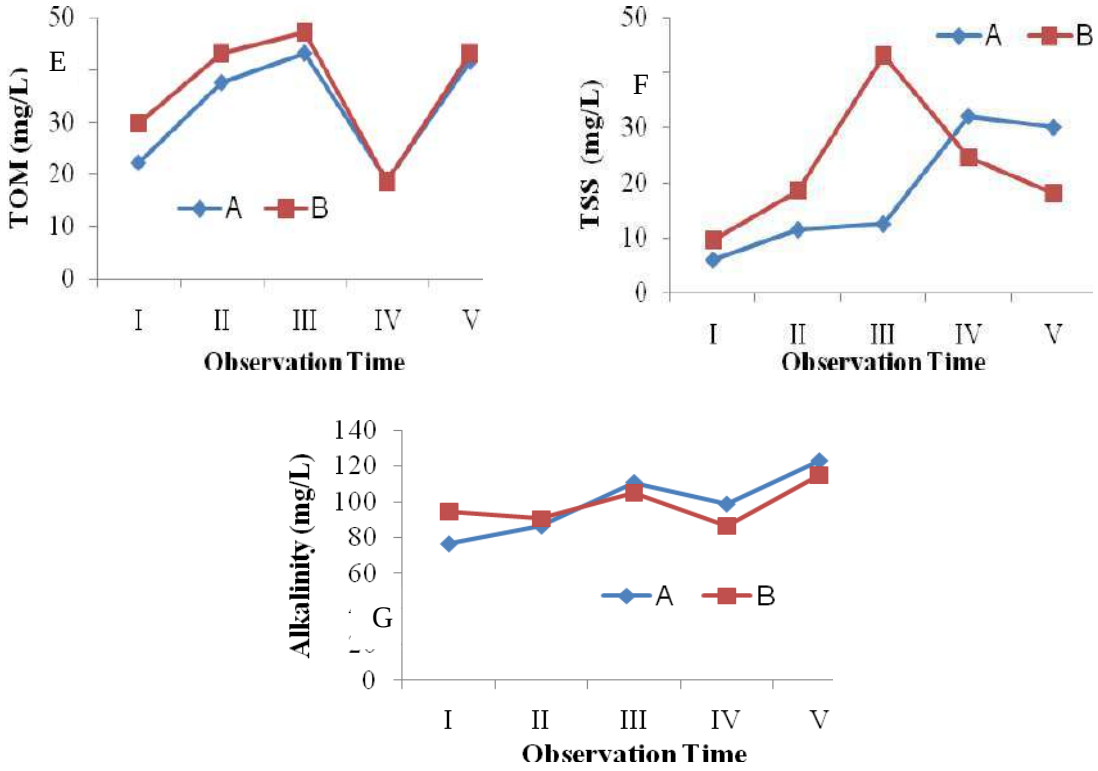

Fig.2: Fluctuation of TOM (E), TSS (F), and Alkalinity (G) in pond water during tiger shrimp (P. monodon) cultivation

The TOM and TSS content increased in both treatments, and decreased after the fifth observation. The average value of TOM in Treatment A was 32.65 mg/L, and treatment B was 36.35 mg/L. Gunarto, *et al*. (2006), states that the use of probiotics can improve the redox potential value of pond sediments, reduce the value of ammonia content, organic matter, and suppress the growth of *Vibrio sp* population in pond water. The TSS content in Treatment A was 18.4 mg/L, and Treatment B was 22.7 mg/L; the high TSS content in shrimp ponds is caused by artificial feed input in the shrimp aquaculture system which is not consumed (leftover), and the metabolism results in the form of feces that are dissolved in the waters. For the shrimp aquaculture that depends entirely on artificial feed (pellets), if the feed is given a lot that is not eaten by shrimp, then it can cause high organic matter (TOM). The average alkalinity obtained was 98.86 mg/L in Treatment A, and in Treatment B it was 36.35 mg/L. Alkalinity is determined by the amount of acid needed to reduce pH. According to Atmomarsono, *et al*. (2013), the value of water alkalinity in ponds is used as pH stabilization and normal growth of phytoplankton.

According to Adiwidjaya, *et al*. (2008), the optimal alkalinity for vannamei shrimp aquaculture activities ranges from 90 to 150 ppm. The alkalinity content of tiger shrimp pond water is recommended > 100 mg/L or in the range of 120 to 160 mg/L.

IV. CONCLUSIONS

Application of liquid and powder formed probiotics in semi-intensive tiger shrimp aquaculture ponds can maintain a better water quality stability.

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Resource use efficiency of mechanized and traditional rice farms in Nepal: A comparative analysis

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Abstract**—** *This paper has compared the resource use efficiency in rice production among mechanized and traditional farmers in Tarai districts (Jhapa, Sunsari and Bardiya) of Nepal in 2018/19. Data were collected from 494 farmers (274 mechanized and 220 traditional) using multistage random sampling. Production function analysis (Cob-Douglas production function) was used to obtain the marginal value productivity of inputs and to examine the resource use efficiency in crop production in mechanized and traditional rice farms. Study showed that machinery cost was highest and significant to the total income from rice in mechanized rice farm, whereas fertilizer cost accounted the highest in traditional rice farm. Increase in human labor was found to decrease the income from crop in mechanized rice farm. Effect of manures, fertilizers and bio and chemical pesticides was positive and significant in both type of farms. The effect of irrigation cost was significant in mechanized farm and it was non-significant for traditional farm. There was decreasing returns to scale in all the farms. Production resources in the study area were found not to be efficiently utilized to optimum economic advantage for both mechanized and traditional rice farmers, respectively. Return to scale was found to be 0.695 and 0.488 for mechanized and traditional rice farm which revealed that inputs used in rice production were ineffectively utilized in which manures, chemical fertilizers, machineries, bio- and chemical pesticides and irrigation resource were under used and human labor, seed and animal labor over used. This situation of underutilization of these resources should be overcome by increasing farmers' access to these inputs and encouraging them to use in higher quantity to realize higher return. Assurance of quality and timely supply of fertilizers, plant protection materials and investment on irrigation infrastructure should be done by government authority to increase in efficiency of resources used by farmers. Moreover, technology packages for adequate and timely application of these inputs should be delivered to the farmers to maximize returns through increased resource use efficiency. Relevant policies should be formulated to encourage the creation of alterative employment opportunities to absorb the excess labor used in rice production. Based on the result obtained it can be concluded that mechanized rice production is more efficient in resource utilization and subsequently more profitable than traditional farming.*

Keywords**— Mechanization, Resource Use Efficiency, Returns to Scale, Efficiency.**

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I. INTRODUCTION

Agriculture sector is considered as an important economic sector to increase income, alleviate poverty and also enhance the living standard of Nepali people (Gauchan and Shrestha, 2017). Agriculture contributes 26.98% of country's GDP (MoALD, 2019) and engages 60.4% of its labor force (NPC, 2020). However, the productivity of agriculture in Nepal is placed the lowest in South Asian countries (FAO, 2019). The indicators, like 21% of population without having access to adequate food and 18.7% of population under absolute poverty line, indicate food insecurity and poverty are major issues of the Nation in the present times.

Rice is placed at the first rank among cereal crops in terms of its area, production and contribution to GDP, AGDP and livelihood of the people in Nepal. (Regmi, 2017). Rice contributes about 20% and 7% to AGDP and GDP, respectively and also supplies about 40% of the food calorie intake in Nepal. (CDD and ASoN, 2017).Currently, from the area of 1.49 million hectares of land, 5.61 million metric tons of rough rice is produced in Nepal. Terai region of the country has highest shares with more than 70% in term of area and production in Nepal (MoALD, 2019). However, the average growth rate in area and production of rice is only about 0.35% and 1%, respectively per year (Regmi, 2017).

Rice is labor intensive crop and thus requires large number of labors during various farm operations (Bhandari *et al*., 2015 and Dhital, 2017). The rice productivity is greatly affected by labor scarcity during crop establishment (Liu et al, 2017). For the successful crop production, the timeliness of farm operations is important and use of improved implements and machineries is more important for undertaking the farm operation in time. In this context, farm mechanization can help address shortage of labor, ease drudgery, enhance productivity and the timeliness of agricultural activities and promote efficiency in resource use (ESCAP, 2018).

The number of of factors of production and their time of applications influence the agricultural production. Farmers adopting new and modern agricultural technology are found to utilize production inputs more efficiently than non-adopters (Idi, 2004). If the resources are utilized efficiently, the output and income of farmers can be increased at the existing technology, and about 28% increase in output of rice is obtained by adoption of the technology and best practices (Ajao, 2005). As Nepal is currently net importer of rice and thus investigation of the status of resource use in rice farms to determine the adjustments needed to increase the output is the indispensable approach at the moment. In order to feed the growing human population, increase in agricultural production by increasing resources use efficiencies through farm mechanization is required. Efficient use of resources and adoption of new technologies are the major emphases to be given to increase agricultural production thereby leading the country to be self-sufficient in rice production. Farm mechanization could be the option for Nepali rice growers to increase the use of machineries in rice cultivation and increase the production by efficient use of resources. For obtaining maximum production from any agricultural commodity, resources must be available and available resources must be used efficiently, and for this purpose, one must have knowledge about whose quantity rate should be increased or decreased (Alimi, 2000). The major factors affecting the technical efficiency of rice production include seed, fertilizer, labor as well as irrigation (Hasnian and Hossain, 2015). For increasing the production of rice, the use of improved farm mechanization and input is the best way (Nargis and Lee, 2013).

Experiences with quantifying the impact of mechanization on agricultural production efficiency in Nepal are very limited. There are no studies till date on comparative analysis of the agricultural production efficiency of rice between mechanized and traditional farms. A study of resource use efficiency on mechanized and traditional rice farms can explain the marginal value productivity of inputs between two categories of farms and justify the investment demands. Thus, the current study examines the resource use efficiency of mechanized and traditional rice farms which would ultimately help Nepali rice growers to compare the efficiencies of resources between two types of rice farms and make appropriate adjustment to existing resources use patterns for increasing the production on time.

II. MATERIALS AND METHODS

Study area: The study was conducted in Jhapa and Sunsari districts of province 1 and Bardiya districts of Lumbini province. The districts were among the most potential districts in rice production in Nepal. The three districts share 12.6% and 14.1% of total national area and production, respectively in Nepal (MoALD, 2019). These districts were also the command areas of Rice Zone and Super Zone units of Prime Minister Agriculture Modernization Project (PMAMP), which is a government owned project being

implemented to facilitate for industrialization of rice sector *via* promotion of mechanization as one of the strategic interventions. Within the selected districts, respondents from one local unit from Jhapa (Kachankawal Rural Municipality), two local units from Sunsari (Duhabi Municipality and Gadi Rural Municipality) and two local units from Bardiya (Rajapur Municipality and Geruwa Rural Municipality) were selected for taking data.

Sampling design: Multistage random sampling technique was adopted for the selection of study area and sample respondents for collection of information required for the study. The rice growing farms were divided into two categories, i.e. Mechanized and Traditional rice farms. Mechanized farm referred to the rice farm that uses at least one or more of agricultural machines for at least one or more farm operation in tillage, transplanting, intercultural operation, harvesting and threshing. The rice growers of the selected rural municipalities and municipalities were considered to be in sampling frame. The data were collected through structured and semi-structured questionnaires. Based on the population size, the sample size of the study was 494, which was constituted of 274 from mechanized rice farms and 220 respondents from traditional (Table 1). The focused group discussion, key informants interview and stakeholders analysis were performed during study. The sample size was determined using the following formula (Daniel and Cross, 2013) and was also verified by using Raosoft software.

Table 1: Sampling frame

District	Population size		Sample size	
	Mechanized	Traditional	Mechanized	Traditional
Jhapa	1895	334	91	75
Sunsari	1760	240	91	69
Bardiya	2007	354	92	76
Total	5662	928	274	220
Total Sample size: 494				

The sample size was determined using the following formula:

$$n = \left[\frac{[N\, z^2\, p\, (1-p)]}{[(N-1)d^2 + z^2\, p(1-p)]}\right]$$

(Daniel and Cross, 2013)

Where:

n = Sample size

N = Total population size/household

p = Estimated proportion of population included

d = Error limit (10%)

The field survey was conducted during 2018/19

Analytical methods: The production function approach was used to find out the productivity of resources used in rice cultivation. For this purpose, Cobb Douglas production function model as described by Gujarati (2009) was adopted to estimate the resource use efficiency of various inputs at different farm categories, i.e. mechanized and traditional rice farms. The present study has compared the marginal value products of the inputs with their respective per unit acquisition cost to arrive at the use efficiency of such inputs. If the marginal value product of certain resources was lesser or greater than its acquisition cost, the input was considered to be over-utilized or under-utilized, respectively. To determine the contribution of different inputs as well as for the estimation of the efficiency of variable production inputs in rice production system in mechanized and traditional rice farms, the general form of Cobb-Douglas production function used was as follow:

$$Y = a\, X_1^{b_1}\, X_2^{b_2} X_3^{b_3} X_4^{b_4} X_5^{b_5} X_6^{b_6} X_7^{b_7} X_8^{b_8} X_8$$

The dependent and explanatory variables were transformed to natural logarithm.

$$\ln Y = \ln a + b_1 \ln X_1 + b_2 \ln X_2 + b_3 \ln X_3 + b_4 \ln X_4 + b_5 \ln X_5 + b_6 \ln X_6 + b_7 \ln X_7 + b_8 \ln X_8 + vi + ui$$

Where,

Ln = Natural logarithm

Y = Total income from rice cultivation (NRs./ha)

X_1 = Seed cost (NRs./ha)

X_2 = Manures/compost cost (NRs/ha)

X_3 = Fertilizer cost (NRs./ha)

X_4 = Chemical/bio pesticides cost (NRs/ha)

X_5 = Irrigation cost (NRs/ha)

X_6 = Machine hours cost (NRs/ha)

X_7 = Human labor cost (NRs./ha)

X_8 = Animal power cost (NRs./ha)

Vi = Error term measuring errors not under the control of farmers

Ui = Error term measuring errors under the control of farmers.

$b_1, b_2 ... b_8$ = Coefficients to be estimated

The resource use efficiency ratio (r), absolute value of percentage change in MVP (D) and return to scale (RTS) was estimated by using the following formula, as coined by Sapkota *et al*. (2018). Enlist this literature in Reference list.

r = MVP/MFC

Where, r= efficiency ratio, MVP = Marginal Value Product of variable input, MFC= Marginal Factor Cost

The Marginal Value Product was estimated using the following formula:

$$MVPi = bi\frac{y}{xi}$$

Where, b_i = estimated regression coefficients, y = Geometric mean of total income from rice cultivation, x_i= Geometric mean of i^{th} input.

Decision criteria:

If, r = 1 indicates the efficient use of the resource

If, r> 1 indicates underuse of the resource

If, r< 1 indicates overuse of the resource

Similarly, the absolute value of percentage change in MVP of each resource was estimated as

$$D = \left(1 - \frac{MFC}{MVP}\right) * 100$$

or

$$D = \left(1 - \frac{1}{r}\right) * 100$$

Where, D= Absolute value of percentage change in MVP of each resource.

The returns to scale (RTS) was calculated as follows:

RTS =Σ bi, Sum of bi from the cobb-Douglas production function gives the value of return to scale.

Where, bi = coefficient of i^{th} explanatory variables obtained from OLS regression of Cobb-Douglas production function

Decision rule:

If, RTS < 1: Decreasing return to scale

If, RTS = 1: Constant return to scale

If, RTS > 1: Increasing return to scale

Note: 1 USD =117.50 NRs. (As of 24th October,2020)

III. RESULTS AND DISCUSSION

Land holding status in study sites

The study showed that the 45.5% of respondents under traditional rice farm categories had land holding of less than 0.66 ha (20 *kattha*) whereas 30.3% of respondents under mechanized rice farms had land holding of less than 0.66 ha. The land holding size of more than 1.33 ha was higher in mechanized farm (21.5%) as compared to traditional rice farm category (17.2%). The average land holding size of respondents in both type of farm was higher in Bardiya. The difference in average land holding in mechanized and traditional rice farms in all districts under study were significantly different at 5% and 1% level of significance. The details of land holding status in study areas is presented in the Table below:

Table 2: Land holding range

Land holding	Traditional	Mechanized	Total
< 0.66 ha	100 (45.5)	83 (30.3)	183 (37.0)
0.66-1.33 ha	82 (37.3)	132 (48.2)	214 (43.3)
>1.33 ha	38(17.2)	59 (21.5)	97(19.7)
Total	220 (100)	274 (100)	494 (100)

Figures in parenthesis indicate percentage

The average land holding was 0.83 ha/HH and 1.06 ha/HH in traditional and mechanized rice farm. The average rice cropped area was 0.77 and 1.02 ha/HH for traditional and mechanized rice farms. The study indicated that mean difference in rice area/HH between traditional and mechanized rice farms was significant at 5% level of significance. The mean differences in rice areas in both type of farm categories in all three districts was also significant at 5% and 1% level of significance as shown in the Table. The respondents from mechanized rice farm category in Bardiya district had highest rice area/HH (1.16 ha/HH) whereas in respondents from traditional rice farms of Jhapa district had highest land area under rice crop (0.78ha/HH) as shown in the Table.

Table 3: Land holding status

Districts	Average land holding (ha/HH)			Average rice area (ha/HH)			t-test	
							Mean difference (rice area)	t-value
	Overall	TF	MF	Overall	TF	MF		
Jhapa	0.92	0.82	1.02	0.88	0.78	0.98	-0.20*	-2.514
Sunsari	0.87	0.77	0.97	0.83	0.75	0.91	-0.16*	-2.413
Bardiya	1.045	0.89	1.20	0.965	0.77	1.16	-0.39**	-2.566
Total	**0.95**	**0.83**	**1.06**	**0.89**	**0.77**	**1.02**	**-0.25***	**-2.443**

TF = Traditional rice farm, MF=Mechanized rice farm

Adoption of variety

The majority of respondents in both the mechanized and traditional rice farms were found to adopt improved varieties. Percentage of the respondents in mechanized and traditional rice farm adopting the improved varieties of rice were 70.90% and 63.64% respectively. The adoption of hybrid varieties was higher in mechanized rice farm (18.25%) in comparison to traditional rice farm (12.23%). The average coverage of improved varieties to total rice area in mechanized and traditional rice farms were 69.14% and 70.61% respectively. This indicates about 70% area coverage by improved varieties in both the mechanized and traditional farm. The area covered by hybrid varieties in mechanized rice farm was 19.79 % of the total rice area cropped whereas it was 12.23% in traditional rice farm. The adoption of local varieties were higher in traditional rice farm (25.0%) as compared to mechanized rice farm (9.85%). The majority of varieties adopted in both type of farms were old varieties like Makawanpur-1 (Bardiya), Radha-4 (Jhapa, Sunsari, Bardiya), Ram Dhan (Jhapa, Sunsari), Sabitri (Jhapa, Sunsari Bardiya), Hardinath-1 (Jhapa, Sunsari, Bardiya), Sarju-52 (Bardiya), Ranjit (Jhapa and Sunsari), Sona Masuli (Sunsari), Sawa Masuli (Jhapa and Sunsari). However, in few cases new and climate resilient varieties like Cheharang sub-1(Bardiya), Sworna Sub-1(Jhapa, Sunsari, Bardiya), Sambha Masuli Sub-1(Jhapa, Sunsari), Bahuguni-1and Bahuguni-2 (Jhapa, Sunsari) etc were found to be adopted by respondent farmers. Hybrid varieties adopted were mainly registered and imported from India. Gorakhnath-509 (Bardiya, Sunsari), Garima (Jhapa,

Sunsari), US-312 (Sunsari) were popular hybrid varieties adopted by the farmers in both mechanized and traditional rice farms. Lalka basmati (Jhapa, Sunsari), *Kariya kamad* (Jhapa, Sunsari), Tilki (Bardiya), *Anadi* (Bardiya) etc varieties were among the local varieties being adopted by the respondents in study sites. The area coverage under local varieties of rice in mechanized and traditional rice farm was 11.04 % and 17.14 % respectively. More specifically, the highest percentage of respondent farmers from mechanized farm category of Bardiya district (73.91%) had adopted improved rice varieties followed by Sunsari (71.73%) and Jhapa (70.33%). Similarly, highest percentage of respondents in Sunsari (21.98%) were adopting hybrid rice varieties followed by Jhapa (16.48%) and Bardiya (16.30%) in mechanized farm category. The result was similar for traditional rice farm with regard to adoption of hybrid rice varieties in traditional rice farm with highest percentage of household adopting hybrid varieties in Sunsari (13.04%) followed by Jhapa (10.67%) and Bardiya (10.53%).

Table 4: Adoption of rice varieties in study sites

District	Farm Category	Descriptions	Improved	Hybrid	Local	Total
Jhapa (N=166)	Mechanized (N=91)	Household No	64 (70.33)	15 (16.48)	12 (13.19)	91 (100)
		Area.	60.17 (67.33)	18.30 (20.48)	10.90 (12.20)	89.37 (100)
	Traditional (N=75)	Household No.	50 (66.67)	8 (10.67)	17 (22.67)	75 (100)
		Area	39.51 (67.42)	7.66 (13.07)	11.43 (19.51)	58.60 (100)
Sunsari (N=160)	Mechanized (N=91)	Household No.	65 (71.43)	20 (21.98)	6 (6.59)	91 (100)
		Area	53.14 (64.17)	24.21 (29.24)	5.46 (6.59)	82.81 (100)
	Traditional (N=69)	Household No.	41 (59.42)	9 (13.04)	19 (27.54)	69 (100)
		Area	36.54 (70.94)	7.42 (14.40)	7.55 (14.66)	51.51 (100)
Bardiya (N=168)	Mechanized (N=92)	Household No.	68 (73.91)	15 (16.30)	9 (9.78)	92 (100)
		Area	79.76 (74.50)	12.75 (11.91)	14.55 (13.59)	107.06 (100)
	Traditional (N=76)	Household No.	49 (64.47)	8 (10.53)	19 (25.0)	76 (100)
		Area	49.50 (73.16)	6.67 (9.86)	11.49 (16.98)	67.66 (100)
Total (N=494)	Mechanized (N=274)	Household No.	197 (71.90)	50 (18.25)	27 (9.85)	274 (100)
		Area	193.07 (69.14)	55.26 (19.79)	30.91 (11.07)	279.24 (100)

District	Farm Category	Descriptions	Improved	Hybrid	Local	Total
	Traditional (N=220)	Household No.	140 (63.64)	25 (11.36)	55 (25.00)	220 (100)
		Area	125.55 (70.62)	21.75 (21.23)	30.47 (17.14)	177.77 (100)

Figures in parenthesis indicate percentage

labor use status in mechanized and traditional rice farms

The study showed the significant differences in status of labor use in performing various farm operations except irrigation in mechanized and traditional rice farms. The labor use in mechanized rice farm was significantly lower than in traditional rice farm at 5% and 1% level of significance. The mean differences was maximum in labor use for land preparation followed by threshing, winnowing and storage of the rice grain. This indicated that the use of farm machines for rice cultivation would significantly displace the human labor thereby removing drudgery and addressing the labor shortage issues while doing various farm operation in rice cultivation. Similarly, there was significant difference in use of bullock labor in mechanized and traditional rice farm. The mechanized rice farm was using lower number of bullock power (5.0 days) than traditional rice farm (22.6 days) and the difference was significant at 1% level of significance. This also clearly indicated that the mechanized rice farm displaces the bullock use for rice cultivation.

Table 5: Mean differences in labor use status in mechanized and traditional rice farms

Description	Pooled (N=494)		Mechanized (N=274)		Traditional (N=220)		Mean difference	t- value
	Mean	Std Dev	Mean	Std Dev	Mean	Std. Dev		
Land preparation	15.00	13.59	3.0	0.72	27.0	9.76	-24.0**	-40.497
Fertilizers and compost transportation	5.50	3.16	3.1	1.63	7.9	2.56	-4.8**	-25.036
Nursery Bed Preparation	4.20	3.29	1.2	.55	7.2	2.10	-6.0**	-44.718
Puddling of field for transplantation	7.30	4.33	3.8	1.17	10.8	3.63	-7.0**	-30.046
Transplanting	25.2	8.42	22.2	9.84	28.2	4.22	-6.0**	-8.635
weed control	19.7	7.97	18.6	10.38	20.8	2.52	-2.2*	-2.994
Irrigation	1.60	.77	1.6	.78	1.6	.76	0.0	0.198
Plant Protection	1.20	.62	1.0	.21	1.4	.85	-0.4**	-7.470
Harvesting, bundling	17.1	8.50	12.5	9.13	21.7	3.49	-9.2**	-13.99
Threshing, Winnowing and storage	10.35	4.98	5.7	2.27	15.0	1.22	-9.3**	-54.571
Total	107.2	15.05	72.7	20.07	141.6	12.27	-68.9**	-44.58
Bullock labor used	13.8	3.11	5.0	2.05	22.6	3.01	-17.3**	-77.389

Description	Pooled (N=494)		Mechanized (N=274)		Traditional (N=220)		Mean difference	t- value
	Mean	Std Dev	Mean	Std Dev	Mean	Std. Dev		
Machine labor used	7.0	14.0	14	6.44	00	00		

∗ and ∗∗ indicate significant at 5% and 1% level of significance

Estimation of elasticity, MVP, returns to scale and efficiency ratio in study areas

Overall pool results including farms from all three sampled districts showed positive and significant impact of manures, fertilizers and plant protection measures to return. Table 6 presents estimation of elasticity in mechanized and traditional rice farms in study areas. The impacts of irrigation and machines were significant and positive, seed was positive but non- significant to the return in case of mechanized farms. This indicated that additional use of seed would not be adding more output. This could be because farmers were using seed rate for rice cultivation more than recommended, as a result of which additional use of seed would not necessarily increase the output. In mechanized farms, 1% increase in machine cost would increase the income by 0.379%. Fertilizers cost was second to machine cost to contribute in total income in mechanized rice farms. Human labor showed negative and significant impact and animal power showed negative, but non-significant impact to the return in case of mechanized farms. That means additional supply of labor in rice production system would decrease the income from the rice crop. Seed and animal power showed negative impact on return, while the impact on return was negative and significant for human labor in case of traditional farms. In traditional rice farms, fertilizer was found to be having the highest impact on return .

Table 6: Estimation of elasticity in mechanized and traditional rice farms in study areas

Variables	Mechanized				Traditional			
	Coefficients	Std. Error	t-value	Sig.	Coefficients	Std. Error	t-value	Sig.
Log seed cost	0.016	0.020	.219	0.827	0.017	0.018	-1.921	0.056
Log manures	0.152∗	0.012	2.607	0.010	0.154∗∗	0.011	9.727	0.000
Log fertilizers	0.307∗∗	0.035	5.825	0.000	0.247∗∗	0.024	5.839	0.000
Log bio/ chemical pesticides	0.095∗	0.010	2.393	0.017	0.096∗∗	0.009	6.687	0.000
Log irrigation cost	0.066∗	0.015	2.181	0.030	0.134	0.017	1.418	0.157
Log machine cost	0.379∗∗	0.020	6.281	0.000	-0.201∗∗	0.008	-3.588	0.000
Log human labor cost	-0.299∗∗	0.007	-3.550	0.000	0.041	0.012	-.486	0.628
Log animal power cost	-0.021	0.016	-1.277	0.203	4.195	0.140	29.961	0.000
Constant	3.616∗∗	0.212	17.074	0.000	-0.201∗∗	0.008	-3.588	0.000
F-Value	31.423				50.96			
Prob>F	0.000				0.000			
R^2	0.700				0.757			
Adjusted R^2	0.491				0.573			
Returns to scale	0.695				0.488			

*and ** indicate significant at 5% and 1% level of significance

The estimation of MVP, efficiency ratio and returns to scale of mechanized and traditional rice farms in the study areas is presented in Table 7. Seed was over-utilized, human labor and animal power were grossly over utilized in mechanized farms, while for traditional farms, these three inputs were grossly over utilized elucidating need of decreasing cost on these resources for return maximization. Manures, fertilizers, plant protection measures, irrigation and machines were underutilized depicting need of increasing cost on these inputs to maximize return. Percent adjustment required for irrigation and plant protection measures were almost similar in both categories of farms, while for mechanized farms, manures needed to be increased by 80.26% compared to 91.2% in traditional farms. But, for fertilizers, mechanized farms needed 81.41% cost increment compared to 76.20% of traditional farms.

Table 7: Estimation of MVP, efficiency ratio and returns to scale of mechanized and traditional rice farms in study areas

Variables	Mechanized				Traditional			
	MVP	MFC	r	D	MVP	MFC	r	D
Log seed cost	0.733	1	0.733	36.33	0.69	1	0.69	44.86
Log manures	5.067	1	5.067	80.26	11.36	1	11.36	91.20
Log fertilizers	5.379	1	5.379	81.41	4.20	1	4.20	76.20
Log bio/ chemical pesticides	16.209	1	16.209	93.83	12.64	1	12.64	92.09
Log irrigation cost	16.454	1	16.454	93.92	32.31	1	32.31	96.91
Log machine cost	3.836	1	3.836	73.93				
Log human labor cost	-3.348	1	-3.348	129.87	-1.06	1	-1.06	194.63
Log animal power cost	-1.581	1	-1.581	163.25	0.61	1	0.61	63.46

Note: MVP = Marginal Value Productivity, MFC= Marginal Factor Cost, r = Ratio

IV. DISCUSSION

The seed cost had non-significant effect in both types of farms because of use of seed by the farmers more than recommended already. Effect of manures, fertilizers and bio- and chemical pesticides was positive and significant in both types of farms, whereas effect of irrigation to total income was found significant in mechanized and it was not significant in traditional rice farms. The reason for significant positive relation of manures and fertilizers, bio and chemical fertilizers was due to lower use of these resources in both types of farms. Suleiman and Ibrahim (2014) who compared the relative economic efficiency of mechanized and non-mechanize rice Farmers Nigeria also revealed fertilizers was under-utilized and seed was over-utilized for both types of farm i.e. mechanized and non-mechanized. The result from current study was also consistent with Suleiman and Ibrahim (2014) for labor in mechanized farm with the status being over utilized. The finding was similar to the study of Dhakal et al (2019) who showed fertilizers, machinery and pesticides were under used resources in Chitwan district of Nepal. Reja (2013) also computed the resource use efficiency in rice production with special focus on mechanization and found similar results for fertilizers and insecticides with the status being under-utilized in power tiller operated farm and bullock operated farm in Bangladesh.

V. CONCLUSION

The study was conducted to assess the resource use efficiency in mechanized and traditional rice farms from 494 respondents (274 mechanized and 220 traditional rice farms). Survey was conducted in Jhapa, Sunsari and Bardiya district of Nepal. The contribution of machinery to total income in mechanized rice farms was highest and was significant. In traditional farms, it was fertilizer cost that had highest contribution to total income from the rice crop. Increase in human labor was found to decrease the income from crop, which indicated the necessity of replacing additional labor cost by machines. The effect of animal power to total income was not significant. There was decreasing returns to scale in

all the farms. Production resources in the study area were found not to be efficiently utilized to optimum economic advantage for both mechanized and traditional rice farmers. Based on the result obtained, it can be concluded that mechanized rice production is more efficient in resource utilization and subsequently more profitable. Findings from this study revealed that rice producers were technically inefficient in the use of farm resources. Overall resource use efficiency illustrated that basic inputs of production (manures, fertilizers, plant protection measures and machines) were underutilized and seeds, human and animal labor were overused.. This situation of underutilization of these resources should be overcome by increasing farmers' access to these inputs and encouraging them to use in higher amount to realize higher return. Assurance of quality and timely supply of fertilizers, plant protection materials and investment on irrigation infrastructure should be done by government authority to increase resources use by farmers. There is a need to provide training for optimum use and awareness to farmers to reduce overuse. Moreover, technology packages for adequate and timely application of these inputs should be delivered to the farmers to maximize returns through increased resource use efficiency.

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Stability of formula *Bacillus* spp. for control *Ralstonia syzygii* subsp. *Indonesiensis* and increase the growth and the yield of chili plants

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Abstract*—Solid formulations of Rhizobacteria Bacillus. Spp. and storage time to control bacterial wilt disease Ralstonia syzygii subsp. indonesiensis. Bacterial wilt disease caused by Ralstonia syzygii subsp. indonesiensis is a major constraint in chili cultivation. Bacillus spp,is capable to suppres bacterial wilt disease, to increase the growth and the yield of chili plants. The aim of this research was to obtain the best formula of Bacillus spp. which was stable to control bacterial wilt disease and to increase the growth and yield of chili plants. To increased its stability and interaction with chili plants, Bacillus spp, was urged to test furthermore especially its formulation with based on solid organic carriers (rice bran, rice straw, bagasse and their combination). The most effective storage time also need to test. Result showed that all formula had ability to decrease incidence of bacterial wilt disease compared to control. Moreover, all the three formula could increase plant growth, total of leaves, total of branch and yields. Decreasing of disease rate and increasing of plant growth rate variated between different formulations.*

***Keywords*—chili, formulation, Ralstonia syzygii subsp. indonesiensis, storage time, Bacillus spp.**

I. INTRODUCTION

Chili is a commodity important horticulture cultivated and consumed by the people of Indonesia. Partly big cultivated and consumed in fresh form or preserved beforehand in the form of sauces, chili powder and dried fruit, and used as ingredients in traditional medicines (Djarwaningsih, 2005). The productivity of chili plants in Indonesia was relatively stable from 2015 to 2017, namely 8.65 tonnes / ha, 8.47 tonnes / ha and 8.46 ton / ha (Central Bureau of Statistics, 2018). However, productivity the still classified low compared optimum productivity which reaches 25-30 tonnes / ha (Rostini, 2011). One of the causes of the low productivity of chili plants in Indonesia is the attack of pests and pathogens that cause disease (Ardianto, 2014).

Some of the main diseases of chili plants in Indonesia include leaf spot *cercospora*, leaf spot *phytopthora*, fusarium wilt (Semangun, 2001), anthracnose, curly yellow virus and bacterial wilt (Wiratama et al., 2013). Bacterial wilt disease caused by *Ralstonia syzygii* subsp. *indonesiensis* (used to be known *Ralstonia solanacearum*) (Safni *et a*l., 2014). *R. syzygii* pathogens because range high genetic diversity (Suryani and Machmud, 2002).

The recommended control for bacterial wilt includes technical culture, mechanics and resistant varieties (Hassan *et al*., 2010). However, this control method is still ineffective, so it is necessary to look for cheap and environmentally friendly alternative controls such as biological control. One of agency group control biological that Lots developed is a group plant growth promoting rhizhobacteria (PGPR) or plant growth promoting rhizobacteria (Yanti *et al*., 2017). Rhizobacteria is a saprophytes that live in the rhizosphere which colonize the root system of plants, become one of the biocontrol agents for disease control and growth promoters plant (plant growth promoting rhizhobacteria) to increase crop production (Silva *et al*., 2004).

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Superior rhizobacteria need to be formulated so that population density can be maintained so that it is effective in controlling disease and make it easy in use and marketing (Nakkeeran *et al*., 2005). The type of formula will be determined by ingredients carrier and too determine the stability of the rhizobacteria in it. Carriers in formula making include peat soil, tapioca starch, charcoal, clay, organic matter (Bashan et al., 2014) and agricultural organic waste (Vandamme, 2009). Material carrier formulations Rhizobacteria are used bagasse, rice straw and bran because they have the following nutrients, bagasse contains 48-52% water, 3.3% sugar, 47.7% fiber and also contains cellulose, hemicellulose and lignin (Idris et al., 1994). Rice straw contains 27% hemicellulose, 39% cellulose, 12% lignin and 11% ash (Karimi *et a*l., 2006). Bran contains metabolic energy of 2980 kcal / kg, 12.9% crude protein, 13% fat, 11.4% crude fiber, 0.07% Ca, 0.22% available P, 0.95% Mg and 9 moisture content. % (Saputra, 2015). Information regarding usage carrier material and storage time for rhizobacterial formula *Bacillus* sp. stain RZ.2.2.AG2 needs to be further investigated to obtain a stable formula in control R. syzygii subsp. indonesiensis. Based on the description above, it is necessary to conduct a study entitled "Stability of the Formula *Bacillus* spp. for Control *Ralstonia syzygii subsp. indonesiensis* and Increased Growth and Yield of Chili. " This research aims to obtain formula *Bacillus* spp., which is stable for controlling bacterial wilt disease and increasing the growth and yield of chili plants.

II. METHOD OF RESEARCH

2.1 Time and Location of Research, and Sampling Method

The research was carried out in the Microbiology Laboratory of the Department of Plant Pests and Diseases, Faculty of Agriculture, Andalas University, Padang and wire house in the Experimental Garden of the Faculty of Agriculture, starting from November 2019-March 2020.

2.2 Formulation *Bacillus* spp.

The study consisted of two phases, namely: stage (1.) Rhizobacterial viability in various organic solid waste formulas, resistant to (2.) Rhizobacterial stability test to control bacterial wilt disease in chilies. This research was an experimental study using a completely randomized design with 24 treatments and 5 replications**.** The treatments consisted of rhizobacteria formulated with various carrier media from organic waste and a combination of organic waste AT + JP, AT + D and D + JP with a ratio of 1: 1 (v / v), stored in time that different (without storage, 2, 4 and 6 weeks). Next 1 ml of results preculture transferred to 49 ml of sterile coconut water in a culture vial flask (100 ml) for mainculture and incubated on shaker for 2x24 hours. Solid formula ingredients (bagasse, straw and bran) used mash in a blender. Each material was put into a Schott bottle with a volume of 100mL of 9.5 g. Then, 0.5 g of sucrose (5% of the total weight of the media) was added and then sterilized by autoclaving at 121 ° C. After cooling it is added 5 mL suspension the result mainculture *Bacillus* sp. strain RZ.2.2.AG2. Formula viability tested for a long time different storage (0, 2, 4, 6 and 8 weeks).

2.3 Stability of Formula *Bacillus* spp. for Bacterial Wilt Control

The composition of the planting medium to be used is a mixture of soil and 2: 1 (v / v) manure. The planting medium is put in 20 g / hole seedtray for seeding and 10 kg into poly bag for planting (Habazar *et al*., 2015). Introduce the formula twice, at the time of seeding and planting. Seed chili sterilized surface. Furthermore, the seeds were put in each treatment according to the standard population formula (10 8 CFU / g). Each treatment was soaked for 15 minutes and dried and dried. Seed sown 2 seeds on seed tray. The seeds are maintained for 3 weeks. Maintenance includes watering chili seeds in the morning or evening using hand sprayer. The introduction of the next formula is at planting 3 week old seeds with the same steps for seeding. *R. syzygii subsp. indonesiensis* inoculated on chilli plants that were 35 days old. Chili plants were inoculated by wounding the roots of chili seeds by cutting the roots on both sides of the plant. Then 10 ml of the bacterial suspension was sent *R. syzygii subsp. indonesiensis* with a population density of 10 7 CFU / mL. Plant maintained by applying fertilizers, weeding weeds, heaping.

Change being observed is Rhizobacteria viability in various formulas Waste Solid Organic, development of bacterial wilt disease (incubation period, disease incidence and severity of attack disease wither bacteria), The growth of the seedling phase (germination capacity, seed yield capacity, seed height, number of seed leaves, seed root length and seed vigor index), vegetative phase growth (plant height, number of leaves, number of branches), generative phase growth (when flowers appear, number of fruit and fruit weight). The effectiveness of disease progression is calculated using formula 1, while the effectiveness of growth is the phase of seedling, phase vegetative and generative phases using formula 2.

$$E = \frac{Kp - p}{Kp} \text{ x } 100\% \qquad (1)$$

$$E = \frac{p - Kn}{Kn} \text{ x } 100\% \qquad (2)$$

Information: E (Effectiveness), P (Treatment), Kn (Negative Control), Kp (Positive Contro).

III. INDENTATIONS AND EQUATIONS

Viability *Bacillus* . ,on storage time that different shows a stable population density (Table 1). The stability of the formula can be assessed based on the standard population determination 10 8 CFU / g. Where is the population density of the formula *Bacillus* spp,ranged between these standard populations. The stability of the bacterial population density in the formula is thought to be due to the presence of nutrients in the carrier materials used so that the bacteria can grow well as long as period storage. Bran contains metabolic energy of 2980 kcal / kg, 12.9% crude protein, 13% fat, 11.4% crude fiber, 0.07% Ca, available P 0.22%, Mg 0.95% and 9% moisture content (Saputra, 2015). Bagasse contains 48-52% water, 3.3% sugar, 47.7% fiber and also contain cellulose, hemicellulose and lignin (Idris *et al*., 1994). Rice straw contains 27% hemicellulose, 39% cellulose, 12% lignin, and 11% ash (Karimi *et al*., 2006) In addition, the carrier material used also has an ideal formulation character. According to Nakkeran, Fernando and Siddiqui (2005), the ideal formulation character for biological agents is to increase shelf life.

Formula introduction *Bacillus* spp. with different storage times in chili plants showed that all the formulas introduced were stable in suppressing the development of bacterial wilt disease (Table 2). All formulas *Bacillus spp*,able push period incubation, disease incidence and severity of bacterial wilt disease in chili plants. There are 8 formulas *Bacillus spp*,from 24 formula that introduced in chili plants which are more stable and suppress the incidence of disease and formula disease severity until the end of the day of observation (42 DAI) with 100% that is formula Bran without 2 weeks storage and storage, Bran + Straw formula without storage, formula Bran + Dregs Cane without storage and storage for 4 weeks, the Straw formula without storage, the Straw formula + Bagasse without storage and the 4 weeks storage sugarcane bagasse formula. This is supposedly a formula *Bacillus* spp. ,produces antibiotic compounds that can directly inhibit development pathogens (Bakker *et al*., 2007). The ability of a biological agent, especially rhizobacteria, to suppress pathogens usually involves one or several inhibitory mechanisms (Mahartha *et al*., 2013). Niu et al. (2011), suggests that the association of rhizobacteria triggers the defense response of plant cells by accumulating hydrogen peroxide, the high peroxide activity will kill the pathogen. Furthermore, Soesanto (2000) said that pathogens are difficult to dopenetration if system the root is dominated by antagonists

Formula *Bacillus* spp. which was introduced to chili seeds was able to increase the yield capacity of the seedlings compared to control with effectiveness sprouts are suspected because these bacteria have the ability of PGPR (Plant Growth Promotong Rhizobacteria) so as to increase seed germination (Table 3). This is in accordance with Muis's research *et al*. (2014), which states that giving antagonistic bacteria to plant seeds can increase seed germination. The results showed that the chili seeds were introduced to the formula *Bacillus* spp. which is more stable in increasing the growth of chili seeds is a formula Straw + Dregs Cane without storage. Formula introduction *Bacillus* spp,was also able to increase the growth of chili plants in the vegetative phase with effectiveness 69.00%. Increase in plant growth chili could seen on increase in plant height, number of leaves and number of branches of chili plants every week. This is thought to be a rhizobacterial formula that used able stimulates increased production of growth hormones in chili plants. According to Sorensen *et al*. (2001) which states that PGPR is able to synthesize auxins and cytokinins and are involved in plant ethylene synthesis. Rhizobacterial ability 0.40%. Enhancement power as a plant growth promoter is indicated by the ability to provide and mobilize the absorption of various nutrients in the soil as well as to synthesize and change the concentration of various phytohormones (Compant *et al*., 2005).

Formula introduction *Bacillus* spp. on chili plants also had an effect to enhancement The growth of chili plants in generative fese (first flower appearance, number

of fruit and fruit weight) was compared to the control (Table 4). There are two best formulas for increasing the growth of chili plants phase generative that is, formula Bran + Straw that is stored for 4 weeks and the formula of Straw + Bagasse which is stored for 4 weeks with an effectiveness of 60.45%. In accordance with the results of Sari's research (2017), chili plants introduced by PGPR can accelerate the appearance of chilies better than the control (51.25 dst). Character urgent rhizobacteria in increasing plant growth is to produce the IAA hormone, giberalin (Joo *et al*., 2004), fixing N (Hafeez *et al*., 2007), dissolving P (Mehrvraz and Chaichi, 2008). Special on dissolve *Bacillus* . spp could excrete organic acids such as formial, acetic and lactic acids which can dissolves the insoluble forms of phosphate so that they become available forms for plants (Rao, 2007). When compared with the optimum production of chili plants (25-30 tonnes / ha), the highest production in this study is said to have not been able to achieve optimum production in the field. This is because, when in the field the chili plants are attacked by whitefly pests (*Bemisia tabaci*) which results in chili production. This is supported by Hoddle's (2003) statement, attack Bemisia tabaci on plants can result in reduced plant height, leaf number, leaf size and yield. Result that, a stable formula that increases the growth of chilies in the seedling phase and plant growth phase has not been able to increase the yield of chili optimally in the fruit weight observation, and vice versa

I. FIGURES AND TABLES

Table 1: Viability of the Bacillus spp. in each formula

Carrier Material	**Formula Viability (CFU/g)**			
	No storage	2 weeks	4 weeks	6 weeks
Sugarcane Dregs	64.50 x 10^7	24.10 x 10^7	20.90 x 10^7	19.40 x 10^7
Bran	68.10 x 10^7	24.40 x 10^7	9.80 x 10^7	9.20 x 10^7
Straw	46.10 x 10^7	24.10 x 10^7	13.50 x 10^7	15.10 x 10^7
Bran + Sugarcane Dregs	77.20 x 10^7	25.60 x 10^7	23.70 x 10^7	11.80 x 10^7
Bran + Straw	52.10 x 10^7	27.00 x 10^7	26.80 x 10^7	12.30 x 10^7
Straw + Sugarcane Dregs	59.80 x 10^7	23.20 x 10^7	18.60 x 10^7	8.20 10^7

Table 2: Development of Bacterial Wilt Disease in Chili Plants Introduced by Bacillus spp.

Treatment		Observation						
Carrier Formula		Incubation Period		Incidence of Disease		Severity of Disease		
		DAI	Effectiveness %)	%	Effectiveness(%)	%	Effectiveness(%)	Reaction
Bran	0	42.0a*-	150.0	0.0d	100.0	0.0b	100.0	Healthy
Bran	2	42.0a*-	150.0	0.0d	100.0	0.0b	100.0	Healthy
Bran	4	37.2abc	121.4	80.0ab	20.0	2.2b	97.5	Healthy
Bran	6	31.2bcd	5.7	60.0abc	40.0	1.4b	98.4	Mild
Sugarcane Dregs	0	33.6abcd	00.0	40.0bcd	60.0	0.4b	99.5	Healthy
Sugarcane Dregs	2	30.0cd	8.6	80.0ab	20.0	2.0b	97.6	Mild
Sugarcane Dregs	4	42.0a*-	50.0	0.0d	100.0	0.0b	100.0	Healthy
Sugarcane Dregs	6	30.6abcd	2.1	80.0ab	20.0	2.0b	97.6	Mild
Straw	0	42.0a*-	50.0	0.0d	100.0	0.0b	100.0	Healthy
Straw	2	37.8abc	25.0	20.0cd	80.0	1.4b	98.3	Mild

Straw	4	39.6ab	35.7	20.0cd	80.0	1.5b	98.2	Mild
Straw	6	35.4abcd	10.7	40.0bcd	60.0	2.8b	96.6	Mild
Bran+Sugarcane Dregs	0	42.0a*-	50.0	0.0d	100.0	0.0b	100.0	Healthy
Bran+Sugarcane Dregs	2	39.6ab	35.7	40.0bcd	60.0	0.4b	99.5	Healthy
Bran+Sugarcane Dregs	4	42.0a*-	50.0	0.0d	100.0	0.0b	100.0	Healthy
Bran+Sugarcane Dregs	6	30.0cd	8.6	80.0ab	20.0	0.8b	98.8	Healthy
Bran + Straw	0	42.0a*-	50.0	0.0d	100.0	0.0b	100.0	Healthy
Bran + Straw	2	33.6abcd	00.0	40.0bcd	60.0	0.4b	99.5	Healthy
Bran + Straw	4	33.6abcd	00.0	40.0bcd	60.0	0.4b	99.5	Healthy
Bran + Straw	6	27.0d	0.7	80.0ab	20.0	5.6b	93.4	Mild
Straw+Sugarcane Dregs	0	42.0a*-	50.0	0.0d	100.0	0.0b	100.0	Healthy
Straw+Sugarcane Dregs	2	33.6abcd	00.0	40.0bcd	60.0	0.8b	99.1	Healthy
Straw+Sugarcane Dregs	4	39.6ab	35.7	20.0cd	80.0	1.0b	99.8	Mild
Straw+Sugarcane Dregs	6	38.4abc	28.6	40.0bcd	60.0	0.6b	99.3	Healthy
Streptomycin	-	42.0a*-	50.0	0.0d	100.0	0.0b	100.0	Healthy
Control (+)	-	42.0a*-	50.0	0.0d	100.0	0.0b	100.0	Healthy
Control (-)	-	16.8e	0.0	100.0a	0.0	84.6a	0.0	Weight
CV		11.95		8.93		11.95		

***** - shows the plant does not cause symptoms until the last day of observation (42HSI)
*** *** asymptomatic repetition plus the rationalization number with the number 42.00
* The numbers followed by the same lowercase letter in the same row are not significantly different according to LSD at 5% level

Table 3. Growth of Vegetative Phase of Chili Plants Introduced by Bacillus spp.

Treatment		Observation					
Carrier Formula		plant height		Number of leaves		Number of Branches	
		cm	Effectiveness (%)	Sheet	Effectiveness (%)	Branch	Effectiveness (%)
Bran	0	53.4cd	4.71	38.0cdef	-19.83	14.2abcd	-10.13
Bran	2	51.2d	0.39	47.4bcde	0.00	15.6ab	-1.27
Bran	4	55.2cd	8.24	79.8a	68.35	14.6abcd	-7.59
Bran	6	59.4abcd	16.47	49.2bcd	3.80	15.6ab	-1.27
Sugarcane Dregs	0	68.2ab	33.73	56.8bc	19.83	16.4ab	3.80
Sugarcane Dregs	2	56.4bcde	10.59	45.8bcdef	-3.38	13.6bcd	-13.92
Sugarcane Dregs	4	53.2cd	4.31	39.2bcdef	-17.30	15.0abcd	-5.06
Sugarcane Dregs	6	59.8abcd	17.23	58.0b	22.36	16.4ab	3.80
Straw	0	51.6d	1.18	46.2bcdef	-2.53	17.6a	11.39
Straw	2	50.8d	-0.39	30.0ef	-36.71	17.4ab	10.13
Straw	4	48.4d	-5.10	53.8bc	13.50	16.6ab	5.06
Straw	6	69.8a	36.86	52.6bc	10.97	13.6bcd	-13.92

Bran+Sugarcane Dregs	0	52.0d	1.96	51.0bcd	7.59	14.2abcd	-10.13
Bran+Sugarcane Dregs	2	53.2cd	4.31	57.8b	21.94	16.0ab	1.27
Bran+Sugarcane Dregs	4	50.4d	-1.18	33.2def	-29.96	12.0 cd	-24.05
Bran+Sugarcane Dregs	6	53.4cd	4.71	32.8def	-30.80	11.8d	-25.32
Bran + Straw	0	53.2cd	4.31	42.0bcdef	-11.39	14.4abcd	-8.86
Bran + Straw	2	53.2cd	4.31	28.2f	-40.51	14.6abcd	-7.59
Bran + Straw	4	57.20abcde	12.16	49.4bcd	4.22	15.4abc	-2.53
Bran + Straw	6	56.20bcde	10.20	49.8bcd	5.06	16.4ab	3.80
Straw+Sugarcane Dregs	0	56.8abc	29.02	50.6bcd	6.75	15.4abc	-2.53
Straw+Sugarcane Dregs	2	51.0d	0.00	46.0bcdef	-2.95	15.2abcd	-3.80
Straw+Sugarcane Dregs	4	54.0cd	5.88	47.6bcde	0.42	16.4ab	3.80
Straw+Sugarcane Dregs	6	69.8a	36.86	44.8bcdef	-5.48	15.0abcd	-5.06
Streptomycin	-	46.4d	-9.02	50.4bcd	6.33	14.2abcd	-10.13
Control (+)	-	51.0d	0.00	47.4bcde	0.00	15.8ab	0.00
Control (-)	-	18.80e	-63.14	46.0bcdef	-2.95	6.0 e	-62.03
CV		8.99		15.44		19.16	

* The numbers followed by the same lowercase letter in the same row are not significantly different according to LSD at 5% level

Table 4. Growth of Generative Phase of Chili Plants Introduced by Bacillus spp.

