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FOREWORD

I am pleased to put into the hands of readers Volume-7; Issue-2: March-April 2022 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: May, 2022

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Analysis of the Ecstasy Rate of Cakalang Fish (Katsuwonus pelamis) in the Flores Sea of South Sulawesi

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Abstract— *The purpose of this study is to analyze the level of exploitation of cakalang fish in the Flores Sea of South Sulawesi. This study will be conducted in September - December 2021, keeping in mind the Covid-19 health protocol. The research method used is the survey method. The sampling method used is the method of taking the effort of the sample capture unit is done random sampling. The data analysis used is the estimation of biological parameters and maximum sustainable yield. The results showed that the condition of the maximum sustainable sustainable potential value in the Flores Sea has not been indicated to experience over fishing with an effort value of 2,215.11 units / year, a yield of 621,343.85 tons / year and Biomass of 306,574.77 tons / year*

Keywords— ***Level of Exploitation, Maximum Sustainable Yield.***

I. INTRODUCTION

The largest production of cakalang fish (Katsuwonus pelamis) in the Flores sea of South Sulawesi is one of them is in Bulukumba Regency and Selayar Islands Regency. Bulukumba regency is one of the most potential districts from the marine and fisheries aspect with an area of 1,154.67 km2 and a coastal length of about 128 km with a catch fishery production of 53,612.3 tons. Cakalang fish (Katsuwonus pelamis) in Bulukumba Regency is one of the marine fishery resources categorized as pelagic fish where the largest cakalang fish production recorded in 2018 amounted to 10,068.2 tons. As for the details of the production of cakalang fish (Katsuwonus pelamis) in Bulukumba Regency, namely in 2010 amounted to 2,528.3 tons, in 2011 amounted to 3,667.6 tons, In 2012 amounted to 5,400.2 tons, in 2013 amounted to 6,465.9 tons, in 2015 amounted to 4,114.5 tons, in 2016 amounted to 3,845.5 tons, in 2017 amounted to 3,995.7 tons, in 2018 amounted to 10,068.2 tons and in 2019 amounted to 3,247.8 tons. Similar to Bulukumba Regency, Selayar Islands Regency itself is estimated to have considerable cakalang fishery resource potential where cakalang fish production recorded in 2014 amounted to 2,010.8 tons.

Utilization of cakalang fish (Katsuwonus pelamis) with a dense intensity can cause several problems such as catches experiencing fluctuations every year, the time of catching is getting longer, the fishing area is getting farther and fishermen no longer choose the catch, for example cakalang fish that are still small in size. The basis in the management of fish resources is how to utilize resources so as to produce high economic benefits for business actors, but their sustainability is maintained.

Data on the utilization rate of a fish resource is very important, because it will determine whether the utilization of these resources is less than optimal, optimal, or excessive. Excessive utilization of fish resources will interfere with the level of sustainability. By knowing the level of utilization of fish resources is expected to be carried out planned and sustainable management. Existing problems need to be studied both in terms of exploitation of fishing activities using calculations of catch per unit effort (CPUE) utilization rate analysis, determination of Sustainable Potential Level, Optimum Effort, and

Utilization Rate of cakalang fish resources in the Flores Sea of South Sulawesi, where the information is needed in sustainable fisheries management in order to use cakalang fish (Katsuwonus pelamis) in the sea waters of Flores Sulawesi. South can run optimally (Budiasih and Dian, 2015)..

II. METHODOLOGICAL RESEARCH

A. Time and Location of Research

This study will be conducted in September - December 2021, keeping in mind the Covid-19 health protocol. The study was conducted in the waters of the Flores Sea. The areas that include flores sea waters include parts of Takalar Regency, Jeneponto Regency, Bantaeng Regency, Bulukumba Regency, Selayar Islands Regency, and part of Sinjai Regency which can be seen in appendix 1. In this study will use regional representation including Bulukumba Regency and Selayar Islands Regency as research locations. The selection of research locations (Regencies / Cities) was carried out purposively (intentionally) on the grounds that the selected regency could describe the waters of the Flores Sea as a whole where Bulukumba Regency was chosen because it was seen from the aspect of the highest cakalang fish production in the Flores Sea Waters of South Sulawesi as much as (2,528 - 10,068) tons in 2010 - 2018.

B. Research Methods

The research method used is the survey method. According to (Sugiyono, 2014: 7) Survey research is a study conducted on large and small populations, but the data studied is data from samples taken from that population, so that relative events, distribution, and relationships between sociological and psychological variables are found. In the survey method information is collected from respondents using questionnaires as research instruments. Questionnaire is a sheet that contains several questions with a standard structure. This research is both quantitative and analytical analytical. Quantitative is research that data from a sample of the study population is analyzed according to the statistical methods used. Quantative data is an assessment based on the amount of something. Analytical research is research that needs to be studied in several more detailed parts to understand the various relationships, properties and roles of these parts.

C. Sampling Methods

The method of sampling units is done random sampling is a sampling method by randomizing samples simply, in accordance with Prasetiyo (2005) which states that if the population is less than 100 then the sample is better taken all, but if the population is more than 100 then the sample can be taken between 10-15% of the population or depending on the ability of the researcher, the area and the size of the risk borne by researchers.

Respondents for the marketing aspect of cakalang fish using the Stratified random sampling method, is the process of sampling through the process of dividing populations into strata, selecting simple random samples from each stratum and combining them into a sample to estimate the parameters of the population. If the population is homogeneous, then the sample can be taken from any population, but if the population is heterogeneous, then the sample must represent from each heterogeneous part of that population so that the results of the study of the sample can be met with each member of the population. The process of dividing populations into stratum aims so that samples taken from each stratum can represent the characteristics of a large and heterogeneous population. Therefore, the stratum should be formed as well as possible with the characteristic manganesealysis of the population well (Demokrawati, 2014).

D. Data collection techniques

The data collection techniques used in this study are:

1. Interview, which is to conduct a systematic interview method that is an interview conducted by first preparing written guidelines about what to ask respondents.
2. Observation, which is to obtain the main data sourced from respondents in the form of fishermen's activities in the selected district.
3. Documentation, i.e. completes the analysis and strengthens the conclusions, all data and activities in the research are documented in the form of images.
4. Library Studies, which is to support the method of interview and observation that has been done. The collection of information needed in finding references related to the research conducted.
5. Questionnaire, which is a list of questions that must be answered or done by respondents

E. Data Source

The data sources used in this study are as follows:

1. Primary data, which is data obtained from interviews with respondents based on questionnaires that will be given later, for cakalang fishing unit business includes: investment and income. As for the marketing system includes: the identity of the respondent, the determination of selling and buying prices, the pattern of marketing channels, and marketing costs.
2. Secondary Data, which is data obtained from the District/City Marine and Fisheries Office that has been selected. Secondary data used in this study are: data on the number of cakalang fishing units and cakalang fish marketing agencies in Selayar and Bulukumba Islands Regencies.

F. Data Analysis

The data analysis used in this study is as follows:

To answer the first problem formulation about the exploitation of fish. Data used in the Surplus Production method in the form of catch and capture effort, then processing data through the Schaefer Model and Fox Model approach which is a regression analysis model from CPUE to the amount of effort

1. Catch per Unit Effort (CPUE)

After the production and effort data (input or effort) were arranged in a time sequence according to the type of fishing gear, the next step is to find the catch per unit effort (CPUE). According to Ghulland (1991), CPUE calculation aims to determine the abundance and utilization rate of fishery resources in a certain water area. The CPUE value can be denoted as follows :

$$\mathbf{CPUE_t = \frac{Catch_1}{Effort_1}}$$

$$\mathbf{t = 1,2,...n}$$

Where :

$CPUE_t$ = catch per catch effort in year t

$Catch_1$ = the catch in year t

2. Standardization of fishing gear

Fishing gear standardization aimed to uniform effort different units, so it can be assumed that the effort to catch a type of fishing gear is the same as that of standard fishing gear. Standard fishing gear is based on the amount of catch obtained and the value of the fishing power index (FPI) with the input (effort/effort) of the standardized tool.

$$\mathbf{E_{std} = Y_{tot} / CPUE_{std}}$$

Where :

E_{std} : Effort/ standard fishing effort

$CPUE_{std}$: CPUE standard fishing gear

Y_{tot} : CPUE which is made the standard

3. Estimation of Biological parameters

Biological parameters include water carrying capacity constants (K), natural growth constants (r), technological parameters (q). Meanwhile, economic parameters include the cost per fishing effort (c / p), the price of Indian Scad fish per unit, the catch (p), and the discount rate. There are several approaches in estimating biological parameters, but in this study, the CYP estimation model (Clark, Yoshimoto and Pooley) is used with the approach and development of the Fox (1970) and Schunate (1977) model formulas, systematically the equation is written as follows: Clark *et al.*, (1992)

$$\mathbf{Ln\ (U_{t+1}) = \frac{2r}{(2+r)} Ln(q,K) - \frac{(2-r)}{(2+r)} Ln(U_t) - \frac{q}{(2+r)}(E_t + E_{t+1})}$$

Where :

U_{t+1} = CPUE at time t+1

U_t = CPUE at time t

E_t = Effort at time t

E_{t+1} = Effort at time t+1

β_0 = regression result intercept coefficient

β_1 = coefficient X variable 1 regression results

β_2 = coefficient X variable 2 regression results

4. Dynamic Bioeconomic Analysis

The output of the bioeconomic model includes optimal stock (X *), optimal catch (Y *) and optimal fishing effort (E *) which are estimated using the equation, (Najamuddin, 2014):

$$\mathbf{X^* = K/4\left[c/qpK + 1 - \sigma/r) + \left\{(c/qpK + 1 - \sigma/r)^2 + 8c\sigma/qpKr\right\}^{1/2}\right]}$$

$$\mathbf{Y^* = rX^* (1 - X^*/K)}$$

$$\mathbf{E^* = Y^*/qX^*}$$

Where :

K : Environmental carrying capacity

c : Operating costs for catching the Cakalang Fish

p : the price of Cakalang Fish per kilogram

r : fish growth rate

q : catching power coefficient and fishing gear

σ : resource cut rate

The calculation of the Maximum Sustainable Yield (MSY) model uses the following equation:

$$\mathbf{E_{MSY} = \frac{r}{2q}}$$

$$\mathbf{Y_{MSY} = \frac{Kr}{4}}$$

$$\mathbf{X_{MSY} = \frac{K}{2}}$$

Where :

E_{MSY} : Efforts to catch MSY's condition

Y_{MSY} : The catch in MSY condition

X_{MSY} : Estimating optimal stock of MSY conditions

III. RESULTS AND DISCUSSION

A. Cakalang Fishing Efforts

Cakalang fishing efforts in the Flores Sea are carried out with the most dominant fishing gear, the Purse Seine. During the period 2011 - 2020 Cakalang fishing efforts in the waters of the Flores Sea can be seen in the following table.

Table 1. Development of Cakalang Fishing Efforts in the Flores Sea Based on Fishing Equipment For The Period 2011-2020

Tahun	Effort (Unit)				
	Rawai Tuna	Rawai Tetap	Pancing Tonda	Pancing Ulur	Pukat Cincin
2011	172	176	595	2.271	244
2012	175	153	635	2.331	168
2013	165	176	696	2.570	164
2014	162	160	729	2.570	171
2015	122	150	478	2.603	263
2016	140	145	478	2.894	452
2017	134	158	486	2.716	692
2018	117	196	666	3.037	207
2019	135	190	688	2.715	216
2020	137	197	700	2.383	213

Source: Secondary data after processing, 2022.

B. *Catch Per Unit Effort* (CPUE)

Catch per Unit Effort (CPUE) is a method used to determine the average amount of marine fishery production in years. Fishery production in an area experiencing an increase or decrease in production can be known from cpue result

Table 2. The magnitude or value of the catch per unit effort (CPUE) describes or reflects the level of productivity of the capture effort.

Tahun	Rawai Tuna	Rawai Tetap	Pancing Tonda	Pancing Ulur	Pukat Cincin
2011	5,156	0,455	0,738	0,208	12,778
2012	6,602	0,164	1,240	0,369	19,761
2013	0,480	0,146	0,935	0,619	31,43
2014	0,429	0,212	1,148	0,084	27,833
2015	15,606	0,109	1,432	0,303	3,084
2016	1,386	0,170	2,130	0,422	2,983
2017	2,012	0,118	0,496	0,539	11,16
2018	2,022	0,118	1,188	0,359	15,726
2019	1,813	0,151	1,064	0,079	12,395
2020	1,738	0,124	0,883	0,311	10,798

Source: Secondary data after processing, 2022.

C. Standardization of fishing gear

Cakalang fishing efforts in the Flores sea use the dominant fishing gear that is purse seine fishing gear. As for the reason for the selection of these fishing gear based on the real circumstances on the ground and input that this fishing gear is the dominant fishing gear for use to catch Cakalang fish in the area. Each unit of fishing gear has different abilities, both against the type and the number of species caught.

Standardization of fishing gear is needed to uniformize Cakalang fishing efforts consisting of various types of fishing gear. The determination of standardization of effort units in this study is the total effort per year of several fishing gear with time series data in 2011-2020 obtained from the Fisheries Office of Selayar Islands Regency and

Bulukumba Regency of South Sulawesi Province. The standard fishing gear used in this study is purse seine on the grounds that this fishing gear has a catch value per unit of effort greater than other fishing gear.

Table 3. Standardization of Cakalang Fishing Gear in the Flores Sea Waters of South Sulawesi in 2011-2020.

Tahun	Rawai Tuna	Rawai Tetap	Pancing Tonda	Pancing Ulur	Pukat Cincin
2011	0,404	0,036	0,058	0,016	1,000
2012	0,334	0,008	0,063	0,019	1,000
2013	0,015	0,005	0,030	0,003	1,000
2014	0,015	0,008	0,041	0,003	1,000
2015	0,524	0,035	0,464	0,098	1,000
2016	0,465	0,057	0,714	0,142	1,000
2017	0,180	0,011	0,044	0,048	1,000
2018	0,129	0,007	0,076	0,023	1,000
2019	0,146	0,012	0,086	0,006	1,000
2020	0,297	0,012	0,085	0,034	1,000

Source: Secondary data after processing, 2022.

Table 4. Standard Efforts of Cakalang Fish in the Waters of the Flores Sea

Tahun	Rawai Tuna	Rawai Tetap	Pancing Tonda	Pancing Ulur	Pukat Cincin
2011	69,405	6,265	34,37	36,929	244
2012	58,461	1,269	39,839	43,546	168
2013	2,519	0,815	20,695	50,622	164
2014	2,499	1,218	30,071	7,735	171
2015	617,392	5,279	221,953	255,928	263
2016	65,045	8,284	341,405	409,893	452
2017	24,162	1,666	21,604	131,226	692
2018	15,04	1,468	50,3	69,244	207
2019	19,748	2,31	59,043	17,24	216
2020	22,053	2,253	57,225	68,526	213

Source: Secondary data after processing, 2022

The following values from (C) that describe the level of productivity of the capture effort (effort) after strandarization of fishing gear can be seen in the following table.

Table 5. Productivity Level of Cakalang Fishing Efforts

Tahun	Produksi (Ton)	Total Effort Standard (Unit)	CPUE (Ton/Unit)
2011	4.024,80	390,97	10,29
2012	6.068,99	311,12	19,51
2013	7.720,80	195,35	39,52
2014	8.475,91	212,52	39,88
2015	4.787,70	810,11	5,91
2016	4.523,91	1.276,62	3,54

2017	4.304,11	870,66	4,94
2018	5.536,29	343,05	16,14
2019	3.896,11	314,34	12,39
2020	3.920,40	363,06	10,80

Source: Secondary data after processing, 2022

D. Estimated Biological Parameters of Cakalang Fish's Dynamic Bioeconomics Model in the Flores Sea

There are several estimation models that can be used to perform biological parameter estimation. but in this research. The estimation model used is an estimation model developed by Clark Yoshimoto and Pooley (1992) or better known as the CYP model. Biological parameters to be estimated include environmental carrying capacity (K). Catch (q) coefficient and fish growth rate (r).

The restoration of biological parameters with the CYP method requires logarithm input data from CPUE at time t + 1 and CPUE logarithm at time t as well as input data t Effort at the time of t and t + 1. To use OLS or regression, Ln CPUE t + 1 / CPUEt as Y, U_t + 1 as X1 and Et + 1 as X2 (Fauzi and Anna, 2010). In table 3 presented regression results from Cakalang Fish using the CYP estimation model.

Table 6. Cakalang Fish Regression Results with CYP Analysis Model

No	Estimasi	Parameter Regresi Ikan Cakalang		
		β_0	β_1	β_2
1	Coefficients	4,05346	0,00723	9,15E-04
2	Standard Error	0,11906	0,00065	1,24E-04
3	t Stat	34,0447	11,1521	7,36984
4	F	318,251		
5	R Square	0,99066		

Source: Secondary data after processing, 2022

The Ordinary Least Squares (OLS) model of Table 6 for Cakalang Fish is as follows Y = y = 34E-05x + 4.0534 R2 0.99.

From the data contained in table 6, the magnitude of the value of R2 of Cakalang Fish is 0.99, this indicates that independent variables in the equation have a strong influence and interrelationship to dependent variables.

The estimation results of the three parameters presented in table 18 are useful for determining sustainable production levels such as maximum sustainable yield (MSY). These values can be seen as follows:

Table 7. Estimated Results of Cakalang Fish Biological Parameters

No	Parameter Biologi	Hasil Estimasi	Satuan
1	Konsanta Laju Pertumbuhan alami ikan (r)	4,05346	ton per tahun
2	Koefisien penangkapan (q)	0,000915	ton per unit
3	Konstanta daya dukung Perairan (K)	613.149,54	ton per tahun

Source: Secondary data after processing, 2022

Biological parameters are one of the factors that greatly affect the survival of Cakalang fish. Because if one of the variables of biological parameters such as environmental carrying capacity is not in accordance with the needs then this will have an impact on the growth rate of Cakalang fish.

E. Sustainable Potential (Maximum Sustainable Yield) of Cakalang Fish

The key assumption of the sustainable potential model or sustainable harvest in maximum sustainable yield (MSY) is that the cakalang population grows and replaces itself, in the sense that the cakalang fish population is a renewable resource. The concept of sustainable catchment or Maximum Sustainability Yield (MSY), aims to maintain population size at the maximum point where the growth rate by harvesting would normally be added to the population,

and allow that population to be productive forever (Hertini et al., 2013). To determine sustainable catchment or sustainable potential, a maximum sustainable yield management regime is used. The management regime model can be determined using analytical solving tools through excell programs. The results of the bioeconomics optimization analysis of each Cakalang Fish management regime in this study are succinctly presented in the following table.

Table 8. Results of Bioeconomics Optimization Analysis of Cakalang Fish Regression Utilization

Model	Effort (Unit)	Yield (Y) (Ton)	Biomass (X) (Ton)
MSY	2.215,11	621.343,85	306.574,77

Source: Secondary data after processing, 2022

IV. CONCLUSION

The level of ecstasy of cakalang fish in the waters of the Flores Sea is seen from the maximum sustainable yield has not been indicated to experience over fishing with an effort value of 35,506.20 Units / year, the yield of 30,161.10 tons / year and the actual production of the sustainable potential of kite fish in the bone bay water of 16,142.04 tons / Year, the fishing efforts carried out have not been optimal and the number of catches produced from 2011-2020 has not reached the maximum number (< MSY).

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Purification of Grey water using the natural method

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Abstract**—** *The Water crisis is a major problem now a day. To solve this problem, there are various methods of water conservation such as rainwater harvesting or water reuse. Grey water treatment is also an option for water conservation. Grey water is the untreated household wastewater that has not come into contact with sewage (WHO -ROEM2006). In this study we have used various flocculating agents like alum, PAC, lime, chitosan, alum+ lime, fuller earth, ferric chloride, ferrous sulfate, PAM, Micro+, soya bean, alum+ soya bean etc. and the one thatgave the most significant results were used for further study. Among the flocculating agents used, alum + soybean powder gave promising results. So this flocculating agent and coagulating aid were used for further experiment. We prepared biochar using groundnut husks which is a waste material and activated it using zinc chloride. We prepared a unit which consisted of a column packed with sand, gravel, activated biochar and vetiver roots. We passed the supernatant obtained after flocculation through this unit at flow rate 5L/hr. The effluent water was disinfected using Medichlor. Using this unit the turbidity was decreased to 0.08 NTU, pH was 6.3, TSS was nil, and TDS reduced upto 75%. The microbial load (bacteria, fungi, coliforms, Thermotolerents), MPN test was also found to be negative. The unit which we have prepared was ecofriendly and economically affordable. This treatment system can be used in the new constructions, bungalows, societies etc. The treated water can be used for various purposes such as gardening, car washing, toilet flushing, road construction, irrigation etc.*

Keywords**— Gray water, flocculation, activated biochar, treatment unit.**

HIGHLIGHTS

- Grey water is the untreated household wastewater that has not come into contact with sewage.
- Microbial contamination of gray water comprises potential risk to health. Grey water also contains some chemical substances which may pollute the natural resources, So grey water needs to be treated.
- During this study, alum + Soybean powder showed best results as coagulation or flocculating agent and coagulation aid.
- Biochar was made using biological or organic material and was activated using $ZnCl_2$
- Water passed through our natural treatment unit followed USFDA norms.
- The treatment unit was made of natural material, hence the unit was ecofriendly and economically affordable.

I. INTRODUCTION

Grey water is spelt and defined differently in different parts of the world. It is the household wastewater that has not come into contact with sewage (WHO ROAM 2006). With an anticipated increase in world population by 2-3 billion people over the next decades (WHO 2010), the water demand is increasing two-fold (64 billion cubic metersper year). Urbanization is growing by 1.5% per year

globally (WHO 2010), and is estimated that by 2050 the percentage of the total population that will live in urban centers is going to increase further. Familiar sources of household gray water include water from showers, baths, sink, water generated from cloth washing, utensil washing, floor washing, hand washing, kitchen washing etc. Wastewater from kitchen sinks and automatic dishwasher have a high concentration of organic matter that encourages the growth of bacteria.This water is sometimes referred to as dark grey water. Grey water can be reclaimed by three main mechanisms; physical treatment, chemical process and biological treatment. Physical treatment is effective in improving the aesthetic quality of the effluent but can be fouled by pollutants and is energy demanding. The organic treatments reduce to some extent all affective components of the gray water but they are costly (Jefferson et al. 2000) variety of gray water treatment units remove microbial load, salts, pollutants etc. but the degree of treatment varies widely.

Grey water can be used for various purposes that don't require potable water such as landscaping, agricultural use, gardening, car washing, toilet flushing, road construction etc. It will reduce the demand of fresh water.(A.Gross. et al. 2007) Grey water may contain contaminants that are present in raw sewage or wastewater but in very low concentration. It contains coliforms, fungus, nutrients like nitrogen and phosphorus, detergents, surfactants and some amounts of oil or grease. Microbial contamination of grey water comprises potential risk to health. Grey water also contains detergents or surfactants, which will affect soil quality. So there is a need to treat the grey water and reuse it for various purposes in the regions where the population is high and scarcity of water. (Eriksson et Al. 2016)

Gray water treatment using natural treatment methods have not been fully explored . Hence we decided to identify natural ways that may be used to treat gray water. our present study aimed to reduce the pollutants by natural treatment system on a laboratory scale. This treatment system will be eco-friendly and economically affordable.

II. MATERIALS AND METHODS

Survey - we carried surveyed to get information regarding water usage, the quantity of wastewater or gray water generated, sources etc. For this purpose, we selected five homes. We prepared a questionnaire and gave it to the owners of the selected houses. Based on the information collected, we procured the gray water. (Pangarkar*etal*. 2010)

Collection - we received the gray water sample from houses. The sources of gray water were wastewater from cloth washing, utensil washing, floor washing, kitchen washing, bathing kitchen sink, hand washing etc. Approximately 5 L Of gray water used for the experiment. (Pangarkar*et al*. 2010)

Characterization of gray water – First, we collected the gray water and characterized it using following physicochemical and biological parameters. (Joonkyu Kim *et al.* 2009)

pH, Turbidity, Total suspended solids, Total dissolved solids, Total solids, Chemical oxygen demand (COD), Biological Oxygen Demand (BOD), Surfactants, oil and grease, Nitrates, Sulphates, Total Viable Count, Most Probable Number (MPN), Coliform count, Thermotolerant organisms.

Coagulation and flocculation - To reduce the load on the treatment system, the preliminary study on the gray water was coagulation and flocculation. For this purpose, we have used various coagulation agents and one amongst them which gave the best result was used for further experiment.

Jar test method - we used the jar test method for the process of coagulation and flocculation. The process was as follows,take 1000 ml of gray water in a jar. Add flocculating agent in the gray water. 3 minutes slow stirring and 9 minutes fast stirring. Allow to settle down the floccs for 30 minutes. Take the supernatant for further experiment.

Characterization of Supernatant - The supernatant collected was analyzed for the physico-chemical biological parameters using following testspH, Turbidity, TDS, TSS, Total viable count, MPN.

Preparation of Biochar

Biochar - charcoal prepared from biological waste materials.

To prepare the biochar we used organic or biological material. We collected groundnut shells. Washed them with water, and then dried them in the sunlight. Then again kept washed anddried shells in the hot air oven to remove moisture if any. The fully dried shells were ground using mortar and pestle. The powder was then placed in Muffle furnace for the charcoal preparation. (M.F.Olmenarejo*et. al*. 2006)

Activation of biochar

The prepared biochar was activated using zinc chloride ($ZnCl_2$) . Following procedure was followed for activation ofbiochar. Wash the prepared biochar with distilled water. Dry it in the hot air oven Prepare a solution of 33 ml distilled water and 2.1 gm of $ZnCl_2$. Take 30 gmofbiochar, add the prepared $ZnCl_2$ solution to it. Boil this mixture on water bath at100°C. Keep it in hot air oven for 20-24

hours. Wash the activated biochar with distilled water, again dry it in the hot air oven to remove moisture. The activated biochar was ready for the use.

Unit preparation

We prepared an unit of 45 cm. It considered of sand layer of 7.5 cm which was present at the bottom, next to it gravels layer of 5cm was present, on the gravels charcoal + activated biochar layer of 5cmwas present, next to it sand + coal layer of 7.5 6cm was present and the upper layer was of vetiver and is about 5 - 10 cm in length. The supernatant collected from flocculation treatment was passed through this improved unit. The effluent from this improved unit subjected to the physico-chemical and biological analysis. (Parjane Saroj and *et. al*. 2011 ;Tan I. A. W. And*et al*.2008).

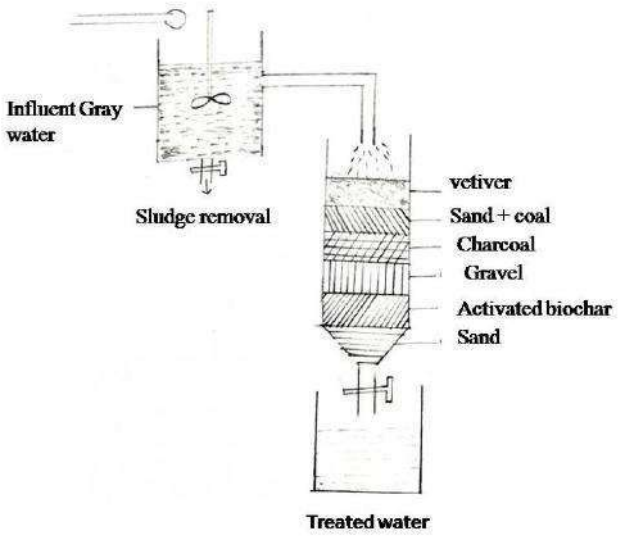

Fig. 1 Schematic representation of Gray water treatment unit

III. RESULTS AND DISCUSSION

To know the water consumption per house per day we did statistical analysis using the data obtained from the questionnaire. We also got the information regarding the use of water per day for various household purposes and generation of waste water. Average 755 L water was used by per family per day.

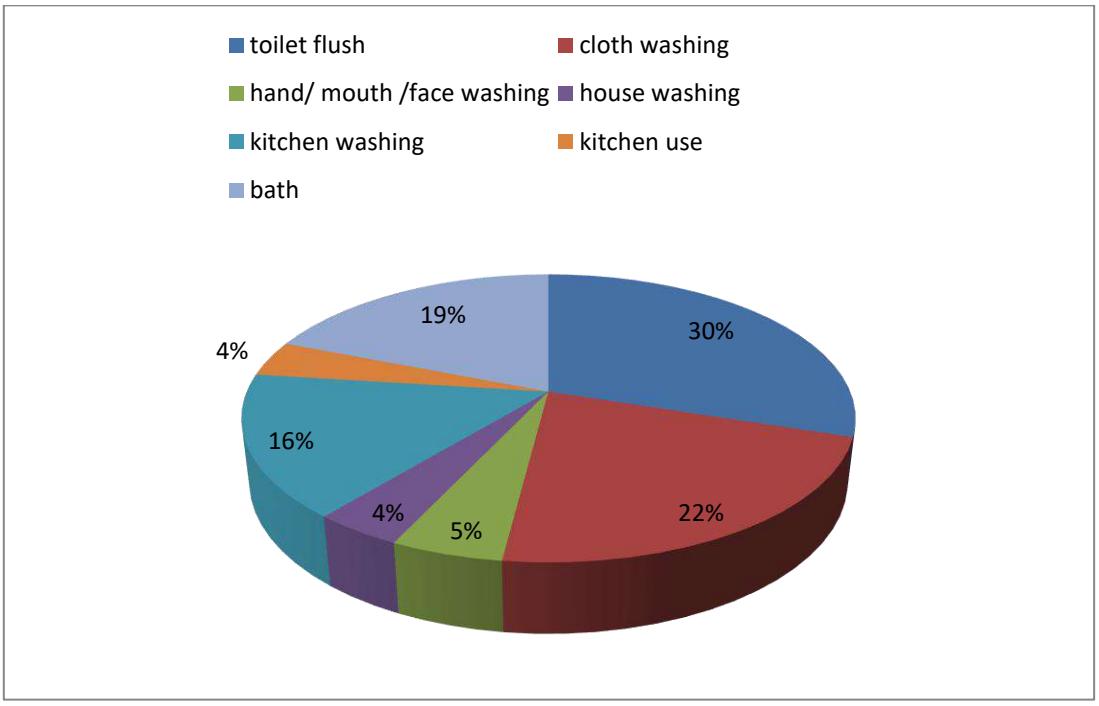

Fig. 2 Percent of water used by per family per day

Color of gray water was grayish black, brown, grayish white. After collection of gray water physico-chemical and biological parameters were analyzed. Physico-chemical and biological characterization of parameters is shown in table.

Table- 1 Physico-chemical and biological characteristics of sampled gray water

Parameters	Observed values
pH	6.9
Turbidity	400-1241 (NTU)
Oil content	370mg/L
Total Dissolved Solids (T.D.S.)	827.5-5700mg/L
Total Suspended Solid (T.S.S.)	282.5-1300mg/L
C.O.D.	2244mg/L
B.O.D.	1100mg/L
Nitrates	3.07mg/L
Sulphates	21.79mg/L
Detergents	8.93mg/L
Most Probable Number(MPN)	16
Thermotolerants	-
E.coli	351×104
Total Viable Count 1)Bacterial 2)Fungal	 96×10^5CFU/mL 32×10^5CFU/mL

- Physico-chemical and biological parameters were very high and pose the pollution threat to the receiving water bodies or natural streams that will receive gray water.
- It indicates that treatment of gray water is essential.

Flocculating agents

Different flocculating agents were used for removal of turbidity. Use of different flocculating agents, their efficiency with respect to turbidity removal, effect on pH, TSS, TDS was analyzed. The flocculating agent which gave promising results was used for further experiment.

During past years research has been carried out natural coagulant for the turbidity removal. To explore the use of soybean powder along with the alum we used different combinations of the two. It is economically affordable and eco-friendly agro-based product used for turbidity removal. It also reduced the amount of alum required.

- 1% alum and 1% soybean powder solution were used.

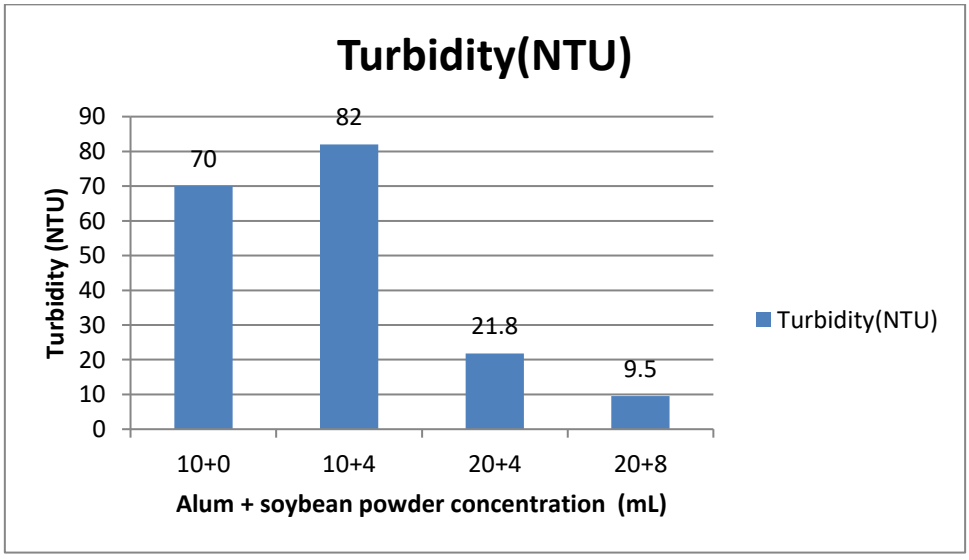

Fig. 3 Turbidity removal by Alum + Soybean powder

Table 2 characterization of gray water after 1st treatment (flocculation and coagulation)

	Turbidity (NTU) **For 500mL of gray water sample**		**pH** **For 500mL of gray water sample**	
Alum + soybean	Initial	After 1st treatment	Initial	After 1st treatment
10 + 0	435	23.7	8.3	5.4
10 + 2	435	22.5	8.3	6.2
10 + 4	435	17.8	8.3	6.9
	Turbidity (NTU) For 1000mL of gray water sample		pH For 1000mL of gray water sample	
Alum + soybean	Initial	After 1st treatment	Initial	After 1st treatment
10 + 0	550	70	7.5	5.4
10 + 4	550	82	7.5	6.2
10 +4	550	21.8	7.5	6.0
20 + 8	550	9.5	7.5	6.3
	T.S.S. (mg/L) 30 mL sample after 1st treatment is used		T.D.S. (mg/L) 30mL sample after 1st treatment	
Alum + soybean	Initial	After 1st treatment	Initial	After 1st treatment
	366.6	33.33	2733.3	333.3
	MPN		Total viable count	
Alum + soybean			Bacterial	Fungal
	-		25×10^6CFU/mL	-

Table -3 Characterization of effluent from unit prepared

Sample	**pH**	**Turbidity (NTU)**	**T.S.S. (mg/L)**	**T.D.S. (mg/L)**	**MPN**	**E.coli**	**Total viable count**
Initial	8	450	433.3	666.6	16	35×10^4CFU/mL	Bacterial Fungal
After 1st treatment	6.3	9.5	33.33	366.6	-	-	25×10^6 – CFU/mL
unit	6.3	0.082	-	166.6	-	-	- -

Table 4- Performance of gray water treatment unit

Parameters	Initial Greywater	Treated Effluent	% of removal Efficiency	Standard of USFDA
pH	6.9	7.2	-	6.5-8.5
Turbidity(NTU)	400-1241	0.08-2	99.82-99.98	5
Color	Grayish	Colorless	-	Colorless
Odour	Soapy, phenolic	Unobjectionable	-	Unobjectionable
Oil content	370	-	100	0.01
TSS(mg/L)	282.5	-	100	*

TDS(mg/L)	827.5	166	80	500
COD(mg/L)	2244	134	94	*
BOD(mg/L)	1100	38	96	*
Nitrates	3.07	3.01	2	45
Sulphates	21.79	141	-	200
Detergents	8.93	-	100	NIL
MPN	<16	-	100	NIL
E.coli	341×10^4	-	100	NIL
Total Viable Count (CFU/ml)				
i. Bacterial	96×10^5	-	100	NIL
ii. Fungal	32×10^5	-	100	NIL

* In case of drinking water standards USFDA, BIS do not define the limits.

The results presented in this study establish the potential applicability of the development of treatment unit. During this research we found soybean powder which acts as an amazing coagulant aid. Naturally and easily available low cost materials were used like sand and gravel, coal etc. for the treatment processes. The ground nut husks are the biological waste material which was used for activated charcoal preparation. It acts as an adsorbent. Similarly Vetiver roots are also easily available. They can act as an adsorbent and remove heavy metals from gray water. In economy of the unit which is an important part of the operation cost. Only forcoagulation or flocculation process stirring is required which consumes electricity. As per Indian Standards, the treated water can be used for landscaping, gardening, toilet flushing, floor washing, car washing and irrigation.

We compared our treatment unit with previously reported treatment units. Physical treatment unit removed T.S.S. (98%), BOD (100%), C.O.D(81%), Coliform (99%). (Gross *et al.*2007). Biological treatment unit removed C.O.D(89%), T.S.S.(95%) (Gros*et al*. 2007). Physical and chemical unit removed Turbidity (18%), C.O.D (25%) (Gual*et al*.2007). Physical and chemical method removed Turbidity (98%), C.O.D (99%) (Kim *et.al*.2007). And our treatment unit removes Turbidity (99.98-99.99%), C.O.D (64%), BOD (96%), T.D.S (80%).

Various benefits of gray water –

1. Reduced use of fresh water
2. Less strain on septic tanks.
3. More effective purification.
4. Reduced use of energy and chemicals.
5. Ground water recharge.
6. Saving consumption of water per day.
7. Saving of drinking water by reuse of gray water.

IV. CONCLUSION

The present study demonstrate the reuse and treatment of gray water efficiently. Based on the findings of the present study, this unit can be considered as a viable alternative to conventional treatment plants. The unit which we have prepared was eco-friendly and economically affordable.

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Extension Methods of Pekarangan Pangan Lestari (P2L) Program Before and During the Covid-19 Pandemic at Parepare City

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Abstract— *Agriculture is a sector that is very important to pay attention to its development because one of the basic needs, namely food, is the main activity of this sector. Therefore, the government formed an activity, namely the Pekarangan Pangan Lestari (P2L) to support the agricultural sector in meeting food availability, especially in urban areas that are vulnerable to food insecurity. The aims of this study were 1) To find out the extension method for the Pekarangan Pangan Lestari (P2L) program in Parepare before and during the Covid-19 pandemic, 2) To analyze the differences in the extension method to the Pekarangan Pangan Lestari (P2L) program in Parepare before and during the Covid-19 pandemic, 3) Analyzing the effect of the Covid-19 pandemic on the extension method to the Pekarangan Pangan Lestari (P2L) program in the City of Parepare. This research was conducted at the Dinas Ketahanan Pangan of Parepare City and used descriptive and quantitative methods. The results showed that there were significant differences between the extension methods of the Pekarangan Pangan Lestari (P2L) program before and during the Covid-19 pandemic. The change in the extension method was influenced by several factors, namely government policies, the quantity of meetings and the extension materials provided. The results showed that there were significant differences between the extension methods of the Pekarangan Pangan Lestari (P2L) program before and during the Covid-19 pandemic. The change in the extension method was influenced by several factors, namely government policies, the quantity of meetings and the extension materials.*

Keywords— ***Pekarangan Pangan Lestari (P2L), Extension Method, Extension, Covid-19***

I. INTRODUCTION

1.1. Background

Agriculture and food are two things that go hand in hand and have a very important role in human life, namely meeting the national food supply. This is because food is produced through the process of cultivating food crops which is one of the activities of agriculture. The agricultural sector is also a source of employment for most of the population. A problem researcher from the Food and Agriculture Organization (FAO), stated that no country can rise from the poverty line without the help and support of a productive agricultural sector. This means that agriculture is one sector that needs more attention. because not only as a supporter of increasing the national economy but also has a mission to provide food for the entire population, especially in some areas where the land is still not sufficient to produce sufficient food for the population living in urban areas. The development of the agricultural sector is carried out by carrying out agricultural development so that it is better in supporting the national economy and following the development of an increasingly fast era(Yodfiatfinda, 2018).

Agricultural development is inseparable from the efforts to develop human resources involved in it, especially farmers as the main actors in agricultural activities. The decline in agricultural development activities was caused by differences in perceptions between two different parties about the role of agriculture and the low priority and budget allocation for agricultural

development, namely between the regions and the center as well as between the executive and local legislatures. In addition, the problem faced is the limited availability of information about agriculture, the capacity and managerial ability of extension workers who are still lacking and rarely visit the farmers and group(Nurdyawati, 2020).

Agricultural extension is an informal education aimed at farmers with the aim of gaining and improving skills, knowledge and skills through a learning process facilitated by extension workers. Extension activities are expected to improve the development of farmers and farmer groups both in terms of quality and quantity and have good relations with all relevant agencies and production results can increase so as to have an impact on increasing economic income for farmers(Halimah & Subari, 2020).Agricultural instructors have several methods or extension programs that are applied to farmers or farmer groups. Agricultural extension methods have been regulated and discussed in the Regulation of the Minister of Agriculture of the Republic of Indonesia Number 03 of 2018 Article 1 Paragraph 8concerning Guidelines for theImplementation of Agricultural Extension, it is stated that the agricultural extension method is a method used by agricultural extension workers to provide agricultural extension materials to farmers or farmer groups. in order to foster curiosity, willingness, and be able to help and regulate themselves in getting the information they need to improve their farming activities such as markets, technology, and other resources to improve their ability to carry out production activities, business efficiency, farmers' income which is accompanied by an increase in the welfare of farmers as well as increasing their awareness in preserving environmental functions. Therefore, to support the agricultural sector, in 2010, the Badan Ketahanan Pangancreated a program namely the Kawasan Rumah Pangan Lestari (KRPL). This activity will continue until 2019 then in 2020 the name of this activity will be changed to Pekarangan Pangan Lestari (P2L) with the aim of expanding the number of beneficiaries and land use.

Pekarangan Pangan Lestari (P2L) are activities carried out by the community in groups by utilizing previously unproductive land such as yards, vacant land and unused land as a source of food in a sustainable manner, namely by producing food to meet household food and nutritional needs and has a selling value to increase household income. This is done with the aim that they are able to increase the availability of food, the ease of obtaining food, as well as the processing and utilization of food to increase their household income. The Pekarangan Pangan Lestari (P2L) activity is held with the aim of supporting the work program that has been carried out by the government for handling stunting-prone areas and handling areas that if not paid attention to will experience food insecurity conditions. This Pekarangan Pangan Lestari (P2L) really helps the community in meeting their respective food needs, especially during the Covid-19 pandemic as it is today.(Badan Ketahanan Pangan Kementerian Pertanian, 2020).

The Covid-19 case has an impact on all sectors, not only the economic sector, the agricultural sector is also affected by this pandemic. The agricultural sector is a priority need in dealing with the spread of Covid-19 in Indonesia. The agricultural sector must be strong in dealing with the Covid-19 pandemic because it is directly related to the basic needs of mankind. If agriculture is not strong enough to face the pandemic, this will have an impact on other sectors and getting worse.

Strengthening the agricultural sector starts from human resources who are at the forefront or can be called the frontline of agriculture, namely farmers. A farmer group is one of the organizations formed with the aim of making it easier for farmers to solve their problems so that production can be carried out optimally and efficiently because of agricultural activities carried out in farmer groups, procurement of production facilities and sale of produce that can be done together(Jamil et al., 2020).In empowering farmers during the Covid-19 pandemic, agricultural extension workers are needed so that they can continue to run their business as usual(Hartati, 2020). In addition to the economy, the current pandemic also has several impacts on extension workers in carrying out their main tasks. These impacts can hinder and become a challenge for extension workers at this time to adjust to the conditions of the Covid-19 pandemic. Not only extension workers, but farmer groups or farmers are alsoaffected by the conditions during the Covid-19 pandemic.

Farmer groups participating in the Pekarangan Pangan Lestari (P2L) program also have various problems that have arisen due to the Covid-19 pandemic. One of them is the implementation of social distancing during the Covid-19 pandemic which hampers the Pekarangan Pangan Lestari (P2L) program. During the Covid-19 pandemic, there were several farmer groups ofPekarangan Pangan Lestari (P2L) program whose activities were stopped. In fact, the Pekarangan Pangan Lestari (P2L) program can be a solution for the availability of food during the Covid-19 pandemic. This problem is one that is commonly faced by agricultural extension workers in carrying out agricultural extension activities. They must cultivate and maintain the interest and desire of farmer groups from the Pekarangan Pangan Lestari (P2L)

program to continue to carry out their activities so that they can feel the benefit greatly from this program. Extension workers want to have several ways or strategies and methods to help farmer groups in dealing with their problems during the current Covid-19 pandemic. Based on the description above, the author wishes to conduct a research with the title "**Extension Methods of Pekarangan Pangan Lestari Program (P2L) Before and During the Covid-19 Pandemic at Parepare City**".

1.2. Formulation of the problem

Based on the description that has been put forward in the background, the formulation of the problem is as follows:

1. How was the extension method for the Pekarangan Pangan Lestari (P2L) program in the City of Parepare before and during the Covid-19 pandemic?
2. Are there differences in the extension methods applied to the Pekarangan Pangan Lestari (P2L) program in the City of Parepare before and during the Covid-19 pandemic?
3. Does the Covid-19 pandemic condition affect the extension method applied to the Pekarangan Pangan Lestari (P2L) program in Parepare City?

1.3. Research Gape (Novelty)

There are several previous studies that discuss extension methods such as research conducted by Andi Nur Imran, Muhanniah and BibianaRiniWidiatiGiono(Imran et al., 2019)in Maroswith the title "Agricultural Extension Methods in Improving Farmers' Knowledge and Skills (Case Study in Maros Baru District, Maros Regency" and "Approaching Agricultural Extension Methods for Chili Farming in Tiwoho Village, Wori District, North Minahasa Regency" by Patrix Juriko Tumurang, Melsje Yellie Memah and Melissa Lady Gisela Tarore(Tumurang et al., 2019). Both of these studies used a qualitative descriptive method with a focus on research on the opinions and views of farmers about the extension methods that have been applied by agricultural extension workers, then from the assessment of the farmers, they found the best, efficient and effective method according to them.

Research on the extension method of the Pekarangan Pangan Lestari (P2L) program is still very limited and it can be said that it does not yet exist. Therefore, research like this is urgently needed to improve the performance of the agricultural sector, especially in urban areas which are areas of agricultural land crisis. The current state of the Covid-19 pandemic has greatly affected the agricultural sector, therefore food security and the agricultural sector must be able to survive. The increase in the agricultural sector was carried out by farmer groups as the main actors and assisted by agricultural extension workers. Extension methods are very influential on the success of an extension worker in providing information to farmer groups. If the extension method used is not appropriate, then the information provided will also not be well received by the farmer group.

1.4. Research purposes

Based on the formulation of the problem that has been stated, the research objectives are as follows:

1. To find out the extension method for the Pekarangan Pangan Lestari (P2L) program in the City of Parepare before and during the Covid-19 pandemic
2. To analyze the differences in the extension methods applied to the Pekarangan Pangan Lestari (P2L) program in the City of Parepare before and during the Covid-19 pandemic.
3. To analyze the effect of the Covid-19 pandemic on the extension method applied to the Pekarangan Pangan Lestari (P2L) program in the City of Parepare

1.5. Research Use

The benefits that can be obtained from this research are as follows:

1. For the government, this research can be used as consideration for the authorities to make decisions in developing the Pekarangan Pangan Lestari (P2L) program in the future.
2. For Pekarangan Pangan Lestari farmer groups, this research can be used as learning material in developing their farming.

1.6. Framework

The agricultural extension method is a method used by agricultural extension workers to convey an extension material to farmers and their families either directly or indirectly, so that they are able to improve their abilities and apply innovations (new technologies). The Pekarangan Pangan Lestari (P2L) activity is one of the government's efforts and efforts, especially the ministry of agriculture, to increase the availability of food, the ease of obtaining food, and the use of food for households in accordance with the needs of diverse, nutritionally balanced, and safe food that has value. good selling to increase the income of a household.

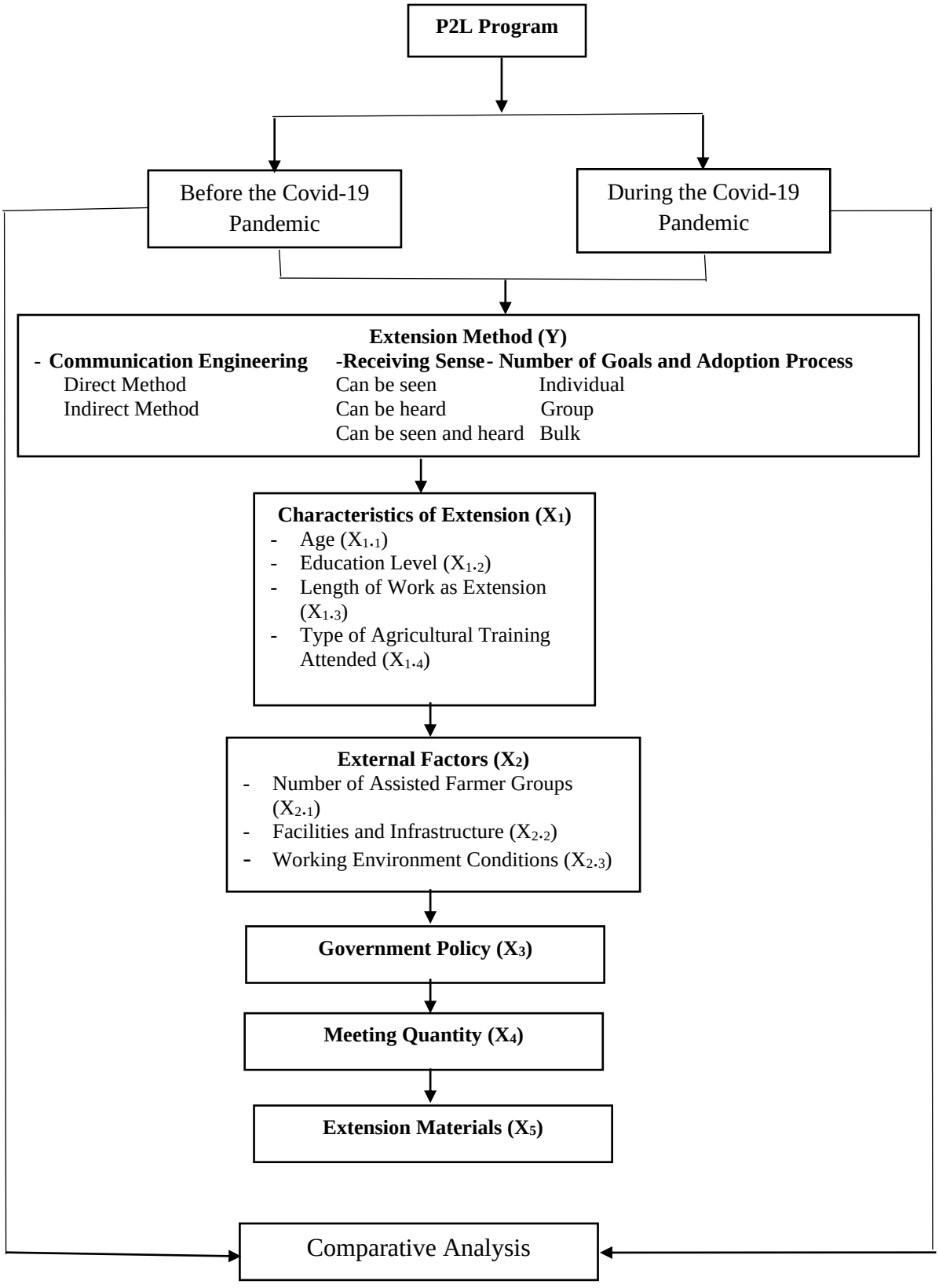

Fig.1. Research Framework

The current Covid-19 pandemic is very influential for several people, including agricultural extension workers. They are required to improve the quality of agriculture through outreach activities during the Covid-19 pandemic like this. Starting from utilizing technology and social media platforms to facilitate their extension activities, adjusting extension methods, and adjusting extension materials for farmers. This is in accordance with the opinion(Calvert, 2020)which states that extension workers should be motivated by every situation they are

facing to be able to lead, adapt, innovate, and find solutions to the problems and challenges they face today and in the future that they will face.

One of the agricultural programs that requires counseling is the Pekarangan Pangan Lestari (P2L) program which has the aim of increasing household income by utilizing yards that were not previously used and used as agricultural land to be planted with various market-oriented crops. This program extension has its own challenges in carrying out its role. At this time, extension workers want to adapt so that they are able to carry out their role and maintain health to avoid the Covid-19 virus. One of them is by adjusting the method of counseling that is carried out. This is in accordance with the opinion(Wibowo & Haryanto, 2020)who stated that during the current Covid-19 pandemic, the important role of agricultural extension workers is expected to be able to make agriculture run well so that it can help the availability of food and ensure the availability of basic materials for 267 million Indonesian people.

There are several factors that can influence the extension method applied to a farmer group, namely the characteristics of the extension worker, starting from age, gender, education level, length of work as an extension worker, and the type of agricultural training he has attended. In addition, the characteristics of the target also affect the extension method in this case the farmer group.

Farmer groups cannot be separated from the head of the farmer group who acts as a leader who directs, fosters, assigns and guides its members to achieve the goals of the farmer group. The role of the leader is not only to direct but also to be an example for its members.In addition to characteristics, external factors such as the number of assisted farmer groups, facilities and infrastructure as well as the work environment also can affect the extension methods used to farmers.

Government policies can also have an influence on extension methods during the Covid-19 pandemic. Especially regarding the health protocols that make us unable to move freely due to the Covid-19 pandemic. As a result, the material and quantity of meetings can also affect the extension method that applied.

II. RESEARCH METHODS

2.1. Research Location and Time

This research was conducted at the DinasKetahananPangan of Parepare City Office. The determination of the research location was carried out intentionally with the consideration that the Pekarangan Pangan Lestari (P2L) in Parepare City has many types of plants and groups that are active in carrying out this program and the Pekarangan Pangan Lestari (P2L) program has received good attention from the government and this program has running for ± 4 years at the study site. During the Covid-19 pandemic, several farmer groups stopped their activities due to several problems. This program instructor at the research location had several challenges, especially during the Covid-19 pandemic with the limitations and experience of farmer groups that ran a few months before the emergence of Covid-19.

2.2. Research methods

The type of research used is descriptive quantitative. As explained in the book(Mukhtar, 2013), that quantitative research is a systematic scientific research on an event or phenomenon that is currently happening. The purpose of quantitative research is to develop and use mathematical models, theories or hypothesis related to or in accordance

$$Y = \beta_0 + \beta_1X_1 + \beta_2X_2 + \beta_3X_3 + \beta_4X_4 + \beta_5X_5 + e$$

with events that occur in the field(Hardani. Ustiawaty, 2017).