Treatment		Observation						
Carrier Formula		Appear flower first		Number of fruit		Fruit weight		
		HST	Effectiveness (%)	Buah	Effectiveness (%)	g/plant	Ton/ha	Effectiveness (%)
Bran	0	40.6ab	0.0	34.6ab	55.9	56.7ab	3.92	55.09
Bran	2	39.2abc	3.5	23.0 f	3.6	39.3defgh	2.63	7.71
Bran	4	36.4bcd	10.3	25.2def	13.5	37.4fgh	2.49	2.30
Bran	6	37.8abcd	6.9	26.6cdef	19.8	37.2gh	2.48	1.64
Sugarcane Dregs	0	37.8abcd	6.9	26.8cdef	20.7	43.1cdefgh	2.88	17.78
Sugarcane Dregs	2	37.8abcd	6.9	23.8f	7.2	37.4 fgh	2.49	2.95
Sugarcane Dregs	4	40.6ab	0.0	24.8def	11.7	38.3 defgh	2.56	4.98
Sugarcane Dregs	6	37.8abcd	6.9	24.4def	9.9	37.9efgh	2.53	4.81
Straw	0	39.2abc	3.5	25.6cdef	15.3	41.9cdefgh	2.80	14.72
Straw	2	39.2abc	3.5	29.2bcdef	31.5	43.4cdefgh	2.90	18.76
Straw	4	33.6d	17.2	26.6cdef	19.8	44.1cdefgh	2.91	20.68
Straw	6	33.6d	17.2	24.0ef	8.1	37.9 efgh	2.51	3.77
Bran+Sugarcane Dregs	0	37.8abcd	6.9	31.6abcd	42.3	48.9abcdefg	3.27	34.14
Bran+Sugarcane Dregs	2	36.4bcd	10.3	32.8abc	47.8	52.2abcd	3.47	43.98
Bran+Sugarcane Dregs	4	40.6ab	0.0	29.6bcdef	33.3	49.0abcdef	3.27	35.83

Bran+Sugarcane Dregs	6	42.0a	-3.3	31.4abcde	41.4	45.7cdefgh	3.05	25.05
Bran + Straw	0	39.2abc	3.5	32.8abc	47.8	52.6abc	3.52	44.37
Bran + Straw	2	37.8abcd	6.9	29.0bcdef	30.6	46.7 bcdefgh	3.12	27.68
Bran + Straw	4	36.4 bcd	10.3	34.4ab	54.9	55.7ab	3.72	52.41
Bran + Straw	6	37.8abcd	6.9	23.8f	72.1	58.7 a	3.92	60.45
Straw+ Sugarcane Dregs	0	33.4d	17.7	33.0abc	48.7	53.2abc	3.55	45.40
Straw+ Sugarcane Dregs	2	39.2abc	3.5	35.2ab	58.6	48.2bcdefgh	3.22	31.84
Straw+ Sugarcane Dregs	4	36.4bcd	10.3	34.4ab	55.0	49.7 abcde	3.27	60.45
Straw+ Sugarcane Dregs	6	35.0cd	13.8	31.4abcde	41.4	48.2bcdefgh	3.22	33.86
Streptomycin	-	39.2abc	3.5	26.6cdef	19.8	37.40fgh	2.95	20.79
Control (+)	-	40.6a	0.0	22.2f	0.0	36.6h	2.44	0.00
Control (-)	-	42.0 a	-3.3	0.00g	-100.0	0.00i	0.00	-100.0
CV		11.95		21.21		20.12		

* The numbers followed by the same lowercase letter in the same row are not significantly different according to LSD at 5% level

IV. CONCLUSION

Formula best in pressing disease progression, namely the formula of Sugarcane Dregs stored 4 weeks and Bran + Sugarcane Dregs stored 4 weeks; increase the phase of plant growth and yield, namely bran + straw stored 4 weeks and sugarcane dregs formula stored 4 weeks. Meanwhile, in general, the most stable formula in suppressing the development of bacterial wilt and increasing the growth and yield of chili plants is the Sugarcane Bagasse Formula is a formula that is stable on the viability test. Formula best in pressing disease progression, namely the formula of Sugarcane Dregs stored 4 weeks and Bran + Sugarcane Dregs stored 4 weeks; increase the phase of plant growth and yield, namely Bran + Straw stored 4 weeks and formula research show Sugarcane dregs stored for 4 weeks. Meanwhile, in general, the most stable formula in suppressing the development of bacterial wilt and increasing the growth and yield of chili plants is the Straw + Pulp of Tebuyang formula which is stored for 4 weeks. Based on the research results it can be concluded that all formulas *Bacillus* spp. ,able push development of bacterial wilt disease and increase the growth and yield of chili plants. Sugarcane Bagasse Formula is a formula that is stable on the viability test.

formula of straw + sugarcane pulp which is stored for 4 weeks

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Production of High Nutritional Set Yoghurt Fortified with Quinoa Flour and Probiotics

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Abstract**—** *There has been highly attention in supplementation of yoghurt with probiotics and bioactive cereal compounds for enhancing their nutritional and therapeutic functions. So, the propose of this research was planned to develop novel set yoghurt supplemented with quinoa flour and probiotics. Four treatments (control, T1, T2 and T3) of yoghurt were manufactured buffalo's milk. Control treatment inoculated with Streptococcus thermophiles and Lactobacillus bulgaricus (1:1) without quinoa flour. The other treatments T1, T2 and T3 were manufactured with adding Streptococcus thermophiles and Lactobacillus brevis NRRLB-4527 (1:1), Streptococcus thermophiles and Lactobacillus reuteri NRRLB-14171 (1:1) and Streptococcus thermophiles and Lactobacillus curvatus NBIMCC-3452 (1:1) and quinoa flour at the level of 0.5, 1. and 1.5 % respectively. Yoghurt samples were analyzed when fresh and during cold storage at 5±2º C., the results showed that the pH values took a reverse trend to acidity and gradually decreased by extending storage period. Moreover, fortification of yoghurt with quinoa flour leads to increase in viscosity because of the high starch contents and binding properties of quinoa flour. Furthermore, diacetyl contents were increased but acetaldehyde decreased by extending storage period. The high nutritive value of quinoa flour lead to improve the yoghurt starter and probiotic counts during all refrigeration storage time. The treatment supplemented with 1.5 quinoa flour had the higher counts of Streptococcus thermophiles and Lactobacillus strains. Organoleptic scores were 95, 96, 96 and 94 in treatments control, T1, T2 and T3 respectively. Coliforms were absent in all yoghurt samples either when fresh or during cold storage period. Finally, it can be recommended that yogurt can be fortified with quinoa flour without any defects and showed adequate potential for future dairy application.*

Keywords**— Set yoghurt, quinoa flour, probiotics, diacetyl and acetaldehyde.**

I. INTRODUCTION

Functional foods are food products containing biologically active components that can improve human health and reducing the risk of diseases by beneficially affecting one or more target functions in the host [1, 2]. Many studies used several flours from different sources such as fruits, fruit skin/peel, different cereals for improving the growth of lactic and/or probiotic bacteria, sensory acceptance, rheological properties and nutritional values of functional foods [3]. Cereals and pseudocereals usually consumed in the form of fermented beverages, cakes, breads or porridges in developed countries. These kinds of food would increase consumption of cereals and contribute to increase probiotic intake, in case of using probiotic strain for fermentation process [4]. The uses of pseudocereals were increased not only in special diets of people allergic to cereals, but also in healthy diets functional foods [5]. Quinoa is an excellent example of pseudocereals rich in fibers, minerals, vitamins, fatty acids, antioxidants and phytonutrients, which contribute to human nutrition and improving neuronal functions [6]. Moreover, the recognition and diffusion of quinoa functional properties such as high water retention capacity, gelation and emulsification have allowed its increasing utilization in value-added products [7]. Quinoa flour used as a food supplement due the high amino acid contents, vitamins, high ratio of linoleic and linolenic acids, minerals and calcium. These characteristics provide the grain great advantage for human nutrition and health

maintenance [8, 9, and 10]**.** Quinoa flour used in many food products such as cookies, biscuits, noodles, pasta, flat bread, white bread and pancakes [11, 12, 13, and 14]. Moreover, quinoa seeds can be fermented to make beer or a traditional ceremonial alcoholic beverage from South America called "chicha" [15]. Quinoa leaves eaten similarly to spinach [16]. The germinated quinoa seedlings quinoa sprouts incorporated in salads [17]. Yoghurt is one of the most popular fermented dairy products that consumed widely worldwide because of its therapeutic, nutritional benefits. This good reputation for yoghurt is due to the presence of many healthy nutrients [18]. In addition, many researchers have tried to add cereals to dairy products for improving the curd properties, sensory quality and nutritional characteristics [19]. The consumption of probiotic fermented dairy products in particular has reached in the last years a new dimension due to their beneficial effects on human health [20, 21]. Addition of various cereals into yogurt formulation is growing nowadays in many countries like lentil [22, 23]**,** chickpea [24]**,** bean [25] and quinoa [26, 27, and 28]. Also, quinoa was added to fermented dairy products such as quinoa-based yoghurt [29]**,** fermented quinoa-based beverages [30], yogurt-like beverages [28]**,** milk based fermented beverage from quinoa extract and buffalo's milk [32]**,** synbiotic fermented beverage [33]**,** processed cheese spread [34] and sprouted quinoa based-yoghurt beverage fermented with lactic acid bacteria [35]**.** The baby foods supplemented with quinoa capable to prevent malnutrition among kids [36]. Moreover, supplementation of diet with quinoa has been demonstrated to prevent cardiovascular disorders in healthy people [37]**.** Quinoa contains bioactive compounds that reduce the risk of different chronic disorders including cancer, cardiovascular diseases, diabetes, and aging [38]. The nutritional quality and protein value of quinoa is similar to casein from milk [8, 10]. From the previous information, it is clear that, the addition of quinoa flour in yogurt will improve the nutritional characteristics and quality of the product. Therefore, the aim of this study is to supplement set yoghurt with different levels of quinoa flour and probiotic cultures for enhancing the therapeutic nutrition of yoghurt and study the properties of resultant product during storage period.

II. MATERIALS AND METHODS

2.1. Yoghurt starter and probiotic origin

The yoghurt starter containing *Streptococcus thermophiles* and *Lactobacillus delbrueckii* subsp. *bulgaricus* were obtained from dairy science dept., (microbiology lab) National Research Centre. *Lactobacillus brevis* NRRLB-4527, *Lactobacillus reuteri* NRRLB-14171 were given from Northern Regional Research Laboratory, Illinois USA (NRRL). *Lactobacillus curvatus* NBIMCC-3452 were obtained from National Bank for Industrial Microorganisms and Cell Cultures, Sofia, Bulgaria (NBIMCC). All strains were activated by successive transfers in sterilized skim milk (12 % TS) for 24 h for preparing fresh mother culture before yoghurt manufacture.

2.2. Yoghurt manufacture

Yoghurt was manufactured from fresh buffalo's milk (6.5 % fat). The milk heated at 90^{o} C for 10 min then cooled to 42^{o} C and inoculated with previously activated yogurt starter and probiotic cultures at the level of 2 %. The treatments were manufactured as follows; control treatment without quinoa flour, only added *Streptococcus thermophilus* and *Lactobacillus bulgaricus* (1:1); treatment T1 supplemented with quinoa flour at the level of 0.5 % and *Streptococcus thermophilus* + *Lactobacillus brevis* NRRLB 4527 (1:1); treatment T2 enriched with quinoa flour at the level of 1 % and *Streptococcus thermophilus* **+** *Lactobacillus reuteri* NRRLB-14171 (1:1). Finally, treatment T3 enriched with quinoa flour at the level of 1.5 % and *Streptococcus thermophilus*+ *Lactobacillus curvatus* NBIMCC 3452 (1:1). Inoculated portions mixed well then incubated at 42^{O} C until the fermentation was completed when pH reached to 4.6-4.7. Yoghurt analyses (acidity, pH values, diacetyl and acetaldehyde, apparent viscosity, *Streptococcus* and *Lactobacilli* counts and sensory evaluation) were carried out an overnight after production and each week of refrigeration storage at 5 ± 2^{o} C.

2.3. Yoghurt analysis:

2.3.1. Acidity development

The titratable acidity % (expressed as lactic acid) of yogurt samples were determined by titration method using 0.1M sodium hydroxide solution and 0.3 ml of phenolphthalein to noticeable pink color as an end point. The titratable acidity percentage = (Volume of NaOH x N/9 x 90 / weight of sample x 100) x 100

2.3.2. pH analysis

The pH values of yoghurt samples were measured using a digital pH meter (Adwa, AD1000, Romania) by immersing the electrode in the yogurt samples.

2.3.3. Acetaldehyde and Diacetyl

Acetaldehyde and diacetyl contents of yoghurt samples were determined according to [39, 40] respectively.

2.3.4. Apparent viscosity (cPs) of set yoghurt

The apparent viscosity of fortified set yoghurt was measured at room temperature using a Brookfield digital viscometer (Middleboro, MA 02346, U.S.A). The sample was subjected to shear rates ranging from 0.3 to 100 S^{-4} for an upward curve. Viscosity measurements expressed as centipoise (cP.s) according to [41].

2.3.5. *Streptococcus* and *Lactobacilli* counts

Ten grams of resultant set yoghurt samples were homogenized in 90 ml of sterile physiological saline (0.85 % NaCl w/v) then; the homogenate was serially diluted up to 10^{-8} [42]. One milliliter from each dilution plated onto sterile petri dishes in duplicate after that, M17 agar and de Mann Rogosa Sharpe (MRS) agar were poured for *Streptococcus thermophilus* and *Lactobacilli* respectively [43]**.** The plates were incubated anaerobically at 37°C for 48 h for *Lactobacillus* strains and aerobically at 37°C for 48 h for *Streptococcus thermophilus.*

2.3.6. Coliform counts

Coliform counts were determined on violet red bile agar according to the method of **APHA (2001)**.

2.3.7. Sensory Evaluation:

Yoghurt fortified with different concentrations of quinoa flour were sensory evaluated when fresh and after 21 days of storage by staff members of dairy department at Food Industry and Nutrition Research Division, National Research Centre, using the score sheet according to [44].

III. RESULTS AND DISCUSSION

3.1. Titratable acidity (%)

Figure (1) shows the changes in titratable acidity of set yoghurt fortified with quinoa flour during storage period at 5° C for 21 days. The titratable acidity in fresh samples recorded 0.81 in control treatment, 0.86 in treatments T1, T2 and 0.88 in T3. The titratable acidity were increased with extending refrigeration storage period reached to 1.17, 1.14, 1.25 and 1.28 in control, T1, T2 and T3 respectively. The continuous development in titratable acidity of all yoghurt treatments by extending the storage time could be attributed to the high metabolic activities of added starters and conversion of residual lactose into lactic acid [45, 46]**.** In addition, the addition of quinoa flour up to 1 % resulted in an increase in the yoghurt acidity with a reduction in pH values. Our findings agree with the data recorded by [26, 27]**.**

3.2. pH values

The pH values of set yoghurt fortified with quinoa flour illustrated in **Figure 2** when fresh and along storage time at 5° C for 21 days. As can be seen, the pH values of fortified set yoghurt ranged from 4.65 to 4.68 at zero time. Meanwhile, the pH values of the examined set yoghurt took a reverse trend to acidity and gradually decreased by extending storage period. The decreasing in pH values is most evident in yoghurt samples with the highest concentration of the quinoa flour and recorded 4.31, 4.23, 4.19 and 4.13 in control, T1, T2 and T3 respectively at the end of storage period. These data suggest that quinoa flour enhance the growth of probiotic and yogurt starter cultures due to high amino acids and minerals [47, 48]**.**

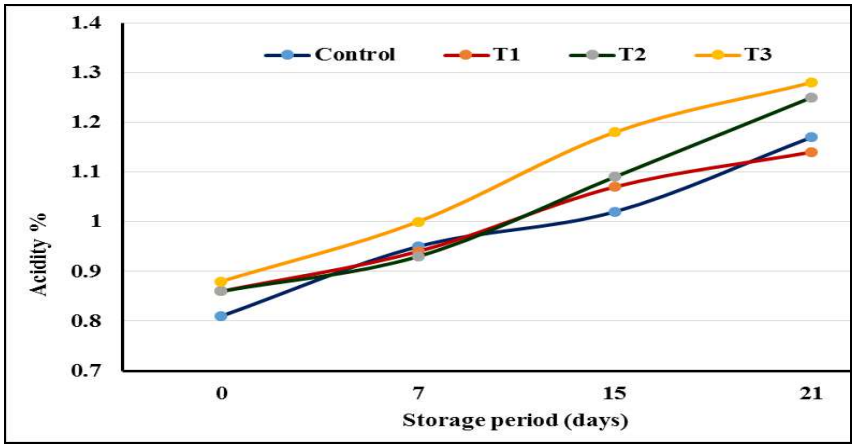

Fig- 1 Titratable acidity of set yoghurt fortified with quinoa flour during refrigerated storage.

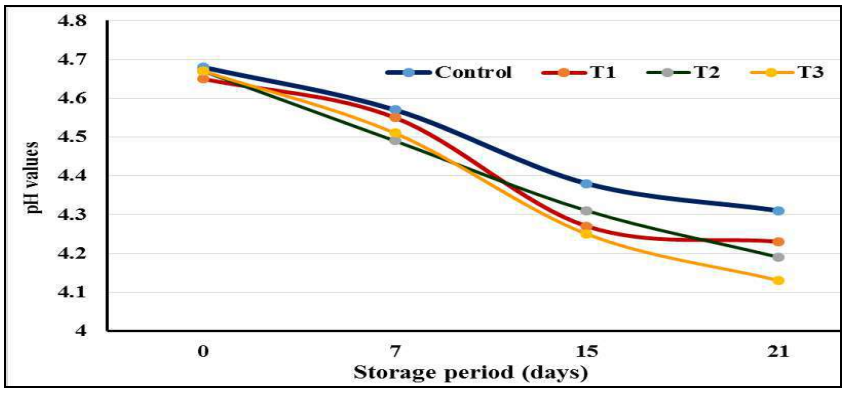

Fig- 2 pH values of set yoghurt fortified with quinoa flour during refrigerated storage.

3.3. Viscosity (cP) of set yoghurt

The data in Figure (3) presents the apparent viscosity of set yoghurt fortified with different concentrations of quinoa flour. There was high viscosity observed in treatments supplemented with quinoa flour. In addition, we found that increasing of quinoa flour levels lead to increase the viscosity of set yoghurt. The highest viscosity values were recorded in T3 and this may be due to the high total solids in set yoghurt fortified with 1.5 % quinoa flour comparing with other treatments. The increase in apparent viscosity is due to the high binding properties of quinoa flour and high starch granules rich in amylopectin used as thickener in frozen and fermented foods [49]**.** In addition, quinoa flour is good for enhancing viscosity, stability and water absorption capacity in yogurt [27, 10]. In addition, quinoa flour used as stabilizing and emulsifying agents because of high protein and starch contents [50].

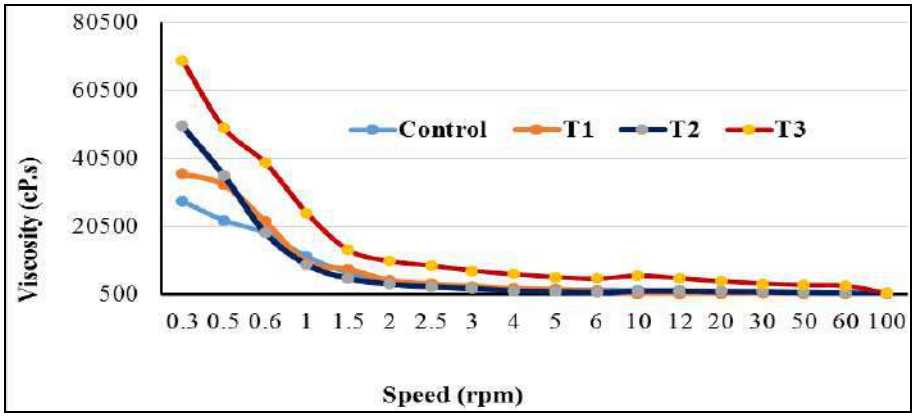

Fig- 3 Effect of quinoa flour levels on apparent viscosity of set yoghurt.

3.4. Acetaldehyde and Diacetyl Contents

The results of acetaldehyde and diacetyl contents of set yoghurt fortified with quinoa flour are presented in Figures (4 and 5) respectively. The diacetyl contents were increased in all treatments with extending storage period. At the end of storage period the diacetyl contents reached to 272.8, 414.5, 416 and 448 microgram/ 100g of the product in treatments control, T1, T2 and T3 respectively. Diacetyl contents increased by increasing the level of quinoa flour addition. On the other hand, acetaldehyde contents were decreased and recorded 25.44, 26.4, 19.82 and 18.39 microgram/100g in treatments control, T1, T2 and T3 respectively. From the results as prolonging the cooled storage of yoghurt samples, the acetaldehyde contents of all treatments were decreased and diacetyl contents took an opposite trend to acetaldehyde. This may be due to the activation of lactic acid starter cultures and their ability to convert the acetaldehyde to ethanol and diacetyl. The results were similar with those obtained by [51, 52].

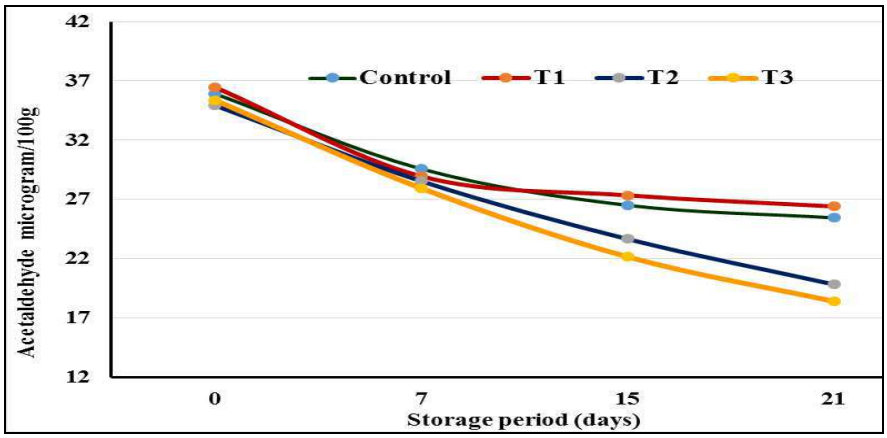

Fig -4 Acetaldehyde contents of set yoghurt fortified with quinoa flour during refrigerated storage.

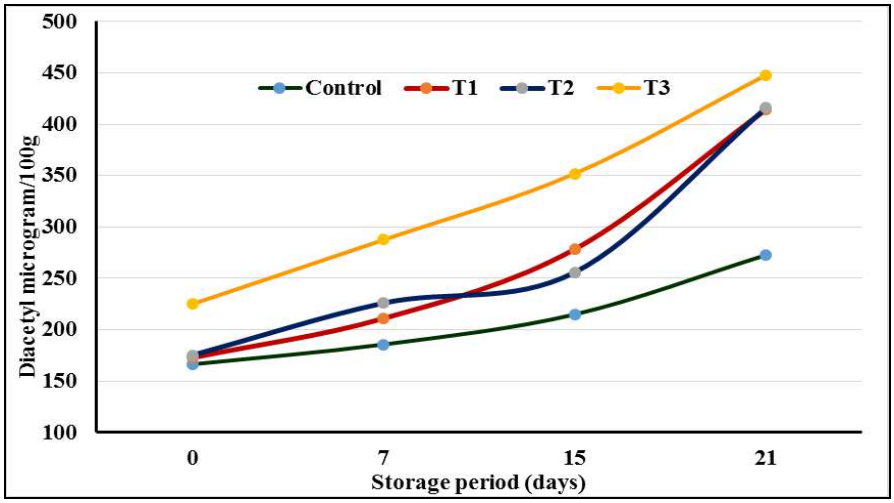

Fig-5 Diacetyl contents of set yoghurt fortified with quinoa flour during refrigerated storage.

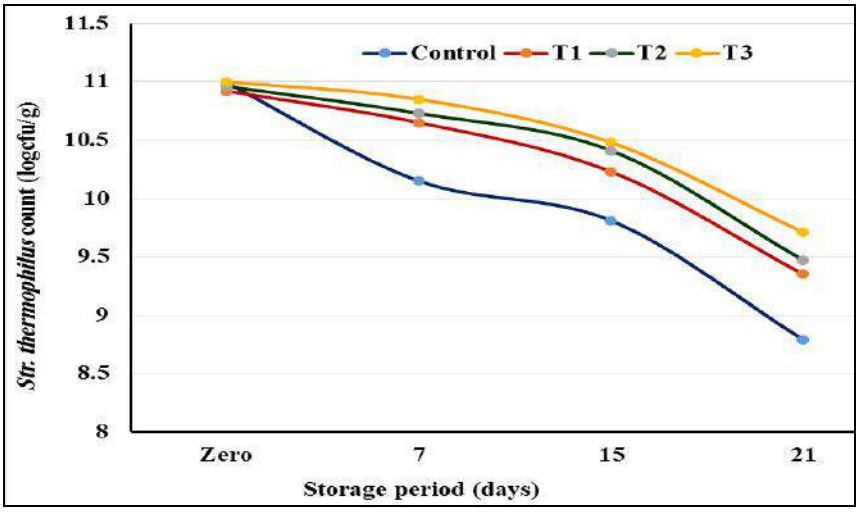

Fig- 6 Streptococcus thermophilus count (Log cfu/ml) in set yoghurt fortified with quinoa flour during refrigerated storage.

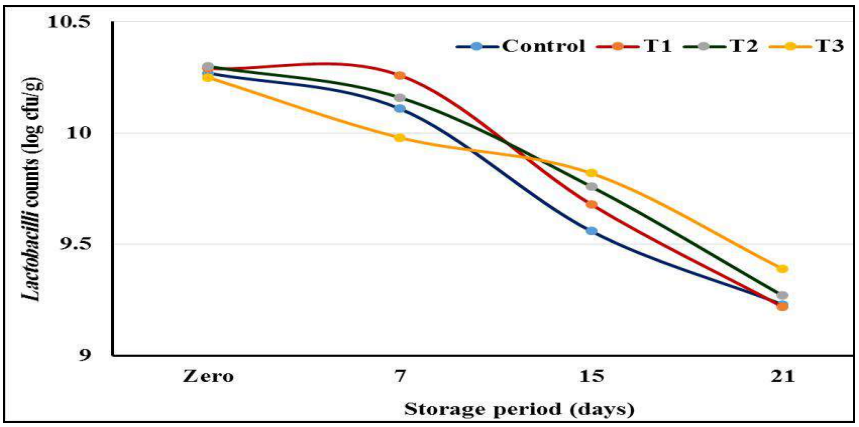

Fig-7 Lactobacilli counts (Log cfu/ml) in set yoghurt fortified with quinoa flour during cooled storage.

3.5. Viable counts of streptococci and lactobacilli

Generally, as shown in Figures (6, 7) the counts of all added starters streptococci and lactobacilli were slightly increased at the first week of storage time then, started to decrease until the end of storage period. The counts of all strains still above the recommended therapeutic level and *Lactobacillus curvatus* NBIMCC 3452 recorded the highest count (9.39) log cfu/g in treatment (T3) supplemented with 1.5 quinoa flour. In addition, *Streptococcus thermophilus* recorded (9.71) log cfu/g in the same treatment. The high ingredients and nutritive value of quinoa flour may be lead to improve the survival and high counts of probiotics in the product. The obtained results were coincide with the reported by **[**26, 53**].** They reported that the fermented milk supplemented with quinoa, oat, rice and barley flours promoted the growth of *L. rhamnosus* GR-1 (10^8 cfu/mL) up to the 14^{th} day of storage and resulted in significantly higher population of *Lactobacillus acidophilus* La-5 and *Bifidobacterium animalis* ssp. *lactis* BB-12 in fermented milk.

3.6. Organoleptic properties

Organoleptic scores of set yoghurt fortified with quinoa flour are shown in Figure (8). The yoghurt samples were evaluated when fresh and after 21 days of storage period and the mean organoleptic scores showed that the adiition of quinoa flour had no effects on the acceptability of set yoghurt when fresh and at the end storage. The total organoleptic scores ranged from 95 to 98 at zero time but at the end storage recorded 94, 95,96 and 96 in treatments T3, control T1 and T2 respectively. The Sensory analyses indicated that the incorporation of quinoa flour in set yoghurt improve the quality, organoleptic profile and nutritional value, which may increase the appeal of the product to consumers. The results are agreement with those reported by [26, 27, 54] they mentioned that, the addition of quinoa flour had desirable effects on yoghurt stability and increases the consumer acceptability. Finally, the addition of quinoa malt or powder into milk makes it more nutritious and increase the acceptability of milk by many people [55].

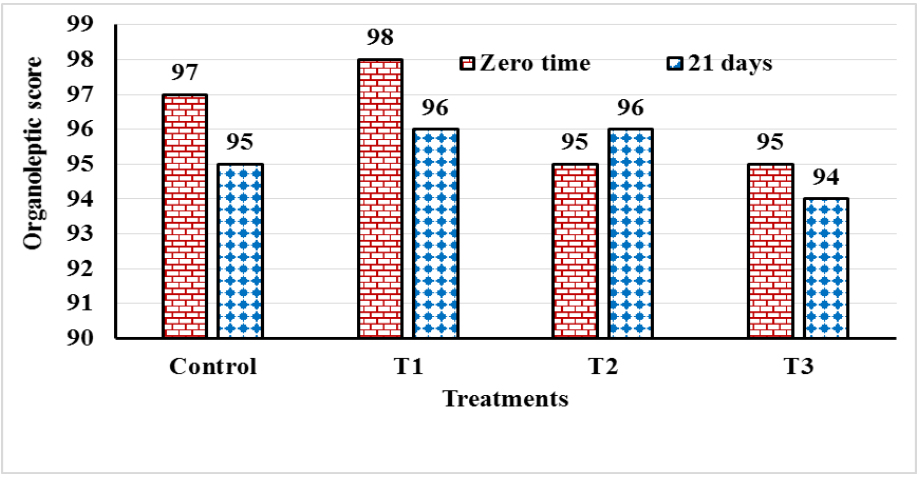

Fig 8- Organoleptic acceptability of set yoghurt fortified with quinoa flour when fresh and after 21 days of refrigerated storage.

IV. CONCLUSIONS

This study concluded that, the use of quinoa flour and probiotics for producing functional set yoghurt gavin positive properties to the product and showed adequate potential for future dairy application.

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Antagonism activity of phosphate solubilizing microbes and nitrogen fixing bacteria toward *Fusarium* sp.

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***Abstract*— *The ability of phosphate solubilizing microbes and N-fixing bacteria as biofertilizers to increase growth and soil P-dissolve, N soil, they also have the potential to suppress disease, The purpose of this experiment to study antagonism activity of phosphate solubilizing microbes and N-fixing bacteria to inhibit pathogen isolate. In vitro antagonism test was carried out to test the ability of isolates biofertilizers as biocontrol The isolate of phosphate solubilizing microbes were Pseudomonas mallei, Pseudomonas cepacea, Bacilkussubtilis, Bacillus megaterium, Penicilliumsp.,and Aspergillusniger while the isolate ofN-fixing bacteria were (Azotobactervinelandii, Azotobacterchroococcum and Azospirillumsp. Fusarium sp. as pathogen was used for antagonism test. The experiment showed that phosphate solubilizing microbes and N-fixing bacteria have ability to inhibit growth of Fusariumsp. as pathogen. The results of the antagonism test of the biofertilizer isolates tested by Azospirillum were able to inhibit the growth of Fusariumsp. higher than other isolates.Furthermore, from this study it can be developed that biofertilizers are not only to increase soil nutrient availability and plant growth but can be used as biocontrol.*

Keywords*— *antagonistic, biocontrol, biofertilizers, and in vitro.

I. INTRODUCTION

The continuous use of inorganic fertilizers can reduce soil quality, including reducing soil organic matter content, destroying soil structure, and reducing the population and biodiversity of soil organisms. The continuous use of synthetic pesticides can also cause negative impacts, including resistance to pathogens, the killing of beneficial non-target organisms, environmental pollution, and the presence of pesticide residues on agricultural products (Aktar et al., 2009). Therefore, it is necessary to develop environmentally friendly fertilizers and pesticides, including a consortium of growth-promoting microbes and biological agents that can function as biofertilizers and biopesticides as biocontrol.

Growth-promoting microbes that act as biological fertilizers have been developed, namely phosphate solubilizing microbe (PSM) *Pseudomonas malei*, *Penicillium* sp. and *Aspergillus Niger* which have known their ability to dissolve phosphate and the production of phosphatase enzymes and are able to produce growth regulators (Fitriatin et al., 2014 ; Fitriatin et al, 2020) . The research by Vassileva et al. (2006) reported that phosphate solubilizing microbes can produce growth regulators, siderophores and lytic enzymes to suppress the growth of pathogens. Further studies have shown the ability of PSM to synthesize

metabolites to chelate metals (Vassileva et al., 2010). Research by Walpola& Yoon (2013) showed that phosphate solubilizing bacteria (*Pantoeaagglomerans* and *Burkholderiaanthina*) can produce IAA, ammonia, hydrogen cyanide (HCN), and siderophore and can increase plant growth and P uptake and resistance to disease.

Fusarium wilt control is recommended such as the use of fungicides and technical culture, but has not been able to suppress the disease (Thangavelu&Mustaffa, 2012).Biological control is directed at the development of research on antagonistic agents. Bacillus is a genus of bacteria that is reported to be able to increase plant resistance (Rebib et al. 2012). Bacillus is able to produce antifungal compounds that cause swelling of Foc hyphae in vitro (Arrebola et al. 2010)

Microbes that promote plant growth are able to control disease (Nakkeeran et al., 2005). Microbes that can be used as biological fertilizers such as *Azotobacterchroococcum* (Mali &Bodhankar, 2009) can also suppress disease. Therefore, research is needed to develop biofertilizer formulations (P-solubilizing microbes and N fixers) that act as biocontrol to increase nutrient availability and plant yields. The objective of research to study antagonism activity of phosphate solubilizing microbes and N-fixing bacteria to inhibit pathogen isolate.

II. MATERIAL AND METHODS

The antagonism test was carried out by invitro to determine the ability of isolates in biofertilizers, namely P-solubilizing microbes and N-fixers in inhibiting disease fungi. The isolates tested as biofertilizers werephosphate solubilizing microbes (*Pseudomonas mallei, Pseudomonas cepacea, Bacilkussubtilis, Bacillus megaterium, Penicilliumsp., and Aspergillusniger)* while the isolate of N-fixing bacteria were (*Azotobactervinelandii, Azotobacterchroococcum*and *Azospirillum*sp. *Fusarium* sp. as pathogen isolate was used for antagonism test.

The antagonism test medium used Nutrient Agar for all isolates. The dilutions of the test isolates (P-solubilizing microbes and N fixers) were 10^{-4} and 10^{-5}). This antagonistic test uses an inhibition zone indicator or clear zone that occurs characterizing the presence of inhibition of the test isolate against *Fusarium* as a pathogen. The test isolates were inoculated by streaking / scratching on petridish with NA media after which disc paper soaked in *Fusarium* isolate was planted. The zone of inhibition that occurs was measured to see how much inhibition of P-solubilizing microbes and N fixer isolates on *Fusarium*growth (Figure 1).

Fig.1: Antagonistic test of Azospirillumsp. isolates sp. towardFusariumsp.

III. RESULTS AND DISCUSSION

Antagonistic tests of biofertilizers (P-solubilizing microbes and N-fixer isolates against disease isolates (*Fusarium*sp.) can be found from Table 1**.**

Table 1. Antagonistic tests between biofertilizer and Fusarium sp. (3 days after inoculation/DAI)

Isolates	Zone of inhibition(mm)
Pseudomonas mallei	1,3
Pseudomonas cepaceae	1,65
Bacillus subtillis	1,9
Bacillus megaterium	2,1
Azotobactervinelandii	1,5
*Azospirillum*sp.	5,1
Azotobacterchroococcum	1,8
*Penicillium*sp.	1,5
Aspergillusniger	0,8

The results of the antagosnistic test on 3 DAI showed that P-solubilizing microbes (*Pseudomonas mallei, P. cepaceaae, Bacillus subtillis, B.megaterium, Penicilliumsp.* and *Aspergillusniger*) and N-fixing bacteria

(*Azotobactervinelandii*, *Azotobacterchroococcum*, *Azospirillum* sp.) inhibited the growth of *Fusarium*sp. The inhibition zone or clear zone found in petridish (ranging from 0.8-5.1 mm), these isolate were able to be a biocontrol because it could be inhibit the growth of *Fusarium*sp. This was presumably because biofertilizers isolates produces secondary metabolites which can inhibit growth of *Fusarium* sp. This is supported by Raaijmakers & Mazzola (2012) that soil beneficial produced antibiotic to suppress disease.

The phosphate solubilizing fungi (*Aspergillusniger*, *Penicillium* sp.) shown their inhibition at DAI have diameter of clear zone were smaller. This is presumably because the growth of fungi is longer than bacteria,Based on the antagosnistic test showed that *Azospirillum* was able to inhibit the growth of *Fusarium* sp. higher than other isolates. Different results were shown by Widyantoro et al (2020) that *Azospirillum* had not been able to prevent the plant wilt.

IV. CONCLUSIONS

Base on in vitro antagonism test showed that biofertiizers (phosphate solubilizing microbes and N-fixing bacteria) have ability to inhibit growth of *Fusarium*sp. as pathogen. The results of the experiment were found that *Azospirillum* were able to inhibit the growth of *Fusarium*sp. higher than other isolates. Furthermore, from this study it can be developed that biofertilizers are not only to increase soil nutrient availability and plant growth but can be used as biocontrol.

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Determination on the level of adoption of IPM Technology in western Nepal

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Abstract*— *Integrated Pest Management (IPM) is as a broad based approach for economic control of pests. A study on determination of level of adoption of IPM technology was conducted in the Banke and Surkhet districts of Nepal. For determining the spread of information and level of adoption, farmers were asked a series of questions during the survey to determine knowledge of IPM and level of IPM adoption. Using descriptive statistics and differences in means, analysis was done on relationships among access to information, IPM knowledge and adoption. This study revealed that 51.13% of the population were male with an average family size was 6.45. Almost half of the respondents had adopted IPM practices for vegetable production by receiving advice or getting training about IPM technology through agriculture officer, Market Planning Committee, I/NGOs, etc. Market Planning Committee has played a vital role in spreading IPM knowledge and information quickly, followed by mass media, as MPCs allow farmers to aggregate smallholder produce to meet market demand. Several farmer organizations join together and elect representatives to serve on the board of the MPC and have regular monthly meeting so that they could discuss on IPM technology and marketing strategy of the products. MPC group has been identified more effective in improving knowledge of IPM than other farmers' groups.

***Keywords*— IPM Technology, Knowledge, level of adoption and dissemination.**

I. INTRODUCTION

Agriculture is the main source and backbone of Nepalese economy. It provides employment opportunities to around 65 percent of the total population and contributes about 31.23 percent in the GDP. Nepalese agriculture has stumpy productivity, depriving farmers of a sustainable livelihood. Especially in the mountain region, people who endure by cultivating cereals on mountain slopes, small valleys and river basins to meet their basic needs, often have low income and suffer from food deficits. Therefore to diminish farm-poverty, the country, through various plans and policies (NPC, 1995; NPC, 1998; NPC, 2003; NPC, 2007; MOAC, 2004; MOICS, 1992), identified vegetables to harness the advantages of agro ecological diversity in Nepal. For reducing the poverty a planned has been made in a vegetable promotion strategy for small holders to capture the comparative advantage of vegetable production and marketing in economic growth and development.

The most restricting factor to accelerated crop production is pests and diseases (Wilson, 2001). In annual, an average of 32.1 percent of the global crop production is lost because of pests (Dhawan *et al*., 2010). Pests are the major constraint to

increased vegetable production. In developing countries, including in total agricultural production and post-harvest losses due to pests is 25-50% of total production (IPM-IL, 2013). The cost of pesticides can be as much as 35% of total agricultural production costs in developing countries (Karim, 2009). Various approaches had been practiced for the diffusion of IPM technologies in Nepal, including farmer field school (FFS), field day, group dissemination through market planning committee (MPC); demonstrations, training, field days, written media (pamphlets), etc. through FAO, Integrated Pest Management Innovation Lab (IPM IL), KISAN, and Caritas Nepal among others. Given only limited involvement of the public sector in technology transfer, decision makers/policy makers should need information about farmers on the depth of knowledge and level of adoption on IPM technology. This understanding can help promote better technology transfer and helps in sustainable vegetable production through IPM in Nepal.

II. METHODOLOGY

2.1 Selection of the study area

The study was conducted in Banke and Surkhet districts of Nepal. These districts were the major vegetable growing areas in Nepal and IPM IL program funded by USAID was promoted in that area for the vegetable production through IPM technology. Altogether, 500 households were randomly selected as the samples comprising of 42 farmers from each of six VDCs of each district, which included farmers and marginalized people.

2.2 Spread and source of information by IPM adoption level

To assess the spread of information, farmers were asked a series of questions during the survey to determine knowledge of IPM and degree of IPM adoption. Using descriptive statistics and differences in means, analysis was done on relationships among access to information, IPM knowledge and adoption, and word-of-mouth diffusion of IPM techniques to neighboring farmers. Estimating the speed at which each method delivers information to farmers is based on information obtained about the different methods but is not based on formal quantitative analysis. If farmers do not receive information in a timely manner, the technology may lose its usefulness by the time it reaches them. Based on expert opinion on the study area, it was assumed that marketing planning committee reaches farmers at the fastest rate of all formal methods. Other methods, such as mass media, collection centre, field day, farmer field school, neighboring farmer, agriculture officer, community business facilitators (CBFs), cooperatives and agro-vets may not transfer information as quickly as market planning committee, but may be more effective at spreading information faster than farmer field school (FFS).

Adoption intensity was defined according to the following five categories:

1 = 0% adoption

2 = 1% - 25% adoption

3 = 26% - 50% adoption

4 = 51% - 75% adoption

5 = 76% - 100% adoption

Adoption percentages was calculated by taking the total number of recommended IPM activities and determining the percentage of activities utilized by each farmer.

2.3 Knowledge on IPM technology

Knowledge of an innovation is usually preceded by awareness of a need, and it is need awareness that precipitates active knowledge seeking behavior in order to address the need. Since IPM is a multi-dimensional concept (Dent, 1995), a summated ratings scale consisting of six attributes, with a score range of 0-11, was devised to measure farmers' knowledge of IPM. Each of these knowledge attributes were considered fundamental to a strong working knowledge of IPM and have been validated in previous IPM studies in Uganda (Erbaugh, et al., 2001; Morse & Buhler, 1997). The coefficient of reliability for the knowledge of IPM scale was 84, indicating an acceptable level of reliability (Nunnally, 1978). The first item was asked whether they heard about IPM on 0-2 scale, where 0 indicated didn't heard about IPM; 1, indicated a partial heard about IPM; and 2, indicated a more complete known about IPM. Partial and more complete known were scored if farmers give description of IPM with examples. The second question was asked to the farmers if they can define the IPM on a 0-2 scale, where o indicated unable to describe IPM; 1, indicated a partial description of IPM; and 2, indicated a more definition of IPM. The third item was asked whether they were aware of merits of IPM on a 0-3 scale, and was coded 0 if they were unaware; and 1-3 if they were aware of one or more of the potential merits. A fourth item was asked to farmers whether they use pheromone traps on a 0-2 scale, and was coded o if they don't use and 1-2 on the basis of partial and complete use of pheromone traps. A fifth item

asked was if they knew alternative pest management practices, with a no (0) response indicating that they were not aware of alternative pest management practices; and coded 1-3 on the basis of partial to complete use of alternative pest management practices, and the last item asked was whether they use mulching practices on a 0-2 scale, and was coded 0 if they don't use and 1-2 on the basis of partial and complete use of mulching practice.

2.4 Scaling technique

Scaling technique is the tool to study the degree and direction of attitude of the respondents towards any proposition. A respondent is asked to choose among various categories indicating his / her strength of agreement and disagreement. The categories are assigned scale values and the sum of the values of the categories is the measure of attitude of respondents (Miah, 1993).

The priority index for the problem was computed by using the following formula:

$$I = \sum f_i s_i / N$$

Where, I= priority index such that $0 \leq I \leq 1$

f_i= frequency of i^{th} priority (category)

s_i= scale value at i^{th} priority

N= Total number of participants $=\sum f_i$

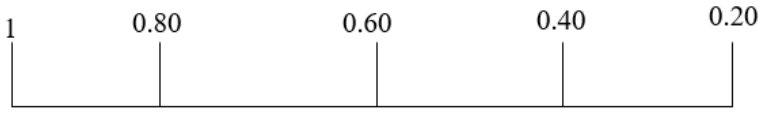

Fig.1: Scale value for intensity of situation.

III. RESULTS AND DISCUSSION

3.1 Area and production of vegetable crops in Nepal

In Nepal, vegetable production is the primary occupation for most of the people who are mostly smallholders and have low incomes. According to the Central Bureau of Statistics (2009-10) & Ministry of Agriculture and Cooperatives (MoAC), vegetable crops are cultivated in only 7.3 percent of the total cultivable land in Nepal. Total worth of vegetables (excluding potatoes) produced during 2009/10 was around Rs. 105 billion, which is 8.8 percent of the country's GDP. Per capita vegetable consumption has increased to 105 kg from 60 kg over last two decades due to massive rise in agriculture and production area.

The study revealed that both area and productivity of the vegetables in Nepal were in increasing trend over the time. The positive value of the slopes indicates the decreasing trend on area allocation over the time. The area and production for vegetables was increasing at 6,902 ha per year and 13,254 mt. ton/year, respectively, in Nepal over the last 10 years as shown in figure 2.

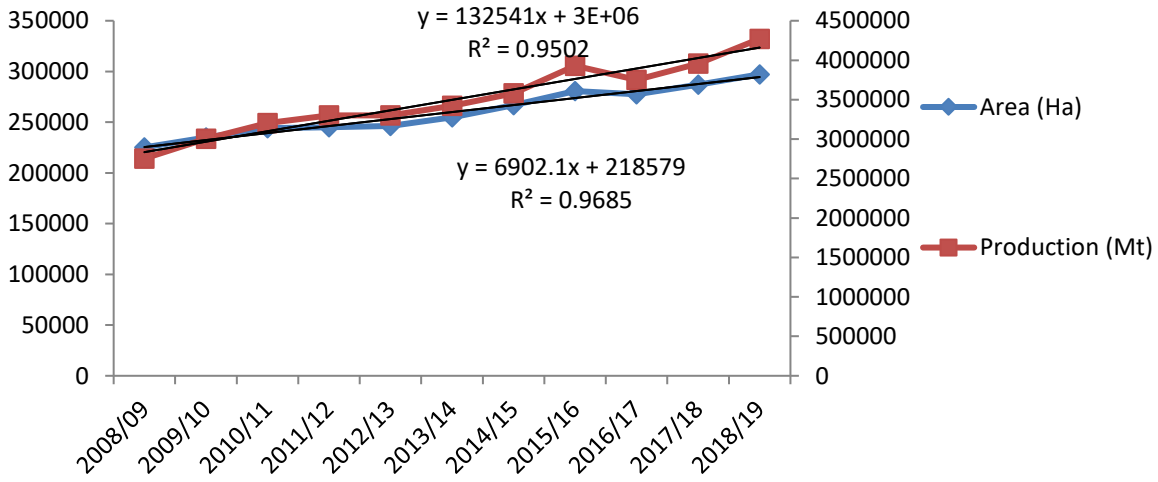

Fig.2: Area and productivity of vegetables over the time in Nepal (2075/76)
Source: Statistical Information on Nepalese Agriculture (2075/76)

3.2 Family size of the respondents

The population distribution showed that the population size was greater in Banke district than Surkhet because of low level of literacy in the Banke district. The average family size of the surveyed area was 6.45, particularly, 7.84, and 5.05 in Banke and Surkhet districts, respectively. The average family size in the study area was greater than 5.18 and 4.81 in Banke and Surkhet districts, respectively CBS, (2011). The higher family size may be due to the lower literacy rate in the study area. The detailed family size is described in table 1.

Table 1. Family size of the respondents in the study area

Average family size Banke(Mean ±SD)	Surkhet(Mean±SD)	Total Average(Mean±SD)	Minim-um	Maxi-mum	Modal size
7.84±2.07	5.05±1.78	6.45±1.92	1	12	5

Source: Field Survey (2019)
SD= Standard Deviation

3.3 Cultivation of vegetables

In the study area it has been found that 30% of the households have cultivated tomato, followed by cauliflower/cabbage (27%), bitter gourd (17%), cucumber (16%) and eggplant (10%). The study revealed that tomato is the major vegetable crops in the study area as given in figure 3.

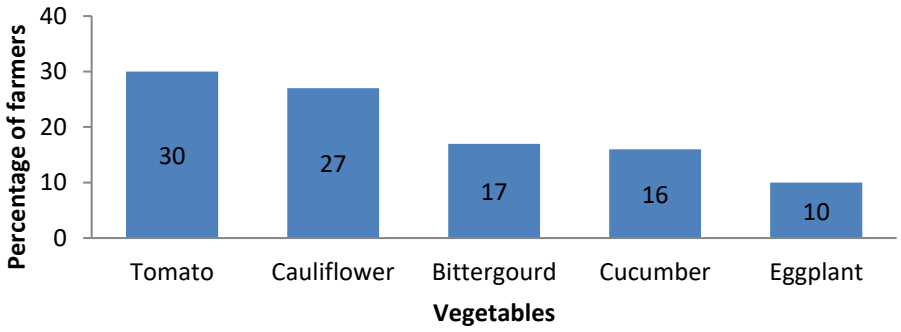

Fig.3: Cultivation of vegetables by the households in the study area.

3.4 Information on pesticide management

Majority of the population approximately 50% of population in the study area received information on pesticide management through agro-vets followed by CBFs/NGOs (27%), DADO (9%), neighbor (9%), family members (2%) and others (2%) respectively as shown in figure 4.

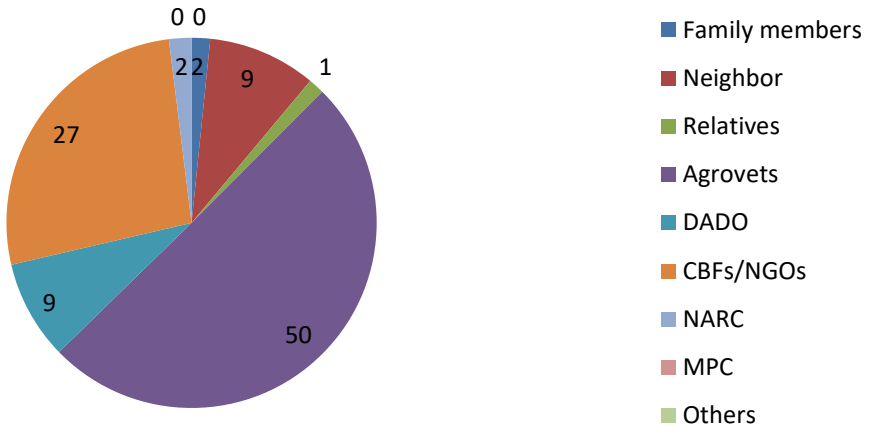

Fig.4: Information on pesticide management.

3.5 Distribution of sample household on IPM technology adoption

In the study area it has been found that 48% of the households have adopted IPM technology for the production of vegetable crops.

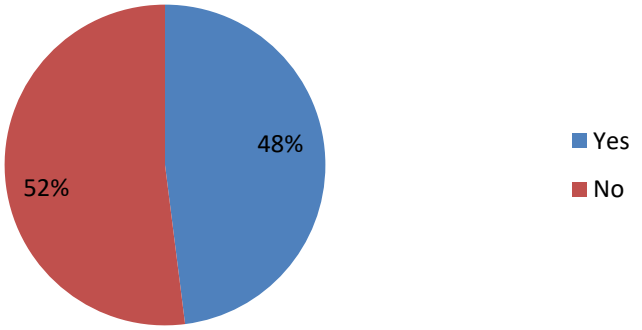

Fig.5: Distribution of sample household on IPM technology adoption.

3.6 Sources of IPM knowledge

The study revealed that most of the respondents (53%) in the study area received advice or learned about IPM technology through agriculture officer (DADO/NGOs), followed by MPC, CBFs, neighboring farmers, mass media, farmer field school, field day, collection centre, Agro-vets and others (cooperatives). In the study area, 35% of the respondents claimed that they had not received advice and/or learned about IPM. The sources or media from where the respondent received or learned about the IPM technology were ranked with index value in table 2.

Table 2. Sources of knowledge for IPM

SN	Sources	Index value	Rank
1	Agriculture officer (DADO/NGOs)	0.97	I
2	MPC	0.95	II
3	CBFs	0.96	III
4	Neighboring farmer	0.93	IV
5	Mass media	0.92	V
6	Farmer field	0.87	VI

	school		
7	Field day	0.85	VII
8	Collection centre	0.81	VIII
9	Agro-vets	0.77	IX
10	Others (Cooperatives)	0.73	X

Source: Field Survey, 2019

3.7 IPM practices used in the study area

Just over 48% of the respondents indicated that they incorporated at least one IPM practice in their production of vegetables. Bio-fertilizers, jholmol and bio-pesticides were the most popular IPM practices, followed by the adoption of pheromone traps, soil amendments, mulching, soil solarization, bagging and grafting. Bio-fertilizers, jholmol and bio-pesticides were adopted most as these practices were easily available, cheap, increase the yield of vegetable and effective in controlling insects and diseases. Similarly, grafting technology was adopted the least, possibly due to the higher level of training and inputs required for successful adoption. In contrasting to the result, Kabir and Rainis, (2015) in their study in Bangladesh had identified among different IPM practices most farmers had adopted sex pheromone traps and soil amendment methods. The findings also indicate that farmers in the study areas hardly used any complex IPM practices, such as bagging, graftings, which is similar to the findings of other studies (Singh *et al*. 2014; Materu *et al*. 2016). Figure 6 depicts the percentage of total respondents adopting each of the nine different IPM practices.

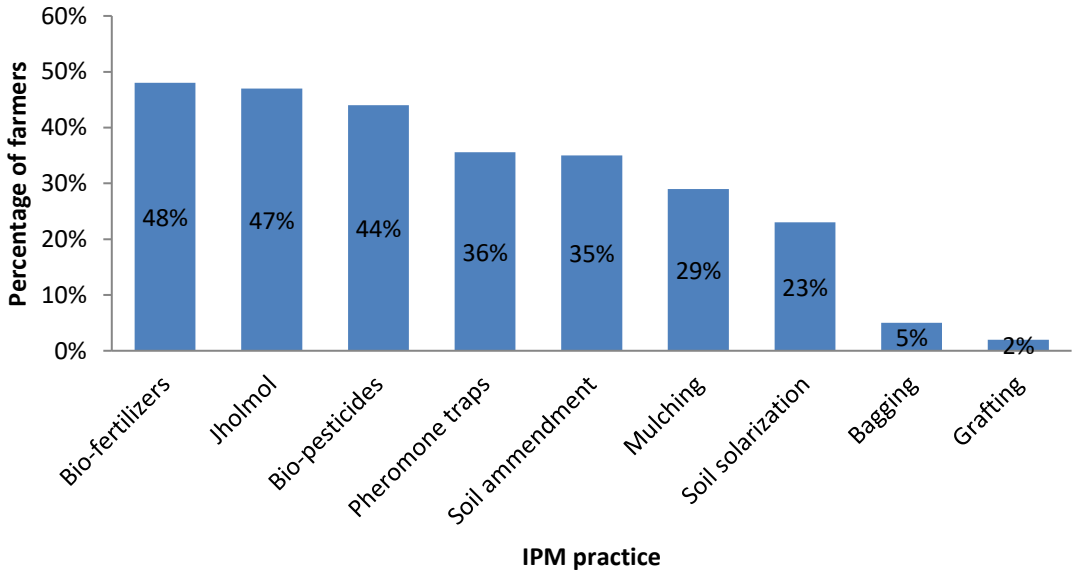

Fig.6: IPM practices used for crop production in the study area

3.8 Knowledge on IPM technology

A t-test was done for assessing differences on IPM knowledge between MPC and other farmers' groups using various scales. The results showed statistically significant difference in IPM knowledge between the two groups at 1% and 5% levels of significance. For all activities, mean scores were higher among farmers who were from MPC group, indicating that MPC group was more effective in improving knowledge of IPM than other farmers' groups (Table 3).

Table 3. Knowledge on IPM of respondents involved in MPC and other farmers' groups

Activities	Range	MPC group (n 90)	Other group (n 90)	df	t-value
IPM knowledge scale	0-11	6.43 (1.45)	0.78 (0.89)	178	-11.12*
Heard about IPM	0-2	2.06 (0.92)	0.86 (0.36)	178	-17.36**
Define IPM	0-2	1.21 (0.58)	0.21 (0.43)	178	-19.32*
Merits of IPM	0-3	1.93 (0.83)	0.57 (0.51)	178	-6.21*
Use of pheromone traps	0-2	1.57 (0.75)	0.86 (0.36)	178	-5.27*
Aware of alternative pest management practices	0-3	1.35 (0.74)	0.64 (0.63)	178	-4.39**
Use of mulching	0-2	1.71 (0.61)	0.43 (0.51)	178	-7.23**

Source: Field Survey 2019

Values in parentheses (-) are standard deviations; * t-test significant at p<.05, ** t-test significant at p<.01.

3.9 Depth of knowledge about IPM technology by information sources

It has been affirmed that agriculture officer Group contributed the highest IPM knowledge scores but CBF, FFS and MPC also contributed to high scores. Neighboring farmer, field day, mass media, collection centers, agro-vets and cooperatives also have some impact on knowledge, but scores are not as high as agriculture officer group and CBFs. In the study of Maucery *et al.* in 2007 also had identified that FFS had contributed the highest IPM knowledge scores as compared to field day, mass media, pamphlets, etc.

Table 4. Depth of knowledge about IPM technology by information sources.

(Knowledge category was determined by the percentage of IPM question answered correctly by farmers)

IPM knowledge by category	Information Source									
	MPC	Mass media	CC	Field day	FFS	Neighbor	Officer	CBFs	Agrovets	Cops
Category I (0%)	0.0	4.4	0.0	3.5	0.0	16.7	0.0	1.2	11.5	13.5
Category II (1-25%)	15.5	32.3	32.3	27.5	14.5	43.3	0.0	6.5	48.4	39.4
Category III (26-50%)	27.3	42.4	44.6	38.5	16.3	31.4	11.2	17.4	31.6	36.5
Category IV (51-75%)	36.4	17.6	20.4	19.4	44.2	4.7	23.5	47.7	6.3	7.1
Category V (76-100%)	20.8	3.3	2.7	11.1	25.0	3.9	65.3	26.2	2.2	3.5

Source: Survey Data, 2019

Note: A pearson chi^2 test showed significant difference between information sources at 1% level.