2.3. Analysis Method

2.3.1. T test

The T-test formula is used to answer the researcher's goals, namely the first objective which discusses the differences in counseling methods before and during the Covid-19 pandemic. The T-test formula used in this study is the Paired T-test formula. The following formula is used:

$$t = \frac{\overline{D}}{SD/\sqrt{N}} \qquad \overline{D} = \frac{\sum_{i=1}^{n} d_i}{n}$$

Information :

t = value t count
$\overline{D}$= Average difference in measurement 1 and 2
SD = Standard deviation of the difference in measurement of 1 and 2
N = Number of samples
To get the value of the deviation and standard deviation used the formula:

$$SD = \frac{\sqrt{\sum_{i=1}^{n}(d_i - d)^2}}{n-1}$$

Information :
di = difference of each pair
d = Mean

2.3.2. Multiple linear regression

Multiple linear regression was used to answer the second objective of this study, namely to examine the effect of the independent or independent variables on the dependent variable. The equation of the form of multiple linear regression that canbe formulated as:

Information :

Y = Extension method

β_0 =Regression intercept coefficient

$\beta_1, \beta_2, \beta_3, \beta_4, \beta_4$= Regression coefficient

X_1 = Characteristics of the instructor

X_2 = External factor

X_3 = Government Policy

X_4 = Number of encounters

X_5 = Extension material

e = Error term (interference factor) or residue.

2.6. Hypothesis

Based on the formulation of the problem, the purpose of the research, the frame of mind in the description above, the hypothesis of this study is:

1. H_0: There is no changes in extension methods made to the Pekarangan Pangan Lestari (P2L) program group during the Covid-19 pandemic
2. H_1: There was a change in the extension method made to the Pekarangan Pangan Lestari (P2L) program group during the Covid-19 pandemic

III. RESULTS AND DISCUSSION

3.1. Pekarangan Pangan Lestari Extension Methods (P2L) Before the Covid-19 Pandemic

The Pekarangan Pangan Lestari(P2L) extension method before the Covid-19 pandemic was carried out face-to-face between the extension worker and the group concerned. The meeting was conducted and planned by the instructor for each group. The extension process begins with planning the extension activities made by the extension worker before going to the field. Generally, the method used by extension workers is face-to-face, which is a direct visit to their location with the aim of directly observing group activities. Usually, they visit 2 times a week. At these meetings, they usually evaluate the activities that have been carried out by the group. In addition, extension workers usually go directly if there are new activities held by Pekarangan Pangan Lestari (P2L) farmer groups.

Counseling is given starting from the upstream to downstream process. The selection of the commodity to be planted is determined by the group itself based on the needs of the general public by considering their skills in caring for the plant. After conducting the counseling, the extension worker will make a report regarding the progress and obstacles encountered during the extension. During the extension activities, the extension workers will monitor and help the participants solve their problems if there are problems encountered during the week. In addition, all types of assistance provided to participants by the government through the Food Security Service will be provided directly, without any intermediary, either extension workers or other people. This is done in order to minimize any misunderstanding between the recipient and the aid provider.

The extension media used by most of the extension workers is a mobile phone. This media is one of the tools used to facilitate their daily activities. Whether it's contacting each other when problems arise or learning from the internet about the activities they are currently doing. However, before Covid-19, they rarely held meetings by cell phone, but went directly to the field and taught about new things they wanted to know for the smooth running of their activities and the achievement of the goals of the Pekarangan Pangan Lestari (P2L) program during Covid-19 pandemic.

The extension of the Pekarangan Pangan Lestari Program (P2L) will accompany their group until they become an independent group. In this case, the group has been able to manage their finances and the smooth running of their activities. During the process, from growth to development, extension workers face various obstacles and challenges in carrying out their duties. One of them is, there are still some group members who have not actively contributed to their group. They only want to participate in receiving the assistance provided by the government, not to take advantage of this activity.

3.2. Pekarangan Pangan Lestari Extension Methods (P2L) During the Covid-19 Pandemic Masa

When the Covid-19 pandemic spreads to all parts of the world including Indonesia, all activities we do are limited in order to prevent the spread of the corona virus in every country, especially Indonesia. The government is doing everything possible so that the spread of the corona virus or Covid-19 can be immediately resolved and no more victims will be affected by the virus, because this virus is a dangerous virus and can be transmitted from one human to another. Thus, the government makes several policies that must be obeyed by the community with the first step being to do a lockdown and we are also encouraged to do physical quarantine for areas that are already included in the dangerous zone or red zone. To reduce the risk of contracting the Covid-19 virus(Habibah et al., 2020).

The method of counseling the Pekarangan Pangan Lestari (P2L) in Parepare City during the Covid-19 pandemic did not have many significant changes. They continue to carry out the counseling method as usual.

However, because the Covid-19 virus is spreading very quickly, they still pay attention to the health protocols that have been set by the government. This is implemented for the common safety of both extension workers and participants of the Pekarangan Pangan Lestari (P2L) program to avoid the Covid-19 disease. In addition to the direct meetings that are used, namely LAKU (Training and Visits), they also usually hold discussions together through the WhatsApp group chat that they had previously created. So, there is no specific time to have a discussion with the extension worker. They can do it anywhere and anytime.

The existence of a virus that appeared suddenly that attacked almost all over the world, including Indonesia, made every human activity, both economic and social, also experience a very significant impact. All activities carried out will be carried out online. Online is a meeting system that is carried out using pedagogical tools or tools that allow you to use internet access and good information technology to facilitate the formation of processes and knowledge through interactions carried out. With the development of increasingly advanced technology, so that it also has an impact on the progress of the media used today, although with different plantings.

Technology and information is a solution for the activities carried out today. During the current Covid-19 pandemic, extension workers are greatly helped by the presence of mobile phones and several supporting communication applications that can make it easier for them to carry out the tasks that must be done. By utilizing existing media, they can work anywhere and anytime. Since the introduction of social distancing, they have to do everything from home and reduce face-to-face meetings. Therefore, the intensity of their meetings is also reduced, because they want to follow the existing regulations.

In addition to conducting online or online counseling, extension workers also carry out several other ways by utilizing existing media. One of them is collaborating with TV stations owned by the local government, in this case the Mayor of Parepare. They broadcast videos that can educate the public at large, not just farmer groups for the Pekarangan Pangan Lestari (P2L) program. The schedule for broadcasting the video is 2 times a week. Through this video, extension workers can evaluate the activities carried out by farmer groups. The video shown is a personal video of the activities of the Pekarangan Pangan Lestari (P2L) farmer group and an explanation video from agricultural extension workers containing extension materials. With this broadcast, it is able to motivate the wider community about agriculture and plant cultivation carried out by farmer groups for the Pekarangan Pangan Lestari (P2L) program and grow their interest in participating in this program and feeling the benefits. In addition, farmer groups from the Pekarangan Pangan Lestari (P2L) program and agricultural extension workers can be more creative in making videos of their respective activities.

However, there are several problems faced by extension workers during the Covid-19 pandemic, namely the lack of group members who do not yet have mobile phones to maximize their participation in this activity. As a result, there are some members who still don't understand what they have to do and miss the material that has been given by the extension worker and they will be increasingly lazy to take part in the Pekarangan Pangan Lestari (P2L) activity. As a result, the members' interest in continuing their participation in the program is decreasing. In addition, with their many activities apart from participating in the Pekarangan Pangan Lestari (P2L) program.

The majority of farmer group members who participate in this program are housewives who have many other activities. according to(Chairani, 2020), women experience higher difficulties in dealing with Covid-19 compared to men, both from a health and economic perspective. The role of women in the care economy requires more attention to anticipate the surge in cases of Covid-19 for women. Women are overshadowed by conditions of termination of employment due to the cessation of economic activity in a sector dominated by women. Women who are still working with the Work From Home (WFH) policy also experience a double burden in the household.

During the Covid-19 pandemic, there were several farmer groups whose activities were stopped due to several problems. Extension workers do some several things to motivate farmer groups that are still active. One of them is holding a competition or competition between farmer groups for the Pekarangan Pangan Lestari (P2L) program. This competition aims to grow and maintain the spirit of the Farmer Group's Sustainable Food Program (P2L) in carrying out their respective activities.

3.3. T Test (Different Test)

The difference test is one of the analytical tools used to determine whether or not there are differences between two objects that are the same but in different circumstances or situations. In this study, a paired difference test was used, namely with the same object, namely the Pekarangan Pangan Lestari instructor (P2L) but in a different situation, namely before Covid-19 and after the Covid-19 pandemic. Their differences can be seen from the counseling methods they applied both before the Covid-19 pandemic and after the emergence of the Covid-19 pandemic. This is assessed from several factors that have changed and are able to influence changes in the

extension method applied to both conditions. The following table shows the results of the analysis of the different tests in this study.

Table 3.3. Pairwise Difference Test Analysis Results

		Paired Differences					T	df	Sig. (2-tailed)
		Mean	*Std. Deviation*	*Std. Error Mean*	*95% Confidence Interval of the Difference*				
					Lower	*Upper*			
Pair 1	Before - During	-21.50000	3.33167	1.36015	-24.99637	-18,0363	15,807	5	.000

Source: Processed Primary Data (2021)

From the table above, it can be seen that the significance value is less than 0.05. This means that H_0 is rejected and H_1 is accepted, namely there is a significant difference between the extension method before the Covid-19 pandemic and the extension method during the Covid-19 pandemic. This can be seen from the use of social media and other platforms to conduct discussions between extension workers and target groups. In addition, the number of meetings has also decreased due to several government policies which have resulted in a lack of direct meetings in order to avoid crowds and the risk of contracting the Covid-19 disease. In addition, the t-count value is greater than the t-table(15,807>2,015) which means that there is a significant difference between the extension methods that appliedto PekaranganPangan Lestari (P2L) farmer group at Parepare City before and during the Covid-19 pandemic.

3.4. Multiple linear regression

Multiple linear regression uses several independent variables (X) to determine whether or not these variables have an effect on the dependent variable (Y). In this study, the dependent variable was used, namely Y (extension method) and 5 independent variables including X_1 (characteristics of extension workers), X_2 (external factors), X_3 (government policy), X_4 (quantity of meetings) and X_5 (extension materials). The results of multiple linear regression can be seen in table 3.4 below

Table 3.4. Multiple Linear Regression Coefficient Results

Coefficients

Model		*Unstandardized Coefficients*		*Standardized Coefficients*		
		B	Std. Error	Beta	T	Sig.
1	Y (Constant)	5.787	13.175		.439	.664
	X1	-.052	.441	-.028	-.117	.908
	X2	-.136	.182	-177	-744	.464
	X3	.355	.278	.259	2.278	.013
	X4	.242	.313	.167	1,774	.046
	X5	.322	.281	.265	2.145	.033

Source: Processed Primary Data (2021)

In the table, it can be seen that of the five variables, but there are onlythree variables that have a significant influence on the Pekarangan Pangan Lestari (P2L) extension method in Parepare City during the Covid-19 pandemic, namely X_3 (Quantity of Meetings), X_4 (Government Policy) and X_5 (Extension Material). This is evidenced by the ongoing implementation of outreach to members of the Pekarangan Pangan Lestari (P2L) group during the Covid-19 pandemic, although it is carried out using a different method from the previous one due to the influence of these three variables. The following is an explanation of the influence of the five variables above is:

1. The influence of the characteristics of the extension agent on the extension method

Characteristics of extension agents are one of the factors that can influence extension methods such as age, last education, main and side jobs, making them choose different extension methods. The results of the analysis of the data obtained indicate that the value of the X_1 variable

(characteristics of the extension agent) has no effect on the extension method carried out by the Pekarangan Pangan Lestari (P2L) extension during the Covid-19 pandemic. This is in accordance with the significance value obtained, which is 0.908, meaning that the significance value is greater than the significance level value in the t table (0.908 > 0.05) while the t arithmetic value obtained is -0.117 which means it is smaller than the t table value. (-0.117<1.711).

This can be proven directly by looking at the extension activities carried out at the Pekarangan Pangan Lestari (P2L), namely by carrying out the extension method in accordance with the agreement taken and has been adapted to the conditions of the Covid-19 pandemic by the extension workers. This decision is a form of mutual agreement made by the extension workers before carrying out extension activities, namely every three months a meeting will be held to discuss the design of the extension method that they will apply to the Pekarangan Pangan Lestari (P2L) group.

2. The influence of external factors on the extension method

A person's external factors will affect the extension method to be used. Usually, extension workers consider their external factors, such as the distance between the location where the extension worker lives and the location where the assisted members live. If their house is far away, they usually use indirect extension methods. However, the results of the study show that the X_2 variable has no significant effect on the extension method during the Covid-19 pandemic. It can be seen from the significance value which is greater than the value of the significance level in the t table (0.464>0.05) while the calculated t value obtained is smaller than the t table value (-0.744<1.711). This means that external factors have no significant effect on the extension method during the Covid-19 pandemic. With the current social media platforms and mobile phones, external factors do not affect the extension method. This is because it is easier to access various activities through social media so that they can carry out more than one activity and control more than one farmer group.

3. The influence of government policies on extension methods

In general, every activity carried out by citizens still pays attention to government policies that have been previously regulated.The facts of the research results are different from general conditions, based on the results of data analysis, it shows that the extension method variable has a positive and significant effect on the extension method during the Covid-19 pandemic. It can be seen from the significance value which has a greater value than the significance level value in the t table (0.013<0.05) while the t arithmetic value obtained is greater than the t table value (2.278>1.711).This is because during the Covid-19 pandemic, the government issued new regulations or policies to suppress the spread of the Covid-19 virus. Such as, implementing social distancing or keeping a distance, wearing a mask when doing activities outside the home, and other new rules that must be obeyed. This affects the extension method applied by the extension worker to the Pekarangan Pangan Lestari (P2L) program.

4. The Influence of Meeting Quantity on Extension Methods

The number of meetings is very influential on the method of counseling. This is because the more efficient the extension methods used by the extension workers, the more often they carry out discussions because it does not take up a lot of time from each group member and the extension workers have their own activities and activities apart from participating in the Pekarangan Pangan Lestari (P2L) program.

The results of the data analysis show that the variable quantity of meetings has an effect and is significant on the extension method during the Covid-19 pandemic.It can be seen from the value of its significance which has a smaller value compared to the value of the level of significance in the table t (0.046<0.05) and the value of the calculation t which is greater than the value of the table t (1,774>1,711).This means, with the dense quantity of meetings during the Covid-19 pandemic, they prefer to implement virtual meetings or via mobile phones and social media. In addition to making it easier to convey information to the Pekarangan Pangan Lestari (P2L) program groups, they are also easier to control and evaluate the activities of the Pekarangan Pangan Lestari (P2L) groups assisted by each extension. They only make direct visits if there are certain and urgent circumstances or there are problems that cannot be resolved by telephone. However, things like this are still rare and group members can still resolve their respective problems by deliberation and prioritize mutual agreement.

5. The Influence of Extension Materials on Extension Methods

The counseling materials provided to the Pekarangan Pangan Lestari's (P2L) group program are adapted to current technological developments.With the variety of extension materials provided by extension workers, the extension methods used are also different. This is because the extension method is a way to convey information to their target groups. The results of data analysis showed that the extension material variable had a positive and significant effect on the extension method

during the Covid-19 pandemic. It can be seen from the significance value which has a value of (0.033<0.05) and the value of t count > t table (2.145>1.711). It means, The extension method during the Covid-19 pandemic has more material than the previous material that has been designed. With the current situation, special materials were added, such as how to farm during the Covid-19 pandemic.

IV. CONCLUSIONS AND RECOMMENDATIONS

4.1. Conclusion

Based on the results and discussion of the research that has been done, the following conclusions can be drawn:

1. There is a significant difference between the extension method before the Covid-19 pandemic and the extension method during the Covid-19 pandemic.

2. Changes in counseling methods during the Covid-19 pandemic were caused by government policy factors, the quantity of meetings and extension materials

4.2. Recommendation

After analyzing the P2L counseling method before and during the Covid-19 pandemic, here are some suggestions and recommendations that can be taken into consideration by the parties concerned:

1. It is better to apply extension methods that are more effective and can reach all members of the Pekarangan Pangan Lestari (P2L) group and not just monitor through their respective group leaders.
2. It is recommended that the extension activities of the Pekarangan Pangan Lestari (P2L) are carried out regularly and on a scheduled basis so that participants or group members can adjust their schedule to activities related to this activity.

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Arid Lands Flood Evaluation and Mitigation Measures Using HEC-HMS Model and Best Management Practices (BMPs)

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Abstract— *This study emphasis on draining and slowing runoff volumes in arid and semi-arid lands regions using HEC-HMS model linked with various Best Management Practices (BMPs) alternatives. The proposed methods may help significantly in reducing flash flood precipitation and improving instream water quality. It provides facilities to increase flood retention times and infiltration rates. This study proposed a modern and progressive flash flood management scheme that would emphasize on enhancing soil infiltration and runoff attenuation by construction facilities that would absorb runoff water. We formulated five proposals for flood mitigation derived from BMP techniques for the purposes of stormwater management. Results for investigating the construction of dry dams' option showed a significant reduction in both peak volumes and time to peak and consequently, the mitigated flood spreads longer. Furthermore, downstream calculations prove the contributions of other events on increasing flood hazards downstream. Outlet bottom size and storage capacity are the major parameters that control flood hazards. Outcomes of this study would help water resources managers and decision-makers who need immediate action plans and operational responses when floods occur. Discharge outflow rates were assessed using HEC-HMS and CN methods, and modeling the studied area using Geographic Information Systems (GIS). This study reveals runoff volumes ranges of 0.04 to 0.77 cm/hr.*

Keywords— ***Flash Flood, Arid Land, HEC-HMS model, Best Management Practices, CN method.***

I. INTRODUCTION

Water-powered engineers' endeavors have been embraced for quite a long time to lessen flood harm. More often, the point was to forestall floods and guarantee a fast outpouring of runoff volumes. Streams were channelized, redirected, fixed, and corseted in levees, with next to zero ideas for waterway elements and biodiversity protection. This methodology is presently generally reprimanded (Loucks, 2006). Initially, speeding up the stream regularly brings about exasperating floods downstream. Besides, the interruption of the regular examples can upset the residue balance, thus causing disintegration or stores. Lastly, the results on biological systems are regularly terrible (Lowe et al., 2020). The most effective method to both shield residents from floods and biodiversity from flood-the executives' plans is a hot issue (Jenkins et al., 2017). We promote that ecologist and hydraulic practitioners should work intently together to supplement applicable flood mitigation solutions with eased effects on the environment.

In arid environments like the east region in Jordan, precipitation storms display solid spatial fluctuation, particularly during thunderstorms and localized torrential rainstorms (Almazroui, 2010). All through the most recent couple of years, flooding has been one of the most exorbitant calamities as far as both property harm and human setbacks in the Kingdom. In 2018 almost all of the kingdom witnessed heavy rain and flash floods. The hot springs the surroundings Dead Sea flood was the most

influenced. Approximately 21 individuals are accounted for to have been killed and the number of missing people remains unknown. Many other cities in the country have witnessed streets were under a meter (two feet) of water on 26 October, and a large number of the casualties were accepted to have suffocated in their vehicles. No less than 1200 vehicles were cleared away or harmed.

The climatological dispersion of yearly precipitation saw by downpour measures across Saudi Arabia is displayed in Fig. 1 and the 10-year climatological satellite image is displayed in Fig. 2 (Habib and Nasrollahi, 2009). Dryness is the predominant climatic attribute of Saudi Arabia besides in the Asir district (shown in Abha in Fig. 1), which gets yearly precipitation > 300 mm because of its special geological setup and the nearby mountains, and an optional pinnacle situated in the upper east, which is related with winter precipitation. Precipitation in the majority of Saudi Arabia is < 200 mm.

In arid environment like Saudi Arabia and Jordan, rainfall patterns exhibit strong temporal and spatial variability, particularly during heavy rainfall storm event and localized flash floods (Almazroui, 2010). Throughout the last few decades, the kingdom has witnessed several flood events and it has been cost a lots of damages to public and private properties and deaths and human causalities. For example, the most intense flood was the 2009 event in Jeddah and other areas of Makkah Province (western coast of the kingdom) (Huffman, Bolvin, 2009). Civil defence officials described the flood as the worst in the 30 years.

Rainfall gauge stations are distributed across the kingdom as shown in Fig. 1. Fig. 2 shows 10-year climatology observed by satellite (Habib and Nasrollahi, 2009). Most areas of the kingdom have dry climate characteristics except Asir region in the southern part of the kingdom, where it has special topography and local mountains, which usually receives annual rainfall greater that 300 mm. On the contrary, most areas in Saudi Arabia receive annual rainfall less than 200 mm.

Meteorological parameters are critical for any hydrological studies. Rainfall intensities and distribution should be measured accurately for any application studies of rainfall data. Additionally, assessing runoff volumes and surface storage are important to supply domestic, agricultural, and industrial demands (Goodrich et al., 2004).

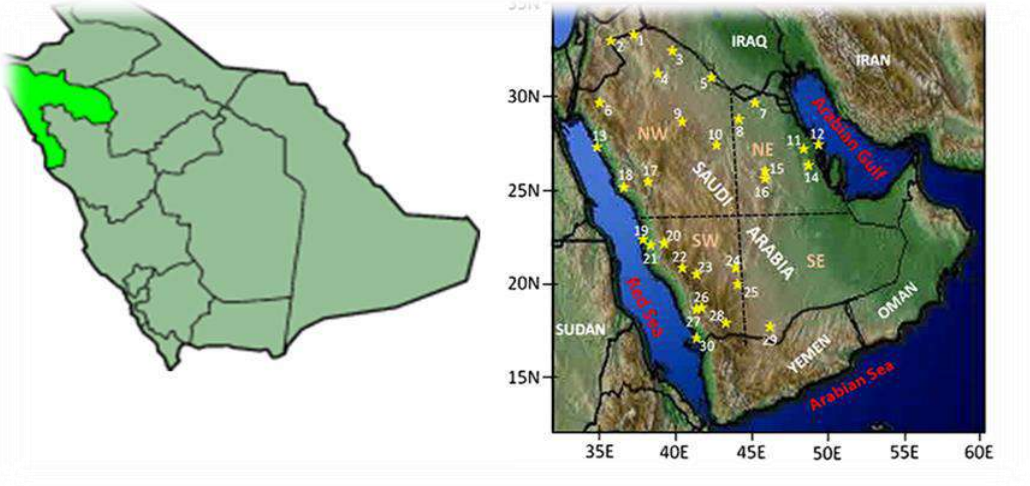

Fig1. (a) Vicinity of Tabuk Region. (b) Rainfall gauge stations cover entire Saudi Arabia (after Almazroui, 2010).

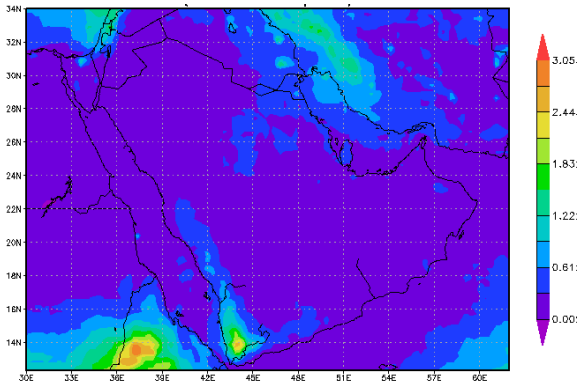

Fig. 2. 10-year climatology observed by satellite for Saudi Arabia.

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This study proposed a modern and progressive flash flood management scheme that would emphasize on enhancing soil infiltration and runoff attenuation by construction facilities that would absorb water such as rain gardens and bio swales, rainwater collection systems, and water reuse systems. We formulated five proposals for flood mitigation derived from BMP techniques for the purposes of storm water management. Various flood mitigation structural measures proposed by hydraulic engineers and biologists will be checked for the purpose of proposing operational plans responses to be implemented once flash flood occur. The implementations of these proposal required a coordinated effort between all parties involved and to be conducted on a long-term policy.

II. RESEARCH METHODOLOGY

2.1 Data Collection

Runoff Volume Calculations

Commonly, the Rational Method used for hydrological analysis of runoff. It uses the general form of the equation Q=CiA, in which Q is the runoff volume in acre-in/hr or cubic feet per second (cfs), C is the runoff coefficient for a given surface material and condition, i is the rainfall intensity in in/hr, and A is the catchment area in acres (Gupta, 2001). The runoff coefficient C is requiring accurate evaluation, hence, it depends on soil type, land use, surface cover and slop of drainage basin. Agricultural and forest land usually have low runoff coefficients in the range of 0.05-0.4 (Alsharhan et al., 2001). On the contrary, paved and concrete surfaces have much higher runoff coefficients of about 0.9 (Alsharhan et al., 2001).

NRCS TR-55 is another common method for estimating surface runoff, more complicated than Rational Method since it employs several equations to calculate runoff peak volumes (Gupta, 2001). CN is the most critical parameter that affect the runoff calculations, it is a function of land use and soil group. Similar to runoff coefficient, a higher CN value associated with high runoff volumes, and lower CN value indicates low runoff. Typical CN value for arid lands is 91, while for agricultural land CN ranges of 30 to 84, depending on soil surface conditions (Mays, 2001).

In this study, city area will be subdivided to assess runoff volumes and contributions in the overall flood. Also, soil infiltration capacity and infiltration rates will be evaluated. This analysis is necessary to identify areas where mitigation action plans should be focused.

2.2 Mapping and Modeling

In this study, Geographic Information System (GIS) was employed to calculate the area of each surface group type. We coupled GIS with Hydrological Modeling System (HEC-GeoHMS) (Mays, 2001). HEC-HMS model was developed by US Army Corps of Engineers, Hydrological Engineering Center. The coupled version of these models contains Arc Hydro tool, which has been used to delineate two sub watersheds for Tabouk watershed. Inputs for GIS modeling are surface topography, land cover, land use, soil type, stream flows data, and physical characteristics of the watershed. Various HEC-HMS input models were created for Tabouk Sub watersheds. These models will be used later on to simulate rainfall and runoff volumes produced from storm water event.

2.3 Soil Properties Investigation

Soil infiltration rates in the study area are evaluated using Darcy's law. This law contains parameters of hydraulic conductivity, soil porosity, and hydraulic gradient. Such parameters are best describe soil nature, land cover, porosity, and hydraulic conductivity of the soil column.

$$Qz = \frac{ka\Delta H}{nL}$$

In which Q_z is the volumetric seepage rate, k is the hydraulic conductivity, A is the area of watershed of interest perpendicular to the direction of flow, Δ H / L is the change in hydraulic head divided by the seepage depth, and n is the soil porosity.

III. STUDY AREA

3.1 Topography of the Tabuk Area; Tabouk city has a desert climate surrounded by low relief of local mountains from east, west, and south, while the city is open to the north. The city has an elevation of 700-800 m above main sea level. Mountains in the west of study area has an average elevation of 1200 m above main sea level. Most of the catchment drainage area is located in the north side of the city. Basically, the Tabouk basin is flat, drainage network extends southwards to Harrah, three main wadis are located in the basin, namely Wadi Al-Khader (east) and Wadi Abu Nishaiah (east), and Wadi Al-Baggar (west) (Al-Baradi, 2000). Tabouk city is underlain by a shale formation where silt and sandy soils are prevailed in

several parts of the province. Fig. 3 shows geological map of the study area (Masoud, 2009).

3.2 Climate of Tabuk; the climate of Tabouk city is dry most of the year, it has occasionally influenced by Mediterranean Sea climate. Mean annual temperature in summer is about 28 C° and 12 C° in winter. Average annual rainfall is about 20 mm as shown in Fig. 5 and Fig. 6. Evaporation rates are high and is almost about 40 mm. In winter season, sometimes the penetration of cool Mediterranean air mass brings frontal rainfall events with high intensities (Alsharan and others, 2001).

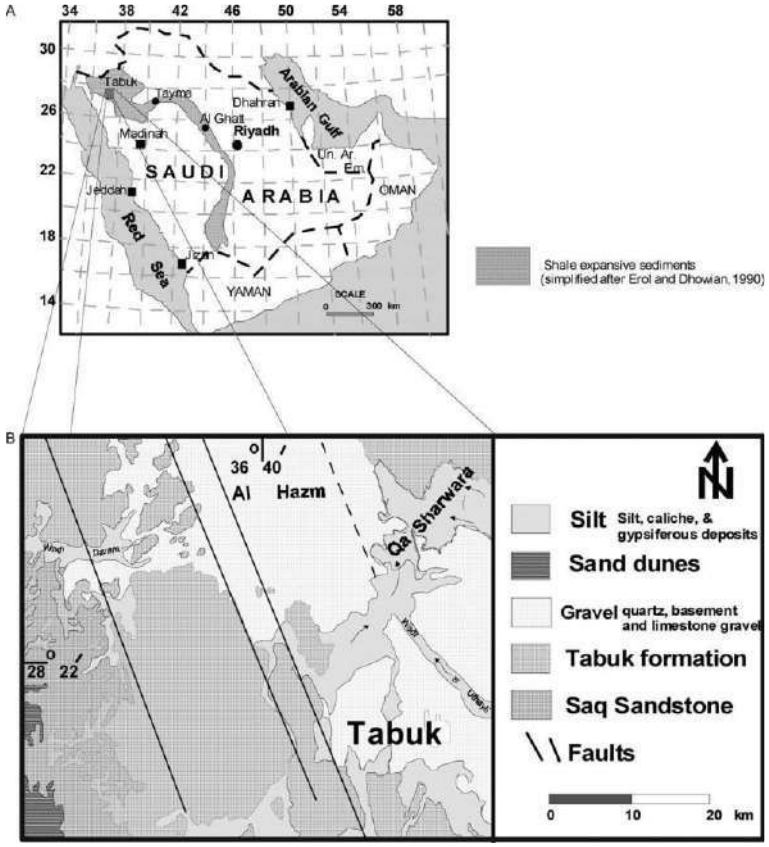
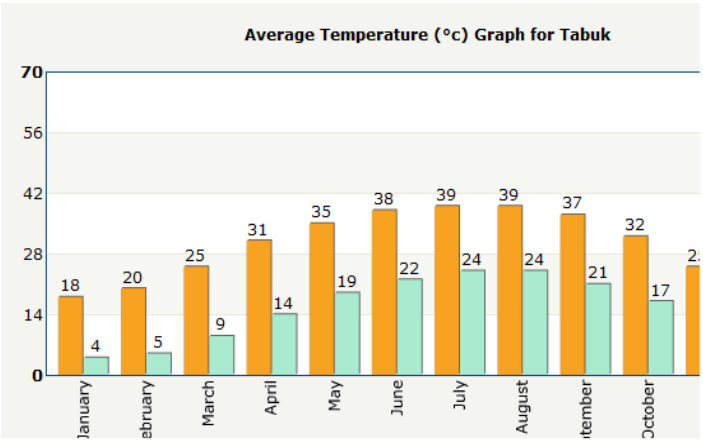


Fig. 3. Geological map for the Tabouk area.



Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
18°c	20°c	25°c	31°c	35°c	38°c	39°c	39°c	37°c	32°c	25°c	20°c
4°c	5°c	9°c	14°c	19°c	22°c	24°c	24°c	21°c	17°c	10°c	5°c

Fig. 4. Average Temperatures for Tabouk city

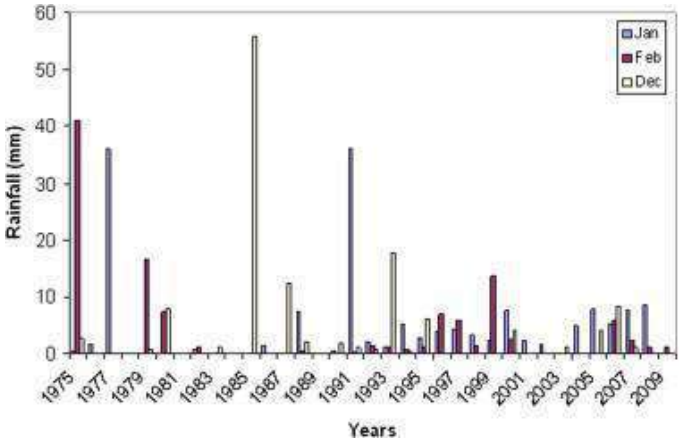


Fig. 5. Annual rainfall values for Tabouk City in mm (1975-2019)

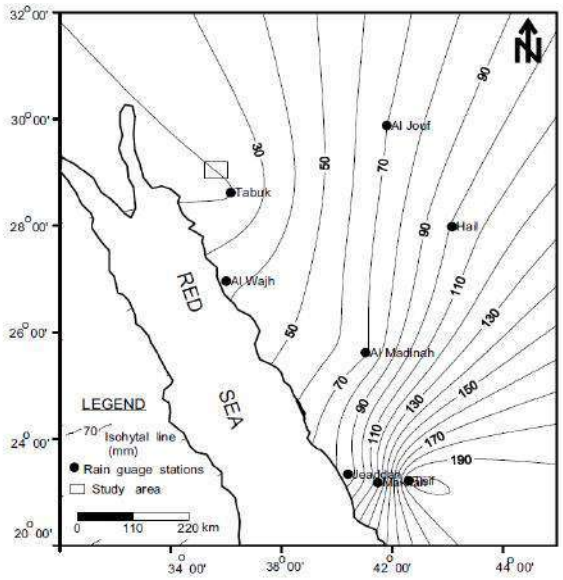


Fig. 6. Isohyetal map shows average annual rainfall for Tabouk city

IV. RESULTS AND DISCUSSION

Low cost and nonstructural structures used in mitigation floods in urban settings are commonly known as Best Management Practice (BMP). Non-structural measures are used in rural areas. These structural measures are used to reverse the disturbances caused by urbanization, many of these structures proven promising efficiency for controlling and lessen damages caused by flash flooding and combined sewer systems (Glas et al., 2020). Selection of these mitigation structures are usually consider entire watershed characteristics, technical feasibility, economical aspect, sustainability, environmental integrity, and public acceptance. Determination of the BMPs to propose will be based on past and practical experience gained upon the implementation and monitoring of these mitigation measures. In addition, theoretical and common engineering sense will be used for the purpose of the assessment. It is a learning by doing process based as shown in Fig. 7. Two main principles are embedded in this practice, namely promoting natural drainage systems and urbanized surfaces. It combines both storm water components and flood control components (Bathrellos et al., 2016).

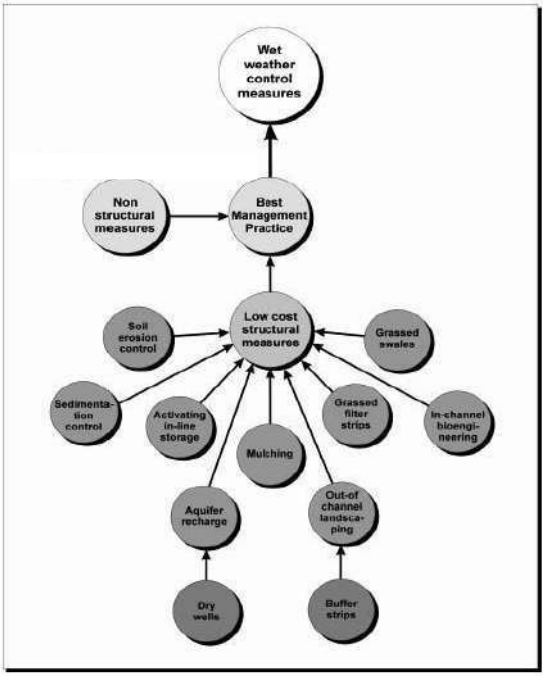


Fig. 7. BMP low cost structural measures

Herein, we formulated five proposals for flood mitigation techniques for the purposes of storm water management;

4.1 Construction of dray dams

This study investigated the constructing a dray dam across floodplain, the dam has a bottom outlet for the purpose of discharging main channel flow. All hydraulic, hydrological, and civil engineering characteristics were described accordingly with typology thus minimize impacts of river biodiversity. Outflows that exceed conduit capacity have been modified.

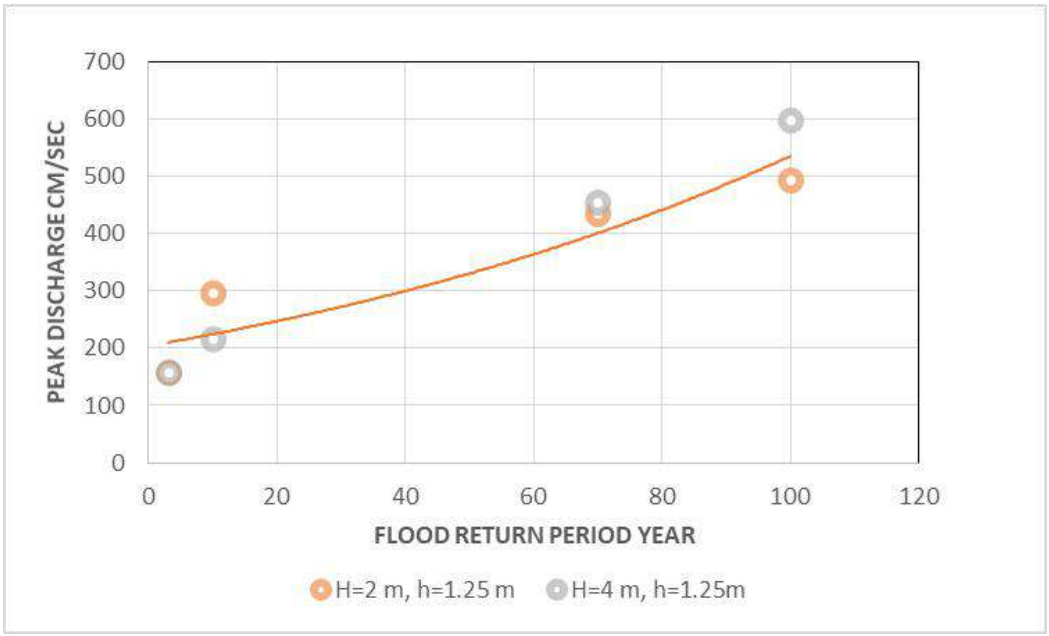
Fig. 8a shows the dry dams' responses to various flood magnitudes differently. Two types of dry dams are proposed, dam#1 with H=2 m, h= 1.25 m, and dam32; H=4 m, h= 1.25 m. All calculations were taken at 1000 m downstream of the dry dams. Outflow discharges are shown in Fig. 8b. Primarily, the existence dry dams affect the hydraulics of flash flood by delaying peak flows while it's obvious that flood waves last longer. The adverse impact of this response that in some cases of delayed peak may become associated with other runoff minor events which ultimately will increase flood hazards downstream. Commonly, two significant parameters in flood mitigation; size of bottom outlet and its storage capacity. Large runoff volumes can fill up storage capacity quickly and thus there is a great need for flow control structures (flow weirs and over spillway devices) to be provided to drain excess flows (Liu et al., 2021). Usually, flood risks drop consequently after overflows happen (Lai et al., 2020).

On the other hand, small flow outlet reaches faster the maximum storage capacity which permit earlier mitigation actions.

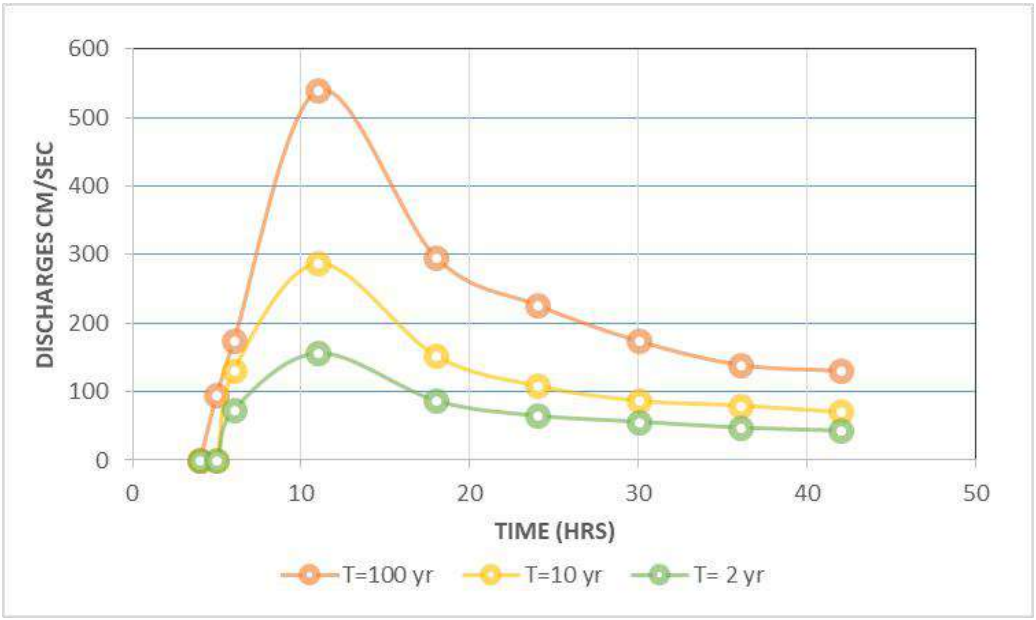
4.2 An Extended Detention Basins (EDB) Design Criteria

An extended detention basin is a structure that permit a temporal storage for runoff. The design of such structure with outlet device detains and attenuated the outflow runoff and allow settling of sediments. Usually, EDB has multi stage facility for storage and attenuation. EDB provides a better management tool for storm water quantitatively and qualitatively. Expected removal of suspended solids varies from 40-70 percent, mainly depending on the designed settling time of the basin.

Typical components of EDB are shown in Fig. 9. Storage volume and detention time parameters must be designed carefully for an extended detention basin. Detention time usually defined as the time from maximum storage is reached in the basin till 10 percent of that storage remains in the basin. The combination of the two parameters would control the outflow runoffs velocities and travel times. Eventually, the construction of such structure must slowly drain outflows and consequently increase detention time. Furthermore, design should provide enough detention time to ensure the treatment of runoff volumes generated by storm event. Better efficiency can be achieved for orifice diameter greater than 9 cm.



(a)



(b)

Fig. 8. (a) Dry dams construction effects; (b) Computed hydrographs delay and attenuation.

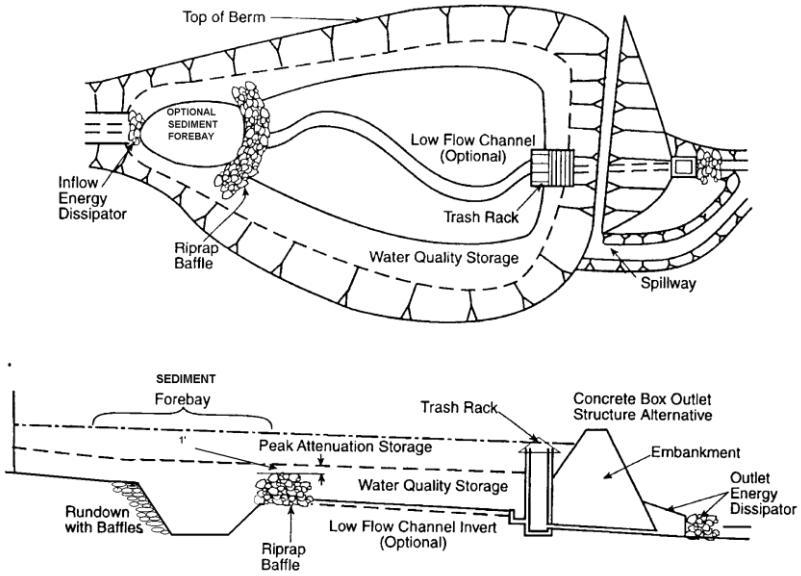


Fig. 9. An extended detention basin basic Components

4.1 Land Erosion Control

Sedimentation and soil erosion control measures should be coordinated with any construction of land undergoing development. Control measures like berms, mulching, silt traps and others are recommended to put into service in conjunction with infrastructure grading operations and before the construction of any increment of urbanization or land development. These measures are crucial for the removal of suspended sediments from runoff storm waters draining from urban areas. Furthermore, stream banks should be maintained and stabilized, removal of vegetation covers and cut of trees should be prohibited. Construction of roads, drainage improvements works, and utility rights of way may consider as exceptional case. Pavement with concrete or asphalt within trees zones should not be allowed. Installation of permanent vegetation cover should be installed immediately as soon as all utilities are in place and final grading phase is achieved.

Runoff attenuation and retention structures should preserve natural topography and vegetation, where possible. All constructed mitigation measure (on-site) should be properly monitored and maintained by the owner of this site. This practice is important to operate these facilities appropriately and not become a source of nuisance to public. For example static water and improper storage may result excessive algae growth and uncontrolled runoff volumes, respectively. Outlets conduits should be designed to prevent or to minimize stream bed erosion.

4.2 Source control measures

In rural areas, where a natural and undeveloped watershed exists, the spatial distribution of runoff are in the form of mobilized small volumes which result in slowing down the runoff flow volumes. Usually, wetlands, flood plains, previous soils, and natural depressions are good storages for such small waters. Urbanization and development may cause serious damage to these natural ways of attenuation and cleaning storm runoff though decreasing permeability of soil, removing vegetation cover, altering these natural way and transforming into culverts and channels, and finally by leveling off the irregular natural ground surface (Poulard et al., 2010).

Source control measure are considered as an extra effort to improve methods of maintaining natural drainage system behavior. It's a supplementary tool and does not cancel the role of other conventional structural practices. Figs. 10-12 represent number of urban low-cost effective structural measures that proved to be very efficient as flood mitigation measures.

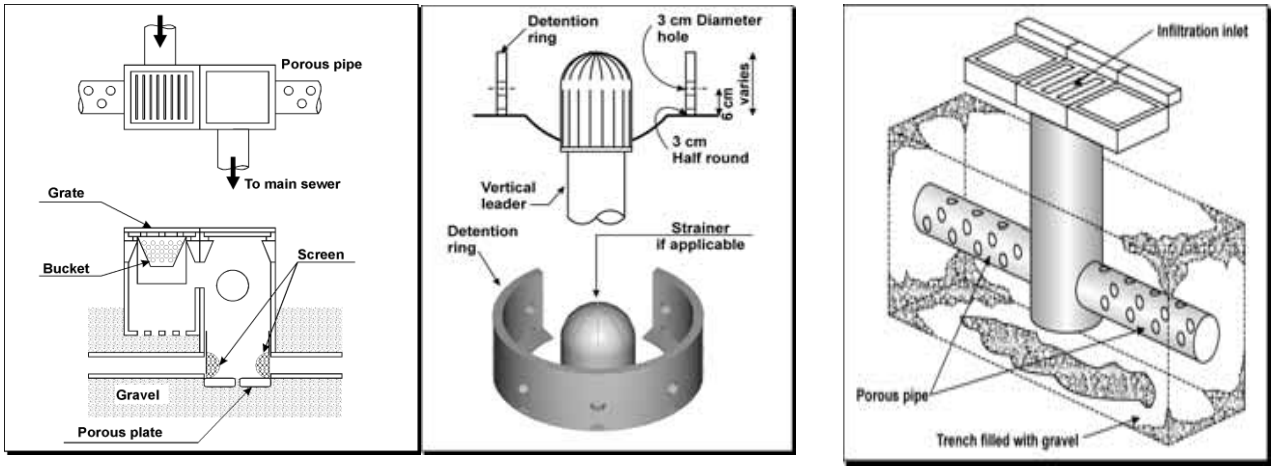


Fig. 10. Examples on source control measures (Rooftop, infiltration inlet and trench)

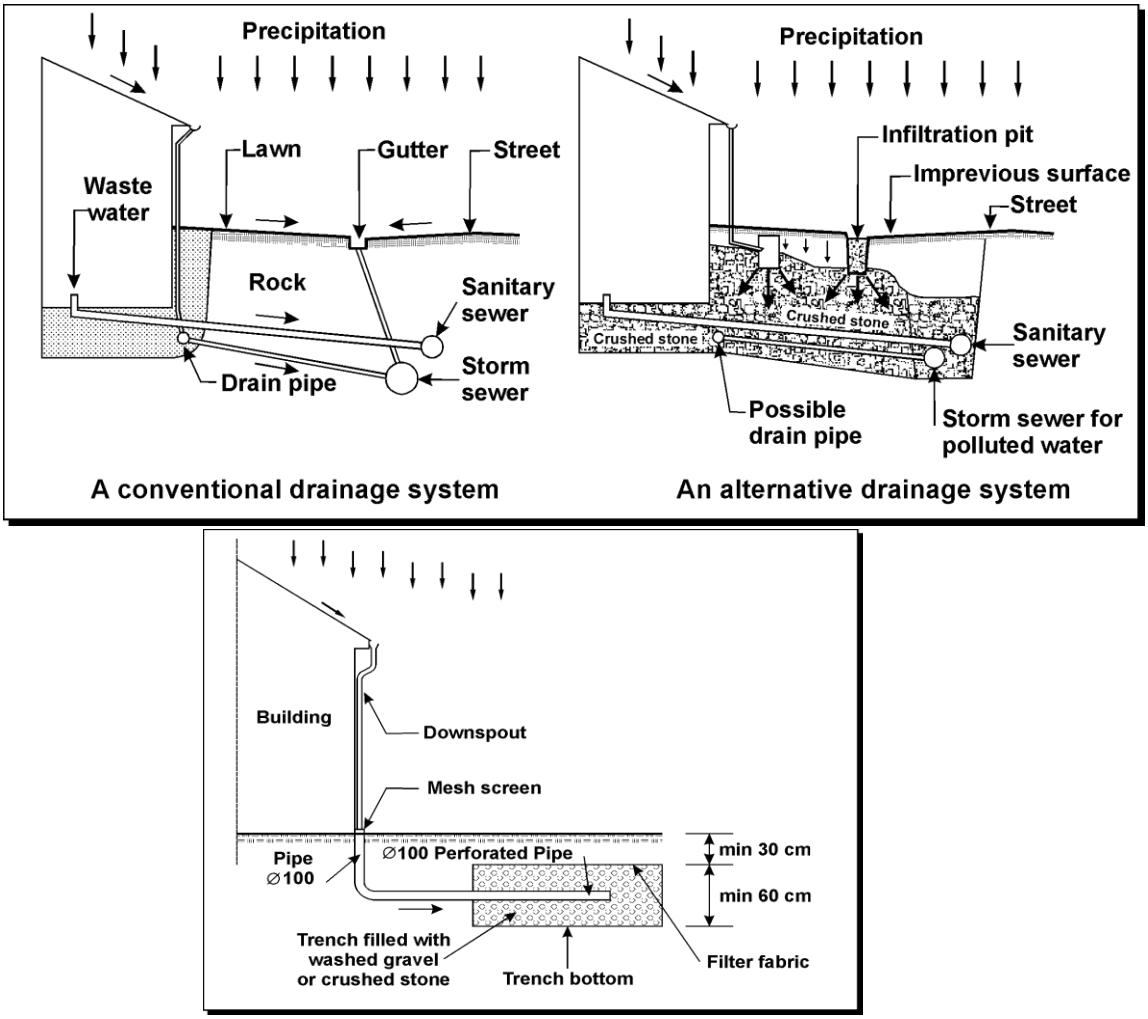


Fig. 11. Infiltration trenches as an example on source control measures

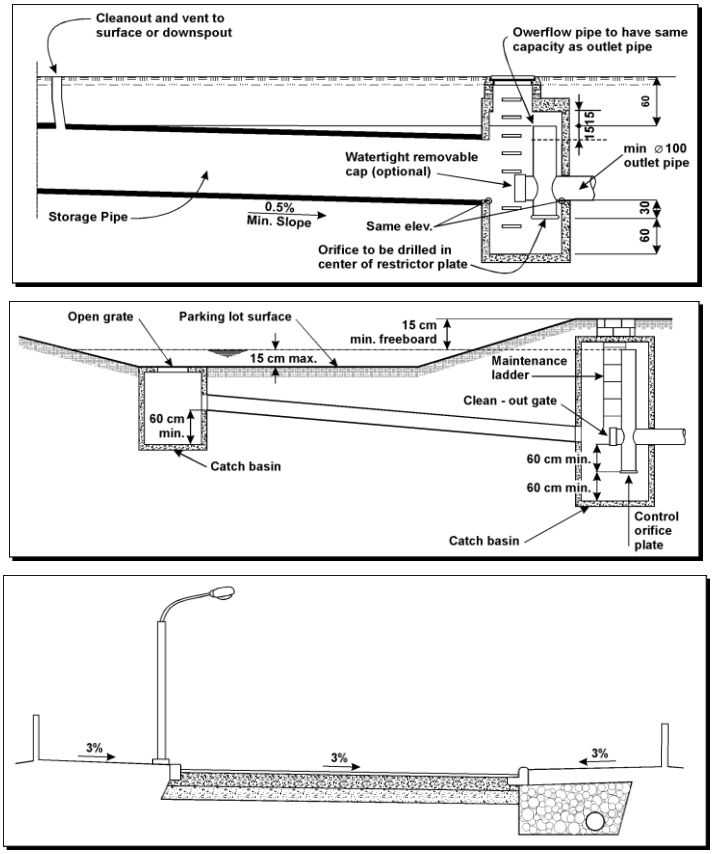


Fig. 12. Source control measures examples (pipe storage, parking lot, porous pavement)

4.5 HEC-HMS Modeling

Nowadays engineers are capable to study and analyze impacts of various floods with various intensities on a hydraulic structure. The analysis involved calculations for all hydrological parameters like peak flows and flood hazards maps. In addition, modelers are able to do economical assessment by comparing several technical solution, estimating the costs of avoided damages. Various flood scenarios with different return period (different probabilities) can be tested. Such analysis is invaluable to predict flood occurrence and to protect human lives and their properties. Also, it helps in protecting flood mitigation structures by considering the safety check floods (i.e., maximum peak flow which the dam can withstand without risk of failure and low-probability but devastating dam-breaks).

In this project, HEC-HMS has been employed to estimate various runoff volumes resulted for the several scenarios we proposed. HEC-HMS is a commercial model developed by US Army Corps of Engineers, several sub-models are embedded inside the model to compute hydrological parameters such as channel routing values, rainfall losses, runoff generations, and base flows. HEC-HMS package comprises of three main model, namely basin model, precipitation model, and control; model (Romali et al., 2018). Basin model calculates watershed and routing variables, and basin connectivity data as well. All rainfall data and computations are embedded in the precipitation model. Timing information are included in control model. Uses has the option to assign the proper data sets for each model, then the whole run is executed using all data sets from the basin model, precipitation model, and control model. Model structures and processes are given in the technical manual (USACE-HEC, 2000, 2008, and 2018). Follow to this section a brief description of the model employed in this project is provided.

The HEC-HMS model contains three main sub-models. The basin model provides watershed physical elements (i.e., subwatershed areas, reaches, junctions, diversions, reservoir, sources and sinks, and hydrologic model for each element as shown in Fig. 12. Terrain processing module (ArcHydro) was used to delineate basin and sub-basin boundaries as networks as needed for the basin model. DEMs data was obtained using an existing contour map 1:100,000 scale. HEC-HMS model was employed to calculate initial model variables using model default

values. HEC-GeoHMS then was used to develop soil maps and land use maps, CN for each grid was assigned (10 m x 10 m resolution). Averaging method linked within spatial analyst module of ArcGIS was used to evaluate the weighted CN values for each sub watershed. CN values were approximately in the range of 83-95 for studied sub watersheds.

This study shows that basin-hydraulic responses are in the form of runoff hydrographs and tables for hydrologic characteristics (such as, peak discharges, time to peak and lag time). Runoff, infiltration rates and interception are presented in the form of a numeric form. Table 2 display the main hydrological values used in this modeling. Repeated revisions for storms of return periods 2, 10, and 100 years at different durations of 0.75, 1.25 and 1.75 hours were employed and analyzed. Analysis proved that least frequent storms (large return period) bring rain more than the most frequent ones (small return period). HEC-HMS model calculate runoff volume rates in the range from 0.04 to 0.77 cm/hr. Fig. 13 show the runoff distribution for two sub-basins within the study area.

Table 2. Primary values used in the HEC-HMS model runoff calculations

Variable	Value
Rain fall duration (hr)	2.20
Rainfall intensity (cm/hr)	0.87
Area ratio (R_A)	0.25
Length ratio (R_L)	2.00
Bifurcation ratio (R_B)	2.00

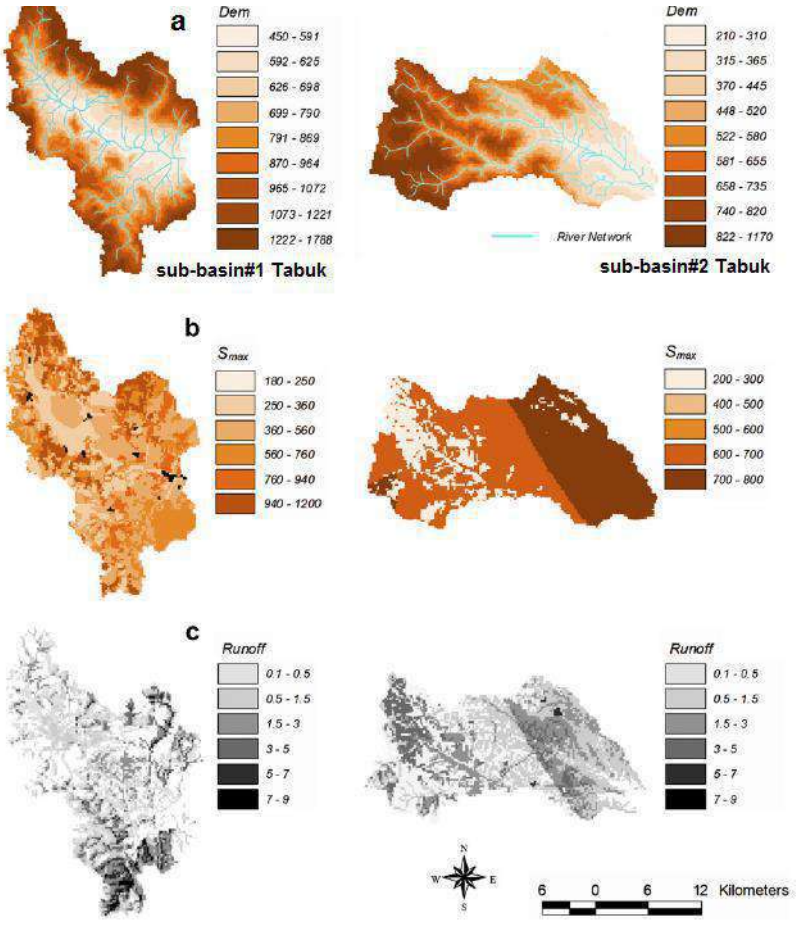


Fig. 13: Physical characteristics for selected sub-basins considered in the HEC-HEM modeling. (a) Stream network, (b) Soil type and (c) Runoff values

V. CONCLUSIONS

Nonstructural flood control and mitigation measures provided useful tools for prevent or avoid serious damage consequences of flash floods in arid lands. Traditional plans treat the results of flood rather than focus on operational or action plans to avoid floods. Five proposals of BMPs were described in this study and general concepts

were introduces, as follows:

- Optimal technical engineering solutions may not the best due to social and political constrains
- Urbanization should consider hydrological principles for planning in addition to the administrative instructions
- To prevent flood consequences in arid lands, the management plans should be comprehensiveness in terms of storm water drainage, pollution control, and flood mitigation as well.
- Public participation and data accessibility are key practices to build the trust between officials and the public to reach a successful management tool.

Repeated revisions for storms of return periods 2, 10, and 100 years at different durations of 0.75, 1.25 and 1.75 hours indicate that least frequent storms bring rain more than the most frequent ones.

Eventually, dry dams' construction may create unexpected new flooding zone in area where floods were not witnessed before. This anthropogenic disturbance to ecosystem may have a serious consequences and possible damages on the ecosystem and river basin dynamic system. Also, a significant change in the river water table may occur. Remediation measures for river repair is substantial, an interdisciplinary collaboration with specialists from various fields such as ecology, sociology, restoration and river engineers, and environmentalists are involved.

This study assessed multi-alternative tools which may help in applying flood mitigation measures using well-defined structures/methods. Certainly, managers need immediate responses and operational plans – when a flood occurs, the demand for such action is robust. Managers and decision-makers need quick and efficient plans to respond to flood prior to their occurrence. More researches are needed on a case-by-case basis to find the most appropriate and efficient management scheme. The linkage between the conceptual approach and the operational plans is necessary to facilitate the transfer of knowledge, the exchange of technicalities, and promote sharing progress developed by all involved parties.

VI. ACKNOWLEDGEMENT

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Emerging developments and innovations in the construction of microbial cell factories for the improved production of S-adenosylmethionine

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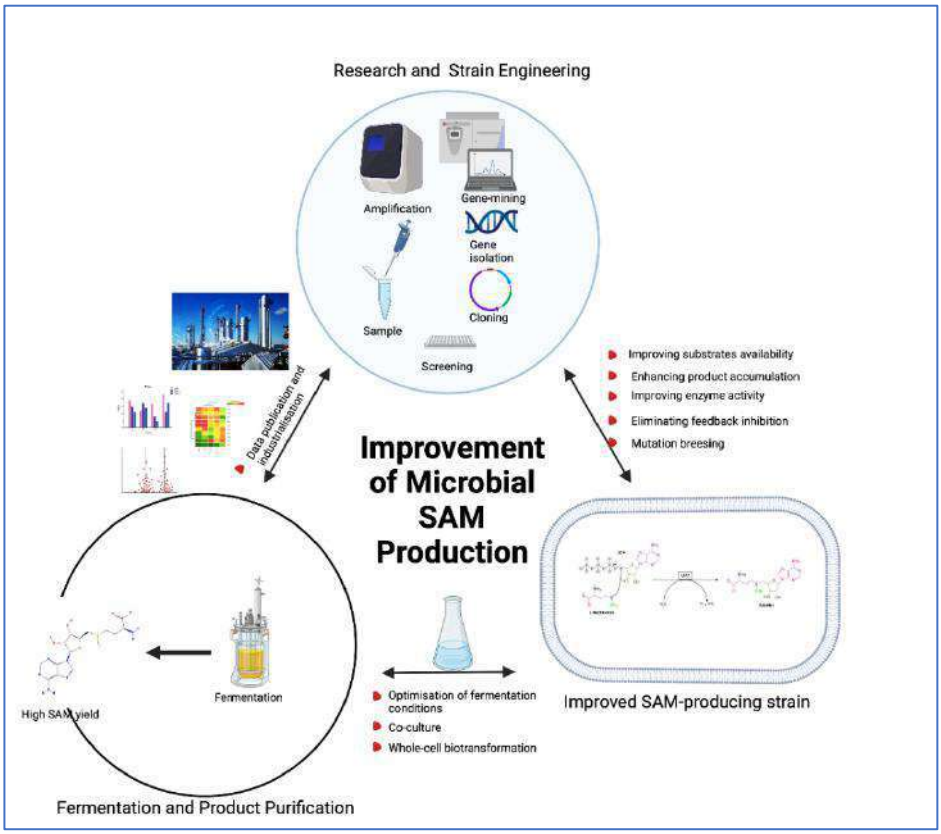
Abstract— *S-adenosylmethionine (SAM) is a metabolite of great biological and pharmacological significance. Its biochemical roles can only be described as central for life. It is produced in almost every organism, and its chemical structure enables it to participate in at least three classes of group transfer reactions as the group donor. It has been employed in treatment of osteoarthritis, depression, Alzheimer's disease, liver disease and fibromyalgia. Its enzymatic synthesis requires the participation of L-methionine and ATP as substrates and methionine adenosyltransferase the enzyme. Meeting the ever-increasing demand for the compound requires increasing production. A number of strategies have been employed to achieve sustainable SAM production. Principal focus has been on improving its production by microbial fermentation. Enzyme engineering, metabolic engineering, conditions optimisation are some of the biotechnological approaches the have been explored to achieve improved microbial SAM production. The successes achieved by the methods have mostly been mixed. Presently industrial SAM production is by yeast fermentation, applying high density cultures of Pichia pastoris or Saccharomyces cerevisiae. This review provides a concise report of the present achievements of methods that have been applied in Escherichia coli, P. pastoris and S. cerevisiae to achieve sustainable improvement in industrial SAM production, highlights contemporary strategies and also suggests potential methods that could be channelled to the same goal.*

Keywords— ***S-adenosylmethionine, microbial fermentation, methionine adenosyltransferase, bacteria, yeast.***

Graphical Abstract



Highlights:

- Coupling SAM production with supply of energy and/or substrate in a co-culture microbial consortium can enhance its production.
- Largely unexplored, mutation breeding exploring the unconventional space-flight microbial culturing can have beneficial effects on SAM production.
- Due to its high cost, strategies employing ATP generation or regeneration can greatly reduce the cost of SAM production.
- *Saccharomyces cerevisiae* expresses a SAM exporter that has heretofore remained largely unemployed in microbial construction for SAM production.
- We recommend system-wide metabolic engineering especially with relation to homogenous ATP availability, however, with consideration for strain viability alongside fermentation optimisation as valuable towards improving microbial SAM production.

I. INTRODUCTION

In all living organisms, reactions which require the transfer of methyl-, sulphur- or propylamine- groups occur, and very importantly, DNA methylation is a critical survival adaptation for most organisms. Such reactions require the participation of a group donor and a group recipient. S-adenosylmethionine (SAM) (Fig. 1), a small compound synthesized in almost every living organism serves as donor of these groups for most reactions. It is in fact able to donate all the groups surrounding its sulphur atom for participation in biochemical reactions requiring them. It is also a precursor in the production of glutathione [1], polyamines such as spermine and spermidine, some plant compounds (phytosiderophores and nicotianamine) that serve to chelate metal ions, and of ethylene, the important gaseous plant hormone responsible for some fruit ripening. SAM provides the 5′-deoxyadenosyl radicals which bear catalytic functions, generated as reaction intermediates by the radical SAM enzyme superfamily [2]. It is also the amino group donor in Gram-negative bacteria's manufacture of N-Acyl Homoserine Lactones (AHLs), where its amino group is employed to synthesize the homoserine lactone ring moiety of the chemical [3].

SAM physiologically functions in the synthesis of coenzyme Q10, creatine, melatonin, phosphatidylcholine, methylcobalamin, norepinephrine, and carnitine. It also participates in the metabolism of serotonin and

niacinamide [4]. Pharmacologically, it possesses anti-inflammatory activity [5] and has also been used in the therapy for disease including Alzheimer's disease [6], liver disease [7], depression [8, 9], fibromyalgia [5], osteoarthritis [10, 11], and colon cancer [12]. SAM has also been suggested to function in improvement of sleep quality [13], and in the regulation of lifespan in yeasts, flies and worms [14-16].

Due to the vast pharmacological applications of SAM, it has become expensive, requiring sustainable improved industrial production to keep it affordable. A variety of approaches to improve and provide efficient and sustainable production of the compound have been explored by different research groups. Chemical synthesis of SAM has applied reactions that chemically couple adenosyl homocysteine and the methyl group of a donor to produce biologically active SAM [17]. However poor diastereomeric excess, a key limitation of the method makes it unsustainable. Enzymatic synthesis presents as an alternative method for increasing production of SAM.

Methionine adenosyltransferase, a.k.a. MAT (E.C 2.5.1.6), otherwise referred to as S-adenosylmethionine synthase or S-adenosylmethionine synthetase is the enzyme that produces SAM from the substrates, L-methionine and ATP. Its catalysis occurs in a rare-kind two-step reaction in which while SAM is formed, the triphosphate chain of ATP is cleaved and then, the triphosphate is further hydrolysed to inorganic pyrophosphate (PPi) and inorganic phosphate (Pi) before the sulfonium product is released [18]. This enzyme has been exploited for enzymatic synthesis of SAM form L-methionine and ATP with varying fortunes in terms of productivity. Notable limitations of the enzymatic synthesis procedure is the requirement of too much effort to yield even small amounts of product [19] and product inhibition [17] when SAM accumulates to high amounts. MAT has been purified and characterised from several organisms including *Escherichia coli* [20], *Saccharomyces cerevisiae* [21], *Bacillus subtilis* [22, 23], *Solfolobus solfataricus* [24], *Leishmania infantum* [25], *Rattus norvegicus* [26] and *Homo sapiens* [27]. These enzymes have although only shown to have quite low specific activity. However, comparatively, MAT from the hyperthermophilic archaeon, *Pyrococcus furiosus* upon purification demonstrated significantly high activity, temperature and pH tolerance as well as it did upon treatment with organic solvent [28]. Also, the MAT from the thermo-acidophile, *Methanococcus jannaschii* has been characterised [29]. Thomas and Surdin-kerjan have reported that in their study that all eukaryotic organisms studied presented with multiple forms of the enzyme, which is believed to be well conserved through evolution [30]. In *S. cerevisiae*, two forms of the enzyme MAT I and MAT II have been reported. Overexpression of MAT in *B. subtilis* has reportedly led to methionine auxotrophy [22, 30].

Owing to the limitations of chemical synthesis and enzymatic synthesis, as well as the dawning of the age of metabolic engineering and synthetic biology with microbial fermentation of valuable products, the direction of recent research has now focused on using these tools to accelerate microbial fermentation of SAM. Efforts have applied several metabolic strategies including those directed at substrate and cofactor availability, improvement of enzyme availability and activity. A number of microbial strains for improved SAM production have also been engineered. Notably, *E. coli*, *Pichia pastoris*, *S. cerevisiae*, *Kluyveromyces lactis*, *Kluyveromyces marxianus*, *Candida utilis*, *Corynebacterium glutamicum*, *Scheffersomyces stipitis*, to mention but a few, have been used for extensive investigations towards improving microbial production of SAM. For specific direction, this review focus on trends involving *E. coli*, *S. cerevisiae* and *P. pastoris*. Several techniques have also been employed to improve SAM fermentation. Table 2 summarizes the strategies that have been employed to improve SAM production by industrially-relevant microorganisms. Most of the techniques have been based on enhancement of enzyme expression, improvement of enzyme specific activity, modulation and fine-tuning of enzyme expression, increasing ATP generation, elevation of substrate conversion ratio and blocking SAM consumption.

This review is intended to provide a condensed evaluation of recent results and progress towards sustainable increase in S-adenosylmethionine production by the application of metabolic engineering and synthetic biology.

1.1 Molecular Structure of SAM

Critical to its diverse functions is the molecular structure of SAM. The metabolite is a sulfonium compound. Its molecular structure (Fig. 1) is a ligation of the methionine structure and the adenosyl moiety of ATP via the sulphur atom of methionine, which subsequently acquires a positive charge, making it willing to donate all the groups surrounding it.

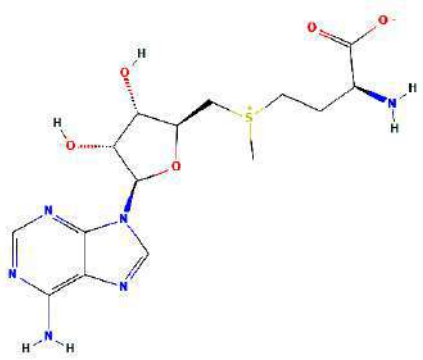


Fig 1. Molecular structure of SAM

1.2 Biosynthesis of SAM

The enzyme methionine adenosyltransferase (MAT) is a fascinating enzyme with diverse behaviours which can vary from organism to organism [31]. The enzyme requires methionine, ATP and water to form SAM, pyrophosphate and inorganic phosphate (Fig. 2a) in the presence of K^+ and Mg^{2+} ions as cofactors [31, 32] (Fig. 2a). MAT is highly conserved across species with up to 85% catalytic sequence amino acid sequence conservation reportedly seen between rat liver MAT and *E. coli* MAT [32]. Regulation of methionine adenosyltransferases vary across species, across organisms and across cells. The mammalian isozymes vary in their K_m for methionine. While MAT II has K_m of about 30 μM [27], it is about 100 μM for MAT I and that of MAT III about 1 mM [33]. In addition, MAT III seems to be greatly activated by DMSO through a mechanism that is unclear [33]. When MAT is overexpressed in *B. subtilis* methionine auxotrophy results [22, 30]. *S. cerevisiae* exhibits two isoforms of the enzyme which are regulated differently. While the isoform *sam1* (like most other MATs) has its activity repressed by accumulation of SAM, *sam2* is insensitive to this phenomenon [30]. In mammals, there are two MAT-coding genes, one of which is expressed exclusively in the liver, and the other in all tissues [33].

SAM synthesis catalysed by methionine adenosyltransferase (MAT) occurs in a reaction between L-methionine and ATP. It occurs in two reaction steps. SAM is produced in the first reaction step, but is however held by the enzyme until the second reaction step is completed. The SAM synthetic reaction is typically cytosolic, occurring via the one-carbon metabolic pathway that involves both methionine and folate cycles [34]. The generation of SAM via the methionine cycle (otherwise called the Activated Methyl Cycle) (Fig. 2b) can be facilitated by several amino acids, including serine, threonine and glycine initiating the folate cycle. During the process of SAM formation, methionine is transformed into a high energy reagent, carrying a sulfonium ion (positive charge) by its combination with the adenosyl moiety to ATP. In the synthesis of SAM, while methionine is a critical substrate, ATP, often regarded as co-factor in reactions wherein it participates, is also a substrate, and could become a rate-limiting substrate when supply of methionine is in excess [35]. In the activated methyl cycle (Fig. 2b) which ensures consistent regeneration of SAM, the methyl-transfer that generates S-adenosylhomocysteine (SAH) from SAM occurs in a typical S_N2-like fashion of nucleophilic substitution. In the reaction, at the expense of ATP, a high energy sulfonium compound is formed. ATP is completely dephosphorylated, and its 5'-deoxyadenosyl moiety transferred to a free electron pair carried by the sulphur atom. This is a resemblance to vitamin B_{12} adenosylation. Although the two innermost phosphate groups of ATP are released as inorganic pyrophosphate (PPi) and the outermost as inorganic phosphate (Pi), a compulsory reaction intermediate formed in the reaction is enzyme-bound tripolyphosphate (PPPi). The MAT enzyme exhibits tripolyphosphatase activity, which is significantly and specifically stimulated by low SAM levels [36]. In the reaction catalysed by the yeast MAT enzyme as described by Greene [37], there seems to be the initial formation of a ternary complex between the enzyme and both substrates, ATP and methionine prior to product formation.

1.3 Regulation of Methionine Adenosyltransferases

Methionine adenosyltransferase is a highly conserved enzyme across the three domains of life. Even though there is evolutionary divergence at some point, reports have suggested that all extant MATs evolved from a common ancestry [38], with most organisms having the enzyme existing in multiple forms [31]. Regulatory mechanisms for the enzyme are however variable across species. SAM metabolism is in *E. coli* greatly controlled and is subject to feedback inhibition [39]. Holloway and colleagues in their 1970 publication [40] have reported the regulatory mechanisms for the enzyme in *E. coli*. In *E. coli*, MAT specific activity is reduced when methionine is added to growth medium, implying that the enzyme is rather repressible than inducible, suggesting that the enzyme is constitutively expressed. SAM biosynthesis is genetically regulated by *metJ*, a repressor. In conditions of its intracellular abundance, SAM binds to *metJ* which subsequently represses transcription of represses responsible for SAM biosynthesis [41]. However, ethionine, nor-leucine, and α-methylmethionine which are poor substrates or non-substrates for MAT have shown to be ineffective as repressors of the enzyme. The methionine analogue selenomethionine, which is a better MAT

substrate than methionine has also demonstrated a slightly higher repressive potential for the enzyme than even methionine. According to [37] tripolyphosphate is a non-competitive inhibitor of the yeast enzyme with respect to methionine at constant ATP concentration. By comparison, SAM is a much weaker inhibitor of the entire reaction than tripolyphosphate and is reportedly an uncompetitive inhibitor in relation to both ATP and L-methionine. While the substrate preference of this MAT is almost inelastic, with closely related L-methionine analogues unable to be activated by the enzyme owing to their very minimal, if any affinity to the enzyme, and thus negligible inhibitors of the enzyme. There are reports that one exception is S-trifluoromethyl-L-homocysteine. This compound exhibits a moderately good inhibition of the yeast enzyme [36]. Other inhibitors of the enzyme of both *E. coli* and yeast, according to the same report include 1-aminocyclopentanecarboxylic acid and 1-amino-3-methylcyclopentanecarboxylic acid [36].

II. CONSTRAINTS TO ENZYMATIC AND MICROBIAL S-ADENOSYLMETHIONINE PRODUCTION

A variety of microbial hosts such as *S. cerevisiae*, *P. pastoris* and *E. coli* have been engineered for SAM production. Despite some successes, there exists a number of bottlenecks hindering the SAM production by microbial or enzymatic strategies on a grand scale. The most concerning constraint to enzymatic SAM production is product inhibition. Reports have suggested severe inhibition of the MAT enzyme in systems accumulation the product to concentrations that exceed 0.1 mM [42-44]. Strategies that have been directed towards relieving this constrain include the use of high concentration of sodium *p*-toluenesulfonate (*p*TsONa) [45, 46]. In a recent report, Yin and colleagues have published that *p*TsONa concentrations up to 0.8M can completely alleviate product inhibition of the *E. coli* enzyme, and in one variant created by site-directed mutagenesis where the 303rd isoleucine residue was substituted for valine, only about 0.3 M *p*TsONa was required for complete alleviation of product feedback inhibition, and over 80% ATP conversion on a 40 mM- scale [45] was achieved.

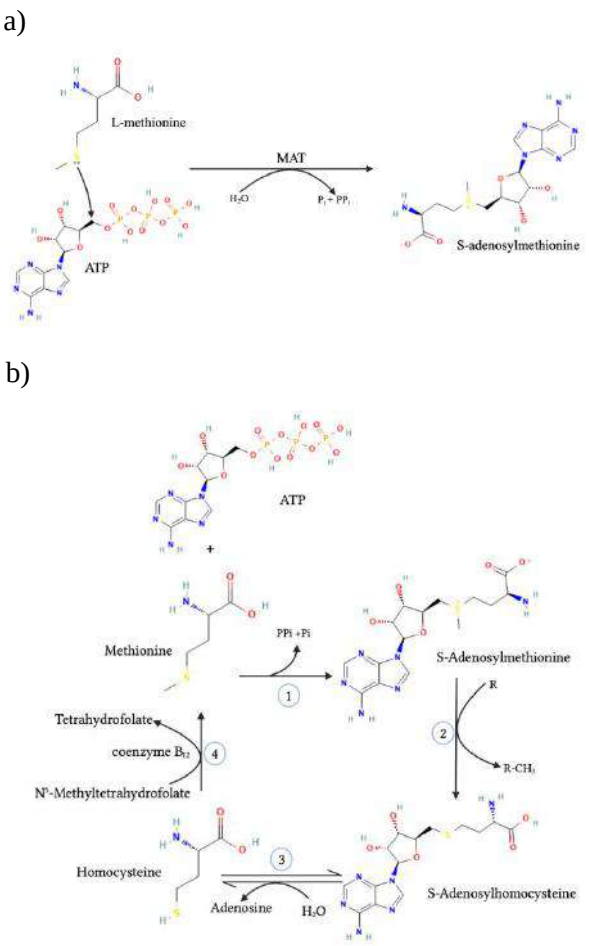


Fig. 2 Biosynthesis of SAM. a) Production of S-adenosylmethionine (SAM) from L-methionine and ATP, catalysed by Methionine adenosyltransferase (MAT) with release of inorganic phosphate (Pi) and inorganic pyrophosphate (PPi). b) Activated methyl cycle for the continuous cycling of SAM (1)methionine adenosyltransferase (2) various SAM-dependent methyltransferases (3) hydrolase (4) methionine synthase

Also, fermentative SAM production by yeast cells is hindered by uptake of L-methionine. When culture medium contains more than 1% of L-methionine, cell growth and transcription of MAT gene is repressed [47]. Expressing the enzyme using a strong promoter can easily manage this concern. Another constraint that requires mention is that when methionine is available in sufficient to high concentrations, ATP becomes a rate-limiting substrate for SAM synthesis [35].

Some other drawbacks of SAM fermentation associated with the use of yeast include very long fermentation time (at least 48 h and up to 120 h), which require very high energy consumption and often leads to yields not more than 15 g/L medium. Yeasts have a low L-met conversion rate [47]. Also, extraction and purification of SAM from

yeast and other downstream fermentation processing usually require some environmentally unfriendly procedures for cell disruption, as well as the excessive use of perchloric acid for product extraction.

III. STRATEGIES FOR IMPROVING MICROBIAL SAM PRODUCTION

3.1 Improving endogenous substrate availability and enhancing substrate uptake

Very often, the route to fermentative production of SAM is through the addition of L-methionine to the fermentation medium, with the utilisation of endogenous ATP as second substrate. [48] reported that when proper concentration is applied, the precursor methionine is favourable for improving production of SAM by *C. utilis*. Surprisingly however, in some microbial strains, especially yeast, cell growth and transcription of MAT genes is repressed when culture medium contains more than 1% methionine, as previously mentioned [47]. This MAT repression ultimately results in reduced SAM yield. A proposed mechanism for this yeast methionine uptake constraint is the repression, by excess methionine, of two out of seven important permeases that allow uptake of methionine into yeast cells, namely MUP1 and MUP3 [49]. In addition to this reported methionine uptake constraint, ATP the second substrate is important for a diverse variety of energy-dependent cellular functions, and in the abundance of methionine could become the rate-limiting substrate in the bioconversion of methionine to SAM [35]. When this happens, exogenous ATP supply might become necessary to sustain SAM production. Considering how expensive commercial ATP is, it is imperative to apply a cheaper alternative to its supplementation for more cost-effective SAM production. Several groups have attempted to improve microbial SAM production by increasing available endogenous ATP. There have been reports of improved microbial SAM production when either or both MAT substrates are endogenously generated by the cell. In one study with *S. cerevisiae*, Chen and colleagues reported that co-expressing *met6* (encoding yeast methionine synthase) and *sam2* (encoding yeast MAT2) combined with sodium citrate feeding; for increasing methionine availability, MAT expression and provision of auxiliary energy respectively. Their results showed the SAM production of the recombinant had increased by 2.34 folds from that of the wild-type, and sodium citrate feeding also led to 19% increase in SAM accumulation [50]. In a similar study aimed at improving the availability of ATP for SAM synthesis in *P. pastoris*, *Vitreoscilla* haemoglobin was expressed along with the MAT gene from *Streptomyces spectabilis.* Expression of the enzyme with the enhancement of ATP availability by increasing cellular oxygen delivery and oxidative respiration intracellularly resulted in higher MAT activity by a value of 27 times and an increase in SAM production about 19 times compared to wild-type [35]. Also, in *P. pastoris*, a recombinant overexpressing the *sam2* gene, methionine permease *mup1*, and adenylate kinase *adk1* for MAT, methionine uptake and ATP improvement respectively. While expressing the genes individually had only the *sam2* recombinant showing marked SAM production, there was great improvement of up to 77% with all three genes synergistically expressed [51]. To produce SAM in *C. glutamicum* without the addition of exogenous methionine, *thrB*, *metB*, *mcbR* and *Ncgl2640* were deleted, and *metK*, *vgb*, *lysCm*, *homm*, *metX* and *metY* were overexpressed in *C. glutamicum*ATCC13032. The recombinant strain was able to produce SAM as much as about 0.2 g/L after fermentation for 48 h [52]. Methionine biosynthesis in yeast typically requires consumption of acetyl-CoA in the cell cytosol which is mainly produced from acetate in a reaction which is catalysed by acetyl-CoA synthase (*acs2* encoded). Furthermore, there are also two enzymes of the glyoxylate which play significant roles in cytosolic acetyl-CoA homeostasis, namely malate synthase (encoded by *mls1*) and citrate synthase of the peroxisome (encoded by *cit2*). [53] demonstrated that over-expression of *acs2* and deletion of both *cit2* and *mls1* in a *S. cerevisiae* strain which has ethionine tolerance caused increase in levels of acetyl-CoA, and also elevated methionine level, and in turn SAM accumulation in the yeast strain was enhanced.

To improve the amount of ATP available intracellularly for SAM production, Wang and colleagues [48] further reported that addition of amino acids oriented towards ATP production could improve accumulation of SAM and glutathione, which also stresses the fact that SAM biosynthesis could be enhanced by manipulating the ATP supply. Furthermore, sodium citrate has been reported to stimulated the production of ATP and further enhancing production of SAM by *C. utilis* [54]. A similar phenomenon has also been reported in *S. cerevisiae*[50]. [55] reports enhanced *E. coli* SAM production by using srRNAs for the control of ATP concentration. The SAM titre as well as the yield of the recombinant strain increased nearly two folds in comparison with control. In the study, genes involved in the synthesis of by-products requiring ATP in the pathways for SAM production were repressed. These genes include *argB*, *glnA* and *proB*. The highest SAM titre and yield were respectively obtained with strain Anti-*argB* (0.121 g/L) and the Anti-*glnA* strain (0.13 mg/g, 12 h). In another report, by combining PPK from *C. glutamicum*, and *V*Hb with *ydaO* motif riboswitch for sensing ATP riboswitch in separate experiments [56]

developed a dynamic ATP regenerating strain of *E. coli* which was able to accumulate SAM up to 82% more than the wild type. A recent publication has shown improved ATP and SAM synthesis by application of engineered multi-domain scaffold proteins in *E. coli* [57]. The study assembled an artificial protein scaffold having a CBM3 domain, CL-labelled proteins and IM proteins. This scaffold complex would serve to boost ATP production. The results demonstrated ATP production up to about 25 g/L with ADP concentration of 15 g/L; AMP 5 g/L using adenosine 12.5 g/L and sodium hexametaphosphate 40 g/L. Combined with the already established complex for ATP generation, SAM synthesis was achieved by another scaffold complex employing two CL-labelled methionine adenosyltransferases (CL9-MAT4 and CL9-MAT5). MAT4, in the presence of 0.5 g/L methionine was able to yield about 0.9 g/L SAM while MAT5 could produce 1.2 g/L.

Chen and Tan in 2018 also posits enhanced SAM production in *S. cerevisiae* by increasing ATP levels [42]. Here, they adopted ATP-regulation strategies which included manipulating the availability of NADH while also regulating the supply of oxygen. A variety of ATP-regulation systems were designed by employing NADH oxidase, *Vitreoscilla* haemoglobin and phosphite dehydrogenase. Improved SAM production was attained by combination of the system with overexpression of the *sam2* gene. With the collective system, the SAM titre of the resulting strain ABYSM-2 reached about 55 mg/L, amounting to about 67% increase relative to the control strain.

Oxygen supply and source of carbon limitations are causes of serious concern during fermentation when yeast cells grow rapidly. To improve the yield of SAM by recombinant *P. pastoris*, a technique which requires addition of 1 % n-heptane, to function as oxygen vector, and 1.2 % sorbitol, for supply of supplemental carbon has been described. The method led to significant improvement in SAM productivity (53.26 %) when 1.0 % n-heptane was added at 72 h, 1.2 % sorbitol at 72 h, 96 h, and 120 h of incubation, with 1.0 % methanol every 24 h during the fermentation process. This study confirms that carbon source supplementation and application of oxygen vectors is effective to overcome intracellular scarcity of energy by improving respiration and the subsequent ATP generation [58]. Table 1 summarises the substrates-focused improvements towards improving microbial SAM production.

3.2 Enhancing substrate conversion

Generally, a very simple way to improve bioconversion of substrate to product especially for a single-enzyme catalysed biotransformation such as that leading to SAM production would be increasing the cell saturation status of the enzyme: more enzyme equals more bioconversion. Typically, most methionine adenosyltransferases demonstrate great sensitivity to the product S-adenosylmethionine. They are inhibited when the product has accumulated to high concentration. Significantly, [20] had characterised the *E. coli* enzyme, stating that SAM is a competitive inhibitor against ATP (K_i = 0.01 mM) and a non-competitive inhibitor against methionine (K_i = 0.01 mM), and the other products pyrophosphate (PPi) and orthophosphate (Pi) also serve as non-competitive inhibitors against ATP (K_i, ppi = 0.4 mM & K_i, pi = 8 mM) and methionine (K_i, ppi = 0.4 mM & K_i, pi = 12 mM). Their publication suggests therefore that the *E. coli* enzyme is severely inhibited by SAM in incubations containing ≥ 1mM methionine. Notably, one variety of the MAT enzyme has been identified to resist inhibition by the product. This is the enzyme product of the *sam2* gene of *S. cerevisiae*. Several research groups have attempted to overcome this important problem, a cog towards enhanced microbial or enzymatic SAM production. A common strategy is the use of high concentration of a salt of an organic acid such as sodium *p*-toluenesulfonate (*p*TsONa) [45, 46]. In one of such studies that could be considered a pioneering one, Park and colleagues proved that when cell free catalysis is carried out in a system containing 400 mM *p*TsONa with 10 mM methionine, product inhibition was completely overcome. The effect of *p*TsONa in alleviating such product feedback inhibition was determined to be dependent on its concentration. And it was able to enhance permit the complete conversion of substrates to SAM within 5 hr when methionine concentration was about up to 30 mM and ATP 1.3 equivalents of that and $MgC1_2$ 2.6 equivalents [46]. Another strategy that has been employed to improve substrate conversion by MAT is enzyme engineering. Yin and colleagues have in 2017 reportedly improved the activity and substrate conversion strength of recombinant *E. coli* MAT overexpressed in the same organism by a site-directed mutagenesis wherein I303 residue was replaced with a less bulky valine residue. The resulting variant exhibited a significantly reduced susceptibility to product inhibition and therefore marked improvement in substrate conversion [45]. Also, in line with this strategy, semi-rationally modified the MAT enzyme from *E. coli* to produce a variant with triple amino acid substitution– Ile303Val/Ile65Val/Leu186Val– resulted in a 42-fold improvement in its K_i for ATP and a 2.08-fold improvement in the enzyme's specific activity in comparison with the native unedited MAT [59]. Its K_i for ATP was 0.42mM and specific activity was 3.78 ±0.19 U/mg. The increased K_i for ATP implies less susceptibility

to product inhibition and a concomitant enhancement of SAM synthesis. The amount of SAM produced by this variant could reach 3.27 mM which is about twice that of the wild-type MAT when the concentration of substrates was 10 mM. Further optimization of the 104th residue of the variant, Asn104Lys by site-saturated mutagenesis, increased the enzyme's specific activity to about 6.02 U/mg at 37 °C. However, the amount of SAM accumulated by the resulting variant dropped to 2.68 mM with the same substrate concentration. When the endogenous *S. cerevisiae sam2* gene was integrated into the genome of an industrial *S. cerevisiae* strain in other to improve its SAM productivity while also barring product inhibition [30, 60], the application of a tri-phasic fed-batch fermentation module in 15 L fermenter led to 8.81 g/L SAM production after 52 h of cultivation. This result amounts to about 27.1 % better performance than the parent strain [61]. Similarly, overexpression of the same gene in *P. pastoris* led to the development of a strain that demonstrated a 17-fold higher MAT enzyme activity than the control [60]. [62] submits that an *E. coli* strain that could effectively produce SAM was constructed by overexpressing the *metK*. In shake flask and basic fermentation medium, the strain was able to produce about 34.5 mg/L of SAM.

Having expressed the enzyme, a report [25] has also described the characteristics of *Leishmania infantum* MAT (encoded by *metK1*). In contrast to the behaviour of the same enzyme from mammals and yeast, the activity of *Leishmania* MAT is only weakly subject to regulation by the product. To overcome possible feedback inhibition of MAT with the goal to improve SAM production, the *metK1* gene from *L. infantum* (codon-optimised) was expressed in *S. cerevisiae*. The resulting strain could accumulated up to 1.62 g/L of SAM, 2.45 times better than the untransformed wild type, and its calculated MAT activity was shown to be much greater in comparison with the wild type and a parallel strain which expressed *sam2* [53].

3.3 Application of unusual substrates

Production cost exerts heavy impact on sustainable SAM production. Thus, heralded by the constant quest to reduce cost of production, the use of cheaper or more readily available substrates presents an option for relieving this bottleneck. As has already been established, SAM is synthesised from methionine and ATP. Typically, most MATs prefer the L- isomer of methionine. DL-methionine, a racemic mixture consisting of both the D- and L- isomer has been used to successfully synthesise SAM, while also some promiscuous MAT enzymes with extended substrate specificity have been engineered or isolated. Based on a survey of the market in China, L-methionine was found to cost about 3-times more that of DL-methionine [63]. To reduce the cost of production, [64] by metabolic engineering developed an industrial yeast strain for conversion of D-methionine to L-methionine by disrupting D-amino acid-N-acetyltransferase (HPA3), and up-regulating expression of both *Trigonopsis variabilis* D-amino acid oxidase (DAAO) and *Rhodococcusjostii* L-phenylalanine dehydrogenase (L-PheDH) in the yeast strain. The resulting strain, when *sam2* was also overexpressed, could by such modification convert the cheaper racemic DL-methionine to SAM. When this strategy was combined with other genetic modifications to lessen SAM degradation, SAM was effectively produced to 10.3 g/L concentration by the resulting strain in 10 L fermenter when DL-methionine (16 g/L) was supplied. [65] also describes a method for producing SAM from pilot scale to industrial scale using DL-methionine as substrate by yeast fermentation applying *S. cerevisiae* SAM0801, a mutant displaying high MAT activity. Interestingly, while serving as a useful method for improved industrial SAM production, the method proves to also be effective for chiral separation of the racemic mixture of DL-methionine and thus, the by-product D-methionine might as well be treated as completely new product, effectively implying co-production of SAM and D-methionine. In a 5-L fermentation with 80 g/L of DL-methionine, the SAM concentration reached was about 13.74 g/L, with the rate of conversion of L-methionine being 32.15 %. Upon scaling up the process to more realistic industrial capacity in a 300-L pilot scale of the SAM-producing fermentation system, 10.76 g/L SAM was generated, with the rate of conversion of L-methionine approaching 30.22 %. The amount of D-methionine left behind in the fermentation broth was 76.89 %. This therefore implies the feasibility of this process for commercial scale SAM production.

3.4 Co-culture

When it comes to microbial production of useful chemicals, the monoculture strategy has been the mainstay. Production has traditionally focused on engineering a single microbial species for the total conversion of substrate to product. However, there are reports of the application of a divide-and-conquer strategy that involves modular co-culture which applies multiple microorganisms synergistically thriving in one system to enhance chemical biosynthesis. A key advantage of the latter strategy is that one can streamline genetic optimization of the individual modules to the requirements of the specific points of biosynthesis in the production process. Systems biology and certain integrated genetic engineering approaches on a single microbial strain would usually involve specific genes knockout or up-regulation which could concertedly

direct metabolic flux to production and withdraw nutrient from microbial thriving and formation of cell biomass, ultimately yielding detrimental effects on the life of the engineered microbe. Another feature of monoculture system that poses significant threat to productivity is that sometimes, some engineering strategies can lead to the development of redox imbalance to the detriment of cell survival [66]. This burden can be relieved by the latter modular approach that combines several engineered strains in a microbial consortium to achieve a final product while trying to maintain strains survival. Jones and Wang have a rich summary of the application of this model [67]. The co-culture approach has been successful in producing several chemicals of industrial interest [68, 69]. Despite this increasing popularity, there is scarcity of data reporting application of the method to SAM production.

A 2015 report [70] has suggested improved *E. coli* production of SAM in a co-culture system with *S. cerevisiae*. While *E. coli* was the producer strain overexpressing the MAT enzyme, the *S. cerevisiae* in the system provided the ATP substrate required for SAM production. The SAM concentration of the coupled system was able to reach about 1.7 g/L a 10-fold increase from the *E. coli* alone system which could only produce about 0.17 g/L. This points to the possible success of further exploration of the co-culture metabolic engineering model for application in the microbial production of SAM.

3.5 Optimization of fermentation conditions and feeding strategy

The effect of a variety of fermentation conditions and substrate feeding modes on the production of SAM by recombinant microbes have been investigated by several groups. Despite the diversity of methods tried, the goal remains to achieve sustainable improved production of SAM by the microbial machines at industrial capacity. Using recombinant *P. pastoris* harbouring shuffled genes for the MAT enzyme from *S. cerevisiae* and *E. coli*, Hu and colleagues [71, 72] explored a variety of L-methionine feeding strategies for improving SAM synthesis. They explored fed-batch and continuous L-methionine feeding in 5 L and 30 L bioreactors. The batch strategy involved addition of 15 g/L culture broth L-methionine supplementation independently delivered at three various times at a concentration of 5 g/L/20 hour from the 10^{th} to 50^{th} hour post-induction. Whereas, for continuous feeding of L-Methionine, a variety of concentrations of the substrate was applied. Concentrations of 8 g/L, 16 g/L, and 40 g/L were fed into the fermentation set-up continuously from 10 h post-induction at the same volumetric rate of 12.5 ml/L/h. They report that SAM concentration rose to much higher concentration when the continuous feeding mode was employed, in contrast to the fed-batch mode. Continuous feeding of 0.2 g/L/h L-methionine produced the greatest amount of SAM, about 8.5 g/L, which was about 49% better than what could be obtained by the batch- methionine addition strategy. In a 2008 report also using *P. pastoris* for SAM fermentation, Xiao-Qin Hu and their team examined how five modules of glycerol supply (based on limited and unlimited feeding modes) affected the production of SAM by *P. pastoris* overexpressing MAT under the P_{GAP} promoter [73]. They submit that SAM production was enhanced when glycerol concentration was kept at 2% by unlimited supplementation in fed-batch cultivation, reaching up to 9.26 g/L SAM volumetric accumulation, a yield of 0.058 g/g. Tofu yellow serofluid is a nutritionally rich, yet cheap microbial feedstock which makes it a reliable fermentation material. Its application in the fermentation of SAM is quite limited. However, one research group [74] in a recent publication have demonstrated its applicability as cheap nutrition source for SAM fermentation. The reported optimum fermentation medium with tofu yellow serofluid for SAM fermentation contained, 30 % yellow serofluid, 70 g/L glucose, 2.5 g/L ammonium citrate and 20 g/L L-methionine. Results showed high cell biomass, reaching 123.1 g/L dry cell weight, and SAM production reaching 16.14 g/L, with overall productivity of up to 1.05 g/L/h. It was also estimated that addition of tofu yellow serofluid to the fermentation was able to reduce the mean commercial SAM cost by about 31.9 % in contrast to fermentation without its addition.

Another uncommon nutrition source that has been used in the fermentative production of SAM is unpolished rice from aging paddy along with medium optimisation and *S. cerevisiae* fed-batch fermentation. In the study aimed to cut down overall SAM fermentation cost, the major source of nutrient provided was unpolished rice gotten from aging paddy. Carbon and nitrogen were supplied by the unpolished rice saccharificate (URS) and yeast extract, respectively. The adequate dosages of these nutrient sources were optimised using response surface methodology (RSM) and 51.4 g/L URS and 4.74 g/L yeast extract was determined as optimum and applied for fermentation. This led to SAM yield of 2.61 g/L. One-time methionine supplementation when cell density was 80 g/L led to SAM production of 5.3 g/L and cell biomass 89 g

Table 1: Summary of strategies for improving SAM production by substrates-focused modifications

Reference	Substrate targeted	Microbe employed	Strategy	Result
[50]	Methionine	*Saccharomyces cerevisiae*	co-expressing *met6* (encoding yeast methionine synthase) and *sam2* (encoding yeast MAT2) combined with sodium citrate feeding	2.34-fold increased SAM production. Sodium citrate feeding also led to 19% increase in SAM accumulation
[35]	ATP	*Pichia pastoris*	Expression of *Vitreoscilla*haemoglobin for improved oxygen delivery and MAT gene from *Streptomyces spectabilis*	19 times improved SAM production compared to wild type
[51]	Methionine, ATP	*P. pastoris*	Overexpressing the sam2 gene, methionine permease *mup1*, and adenylate kinase *adk1* for MAT, methionine uptake and ATP improvement respectively	77% improved SAM production with all three genes synergistically expressed
[52]	Methionine, ATP	*Corynebacterium glutamicum*	Deletion of *thrB*, *metB*, *mcbR* and *Ncgl2640* and overexpression of *metK*, *vgb*, *lysCm*, *homm*, *metX* and *metY*	196.7 mg/L (12.15 mg/g DCW)
[53]	Methionine, ATP	*S. cerevisiae*	Overexpressing *acs2* and deleting *mls1* and *cit2*	6.06 g/L SAM in 10-L fermenter by fed-batch fermentation
[54]	ATP	*Candida utilis*	Sodium citrate feeding	27.5 % increase compared to control
[55]	ATP	*E. coli*	Application of srRNAs for control of intracellular ATP concentration	2-fold increase in yield of SAM
[56]	ATP	*E. coli*	Combining PPK from *C. glutamicum*, and *V*Hb with *ydaO* motif riboswitch for sensing ATP	82% better SAM accumulation than wild type
[57, 75]	ATP	*E. coli*	Assembly of an artificial scaffold protein containing CBM3 domain, IM proteins and CL-labelled proteins for ATP improvement and scaffold protein construction comprising two CL-labelled methionine	1.2 g/L SAM accumulation

			adenosyltransferases (CL9-MAT4 and CL9-MAT5) to achieve SAM synthesis.	
[42]	ATP	*S. cerevisiae*	Introduction of NADH oxidase, phosphite dehydrogenase and *Vitreoscilla* hemoglobin in combination with *sam2* overexpression	67% increase in SAM accumulation
[58]	ATP	*P. pastoris*	Addition of 1 % n-heptane (oxygen vector) and 1.2 % sorbitol (supplemental carbon source)	53.26 % improvement in SAM productivity by adding 1.0 % n-heptane

However, slight improvement in SAM resulted when methionine was fed in the broth at 2 g per hour for 5 hours when the cell density had reached 80 g/L. With this method, SAM production reached 5.82 g/L and the cell biomass got up to 90.2 g/L. [76]. According to Yu and Zhu [77], *E. coli* overexpressing homologous *metK* had its SAM yield affected by pH, yeast extract and loaded volume. The study determined the optimal values of the parameters to be 7.5, 35 g/L and 30 mL, respectively. The final optimum medium condition was subsequently determined to be: 40 g/L peptone, 20 g/L glucose, 35 g/L yeast extract, 1.2 g/L $MgSO_4$, 10 g/L NaCl, 1 g/L L-methionine, rotation speed of 220 rpm, 30 mL loaded volume, inoculation of 1%, temperature and initial medium pH of 37 ºC and 7.5 respectively. Under these determined optimum conditions, the recombinant *E. coli* was able to produced SAM up to 128.2 mg/L during shake flask fermentation. In 5 L fermenter, cell biomass reached 60.4 g/L after 20 h of culture, and the SAM yield reached 300 mg/L after 8 h of induction [77].

3.6 Mutation breeding: UV mutagenesis, gene shuffling and spaceflight culture

In contemporary biotechnology, mutation breeding has been employed to improve performance of microbial strains in various biotechnological applications. UV mutagenesis, ARTP mutagenesis, DNA shuffling and spaceflight culture are some methods that have been used in introducing desirable genetic changes in microbes for strain improvement. UV mutagenesis has been used to significantly improve production of SAM by *S. cerevisiae* Sake K6 which overexpressed the *sam2* gene [43]. Yeast MTHFR (met13 gene product) is inhibited by SAM and requires NADPH to function, unlike plant MTHFRs, which are SAM-insensitive and rather require NADH. For ameliorating SAM feedback inhibition of MTHFR, expressing an chimeric MTHFR made up of the N-terminal domain of yeast MTHFR and C-terminal domain of Arabidopsis MTHFR, led to elevated methionine and SAM synthesis by 7- and 140-fold, respectively [2].

New and often interesting or desirable phenotypes have been successfully developed through exposure of cells to Ultraviolet (UV) irradiation, lithium chloride and ethyl methane sulfonate [78, 79]. Ethionine is able to regulate the synthesis of sulphur-containing amino acids and is also an analogue of methionine that is competitive with it in protein composition [80]. Employing the budding yeast as chassis and introducing genetic variations by UV irradiation, [81] achieved a high SAM-accumulating strain which was isolated by ethionine-resistance screening which was quick and effective for their selection. The strain demonstrated improved SAM production and MAT activity by about 4.3-fold and 2.7-fold compared to each of their starting strains. SAM production by the mutant *S. cerevisiae* CGMCC 2842 reached 6 g/L in a 15-L fermenter following 36-h fed-batch fermentation. *In vitro* recombination has also been employed towards improving microbial production of SAM. Enhanced MAT activity and SAM production by recombinant *P. Pastoris* expressing a chimeric MAT gene that consisted of the gene from *E. coli*, *S. cerevisiae* and *S. spectabilis* recombined by DNA shuffling for enhancement of MAT activity, and in turn, accumulation of SAM has been reported [71]. The recombinant strain which carried the shuffled gene demonstrated greatly improved MAT activity at least 200% more and SAM accumulation at least 103% more than any recombinant with a native MAT gene merely overexpressed. Upon scaling up fermentation to a 500-L bioreactor, SAM production of up to 6.14 g/L was recorded. A genetic recombination strategy that increased SAM production alongside that of S-adenosylhomocysteine in sake yeast has been reported [82]. *Sah 1* is required for growth in yeast, and a *Sah1-1*

mutant engineered in two steps via genetic recombination showed increased SAM production 0.1 g/g DCW compared with wild type, 0.04 g/g DCW [82, 83]. Most industrial strains of yeast exhibit diploidy and are often sporulation- defective. Consequently, genetic engineering and/or manipulation of the strains are somewhat thought to be less manageable. The diploid Japanaese sake yeast is one of such strains. To make it more tractable for genetic manipulation, variants carrying homozygous deletion of *ura3*, showing homozygousity for either MATα or MATa, and homozygousity for either the *his3* or the *lys4* mutation were engineered. Gene targeting was enhanced in the *ura3*-null genotype variant. To enable their overexpression constitutively, the TDH3 promoter was placed upstream of six yeast genes known to participate in the control of flavour. Tetraploid strains simultaneously overexpressing two different genes were combinatorially constructed by allowing mating between homozygous MAT allele carries with non-complementary auxotrophic mutants and selecting by the resulting tetraploidy. Furthermore, by replacing one wild-type allele and subsequent disruption of the other, a recessive mutant *sah1-1* known to SAM in high amounts was introduced into the diploid sake yeast strain. The strain resulting from the construct was determined to produce much higher quantities of SAM compared with the wild-type

During mutation breeding, it is imperative to apply mutation techniques with high mutation rate and ability to introduce a broad range of mutations. Spaceflight environment represents a complex environmental state, involving the interplay of a variety of forces, including, *inter alia*, microgravity, space magnetic fields, cosmic radiation, high charge and high energy (HZE) particles and high vacuum [84, 85]. Within the last five decades, several natural or simulated spaceflight experimental procedures have been intentionally performed on terrestrial life on the basis of spaceflight biological research platform [84, 86]. DNA mutation [87-89], changes in gene expression [90, 91], changes in the production profiles of secondary metabolites [92, 93], physiological and morphological characteristics change [94, 95] are some observed modifications due to spaceflight. A high SAM-accumulating *S. cerevisiae* strain has been isolated through spaceflight culture. Upon isolation, this strain was reported to synthesize SAM 86.89% more than the control strain ground. By application of Amplified fragment length polymorphism (AFLP) analytical method, it was confirmed that demonstrated that genetic variations had occurred between spaceflight cultured strain and the control on ground. By means of recombinant DNA technology, a heterologous MAT gene was inserted into the spaceflight strain genome. This generated a recombinant with 42.98% improved SAM production. On further optimisation of cultivation conditions using Taguchi and one-factor-at-a-time methods, the final strain produced SAM up to 7.76 g/L in shake flask, representing about 536% improvement from the initial ground control strain. In fermenter cultivation, about 9.64 g/L SAM resulted [96].

3.7 Whole-cell biotransformation

In classical fermentation, a palpable demerit is that while being hopeful of great productivity due to channelling of pathways and overexpression of key enzyme(s), often, the microbial strain could convert some substrate into biomass if they remain in growing state. To overcome this perceived limitation, cells could be used to make products in biotransformation or bioconversion in their resting state. In recent years, biocatalysis has gained much attention as an environmentally friendly and sustainable way to synthesize valuable organic compounds [97]. The application of whole-cells for biocatalysis is an area that is still gaining popularity in synthetic biology. Besides its offering of a channel for the conversion of substrates to product without diversion to growth and cell maintenance, whole-cell application for biocatalysis or biotransformation also provides the advantages of reduced undesired reactions, easier products purification, and less environmental harm [98]. The application of whole-cells in resting phase for biotransformation or bioconversion outmanoeuvres the requirement of intracellular enzyme purification for catalysis and also provides a more favourable environment for the functioning of enzymes. An advancement of the method is the use of immobilised whole-cells. The immobilization of cells for biocatalysis can enhance product purification and allow for several re-uses of the biocatalysts, thus significantly cutting down the production cost. Despite these merits, this method remains largely unexplored for the production of SAM as there is scarcity of data reporting its application. However, one recent publication where *E. coli* cells overexpressing a variant of its MAT enzyme was used for whole-cell biotransformation, high activity was demonstrated by both the mobilised and immobilised cells, and immobilised cells kept about 90% of the initial activity they demonstrated after being reused five times, and 67% being reused 10 times. In addition, the cells, immobilised magnetically could produce SAM using 40-mM ATP effectively even when re-used 10 times, with a very efficient rate of conversion of ATP greater than 95%. There was 100% ATP conversion within the first four cycles within four hours while in the fifth to tenth cycles, complete conversion required about 5-6 hours [45].