3.10 Level of adoption of IPM Knowledge by information source

It has been revealed that out of 500 respondents, 41.5% of the respondents had moderately high to high level of adoption of IPM technology (Category IV and V), 38.2% of the respondents had low to moderate level of adoption (category II and III) and 20.4% of the respondents had not adopted IPM technology (category I) as shown in table 5. The majority of the high level adopters had received information of IPM technology from MPC (68.6%). Farmers

who were the member of MPC adopted more IPM technology rather than random farmers. MPC were associated with highest level of adoption. Category V adoption was mainly observed in MPC followed by agriculture officer, farmer field school, field day. The highest adoption rate in category IV was also observed in MPC, farmer field school, agriculture officer and field day (partially attributed to correspondingly high knowledge scores). Diffusion from neighboring farmer-farmer seemed to be less effective as both knowledge scores and level of adoption rates were lower. The lowest rates of adoption were observed in the farmers who had heard of IPM from cooperatives and claimed they had not received information on IPM. Neighboring farmers and cooperatives may lack the expertise to transfer IPM knowledge effectively. In the study of Maucery *et al.* in 2007 also had identified that FFS, field day participants were associated with highest level of adoption. When learning IPM technology, farmers show a preference for more experienced individuals. (Owens and Simpson, 2002)

Table 5. Level of adoption of IPM knowledge by information source

IPM knowledge by category*	Information Source								
	MPC	Mass media	Field day	FFS	Neighbor	Officer	Agro-vets	Cops	Total
Category I (0%)	2.5	27.6	12.6	16.4	32.6	9.2	19.6	42.6	20.4
Category II (1-25%)	11.3	24.2	19.5	13.2	21.6	17.4	29.3	27.2	20.5
Category III (26-50%)	17.6	19.2	19.2	14.4	16.7	14.6	18.2	21.3	17.7
Category IV (51-75%)	35.2	26.7	29.4	31.2	27.1	31.2	24.4	8.9	26.8
Category V (76-100%)	33.4	2.3	19.3	24.8	2	27.6	8.5	0	14.7
Total	100	100	100	100	100	100	100	100	100

Source: Survey Data, 2019

Note: A pearson chi^2 test showed significant difference between information sources at 1% level.

*Adopted categories are defined by % of IPM practices adopted

3.11. Farmers' perception about IPM Practice

The study revealed that 28.2% of the respondent affirmed that IPM practice is good for health as compared to conventional practice which confirms with the finding of Roy *et al.*, (2009).This may be due to the fact that IPM may discourages excess use of harmful chemicals which can cause health problems. 25.8% of the respondent said that it is eco friendly method, 19.7% of the respondent thought that it yields more as compared to conventional practice, 16.5% of the respondent had opinion that it takes more time to implement and 9.2% of the respondent had opinion about that it is more costly practice as compared to traditional practice. 0.7% of the respondents said that they do not know about beneficial effects of the IPM, which indicates that the IPM concept is still not clearly understood by farmers although IPM IL program was running over there. More awareness should be done in the study areas to encourage farmers to reduce harmful pesticide applications. Previous studies done by Allahyari *et al.* in 2016 and 2017 have also identified that improving farmers' technical knowledge of IPM by extension services can minimize excessive spraying of insecticide. Figure 7 depicts the percentage farmers' perception about IPM practices.

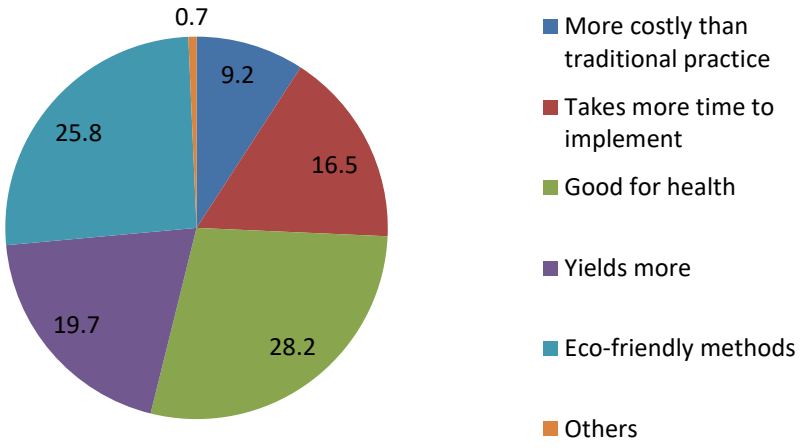

Fig.8: Farmers' opinion about IPM Practice

IV. CONCLUSION

Integrated Pest Management (IPM) is as a broad based approach for economic control of pests. A study on determination of level of adoption of IPM technology was conducted in the Banke and Surkhet districts of Nepal. For determining the spread of information and level of adoption, farmers were asked a series of questions during the survey to determine knowledge of IPM and degree of IPM adoption. Using descriptive statistics and differences in means, analysis was done on relationships among access to information, IPM knowledge and adoption. This study revealed that 51.13% of the population were male with an average family size was 6.45. Almost half of the respondents had adopted IPM practices for vegetable production by receiving advice or getting training about IPM technology through agriculture officer, Market Planning Committee, I/NGOs, etc. Market Planning Committee has played a vital role in spreading IPM knowledge and information quickly, followed by mass media, as MPCs allow farmers to aggregate smallholder produce to meet market demand. Several farmer organizations join together and elect representatives to serve on the board of the MPC and have regular monthly meeting so that they could discuss on IPM technology and marketing strategy of the products. MPC group has been identified more effective in improving knowledge of IPM than other farmers' groups. Farmers who were the member of MPC adopted more IPM technology rather than random farmers. MPC were associated with highest level of adoption.

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Risks and Adaptation Strategies for Disaster Readiness: Basis for School Sustainability Plan

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Abstract**—** *This study was conducted to determine the risks and adaptation strategies for disaster readiness as a basis for the school sustainability plan of eight (8) medium public elementary schools in Licab District, Nueva Ecija with the use of descriptive-correlational research design. A total of 103 employees served as respondents of the study.*

The yearly disasters encountered by the school employees are floods and typhoons. They are aware of flood risks thus, they always ensure the safekeeping of school records. As to their experience in a typhoon, "their understanding of the warning signal was very evident and always adopt measures of disaster prevention and facilitate the immediate resumption of classes. In their risk adaptation strategies, the respondents prepare the best response during an emergency, they know the emergency hotline numbers, they are aware of the emergency evacuation plan and they actively joined any disaster prevention, relief action, or emergency drill.

As to their knowledge about the disaster, the respondents always get information from TV or Radio, electronic messages (Mobile SMS, etc.), and social networking sites (e.g. Facebook, Twitter). In terms of their readiness, they claimed that they always: have medication and first aid kit ready and store emergency kits or Go bag (including documents, water, foods, flashlight or candle, radio, cellphone, rope and etc.). The risk adaptation strategies of the respondents were positively related to their disaster readiness which implies that activities such as conducting drills, emergency plans, and attending seminars and trainings increase the preparedness for a disaster of the respondents.

Keywords**— Adaptation strategies, disaster readiness, emergency, school employees, sustainability plan.**

I. INTRODUCTION

The Philippines is exposed to disasters and hazards [1] because of its geographical and geological position. "It has been ranked third (3rd) among 173 countries in terms of exposure to hazards, such as typhoon, earthquake, flooding, landslide, volcanic eruption, and tsunami, according to the World Risk Index 2012 released by the United Nations International Strategy for Disaster Reduction [2]". In the R.A. 10121 Orientation Seminar conducted by the Office of Civil Defense, it was presented that the Philippines' location along the Pacific Typhoon Belt makes it more prone to typhoons or hydro-meteorological hazards. Based on the 2002 to 2012 data of the National Disaster Risk Reduction and Management Council (NDRRMC), the country experiences an average of twenty (20) typhoons a year, and 50% of it has damaging effects on lives and properties. At the same time, the Philippines is also situated along the Pacific Ring of Fire which makes it susceptible to earthquakes and volcanic eruptions. These risks prompted the Philippine Disaster Management System to undertake a paradigm shift. From Presidential Decree 1566 to Republic Act 10121, from reactive to pro-active, from top-down and

centralized management to bottom-up and participatory disaster risk reduction process [3].

"Republic Act 10121, also known as the Philippines Disaster Risk Reduction and Management (DRRM) Act of 2010, is an Act focusing on strengthening the Philippine Disaster Risk Reduction and Management System". Through this Act, the National DRRM Framework (NDRRMF) and National DRRM Plan (NDRRMP) were developed. Both the NDRRMF and NDRRMP foresee a country which has "safer, adaptive and disaster-resilient Filipino communities toward sustainable development". Together with the paradigm shift is the creation of the four thematic areas namely; a) Disaster Prevention and Mitigation, b) Disaster Preparedness, c) Disaster Response, and d) Disaster Rehabilitation and Recovery [4].

The performance of each locality, city, or municipality in preventing the adverse impacts of hazards and related disasters, counts on the way local government units, in particular, have prepared for disaster possibilities in their respective jurisdictions. "Contingency planning is actually a fundamental tool, but a good plan cannot stand alone without having an empowered citizenry, infrastructures, emergency response mechanisms, rehabilitation, and other important logistics" [5]. According to authors in [6] "a focus on service-delivery is important in the local government because it represents the public face of the state and is where citizens' experience of government is derived on a day-to-day basis".

Therefore, this study was conducted to hopefully improve the current strategies and preparedness that are being implemented in the event that any disaster occurs. Also, to develop a new plan of strategies based on the findings and observations which can be useful for everyone. Through this, the respondents may be given sufficient, meaningful, and empirical basis in disaster readiness and adaptation strategies. Moreover, this may help enhance, enrich, and develop preparedness for disasters.

II. METHODOLOGY

This study used a descriptive-correlational research design with 103 personnel from eight (8) medium public elementary schools in Licab District, Nueva Ecija, Philippines served as respondents. According to [7], as cited by [8], "Correlational research is employed to test the degree of relationship between two variables". The researchers assessed the risks, adaptation strategies and disaster readiness of the schools using a Likert-scale questionnaire and the result served as a basis for the school sustainability plan. Frequency count, percentage, weighted mean, and correlational formulas were utilized to perform statistical computations of the data gathered.

III. RESULTS AND DISCUSSION

1.Annual Disaster Encountered by the Employees

The yearly disasters encountered by the school employees are floods and typhoons. According to the respondents, they are always aware of flood risks (WM=3.78), thus, always ensure the safekeeping of school records. As to their experience in a typhoon, "their understanding of the warning signal was very evident (WM = 3.85) and therefore, always adopt measures of disaster prevention and facilitate the immediate resumption of classes. The results indicate that respondents are always ready during floods and typhoons. This preparedness can be noted due to their experiences.

2. Schools Disaster Risk Reduction Management (DRRM) Adaptation Strategies

Table 1. School DRRM Adaptation Strategies

Conducting Mock Drills	Mean	*VD*
I prepare the best response during an emergency	3.65	*Always*
I am providing the best strategy without any panic	3.45	*Always*
I give an opportunity to practice emergency procedures in a simulated but safe environment	3.56	*Always*
I implement mock drill experiences	3.62	*Always*
I conduct mock exercise or emergency drill	3.58	*Always*
Overall Weighted Mean	***3.57***	***Always***

Emergency Evacuation Plan	Mean	*VD*
I am aware of the emergency evacuation plan	3.69	*Always*
I know the emergency hotline numbers	3.72	*Always*
I am aware of the local emergency plans	3.62	*Always*
I review any existing emergency plans to incorporate what's worked in the past	3.53	*Always*
I identify evacuation routes	3.57	*Always*
Overall Weighted Mean	***3.63***	***Always***
Seminars and Trainings	Mean	*VD*
I attend seminar/training/drills about disaster risk reduction management	3.46	*Always*
I actively joined any disaster prevention, relief action or emergency drill	3.50	*Always*
I increase my training and drills	3.47	*Always*
I conduct a risk assessment to prioritize various scenarios	3.44	*Always*
I conduct a relational training about disaster risk reduction management (DRRM)	3.44	*Always*
Overall Weighted Mean	***3.46***	***Always***

2.1 Conducting Mock Drills

The table shows that the overall weighted mean for the conduct of mock drills was 3.57, which meant that the respondents "always" apply these strategies inside the school. The data further revealed that the item "*I prepare the best response during an emergency*" had the highest weighted mean of 3.65 verbally interpreted as "always". The lowest item is on "*Providing best strategy without panic*" with a weighted mean of 3.45 which was also interpreted as "always". Preparedness activities have the potential to enhance response and improve operations as long as the context of disaster is properly considered, because different types of disasters require different types of the response given the varying features of each type of hazard [2].

2.2 Emergency Evacuation Plan

It can be gleaned from the table that the overall weighted mean was 3.63 and was verbally interpreted as "always". The data also reveals that the item "*I know the emergency hotline numbers*" had the highest weighted mean of 3.72 verbally interpreted as "always". followed by the item "*I am aware of the emergency evacuation plan*" WM 3.69. verbally interpreted as "always". As all the items indicated were interpreted as "always", it implies that all respondents know what to do during a disaster.

2.3 Seminars and Trainings

Exchanging information at all levels of the society through symposia, workshops, seminars, and training can increase the intensity of awareness and participation of the communities in the prevention and preparedness programmed [9]**.** The result showed that the item "*I actively joined any disaster prevention, relief action or emergency drill*" had the highest weighted mean of 3.50 verbally interpreted as "always". The donor base for disaster relief today is more diverse than ever before, and the amount of philanthropic funding being directed toward disaster relief has increased significantly. For a response to disasters to be as swift and efficient as possible, the financial aspect is very important. Information sharing can retain the skills learned and will make us ready when facing disaster [10].

3. Disaster Readiness

In terms of knowledge of about disaster, the respondents always get information from TV or Radio (WM =3.89), electronic messages (Mobile SMS, etc.) directly for individuals (WM=3.78), social networking sites (e.g. Facebook, Twitter) (WM=3.75) and official weather bulleting (WM=3.75). As to their readiness, the respondents claimed that they always: "*have medication and first aid kit ready (WM=3.74)*" and "*store emergency kits or Go bag*"

(including documents, water, foods, flashlight or candle, radio, cellphone, rope and etc.) (WM=3.72)".

4. Relationship between DRRM Adaptation Strategies and Disaster Readiness

Table 2 shows the relationship between disaster risk reduction management (DRRM) adaptation strategies and disaster readiness. This means that DRRM in terms of conducting mock drills, emergency evacuation plans and seminars and trainings are significantly related to disaster readiness and therefore, the null hypothesis was rejected.

Table 2. Relationship Between DRMM Adaptation Strategies and Disaster Readiness

DRRM Adaptation Strategies		Disaster Readiness
Conducting Mock Drills	r	**.552****
	p-value	**0.000**
Emergency Evacuation Plan	r	**.375****
	p-value	**0.000**
Seminars and Trainings	r	**.432****
	p-value	**0.000**

This implies that activities such as conducting drills, emergency plan and attending seminars and trainings related to disaster increases the readiness to a disaster of the respondents. This cluster of activities includes learning and practicing skills that may be useful during an emergency. Such activities include practicing "drop, cover, and hold on," practicing household evacuation plans, and learning how to assist special-needs populations such as infants, elderly individuals, and those with disabilities [9]. Promoting acquisition, storage, and maintenance of disaster survival items is often the focal activities in preparedness campaigns. Lists of essential supplies and equipment are available from several sources, including the Red Cross and government agencies [9]. Training should also be given to workers in the focused area where flood usually occurs during the rainy season. This initiative will also enhance the knowledge and make the workers aware of how to prevent and what are to be done in real situations.

5. Proposed Sustainability Plan

Rationale:

A proposed sustainability plan for disaster readiness is anchored on the result of the responses of the respondents of eight (8) medium public elementary schools in Licab District. In addition, the proposed plan entails the school profile, disaster risks, and DRRM adaptation strategies.

Proposed Sustainability Plan

OBJECTIVES	KEY ACTIVITIES	PERSON INVOLVED/ RESOURCES	TIME FRAME	SUCCESS INDICATOR
1. To demonstrate understanding and awareness of disaster readiness and its importance among employees	-Drop, Cover and Hold -Forums about disaster preparedness and disaster warning -DRRM Training	Teachers, School Head, Resource Speaker, and LGU	Quarter 1-2 S.Y 2020-2021	Integrate a more realistic perception of risks into their materials and curricula
2. To increase knowledge of protective behaviors.	Trainings and simulation exercises on disaster preparedness and response	Teachers, School Head, Resource Speaker, and LGU	Quarter 1-2 S.Y 2020-2021	Improves the skills of the employees to drills and participate in the accomplishment of their goal
3. To further increase understanding of disaster readiness and appropriate strategies for employees	-Seminar/ Training/ Drills about DRRM -Role-playing of response operations	Teachers, School Head, Resource Speaker, and LGU	Quarter 2-3 S.Y 2020-2021	Learning and practicing skills that may be useful during an emergency

4. To make an access plan that addresses the safety of everyone	-Identify evacuation routes -Identifying the hotline numbers -Emergency evacuation plan -Development or enhancement guidelines for emergency response teams	Teachers, School Head, and LGU	Quarter 2-3 S.Y 2020-2021	Makes a decision to evacuate or not depends on the perception of danger
5. To continue the assessment of the strengths and weaknesses of the sustainability plan	Risk assessment to prioritizing various scenarios	School Heads, Monitoring and Evaluation Coordinator and Teachers	Quarter 3-4 S.Y 2020-2021	To become prepare on how they can protect themselves, and the cost of various readiness actions
6. To strengthen further the partnership and coordination among all key players and stakeholders	Formulate coordination mechanism and guidelines for partnership arrangements in the contingency plan	Stakeholders, Teachers, and School Heads	Quarter 1-4 S.Y 2020-2021	Partnerships arrangement among stakeholders established

IV. CONCLUSIONS AND RECOMMENDATIONS

In the light of the findings, the following conclusions and recommendations were drawn: The respondents were always prepared for a disaster such as: being aware of the flood risks and also understand the typhoon warning signal. The respondents always apply the Disaster Risk Reduction Management (DRRM) Adaptation Strategies such as: preparing the best response during an emergency, knowing the emergency hotline numbers, and actively join any disaster prevention, relief action, or emergency drill. In Disaster Readiness, they follow the official weather bulletin and having medication and first aid kit ready. There is a significant relationship between disaster risk reduction management (DRRM) adaptation strategies and disaster readiness. Based on the summary of findings and conclusions, the following recommendations are offered: 1). Employees must continue to be more creative, innovative, and resourceful in devising improvised materials to help promote effective readiness in case of disaster. 2.) Teachers must continue to be guided and be familiar with various kinds of adaptation strategies that they can use inside and outside the classroom. Seminars, workshops, and in-service trainings should continue to be provided by the school heads to uplift employees' disaster readiness and appropriate coordination with community and government authorities, and a better flood prevention plan should be implemented by the Local Government Units [11].

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Potential of salicylic acid rhizobacteria indigenous chili which is able to suppress Bacterial

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Abstract**—***Plant growth promoting rhizobacteria are a group of bacteria that actively colonize plant roots, increase plant growth and control plant pathogens. Some strains of rhizobacteria can produce salicylic acid and are responsible for the induction of ISR in plants. Salicylic acid is widely recognized as the key to plant resistance. This study aims to analyze the potential of salicylic acid from indigenous rhizobacteria isolates from the roots of chili plants. Rhizobacteria isolates were isolated from the roots of healthy chili plants and endemic bacterial wilt disease. The isolation results were tested in planta for the ability to control bacterial wilt disease (Ralstonia solanacearum). Salicylic acid levels were analyzed using the HPLC method. Results of the test twenty chili ingenus rizobakteria isolates were able to suppress the attack of bacterial wilt. The analysis showed that only nine of the twenty isolates contained salicylic RZ.1.1.AG4, RZ.1.1AP1, RZ.1.2.AP1, RZ.1.3.AG4, RZ.2.1.AG1, RZ.2.1.AP1, RZ.2.1.AP3, RZ.2.1.AP4, dan RZ.2.2.AG2. Eleven other isolates namely namely RZ.1.3.AP1, RZ.1.4AG4, RZ1.4.AP4, RZ.2.1.AP2, RZ.1.5.AP4, RZ.2.3AG4, RZ.2.1.AG3, RZ.2.2.AG4, RZ.2.5.AP4, RZ.2.1.AG2 and RZ.2.3.AP1 do not produce salicylic acid. The highest concentration of salicylic acid from isolate RZ.1.1.AG4 at 20,95 ppm followed by RZ.1.1AP1 at 19.27 ppm, RZ.1.2.AP1 at 18.05 ppm, RZ.1.3.AG4 at 16.96 ppm, RZ.2.1.AG1 at 15.45, RZ.2.1.AP1 at 15.25, RZ.2.1.AP3 at 14.28, RZ.2.1.AP4 at 14.09, and RZ.2.2.AG2 at 13.45 ppm.*

Keywords**—** ***bacterial wilt disease, HPLC, rhizobacteria, salicylic acid.***

I. INTRODUCTION

Bacterial wilt disease caused by *Ralstonia solanacearum* is one of the most dangerous plant diseases that is widespread in the tropics and subtropics, and attacks many agricultural crops including tomatoes, peanuts, bananas, potatoes, tobacco and other *Solanaceae* tribes (Persley, 1985). Attack R. solanacearum on chilies can cause losses ranging from 0.8-10% (Simanjuntak *et al.*, 2014). Bacterial wilt disease is difficult to control because these bacteria are classified as soil-borne pathogens and have a wide host range, high genetic diversity (Suryadi and Machmud, 2002). Control *R. solanacearum* which have been recommended include the use of pathogen-free soil, crop rotation with resistant crops and non-host plants (Gnanamanickam, 2006), and the use of bactericides such as streptomycin (Rahaju and Sucahyono, 2000). However, these control techniques are still ineffective. As an alternative to control Xag is with microorganisms as biocontrol agents (Manuela, *et al.*, 1997), like a group Plant growth promoting rhizobacteria (PGPR). PGPR is a heterogeneous group of bacteria in the rhizosphere complex, on the root surface (rizoplan) and associated in the root (endophyte) (Soesanto, 2008)

The plant rhizosphere is home to various species of bacteria which are generally known as rhizobacteria. Microorganisms that have been widely reported to be capable of being biocontrol agents are the group plant growth promoting rhizobacteria (plant growth promoting rhizobacteria) known as PGPR. PGPR has the ability to antagonize plant pathogens in several ways, namely the production of antibiotics, siderophores, chitinase enzymes, β-1,3-glucanase, cyanide, parasitism, competition for nutritional sources and ecological niches and induces systemic plant resistance (Fernando *et al*., 2005). Besides

being able to control plant pathogens, PGPR is also a group of bacteria that can improve the quality of plant growth either directly or indirect (Joseph *et al*., 2007). PGPR is capable of producing or changing the concentration of plant hormones such as indolasetic acid (indoleasetic acid = IAA), gibberellic acid, cytokinins, and ethylene or their precursors (1-aminocyclopropene 1 carboxylate deaminase (ACC deaminase)) in plants, are not symbiotic in N2 fixation and dissolve phosphate. Rhizobacterial isolates can function as growth promoters or plants Plant Growth Promoting Rhizobacteria (PGPR) and as an antagonist against plant pathogens (Timmusk, 2003).

PGPR is a heterogeneous group of bacteria found in the rhizosphere complex on the root surface and associated in the roots, which can improve the quality of plant growth directly or indirectly (Joseph *et al*., 2007). The application of PGPR to the rhizosphere is closely related to its ability to colonize plant roots. Due to the activeness of root colonization, roots absorb microbial products which directly affect root growth and physiology, in addition to influencing pathogen invasion (Soesanto, 2008). Colonization by PGPR can occur through seed cloaking or the addition of a PGPR suspension into the soil at the time of planting (Kloepper and Tuzun, 1996). Rhizobacteria can be applied through seed covering, soaking the seeds in suspension, and watering the PGPR suspension into the soil (Widodo, 2007). The ability of biological agents to control pathogens when applied in the field depends on factors, among others (1) The host plant must provide a suitable environmental niche and dissolved and evaporated nutrients from root, seed, flower, and leaf exudates required for the production of antibiotics for RB, (2) Dry environmental conditions, and (3) The plant surface is not covered by a hydrophobic wax layer (Soesanto, 2008).

Salicylic acid (SA) has an important role in plant defense signaling networks (Pieterse *et al*., 2012). Salicylic acid in general can be known to play a role in plant resistance to disease, including basal resistance, effects trigger immunity and induction of systemic resistance (Induce Systemic Resiatance = ISR) (Vlot *et al*, 2009). In general, it can be stated that biotropic pathogens are more sensitive to the induction of salicylic acid defense, whereas herbivorous pathogens and insects are necrotrophic by the induction of jasmona acid defense. Other growth regulating hormones, such as ethylene, abscisic acid, gibberellin, auxins, cytokinins, and brassinosteroids, are also involved in the regulation of network signaling immune or plant resistance (Pieterse *et al*, 2012), this activity shows that the regulators of plant growth and defense are closely related to signal transduction (Rasmunsoll., *et al* 2013)

This research has analyzed the potential of indigenic rhizobacteria of chili roots to produce salicylic acid from soybean plants. Indigenous rhizobacteria isolates are selected isolates that have been tested for their ability to suppress attacks (*Ralstonia solanacearum*) causes bacterial wilt disease in chili plants. The research objective was to analyze the potential of indigenic rhizobacteria isolates of chili roots in producing salicylic acid in plants.

II. METHOD OF RESEARCH

2.1 Time and Location of Research, and Sampling Method

The research was conducted at the Laboratory of Plant Bacteriology, Department of Plant Pests and Diseases, Greenhouse, Faculty of Agriculture, Andalas University, Laboratory of Natural Material Chemistry, Faculty of Pharmacy, Andalas University. The research took place from July to September 2020.

2.2 Ability Test of Rhizobacteria Isolates to Control Bacterial Wilt Disease in Planta

Selection is carried out in planta using a completely randomized design (CRD). The treatment used was the introduction of 42 RBI isolates on chili seeds with 3 replications. Rhizobacteria isolates introduced to chili and control plants. The data were analyzed by using variance, if they were significantly different, it was followed by testing Least Significance Difference (LSD) at the 5% real level.Root samples of chili plants from endemic areas of bacterial wilt disease (Nagari Sungai Nanam, Lembah Gumanti District, Solok District and Nagari Taluak IV Suku, Banuhampu District Agam Regency), with criteria: 2-3 months old, fruitful and healthy. RBI was isolated by serial dilution, 1 g of roots was washed with distilled water, sterilized the surface with 70% alcohol for 1 minute, rinsed again with sterile distilled water and macerated. The root suspension is diluted to 10- 6, 0.1 ml suspension from a 10- dilution 5 and 10- 6 cultured on Nutrient Agar (NA) media and incubated at room temperature for 48 hours. Indigenous rhizobacteria isolates were selected with the following criteria: dominant colonies with different morphologies, purified on the media and incubated in the same way. Single colonies of indigenous rhizobacterial isolates were transferred inside microtube contains 1 ml of sterile distilled water and stored in the refrigerator.

Indigenus rhizobacteria isolates were rejuvenated on NA medium and incubated for 48 hours. For

introduction to seeds, indigenous rhizobacterial isolates were reproduced on the media in the same way as rejuvenation. Indigenous rhizobacterial cultures were suspended with sterile distilled water, the population density was determined by comparison with the solution McFarland scale 8 (bacterial population 10 8 sel / ml) (Yanti and Resti, 2010). For introduction to plants, indigenous rhizobacteria are reproduced in Nutrient Broth (NB). To preculture, 1 indigenous endophytic bacterial colony was transferred to 25 ml NB medium and incubated 18 hours on rotary shaker horizontal at 150 rpm. To mainculture, 1 ml of rhizobacterial suspension indigenus from preculture transferred into 150 ml NB and incubated for 48 hours in the same way (Trisno, 2010). Indigenous rhizobacteria suspension from mainculture population in the same way as seeds.

The planting medium is a mixture of soil and sterile manure (2: 1 v / v). The chili seeds used are varieties from the same location as the source of the indigenous rhizobacteria isolates. The germination of the seeds was tested by method Standard Germination Test (96%). Chili was introduced with rhizobacteria isolates indigenus twice, namely: 1). In the seeds, the seeds were sterilized with 1% NaOCl, washed with sterile distilled water and dried. The seeds were immersed in a suspension of rhizobacterial isolates indigenus 10 minutes and planted in polybags. 2) For chilies 3 weeks after planting (mst), by pouring 10 ml of rhizobacterial suspension on the soil. Chili plants Inoculation of bacteria was carried out by inserting a bacterial suspension with a bacterial population density of 106 CFU / mL using an inoculation needle at the base of the 35 day old chili seedling. The variables observed were the development of bacterial pustules (incubation period, incidence and severity of leaves). The percentage of disease detection is determined by a formula:

$$E = K - P / K \times 100\%$$

where: E = percentage of emphasis, P = treatment, K = control

2.3 Production of salicylic acid in plants introduced by rhizobacteria

Chili plants (roots) that showed a resistant reaction to bacterial wilt were tracked for theirv salicylic acid levels using the method (Chen et al, 1995) which is modified. Comparators were used as well as controls (without rhizobacteria). 1 g of chili soybean root homogenized with liquid nitrogen in a culture tube measuring 10 x 130 mm, the frozen tissue was washed with 2.5 ml 90% metaol, sonicated for 20 minutes and centrifuged at 2800 g. The pellets were extracted again with 2 ml 100% methanol. The supernatant from the extraction was dried using a liquid nitrogen stream, the residue was suspended in 2 ml 5% Trichlor acetid acid (TCA) and centrifuged at 2500 g for 15 minutes. The supernatant was separated twice with the extraction medium (ethyl acetate: cyclopentane: isopropanol with a ratio of 100: 99: 1). The upper phase is combined and dried with liquid nitrogen. The residue was suspended in 1 ml 20% methanol in 20 mM sodium acetate buffer solution, the solution was filtered using a nylon membrane with a pore of 0.2 μm through a vacuum under pressure of 250 mm Hg. Samples were stored at -20 0 C, and ready to be analyzed by HPLC. The salicylic acid extract was measured by a spectrophotometer and its spectrophotometer was compared with standard salicylic acid (Sigma).

III. INDENTATIONS AND EQUATIONS

46 isolates of ingenus rhizobacteria from chili roots were obtained. Indogenous endophytic bacteria isolates of various colony morphologies. The 46 isolates were tested in planta to see the ability of each isolate in suppressing the attack of bacterial pustules in the greenhouse. Symptoms of an attack *R. solanacearum* characterized by early symptoms in the form of wilting in chili plants starting from the top leaves. Advanced symptoms are characterized by wilting of all plant leaves. Chili plants that were introduced with RBI isolates showed a suppression of the incubation period R. solanacearum which differed significantly among all treatments. The incubation period of bacterial wilt disease in the treatment of each RBI isolate varied between 11.33 days to 27.33 days after inoculation (hsi) compared to negative controls, namely 12.67 his (Table 1). From the observations, there were 13 RBI isolates that did not show any symptoms until the end of the observation (42 dd) with effectiveness 233.33% compared to negative controls. The isolates were RZ.1.1.AG4, RZ.1.1.AP1, RZ.1.2.AP1, RZ.1.3.AG4, RZ.1.3.AP1, RZ.1.4.AG4, RZ.1.4.AP4, RZ.2.1.AG1 , RZ.2.1.AP1, RZ.2.1.AP2, RZ.2.1.AP3, RZ.2.1.AP4 and RZ.2.2.AG2.

Chili plants introduced with RBI isolates showed a decrease in the incidence of bacterial wilt disease (Table 1). The introduction of RZ.1.5.AP4 isolates and RZ.2.3.AG4 isolates can reduce disease incidence by up to 67% with an effectiveness of 33%. RBI isolates that are able to reduce the incubation period *R. solanacearum* able to reduce the incidence of disease up to 0% with 100% effectiveness (RZ.1.1.AG4, RZ.1.1.AP1, RZ.1.2.AP1, RZ.1.3.AG4, RZ.1.3.AP1, RZ.1.4.AG4, RZ. 1.4.AP4,

RZ.2.1.AG1, RZ.2.1.AP1, RZ.2.1.AP2, RZ.2.1.AP3, RZ.2.1.AP4 and RZ.2.2.AG2). The introduction of RBI isolates can reduce the severity of bacterial wilt disease in chili plants (Table 1). The disease severity level of each isolate can be seen in Table 4. Isolates RZ.2.3.AG4 and RZ.1.5.AP4 showed disease suppression with an effectiveness of 33%. RBI isolates that were able to suppress the incubation period and disease incidence were the best (isolates RZ.1.1.AG4, RZ.1.1.AP1, RZ.1.2.AP1, RZ.1.3.AG4, RZ.1.3.AP1, RZ.1.4. AG4, RZ.1.4.AP4, RZ.2.1.AG1, RZ.2.1.AP1, RZ.2.1.AP2, RZ.2.1.AP3, RZ.2.1.AP4 and RZ.2.2.AG2) also did not show an emphasis on severity. disease up to a scale of 0 (no symptoms) until the end of the observation with 100% effectiveness and was able to reduce the severity of the disease until the end of the observation (42 dd).

The results of this study are in line with Sutariati's research et al. (2006), that is reported the use of PGPR was able to trigger seed viability and growth of chili plant seeds. The results of the study (Rahni, 2012) also showed an increase in growth in maize and sorghum (Sutariati et al., 2011) and Jatropha (Wibowo, 2010). The ability of rhizobacterial isolates to increase plant growth has been widely reported (Taufik et al., 2005). Currently, rhizobacteria are increasingly being developed, especially in an effort to increase horticultural production and improve the quality of the environment. Rhizobacteria are ideal for use as biocontrol agents because rhizobacteria can serve as the front line defense for plant roots against various pathogens (Pal & Jalali, 1998).

The use of RBI to increase emphasis on pathogen development has been developed. RBI isolates that have been reported to be effective in increasing plant resistance include Isolate P12Rz2.1. and P14Rz1 isolate was able to control bacterial pustules (Yanti *et al*. 2013), ST26c isolate was able to suppress Phytophthora capsici in chili plants (Khaeruni *et al*., 2011), RB.2.4 isolate was able to reduce the incidence and severity of wilt disease fusarium on tomatoes (Khaeruni *et al*. 2013), PKLK5 and P11a isolates were able to suppress Xanthomonas oryzae pv. oryzae in rice plants (Khaeruni *et al*, 2014b), CRb-26, CRb-39, CRb-17, CRb-9 and CRb-14 isolates were able to increase germination, growth and suppress blight in cotton (Mondal *et al*., 1999) and isolates S188, s215 and s288 were able to control R. solanacearum on the tomato plant (Ramadasappa *et al*., 2012).

The ability of 20 rizobacteria isolates to produce salicylic acid and the resulting salicylic acid concentration can be seen in Table 2. nine of the twenty isolates tested were able to produce salicylic acid and the other eleven isolates did not produce salicylic acid Table 2. The isolates were RZ.1.1.AG4, RZ.1.1AP1, RZ.1.2.AP1, RZ.1.3.AG4, RZ.2.1.AG1, RZ.2.1.AP1, RZ.2.1.AP3, RZ.2.1.AP4, and RZ.2.2.AG2 were shown with electrophoregram peaks at relatively similar migration times with standard salicylic acid. The other four isolates, namely RZ.1.3.AP1, RZ.1.4AG4, RZ1.4.AP4, RZ.2.1.AP2, RZ.1.5.AP4, RZ.2.3AG4, RZ.2.1.AG3, RZ.2.2.AG4, RZ.2.5.AP4, RZ.2.1.AG2 and RZ.2.3.AP1 were unable to produce salicylic acid.

The existence of isolates that were successfully analyzed for their salicylic acid content proved that indigenous rhizobacteria of chili roots were able to produce salicylic acid (Table 2). Isolate RZ.1.1.AG4 at 20,95 ppm followed by RZ.1.1AP1 at 19.27 ppm, RZ.1.2.AP1 at 18.05 ppm, RZ.1.3.AG4 at 16.96 ppm, RZ.2.1.AG1 at 15.45, RZ.2.1.AP1 at 15.25, RZ.2.1.AP3 at 14.28, RZ.2.1.AP4 at 14.09, dan RZ.2.2.AG2 at 13.45 ppm. The high content of salicylic acid in ST4E1.1 isolates was related to the level of plant resistance to bacterial pustules. According to Silverman *et al.* (1995) that plant resistance has a positive correlation with salicylic acid content. Furthermore, Kloepper & Ryu (2006) stated that the colonization of endophytic bacteria Bacillus pumilus SE34 on Arabidopsis triggering the induction of systemic resistance to *Pseudomonas syringae* pv. *Maculicola* that associated with the salicylic acid pathway. Salicylic acid production has an important role in the transduction signal pathway that triggers the induction of systemic resistance and is associated with the accumulation of PR proteins (pathogenesis-related), like PR1 (Lyon 2007). Salicylic acid production by P. aeruginosa 7NSK2 plays an important role in the induction of resistance *Botrytis cinerea*. Mutants of the same strains that do not produce salicylic acid have lost their ability to induce systemic resistance in green beans (De Meyer and Hofte, 1997). *P. fluorescens* Natural CHAO produces salicylic acid under iron-limited conditions and also induces ISR in tobacco against TMV (Tobacco Mosaid Virus).

IV. FIGURES AND TABLES

Table 1. Incubation period of bacterial wilt disease introduced with several isolates RBI

Isolate	Period Incubation (hsi)	Effectiveness (%)	disease Incident	Effectiveness (%)	Severity	Effectiveness (%)
RZ.1.1.AG4	42.00*a	233.33	0	100	0 c	100
RZ.1.1.AP1	42.00*a	233.33	0	100	0 c	100
RZ.1.2.AP1	42.00*a	233.33	0	100	0 c	100
RZ.1.3.AG4	42.00*a	233.33	0	100	0 c	100
RZ.1.3.AP1	42.00*a	233.33	0	100	0 c	100
RZ.1.4.AG4	42.00*a	233.33	0	100	0 c	100
RZ.1.4.AP4	42.00*a	233.33	0	100	0 c	100
RZ.2.1.AG1	42.00*a	233.33	0	100	0 c	100
RZ.2.1.AP1	42.00*a	233.33	0	100	0 c	100
RZ.2.1.AP2	42.00*a	233.33	0	100	0 c	100
RZ.2.1.AP3	42.00*a	233.33	0	100	0 c	100
RZ.2.1.AP4	42.00*a	233.33	0	100	0 c	100
RZ.2.2.AG2	42.00*a	233.33	0	100	0 c	100
RZ.1.5.AP4	27.33b	116.93	67	33	2 b	33
RZ.2.3.AG4	26.33b	108.99	67	33	2 b	33
RZ.2.1.AG3	21.67** b	71.96	100	0	3 a	0
RZ.2.2.AG4	21.00**b	66.67	100	0	3 a	0
RZ.2.5.AP4	20.33**bc	61.38	100	0	3 a	0
RZ.2.1.AG2	19.67**bc	56.08	100	0	3 a	0
RZ.2.3.AP1	11.33**d	0.00	100	0	3 a	0
Kontrol-	12.67**cd	100	0		3 a	

* Figures followed by the same lowercase letter in the same row are not significantly different according to LSD at the 5% level.

* The incubation period of 42 days showed no symptoms until the last observation day.

* * * All repetitions in the treatment were off so that data analysis was not continued on the parameters further observations of these isolates.

Table 2: Salicylic acid concentration of selected indigenous rhizobacteria isolates suppress bacterial wilt disease in chili plants

Isolate code	Ability produce acid salicylates	Salicylic acid concentration (ppm)
RZ.1.1.AG4	+	20.95
RZ.1.1.AP1	+	19.27
RZ.1.2.AP1	+	18.05
RZ.1.3.AG4	+	16.96
RZ.1.3.AP1	-	-

RZ.1.4.AG4	-	-
RZ.1.4.AP4	-	-
RZ.2.1.AG1	+	15.45
RZ.2.1.AP1	+	15.25
RZ.2.1.AP2	-	-
RZ.2.1.AP3	+	14.28
RZ.2.1.AP4	+	14.09
RZ.2.2.AG2	+	13.45
RZ.1.5.AP4	-	-
RZ.2.3.AG4	-	-
RZ.2.1.AG3	-	-
RZ.2.2.AG4	-	-
RZ.2.5.AP4	-	-
RZ.2.1.AG2	-	-
RZ.2.3.AP1	-	-
Kontrol-	-	-

V. CONCLUSION

Results of the test twenty chili ingenus rizobakteria isolates were able to suppress the attack of bacterial wilt. The analysis showed that only nine of the twenty isolates contained salicylic RZ.1.1.AG4, RZ.1.1AP1, RZ.1.2.AP1, RZ.1.3.AG4, RZ.2.1.AG1, RZ.2.1.AP1, RZ.2.1.AP3, RZ.2.1.AP4, dan RZ.2.2.AG2. Eleven other isolates namely namely RZ.1.3.AP1, RZ.1.4AG4, RZ1.4.AP4, RZ.2.1.AP2, RZ.1.5.AP4, RZ.2.3AG4, RZ.2.1.AG3, RZ.2.2.AG4, RZ.2.5.AP4, RZ.2.1.AG2 and RZ.2.3.AP1 do not produce salicylic acid. The highest concentration of salicylic acid from isolate RZ.1.1.AG4 at 20,95 ppm followed by RZ.1.1AP1 at 19.27 ppm, RZ.1.2.AP1 at 18.05 ppm, RZ.1.3.AG4 at 16.96 ppm, RZ.2.1.AG1 at 15.45, RZ.2.1.AP1 at 15.25, RZ.2.1.AP3 at 14.28, RZ.2.1.AP4 at 14.09, dan RZ.2.2.AG2 at 13.45 ppm.

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Effectiveness of AM Fungi to Increase The Growth and Production of Peanuts Plant Infected by *Sclerotium rolfsii*

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Abstract— *Arbuscular Mycorrhizal Fungi (AM Fungi) are known to have the potential to increase plant growth. This study aims to find the best isolates of Indigenous Arbuscular Mycorrhizal Fungi (AM Fungi) that can increase the growth and production of peanuts plant infected by Sclerotium rolfsii caused stem rot disease. The method used is an experimental method with a Completely Randomized Design with 5 treatments, and 5 replication, namely A: AM Fungi Glomus sp-3 + S. rolfsii; B: AM Fungi Acaulospora sp + S. rolfsii; C: AM Fungi Gigaspora sp + S. rolfsii; D: Combined AM Fungi Glomus sp-3, Acaulospora sp, and Gigaspora sp + S. rolfsii; E: Without AM Fungi + S. rolfsii (Control). The data were analyzed using Analysis of Variance (ANOVA) using the Statistix 8 program and the Least Significance Different (LSD) test at a 5% significance level. The results showed that the Isolates of In general, Glomus sp-3 was able to increase the growth and production of peanuts.*

Keywords— ***Acaulospora, Gigaspora, Glomus, indigenous.***

I. INTRODUCTION

Indonesia's peanut productivity in 2020in 2011, 2012, 2013, 2014 and 2015 tended to fluctuate, namely 1,281 tons / ha, 1,274 tons / ha, 1,352 tons / ha, 1,279 tons / ha and 1,333 tons / ha, respectively. Meanwhile, productivity in West Sumatra was 1.509 tonnes / ha, 1.407 tonnes / ha, 1.540 tonnes / ha, 1.362 tonnes / ha and 1.459 tonnes / ha (BPS, 2018). This productivity is still low compared to its potential productivity which can reach 2.5 - 3 tons / ha (Rahmianna et al., 2015). One of the factors causing the low productivity of peanuts is the presence of plant diseases.

Stem rot caused by *S. rolfsii* fungus is the most detrimental disease (able to reduce pod yield by 60%) (Kator *et al.*, 2015). Therefore it is necessary to take control measures that are environmentally friendly besides being able to increase crop production. one of them is by using indigenous Arbuscular Mycorrhizal Fungi (AM Fungi) (Rahman et al, 2017).

AM Fungi is found in almost all habitats throughout the world and can associate with many plants (INVAM, 2020). Mycorrhizal fungi are one of the living organisms that can increase plant growth and development, reduce stress, remediate soil pollution, help C absorption, and increase plant resistance. Mycorrhizal fungi help plants absorb nutrients by expanding the network of mycorrhizal hyphae in the rhizosphere. Mycorrhizal inoculation changes the root architecture so that the nutrient absorption capacity of the inoculated roots is much better than those that are not inoculated (Ortas and Rafique, 2017).

The study aimed to obtain Indigenous Arbuscular Mycorrhizal Fungi (AM Fungi) isolates that can increase the growth and production of peanuts attacked by *Sclerotium rolfsii* which causes stem rot disease

II. MATERIAL AND METHOD

2.1. Time and Location of Research

This research has been carried out from July 2019 to April 2020 in the greenhouse and the Phytopathology Laboratory, Faculty of Agriculture, Andalas University.

2.2. Research Materials

Local cultivar (*Arachis hypogeae* L.) was used as plant material. *S. rolfsii* isolated from naturally infected peanut plants was maintained on the Potatoes Dextrose Agar (PDA) were then cultured on Corn Meal Sand (CMS) media. This study used four indigenous AM Fungi isolates namely A (*Glomus* sp-3), B (*Acaulospora* sp), C (*Gigaspora* sp), D (Combined *Glomus* sp-3, *Acaulospora* sp, and *Gigaspora*).

2.3. Experiment Details and Statistics Analysis

Experiment design in this study was Completely Random Design (CRD) with 5 treatments and 5 replication. Data were analyzed using Statistix 8 program. Analysis of variance (ANOVA) was used to determine the treatment effects and the differences between treatments were determined using LSD Test on 5%. Treatments were as follow A : AM Fungi *Glomus* sp-3 + *S. rolfsii*; B: AM Fungi *Acaulospora* sp + *S. rolfsii*; C: AM Fungi *Gigaspora* sp + *S. rolfsii*; D: Combined AM Fungi *Glomus* sp-3, *Acaulospora* sp, and *Gigaspora* sp + *S. rolfsii*; E: Without AM Fungi + *S. rolfsii* (Control).

2.4. Experiment Procedure

The planting medium is a mixture of ultisol soil with manure (2:1 v/ v). The mixture medium then was mashed and sieved. The mixture is sterilized for 2 hours at 100ºC and then dried at room temperature for 1 day. The medium was put into 45 x 50 cm polybags. AM Fungi inoculum (100 spores/plant) was introduced at planting time (seeds are ± 7 days old after germinated). Synthetic fertilizer was applied to the planting medium with a half recommendation dose (Urea 0.1 g / polybag, TSP 0.2 g / polybag, and KCl 0.2 g / polybag). Eight weeks after introducing AM Fungi, all plant was inoculated with 50 g inoculum of *S. rolfsii.*

2.5. Observations

2.5.1. Plant height

Plant height measurements are carried out from the base of the marked stem to the highest growth point. Plant height measurements were carried out at the age of 30, 37, 44, 51, 58, 65 and 72 DAP.

2.5.2. Number of leaves

The number of leaves was counted from leaves that opened completely and were done by counting the total number of peanut leaves in each plant sample at the age of 30, 37, 44, 51, 58, 65, and 72 DAP.

2.5.3. Number of branches

The calculation of the number of branches is done by counting all clump branches of each sample plant at the age of 30, 37, 44, 51, 58, 65, and 72 DAP.

2.5.4. Number and weight of pods

Each plant per treatment was counted the number and weight of pods by shedding the pods from the plant, then counted and weighed and differentiated which treatment was the best.

The effectiveness of each AM Fungi treatment on the number and weight of peanut pods was calculated using the formula:

$$EP = \frac{NM - NC}{NM} \; x \; 100\ \% \qquad (1)$$

EP = The effectiveness of peanut growth (number of pods)

NM = number of pods in plants with mycorrhizal treatment

NC = Number of pods in control plants

$$EP = \frac{PM - PC}{PM} \; x \; 100\ \% \qquad (2)$$

EP = effectiveness of peanut growth (pod weight)

PM = pod weight in plants with mycorrhizal treatment

PC = pod weight in control plants

2.5.5. Canopy fresh weight and dry weight

Each plant (canopy to root neck) per treatment was weighed to obtain fresh weight. Then it was dried using an oven at 70ºC until the weight was constant and then weighed (to get dry weight).

2.5.6. Root length

Each plant root (neck to root) per treatment was measured using a ruler. Plant roots are measured when the plants are finished harvesting.

2.5.7. Root fresh weight and dry weight

Each plant root (neck to root) per treatment was weighed to obtain fresh weight. Furthermore, the roots of the plants were dried using an oven at 70ºC until the weight was constant and then weighed (to get dry weight).

The effectiveness of each AM Fungi treatment on fresh weight and dry weight of peanut plant shoots and roots was calculated using the formula:

$$EP = \frac{WM - WC}{WM} \times 100\ \% \quad (3)$$

EP = effectiveness of peanut growth (fresh weight)

WM = weight of fresh roots / plant canopy in mycorrhizal treatment

WC = weight of fresh roots / plant canopy of plants in control

$$EP = \frac{DM - DC}{DM} \times 100\ \% \quad (4)$$

EP = effectiveness of peanut growth (dry weight)

DM = Dry weight of roots/plant canopy in mycorrhizal treatment

DC = Dry weight of the roots/canopy of the plant in the control

III. RESULTS AND DISCUSSION

Plant Height

Combined indigenous AM Fungi was able to increase plant height with the effectiveness of 31.42%. The administration of AM Fungi *Glomus* sp-3 showed results that were not significantly different than the combine with an effectiveness of 15.01% but also not significantly different from other isolates and also controls (Table 1). The comparison of the growth of peanut plants can be seen in Figure 1.

Table 1. Height of peanut plants (72 DAP)

Treatment	Height of plant (cm)		Effectiveness (%)
D (Combine)	51.36	a	31.42
A (*Glomus* sp-3)	45.98	ab	15.01
B (*Acaulospora* sp)	43.02	b	9.16
C (*Gigaspora* sp)	39.46	b	0.96
E (Control)	39.08	b	0.00
Cv = 12.75			

The numbers followed by the same lowercase letters in the same column indicate no significant results among treatments at 5% level by LSD test

3.2. Number of leaves

The number of peanut leaves with the treatment of several types of indigenous AM Fungi can be seen in Table 2. *Glomus* sp-3 indigenus AM Fungi was able to increase the number of leaves with an effectiveness of 39.88% and AM Fungi *Acaulospora* sp also increased the number of leaves with 22.83% effectiveness, however, has not given significantly different results compared to other isolates and also controls.

Table 2. Number of peanut leaves at 72 DAP.

Treatment	Number of leaves (sheet)		Effectiveness (%)
A (*Glomus* sp-3)	32.60	a	39.88
B (*Acaulospora* sp)	25.40	ab	22.83
D (Combine)	24.00	b	18.33
C (*Gigaspora* sp)	22.60	b	13.27
E (Control)	19.60	b	0.00
Cv = 22.57			

The numbers followed by the same lowercase letters in the same column indicate no significant results among treatments at 5% level by LSD test

3.3. Number of branches

The number of peanut branches can be seen in Table 3. The provision of various types of indigenous AM Fungi can increase the number of peanut branches with an effectiveness of 21.43 - 50%. AM Fungi *Glomus* sp-3 gave the best results in increasing the number of peanut branches with 50% effectiveness.

3.4. Number and weight of pods

The number and weight of peanut pods in each treatment can be seen in Tables 4 and 5. Applications of various types of indigenous AM Fungi can increase the number and weight of peanut pods (Tables 4 and 5). The highest number of pods was found in the provision of AM Fungi *Glomus* sp-3, namely 5.40 with 51.85% effectiveness and the highest pod weight was also found in the provision of AM Fungi *Glomus* sp-3, namely 12.35 grams with an effectiveness of 53.04% compared to the control.

Table 3. Number of peanut branches (72 DAP) (Transformation to √ (x + 1)).

Treatment	Number of branches	Transformation to $\sqrt{x+1}$)		Effectiveness (%)
A (*Glomus* sp-3)	4.40	2.3214	a	50.00
B (*Acaulospora* sp)	3.60	2.1307	ab	38.89
D (Combine)	2.80	1.8944	ab	21.43
C (*Gigaspora* sp)	2.40	1.7664	ab	8.33
E (Control)	2.20	1.7301	b	0.00
Cv = 22.27				

The numbers followed by the same lowercase letters in the same column indicate no significant results among treatments at 5% level by LSD test

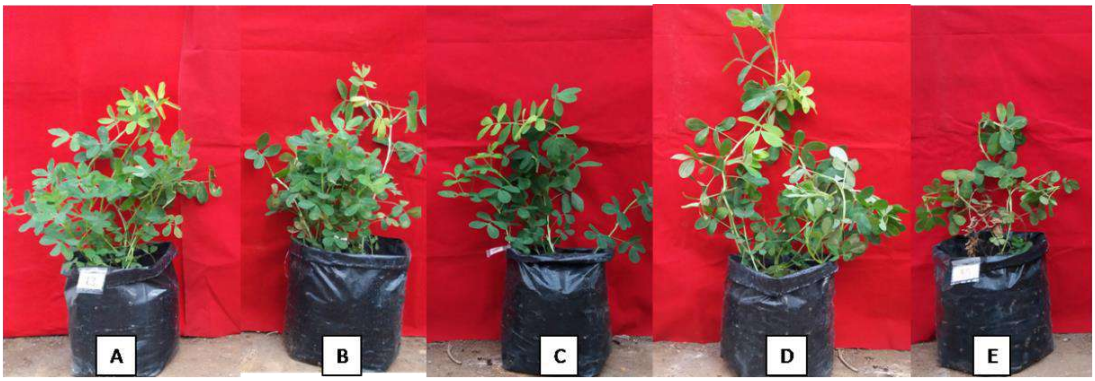

Fig 1. Growth comparison of peanut plants inoculated with indigenous and control AM Fungi. A. Glomus sp-3, B. Acaulospora sp, C. Gigaspora sp, D. Combine, E. Control

Table 4. Number of peanut pods (110 DAP) (Transformation to Log (x + 1)).

Treatment	Number of pods (grain)	Transformation to Log (x + 1)	Effectiveness (%)
A (*Glomus* sp-3)	5.40	0.7947 a	51.85
B (*Acaulospora* sp)	4.80	0.7373 ab	45.83
C (*Gigaspora* sp)	4.40	0.7282 ab	40.91
D (Combine)	4.40	0.6816 ab	40.91
E (Control)	2.60	0.5362 b	0.00
Cv= 23.50			

The numbers followed by the same lowercase letters in the same column indicate no significant results among treatments at 5% level by LSD test

Table 5. Weights of peanut pods at 110 DAP (Transformation to Log x).

Treatment	Weights of pods (gram)	Transformation to Log x	Effectiveness (%)
A (*Glomus* sp-3)	12.35	1.0347 a	53.04
B (*Acaulospora* sp)	11.62	0.9868 ab	50.09
D (Combine)	10.50	0.9864 ab	44.76
C (*Gigaspora* sp)	9.78	0.9831 ab	40.70
E (Control)	5.80	0.7453 b	0.00
CV = 22.96			

The numbers followed by the same lowercase letters in the same column indicate no significant results among treatments at 5% level by LSD tes

3.5. Canopy fresh weight and dry weight

The fresh and dry weight of peanut canopy in each treatment can be seen in Tables 6 and 7.

Table 6. Fresh weight of peanut canopy (110 DAP)

Treatment	Fresh weight of canopy (gram)		Effectiveness (%)
A (*Glomus* sp-3)	57.53	a	40.17
B (*Acaulospora* sp)	57.06	a	39.68
C (*Gigaspora* sp)	49.78	ab	30.86
D (Combine)	46.07	ab	25.29
E (Control)	34.42	b	0.00
Cv= 16.72			

The numbers followed by the same lowercase letters in the same column indicate no significant results among treatments at 5% level by LSD test

The application of various types of indigenous AM Fungi can increase the fresh weight of peanut canopy (Table 6). The highest canopy fresh weight was found in the provision of AM Fungi *Glomus* sp-3, namely 57.53 grams with an effectiveness of 40.17% and the highest canopy dry weight was also found in AM Fungi *Glomus* sp-3, namely 16.81 grams with an effectiveness of 55.21% compared to control (Table 7).

3.6. Root length

The root length of peanut in each treatment can be seen in Table 8 and Figure 2. The longest roots were found in plants given AM Fungi *Glomus* sp-3 with a root length of 37.40 cm and an effectiveness of 29.95%.