3.8 Enhancing product accumulation by metabolic engineering

A study of the metabolic fluxes towards and away from SAM would reveal that besides driving substrates towards SAM production, it is also imperative to keep SAM from being used up if high intracellular accumulation of SAM is to be achieved. Homocysteine is a critical metabolite in the flux map of SAM metabolism. This compound has three alternative fates that somewhat directly imparts SAM: it could be converted to methionine, or S-adenosylhomocysteine or to cystathionine by cystathionine-ß-synthase. The latter takes homocysteine away from the SAM, and therefore is of negative influence towards accumulation SAM. In a bid to enhance *P. pastoris* capacity to produce SAM, He and colleagues knocked-in the *S. cerevisiae sam2* gene and knocked out the CBS gene [47]. The result was a significant rise in SAM accumulation by the recombinant strain which accumulated SAM up to 3.6 g/L at shake flask level, and 13.5 g/L in 5 L fermenter. In order to produce SAM in without addition of the principal substrate methionine, [52] has described a metabolic engineering strategy that utilises the metabolic system of the popular amino acids-producing *C. glutamicum.* The resulting strain from SAM-centred gene deletions and knock-ins was able to produce 196.7 mg/L SAM (12.15 mg/g DCW) after 48 h. Similarly, the SAM productivity of *B. amyloliquefaciens* has been significantly improved by metabolic engineering [99]. In the publication, four genes thought to affect SAM production, including *S. cerevisiae sam2, E. coli metA* and *metB* and *B. amyloliquefaciens mccA* were applied together in engineering the SAM synthetic pathway. This resulted in a 1.42-fold SAM titre increase in the recombinant. A subsequent coupling of this pathway with the TCA cycle by deleting the succinyl-coA synthetase gene (*sucC*) led to a 0.59-fold increase in SAM titre, producing up to 107.74 mg/L SAM. Further expression of *sam2* in the strain generated 0.65 g/mL SAM in semi-continuous batch fermentation, a comparatively high SAM yield when methionine is not applied in fermentation medium.

Also, in *E. coli*, deletion of *metJ* alongside overexpression of genes that play part in the pathway for biosynthesis of methionine led to a 33% increase in SAM concentration [100].

3.9 Sundry Technologies

These days, genes can be switched on and off in similar fashion to light switch control by a simple circuit. One report [101] suggests the effectiveness of a constructed genetic circuit that reports the concentration of SAM available intracellularly in *S. cerevisiae*, simply by applying the *metJ-metO* system and SAM feedback regulation. To construct the circuit, *metJ* was fused with transcriptional activator domain (AD) B42 in addition to a reporter gene incorporated downstream of *metO*, a methionine operator. The SAM-*metJ*-B42 complex will usually bind to *metO* when SAM is present, leading to the activation of the downstream reporter gene by B42. This circuit has the ability to perceive SAM concentrations as little as 5 µM. The study reports that with this circuit enabling high-throughput screening for SAM production, the authors were able to identify that SAM production improved in *S. cerevisiae* by 3.3-fold when *gal11* was overexpressed. CRISPR technologies have exposed almost every field in biotechnology to a world of unending possibilities. Dong and their colleagues [102] reportedly applied one such CRISPR-based technologies to improve SAM production of industrial *S. cerevisiae*. They describe MAGIC (multi-functional genome-wide CRISPR), a method by which transcription was simultaneously activated and interfered with, and genes deleted. They combined this method with Umeyama and collegue's circuit, and by several cycles of transformation of guide RNA libraries into yeast harbouring the SAM circuit, they identified new mutable targets for improving SAM accumulation by the yeast strain. Upregulating RPS18B, SNZ3, and RFC4 improved SAM production by 2.2- and 1.6-fold respectively, in laboratory strain and industrial yeast strain.

IV. CONCLUSION AND RECOMMENDATION

The existing wide applications of SAM necessitates the need for its sustainable production. Presently, its market capacity is quite unsatisfactory and requirement constantly increasing, hence it is unable to quench existing demand. Herein, we have described several strategies that have been employed to boost production of SAM which can translate into commercial scale sustainable production. These strategies comprise system metabolic engineering and mutation breeding, encompassing carbon source and nutrients feeding, endogenous substrates generation, and energy supply, enzyme evolution and conditions optimisation, system metabolic engineering. While successes have been mixed, previous and ongoing studies on engineering of microbial cells for SAM production suggests great promise for sustained availability of the product in the near future, as it is expected that application of existing and novel strategies can fuel success. While most research has focused on improving accumulation, there is scarcity of data on improving SAM efflux. *S. cerevisiae* has been reported to possess a high-affinity S-adenosylmethionine permease, a SAM transmembrane transporter encoded by *sam3* gene [103]. Successful

exploration and application of this transporter to improve product efflux could prove to be game-changing in improving microbial SAM production. Many reports have applied chemicals and surfactants such as Cetyl trimethyl ammonium bromide (CTAB), ethyl acetate, hexane and triton X-100 to enhance cell permeability and efflux of the product. Most of these chemicals are potentially toxic both to the cells and to the handlers. Transporter engineering and/or application of environmentally safe cell cytoplasm permeabilizers in contrast to toxic surfactants currently in use, could represent a potential defining moment in SAM production research as it can serve two purposes. While eliminating the arduous task of intracellular SAM extraction, it also has the potential to ameliorate product feedback inhibition of the MAT enzyme. Breakthrough in enhancing product efflux could be tantamount to unprecedented success in enhancing largescale SAM production by the application of microbial cell factories. *P. pastoris* and *S. cerevisiae* have the capacity to accumulate SAM contents to high amounts because of their possession of large vacuoles which are able to accommodate sequestration of SAM within and could hence provide effective starting strains for improved SAM production. In addition, a potentially useful starting strain for SAM fermentation might be one that produces high amounts of substrates available for conversion to product. This might be cost-saving when such strains are applied as starting strains or as part of a SAM-producing microbial consortium. Wilke [104] has summarised microbes engineered

Table 2: General strategies employed to improve the production of SAM by industrially relevant microbes

Microbe	Strategy	SAM Titre Attained	Reference
Escherichia coli	Control of intracellular ATP concentration using ATP-sensing riboswitch	1.23 mg/L	[105]
E. coli (coupled with *Saccharomyces cerevisiae*)	Overexpression of *Pichia pastoris* MAT in *E. coli* and co-culture with *S. cerevisiae*	1.7 g/L	[70]
E. coli	Magnetically immobilised recombinant cells overexpressing a variant of the E. coli MAT enzyme	3.8 mM	[45]
S. cerevisiae	Metabolic engineering to overcome SAM decarboxylation, glycogen branching and ergosterol biosynthesis combined with a pseudo-exponential fed-batch cultivation of a SAM-producing strain	12.47 g/L	[106]
P. pastoris	Overexpression of MAT from *S. cerevisiae*	0.74 g/L (20-fold increase)	[47]
S. cerevisiae	Overexpression of homologous MAT	7.76 g/L (flask); 9.64 g/L (fermenter)	[96]
P. pastoris	Maintaining 2% broth glycerol concentration to increase glycerol consumption rate by enhanced oxygen transfer rate in broth	9.26 g/L (77.39% increase)	[73]
S. cerevisiae	Overexpression of homologous MAT, and ERC1 to enhance ethionine tolerance	2 g/L	[107]
P. pastoris	Overexpression of MAT from *S. cerevisiae* combined with gene shuffling	6.14 g/L	[71]
P. pastoris	Modification of promoter for driving overexpression of MAT from *S. cerevisiae*	11.04 g/L	[108]
P. pastoris	Alternative feeding of methanol and glycerol in strain transformed with MAT from *S. cerevisiae*	13.24 g/L (34.3% increase)	[109]
P. pastoris	Continuous L-methionine feeding at 0.5 g/L/h to prevent heterogenous L-methionine inhibition while sustaining substrate supply	8.46 g/L (48.9% increase)	[72]

P. pastoris	*cbs* knockout	13.5 g/L (2.8-fold increase	[47]

for effective methionine production, some with theoretical yield greater than 100 g/L methionine.

CRISPR technology and its derivative have had tremendous impact on biotechnology. Its derivative, CRISPRi is a potent tool for gene regulation by synthetic circuits. The potentials of CRISPRi as a technology tool to develop regulatory networks of genes is limitless. With this tool and further advancement of new technologies in the field of system metabolic engineering, achieving optimal SAM production on and industrial scale can now be possible by application of CRISPRi in the construct of regulatory circuits for its precursors especially the versatile high energy compound ATP which cells require for viability. Also as has previously been highlighted, another derivative of CRISPR named MAGIC holds great potential for gene deletion and the simultaneous activation and manipulation of transcription and thus useful application for system metabolic engineering for improving SAM production by microbial species. Furthermore, application of omics technologies such as metabolomics, transcriptomics and proteomics can reveal physiological information about a engineered microbes which can be applied for insights into hidden constraints and development of methods to relieve such constraints.

Finally, sustainable industrial production of SAM requires both system-wide engineering and also some culture and fermentation conditions optimization for best results. However, care must be taken not to affect cell viability. We expect that adoption and implementation of some of the strategies proffered in this review might to a great extent exert positive effects on the construction of microbial cell chasses for the sustainable production of SAM and in turn increase its market availability while also reducing price.

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ABBREVIATIONS

AFLP: Amplified fragment length polymorphism

ARTP: Atmospheric Room Temperature Plasma

CRISPR: Clustered Regularly Interspaced Short Palindromic Repeat

CRISPRi: CRISPR interference

CTAB: Cetyl Trimethyl Ammonium Bromide

DCW: Dry Cell Weight

MAGIC: multi-functional genome-wide CRISPR

MAT: methionine adenosyltransferase

MTHFR: methylenetetrahydrofolate reductase

***p*TsONa:** Sodium *p*-toluenesulfonate

RSM: Response Surface Methodology

SAH: S-adenosylhomocysteine

SAM: S-adenosylmethionine

srRNA: small regulatory RNA

URS: Unpolished Rice Saccharificate

UV: ultraviolet

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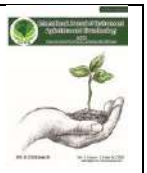

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Antioxidant and antimicrobial properties of cranberry juice and lemon essential oil

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Abstract— *The growing demand for non synthetic preservatives has increased research interest in natural substances with bioactivity. Among recent natural substances investigated for their bio properties are cranberry juice and lemon essential oil. This review discussed the antioxidant and antimicrobial properties of cranberry juice and lemon essential oil.*

Keywords— ***Cranberry juice; Lemon essential oil; Antioxidant property; Antimicrobial property; Preservatives.***

I. INTRODUCTION

For decades, food industries have been using synthetic food preservatives to prevent oxidation and microbial contamination. However, due to the adverse effects of synthetic compounds on health and environment and the growing problem of emergence of multi-drug resistant strains, the food industries are shifting their focus towards the use of natural antioxidant and antimicrobial compounds extracted from plants as preservatives [1]. Therefore, efforts to replace synthetic preservatives with natural compounds with antioxidant and antimicrobial activity have been becoming an important research direction [2]. Natural food preservatives are safe, ecofriendly, cost-effective and have broad spectrum in contrast to synthetic compounds [3]. Natural antioxidants are produced in living cells to protect them from the damage due to free radicals produced in chain reactions. In this sense, some fruits and vegetables and their derivatives are good sources of antioxidants [4]. Moreover, natural extracts can also contain compounds with antimicrobial properties [5].

Essential oils extracted from *Citrus* and juice from cranberry (*Vaccinium* spp.) contain various bioactive compounds. Specifically, lemon (*Citrus limon*) essential oil, which is generally recognized as safe (GRAS), is reported of comprising a complex mixture of volatile components including limonene, β-pinene, geranial, and linalool, etc. that exhibit antioxidant and antimicrobial activity [6,7]. Similarly, cranberry juice is reportedly a rich source of valuable flavonols, terpenes, flavanones, and other phenolic acids, and previous studies have demonstrated its antioxidant and antimicrobial properties [8–10]. Thus, both lemon essential oil and cranberry juice possess antioxidant and antimicrobial properties that can be useful in the food industry.

To the best of our knowledge the current state of researches regarding the antioxidant and antimicrobial properties of lemon essential oil and cranberry is not yet reported. Hence, this review aimed mainly at discussing the antioxidant and antimicrobial properties of lemon essential oil and cranberry juice. Prior, a brief description about their source, extraction and composition is provided.

II. CRANBERRY JUICE

2.1.Source, extraction and composition

Cranberry belongs to the *Ericaceae* or *heath* family, to which plants in the genera *Rhododendron* and *Kalmia* (laurels) also belong [11]. Members of this family prefer acidic soils (pH 4-5) that are moist, well drained and high in organic matter (3-15%). Cranberry is usually placed in the genus, *Vaccinium*, which has 22 species [12]. Some botanists also place cranberry in the genus, *Oxycoccus*, leading to some confusion in the literature with regards to nomenclature.

Cranberry fruits are mainly found in Northern American and some countries in Europe and Asia [13]. The North American cranberry (*Vaccinium macrocarpon*) is recognized by the US Department of Agriculture, USDA, as the standard for fresh cranberries and cranberry juice cocktail. The European variety, grown in parts of central Europe, Finland, and Germany known as *Vaccinium oxycoccus* is a smaller fruit with anthocyanins and acid profiles slightly different to that of the North American variety [14].

Cranberry has become the subject of interest of the food industry in the last two decades due to the increased awareness of consumers about functional food and its preventive and positive effects on human health [15]. Cranberry is a rich source of valuable phytochemicals, including vitamins and phenolic compounds [16]. The profile of bioactive compounds in cranberry differs from other types of berries, as it is rich in proanthocyanidins type A, contrary to the majority of fruits in which proanthocyanidins type B are predominate. Cranberries are mostly consumed in processed form (juices, jams, syrups, or dried) since the sour taste of fresh cranberries is widely unacceptable for consumers [15]. Figure 1 show the tree, fruits and juice of cranberry.

Fig.1. Cranberry (Vaccinium macrocarpon) tree and fruit and juice. Source: Adapted from [17]

Freeze-thaw treatments are widely used in the cranberry processing industry to increase anthocyanin yield to ~ 50% (vs 7% in untreated fruit) as well as total juice yield [18]. Cell wall deterioration due to ice crystal formation releases phytochemicals from normal cellular compartmentalization, resulting in higher extraction efficiency of anthocyanin pigments. It is likely that freeze-thaw treatments similarly release other classes of phytochemicals, such as the other flavonoids, but no experimental verification of this is available [19].

There are three main juice extractions methods used in the cranberry juice industry [20]. The first one uses a mechanical press to extract the juice where no heat is needed, preventing deterioration. The second one is mash depectinization, which consists in the addition of enzymes with the aim to reduce the fruit into a mash and then pressed. The last one is a countercurrent extraction of the sliced fruit and water, involving the use of a large screw. These processes yields are 75%, 100% and 90%, respectively [14]. Pectinase is obtained when fermenting *Asperillus niger* with carbon sources, such as glucose, sucrose and galacturonic acid [21].

Processing variables such as heat, increased pH, light, dissolved oxygen, and ascorbic acid, and the presence of certain enzymes (polyphenol oxidases and glycosidases) markedly destabilize cranberry juice color and anthocyanin content, while pigment co-factors (including cinnamic acid derivatives and flavonoids) as well as certain metals (copper, iron, and tin) improve cranberry anthocyanin and color retention during storage [19]. Phenolic condensation via several mechanisms seems likely to be a major mode of phytochemical loss during processing and storage in cranberry products.

Cranberry juice, the most common form in which cranberries are consumed is a food product with attractive red color and high content of acids. The bright red color in cranberries originates from the anthocyanins cyanidin-3-monogalactoside, cyanidin-3-monoarabinoside, peonidin-3-monogalactoside and peonidin-3-monoarabinoside [22]. Historically, cranberry juice has been consumed to prevent urinary tract infections. These health benefits, including reduced risks of cancer and cardiovascular disease associated with the consumption of cranberry juice, are believed to be due to the presence of various polyphenolic compounds, including anthocyanins, flavonols, and procyanidins, and the synergistic effects among them. According to Brown et al. [23] there are approximately 8000–10,000 phytochemicals detected in *V. macrocarpon*, and *V. oxycoccos*. The phenolic compounds are important for plants for their normal growth and defense against biological and environmental stresses, infection, and injury [24]. Generally, cranberries have a diverse phytochemical profile with phenolic acids such as hydroxycinnamic acid, three classes of flavonoids (i.e., flavonols, anthocyanins, and proanthocyanidins), catechins, and triterpenoids [25]. Česoniene et al. [26] compared the amount of biologically active compounds among 40 genotypes (13 certified

cultivars and 27 wild clones) of *V. oxycoccos* fruit of different origins (Estonian, Russian, and Lithuanian), grown under uniform ecological conditions in Lithuania. They found great variation in anthocyanin content, organic acids, and sugar content in fruits of cultivated types and wild clones, therefore the content of presented compounds differs depending on the cultivars. Analogously to the berries of *V. macrocarpon*, *V. oxycoccos* berries also contain citric acid (10.8 to 54.3 g/kg), malic (14.1 to 43.3 g/kg), and quinic (3.81 to 13.3 g/kg) acids as the main organic acids. Figure 2 provides a detailed bioactive compounds description in berry and cranberry.

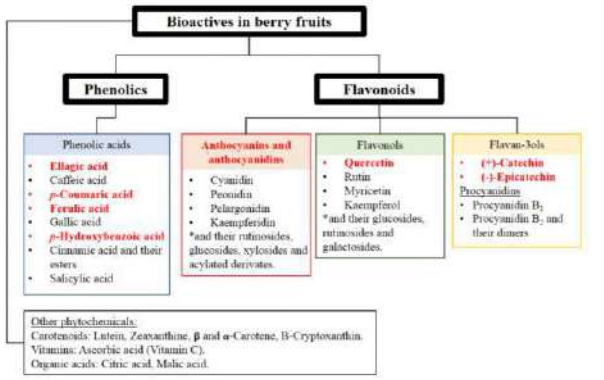

Fig.2. Bioactive compounds in berry. (Bioactive compounds peculiar to cranberry are in red). Source: [17]

2.2. Antioxidant properties

In biology, compounds that can retard or prevent the effects of oxidation have been broadly considered as antioxidants, including compounds that either inhibit specific oxidizing enzymes or react with oxidants before they damage critical biological molecules [27]. Effective antioxidants are radical scavengers that break down radical chain reactions caused by reactive oxygen/nitrogen species or that can inhibit the reactive oxidants from being formed in the first place [28]. These reactions are based on a compound's ability to donate hydrogen as well as to stabilize the resulting antioxidant radical by electron delocalization (resonance) and/or intramolecular hydrogen bonding or by further oxidation. The loss of hydrogen may take place by donation of an electron followed by deprotonation [29,30].

The antioxidant activity of cranberry juice derives from cranberry phenolics and considerable in vitro evidence exists, showing that cranberry phenolics are powerful antioxidants. However, the great diversity of methods applied to studying both the radical-scavenging activity and the antioxidant activity has resulted in great differences in the outcome [31]. Still, cranberry phenolics appear to have free radical-scavenging properties against superoxide radical (O_{-2}), hydrogen peroxide (H_2O_2), hydroxyl radicals (-OH), and singlet oxygen ($^1O_2$), and can also inhibit lipid peroxidation, as well as protein and lipid oxidation in liposomes [32–34]. This could explain why they prevent the oxidation of bulk lipids. The formation of methyl linoleate hydroperoxides for example, was inhibited over 90% by cranberry phenolics at concentrations as low as 500 µg/mL [31]. The effect of cranberry juice on oxidative changes occurring in meat products was determined also by Tyburcy et al. [35]. Cranberry juice, in the amount of 5% of the meat weight, was added to the thermally processed pork burgers, which were stored for seven days at 3 to 7◦C, and juice was added also to a raw beef stuffing. A 5% addition of the cranberry juice caused decreasing of TBARS values, which are formed as a byproduct of lipid peroxidation of burgers, to twice or by three times the value of the control sample. As mentioned by the authors, cranberry juice was a good color stabilizer of the raw beef stuffing. Another study evaluated the degree of antiradical activity against DPPH in incubated and non-incubated juice and reported an increase in antiradical activity against DPPH• with the increase of the concentration of the juice. The incubated samples showed more expressed antioxidant activity due to the longer exposure to radicals. The determined EC_{50} value of the incubated juice is 5.94 mg/L [15]. These results clearly demonstrated that constituents in cranberry juice exhibit antioxidant activity. In literature however, conflicting reports about the main constituents responsible of antioxidant activity among hydroxycinnamates, flavonols, and proanthocyanidins, etc are found. Indeed, Zheng et al. [36] have reported that the anthocyanins contributed 54.2% of the antioxidant activity of cranberries, whereas the flavonols contributed 34.6% of the observed antioxidant activity. According to Yan et al. [37], the cranberry extract composed primarily of flavonol glycosides showed the best inhibition of oxidative processes measured by DPPH assay (EC_{50} at 30-40 µg/mL) compared to anthocyanin-rich cranberry extract or to crude phenolic cranberry extract. From the compounds isolated from cranberry extract by He et al. [38], quercetin,3,5,7,3',4'-pentahydroxyflavonol-3-O-β-D-glucopyranoside,3,5,7,3',4'-pentahydroxyflavonol-3-O-β-D-galactopyranoside, and 3,5,7,3',4' pentahydroxyflavonol-3-O-α-L-arabinofuranoside, showed potent antioxidant activities, with lower median effective concentration, EC_{50}, values of approximately 10 µM. Polyphenol and volatile extracts from cranberry were also reportedly effective in reducing nitric oxide production [39].

2.3. Antimicrobial properties

In the past decade, cranberry juice antimicrobial activity was demonstrated toward various groups of bacteria and fungi. Although the antimicrobial activity of cranberry is generally attributed to the presence of phenolic compounds which activity is accredited to the acidic nature of hydroxyl groups, other polymeric tannins and in particular, the proanthocyanidins consisting primarily of epicatechin tetramers and pentamers with at least one A-type linkage, seem to be the protecting element against pathogenic bacteria [31,34,40]. Several mechanisms could explain the growth inhibition of pathogens in presence of cranberry juice—the destabilization of cytoplasmic membrane, the permeabilization of cell membrane, the inhibition of extracellular microbial enzymes, the direct actions on microbial metabolism, and the deprivation of the substrates required for microbial growth [31]. In an experiment by Ermis et al. [10], there was shown a possibility to inhibit the growth of visible colonies of several fungi with cranberry juice concentrate in fruit spreads (raspberry–aloe vera; strawberry–lime) with reduced sugar, which is a main reason for a growth of microorganisms in low-calorie jams. The antifungal activities of cranberry concentrate were studied in vitro against selected fungi *Absidia glauca*, *Penicillium brevicompactum*, *Saccharomyces cerevisiae*, and *Zygosaccharomyces bailii*. The concentrate was able to inhibit growth of visible colonies of most xerophilic and non-xerophilic fungi. For both fruit spreads with cranberry concentrate *A. glauca* was not able to grow, the growth of P. *brevicompactum* on the spread was inhibited at 3% cranberry concentrate, and S. *cerevisiae* could not grow at a concentration of 18%. *Z. bailii* was the most resistant fungus, the highest concentration (24%) was able to inhibit its growth by 29.8% only for raspberry–Aloe vera spread. The antibacterial effects of American cranberry (*Vaccinium macrocarpon*) juice concentrate on foodborne pathogens, *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella Typhimurium*, and *Staphylococcus aureus* in vitro were also investigated. BHI data indicated that the 100 µl/ml treatment reduced the four pathogens by 3–8 log compared with the control on day 5 at 21 and 4 °C. TEM revealed damage to the bacterial cell walls and membranes. Cranberry concentrate has antibacterial effects on the four foodborne pathogens. Based on potential health benefits and proven antimicrobial effects, the authors suggested that American cranberry concentrate may have dual applications as a food preservative [41]. Ilić et al. [15] investigated the antibacterial activity of cranberry juice against seven bacteria. Among all tested bacteria, *S. aureus* was the most sensitive. In addition, the juice was more efficient against *E. faecalis* than ampicillin, as well as more efficient against *S. aureus*, *S. enteritidis* and *K. pneumoniae* than cefalexin. Moreover, the juice was more efficient against *E. coli* than both tested conventional antibiotics. The authors ascribed the antibacterial activity to the presence of organic acids and phenols in cranberries that cause cell lysis and facilitate leakage of cell content, therefore assuring inactivation [15]. In another study the antibacterial activity of cranberry juice concentrate has been evaluated in vitro and in situ against three foodborne pathogenic bacteria. Results showed a high antimicrobial effect with a noticeable inhibition capacity against *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella typhimurium*. In situ studies showed 2.5, 1.8 and 5 log reduction of *E. coli, L. monocytogenes* and *S. typhimurium*, respectively in presence of cranberry juice concentrate, on pre-cut red peppers after 7 days of storage at 4 °C. A total inhibition of *L. monocytogenes* on fresh cranberry fruits in primary day of storage, was observed. Cranberries treated with cranberry juice concentrate also showed a 3 log reduction of *S. typhimurium* after 4 days of storage at 4 °C. The results suggest that cranberry juice concentrate can be an effective preservative, source of natural antibacterial, to protect ready to eat foods from foodborne pathogens contamination without effecting on sensorial properties of treated samples and allow to maintain the freshness, sensory and the nutritional quality [42].

III. LEMON ESSENTIAL OIL

3.1. Source, extraction and composition

Citrus are the most important crops in the world in terms of production according to the Food and Agricultural Organisation (FAO), with 13 735 357 million tons produced in 2020 [43]. The genus *Citrus* belongs to the *Rutaceae* family that comprises of about 140 genera and 1300 species [44]. One of the species *Citrus limon* is scattered in tropical and sub-tropical regions of South east Asia and the top producers are India, China, Mexico, Argentina, Brazil, Spain, United States, Iran, Turkey, Spain, Greece and some parts of Italy [45]. This is a small thorny tree bearing oval juicy fruit which is acidic and aromatic in nature. It grows up to a height of about 10-20 feet. Leaves of this tree are elliptical and shiny in nature and the flowers possess a strong fragrance. As compared to the other varieties of lemon, *C. limon* is quite larger in size (Figure 3). Furthermore, this species contains many important bioactive compounds such as essential oil, which are complex mixtures of volatile organic substances produced as secondary metabolites [46]. EOs are highly soluble in volatile compounds such as alcohol, ether, and fixed oils but insoluble in water [47]. Also, they can easily

degrade due to environmental factors such as light and heat and quickly oxidize.

Fig.3. Lemon (Citrus limon) tree, fruits and essential oils

Essentials oils from *Citrus* are extracted by methods such as steam distillation, solvent extraction, cold pressing and supercritical fluid extraction and subcritical water [48]. High proportion (93%) of citrus is extracted commercially by traditional methods including hydro-distillation and steam distillation and remaining (7%) by other methods [49]. The yield is a watery emulsion, which is then centrifuged to recover the EOs [50]. CO_2 is the most popular solvent of the supercritical fluid extraction because it is a non-toxic, cheap, readily available, and generally recognized as safe (GRAS) [51]. However, some compounds cannot be fully extracted by using CO_2 only [52]. Therefore, the extraction can be improved by increasing pressure or adding a polar modifier, such as ethanol. The subcritical water or pressurized hot water has been introduced as an extractant under dynamic conditions (pressure high enough to maintain water under liquid state and temperature in the range of 100 to 374 °C). This method is quicker, provides a more valuable essential oil (with higher amounts of oxygenated compounds and no significant presence of terpenes), and allows substantial savings of costs, in terms of both energy and plant material [53]. New extraction method such as solvent-free microwave extraction (SFME) has received increasing attention over the years due to higher yields of EOs, shorter extraction time and less solvent consumption. SFME is a combination of microwave heating and dry distillation, performed at atmospheric pressure without any solvent or water. Isolation and concentration of volatile compounds are performed by a single stage [54]. A novel design for shortening the extraction time to around 20–60 min was documented in a recent study [55]. They reported that solvent-free microwave extraction was an effective method for EO extraction.

Essential oil of *Citrus limon* consist of various compounds such as aliphatic sesquiterpenes, terpenes, oxygenated derivatives and also aromatic hydrocarbons [6,7,56]. Specifically, limonene, α-pinene, geranial, neral, myrcene, linalool, and terpinene are considered to be the major constituents (Figure 4) of the essential oil of *Citrus limon* [6,7,56]. However, great variability may occur in the composition due to several factors, among others the geographical location, season and environmental factors, such as soil type and climate, genetic factors processing and extraction method and the part of the plant used to extract the oil [57].

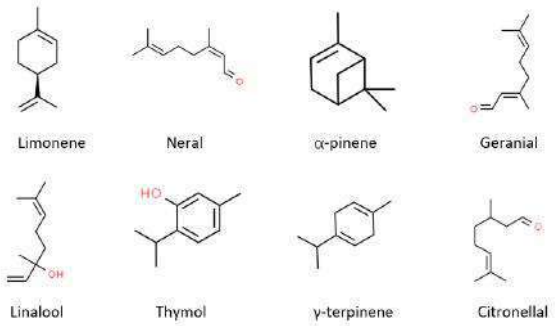

Fig.4. Chemical structures of some popular constituents of lemon essential oil

For instance, in a recent study, it is identified a total of forty-three compounds in *Citrus limon* essential oil, of which limonene (55.40 %) and neral (10.39 %) were found as major compounds followed by trans-verbenol (6.43 %) and decanal (3.25 %) [58]. In another study, the chemical composition analysis by Gas chromatography-mass spectrometry (GC-MS) of *Citrus limon* essential oil from China revealed d-limonene (61.72%), Carene (13.97%), α-pinene (13.67%), Citral (1.88%), geranial (1.29%) as major compounds while the major constituents of essential oil of *C. limon* from Irak were limonene (29.52 %), β-pinene (23.89 %), citronellal (11.53 %) and thymol (9.79 %) [6,46]. Furthermore, limonene (71.81%, 70.36% and 72.48%) with γ-terpinene (8.72%, 8.91% and 8.88%), β-pinene (6.61%, 6.72% and 6.60%), were identified as major monoterpene hydrocarbons in *Citrus limon* extracted by supercritical CO_2 extraction (SFE), cold pressing (CP) and hydrodistillation (HD) methods whereas β-bisabolene (1.42%, 1.41% and 1.22%) and neryl acetate (1.25%, 1.28% and 1.21%) were identified as principal sesquiterpene hydrocarbon and oxygenated compounds respectively in SFE, CP and HD [59]. Extracted essential oil from *C. limon* possesses many medicinal properties. It can be used in different cosmetic industries such as hair oil, beauty soaps, deodorants, and it is most widely used in making soft drinks and in pharmaceutical industries [60,61]. It possesses different anti-microbial, anti-fungal, rich in anti-oxidants and contains anti-cancer properties [7,62]. It also has the great potential for the treatment against cancer [61,63].

3.2. Antioxidant properties

Citrus limon essential oil is a source of natural antioxidants that help in the prevention of oxidative stress and related diseases. It is a good substitute for chemical antioxidants in the food processing industry. The mechanisms by which EOs demonstrate their antioxidant activities depend on the content and composition of active constituents present in them. Due to the large variety of compounds, its antioxidant activity cannot be attributed only to a single mechanism of action [64]. Mainly, their activities are related to the presence of phenolic compounds that have significant redox properties and play important roles in neutralizing free radicals and in peroxide decomposition [65]. The other components such as certain alcohols, ethers, ketones, aldehydes, and monoterpenes: linalool, 1,8-cineole, geranial/neral, citronellal, isomenthone, menthone, and some monoterpenes also play a key role in the antioxidant properties of EOs [66]. Different mechanisms (direct or indirect) to slow down the oxidation reactions including prevention of chain initiation and free-radical scavenging activity are reported [48,67]. Also, continued hydrogen abstraction and terminators, quenchers of singlet oxygen formation, and binding of transition metal ion catalysts are between their modes of actions [53]. EOs activity as antioxidants occurs in three phases: initiation, propagation, and termination as shown in Figure 5.

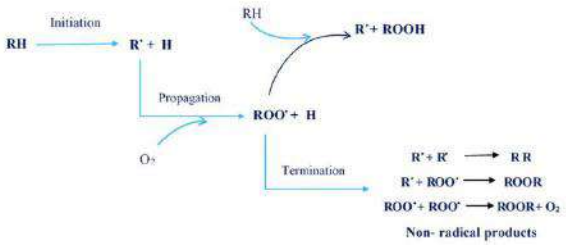

Fig.5. Mechanism of action of essential oil against lipid oxidation. Source : [68]

In vitro DPPH and ABTS assays, conducted on the *Citrus limon* EO, demonstrate its ability to act as an antioxidant [69] while Ben Hsouna et al. [7] reported excellent DPPH scavenging ability of *Citrus limon* EO with IC_{50} of 15.056 µg/ml and a strong β-carotene bleaching inhibition after 120 min of incubation with IC_{50} of 40.147 µg/ml. Ben Miri et al. [70] reported IC_{50} and β-carotene/linoleic acid inhibition percentage of *Citrus limon* essential oils were 1570.10 and 752.26 µg/mL, while the total phenolic were 16.90 and 10.53 µg/mg. The authors concluded that LEO could be good alternative to protect food. Antioxidant activity of EO of *Citrus limon* and other *Citrus* cultivated in China was tested by DPPH and ABTS assays [6]. The study reported that the essential oil exhibited good antioxidant activity and have a potential to be used as a natural food preservative to prevent oxidation. The antioxidant and antiradical scavenging properties of the six different oils including LEO were tested by means of 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay. All examined oils exhibited a free radical scavenging activity, ranging 20–70% of DPPH inhibition. Lemon oil showed the most antioxidant capacity, with DPPH inhibition rate of 70% [71]. Antioxidant assays based on the consumption of stable free radicals (ABTS and DPPH) and assays based in the capacity of antioxidants to reduce ions (FRAP and CUPRAC), were carried out to evaluate the antioxidant capacity of essential oils from *Citrus* species including *Citrus limon* from Argentina and the United States [72]. All essential oils including lemon essential oil showed consistently strong antioxidant activity. Yang et al. [73] compared the antioxidant activities of six popular and commercially available herb essential oils, including lavender (*Lavendular angustifolia*), peppermint (*Mentha piperita*), rosemary (*Rosmarius officinalis*), lemon (*Citrus limon*), grapefruit (*Citrus paradise*), and frankincense (*Boswellia carteri*). *Citrus limon* essential oil showed one of the highest DPPH radical-scavenging activity with RC_{50} values of 2.1 ± 0.04%. Spadaro et al. [74] compared the volatile fraction composition and biological activity of *Citrus limon* essential oils extracted from conventionally grown and biological fruit. Results revealed differences in both oil composition, especially in the content of oxygenated compounds and biological activity with *Citrus limon* from biological production demonstrating greater antiproliferative effect. The authors suggested the antiproliferative effect of the lemon oils could be related to monoterpene hydrocarbons. Dawidowicz et al. [75] reported good antioxidant activity with different essential oils including *Citrus limon* using 2,2′-Diphenyl-1-picrylhydrazyl, 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt and β-carotene bleaching assays. Nevertheless, the obtained data show that the antioxidant properties of essential oils do not always depend on the antioxidant activity of its main component, and that they can be modulated by their other components. Therefore, when comparing the antioxidant properties of essential oils and their main components, the concepts of synergism, antagonism and additivity are very relevant. Also, the conclusions concerning the interaction of essential oil components may depend on the type of method applied for assessing the antioxidant activity.

3.3. Antimicrobial properties

Investigations have proved that EO of *Citrus limon* has potential application as antimicrobial agents or food additives in the food industry due to their antimicrobial

potential against common food-borne and spoilage microorganisms [6]. The antimicrobial mechanism of essential oils depends mainly on the type and concentration of the chemicals it contains. Different chemical components can act through different mechanisms. For example, the main function of phenolic compounds is to disrupt the structure and permeability of cell membranes and the hydroxyl groups carried in phenolic compounds can impair the activity of enzymes in microorganisms [76]. Also the lipophilic or hydrophobic properties of the components of EOs allow them to interact with the lipids of the microbial cell membrane and mitochondria, thereby making cell structures less organized and thus more permeable. This increased permeability allows the outflow of ions and other cell contents. Although a certain amount of outflow from microbial cells may be tolerated with no loss of viability, substantial loss of cell contents or the loss of vital ions and molecules will lead to cell death [77]. In addition, the EOs activity as an antimicrobial is also varying due to the difference in the cellular structure of the bacterial cell, such as Gram (+) and Gram (-) bacteria, which differ in the structure of the cell membrane [68]. Thus, the action route of essential oil antimicrobial mechanism is no single, but two or more routes exist at the same time [76]. The possible action mechanisms of EO are shown in Figure 6, which illustrates them from four aspects of cell wall, cell membrane, DNA, respiration and energy metabolism, respectively.

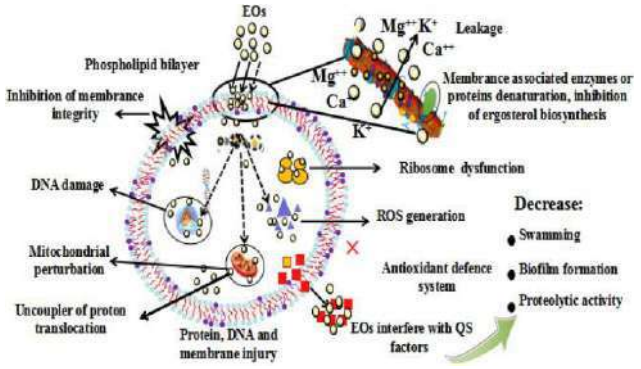

Fig.6. The possible action mechanisms of essential oil. Source: [76]

Frassinetti et al. [71] investigated the antimicrobial activity of four *Citrus* species essential oils including *Citrus limon* against ten strains of gram-negative and gram-positive bacteria and reported all oils showed good antibacterial activity against both gram-negative and gram-positive bacteria. The minimum inhibitory concentration (MIC) for *Citrus limon* oils ranged 20–200 µg/Ml depending on the microorganisms. Ben Hsouna et al. [7] assessed the antimicrobial activities of *C. limon* essential oil along with its preservative effect against *Listeria monocytogenes* inoculated in minced beef meat. The MICs varied from 0.039 to 1.25 mg/ml for gram positive bacteria and from 0.25 to 2.5 mg/ml for Gram-negative bacteria. Additionally, *C. limon* essential oil successfully inhibited the development of L. monocytogenes in minced beef meat. Antibacterial activity of lemon essential oil was determined against six different fish pathogens (*Yersinia ruckeri, Aeromonas hydrophila, Listonella anguillarum, Edwarsiella tarda, Citrobacter freundii* and *Lactococcus garvieae*). The disc diffusion results indicated that essential oil of lemon significantly inhibited the growth of *Y. ruckeri, A. hydrophila, L. anguillarum* and *C. freundii* [78]. The antimicrobial activity of LEO against 12 bacteria and 4 yeasts was tested by a microdilution broth test [69]. Results showed a low inhibitory activity against food grade and *Lactobacillus* strains, whereas *Listeria monocytogenes* and *Staphylococcus aureus* were inhibited at low EO concentration. Also, *Citrus limon* essential oil demonstrated an antimicrobial activity against yeast, with *Saccharomyces cerevisiae* being the most sensitive strain. Akarca et al. [79] investigated the biological activities of LEO on foodborne pathogenic bacteria and food-borne saprophytic yeasts and molds and reported higher antibacterial effect on gram-positive bacteria used in the study compared to gram-negative bacteria. The highest antibacterial activity was detected on *Staphylococcus aureus* with 22.55 mm. The highest anti-fungal effects were determined on *Candida tropicalis* (23.61 mm) and *Rhizopus nigricans* (14.15 mm). Greater antibacterial activity of EO of *Citrus limon* against gram-positive bacteria compared to gram-negative bacteria were also reported in a recent study [6]. The yeasts were also more susceptible than bacteria to the essential oils. Brahmi et al. [80] reported strongest antifungal power with values of 35 mm for lemon essential among four essential tested. However, no antibacterial activity was detected for the four types of EOs. The authors concluded these EOs would be of interest in both agriculture and the food industry by acting as bio-fungicide but also as additives for compounds of medical and cosmetic interest. Antibacterial activities of essential oils from unripe and ripened lemon were carried out by Mehmood et al. [81]. Results showed that both the isolated LEO exhibited considerable antibacterial activity against *E. coli, Bacillus subtilis, Salmonella typhimurium* and *Staphylococcus aureus* with no cytotoxic effects. Nevertheless, a significant variation in biological activities of LEO were observed that can be linked to lemon fruit ripening stages. Yazgan et al. [82] compared the antimicrobial activities of lemon oil based nanoemulsion and two different concentrations of lemon

essential oil (100% and 10%) on food-borne pathogens. According to value of MIC, both nanoemulsion and 100% essential oil inhibited bacterial growth of all of the pathogen bacteria tested whereas they were less effective on inhibition of fish spoilage bacteria. However, 10% essential oil was more effective on spoilage bacteria than pathogens. The minimum bactericidal concentration (MBC) showed that nanoemulsion and 100% lemon essential oil presented a noticeable bactericidal activity against *S. paratyphi* whereas 10% lemon essential oil was found as ≥25 mg/mL against pathogens and spoilage bacteria. The authors concluded that the use of nanoemulsion based on lemon essential oil can have potential as a natural antimicrobial agent against food-borne pathogen and spoilage bacteria for fish processing industry.

IV. CONCLUSION

This review focused on antioxidant and antimicrobial properties of lemon essential and cranberry. Our results indicated that the antioxidant and antimicrobial properties of lemon essential oil and cranberry juice depends on the composition and concentration of active compounds. While major components can play a leading role, the antioxidant and antimicrobial effect do not always depend on the antioxidant and antimicrobial activity of their main components, but can be modulated by their other components. Therefore, when comparing the antioxidant and antimicrobial properties of both lemon essential oil and cranberry juice and their main components, the concepts of synergism, antagonism and additivity are very relevant. Compared to the gram-negative bacteria, the gram-positive bacterial strains are more sensitive to their bioactive compounds. Fungal strains on the other hand seem more susceptible than the bacteria. Overall, this review confirmed that the lemon essential oil and cranberry juice could be used not only as source of natural antioxidants and antimicrobials, but also as possible food natural supplements to extend food shelf life.

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ABBREVIATIONS

ABTS	Radical scavenging assay using 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid
BHI	Brain Heart Infusion
CP	Cold pressing
CUPRAC	CUPric reducing antioxidant capacity
DNA	Deoxyribonucleic Acid
DPPH	Radical scavenging assay using 2,2-diphenyl-1-picrylhydrazyl
EO	Essential oil
EC50	Half maximal effective concentration
FAO	Food and Agricultural Organisation
FRAP	Ferric reducing ability of plasma
GC-MS	Gas chromatography/mass spectrometry
GRAS	Generally recognized as safe
HD	Hydrodistillation
IC50	Half maximal inhibitory concentration
MIC	Minimum inhibitory concentration
MBC	Minimum bactericidal concentration
RC50	Concentration of antioxidant required to achieve absorbance equal to 50% that of control containing no antioxidant
SEM	Scanning Electron Microscopy
SFE	Supercritical fluid extraction
SFME	Solvent-free microwave extraction
TBARS	Thiobarbituric acid reactive substances
TEM	Transmission electron microscopy

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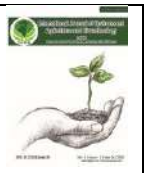

Peer Reviewed

Socioeconomic Characteristics of Farmers, Profitability and Militating Factors Affecting Small Ruminant Production in Ondo State, South-West, Nigeria

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Abstract— *The study investigated the socio-economic characteristics of small ruminant farmers, profitability and militating factors affecting small ruminant production in Ondo State, South-West, Nigeria. A multi-stage sampling technique was used to sample Two Hundred respondents (200) from the four Agricultural Development Programme (ADP) Zones in the study area, in which 25 farmers were randomly selected from 8 different communities. The respondents were accessed and interviewed using a well-structured questionnaire and interview guide, and data collected were analyzed using descriptive statistics such as frequencies and percentages, budgetary analysis and 4-Point Likert type scale. Findings revealed that 60.5% of the respondents were practising semi-intensive management systems, goats were the commonest animals reared by the farmers. The Return on Investment (ROI) was found to be 1.54 which implies that small ruminant producers will realize 1.54 on each naira expended, the gross margin and net farm income shows ₦253, 692.39 and ₦204, 327.08 respectively, Expense Structure Ratio (ESR) of 0.42 and gross ratio (0.45) which also revealed that total revenue accrued from small ruminant production is greater than total cost expended in the course of the business by 55. The study as well identified inadequate capital and high-interest rate of capital as the two most challenging constraints faced by the small ruminant farmers in the study area. Results obtained indicated that small ruminant production is a profitable venture mostly managed under the Semi-Intensive system and requires adequate capital for proper management of the animals.*

Keywords— ***challenges, enterprise, farming, profitability, small ruminant.***

I. INTRODUCTION

Among all the livestock that make up the farm animals in Nigeria, small ruminants, comprising sheep and goat constitute the farm animals largely reared by farm families in the country's agricultural systemThe majority of rural owners of small ruminants are farmers involved in food and tree crop production, or women involved in food processing and marketing [1]. The importance of small ruminants (i.e. sheep and goats) to the socio-economic well-being of people in developing countries in the tropics in terms of nutrition, income and intangible benefits (e.g. savings, insurance against emergencies, cultural and ceremonial purposes) cannot be overemphasized [2]. Sheep and goats are important livestock species in developing countries because of their ability to convert forages, and crop and household residues into meat, fibre, skin, and milk [3]. Sheep and goats play important roles in the socioeconomic and cultural lives of the people in the following ways: they provide meat and milk to humans, they produce wool, hair and skin and manure, they serve as a form of investment and in some cultures or communities in Nigeria, sheep and goats are used to as means of measuring someone's wealth and

they are used during burial ceremonies [4]. Diseases and inadequate nutrition (in terms of quality or quantity) constitute serious constraints to small ruminant production in Africa [5]. Nigeria, in recent times, is being faced by Fulani herdsmen's attack on farmers, thereby posing a great risk on the management and production of small ruminants. Good management practices in terms of adequate nutrition, disease prevention and control and breeding, are essential for improved small ruminant production.

In general, farm animals are poorly managed in Nigeria's agricultural system owing to the fact that the animals are mostly managed on free-range/extensive systems and semi-intensive systems. These management systems are influenced by cheap means of feeding the stock all year round. Based on this, the animals are thus allowed to roam the streets and neighbourhoods to fend for themselves with little or no special or conscientious provision of supplements for them. Although, commonly raised farm animals under the free-range and semi-intensive systems include sheep and goats, alongside chicken constitutes the major farm animals largely raised in these systems of livestock management by the Nigerian rural households or livestock farmers. This practice is believed to have some constraints which are believed to influence the profitability of the enterprise. Thus, this study will provide insight into the socioeconomic characteristics of the farmers, profitability and constraints of small ruminant production in Ondo State.

II. METHODOLOGY

The study was carried out in Ondo State, South-West Nigeria. The State has eighteen local governments which cover a land area of 14,793 square kilometres. Based on the result of 2006 census, the population was 3,441,024 [6]. Data used in this study was from the primary source and a total of 200 respondents were accessed and interviewed using a well-structured questionnaire and interview guide. A multi-stage sampling technique was used in selecting respondents for the study through the four Agricultural Development Programme (ADP) zones in the State. The purposive selection was used to select one local government from each of the four ADP zones (namely: Owo, Okitipupa, Ondo and Ikare zone) in the state. Two communities were purposively selected from the local governments in the second stage, while a random selection of 25 farmers from each of the eight communities earlier selected bringing was done in the third stage, making it a total of 200 respondents that were accessed and interviewed. Data from the study were analysed using descriptive statistics, budgetary analysis and a 4-Point Likert type scale.

III. RESULT

3.1 SOCIO-ECONOMIC CHARACTERISTICS OF RUMINANT FARMERS

Table 1 shows the sex distribution of the sampled ruminant farmers. The sampled small ruminant farmers are made up of 106 males and 94 females which are 53% and 47% respectively. This indicates that more males are practising small ruminant production than females. 1% of the farmers are less than 20 years of age, while 3.5% are between 21-30 years of age and 12.5% are between 31-40 years of age. The age range with the highest percentage of 32.5% were the small ruminant farmers between the age of 41-50 years of age, followed by farmers between the age of 51-60 years of age with 28.5% and 22% of them are above 60 years of age. The mean age of all the sampled small ruminant farmers is 51.4 years. The result revealed that 3.5% of the respondents were single, 71% were married, 3.5% were divorced, 19.5% were widowed and 2.5% were separated. This implies that majority of the small ruminants were married, some were widowed while very few were single, divorced and separated. The largest houshold size had within 1-5 members which is 50.5% of the total respondents, 43.5% had between 6-10 members and 6% had above 10 members. The average household size is 6, which implies that having a large household size helps to support small ruminant farming. 64.5% of the respondents were Christians, 34.50% of them were Islam and 1% of them practised other religions. This indicates that the majority of the small ruminant farmers practice Christianity, followed by Islamic religious practice. The educational level of small ruminant farmers from the result showed that 35% of the respondents had tertiary education, 27% had secondary education, while 12.5% had primary education. 4% of the respondents had no formal education and 4% also attempted primary education. The average years of education of the respondents is 11.2 years.

Table 1 shows the primary occupation of the respondents. The table reveals that 44.5% of the respondents were primarily farmers, 21.5% were Civil Servants, 8% were pensioners, 14% were Artisans, 1.5% were students while 10.5% of them had unspecified occupations (others). Majority of the respondents were primarily farmers, while civil servants and artisans. 53.5% of the respondents were engaged in off-farm income-generating activities while 46.5% were not. 83.5% of the respondents were not a member of any Cooperative Society while 16.5% were members of a Cooperative Society. The result revealed that 92% of the respondents did not take loan in the last production season while 8% of them took. Futhermore, Table 1 shows that 35% of the respondents had 1-10 years of farming experience, 32.5% of them had 11-20 years of farming experience, while 20% had 21-30 years of farming experience. Only 13.5% of the respondents had

more than 30 years of farming experience. 63.5% of the respondents were crop farmers, 22% of them were poultry farmers, 10.5% were fishery farmers, while the remaining 4% of the respondents were involved in snailry, rabbitry, and other farm activities. From the result, majority of the respondents were also engaging in crop farming, followed by poultry farming.

3.2 PROFITABILITY OF SHEEP AND GOAT PRODUCTION

Table 2 shows the budgetary analysis of sheep and goat production. The Budgetary analysis comprises total revenue and total cost of production. The table revealed that the cost of Nanny, Billy, Ewe, Kid and Ram with 30.70%, 15.34%, 10.80%, 9.41% and 8.70% respectively had high percentages of variable costs, while the cost of others, feed and fuel with 0.09%, 0.29% and 0.29% had the least. For fixed costs, the cost of land had the highest with 41.77% while the cost of others with 0.13% has the least. The table shows that the total variable cost was ₦115,648.38, the total fixed cost was ₦49,365.30 which made the total cost to be ₦165,013.68, while the total revenue was ₦369,340.76. The gross margin of the small ruminant production was ₦253,692.39 and the benefit-cost ratio was 2.24, which indicates that small ruminant enterprise is profitable.

3.3 CONSTRAINTS MILITATING AGAINST SMALL RUMINANT'S PRODUCTION IN THE STUDY AREA

Table 3 shows the constraints militating against small ruminant farmers in the study area ranked in order of the most challenging to the less challenging. The small ruminant farmers were faced with challenges which range from inadequate capital (Weighted Mean Score 3.51), High-interest rate of capital (WMS 3.46), Scarcity or high cost of land (WMS 3.29), High cost of transport (WMS 3.16), Vagaries of weather (WMS 3.12), and Theft (WMS 3.11) being the first ranked 6 challenges. The table indicates that the small ruminant farmers were majorly faced with the lack of capital as their major challenge. Theft posed a great challenge to the small ruminant production enterprise and this indicates that most of the farmers had experienced the theft of their stocks at one particular point or the other.

Too much rainfall (WMS 2.62), poisoning (WMS 2.59), Lack of proper care (WMS 2.52), High mortality rate (2.47), fluctuating quality of concentrates and forages (WMS 2.40) and Lack of rainfall (WMS 2.37) ranked the least 6 constraints in the table. Scarcity of skilled and diligent workers (WMS 2.90) was ranked 10th on the table, this could be attributed to the fact that most of the farmers made use of unskilled family labour in the small ruminant production, thereby posing a challenge to the enterprise. Also, Fulani herdsmen (WMS 2.92) was ranked 11th on the table. This showed that Fulani herdsmen insurgency within the country also affect the small ruminant production.

Table 1: Socio-Economic Characteristics of Small Ruminant Farmers in Ondo State, Nigeria

Variables	**Freq.**	**%**	**Mean**	**Variables**	**Freq.**	**%**	**Mean**
Sex				Off-Farm Activity			
Male	106	53.0		Yes	107	53.5	
Female	94	47.0		No	93	46.5	
Age				Agricultural Enterprise			
<20	2	1.0	51.4 Years	Crop Farming	127	63.5	127
21-30	7	3.5		Fishery	21	10.5	21
31-40	25	12.5		Poultry	44	22.0	44
41-50	65	32.5		Snailry	3	1.5	3
51-60	57	28.5		Rabbitry	2	1.0	2
>60	44	22.0		Others	3	1.5	3
Marital Status				Farming Experience (in years)			
Single	7	3.5		≤10	70	35.0	18.8 Years
Married	142	71.0		11-20	63	31.5	

Divorced	7	3.5		21-30	40	20.0	
Widowed	39	19.5		>30	27	13.5	
Separated	5	2.5					
Household Size (in No.)				Management System			
1-5	101	50.5	6 members	Intensive	38	19	
6-10	87	43.5		Semi-Intensive	121	60.5	
>10	12	6.0		Extensive	41	20.5	
Religion				Type of Small Ruminant			
Christianity	129	64.5		Sheep	15	7.5	
Islamic	69	34.5		Goat	140	70.0	
Others	2	1.0		Both	45	22.5	
Education (in level)				Type of Labour			
No Formal Education	8	4.0	11.2 years	Hired Labour	25	12.5	
Attempted Primary	8	4.0		Family Labour	170	85.5	
Primary	25	12.5		Contractual Labour	4	2.0	
Attempted Secondary	7	3.5		Communal Labour	1	0.5	
Secondary	55	27.5		Cooperative Membership			
Attempted Tertiary	27	13.5		Yes	33	16.5	
Tertiary	70	35.0		No	167	83.5	
Primary Occupation				Monthly Income (in Naira)			
Farmer	89	44.5		< 10,000.00	27	13.5	₦26,146.50
Civil Servant	43	21.5		10,000.01 - 20,000.00	45	22.5	
Pensioner	16	8.0		20,000.01 - 30,000.00	60	30.5	
Artisan	28	14.0		30,000.01 - 40,000.00	25	12.5	
Student	3	1.5		40,000.01-50,000.00	15	7.5	
Others	21	10.5		>50,000.01	28	14.0	
Method of Acquisition				Land Acquisition			
Contractual	11	5.5		Inheritance	40	20.0	
Purchased	140	70.0		Purchased	133	66.5	
Inherited	20	10.0		Rent or Lease	19	9.5	
Gift	29	14.5		Gift	8	4.0	
Loan Acquisition							
Yes	16	8					
No	184	92					

Source: Field Survey, 2021

Table 2: Budgetary Analysis of Small Ruminant Production

Item	Average (in Naira)	Percentage of Cost
Variable Cost		
Cost of Ram	10,057.50	8.7
Cost of Ewe	12,492.50	10.8
Cost of Lamb	4,700.00	4.1
Cost of Billy	17,742.50	15.3
Cost of Nanny	35,502.50	30.7
Cost of Kid	10,885.00	9.4
CST of Medicine	4,612.60	4
Cost of Vaccine	1,269.00	1.1
Cost of Veterinary Service	1,982.40	1.7
Cost of Feeds	7,544.25	6.5
Cost of Fuel	335.00	0.3
Cost of Labour	3,505.00	3.0
Cost of Transportation	2,980.125	2.6
Cost of Electricity	330.00	0.3
Cost of Rent	1,610.00	1.4
Cost Others	100.00	0.1
Total Variable Cost	**115,648.38**	**100**
Fixed Cost		
Cost of land	20,620.00	41.8
Cost of fencing	6,975.00	14.1
Cost of pen	6,857.50	13.9
Cost of drinkers	1,546.00	3.1
Cost of feeders	2,169.30	4.4
Cost of gen	2,125.00	4.3
Cost of BHTC	4,505.00	9.1
Cost of electricity	277.50	0.6
Cost of water tank	1,425.00	2.9
Cost of farm vehicle	2,800.00	5.7
Cost of others	65.00	0.1
Total Fixed Cost	**49,365.30**	**100**
Total Cost (TFC+TVC)	165,013.68	
Total Revenue (TR)	369,340.76	
Gross Margin (TR-TVC)	253,692.39	
Net Farm Income (GM-TFC)	204,327.09	
Benefit Cost Ratio (TR/TC)	2.2382	
Rate Of Return of Investment (GM/TC)	1.5374	
Expense Ratio (TFC/TVC)	0.4269	

Gross Ratio (GM/TR)	0.4468

Source: Field Survey, 2021.