Table 7. Dry weight of peanut canopy (110 DAP)

Treatment	canopy dry weight (gram)		Effectiveness (%)
A (*Glomus* sp-3)	16.81	a	55.21
B (*Acaulospora* sp)	12.40	ab	39.27
D (Combine)	11.37	bc	3377
C (*Gigaspora* sp)	11.26	bc	33.13
E (Control)	7.53	c	0.00
Cv = 28.71			

The numbers followed by the same lowercase letters in the same column indicate no significant results among treatments at 5% level by LSD test

Table 8. Length of peanut roots (110 DAP)

Treatment	Length of roots (cm)		Effectiveness (%)
A (*Glomus* sp-3)	37.40	a	29,95
B (*Acaulospora* sp)	32.60	ab	19,63
C (*Gigaspora* sp)	29.90	ab	12,37
D (Combine)	28.20	b	7,09
E (Control)	26,20	b	0,00
Cv = 21.03			

The numbers followed by the same lowercase letters in the same column indicate no significant results among treatments at 5% level by LSD test

The administration of AM Fungi *Acaulospora* sp and *Gigaspora* sp also increased plant root length but it was not significantly different compared to control. Meanwhile, giving combined AM Fungi did not show significantly different results from the control (Table 8).

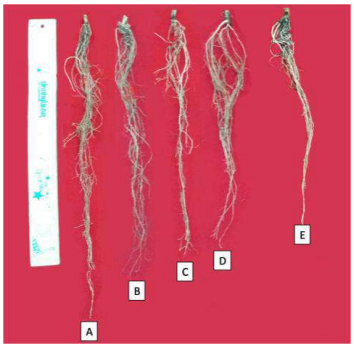

Fig 2. Comparison of root lengths of peanut plants inoculated with indigenous and control AM Fungi. A. Glomus sp-3, B. Acaulospora sp, C. Gigaspora sp, D. Combine, E. Control.

3.7. Root fresh weight and dry weight

The fresh weight and dry weight of peanut roots in each treatment can be seen in Tables 9 and 10. The application of AM Fungi *Glomus* sp-3 and *Acaulospora* sp gave significantly different effects on root fresh weight with the effectiveness of 49.34 and 44.71% respectively (Table 9). AM Fungi *Glomus* sp-3 was also able to increase root dry weight with 43.14% effectiveness compared to control plants. Other isolates did not give significantly different results compared to controls (Table 10).

Table 9. Fresh weight of peanut roots (Transformation to $\sqrt{x}$).

Treatment	Fresh weight of roots (gram)	(Transformation to $\sqrt{x}$).	Effectiveness (%)
A (Glomus sp-3)	2.27	1.4670 a	49.34
B (Acaulospora sp)	2.08	1.4252 a	44.71
C (Gigaspora sp)	1.74	1.3019 ab	33.91
D (Combine)	1.48	1.2160 ab	22.30
E (Control)	1.15	1.0575 b	0.00
Cv = 19.44			

The numbers followed by the same lowercase letters in the same column indicate no significant results among treatments at 5% level by LSD test

Table 10. The dry weight of peanut roots (110 DAP)

Treatment	dry weight of roots (gram)	Effectiveness (%)
A (*Glomus* sp-3)	0.51 a	43.14
B (*Acaulospora* sp)	0.44 ab	34.09
C (*Gigaspora* sp)	0.39 ab	25.64
D (Combine)	0.39 ab	25.64
E (Control)	0.29 b	0.00
Cv = 28.40		

The numbers followed by the same lowercase letters in the same column indicate no significant results among treatments at 5% level by LSD test

Observations on all plant growth parameters showed varied results. In general, indigenous AM Fungi has a positive effect on the growth of peanut plants compared to controls. In this study, the application of combined AM Fungi and *Glomus* sp-3 was able to increase the height of peanut plants with the effectiveness of 31.42% and 15.01% (Table 1). The results of Delvian's (2003) study also found that thecombined inoculum of *Acaulospora* sp-1 and *Gigaspora* sp (S1M6) was more able to increase plant height than the inoculum of one type of *Gigaspora* sp (S1M2) in Leucaena leucocephala plants. The ability of combined AM Fungi isolates to increase plant height was thought to be related to the ability of AM Fungi to help plants absorb nutrients through external hyphae networks. Prasasti et al., (2013) stated that the capacity of plants to absorb water and nutrients, especially P elements, will increase along with the development of external hyphae tissue in plant roots due to infection from AM Fungi. In addition, AM Fungi can also stimulate the formation of plant growth hormones such as cytokinins and auxins which play a role in cell division and elongation, thereby optimizing plant height growth (Talanca, 2010). The same thing was stated by Ortas and Rafique (2017) that AM Fungi helps plants absorb nutrients by expanding the hyphae network in the rhizosphere. Mycorrhizal inoculation changes the root architecture and studies have shown that the nutrient absorption capacity of inoculated roots is much better than those that are not inoculated.

AM Fungi *Glomus* sp-3 was the best in increasing the number of leaves, number of branches, number of pods, pod weight, shoot fresh weight, shoot dry weight, root length, root fresh weight, and root dry weight (Tables 2 - 10). AM Fungi *Glomus* sp-3 gave significantly different results to all observed parameters of peanut plant growth compared to control. AM F *Glomus* sp-3 was able to increase canopy dry weight (55.21%) and roots (43.14%) (Tables 7 and 10). This is because AM Fungi can increase nutrient uptake by plants. Lizawati et al., (2014) stated that dry weight is an indication of the success of plant growth because the dry weight of plants shows the presence of protein content, and photosynthesis results in the form of organic compounds that can be deposited after the moisture content are dried. The greater the dry weight of the plant, the more efficient the photosynthesis process is and the higher and faster the productivity and development of tissue cells, so that plant growth is better, which ultimately increases the dry weight of the plant. In addition, root length, fresh weight, and root dry weight of peanut plants can also increase because the root volume increases due to colonization of AM Fungi. This is consistent with the statement of Prasasti et al., (2013) that mycorrhizae affects the dry weight of the roots because plants infected with mycorrhizae will make the volume and length of the roots wider so that the dry weight of the roots will increase.

AM Fungi *Glomus* sp-3 was also able to increase the number and weight of peanut pods with the effectiveness of 51.85% and 53.03%, respectively (Tables 4 and 5). The ability of AM Fungi in increasing the number and weight

of peanut pods was related to the ability of AM Fungi in colonizing plant roots, especially in the planting medium used, namely ultisol soil. Rajmi et al., (2018) stated that ultisols are acid dry land that has low fertility and productivity, one of which is the problem of availability of elements P, stunted plants, small leaf size, and the development of roots, pods, and seeds are hampered. Meanwhile, the results showed that AM Fungi was able to increase the growth and production of peanut plants, which indicated that AM Fungi was able to colonize peanut roots well so that it could help the roots absorb nutrients.

IV. CONCLUSION

In general, the AM Fungi *Glomus* sp-3 was able to increase the growth and production of peanuts with the effectiveness of 15.01 – 55.21%.

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Evaluation of sublethal phyto-toxic effects of herbicides using biochemical indices

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Abstract**—** *The study assessed the biochemical alterations of three commonly used herbicides (Starforce®, Dragon® and Force Uron®) exposed to anon-target environmental receptor -Allium cepa L at sub-lethal levels. The oxidant activity - measured as malondialdehyde and the anti-defensive mechanism (superoxide dismutase and catalase were used to evaluate the deleterious effects of the test herbicides. At higher percentage of the test herbicides, there was elevated levels of MDA in the exposed Allium cepa Linn with respect to the control (P<0.05). This was followed bya decrease in the antioxidant activities (SOD and CAT) as concentrations increased (P<0.05).The study concluded that the test herbicides generated reactive oxygen species (ROS) resulting in elevated levels of lipid peroxidation, which may possibly havealtered the activities of SOD and CAT, thereby leading to oxidative stress in Allium cepa Linn at levels below and at the presumed safe limit (10% of EC_{50}) and this should be a concern to human who are the end users of this edible non-target plant.*

Keywords**— Allium cepa Linn, herbicides, oxidative stress, reactive oxygen species (ROS).**

I. INTRODUCTION

In the last few decades, more than eight(8) billion kilograms of different herbicides have been applied yearly to control weeds and increase crop yield. However, exposure to herbicide scan lead to perturbations in non-target plants, which may generate reactive oxygen species (ROS) resulting in oxidative stress manifested as alteration in lipid peroxidation (LPO)(Caverzan *et al*., 2014).Reactive oxygen species (ROS) are produced in both unstressed and stressed cells, however when there is an imbalance between oxidative and anti-oxidative mechanism in the specie, oxidative stress sets in. Plants have well developed defense systems against ROS, involving both limiting the formation of ROS as well as instituting its removal (Alscher *et al*., 2002). The defense systems (anti-oxidants) include enzymes such assuperoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX), which help in protecting cells from the damaging effects of ROS.

Overproduction and accumulation of ROS can be a threat to cells leading to peroxidation of lipids, oxidation of proteins, damage to nucleic acids, enzyme inhibition and ultimately leading to death of cells (Sharma *et al*., 2012; Gurvinder, 2019).Oxidative stress and alteration in oxidant/antioxidant status in living organisms have been considered as an important tool in assessing pesticide toxicity especially at sub-lethal levels (Mansour *et al*., 2017). Most of the perturbations caused by herbicide treatment in plants are related to ROS generation. The use of biomarkers for studying contaminant exposures and effects on living organisms has been identified as a useful environmental tool. Biomarkers are used in toxicity bioassay to give a supplementary and more accurate approach and picture, than the chemical analysis because of the need to integrate all

environmental variables and stressors. These are used to indicate the health status of organisms and to obtain early warning signs and responses (Ogeleka and Okieimen, 2019).

Starforce® is afluazifop-b-butyl herbicide(Butyl 2-[4-[5-(trifluoromethyl) pyridin–2-y] oxyphenoxylpropanoate) used to control perennial and annual grass weeds (Cieslik *et al*.,2017). It acts as an inhibitor of acetyl CoA carboxylase (Accase) that catalyzes the formation of matonyl-CoA during metabolism of lipids and/or of some secondary compounds. Dragon® is a paraquat dichloride herbicide(1,1-dimethyl-4,4-bipyridinium dichloride)used to control broad-leaved weeds and grasses. It is a quick acting, non-selective compound that destroys green plant tissue on contact and by translocation within the plant (Janaki *et al*., 2017). Similarly, Force Uron®, a diuron herbicide (3-(3,4-dicholophenyl)-1,1-dimethylurea) is a potent inhibitor of photosynthesis (Kumar *et al*., 2010).Fluazifop-P-butyl, paraquat dichloride and diuron herbicides have not only been associated with high degree of toxicity but also have been known to induce oxidative stress on living organisms. Some researchers have linked induction of ROS with fluazifop-p-butyl (Fayez *et al*., 2014; Liu *et al*., 2017).Several studies have also shown that Paraquat, a commonly used herbicide, induces oxidative stress via ROS generation (Somayajulu-Nitu *et al*., 2009; Moustaka *et al*., 2015; Orti *et al*., 2016). Diuron herbicide used in champagne vineyard was found to generate reactive oxygen species which induced a shift of the balance between pro-oxidative and anti-oxidative reactions leading to oxidative stress (Geoffroy *et al*., 2002).

In this study, the phyto-toxic effects of three commonly used herbicidesin Nigeria (Starforce®, Dragon® and Force Uron®) were evaluated on *Allium cepa Linn*at sublethal levels using an oxidant index (lipid peroxidation) and two anti-oxidants parameters(superoxide dismutase and catalase) as biomarkers.

II. MATERIALS AND METHODS

2.1 Test chemicals

Sublethal concentrations of the test herbicides (Starforce®, Dragon® and Force Uron®) was used in this assessment after a lethal evaluation (Okieimen *et al*., 2020). Sublethal concentrations of these three herbicides at2%, 5% and 10% of their respective EC_{50} values was used for this study (Table 1).

Table 1: EC_{50} and the concentrations used for this study

Chemical	Herbicide formulation (active ingredient)	Effective concentrations(EC_{50}) mg/L	2%	5%	10%
Starforce®	Fluazifop-p-butyl herbicide,	0.237	0.00474	0.01185	0.0237
Dragon®	Paraquat dichloride	0.042	0.00084	0.0021	0.0042
Force Uron®	Diuron	0.169	0.00338	0.00845	0.0169

2.2 Test species

Allium cepa has been commonly used in the laboratory to determine the toxicity of xenobiotics and environmental risks. The *Allium cepa* test is a simple, sensitive and rapid bio-tool that shows high sensitivity to toxic chemicals /substances. Roots have been found to the most vulnerable and reliable system to study the mechanism of herbicide interaction as a primary receptor (Igbal *et al*., 2019; Srivastava and Singh, 2020).

2.3 Bioassay for biochemical indices in *Allium cepa L*

Onion bulbs (*Allium cepa L*) of the purple variety, with an average weight and length of 73.00 ± 0.24g and 6.30 ± 0.07 respectively were obtained from vendors inWarri, Delta state, Nigeria. The bioassay was conducted using the Organization for Economic Co-operation and Development (OECD) protocol #208 for root growth inhibition (OECD, 2003) before the biochemical evaluation. At the end of 48 hours when the mitotic activity was presumed optimal, the root tips were excised from each bulb and used for the analysis. Homogenates of the onions were prepared by homogenizing 0.5 g of the onion root tips in ice-cold phosphate buffer at pH 7.2. The homogenates were centrifuged at 4000 rpm for 10 minutes and the supernatant used for the biochemical analysis.

2.4 Lipid peroxidation assay

Peroxidation was estimated using the method of Hodges *et al*., (1999) based on malondialdehyde assay. MDA, a product of lipid peroxidation and a biomarker for oxidative stress, when heated with 2-thiobarbituric under acidic conditions forms a pink coloured product which has a maximum absorbance at 532nm. Malondialdehyde content was expressed as mmol/MDA wet root.

2.5 Antioxidant enzymes (superoxide dismutase and catalase)

The activity of SOD in *Allium cepa Linn* was estimated spectrophotometrically using the method of Misra and Fridovich (1972). The assay of SOD is an indirect method based on the inhibitory effect of SOD in the initial rate of epinephrine (adrenaline) auto-oxidation. SOD was expressed in unit/mg onion root.The activity of CAT was determined by the method of Hasanuzzaman *et al*., (2011). It was based on the measurement of the rate of decomposition of hydrogen peroxide (H_2O_2) after the addition of the material containing the enzyme and was expressed asunit/mg onion root.

2.6 Statistical Analysis

Values of the enzymological results were expressed as mean ± standard deviation. These were statistically analyzed for significant differences between treated and control groups using Student's t test in analysis of variance (ANOVA), where P values ≤ 0.05 were considered statistically significant.

III. RESULTS

The results for the biochemical indicators (MDA, SOD and CAT) are presented in Table 2 and Figures 1-3. The results in Table 2 indicated that there was increase in the values for MDA (lipid peroxidation) while a decrease was observed in SOD and CAT activities as the concentrations of the test herbicides increases. However, there was significant difference between the various activities in some of the treatment groups and the control.

Table 2: Mean values ±standard deviation of the enzyme analysis for Allium cepa L

Test chemical	% of EC_{50}	Concentration, mg/L	MDA (mmol/MDA)	SOD (unit/mgprotein)	CAT (unit/mgprotein)
		Control	0.89 ± 0.24^a	41.05 ± 5.00^a	23.38 ± 1.99^a
Starforce®	2	0.00474	0.95 ± 0.15^a	39.88 ± 3.91^a	15.46 ± 0.60^b
	5	0.01185	1.44 ± 0.07^b	28.32 ± 1.30^a	14.04 ± 0.45^b
	10	0.0237	1.88 ± 0.26^b	16.88 ± 0.01^b	12.45 ± 0.37^b
Dragon®	2	0.00084	1.26 ± 0.14^a	32.34 ± 0.10^a	15.78 ± 0.76^b
	5	0.0021	1.63 ± 0.18^b	16.89 ± 0.01^b	14.78 ± 0.15^b
	10	0.0042	2.38 ± 0.22^b	16.85 ± 0.03^b	14.02 ± 0.60^b
Force Uron®	2	0.00338	1.25 ± 0.11^a	38.65 ± 1.88^a	15.12 ± 0.09^b
	5	0.00845	1.48 ± 0.13^b	35.23 ± 1.23^a	14.58 ± 0.31^b
	10	0.0169	1.82 ± 0.14^b	29.44 ± 4.22^a	12.87 ± 1.34^b

Values are means± standard deviations of triplicate determinations. Values not sharing a common superscript on the same column differ significantly (P<0.05)

3.1 Malondialdehyde activities

The values of malondialdehyde increased with respect to the control for all the test chemicals with increasing concentrations (Figure 1). Although MDA values increased in all concentrations, they were not statistically significant only in 2% of the EC_{50}. The effects induced by the test herbicide on lipid peroxidation expressed as MDA was higher at 10% of the $EC_{50}$and decreased down as the concentration reduces from 5% to 2%, which implied that as you progress further from the safe limit of 10% of the EC_{50}, the effect of the test herbicides with respect to oxidative stress reduces.

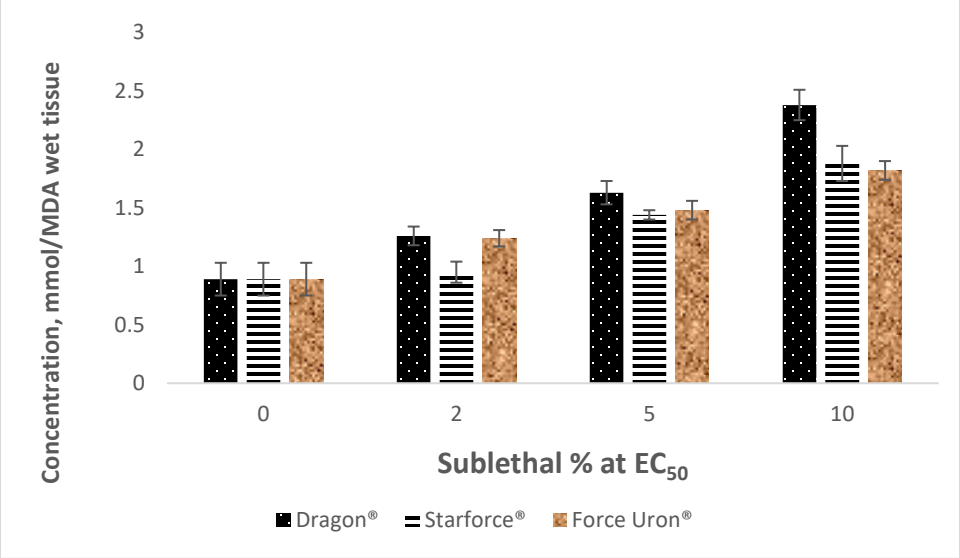

Fig.1: Malondialdehyde concentrations(mean ± SE) inAllium cepa L exposed to sublethal levels of test herbicides

3.2 Superoxide dismutase activities

Superoxide dismutase activities decreased with respect to control for the test herbicide, however, the decrease in SOD activities for some concentrations were not significant. The effect SOD induced by the herbicide was more at the lowest concentration of 2% of the EC_{50} and decreased down through 5% to 10%, which implied that as you move up away from the safe limit, the more reduced the levels of SOD and the weaker the anti-oxidant defense mechanism (Figure 2).

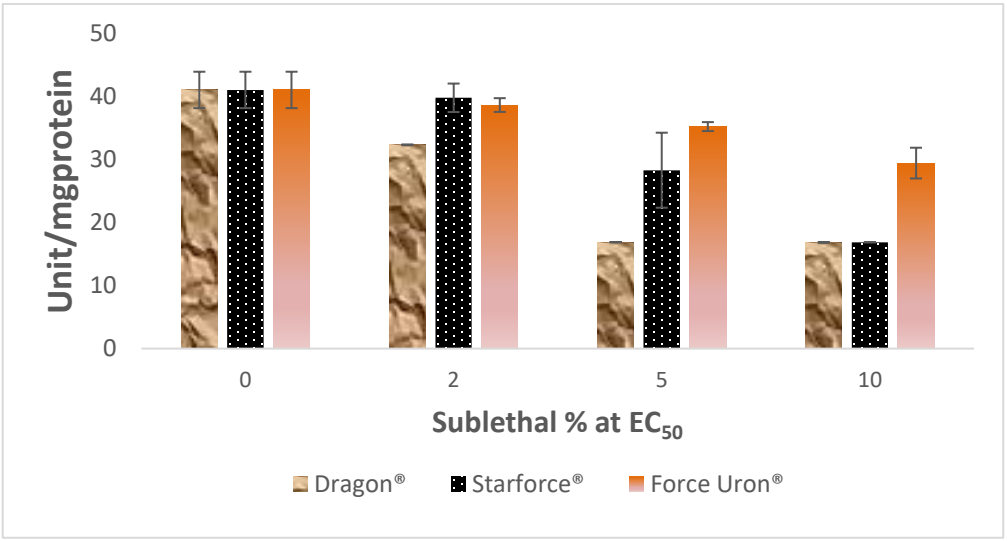

Fig.2: Superoxide dismutase concentrations (mean ± SE) in Allium cepa L exposed to sublethal levels of test herbicides

3.3 Catalase activities

Catalase activities decreased appreciably in the test herbicides for all concentrations with respect to the control.Thus, it can be seen that the effect on CAT induced by the test herbicides was more prominent at the lowest concentration of 2% of the $EC_{50}$and decreased down to 5% and finally 10%, which implied that as you more up away from the safe limit, the more reduced the levels of CAT and more vulnerable (exposed) the specie to oxidative stress from toxicants like the test herbicides (Figure 3).

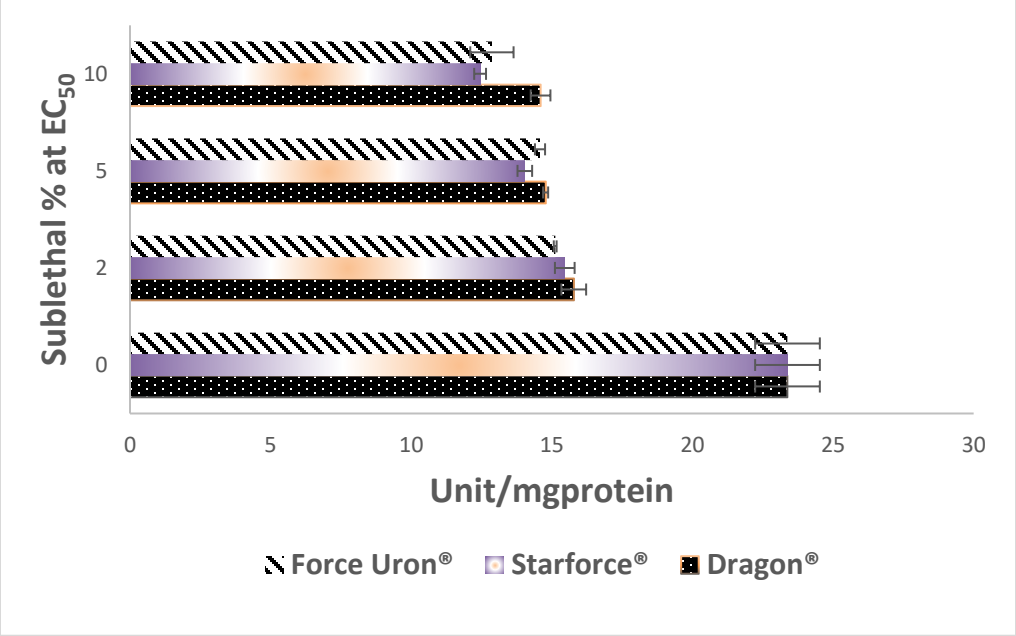

Fig.3: Catalase concentrations (mean ± SE) in Allium cepa L exposed to sublethal levels of test herbicides

IV. DISCUSSION

Plants activate antioxidant defense mechanisms under stresses, which helps in the maintenance of the structural integrity of the cell components and presumably alleviates oxidative damage. Several antioxidant enzymes contribute to plant defense such as superoxide dismutases, catalase, glutathione peroxidase and non-enzymatic antioxidants. (ascorbic acid, glutathione and phenolics) (Caverzan *et al*., 2016).

Recently, lipid peroxidation (LPO)has been gaining attention as a potential toxicological hazard and several pesticides including herbicides have been shown to stimulate peroxidation of cellular membranes. In this assessment, there was significant increase in the level of MDA of *Allium cepa Linn* roots exposed to the test herbicides with respect to the controland this increment was concentration dependent. Elevated level of lipid peroxidation may be an indication that the herbicides at higher concentrations generated ROS which could possibly led to oxidative stress in the exposed *Allium cepa Linn* (Nohatto *et al*., 2016). These observations are in accordance with reports by several researchers (Çavuşoğlu *et al*., 2011; Nohatto *et al*., 2016; Singh and Roy, 2017; Radwan *et al*., 2019; Srivastava and Singh, 2020).

The enzyme superoxide dismutase plays a significant role in defense against reactive oxygen species (ROS)in living organisms bycatalyzing the dismutation of most reactive and dangerous free radicals, superoxide radicals (O^{2-}) to molecular oxygen (O_2) and hydrogen peroxide (H_2O_2) that would be less reactive. In this assessment, the activity of SOD decreased with respect to the control, which could possibly be as a result of the failure of SOD to scavenge the rapidly generated ROS. Liu *et al*., (2012), exposed maize and rice roots to chloracetanilide herbicide and found that the activities of superoxide dismutase, peroxidase and catalase were lower after the exposure while a lower activity of SOD was also corroborated byStajner *et al*., (2003) and Kumar *et al*., (2010).

Catalase activity is also an important indicator to evaluate herbicide-induced oxidative stress. Catalase catalyzes the decomposition of two hydrogen peroxide (H_2O_2) molecules to water (H_2O) and oxygen (O_2).In this appraisal, catalase activities were reduced as a result of exposure of *Allium cepa Linn* roots to the test herbicides.The reduction of CAT activity might be as aresult of inhibition of the enzyme synthesis or probably the herbicides caused stress conditions which changed the assembly of the enzyme subunits (Abedi and Pakniyat, 2010). Alterations in enzymatic antioxidants such as reduction in catalase activity have been documented (Stajner *et al*., 2003; Fakhari *et al*., 2020).

In addition, the increased in the levels of MDA which was used to assess the LPO may possibly be associated with decreased activity of scavenging enzymes such as catalase and superoxide dismutase. A decrease in the activities of these enzymes can lead to excessive availability of superoxides and peroxy radicals resulting in the initiation and propagation of LPO. Hence as you move up away from the safe limit of 10% of the EC_{50} values, the more reduced the levels of the anti-oxidant defense mechanisms (SOD and CAT) and thus enhanced probability of oxidative stress, which some species may likely not be able to overcome and may possible result to sub-cellular alterations that could have long term deleterious effects.

V. CONCLUSION

This study revealed that the test herbicides activated the enzymatic defense systems due to imbalance between production of ROS and scavengy tendency of the antioxidants leading to perturbation in *Allium cepa Linn* mechanism. This action therefore altered the status of lipid peroxidation (increase) and the enzymatic antioxidants – SOD and CAT (decrease). Herbicides exposure therefore can cause toxic effects such as alteration in the oxidants and antioxidants concentrations in *Allium cepa Linn* even at levels beyond the presumed safe limit (10% of EC_{50}). This should be of considerable public health and safety concerns to human who are the consumers of this edible non-target plant.

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Safety House: A developed Framework to Improve Safety Performance among Highly Risky Industries

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Abstract*— *The concept and importance of Occupational Health and Safety Management Systems (OHSM) have extensively been discussed in previous literature. This study, however, focuses on reviewing and addressing gaps in some well-known industry-accepted OHSMS models developed between the periods 1990 – 2018 to design a new OHSM framework known as the "Safety House" that addresses all significant gaps in previous models.

The findings of this study show that most previous models have either ignored safety leadership or safety commitment as one of the most pivotal elements that can improve the effectiveness of OHSMS and promote workplace safety. The concept of integration and relationships among all previous models was not clearly established.

The innovation of this study highlighted the importance of integrating safety leadership and safety commitment into all phases of continuous OHSMS models. The study as well addresses the issue of employees' involvement in safety planning before and after implementation of OHSMS. This is an initiation that has not benefitted the needed attention in almost all previous safety models.

***Keywords*— *Safety Performance; Occupational Health and Safety Management; Risk; Safety Models*.**

HIGHLIGHTS

1. The importance of integrating safety leadership and safety commitment into all phases of continuous OHSMS models
2. Addressing gaps in previous well-known industry-accepted safety models
3. Addressing gaps in previous well-known industry-accepted safety models

I. INTRODUCTION

The concept of the Occupational Health and Safety Management System (OHSMS) can be traced as far back as the 1400s when physicians advocated for the need for employee safety and disease prevention among mineworkers. However, the adoption, development, and international conceptualization of the OHSMS concept gained momentum in the 20th century after the International Commission for Occupational Health (ICOH) and the Industrial Labor Organization (ILO) was established in 1906. Today, the concepts of OHSM have been expanded by various studies to include the promotion and provision of a wide range of employee health and safety concerns (ILO, 1985). Thus, the concept of OHSMS has been widely recognized and adopted

by many industries to provide or improve health and safety at the workplace.

As Kuusisto (2000) put it, safety is a "reliable control of harm" whiles health refers to the affirmation of physical, mental, and social wellbeing of workers (WHO, 1948). Burrage (1995) described OHSM as "the measures, procedures, and controls applied to working activities that minimize risks and maximize safety". Mitchison and Papadakis (1999) viewed OHSM as an aspect of the overall management function that determines and implements the organization's safety policies. The Hong Kong Labor Department (1999) further described OHSM as "planning, developing, organizing, and the implementing of safety policy which can measure and audit the performance of those functions". From an integration perspective, the European Council Directive 96/82/EC viewed OHSM as an accident prevention mechanism, which should be part of the organizational structure. The council defined OHSM to include the entire organizational structure, responsibilities, practices, procedures, processes, and resources for determining and implementing the major-accident prevention policy (Mitchison and Papadakis, 1999).

As a global organization, WHO defined OHSM as the maintenance of employee wellbeing over a longer period by focusing on occupational, environmental, and social culture determinants of health (WHO, 1999). Thus, WHO emphasized the need for collaboration between employees and managers for the continuous process of improving and promoting the health, safety, and sustenance of employees' wellbeing at the workplace.

Quite recently, Fernandez-Muniz et al. (2007) considered OHSM as "a set of policies, strategies, practices, procedures, roles, and functions allied to safety". Granerud and Rocha, (2011) added that OHSMSs are systematic risk eradication tools used to control health and safety challenges at the workplace. More significantly, OHSMS are supportive tools that promote the wellbeing of employees at the workplace (Ramli, Watada, and Pedrycz, 2011).

The several concepts and definitions of OHSM may differ in the content; however, the focus and motivation behind OHSM remain similar. Clearly, OHSM directly focuses on initializing, developing, and implementing policies that will govern work operations by enhancing work safety and promote employees' wellbeing by reducing or preventing risk and hazardous exposures at the workplace. As briefly described by Koehn, (2000) OHSMS is "a method of controlling the safety policies, procedures, and practices".

In this current study, the definitions by the European Council Directive 96/82/EC, SEVESO II, and WHO, (1999) were integrated. Thus, OHSMS is defined as management responsibilities, practices, procedures, processes, and resources focusing on occupational, environmental, and social culture determinants of health that guide the implementation of accident prevention policies and can maintain the wellbeing of workers over a sustainable period. This definition is in line with all other definitions of OHSMS and as well, captures the research objectives, which focus on the development of a safety framework that can improve safety performance in highly risky industries.

II. REVIEW OF OCCUPATIONAL HEALTH AND SAFETY MODELS

The OHSM framework is a conceptual structure of ideas established, adopted, or implemented by an organization with an intention to support, guide, and improve the continuance work safety of employees. Over the past 20years, several OHSM frameworks, also known as OHSM standards, guidelines, or models have been developed and disseminated by health and safety experts, organizations, and government as a whole. In this current study, we explored the different forms of models chronologically, highlighted the gaps, and presented a recommendation by developing a safety framework known as the "Safety House" that addresses all gaps in previous models.

2.1 The Accident Prevention Advisory Unit (APAU) of the UK Health and Safety Executive (HSE)

The Accident Prevention Advisory Unit (APAU) of the UK Health and Safety Executive (HSE) in 1991 developed one of the first safety frameworks known as 'Successful Health and Safety Management (HSG65) to be used as a practical guide for directors, managers, safety professionals, and employee representatives. The 'Successful Health and Safety Management (HSG65)' OHSM framework proposes a continuous and interconnection of five (5) different areas of safety plans. These include safety policy development, organizational development, safety planning, measuring performance, and review of performance. The framework further proposes feedback loop and safety auditing as key factors in improving organizational safety. Although the model is quite extensive in terms of the coordination among the decision processes in the cycle, it failed to integrate

employee involvement in safety decisions and safety planning. The model is superior centered, thus, it focuses more on implementing and executing safety decisions by management with complete disregard to the role employees play in improving safety results apart from feedback loops. Feedback alone is not enough, particularly, workers who are highly exposed to work-related accidents must be given the channel to move beyond reporting but rather be involved in key safety management decisions. The domino theory of accident causation asserts that workers cause more than 88% of work-related accidents; hence, organizations may fail in the quest to improve the health and safety of employees are not highly consulted. In as much as the emphasis is on management's ability to execute safety plans, the model fails to address managerial commitment level and safety leadership as a major determinant for improving safety outcomes (Clarke, 2013; Yagil and Luria, 2010; Zohar and Luria, 2010; Amponsah-Tawiah, 2016; Zohar and Luria, 2005).

Basically, safety leadership is the exhibition of inspiration and motivation of achievement by leaders to followers in order to promote good safety behavior (Burns, 1978; Chemers, 1997) whiles management commitment has been used as dimensions to predict employees' perceptions on organizations safety climate (Zohar, 2008; Zohar, 2000; Zohar and Luria, 2005). Although current research seems to focus on the effectiveness of individual characteristics of leadership, safety leadership has been identified as one of the major factors that predict positive outcomes (Donovan et al., 2016; Clarke and Ward, 2006, Martínez-Córcoles et al., 2012; Nielsen et al., 2013). The theory of perceived organizational support has been used as well to demonstrate the relationship between organizational commitment to safety and work outcomes (Eisenberger and colleagues', 1986; Judd et al, 2005). This makes safety leadership and management commitment to safety a significant determinant of safety outcomes and production outputs as a whole hence should have merited the needed locus in the APAU model. More importantly, safety training, orientation, and education have long been held as a mechanism to reduce work-related accidents and injuries yet its emphasis has heavily been ignored in the model (Florio, 1979).

2.2 The continuous Improvement Model

The National Safety Council (NSC 1994) of the United States developed the continuous Improvement Model on Safety Management Systems also focusing on 5 key phases similar to the UK's Successful Health and Safety Management (HSG65)'. However, in anticipation to achieve the best safety performance outcomes, the NSC continuous improvement model advanced a step further to integrate management commitment and involvement as a key phase in the cycle. The framework viewed safety systems as a cycle that must be continuously improved through review and adjustment. The model further proposed the integration of safety, health, and environmental professionals as the center holding all the five phases of the cycle. Thus, the emphasis is on the involvement of safety, health, and environmental experts in the planning, goal settings, implementations, and review of safety policies. To some extent, the NSC model faces similar drawbacks just as HSG65. Thus, in as much as safety commitment and involvement forms part of the phases in the cycle, safety leadership has been completely ignored. More significantly, safety commitment and involvement do not seem to be coordinating with the other four phases in the cycle.

2.3 The BSI model

The British Standard Institute (BSI) developed the Elements of Successful Safety Management System in 1999. The BSI model is most similar to NSC 1994 as it proposes the same continuous cycle of improving occupational health and safety. BSI however omitted the level of management involvement and commitment in promoting safety, a lapse that was filled by the NSC 1994. The BSI also suffers the drawbacks of emphasizing the integration of safety leadership and management commitment level in the continuous cycle. Other relative models that as well viewed safety frameworks as a continuous cycle include the Australian/New Zealand Standard 4804 (AS/NZS 4804) and the International Labor Organization (ILO) "Elements of the Safety Management Systems." It is worth noting that, all five-safety framework concepts are the same by structure. Thus, the models are more or less proposing continuance safety activities through safety policies; planning, organizing, and implementations; monitoring; review, and audit. As opined by Raglan, (2003), achieving the best safety outcome entails effective planning, monitoring, and evaluation of safety policies. Though these phases in the models are significant determinants of promoting safety behaviors or improving the work environment, they lack some significant parameters deemed necessary to archive the best safety outcomes.

2.4 The WHO Safety Model

More constructively and quite recently, the World Health Organization combined five different continual health and safety models (i.e. OHSAS 18001, WHO-WPRG, ILO OSHM, CCOHS, Deming-PDCA) to develop an eight

continual process health and safety framework. This framework focuses on four safety thematic areas. These include the physical work environment, psychosocial work environment, health resource administration at the workplace, and communal health and safety participation (WHO, 2014). The model is known as the healthy workplace continual improvement process and it extensively advanced the Deming-PDCA framework and the concepts propounded by other continuance models by addressing their existing drawbacks. These include mobilizing, assemble, assess, prioritize, plan, do, evaluate, and improve as the key phases in the continuance safety cycle. The need for organizations to improve work engagements with employees through effective leadership and the promotion of workplace culture, values, and promoting sustainable wellbeing was highly emphasized. The framework also traveled a step further to propose the inclusion of non-management employees with equitable gender balance into teams. These groups of workers in teams are to report all safety concerns of workers to their supervisors or managers and highly sort or involved in all aspects of safety planning.

What makes the WHO framework more extensive is the integration of stakeholder commitment, leadership engagement, workers involvement, ethics, and values as integral factors that coordinate all the phases in the cycle. More so, unlike the other continuance safety models that focus on improving employees' wellbeing within the work settings, the WHO framework goes beyond the organization by widening the safety scope to include physical work environment, psychosocial work environment, and communal health. The proposal of mobilizing, assessing, assembling, and prioritizing the interest and ideas of work safety from managers, employees, opinion leaders, union members, and all other stakeholders in addressing health and safety issues within the four thematic areas is a step ahead of all previous models. Distinctively, commitment among all the stakeholders in safety decisions was highly emphasized and elaborated. The strength and complacency of the WHO models lie in the integration of the concept of the five-continuance model into one major framework. Never the less, this framework is anticipated to be time-consuming due to its nature and scope of implementation. As well, the involvement of all stakeholders is expected to create some bureaucracies that management may not be able to control hence may cause delays in achieving the necessary safety objectives.

2.5 The (ISO) 45001 International Standard for Occupational health and safety management

Irrespective of the strengths and extensive concepts and ideas proposed by WHO continuance model, the International

Organization for Standardization (ISO) 45001 known as the "ISO 45001" is the world's first International Standard dealing with Occupational health and safety management systems. The ISO 45001 is as well a continuous international framework specifically developed and published in 2018 to meet the standard of safety compliance, legislations, and the dynamic business environment across the world. The core concepts for building the framework was based on the improvement of the existing OHSAS 18001, the conventions and guidelines of ILO OSH 2001, and several national standards. It focuses on seven thematic areas, which include leadership, safety scope, safety planning, safety resourcing specification, implementation of safety policies, safety evaluation, and safety improvement.

Moreover, ISO 45001 addresses the issue of controlling OHS risk exposures through the development and provision of safe and healthy workplaces for employees and other interested parties; improve OHS performance; prevent deaths; reduce work-related injuries and ill-health all over the world. Similar to the WHO framework, ISO 45001 also focuses on the interrelationship between the enterprise and its business environment. It proposes the embedment of OHSMS as a core objective that should be pursued and be coordinated with all other organizational objectives rather than making it a stand-alone objective. The study of Mitchison and Papadakos, (1999); Cheng et al., (2004) supports the integration of safety policies and procedures together with other organizational activities. Most chemical and petrochemical companies have adopted integrated health, safety and environment (HSE) management systems whiles some integrate safety with quality management (Shen and Walker, 2001, Yu and Hunt, 2002; Koehn and Datta, 2003; Yu and Hunt, 2004; Yu et al., 2004) and safety with project management (Cheng et al., 2004). The integration approach has currently gained recognition among large companies such as processing plant clients (e.g. BP, Texaco, Shell, and Exxon Mobil). It has also been found to be effective in reducing workplace accident rates and improving of firm's productivity, economic and financial performance (Health and Safety Executive, 1997; Smallman and John, 2001; Rechenthin, 2004). The additional concept the ISO 45001 introduced which other models failed to address is linking all OHSM responsibilities to organizational leadership. Table 2.1 below presents a brief overview of all the reviewed OHSM frameworks.

Table 2.1 Overview of Occupational Health and Safety Models

OHSM Framework	Year	OHSM Cycle
The Accident Prevention Advisory Unit (APAU) Successful Health and Safety Management (HSG65)	1991	1. Policy 2. Organizing 3. Planning and Implementing; 4. Measuring Performance 5. Auditing and Reviewing Performance
National Safety Council (NSC): The Continuous Improvement Model for Safety Management Systems	1994	1. Management commitment and involvement 2. Establish a baseline 3. Set goals 4. Implement strategies 5. Review and adjust.
British Standard Institute (BSI), BS8800: 2004	2004	1. Policy 2. Planning 3. Implementing 4. Measuring Performance 5. Management Review.
Australian/New Zealand Standard 4804 (AS/NZS 4804)	2001	1. Commitment and Policy 2. Planning 3. Implementation 4. Measurement and Evaluation 5. Review and Improvement.
Deming PDCA	1986	1. Plan 2. Do 3. Check 4. Act
WHO WPRG - Western Pacific Regional Guideline	1999	1. Ensure management support 2. Establish a coordinating body 3. Conduct a needs assessment 4. Prioritize needs 5. Develop an action plan 6. Implement the action plan 7. Evaluate the process and outcome 8. Revise and update the programme
ILO-OSHM - Guidelines on Occupational Safety and Health Management Systems	2001	1. Policy 2. Organizing 3. Planning and implementation 4. Evaluation 5. Action for improvement

OHSAS 18001 - Occupational Health and Safety Assessment Series	2007	1. OHS policy 2. Planning 3. Implementation and operation 4. Checking and corrective action Management 5. Review
CCOHS - Canadian Centre for Occupational Health and Safety	2009	1. Lead: management commitment, worker participation, OHS Policy 2. Plan: legal and other, hazards and risks, workplace health, objectives and 3. Do: prevent and protect, emergency plans, train, communicate, procure, contract, manage change, document control, and record control. 4. Check: measure and monitor, investigate incidents, audit and inspect, evaluate and correct 5. Act: review and improve
WHO - Model of Healthy Workplace Continual Improvement Process.	2014	1. Mobilize 2. Assemble 3. Assess 4. Prioritize 5. Plan 6. Do 7. Evaluate 8. Improve
ISO 45001 - International Organization for Standardization (ISO)	2018	1. Leadership 2. Safety scope 3. Safety planning 4. Safety resourcing specification 5. Implementation of safety policies 6. Safety evaluation 7. Safety improvement

III. DEVELOPMENT OF THE "SAFETY HOUSE" FRAMEWORK

It is quite conclusive that, the several OHSMS models or frameworks discussed differ in approaches and guidelines towards improving organizational health and safety yet there exist significant similarities running through them. Thus, safety planning, implementation of safety policies, and review of safety systems to ascertain the required safety outcomes remain pivotal throughout the frameworks. This insinuates that, achieving the best health and safety outcomes need proper planning of safety policies, effective implementation of the right safety programs, and the review of the effectiveness of the implemented safety programs through feedbacks. The WHO model for healthy workplace continual improvement process and ISO 45001 were however extensive in scope, concepts, structure, and focus. They both addressed issues of organizational leadership, support, and commitments towards occupational safety. Again, the emphasis for prioritizing the sustainability of the balanced wellbeing of the worker at the workplace and the work environment were also highlighted.

On the contrary, this current study argues that an effective OHSM framework is a safety management system, which is built on a high level of managerial leadership and commitment

by both superiors and subordinates. Thus, to achieve the best safety outcome, this study proposes that each phase in the safety cycle must coordinate with safety commitment and leadership. Hsu et al. (2007) explain safety commitment as the degree of attention and support exhibited by organizations' top management towards employees' work safety. As the promotion of effective OHSM remains a complex managerial issue, top management must prioritize safety needs and duly intervene in all aspects of safety administration (Steenkamp and Van Schoor, 2002). A positive perception among employees towards a managerial interest in safety needs positively affects safety outcomes (Yule et al., 2007; Ali et al., 2009).

Further, Heinrich in the late 1920s collected and studied a number of industrial accidents–a total of 75,000 accidents studied revealed that 88% of 75,000 accidents were triggered by risky workers' behavior. Likewise, the Human Factor Theory of Accident Causation, accident/incident theory, Behavior-Based Safety (BBS), Turner's model of accident causation, and the Swiss Cheese model confirms the assertion of Heinrich (Andersson, 2012). Clearly, the foundation for most accident causation among industries can be attributed to human errors, hence, the burden of accident prevention lies on both management and employees to co-operate at work and improve work safety. Thus, as management strives to provide safety systems to improve job safety, employees must also be prepared to participate and comply with these systems. Most of the burden however lies on the organization to support employees through training and education to improve safety knowledge (Shamsul and Juliana, 2018; Vinodkumar and Bhasi, 2010; Abad, Lafuente and Vilajosana, 2013). It is therefore expected that the progress made in each phase of the cycle (i.e. plan, organize, implement, evaluate, feedback, etc.) will be determined by the level of commitment and leadership exhibited by managers to engage, educate and train workers to understand the organization safety systems.

This current study therefore proposes and advances the continuous OHSM framework by prioritizing and integrating safety commitment and leadership as significant factors that must coordinate with six other phases in the occupational safety cycle to aid achieve the best safety outcome. It is worth noting that, both safety commitment and leadership in the model must be pursued simultaneously. Fig 3.1 presents the author's own framework known as the "Safety House."

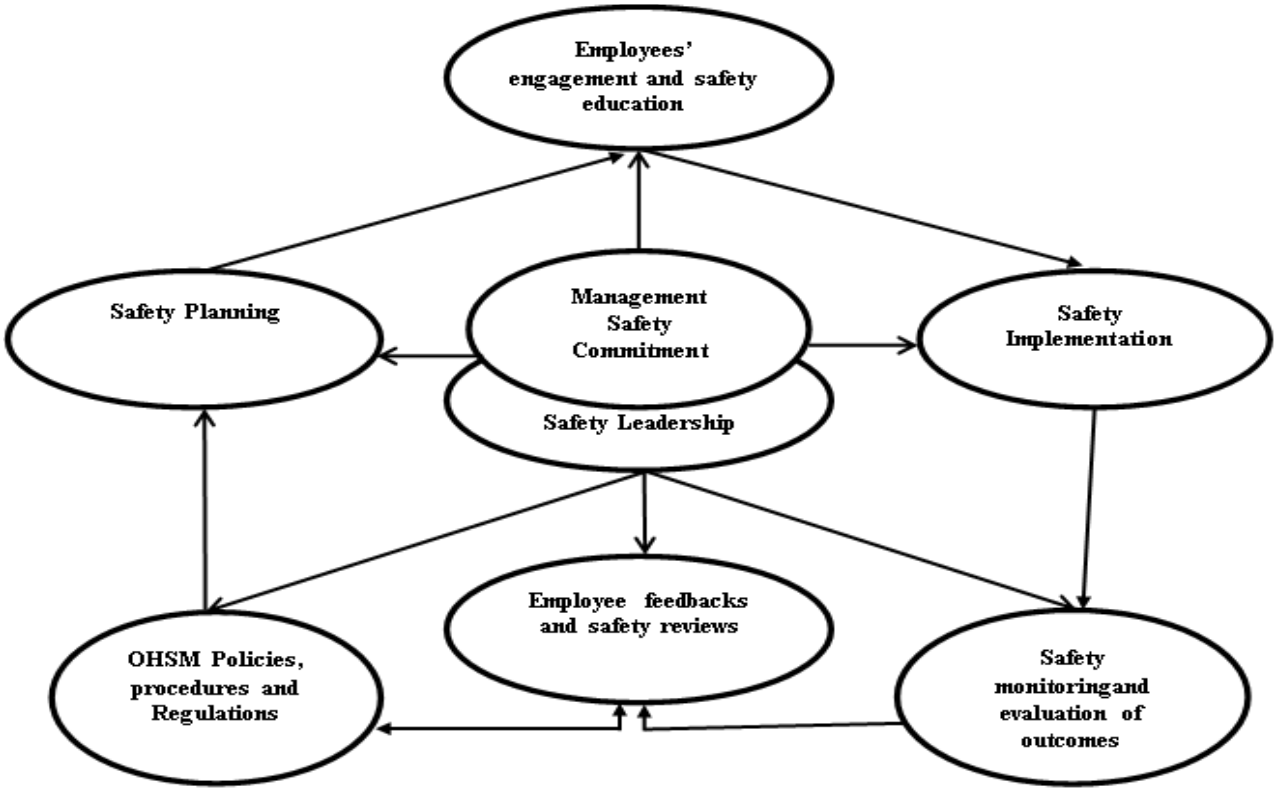

Fig 3.1 Safety House – The New Developed Safety Framework

IV. DISCUSSIONS

The study proposes a health and safety management framework that views safety commitment as a universal factor in safety leadership, which has a direct influence on the safety management processes and administration of the organization. Leadership quality in the organization is expected to influence the work climate, which may directly or indirectly affect the overall performance of the company. This is possible because of group behavior norms about leadership perceptions and the level of interaction between workers and management.

Huang et al. (2006) as well viewed management commitment to safety as a significant pillar of an organization's safety climate that predicts safety performance. Wu et al. (2008) further explained that the safety commitments of the CEOs, the managers, and the employees are essential constituents of health and safety systems. Thus, Occupational health and safety systems with strong management support and employees' engagement at all stages of safety decision making determine the degree of safety outcome at work. Likewise, safety outcomes improve when safety systems are clearly linked to the organization's vision and pursued simultaneously (WHO, World Economic Forum Report, 2008).

Undoubtedly, employees are mostly the victims of occupational injuries and accidents hence their involvement in safety decision-making will yield significant safety improvement and outcomes. In as much as managers assign job responsibilities, employees understand the nature of their job tasks better hence the assembling of workers into safety teams will ease the assessment of workplace safety and improve safety outcomes. Moreover, as safety programs exist to improve job safety, feedback from employees on OHSM system implementation is highly necessary. It allows management to improve existing OHSM policies by integrating the safety outcomes from their point of view and the point of view of the employees.

What makes this newly developed framework quite distinct from other extensive frameworks like the WHO Model of Healthy Workplace Continual improvement process and the ISO 45001 is the focus and attention directed towards the degree of workers' opinions, voice, and involvement in occupational health and safety decisions making at the top level. In most cases, employees understand the nature of risk involved in their job better hence the assembling of workers into safety teams to discuss and report safety issues is expected to ease the assessment of workplace safety systems and improve safety outcomes. Moreover, as safety programs exist to improve job safety and wellbeing, frequent feedback from employees on OHSM system implementation is highly necessary. It allows management to improve existing OHSM policies by integrating the safety outcomes from their point of view and the point of view of the employees.

Similar to the assumptions of the continual models, this current study supports the assertion that understanding of existing Occupational Health and Safety (OHS) policies, procedures, and regulations precede all other significant factors in the safety framework. Thus, before any effective safety planning can be conducted, managers, policymakers, employees, and all other stakeholders must understand the scope and nature of existing safety policies. More importantly, the safety policies must conform to both national and global standards of improving workplace safety or wellbeing of employees' (i.e. ILO conventions on and standards on Occupational Safety and Health; Factories, Offices and Shops Act 1970 (Act 328). Thus, safety plans should be drawn and built from these health and safety global standards and if possible be immediately communicated to employees. Parker et al., (2001) found safety communication to be significantly associated with management commitment. The sharing of information, persuasion, and workers' engagement about their work responsibilities and the potential risk they face may improve safety outcomes. Siu et al. (2004) as well identified safety attitudes and communication as significant variables that predict the relationship between organizations' safety climate and safety performance.

Again. In most cases, the continual models sought employees' opinions through feedbacks and reviews after the safety plan has been initiated and implemented. This mostly falls within the annual or semi-annual safety reviews hence it takes longer than necessary to engage employees in safety decision making. If this continues, it will take a year to correct the safety lapses and existing risk exposures caused by the implementation of the safety plan. The current framework, therefore, proposes that, before the implementation of safety plans as exhibited by almost all the continual models, employees must be engaged and educated on the objectives of the intended plan. Safety education on safety plans is expected to improve safety outcomes. Employees' engagement and education have been found to mediate the relationship between OHS and safety performance (Griffin and Neal, 2006). In the absence of workers' involvement in the enactment of safety plans, management should educate and

train them before implementations. Thus, workers must understand the scope and specifications of safety plans before implementation, if not; the plan may yield conflicting outcomes. This is an interest, which was not highly addressed by the existing continual models.

Finally, instead of the periodic review and solicitation of feedback immediately after the implementation of safety plans, it is ideal for organizations to monitor the safety behaviors and outcomes produced by the safety plan. Continuance safety monitoring is the frequent inspection of implemented health and safety systems or plans. In most organizations, there exist several electronic systems that monitor workers' safety and prompt supervisors of dangers or hazardous exposures that may occur, however, competent safety officers must as well be involved in continuous safety monitoring during work supervisions. This may serve as an intervention measure that will continuously address key safety issues that need prompt attention. The absence of continuance safety monitoring may alter the expected directions of the implemented safety plans if reviews and feedbacks take longer than necessary.

V. CONCLUSIONS

In conclusion, there have been extensive contributions and discussions on safety models or frameworks. However, this current framework as proposed was built on the integration of safety leadership and commitment, employees' engagement in safety plans, and inclusion of safety education between safety plans and implementations. Finally, the emphasis on safety monitoring immediately after the implementation of safety plans to serve, as a risk control intervention tool is a significant strength that was ignored in previous models. This current framework is the projection of the reflection of all other health and safety continual frameworks and is expected to enhance and improve workplace safety if well implemented. It is also important to reiterate that, organizations can improve work performance when accidents and injuries are low hence this framework fits the description of accident prevention and hazard control tool.

CONFLICT OF INTEREST

The authors declare no conflict of interest throughout the conduct of this study.

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The Effect of Production Factors on the Productivity of Seaweed *Gracilaria sp.* in the policulture cultivation system with Milkfish (*Chanos chanos)* in Luwu District

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Abstract— *This research was conducted to see to what extent the production factors used by the polyculture system of Gracilaria sp and milkfish farmers in Luwu Regency affect the production of Gracilaria sp. This research was conducted by interviewing Gracilaria sp cultivators in Luwu Regency by taking samples from three districts. The results show that the R2 value is 0.689 which means that 68.9% of the production / harvest can be explained by the production input, while the remaining 31.1% is influenced by other factors. In addition, it is also seen that the Fcount value obtained is 38.294 while Ftable (0.05) is 2.50 at the 95% confidence level, which means that the production input has a significant effect on the production of Gracilaria sp. Meanwhile, partially each production input has an effect on production, except for fertilizer production input.*

Keywords— ***Cobb-Douglas, Gracilaria sp., Polyculture, Production factors.***

I. INTRODUCTION

Seaweed is a fishery commodity that has economic value and is widely practiced by people in Indonesia. The territory of Indonesia which is dominated by marine waters which is the land for seaweed cultivation and relatively easy cultivation techniques from seaweed have made seaweed become one of the stars. Besides, the determination of seaweed as a priority commodity in fisheries revitalization has a very large development prospect, namely the availability of 1.1 million ha of land spread across almost all provinces in Indonesia (Bhakti FK, 2016).

The world's need for seaweed increases every year, causing the development of seaweed cultivation to be something that needs to be done. In 2015, the Ministry of Marine and Fisheries is committed to strengthening Indonesia's position as a major player in the world's seaweed industry and has set a target of increasing seaweed production by 45% in 5 years, namely 19.5 million tonnes by 2019 (Dirjen Perikanan Budidaya, 2015). FAO data 2015 shows that the total world seaweed production in 2013 reached 26.98 million wet tons, and Indonesia contributed 34.47 percent of this production, which is around 9.30 million wet tons (KKP, 2018).

One of the fisheries production centers in Indonesia is South Sulawesi. As one of the largest seaweed producing areas in Indonesia, South Sulawesi contributed 30% of the total seaweed produced by Indonesia in 2016 amounting to 3,409,048.20 tons (BSN, 2017). The types of seaweed that are widely cultivated by people in South Sulawesi are *Euchema Cottonii* and *Gracilaria sp.*

Gracillaria sp is one type of seaweed that is widely cultivated by cultivators in Luwu District. Seaweed cultivation is increasingly attractive to people living in coastal areas for several reasons. First, it does not require relatively high capital. Second, the cultivation technology applied is simple so that it is easily adopted by the small community. Third, in terms of time, it is relatively efficient. Fourth, a short cultivation cycle. Cultivator farmers can harvest the results within 45 days. Fifth, it can be done by anyone, including housewives (Yayasan WWF Indonesia, 2014).