Table 3: Constraints Militating against Small Ruminant's Production in the Study Area

Constraints	Strongly Disagree		Disagree		Agree		Strongly agree		Total Score	Weighted Mean Score	Rank
	Freq	%	Freq	%	Freq	%	Freq	%			
Inadequate capital	1	0.5	6	3	84	42	109	54.5	701	3.51	1st
High-interest rate of capital	1	0.5	6	3	94	47	99	49.5	691	3.46	2nd
Scarcity or high cost of land			31	15.5	81	40.5	88	44	657	3.29	3rd
High cost of transport	1	0.5	37	18.5	91	45.5	71	35.5	632	3.16	4th
Vagaries of weather	4	2	16	8	132	66	48	24	624	3.12	5th
Theft	6	3	40	20	81	40.5	73	36.5	621	3.11	6th
Scarcity/poor drug quality	1	0.5	25	12.5	130	65	44	22	617	3.09	7th
Scarcity of quality foundation stock	2	1	44	22	104	52	50	25	602	3.01	8th
Scarcity of suitable equipment			56	28	103	51.5	41	20.5	585	2.93	9th
Scarcity of skilled and diligent workers			51	25.5	115	57.5	34	17	583	2.92	10th
Fulani herdsmen	5	2.5	52	26	102	51	41	20.5	579	2.90	11th
Scarcity of water	6	3	52	26	100	50	42	21	578	2.89	12th
Low or fluctuating price of stock	4	2	50	25	113	56.5	33	16.5	575	2.88	13th
Scarcity of concentrates	5	2.5	61	30.5	91	45.5	43	21.5	572	2.86	14th
Poor market information			83	41.5	72	36	45	22.5	562	2.81	15th
Occasional market	6	3	68	34	88	44	38	19	558	2.79	16th
High cost of forages	6	3	77	38.5	74	37	43	21.5	554	2.77	17th
Outbreak diseases	8	4	70	35	87	43.5	35	17.5	549	2.75	18th
Problem of litter	14	7	73	36.5	73	36.5	40	20	539	2.70	19th
Restriction by environmental law	11	5.5	82	41	72	36	35	17.5	531	2.66	20th
Too much rainfall	11	5.5	90	45	64	32	35	17.5	523	2.62	21st
Poisoning	9	4.5	92	46	71	35.5	28	14	518	2.59	22nd
Lack of proper care	9	4.5	105	52.5	59	29.5	27	13.5	504	2.52	23rd
High mortality	12	6	111	55.5	48	24	29	14.5	494	2.47	24th
Fluctuating quality of concentrates and forages			39	19.5	120	60	41	20.5	479	2.40	25th
Lack of rainfall	17	8.5	115	57.5	45	22.5	23	11.5	474	2.37	26th

Source: Field Survey, 2021.

IV. DISCUSSION

4.1 SOCIO-ECONOMIC CHARACTERISTICS OF RUMINANT FARMERS

The result shows that 53% of the respondents were male while 47% of them were females. The average age distribution of the respondents was 51.4 years. This implies that the matured ones having known the importance of farming enterprise are mostly involved in small ruminant production while the younger ones were less involved in livestock production. This could be attributed to rural-urban migration by the young men for white-collar jobs while some are basically involved in food crop production only. This is in line with the previous that the mean of small ruminant farmers is within the range of 51-60 years [7] and that which states that the majority of the farmers were males (57.87%) as against 42.6% being females and the mean active farming age of small ruminant farmers falls between 45-60 because this group of persons are either retired from other businesses or are tired of the life-styles the city does offer [8]. 71% of the respondents were married, 50.5% were from the household size within range 1-5 while the average household size was 6. This implies that the farmers in the area could readily use family labour in carrying out their farm activities thereby reducing the cost that would have been incurred by hiring labour [9]. This study reports that 35% of the respondents had tertiary education while 27% of them attended secondary school, this made the total of 96% of the respondents had one form of education or the other. This means that majority of the respondents can read and write and this would enable them to easily adopt new innovations and expose them to information that could lead to more efficient farming activities. This corroborates previous studies on the level of education of small ruminant farmers conducted in Ondo State [10,11,12].

Christianity is the religion mostly practised as 64.5% of the respondents were Christians while 35.5% of them were Muslims. The majority of the farmers (44%) were primarily farmers, 21.5% of them were civil servants, 14% were artisans and 8% were pensioners. From this study, 46.5% of the respondents depended solely on net farm income while 53.5% were engaged in off-farm work. The majority of the respondents (86%) earned less than ₦50,000 as their monthly income from off-farm activities. 83.5% of the respondents did not belong to any cooperative society while only 16.5% of them belong to at least one cooperative society, 92% of the respondents have not previously had access to credit loans to support their small production enterprise, while 8% of them had. This could be attributed to the fact that most of the farmers have other sources of income being generated from off-farm activities which serve as household income and from which they use as input to their small ruminant production enterprise. This could also be said that most of the respondents did not belong to any cooperative membership because of lack of interest, lack of awareness or difference in ideology. This implies that most of the small ruminant farmers who do not belong to any cooperative society might find it difficult to get access to credit loans that could be used to help enlarge their production enterprise, hence limiting their profit margin. The finding from this study corroborates with a previous study which states that about 67.3% of the respondents are not members of any group while the remaining 32.7% belong to one form of association or the other [10].

The result of this study revealed that 63.5% of the respondents were into crop farming, 22% of them were into poultry farming and 10.5% of them were into fishery farming. 35% of the small ruminant farmers had less than 10 years of farming experience and 13.5% had above 30 years experience. 31.5% and 20% of the respondents had 11-20 and 21-30 years of farming experience with an average of 18 years and 8 months of farming experience. This showed that farming is not strange among the respondents in the study area. This finding of this study corroborates with the study that reported that 29.4% of respondents had below 10 years of farming experience, 38.1 had 12-20 years of experience, while 32.6% had more than 20 years of farming experience with the mean within the range of 11-20 farming year experience [8].

This study reports that 7.5% of the respondents reared sheep, 70% reared goats while 22% had both sheep and goats on their farm, and 70% of the animals were purchased. These data imply that goat was the commonest animal reared by the farmers. The ratio of small ruminants agrees with the World Almanac Education Group that Nigeria has a livestock population of 24 million goats, 13.5 million sheep [13]. It was revealed that the majority (75%) of the respondents kept goats, sheep were kept by 10% of the respondents while goats and sheep (combine) accounts for 13.3% [14]. However, 60.5% of the respondent were practising semi-intensive management systems while the least percentage 19% practised intensive management systems. This implies that the farmers provide basic needs such as shelter and limited feeding for the animals while they left the animals to scavenge to meet up with their requirements. The findings of this study contradict a study that showed that 44% of the farmers practised permanent confinement (intensive system), 41.25% of the farmers practised partial confinement or tethering (semi Intensive system) and 15% of the sampled respondents used the free-range (extensive) management system where the animals are allowed to roam about and feed themselves [12].

4.2 PROFITABILITY OF SHEEP AND GOAT PRODUCTION

The result from the budgetary analysis of this study revealed that variable cost takes more than 50% of the total cost of production of small ruminants. The outcome of the cost analysis is in line with previous findings where it was reported that variable costs always take more than 50% of the cost of marketing of most agricultural enterprises [15,16,17]. Again, the value of Return on Investment (ROI) of 1.54 implies that small ruminant producers will realize 1.54 on each naira expended. The value of Expense Structure Ratio (ESR) of 0.42 indicated that the fixed cost incurred in the business is less than money expended on the variable cost by 0.58%, while gross ratio (0.45) also revealed that total revenue accrued from small ruminants production is greater than total cost expended in the course of the business by 55%. The result of the gross margin and net farm income shows ₦253, 692.39 and ₦204, 327.08 respectively. All these profitability measures confirmed and reiterated the profitability of small ruminants' production in the study area.

4.3 CONSTRAINTS MILITATING AGAINST SMALL RUMINANT'S PRODUCTION IN THE STUDY AREA

Inadequate capital and high-interest rate of capital were the two most challenging constraints faced by the small ruminant farmers in the study area. This implies that some people do not venture into small ruminant production or practiced it on large scale because of inaccessibility to fund (capital) and a high rate of interest on capital. This aligns with previous findings where it was reported that the respondents identified the inadequate fund as their major constraints that militated against small ruminant production in the study area [12,14]. Unavailability of funds (38.6%), theft (60.4%), accessibility to the market (50.5%), no credit facilities (70.3%) and transportation problems (74.3%) were seen as constraints facing small ruminant livestock animals [11]. It was stated in a study that pilfering (theft) was ranked the 8th constraint faced by the respondents, and this is almost in tandem with that of this study as theft was ranked 6th [12]. The result of this study also agrees with a previous study which states that the cost of labour, theft and unavailability of funds are major constraints affecting small ruminant livestock animals [18]. In this study, high cost of transportation was ranked 4th which indicates that it is one of the challenges faced by the respondents, but the finding in study [11] negates this as it was recorded there that 74.3% of the respondents felt that transportation was never a problem for them in the study area.

V. CONCLUSION

In this study, it was observed that small ruminant production is profitable and viable in the study area and the majority of the small ruminant farmers in the study area reared goats using intensive management systems, this could be attributed to the fact that it is possible to rear goats on free-range which is less costly and with less managerial involvement. This study also revealed that the majority of the small ruminant farmers in the study area were Christians, married, male with an average age of 51.4years and household size 6. From this study, 96% of the respondents had one form of education or the other, therefore, they will be able to read and understand instructions required for technical expertise in animal husbandry. The majority of the respondents were primarily farmers, they mostly practised crop farming alongside small ruminant production, while 53.5% of them were involved in other off-farm generating income activities. This study revealed that 83.5% of the respondents did not belong to any cooperative society which implied that there was a limited number of respondents that had access to credit loans to just 8%.

It could also be concluded from this study that small ruminant production is profitable and viable in the study area with; Return on Investment (ROI) of 1.54 which implies that small ruminant producers will realize 1.54 on each naira expended, the gross margin and net farm income are ₦253, 692.39 and ₦204, 327.08 respectively, Expense Structure Ratio (ESR) of 0.42 and gross ratio (0.45) which also revealed that total revenue accrued from small ruminants production is greater than total cost expended in the course of the business by 55%. The study as well identified inadequate capital and high-interest rate of capital as the two most challenging constraints faced by the small ruminant farmers in the study area.

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Resource use efficiency among Sweet Potato Farmers in Ifedore Local Government Area of Ondo State

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Abstract— *The study examines the resource use efficiency among sweet potato farmers in Ifedore Local Government Area of Ondo State. Specifically, it described the socio economic characteristics of the respondents, determine allocative efficiency of sweet potato production, determine factors that affect sweet potato production in the study area. Multistage sampling techniques was used to select two districts from the study area, ninety eight (98) respondents was randomly selected. Data were collected through the aid of a well-structured questionnaire and analyzed using descriptive and inferential statistics. The result of the findings revealed that the respondents were mostly male (71.4%), 78.6% were married, 36.7% had secondary education, 78.6% had years of experience of between 1-5years, 41.8% are between the age of 30-40years. Land and fertilizer were underutilized, output can increase if the quantities of these resources are increased and capital input was over utilized. Linear regression showed that there is significant relationship between educational levels, quantity of fertilizer, improved tillage practice and yield per hectare. Stochastic frontier result revealed that labor and farm size was significant at 1%. Pest and disease is the major constraints faced by the farmers. Therefore, it was recommended that farmers should use disease resistant varieties.*

Keywords— ***Sweet Potato, Production, Ifedore and Resource use.***

I. INTRODUCTION

The persistent food crisis in Nigeria has been a thing of concern for the government, development partners and the ordinary citizens. Over 40% of the Nigeria's estimated population of 133 million people is food insecure (Idachaba, 2004). This is partly because the food production in the country has failed to respond adequately to increase in demand for food by the ever-increasing population (Babatunde, *et al.*, 2005).

Sweet Potato is an important food crop of both tropical and sub-tropical regions with a cultivation spread across 100 countries according to Nnamdi *et al., (2015)*. Ndukwu (2010) noted that the crop has high photosynthetic efficiency. It has a high yielding capacity per unit area and ability to sustain human livelihood during periods of food shortage. Farmers engage in massive production of this crop because of its short gestation period and its ability to suppress weeds once it has been fully established, thus reducing the overhead cost of production more than that incurred by cassava and yam (Nnamdi *et al* 2015).

Sweet potatoes offer a particularly significant potential for increasing food production and income thereby reducing poverty and improving food security level in Nigeria. Sweet potatoes are consumed without much processing in most parts of the tropics (Ahmad *et al.*, 2014).

Sweet potatoes present diverse industrial uses, some of which are potentially highly profitable, such as sweet potato snacks (Adewumi and Adebayo, 2008). They are extremely adaptable to adverse environmental conditions; they can help increase food security in times of

drought and famine, particularly in post-conflict areas for displaced persons. (Andrade *et al.*,2009).

The importance of sweet potato is increasing in Nigeria's farming and food systems because its production has recorded good profit margin and is suitable for income generation. It has the potential for food security as well as serving as a cash crop (Adekoya *et al.*, 2010). It has edible tubers which can be eaten boiled, fried, or baked. The tubers can be consumed by man, the leaves and stems can provide important fodder sources for domesticated animals. Sweet potatoes produce carbohydrates much faster and require less labor than other crops. Sweet potatoes are used to restore access to food for resetting populations and alleviate future agro-climatic or political shocks. The challenge with using sweet potatoes in emergency response situations is the crop's low multiplication rate. Vine material needs to be ready to go and mechanisms in place to distribute vine materials to needy farmers. (Andrade *et al.*, 2009)

Spent fields of sweet potato have been widely noted as supplementary pig forage. The leaves are also consumed as vegetables because its leaf contains (on dry matter basis) about 8% starch, 4% sugar, 27% protein and 10% ash (Adekoya *et al.*, 2010). The leaves are much richer (than the root) in protein, minerals and vitamins and therefore are more nutritious (Adewunmi and Adebayo, 2008). Industrially, sweet potato flour can be used to substitute wheat bread making or maize flour in balanced feeds. Baby foods have been formulated using sweet potato while some bakeries blend 15-30% of sweet potato flour for making bread and 20-30% for pastries. It is also used in the brewing of alcoholic drinks and as sweeteners in non-alcoholics drinks (Agbo and Ene,1992). Sweet potato starch can also have medicinal value. According to Aboajah, *et al.*, (2018), the leaves decoction is used in folk remedies for tumor of the mouth and throat. Reported to be alternative, aphrodisiac, astringent, bactericide, demulcent, fungicide, laxative and tonic, industrial potentials of sweet potato have not been fully exploited due mainly to a chronic lack of awareness of the commercial benefits derivable from sweet potato (Azogu and Olomo, 2002).

Sweet potato has also been used in Africa to fight vitamin A deficiency that result in blindness and even death of about 25,000 – 500,000 African children per year (CIP, 2009). The leaves contain vitamin A with sufficient quantities of *beta-carotene***.** Vitamin A deficiency is a particular problem with children under five and for pregnant and lactating women (Sanusi and Adesogan, 2014).

Despite all these importance of sweet potatoes, its production has been low, these could be due to inadequate use of disease resistant varieties and high yielding sweet potato clones from National Root Crops Research Institute (NRCRI) and International Institute of Tropical Agriculture (IITA). The wide use of unimproved varieties such as *Cylaspuncticollis* by farmers due to shortage of improved planting materials at the beginning of every cropping season could be a reason for the low output of sweet potatoes.

It has been reported in several studies such as Nnamdi *et al.*, (2015), Ahmad *et al.*, (2014) and Nwaru *et al.*,(2011) that resources are not adequately utilized by sweet potato farmers leading to low yield and low income. Poor resource farmers' find it difficult to purchase inputs and adopts technology faster leading to low productivity. Therefore, this research aim to answer the following research questions to determine the resource use efficiency of potato production in Ifedore Local Government Area of Ondo State. What are the socio economic characteristics of the respondents? How technically efficient is sweet potato farming in the study area? How do farmers allocate their resources during production? What factors affect sweet potato production in the study area?

Specifically, the study sought to:

i. describe the socio economic characteristics of the respondents
ii. estimate the technical efficiency of potato production in the study area.
iii. determine the allocative efficiency of potato production in the study area.
iv. determine factors affecting potato production in the study area.

II. METHODOLOGY

The study was carried out in Ifedore Local Government Area of Ondo State, Nigeria. It lies between latitude 7.34°N and longitude 5.08°E within the tropical rainforest zone of Nigeria.

It has land area of 295km^2 and population of 176,327 at the 2006 census. It has an annual rainfall often exceeding 2000mm, while soil temperature has narrow range of 27°C and 28°C.

The inhabitants are majorly civil servant and farmers. Crops majorly grown include sweet potato, plantain, oil palm and yam. The Local Government is divided into five districts of Ipogun, Ijare, Ilara and Igbara Oke districts. These districts are further divided into towns and villages.

Sampling Procedure

Multistage sampling technique was used for the study. First stage was the random selection of two districts

from the five districts due high level of sweet potato production in the districts. The second stage involved random selection of five villages from the two districts and the third stage was the random selection of the ten (10) respondents from the villages however, 98 questionnaire was retrieved for further analysis.

Data Collection

Primary data was used for this study. A well-structured questionnaire was administered to the farmers since they were considered literate.

Demographic and non-demographic data was collected from the farmers. Information on age, gender, marital status, educational status, Area cultivated, input used and output etc. was also collected.

Analytical Techniques

Descriptive statistics such as percentage, frequency and mean were employed to describe the socio-economic characteristics of the respondents. Inferential statistics such as Cobb-Douglas stochastic frontier production function was used for objective ii, Allocative Efficiency Index was used for objective iii, while simple regression analysis was also used for objective iv

Simple Linear Regression

To determine factors affecting sweet potato production in the study area, the simple linear regression was used. The model is specified below

$Y = f(X_1\ X_2\ X_3\ X_4\ X_5\ X_6X_7\ X_8\ X_9)$……...(i)

Where;

Y_i= Sweet potato production in kilograms per hectare

X_1= Gender

X_2= Age in years

X_3=Educational level in years

X_4 = Farm size measured in hectares

X_5= Years of experience

X_6 = Access to credit

X_7 = Fertilizer application

X_8 = Extension visit

X_9 = Tillage practice

Allocative Efficiency Index

The allocative efficiency index (AEI) was used to determine the efficiency of resources used in production of sweet potato. This was done by computing the ratio of the marginal value product (MVP) to the marginal factor cost (MFC) used in production. The ratio used in determining the efficiency of resources was calculated as:

AEI=MVP or K= MVP, MFC Px ……. (ii)

Where: AEI or K= Allocative efficiency index, MVP=Marginal value product of the various inputs. (MPP x Py) MPP= marginal physical product, Py = unit price of output, MFC= Marginal factor cost (cost of unit input) (Px).

Rule of thumb: if the ratio is equal to one, it indicates that the resource is efficiently or optimally utilized. If the ratio is greater than one, it is indicative that the resource is underutilized, if the ratio is less than one, it indicates that resource is excessively utilized. Mathematically, this can be represented as:

MVP =1; the resource used is optimally efficient,

MVP >1; the resource used is under utilized

MVP < 1; the resource used is over utilized

The frontier model

The stochastic frontier production function for Sweet Potato production adopted in this study as specified by the Cobb-Douglas functional form (*Ahmad et al, 2014*) was defined thus:

$\text{Log } Y_i = b + b_1\log X_1 + b_2\log X_2 + b_3\log X_3 + b_4\log X_4 + b_5\log X_5 + b_6\log X_6 + e$….(iii)

Where Y_i = output of the farmer (kg)

X_1 = is labour

X_2 = is sweet potato vine

X_3 = is fertilizer in kg

X_4 = is capital inputs

X_5 = is farm size

Sweet potato output was expected to be influenced positively by labour, sweet potato vine, fertilizer used, capital inputs, and farm size. The functional form for the stochastic frontier is defined by equation (iii).

III. RESULTS AND DISCUSSION

Table 1: socio-economic characteristics of the Respondents

Characteristics	Frequency	Percentage (%)
Gender		
Male	70	71.4
Female	28	28.6
Age (years)		
Below 30	19	19.4
30-40	41	41.8
41-50	26	26.5
Above 50	12	12.2
Marital Status		
Single	13	13.6
Married	77	78.6
Divorced	5	5.1
Separated	1	1.0
Widowed	2	2.0
Education Level		
Primary Education	17	17.3
Secondary Education	36	36.7
Tertiary Education	32	32.7
Adult Education	13	13.3
Years of Experience		
1-5	77	78.6
6-10	13	13.3
11-15	7	7.1
16-20	1	1.0
Total	**98**	**100.0**

Source: Field Survey, 2021

Table 1 showed that 41.8% of the respondents are aged between 30 – 40 years, 26.5% are aged between 41 – 50 years, 19.4% are below 30 years while only 12.2% are above 50 years of age. According to Okoye *et al.*, (2008), the result showed that young people are involved in sweet potato production in the study area. This could be as a result of unemployment in the country and this serve as a means of employment to able bodied youths. Furthermore, 71.4 of the respondent are male while 28.6% of the respondents were female; According to Bonabana-Wabbi *et al.*, (2013), male are more involved in the production of sweet potato because male have more strength and energy to carry out agricultural activities than female. The result also shows that 78.6% of the respondents are married, 13.6% of the respondents are single. This shows that majority of the sweet potato farmers are married and have family responsibilities which sweet potato can cater for. Also they will have more helping hands on the farm. Also, 36.7% of the respondents had secondary education, 32.7% of the respondents had tertiary education, 17.3% of the respondents had primary education. According to Okoye *et al.*, (2008), the result shows that all the respondents are educated and can read and write which will enhance production through adoption of innovation. 78.6% of the respondents had their years of experience ranging between 1 – 5 years, 13.3% of the respondents had their years of experience ranging between 6 – 10 years, 7.1% had 11 – 15years experience while 1.0% of the farmers had16 – 20years experience. The results showed that majority don't have enough experience in sweet potato production which may affect their productivity.

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Table 2: Linear Regression Result

Unstandardized coefficient and standardized coefficient

Variable	Co efficient	Std. error	Beta	t – value
Constant	935.298	304.401		3.073
Gender (X_1)	-126.514	86.519	-152	-1.462
Age of respondents (X_2)	-48.279	46.239	-1181	-1.044
Educational level (X_3)	-91.506	38.503	-271	-2.377 **
Farm size (X_4)	-1.166	23.101	-006	-005
Years of experience (X_5)	-6.502	11.603	-063	-560
Access to credit (X_6)	-6.733	93.236	-079	-072
Fertilizer application (X_7)	-140. 420	88.759	-179	1.582
Extension visit (X_8)	-68.764	88.211	-091	-780
Tillage practice (X_9)	-136.754	84.976	-181	-1.609 **

Source: Field Survey, 2019.

**Significant at 10%,

Dependent variable yield: Productivity

$R^2 = 0.587$

$R^{-2} = 0.371$

F value = 1.416

The result of the regression analysis for factors affecting sweet potato output is presented in table 2. The R^2 value of 0.587 indicates 58.7% variability in sweet potato output is explained by the independent variables. The coefficient of Educational level (X_3) was negatively signed and significant at 10% level of probability. The coefficient of quality of fertilizer (X_7) was positively signed and significant at 10% level of probability, also the coefficient of Improved tillage practice (X_9) was negatively signed and significant at 10% level of probability For fertilizer, the result implies that any increase in the amount of fertilizer will lead to a corresponding increase in sweet potato output. The result for educational level and improved tillage practice do not follow appropriate expectation as they were negative and an increase in their level will reduce output. The co efficient of Gender (X_1), Age (X_2), Farm size (X_4), Years of experience (X_5), access to credit (X_6), Extension visit (X_8) were all negatively signed and not significant at any level of probability. This indicates that any increase in the level of these variables will reduce sweet potato output in the study area.

Table 3: Allocative Efficiency Index

Resource	App (Kg)	Mpp (Kg)	MVP (N)	MFC(N)	MVP/MFC	Decision
Land	529.69	5855.21	23881.54	2200	10.85	Under utilized
Sweet potato vine	80.73	12.84	476.727	195	2.44	Under utilized
Fertilizer	21.25	2.54	241.889	82	2.949	Under utilized
Capital input	33.20	123.56	505.02	600	0.842	Over utilized
Labour	823.25	2.65	2658.01	1800	1.477	Under utilized

Source: Field Survey, 2019.

Table 3 shows the result of the estimated allocative efficiency index of the sweet potato production in the study area. The result indicated that land, sweet potato vine, fertilizer and labor were under-utilized resources as their allocative efficiency indexes were found to be greater than one. This means that the farmers should increase the level of quantities of these resources used in production. Capital input was over utilized as the allocative efficiency index was found to be less than one. This means that the farmers should reduce the level of capital input used in production in order to maximize output level.

Table 4: Cobb Douglas Stochastic Frontier Production Function

Production factors	parameter	Co efficient	Standard error	t. value
Constant term	β_O	5228	0.6248	11.6412
Labor	β_1	0.0549	0.0211	4.0537
Sweet potato vine	β_2	0.1337	0.0597	2.4317
Fertilizer	β_3	0.0279	0.0139	2.6145
Capital inputs	β_4	0.0627	0.0505	1.237
Farm size	β_5	0.5101	0.0356	12.4271
Diagnostic statistics				
Log – livelihood function		36.5761		
Total variance	σ	0.1223	0.0592	2.2849**
Variance ratio	γ	0.8492	0.0308	31.7151
LR Test		61.3101		
Sigma squared	σ	0.46		

Significant at 10%, 5%, and 1% respectively

The maximum likelihood parameter estimates of the stochastic frontier Cobb Douglas production function were presented in Table 4 for the production function. The sigma squared (0 = 0.46) was statistically significant at 5% indicating goodness of stand the correctness of the specified distribution assumption of the composite error term.

Gamme was estimated at 0.85 and is highly statistically at 1% indicating that only 0.5% of the total variation in sweet potato output was due to technical efficiency.

The co efficient of labor and farm size was statistically significant at 1% level of probability showing direct relationship with output. This implies that a 1% increase in labor and farm size would increase output by 0.0549% and 0.5101% respectively. The co efficient for sweet potato vines and fertilizer were also statistically significant at 5% level of probability. This implies that 0.1% increase in sweet potato and fertilizer would lead to a 0.1337% and 0.0279% increase in output respectively. The coefficient of capital input was not significant.

IV. CONCLUSION

Findings from the study reveals the following, the farmers are young and energetic, they have the energy to carry out farming activities. The studies revealed that majority of the sweet potato farmers are married and have family responsibilities. Capital inputs were over utilized in the study area so farmers can reduce the level of capital input. The farmers should be encouraged to take advantage of the various private and government farm credit schemes to enable them acquire production resources necessary to expand the farm land.

RECOMMENDATIONS

Based on the findings, the following recommendations are made

i. Government should revisit the land use act which will make land ready available for the sweet potato farmers.

ii. Farmers should apply pesticide in other to reduce pest infestation and increase the production yield

iii. Farmers should organize themselves into agricultural cooperative to pull their resources together and purchase inputs for their farming activities.

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Use of Cost-Effective Biofertilizers Interventions for Enhanced food Security and Soil Management Amidst Covid-19 Crisis. Review

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Abstract— *Current soil management strategies are mainly dependent on inorganic chemical-based fertilizers, which are expensive and have led to a serious threat to human health and environment. Moreover, COVID-19 pandemic caused economic recession coupling with the effects of climate change. The effect of the pandemic has led to increased prices of inputs particularly chemical fertilizers, resulting in a reduction in crop production. Soil fertility which is a function of agricultural production has declined overtime due to nutrient depletion. The exploitation of cost effective and beneficial microbes as a biofertilizer has become paramount importance in agriculture sector for their potential role in food safety, sustainable crop production and soil fertility improvement. The eco-friendly approaches inspire a wide range of application of plant growth promoting rhizobacteria (PGPRs), endo- and ectomycorrhizal fungi, cyanobacteria and many other useful microscopic organisms that have led to improved nutrient uptake, plant growth and plant tolerance to abiotic and biotic stress. The present review highlights; biofertilizers relevance and plant tolerance to environmental stress, biofertilizer exploitation and nutrient profile of crops, potential significance of beneficial microbes in sustainable agriculture. The knowledge gained from the literature appraised herein will help in understand the benefits of biofertilizers towards sustainable agriculture in reducing problems associated with the use of chemicals fertilizers.*

Keywords— ***Biofertilizer, Crop improvement, Food security, Soil fertility management, Covid-19 pandemic, Sustainable agriculture***

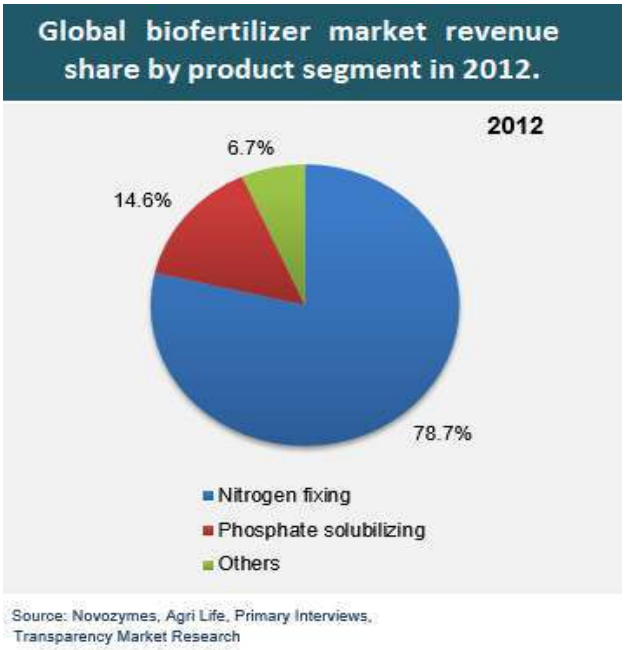
I. INTRODUCTION

The world population is estimated to reach about 9 billion by 2050 and Africa, especially sub-Saharan Africa, has been predicted to contribute to bulk of the increase (Godfray et al., 2010; United Nations, Department of Economics and Social Affairs, (UN DESA, 2015). In this context, sub-Saharan Africa needs to increase food production to feed her growing population adequately. This growing population has triggered competition in all forms of resources required for human survival such as land, water, energy and food. Perhaps, the most essential is the food resources that have become insufficient, and consequently, its increased production cannot be compromised (Asenso and Jemaneh, 2012).

Sadly, many African countries lack political will that can drive agricultural policies, and this has caused low agricultural productivity. Lack of attention for improving agricultural productivity has degenerated to a more complex situation of food insecurity, which has caused economic, environmental and financial losses in sub-Saharan Africa (Rosegrant et al., 2015). Therefore, sub-Saharan African nations need to combat food security situation with a scientific, economic and technologically based approach which will increasing agricultural productivity at a rate higher than the population growth

rate, which will in turn trigger economic growth and development (Muzari and Muvhunzi, 2016). Many economic, scientific and technological measures have been designed to support farmers and increase food production in developed countries.

Biofertilizers are considered as strong alternative for minimizing the use of various types of synthetic fertilizers which are not only costly but also cause adverse effects on soil health. In general, biofertilizers include living microorganisms which provide nutrients and promote growth and development of plants via the natural processes such as nitrogen fixation, phosphate solubilization, production of hormones, and other plant growth-promoting substances (Rai, 2006; Kumar et al., 2017). Biofertilizers are ecofriendly, inexpensive, important source of essential nutrients for plants, and increase soil fertility as well as play a vital role in improving soil nutrient status and, thus, crop productivity. Biofertilizers are living formulations consisting of advantageous microorganisms, including fungi, bacteria, and actinomycetes, that can be applied successfully to seeds, seedlings, plant roots, or soil and which help in the mobilization as well as the accessibility of nutrients due to their inherent biological activities (Pal et al., 2015).



1.2 OBJECTIVE OF THE REVIEW

This review was aimed at highlighting the huge economic importance associated with the use of biofertilizers in improving crop productivity and soil fertility management sustainably among in sub-Saharan Africa.

II. METHODOLOGY

The search strategy was designed by the author to identify articles that address the effects of biofertilizers with regard to sustainability in soil management and agricultural production. A filter of subject-related key words was used to obtain relevant articles. The filter for defining biofertilizers was comprised of the following phrase and words; as major types of biofertilizers in soil fertility management, including nitrogen fixation, mycorrhizal Fungi, plant growth promoting rhizobacteria, potassium solubilizing bacteria, contribution of biofertilizers in agriculture such as increased yield and nutrient availability, prevention of plant pests and diseases, water stress resistance. Some of the parameters of concern were obtained from individual researchers' publications that outlined in-depth the use of biofertilizers in agriculture.

2.1 SELECTION OF ARTICLES

The study undertook the article selection process following two steps; i) Preliminary review of titles using the key words. (ii) Review of article abstracts. The selected abstracts had to address the contributions of biofertilizers in soil management and agricultural production. Thus the articles were eliminated based on inconsistency with the search strategy and criteria, this followed the article review form to classify and describe the characteristics of each article including study aims, parameters discussed and the mentioned effects of biofertilizers in agriculture. However, the effects and results of the different biofertilizers varied slightly across the articles reviewed.

III. RESULTS AND DISCUSSIONS

3.1: MICROBES-SOIL-PLANT INTERACTIONS

Soil is a complex mixture of minerals, water, air, organic matter, billions of organisms, and the changes taking place in its composition (biogeochemical transformations). Soil fertility refers to the capacity of the soil to supply essential plant nutrients such as N, P, K and micronutrients, which are often not available in free form or are in limited quantities in the soil. This is where root-associated beneficial microbes are important partners. It is known that microorganisms can make nutrients available to plant by different mechanisms (Ahemad & Kibret 2014). In the soil, it is possible to find various types of microorganisms such as bacteria, fungi, actinomycetes, protozoa, and algae which bacteria are by far the most common (i.e., ~ 95%).

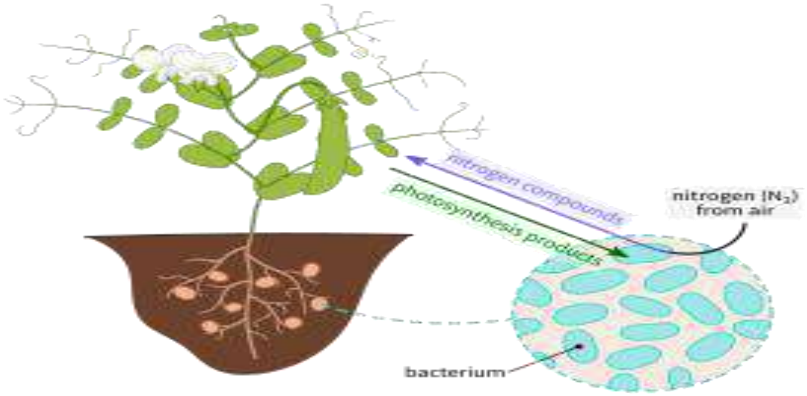
There is an estimated 60,000 different type of bacteria that reside in the soil, most of which have yet to be even named, and each has its own particular roles and capabilities. The number and diversity of bacteria are influenced by the soil conditions such as organic carbon, temperature, moisture, electrical conductivity and other

chemicals as well as by the number and types of plants found in those soils. Therefore, soil-grown plants are immersed in a sea of microorganisms especially bacteria (Chianu et. Al., 2010). Recent studies show that most plant species require microbial associations for survival (Balestrini, 2015). In addition, plants possess the ability to select their own root microflora from the surrounding soil. In other words, each particular plant species has a characteristic group of associated microbes. The establishment of beneficial plant–microbial interactions needs a mutual recognition and a considerable orchestration of the responses at both the plant and the microbial side (Beneduzi et al., 2012). By exuding chemicals or signals, plants can effectively communicate with the rhizosphere microorganisms, while their associated microbes may establish an efficient associative symbiosis with plants by triggering host functional signals (e.g., microbial chemotaxis and colonization). The interactions established between bacteria and plant may be beneficial (e.g., plant growth promoting rhizobacteria, PGPRs), harmful (e.g. pathogens), or neutral for the plant, and sometimes the impact of a bacterium may vary as the soil conditions change (Han, 2006). The microbes that provide some benefits to plants are: (i) those that form nodules on host plant roots (symbiotic relationship) and fix nitrogen; (ii) those that are endophytic and colonize the internal plant tissues without pathogenic effects in host; (iii) those that have ability to competitively colonize the rhizosphere and plant root surface; and (iv) those that are free living in the soil (Gupta et al., 2007). In agriculture, beneficial bacteria are defined as any bacteria that colonize the roots of plants following inoculation onto seed and improve plant growth by increasing seed emergence, plant weight, and crop yields.

3.2: MAJOR TYPES OF BIOFERTILIZERS AND THEIR EFFECT ON SOIL FERTILITY MANAGEMENT

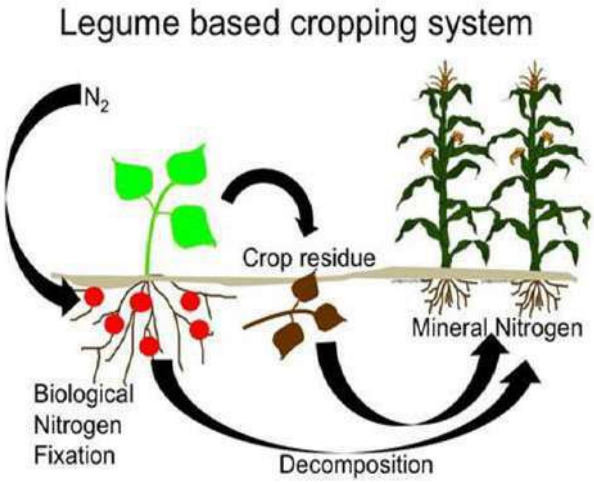
3.2.1 Biological Nitrogen Fixation (BNF)

Bacterial symbionts are well-recognized natural donor of fixed nitrogen as they enrich soil fertility by fixing atmospheric dinitrogen (N_2) resulting in increased growth and yield of rice. Besides N_2 fixation, they produce various types of growth hormones, vitamins, bioactive compounds, organic acids, antagonistic compounds, and play an important role in nutrient cycling (Singh et al., 2016). Cyanobacteria also increase the soil fertility by increasing soil pore size and water holding capacity due to excess mucilage production and can tolerate high dose of pesticides over the recommended levels applied in the field (Kaushik, 2013; Cohen, 2008). In addition, BNF form symbiotic associations with a number of eukaryotic hosts including plants, and cyanobiont present therein fixes N_2, the fixed nitrogen is utilized by the respective hosts (Adams, 2013). The fixed nitrogen may be excreted in the form of ammonia, amino acids, short nitrogenous polypeptides, certain vitamins, and hormones as well as other bioactive compounds.



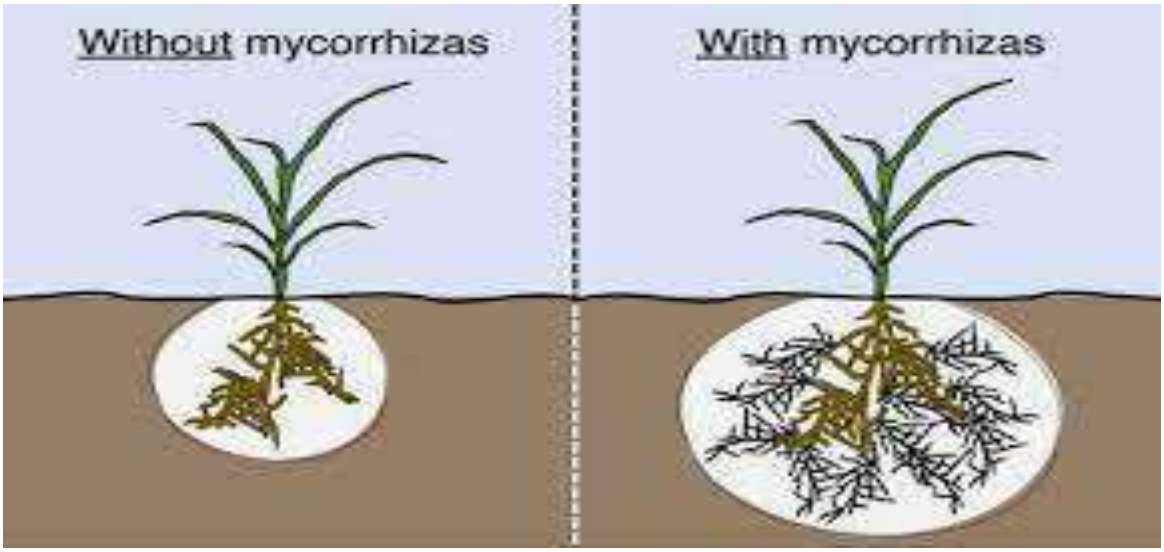
However, bulk of the fixed nitrogen is released after death and decay (autolysis) of cultures followed by decomposition. Potentials of a number of free-living species of cyanobacteria as biofertilizer have been tested in different crops, the results are encouraging (Karthikeyan et al., 2007; Singh et al., 2016). Studies based on $^{15}N_2$ experiments and N-balance suggested that cyanobacteria can fix up to 20-40 kg N ha^{-1} $crop^{-1}$ and the fixed nitrogen is readily available for plants especially the rice plant. $^{15}N_2$ experiments carried out in the field have demonstrated the exchange and bulk accumulation of fixed $^{15}N_2$ in rice plants (Ladha and Reddy, 2011). It has been proposed that BNF especially cyanobacteria can solubilize and mobilize the insoluble P by the enzyme phosphatase. In addition, Ca^{2+} bound P may be dissociated either by the formation of a Ca-specific chelator or by the formation of organic acids (Cameron and Julian, 2010). Subsequently, solubilized P may be directly used by the

plants or indirectly after death and decay of cyanobacteria which had utilized the solubilized P for their own metabolism. Recently, Teikari et al. (2015) have reported the upregulation of phosphorous.



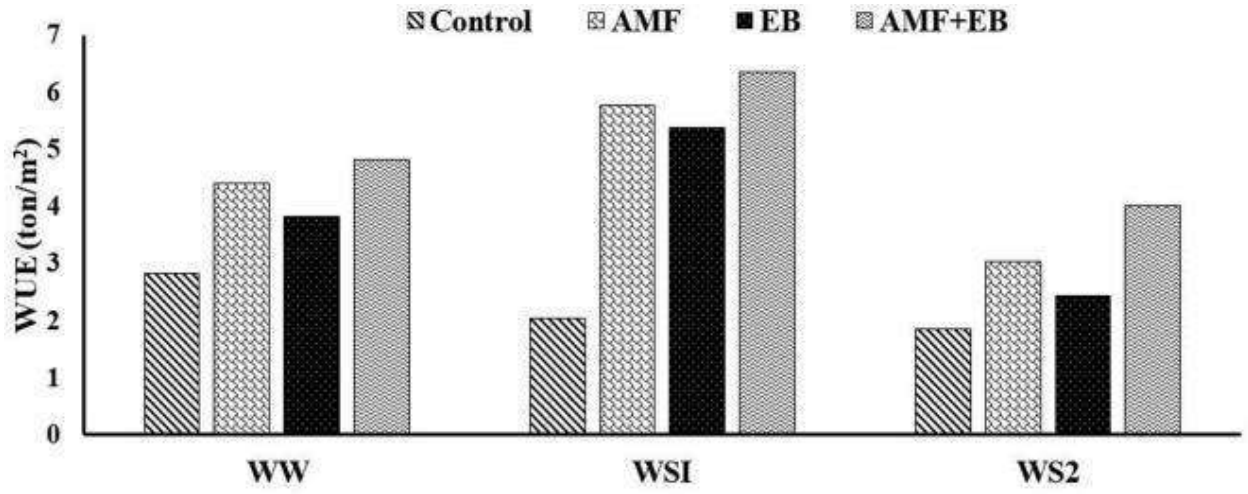
3.2.2: Mycorrhizal Fungi as a Biofertilizer

Fungal biofertilizers, when applied in a natural field system either alone or in combination, are known to cause a direct or indirect beneficial impact on plant development, growth, and yield through several methods (Rai et al., 2013). The roots of different plant groups, such as herbs, shrubs, trees, aquatics, xerophytes, epiphytes, hydrophytes, and terrestrial plants, growing in natural conditions, have been reported to develop mycorrhizal associations when grown in conditions with a low bioavailability of essential elements, including phosphorus, nitrogen, zinc, copper, iron, sulfur, and boron (Zhu et al., 2008).Phosphate solubilizing fungal biofertilizers are one of the most commonly employed biological agents for improving plant growth and development by facilitating phosphorus uptake in plants. Fungi possessing a phosphate solubilizing property contribute significantly to the availability of soil phosphates to plants.



The amendment of yeasts (either live or dead) to soil has been demonstrated to substantially enhance the nitrogen and phosphorus availability to the roots and shoots of *Solanum lycopersicum* L. and sugarcane plants (Mohammadi, 2012. Moreover, yeast amendment to soil was also reported to enhance the root to shoot ratio in both plants and the induction of species-specific morphological alterations leading to enhanced tillering in sugarcane and higher shoot biomass in *S. lycopersicum* L. Brewer's yeast is an inexpensive biofertilizer that enhances plant nutrient status and plant potential, thus, aiding plant growth and nutrient uptake (Lonhienne et al., 2014). Approximately 80% of plant species are reported to have mutualistic association with arbuscular mycorrhizal fungi (AMF). Partial or complete degeneration of AMF activity in soil can lead to significant changes in soil

properties, that directly or indirectly helped in the enhancement of agriculture production.



Effect of arbuscular mycorrhizal fungi(AMF) alone, endophytic bacteria(EB) in combination on seed yield of common bean plants grown under different levels of water stress (WW, WS1, WS2)

3.2.3: Plant Growth Promoting Rhizobacteria as Biofertilizer

Various species of beneficial microorganisms grow in the rhizosphere, participating in nutrient cycling and the production of plant growth promoting substances. Hence, are called plant growth promoting rhizobacteria (PGPR) (Ahemad and Kibret, 2014). Similarly, bacteria such Bacillus and Paenibacillus have been found to cause an increase in plant-mycorrhizal colonization, therefore, are referred to as mycorrhizal helper bacteria (MHB) (Adeleke and Dames, 2014). Some biofertilizing-PGPRs produce phytohormones such as indole acetic acid, gibberellins and cytokinins that cause an increase in plant foliage, root elongation, fruit yield and plant-microbe symbiosis (Hassen et al., 2016; Vacheron et al., 2013). Indole acetic acid (IAA) affects plant root architecture; leading to increased root surface area and root tip elongation (Ahmad, Ahmad, & Khan, 2005; Lu et al., 2015) while gibberellic acid induces increased flowering, stem and internode elongation, fruit setting and growth in plants (Zalewska and Antkowiak, 2013). Therefore, beneficial microbes that promote root development have an immense impact on nutrient uptake ability.

3.2.4: Potassium Solubilizing Biofertilizer (KSB)

Potassium solubilizes aids in solubilizing potassium from compounds such as mica, illite, orthoclase and biotite by producing organic ligands, hydroxyl anions, enzymes and biofilms (Bahadur et al., 2014; Shanware et al., 2014). The ability to solubilize potassium effectively depends on the soil type and microbial strain, as well as the form of K compounds (Bhai and Srinivasan, 2012). Potassium solubilizing biofertilizer include; Pseudomonas, Burkholderia, Bacillus, and Paenibacillus genera (Ahmed and El-Araby, 2012; Liu et al., 2010; Yasin et al., 2016)

IV. ONTRIBUTION OF BIOFERTILISER IN AGRICULTURE

4.1 Increased Yield and Nutrient Availability

Legume yield among smallholder farmers can be increased by using N-fixing biofertilizers such as Rhizobium and Bradyrhizobium (Hassen et al., 2016). For example, inoculation of soybean causes an increase in yield, improves soil organic matter while also fixing about 80% of soybean N need (Giller et al., 2011). Rose et al. (2014) reported that biofertiliser could replace about 52% of N-fertiliser and cause an increase in rice yield over the control. Rhizobium biofertiliser alone can supplement about 50% of the fertilizer need of crops in most arid and semiarid marginal lands of Zimbabwe, Tanzania, and Kenya, which are deficient in N (Chianu et al., 2014). Ronner et al. (2016) also found that crop yield increased by 447 kg/ha over the control due to inoculation in northern Nigeria. Azolla soaked in 50 ppm of superphosphate when inoculated in a paddy field fixed about 40–55 kg N/ha, 15–20 kg P/ha and 20–25 kg K/ha in a month per 1 kg of Azolla applied, bringing the yield of flooded paddy to about 10–20% over the control (Wagner, 2012).

The use of biofertilizers leads to separate accumulation of N, P and K in the soil, thereby maintaining soil nutrient balance (Adesemoye et al., 2008; Egamberdiyeva, 2007). Sundara et al. (2002) observed an increased sugarcane and

sugar yield when the plant was inoculated with PSB, Bacillus magisterium var. Phosphaticum. Similarly, the use of biofertilisers with cheap rock phosphate increased crop yield by 74% over the control, while peanut and sunflower plants inoculated with PSB recorded a significant yield over the control (Ahmed & El-Araby, 2012). According to Bambara and Ndakidemi (2010), the concentrations of Fe, Cu and Zn in the rhizosphere of Phaseolus Vulgaris when inoculated with Rhizobium increased by 28, 20 and 67% respectively compared to the control. This could be due to the role of biofertiliser in improving soil nutrient content and nutrient uptake of the plant (Guimarães et al., 2016).

Some biofertilizing particularly PGPRs produce phytohormones such as indole acetic acid, gibberellins and cytokinins that cause an increase in plant foliage, root elongation, fruit yield and plant-microbe symbiosis (Hassen et al., 2016; Vacheron et al., 2013). Indole acetic acid (IAA) affects plant root architecture; leading to increased root surface area and root tip elongation (Ahmad, Ahmad, & Khan, 2005; Lu et al., 2015) while gibberellic acid induces increased flowering, stem and internode elongation, fruit setting and growth in plants (Food and Agriculture (2017). Therefore, beneficial microbes that promote root development have an immense impact on nutrient uptake ability.

4.2 Prevention of Plant Pests and Diseases

Some biofertilizers prevent plant diseases by directly inhibiting pathogens through their metabolic activities or indirect competition (García et al., 2015; Rudrappa et al., 2008). The nodule-forming symbiotic association of legumes with Rhizobium has been established to enhance the synthesis of cyanogenic defense substances, which increases plant resistance to herbivore attack (Mazid et al., 2011; Megali et al., 2015; Thamer et al., 2018). Bacterial and fungal attacks are major factors affecting smallholder productivity, especially in sub-Saharan Africa (Strange & Scott, 2005). Fusarium wilt of pigeon pea and soft rot of potato caused by Fusarium and Erwinia Carotovora can be controlled by Pseudomonas fluorescent and sinorhizobium, both producing chitinase and β-glucanases (Guo, et al., 2013; Kumar et al., 2017).

Bacillus sp. inhibit important pathogens such as Rhizoctonia solani in tomatoes and Phytophthora capsici in pepper (Solanki et al., 2012). Some biofertilizers produce siderophore, a Fe-chelating agent, which limits the available Fe in the soil. This indirect competition for nutrients suppresses the pathogen's ability to cause diseases (Arora, Khare, & Maheshwari, 2010; Solanki et al., 2014). Siderophores produced by Pseudomonas and Bacillus attack the popular fusarium wilt of potato and maize, thereby increasing potato and maize yield of smallholder farmers (Beneduzi et al., 2012). Similarly, Pseudomonas aeruginosa is used against bacterial blight caused by Xanthomonas oryzae and Rhizoctonia solani, which are major rice diseases in West Africa (Mali, Senegal, Nigeria, Niger etc.) (Nga et al., 2013)

4.3 Water Stress Resistance

Many African countries, especially the arid and semi-arid areas, have long drought season and this has caused limitation to plant growth (Falkenmark & Rockström, 2008). In this situation, biofertilizers, which enhance plant water-stress tolerance, is of immeasurable importance (Dimkpa, Weinand, and Asch, 2009; Hassen et al., 2016). The production of auxins, cytokinins, gibberellins and 1-aminocyclopropane-1-carboxylate (ACC) deaminase by some biofertilizers has been reported to improve plant water stress tolerance (Khalil and El-Noemani, 2015; Mayak, Tirosh, and Glick, 2009). Similarly, Aroca and Ruiz-Lozano (2009) and Mayak et al. (2004) reported an increase in water resistance of pepper and tomato plant grown on water deficient soil when inoculated with PGPRs. Essentially, under water-stressed conditions, AMF with their hyphae make available substantial amounts of ammonium and nitrate to the host plant (Wu & Xia, 2006). Therefore, biofertiliser has a great economic importance in improving the productivity of smallholder farmers in seasons of drought, especially in drought-prone sub-Saharan African countries such as South Africa, Kenya, Uganda, Ethiopia and Somalia (Kaushal andWani, 2016).

V. CHALLENGES FACING UTILIZATION OF BIOFERTILIZERS

- Lack of awareness amongst farmers
- Lack of government support on the production of biofertilizers
- Lack of trained staff in the production of biofertilizers
- Low acceptance on the use of biofertilizers by most of the large scale farmers
- In availability of commercial biofertilizers

VI. CONCLUSION

From the different literature reviewed, the use of biofertilizer has been established to increase plant growth and yield, as well as improve soil quality. In addition, biofertilizer could also protect the natural environment and soil biodiversity. Certain biofertilizers produce metabolites that protect plants from pest and disease attack. The environmentally friendly property of biofertiliser, as well as its great potential in sustainable agriculture, have accentuated the need to reduce, if not replace, the use of

agrochemical inputs with biofertilizers. The resource-poor farmers who cultivate on nutrient-poor sub-Saharan African soil need a costeffective and efficient technology to increase yield and profitability. It is uninteresting that the intensification in the use of chemical fertilizers has mainly focused on productivity with little or no concern about the increasing cost and ecological damage. This review revealed that the cost-benefit ratio in using biofertiliser is higher than any other nutrient management practice especially the inorganic fertilizers.