Production factors consist of natural resources, human resources, capital, entrepreneurship, and information. A study conducted by (Inrise, 2015 in Pratomo N, 2018) shows that farmers' knowledge of seaweed production is also still limited. Besides, the lack of capital is also a problem in the production and increasing income of seaweed cultivators (Sari R.R and Dewi M.H.U, 2017).

Knowledge and capital lowness cause farmers in Luwu District to speculate in the process of cultivating Gracilaria seaweed. This speculation is an attempt by farmers to reduce production costs due to a lack of capital. However, due to the lack of knowledge of farmers about seaweed production, it is feared that the speculation will cause low income of Gracilaria seaweed farmers. A study conducted by (Patawari A.M.Y., 2018) shows the income of Gracilaria seaweed farmers in Seppong Village, North Belopa Sub-district, Luwu District is classified as lacking.

Based on the above, it can be seen that the use of production factors is not appropriate, so it is necessary to do research on the effect of production factors on the productivity of Gracilaria sp in polyculture cultivation with milkfish in Luwu Regency.

II. METHODS

2.1. Time and Location of Research

This research has done in November 2020 in 3 sub-districts, namely Suli Sub-district, South Ponrang Sub-district, and East Walenrang Sub-district in Luwu District, South Sulawesi Province.

2.2. Collecting Data Method

The collected data consists of 2 (two), namely primary data and secondary data. Secondary data is obtained through related offices or agencies. Primary data is obtained through surveys and interviews with *Gracilaria* seaweed farmers in the Luwu District. Seaweed cultivators who are used as respondents are those who live and carry out *Gracilaria* seaweed cultivation in Luwu District. Based on data from the Dinas Perikanan Kabupaten Luwu, it is known that there are 80 groups of *Gracilaria sp* cultivators, spread across 9 (nine) sub-districts. Of the 9 (nine) Sub-districts in Luwu Regency, it was divided into three sampling areas based on the similarity of characteristics and proximity of the sub-districts. The sub-districts of Larompong, Suli, Belopa, and North Belopa become region I, Kamanre, South Ponrang, and Bua sub-districts become region II, and East Lamasi and East Walenrang sub-districts become region III. Each area was taken 1 sub-district as a sample and all groups in the sub-district were taken representatives, they were the chairman and secretary to be interviewed as respondents. Interviews were conducted with *Gracilaria* seaweed farmers using a pre-arranged list of questions (questionnaire). These aim to determine the production factors of *Gracilaria* cultivation carried out by respondents.

2.3. Data Analysis Method

To see how much influence each production factor has on the production of *Gracilaria sp.* and whether production factors have the most effect on the production of the data obtained were analyzed using Cobb-Douglas analysis with the help of SPSS 20 and Microsoft Excel applications. The Cobb-Douglas production function can be written with the following formula (Soekartawi, 2003).

$$Y = b_0 X_1^{b1} X_2^{b2} X_3^{b3} X_4^{b4} e^{u}$$

Y: Dry Seaweed Gracilaria sp. production (kg)
X1: Land Area (m^2)
X2: Seed weight of Gracilaria sp. (kg)
X3: The amount of fertilizer used (kg)
X4: Number of milkfish seeds spread (unit)

Furthermore, to determine the closeness of the relationship between production factors (Xi) and production (Y), the correlation coefficient (R) is used and to find out the extent to which the factors of production (Xi) can explain production (Y), the coefficient of determination (R^2) is used.

III. RESULTS AND DISCUSSION

3.1. Profile of Respondents

1. Land Ownership

Based on the pattern of land ownership for business, it is divided into 3, namely: self-owned land, leasehold land, and production sharing land (Hayami and Oksuka, 1993). Based on research by Koirala, Mishra, and Muhanty, 2016, shows that this land ownership pattern has a significant effect on farmers' income.

Table 1. Pattern of Land Ownership

Worker Status	Number of Respondents	Percentage
Owner	51 persons	69%
Worker	14 persons	19%
Tenant	9 persons	12%

Source: Primary Data

From the table above, it can be seen that the land management status of seaweed cultivators as respondents is 69% are own owners, 19% are workers with profit-sharing systems and the remaining 12% are land tenants.

2. Age

Age is an important factor influencing people to think and act. At a young age, someone will be more open to accepting environmental changes, and also the level of acceptance of innovation will be faster and easier. Agricultural activities, in this case, seaweed cultivation, also require stronger energy. This is because in addition to having experience, knowledge, and seriousness, the cultivation of seaweed *Gracilaria sp*. also requires a resilient workforce.

Table 2. Age Range of Respondents for Gracillaria sp.

Age Range	Number of Respondents	Percentage
≤ 30 years old	4	5,4%
31-59 years old	61	82,4%
≥ 60 years old	9	12,2%

Source: Primary Data

Based on the table above, it is showed that the majority sample is still classified as productive, namely, 82.4% in the age range of 30-59 years old, while those in the ≤ 30 years age range are only 5% and those in the age range ≥ 60 are 12.2%. According to Samun et al.(2011), farmers at the age of 30-59 years old still have a strong physique that can support their farming activities and are easy to accept changes in technological innovation. Meanwhile, those aged >59 years old are often considered not agile and not fast enough to accept new technology (Sunar, 2012).

3. Work Experience

Success in managing *Gracilaria sp*. Also influenced by experience in cultivating seaweed *Gracilaria sp*.. According to Hilgard and Bowder, (1975), repeated experiences in certain situations lead to changes in a person's behavior in certain situations as a learning process.

Table 3. Experiences of Cultivators of Gracilaria sp.

Experience	Number of Respondents	Percentage
1-5 years	5	6,8%
6-10 years	13	17,6%
11-15 years	21	28,4%
16-20 years	20	27,0%
21-25 years	5	6,8%
26-30 years	10	13,5%

Source: Primary Data

From the table above, it is found that the largest number of respondents who have 10-15 years of experience with a percentage of 28.4%, but slightly different from those who have 16-20 years of experience with a percentage of 27.0%. Besides, some respondents had 1-5 years of experience as much as 6.8%, 6-10 years as much as 17.6%, 21-25 years as much as 6.8%, and 26-30 years as much as 13.5%.

4. Level of Education

According to Soekartawi (2001), the level of education will greatly affect the way of thinking and one's absorption of new technology. The level of education affects the way business owners think and act in doing their business. Based on the data obtained in the field, it was found that the research sample education was very diverse from elementary school (SD) to senior high school (SLTA). For more details, it can be seen in Table 6 below:

Table 4. Educational Level of Gracilaria sp. Cultivators.

Educational Level	Number of Respondesnts	percentage
Not SD	1	1%
Elementary School (SD)	21	29%

Junior High School (SMP)	25	34%
Senior High School (SMA)	26	35%
Undergraduate	1	1%

Source: Primary Data

From the table above, it is found that the educational level of Gracilaria sp. At the SMA and SMP levels are the most with a percentage level of 35% and 34%, then some do not complete elementary school with a percentage of 1%, then graduate from elementary school with a percentage of 29% but there are also undergraduate at 1%

5. Other Occupation

A person's steps or actions to increase their household income are to get other jobs besides their regular jobs. For farmers, their regular job is farming. However, there are still other jobs they do to increase family income to fulfill household consumption.

Table 5. List of other occupations of respondents

Other Occupation	Number of Respondents	Percentage
None	36	49%
Farmer	31	42%
Trader	5	7%
Breeder	1	1%
Seaweed E.Cottonii Cultivator	1	1%

Source: Primary Data

From the table above it is known that the number of respondents who do not have other occupations is 49% and those who have other occupations are 51% with each percentage of the occupation, namely farmers by 42%, traders by 7%, breeders by 1% and seaweed E. Cottonii cultivator at 1%. This shows that the majority of Gracilaria cultivators have other occupations to get additional income for their household.

3.2. Production Factor

Based on the results of production input data processing, it can be seen the effect of production input on seaweed production Gracilaria sp. in the Luwu District. The amount of influence can be seen in the following table

Table 6. Summary of Data Processing Results using SPSS

Model Summary[b]

Model	Change Statistics					Durbin-Watson
	R Square Change	F Change	df1	df2	Sig. F Change	
1	.689[a]	38.294	4	69	.000	1.813

a. Predictors: (Constant), Milkfish, Fertilizer, Land_Area, Number_of_seed

b. Dependent Variable: Harvest

Source: Primary Data Processing

From the table above, it can be seen that the R^2 value is 0.689 which means that 68.9% of the production/harvest can be explained by the production input, while the remaining 31.1% is influenced by other factors. Besides, it is also seen that there is an effect of production input on the production of *Gracilaria* sp. This can be seen from the F_{count} value obtained is 38.294 while F_{table} (0.05) is 2.50 at the 95% confidence level, which means that the production input has a significant effect on the production of seaweed *Gracilaria* sp.

From the analysis, the coefficient value of each independent variable (land, seed weight, fertilizer, and milkfish) was also obtained. The coefficient value aims to determine which independent variable has a significant effect on the

dependent variable, namely seaweed production *Gracillaria* sp. If t significance is used as a measure, the t significance value must be compared with the alpha level (α = 0.05). If the significance of t count<table, it is declared significant. However, if the significance of t count>table, it is declared insignificant.

Table 7. Coefficient Table of Each Production Factor

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	-.069	.616		-.112	.911
	Land_area	.456	.071	.537	6.450	.000
	seed_weight	.239	.078	.257	3.074	.003
	Fertilizer	-.055	.024	-.164	-2.241	.028
	Milkfish	.194	.059	.248	3.266	.002

a. Dependent Variable: Harvest

Source: Primary Data Processing

The results of the multiple linear regression data processing above can simply be written through the following equation:

$$Y=-0{,}069X1^{0{,}456}X2^{0{,}239}X3^{-0{,}055}X4^{0{,}194} + e \text{ …….. } (1)$$

With:

Y= Dry Seaweed Gracilaria sp. production (kg)

X1= Land Area (m^2)

X2= Seeds weight (kg)

X3= Fertilizer (kg)

X4= Milkfish (unit)

From the table above information is obtained about the effect of each production input on the production of *Gracilaria sp.* as follows:

a. Land Area

In this variable, T_{count} is 6,450, which is greater than T_{table}, which is 1.995, so that the land area has a significant effect on the production of *Gracilaria sp*. The regression coefficient for the land area is 0.456, which means that each additional 1 m^2 of land will increase seaweed *Gracilaria sp*. production up to 0.456 kg. This is consistent with the results of research by Wibawa M A (2017) and Risna, Munarka, & Surullah (2018) that state that land area affects the production and income of cultivators.

b. Seeds Weight

In this variable, the T_{count} is 3.074, which is greater than the T_{table}, which is 1.995, so that the seed weight has a significant effect on the production of *Gracilaria sp*. Seaweed. The regression coefficient value for Seed Weight is 0.239 which means that every 1 kg of *Gracilaria sp*. will increase the production of *Gracilaria sp*. up to 0.239 kg. This is following the research of Sabarno A., et al. (2018) which states that seed weight greatly affects production.

c. Fertilizer

In this variable, the T_{count} is -2,241, which is smaller than T_{table}, which is 1.995, so that fertilizer has no significant effect on *Gracilaria sp*. Seaweed production. The regression coefficient for fertilizer is -0.055, which means that the addition of 1 kg of fertilizer will reduce seaweed *Gracilaria sp*. production down to 0.055 kg.

It shows that, statistically, fertilizer does not affect the production of *Gracilaria sp*. However, the conditions in the field are still many people using fertilizers, especially SP 36 fertilizers. This is due to the soil conditions in the Bone Bay area, Luwu District which is acidic (Mustafa A & E Ratnawati, 2005), and the phosphate levels in the waters of Luwu District is very low (Patahiruddin, 2015). Phosphate is one of the essential elements for plants and algae and strongly affects the level of aquatic productivity (Yunus et

al., 2010). The use of fertilizer in question is based on (Halid I & Patahiruddin, 2019) which states that one of the diseases that attack seaweed, namely "ice-ice" can be prevented and stopped by using fertilizers containing phosphate. However, public knowledge of the way and the right dose of fertilizer use is not enough to cause people to speculate on this. Apart from the relatively low level of formal education, the education program about *Gracilaria sp* cultivation to the community is also lacking. This is evidenced by the results of interviews with cultivators whom 81% said there was no education program on *Gracilaria sp* cultivation, 14% said sometimes, and 5% said there was.

Table 8. Education Program about Gracilaria sp *cultivation*

Education Program	Number of Respondent	Percentage
There is	4	5%
Sometimes	10	14%
Nothing	60	81%

Source: Primary Data Processing

The use of fertilizers in the cultivation of *Gracilaria sp.* in Luwu District has similarities with several other areas. However, it should be noted that excessive use of inorganic fertilizers or in inappropriate doses can endanger the environment and *Gracilaria sp.* cultivated (Halid I & Patahiruddin, 2019).

d. Milkfish

In this variable, the T_{count} is 3.266, which is greater than the T_{table}, which is 1.995, so that milkfish has a significant effect on *Gracilaria sp.* seaweed production. The value of the milkfish regression coefficient is 0.194, which means that every addition of 1 milkfish will increase *Gracilaria sp.* seaweed production up to 0.194 kg. This is following the results of research by D Fidyansari & W Rafli (2015) that states that the production of seaweed with milkfish reaches 800 kg, while those without milkfish are only 586 kg.

According to Priono B., Septyan A., & Irsyaphiani I. (2012), that the cultivation of *Gracilaria sp.* and milkfish simultaneously can be done even *Gracilaria sp.* can grow to more than 375% for more than two months of maintenance. This is because of the presence of milkfish in *Gracilaria sp.* seaweed ponds will help supply nutrients from the secretions of milkfish and also help prevent algae growth through its movement on the bottom of the water while looking for food (W. Isroni et al., 2020)

IV. CONCLUSIONS AND SUGGESTIONS

4.1. Conclusions

In the Polyculture Gracilaria sp and milkfish culture system, it is known that holistically, the production of Gracilaria sp. can be explained by the production input of 68.9% while 31.1% is influenced by other factors. However, partially, the input of fertilizer production does not have an effect on productivity, while the input production of land area, weight of seedlings and number of milkfish seeds have an effect on the productivity of seaweed Gracilaria sp in the polyculture system with milkfish. So that to save capital, cultivators can reduce or even need to use fertilizers.

4.2. Suggestions

Nutrition for the growth of Gracilaria sp seaweed can be fulfilled by changing the water at a certain time. In addition, it is also necessary to conduct research on the nutritional requirements for the growth of Gracilaria sp. Seaweed and its availability in Bone Bay, Luwu Regency.

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Modelling Determinants of Farmers' Choice of Adaptation Strategies to Climate Variability and Extreme Events in Kitui County, Kenya

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Abstract*—The study was carried out to assess determinants of farmers' choice of specific adaptation strategies to climate variability and extreme events in selected agro-ecological zones in Kitui County. Descriptive survey design was used. The study area was stratified into four study sites with respect to four different agro-ecological zones and a total of 341 households selected to constitute the sample size. Multivariate probit regression model was run in Stata version 12 to determine the influence of different socio-economic characteristics on farmers' choice of specific adaptation strategies. The model results indicated that age, gender, farming experience, membership to farmers' organization, education level, access to extension services and proximity to market had a significant varying influence on farmers' choice of several adaptation strategies. The study established that different socio-economic characteristics had a different influence on the farmers' choice of specific adaptation strategies. The study therefore recommends that climate variability adaptation policies, programs and projects by governmental and non-governmental development agencies should target specific socio-economic characteristics that are relevant to the adaptation strategies in question.*

Keywords*—*Socio-economic characteristics, multivariate probit regression, agro-ecological zones, adaptive capacity.

I. INTRODUCTION

Changes in the climate system in recent decades have caused significant impacts on the natural and human systems on all continents and across the oceans (IPCC, 2014). According to IPCC (2007), variability in temperature and rainfall patterns have been predicted to cause significant effects on global agriculture, due to extreme weather events such as droughts and floods and changes in patterns of pests and diseases. The effects of changing temperature and rainfall patterns are more pronounced in developing countries owing to their geographic exposure, low income, greater reliance on rain-fed agriculture and other climate sensitive sectors coupled with its weak capacity to adapt to the changing climate (Belloumi, 2014; Thomas et al., 2005; Slingo et al., 2005).

The IPCC (2007) report estimated that Africa will be the most vulnerable continent to the progressive changes in climate globally, due to its low adaptive capacity resulting from the multiple stresses of poor infrastructure, poverty and governance. Kenya is one of the most vulnerable countries to climate variability and extreme events in Africa due to its low adaptive capacity and dependence on climate-sensitive sectors such as agriculture and fisheries as the key drivers of its economy (FAO, 2011; Herrero et al., 2010;

Kurukulasuriya et al., 2006; Kabubo-Mariara and Karanja, 2007). Climatic variability is therefore expected to have adverse effects in Kenya's economy because of her dependency on climate sensitive natural resources, and thus recurring droughts, erratic rainfall patterns and floods will continue to negatively impact livelihoods and community assets (Government of Kenya, 2016).

Since agriculture is the mainstay of most rural communities in Kenya (Republic of Kenya, 2005) negative developments in agriculture would adversely affect the rest of the livelihoods that are depended on agricultural production. The cumulative effects of climate variability and extreme events in Kenya therefore pose a significant threat towards the attainment of the country's Vision 2030 (Parry et al., 2012) as well as the implementation of the Sustainable Development Goals (UNECA, 2018; UNCCS, 2017). Implementation of adaptation strategies will therefore be paramount to cushion communities from the effects of climate variability and extreme events and promote sustainable livelihoods in the advent of a changing climate (Akinnagbe and Irohibe, 2014; Schipper et al., 2008; IPCC, 2007).

According to Maddison (2006), the ability and decision to adopt a particular adaptation strategy is determined by several institutional and socio-economic factors. In the face of climatic variability, farmers may opt to adopt several strategies instead of relying on a single strategy to exploit complementarities or substitutability among alternatives (Ojo and Baiyegunhi, 2018). The current study therefore sought to examine the determinants of farmers' choice of specific adaptation strategies in different agro-ecological zones in Kitui County.

II. MATERIALS AND METHODS

2.1 Profile of the study area

The study was carried out along a transect line (in a buffer zone of 5km radius on both sides of the line) in semi-humid, transitional semi-humid to semi- arid, semi-arid and arid zones in Kitui County. The study sites are shown in Fig. 1.

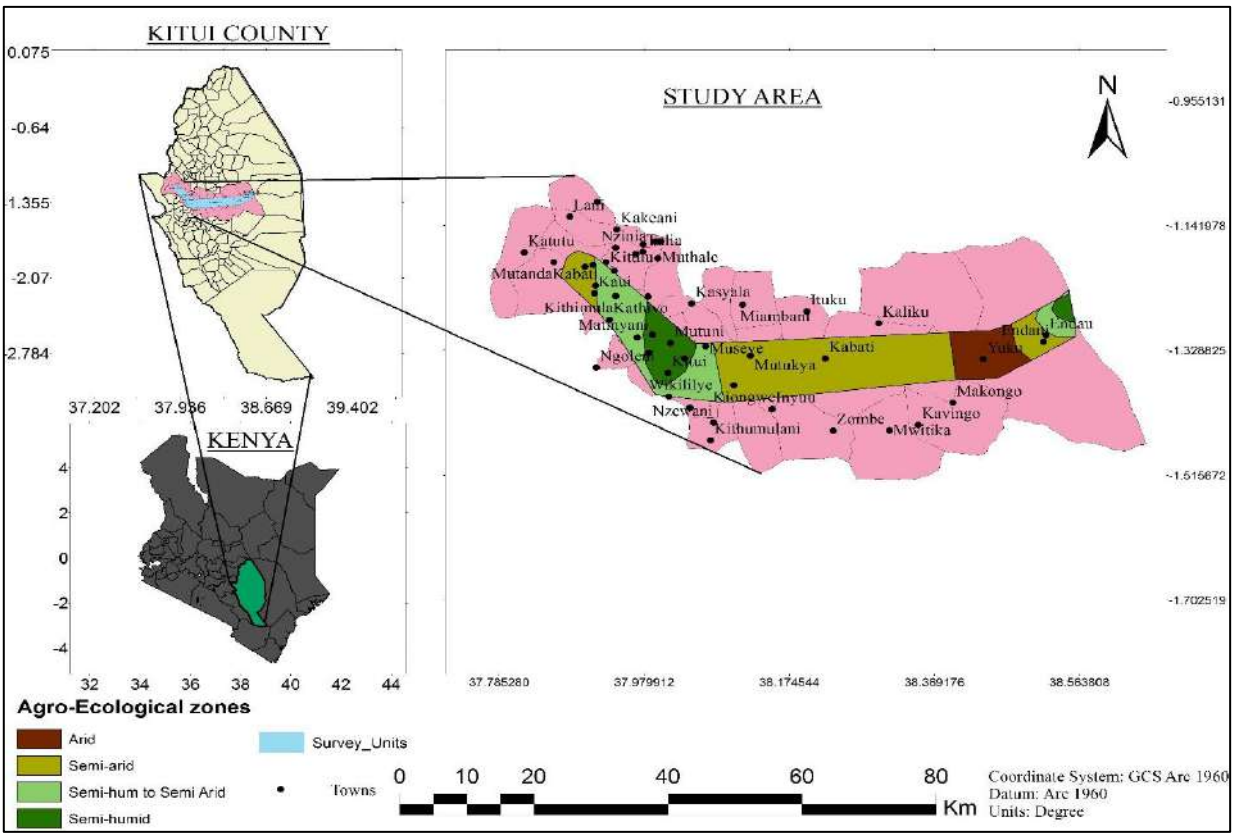

Fig.1: Map of Kitui County showing the study area in four agro-ecological zones

Source: ILRIS GIS Database

Kitui County lies between 400m to 1,830m above sea level and generally slopes from the west to east with the highest regions being Kitui Central and Mutitu Hills (KCIDP, 2018). The climate of the area is semi-arid with very erratic

and unreliable rainfall. The area is hot and dry throughout the year with temperatures ranging from a minimum of 14-22° centigrade to a maximum of 26-34° centigrade. The months of February and September are the hottest months in the year (KCIDP, 2018). Rainfall is distributed within two seasons annually and varies from 500-1050mm with about 40% reliability. The long rains are experienced between March and May and short rains between October and December. The short rains are considered more reliable than the long rains since it is during the short rains that farmers get their main food production opportunity (NDMA, 2017).

The soil types range from red sandy soils, to clay black cotton soils which are generally low in fertility (Republic of Kenya, 2005). The County's population is approximately 1,136,187 according to the population and housing census report of 2019 (Government of Kenya, 2019). Livestock production and crop farming are the back bone of the people's economy in the area contributing to nearly three quarters of the household earnings (KCIDP,2018; Republic of Kenya, 2005). The main livestock types kept in the County are cattle (beef and dairy), goats (meat and dairy), sheep and poultry (indigenous and exotic) (KCIDP, 2018). Various crops such as maize, beans, sorghum, pigeon peas, millet and cassava are cultivated mainly for subsistence while green grams, sweet potatoes, vegetables such as tomatoes, kales, spinach, pawpaw, onions and fruits (mangoes, bananas, water melons) are grown for sale and household consumption (KCIDP, 2018; NDMA, 2017; Republic of Kenya, 2005).

2.2 Study Design and Sampling Techniques

Descriptive survey design was used. The target population for the study was the agro-pastoral farmers in the study area. The unit of study was the household while the respondents comprised of the head of the households. Stratified sampling method was used to classify the study sites with reference to four different agro-ecological zones in Kitui County. One sub-location in each agro-ecological zone was randomly selected along a transect line (in a buffer zone of 5km radius on both sides of the line). Systematic random sampling method was used to identify respondents in the selected sub-location.

The sample size for the study was determined by calculating 10% of the number of households in each of the four sub-locations. According to Mugenda and Mugenda (2003), a sample size of 10% provides an adequate representation of the target population in descriptive research. The total sample size for the study was 341 households with 39,160, 38 and 104 households from the arid, semi-arid, transitional semi-arid to semi-humid and the arid zones, respectively.

2.3 Data Collection and Analysis

Primary data was collected through administration of questionnaires to 341 respondents. Interviews with key informants were also conducted. Multivariate Probit (MVP) regression model was run in Stata version 12 to assess the determinants of farmers' choice of different adaptation strategies in the study area.

The MVP decision model is guided by the random utility theoretical model which describes a choice decision in which an individual has a set of alternative adaptation strategies from which to choose (McFadden, 1978). The model assumes that each adaptation option has distinct attributes that influence a farmer's choice over another alternative and is based on the notion that the utility is derived by choosing several alternatives.

The utility random model is described below as applied by Feleke et al. (2016).

Assuming that U_j is the expected utility that a farmer will gain from using adaptation strategy j whereas U_k is the expected utility for not choosing adaptation strategy j but rather k.

The linear random utility model of adapting to climate variability by choosing j_{th} adaptation strategy (U_j) can be expressed as a function of explanatory variables X_i as shown below:

$$U_{ij} = x_i\beta_j + \mu_j \ (1)$$

The linear random utility model for i_{th} farmer who does not use j_{th} adaptation strategy but rather k_{th} adaptation strategy is given by:

$$U_{ik} = x_i\beta_k + \mu_k \ (2)$$

Where x_i is a vector of explanatory variables β_j and β_k are vectors of parameters for choosing j_{th} and k_{th} adaptation strategy respectively, μ_j and μ_k are error terms for choosing j_{th} and k_{th} adaptation strategy, respectively. According to Gujarati (2006), the error terms in the above equations are assumed to be normally independently and identically distributed.

If a farmer chooses to adopt j_{th} adaptation strategy to climate variability, then the expected utility that the farmer gets is greater than the expected utility for not using that strategy and according to Falco et al. (2011), a farmer chooses adaptation strategy j over adaptation strategy k if and only if the expected utility from adaptation strategy j is greater than that of k as expressed in equation 3:

$$U_{ij} = x_i\beta_j + \mu_j > U_{ik} = x_i\beta_k + \mu_k \ (3)$$

Following Mihiretu et al. (2019) and Piya et al. (2012), the MVP model assumes that each subject has J distinct binary responses. Let i=1,...n be the independent observations, j=1,...J be the available options of binary responses, and X_i be a matrix of covariates composed of any discrete or continuous variables.

Let $Y_{ij} = Y_{i1} \dots Y_{ij}$ denote the J-dimensional vector of observed binary responses taking values {0;1} on the i^{th} household and $Z_{ij} = Z_{i1} \dots Z_{ij}$ denote a J-variate normal vector of latent variables such that:

$$Z_{ij} = X_{i\beta} + \varepsilon_i 1 = 1 \dots n \quad (4)$$

where $\beta = \beta_1 \dots \beta_j$ is a matrix of unknown regression coefficient, ε_i is a vector of residual error distributed as multivariate normal distribution with zero means and unitary variance;

$$\varepsilon_i \sim N(0, \sum),$$

where Σ is the variance-covariance matrix.

The off-diagonal elements in the correlation matrix, $\rho_{kj} = \rho_{jk}$ represent the unobserved correlation between the stochastic components of k^{th} and J^{th} options (Cappellari and Jenkins, 2003).

The relationship between Z_{ij} and Y_{ij} is:

$$Y_{ij} = \{1 \; if > 0; 0 \; otherwise\} i = 1 \dots n \; and \; j = 1 \dots J \quad (5)$$

The likelihood of the observed discrete data is then obtained by integrating over the latent variables:

$$Z: P(Y_{ij} = {}^{1}/_{X_i\beta\sum}) \int A_{i1} \Phi_T \, (Z_{ij} = {}^{1}/_{X_i\beta\sum}) dZ_{ij} \quad (6)$$

Where, A_{i1} is the interval (0, ∞) if Y_{ij}=1 and the interval (-∞, 0) otherwise and

$A_{i1}\Phi_T(Z_{ij} = {}^{1}/_{X_i\beta\sum})dZ_{ij}$ is the probability density function of the standard normal distribution.

Since the coefficient estimates from MVP regression show the direction of influence rather than the magnitude (Mullahy, 2017), to interpret the effects of explanatory variables on the probabilities, marginal effects were derived as follows:

$$\partial ij = \frac{\partial Pij}{\partial x_i} Pij \left[\beta j - \sum_{k=0}^{j} P_{kj} \, \beta_k \right] = P_{ij} [\beta j - \beta] \; (7)$$

where, δij-denotes the marginal effect of the explanatory variable on the probability that alternative j is chosen. The marginal effects measure the expected change in probability of a particular choice with respect to a unit change in an explanatory variable (Amdu et al., 2012).

The multivariate probit (MVP) regression model was chosen for this study since it simultaneously models the influence of the set of explanatory variables on each of the adaptation strategies, while allowing the unobserved factors (error terms) to be freely correlated (Belderbos et al., 2004; Lin et al., 2005). According to Belderbos et al. (2004), the source of correlation may be complementarities (positive correlation) and substitutability (negative correlation) between different adaptation strategies.

For the purpose of this model, selected adaptation strategies to climate variability and extreme events adopted by farmers were used as the dependent variables while farmers' socio-economic characteristics were used as the explanatory variables for the model as described in Table 1 and Table 2, respectively.

Table 1 Description and summary statistics of dependent variables used in the Multivariate Probit model

Dependent variables (Adaptation Strategies)	**Description of Variables**	**Mean**	**SD**
Crop diversification	Dummy=1 if household adopts crops diversification, 0=otherwise	0.70	0.46
Planting drought resilient crops	Dummy=1 if household adopts planting drought resilient crops, 0=otherwise	0.66	0.47
Planting hybrid crop varieties	Dummy=1if household adopts planting hybrid crop varieties, 0=otherwise	0.60	0.49
Use of soil conservation techniques	Dummy=1if household adopts soil conservation techniques, 0=otherwise	0.72	0.45
Use of inorganic fertilizers	Dummy=1if household adopts use of fertilizers, 0= otherwise	0.27	0.46

Use of manure	Dummy=1if household adopts use of manure, 0=otherwise	0.81	0.40
Agroforestry	Dummy=1if household adopts agroforestry, 0=otherwise	0.47	0.50
Use pesticides	Dummy=1if household adopts use of pesticides, 0=otherwise	0.72	0.45

Table 2 Description and summary statistics of explanatory variables used in theMultivariate Probit Model

Variable	Description	Mean	SD	Expected sign
X_1	Gender of household head (1= male; 0= female)	1.29	0.46	+/-
X_2	Age of the household head (number of years of the household head)	55.86	15.11	+/-
X_3	Household size (number of family members in the household)	5.88	2.64	+/-
X_4	Membership in a farmers' cooperative/group (1= yes; 0= otherwise)	0.20	0.40	+
X_5	Farming experience (number of years household head involved in farming)	25.66	16.52	+
X_6	Education level of the household head (years of schooling of the household head)	12.43	4.41	+
X_7	Access to credit (1= yes; 0= otherwise)	0.35	0.48	+
X_8	Access to extension services (1=yes, 0=otherwise)	0.20	0.40	+
X_9	Distance from the market (how far the household is from the market in Km)	2.79	3.24	+
X_{10}	Access to early warning weather information (1=yes, 0=otherwise)	0.74	0.44	+
X_{11}	Land size(Number of acres owned by the household)	5.82	8.07	+/-

III. RESULTS

3.1 Determinants of farmers' choice of specific adaptation strategies to climate variability and extreme events in the study area

The coefficient estimates of the multivariate probit model are presented in Table 3. The null hypothesis for test of independence in the model was rejected since the likelihood ratio test (*Log likelihood = -1394.05*; Prob > $\chi 2$ = 0.00) of independence of error terms was significant implying that there is a mutual interdependence among the adaptation strategies and thereby justifying the use of multivariate probit regression model in assessing the determinants of farmers' choice of different adaptation strategies as it captures wider effects than a univariate probit model could obtain.

The pairwise correlation coefficients (Rho) shown in Table 3 also indicated a positive correlation between the pairs most of which are highly significant implying that the sets of adaptation strategies are complimentary. Variance inflation factor (VIF) values for all the explanatory variables were between 1 and 3 implying that multicollinearity was not a concern since according to Yoo et al. (2014), multicollinearity concerns exist when the VIF value is greater than 10.

Table 3 Coefficient estimates of Multivariate Probit regression results on determinants of farmers' adoption of specific adaptation strategies in the study area

Explanatory variables	Dependent Variables							
	Crop diversification	**Drought resilient crops**	**Hybrid crop varieties**	**Soil conservation techniques**	**Use of fertilizer**	**Use of manure**	**Agroforestry**	**Use of Pesticides**
Age	-0.02 (0.01)**	-0.01 (0.01)	-0.00 (0.01)	-0.02 (0.01)**	-0.00 (0.00)	0.00 (0.01)	-0.01 (0.01)*	-0.01 (0.01)
Gender	0.32 (0.18)**	0.20 (0.17)	-0.11 (0.17)	0.25 (0.19)*	-0.46 (0.18)***	0.30 (0.19)*	0.25 (0.16)*	-0.37 (0.17)**
Household size	-0.05 (0.03)	0.04 (0.03)	-0.03 (0.03)	-0.06 (0.03)**	-0.01 (0.03)	0.00 (0.03)	-0.04 (0.03)	-0.03 (0.03)
Farmers' group membership	0.16 (0.23)	-0.31 (0.21)	-0.42 (0.21)**	0.57 (0.27)**	-0.09 (0.22)	-0.42 (0.24)*	0.21 (0.21)	0.27 (0.24)
Farming experience	0.01 (0.01)*	0.01 (0.01)	0.00 (0.01)	0.01 (0.01)*	0.00 (0.01)	-0.01 (0.01)	0.01 (0.01)	0.00 (0.01)
Education level	-0.00 (0.17)	0.02 (0.02)	0.07 (0.02)***	0.06 (0.02)***	0.01 (0.17)	0.06 (0.02)***	0.05 (0.02)***	0.05 (0.02)**
Access to credit	0.19 (0.17)	0.30 (0.16)*	0.08 (0.16)	-0.01 (0.17)	0.05 (0.16)	0.14 (0.19)	0.04 (0.15)	0.15 (0.17)
Access to extension services	0.17 (0.23)	0.51 (0.23)**	-0.30 (0.21)	0.43 (0.26)*	0.31 (0.21)*	0.34 (0.26)	0.41 (0.21)**	0.28 (0.23)
Distance to market	-0.03 (0.02)*	-0.02 (0.02)	-0.13 (0.03)***	-0.06 (0.02)***	-0.04 (0.03)*	0.01 (0.40)	-0.02 (0.02)	-0.09 (0.03)***
Access to weather information	-0.37 (0.18)	-0.11 (0.17)	-0.04 (0.17)	-0.38 (0.19)	0.27 (0.19)	-0.10 (0.19)	0.15 (0.16)	-0.07 (0.17)
Land size	0.01 (0.01)	0.03 (0.01)**	-0.00 (0.01)	0.01 (0.01)	-0.03 (0.01)**	-0.00 (0.01)	-0.01 (0.01)	-0.02 (0.02
Constant	1.18 (0.45)***	-0.10 (0.43)	0.47 (0.43)	0.91 (0.47)**	-0.45 (0.46)	-0.08 (0.48)	-0.52 (0.43)**	0.91 (0.46)**
	Rho 1	**Rho 2**	**Rho 3**	**Rho 4**	**Rho 5**	**Rho 6**	**Rho 7**	**Rho 8**
Rho 2	0.30 ***							
Rho 3	0.29***	0.19***						

Rho 4	0.01	0.10*	0.14*					
Rho 5	0.50***	0.31***	0.21***	0.08*				
Rho 6	0.01	0.13*	0.25***	0.16***	0.05			
Rho 7	0.26***	0.08*	0.13***	0.19***	0.24***	0.11**		
Rho 8	0.31	0.10*	0.28***	0.06	0.32***	0.10*	0.31***	

*Number of observations = 341; Wald chi^2 (88) = 204.71; Log likelihood = -1394.05; Prob > chi2 = 0.00; Figures in parentheses are standard errors; ***, **, * significant at 99%, 95% and 90% confidence levels, respectively*.

The marginal effects presented in Table 4 were used to quantify the influence of explanatory variables on the dependent variables in the model.

Table 4 Marginal effects of explanatory variables on dependent variables

Explanatory variables	**Dependent Variables**							
	Crop diversification	**Drought resilient crops**	**Hybrid crop varieties**	**Soil conservation techniques**	**Use of fertilizer**	**Use of manure**	**Agroforestry**	**Use of pesticides**
Age	-0.01 (0.05)**	-0.01 (0.01)	0.01 (0.01)	0.08 (0.05)	0.00 (0.00)	0.00 (0.00)	-0.01 (0.01)**	-0.01 (0.01)
Gender	0.12 (0.04)**	0.07 (0.06)	0.04 (0.06)	-0.01 (0.01)**	-0.15 (0.06)***	0.07 (0.05)*	0.09 (0.16)*	-0.11 (0.01)**
Household size	-0.02 (0.06)*	0.01 (0.01)	-0.01 (0.01)	-0.02 (0.01)**	-0.01 (0.01)	0.01 (0.01)	-0.02 (0.01)	-0.01 (0.01)
Farmers' group membership	0.06 (0.44)	-0.11 (0.07)	-0.13 (0.07)	0.17 (0.07)**	-0.01 (0.07)	-0.10 (0.06)*	0.08 (0.08)	0.08 (0.07)
Farming experience	0.01 (0.01)**	0.01 (0.01)*	0.01 (0.01)	0.01 (0.01)	0.00 (0.00)	-0.01 (0.01)	0.01 (0.01)	0.01 (0.01)
Education level	-0.01 (0.01)	0.01 (0.01)	0.02 (0.01)***	0.01 (0.01)***	0.01 (0.01)*	0.02 (0.01)***	0.02 (0.01)***	0.01 (0.01)**
Access to credit	0.05 (0.05)	0.09 (0.05)*	0.03 (0.05)	0.01 (0.05)	0.01 (0.05)	0.03 (0.05)	0.00 (0.00)	0.04 (0.05)
Access to extension services	0.06 (0.07)	0.17 (0.08)**	-0.11 (0.07)	0.10 (0.07)	0.09 (0.07)	0.08 (0.07)	0.16 (0.07)**	0.09 (0.07)
Distance to market	-0.01 (0.06)*	-0.01 (0.01)	-0.05 (0.01)	-0.02 (0.01)***	-0.01 (0.01)	-0.01 (0.01)	-0.01 (0.01)	-0.04 (0.01)***
Access to weather information	-0.12 (0.01)	-0.03 (0.06)	-0.01 (0.06)	-0.10 (0.05)	0.01 (0.01)	-0.02 (0.05)	0.06 (0.06)	0.05 (0.05)

Land size	0.01 (0.01)	0.01 (0.01)**	-0.01 (0.01)	0.01 -(0.01)	-0.01 (0.01)	$7.84e^{-06}$ (0.00)	-0.01 (0.01)	0.01 (0.00)

The multivariate probit regression results indicated that age of the household head had a positive but insignificant influence on the adoption of use of manure. There was however a negative influence of age of the household head on the adoption of crop diversification, planting drought resilient crops, planting hybrid crop varieties, soil conservation techniques, agroforestry and use of pesticides. The negative influence of age of the household head was significant on the adoption of crop diversification, soil conservation techniques and agroforestry. Marginal effects results showed that a unit increase in age reduced the probability of adopting crop diversification, soil conservation techniques and agroforestry by a factor of 0.01, 0.08 and 0.01, respectively.

Gender of the household head had a positive influence on the adoption of crop diversification, drought resilient, soil conservation techniques, use of manure and agroforestry which was significant on the adoption of crop diversification, use of manure and agroforestry. The marginal effects results indicated that male headed households were 12%, 7% and 9% more likely to adopt crop diversification, use of manure and agroforestry, respectively than their female counterparts. In regards to the adoption of use of fertilizers and pesticides, gender of the household head had a significant negative influence with marginal effects of 0.15 and 0.11, respectively. This implies that female headed households were 15% and 11% more likely to use fertilizers and pesticides, respectively than their male counterparts. The results indicated a positive but insignificant influence of household size on the adoption of drought resilient crop varieties. A negative influence of household size was however noted on the adoption of crop diversification, hybrid crop varieties, soil conservation techniques, agroforestry and use of pesticides and fertilizers with the influence being significant only on the adoption of soil conservation techniques with a marginal effect of 0.02. This implies that a unit increase in household size reduced the probability of adopting soil conservation techniques by 2%.

Membership in a farmers' organization had a negative insignificant influence on the adoption of drought resilient crops, hybrid crop varieties, manure and fertilizers. There was however a positive influence on the adoption of crop diversification, soil conservation techniques, pesticides and agroforestry. The significant negative influence of membership in a farmers' organization had a marginal effects 0.13 implying that membership to farmers' organization decreased the probability of adopting hybrid crop varieties by 13%. On the other hand, membership in a farmers' organization significantly increased the probability of adopting soil conservation techniques by 17%.

The results indicated that farming experience had a positive influence on all the adaption strategies except for adoption of use of manure, which was negative but insignificant. The positive influence of farming experience was significant on the adoption of crop diversification and soil conservation techniques with marginal effects of 0.01 on each, implying that a unit increase in farming experience increased the probability of adopting crop diversification and soil conservation techniques by 1%.

Further, the results indicated that education level of the household head had a positive influence on the adoption of all the strategies except for crop diversification. The results indicated a statistically significant positive influence on the adoption of use of hybrid crop varieties, soil conservation techniques, use of manure, agroforestry and use of pesticides with marginal effects of 0.02, 0.01, 0.02, 0.02 and 0.01, respectively. The results implied that a unit increase in education level of the household head increased the probability of adopting hybrid crop varieties, soil conservation techniques, use of manure, agroforestry and use of pesticides by 2%, 1%, 2%, 2% and 1%, respectively.

Access to credit facilities had a positive influence on the adoption of all the strategies except for soil conservation techniques. The influence of access to credit facilities was significant on the adoption of drought resilient crops with a marginal effect of 0.09 implying that farmers with access to credit facilities were 0.09 more likely to adopt drought resilient crops than those without access to credit facilities.

In regards to access to extension services, a positive influence was noted on the adoption of all the adaption strategies except for use of hybrid crop varieties. The results indicated a significant positive influence on the adoption of drought resilient crops, soil conservation techniques, use of fertilizers and agroforestry. The marginal effects results indicated that access to extension services increased the farmers' probability of adopting the drought resilient crops, soil conservation techniques, use of fertilizers and agroforestry by 17%, 10%, 9% and 16 %, respectively. Access to weather information however had an insignificant

negative influence on the adoption of all the adaptation strategies except for the use of fertilizers and agroforestry.

The results further indicated that distance to market had a negative influence on the adoption of all the adaption strategies except for use of manure implying that ease of access to the market increased farmers probability of adopting the different adaption strategies. The negative influence of distance to market was significant on adoption of crop diversification, use of hybrid crop varieties, soil conservation techniques, fertilizers, agroforestry and pesticides whose marginal effects implied that a unit increase in distance to the market reduced farmers' probability of adopting crop diversification, use of hybrid crop varieties, soil conservation techniques, fertilizers, agroforestry and pesticides by a factor of 0.01, 0.05, 0.02, 0.01, 0.01 and 0.04, respectively.

The influence of land size was positive on the adoption of crop diversification, drought resilient crops and soil conservation techniques. There was however a negative influence of land size on the adoption of hybrid crop varieties, use of fertilizers, manure, agroforestry and pesticides. A significant influence of land size was noted on the adoption of drought resilient crops and use of fertilizers implying that an increase unit in land size increased the probability of adopting drought resilient crops while decreasing that of adopting use of fertilizers by 1%.

IV. DISCUSSION

The ability and decision to adopt a particular adaptation strategy is determined by several socio-economic factors (Maddison, 2006). Results from the present study indicated that different socio-economic characteristics of farmers had a different influence on the farmers' choice of specific adaptation strategies to climate variability and extremes. The results showed that there was a significant negative influence of age on the adoption of crop diversification, soil conservation techniques and agroforestry which implies that younger farmers in the study area were more likely to adopt the adaptation strategies as compared to older farmers. This could be because younger farmers are innovative and likely to try new technologies and methods to improve agricultural productivity. Conversely, in most cases older farmers are often not aware of recent innovations in agriculture and/or are reluctant to try new methods. Similar findings where there was a significant negative influence of age on the adoption of mixed cropping and improved crop varieties were reported in other studies (Ojo and Baiyegunhi, 2018; Ali and Erenstein, 2016).

With regards to gender of the household head, female headed households were more likely to use fertilizers and pesticides compared to their male counterparts. This could be attributed to the fact that female headed households have less access to resources such as land and therefore resort to invest in use of fertilizers and pesticides to boost their agricultural productivity in their small pieces of land. On the other hand, the results indicated that male headed households were more likely to adopt crop diversification, use of manure and agroforestry as opposed to female households. This could be because women-headed households are usually constrained by family labor since they are culturally assigned responsibility in domestic activities and also have less access to resources and information compared to male headed households which limit their ability to carry out labor-intensive activities. The easiness with which male headed households adapt to climate change compared to female headed ones was also highlighted by Tenge De Graffe and Heller (2004) while working on the social and economic factors that influence adoption of soil and water conservation (SWC) measures in the West Usambara highlands, Tanzania. Further, Deressa et al. (2009) noted that male headed households are more likely to have access to technologies and climate change information than female-headed households and therefore better placed in adopting diverse adaptation strategies than female- headed households. In addition, Mihiretu et al. (2019), Asrat and Simane, (2018) and Belay et al. (2017) also reported that male-headed households had a higher probability of adopting new agricultural technologies compared to their female counterparts. The results of the current study are however contrary to findings by Nhemachena and Hassan (2007) who noted that female-headed households in Southern Africa were more likely to take up climate change adaptation practices since they are responsible for much of the agricultural work in the region and therefore have greater experience and access to information on various management and farming practices.

The negative influence of household size noted on the adoption of soil conservation techniques could be explained by the fact that since not all members in the family are actively engaged in agricultural activities, a bigger household size would increase demand for resources thereby diverting family labor to off-farm jobs to supplement households' food and economic needs. The results are in agreement with findings from Arun and Yeo (2019) who reported a negative influence of household size on farmers' adoption of adaptation strategies such as crop irrigation, changing of crop date crop type, and crop varieties. In their study, Dumenu and Tiamgne (2020) also

noted that a household with more dependents was more likely to direct a larger proportion of its resources towards the household's welfare leaving it with little resources for adapting to climate change and variability thereby increasing its vulnerability to climate variablilty and extremes. Similarly, Tizale (2007) found that there was a possibility that households with large families diverted part of their labour to non-farm activities to earn income to ease the consumption pressure imposed by a large family. The results however contradict findings from similar studies by Asrat and Simane (2018), Belay et al. (2017) and Jiri et al. (2015) who noted a positive influence of household size on adoption of labour intensive adaptation strategies.

From the study, membership in a farmers' organization reduced farmers' probability of adopting hybrid crop varieties. Discussions with farmers and key informants from relevant institutions revealed that high cost of hybrid crop varieties discouraged households from adopting the strategy thus gaining knowledge about the hybrid crop varieties without financial facilitation from the organizations was not adequate in enabling farmers to adopt the strategy. It was however noted that membership in a farmers' organization significantly increased the probability of adopting soil conservation techniques. This could be because farmers' organizations in form of cooperatives, self-help groups or market groups function as sources of information, learning platforms and social support systems that are critical in creating linkages with other actors, providing space for knowledge generation and sharing, discussion of innovation and information necessary in adapting to changes in climatic conditions. The current trend of results is concurrent with findings of similar studies (Borda-Rodriguez and Vicari, 2015; Kearney and Berkes, 2007). In addition, studies by Kangogo et al. (2020), Žurovec and Vedeld (2019) and Bryan et al. (2011) indicated that farmers belonging to farmers' organizations were more likely to adopt different adaption strategies since social networks facilitate information flows through discussion of problems, sharing new innovations and technologies as well as taking collaborative decisions which enhance their capacity to adapt to climate variability and extreme events.

Farming experience of the household head was found to have a positive influence on the adoption of crop diversification and soil conservation techniques. This can be ascribed to the fact that experienced farmers have high skills in farming techniques and management and are able to spread risk when facing climate variability and extreme events by exploiting strategic complementarities in different adaptation strategies. The results are in agreement with findings from similar studies by Asrat and Simane (2018) and Belay et al. (2017) who noted a positive influence of farming experience on the adoption of several adaptation strategies. Further, the current trend of results corroborates findings of similar work by Nhemachena and Hassan (2007).

The education level of the household head increased the probability of adopting hybrid crop varieties, soil conservation techniques, use of manure, agroforestry and use of pesticides. This could be attributed to the fact that educated farmers are more likely to perceive changes in climatic conditions, better recognize the risks associated with the climatic changes and have better reasoning capability and awareness about new technologies. The results are in consonance with findings from similar studies (Asrat and Simane, 2018; Fagariba et al., 2018; Belay et al., 2017; Deresa et al., 2009 and Nhemachena and Hassan, 2007).

Access to credit facilities had a positive influence on the adoption of all the strategies except for soil conservation techniques. The results imply that access to credit facilities increased the probability of farmers to adopt different adaptation strategies. Accordingly, access to credit facilities increases farmers' financial capacity to meet transaction costs associated with several adaptation strategies. The results are in agreement with findings from similar studies by Arun and Yeo (2019), Fagariba et al. (2018) and Tesfaye and Seifu (2016) who noted a positive relationship between access to credit facilities and adoption of different adaptation strategies.

In regards to access to extension services, the results indicated a significant positive influence on the adoption of drought resilient crops, soil conservation techniques, use of fertilizers and agroforestry. This is because agricultural extension services provide farmers an opportunity to acquire information and trainings on climatic variations, new technologies and innovations as well as new skills and technical capacity for sustainable implementation of adaptation strategies. The results are in agreement with findings from similar studies which reported a positive influence of access to extension services on farmers' adoption of different adaptation strategies (Teklewoldet al., 2019; Fagariba et al., 2018; Belay et al., 2017 and Nhemachena et al., 2014).

Contrary to the expectation, access to weather information had an insignificant negative influence on the adoption of all the adaptation strategies except for the use of fertilizers and agroforestry. Similar studies noted that access to weather information increases farmers awareness on climatic changes which is essential in making informed

decisions on preparedness to reduce agricultural losses that might occur from climate variability and extreme events thereby increasing the probability of farmers' to adopt different adaption strategies (Asrat and Simane, 2018; Fagariba et al., 2018; Belay et al., 2017; Nhemachena et al., 2014). The negative influence of access to weather information on adoption of the different adaptation strategies in this study could suggest that farmers are more likely to invest in off-farm livelihood options as opposed to agriculture upon noting the possibility of occurrence of extreme weather events.

The results further indicated that distance to market had a negative influence on the adoption of all the adaption strategies except for use of manure implying that ease of access to the market increased farmers probability of adopting the different adaption strategies. Proximity to market facilitates farmers' access to information and agricultural inputs such as hybrid crop varieties, fertilizers and pesticides as well as a market for selling agricultural outputs increasing the likelihood of adopting different adaptation strategies. The results are in consonance with findings by Marie et al. (2020) who noted that farmers with access to market were 0.34 times more likely to adopt climate change adaptation strategies than those without. Further, the results corroborate findings by Belay et al. (2017) who found a positive and significant effect of distance to market on farmer input intensity and crop diversification.

Lastly, land size increased the probability of adopting drought resilient crops while reducing the probability of adopting use of fertilizers. The mixed effect of land size on adoption of the different strategies could be because a large farm size allows farmers space to practice crop diversification and also discourage adoption of high cost strategies. The results of the study are in consonance with findings from Žurovec and Vedeld (2019). A positive and significant relationship between land size and farmers' adoption of a combination of several adaptation strategies such as agroforestry, perennial plantation, crop–livestock diversification and improved varieties was also reported by Fadina and Barjolle (2018).

V. CONCLUSION AND RECOMMENDATIONS

The study established that age, gender, farming experience, membership to farmers' organization, education level of the household head, access to extension services and proximity to market had a significant varying influence on farmers' choice of different adaptation strategies. The study therefore recommends that policies, programs and projects by governmental and non-governmental development agencies aimed at helping farmers adapt to climate variability and extreme events should target specific socio-economic characteristics that are relevant to the adaptation strategies in question.

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Introduction Prospect of artificial Insemination Technology in Supporting Beef Cattle Development

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Abstract**—** *The beef cattle business currently has the prospect to be developed due to the increasing demand for livestock products.The increase in population, economic level and fulfillment of people's nutritional needs had an impact on the tendency of increasing demand and consumption of beef as a source of animal protein.On the other hand, the increase in demand for meat was not matched by availability from beef cattle producers. The problem was that the development of beef cattle in the research area was slow, so the introduction of technology is needed which has an impact on increasing the population of beef cattle.The purpose of this study was to examine the prospects for the introduction of artificial insemination technology. The research method used was a survey method.The sample location was Sangkub District which was determined by purposive sampling, namely as a beef cattle development area. Respondents were beef cattle farmers who have participated in the Artificial Insemination program, as many as 33 people. The data analysis used was descriptive analysis.The prospect of introducing Artificial Insemination at the research location depends on the number of beef cattle, the number of instructors and inseminators, and the response of farmers to the program.The results showed that the beef cattle population in North Bolaang Mongondow Regency from 2018 to 2019 had increased by 7.68 percent.The largest population of beef cattle was in Sangkub District, namely 18.97 percent.The number of instructors in North Bolaang Mongondow Regency were 54 people and inseminators were 6 people. Based on the research results, it can be concluded that Artificial Insemination has the prospect of being introduced even though the success rate was considered sufficient. Suggestions need to increase the socialization of the Artificial Insemination program by the government.*

Keywords**— introduction, artificial insemination, beef cattle.**

I. INTRODUCTION

The beef cattle business currently has a very big prospect because the demand for livestock products continues to increase from year to year.The increase in population, increase in economic level and fulfillment of people's nutritional needs have an impact on the tendency of increasing demand and consumption of beef as a source of animal protein.On the other hand, the increase in demand for meat was not matched by availability from beef cattle producers. As stated by Yendraliza (2018), Indonesia had not been able to supply all the needs of beef. In connection with the fulfillment of animal protein, the government program was still mainly focused on increasing the productivity and population of beef cattle (Romjali, 2018).

The problem was that the development of beef cattle in the research area was slow, so the introduction of technology was needed that can encourage an increase in the population of cattle. Artificial insemination technology (AI) is a reproductive technology that can be applied in

developing beef cattle. The AI technology is in accordance with the current farmers' objective conditions. Based on the researchers, the success of AI technology must be supported by improvements in livestock management as a whole and carried out simultaneously. The implementation of the application of AI technology cannot stand alone. The implementation of the application of AI technology is carried out in an integrated manner with several activities directly related to the success of the AI program. Integrated activities are intended to minimize the constraints faced in the process of implementing AI. These activities include: efforts to increase farmers' knowledge and skills in the field of reproduction, increase in inseminator skills, quality of liquid and frozen semen, and pregnancy examinations. The minimization of constraints has an impact on achieving population increase and improving genetic quality.

AI technology was important because it plays a very important role in increasing livestock development which further supports the development of livestock in the research area, even in Indonesia in general. In this case, livestock development had the aim of increasing the income and welfare of farmers, food security, environmental preservation, and regional and even state foreign exchange. Based on these thoughts, a study was conducted with the aim of examining the extent of the prospects for the introduction of artificial insemination technology.

II. RESEARCH METHODS

The research method used was a survey method in North Bolaang Mongondow Regency. The data sources of this research were primary and secondary data. The sample location was Sangkub District which was determined by purposive sampling, namely the district of beef cattle development. Respondents were cattle farmers who have obtained calves through the Artificial Insemination program, as many as 33 people. The data analysis used was descriptive analysis.

III. RESULTS AND DISCUSSION

The North Bolaang Mongondow Regency government was very concerned about the development of beef cattle in the area. This was because beef cattle were a source of income for farmers. One of the goals of the local government, in this case the Department of Agriculture and Animal Husbandry, was to increase farmers' income. Various central government programs are applied in the regions, including the SIWAB (Sapi Induk Wajib Bunting) program. The SIWAB program includes the realization of the introduction of artificial insemination (AI) technology. The introduction of AI technology was also applied in other regions in Indonesia. But based on some researchers that the development of IB has not been significant for increasing population. In fact, AI was one of the reproductive technologies that was capable of and had succeeded in improving the genetic quality of beef cattle. The use of AI technology allows farmers to produce calves of better quality in large quantities and in a short time. According to Widjaja et al (2017), AI as a form of biotechnology in the field of reproduction allows farmers to do AI without a male. The AI program was carried out by inserting semen into the genital tract and using a device called an artificial insemination gun (AI Gun).

Beef cattle breeding in North Bolaang Mongondow was introduced to beef cattle farmers, and many farmers have even joined the AI program. The prospect of introducing AI in the research location depends on the number of beef cattle, the number of extension agents and inseminators, and the response of farmers to the program. The population of cattle in North Bolaang Mogondow Regency according to BPS for North Bolaang Mongondow Regency (2019) can be seen in Table 1.

Table 1. Cattle Population Development in North Bolaang Mongondow Regency

No.	District	Year	
		2017	2018
1.	Sangkub	2471	3570
2.	Bintauna	3446	2595
3.	Bolangitang Timur	2616	2740
4.	Bolangitang Barat	2824	2948
5.	Kaidipang	3070	3194
6.	Pinogaluman	3051	3147
	Total	17478	18821

The results showed that the largest population of beef cattle was found in Sangkub District, namely 18.97 percent of the population in North Bolaang Mongondow Regency. The population of beef cattle in this region had increased from 2018 to 7.68 percent in 2019. This increase can still be increased through technological innovation. The intended technological innovation was through the introduction of AI technology. This program has been carried out by the government in the research area. The development of beef cattle innovation initially began with technology dissemination since 2017. Dissemination was carried out by the government, extension agents and universities through empowerment activities. According to

Widjaja (2017), AI technology has long been introduced and applied to cattle farms in Indonesia. However, the introduction of AI technology has not yielded maximum results. This was because the success of the AI program was influenced by the detection of lust, post thawing motility, cement handling, timing of AI, inseminator skills, cement quality, cement deposition, and the livestock itself (Widjaja et al. 2017 and Tiro, 2017). The data in Table 1 shows that the cattle population in Sangkub District had increased from 2017 by 44.48 percent in 2018. This was because Sangkub District was one of the areas for developing beef cattle. The condition of the cattle population in Sangkub District shows that this area had prospects for the introduction of AI technology.

The number of extension agents in North Bolaang Mongondow Regency was 54 people. Extension workers in the research area who deliver the introduction of AI technology have prospects for application in this area. This was because extension agents act as a bridge between technological innovation and farmers. Technological innovation can reach farmers through extension workers who carry out extension activities in the research area.

The number of inseminators in the study area was 6 people. The government, in this case the Department of Animal Husbandry, North Bolaang Mongondow District, was trying to recruit inseminators and these prospective inseminators were funded to take part in training as inseminators in order to improve their skills. This condition had an impact on the prospects for applying AI technology in the research area.

In this case, the extension agents and inseminators play a role in introducing AI technology innovations. Although in the field the application of AI technology innovation was influenced by various factors.Fast or not, farmers in adopting innovation were influenced by: fast or not, the process that occurs in the spread / diffusion of innovation.The diffusion of agricultural technology was an important element in supporting the progress of beef cattle farming. In theory, the adoption of AI technology was important to understand the factors associated with technology adoption.

The participation of farmers was expected to respond to the implementation of AI which was proclaimed as a government program. The participation of beef cattle farmers, among others, plays an active role in empowerment activities as the implementation of government programs. The results showed that farmers in Sangkub District responded highly to the AI technology innovation and had adopted and succeeded in utilizing the AI technology. An example of beef cattle produced by farmer's AI is shown in Figure 1.

Fig.1: Artificially Inseminated calves

Figure 1 shows that farmers had responded well to the introduction of AI technology by the inseminator. The results were very satisfying to farmers. The results showed that all of the farmer's beef cattle (Figure 1) had been bred through the AI program. The farmer who owns the beef cattle had knowledge about when his cattle were in a state of heat so that in this condition the farmer directly contacts the inseminator. Farmers understand that if 21 days after AI and cattle do not experience the first lust, and also do not experience the second cycle of lust, then the cattle are declared pregnant at 42 days. The results showed that the farmers knew the objectives of AI, namely: (i) Improving the quality of cattle for the better; (ii) Reducing costs because superior bulls do not need to be brought to the farmer's place; (iii) Utilizing breed from superior bulls optimally and more widely in a longer period of time; (iv) Increase the birth rate rapidly and regularly; (v) Prevent transmission / spread of venereal disease. According to Widjaja et al (2017), in terms of benefits obtained, the advantages of AI technology were shortening the calving interval, increasing the use of superior bulls, overcoming distance and time constraints, preventing the transmission of infectious animal diseases through the genital tract, saving funds because there was no need. raising males, improving the genetic quality of cattle through superior bulls.

IV. CONCLUSION AND SUGGESTION

Based on the research results, it can be concluded that Artificial Insemination has the prospect of being introduced even though the success rate was considered sufficient. Suggestions need to increase the socialization of the Artificial Insemination program by the government.

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Effect of anthropization on macroinvertebrate communities in the Kou River, Burkina Faso.

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Abstract**—** *The structuring of aquatic macroinvertebrate communities is used as an indicator of the effects of human activities on river ecosystems. This study focused on the distribution of macroinvertebrate communities along the Kou River in western Burkina Faso. Its objective was to characterize macroinvertebrate communities and water quality in the Kou River protected-site-manipulated site continuum in order to develop biological indicators for monitoring and assessing the overall health of aquatic ecosystems. Macroinvertebrate sampling, carried out using a cloud net and a Suber net, was carried out during the low-water period from January to April 2018. Their identification was carried out using a binocular magnifying glass and reference determination keys, and was limited to the systematic Family level. The study identified 04 Classes, 14 Orders and 54 Families of macroinvertebrates. The analysis of these results showed that 100% of the identified Orders and 98.15% of the identified Families were found in the protected site, compared to 64.29% of the Orders and 59.26% of the Families in the anthropized site. It also showed that the protected site is taxonomically richer than the anthropized site with the presence of 53 Families (98.15% of the Families identified), compared to 32 Families representing 59.26% of the Families identified for the anthropized site. Also, 22 taxa are specific to the protected site and remain absent in the anthropized site. This study also allowed the identification of 04 potential taxa bioindicators that would constitute excellent biological tools for monitoring aquatic systems. The agro-demographic pressure on natural resources has a negative impact on the diversity of species, the dynamics of which must be better monitored. The extension of the tools tested in the present study to river managers will strengthen their technical capacity for monitoring surface water quality.*

Keywords**— *Identification, Reference macroinvertebrates, Bioindicators, Continuum protected site, anthropized site, Specific diversity, Burkina Faso.***

I. INTRODUCTION

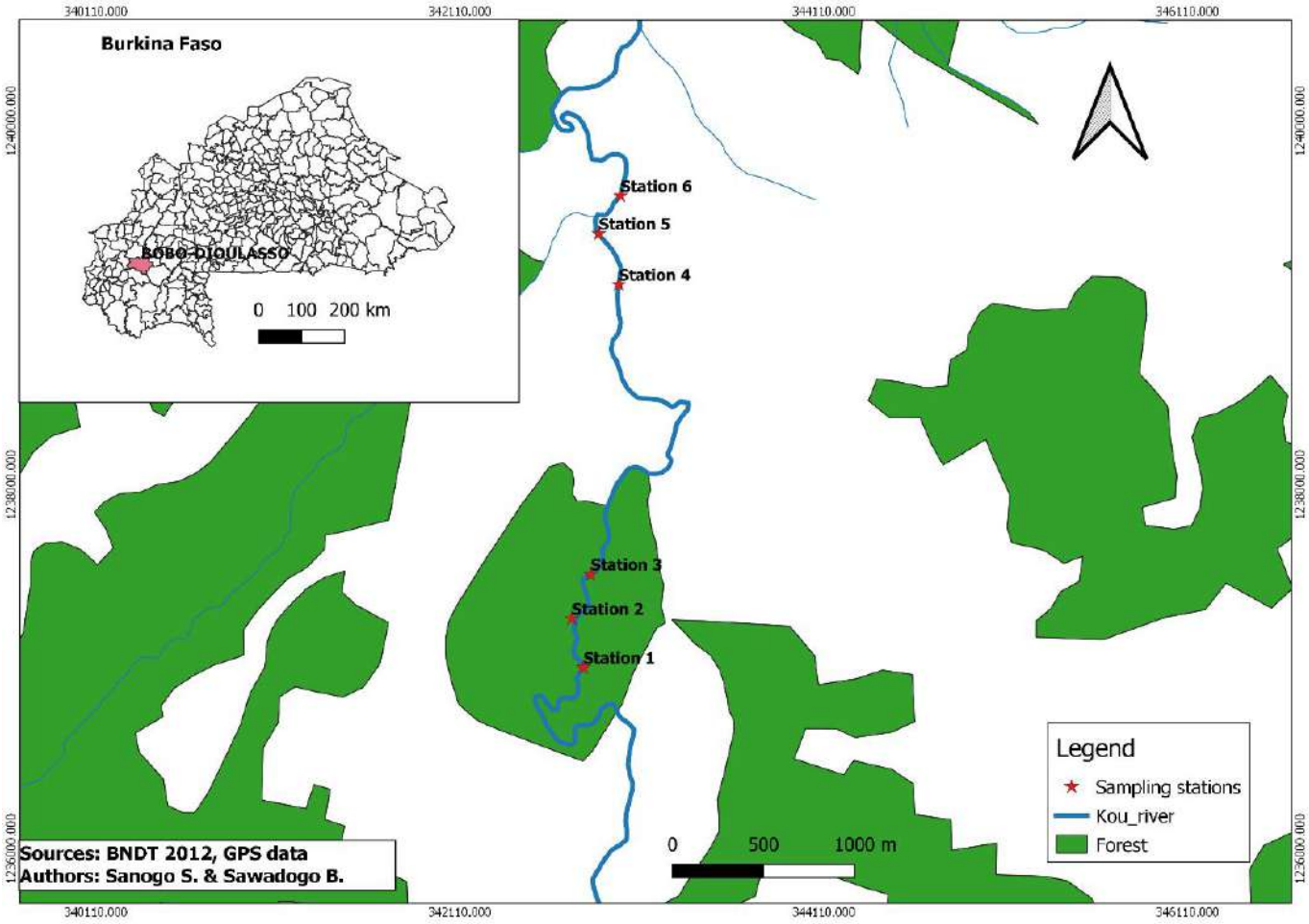
Located on the outskirts of the city of Bobo - Dioulasso (Western Burkina Faso), the Kou River offers many goods and services to the local population. The spring that feeds it provides drinking water to the population. Peri-urban and a rare perennial watercourse in the region, the Kou is suffering from the effects of anthropization like most of the watercourses that run through major African cities. According to Alhou (2007), these watercourses are used for watering market gardening crops, bathing, laundry, and also for the disposal of domestic and industrial effluents. These multiple uses of water alter its quality and disrupt the balance of the local biocenosis as well as the general functioning of this ecosystem (Bruslé and Quignard, 2004;

Hepp et *al.*, 2010). Therefore, special attention must be paid to monitoring these aquatic ecosystems to ensure their sustainability. This monitoring must be done through the use of reliable and adequate indicators such as biological indicators (Ben Moussa et *al.*, 2014). Today, macroinvertebrates are the most commonly used organisms for biomonitoring and assessment of the overall health of aquatic ecosystems (Adandedjan, 2012; Ben Moussa et *al.*, 2014; Camara et *al.*, 2014; Sanogo et *al.*, 2014). In Burkina Faso, despite multiple studies on freshwater macroinvertebrates, none have provided the data needed to develop biological indicators based on these integrative groups (Kaboré et *al.*, 2015). This study is part of this dynamic, with the general objective of identifying reference macroinvertebrate taxa in a comparative study between differently used sections of a river.

II. MATERIALS AND METHODS

Study area

The study was carried out at the Kou River (located between latitudes 11° 05' and 11° 25' North and longitudes 4° 20' and 4° 30' West) where the water is always transparent. Two study sites were established. A first site, called a protected site, is located in the portion of the watercourse located in the classified forest of Kou which is free of all anthropic activities. The second site, known as a man-made site, is located in the portion of the watercourse downstream of the Kou classified forest where various agricultural, pastoral, domestic and leisure (swimming) activities are carried out. In each of the two (2) sites, three (3) stations have been established for macroinvertebrate sampling (map 1). Table 1 provides the characteristics of each station.



Map 1: Presentation of the stations in the study area

Table 1: Characteristics of Different Sampling Stations

Sites	Stations	geographical coordinates	characteristics
Protected site	Station 1	11°11'11,81''N 04°26'23,48"W	Significant presence of trees and shrubs on the banks. Bottom substrate composed of silt, sand, wood and dead leaves.
	Station 2	11°11'20,59"N 04°26'25,63" W	Lots of trees on the banks Substrate of silt compound, dead leaves and dead woods
	Station 3	11°11'28,5"N 04°26'22,2" W	lots of trees on the banks Bottom substrate composed of sand, gravel, blocks and dead woods
Anthropised site	Station 4	11°12'20,35"N 04°26'17,4" W	Garden production at the riverbank level Bottom substrate composed of sand
	Station 5	11°12'29,45"N 04°26'21,06" W	Agricultural and pastoral activities on the banks. Bottom substrate composed of sand
	Station 6	11°12'36,24"N 04°26'17,17" W	Agricultural, pastoral and domestic activities at the riverbank level Bottom substrate composed of sand

Sampling

The methods adopted in this study for the sampling, processing and analysis of macroinvertebrate samples are those developed by the European Water Framework Directive (2010, 2016). Based on these methods, the NF T90-333 standard (September 2016) was applied for sampling, and the XP T90-388 standard (June 2010) was used for the treatment and analysis of macroinvertebrate samples.

Sampling plan

The sampling plan was established based on the gridding technique which consisted of nine (9) samples taken at each station. The nine (9) sampling points were distributed on either side of the watercourse, from the edge to the center along transects perpendicular to the direction of the water flow. At each sampling point, three (3) microhabitats (macrophytes, silt and water surface) were excavated for the collection of macroinvertebrates; and within each microhabitat, twelve (12) net hauls (elemental sampling) were carried out to capture macroinvertebrates. Elemental sampling was carried out from downstream to upstream to avoid any disturbance that could be caused by the possible disturbance of the water and to avoid damaging habitats not yet sampled.To ensure the representativeness of the sampling stations in relation to the study area, the size of each station was set according to the formula Length = 10*Width as recommended by the DCE (2016). Thus, the average width measured at the protected site stations is 3.6 meters, representing an average sampling area of 129.6 m². The average width of the stations at the anthropized site is 4.8 meters, corresponding to an average sampling area of 230.4 m².

Sample and data collection

From January to April 2018, six outings were organized with an interval of 14 days. Three microhabitats were the subject of macroinvertebrate collection at each station. These were mud, water surface and macrophytes (herbaceous plants, dead leaves and dead wood). The collection material consisted of a Surber-type net with a 25 cm diameter opening and a muddy net with a 30 cm diameter opening.

The Surber net for collecting benthic species was placed in the streambed facing the water current and then pulled over a distance of 01 meters by sampling. After capture, the macroinvertebrate species were sorted and preserved in 70% alcohol (DCE, 2016).

The cloud net was used to collect the species inferred to macrophytes. For 30 seconds the net was passed under the plants with back and forth movements. The contents of the net, consisting of plant parts, macroinvertebrates and mud, were rinsed with water, after which the macroinvertebrates were removed and stored in bottles with 70% alcohol; the remainder was stored in jars with 70% alcohol for sorting in the laboratory. This net was also used for the capture of surface species.

Identification and taxonomic analysis

Identification

The identification of macroinvertebrates was carried out using a binocular microscope and sometimes the naked eye. Insects have been identified using the identification keys of Durand and Levêque (1981), Merritt and Cummins (1984), Tachet et *al.* (2000), Stals and De Moor (2007), Moisan (2006). Molluscs were identified using the identification keys of Moisan (2010) and Brown (1980).

Annelids were identified using the identification keys of Lafon (1983) and Moisan (2010). Crustaceans were identified with the identification key of Tachet et*al.* (2000) and Moisan (2010). After identification, the individuals of each macroinvertebrate species were kept in a labelled bottle containing 70% alcohol. All vials containing macroinvertebrates from the same station were collected.

Taxonomic analysis

The taxonomic analysis of macroinvertebrate communities was based on the determination of taxa diversity and their occurrence (presence-absence of observed families). This analysis allowed the estimation of different metrics (taxonomic richness, taxonomic diversity, frequency of occurrence and biological indices) to assess the biological quality of the water at the two study sites.The level of identification and taxonomic analysis was limited to the family. Following identification, the reference taxa bioindicators were determined using the pollution tolerance scale for major taxonomic groups defined by Zimmerman (1993) and Moisan (2010) (Table 2). On the basis of this scale, all macroinvertebrates belonging to the sensitive orders Ephemeroptera, Plecoptera and Trichoptera (EPT) were considered to be reference pollution-sensitive bioindicator taxa.

Table 2: Pollution Tolerance Scale for Major Taxonomic Groups (Moisan, 2010)

Tolerance scale	**Taxonomic groups**
Sensitive	Ephemeroptera Trichoptera Plecoptera
Intermediate	Crustaceans (Decapods (Cambaridae)) Molluscs (Bivalves, gastropods with a lid) Odonates (Anisoptera and Zygoptera) Beetles Hemiptera Lepidoptera Megaloptera Diptera except Chironomidae Hydracarans
Tolerant	Crustaceans (Isopods, Amphipods, Ostracods, Cladocerans, Copepods) Molluscs (Gastropods without lid) Chironomidae (Diptera) Annelids (Oligochaetes, Leeches)

In order to analyze ecological diversity, the frequency of occurrence (F) of Dajoz (1985) and some ecological indices were calculated:

- **Frequency of occurrence (F)** of a taxon is the ratio between the number of samples (Pa) from a station where the taxon is present and the total number (P) of samples. $$\mathbf{F = \frac{Pa}{P} * 100}$$

Pa: number of samples; P: total number of samples.

Three groups are thus defined by Dajoz (1985). The first concerns very frequent taxa with F ≥50%; the second group corresponds to frequent taxa with 25%≤F<50%; rare taxa form the third group with F<25%.

In this study, a fourth group is also defined: these are the absent taxa with F= 0%.

- ***The Shannon-Wiener diversity index H'*** was calculated as it is suitable for the comparative study of stands. It is independent of sample size and takes into account both the taxonomic richness and the relative abundance of each taxon (Peet, 1975) to characterise the equilibrium of the stand in an ecosystem. H' has the advantage of not being subject to any prior hypothesis on the distribution of species (families) and individuals (Blondel, 1979). Its expression is :

$$H' = -\sum P_i * \log_2 P_i$$

Pi = proportional abundance or percentage of importance of the species (family) :

Pi = ni /N; S, total number of species (here taxa); ni, the number of individuals of a species (family) in the sample and N, total number of individuals of all species (family) in the sample.

Pi is the proportion of taxon i in the sample considered.

- ***Coefficient de similitude (Cs) de Sorensen (1948)***:

$$Cs = \frac{2C}{A + B} * 100$$

CS: Sorensen's similarity coefficient; A: number of taxa from site A; B: number of taxa from site B; C: number of taxa common to A and B. Sorensen's coefficient of similarity (Cs) made it possible to compare macroinvertebrate populations between sites, taking into account the presence or absence of taxa. This index varies between 0 and 1 ;

Cs=0: There is no similarity between the two sites, and the two sites considered have no common species, and, Cs=1: There is total similarity between the two sites studied.

The venn software (Lin et *al*., 2016) made it possible to make a comparison chart of the two sites.

III. RESULTS AND DISCUSSION

Results

Taxonomic composition

The total sampling resulted in the collection of two thousand seven hundred and eighty-eight (2788) macroinvertebrate individuals, including two thousand one hundred and sixty-six (2166) in the protected site and six hundred and twenty-two (622) in the anthropized site.

The captured macroinvertebrates are divided into four (4) classes: Insects, Clitellates, Malacostracans and Molluscs. Within these four classes, fourteen (14) orders and fifty-four (54) families were counted.The Insects that are the most represented include forty-eight (48) families and nine (9) Orders which are: the Coleoptera, the Diptera, the Ephemeroptera, the Heteroptera, the Lepidoptera, the Odonata, the Orthoptera, the Plecoptera and the Trichoptera. Beetles and Heteroptera were the most representative in this class of insects with respective rates of 18.97% and 25%. The Clitellates include three (3) families and two (2) orders which are the Purchasers and the Oligochaete. The Malacostracans are composed of two (2) families and two (2) orders which are Amphipods and Decapods. The Molluscs are represented by a single family and the only Order of Gasteropods.

Among the four (4) Classes, the Insects are the most represented with a rate of 64% of Orders and 89% of Families. At the level of Orders, Beetles and Heteroptera are dominant in terms of Families with respective rates of 20% and 22%.

Taxonomic richness

The number of families varies from station to station and site to site. Stations 1, 2 and 3, located in the protected site, have 53 families, while stations 4, 5 and 6, located in the anthropized site, have 32 families (Figure 1).

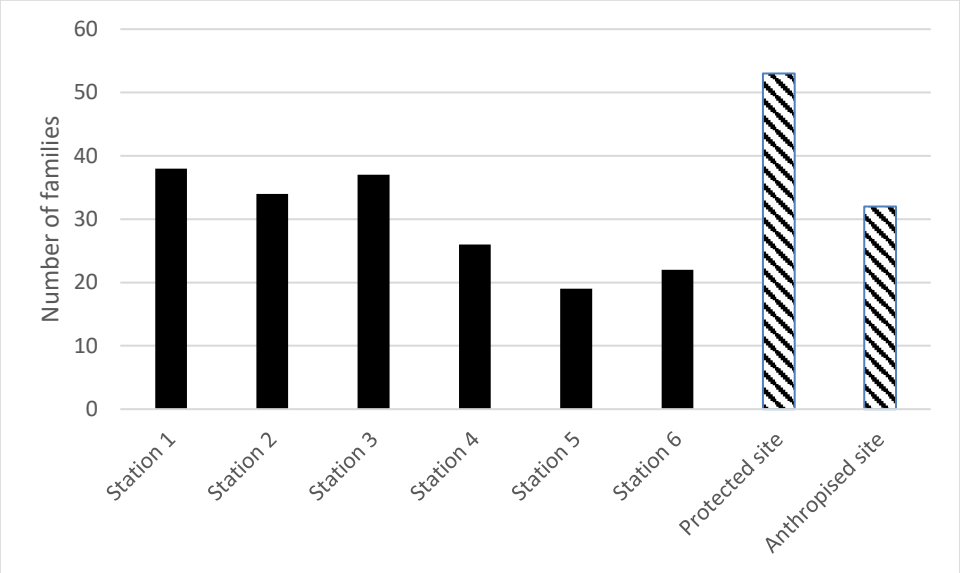


Fig.1: Relative Taxonomic Richness at Station and Site Levels

Taxonomic diversity of stations and sites

The identifications identified fifty-four (54) families of macroinvertebrates, which are diversely distributed among the stations and sites. Thus, analysis of these families based on the diversity index of Shannon and Weaver (1949) and the frequency of occurrence of Dajoz (1985) made it possible to assess the distribution of macroinvertebrate families according to stations and sites.

It emerges that stations 1, 2 and 3 have 70%, 63% and 68% of the families identified, respectively, while stations 4, 5 and 6 have only 48%, 39% and 37% of the families identified, respectively. Thus, the protected site, consisting of stations 1, 2 and 3, has the greatest taxonomic diversity with the presence of 98% of the families identified, while the anthropized site has only 59% of the families identified.

The Shannon - Weaver (1949) diversity indices calculated for the two sites and the six stations that comprise them are shown in Table 3.

Table 3: Shannon-Weaver (1949) Diversity Indices for Stations and Sites

	S1	S2	S3	S4	S5	S6	SP	SA
H	2,769	2,841	2,897	2,617	2,358	2,666	3,076	2,927

H : Index of Shannon et Weaver (1949) S : Station SP : protected Site SA : Anthropised Site

Analysis of the indices shows that the diversity of taxa is average in the two study sites.

However, these indices show greater taxonomic diversity at the protected site level (Figure 2). Indeed, stations S1, S2 and S3 (located in the protected site) present the best indices showing that the taxa in the protected site are more diverse than those in the anthropized site.

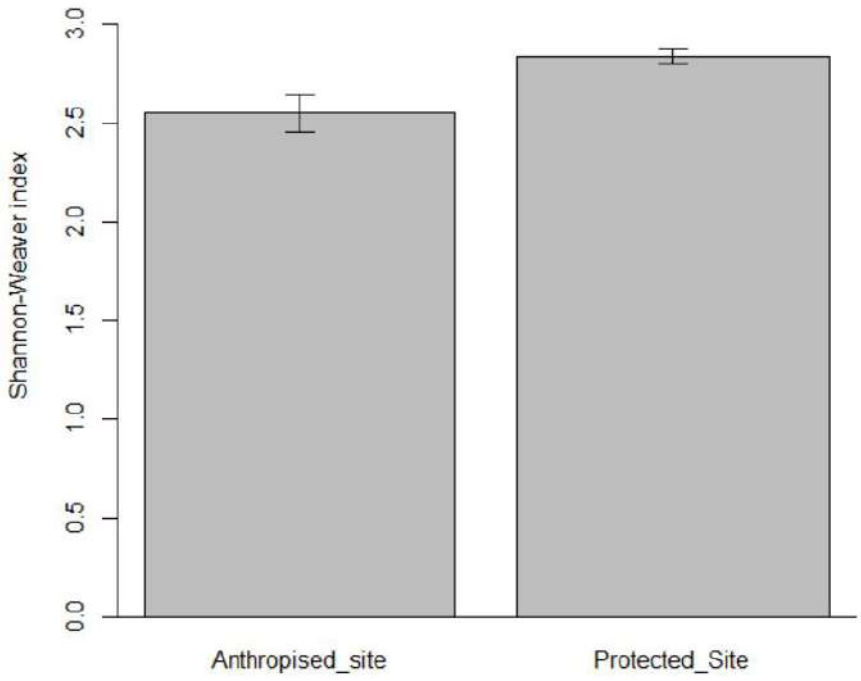


Fig.2: Comparison of Shannon Index Means at the Two Sites

Taxonomic classification

The taxonomic classification allowed to establish the state of taxonomic similarity between the protected site and the anthropized site, through the determination of Sorensen's coefficient of similarity. From the inventory of taxa, thirty-one (31) families common to the two sites, fifty-three (53) families in the protected site, and thirty-two (32) families in the anthropized site emerged. Calculating Sorensen's (1948) coefficient of similarity, a value of 0.73 is found. This value indicates that there is a taxonomic similarity between the two sites, so that this similarity is partial, as shown in the diagram in Figure 3, which presents twenty-two (22) taxa specific to the protected site, one (1) taxon specific to the anthropized site and thirty-one (31) taxa common to both sites. This classification shows that the protected site is richer and more diversified in taxa than the anthropized site.

Fig.3: Distribution Chart of Families in the Two Study Sites

Characterization of taxa bioindicators of water quality

Out of all identified macroinvertebrates, ten (10) orders belong to the group of bioindicators. Among these ten (10) orders, the tolerance levels are the following:

- three (03) orders are sensitive: Ephemeroptera, Trichoptera and Plecoptera;
- four (04) orders are medium: Odonates, Coleoptera, Heteroptera and Lepidoptera;
- three (03) orders are tolerant: Amphipods, Diptera and Oligochaetes.

Sensitive bioindicators

In the order of Ephemeroptera, five (05) families of sensitive bioindicators have been identified. They are Ephemerellidae, Heptageniidae, Baetidae, Caenidae and Leptophlebiidae. In Trichoptera, the two (2) families identified are Lepidostomatidae and Philopotamidae. The only family of Capniidae was observed in the Plecoptera.

Table 4 gives the frequency classes of occurrence of the different families of sensitive bioindicators found in the two study sites.

Table 4: Sensitive bioindicators

Order	Families	Protected site	Anthropised site
Ephemeroptera	Ephemerellidae	+++	+++
	Heptageniidae	+	+
	Baetidae	++	+
	Caenidae	-	++
	Leptophlebiidae	+	-
Plecoptera	Capniidae	+	-
Trichoptera	Lepidostomatidae	+	-
	Philopotamidae	+++	-

- : F = 0% (taxa absent) + : F ≠ 0% and less than 25% (rare taxa)

++ : 25%≤F< 50%(frequent taxa) +++ : 50% ≥ F (very frequent taxa)

Moreover, among the sensitive bioindicators identified in this study, the Ephemeroptera (62.5% of the EPT group) are the most represented, followed by the Trichoptera (25% of the EPT group) and the Plecoptera (12.5% of the EPT group).

The protected site has many more sensitive bioindicators than the anthropized site (87.5% of FTE families in the protected site versus 50% of EPT families in the anthropized site). Also, the four (04) EPT families (Leptophlebiidae, Capniidae, Lepidostomatidae and Philopotamidae) found in the protected site and absent in the anthropized site can be considered as potential sensitive taxa. In general, we note the absence of Trichoptera, Plecoptera and Leptophlebiidae (Ephemeroptera) in the anthropised site.

Medium bioindicators

The average bioindicators found in the order of Odonates, numbering four (04) families, are Calopterygidae, Coenagrionidae, Gomphidae and Libellulidae.

Among the Beetles, the eleven (11) families encountered are the Carabidae, Dystiscidae, Gyrinidae, Haliplidae, Hydraenidae, Hydrophilidae, Limnichidae, Staphylinidae, Elmidae, Noteridae and Scirtidae.

The Heteropterans recorded, twelve (12) families, are the Belostomatidae, Gerridae, Herbridae, Hydrometridae, Mesoveliidae, Nepidae, Notonectidae, Pleidae, Veliidae, Corixidae, Naucoridae and Saldidae.

In the order of Lepidoptera, there are two (02) families that are the Cossidae and Pyralidae.

In Diptera (except Chironomidae), the eight (08) families encountered are Ceratopogonidae, Stratiomyidae, Canaceidae, Chaoboridae, Culicidae, Tabanidae, Tipulidae and Syrphidae.

Table 5 gives the frequency classes of occurrence of the different families of average bioindicators found in the two study sites.

Table 5: Medium bioindicators

Order	Families	Protected site	Anthropised site
Odonata	Calopterygidae	+++	+
	Coenagrionidae	+++	+++
	Gomphidae	+++	+++
	Libellulidae	+++	+++
Coleoptera	Carabidae	+++	++
	Dystiscidae	+++	+++
	Gyrinidae	+++	+++
	Haliplidae	+	-
	Hydraenidae	+++	+++
	Hydrophilidae	+++	+++
	Limnichidae	++	-
	Staphylinidae	++	-
	Elmidae	++	-
	Noteridae	+++	+++
	Scirtidae	+++	-
Heteroptera	Belostomatidae	+++	+++
	Gerridae	+++	+++
	Herbridae	+++	-
	Hydrometridae	++	+
	Mesoveliidae	+++	++
	Nepidae	+++	++

	Notonectidae	+++	+++
	Pleidae	+++	+++
	Veliidae	+++	+++
	Corixidae	+	++
	Naucoridae	++	+++
	Saldidae	+	-
Lepidoptera	Cossidae	+	-
	Pyralidae	+	+
Diptera except Chironomidae	Ceratopogonidae	+++	++
	Stratiomyidae	+	-
	Canaceidae	+	-
	Chaoboridae	+	+
	Culicidae	+++	+++
	Tabanidae	+++	-
	Tipulidae	+++	-
	Syrphidae	+	-

- : F = 0% (taxa absent) + : F ≠ 0% and less than 25% (rare taxa)

++ : 25%≤F< 50% (frequent taxa) +++ : 50% ≥ F (very frequent taxa)

In this group of average bioindicators identified, Heteroptera (36.36%) are dominant, followed by Beetles (33.33%), Diptera (24.24%), and Lepidoptera (06.06%).The protected site contains all the average bioindicators listed, while the anthropized site has only 60.61%.

Tolerant bioindicators

In the order of Diptera, the only family of Chironomidae is considered as tolerant bioindicators. It was encountered in this study.In Amphipods, the only family identified is represented by the Gammaridae.Oligochaetes are also represented by the single family Naididae.Table 6 gives the frequency classes of occurrence of the different families of tolerant bioindicators found in the two study sites.

Table 6: Tolerant bioindicators

Order	Familles	SP	SA
Diptera	Chironomidae	+++	++
Amphipods	Gammaridae	+++	+++
Oligochaete	Naididae	+	-

- : F = 0% (taxa absent) + : F ≠ 0% and less than 25% (rare taxa)

++ : 25%≤F< 50% (frequent taxa) +++ : 50% ≥ F (very frequent taxa)

The orders of tolerant bioindicators identified in this study each have a single family. However, the anthropized site has one family less than the protected site.

Identification of taxa bioindicators

From the analysis of sensitive bio-indicator taxa, it appears that 87.5% of ETP families were found in the protected site, compared to 50% of ETP families in the anthropized site.

Among these ETP families, four (04) were specific to the protected site and are considered as potential sensitive taxa. These are the Leptophlebiidae, Capniidae, Lepidostomatidae and Philopotamidae. These four families

of ETP would be reference bioindicators that can attest, by their presence in a watercourse, to the good biological health of that watercourse.

In addition, two other taxa, namely Caenidae and Elmidae, can be associated with this list of reference taxa, as these two taxa have been particularly marked by their presence and absence on either side of the two sites. The Elmidae were specifically found in the protected site, while the Caenidae were found in the anthropized site.

IV. DISCUSSION

Characterization of taxa bioindicators of water quality

In this study, the level of identification is the family. Indeed, several studies or environmental monitoring programmes based on the occurrence (presence-absence) or abundance of macroinvertebrates have shown that a taxonomic analysis at the family level can provide similar information to that obtained with a finer analysis at the genus level, even if more indicator taxa are found at the genus level (Jones, 2008; Masson et *al.*, 2010; Neeson et *al.*, 2013). Furthermore, according to Usseglio-Polatera and Beisel (2003), the family is the systematic level generally recommended for standardised methods for assessing the biological quality of watercourses.

The analysis of taxonomic diversity shows a low number of sensitive bioindicator taxa in the anthropized site (50% of ETP families), compared to the protected site where this number is high (87% of ETP families in the protected site). The same finding was made by Kaboré et *al* (2016) in semi-arid rivers (the Volta River and the Comoé dam lake) in Burkina Faso, where there is a gradual decrease in sensitive ETP taxa between protected, agricultural and urban sites: ETP taxa were dominant in protected sites, less dominant in agricultural sites, and completely absent in urban sites. Grenier (2007) also showed that reference conditions are characterized by the abundance of sensitive families of ETP such as Philopotamidae, Rhyacophilidae, Leptophlebiidae and Chloroperlidae, and that this type of reference environment favors a greater abundance of taxa, especially Trichoptera and Plecoptera. The same is true for Foto et *al* (2010), who noted that the percentage of ETP taxa in the Nga River (reference site) is much higher than that observed on the Biyémé in Cameroon, which is a man-made river in the same ecological region.The results of our study are therefore in line with those of these authors. In our study, the low representativeness of ETP could be explained by the fact that agricultural activities carried out along the river at the anthropized site, with the use of chemical fertilizers and pesticides, would degrade the quality of the water which no longer offers favorable conditions for the existence of these sensitive taxa. In addition, these practices, which are carried out along the banks of the watercourse at the level of the anthropized site, are increasingly causing the silting up of this area, thus depriving these taxa of their privileged habitat.

Trichoptera, Plecoptera and Leptophlebiidae (Ephemeroptera), which are generally cited among the groups sensitive to pollution (Muli and Mavuti, 2001), were present in the protected site and absent in the anthropized site. This confirms the sensitivity of these taxa to water pollution. This confirmation is consolidated by considering the work of other authors who have found that Ephemeroptera, Plecoptera and Trichoptera are particularly sensitive to variations in environmental conditions to be found (Lenat, 1988) and to chemical and organic pollution (Rosenberg and Resh, 1993).

In addition, the 04 families of Beetles (consisting of Dystiscidae, Gyrinidae, Hydraenidae and Hydrophilidae) and the 02 families of Odonates (Gomphidae and Libellulidae), which recorded 100% frequency of occurrence in the protected site, would be excellent bioindicators of water quality. These results corroborate those of Oertli et al (2005; 2010a; 2010b), for the characterization of good water quality in European ponds and ponds, which associated Beetles and Odonates in good water quality.

The tolerant bioindicators were much more present in the protected site than in the anthropized site. This could be explained by the fact that the latter have their preferred habitat in the protected site. Indeed, the bottom substrate in the protected site is of muddy nature (preferred habitat of Chironomidae larvae) while in the anthropized site the bottom substrate is of sandy nature.

Taxa bioindicators reference

For this first inventory study of macroinvertebrates during the low-water period in the Kou River, the identification of taxa at the two sites, coupled with their frequency of occurrence, allowed the identification of potential pollution-sensitive taxa. Indeed, these taxa are only present at the level of the protected site, and totally absent in the anthropized site; they are the Leptophlebiidae, Capniidae, Lepidostomatidae and Philopotamidae). These taxa could therefore serve as a reference to attest to the good biological health of aquatic ecosystems. In addition, Baetidae, Philopotamidae, Leptophlebiidae, Capniidae (Plecoptera) and Heptageniidae are relatively more represented in the protected site. Our results corroborate those of Grenier (2007) who found that reference sites are characterized by the abundance of susceptible families of EPT such as Philopotamidae, Rhyacophilidae,

Leptophlebiidae and Chloroperlidae and the abundance of mayflies, such as the moderately susceptible families Baetidae and Heptageniidae, and a susceptible family of Plecoptera.

In addition, Elmidae that have marked their absence in the anthropized site can be associated with this list of reference taxa. This result corroborates that of Rosenberg and Resh (1993), who found that the presence of various EPT larval communities, as well as Elmidae and Psephenidae, is associated with low-polluted and well-oxygenated streams. It also corroborates that of Bispo et *al*. (2006) who also found that Elmidae can also be associated with well-preserved riparian vegetation.

Furthermore, analysis of the distribution of bioindicator macroinvertebrates between the protected site and the anthropized site shows that Caenidae are specific to the anthropized site. This result confirms Grenier's (2007) finding that Caenidae occupy the biotypes of the more altered environments. This specificity of the Caenidae to the anthropized site allows us to confirm the status of the reference site that we propose to the protected site.

V. CONCLUSION

The aquatic macroinvertebrates of the Kou River are extremely diverse and abound in potential sentinel species (pollution-sensitive and pollution-tolerant). This comparative study between contrasting sites of the same river also allowed the identification of reference taxa, namely Leptophlebiidae, Capniidae, Lepidostomatidae and Philopotamidae. These bioindicators identified in the protected site could constitute excellent tools for an assessment of the state of health of the hydrosystems. Their association with traditional monitoring tools (measurements of physico-chemical parameters) of aquatic environments would allow to report on the overall level of health of aquatic ecosystems.

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The behavior of Coastal Families Related to the Household Waste Management for the Sustainability of Marine Resources using gender Approach in Kapoposang Islands, South Sulawesi

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Abstract— *Kapoposang Islands is one of the many islands in South Sulawesi which has small island development characteristics and a marine tourism park that serves as a marine conservation area and marine tourism site. These main features lead to the many activities performed by the community that may potentially damage the marine ecosystem. This study aims to understand the behavior of coastal families, both men and women through their roles and activities in household waste management that may support the sustainability of marine resources. Samples were determined through random sampling and a total of 18 families from 4 villages was selected. This study used a qualitative approach and descriptive correlational analysis. The results showed that there were two family activities that may potentially produce household waste: (1) domestic activity and (2) economic activity such as fishing and home industry such as fish processing and coconut oil production. The family's knowledge about household waste (38.8%) and plastic that does not decompose (11.1%) was low. This affected family behavior where 72.2% of women disposed their household waste into the sea compared to 30% of men disposing their fishing waste into the sea. Little support from the village government in household waste management contributed to these behaviors, with only 27.8% samples ever attended recycling training and there was no final waste disposal available on the islands. The conclusion is that the predominant behavior of coastal families in household waste management remains harmful to the environment because of the low support from the village government in providing training activities and facilitation.*

Keywords— ***Behavior, coastal families, household waste, marine resource sustainability.***

I. INTRODUCTION

Waste and trash is a growing problem in today's world, including in Indonesia. According to the data from the Ministry of Environment and Forestry in[1] the average waste generated per day in Indonesia is 67.8 tons a day. While South Sulawesi is estimated to generate waste as 1200 tons a day. This number continues to show an increase from year to year, even though the government has issued Law No. 18 of 2008 regarding waste management that needs to be carried out comprehensively and integrated from upstream to downstream[2]. The mentioned regulation is in line with the regional development objectives which try some efforts to optimizing the local resources for the society welfare by taking into account the preservation of environmental functions. Therefore, the local resources have a dual roles as a resource based economy and as a life support system. Hence, the local resources need to managed in a balanced manner to ensure the sustainability of development[3]

There are several factors that may influence the increase in waste and trash produced by a country, one of which is human behavior [4]. Behavior is a human act, whether it is over behavior or covert behavior. Behavior can be captured directly through the senses, for example garbage activity and picking up scattered trash. Meanwhile, motivation, attitudes, interests, and feelings cannot be directly captured by the senses.

In coastal communities, the behavior of disposing of garbage at sea is still a bad habit that difficult to change. As a consequence, littering and poor waste management results in marine pollution and poses dangers to marine ecosystems. Marine pollution is also caused by fishing activities that produce diesel fuel and net waste. In addition, the polluted sea is also caused by fishing activities that produce diesel fuel and net waste. However, the role of the family is very important, considering the household waste contributes to a large amount of waste in Indonesia. When it is related to gender roles, the role of women in domestic is very large[5]. Based on this, the study tries to raise the problem of family behavior in coastal communities in waste management and household trash through a gender approach in the Kapoposang Islands, Pangkep Regency, South Sulawesi.

II. METHODOLOGY

2.1 Research Sites

The research was conducted in Kapoposang Island, Mattiro Ujung Village, Liukang Tupabiring District, Pangkajene Regency, South Sulawesi Province. Kapoposang Island was selected as the research location was carried out with the consideration that Kapoposang Island is one of the islands that has polluted coastal conditions due to waste and household waste.

2.2 Technique for Determining Samples and Data Sources

The sample was selected through the Stratified Random Sampling technique as much as 15% of the total population of 117 families who were in the neighbourhood 1 to 4 (RT I, RT II, RT III and RT IV). The total sample was 18 people. The data consisted of primary data which obtained from interviews and secondary data from the Kapoposang Village office data and various sources related to the research objectives.

2.3 Research methods and data analysis

The research was done in qualitative using descriptive correlation analysis[6], which describe the relationship between family behavior and activities related to waste and trash management along with the government support, Harvad Theory is used to answer the gender roles[7].

III. RESULTS AND DISCUSSION

3.1 Results

a. The overview of the Kapoposang Island

Kapoposang Islands is designed as a marine tourism park located in the westernmost part of the Spermonde Archipelago and is more precisely part of the Liukang Tuppabiring District area. In general, the facilities and infrastructure in Kapoposang Island are relatively limited. The only educational facility in the island is an elementary school. The land area is about 42 hectares. The total population of Mattiro Ujung Village is 522 people including 249 men and 273 women. There are 117 head of households. Most of the Kapoposang Island residents are fishermen. Their activities include fishing, collectors, boat builders, and house builders[8].

b. Respondent Characteristics

Age and Education background are elements that are closely related to one's behavior in acting which are described as follows:

c. Age

Age according to[9] can be described as follows: early adulthood, with ages between 18-29 years, middle adults between 30-50 years old, and old adults with more than 50 years of age. In table 1, the average age of the respondents, both male and female, is mostly classified as early to middle adulthood, namely the ages of 25-45 years, and older adults who are more than 50 years old. The percentage of males in early adulthood to middle adulthood was 9 people (50.0%) and 6 women (33.3%), and the percentage of older adults over 50 years of age were male respondents - 1 person (5.6%) and 2 women (11.1%). In early and middle adulthood, the number of male respondents was more than the number of female respondents, and the same was true for the number of elderly adults. shown in table 1.

Table 1. The number and percentage of respondents by age in Kapoposang Island

No	Age	Total(person)	Percentage (%)
1	25-35	6	33,3
2	36-45	5	27,8
3	46-55	4	22,2
4	> 55	3	16,7
Total		18	100

Source: Primary data processed in 2020

d. Education

Education has become a necessity to improve the quality of human resources, education in coastal families is still consider low. It is related to knowledge and limitations in accessing education caused by economic factors as well as the distance to schools and locations on the island. As a result, the island community in general experienced a basic education of 77.7% and higher education of 22.3%. This is shown in table 2.

Table 2. The number and percentage of respondents based on education in Kapoposang Island

No	Education	Total (person)	Percentage (%)
1	Not completed in Elementary School	8	44,4
2	Elementary School	6	33,3
3	Junior High School	3	16,7
4	Senior High School	1	5,6
		18	100

Source: Primary data processed in 2020

This is in accordance with[10], which states that people who have better human resources, in the sense that a higher level of education, will easily understand the benefits of something.

3.2 Discussion

a. Coastal family activities in waste and trash management

Study shows the household activities that potentially generate waste and household trash, which are 1) domestic activities 2) fishing activities 3) Industry activities. All of these activities represents the gender roles, men and women contribution in waste and trash management related to the knowledge and facility availability.

b. Family knowledge about waste and trash

It is necessary to increase society knowledge about waste and trash management to make them aware to protect their clean environment from waste and trash. Knowledge can be obtained through training, socialization from institutions, electronic media such as radio and television as expressed by one respondent:

"… I don't remember…, if there was counseling about waste from the government, maybe in the next village but we never get it here…." (dg. Ma, 35 years old).

The results showed that the less knowledge level of coastal families about waste and trash about 11.1%, including knowledge that plastics are not easily degraded (88.8%) as shown in Table 3 in the appendix.

Table 3 The number and percentage of family knowledge level in waste and household trash management.

Fisherman Family Knowledge	Understand			Quite Understand			Not Understand		
	Total (person)		Percentage (%)	Total (person)		Percentage (%)	Total (persons)		Percentage (%)
	L	P		L	P		L	P	
Able to differentiate waste and trash	0	0	0,0	4	3	38,8	6	7	61,2
Non-degradable plastic waste	0	2	11,1	1	3	22,2	5	7	66,7
Residual ship engine oil can pollute the sea	2	2	22,2	8	6	77,7	0	0	0,0
Waste nets can destroy the sea	0	0	0,0	3	2	27,7	7	6	72,3
Throwing waste and rubbish into the sea will damages the ocean	5	5	55,6	3	1	22,2	2	2	22,2
Sorting out wet, dry organic and inorganic waste	0	0	0,0	3	6	50,0	7	2	50,0

Source: Primary data processed in 2020

c. Domestic activities and waste and trash management

[11]Suggest that before most women were born, domestic activities had long been attached to women and had become a culture and customs. Women are always in the connotation of human domestic workers (homemakers). They got a negative perception where they were unable to

contribute actively outside the house so that their role is not just an activity in the home. This can be seen in Kapoposang Island where domestic activities are related to household affairs, cooking, washing, taking care of the house, children and husbands. Domestic activities are roles that are culturally entrusted to women. Therefore women have the potential to produce waste and garbage.

d. Fishing Activity

In carrying out fishing activities, fishermen have produced waste and trash in the fishing area in the form of plastic waste (90%), additional fuel/oil, diesel (80%) and fishing gear/ trawling /trawl waste (70%). In this case, unfortunately most fisherman throw their waste to the sea, as shown in Table 4.

Table 4. The Number and Percentage of waste types and trash generated in economic activities (fishing)

No	Types of waste and trash related to fishing activities	Total in person (orang)	Percentage (%)
1	Plastic waste/food wrappers (eg noodles packages)	9	90,0
2	Used supplies trash	6	60,0
3	Cigarette butts	10	100
4	Toxic waste	2	20,0
5	Fishing gear waste	7	70,0
6	Waste fuel (eg oil and diesel)	8	80,0

Source: Primary data processed in 2020

Table 5 Number and percentage of respondent who is littering during the fishery activities

No	Behaviour while at sea	Total (person)	Percentage %)
1	Throwing plastic waste/food wrappers to the sea	3	30,0
2	Throwing used supplies trash to the sea	2	20,0
3	Throwing cigarette butts to the sea	1	10,0
4	Throwing toxic waste to the sea	0	0,0
5	Throwing fishing gear to the sea	2	20,0
6	Throwing waste fuel, oil and diesel to the sea	2	20,0
Total		10	100

Source: Primary data processed in 2020

To find out the behavior of fishermen when carrying out fishing activities on waste management and waste in the sea is shown in table 5 in the appendix, it is quite high positive behavior of fishermen when fishing, it is seen that only 30% have ever thrown plastic waste and 20% disposed of remaining supplies, tool waste catch and waste oil. This is because there is already awareness not to throw garbage and waste in the sea. There are even fishing boats that prepare trash bin, even from diesel drums or plastic bags where food is carried.

Fish production, coconut oil production and raising poultry in the form of chickens and ducks is kind of activities by fishermen's family which handle by women. The production process is still in simple way only sell around the island and for their own family regular consumption. The number of coconut trees in the island is become the reason behind the coconut oil making activities. Another activity of fishermen's wives that has economic value is manage their husband's fish catches result. In fact, some of the husband's work is taken over by women. So there is a balance of jobs for women and men in meeting economic needs. Women's activities in helping their husbands to provide or repair boat equipment such as provision of "pakkaja", 80% of fishermen's wives carry out these activities in the season of "torani" flying fish eggs, yet this kind of activity poses the increased waste in Kapoposang Island in the last two years, as a consequence, the Kapoposang Island area was experience environmental damage

e. Home industry activities

Regarding the category of types of business carried out by fishermen's wives in Kapoposang Island can be seen from the aspect of community interaction with the available economic resources in Kapoposang Island, the fishermen's wives of Kapoposang Island fall into two of the three categories of community groups put forward by[12]. where coastal communities can be grouped into three categories as; 1) Direct users of environmental resources, such as fishermen, fish cultivators in coastal waters (using floating nets or cages); 2) Processors of fish or other marine products, such as fish dryers, coconut oil entrepreneurs and so on; 3) Supporting fishery economic activities, such as

shop owners or boatmen entrepreneurs. Based on these categories, women in Kapoposang Island correspond to points two and three.

The condition of fishermen who do not have large capital is generally only able to meet household consumption needs. It is not uncommon for one month to go to sea without getting paid due to a lack of catch[13]. Fishermen's wives has an important role in supporting family financial by helping their husband. It is known that the fisherman income is relatively low income and uncertainty. Apart from their roles as wives and mothers in domestic activities, fishermen's wives have a productive economic role to help meet household needs[14]. Coastal women have the potential to be empowered in the productive economic sector because they have enough free time and abundant availability of natural resources[15]. In traditional fishermen groups, the role of fishermen's wives is demanded to be greater in looking for other income alternatives to meet household economic needs. The smaller the household income generated by the husband, the greater the role of the wife in contributing income to meet household needs[16]. The results of the *Coastal Community Development Project-International Fund for Agricultural Development* (CCDP-IFAD) program regarding poverty alleviation in the processing sector by fishermen's wives which contribute to an increase in fishermen household income[17]. This common phenomenon is also happens in Kapoposang Island, as household industrial activities on economic/productive activities on a household scale also affect the waste producers on Kapoposang Island in the form of "biodegradable coconut dregs". However, the number of the piles up in one landfill potentially contribute to marine pollution due to the remaining oil from the coconut dregs. The waste produced during the wave season is the remnants of torn nets and parts of ships that are no longer fit for use that are disposed of at sea which are very dangerous for the sustainability of the marine ecosystem. Nets that are often found in the sea, when it goes down to the seabed it will cover quite a lot of coral reefs on the Kapoposang Island, as a result the coral is covered and causes the coral to die.

f. Gender roles and waste and trash management behavior

According to the data analysis, 11.1% of respondents who recycled waste and trash. The remaining 90% did not treat but throw away and burn or stockpile. It was also found that the largest type of waste in Kapoposang Island was plastic. Plastic waste is particularly problematic as a pollutant because it is so long-lasting. Plastic items can take hundreds of years to decompose, therefore it is better to recycle it so that it can be reused. However, it was found that only 11.1% of women had attended recycling training, by utilising plastic bottles. In terms of gender roles, it is known that men are only involved in activities of lifting trash and piling up garbage (11.1%). The rest of the activities related to waste and garbage are done by women. As shown in table 6 in the appendix.

Table 6 Gender role in the related activities to wate and trash in fishermen family in Kapoposang Island.

No	Type of Activities	L	%	P	%	Notes
1	Washing clothes	3	16,6	8	44,4	Three times a week in average
2	Cooking	2	11,1	8	44,4	Done everyday by women while men done it occasionaly
3	Going to the market	3	16,6	8	44,4	Weekly based on market schedule. Fishermen going to market to buy fishing gear
4	Throwing garbage	2	11,1	8	44,4	Every day
5	Burn garbage	0	0,0	8	44,4	According to the waste type
6	Pile up trash	2	11,1	8	44,4	Not sure
7	Trash sorting	0	0,0	8	44,4	Unsorted

Source: Primary data processed in 2020

IV. CONCLUSION

The behavior of coastal families in waste and trash management has not supported the sustainability of marine resources, which is due to the lack of knowledge and support from the government in providing waste disposal facilities and waste recycling training programs.

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Effect of Temperature Treatments on Seed Germination and Seedling Growth of Jute Mallow *(Corchorus olitorius)*

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***Abstract*— *Jute mallow (Corchorus olitorius) is one of the common green leafy vegetables used widely throughout Ghana. Jute mallow is cultivated by seeds and the demand for the crop is year-round. Despite the high demand, its efficient production is marred by poor seed germination. Thus, this study seeks to determine the effect of pre-chill, dry and wet heat on germination, seedling emergence and seedling vigor of jute mallow.*

Results revealed that hot water at 70ºC with combination of 70ºC oven heat for thirty (30) minutes produced the best germination, emergence and seedling vigour. The effect of hot water at 70ºC only was also significantly higher than other treatments.

Jute mallow farmers who produce on small scale could use hot water at 70ºC to treat seeds before sowing. Large scale producers, however, could use a combination of hot water at 70ºC and oven heat at 70ºC to treat seeds before sowing.

Keywords*— *temperature treatment, seed germination, seedling vigour, jute mallow, Corchorus olitorius.

I. INTRODUCTION

Corchorus olitorius, also known as Jew or jute mallow or bush okra is one of the popular tropical leafy vegetables in Africa, Asia and some part of the Middle East (Olabode and Sangodele, 2014). Studies have shown that jute mallow is considered as a vegetable in most parts of Africa and is widely consumed among rural communities (Velempini *et al.*, 2003). It is an annual crop popularly known in southern Ghana as "Ademe" and northern Ghana as "Ayoyo". It plays very significant role in household nutrition and it is very affordable. It is used to prepare sauce and soup delicacies and its mucilaginous property when cooked facilitate swallowing of solid foods. Hence most parents introduce it to babies who are learning to eat solid foods for the first time. In Ghana, jute mallow is eaten with starchy foods such as "banku", "akple" and "tuo-zafi" due to its ability to enhance swallowing.

Jute mallow was usually found in the wild but recently the health consciousness of many Ghanaians to eat healthy has increased the demand for its consumption hence making it necessary for farmers to deliberately grow them on commercial scale. However, the hard seed coat of jute mallow seeds cause physical dormancy hence, its delay in seed germination (Tareq *et al.*, 2015). Attempts at breaking seed dormancy in jute mallow include the use of heat under constant temperatures (Wahab, 2011), seed scarification (Emongor *et al* 2004) and use of

chemicals such as sulphuric acid (M. Palada and Chang, 2003). Palada and Chang (2003), reported that when jute mallow seeds are steeped in boiled water, seed germination

and seedling emergence are enhanced. These studies produced germination percentage between 40%-80%.