In conclusion, there is an urgent need to improve the awareness and use of biofertiliser among sub-Saharan smallholder farmers. Research studies on efficient microbial strain production, optimization of product design and biofertiliser business management as well as extension programs and product-marketing strategies are essential to achieving these objectives. It is pertinent to emphasize that sub-Saharan African government have crucial roles to play in ensuring biofertiliser technology is fully adopted as the first choice in our quest to address soil fertility challenges. Their support can be in the form of subsidy or materials to farmers. Apart from training the smallholder farmers, the accessibility of the product is also essential

AUTHORS' CONTRIBUTIONS

This review work was carried out in collaboration among all authors. Author FKM come up with the review gap, designed the study and wrote the first draft of the manuscript. Author HON and GOOA managed the literature searches and editing of the manuscript. All authors read and approved the final review manuscript

CONFLICT OF INTEREST STATEMENT

The authors declare that the review was conducted in absence of any commercial or financial relationships that could lead to a potential conflict of interest.

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Practical method for assessing the vulnerability of the agricultural production system to the effects of climate change in rainfed areas "MEVSPA-CC"

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Abstract— *According to the literature, various methods have been used to assess vulnerability to the effectof climate change, few of them have addressed the degree of vulnerability of agricultural production systems to climate change effects. In this paper we propose a new method appropriate to the Evaluation of theVulnerability of an Agricultural Production System to the effects of Climate Change in rainfed areas noted « MEVSPA-CC ».*

This method refers to the concept of vulnerability to climate change as defined by the IPCC.This method builds on practical approaches and methods of climate change vulnerability assessment that have already been implemented in other contexts.This method consists of measuring the intensity of each component of vulnerability (sensitivity, exposure and adaptive capacity) of an agricultural production system based on the causal relationships between its internal and external factors (human, physical, financial, natural, economic, and social) and the potential effects of a climate event.

Keywords— ***Adaptation, Assessment, Climate, Exposure, Method, Sensitivity, Vulnerability.***

I. INTRODUCTION

Without a doubt, climate change has become a reality. Indeed, during the last 50 years, the world has experienced an increase in the frequency and intensity of extreme weather events, including droughts, floods, tsunamis, and storms (***EM-DT, 2014)***.

Thus, the evolution of the average temperature of our planet has recorded an upward trend and the precipitation regime has been modified with a downward trend, especially in arid areas. These climatic fluctuations have had adverse effects on all human activities and particularly on agriculture, since the latter is directly linked to the climate in most countries and particularly in poor countries.

In addition to these exogenous climatic factors, illiteracy, lack of support, supervision and awareness, the fragility of infrastructure and land fragmentation are among the main factors that hinder the adaptation of agricultural production systems to the effects of climate change and consequently increase the degree of vulnerability of these systems (***Laamari,A., and al., 2016)***.

Furthermore, according to the literature***(world Bank, 2013)***on vulnerability assessment, researchers and international organizations have developed several indicators and indices to assess vulnerability to climate change.

Thus, two classifications have been distinguished: the first classification consists of assigning the main general indicators according to the three components that define vulnerability to climate change, namely: sensitivity, exposure and adaptive capacity. The second classification borrows the nature and type of climate event as the main criterion for assigning indicators.

In addition, the Livelihoods Vulnerability Index (LVI), the Environmental and Climate Vulnerability Index (EVI), and Climate Change Vulnerability Mapping (CCVM) are among the most widely used methods for assessing ecosystem vulnerability to the effects of climate change. It should be

noted that, despite this diversity of existing methods for assessing vulnerability to the effects of climate change, specific methods for measuring the degree of vulnerability of agricultural production systems to the effects of climate change are still little studied. Therefore, in order to master the main determinants of vulnerability to the effects of climate change, is it possible to measure the degree of vulnerability of an agricultural production system to the potential effects of a climatic event?

In order to contribute to the answer to this question, we try, in this article, to propose an appropriate Method for the Evaluation of the Vulnerability of an Agricultural Production System to the effects of Climate Change in rainfed areas, known as "MEVSPA-CC".

This method refers to the definition of vulnerability to climate change as put forward by the IPCC - "vulnerability is the degree to which a system is susceptible to, or affected adversely by, adverse effects of CC, including climate variability and extremes. Vulnerability depends on the character, magnitude and rate of CC to which a system is exposed, as well as its sensitivity and adaptive capacity"***(GIEC, 2001)***.And it draws on practical approaches and methods for assessing vulnerability to climate change that have already been implemented in practice in other contexts.

I- Conceptual framework of the "MEVSPA-CC" method

According to the literature***(Barbut, L. and al., 2004)***, the vulnerability can be evaluated, in a quantitative or qualitative way through the analysis of the sensitivity to the damage or through the characterization of the damage or through the capacity of response to the damage. Indeed, the assessment of the sensitivity to damage is essentially based on the study of the main factors (quantitative and/or qualitative) that can directly or indirectly influence the vulnerable system. The assessment of vulnerability through the capacity to respond to damage is often translated into an analysis of the effectiveness of the actions and means implemented to mitigate or reduce the damage. It is based on qualitative description and analysis tools (SWOT, Scorecard, GANTT chart, Evaluation sheets...). Vulnerability assessment through damage characterization is based on damage assessment.

Generally, the characterization of the damage is done according to a quantitative measurement. Vulnerability is expressed either by a potential damage coefficient that varies from 0 to 1, or by a potential loss rate that varies from 0 to 100%. Thus, "The risk corresponds to the mathematical expectation of damage or loss and its analytical expression is based on the product of the temporal component of the hazard (probability of occurrence), the values of the issues and the measure of their vulnerability. The damage or loss rates depend on the nature of the exposed elements (their resistance or resilience) and the magnitude of the natural phenomena involved (their effects). They are given in the best of cases by functions called damage (vulnerability) or losses***(LEONE, F. and al.,2006)***.

1-1- Presentation of the "MEVSPA-CC" method

The idea behind this new vision is that the degree of vulnerability of an agricultural production system to the effects of climate change can be calculated by measuring the ratio of weights between the components of vulnerability, i.e. exposure and sensitivity on the one hand and adaptive capacity on the other. Thus, if the adaptive capacity of an agricultural production system is greater than its exposure and sensitivity, we say that the system is not vulnerable, otherwise, we can say that the system is vulnerable. Thus, we can schematize this idea in Figure 1:

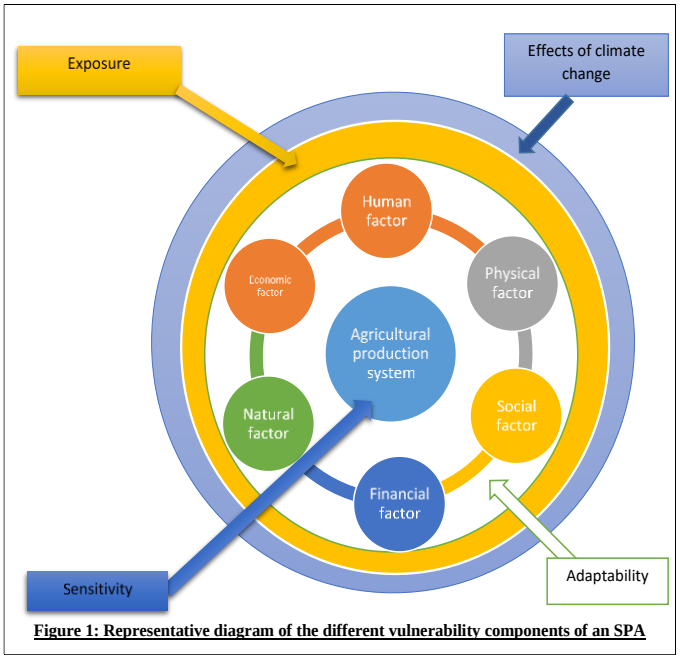


Figure 1: Representative diagram of the different vulnerability components of an SPA

This figure summarizes the key factors that determine the vulnerability of an agricultural production system.

Then, we analyse the causal relationships between the determining spheres of vulnerability in Figure 1, we obtain: The first sphere represents the sensitivity of the agricultural production system to climate change. The measure of the degree of sensitivity of an agricultural production system is a function of its specific internal characteristics. So, if the intensity of sensitivity is very high, this sphere grows while absorbing the sphere that represents the system's capacity to adapt. The second sphere represents the degree of adaptability of the agricultural production system to the effects of climate change.

This degree is measured in terms of the capacity of the production factors (human, physical, economic, social, natural and financial) to reduce or mitigate the adverse effects of climate change. If the degree of adaptive capacity is very important, the white sphere dominates the other spheres. The third sphere represents the degree of exposure of the agricultural production system to climate hazards. The measure of the degree of exposure of a system is a function of its external environment (the magnitude of the effects) and internal environment (the tolerance of its production factors).

The fourth sphere represents the magnitude of the effects of climate change. The degree of the magnitude of these effects depends on the type of climatic event itself (drought, floods, storms,...) and its duration of occurrence.

1-2- Rationale for the choice of factors

Referring to the literature(***MFACED(Zig),2017)***) on the assessment of vulnerability to the effects of CC, and like any other system, the analysis of the vulnerability of an agricultural production system requires us to study its interaction with its internal and external environment. In fact, there are six determining factors(***D'Ercole,R., and al., 1994)***) that can characterize the study of the assessment of each component of the vulnerability of an APS to the effects of CC.

a- Human factor

This factor is very important to assess the degree of vulnerability of an agricultural production system because human capital is the axis on which all other variables that can influence this system are articulated. Thus, an ecosystem can only be developed if great importance is given to this pivotal factor. So to measure the human factor of an agricultural production system, we analyze the following criteria: level of education, age, experience in agriculture, household size and immigration.

b- Social Factor

The social factor is an essential index for any evaluation of the socio-economic and demographic development of ecosystems. Thus, according to **Dugarova**,***(2014)***. "social factors here refer to structures (class, gender, ethnicity, religion and domicile), institutions (laws and norms) and social agency (the ability of individuals and groups to make their own choices and influence decisions that affect their lives) that are based on social norms and values and that determine the directions and modalities of change"**.** Thus, for our study, the criteria assigned to measure this factor are social stability, social membership, family relationship, social supports, and participation in decision making.

c- Physical factor

The physical factor informs us about the wealth of the system, the element or a group of elements to be evaluated. Thus, in our analysis, we have assigned the following criteria to this factor: the size of the farm, the number of livestock, the material and equipment.

d- Economic factor

The economic factor plays a very important role in the evaluation of agricultural production systems. Indeed, the expenses, costs and yields allow us to calculate the profits or benefits of an agricultural activity. And on the basis of these calculations, the decision of profitability of this activity can be taken in order to continue to exploit it, to stop it or to modify the exploitation techniques to improve it. Moreover, these calculations must take into account the potential losses and damages related to climatic risks.

e- Financial factor

The availability and diversification of financial resources are key indicators of an economic activity's ability to succeed.

f- Natural factor

The scarcity of natural resources, the degradation of fertile land and the lack of energy resources undoubtedly increase the vulnerability of an agricultural production system to the effects of climate change. Thus, we have assigned the following evaluation criteria to this factor: soil quality, availability of water resources and energy resources used.

II. PRESENTATION OF THE GRIDS FOR ASSESSING THE VULNERABILITY OF AN SPA

In order to contribute to the bank of tools for measuring the degree of vulnerability to CC, we have invented a specific grid for each component, to which we have assigned specific and quantifiable criteria and indicators to measure its contribution to the intensity of the component in question.

2-1- Evaluation grid for the sensitivity of an SPA to the effects of CC

For the constitution of the sensitivity evaluation grid, the choice of indicators for each criterion followed the following reasoning: For each factor, we asked the following starting question: For each factor, we asked the following starting question: What are the internal parameters related to this factor that can make it sensitive to climatic hazards? Thus, the answers to this question were summarized in the following grid:

Grid 1: Assessment of the degree of sensitivity of the SPA to CC						
Component	Factor	Criteria	S	MS	NS	Note
Sensitivity	Human factor	Level of education of the head of the household				
		Qualification, training				
		Immigration				
		Work availability				
	Physical factor	Size of the exploited area				
		Land tenure				
		Farmmaterials and equipment				
		Housing and storage				
		Livestocknumbers				
	Social Factor	Access to associations or cooperatives				
		Access to social assistance				
	Natural factor	Soilquality				
		Historicalprecipitation				
		Availability of water resources for irrigation				
		Energy resources				
	Financial factor	Self-financing				
		Access to financingcredits				
		Other sources of financing				
	Economic factor	Income				
		Savings/Agricultural product stock				
S: highly sensitive; MS: moderately sensitive; NS: not sensitive.			Total score = (1*S)+(0,5 *MS)+ (0*NS)			

2-2- Evaluation grid of the exposure of an agricultural system to climate change

In order to establish the evaluation grid for the exposure of an APS to CC, we proceeded as follows: the choice of indicators for each criterion is made through the answer to the question: What are the external parameters related to this factor and which can make it fragile to climatic hazards? Thus, the answers were summarized in the following grid:

2-1- Grid for assessing the capacity to adapt to climate change

For the construction of the evaluation grid of the adaptive capacity of an SPA to CC, we asked for each factor the following question: What are the internal and external parameters related to this factor that can make it resistant to climate hazards. Thus, the answers were summarized in the following grid:

Several authors have tried to concretize the function that links vulnerability to its components (sensitivity, exposure and adaptation). Indeed, Hahn et al. have put forward a formula attempting to express the relationship between these three components of vulnerability. Thus, the formula was presented as follows:

$$Vulnerability = (Exposure - Adaptation) \times Sensitivity$$

However, the reliability of this last formula requires an explanation of the mathematical operations linking the three components of vulnerability. Indeed, the difference, if possible, between "exposure" and "adaptive capacity" gives an incomprehensible measure and the multiplication of the latter with "sensitivity" makes the result difficult to interpret.

This ambiguity led us to consider two other alternative formulas for measuring the degree of vulnerability of an agricultural production system, namely a historical formula and a probabilistic formula.

3-1- Historical formula

This formula uses historical data for the three vulnerability components. It calculates the ratio of the degree of sensitivity and exposure to the degree of adaptive capacity. This formula is based on the comparison between the weights of the vulnerability components by putting them on two scales with one containing the weight of the adaptive capacity and the other tray carrying the other two vulnerability components (sensitivity and exposure). Thus, the concept of resilience has been introduced as a complement to vulnerability because "resilience is the ability of a body, organism, organization or system to recover its initial properties after an alteration"***(Wieland, A., and,Wallenburg, C.M. ,2013)***.

Furthermore, resilience can be conceptualized as both proactive and reactive capacity. It includes both the ability to

prevent or resist an event and the ability to return to an acceptable level of performance after being affected by an external event.

Grid 3: Assessment of the adaptability of the SPA to CCs

Component	Factor	Criteria	A	MA	NA	Note
Adaptability	Human factor	Local know-how				
		Local agricultural practices				
		Awareness of CC				
		Participation				
	Physical factor	Efficiency				
		Sustainability				
		Profitability				
		Resistivity				
		Compatibility				
	Social Factor	Laws, regulations, standards				
		Traditions, social culture,				
	Natural factor	Available				
		Accessible				
		Conservable				
		Renewable				
	Financial factor	Diversification				
		Liquid assets				
		Financingguarantees				
	Economic factor	Wealth/Savings				
		Permanent income				
A: Adaptable ; MA:Moderately Adaptable; NA: Not Adaptable.			Total score = (1*A)+(0,5 *MA)+(0*NA)			

Grid 2: Assessment of the degree of exposure of the SPA to CC

Component	Factor	Criteria	E	ME	NE	Note
Exposure	Human factor	Illiteracy/non-education				
		Healthinsecurity				
		Unemployment				
		Technological innovation				
	Physical factor	Urbanization				
		Degradation of cultivated land				
		Deterioration of material and equipment				
		Deconstruction of dwellings and depots				
		Livestock diseases				
	Social Factor	Social conflicts				
		Social and political instability				
	Natural factor	Geographic location				
		Frequenthazards				
		Degradation of resources				
		Pests (viruses, diseases,..)				
	Financial factor	Financial crises				
		Financial charges				
		Insurancecosts				
	Economic factor	Variation in prices (seed, raw materials,...)				
		Variation of the selling prices of the products				
E: highly exposed; ME : moderately exposed; NE : not exposed.			Total score = (1*E)+(0,5 *ME)+(0*NE)			

III. PRESENTATION OF THE FORMULA FOR MEASURING THE DEGREE OF VULNERABILITY OF AN SPA TO CC.

So, we can borrow the definition put forward above and apply it to the behavior of an agricultural production system towards the effects of climate change by stating that the resilience of an agricultural production system to the effects of climate change is the capacity of this system to behave proactively and reactively towards a climate event in order to mitigate its effects, avoid them or transform them into opportunities.

So, this last definition allowed us to write the following formula:

$$Degree\ of\ Resilience = \frac{Adaptation}{(Exposure\ + Sensitivity)}$$

with

$$Degree\ of\ vulnerability\ = 1 -\ degree\ of\ resilience$$

If the ratio of the degree of resilience is close to 0, this implies that the sum of the weights of the sensitivity and exposure of the agricultural production system is very high compared to the weight of the capacity to adapt. This means that the degree of resilience of this system is very low and consequently its degree of vulnerability to climate change is very high. On the contrary, if the resilience ratio is close to 1, we say that the system is able to face the potential effects of climate change and therefore the system is not vulnerable.

3-2- Probabilistic formula for assessing the vulnerability of APS to CC

To measure the intensity of the potential vulnerability of an agricultural production system to climate change, we propose a second alternative formula. The latter calculates the probable degree of vulnerability of an APS to CC according to two conditional probabilities. The probability of the system's capacity to adapt to climate change knowing its degree of exposure and the probability of the system's capacity to adapt to climate change knowing its degree of sensitivity. Then this second formula is written as follows:

$$\pi(RSPA)_{/CC}\ = (P(Adaptation\_CC)_{/(Sensitivity)}\ ) \cap (P(Adaptation\_CC)_{/(Exposre)})$$

With

- $\pi(RSPA)_{/CC}$: The probable intensity of resilience of the agricultural production system to climate change..
- $(P(Adaptation\_CC)_{/(Sensitivity)}$: the conditional probability of the capacity of an agricultural production system to adapt to climate change knowing its degree of sensitivity.
- $P(Adaptation\_CC)_{/(Exposure)}$)The conditional probability of an agricultural production system's capacity to adapt to climate change given its degree of exposure.

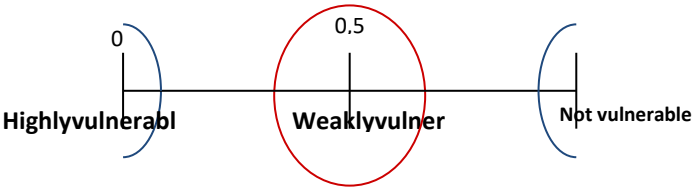
And since these two probabilities are independent, then we can write the said formula in this way:

$\pi(RSPA)_{/CC} = (P(Adaptation\_CC)_{/(Sensitivity\ )}\ ) \times (P(Adaptation\_CC)_{/(Exposure)})$

With

$$Degree\ delaVulnerability = 1 - \pi_{RSPA/CC}$$

And since the result of this formula is always between 0 and 1, it can be interpreted as follows:



$$\begin{cases} If\ \pi_{\frac{RSPA}{CC}}\ is closeto\ 0: the systemis\ highlyvulnerable toCC \\ If\ \pi_{RSPA/CC}\ is closeto\ 0{,}5\ ;\ the systemismoderatelyvulnerable toCC \\ If\ \pi_{RSPA/CC}\ is closeto\ 1\ :\ the systemisadaptable\ (notvulnerable)\ toCC \end{cases}$$

IV. CONCLUSION

With this method we intend to contribute to the enrichment of the bank of existing methods related to the assessment of vulnerability to climate change. Thus, this method is the first specific method to assess the vulnerability of APS to the effects of CC. Moreover, this method allows to measure in a historical or preliminary way the intensity of the vulnerability of an agricultural production system to the effects of climate change, which will help, in advance, the clarification of the vision of the different actors in order to improve their interventions to strengthen the resilience of vulnerable systems and in particular the small farmers operating in countries where the climate is arid or semi-arid Thus, we plan to cooperate with national and international bodies operating in the field of vulnerability analysis of ecosystems to the effects of CC in order to develop this method through experimentation for other agricultural production systems in larger areas.

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Analysis of Heritability and Correlation for Yield and Yield Attributing Traits in Single Cross Hybrids of Maize

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Abstract— *It is very important to study and understand the inter-relationships among the yield and yield attributing traits as well as heritability for the increased efficiency of the breeding programs. Thus, this research is meant to examine economic and biological performance and then measure the correlation between these traits and their heritability. A field experiment was performed in a Randomized Block layout with three replications, every assigned with fifteen treatments in Bharatpur, Chitwan. Effects confirmed considerable variants among all the found quantitative data. Days to tassel initiation were positively and significantly correlated with days to silking (0.83***), days to maturity (0.85**), and days to anthesis (0.87**). Thousand seed weight was significantly and positively correlated with shelling percentage (0.34*). Grain yield was significantly and positively correlated with cob length (0.32*) and number of grains per row (0.33*) whereas highly significant and positively correlated with circumference (0.43***). Traits like days to maturity (0.3), shelling percentage (0.3), and grain yield (0.6) exhibited moderate heritability while others exhibited low heritability. RL-294/CML-226 had the highest grain yield (7.7 t/ha) and grain per row (15.2) with medium performance for other characters. RML-57/RL-174 recorded the highest rows per cob (39.7), cob length (18cm), and shelling percentage (80%) whereas the lowest days to maturity (116.7 DAS) and fluctuating performance in others. Variety RML-86/RML-146 showed better consistent performance for all the traits with the third-highest grain yield (7.1 t/ha) except for shelling percentage (which was the lowest) and days to maturity (which was the longest). This depicts that the two varieties RL-294/CML-226 and RML-86/RML-146 have good possibilities for improvement and cultivation in that area.*

Keywords— ***Positively, Significant, Performance, Grain yield, Shelling percentage, Consistent.***

I. INTRODUCTION

Maize (*Zea mays*) is a monocot cereal grain belonging to the family *Poaceae* and tribe *Maydae*. Maize is considered the queen of cereals as it possesses the highest genetic yield potential rather than other cereals (Gami et al., 2018). Along with the traditional way of use as food, feed, and fodder, maize can also be used as raw material in a variety of food and business products such as starch, sweeteners, oil, drinks, glue, Industrial alcohol, and gas ethanol. Maize is taken into consideration as stable food throughout the globe because of its high nutritional importance enriched with an abundant amount of macronutrients like starch, fiber, protein, and fat along with micronutrients like B-complex vitamins, ß-carotene, and essential minerals, i.e. magnesium, zinc, phosphorus, copper, etc. (Bathla et al., 2019). Maize cultivation and consumption has been a way of life for Nepalese farmers as it is included in most of the crop rotations practiced in Nepal. Most of the hilly

farmers here solely depend on maize for cereal consumption for about 1/3rd of the year.

The majority of the repeated maize cultivators belong to the hills of Nepal and are popular for winter cultivation on plains. The area under maize production in plains when increased can serve as an economic boon to the country by reducing the amount of maize imported every year and increasing livestock and poultry production. Thus, it is very important to assign suitable varieties for winter to the specific area in plains for incline in maize yield.

Being a developing country, maize cultivation in Nepal faces different infrastructural, mechanical, and economic challenges. The productivity and achievable yield of maize in Nepal is 2.55mt/ha and 5.70mt/ha respectively (Thapa, 2021), but this figure is hard to accomplish due to various reasons. the highest rate of hybrid seed is the important trouble followed by untimely availability of inputs, seed replacement for every season, and excessive irrigation requirements (Dawadi & Maize, 2015). In Nepal, farmers started to develop hybrid maize because in the Eighties importing seeds from India as an open border among the international locations makes imports smooth (Thapa, 2013). The farmers do not have access to sufficient input supplies in a time of need. Sometimes even getting the seed of desired varieties is a very difficult task for them which compel them to cultivate any random seeds available in the locality. As such, they cannot increase their production by increasing the number of fertilizers, mechanization, and other options which results in less utilization of the production potential. Even if more cannot be accomplished, just a simple assessment of varieties suitable for the specific maize growing areas helps to increase production and productivity as well as adds to farm family income to increase their living standard.

The hybrid maize variety is correctly grown in the plains, inner plains, valleys, foothills, and mid-hill of Nepal (Sharma et al., 2007). But plains and inner plains are considered to have high scope for hybrid maize production during winter and spring (Dawadi & Sah, 2012) and (Sharma et al., 2004). There are altogether 59 registered hybrids, among which five were released and two were registered for release by NMRP Rampur, and 52 are multinational companies' hybrids(Dhakal et al., 2020). Hybrid maize seed advertising and marketing is prospering every year however constrained commercial hy brids are perfect for cultivation as a result of the present diverse agro-ecological regime of the count (Sharma et al., 2016). The varietal options for the farmers for hybrid seeds are very limited (Kunwar & Shrestha, 2014), which is why this research was conducted to assign the farmers all suitable hybrids for the inner plains. The seed industries are slowly establishing and developing to provide the hybrid seeds for farmers. But, the dependency over imported hybrid maize seed increased each year due to unavailability of aggressive cultivars within nation and underdeveloped seed industries (Joshi et al., 2016).

Varietal evaluation of the self-produced hybrids enables to increase in a competitive marketplace for the imported hybrid seeds and also gives a reliable option to the farmers seeking higher yield. Systematic research on maize hybrids started in 1997 after comparing 9 Indian hybrids in Rampur (Kandel, 2021). Hybrid cultivation is the best alternative option for Nepalese farmers to increase their crop productivity even with their current economic status to meet the growing demand for maize as a result of the flourishing poultry industry. To meet this, there should be a significant improvement in maize production which requires the introduction of more improved and hybrid varieties (Adhikari et al., 2018). This helps the GDP of the country to rise and also reduces the reliance on imported maize from India for food, feed, seed, and other industrial purposes. From the knowledge of the inter-relationship between yield and its contributing components the efficiency of the maize breeding programs can be improved(Mohammadi et al., 2003).

II. MATERIALS AND METHODS

Seeds of 15 single cross hybrids of maize had been obtained from the National Maize Research Program (NMRP), Rampur, and Chitwan for this experiment which was conducted in Bharatpur, Chitwan on the research farm of Nepal Polytechnic Institute. The crop was planted in late winter (early February). The average annual temperature of Bharatpur is 24 degrees Celsius and rainfall here is around 1993mm. The field experiment was conducted in a Completely Randomized Block layout with three replications. Three blocks were made with 15 experimental units in each and all 15 treatments were randomly assigned in each experimental unit of each block so that all three blocks have all treatments placed randomly. The size of each plot was 5-meter squares (2m*2.5m). The spacing between rows was 50cm and plant to plant 25cm. Each experimental unit contained 40 plants. The single cross hybrids were allotted into treatments as below:

Table 1: Allotment of single cross hybrids into treatments

Treatments	Genotype
1	RH-6
2	RH-10
3	RML-86/RML-96
4	RML-95/RML-96
5	RML-89/RML-140
6	RML-57/RML-17
7	RML-138/RML-96
8	RML-234/RML-96
9	RML-87/RL-105
10	RL-243/RML-140
11	RML-86/RML-146
12	RL-248/RML-25
13	RML-94/NL-1
14	RL-294/CML-226
15	RML-57/RL-174

The field was prepared with two harrowing followed by leveling. National Agricultural Research Council (NARC) recommended dose of 180:60:40kg NPK/ha was applied where the Nitrogen fertilizer was divided 3 times among which half of the dose was applied as basal application and the remaining half was divided equally again to be applied on 60DAS and during flowering. Five plants were sampled randomly for recording observation for each entry and 50 percent completion out of the total plants was taken for days to germination, silking, tasseling, and maturity. Plant height was taken at the time of maturity by measuring the length above from the ground to the base of the tassel. The cobs obtained after the maturity were shelled and sundried up to 15% of moisture after which the necessary measurements were recorded. The yield was recorded after the winnowing of the shelled cobs as it removes the other impurities. The shelling percentage was calculated using five sample cobs and formulae as:

$$\text{Shelling Percentage} = \frac{\text{Grain Yield (kg)} \times 100}{\text{Cob yield (kg)}}$$

Data entry and processing were carried out using the Microsoft Office Excel 2010 software and means and standard deviation for all traits were compared. The testing of the hypothesis and analysis of variance was calculated using R-software.

III. RESULTS AND DISCUSSION

Growth parameter

Plant height was observed to evaluate the growth parameter. In the experiment, highly significant variation was found from the statistical analysis for plant height among the tested single cross hybrids. Maximum plant height was exhibited by the treatment RML-86/RML-146 with 209cm and the lowest plant height was shown by the single cross hybrid RML-234/RML-96 with 152cm. The CV value for plant height was 7.519921; the LSD value among the tested single cross hybrids was 21.42786 and the mean plant height for the treatments was 170cm. The heritability value of plant height was 0.8 which indicates only 80% of the variability in the plant height is due to the genetic differences among the single cross hybrids and the other 20% is due to environmental factors.

Table 2: Growth trait for fifteen single cross hybrids of maize

Genotype	Plant height
RH-6	161.7def
RH-10	167.4cdef
RML-86/RML-96	188.9ab
RML-95/RML-96	168.3bcdef
RML-89/RML-140	159.3ef
RML-57/RML-17	166.0cdef
RML-138/RML-96	171.5bcdef
RML-234/RML-96	151.6f
RML-87/RL-105	160.9ef
RL-243/RML-140	152.4f
RML-86/RML-146	208.9a
RL-248/RML-25	182.6bcd
RML-94/NL-1	183.8bc
RL-294/CML-226	158.1ef
RML-57/RL-174	174.0bcde
CV	**7.5**
LSD	**21.4**
MEAN	**170.4**
Heritability	**0.2**
F-test	***

(Kandel et al., 2018) their article mentioned a mean plant of 201cm. This means the height is much higher than our result which is only 152cm. This difference might have occurred due to the difference in growing degree days and also the use of different hybrids with different genotypic components. The plant height variation among the

treatments could be the result of variation in genotypic components of hybrids, photosynthetic rate, and leaf size.

PHENOLOGICAL TRAITS

Phenological traits such as days to germination, silking, tasseling, anthesis, and maturity were recorded. In our experiment, there was significant variation for days to germination among the tested single cross hybrids. A maximum day for germination was observed in the treatment RL-243/RML-140 in 16 days and a minimum day for germination was observed in the single cross hybrid RML-138/RML-96 in 14 days. The heritability value of days of germination was 0.8.

A highly significant variation for days of cob tassel initiation was observed among the tested single cross hybrids. A maximum day for tassel initiation was observed in the treatment RML-87/RML-105 in 86 days and a minimum day of tassel initiation was observed in the single cross hybrid RL-243/RML-140 in 75 days. The heritability value of days of tassel initiation was 0.8. (Ghimire & Timsina, 2015), in their research reported similar variation for days to silking which ranged from 70-91 days. The difference in days to variation among different single cross hybrids might have resulted due to the difference in the duration of the vegetative phase among the germplasms.

There occurred significant variation for days of silking among the tested single cross hybrids. A maximum day for silking was observed in the treatment RML-87/RML-105 in 88 days and a minimum day for silking was observed in the single cross hybrid RL-248/RML-25 in 79 days. The heritability value of days of silking was 0.7. (Ghimire & Timsina, 2015) found the days for silking to vary within the range of 72-92 days. This result is slightly greater than our result which might have been caused due to the different genotypic components of the germplasms used in these two studies. The difference in days of silking is also affected by the duration of the vegetative phase as well as tassel and cob initiation days among the germplasms.

Moderately significant variation was found for days of cob anthesis among the tested single cross hybrids. A maximum day for anthesis was observed in the treatment RML-87/RML-105 in 88 days and a minimum day for anthesis was observed in the single cross hybrid RL-243/RML-140 in 77 days. The heritability value of days of anthesis was 0.8. (B P Kandel et al., 2018) the recorded mean of 108 days for days to anthesis in different hybrids is a little higher than the result we got. This difference in the days to anthesis might have occurred due to the difference in growing degree days between the two types of research, ours which was conducted in spring and summer, and the other one was conducted in winter. Different treatments vary for days to anthesis due to the difference in the vegetative cycle and days to tassel initiation.

There was highly significant variation for days to maturity among the tested single cross hybrids. A maximum day for maturity was observed in the treatment RML-86/RML-146 in 127 days and a minimum day for maturity was observed in the single cross hybrid RML-57/RML-174 in 117 days. The heritability value of days of maturity was 0.6. (Ghimire & Timsina, 2015) reported the days to physiological maturity in maize ranged from 143-151days in their article. Their range of days to maturity is slightly higher than our result, which might be due to the difference in the germplasm used in these two types of research. The variation in days to physiological maturity depends upon the variation in the duration of the vegetative stage, time of tassel initiation, cob formation, silking, and anthesis as well the time for cob filling and kernel development.

Table 3: Phenological traits for fifteen single cross hybrids of maize (1)

Genotype	Days of germination	Days of tasseling	Days of silking
RH-6	15.3ab	81.7bc	83.7bc
RH-10	14.0c	79.0cde	81.7cd
RML-86/RML-96	14.0c	81.7bc	83.7bc
RML-95/RML-96	14.7bc	80.3cde	83.7bc
RML-89/RML-140	14.0c	81.0cd	83.0bcd
RML-57/RML-17	14.7bc	81.0cd	83.0bcd
RML-138/RML-96	14.0c	79.7cde	84.3abc
RML-234/RML-96	16.0a	79.0cde	81.7cd
RML-87/RL-105	15.3ab	86.3a	88.3a

RL-243/RML-140	16.0a	75.0f	79.0d
RML-86/RML-146	14.0c	85.0ab	87.0ab
RL-248/RML-25	14.7bc	77.0ef	79.0d
RML-94/NL-1	14.0c	82.3bc	83.0bcd
RL-294/CML-226	14.0c	81.7c	83.0bcd
RML-57/RL-174	14.7bc	77.7def	81.7cd
CV	**4.6**	**2.7**	**3.3**
LSD	**1.1**	**3.6**	**4.6**
MEAN	**14.6**	**80.6**	**83.0**
Heritability	**0.2**	**0.2**	**0.1**
F-test	**	***	*

Note: Mean separated by DMRT and columns represented with the same letter (s) are non-significant at a 5% level of significance, CV= Coefficient of Variation, LSD= Least Significant Difference at 0.05 level of significance, and SD= Standard Deviation

Table 3: Phenological traits for fifteen single cross hybrids of maize (2)

Genotype	Days of anthesis	Days of maturity
RH-6	85.0abc	121.3bcd
RH-10	84.3bc	122.7bcd
RML-86/RML-96	85.0abc	125.3ab
RML-95/RML-96	84.3bc	122.7bcd
RML-89/RML-140	83.7c	123.3bc
RML-57/RML-17	84.3bc	118.7ef
RML-138/RML-96	83.7c	123.3bc
RML-234/RML-96	82.3c	122.7bcd
RML-87/RL-105	88.3a	124.7abc
RL-243/RML-140	77.7d	125.3ab
RML-86/RML-146	87.7ab	127.3a
RL-248/RML-25	81.7c	119.3def
RML-94/NL-1	85.0abc	125.3ab
RL-294/CML-226	83.7c	127.3a
RML-57/RL-174	82.3c	116.7f
CV	**2.6**	**1.9**
LSD	**3.7**	**3.9**
MEAN	**83.9**	**123.1**
Heritability	**0.2**	**0.3**
F-test	**	***

Note: Mean separated by DMRT and columns represented with the same letter (s) are non-significant at a 5% level of significance, CV= Coefficient of Variation, LSD= Least Significant Difference at 0.05 level of significance, and SD= Standard Deviation

YIELD COMPONENTS

Different yield components such as row per cob, grain per row, cob length, circumference, shelling percentage, thousand seed weight, and grain yield were studied. There was moderately significant variation for grain per row among the tested single cross hybrids. A maximum grain per row was exhibited by the treatment RML-57/RL-174with 40 and the lowest grain per row was shown by the single cross hybrid RML-234/RML-96 with 30. The heritability value of grain per row was 0.8. (Kandel et al., 2018) reported mean grain per row of 28cm which is a little less than our result. The use of different hybrids with varying genotypic components might have resulted in this. Variation in grain per row among the treatments might be the result of the difference in photosynthetic rate and also genetic components among them.

In our experiment, there was significant variation for row per cob among the tested single cross hybrids. A maximum row per cob was exhibited by the treatment RL-294/CML-226 with 15 grains and the lowest row per cob was shown by the single cross hybrid RML-94/NL-1 with 13 grains. The heritability value of row per cob was 0.7. (Kandel et al., 2018) reported mean row per cob of 13rows which is similar to our result. Variation in a row per cob among the treatments might be the result of the difference in photosynthetic rate and also genetic components among them.

Significant variation was observed for row per cob among the tested single cross hybrids. A maximum row per cob was exhibited by the treatment RL-294/CML-226 with 15 grains and the lowest row per cob was shown by the single cross hybrid RML-94/NL-1 with 13 grains. The heritability value of row per cob was 0.7. (Kandel et al., 2018) reported mean row per cob of 13rows which is similar to our result. Variation in a row per cob among the treatments might be the result of the difference in photosynthetic rate and also genetic components among them.

Cob length exhibited significant variation among the tested single cross hybrids. Maximum cob length was exhibited by the treatment RH-10 with 19cm and cob length was shown by the single cross hybrid RML-95/RML-96 with 16cm. The heritability value of cob length was 0.6. (Kandel et al., 2018) also recorded the mean cob length of 17cm in their research of performance check of hybrids in Rampur, Chitwan. The variation of cob length among the treatments could have been the result of the difference in the duration of reproductive stage, photosynthetic rate, number of grains per row, and also the genetic components.

Moderately significant variation for circumference among the tested single cross hybrids was found. Maximum circumference was observed in the treatment RL-243/RML-140 with 16cm and the lowest circumference was shown by the single cross hybrid RML-57/RL-174 with 14cm. The heritability value of circumference was 0.9. (Neupane et al., 2019) also recorded a similar result of a mean of 15cm for the circumference among maize hybrids in his result. The variation of circumference among the single-cross hybrids could have resulted due to the difference in grain per row, grain size, cob length, and genotypic components.

A highly significant variation for shelling percentage was observed among the tested single cross hybrids. Maximum shelling percentage was exhibited by the treatment RML-57/Rl-174 with 80% and the lowest shelling percentage was shown by the single cross hybrid RML-86/RML-146 with 70%. The heritability value of the shelling percentage was 0.9. (Neupane et al., 2019), in their article reported an average shelling percentage of 72% among the maize hybrids. This result is slightly lower than ours. The difference in grain size, number of rows per cob, number of cob per row, cob diameter, and also genotype of the hybrids might have contributed to the difference in shelling percentage among the genotypes.

In our experiment, there was a highly significant variation for thousand seed weights among the tested single cross hybrids. Maximum thousand seed weight was exhibited by the treatment RH-10 with 375gm and the lowest thousand seed weight was shown by the single cross hybrid RML-89/RML-140 with 255gm. The heritability value of thousand seed weight was 0.9.

There was a highly significant variation in grain yield among the tested single cross hybrids. Maximum grain yield was observed in the treatment RL-294/CML-226 with 7.7t/ha and minimum grain yield was observed in the single cross hybrid RH-6 with 2.1t/ha. The heritability value of grain yield was 0.6. (Kandel et al., 2018) the reported grand mean of 9.4t/ha for grain yield. This is higher than the result we derived. The use of different hybrids with varying genotypic components might have resulted in this. The germination percentage, number of cobs per plant, cob length, circumference, grains per cob, and the genotypic components of different treatments could be the cause for variation in grain yield.

Table 5: Yield attributing traits of single-cross hybrids of maize

Genotype	Rows per cob	Grain per row	Cob length
RH-6	32.9cdef	13.9abcd	15.8cd
RH-10	33.7cdef	13.9abcd	18.9a
RML-86/RML-96	32.2ef	15.2a	17.7abc
RML-95/RML-96	30.9f	14.5ab	15.6d
RML-89/RML-140	33.1cdef	13.7abcd	16.0bcd
RML-57/RML-17	38.3ab	14.1abcd	17.3abcd
RML-138/RML-96	32.3def	14.9a	15.7cd
RML-234/RML-96	30.5f	14.4abc	16.2bcd
RML-87/RL-105	35.7abcde	12.9bcd	16.3bcd
RL-243/RML-140	37.1abc	14.7a	17.6abcd
RML-86/RML-146	35.4abcde	14.8a	16.7bcd
RL-248/RML-25	33.5cdef	14.3abcd	16.4bcd
RML-94/NL-1	36.5abcd	12.7d	16.6bcd
RL-294/CML-226	34.5bcdef	15.2a	16.8bcd
RML-57/RL-174	39.7a	12.8cd	18.0ab
CV	**7.5**	**6.8**	**7.3**
LSD	**4.3**	**1.6**	**2.1**
MEAN	**34.4**	**14.1**	**16.8**
Heritability	**0.2**	**0.1**	**0.1**
F-test	**	*	.

Note: Mean separated by DMRT and columns represented with the same letter (s) are non-significant at a 5% level of significance, CV= Coefficient of Variation, LSD= Least Significant Difference at 0.05 level of significance, and SD= Standard Deviation

Table 6: Yield attributing traits of single-cross hybrids of maize

Genotype	Rows per cob	Grain per row	Cob length
RH-6	32.9cdef	13.9abcd	15.8cd
RH-10	33.7cdef	13.9abcd	18.9a
RML-86/RML-96	32.2ef	15.2a	17.7abc
RML-95/RML-96	30.9f	14.5ab	15.6d
RML-89/RML-140	33.1cdef	13.7abcd	16.0bcd
RML-57/RML-17	38.3ab	14.1abcd	17.3abcd
RML-138/RML-96	32.3def	14.9a	15.7cd
RML-234/RML-96	30.5f	14.4abc	16.2bcd
RML-87/RL-105	35.7abcde	12.9bcd	16.3bcd
RL-243/RML-140	37.1abc	14.7a	17.6abcd
RML-86/RML-146	35.4abcde	14.8a	16.7bcd
RL-248/RML-25	33.5cdef	14.3abcd	16.4bcd

RML-94/NL-1	36.5abcd	12.7d	16.6bcd
RL-294/CML-226	34.5bcdef	15.2a	16.8bcd
RML-57/RL-174	39.7a	12.8cd	18.0ab
CV	**7.5**	**6.8**	**7.3**
LSD	**4.3**	**1.6**	**2.1**
MEAN	**34.4**	**14.1**	**16.8**
Heritability	**0.2**	**0.1**	**0.1**
F-test	**	*	.

Note: Mean separated by DMRT and columns represented with the same letter (s) are non-significant at a 5% level of significance, CV= Coefficient of Variation, LSD= Least Significant Difference at 0.05 level of significance, and SD= Standard Deviation

Table 7: Statistical parameters and testing of hypothesis for 15 quantitative traits of 15 single cross hybrids of maize

Traits	Range (min-max)	Mean	SEm	LSD0.05	SD	CV%	Significance
Germination percentage	61-88	78.16667	58.39286	12.78058	9.4	9.775934	*
Days to germination (days)	14-16	14.62222	0.4507937	1.122948	0.9	4.5917121	**
Days to cob formation (days)	83-105	79.97778	6.736508	4.340983	2.6	3.245249	Ns
Days to tassel initiation (days)	75-86	80.55556	4.71746	3.63266	3.3	2.696241	***
Days to silking (days)	79-88	83.04444	7.688889	4.637698	3.3	3.339037	*
Days to anthesis (days)	77-88	83.93333	4.838095	3.678814	3.0	2.620612	**
Days to maturity (days)	117-127	123.0667	5.314286	3.85561	3.6	1.873191	***
Rows per cob	30-40	34.4133	6.63847	4.309281	1.1	7.486994	**
Grain per row	13-15	14.133	0.9179048	1.602395	3.5	6.778822	*
Plant height (cm)	152-209	170.3707	164.1407	21.42786	18.4	7.519921	***
Cob length (cm)	16-19	16.77311	1.515709	2.059105	1.4	7.33997	.
Circumference (cm)	14-16	14.59178	0.3969089	1.5053698	0.9	4.317548	**
Shelling percentage (%)	70-80	75.65022	1.85035	2.275198	2.9	1.798202	***
Thousand seed weight (gm)	255-375	293.6444	77.16032	14.69155	35.18	2.991405	***
Grain Yield (t/ha)	2.0-7.7	5.7	20.8330	0.8811411	1.4	9.344735	***

Note: Mean separated by DMRT and columns represented with the same letter (s) are non-significant at 5% level of significance, CV= Coefficient of Variation, LSD= Least Significant Difference at 0.05 level of significance, and SD= Standard Deviation, SeM= Mean sum of square

Correlation among quantitative traits

Table 8: Pearson's correlation coefficient among fourteen quantitative traits in single cross hybrids of maize

	DAG	GP	DTT	DTS	DTA	DTM	PH	SP	GPR	RPC	CL	C	TSWT	GY
DAG		-0.51***	-0.2	-0.18	-0.24	-0.11	-0.31	0.03	0.02	0.1	0.03	0.21	-0.02	-0.07
GP			0.05	0.02	0.03	0.03	0.26	0.16	0.05	0.06	-0.07	-0.06	-0.13	0.03

DTT	0.83***	0.87***	0.53**	0.13	-0.13	0.01	0.03	-0.16	-0.22	-0.23	-0.05
DTS		0.83***	0.61***	0	-0.07	0.14	-0.02	-0.2	-0.19	-0.17	-0.01
DTA			0.49**	0.16	-0.04	-0.02	0.02	-0.05	-0.25	-0.24	-0.14
DTM				0.27	0.14	0.37**	-0.16	-0.06	0.38**	0.39***	0.25
PH					0.1	0.15	0.12	0.18	0.18	0.3	0.18
SP						-0.14	0.03	0.21	0.1	0.34*	-0.01
GPR							-0.17	0.05	0.46	0.14	0.33*
RPC								0.65***	0	-0.34*	0.22
CL									0.36**	0	0.32*
C										0.49***	0.43***
TSWT											0.09*
GY											

DTG- Days to Germination, GP- Germination Percentage, DTT= Days to Tassel Initiation, DTS- Days to Silking, DTA- Days to Anthesis, DTM- Days to Maturity, PH- Plant Height, SP- Shelling Percentage, GPR- Grain Per Row, RPC- Row Per Cob, CL- Cob length, C- Circumference, TSWT- Thousand Seed Weight, GY- Grain Yield, ***p=0.001>, **p= 0.01-0.001, *p=0.04-0.01

Correlation between all quantitative traits was analyzed using R-software. Days to germination and plant height were significantly and negatively (-0.31*) associated with each other whereas days to germination and germination percentage were negatively and high significantly (-0.5***) associated with each other. Moreover, days to germination were negatively and non-significantly correlated with days to cob formation (-0.03ns), days to tassel initiation (-0.2ns), days to silking (-0.18ns), days to anthesis (-0.24ns), days to maturity (-0.11ns) and plant height (-0.31ns).

Days to cob formation were strong undoubtedly and significantly correlated with days to tassel initiation (0.78***) and days to silking (0.86***) whereas considerable and sturdy related to days to anthesis (0.79**) and days to maturity (0.85**). Days to tassel initiation had fairly tremendous and became robust positively correlated with days to silking (0.83 ***) and days to maturity (0.85**) while extensive and positively correlated with days to anthesis (zero.87**). Days to silking became distinctly vast and positively associated with days to anthesis (0.83**) and days to maturity (0.53***). Days to anthesis and days to maturity have been fairly substantial mild positively correlated with each other (0.6**).

Thousand seed weight changed into exceedingly huge and correlated with days to adulthood (0.39***) and circumference (0.49***) while extensive and undoubtedly correlated with shelling percentage (0.34*). Likewise, it changed into full-size and negatively related to row in step with cob (-0.34*).

Grain yield becomes large and undoubtedly correlated with cob period (0.32*) and number of grain in keeping with row (0.33*) while particularly good sized and correlated with circumference (0.43***). Likewise, grain yield changed into non-large and undoubtedly correlated with days to maturity (0.25ns), plant peak (0.18ns), range of row according to cob (0.22ns), and thousand seed weight (0.09ns). Grain yield changed into non-extensive and negatively correlated with days to germination (-0.07ns), days to cob formation (-0.06ns), days to tasseling (-0.01ns), days to anthesis (-0.14ns) and non-significant but positive correlation with days to maturity (0.25).

Characters like plant height and days to silking (which was significant and negative in (Ghimire 2015)), circumference and row per cob (which was found positive and incredibly substantial in (Ghimire 2015) as well as

thousand seed weight and cob length were not associated at all (r=0.00).

Grain yield ha-1 showed a positive correlation with plant height, ear height, ear length, ear girth, number of kernel rows per ear, number of kernel per row, ear weight and five hundred kernel weight (Ghimire & Timsina, 2015), just like our result. This approach that grain yield in keeping with hectare will increase with the boom in the price of plant top, ear height, ear length, ear girth, range of kernel rows in step with ear, a wide variety of kernel according to row, ear weight and five hundred kernel weight. Further, days to 50% silking, days to 50% tasseling and days to maturity confirmed a terrible correlation with grain yield ha-1(Ghimire & Timsina, 2015), that's much like our result except for days to maturity.

Thousand kernel weight exhibited a pretty huge and powerful correlation (r=0.753**) with cob circumference (Bonea & Bonciu, 2019) much like our result. Plant height showed a negative correlation with days to tasseling and days to silking (Bonia & Bonciu, 2019) which is not much like our result.

IV. CONCLUSION

Traits like days to germination, days to tassel initiation, days to silking, days to anthesis, plant height, grain per row, a row per cob, circumference, shelling percentage and thousand seed weight exhibited high heritability whereas traits like days to maturity, cob length and grain yield exhibited moderate heritability. Heritability of different traits and characterization of 14 maize genotypes in this study helps to describe the various traits along with their ability to transfer to their off-springs which will be very useful to the plant breeders for the crop improvement program.

The positive correlation among the traits signifies that with one unit increment in a trait another positively correlated trait also increases like days to tassel initiation and days to anthesis which have the highest positive correlation in our research. Among the tested single cross hybrids, RL-294/CML-226, RL-243/RML-140, RML-86/RML-146 and RML-138/RML-96 produced higher yields as compared to other genotypes. The superior parental lines are RML-86, RML-96, RML-146, RL-294 and CML-226. Variety RML-86/RML-146 showed comparatively better performance in most of the traits except for shelling percentage. Hence, these varieties of single cross hybrid of maize can be used for further studies for its possibilities and potential in Bharatpur, Chitwan. Single cross hybrids RML-234/RML-96 and RL-294/CML-226 had the smallest plant height but better yield production which implies they are possibly suitable hybrids for cultivation in Bharatpur, Chitwan.

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Hygienic condition of beef meat sold in the markets of the city of Man (Côte d'Ivoire)

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Abstract— *Food-borne illnesses represent a real public health problem, due to their severity and frequency. These are common pathologies that can be dangerous and sometimes fatal, due to the consumption of food contaminated by pathogenic microorganisms or toxic substances such as pesticides. The risk of poisoning increases when the most elementary rules of hygiene are not respected. Among the foods often affected is meat, which is an extremely perishable commodity. The objective of this study was to show, through a survey of beef sellers in three markets in the city of Man (Côte d'Ivoire) as well as consumers and, through microbiological tests, the danger that consumption of beef sold in these informal markets can represent. The surveys carried out showed that the most basic hygiene rules were not respected by the beef meat sellers. And even, for the sellers who say they respect them, that leaves something to be desired. As for the microbiological tests which consisted in determining the presence of contaminating germs on pieces of meat taken during the investigation, they revealed the presence of germs such as total aerobic mesophilic flora (TAMF), Escherichia coli and Salmonella spp. The bacterial load of E. coli varied between 4.105 CFU/g and 15.105 CFU/g depending on the market and Salmonalla spp. was present in all meat samples taken from the three markets. These results suggest that the meat sold in the markets of Man is indeed a risk factor for food poisoning for the populations that consume it.*

Keywords— ***Beef meat, food poisoning, informal markets, Microbiology quality, city of Man***

I. INTRODUCTION

Throughout the world, food poisoning remains a real public health problem. Their impact on health and the global economy is increasingly recognized (WHO, 2002). Particularly susceptible populations are young children, the elderly, pregnant women and immunocompromised people (Kouamé-Sina, 2013). The quality of food hygiene is essential for human health, it is an important index to assess the socio-economic performance of a country. Therefore, food safety becomes a necessity for human surveillance (WHO, 2002).

In Côte d'Ivoire, we are witnessing a resurgence of food poisoning and the emergence of certain diseases such as digestive disorders and especially cholera. In 2011, the country experienced several cholera outbreaks that caused 22 deaths out of a total of 933 recorded cases (Tra and Konan, 2018). In the Department of Yakassé-Attobrou, in 2018, 12 people died of food poisoning. Recently, in the Department of Vavoua, in september 2021, nine people died of food poisoning. The recrudescence of these diseases is the majority of the consequent cases of the extreme unhealthiness of the environment of production and sale of products intended for human consumption. The

sale of food in an unsanitary environment is the first element of microbiological transmission, which can lead to health risks for consumers (Tra and Konan, 2018). According to the official health control services, germs of the genus *Salmonella*, *Clostridium*, *Staphylococcus*, coliforms, yeasts and molds have been identified in foodstuffs intended for human consumption. These potentially pathogenic germs are involved in most of the poisonings encountered (Michel *et al.*, 2005). In Abidjan, for example, between 2006 and 2008, the prevalence of *Salmonella typhi* increased from 3% to 22% and the microorganism was responsible for the death of 13 people following the consumption of contaminated maize porridge in Bongouanou. In the same year, nine people fell ill in Alépé after eating cassava contaminated with three types of dangerous microbes: *Salmonella*, *Staphylococcus* and *Clostridium* perfringens (Roesel and Grace, 2016). These species, namely: *Salmonella* spp, *Staphylococcus* spp and *Clostridium* spp are bacteria responsible for zoonoses, i.e., diseases present in animal hosts and which can be transmitted naturally to humans (Roesel and Grace, 2016).

However, in sub-saharan Africa, animal products are an essential component of the food and the means of subsistence of the populations, in addition to the fact that these animal products and in particular beef are extremely perishable and contaminate foodstuffs, several different pathogens along the production chain (Hajaniaina, 2017). In addition, the contamination of beef is accentuated by the lack of and non-compliance with hygiene conditions in the markets. In fact, in developing countries, and particularly in sub-saharan Africa, meat is most often sold on informal markets, which are traditional markets with infrastructures that escape any food safety regulations and controls. (Roesel and Grace, 2016). In Man, a town located in the mountainous west of Côte d'Ivoire, as everywhere else on ivorian territory, beef is sold in informal markets. The assessment of the health risks associated with the consumption of this food is limited because there is little information on the epidemiology of the contamination of the ivorian territory and more particularly of the city of Man (Côte d'Ivoire).

Thus, one could ask the question: is the beef produced and sold on the Man market safe for consumers of this commodity?

Controlling beef contamination is now a major concern for actors in the production to consumption chain (Koffi-Nevry *et al.*, 2012). This study is, to our knowledge, the first carried out in the city of Man. The objective of this study is therefore to contribute to the improvement of the hygienic quality of foodstuffs: the case of meat, sold on the markets of the city of Man. The answer to our hypothesis will consist of:

- carry out a survey of beef sellers and consumers to get an idea of their opinion on the health risks associated with the marketing of beef;

- determine the presence of any contaminating germs in beef from pieces of meat taken from the market.