Mechanical scarification usually makes the surface of seeds permeable to water but reduces seedling vigor. Apart from reducing seedling vigor, the size(small) of the seed makes it difficult to carry out mechanical scarification on jute mallow seeds (Velempini *et al.*, 2003). Some of these treatments involve chemicals which do not only overburden small scale farmers with high production cost but these chemicals are not easily accessible. It is important to exploit feasible and effective methods that can be used by small scale farmers as well as commercial farmers to enhance germination and seedling vigor. Application of heat may be easier than earlier attempts that are more difficult to apply especially by small scale farmers. Thus, this study seeks to assess seed quality of jute mallow in terms of health, purity and viability while assessing rate of seed germination and seedling vigor, following different methods of breaking dormancy using heat treatments.

II. MATERIALS AND METHODS

The experiment was conducted at the laboratory of Ghana Seed Inspection Division (GSID) located at Pokuase, Accra and the Pathology Laboratory of Crop Science Department, University of Ghana. Jute mallow seeds used for the study were farmer saved seeds and were purchased from seed shop in Accra.

Seed viability test was conducted by using floatation method (www.agrifarming.in). In this process, seeds were poured into beaker containing water and allowed to settle for 20 minutes. Viable seeds are usually heavier than unviable seeds hence they settled at the bottom of the glass beaker while the lighter seeds with other impurities floated on the water.

The purity test was conducted according to the rule of International Seed Testing Association (ISTA, 2017). The aim of the purity test was to determine the percentage composition by weight of the seed being used. Two hundred grams (200g) of seeds were weighed and mixed thoroughly by hand. The seeds were poured onto the purity working board with reflected light to enhance vision; this was done with about 150 seeds at a time. Magnifiers were used to aid the separation of seeds into various components by magnifying the seeds and other components. The components were pure seeds, other seeds and inert matter. Pure seeds were seeds with all the features of jute mallow. Other seeds were defined as seeds of other plants that may be present. Inert matter was the materials that were neither pure seeds nor other seeds.

Purity percentage was calculated by dividing the weight in grams of pure seeds by the total seed weight and multiplied by 100 as shown in the equation 1 below:

$$P = \left(\frac{P_{ws}}{T_{ws}} \right) x100\% \qquad \text{Equation 1}$$

Where purity percentage is denoted by P, weight of pure seeds as P_{ws} and total seed weight denoted by $T_{ws.}$

Seed Moisture Test

Seed moisture test was done by weighing one hundred grams (100g) of seeds on a weighing scale. The seeds were poured into a digital moisture meter and calibrated to shallot seed. The calibration did not have jute mallow seeds on the machine so shallot was used since it has similar seed size.

To conduct health test on the seeds, ten (10) glass petri dishes were sterilized in a hot oven sterilizer at 175ºC for 90 minutes. Ten milliliters (10 ml) of Potato Dextrose Agar (PDA) media was poured into each sterilized petri dish. Ten (10) seeds were placed in each petri dish and covered. This was replicated ten (10) times, making a total of hundred (100) seeds. The petri dishes were labeled and incubated for five days to observe the presence of pathogens on the seed surface.

Two grams (2g) of seeds were placed in a conical flask and 1% sodium hypochlorite was poured into the flask for sixty (60) seconds to sterilize the surface of the seed.

The contents in the flask were sieved and the seeds were poured onto a tissue paper to absorb the remaining moisture on it. Ten (10) glass petri dishes were sterilized in a hot oven sterilizer at 175ºC for 90 minutes. Ten milliliters (10 ml) of PDA media was poured into each sterilized petri dish. Ten (10) seeds were placed in each petri dish and covered. This was replicated ten (10) times, making a total of hundred (100) seeds. The petri dishes were labeled and incubated for five days to observe the presence of pathogens within the seed.

Seed Treatments to Break Dormancy

Seeds were subjected to Ten (10) temperature treatments (T) and assessed for germination, emergence and seedling vigor as shown in table 1. A control, where seeds

were sown without temperature treatment was included in the study. For oven treatments, the oven was pre-heated.

Table 1: Seed Treatments Evaluated for Ability to Break Dormancy

Treatment	Procedure
Treatment 1	Soak seeds for 15 hours at 21ºC
Treatment 2	Soak seeds for 15 hours at 21ºC, followed by heating at 70ºC for 30 minutes
Treatment 3	Dip seed in hot water at 70ºC for 5minutes
Treatment 4	Dip seeds in hot water at 70ºC for 5minutes, followed by heating at 70ºC for 30 minutes
Treatment 5	Pre-chill seeds at 5ºC for 24 hours
Treatment 6	Pre-chill seeds at 5ºC for 24 hours, followed by heating at 70ºC for 30minutes
Treatment 7	Oven heat seeds at 70ºC for 30 minutes
Treatment 8	Oven heat seeds at 80ºC for 30 minutes
Treatment 9	Oven heat seeds at 90 ºC for 30 minutes
Treatment 10 (control)	Sowing seeds directly

Germination Test

Germination was done by using the top of paper method. Hundred seeds from each treatment were sown on filter paper. This procedure was replicated four (4) times. Germination counts started on the third day after planting and continued for a total period of 14 days when most of the seeds had germinated. Radicle (root) emergence was used as the criterion for germination (Denton *et al*., 2013). The percentage germination was calculated by dividing the total number of seeds that germinated over the number of seeds sown and multiplied by hundred (Denton *et al*., 2013) as shown in equation 2 below.

$$GP(\%) = \frac{T_{sg}}{T_{ssn}} x100 \quad \text{Equation 2}$$

where germination percentage is denoted by *GP*, total number of seeds germinated is denoted by T_{sg}, and total number of seeds sown denoted by T_{ssn}.

Seedling Emergence and Seedling Vigor Index

The experiment was a complete randomized design. One hundred seeds were taken from each treatment and sown in a germination tray which was filled with sand to about 3cm thick. Each treatment had three (3) replications. The set up was irrigated lightly every day to ensure adequate water supply for germination and to prevent the seed from drifting away from the planted rows.

The numbers of seedling emergence were recorded on daily basis, starting from the third day after sowing until 14 days after sowing. Seedling was scored as emerged when the cotyledons break through the soil surface and the percentage seedling emergence was calculated by dividing the total number of seedlings that emerged by the number of seeds sown and multiplied by hundred.

On the 14th day, measurements of seedling length were carried out on 10 randomly selected seedlings from each replicated tray (Denton *et al*., 2013). The seedling vigor index was calculated by multiplying the seedling length by percentage seedling emergence (Denton *et al*., 2013).

$$EP(\%) = \frac{T_{se}}{T_{ssn}} x100 \quad \text{Equation 3}$$

where emergence percentage (%) is denoted by *EP*, total number of seeds emerged denoted by T_{se}, and total number of seeds sown denoted by T_{ssn}

III. RESULTS

Seed Quality

The seed quality tests revealed that ninety two percent (92%) of the jute mallow seeds were viable and eighty seven percent (87%) pure. Seed health test within the seed revealed no contaminants however test on the seed surface showed that seeds carried 10% fungal pathogens and 5% bacterial pathogens refer to table 2.

Table 2: Results of Seed Quality Tests

Seed Quality test	Results in percentage (%)
Seed viability	92
Seed moisture	11
Seed purity	87
Seed health (within seed)	100
Seed health (seed surface)	85

Effect of Treatments on Percentage Germination and Emergence

Seeds that were treated with hot water and oven heat combination resulted in the highest percentage of

germination of 92%, as well as the highest emergence of 87% (see table 3).

The untreated seeds resulted in the lowest percentage germination (2.5%) and there was no seedling emergence.

Table 2: Effect of temperature treatments on percentage germination and emergence

Treatments	Percent (%) Germination	Percent (%) Emergence
Soak seeds in water at 21ºC for 15 hours	5	1
Soaking in water at 21ºC + oven heat	26	19
Hot water	77	61
Hot water + oven heat	92	86
Pre-chill seeds at 5ºC for 24 hours	8	7
Pre chilling+ oven heat	49	38
Oven heat seeds at 70ºC for 30 minutes	38	33
Oven heat seeds at 80ºC for 30 minutes	42	40
Oven heat seeds at 90 ºC for 30 minutes	48	41
Control	2.5	0

Table 4: Effect of temperature treatment on seedling length (cm) and seedling vigor of jute mallow seeds

Treatments	Seedling length	Vigour
Soak seeds in water at 21ºC for 15 hours	3.2^{a}	7.2^{a}
Soaking seeds in water at 21ºC + oven heat	3.1^{a}	82.7^{b}
Hot water	2.9^{a}	180.6^{d}
Hot water + oven heat	3.8^{b}	293.2^{e}
Pre-chill seeds at 5ºC for 24 hours	3.1^{a}	22.5^{a}
Pre chilling+ oven heat	3.2^{a}	157.5^{cd}
Oven heat seeds at 70ºC for 30 minutes	4.1^{b}	154^{cd}
Oven heat seeds at 80ºC for 30 minutes	3.1^{a}	135.9^{c}
Oven heat seeds at 90 ºC for 30 minutes	2.7^{a}	134.9^{c}
Control	3.0^{a}	3.0^{a}

Means followed by the same Superscript along the column are not significantly different at 5 % significant level by Duncan Multiple range test. All values in brackets were transformed by Arcsine transformation.

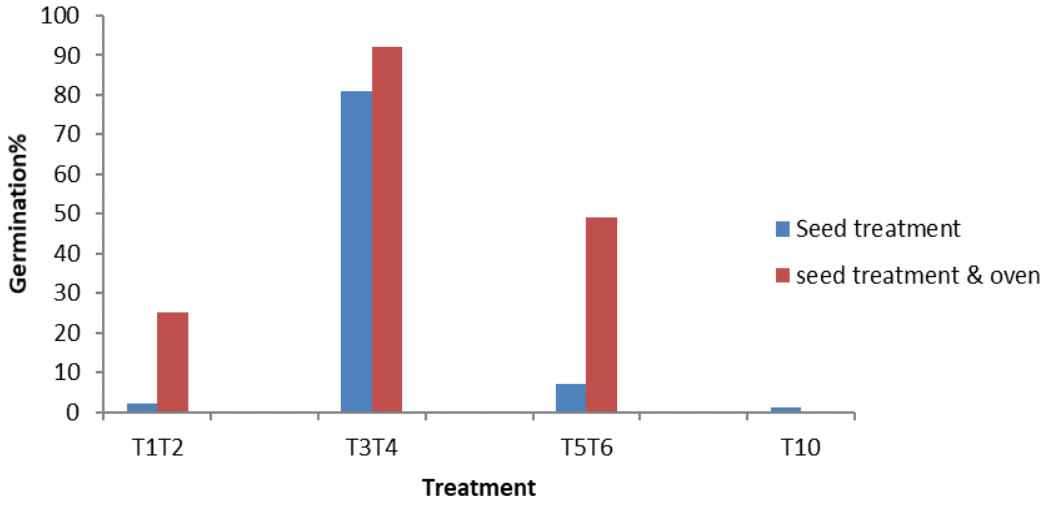

Fig.1: Germination relationship between seeds followed by oven treatments and non-oven treatments

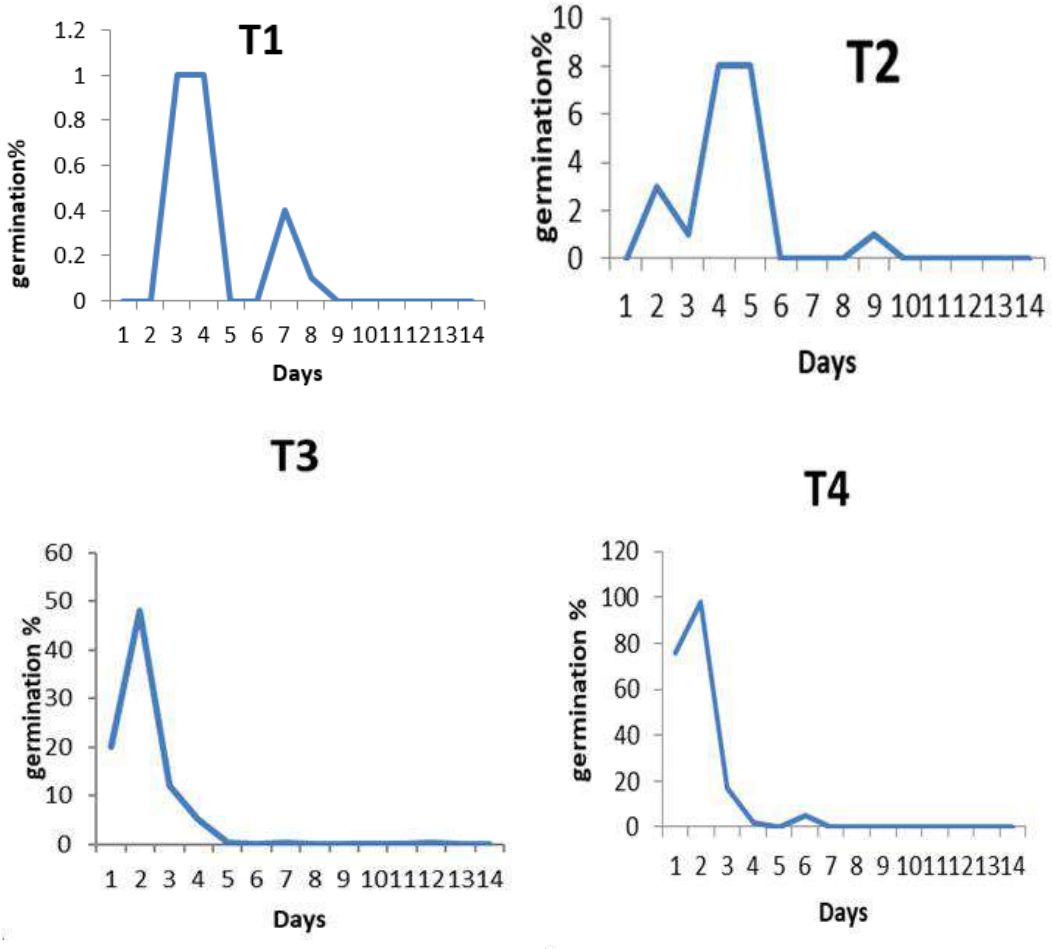

Fig.2: Germination rate of jute mallow following various heat treatments (T1-T4)

T1: soak seeds in water at 21ºC for 15hours, T2: Soak seeds in water at 21ºC for 15 hours followed by oven heat, T3: Hot water at 70ºC, T4: Hot water at 70ºCFollowed by oven heat at 70ºC, T5: Pre chill at 5ºC for 24 hours, T6: Pre-chill at 5ºC for 24 hours followed by oven heat at 70ºC, T10: Control).

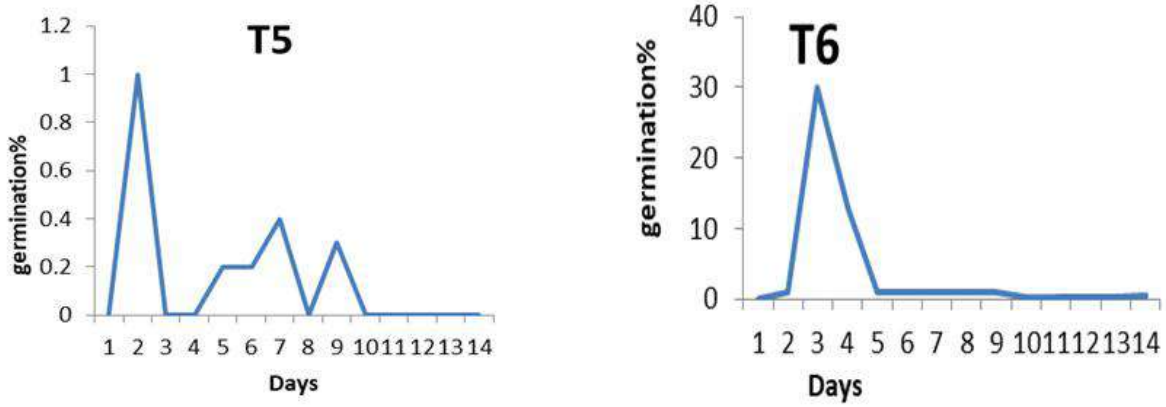

Fig.3: Germination rate of jute mallow following various heat treatments (T5-T6)

(T1: soak seeds in water at 21ºC for 15hours, T2: Soak seeds in water at 21ºC for 15hours followed by oven heat, T3: Hot water at 70ºC, T4: Hot water at 70ºC followed by oven heat at 70ºC, T5: Pre chill at 5ºC for 24 hours, T6: Pre chill at 5ºC for 24 hours followed by oven heat at 70ºC)

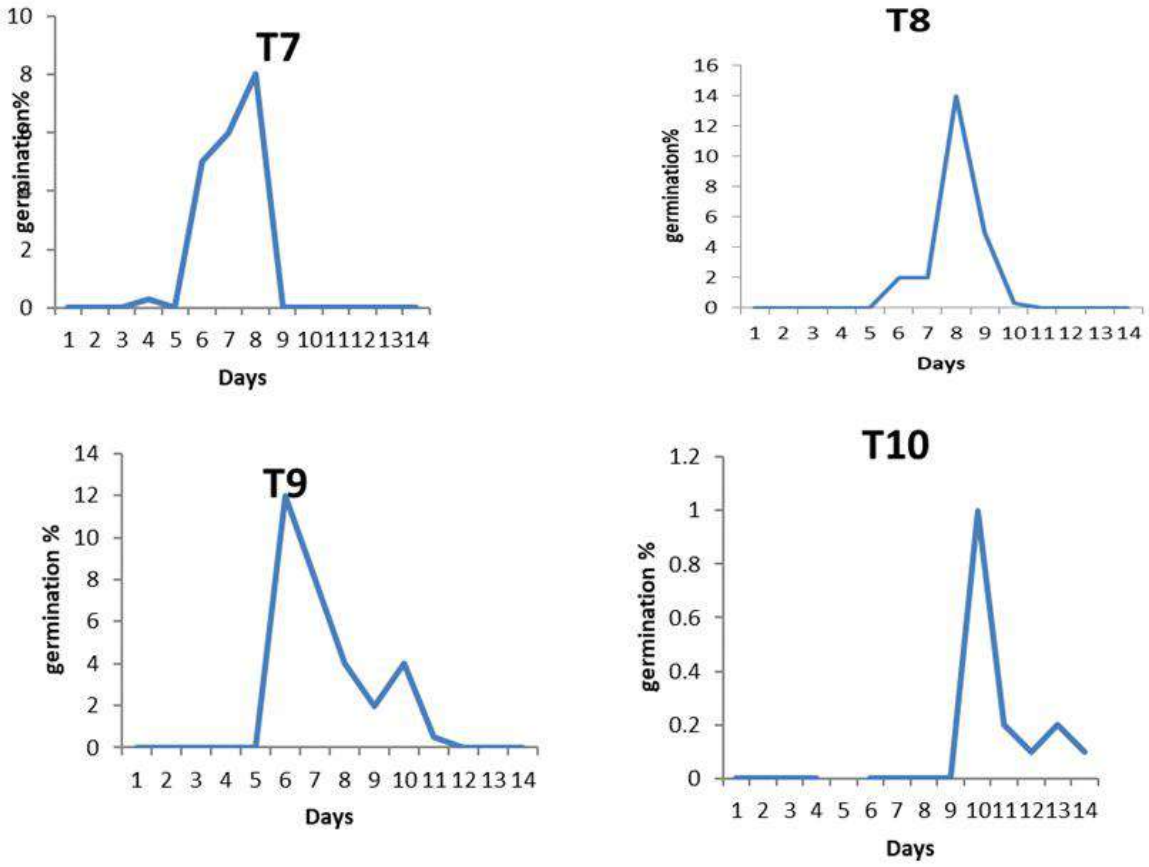

Fig.4: Germination rate of jute mallow following various heat treatments (T7-T10)

(T7: oven heat at 70ºC, T8**:** oven heat at 80ºC, T9: oven heat at 90ºC, T10: Control)

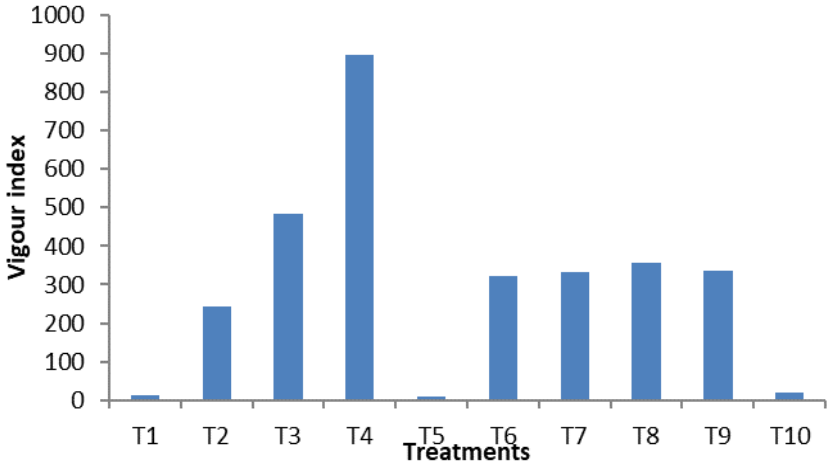

Fig.5: Effect of treatments on seedling vigor of emerged jute mallow seedlings

T1: soak seeds in water at 21ºC for 15hours, T2: Soak seeds in water 21ºC for 15hours followed by oven heat, T3: Hot water at 70ºC, T4: Hot water at 70ºC followed by oven heat at 70ºC, T5: Pre chill at 5ºC for 24 hours, T6: Pre chill at 5ºC for 24 hours followed by oven heat at 70ºC, T7: Oven heat at 70ºC, T8: Oven heat at 80ºC, T9: Oven heat at 90ºC, T10: Control)

IV. DISCUSSION

Health Status and Contributing Factors of Farmer Saved Seeds

African indigenous vegetables are usually cultivated with other crops where the same plot is divided into sub plots and different crops are cultivated in each subplot; this agrees with (Maseko *et al.,* 2018) who reported that African leafy vegetables are cultivated under a mixed cropping system. Farmers with a variety of crops are able to make sales every time compared to farmers who grow single crops because a crop is always available for sale notwithstanding, it also comes along with its challenges. It is however easy for pest and diseases to spread on such farms, especially when crops from the same family are found on the subplots. Pest easily migrate from one crop to another. The presence of fungi on the seed but not within the seed indicated that the seeds may have been kept with other contaminated seeds during storage. Seeds may also have been contaminated during the extraction process or through harvesting equipment or even via storage environments as seeds were not certified. Fungus is usually associated with stored grains and legumes; it also grows well where there is water (moist) and temperature interaction. Fungal infestation of seed coat decreased viability of seeds causing abnormal seedlings.

However, methods of sterilization such as hot water, natural compounds, commercial bleach and ethylene are able to get rid of the seed's infestation. Farmers are able to eradicate the fungal contamination on the seed coat of jute mallow unconsciously during the period of breaking dormancy by steeping the seeds in hot water (Selcuk *et al.*, 2008). The seed is thus sterilized while dormancy is being broken and this reduces the risk of disease infestation in jute mallow. Disease causing bacteria present on the seed coat showed that the farmer saved-seeds did not go through any standard check, therefore, there is potential yield loss as a result of disease infestation in the life of the plant.

Efficient and Effective Methods of Breaking Dormancy on Germination and Seedling Vigor of Jute Mallow

Hot water caused thermal shock to the embryo or leaching of inhibitors to enhance germination, however the embryo may be destroyed as a result of prolong contact with high temperatures which is consistent with (Velempini *et al.*, 2003) who reported that, longer soaking times result in drastic reduction in germination.

Treatments included dipping jute mallow seeds into hot water when it starts to bubble and this caused germination. Based on the observations, the effect of temperature treatments on germination and seedling emergence followed similar trend with seeds dipped into hot water at 70ºC followed by oven heat at 70ºC for 30minutes showing the highest germination and seedling emergence. Seed dormancy in jute mallow is physical dormancy and is usually as a result of hard seed covering which prevent water from entering into the seed (Abukutsa-Onyango, 2005). The hot water thus scarified the seed coat and caused water imbibition hence facilitating germination and emergence. The high temperature also provided good medium for enzymes to catalyze the breakdown of seed coat and this allowed the water imbibition and gaseous exchange thereby enhancing germination and emergence.

Exposing seeds to oven heat at 70ºC showed moderate seedling emergence, which increased with temperature at 80ºC. Dry heat produced may have cracked the hard seed coat making it permeable to water when moistened similar to method of breaking dormancy in some *Acacia* tree species (Walters *et al*., 2004). This explains why seeds in the wild readily germinate and produce seedlings during the rains on farms that were burned during land preparation (Denton *et al.,* 2013). No significant increase in seedling emergence and germination were obtained when oven heat temperature was increased from 80ºC to 90ºC for 30minutes. This could be as a result of embryo damage due to the excessive high temperature and this observation was contrary to Denton *et al., (*2013) who reported that irrespective of how long jute mallow seeds were subjected to bush fire, there were seedling emergence on the field after the first rain. The difference in germination and emergence when temperature increased from 80ºC to 90ºC may also be as a result of embryo damage due to the prolong exposure of the seed to dry heat causing seeds to dehydrate hence increased mortality.

The control treatment and seeds soaked in water at 21ºC for 15 hours did not enhance germination at all. The seed coat could not be modified to imbibe water for germination to occur due to physical dormancy. Pre chilling

seeds at 5ºC for 24 hours resulted in poor germination and emergence which agrees with (Nkomo *et al.*, 2009b) who reported that there was no germination for seeds that were pre-chilled for one(1) day. However, seeds that were pre-chilled with oven heat combination showed significant difference in germination. This finding agrees with (Nkomo & Kambizi, 2009b) who reported that germination occurred when seeds were exposed to temperature of 35ºC and above.

Hot water followed by oven heat treatment showed high seedling vigor for both germination and emergence indicating that, hot water and dry heat enhanced germination, emergence and seedling length. Though the seeds had high viability, the treatments for breaking dormancy favored the plant establishment. The term "seed vigor" has a concept associated with aspects of seed performance which include rate and uniformity of seed germination, seedling growth and emergence. Seeds soaked in water at 21ºC (T1), seeds soaked in water followed with oven heat (T2) and control (T10) produced low seed vigor. This was as a result of poor seedling performance in terms of height and germination which agrees with the International rules for seed testing's explanation of seedling vigor as the total properties that determines the activity and acceptable germination performance of seed (ISTA, 2015).

Germination rate is a necessary parameter in crop establishment on the field. The effect of treatments on the seed varied significantly, seeds dipped into hot water only (T3) and seeds dipped into hot water followed by oven treatments (T4) were the only treatments that started germination from the first day of germination count. This is consistent with other findings that hot water treatments enhance germination (Maina *et al.*, 2011). Germination started on day one and increased by day two but the rate of germination decreased in the subsequent days. About 85% of the total seeds' germinations occurred from day one (1) to two (2) while the remaining treatments started germinating between day two (2) and fourteen (14). The trends of germination rates of the various treatments indicated their effectiveness in breaking dormancy with the control taking the longest time to germinate. A low germination of 2.5% is an indication of the importance of treating seeds of jute mallow prior to planting.

Also soaking seeds in water at 21ºC alone is equally not very effective. Maximum germination rate for treatment (6 and 1) occurred on day three, treatment (2) was on day four, treatment (7and 8) was on day eight, treatment (9) was on day six and treatment (10) started germinating on day fourteen. This revealed that, though the other treatment enhanced germination at some point, there was delay in germination which makes the treatment method ineffective for breaking dormancy.

Treatment 10 which was the control had its maximum germination on day fourteen with germination percentage of 2.5% revealing that jute mallow seeds will not germinate at all or have poor germination and seedling vigor when it is not treated before sowing. Soaking seeds in ordinary water at room temperature (T1) resulted in 5% germination and was not an effective method of breaking dormancy. It agrees with the report by (Maina *et al.*, 2011) that soaking jute mallow seeds in ordinary water will not break dormancy.

V. CONCLUSION

The quality of farmer saved-seeds in terms of purity and viability is adequate as it was above 80% for both 87% and 92% respectively. For seed health quality, no seed borne pathogen were observed. Hot water followed by oven heat treatment can be adopted on large scale production while small scale producers can employ hot water treatment for uniform growth and development.

The best treatment for breaking dormancy in jute mallow from the study was dipping seeds in hot water treatment at 70ºC for five (5) minutes followed by oven heat at temperature of 70ºC for thirty (30) minutes as it gave the highest seedling germination, emergence and vigour. Hot water treatment and hot water combination with oven heat enhanced germination and good germination rate.

Hot water followed by oven heat treatment should be used by large scale producers while small scale producers use only hot water treatment.

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DISCLOSURE

The authors declare that there are no financial supports or relationship that may pose conflicts of interest.

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Appendix

Anova table for percentage germination

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	123.28	41.09	1.60	
Treatment	9	23320.62	2591.18	100.60	<.001
Residual	27	695.48	25.76		
Total	39	24139.38			

Anova table for germination vigor

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	3787.3	1262.4	2.89	
Treatment	9	286451.0	31827.9	72.93	<.001
Residual	27	11782.5	436.4		
Total	39	302020.9			

Anova table for seedling emergence

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.012729	0.006365	1.93	0.173
Treatment	9	2.919484	0.324387	98.61	< 0.001
Residual	18	0.059211	0.003289		
Total	29	2.991424	0.103153		

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Monitoring of Heavy Metal Content in Leafy Vegetables Irrigated with Different Water Sources

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Abstract*— *The present research was conducted to calculate levels of different heavy metals like Fe, Mn, Cu, and Zn in leafy vegetables when grown with different water sources. The result showed quite high range of accumulation when the vegetables were irrigated with wastewater. Wastewater irrigated vegetables showed variations (115-377), (12-68), (5.2-16.8), (21-45) mg/kg for iron, manganese, copper and zinc. Highest toxic level of iron and manganese were detected in mint and spinach and carrot showed highest value of copper and zinc. From the present study we can conclude that the vegetables grown from these water resources will lead to heavy metal accumulation in human body i.e for both adult and children if they continuously consume these vegetables. For this regular monitoring of these heavy metals is needed in order to prevent excessive metal accumulation in the body. However the results obtained from the present study shows that the ranges of heavy metal were below the permissible limitsset by WHO/FAO.

Keywords*— *Heavy metals, vegetables, daily requirement, waste water effluent, human health.

I. INTRODUCTION

Due to easy availability of wastewater and scarcity of freshwater, it is mostly used for irrigation of vegetables[1] . Waste irrigation is thought to make a considerable contribution to heavy wastewater. The metal content of the soil are very harmful due their non- biodegradable nature and they can easily get assemble indifferent parts of the body. These metals are harmful because they can easily dissolve in water.A small amount of these metals is harmful because there is no proper method to remove these metals from our body.Heavy metals find their use in many industrial applications and so there are widely spread. Due to this reason the available waste water have large unit of these heavy metals in them as which when indirectly used for irrigation severely effects human body[2]. Excess amount of accumulation in agricultural land irrigated by wastewater affects the food quality The metal requirement in our body is obtained from the food and water that we consume and this in turn directly exposes us from the entry of toxic heavy metals. Vegetables are an important ingredient of human diet that contains essentials nutrients like vitamins, minerals dietary fiber and antioxidants[3]. Leaves from different plant species such as perennial, annuals are consumed especially in rural areas, and there has been an increased trend of the consumption among the persons living in metro cities. Vegetables which have leaves as edible part are an economic source to ensure the micronutrient intake. Examples include Cabbage, Cauliflower, Broccoli, Lettuce, Coriander, Spinach and Turnip. Rapid industrialization and the use of natural resources have increased the accumulation of toxic substances like heavy metals in the soil. The required protein and vitamin which are supplied by vegetables are best to act against rough digestion and prevents constipation are supplied by vegetables[4].The current research work was conducted with a view to calculate the amount of heavy metals that enter in our body through the agricultural practices that involves the use of wastewater irrigation. The various disadvantage of using wastewater was noticed and

daily intake of heavy metals were calculated with regard to different section of society[5].

II. MATERIALS AND METHODS

2.1. Area of research and analysis

All the experimental findings were conducted in the Environmental science department of SHUATS, Allahabad. Leafy vegetables included in the research were radish , spinach, turnip, cauliflower, mint, coriander , and carrot were collected from three different selected areas in Allahabad. For the purpose of metal analysis edible parts of the plant were taken.

2.2. Preparation of samples

To remove harmful chemicals from the different vegetable samples double distilled water was used. Water content from the edible parts of the plant was removed by weighing the plant sample and then air-drying it. Vegetable samples were dried in oven at 70 °C- 80 °C for 24 hrs to remove moisture from it. Dry vegetable samples were crushed with mortar and pestle and filtered through cotton fabric.

2.2.1. Digestion of the vegetable samples

From different irrigation method three powder samples weighing 0.5 g was prepared for each leafy vegetables and three replicates were made. Crushing of ash was done with the help of per chloric acid and HNO_3 which was in the ratio 1:4. The sample wasleft to cool down and it was then filtered using Whatman filtrate paper No 42. A final volume of solution wasmade with 25 ml of distilled water and was sent for Atomic absorption spectrophotometry.

2.3. Standards

Standard solution used (1000 mg/l) (Merck, Germany). Different concentration solution for various metals were also prepared.

2.4. Data analysis

The daily intake of metals (DIM) wasdetermined by the following formula:

$$DIM = \frac{M * K * I}{W}$$

$\boldsymbol{DIM}$ =M.K,Iand W represent the heavy metal concentration found in plants (mg/kg), conversion factor, daily intake of vegetables and average body weight respectively. The conversion factor used to convert fresh green vegetables weight to dry weight was 0.085.

As investigated we have found the following facts average adult body weight for adult was 55.9 kg and child body weight was found to be 32.7 kg. Average daily vegetable intakes for Adults was 0.345 kg/day and for children daily vegetable requirement was 0.0232 kg/ day[6].

2.5 Statistical Analysis

All data are presented in terms of the means and standard triplicate error. Observations on heavy metal concentrations in response to different sources of irrigation were evaluated for the significance of the different using the t- test.

III. RESULTS AND DISCUSSION

A. Metal accumulation in plants

When any form of wastewater is added to the soil it changes the physical and chemical properties of the soil. We all know that heavy metals intake by the vegetables are not only affecting the soil profile but also causing serious health issues.. Heavy metal contamination was much higher than freshwater irrigated vegetable samples. The concentration was in the ascending order starting with copper, zinc, manganese and iron in spinach, cauliflower, mint and coriander. In radish, turnip and carrot it was Fe>Zn>Mn>Cu. The heavy metal concentration for different vegetables irrigated with wastewater is shown in Table 1 ..Due to scarcity of fresh water most of the vegetable samples indicated the use of wastewater for irrigation[1]. Minimum heavy metal accumulation was seen in freshwater Table 3 and unknown source of water Figure 1 as compared to wastewater irrigation. The observed differences may also depend on the different properties of soil[7]. Maximum accumulation of Manganese was in spinach (44-68 mg/kg) and carrot showed maximum concentration of copper. All the leafy vegetables have lower values of Zn and Cu as compared to maximum acceptable limit (61 mg/kg and 41 mg/kg). Following result was also reported by[8].When a large quantities of these heavy metals get accumulate in the soil and plants they result in various health issues in human being[9].

B. Daily requirement of metals (DIM)

To understand the intake of metals we need to understand the amount of exposure a certain heavy metal is causing by knowing its route into human body. Food chain is the easiest route for heavy metals to enter into our body. We all know that certain amount of minerals are essential for our metabolic activity but when the metals accumulate in larger quantity, they can result in health issues. Table 2 and 3 shows the daily consumption of metals from the selected vegetables for both adult and children. Waste water Table 2

and Figure 2 showed maximum DIM values for heavy metals as compared to fresh water. Following study results that consumption of vegetables grown from wastewater is high compared to other water treatment but still the values are under recommended limits (WHO)

Table 1 Heavy metal content in plants grown in wastewater irrigated soil.

Plants	Zn		Cu		Fe		Mn	
	A	C	A	C	A	C	A	C
Radish	0.011	0.0013	0.0031	0.0035	0.0612	0.06	0.0066	0.0076
Spinach	0.016	0.01	0.0086	0.01	0.162	0.185	0.0365	0.0418
Turnip	0.014	0.017	0.0084	0.0096	0.1032	0.118	0.0095	0.01
Cauliflower	0.02	0.023	0.0026	0.0031	0.0027	0.12	0.0216	0.0248
Mint	0.023	0.026	0.0066	0.0076	0.1984	0.227	0.0353	0.0403
Coriander	0.016	0.018	0.0064	0.0074	0.1643	0.189	0.024	0.0264
Carrot	0.023	0.027	0.0087	0.01	0.1132	0.12	0.009	0.0104

Table 2 Daily consumption of heavy metals in wastewater source of irrigation

A-ADULT C-CHILDREN

Plants	Zn		Cu		Fe		Mn	
	A	C	A	C	A	C	A	C
Radish	0.012	0.013	0.003	0.003	0.06	0.069	0.0063	0.0072
Spinach	0.017	0.015	0.004	0.004	0.158	0.0181	0.0266	0.0306
Turnip	0.015	0.016	0.005	0.006	0.096	0.11	0.0082	0.0094
Cauliflower	0.019	0.022	0.002	0.002	0.077	0.089	0.0135	0.0155
Mint	0.023	0.027	0.008	0.009	0.189	0.217	0.0236	0.0271
Coriander	0.014	0.017	0.006	0.006	0.158	0.182	0.0198	0.0228
Carrot	0.024	0.027	0.008	0.01	0.097	0.112	0.0083	0.0096

Table 3 Daily consumption of heavy metals in freshwater source of irrigation

A-ADULT C-CHILDREN

Plants	Fe	Zn	Mn	Cu
Radish	111–122	21.1–24.3	10.0–17.0	5.21–6.42
	117 ± 5.4	22.5 ± 1.6	12.8 ± 3.7	5.96 ± 0.7
Spinach	279–333	31.2–34.9	64.3–73.8	15.9–17.4
	309 ± 27.0	33.1 ± 1.9	69.4 ± 4.8	16.5 ± 0.8
Turnip	176–212.4	28.8–30.3	11.8–23.3	12.4–20.1
	197 ± 19	29.3 ± 0.8	18.2 ± 5.9	16.1 ± 3.9

Cauliflower	198–232	38.2–41.8	33.5–47.5	4.8–5.5
	215 ± 17.0	40.2 ± 1.9	41.3 ± 7.2	5.23 ± 0.4
Mint	335–412	41.4–47.4	61.0–70.8	11.8–14.1
	378 ± 39.0	45.0 ± 3.2	67.0 ± 5.2	12.7 ± 1.2
Coriander	292–326	29.8–32.8	41.4–47.6	10.9–12.7
	313 ± 18.0	30.9 ± 1.6	43.9 ± 3.2	12.1 ± 1.0
Carrot	200–235	40.4–50.7	14.0–20.4	12.5–21.6
	216 ± 18.0	46.4 ± 5.3	17.4 ± 3.2	16.8 ± 4.6

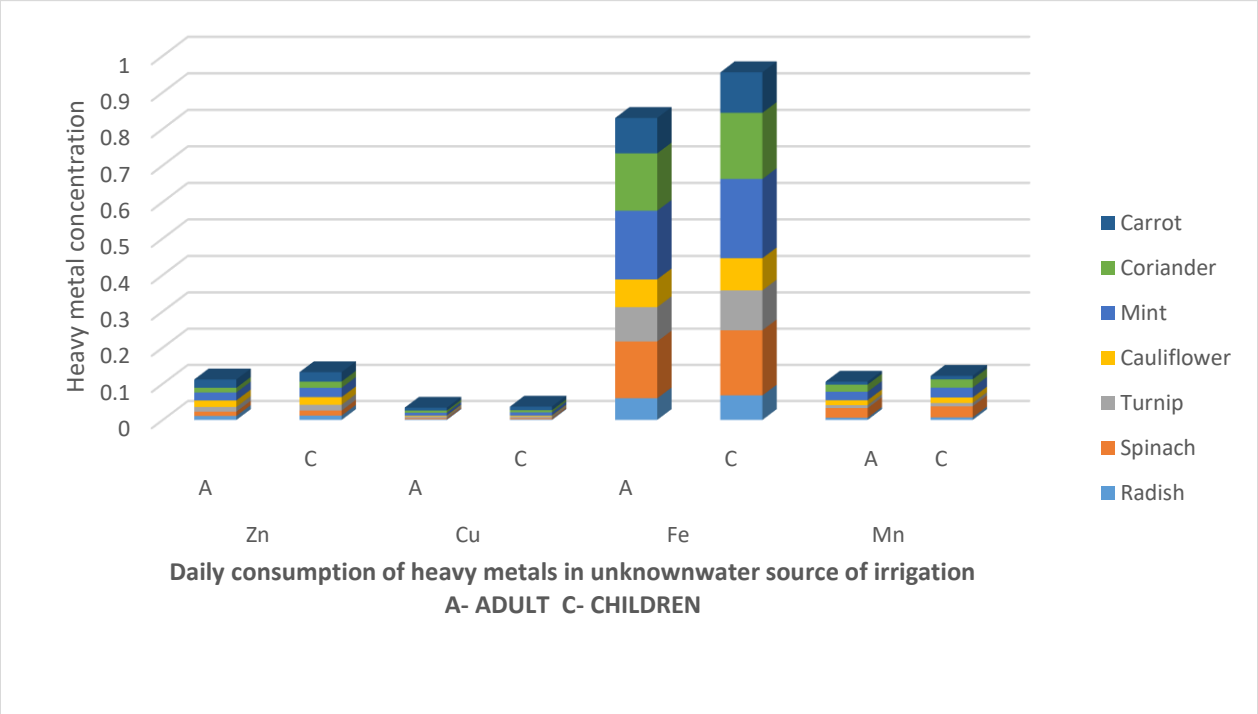

Fig.1: Daily consumption of heavy metals(mg) in unknown water source of irrigation

A- ADULT C- CHILDREN

IV. CONCLUSION

The main source of wastewater contamination are human and animal waste. Presence of phosphorus and nitrogen has also resulted in eutrophication of water resources and also has resulted in high amount of heavy metals in soil and vegetation resulting in potential health hazards. From the present study we conclude that heavy metals showed higher presence in waste water mode of irrigation. This study may also help other researchers to study different affected areas of Allahabad. Heavy metals showed their presence because of wastewater irrigation system practiced in the selected areas. Heavy metals showed their presence could be due to following reasons i.e agricultural practices, geographic position and ability of the plant to absorb heavy metals. Suggested measure may include regular examination of heavy metals in all the food commodities grown in and out.Daily consumption of food results in long term low level body accumulation of heavy metals, with negative impacts only after certain time interval of metal exposure. Therefore, regular inspection of these heavy metals from different water sources, leafy vegetables and other daily intake is necessary to their entry in our food chain.

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Initial Survey of Composition, Generation, and Proposing Management Solutions for Domestic Solid Waste in Cai Khe Ward, Ninh Kieu District, Can Tho City, Vietnam

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Abstract**—** *This study was carried out to survey the composition and amount of domestic solid waste generated and management work in Cai Khe ward, Ninh Kieu district, Can Tho city. Households and staff in Cai Khe ward were interviewed to collect information on management assessment while waste collection tools were placed in the households to calculate generation rate and waste composition. The research results showed that the rate of domestic solid waste generation of the household and individual were 0.74 kg/household/day and 0.2 kg/person/day, respectively. Domestic solid waste in the study area has a a veriety of composition, of which organic waste accounted for the highest proportion (72.7%), plastic component accounted for 13.4%, and glass tookup 2.7%, hazardous waste occupied 1.4% and other components accounted for 3.6%. Currently, only 90% of domestic solid waste was properly collected. Odor and leachate remained the problems during waste collection and transportation. In overall, the management of domestic solid wastes in Cai Khe ward is relatively good. In order to ensure a clean and beautiful environment in the study area, the environmental management agency should continue to propagate to raise people's awareness of environmental protection, creating favorable conditions for sorting wastes at source for the improvement of solid waste collection, transportation, and treatment facilities.*

Keywords**— domestic solid waste, organic waste, waste sorting, collection, Ninh Kieu.**

I. INTRODUCTION

The country develops in the direction of industrialization and modernization, but the urbanization process takes place strongly everywhere in terms of scale, quantity, and quality of life of people increasingly improved. The higher people's living standards, the higher the demand for social products, which means an increase in domestic waste. Domestic waste generated in the process of eating, living and consumption of people, being discharged into the environment exceeds the ability of the environment to self-clean, leading to a polluted environment. Along with the development of the country, in recent years, Can Tho city has affirmed its role as a central city of the Mekong Delta and recognized by the Prime Minister as Class I city in June 24, 2009. With its convenient location and abundant potential, the city has continuously accelerated economic development, production and business activities, trade and urbanization in order to improve life. and spiritual material for the people in the area.But in parallel with the growth of the city, at present, the amount of solid waste from people's daily lives will increase and the garbage classification is still very limited, the awareness of the people is not high in littering. The amount of waste is relatively large while the investment budget is still difficult. Most of the waste is directly discharged to the environment, if there is a collection, only the mixture can be transported to the landfill, which is the cause of serious pollution, affecting human health.Composition, generation, and separation of waste at source play an important role in the effective

collection, treatment and management of domestic solid waste. The study selected Cai Khe ward, Ninh Kieu district, Can Tho city to investigate these issues, providing important information for the environmental managersto conduct more in-depth studies, thereby solving urban environmental management issues.

II. METHODOLOGY

Information on the current situation of generation, collection, transportation and solid waste management in Cai Khe ward, Ninh Kieu district, Can Tho city was collected by interviewing the households. The questionnaire content included general information about the interviewee such as gender, age, education level, occupation; information on current situation, source separation, collection time, impacts of solid waste on environment and health, assessment of current status of solid waste management in the study area. To assess the rate of generation and composition of solid waste, thirty households in the surveyed area were selected for providing plastic bags to store all solid waste in the family. Each day the placed plastic bags were collected at 5:00 PM to classify and calculate solid waste composition and rate of generation of solid wastes. Waste separation was conducted in accordance with the guidance of the Department of Natural Resources and Environment of Ho Chi Minh City. Specifically, organic waste includes easily biodegradable waste including uneaten food, vegetables, tubers, fruits, leaves, twigs and recalcitrant including nylon bags, straws, bottles, glasses, plastic jars; inorganic wastes includes glass, bottles and jars; toxic wastes includes battery, light bulb, rubber, empty pesticide bottles; and other waste includes soil, stone, rubble. The interview data were imported into Excel spreadsheets (Microsoft Excel 2016, Microsoft, USA) to aggregate, calculate percentages and present data in the form of simple tables and charts.The following equation was use to estimate the amount of domestic solid wastes in Cai Khe ward to the year of 2025.

Euler's equation: $N^*_{i+1} = N_i + r.N_i.\Delta t$,in which N_i: initial polulation (persons); N^*_{i+1}: population one year later; r: population growth rate (%/year); Δt: time (year).

III. RESULTS AND DISCUSSION

3.1 Solid waste generation rate

The research results showed that the daily amount of garbage generated by the households was the highest at 1.5-2 kg/day and the lowest was less than 0.5 kg/day. The amount of waste generated by each household was different depending on occupation, income level and living and production habits of the households. However, this difference is mainly due to the level of income, a higher standard of living, eating, and household activities generate more waste. Figure 1 showed that the amount of waste generated per day: <0.5kg/day accounted for 40%, 0.5-1kg/day accounted for 46.67%, 1-1.5kg/day accounted for 6.67%, and 1.5-2kg/day accounted for 6.67%.

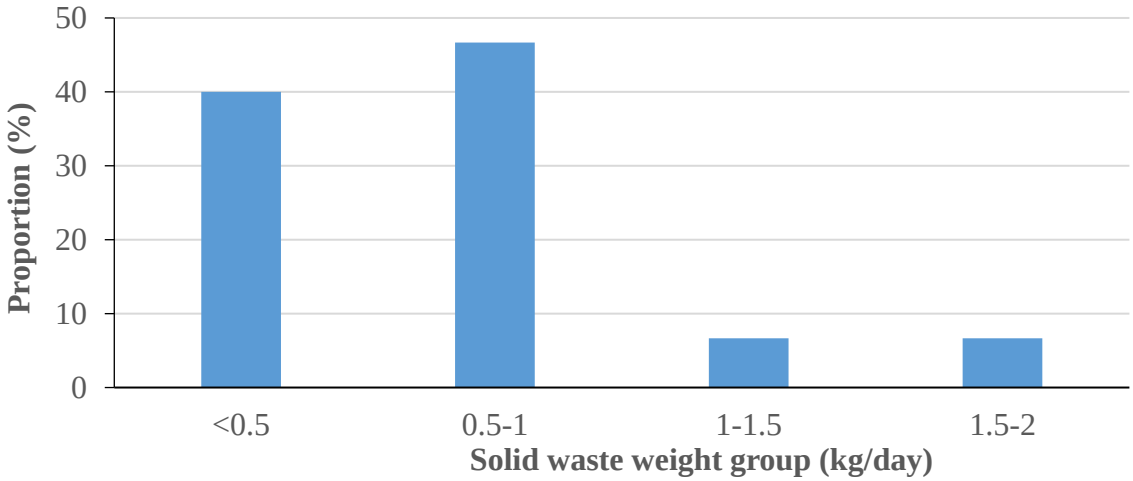

Fig.1: The mean weight of solid waste generated

The solid waste generation in Cai Khe ward was presented in Table 1. It was found that, on average, each household in Cai Khe ward dischargeed about 0.74kg/household/day and 0.2kg/person/day. The highest amount of waste per capita was 0.23kg/person/day and the lowest was 0.12kg/person/day (table 1).The urban solid waste generation per capita increases with living standards. According to the 2011 environmental report, the average

domestic solid waste generated per capita for urban areas nationwide was about 0.75kg/person/day in 2007. In 2008, according to the Ministry of Construction, the solid waste generation was 1.45 kg/person/day that was much larger than in that in rural areas (0.4 kg/person/day). However, according to reports of the local environmental authorities, the mean domestic waste generation rate was less than 1kg/person/day. The inconsistent statistics of urban domestic solid waste generation are one of the challenges for calculating and forecasting urban solid waste emissions in Vietnam. In the current study, the average volume of waste per capita of the households in the sampling area was relatively low at 0.2 kg/person/day, due to small-scale sampling, more in-depth studies are needed to obtain more presentative data.

Table1. Domestic solid waste generation in Cai Khe ward

Household (HH)	Members in HH	Total amount of solid waste (kg/week)	Amount of solid waste per HH (kg/HH/day)	Amount of solid waste per capita (kg/person/day)
A1	2	3.22	0.46	0.23
A2	3	4.82	0.69	0.23
A3	3	3.04	0.43	0.14
A4	7	10.7	1.53	0.22
A5	3	4.4	0.63	0.21
A6	7	10.91	1.56	0.22
A7	4	5.63	0.8	0.2
A8	4	5.85	0.84	0.21
A9	2	2.84	0.41	0.21
A10	4	3.43	0.58	0.12
A11	2	2.69	0.38	0.19
A12	2	2.8	0.42	0.2
A13	3	2.98	0.49	0.14
B1	3	4.93	0.6	0.2
B2	3	4.09	0.58	0.19
Mean	**3.47**	**4.82**	**0.74**	**0.2**

3.3 Solid waste compostion

The composition of the domestic solid wastes at Cai Khe ward was indicated in Table 2. As could be seen that the solid waste in the ward has a diversity of components in which organic waste accounted for the highest proportion (72.7%), plastic components accounted for 13.4%, glass for 2.7%, hazardous waste for 1.4% and other wastes for 3.6%. If separation at source was done well, the amount of organic wastes would be separated and treated by biological methods, which could reduce the amount of wastes transported to the landfills and reuse and recycle could be strongly faciliated.

Table 2. Composition of solid wastes in the study area

No.	Component	Weight (kg/day)	(%)
1	Organics	0.480	72.7
2	Paper	0.480	7.2
3	Plastic	0.089	13.4
4	Glass	0.018	2.7
5	Batteries, light bulbs	0.009	1.4
6	Others	0.024	3.6
	Total	**0.661**	**100**

3.4 Current status of solid waste management

3.4.1. Classification

At presnt, Cai Khe ward has implemented the work of classifying solid waste at source. However, after conducting interviews with 30 households, it was found that households mainly classify between things that can be sold or reusable and those that cannot be sold. With toxic and difficult to decompose wastes such as batteries, blades, wrap, ... are still disposed of with daily household wastes.Due to the new solid waste classification currently applied, people have not done well, they still have the habit of not sorting waste at source. The amount of non-biodegradable and toxic wastes is still disposed of with organic wastes, so the treatment of solid waste is not yet effective, adversely affecting the quality of the surrounding environment.

3.4.2 Storage

Domestic solid waste from generated sources is stored in containers. The survey results showed that households storing garbage in plastic containers accounted for 63.33%, using plastic wrap to store household waste accounted for 36.67%. Household waste is stored in a 240 liter garbage can located on the roads. Once a day, the garbage will be collected by specialized vehicles.

3.4.3 Collection, transport and fee

In Cai Khe ward, there are 17 garbage collectors with one large and 11 small special-use vehicles for garbage collection in small alleys. The process of garbage collection in Cai Khe is in the form of both manual and motorized. In public buildings such as markets, shopping centers, large trash bins are arranged, all waste is dumped into the waste bins and the large garbage collecting truck arrives and collects the bins. For households, each household's waste is placed in a location in front of the house, a small vehicle will be used to go to each house to collect garbage. These small vehicles are gathered at transfer stations, then large vehicles come to collect and transport to landfills or disposal sites. In Cai Khe ward, garbage collection time is usually at 16:00, with a frequency of once a day. Waste collection fee is considered as a fee to offset part or all of the investment cost for waste collection, transportation and treatment in the locality. This fee is spent on activities such as the organization and operation of the waste collection, transportation and treatment unit according to the technical process of the authorized agency. The current collection fee ranges from 10,000-20,000 VND/month/household. People have not paid the waste treatment fee. This is also the reason for the lack of funds to effectively treat domestic solid wastes.

3.4.4 Solid waste treatment

Cai Khe ward does not organize the treatment of domestic solid waste. All waste, after being collected, will be gathered to the centralized waste treatment site of Can Tho city. In terms of planning, Can Tho city has a waste treatment plan as follows:

Zone 1 is the solid waste treatment area in Phuoc Thoi and Thoi An wards, O Mon district, with an area of about 47.0 ha, a treatment capacity of about 1,500 tons/day. This area processes domestic, construction, industrial and conventional solid waste, septic tank sludge and sewer sludge, medical solid waste using modern, advanced technology. Priority is given to recycling, renewable energy in accordance with technological conditions and ensuring environmental safety. This plant will treat solid waste within the area of Ninh Kieu, Binh Thuy, O Mon districts and surrounding areas. It is also treating hazardous medical solid waste for the whole city.

Zone 2 is the solid waste treatment area in Truong Xuan commune, Thoi Lai district, with an area of about 30.0 ha. The solid waste treatment capacity of this plant is about 750 tons/day and about 1,000 tons/day by 2030. This plant handles domestic and construction solid waste, sludge from septic tanks and sewage sludge; hazardous medical solid waste treatment; hazardous industrial solid waste. In addition, this area also supports the post-treatment landfill for the solid waste disposal complexes in the city. The treatment scope of this solid waste treatment facility includes Cai Rang district, Thoi Lai district, Phong Dien district and surrounding areas; at the same time, to support solid waste disposal complexes throughout the city.

Zone 3 is the solid waste treatment area in Thanh Quoi commune, Vinh Thanh district, with an area of about 15.0ha, about 40.0ha by 2030. Treatment capacity of the plant in the first phase is about 400 tons/day and about 1,000 tons/day by 2030. This plant will treat domestic and construction solid waste, sludge from septic tanks and sludge from the drainage system; conventional industrial solid waste. The scope of the plant's treatment is Thot Not district, Co Do district, Vinh Thanh district and surrounding areas.