II. MATERIAL AND METHODS

2.1. Study area

This study was carried out in the city of Man, about 578 km from the Autonomous District of Abidjan. Man is the regional capital of the Tonkpi Region. Tonkpi Region is located in the mountainous west of Côte d'Ivoire between 07°20 and 07°35 North latitude and 07°25 and 07°45 West longitude. This Region is bordered to the West by the State of Liberia and to the North-East by the State of Guinea. In Côte d'Ivoire, it admits as administrative limits, the Bafing Region in the North, the Woroba Region in the North-East, the Guémon and Haut-Sassandra Regions in the East and in the South, it admits as limits the Cavally Region and the western part of the Guémon Region. Tonkpi Region constitutes with the regions of Cavally and Guémon, the District of the Mountains. It brings together five departments and thirty-three sub-prefectures. The five departments are: the Departments of Biankouma, Danané, Sipilou, Zouan-Hounien and the Department of Man (Figure 1).

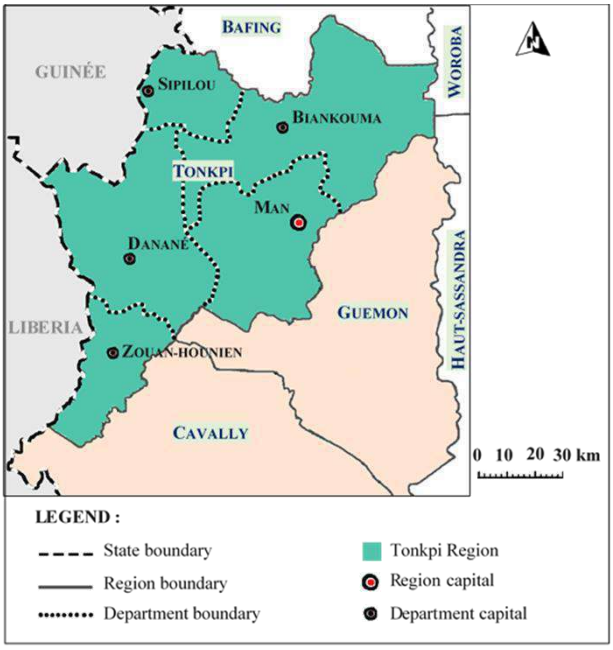

Fig.1: Administrative map of the Tonkpi Region (Source : INS, 2016)

The region covers 14,540 km^2 and has a population of 1,047,769 according to the last population census carried out in 2014. The lowlands have the highest rural population densities in the region, with many localities having more of 200 inhabitants per km^2. The Dan or Yacouba are the dominant indigenous ethnic group in the territory, which is also home to several non-indigenous ivorian populations as well as foreigners of african origin, notably guineans, malians and Burkinabe people. Non-africans constitute a marginal population (RGPH, 2014).

2.2. Survey of beef sellers and consumers on the health status of beef

Three markets were randomly selected to conduct the surveys. First of all, there is the big market of Man in addition to neighborhood markets of Doyaguiné and Cacasport. The survey was carried out in april 2021. Two questionnaires intended for meat sellers and one for meat consumers were developed and administered in the form of interviews to these different actors. The sellers are questioned stand by stand while the consumers were questioned on the place of sale of the meat or taken randomly on the market. The information sought concerning beef sellers related, among other things, to socio-demographic status (sex, age and level of education) and their knowledge of the rules of good hygiene practices. It was also necessary to know whether the beef sellers cleaned their working premises daily and what was the method of preserving the meat that had not been purchased. Among meat consumers, the information sought was to know, on the one hand, the reason for which they consumed beef despite the health risks incurred, and on the other hand, whether they had suffered from digestive disorders after meat consumption and possibly the various symptoms.

2.3. Microbiological analyzes

Three markets in order to carry out the analyses, samples of pieces of meat were taken beforehand. Samples were taken from randomly selected outlets in these markets. The sample was taken by the sellers under the same purchasing conditions as the consumers. After purchase, the samples are put in stomacher bags and then transported as quickly as possible to the laboratory using a cooler for microbiological analyses. A total of thirty (30) meat samples were taken: fifteen (15) samples from the Man market, nine (9) from the market in the Cacasport neighborhood and six (6) from the market in the Doyaguiné neighborhood. For the enumeration of each type of microorganism, one proceeds by the method of inoculation by incorporation into the agar which consists in counting the viable microorganisms present in the sample. And each type of germ is specific to a given agar.

Preparation of the sample to be analyzed

25 g of each sample was placed in 250 mL of previously sterilized buffered peptone water for the preparation of a 10^{-1} stock solution. Everything was stirred in a stomacher to ensure the dispersal of germs. From the solution thus obtained, decimal dilutions were carried out, by aseptically transferring 1 mL of this stock solution into tubes containing 9 mL of sterile physiological water.

Enumeration of the total aerobic mesophilic flora (TAMF)

The count was carried out by inoculation in the mass. A volume of 1 mL of the stock suspension and its decimal dilutions in duplicate were inoculated into PCA agar (Plate Count Agar) previously maintained under supercooling at 40° C. The incubation was carried out at 30°C for 72 hours, then the counting and calculation of the average of the germs in Colony Forming Unit (CFU)/g of sample analyzed were carried out according to the method specified by the NF V08-051 standard. Only Petri dishes with a number of colonies between 30 and 300 were taken into account for counting.

Enumeration of total coliforms, thermotolerant coliforms and determination of Escherichia coli

The search for these different germs was carried out by sowing streaks for *Escherichia coli*. One (1) mL of the stock suspension and its duplicate dilutions were inoculated into the bile lactose medium with crystal violet and neutral red (VRBL) for the detection of total and thermotolerant coliforms. Tryptone Bile X Glucuronide (TBX) medium was used for the detection of E. coli. Incubation was carried out at 30°C for 24 hours for total coliforms, 44°C for thermotolerant coliforms and E. coli. The counting and calculation of the average of the germs in Colony Forming Unit (CFU)/g of sample analyzed were carried out using Petri dishes containing 15 to 150 colonies for thermotolerant coliforms and between 30 and 300 for total coliforms, according to the method specified by standards NF ISO 4831 and NF V 08-060. A qualitative search for Escherichia coli was performed. The *Escherichia coli* test was performed using the Leminor reduced rack. After observation of suspicious coliform colonies on VRBL, five colonies are chosen and inoculated on bromocresol purple medium (BCP). The presence of yellow colonies after incubation at 37°C reflects the fermentation of lactose by coliforms and confirms the presence of these germs.

Enumeration of staphylococci

The method used is that described by standard NF EN ISO 6888-1. The surface spreading technique of 0.1 mL of

inoculum (samples and decimal dilutions) on complete Baird Parker medium was used. The incubated media were incubated at 37°C for 48 h. Inoculations were carried out in duplicate. Petri dishes containing a number of colonies between 15 and 150 were examined. The characteristic shiny black colonies surrounded by a clear halo were counted.

Salmonella research

Buffered peptone water and Rappapport-Vassiliadis broth were used as the pre-enrichment and enrichment broth, respectively. Xylose-lysine-deoxycholate (XLD) and Hektoen agar served as the isolation medium at 37°C. The method used for this search for salmonella is that specified by standard NF EN ISO 6579. The pre-enrichment is carried out directly from the stock suspension which is incubated at 37°C for 24 hours. It allows the growth of bacteria under stress or damaged by factors such as freezing, dehydration or preservatives. For enrichment, a volume of 10 mL per tube of Rappaport Vassiliadis Broth (RV10) was dispensed into screw-capped tubes. Into this broth, 0.1 mL of the pre-enrichment solution is added using a sterile pipette. The whole is incubated in an oven at 37° C. for 24 hours. The selectivity of the broth and the relatively high incubation temperature result in the elimination of much of the accompanying flora and promote the growth of salmonella strains. For the isolation of salmonella, the enrichment broth was inoculated onto Hektoen agar by the exhaustion streak technique. The plates are then incubated at 37°C.

Microbiological criteria for the evaluation of the hygienic quality of meat samples

The criteria for assessing the quality of the samples used are the French standards relating to the criteria which the ready meals must meet (EC regulation, 2005). These criteria are presented in Table 1. The interpretation of the results stems from a three-class plan and is carried out as follows: when the values obtained are lower than the criteria and up to three (3) times the criterion, the product is of satisfactory microbiological quality (SMQ). The product is of acceptable microbiological quality (AMQ); when the values obtained are between three (3) and ten (10) times the criterion. Finally, the microbiological quality is unsatisfactory (UMQ); when the values obtained are greater than ten (10) times the criterion.

Table 1: Microbiological criteria (EC Regulation, 2005)

Microorganisms	Criteria CFU/g
TAMF	**3.10^5**
Escherichia coli	**1.0.10**
Staphylocoque spp	**1.10^2**
Salmonella spp	**Absence in 25 g**

2.4. Microbiological analyzes

The analyzes of the data concerning the survey on health conditions among beef sellers and consumers and the microbiological analyses, the construction of tables and the various graphs (histograms and pie charts), were carried out using the EXCEL 2019 software.

III. RESULTS AND DISCUSSION

3.1. Results

Results of survey of beef meat sellers and consumers

A total of 37 beef meat sellers and 101 beef consumers were interviewed. Concerning the meat sellers, the socio-demographic characteristics showed that the interviewed beef sellers are all men (Figure 2). Figure 3, which presents the age range of sellers, shows that 10.9% of them are between 18 and 24 years old, 37.8% are between 25 and 34 years old, 37.8% are also between 35 and 49 years old; sellers aged 50 to 64 are present with a percentage of 13.5%. Figure 3 also shows that no seller is over 64 years old. The analysis of this figure shows us that in Man, beef sellers are mostly young people. The level of education of beef sellers is shown in Figure 4. In this figure, we note that no seller has reached the higher level, 27.0% have reached secondary level, 10.8% have at the primary level, 48.7% have rather made Koranic studies while 13.5% are illiterate. After this analysis, we can deduce that the beef is sold by men, young in age and not having done the conventional school.

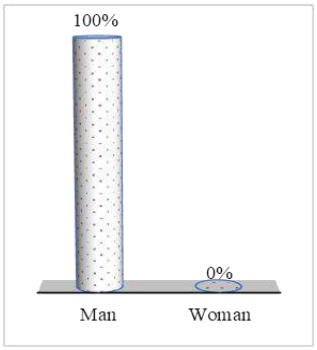

Fig.2: Distribution of beef meat sellers by sex

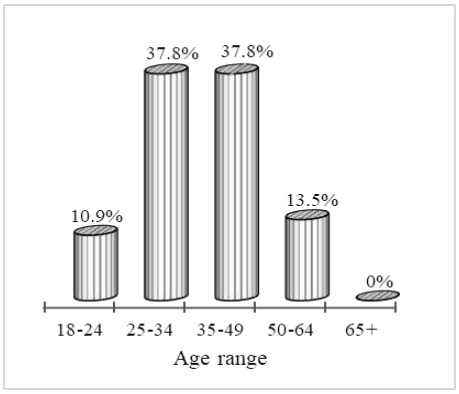

Fig.3: Distribution of beef meat sellers by age range

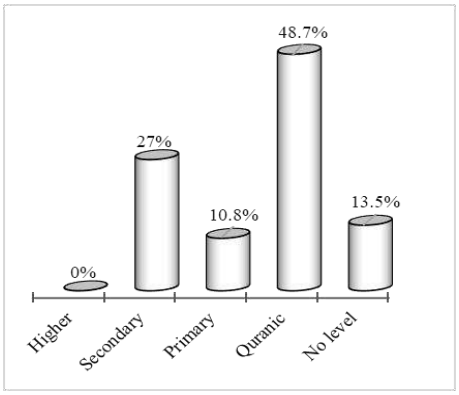

Fig.3: Distribution of beef meat sellers by education level

The results of the survey on the knowledge or not of beef sellers good hygiene practices are presented in Figure 5. In this figure, we can see that of the 37 sellers questioned, 59.5% received a training on hygiene rules while the remaining 40.5% received none. Also, according to Figure 6, the majority of sellers, approximately 83.8%, believe that they take care of the cleaning of points of sale on a daily basis and only 16.2% do not do it daily. The results obtained also show that all sellers without exception declare sending the rest of their meat to cold rooms or, failing that, to freezers for post-sale storage (Figure 7).

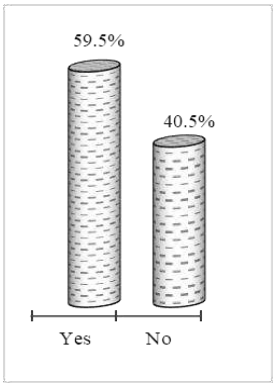

Fig.5: Proportion of beef meat sellers knowing or not the rules of hygiene

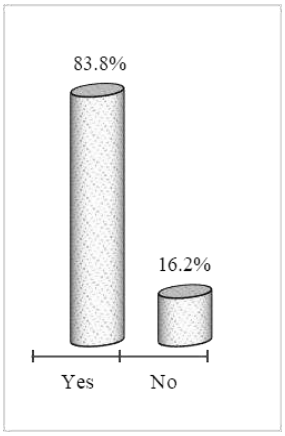

Fig.6: Proportion of beef meat sellers cleaning or not their place of sale

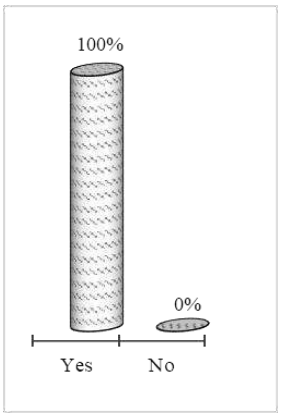

Fig.7: Proportion of beef meat sellers keeping or not the rest of the meat after sale

With consumers, the survey carried out aimed to find out the main reasons that led them to consume beef, whether there were any digestive disorders after eating meat and the main symptoms when they were having trouble. The results showed that the main reason for consuming beef in the city of Man was for its good taste, i.e., 43.9% of the people interviewed, then followed by those who consume it for simple pleasure (26.2%), followed by people who eat meat because it is nutritious (25.2%). Finally, only 3.9% of people interviewed believe that the cost of meat is cheaper and very few people, i.e., 0.9%, believe they buy meat from sellers because it is hygienic (Figure 8). Of all the consumers, 28.7% said they had goten digestive problems after eating meat and 71.3% said they had no problems (Figure 9). Among the people who presented with a disorder, 44.7% revealed that they had goten diarrhea as symptoms and 55.3% had stomach aches as symptoms (Figure 10).

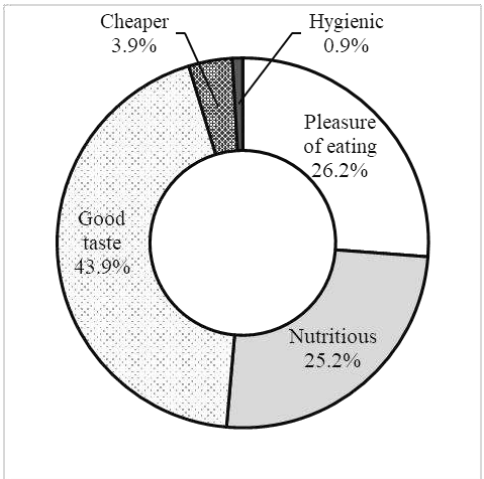

Fig.8: Reasons for beef meat consumption in the city of Man

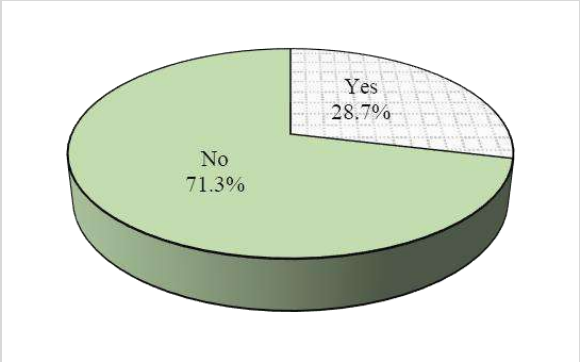

Fig.9: Proportion of people having had or not digestive disorder after eating beef meat

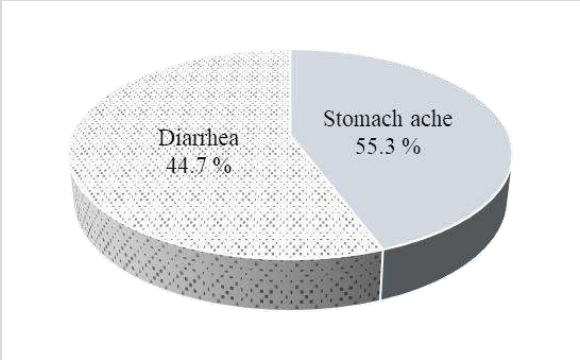

Fig.10: Symptoms presented in people with a digestive disorder

Results of microbiological analyzes

The microbiological analysis carried out on the meat samples from the markets of Doyaguiné, Cacasport and the large market of Man gave the results recorded in Table 2.

This table shows the average bacterial load in CFU/g of the different germs tested for on the meat samples taken from the three markets. The germs which were sought are the total aerobic mesophilic flora (TAMF), *Escherichia coli*, *Staphylococcus aureus* and *Salmonella* spp.

Concerning the TAMF, the microbiological analysis showed the presence of this germ on the meat samples taken from the three markets. The values of the bacterial load observed for this germ are $2.65.10^6$ CFU/g, $2.76.10^6$ CFU/g and $2.1.10^6$ CFU/g respectively for the large market of Man, the market of Cacasport and the market of Doyaguiné. The comparison of the different bacterial loads obtained for this germ with the reference microbiological load (3.10^5) could lead to the conclusion that the samples taken were of acceptable microbiological quality. Indeed, the bacterial loads obtained are between three (3) and ten (10) times the reference criterion.

For *E. coli*, the analysis also showed its presence in all the samples taken. The very high bacterial loads were $1.5.10^6$ CFU/g on average for the samples from the large market in Man, $3.42.10^5$ CFU/g for those from the Cacasport market and 4.10^5 CFU/g for the samples from Doyaguine. Regarding *E. coli*, the reference microbiological criterion is 10^2. The values obtained are well above (10) times the reference criterion. The meat samples analyzed on these three markets are therefore of unsatisfactory microbiological quality.

As for the *S. aureus*, the analysis of meat samples taken from the three markets showed a total absence of this germ. The microbiological quality of the samples with regard to *S. aureus* is therefore satisfactory.

For *Salmonella* spp. germ, the microbiological analyzes showed that it was present in all the samples taken. The very high bacterial loads observed are $7.13.10^5$ CFU/g for samples from the big Man market, $1.12.10^6$ CFU/g for the Cacasport market and $6.45.10^5$ CFU/g for the Doyaguiné market. The presence of this germ on the meat samples taken shows that these samples have an unsatisfactory microbiological quality. Indeed, according to the reference microbiological criterion relating to this germ, a sample is of satisfactory microbiological quality when this germ is absent from 25 g of this sample.

Overall, the microbiological quality of the meat samples taken from the three Man markets is unsatisfactory given the presence of salmonella and the high bacterial load of *Escherichia coli* detected without exception in all the samples analyzed.

Table 2: Microbiological quality of meat samples taken from the three markets

Germs studied		Samples taken from the markets			
		Large market of Man	**Cacasport market**	**Doyaguiné Market**	**Microbiological criteria**
TAMF	Load in CFU/g	$26,5.10^5 \pm 6,4$	$27,6.10^5 \pm 0,6$	$21.10^5 \pm 1,0$	3.10^5
	Quality	AMQ	AMQ	AMQ	
Escherichia coli	Load in CFU/g	$15.10^5 \pm 1,35$	$3,42.10^5 \pm 0,74$	$4.10^5 \pm 0,8$	10^2
	Quality	UMQ	UMQ	UMQ	
Staphylococcus aureus	Load in CFU/g	Absent	Absent	Absent	10^2
	Quality	SMQ	SMQ	SMQ	
Salmonella spp	Load in CFU/g	$7,13.10^5 \pm 8,5$	$11,19.10^5 \pm 7,78$	$6,45.10^5 \pm 0,85$	Absent/25g
	Quality	UMQ	UMQ	UMQ	

TAMF : total aerobic mesophilic flora; SMQ : satisfactory microbiological quality; AMQ : acceptable microbiological quality; UMQ : unsatisfactory microbiological quality; CFU : colony forming unit

3.2. Discussion

This study was first conducted to get an idea of the opinion of beef sellers and consumers regarding the health risks around the marketing of this food. It appears from this survey that these actors are well and truly aware of the risks incurred for the consumer. Regarding meat sellers, the socio-demographic characteristics show that the sellers surveyed are all men and mostly young (86.4%). This result was expected, because cutting and processing beef carcasses requires a lot of physical effort, which a young man can easily do. According to the results observed, the majority of meat sellers believe that they clean their work environment daily and have even received training on the rules and respect for good hygiene practices. However, this does not reflect the observation made during the surveys. This paradox is explained by the negligence of these sellers as to the practice and respect of hygiene rules. To this end, according to Tra and Konan (2018), in a study conducted on the environment and sanitary awareness of traders and consumers from Wassakara to Yopougon (Côte d'Ivoire), these actors are aware of the health risk of food sold in such an environment but they mobilize the ideology of habit and social obligation to stay in this market and exercise their economic activity there. As for the survey carried out among consumers, to find out their appreciation of the hygienic quality of the meat made available to them on the markets, it appears that the real reasons which lead them to consume meat are the good taste of the meat and also for the simple pleasure, but not because the meat sold is hygienically clean. This attitude is reflected in the fact that, despite the social realities of the markets in terms of food hygiene, these actors (traders and consumers) develop a capacity to adapt to the environmental conditions imposed on them, even if health risks may result (Tra and Konan, 2018). Some consumers surveyed (28.7%) claimed to have had digestive problems following the consumption of purchased beef, and the main symptoms were diarrhea and stomach aches. However, according to Farthing (2000), the presence of such disorders is generally attributed to the ingestion of undercooked foods, especially beef. These diarrheas and stomach aches are most often characteristic of toxi-infections caused by pathogenic microorganisms (Cassin *et al.*, 1998).

The study on the determination of possible contaminating germs of meat intended for consumption by human populations has only confirmed our concerns. Indeed, the microbiological analyzes carried out showed the presence of total aerobic mesophilic flora (TAMF), *Escherichia coli* and above all, the presence of *Salmonella* spp in all the meat samples taken from the three markets in the city of Man selected. However, among the germs sought, the absence of the *Staphylococcus aureus* germ was observed.

Concerning the detected TAMF, although this germ is not dangerous for health, its detection in the meat can however reflect an alteration of this foodstuff. According to Kneifel *et al.* (2002), this flora reduces the intrinsic quality (taste, smell, physical aspect) of the food. And for the WHO (2002), a high bacterial load of TAMF in the samples may indicate that the meats have been exposed to poor handling, inadequate processing methods and inappropriate storage conditions.

For contamination of meat samples with *Escherichia coli*, this is confirmation of fecal soiling, which is directly associated with unsanitary conditions. This bacterium comes exclusively from the intestines of humans and animals. Some serotypes can cause gastrointestinal illnesses. Infections with this microorganism are most often caused by eating contaminated, undercooked beef. In such a context, the education of meat sellers and consumers is essential to prevent foodborne illnesses through the application of good hygiene practices in the markets.

As for *Salmonella* spp., a highly pathogenic germ, a high bacterial load was observed in all the meat samples taken. However, the presence of this contaminating agent stipulates that the microbiological quality of a food is not satisfactory. According to Bornert (2000), the main reservoir in which salmonella actively multiply is the digestive tract of their potential hosts, to the point that they are considered normal hosts of the digestive tract and their only natural habitat. Thus, all animals including cattle are potential carriers of salmonella in the digestive tract (Peter *et al.*, 2010). Thus, the presence of these bacteria elsewhere in the environment or in water would only be due to fecal contamination. The presence of this microorganism is most of the time associated with the existence of food poisoning. Indeed, the main strains of salmonella are responsible for typhoid fevers but also paratyphoid fevers (Euzeby, 2007). These fevers are serious, often fatal if left untreated. This illustrates the need for good hygienic practices when slaughtering, processing, storing, cutting, marketing and even preserving beef.

However, according to Roesel and Grace (2016), in the informal markets of most african countries, dangers are frequent but they do not necessarily turn into risks. Even if the dangers are often present, the risk for human health is not necessarily high. The danger is that contaminating germs are present on the food. However, the risks may be lower due to african consumption practices such as boiling meat long before human consumption, which effectively reduces the danger.

IV. CONCLUSION

Around the world, food-borne illnesses have a serious impact on the health of populations. They are of increasing concern to consumers, producers and policy makers. The study on the hygienic conditions of the meat sold on the market of the city of Man showed that this locality is not immune to this health problem. Indeed, the results of the survey conducted among beef sellers and consumers of this foodstuff as well as the microbiological tests carried out revealed that the populations of the city of Man are exposed to real dangers. The determination of contaminating germs such as *Escherichia coli* and *Salmonella* spp incriminated in several cases of toxi-infection justifies this assertion. Even if it is difficult to control the epidemiology and the frequency of contamination of these affections, it would be interesting to sensitize the actors of the beef production chain to the respect of the elementary rules of hygiene in order to reduce health risks incurred by the people.

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The Effectiveness of Poly Alumunium Chloride (PAC) on Chemical Oxygen Demand (COD) Levels of Laundry Wastewater in Batam City, Indonesia

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Abstract— *Laundry activities produces waste water contains high pollutants such as Chemical Oxygen Demand (COD). The COD level of sample from a laundry wastewater was 541 mg/L, which over the wastewater quality standard the Indonesian Minister of Environment Regulation no 5/2014 (100 mg/L), therefore wastewater treatment was required to prevent any adverse effect. One of the parameters which is able to describe the pollutants in wastewater is COD. Wastewater treatment can be done by coagulation-flocculation process with Poly Aluminum Chloride (PAC) coagulant. The purpose of this study was to determine the efficiency of PAC in reducing COD levels in laundry wastewater. This study was a true experimental study with a pre-test post-test with control group design, used jar test to simulate the coagulation-flocculation process in a laboratory scale. PAC variations concentration were 300 mg/L, 350 mg/L, 400 mg/L, and 450 mg/L with additional samples of pretest and control. The treatments were repeated in 6 times. Kruskal Wallis test showed population of COD level difference before and after treatment ($p=0.008$). Mann Whitney test showed that groups which had significant difference in COD reduction were between the control group and each of PAC-treated group ($p<0.05$). The optimum concentration of PAC in reducing COD levels was 300 mg/L with an average effectiveness of 58.97%. The final COD levels in this study still requires further wastewater treatment before the final disposal.*

Keywords— ***Chemical Oxygen Demand (COD), Laundry, Wastewater, Poly Aluminum Chloride (PAC).***

I. INTRODUCTION

The laundry activities were widely used by the community to help with household activities, along with the increasing demands in the communities. The development of a laundry activities must be equipped by waste management before wastewater diposal to water bodies.(Wicheisa et al., 2018; Yusmidiarti, 2018) Laundry wastewater has high pollutant levels, which can be seen from chemical parameters such as Biological oxygen demand (BOD), chemical oxygen demand (COD), phosphate, physical parameters such as total suspended solid (TSS), and total dissolved solid (TDS) (Pratiwi et al., 2012). The results of COD measurements carried out on laundry wastewater X showed a COD level of 541 mg/L and there was a wastewater treatment process. This figure exceeds the quality standard of the Indonesian Minister of Environment Regulation Number 5 year of 2014 concerning Wastewater Quality Standards, which is 100 mg/L for group I wastewater (Menteri Negara Lingkungan Hidup, 2014)

Chemical substances derived from detergents contain complex chemical compounds including surfactants, builders, bleaches, and additives (Apriyani & Nani, 2017) These substances can increase the COD level of wastewater. COD is defined as the equivalent amount of oxygen used in the chemical oxidation of organic matter. COD measurements were measured using strong oxidants to oxidize organic matter which could only be partially oxidized by microorganisms. Organic and inorganic components can be oxidized during COD measurement.(Hu & Grasso, 2004)

Wastewater with high levels of COD if discharged into the environment without going through several treatment will exceed the water's ability to adjust with the conditions in the water, so that bacteria grow rapidly and consume dissolved oxygen which causes a decrease in dissolved oxygen levels. Organisms in water such as protozoa and fish will find it difficult to live without dissolved oxygen.(Aini et al., 2017) Laundry waste also contains phosphate which can cause eutrophication, because phosphate is a nutrient for algae. Leftover detergents also contain toxic compounds that can be harmful to life in the water and to humans who use the water.(Yusmidiarti, 2018)

Poly aluminum chloride (PAC) is one of the coagulants commonly used in the coagulation and flocculation processes of wastewater treatment because it is safe and economical. PAC is a collection of complex inorganic compounds, hydroxyl ions, and aluminum ions with different degrees of chlorination that form polynuclear. The general formula for PAC is $Al_m(OH)_nCl_{(3m-n)}$. PAC can reduce COD levels up to 62%-83% in a study conducted by Yustinawati in 2014 with oil well drilling waste (Yustinawati, 2014). Some of the advantages of PAC are it can coagulate water with different turbidity in a short time, produces less sludge, and only leaves a small amount of aluminum residue in the water. Another advantage of PAC is that it can work in a wide pH range, strong adsorption rate, and high floc formation rate even though the dose given is small.(Murwanto, 2018; Rahimah et al., 2016) This study aims to determine PAC effectiveness in decreasing COD levels in laundry wastewater X.

II. METHOD

This research was using true experiment, pretest post-test with control group design. The sample used was wastewater produced by laundry X which located in Batam City, Indonesia. Samples were taken by grab sampling for 6 times repetition in different days. The total samples taken were 36 samples consisting of 4 treatment samples, 1 control sample, 1 pretest sample, and repeated 6 times. The pretest sample was first measured for temperature, pH, and COD levels and used as data before treatment. The measurement results of temperature, pH, and COD levels of control and treatment groups were used as data after treatment (post test).

The independent variables used were variations in PAC concentration of 300 mg/L, 350 mg/L, 400 mg/L, and 450 mg/L. Dosing of PAC was done by making a 1% PAC solution and calculating the volume of the PAC solution needed to reach 300 mg/L, 350 mg/L, 400 mg/L, and 450 mg/L concentration according to the dilution formula, then adding it to 500 mL of the wastewater sample. The control sample was not treated with PAC coagulant, but the stirring process was still conducted. The dependent variable was the COD level of laundry wastewater X which will be proceeded by calculating the reduction in COD levels and the efficiency of the reduction. There were confounding variables in this study. The pH value and temperature were confounding variables measured, while the confounding variables which controlled were mixing speed, mixing time, and processing capacity.

Samples were stirred using a jar test. The stirring speed for rapid mixing was 100 rpm with a stirring time of 1 minute, followed by slow mixing of 40 rpm with a stirring time of 20 minutes. Precipitation was done for 1 hour. Then the samples were examined for COD levels using the open reflux method based on APHA-5220-B standard.(Rodger B. Baird, Andrew D. Eaton, 2018)

Calculation of COD reduction efficiency using the formula:

$$\% \text{ Efficiency} = \frac{\text{COD level pretest} - \text{COD level post test}}{\text{COD level pretest}} \text{ X } 100\%$$

Data analysis of the difference in COD levels before and after various treatments was carried out using the Saphiro Wilk normality test, followed by a homogeneity test as a fulfillment of assumptions. The Kruskal Wallis test was conducted with the hypothesis that there were differences in the decrease in COD levels in each group, and the Mann Whitney follow-up test was used to determine the pairs of groups with significant and non-significant differences.

III. RESULTS AND DISCUSSION

Laundry X served 5-30 customers every day. In one time wash, 30-100 mL of liquid detergent was used, and 50-100L of water was used. In one time wash, the amount of dirty laundry could reach 5 kg.

The results of testing the COD levels of the laundry wastewater pretest sample X for 6 days had an average of 442.83 mg/L (see Table 1.). This figure exceeds the quality standard of the Minister of Environment Regulation Number 5 of 2014 for group I wastewater, which is 100 mg/L for COD. Fluctuating COD levels were influenced by the amount of detergent used, water usage, and the presence of impurities in the laundry. The results of the pH value of the pretest group tended to be neutral and still met the quality standard of 6-9, except for the 1st and 6th repetitions which did not qualify the quality standard (see Table 3.). In this study, no pH adjustment was made with the consideration that PAC can work in a wide pH range. The average temperature for pretest samples were 26,88ºC, with the minimum temperature of 25,9ºC dan maximum temperature of 27,9ºC (see Table 4). The temperature measurement for 6 times repetition was considered normal

and still meets the 38ºC temperature quality standard treshold according to Regulation of the Minister of Environment 5/2014.

A. Chemical Oxygen Demand (COD)

After the rapid mixing process and slow mixing, the floc formed was precipitated for 1 hour, so that the floc gathered at the bottom of the beaker glass due to gravity. The floc formed was less than 1 mm in size. The formation of flocs in the samples treated with PAC coagulant can be occurred after the wastewater was stirred.

Flocs were formed as a result of PAC mixed in. There was a destabilization of colloids and suspended particles through neutralization of electric charges so that the force of repulsion of similar electric charges between particles was reduced. PAC had a high positive electric charge, as well as a long polymer chain so that it easily increased the covalent attraction between particles.(Firra & Mohamad, 2013) The electrolyte that played a role in this coagulation process is Al^{3+} cation. In the study, laundry wastewater which was originally turbid and colored, after the addition of PAC coagulant became clear, because the positive ions Al^{3+} bind organic ions that were negatively charged in the wastewater.(Susanti & Hartati, 2003)

The average post-test COD levels in the control group, with variations in the treatment doses of 300 mg/L, 350 mg/L, 400 mg/L, and 450 mg/L, respectively, were 397.83 mg/L, 172.50 mg. /L, 181.50 mg/L, 194.83 mg/L, and 220.67 mg/L. This showed that in laundry wastewater X after being given a variety of treatments, the COD level has decreased. However, the average COD content of all groups still exceeded the specified quality standard stated in Regulation of the Minister of Environment 5/2014 which is 100 mg/L.

Table 1. COD Level Measurement Results

Repetition	pretest (mg/L)	Post test (mg/L)				
		Control	300 mg/L	350 mg/L	400 mg/L	450 mg/L
1	541	559	263	311	289	400
2	337	407	187	192	184	198
3	483	372	88	133	156	220
4	584	445	129	165	152	139
5	348	262	137	108	209	174
6	364	342	231	180	179	193
Average	442.83	397.83	172.50	181.50	194.83	220.67

In the control group, COD was increase, it could be seen in the 1st and 2nd repetitions that the COD post-test levels in the control group increased. This can be due to changes in temperature that occur. Temperature affects the physical, chemical, and biological properties of wastewater. Changes in temperature cause an increase in viscosity, evaporation, and volatilization. As a result, the structure of the colloidal particles became smaller. This caused the ability for deposition to decrease. In the control group, only mixing and precipitation was done. The increase in COD was due to the fact that the colloidal particles cannot be deposited, so that when measuring COD levels, organic matter was carried in the sample. This was in line with Ayu Larasati's 2017 research in the study of decreasing COD levels of laundry wastewater using $FeCl_3$ coagulant which study showed that the control group in the 2nd and 3rd repetitions experienced an increase in COD levels from an initial level of 1.012 mg/L to 1,680 mg/L, and from an initial level of 717 mg/L to 979 mg/L.(Larasati et al., 2017)

The mixing process with the jar test in the control group without PAC treatment can also reduce COD levels because the stirring process could increase the dissolved oxygen content in the water. With the availability of oxygen in the water, the need for oxygen decreased, so that the COD level decreased. A similar thing happened in Islamawati's 2018 study which showed that the COD level of control group in the study of decreasing COD levels of tapioca wastewater that was treated with jar test mixing was indeed decreasing.(Islamawati et al., 2018)

Table 2. COD Levels Reduction

Group	COD levels average (mg/L)		Difference (mg/L)	Efficiency (%)
	Pretest	Post test		
300 mg/L	442.83	172.50	270.33	58.79
350 mg/L	442.83	181.50	261.33	58.21
400 mg/L	442.83	194.83	248.00	54.07
450 mg/L	442.83	220.67	222.17	49.16

The decrease in COD levels in the treatment group showed in Table 2 was due to the negative ionized organic matter already bound with the positive ions from the PAC coagulant and forming flocs. The flocs settle to the bottom of the beaker glass due to gravity and were separated from water which has a lower density and clearer color. The clearer water has lower COD levels due to the process of removing organic matter. This was corresponding with the 2018 study by Ulima where PAC in slaughterhouse wastewater samples can cause COD levels to decrease by up to 40%.(Salsabila et al., 2018)

B. pH value

The results of pH value in the pretest and posttest treatment samples in 6 times repetition were as follows:

Table 3. pH Measurement Results

Repetition	Pretest	Post test				
		Control	300 mg/L	350 mg/L	400 mg/L	450 mg/L
1	5.98	5.8	4.61	4.55	4.5	4.48
2	6.53	6.6	4.4	4.4	4.39	4.38
3	6.97	6.86	4.31	4.33	4.33	4.37
4	6.96	6.98	4.6	4.56	4.53	4.51
5	6.69	6.8	4.44	4.42	4.42	4.41
6	9.95	10.08	8.95	8.69	8.4	8.04

The results of measuring pH value before and after treatment with 6 times repetition for the control group, variations in the treatment doses of 300 mg/L, 350 mg/L, 400 mg/L, and 450 mg/L in the table show that PAC can cause a decrease in pH. The more PAC given, the more acidic the pH of the wastewater. This was in line with Yustinawati's research in 2014 where the pH level of the drilling mud waste after being treated with PAC decreased by ±2 from the initial pH.(Yustinawati, 2014) Husaini's 2018 research also showed that PAC could lower the pH level in gold processing wastewater.(Husaini et al., 2018)

The decrease in pH was caused by the addition of PAC. H^+ ions were released into the water for each hydrogen group produced. The PAC reaction in water can be described as follows: $Al_2(OH)_3Cl_3 \rightarrow Al_2(OH)_3^{3+} + 3Cl^- + 3H_2O \rightarrow 2Al(OH)_3 + 3H^+ + 3Cl^-$. Product of $3H^+$ ions causes pH level to decrease became more acidic.(Yustinawati, 2014) This can affect the next wastewater treatment, thus neutralization is required.

C. Temperature Measurement

The results of temperature measurements on the pretest and post test samples of treatment in 6 times repetition were as follows:

Table 4. Temperature Measurement Results

Repetition	Pretest (ºC)	Post test (ºC)				
		Control	300 mg/L	350 mg/L	400 mg/L	450 mg/L
1	26.4	25.5	25.5	25.3	25.3	25.2
2	27.4	26.7	26.6	26.4	26.5	26.5
3	27.2	25.5	25.2	25.2	25.1	25.2
4	26.5	24.3	24.5	24.6	24.4	24.4
5	25.9	24.6	24.5	24.4	24.4	24.3
6	27.9	27.9	27.6	27.3	27.1	27.2

The results of the measurement of the temperature of the wastewater sample can be seen in the table above. The temperature of the treatment group decreased from the results of the temperature measurement of the pretest group with a difference of ±1ºC. All temperatures of the group qualify the quality standard according to Regulation of the Minister of Environment 5/2014 which was 38ºC because they were still adjusting to room temperature.

D. COD Reduction Efficiency

The following was a graph of the efficiency of reducing COD levels in the control group and various doses of PAC treatment:

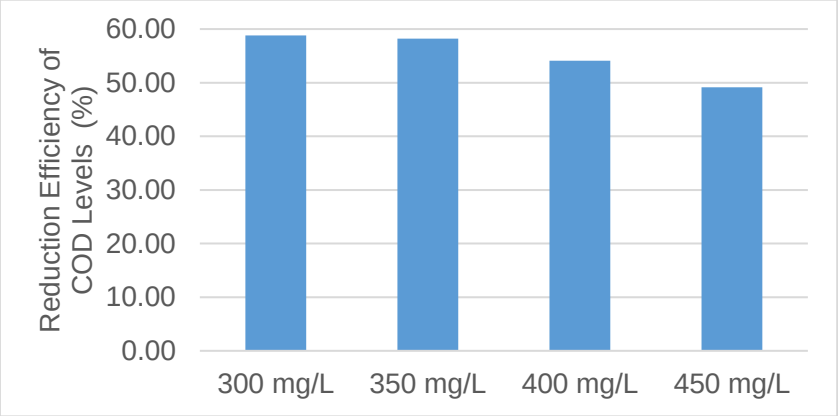

Fig. 1 COD level Reduction Efficiency

According to the COD levels in the pretest and posttest groups in Table 2, there was a decrease in COD levels. The average decrease in COD levels for the group with PAC doses variation of 300 mg/L, 350 mg/L, 400 mg/L, and 450 mg/L were 270.33 mg/L, 261.33 mg/L, 248 mg/L, and 222.17 mg/L. The COD reduction efficiency in the four groups were 58.79%, 58.21%, 54.07%, and 49.16%, respectively. From these results, it can be concluded that laundry X wastewater treated with PAC coagulant causes a decrease in the level of COD parameters.

Treatments with doses of 300 mg/L, 350 mg/L, 400 mg/L, and 450 mg/L showed lower efficiency and lower COD levels decrease at higher dose. This was caused by colloid re stabilization that occurs when high doses of coagulant were given to wastewater, there was excessive absorption of cations by colloidal particles. So that the positive ions of the coagulant were no longer able to adsorb the negative ions of colloidal particles. This also caused the wastewater that was given a dose of coagulant that has passed the optimum dose to increase turbidity.(Susanti & Hartati, 2003; Yustinawati, 2014) So that the decrease in COD levels was more significant at lower dose of coagulant. The PAC dose of 300 mg/L was the optimum dose for this study.

Similar result occurred in Adysti's research in 2014 for treating laundry wastewater using combination wastewater treatment using PAC and active carbon filtration. In the research, the optimum dose was 55 mL/L, whereas the higher doses showed the decreasing efficiency of COD removal.(Maretha N et al., 2014)

COD levels reduction data before and after treatment did not meet the normality assumption. One of the data category, which was group of 450 mg/L PAC variation was not distributed normally. Meanwhile homogeneity assumption terted with Levene test shown that the COD levels reduction data were homogen. Further test conducted with non parametric tests.

Based on a test using the Kruskal Wallis test, it was concluded that there were differences in the pretest and posttest COD levels in the control group, and the treatment group with variations in PAC doses of 300 mg/L, 350 mg/L, 400 mg/L and 450 mg/L with a significance 0.008 (significance <0.05, H0 rejected). Thus, further tests were conducted with the Mann Whitney test. The results showed that the groups that had significant differences were between the control group and the PAC dose of 300 mg/L, the control group and the PAC dose group of 350 mg/L, the control group and the PAC dose group of 400 mg/L, and the control group and the PAC 450 mg/L dose group. Meanwhile, between each of the groups of treatment dose variations, there was no significant difference.

The ability of PAC in reducing COD levels of laundry wastewater X was still not optimal, because post-treatment COD levels still exceeded the quality standards set by Ministerial Regulation Number 5 year of 2014. Therefore, further wastewater treatment is required to result laundry wastewater with COD levels that qualify the requirements for discharge into water bodies.

IV. CONCLUSION AND RECOMMENDATIONS

The average efficiency of COD levels reduction in the treatment group of PAC coagulant doses of 300 mg/L, 350 mg/L, 400 mg/L, and 450 mg/L respectively were 58.79%, 58.21 %, 54.07%, and 49.16%. The optimum dose of PAC in this study was a dose of 300 mg/L with an average effectiveness of 58.79%.

The test using the Kruskal Wallis test on the decrease in COD levels of laundry wastewater X showed a significance of 0.008 (significance <0.05, H1 was accepted), so it was concluded that there were differences in the decrease in COD levels of laundry wastewater X between groups. Mann Whitney follow-up test showed that the groups that had significant differences were the control group with each every one of treatment dose variation.

The COD level of laundry wastewater after treatment with various doses of PAC coagulant still exceeded the wastewater quality standard stipulated by the Minister of Environment Regulation No. 5 year of 2014. Further treatment is required so that the quality of wastewater is feasible to be discharged into water bodies.

Suggestions for future researchers is to expand the range of pollutant parameters tested for laundry wastewater such as phosphates, detergents, and others. The next research may conduct pH control to optimize the ability of the coagulant used and the next research also may do a combination of wastewater treatment with biological treatment so that the final pollutant parameter qualified the referenced quality standard.

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Comparative Study of the Physicochemical Properties, of the minerals of two varieties of *Capsicum annuum*: The Hot Pepper and the Sweet Pepper cultivated in Korhogo in the North of Côte d'Ivoire

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Abstract— *The Capsicum annuum pepper species consists of two (2) main varieties: hot pepper and sweet pepper. These two (2) varieties are eaten in Korhogo, in the north of Côte d'Ivoire. However, the hot pepper is consumed much more than the sweet pepper only because of its pungent taste due to the presence of capsaicin. These two (2) varieties of Capsicum annuum encounter problems of distribution and conservation. In addition, the populations do not know their compositions nor their nutritional values. This study is a valuation of the two (2) varieties of Capsicum annuum. Also, it will allow people to consume more sweet pepper if its nutritional value is good. The comparative study of their physicochemical properties, antioxidant and anti-nutrient contents revealed that the sweet pepper has a higher length (6.48cm ± 0.6), circumference (14.51cm ± 0.45) , mass (37.61g ± 4.41), humidity rate (91.48% ± 0.3), oxalate content (377.66 mg / 100g ± 13.22). Hot pepper, for its part, has a higher ash content (0.95% ± 0.11), lipids content (0.45% ± 0.04), proteins content (1.46% ± 0.16), fiber content (5.53% ± 0.06), total carbohydrate (9.14% ± 0.57), energy value (46.45Kcal / 100g ± 2.59), polyphenol content (200.41mg / 100g ± 40.07),phytates content (25.31mg / 100g ± 0.69).In addition, the pH (5.46 ± 0.04) of these two (2) varieties, the titratable acidity (3 meq / 100g ± 0.19), the content of reducing sugars (196.52mg / 100g ± 61 , 46), the total sugars content (3.58% ± 0.52), the vitamin C content (44.16mg / 100g ± 19.02), the flavonoids content (3.65mg / 100g ± 1, 4), the tannins content (19mg / 100g ± 3.62), are not significantly different at the 5% level. Regarding minerals, hot pepper and sweet pepper contain phosphorus (0.15% dm ± 0.015), potassium (0.34% dm ± 0.017), calcium (0.31% dm ± 0.006), magnesium (0.17% dm ± 0.003), copper (3.45ppm ± 0.62), iron (7.53ppm ± 0.488), manganese (0.64ppm ± 0.13), zinc (16.74ppm ± 1.155), sodium (11.13ppm ± 5.261), the contents of which do not vary significantly from one variety to another. This study showed that these two (2) varieties of Capsicum annuum have dietary fiber, vitamin C, minerals, natural polyphenols which are beneficial for the local population. Anti-nutrients can be inactivated by cooking.*

Keywords— ***Hot pepper, Sweet pepper, Capsicum annuum, Physicochemical properties, minerals.***

I. INTRODUCTION

Urban food is a major challenge for African cities today. To this end, in West Africa, rapid urbanization has favored the development of artisanal sectors for processing and marketing local agricultural products (Cheyns and Bricas, 2003) [1]. Among these products, condiments, spices, aromatics are widely used in the traditional diet of countries on the west coast of Africa. They are the subject of a large number of commercial transactions between different countries and are found in all local markets (Ndir et *al*., 2000) [2]. This is why today peppers are grown everywhere whenever conditions permit.

Pepper is a vegetable plant which represents a great interest at the global level both in terms of production and consumption (Bharath et al., 2013) [3]. For its generally spicy taste, pepper is well appreciated in human food. It is a fruit very rich in vitamin C depending on the variety of the pepper. It is adapted to tropical and subtropical conditions (tolerates heat well). Peppers are grown year round, but irrigation is necessary in the dry season. In Côte d'Ivoire, pepper cultivation is generally rainfed.

Pepper belongs to the genus Capsicum. The genus *Capsicum* includes 25 species, 5 of which are cultivated (*C. annuum*, *C. baccatum*, *C. pubescens*, *C. fructescens*, *C.chinense*). The *C. annuum* species is the most cultivated in the world and the most economically profitable (Chaux and Foury, 1994) [4].

Capsicum annuum is a species comprising various varieties producing fruits with the sweetest flavors with the most pungent flavors. Thus, this species is the source of sweet peppers and a large number of hot peppers (Perry et *al*., 2007) (5].

Sweet peppers are distinguished from hot peppers by their fruits that are generally larger (3 to 12 cm in diameter) and fleshy, and above all devoid of any pungent substance (capsaisin) (Pegon, 2009; Zaman, 2009) [6,7] . These fruits are swollen, unripe green, and red, orange, yellow, purple, white, blue, or brown when ripe. They have a sweet taste and aroma (Grubben and Denton, 2004) [8]. The hot peppers of this species vary in shape, but are usually elongated and ovoid, 2 to 16 cm long, or globose. They are generally red, sometimes orange or yellow, when ripe, with a smooth or slightly wrinkled wall, with a mild to very pungent flavor (Csilléry, 2006) [9].

However, in Korhogo in the north of Côte d'Ivoire, the two (2) varieties (hot peppers and sweet peppers) of the species *Capsicum annuum* are underexploited and the income collected by the actors, namely farmers and the market sellers are thin. Significant amounts of the two (2) varieties of *Capsicum annuum* rot in the distribution chain before being purchased by consumers. Conservation issues prevail in the distribution chain; better control of the physico-chemical and nutritional parameters could help ensure better profitability of the two (2) varieties of *Capsicum annuum*. In addition, the populations do not know the nutrients provided by these two (2) varieties nor their composition. In addition, hot pepper is consumed more than sweet pepper only because of its pungent taste due to capsaicin. This study will also allow people who can't stand the hot taste of hot peppers to consume more sweet peppers if it turns out that their nutritional value is as good as that of hot peppers.

The general objective of our study is to promote the two (2) varieties of the species *Capsicum annuum*: hot pepper and sweet pepper.

This study will consist of:

- Determine the physico-chemical properties of these two varieties of *Capsicum annuum*

-Determine their antioxidant, anti-nutrient and mineral content

-Compare the two varieties of the species *Capsicum annuum*

-Evaluate the nutritional value of the two peppers through these results

II. MATERIAL AND METHODS

2.1 Biological material

The biological material used consists of two varieties of the *Capsicum annuum* species: hot pepper and sweet pepper.

2.2 Methods

2.2.1 Sampling

The samples of the two (2) varieties of *Capsicum annuum* were purchased in three (3) markets of the city of Korhogo specifically the Sinistré market, the Koko market and the big market. For each market, 4kg of samples of each variety of *Capsicum annuum* were purchased from three different vendors (traders), which makes 12kg of each variety of peppers purchased per market, resulting in 36 kg of each variety of *Capsicum annuum* for all three markets. The samples were then sent to the laboratory for the various analyzes.

2.2.2 Physical Characterization of Hot and Sweet pepper (*Capsicum annuum*)

Five (5) physical parameters were assessed on the Hot or Sweet pepper fruits, namely length, circumference, mass, moisture, and ash. The length and the circumference of the full fruit were estimated using a meter tape. The fruit's weight was measured using a 2 digits scale (Sartorius,).

The method of determining moisture is that proposed by **AOAC (1990)** [10]. The moisture was assessed by drying 5 g of sweet pepper into an oven at 105 °C till constant weight resulted after 24 h. The ash content was measured by incinerating five (5) g of oven-dried pepper into a muffle furnace at 550 °C for 12 h (**AOAC (1990)**[10].

2 2 3 Chemical trend of the hot andsweet pepper fruits

2.2.3.1 Acidity

The acidity traits (pH and titratable acidity) were measured using **AOAC (1990)** method [10]. Ten (10) grams of crushed sample are slurried in 100 mL of distilled water. The solution obtained is filtered on filter paper (Whatman). The pH measurement is carried out directly by immersing the previously calibrated pH meter (HANNA) electrode in the filtrate obtained. Then, 10 mL of the filtrate are taken and this test sample is titrated with a solution of NaOH (0.1 N) in the presence of phenolphthalein until turning pink. The titratable acidity is given in mEq/100g of dried sample.

2.2.3.2 Total soluble carbohydrates and reducing carbohydrates contents

Ethanosoluble carbobydrates were extracted from 1 g of ground dried hot orsweet pepper with 20 mL of 80% (v/v) ethanol, 2 mL of 10% (m/v) zinc acetate and 2 mL of 10% (m/v) oxalic acid, according to the method of **Agbo et *al.* (1985)** [11]. The extract was centrifuged at speed of 3,000 rpm for 10 min. The ethanol residue was evaporated from the extract upon a hot sand bath. Then, the extracted total soluble carbohydrates were measured out using the method of **Dubois et *al.* (1956)** [12]. The operation consisted in adding 0.9 mL of distilled water, 1 mL of 5 % (m/v) phenol, and 5 mL of 96% sulfuric acid into 100 µL of extract, then measuring the absorbance at 490 nm with a spectrophotometer (PG instruments). For the reducing sugars, 1 mL of extract was processed with 0.5 mL of distilled water and 0.5 mL of 3, 5- dinitrosalycilic acid (**Bernfeld, 1955**) **[13].**prior to the recording of the absorbance from the final solution at 540 nm with a spectrophotometer (PG instruments). Calibrations were performed with standard solutions of glucose and sucrose for recovering the final total carbohydrates and reducing carbohydrates contents in the studied samples.

2.2.3.3 Lipids content

Lipids were quantified from 10 g of ground dried hot or sweet pepper sample by solvent extraction using 300 mL of n-hexane reagent and a Soxhlet device for 7 h (**AFNOR, 1986)[14].** The hexan-oil mixture resulted from the extraction was recovered and separated with a rotavapor apparatus (Heidolph). The difference between the sample weight before and after the experiment allowed the estimation of the lipids content.

2.2.3.4 Proteins content

Crude proteins content was determined as the total nitrogen using the Kjeldhal method **(AOAC, 1990)** [10]. Thus, 1 g of hot or sweet pepper mash was mineralized at 400 °C for 2 h, with adding of concentrated sulfuric acid (H_2SO_4) and potassium sulfate (K_2SO_4) catalyst. The mineralizate was diluted and distilled for 10 min. Thereafter, the distillate collected into a flask containing boric acid and methylen bromocresol reagents ion, was titrated for the total nitrogen using ammonium sulfate ($(NH_4)_2SO_4$). The crude protein content of the hot or sweet pepper was deduced from the nitrogen level using 6.25 as conversion coefficient.

2.2.3.5 Fibers content

The determination of the crude fibers content consisted in treatment of 2 g of ground hot orsweet pepper sample with 50 mL of 0.25 N sulfuric acid and 50 mL of 0.31 N sodium hydroxide and filtration of the resulting solution upon Whatman paper. The residue was dried for 8 h at 105 °C then incinerated at 550 °C for 3 h into ovens (**Wolff JP, 1968)[15].** The final residue was weighed as crude fibers and expressed in percentage.