3.5 Assessment of solid waste management in Cai Khe district

The management of domestic solid wastes in Cai Khe is relatively good, the collection rate is about 85-90%. However, the proportion of solid waste that is not collected still accounting for 10-15%, mainly on households living along canals (Cai Khe market), dumping rubbish directly into canals, and burning at home. These habits cause

unsanitary conditions and affect public health. The research results showed that about 20% of people are still dissatisfied with the waste management because the waste collection is slow, the smell and leachate arising from the garbage gathering point polluteing the environment of the area.

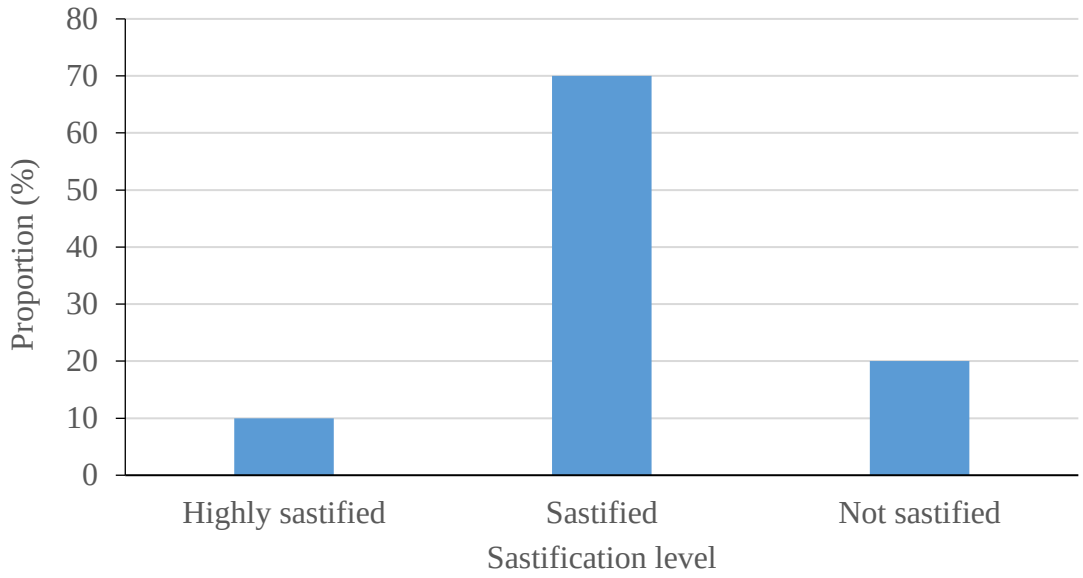

Fig.3: Sastifation levels of the households regarding solid waste management in the study area

3.6 Prediction of solid waste generation in the study area

Based on the local population and annual population growth rate to calculate the current domestic waste volume and estimate the future generation volume. The average population growth rate of Cai Khe ward in 2016 was 3.19%. Based on data collected the population growth rate over the previous years (from 2010 to 2015), it is predicted that the coming period until 2025 will remain at the growth level of 3.19%. It is possible to forecast the population of Cai Khe ward until 2025 as in Table 3.

Table 3. Population of Cai Khe ward in 2025

Year	Population growth rate (%)	Predicted polulation (persons)
2016	3.19	21,931
2017	3.19	22,631
2018	3.19	33,202
2019	3.19	33,294
2020	3.19	33,387
2021	0,28	33,480
2022	0,28	33,574
2023	0,28	33,668
2024	0,28	33,762
2025	0,28	33,857

Table 4. Expected solid waste in Can Khi in 2025

Year	Population	Rate of solid waste generation (kg/person/day)	Collection proportion (%)	Predicted solid waste (tons/day)	Predicted solid waste (tons/year)
2016	33,018	0.2	90	5.943	2169.2
2017	33,110	0.2	90	5.960	2175.4
2018	33,202	0.2	90	5.976	2181.2
2019	33,294	0.2	90	5.993	2187.4
2020	33,387	0.2	90	6.001	2190.4
2021	33,480	0.2	90	6.026	2199.5
2022	33,574	0.2	90	6.043	2205.7
2023	33,668	0.2	90	6.060	2211.9
2024	33,762	0.2	90	6.077	2218.1
2025	33,857	0.2	90	6.094	2224.3

The forecast results show that the amount of domestic solid waste in Cai Khe up to 2025 is 6.094 tons/day or 2224 tons/year. It is necessary to have a strategic solution to well manage a large amount of domestic solid waste generated.

3.7 Solutions to solid waste management

3.7.1 Law and policy

Master plan for solid waste collection and treatment has oriented investment in solid waste collection, transportation and treatment. Socio-economic development planning must be integrated with environmental protection contents. There should be a close coordination between all levels of sectors from cities, districts, wards, state agencies in the environment in the management of waste collection, transportation and treatment. There is a need to have a long-term plan for solid waste collection, transportation and treatment, defining specific goals and targets to be achieved in waste management and the tasks and solutions to be performed. Solid waste recycling has reduced a large amount of waste to be disposed of. Most of the recyclable waste is persistent or non-biodegradable such as nylon, glass bottles, materials PP, PE, ... Recycling and reuse activities are prioritized by the state when encouraging investment. However, recycling facilities need to be well managed because in fact they are all outdated and also one of the polluting facilities. Encouraging development also means close supervision. It is important to consider solving domestic waste issues a priority issue. The amount of waste is increasing in quantity as well as types, so it is necessary to improve the capacity of the management agencies. Develop a plan and establish a fund for a waste separation program at source. Develop regulations on solid waste management in general and domestic solid waste in particular. Encourage participation of private companies in the field of environmental protection. The general direction is to combine state-owned enterprises with other private economic sectors to participate in solid waste management in general and domestic solid waste in particular. Joint venture with foreign direct investment, 100% foreign owned enterprise, in charge of solid waste treatment with high technology and large investment capital, modern and centralized recovery and recycling technologies.

3.7.2 Reduction at sources

Generation reduction is not only about reducing the amount of waste, but also about reducing the concentration and toxicity of the waste at the source. Reducing solid waste generation in industry includes reducing generation in the manufacturing process, producing products that generate less wastes, products that easily decompose when discarded, products with little or no hazardous waste, etc. Even changing daily consumption habits towards environmentally friendly products, products with less packaging, less active ingredientsis also an effective solution to reduce solid waste generation. In addition, the waste reduction of a production process is also implemented by many different solutions, in which assessment of cleaner production is also a positive and effective solution in recent years. The application of cleaner production programs in factories and manufacturing enterprises increases the economic efficiency of their production processes by reducing

pollution at the source. In addition, other strategic solutions such as the imposition of disposal fees, waste treatment taxes for electronic waste, refrigeration, ... have been successfully applied in many developed countries but are not suitable for Vietnamese conditions where waste management and treatment solutions are only in improving process.

3.7.2 Reuse and recycle

Reuse and recycle are terms synonymous with reducing waste. Reuse is the reuse of products or materials without significant modification, which only need to be cleaned or repaired before reuse. Recycling is different from reuse in that it requires certain modification of the composition, physical, chemical, or biological properties of the waste to become a usable product. For industrial wastes, after being sorted at source, collected and transported to the solid waste treatment complex for treatment. In the treatment area, useful materials for recycling are classified, the hazardous wastes are treated according to solidification and stabilization technology, and burning. Strategic solutions to promote industrial reuse and recycling can take the form of an industrial waste exchange program. In this program, waste from one manufacturing industry but as raw material for another, can be communicated and exchanged for efficient and optimal use of resources before disposal.

3.7.3 Solid waste processing

It is the physical, chemical and biological transformation process of generated solid waste, improving the efficiency of the management system of recycled and reusing solid waste, using recycled products (compost) and recovering energy in the form of heat and biogas. The process of converting waste into an original product or a product that serves human life. This is the stage after classification has been done. Recyclable and reusable wastes are used as input to new product (recycled products) processes.

IV. CONCLUSIONS

The findings showed that the rate of solid waste generation per household was 0.74 kg/household/day and for each individual was 0.2 kg/person/day. Domestic solid waste in the study area has various composition, of which organic wastes accounted for the highest proportion (72.7%), plastic components accounted for 13.4%, glass accounted for 2.7%, hazardous waste occupied 1.4% and other components took up 3.6%. The separation of waste at source has not been implemented. Tools to store domestic waste in households are mainly plastic bins and plastic bags. The rate of garbage collection was about 90%. The odor and leachate were not well managed, causing pollution to the urban environment. The cause of this problem is probably the collection work is not effective and the awareness of household is still inappropriate. The effective management of domestic solid waste in Cai Khe requires the attention of the local authorities, the participation of the people. Therefore, community consensus is an important issue that needs to be addressed.

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Improvement of Red Rice Yield by Changing from Conventional to Aerobic Irrigation Systems Intercropped with Soybean at Different Dates

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Abstract— *Previous studies reported that intercropping rice with legume crops under aerobic systems increased growth and yield of rice compared with monocropped rice. This study aimed to examine the effects of rice cultivation techniques and patterns of rows on growth and yield of red rice with special objective to examine if intercropping red rice with soybean relay-planted between double or triple rows of rice on raised-beds under aerobic irrigation system can produce higher yield than the conventional one. The experiment was conducted in Narmada (West Lombok, Indonesia), arranged according to Split Plot design with two treatment factors, i.e. rice row patterns as the main plots (P1= double, P2= triple rows) and techniques of rice cultivation (Tc= conventional rice, T0= irrigated aerobic rice system (ARS) without intercropping; T1= relay-planting soybean 1 week, T2= 2 weeks, T3= 3 weeks after seeding the pre-germinated red rice seeds). Results indicated that between the two treatment factors, techniques of growing rice resulted in significant effects on more variables compared with the patterns of rice rows, but there were significant interaction effects on leaf number per clump at anthesis and grain yield per clump. Based on the main effects, tiller number, filled panicle number and grain yield per clump and harvest index were significantly higher on red rice plants grown in irrigated aerobic systems intercropped with soybean compared with those in aerobic monocropped or conventional systems, with the highest grain yield average of 53.76 g/clump in T3, 29.04 g/clump in T0 and only 17.45 g/clump in Tc treatment, and based on the interaction effects, the highest average of grain yield was on red rice plants in the T3 treatment under triple-row pattern (57.58 g/clump) and the lowest was on conventional plot (Tc) under double-row pattern (16.53 g/clump).*

Keywords— ***Soybean, red rice, intercropping, aerobic irrigation systems, comventional, row patterns.***

I. INTRODUCTION

Rice (*Oryza sativa* L.) is normally cultivated under the conventional technique that is referred as to paddy rice, in which rice is grown on puddled and flooded soil. Therefore, the root systems are in an anaerobic soil condition. In contrast, under aerobic rice systems, rice is grown on non-puddled, non-flooded and non-saturated soil [1, 2]. One of the advantages of growing rice under aerobic systems is that rice plants can be grown together or intercropped with a legume crop such as soybean [3].

Intercropping cereal crops, such as rice and maize with legume crops such as peanut and soybean was reported to provide some advantages for both types of crops, but in more specially for the cereal crops. Inal *et al.* [4] found that the rhizosphere of maize-peanut intercrop contains higher concentration of several macro and micro nutrients compared with in the rhizosphere of maize or peanut in monocrop. Chu *et al.* [5] also reported significant N transfer from peanut to rice in intercropping system under aerobic conditions. In addition, Fujita *et al.* [6] also reported N transfer from soybean to sorghum in intercropping system, and the amount of N transfer was higher as the planting distance was closer.

Leaves of the red rice plants grown together with soybean in pot culture under aerobic irrigation systems were also much greener than those grown in monocrop [3], which also indicated some N transfer from soybean to rice plants. The red rice plants grown in intercropping with soybean on raised-bed under aerobic irrigation system was also reported to show better growth and to produce higher grain yield [7] and higher anthocyanin contents in the rice grains [8], when compared with those grown on the monocrop beds. Wangiyana *et al.* [9] also reported that additive intercropping with peanut relay-planted between double or triple rows of rice either one or two or three weeks after planting the pre-germinated seeds of rice significantly increased panicle number and grain yield of red rice per clump.

In addition, relay-planting dates of the peanut plants relative to planting dates of the rice plants also showed significant effects on panicle number and grain yield of the red rice per clump, in which grain yield per clump was higher on the red rice plants relay-planted with peanut at two or three weeks compared with one week after planting the pre-germinated seeds of the red rice [9]. Arifuddin *et al.* [10] also reported significant effects of relay-planting dates of legume crops (peanut, soybean, mungbean)

relative to planting dates of red rice pre-germinated seeds in pot culture on grain yield of the red rice plants, in which grain yield was higher on rice plants relay-planted with the legume crops at two or three or four weeks compared with one week after planting red rice. However, grain yield of the red rice per pot was not significantly different among rice plants relay-planted with the legume crops at two or three or four weeks after planting the red rice seeds [10].

This study aimed to examine the effects of relay-planting dates of soybean between double or triple rows of red rice plants grown on raised-beds under an aerobic irrigation system. It was also aimed to examine the potential of these aerobic rice systems in increasing yield of the red rice compared with those grown under the conventional technique of rice cultivation.

II. MATERIALS AND METHODS

The field experiment in this study was conducted on paddy field in the Experimental Farm of the Faculty of Agriculture, University of Mataram, located in Narmada (Lombok, Indonesia) from June to October 2016. The experiment was arranged according to Split Plot design, testing two treatment factors, namely: the patterns of rice rows (P) as the main plots (P1= double row, P2= triple row), and techniques (T) of rice cultivation as the subplots (Tc= conventional (flooded) rice; T0= irrigated aerobic rice system (ARS) on raised-beds without intercropping, T1= ARS intercropped with soybean ("Anjasmoro" variety) relay-planted at 1, T2= 2, T3= 3 weeks after planting (WAP) pre-germinated rice seeds). The conventional rice systems were established in each block surrounded with a dike to maintain 5-10 cm standing water and rice seedlings (seeded on the same day of planting aerobic rice) were transplanted to the conventional plots at 3 weeks old, while the aerobic rice plants were grown on raised-beds of 25 cm height, and irrigation water was flowed through the furrow surrounding the raised-beds by maintaining the surface of the irrigation water 15 cm lower than the surface of the raised-beds. Each treatment combination was made in three blocks. The details of the treatments of aerobic irrigation systems and the implementation of the experiment were as described in Farida *et al*. [11], except for the legume crop used in this study was soybean instead of peanut in previous report [11].

The observation variables include plant height, leaf number and tiller number at 12 WAP, panicle number, dry straw weight and grain yield per clump, weight of 100 grains, and harvest index, which was based on Wangiyana *et al.* [9]. Data were analyzed with analysis of variance (ANOVA) and Tukey's HSD at 5% level of significance using CoStat for Windows ver. 6.303 and correlation analysis using Minitab for Windows Rel. 13.

III. RESULTS AND DISCUSSION

The summary of ANOVA results in Table 1 shows that between the two treatment factors tested, techniques of rice cultivation had more significant effects on growth and yield of red rice compared with the effects of row patterns, but there were significant interaction effects on grain yield and leaf number per clump at anthesis.

Based on the main effects in Table 1, except for the weight of 100 dry grains, all other observation variables were affected by the techniques of rice cultivation, especially between the conventional rice and aerobic rice on raised-beds intercropped with soybean, in which yield and yield components of the red rice were on average significantly lower on the red rice plants cultivated under the conventional technique. As can be seen from Table 1, grain yield per clump is the most significantly different between techniques of growing rice, being the highest average is in the aerobic red rice plants intercropped with soybean plants relay-planted three weeks after seeding the pre-germinated red rice seeds (T3 treatment).

This highest average of grain yield per clump in the T3 treatment is also supported by the highest leaf and tiller number per clump at anthesis, filled-panicle number and harvest index. The highest harvest index means that the highest proportion of the biomass that was partitioned to the growing seeds during the seed-filling stage, and with the highest filled-panicle number supported with the highest leaf number at anthesis, these conditions would result in the highest grain yield per clump in the red rice plants in T3 treatment. These observation variables had positive and significant correlation coefficients with grain yield per clump, and among those observation variables, harvest index showed the highest correlation coefficient ($r = + 0.961$) with grain yield per clump (Table 2).

In relation to leaf number, the highest average at anthesis was in the T3 treatment, which means that the average number of green leaves at anthesis was in the T3 treatment, in which the red rice plants were grown on raised-beds intercropped with soybean relay-planted three weeks after seeding the pre-germinated rice seeds. Wangiyana *et al.* [3] also reported that red rice plants grown together in one pot with soybean showed much greener leaves than those grown in monocrop. Higher number of green leaves after anthesis would increase photosynthesis during the grain filling process.

Table 1: Summary of ANOVA results for the effects of the treatment factors on all observation variables

Treatments:	Plant height (cm)	Leaf number per clump	Tiller number per clump	Panicle number per clump	Weight of 100 grains (g)	Grain yield (g/clump)	Dry straw weight (g/clump)	Harvest index (%)
Rice cultivation techniques:								
Tc: conventional rice	85.33 b	46.08 c	16.94 b	14.00 b	2.53 a	17.45 e	50.30 c	25.59 c[1)]
T_0: ARS monocrop	103.30 a	97.73 ab	23.43 a	16.50 b	2.37 a	29.04 d	51.10 bc	36.22 b
T_1: ARS+soybean 1wk	106.77 a	95.80 b	25.17 a	21.00 a	2.37 a	43.83 c	53.66 abc	45.13 a
T_2: ARS+soybean 2wk	107.70 a	93.40 b	26.70 a	22.25 a	2.44 a	50.19 b	59.14 a	45.87 a
T_3: ARS+soybean 3wk	103.93 a	108.10 a	28.33 a	21.67 a	2.37 a	53.76 a	58.81 ab	47.74 a
HSD 5%	8.86	12.03	4.92	4.17	ns	3.07	7.80	3.45 [2)]
Rice row-patterns:								
P_1: double-row	99.85 b	95.12 a	24.45 a	18.53 a	2.43 a	37.36 a	53.23 a	39.84 a
P_2: triple-row	102.96 a	81.33 b	23.78 a	19.63 a	2.40 a	40.35 a	55.98 a	40.37 a
HSD 5%	2.16	7.53	ns	ns	ns	ns	ns	ns [2)]
Interactions:	ns	***	ns	ns	ns	**	ns	ns [2)]

[1)] Mean in each column with same letters indicates non-significant differences between levels of a treatment factor.
[2)] ANOVA results: ns = non-significant; **, *** = significant at *p-value* < 0.01 and *p-value* < 0.001, respectively.

Table 2: Correlation coefficients between observation variables and their p-values

Observation variables	Plant height	Leaf number	Tiller number	Panicle number	Weight of 100 grains	Straw dry weight	Grain yield
Leaf number per clump	0.604						
p-value	**0.000**						
Tiller number per clump	0.712	0.738					
p-value	**0.000**	**0.000**					
Panicle number per clump	0.638	0.600	0.803				
p-value	**0.000**	**0.000**	**0.000**				
Weight of 100 grains	-0.290	-0.260	-0.133	-0.090			
p-value	0.120	0.165	0.485	0.641			
Weight of dry straw	0.557	0.174	0.502	0.496	-0.022		
p-value	**0.001**	0.358	**0.005**	**0.005**	0.909		
Dry grain yield per clump	0.724	0.590	0.803	0.828	-0.139	0.675	
p-value	**0.000**	**0.001**	**0.000**	**0.000**	0.465	**0.000**	
Harvest index	0.745	0.715	0.826	0.842	-0.167	0.485	0.961
p-value	**0.000**	**0.000**	**0.000**	**0.000**	0.378	**0.007**	**0.000**

Leaf number at anthesis and grain yield per clump also showed a significant interaction between the two treatments factors, which means that the differences in leaf number and grain yield of the red rice between patterns of rice rows depend on the techniques of rice cultivation practiced. From Fig. 1 it can be seen that average number of green leaves per clump was very low in the conventional rice plants under double-row pattern, which was only around one-third of the highest number of leaves in the aerobic rice plants, which was in the T3 treatment, but under the triple-row pattern, leaf number of the conventional rice was higher than that of the rice plants in the double-row pattern.

If we compare Fig. 1 and Fig. 2, it seems that the patterns of the interaction between the two treatment factors are slightly different. The average grain yield per clump in Fig. 2 is highest on the red rice plants in the T3 treatment under triple-row pattern, while leaf number per clump is highest on the red rice plants in the T3 treatment under double-row pattern. This means that the strongest determinant of the highest grain yield in the context of these treatments was not the highest leaf number at anthesis, as it can also be seen from Table 2 that the correlation coefficient with grain yield is only +0.590, which is much lower than that of harvest index, which is +0.961, although both are significant (p-value < 0.01).

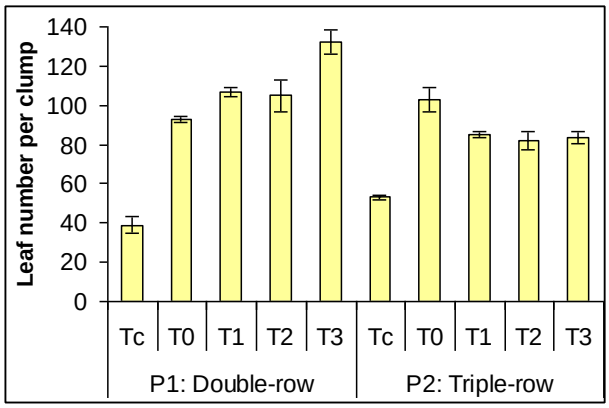

Fig.1: Interaction effects of the treatments on leaf number per clump

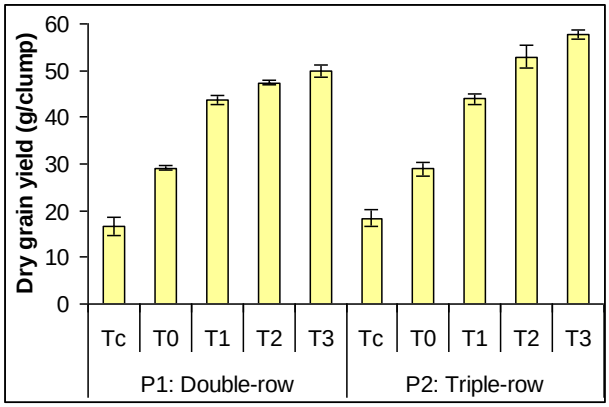

Fig.2. Interaction effects of the treatments on grain yield per clump

Based on the correlation coefficients in Table 2, it seems that the highest determinant for grain yield was harvest index, with an R^2 = 92.35%. According the results of analysis by Sinclair and de Wit [12], seed plants require high supply of nitrogen during the seed-filling stage to maintain high photosynthate and nitrogen supply to the growing seeds for higher grain yield, and if nitrogen supply to the plants is lower than these requirements, then the plants will remobilize N content of the leaves, which accelerates leaf senescence and decreases grain yield. This means that rice plants also require higher N supplying capacity for higher rates of photosynthate production and partition to the growing seeds during the seed-filling stage.

It can also be seen from Fig. 3 that the average harvest index is highest on the red rice plants in the T3 treatment under triple-row pattern, followed by those in the T3 treatment under double-row pattern and those in T2 treatment under triple-row pattern. However, dry straw weight per clump and percentage of panicle to tiller number are much lower on the red rice plants in the T3 treatment of double-row pattern compared with on those in the T3 treatment of triple row pattern, but in contrast tiller number at anthesis was higher on the red rice plants in the T3 of double-row than in the T3 of triple-row pattern (Table 3). The lower dry straw weight (Table 3) coupled with lower harvest index (Fig. 3) and lower percentage of filled panicle number (Table 3) would logically result in lower grain yield per clump on the red rice plants in the T3 treatment under double-row than those in the T3 treatment under triple row pattern.

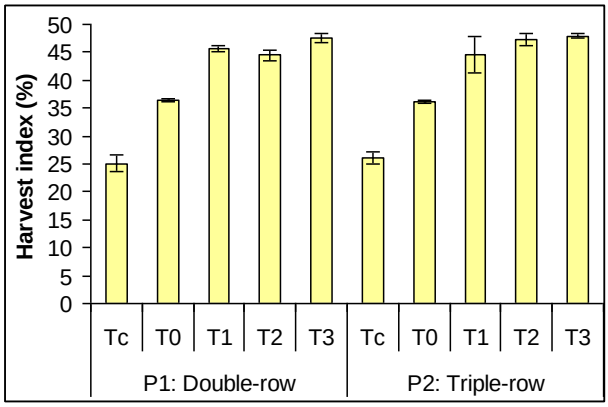

Fig.3. Average (Mean ± SE) harvest index (%) of red rice as affected by relay-planting soybean at different dates between double and triple rows of rice

Table 3: Mean straw dry weight, tiller number, panicle number, and %-panicle number per clump

Row Patterns	Tech	Straw dry weight (g/clump)	Tiller number per clump	Filled panicle number	%-panicle number
P1: Double-row	Tc	48.83	16.9	13.0	77.15
	T0	50.95	23.9	15.3	64.25
	T1	52.02	26.1	20.3	78.01
	T2	59.35	26.1	22.3	85.46
	T3	54.97	29.3	21.7	73.86
P2: Triple-row	Tc	51.77	17.0	15.0	88.06
	T0	51.24	23.0	17.7	76.81
	T1	55.30	24.3	21.7	89.29
	T2	58.93	27.3	22.2	81.30
	T3	62.64	27.3	21.7	79.27

It could also be possible that competition between the red rice and soybean plants was started to occur in the double-row pattern due to higher number of soybean plants per plot and higher number of red rice leaves per clump when compared with those in the triple-row pattern, especially during the seed-filling stage (after anthesis). With higher number of leaves per clump, higher soybean population per bed, and closer planting distance between the red rice rows and soybean row would result in higher shading intensity imposed by the rice plants to the intercropped soybean plants, which in turn would result in lower rates of photosynthesis and N_2 fixation by the

soybean plants under double-row pattern than those under triple-row pattern. This is similar to the results reported by Wangiyana *et al.* [13] that relay-planting higher number of soybean or mungbean plants (i.e. three rows) between two rows of waxy maize plants resulted in lower grain yield of the waxy maize compared with relay-planting two rows of soybean or mungbean between two rows of waxy maize plants.

Many have reported that in an intercropping system, there was a significant N-transfer from legume to cereal crops, such as from peanut to rice plants in an aerobic system [5], from peanut to maize plants [4], and from soybean to sorghum plants [6]. Wangiyana *et al*. [3] also reported that growing together in one pot with soybean under aerobic irrigation system resulted in much greener leaves on red rice plants at anthesis, especially on those inoculated with biofertilizer containing mixed species of Arbuscular Mycorrhizal Fungi (AMF). Several promising lines of red rice plants intercropped with soybean under aerobic irrigation systems on raised-beds were also reported to produce higher number of filled grains and grain yield per clump [7], which also infer higher biomass partitioning rates to seeds under intercropping with soybean compared with under monocropped red rice.

According to the results reported by Inal *et al*. [4], intercropping maize with peanut increased the availability of the nutrients in the rhizosphere of the intercropped plants compared with its monocrop, and this resulted in higher nutrient contents in the plants grown under intercropping system compared with under monocropping system. Therefore, it is clear why grain yield was higher on the red rice plants intercropped with soybean relay-planted at one, two or three weeks after seeding pre-germinated seeds of the red rice, which was probably due to the higher N contents in the leaves of intercropped red rice compared with in the monocropped red rice plants. Higher leaf N content was also found on the red rice plants intercropped with peanut, especially when peanut was relay planted three weeks after seeding the red rice [9]. Arifuddin *et al*. [10] also reported that relay-planting legume crops, including peanut of Hypoma-3 variety, mungbean of Kenari variety, and soybean of Dering-1 variety, at three weeks after seeding red rice pre-germinated seeds was found to result in the highest tiller number, panicle number, filled grain number, and grain yield per clump of the red rice plants grown together with those legume crops in pot culture under aerobic irrigation systems. Leaves of the red rice plants grown together with soybean in pot culture were also reported to be much greener than those growing in monocropped pots, at anthesis of the rice plants [3].

IV. CONCLUSION

It can be concluded that intercropping red rice plants with soybean under aerobic irrigation system significantly increased grain yield compared with monocropped red rice either under aerobic or conventional systems, mostly due to higher panicle number per clump supported with higher harvest index of the rice plants intercropped with soybean especially when soybean was relay-planted at three or two weeks after seeding the pre-germinated seeds of the red rice.

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Comparison of the prevalence of *Cocoa Swollen shoot virus* and the prevalence of *Phytophthora sp* in Petit-Bondoukou, South-West of Côte d'Ivoire

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Abstract— *Background: Swollen shoot and Black pod disease are two major diseases that cause significant damage in Nawa region. In order to compare the prevalence of these two diseases, a study was conducted in Soubré area in the south-west of Côte d'Ivoire, at the site of Petit-Bondoukou. Methods: The observation system consists of a sentinel site of approximately 10 km X 10 km. Each sentinel site is made up of 16 clusters of 2.5 km X 2.5 km containing about 10 plots which constitute the different observation points. In this study, observations were made in 4 plots of the site. The variables that were measured during data collection were the total number of pods on each tree, the total number of pods with black pod disease symptoms and the total number of diseased trees in the swollen shoot outbreaks. The prevalence of each pathogen was deduced from the measured variables and then related using the Bravais-Pearson correlation test. Beforehand, a molecular analysis was carried out to identify the different species of Phytophthora. Results: The results of the molecular analysis showed that the only species identified at this site was Phytophthora palmivora. The descriptive analysis showed that the prevalence of CSSV was higher than that of Black pod disease with 50% and 32% respectively. Statistical analysis showed that there is a positive and significant correlation between the prevalence of swollen shoot and black pod disease. Conclusion: This study shows that most of the test trees at the site of Petit-Bondoukou show both Swollen shoot and Black pod disease symptoms and invites growers contribute to reducing the pressure of both diseases by leading regular activities plot maintenance.*

Keywords— ***Cocoa trees, CSSV, black pod disease, Prevalence, Côte d'Ivoire.***

I. INTRODUCTON

The cocoa tree (*Theobroma cocoa L.*) is a tropical perennial crop of Malvaceae family (Motamayor *et al.,* 2002) formerly classified in the Sterculiaceae family (Metcalfe and Chalk, 1950). The cocoa tree is native to South America and its origin is in the Amazon basin (Motamayor *et al.*, 2002). Cocoa cultivation has been introduced in various West African countries such as Ghana, Cameroon, Nigeria, Togo and Côte d'Ivoire where large plantations have been established (Janny *et al.*, 2003). The extension of cocoa orchards in Côte d'Ivoire is linked to land availability and an attractive and remunerative pricing policy. This is the reason why Côte d'Ivoire has been the world's leading cocoa producer since 1977 (Tano, 2012). This crop is therefore of great importance in the Ivorian economy where it accounts for 40% of export earnings and contributes up to 15% of gross domestic product (GDP) (Tano, 2012; Serges, 2014). However, these important socio-economic achievements related to cocoa production in Côte d'Ivoire should not make us lose sight of the many constraints related to diseases, pests and market price fluctuations (Freud *et al.*, 2000). The consequences of these constraints are lower production and

increased poverty in rural areas. In Côte d'Ivoire, black pod disease and swollen shoot are the two main diseases that pose a major threat to cocoa production (Kouakou *et al.*, 2011).

Black pod disease is caused by *Phytophthora spp*., with 44% crop losses (CNRA, 2017). In areas most favourable to black pod disease, losses can reach 80% of the crop (Koua et al., 2018). In addition, the reappearance of the swollen shoot virus disease in 2003 in the Ivorian orchard (Kébé and N'guessan, 2003) after 60 years, amplifies the threat to cocoa production in Côte d'Ivoire. Swollen shoot is one of the most significant viral diseases in the world (Dzahini-Obiatey *et al.*, 2010). The Cocoa Swollen shoot Virus (CSSV) responsible for this disease belongs to the genre Badnavirus and is transmitted by mealybugs of the Pseudococcidae family (N'guessan *at al.*, 2016). Swollen shoot disease has not progressed since its discovery in 1946 (Alibert, 1946), and it was only in 2003 that new outbreaks were discovered in the central-western part of the country, notably in Sinfra, Issia and Bouaflé (Kébé and N'guessan, 2003). Today, the swollen shoot species discovered in the new outbreaks are gradually spreading in the Ivorian cocoa plots to the point of posing a real threat to the major cocoa-producing basins such as the Soubré region in the south-west of Côte d'Ivoire. In addition to the damage caused by the swollen shoot disease (Fig.1), there is also the damage caused by black pod disease (Fig.2), thus increasing the risk of a drop in cocoa production. The various known pathogens that commonly cause black pod disease are represented by *Phytophthora palmivora* and *Phytophthora megakarya* (Kébé *et al*., 2009).

In Côte d'Ivoire, *Phytophthora megakarya* which did not exist in cocoa plots is also beginning to spread significantly (Coulibaly *et al*., 2013). This species is however the most aggressive (Brasier and Griffin, 1979) unlike *P. palmivora* which is the least aggressive and most widespread species. In addition, the prevalence of *P. megakarya* is poorly known in Côte d'Ivoire and an interaction between *P. megakarya* and *Cocoa Swollen shoot Virus* (CSSV) is suspected. This is the reason this study is being conducted.

The objective of this study is to compare the prevalence of swollen shoot virus and that of *Phytophthora sp* in order to assess the damage caused in plantations by each pathogen. Specifically, the aim is to first characterize the species responsible for black pod disease and then to study the relationship between the prevalence of CSSV and the prevalence of *Phytophthora sp*.

Fig. 1: The different indicators in the plot affected by swollen shoot disease.

***(A):** redness on young leaves of cocoa trees, **(B):** Outbreak of swollen shoot disease, **(C):** Cocoa tree stem swelling (Photo taken by Oro Franck)*

Fig. 2 : Symptoms of black pod disease on immature cocoa pod (Photos taken by Oro Franck)

II. MATERIALS AND METHODS

2.1 Study Area

This study was carried out in Petit Bondoukou site (Fig.3) located 51 kilometers from Soubré town in Côte d'Ivoire. The Soubré region is currently the main cocoa production area in Côte d'Ivoire. This site is characterized by a humid tropical climate with an average rainfall of 1,485 mm per year, and an average temperature of 25.8°C per year. Vegetation and soil are characterized by dense, humid forests and a deep, permeable and well-drained soil that allows for anthropogenic activities related to agriculture (N'go *et al*., 2012). This vegetation is now giving way to shreds of forest and huge plantations of traditional or industrial perennial crops (Le paysan, 2012).

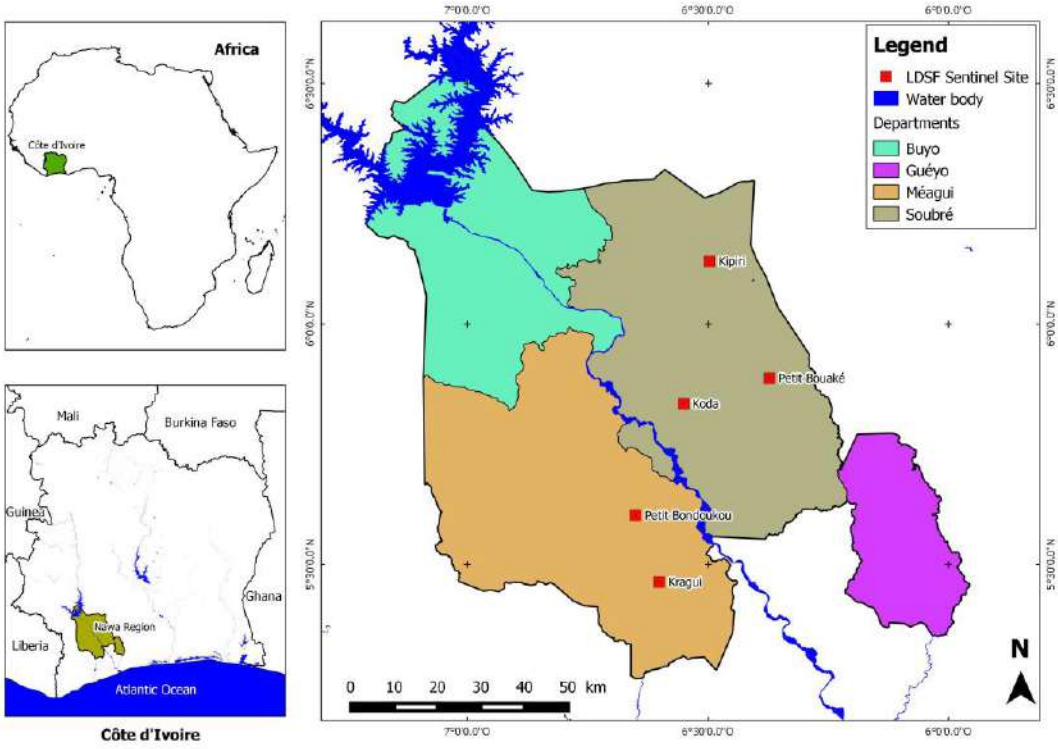

*Fig. 3 : Map of the study area showing the site of Petit-Bondoukou (*Diby *et al.*, 2014*).*

2.2. Experimental device

The experimentation took place in peasant cocoa farms based on prospection surveys. These surveys collected data on swollen shoot and *Phytophthora sp*. These surveys were conducted according to the Land Degradation Surveillance Framework (LDSF) protocol (Diby *et al.*, 2014). The LDSF (Figure 4) is a protocol that was initially developed to monitor soils suitable for agriculture in West Africa. This protocol was adapted in this study for epidemiological investigations of swollen shoot disease and Black pod disease caused by *Phytophthora sp* (Diby *et al.*, 2014). The LDSF represents a sentinel site of 10 Km × 10 Km. The site is divided into 16 clusters of 2.5 Km × 2.5 Km (Fig.4). Each cluster has 10 observations points, called plots.

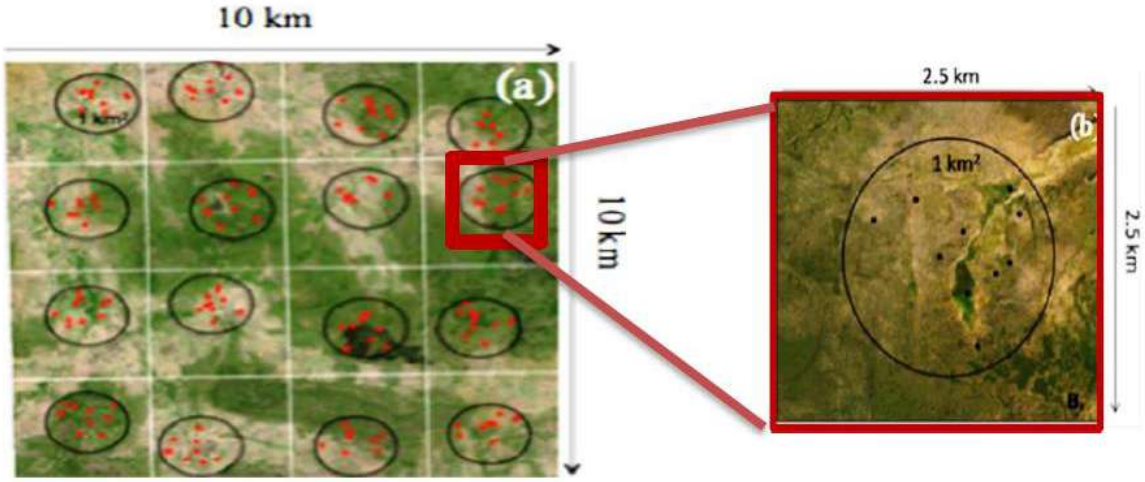

Fig.4 : Land Degradation Surveillance Framework (LDSF) Device (Diby et al., 2014)

2.3. Observation and data collection

The data collected are related to swollen shoot and Black pod disease. These data were collected in 4 plots of the Petit-Bondoukou site that were randomly selected. Observations were made on 10 cocoa trees in each plot, corresponding to 40 test trees.

II.3.1 Collection of CSSV data

The CSSV data collection was carried out at each observation plot, previously identified using the Garmin 64s GPS. Around the plot, an observation area with a radius of 50 m was delimited. In this observation area, infection outbreak were detected on the basis of several indicators including clearings (Fig.1B), leaf symptoms (Fig.1A) and stem symptoms (Fig.1C). Symptoms on young leaves are represented by redness along the veins and discoloration while stem symptoms are related to swellings on young stems. In each observation area several outbreaks were detected in case of presence of swollen shoot disease. Epidemiological data were collected from each outbreak. It is in these outbreaks that the number of healthy and diseased cocoa trees were counted. The areas of the outbreaks were measured using a Garmin 64 S GPS. A prepared survey form was then filled in to compile the physical data.

2.3.2. Collection data of *Phytophthora sp*

The *Phytophthora sp* data were collected in the same area as the CSSV data. In contrast to the CSSV data, epidemiological data on black pod disease were collected within a radius of 300 meters around the same test plots (Fig.5). In the case of this study, surveys were only conducted in four (04) plots on the site of Petit-Bondoukou. For each test tree, the total number of pods was counted, as well as the number of pods affected by black pod disease. For the characterization of isolates of *Phytophthora* species, two samples of pods with symptoms were taken from each test tree. These sampled pods with symptoms were wrapped in newsprint to slow down the development of *Phytophthora sp*, and then labelled with an identifying code. These pod samples were cross-sectioned along the progressing front of the rotten spot, then a rot explant was taken from the cortex and from an eppendorf tube in the presence of a medium culture favourable to the development of *Phytophthora sp*. The different tubes were labelled with the codes corresponding to the identifier of sample pods.

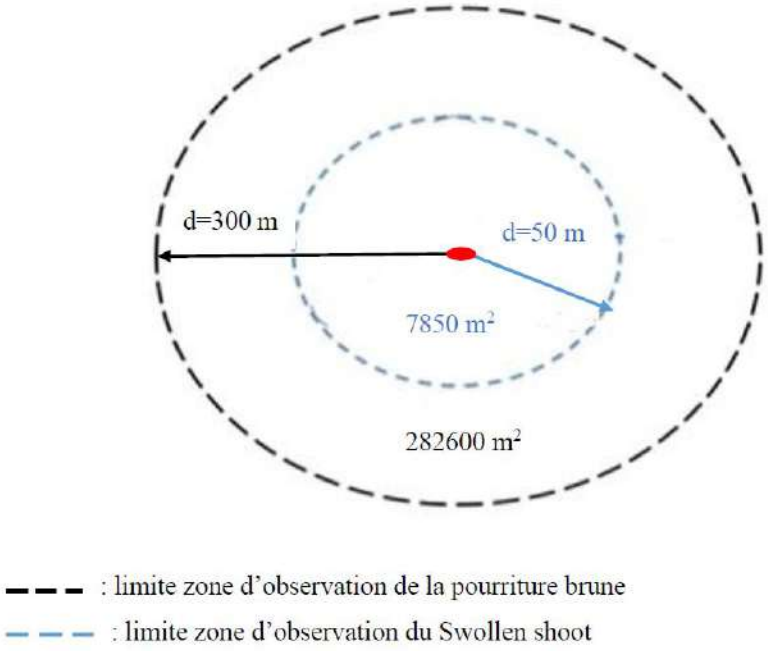

*Fig.5 : Observation areas and data collection for CSSV and Phytophthora sp (*Diby *et al.*, 2014).

2.4. Data Analysis

Data analysis included molecular analysis to identify the different species of *Phytophthora sp* and statistical analysis to assess the relationship between the prevalence of CSSV and that of black pod disease.

2.4.1. Molecular analysis

Molecular analysis of black pod disease data was carried out at the CIRAD Joint Research Unit on Biology and Genetics of Plant-Parasite Interactions in Montpellier, France. This analysis allowed the identification of different *Phytophthora* species. In the laboratory, the sampled explants were grown in Petri dishes on the "V8" medium culture, which consists of 1/10 of a vegetable juice cocktail, agar at 15g / L and CaCO3 at 3g / L for 4 days in the dark, at 25°C. After 4 days, the agar explants containing *Phytophthora sp* mycelium culture were removed from the fungal growth front with a scalpel and then transplanted into the V8 medium culture under the same conditions described above for 7 days. The resulting mycelium is used for DNA extraction. In case the strains were contaminated with other fungi, several transplants on H2O-Agar culture medium at 15g/L were necessary to purify the strain. After purification, the strain is cultured again on "V8" medium at 25°C for 4 days and then 7 days in the dark to obtain a mycelium typical of *Phytophthora sp*. The mycelium obtained after purification is used for DNA extraction. The identification of the different species of *Phytophthora* was performed by PCR (*Polymerase Chain Reaction*) with species-specific ITS primers (*P. megakarya* and *P. palmivora*).

2.4.2. Determination of the prevalence of the two plant pathogens

The prevalence of swollen shoot disease is the ratio of the number of diseased cocoa trees counted to the total number of cocoa trees sampled in the observation area. This prevalence is represented by equation 1. The prevalence of black pod disease is obtained from the ratio of the number of rotten pods to the total number of pods on the selected trees in the plot according to equation 2. In addition, the prevalence of black pod disease for each species of *Phytophthora sp* was also determined from the ratio of the number of pods infected by each species to the total number of pods sampled at the site. This prevalence was obtained according to equation 3.

$$\text{P CSSV (\%)} = \frac{\text{Number of diseased trees sampled}}{\text{Total number of trees sampled from pl}} \times 100 \quad \textbf{(1)}$$

$$\text{P Phyto (\%)} = \frac{\text{Number of rotten pods}}{\text{Total number of pods on the plot}} \times 10 \quad \textbf{(2)}$$

$$\text{P Phyto sp (\%)} = \frac{\text{Number of pods infected by species}}{\text{Total number of pods sampled on site}} \times 100 \quad \textbf{(3)}$$

P CSSV (%): Prevalence of Swollen shoot disease

P Phyto (%): Prevalence of black pod disease

P Phyto sp (%): Prevalence of each *Phytophthora* species

2.4.3. Statistical analysis

Statistical analysis was applied on the prevalence of swollen shoot disease and the prevalence of black pod disease. This analysis first provided a description of these two quantitative variables. Second, it evaluated the relationship between the two variables using the Bravais-Pearson correlation test in SPSS Statistics 20 software.

III. RESULTS AND DISCUSSION

3.1 RESULTS

3.1.1. Different species identified

Molecular analysis revealed a diversity of species of the genre *Phytophthora*, of which only one was identified as *Phytophthora palmivora*. The results of molecular analysis showed that the other *Phytophthora* species are more dominant than *Phytophthora palmivora* at 93% compared to 8% of the pods sampled (Table I). This indicates a prevalence of 23% for other *Phytophthora* species compared to nearly 2% for *Phytophthora palmivora* (Table II). The species *Phytophthora megakarya* was not identified at the Petit-Bondoukou site.

3.1.2. Description of prevalences of Swollen shoot disease and black pod disease

The result of the descriptive analysis (Table III) shows that the prevalence of swollen shoot disease at the site of Petit-Bondoukou is between 0% and 100%, for an average of 50% ± 32%. The prevalence values of Swollen shoot disease rather scattered around the average show that the prevalence of swollen shoot disease differs strongly from one plot to another at the site of Petit-Bondoukou. In addition, more than half of the plots are affected by swollen shoot disease (Table IV). This shows that there are more diseased trees (53%) than healthy trees (48%).

Black pod disease also affects this site with a prevalence of between 21% and 39%. The average prevalence of black pod disease over the whole site is 32 ± 7%. The prevalence of black pod disease less dispersed around the average explains why the Black pod disease differs little or not at all from one plot to another on the site of Petit-Bondoukou.

Table I: Number of trees infected by the different species of Phytophthora identified at the site of Petit-Bondoukou

***Phytophthora* species**	**Number of infected trees**	**Percentage**
P. palmivora	3	7,5
P. megakarya	0	0
Other species	37	92,5
Total	40	100

Table II : Prevalence of each species of Phytophthora identified at the site of Petit-Bondoukou

	Number of infected pods	Prevalence (%)
Phytophthora megakarya	0	**0,00**
Phytophthora palmivora	17	**1,62**
Other species of *Phytophthora*	237	**22,59**
Total number of infected pods	254	**24,21**
Total sampled pods	1049	

Table III : Results of the descriptive analysis of the prevalence of Swollen shoot and Black pod disease

	N	Minimum	Maximum	Average	Standard Deviation
Prevalence CSSV (%)	40	00	100	50	35,81
Prevalence phyto (%)	40	21,36	38,74	31,82	6,98

Table IV : CSSV status of sampled trees

Status of trees	Number	Percentage
Diseased	21	52,5
Healthy	19	47,5
Total	40	100

3.1.3. Relationship between the prevalence of swollen shoot and black pod disease

The result of the Bravais-Pearson correlation test (Table V) showed that there is a positive and significant correlation (r=0.814; p = 0.000) between the prevalence of CSSV and the prevalence of *Phytophthora sp.* This shows that the development of one of these diseases favours the development of the other.

Table V: Result of the correlation test between the prevalence of CSSV and the prevalence of Phytophthora sp.

Correlation		Prevalence CSSV (%)	Prevalence *Phytophthora*
Prevalence CSSV (%)	Correlation of Pearson	1	**0,814****
	Sig. (bilateral)		**0,000**
	N	40	40

** The correlation is significant at the 0.01 level (bilateral).

3.2. DISCUSSION

The result of the descriptive analysis showed that swollen shoot disease is very present in Petit-Bondoukou with a proportion of 50%. This result is in line with the results of the work of Diby *et al* (2014) which showed that the Petit-Bondoukou site is a sentinel site with a high presence of swollen shoot disease with a prevalence rate of 63.4%.The results of molecular analysis also showed that the *Phytophthora* species identified at the study site are *Phytophthora palmivora* and *other Phytophthora* species that were not the subject of this study. The species *Phytophthora megakarya* has not been identified at the site of Petit-Bondoukou. This result is in agreement with the work of Babacauh (1980) which showed that the only *Phytophthora* species responsible for black pod disease were *Phytophthora palmivora* and *Phytophthora citrophthora*. From the above, damage related to other species not identified in this study could be linked to *Phytophthora citrophthora*. Indeed, the absence of *Phytophthora megakarya* at the site of Petit-Bondoukou could be explained by the sampling period which took place towards the end of August. At this period, opportunistic pathogens take over the development of *Phytophthora palmivora* and *P. megakarya* (Kébé *et al.,* 2001; Koné, 1999). Statistical analysis has shown that the presence of CSSV is significantly related to the presence of black pod disease, i.e. the presence of one of these diseases favours the occurrence and/or development of the other. This result shows that both diseases are increasing strongly at the site of Petit-Bondoukou. This means that most of the test trees show both signs of swollen shoot and black pod disease.

Indeed, observations were made on early-infected trees still showing a high level of leaf cover. This high rate of leaf cover could be at the origin of a humid microclimate (Yao et al., 2014; Koua et al., 2018) which could favour the dispersal of the *Phytophthora* pathogen, as well as the development of scale insects that are vectors of swollen shoot disease. This is in line with the studies carried out by Bigger (1972) and Nguyen-ban (1984) which reveal that these climatic conditions favour the outbreak of mealybugs in cocoa trees. These arguments are supported by Brasier and Hansen (1992) who showed that rainfall favours the establishment and development of phycomycetes diseases such as black pod disease due to *Phytophthora sp*. As for mealy bugs of the family Pseudococcidae, the vector species of the swollen shoot virus, have a development cycle which varies according to climatic conditions including temperature, humidity and according to the host plant (Kenza, 2017). According to Goldasteh *et al* (2009) the optimal temperature for development of scales to adulthood is between 15° and 32°C. These development conditions correspond to the climatic parameters of the Soubré region where swollen shoot disease occurs.

IV. CONCLUSION

Swollen shoot and black pod diseases are two major cocoa diseases that cause significant losses in the region of Soubré. At the end of this study, it appears that the site of Petit-Bondoukou is a sentinel site with a high presence of swollen shoot disease. Molecular analysis showed that the only species identified on this site is *Phytophthora palmivora* although there is a strong propensity for other unidentified species responsible for black pod disease. This study also showed that there is a strong positive correlation between the prevalence of swollen shoot and the prevalence of Black pod disease. This shows that most of the test trees at the site of Petit-Bondoukou show both Swollen shoot and Black pod disease symptoms. To limit the spread of these two diseases, firstly, regular plot maintenance should be carried out by regular weeding and regular pruning of the plots to adjust the light and thus break the cycle of *Phytophthora sp*. Secondly, to limit the spread of Swollen shoot, growers should be made aware of the recognition of symptoms so that they can take action by destroying the first trees bearing the symptoms of the disease. This combined action could greatly contribute to reducing the pressure of both diseases.

V. CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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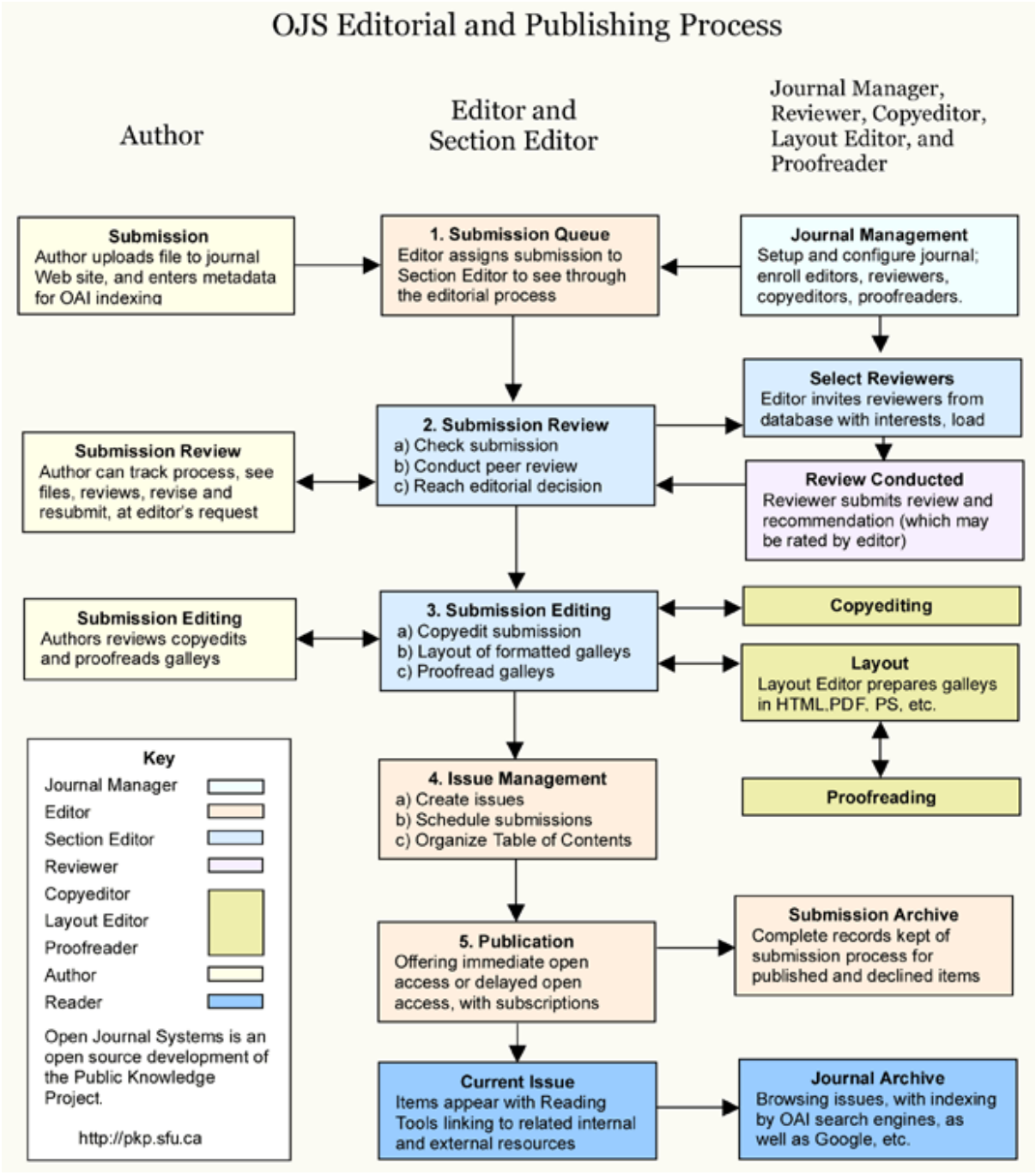

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