2.2.3.6 Total carbohydrates content and energy value

Total carbohydrates and energy values were determined using calculation formulas (**FAO ,2002**)**[16]** accounting the moisture, fat, protein, ash contents and the energy coefficients for macromolecules.

$$TCC\ (\%) = 100-[P(\%) + M(\%) + F(\%)+A(\%)]$$

$$CEV\ (kcal/100g) = [(4\times P) + (9\times F) + (4\times C)]$$

With: TCC, total carbohydrates content; CEV, caloric energy value; P, protein content; M, moisture content; F, fat content; A, ash content; C, total carbohydrates content

2.2.3.7 Vitamin C content

The vitamin C was evaluated from thehot or sweet peppers using 2,6- dichlorophenol-indophenol (DCPIP) reagent (**AOAC, 1984)[17].** Ten (10) grams of ground dried hot or sweet pepper sample were dissolved into 40 mL of metaphosphoric acid-acetic acid solution (2%, w/v). The resulted mixture was centrifuged at 3,000 rpm for 20 min. Thus, the supernatant was recovered, added with boiled distilled water for 50 mL, and titrated with 2, 6- DCPIP solution (0.5 g/L) previously calibrated with a pure vitamin C solution

2.2.3.8 Oxalates content

The oxalate content was determined with the standard **AOAC method (1990)** [10]. Two (2) grams of ground

dried hot or sweet pepper sample were homogenized into 200 mL of distilled water and added with 20 mL of 6N hydrochloric acid (HCl). The mixture was heated in boiling water bath for 1 h, cooled, and filtered. Fifty (50) mL of the filtrate were then homogenized into 20 mL of 6 N HCl, and filtered again. The 2nd filtrate was treated with methyl red (0.1%, w/v), concentrated ammonia, heated, and filtered. The 3rd filtrate was boiled, treated with calcium chloride (5%, w/v) for the formation of calcium oxalate crystals, and then filtered once more. The residues deriving from the filtration steps were successively washed with distilled boiling water, dried into an oven; dissolved into 10 mL of diluted sulfuric acid, and titrated with 0.05N potassium permanganate solution

2.2.3.9 Phytates content

The phytates were measured according to the method processed by **Mohammed et *al.*** (1986) [18]. A slight ground hot or sweet pepper sample (0.5 g) was treated with 25 mL of TCA solution at 3% (w/v) and centrifuged at 3,500 rpm for 15 min. Five (5) mL of the supernatant was removed, treated with 3 mL of ferric chloride 1% (w/v) reagent, heated in a boiling water bath, cooled and also centrifuged at 3,500 rpm for 10 min. The 2nd supernatant was treated with 5 mL of 0.5N hydrochloric acid, 5 mL of 1.5N sodium hydroxide, heated in a boiling water bath and centrifuged once more at 3,500 rpm for 10 min. Thus, 1 mL of the final supernatant was added with 4.5 mL of distilled water and 4.5 mL of orthophenantroline reagent and then measured for the absorbance at 470 nm with a spectrophotometer against standard Mohr salt solution treated likewise and taken as phytates ferric control.

2.2.3.10 Polyphenols contents

The phenol compounds were extracted from hot or sweet pepper with methanol reagent. One gram of dried pepper sample was homogenized in 10 mL of methanol solution 70% (v/v). The resulting mixture was centrifuged at 1,000 rpm for 10 min. The pellet was recovered and treated likewise. The deriving supernatants were thus gathered into a marked flask and added with distilled water at 50 ml.

The total polyphenols content was measured using Folin-ciocalteu reagent, sodium carbonate solution (20% w/v) and distilled water (**Singleton et *al*, 1999)[19].** Essays were measured for their absorbance at 745 nm with a spectrophotometer against standard gallic acid solutions taken as polyphenols control.
The tannins content was deducted from the total polyphenols using vanillin reagent (**Bainbridge et *al*, 1996)[20].** Essays were measured for their absorbance at 500 nm with a spectrophotometer against standard tannic acid solutions taken as tannins control.

Flavonoids content was also determined from the total polyphenols using aluminum chloride (10% w/v), potassium acetate (1 M) and distilled water (**Meda et *al*, 2005)[21].** Essays were measured for their absorbance at 415 nm with a spectrophotometer against standard quercetin solutions taken as flavonoids control

2 2 4 Determination of minerals

The determination of the mineral elements was performed according to the **IITA method (1981)** [22]. Finely ground hot or sweet pepper sample (0.4 g) previously oven dried at 60 ° C was incinerated into a muffle furnace at 550 °C for 3 h. The resulting gray-white ash was cooled, added with 2 mL of half-diluted HCl, placed on a sand bath at 120 °C until full evaporation, and then ovened at 105 °C for a 1 h. The final dry extract was recovered with 2 mL of half-diluted HCl, filtered, and the resulting filtrate added with distilled water, and lanthanum chloride. The mineral elements in the solution were then measured using Atomic Absorption Spectrometry (AAS 20 type VARIAN).

2-2-5-Statistical Analysis

All analyzes were performed in triplicate, then data processed using Statistical Program for Social Sciences software (SPSS version 20.0, SPSS for Windows, USA). For each characteristic, the results were expressed as means followed by their standard deviations as parameters of data dispersion. A one-way analysis of variance (ANOVA 1) was also performed to test the effect of variety on the characteristics assessed, at the statistical significance level of 5%. For statistically different means, classification was performed with the Student-Newman-Keuls test.

III. RESULTS

3-1- Physical properties

The sweet pepper has a length (6.48cm ± 0.6), a circumference (14.52cm ± 0.45), a mass (37.61g ± 4.44), a humidity (91.48% ± 0, 3) higher compared to length (3.37cm ± 0.23), circumference (6.55cm ± 0.28), weight (3.44g ± 0.11), humidity (88 % ± 0.71) of the hot pepper. For the ashes, it is rather the hot pepper which has a higher content (0.95% ± 0.11) than that of the sweet pepper (0.55% ± 0.06). (Table I)

Table I : Physical parameters of the two varieties of the species Capsicum annuum

Parameters	Hot pepper	Sweet pepper	P-value
Length (cm)	3.37± 0.23[a]	6.48 ± 0.6[b]	0.001
Circumferences (cm)	6.55 ± 0.28[a]	14.52± 0.45[b]	0.000
Mass (g)	3.44 ± 0.11[a]	37.61 ± 4.44[b]	0.000
Humidity (%)	88 ± 0.71[a]	91.48 ± 0.3[b]	0.001
Ashes(%)	0.95 ± 0.11[b]	0.55 ± 0.06[a]	0.006

Per row, values followed by different superscript letters are statistically different at 5%. P-value: value of the statistical probability test.

3-2- Chemical properties

Hot pepper has a higher content of lipid (0.45% ± 0.04), of protein (1.46% ± 0.16), of fiber (5.53% ± 0.06), of total carbohydrate (9 , 14% ± 0.57) and a higher energy value (46.45Kcal / 100g) compared to the content of lipid (0.36% ± 0.01), protein (0.87% ± 0.14), fiber (2.43% ± 0.5), in total carbohydrates (6.74% ± 0.17), at the energy value (32.68Kcal / 100g ± 0.82) of sweet pepper.

The two (2) varieties of *Capsicum annuum* did not show significant differences at the 5% threshold for pH, titratable acidity, reducing sugar content and total sugar content. For these parameters, the respective general means are: 5.46 ± 0.04; 3mEq / 100g ± 0.19; 196.52 mg / 100g ± 61.46; 3.58% ± 0.52 (Table II).

TableI : Chemical parameters of varieties of the species Capsicum annuum

Parameters	Hot pepper	Sweet pepper	General average	P value
P^H	5.44 ± 0.01[a]	5.48 ± 0.06[a]	5.46±0,04	0.318
Titratable acidity (mEq/100g)	3.08 ± 0.08[a]	2.92 ± 0.25[a]	3±0,19	0.344
Reducing sugar (mg/100g)	175.13± 81.07[a]	217.92 ± 38.70[a]	196.52±61.46	0.456
Total sugar (%)	3.24± 0,32[a]	3.93 ± 0.45[a]	3.58±0,52	0.095
Lipids (%)	0.45± 0,04[b]	0.36 ± 0.01[a]		0.036
Proteins (%)	1.46± 0,16[b]	0.87 ± 0.14[a]		0.008
Fiber (%)	5.53± 0,06[b]	2.43 ± 0.5[a]		0.000
Total carbohydrate (%)	9.14± 0,57[b]	6.74± 0.17[a]		0.002
Energy value (Kcal/100g)	46.45 ± 2,59[b]	33.68± 0.82[a]		0.002

Per row, values followed by different superscript letters are statistically different at 5%. P-value: value of the statistical probability test.

3-3- Antioxidant and Antinutrient Content

Except for the contents of polyphenols, there are no statistical differences between the contents of antioxidants. However, there is a statistical difference between the levels of anti-nutrients. Indeed, the polyphenol content of hot pepper (200.41 mg / 100g ± 40.07) is higher than that of sweet pepper (125.91mg / 100g ± 8.46). In addition, the two (2) varieties of pepper studied do not show significant differences at the 5% threshold concerning the contents of vitamin C, flavonoids and tannins. For these characteristics, the respective general averages are: 44.16 mg / 100g ± 19.02; 3.65mg / 100g ± 1.40; 19mg / 100g ± 3.62. Regarding anti-nutrients, hot pepper has a higher phytate content (25.31mg / 100g ± 0.69) than that of sweet pepper (21.47mg / 100g ± 1.2) while sweet pepper has a higher oxalate content (377.66 mg / 100g ± 13.22) than that of hot pepper (301.89 mg / 100g ± 23.85) (Table III).

Table II : Antioxidant and anti-nutrient content of varieties of the species Capsicum annuum

Parameters		Hot pepper	Sweet pepper	General average	P-value
Antioxidant content	Vitamin C (mg/100g)	46.94 ± 26.70[a]	41.39 ±12.99[a]	44.16±19.02	0.762
	Polyphenols (mg/100g)	200.41± 40.07[b]	125.91 ± 8.46[a]		0.035
	Flavonoids (mg/100g)	4.36 ± 1,36[a]	2.95 ± 1,3[a]	3.65±1,40	0.256
	Tannins (mg/100g)	21.07 ± 3.90[a]	16.94 ± 2.2[a]	19±3.62	0.185
Anti-nutrient content	Oxalate (mg/100g)	301.89±23.85[a]	377.66±13.22[b]		0.009
	Phytate (mg/100g)	25.31 ± 0,69[b]	21.47± 1,2[a]		0.009

Per row, values followed by different superscript letters are statistically different at 5%. P-value: value of the statistical probability test.

3 4. Mineral content

The hot pepper and the sweet pepper of Capsicum annuum contain phosphorus, potassium, calcium, magnesium, copper, iron, manganese, zinc, sodium, the contents of which do not vary significantly at the threshold of 5 % from one variety to another. The respective general means are (0.15% d.m ± 0.015); (0.34% d.m ± 0.017); (0.31% d.m ± 0.006); (0.17% d.m ± 0.003); (3.45ppm ± 0.62); (7.53ppm ± 0.488); (0.64ppm ± 0.13); (16.74ppm ± 1.155); (11.13ppm ± 5.261).

Table IV : Mineral content

Parameters	Hot pepper	Sweet pepper	General average	P-value
Phosphorus (%d.m)	0.16±0.02[a]	0.14±0.006[a]	0.15±0.015	0.345
Potassium (%d.m)	0.34±0.018[a]	0.35±0.02[a]	0.345±0.017	0.779
Calcium (%d.m)	0.31±0.009[a]	0.31±0.003[a]	0.31±0.006	0.67
Magnesium (%d.m)	0.17±0.003[a]	0.17±0.004[a]	0.17±0.003	0.422
Copper (ppm)	3.6±0.79[a]	3.31±0.52[a]	3.45±0.62	0.59
Iron (ppm)	7.35±0.67[a]	7.71±0.21[a]	7.53±0.488	0.432
Manganese (ppm)	0.66±0.2[a]	0.63±0.05[a]	0.64±0.13	0.812
Zinc (ppm)	17.18±1.66[a]	16.31±0.06[a]	16.74±1.155	0.415
Sodium (ppm)	9.77±3.85[a]	12.49±6.99[a]	11.13±5.261	0.587

Per row, values followed by different superscript letters are statistically different at 5%. P-value: value of the statistical probability test.

IV. DISCUSSION

The study showed a greater size, circumference and mass of the sweet pepper compared to the size, circumference, and mass of the hot pepper. The sweet pepper therefore has larger dimensions than those of the hot pepper; this was also observed by Pegon in 2009 [6] and Zaman in 2009 [7]. However, these dimensions are smaller than those of the eggplant *Solanum melogena* (15.51cm ± 1.73 for the size; 17.77cm ± 0.66 for the circumference; 161.21g ± 33.82 for the mass) studied by Niamke et *al* in 2019 [23].

The two varieties of *Capsicum annuum* have very high humidity percentages, over 80% which are close to that of Lopez-Hernandez et *al.*, 1996) [24] who said that the water content of hot pepper is 91%. These values are also close to that revealed for the onion variety (*Allium cepa*) V1320 (89.06%) by Konate et *al*. 2017 [25]. The moisture content of peppers is disadvantageous for their storage since peppers could be subject to rapid postharvest change such as rotting. This phenomenon was already observed by Ali et *al* in 2010 [26] on the fruits of the palmyrah palm. The difference observed in the moisture percentage of the two varieties could be due to the variety and the water absorption capacity of each of the plants (Kouakou, 2017) [27].

For the pH and titratable acidity values, there is no significant difference at the 5% threshold for the two (2) varieties of Capsicum annuum. However, the pH of hot peppers and sweet peppers are higher than that of the local variety of tomato Gbogan in Benin (4.17) studied by Dossou et al in 2007 [28]. Coliforms of the Escherchia coli type can develop on both varieties of pepper since the minimum pH required for the development of such microorganisms is 4.3 according to Rozier et al, in 1985 [29]. It will therefore be necessary to wash the peppers well before consumption.

The two varieties of capsicum annuum pepper contain lipids (0.45% and 0.36%) whose contents are higher than those given by Ciqual in 2013 [30] for cooked spinach (0.14%) but are lower than that of Okouango et al. (2015) [31] for the leaves of Phytolaccado dodecandra or wild spinach (1.6%). According to Anses in 2021 [32], lipids play two (2) major roles: an energy storage role and a structural role (enter into the composition of cells).

The two varieties of *Capsicum annuum* show a significant difference at the 5% threshold for protein content. These levels are lower than those of the two eggplant varieties *S. aethiopicum gilo* (1.68 ± 0.04) and *S. melogena* (1.81 ± 0.06) given by Niamke et *al* in 2019 [23] and the level obtained by Okouango et *al*. (2015) [31] for cassava leaves which was 7%. Proteins are essential to the body, they play a structural role (at the muscular or even skin level) but are also involved in a large number of processes such as the immune response (antibodies), the transport of oxygen in the organism (hemoglobin) or digestion (digestive enzymes) (ANSES, 2021) [32].The protein contents expressed by the two varieties studied could be explained by the effects of light and the adsorption of N03 by the roots (Guéguen, 1959) [33]. In fact, during the growth of the plant, it draws nitrogen from the soil in the form of N03 through its roots. The amount of nitrogen absorbed will depend on the needs of the plant related to the variety. And this quantity tends to increase or decrease with the degree of adsorption of light, of C02 during respiration, which also takes into account the variety and the climate (Kouakou, 2017) [27].

The total carbohydrate contents of the two varieties (9.14% and 6.74%) are also higher than those recorded by Niamke et *al* in 2019 [23] on the eggplant varieties *S. aethiopicum gilo* and *S. melogena* (5.33% and 4.50%). These total carbohydrate contents are lower than that of sweet potato (28.5% according to the FAO in 1992) [34]. These two (2) varieties of *Capsicum annuum* contain carbohydrates which are compounds that provide energy for the functioning and maintenance of muscle cells, brain, red blood cells and other organs, etc. (Martin, 2000; Folin, 2005; Fredot, 2009). [35,36,37]

Obviously, peppers are vegetables and are not really used as a source of carbohydrates, fat and protein. Therefore, they are recommended in diet, as foods with low calorie content. It is in this sense that Hobbs (1994) [38] reported that consumption of Capsicum helped to lose weight in obese people.

The peppers studied also contain fibers of different contents (5.53% and 2.43%). The fiber contents of cooked spinach (2.7%) and cooked green beans (4%) (Ciqual, 2013) [30] are intermediate between the two fiber contents of peppers. But the fiber content of peppers is lower than those observed by Kouakou in 2017 [27] for the leaves of three varieties of cassava (*Manihot esculenta Crantz*), the average value of which is 8.15%. These two varieties of peppers could be a significant source of dietary fiber, which is eliminated more slowly from the stomach and thus improves intestinal transit. These dietary fibers are absolutely essential for the balance of the digestive tract and that of the body. They represent a factor of good health. Studies have shown an inverse correlation between the consumption of dietary fiber and colon cancer. In fact, fibers have the capacity to complex with carcinogenic molecules, thus preventing their contact with the colon and facilitating their excretion (Jansen et al., 1999; Chene, 2003) [39,40]. Consumption of the two (2) varieties of *Capsicum annuum* could therefore increase gastric volume

and constitute a post-ingestive state making it possible to reach a state of satiety more quickly (Chene, 2003; Al-Dobaib, 2009) [40,41]. Fiber generally reduces blood glucose, HDL-cholesterol and LDL-cholesterol levels and thus helps reduce coronary heart disease (Jalili et al., 2000) [42].

The polyphenol composition differs from the two varieties of peppers. The differences obtained may be due to the variability in chemical composition (Pino et al., 2005) [43]. These discrepancies may also be due to the growth stage of the plants studied and / or the collection season, to the nature of the sampling site (Salem, 2005) [44].

The presence of antioxidants may also reflect a response to stress (scarcity of rainfall, unfavorable soil quality which is associated with an increase in the level of tannins) (Mebirouk-Boudechiche et al., 2014) [45]. Thus, depending on the efforts made to adapt to environmental conditions by the plant, the amount of antioxidant decreases or increases. The measured vitamin C of the two varieties of Capsicum annuum is on average 44.16 mg / 100g. This value is higher than those observed by Niamke et al in 2019 [23] for eggplants S aethiopicum gilo (9.08mg / 100g) and S melogena (9.85mg / 100g). This value is also slightly higher than that of grapefruit (40.9 mg / 100g) and lower than that of lemon (129 mg / 100g) (Pilège, 2021) [46].

Vitamin C plays several roles in the body. It contributes to the health of bones, cartilage, teeth and gums. It also protects against infections, accelerates wound healing and promotes iron absorption. In addition, it participates in the metabolism of hormones and drugs, and in the degradation of cholesterol reported by Mourey, 2004 [47]. Polyphenols have many health benefits, such as reducing cardiovascular, inflammatory or neurodegenerative diseases, preventing cancer, antiplatelet effects, regulating blood pressure, etc. (Achat S, 2013) [48]. The flavonoid contents (3.65 mg / 100g on average) are interesting, flavonoids can neutralize free radicals and reduce the risk of cancer by stopping cell growth in tumors (Wang et al, 2014) [49].

Hot peppers and sweet peppers contain anti-nutritional compounds: oxalates and phytates. These compounds modify the digestibility of nutrients (antiproteases), they are also chelators which reduce the bioavailability of minerals (Weston Petroski and Deanna M. Minich, 2020) [50]. On the other hand, food preparation methods, especially cooking or heat treatment, are the most effective way to inactivate them (Matthias Schulze et al, 2018) [51].

The studies carried out have shown that the two varieties of Capsicum annuum contain minerals (phosphorus, potassium, calcium, magnesium, copper, iron, manganese, zinc, sodium) whose contents do not vary significantly at the 5% threshold of a variety to the other. A diet containing calcium and phosphorus is a factor in preventing osteoporosis and also a factor in reducing the risk of arterial hypertension, colon and prostate cancer (Bonithon-Kopp et al, 2000) [52]. Potassium is a mineral that increases cardiovascular well-being. Just like magnesium recommended for the prevention of certain complications of myocardial infarction (Kannel et al, 1997; Chow, 2009 [53,54]). Iron plays a major role in the production and functioning of hemoglobin, iron is also involved in the constitution of myoglobin, the protein responsible for oxygenating the muscles; zinc is important in the phenomena of cell renewal, healing and immunity; selenium generally contributes to the body's defense reactions (ANSES, 2017) [55]. Manganese is essential for the metabolism of amino acids, carbohydrates and lipids, it participates in the production of insulin (Florence Daine, 2017) [56].

V. CONCLUSION

It emerges from this study on the two varieties of the species *Capsicum annum*, that the hot pepper has a content of ash, lipids, proteins, fibers, total carbohydrates, energy value, polyphenols, phytate, more high. Sweet peppers, on the other hand, have a higher length, circumference, mass, and moisture content, oxalate. Moreover, for these two varieties of peppers, there are no significant differences concerning the contents of pH, titratable acidity, reducing sugars, total sugars, vitamin C, flavonoids, tannins, minerals. Ultimately, these two (2) varieties of *Capsicum annuum* contain macronutrients such as carbohydrates, proteins, lipids and in addition they have fiber, vitamin C, polyphenols, flavonoids, minerals which are beneficial for populations. Anti-nutrients are inactivated by cooking.

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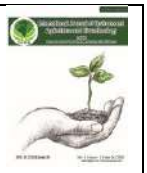

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Implementation of Plant Selection Based-On Plant Growth on Revegetation of Peatland in South Kalimantan

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Abstract—*Tropical peatland is remaining to play a critical geographic, political, and economic role in globalization. Revegetation maintains a vegetation cover on peat and able to increase humidity and decreasing fire risks. The selection of adaptable plants that growing on peatland are the main factors of revegetation accomplishment. The location of the research was in the peat forest of Pulantani village, Haur Gading District, Hulu Sungai Utara Regency, South Kalimantan Province, Indonesia and carried out for 12 months. The plants used are Dyera costulata, Hevea brasiliensis, Melaleuca cajuputi, and Shorea balangeran. Revegetation species were analyzed by calculating percent of plant life and plant growth. Characteristics of revegetation area of the peat forests cover with pure stands of trees and poles of Combretocarpus rotundatus species. Percent of plant life during seed distribution activities are varies between the four species. S.balangeran has the highest percentage of life with a value of 97%, H.brasiliensis has 96%, M.cajuputi has 88% and D.costulata has 77%. There was a decrease in percent growth over time after planting with S.balangeran has the highest percentage compared to other types. Percent growth of S.balangeran is 100% at the beginning of planting (t_0), 96.7% after 1 month of planting (t_1), 93.4% after 3 months of planting (t_2), 84.40% after 8 months of planting (t_3) and decreased up to 55% after 12 months of planting (t_4). Although the phenomenon that occurs is that no tree species achieves 75% growth success , S.balangeran is highly recommended as a selected species in the revegetation of peatlands.*

Keywords—***Peatland, plant species, plant growth, revegetation, S.balangeran.***

I. INTRODUCTION

Peatlands along with tropical forests are providing not only important on the ecological, but also benefits to climate [1] and a wide range of socio-economic from the local to the global scale. However, it is threatened by anthropogenic activities, including agricultural conversion, timber harvesting, peatland drainage and associated fire [2]. Southeast Asian as a region with 60% of global tropical peatland, is continuing to play a critical geographic, political, and economic role in globalization [3].

Indonesian peatlands have been damaged due to various activities. Forest Watch Indonesia in 2014 was reporting that the area of natural forest lost on peatlands reached 1.1 million ha from 2009 - 2013. Kalimantan as one of the largest islands in Indonesia that has peat forest is indicating the area of peatlands had decreased by 2.9% per-year over a period of 20 years. Peat swamp area in Kalimantan has decreased from an area of 4.93 million ha to 2.18 million ha from 1990-2010 million ha [4]. Burnt peat forests generally have low natural regeneration [5]. A

study by [6] shows that the relative humidity contributes most to the burned area in Kalimantan. Meanwhile, revegetation is able to maintain a vegetation cover on peat and increasing humidity in the soil and air, slowing peat decomposition and also decreasing fire risks, thus becoming the second important tenet of peatland restoration after restoration of the hydrology [7]. Moreover, peatland restoration is also maintaining carbon pools (Vasquez, 2021). Furthermore, it is recommended that forest cover should be maintained at the existing level [8]. Peat rehabilitation is a conservation effort in order to save peat forests. Rehabilitation activities can be carried out by using revegetation methods. Revegetation is also expecting to improve damaged peat ecosystems.

An important factor determining the success of revegetation is by selection of the right species and adaptable to the peat environment. The selection of local species (native species) is highly recommended since it has several advantages including aspects of plant health, land productivity, biodiversity and ecosystem services [9] - [12]. Some species recommended in the rehabilitation of peat swamp forest rehabilitation are including *Dyera lowi, Alstonia pneumatophora, Combretocarpus rotundatus, Shorea pauciflora, Tetramerista glabra, Melanorrhoea walichii, Barringtonia racemosa, Syzigum spp*. and *Gonystylus bancanus* [13].

Previously, [14] shows *S.balangeran, M.cajuputi* and *D.costulata* as vegetations that grow on the peatland. *M.cajuputi* dan *S.balangeran* were found to be dominant in the peat forest of Muara Kendawang, West Kalimantan [15]. *M.cajuputi* and *S.balangeran* are the main commodities of paludiculture for peatlands in South Kalimantan and Central Kalimantan [16]. One of the species suitable for planting in burnt peat swamp forests is including *S.balangeran* [17], [18] and *D.costulata* [18]. *D.costulata* is the most suitable species to be developed on deep peatlands due to it has the fastest growth [19]. *Hevea brasiliensis* is an exotic plant that can grow on peatlands. Seedling plant species in this research consist of four species that are three local peat forest species and a type of exotic species. Those selected species as recommended [20] are *Dyera costulata, Melaleuca cajuput, Shorea balangeran* and *Hevea brasiliensis* (as an exotic species).

One of the area in Kalimantan which has peatlands is Pulantani Village, Haur Gading District, Hulu Sungai Utara Regency, South Kalimantan. Peatlands in Pulantani village are being degrading. Repeated fires are a phenomenon that occurs in the Pulantani village peatlands. According to the results of identification of the degradation level of existing peatlands, the Pulantani village area is considered as a priority area for revegetation. The problems of revegetation activities in peatland are limited on the environmental components. The relatively long drought in the dry season triggers land/forest fires. The long period of the rainy season also triggered flooding in the peat forest area. Low pH and soil fertility are also limiting factors in revegetation in peatlands. These constraints can be solved by various effort. One of the efforts is selecting suitable species to plant in peat areas.

II. MATERIAL AND METHODS

The research location is in the peat forest of Pulantani village, Haur Gading District, Hulu Sungai Utara Regency, South Kalimantan Province, Indonesia. The times of data collected were 12 months (December 2018 - December 2019). The times needed in data collection are t_0 = early planting, t_1 = 1 month after planting, t_2 = 3 months after planting, t_3 = 8 months after planting and t_4 = 12 months after planting. Description of research location is shown in Fig.1.

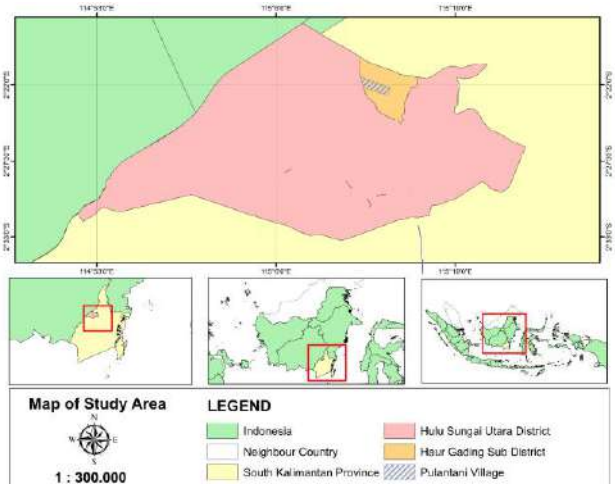

Fig.1: Map are of research location

The research equipment includes field survey tools, distance measuring tools, camera for documentation and a set of computers. The observation of plant composition on peat land for revegetation area is using quadrats plot. There were 15 plots of 10 m x 10 m area measurements and placed in revegetation site. The 10 m × 10 m used to record trees with DBH (Diameter at Breast Height) ≥ 5 cm. The vegetation parameters were number of individuals and species identity. Data of the composition of vegetation types are analyzed descriptively in the revegetation plot. Hypothetically, there are at least 2 species of tree vegetation (woody plants) contained in the planned revegetation location.

The plant species acting as material were *Dyera costulata (D.costulata), Hevea brasiliensis*

(H.brasiliensis), *Melaleuca cajuputi* (*M. cajuputi*), and *Shorea balangeran (S.balangeran)*. The height of the plant seeds used in the study was ± 100 cm. Plant height after early planting in the field was ± 80 cm. During planting plot of peat revegetation, spacing used in planting plot is 4 m x 5 m and equal to 500 seedling/ ha. The planting plot was 21 ha in size, so that there were total of 10,500 seedlings used. The planting hole made with a size of ± 30 x 30 x 30 cm^3. The data recorded is the number of individuals and the number of plant species. The data used are the results of measurements at t_0 = early planting, t_1 = 1 month after planting, t_2 = 3 months after planting, t_3 = 8 months after planting and t_4 = 12 months after planting. Data analyzed by calculating the percentage of plant growth. Percent growth measurement carried out through the following formula:

$$T = \frac{\sum hi}{\sum Ni} \times 100\% \quad (1)$$

where:

T = percent of plant growth

$\sum hi$ = number of living plants

$\sum Ni$ = the total number of plants planted

The standard of success for revegetation growth used is 75% (according to the Regulation of the Minister of Environment and Forestry of the Republic of Indonesia Number P.105/Menlhk/Setjen/Kum.1/12/2018 concerning Procedures for Implementation, Supporting Activities, Providing Incentives, and Guidance and Control Forest and Land Rehabilitation Activities). The research hypothesis is that all species selected have a survival rate > 75%

III. RESULTS

The site of revegetation of peat forest in Pulantani village is dominated by tree (≥ 10 cm dbh) of *Combretocarpus rotundatus* due to repeated fires have limited tree species that can grow on peatlands. These limitations cause only *C.rotundatus* that can live and develop to form a pure stand.

The number of tree species consisting of one species is an indication that the peat revegetation area has been degraded. Repeated fires result in only certain species that can survive. *C.rotundatus* is included in the category of less fire tolerant species that can adapt to repeated fire events [21]. This result is different from the hypothesis that there are at least 2 species found in degraded peat forests. It is also different from [22] that burned peat areas were dominated by pioneer species such as *C.rotundatus* dan *Cratoxylon arborescens.* Meanwhile, *Malaeuca cajuputi* is pioneer wood species that usually occur in degraded peatland [23]. *M.cajuputi* is a species of a tree vegetation that is able to grow on peat swamp soil in South Kalimantan and has a low pH [24]. Based on the density of trees, stands of *C.rotundatus* are included in the rare category. Descriptions of vegetation found in revegetation sites are shown in Fig.2.

Fig.2: C.rotundatus stands on the peatland

There are at least 6 species of undergrowth are found on revegetation area of peatland. Species of undergrowth were including *Eichhornia crassipes*, *Lepironia articulata*, *Melastoma malabathricum*, *Phragmite scarka*, *Salvinia molesta*, *Stenochlaena palustris*. *C.rotundatus* stands which are classified in the rare category cause revegetation patterns using enrichment planting models with a spacing of 4 m x 5 m (target number of plants of 500 stems /ha).

The percentage of seedling life during seed distribution activities are varies between species. The results of the percentage of plant life using Equation (1) are described in Fig.3. Fig.3 shows that the lowest percentage of plant life during seed distribution activities is *D.costulata*. Characteristics of seedlings with weak stems, morphological shapes of leaves and roots that are not firmly formed in the polybags compared to other related species are factors that cause *D.costulata* species to have low resistance during the seed distribution process. The height of *D.costulata* seedling with a size of ≥100 cm is unable be supported properly by the organic media available in the poly bag. Based on the field observations, the use of *D.costulata* seedling with a height of ± 30-40 cm is ideal and supported by planting media in a poly bag. Sufficient organic matter supported by the media in a poly bag is reducing the risk of death during seed distribution.

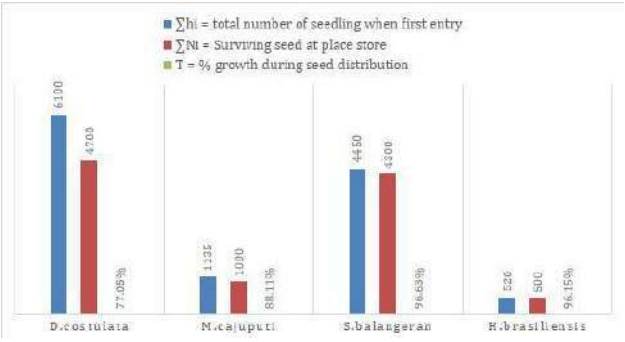

Fig.3: The percentage of seedling life during seed distribution activities

The ability of roots to be firmly supported by the media in a polybag in order to reduce the risk of death during seed distribution. Regulations in revegetation of peatlands require the use of seeds with a height of ≥ 100 cm. This phenomenon is considered during determining the height of the seedlings used in the revegetation in order to deliberate the characteristics of the use of plant species. The percentage of plant life had been measured from the beginning of planting (t_0), 3 months after planting (t_1), 8 months after planting (t_3), and 12 months after planting (t_4). The percentage of life obtained from research results are varies based on the species. The results of measurements of the number of live plants are listed in Table 1.

Table 1. Number of live plants in revegetation activities

Species	T_0	T_1	T_2	T_3	T_4
D.costulata	4700	4375	3520	340	80
M.cajuputi	1000	955	865	218	118
S.balangeran	4300	4157	4018	3645	2372
H.brasiliensis	500	478	470	210	10
Total	10500	9965	8873	4413	2535

The number of plants with the highest survival rate is *S.balangeran*, while the species with low survival rates are *D.costula* and *H.brasiliensis*. The weather factor in this case is the high rainfall that instigated flooding/inundation. It occurred in the 3rd, 4th and 5th month of planting activities. The long period of inundation with a height of ≥ 2 m has increased plant mortality. The dry season period starts from the 7th month to the 11th month. The percentage of plant life in revegetation activities is shown in Fig.4.

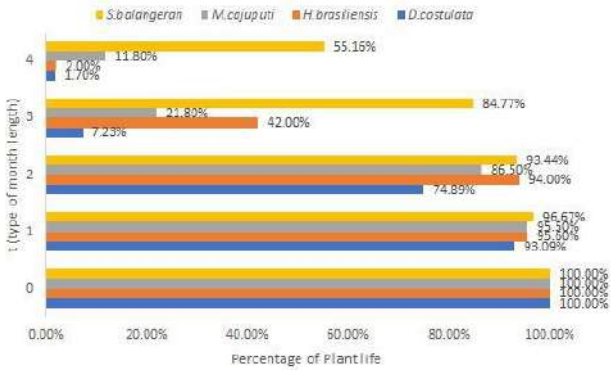

Fig.4: Correlation between number of plants (in percentage) and duration (in type of month length)

Percentage of plant life shows the difference between each plant. *S.balangeran* has the largest growth percentage from 100% at the beginning of planting (t_0), decreasing to 96.7% after 1 month of planting (t_1), decreasing to 93.4% at 3 months after planting (t_2), to 84.40 % after 8 months of planting (t_3) and decreased to 55% at 12 months after planting. Plant growth rates are still quite high at the beginning of planting until the 3rd month after planting (75% -94%). None of the species reached a survival rate of 75%. Species with growth percent > 50%, is *S.balangeran*. The success of growing *S.balangeran* is still higher than the results of other research which shows that the percent growth is only 50% after 8 months of planting (Tata and Pradjadinata, 2016)

The fourth month of the planting activity occurred inundations reaching ≥ 150 cm and exceeded plant height. Height and duration of inundation periods increase plant mortality. Flooded habitation impacted on the disruption of plant physiological processes and resulting in a lack of plant growth and even plant death [25]. Not all type of plants can withstand flood, plants will die after 4-6 weeks of inundation exceeding the height of the seeds planted [26]. *S.balangeran* indicated to be resistant to inundation. [27] stated *S.balangeran* still grow at a water level of 90 cm above the peat soil surface. In this research, *D.costulata* were not able to live well at the study site. However, *D.costula* seedling will develop well if planted with a mound system and not flooded for a long time. *D.costulata* seedling showed the best growth at a water level of 20 cm below the peat soil surface [18]. *H.brasiliensis* can grow well on peatlands if water management and cultivation techniques are carried out properly and correctly. Moreover, *H.brasiliensis* will produce good growth by planting a mound system and the ideal water level in peatlands is 60-100 cm.The 7^{th} to 11^{th} months enters a relatively long period of dry season. A period of drought and a rise in temperature during the dry season results in mortality in revegetation plants. Survival rate of seedlings that planted in reforestation area depends on tree adaptation to water logged, drought and fire [19].

The percentage of life of *S.balangeran* is higher than other species. It is indicating that *S.balangeran* is highly recommended for peat revegetation plants. For ecological value, *S.balangeran* is used as a promising species for restoring degraded peatland [28]. In addition, *S.balangeran* has a wide ecological range, able to grow in a variety of soil and environmental conditions, has adaptability in the open vegetation and able to compete with weeds [29]. [30] confirmed that *S.balangeran* could grow in very deep peat. *S.balangeran* grows on peat areas in which the water management has improved and grows dominantly on peat areas whose water systems have been damaged [31].

The high percentage of plant mortality is a signal that the number of seedlings for enrichment planting should be increased. Additional enrichment seeds in revegetation activities with a number of 20% of the total seeds should

be considered to increase the number. This is related to the relatively low percentage of revegetation plant growth on peatlands.

IV. CONCLUSION

The peatland revegetation accomplishment on the limited capacity of land can be resolved by selecting well adapted species to the peatland environment. *S.balangeran* selection is highly recommended species for peatland revegetation activities. Percent growth above 50% is an indicator of well adaptation of *S.balangeran*. Periodicity of the long rainy season has the potential to cause inundation which can cause plant death. The dry season is a natural factor which has the potential to increase temperatures and fire events. The combination of climatic and edaphic factors from peat forests is a factor that limiting the success of plant growth. The percentage of the prepared seedlings during revegetation activities on peatlands must be in view of the phenomenon of the low success of revegetation of peatlands.

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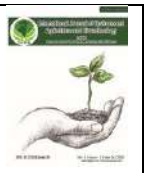

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Fate and Form of Nitrogen under Different Soil Redox Status

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Abstract**—** *The experiment sets out to investigate the effect of oxidation-reduction on nitrogen formsin soil. The trial was a 3 x 2 factorial experiment laid out in a Randomized Complete Block Design (RCBD) and replicated three times. Treatment consisted of two variables which are poultry manure at three levels (0t/ha, 6t/ha and 8t/ha) and water regime at two levels (field capacity and waterlogged).The combinations resulted into six (6) treatments resulting into three oxidation-reduction potential range [oxidized (>300), moderately reduced (-100 to 300), reduced (<-100)]. Data collected pH, Eh, Total nitrogen, Organic matter,NO_3^-N and NH_4^+-N. Data collected were subjected to Analysis of variance (ANOVA) while mean were separated using Tukey Honesty Significant Difference (HSD) Test. Graphs were generated using Microsoft excel. The result showed that nitrogen under oxidize condition is majorly in nitrate form while under reduced condition is ammonia. However, moderately reduced soil had the highest total nitrogen content. This is attributed to the fact that both forms of nitrogen are present in the soil as a result of accumulation of NH_4^+ and progress of nitrification process. It was concluded that the forms and fate of nitrogen in soil also depends on the oxidation-reduction status of the soil.*

Keywords**—** ***Ammonia, Oxidized, Reduced, Nitrate.***

I. INTRODUCTION

Nitrogen (N) is the most abundant element in the atmosphere and is usually the most limiting crop nutrient (USDA, 2014). Nitrogen fixation are necessary to convert N into forms which plants can use. Some of these processes can lead to nitrogen losses such as leaching or volatilization (Aczel, 2019). Nitrogen is added to soil naturally from N fixation by soil bacteria associated with legumes and through atmospheric deposition in rainfall (Brady and Weil, 2010). Additional nitrogen is typically supplied to the crop by fertilizers, manure, or other organic materials. Because nitrogen is so dynamic, it can easily be lost to the environment, making it difficult to determine adequate nitrogen levels throughout the growth cycle of crops. Soil nitrate-N is an excellent indicator of N-cycling in soils, whether carryover nitrogen was used by the previous crop and whether additional nitrogen is needed. Excessive application of N fertilizer can result in leaching of nitrates below the root zone and into groundwater at a shallow depth (Yadav, 1997). Factors such as soil drainage, soil texture, and slope steepness influence N-transport and N-transformation processes that limit availability of crops. Climatic factors such as rainfall and temperature as well as site condition such as moisture, soil aeration (oxygen levels) and salt content (electrical conductivity) affect the rate of N mineralization from organic matter decomposition, nitrogen cycling and nitrogen losses through leaching, runoff, or denitrification (USDA, 2014). The potential for leaching is dependent on soil texture and soil water content among other things (Domnariu*et al*., 2020). Organic matter decomposes releasing nitrogen more quickly in warm humid climate and slower in cool dry climates. This nitrogen release is also quicker in well aerated soils and much slower on wet

saturated soils. Soils that have poor drainage and are saturated with water causes denitrification to occur resulting in loss of nitrogen as a gas resulting in emission of potent greenhouse gases, yield reduction and increased nitrogen fertilizer expense (USDA, 2014).

As both $NO_3^-$and NH_4^+ are soluble, pH mainly influence the form under which N is assimilated by plants (Hinsinger*et al.*, 2003). The form of N assimilated by plants also has a marked effect on cellular regulation of pH (Marschner, 1995). Furthermore, as plants use NH_4^+ to synthesize proteins, the assimilation of NO_3^- N inducesa considerable energy cost for the plant to reduce NO_3^--N to NH_4^+-N (Marschner,1995). In addition, as NO_3^- is highly soluble, there is a risk of loss, and pollution. The form of nitrogen assimilated by plants has a marked effect on rhizospheric pH and on the assimilation by plants of other cations and anions (Hinsinger *et al.*, 2003). This forms of nitrogen is greatly affected by the prevailing conditions in the soil such as aeration and moisture content both of which are function of oxidation-reduction potentials. However, very little is known about how soil redox status affect the forms of nitrogen in the soil. Hence the necessity of this research.

II. MATERIALS AND METHODS

The study was carried out at the screen house of the Department of Crop, Soil and Pest Management, Federal University of Technology Akure, Ondo State, Nigeria.

The experiment was a 3 x 2 factorial experiment laid out in a Randomized Complete Block Design (RCBD) and Replicated 3 times. The two factors were poultry manure at 3 levels (0tha^{-1}, 6tha^{-1} and 8tha^{-1}) and water regimes at two levels (waterlogging and field capacity). Five kilogram homogenous soil was put inside each bucket after which levels of poultry manure was applied. Treatments subjected to field capacity were perforated underneath to allow for easy drainage of water from the bucket.Soil samples were collected, mixed together from which a composite sample was taken for pre-experimental analysis. Subsequent samples were collected based on treatments applied at an interval of 3 weeks (3WAI, 6WAI and 9WAI) samples collected were taken to the laboratory for analysis.

Analysis was done to determine the chemical properties of the soil, some of the properties considered are pH, Eh, organic carbon content, organic matter contents, Total nitrogen, Nitrate and Ammonium content.

pH

In determining the pH, 10g of soil was weighed and 20ml of distilled water was added to it. It was stir and allowed to stand for 30 minutes after which it was measured by using a pH meter.

Determination of Organic Matter

Soil organic matter content was determined using the Walkley-Black oxidation method which measures the active or decomposable organic matter in the soil. The soil sample was ground into fine powder from which 1g soil sample was taken and placed in a 250ml conical flask and 10ml of 0.167M $K_2Cr_2O_7$ was added. Twenty ml of conc. H_2SO_4 was rapidly added to the mixture and the swirled gently until the soil and the reagents mixed properly. The mixture was then allowed to cool for about 30 minutes, 3 drops of ferroin indicator was added and titrated against 0.5M Iron (II) ammonium sulphate. The end product is a brownish red or maroon color solution. Also a blank titration was done without soil.

Calculations

% Organic Carbon= (B-T) x M x 0.003 x 1.33 x 100/wt

Where:

B= Blank titre value

T= Sample titre Value

M= Molarity of Fe $(SO_4)_2$

Wt= Weight ofdriedsample

Percentage Organic Matter is then further calculated as

%OM= % Organic Carbon x 1.724

Soil Redox potential (Eh) determination

Redox potential was measured using the method described by (Rabenhorst*et al.*, 2009). Twenty gram of the soil samples were collected, soaked in water from bottom to top so as to prevent entrapment of air during saturation and allowed to mix for 30 minutes after which 50 ml of the solution was collected and taken to the laboratory for reading. In the laboratory, redox potential (Eh) was measured using a pH/Redox combined meter. Voltage was measured every 10 seconds for 60 seconds and the mean values of the collected measurements were calculated.

AMMONIUM

Five gram of soil was weighed, 50 ml of sodium acetate was added to it allow to stand for 2 hours then filter through filter paper. Then 5 ml of the filtrate was taken and 10 ml of sodium hydroxide was added to it then 25 ml of water. Distillate so that the steam will go to the conical flask where 5 ml of boric acid with 3 drop of ferroin indicator was. The color changes to green as the steam was

dropping and make it up to 25 ml. then titrated against 0.01M hydrochloric acid (HCL).

NITRATE

Five gram of soil was weighed, 50 ml of potassium chloride was added to it allow to stand for 30 minutes then filter through filter paper. Then 5 ml of the filtrate was taken and add 10 ml of sodium hydroxide, 25 ml of distilled water then set up distillation steam, the receiving flask 5 ml of boric acid with 3 drop of ferroin indicator was added. The color changes to green as the steam was dropping and make it up to 25 ml. It was then titrated against 0.01M hydrochloric acid (HCL).

NITROGEN

One gram of soil was weighed into conical flask and 20 ml of concentrated H_2SO_4 with one kjeldahl catalyst tablet was added. It was heated on digestion stand until the solution becomes clear and white. It was then allowed to cool after which 50 ml of distilled water was added. Mixed well then filtered through filter paper. 10 ml sodium hydroxide and 25 ml of distilled water was added to 10ml of the filtrate in a conical flask. To the receiving flask, 5ml of boric acid with 3 drops of ferroin indicator was added. Distillation steam was set up, 25ml of the distillate was collected inside the receiving flask and was titrated with 0.01M hydrochloric acid (HCL).

Statistical Analysis

Data collected were subjected to analysis of variance (ANOVA) using SPSS version 17 and means were compared with Tukey HSD test to verify significant differences among treatment at 5% probability level. Graphs and charts were generated using Microsoft excel 2013 edition.

III. RESULT

Pre Experimental Soil Analysis

Table 1 shows the pre experimental soil analysis of the soil used for the experiment. The soil is a sandy clay loam having a sand content of 64.82%, silt content of 15.14% and clay content of 20.04%. It has a pH of 4.61 making it a strongly acidic soil, the Eh value is 78 mV making it a moderately reduced soil, nitrogen content is 0.19%, organic matter content is 1.77%, nitrate content is 0.04% and ammonium content is 0.06%. Hence the soil can be considered moderately fertile.

Table 1: Pre experimental Soil Analysis

Parameters	Values
Sand (%)	64.82
Silt (%)	15.14
Clay (%)	20.04
Textural class	Sandy loam clay
pH	4.61
Eh (mV)	78
Eh class	Moderately reduced
Nitrogen (%)	0.19
Organic Matter (%)	1.77
NO^-_3 (%)	0.04
NH_4^+ (%)	0.06

Effect of Soil Oxidation-Reduction Potential on N-forms and Other Chemical Properties

Table 2 shows the effect of soil oxidation-reduction on N-forms and other chemical properties. At 3WAI the pH values range from 4.82 to 5.68 which is strongly acidic. Treatment 6 had the highest value of pH 5.68 while treatment 4 (reduced soil) had a pH value of 4.82. Nitrogen content range from 0.10 to 0.28, treatment 5 (oxidized soil) had a nitrogen content of 0.28 while treatment 2 (reduced soil) had a nitrogen content of 0.10.Nitrate content was highest at T1 (oxidized soil) having a nitrate value of 0.22 while T2 (reduced soil) had a nitrate content of 0.04. Ammonium content was highest at T3 and T5 (moderately reduced soils) while the lowest value was recorded at T2 which is 0.02. Organic matter was highest at T6 having an organic matter content of 4.88 the lowest value for organic matter was recorded at T1 (oxidized soil) having an organic matter content of 2.07. At 6WAI T6 recorded the highest value of pH having a value of 5.88 while T1 (oxidized soil) has the lowest of pH 5.03. Nitrogen content was highest on T5 having a value of 0.24 and was lowest on T2 (reduced soil) having a value of 0.07. Nitrate content was highest on T1 having a value of 0.22 and lowest on T2 having a value of 0.02. Ammonium content was highest on T5 having a value of 0.31 (moderately reduced soil) and lowest on T2 (reduced) having a value of 0.04. Organic carbon and organic matter was highest on T4 (reduced) having a value of 2.62 of organic carbon and 4.51 of organic matter and the lowest was recorded at T1 (oxidized) having a value of organic carbon 1.00 and organic matter 1.74. At 9WAI the pH value was highest on T6 (reduced) having a pH value of 6.05 while the lowest

was on T1 (oxidized) having a pH value of 5.00. Nitrogen content was highest on T5 (moderately reduced soil) having a value of 0.27 and lowest on T2 (reduced) having a value of 0.04. Nitrate content was highest on T1 (oxidized) having a value of 0.17and lowest on T2 and T4 both having a nitrate content of 0.00. Ammonium content was highest on T5 (moderately reduced soil) having a value of 0.38 and lowest on T2 (reduced) having an ammonium content of 0.02. Organic matter was highest on T4 (reduced) having organic matter content of 4.44 the lowest organic matter was recorded at T1 (oxidized) having organic matter content of 1.49.

Table 2: Effect of Soil Oxidation-Reduction Potential on N-forms and Other Chemical Properties

Treatments			**3WAI**		
	pH	N	NO_3^-	NH_4^+	OM
T1	5.03d	0.12e	0.22a	0.07b	2.07e
T2	5.10c	0.10f	0.04e	0.02c	2.13e
T3	5.44b	0.26b	0.11d	0.33a	4.64c
T4	4.82f	0.18d	0.09e	0.06bc	4.76b
T5	4.92e	0.28a	0.12c	0.33a	4.41d
T6	5.68a	0.20c	0.19b	0.09b	4.88a
			6WAI		
T1	5.03f	0.11d	0.22a	0.07c	1.74e
T2	5.15e	0.07e	0.02d	0.04c	2.06d
T3	5.76b	0.22b	0.13c	0.30a	3.98c
T4	5.22d	0.15cd	0.03d	0.11b	4.51a
T5	5.42c	0.24a	0.17b	0.31a	3.91c
T6	5.88a	0.17c	0.13c	0.16b	4.31b
			9WAI		
T1	5.00e	0.08c	0.17a	0.05d	1.49e
T2	5.18d	0.04c	0.00c	0.02d	1.57e
T3	5.90b	0.24a	0.16a	0.32a	3.91c
T4	5.74c	0.12b	0.00c	0.11c	4.44a
T5	5.80c	0.27a	0.15a	0.38a	3.76d
T6	6.05a	0.12b	0.09b	0.18b	4.24b

Means followed by the same alphabet are not significantly ($p < 0.05$) different from each other according to Tukey HSD.

Effect of Soil Redox Potential on Nitrogen Forms

Figures 1-6 Show the effect of redox potential on nitrogen forms in soil. T1 (oxidized soil) had a nitrate range from 0.22%, 0.22% and 0.17% and ammonia range from 0.07%, 0.07% and 0.05% at 3WAI, 6WAI and 9WAI respectively. T2 (reduced soil) had a nitrate range from 0.04%, 0.02% and 0.00% and ammonia range from 0.02%, 0.04% and 0.02% at 3WAI, 6WA1, and 9WAI respectively. T3 (moderately reduced soil) had a nitrate range from 0.11%, 0.13%, and 0.16% and ammonia range from 0.33%, 0.30% and 0.32% at 3WAI, 6WAI and 9WAI respectively. T4 (reduced soil) had a Nitrate range from 0.33%, 0.30% and 0.32% and ammonia range from 0.33%, 0.30% and 0.32% at 3WAI, .6WAI and 9WAI respectively. T5 (moderately reduced soil) had a nitrate range from 0.12%, 0.17%, 0.15% and ammonia range from 0.33%, 0.30% and 0.32% at 3WAI, 6WAI and 9WAI respectively. T6 (reduced soil) had a nitrate range from 0.19%, 0.13% and 0.09% and ammonia range from 0.09%, 0.16% and 0.18% at 3WAI, 6WAI and 9WAI respectively.

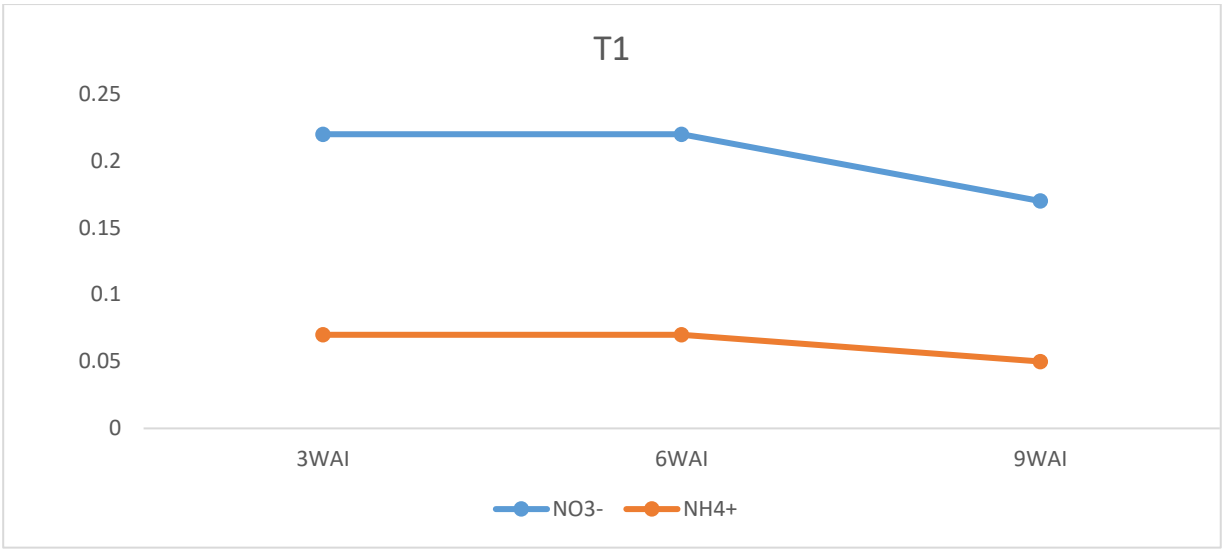

Fig.1: Effect of redox potential on nitrogen forms (oxidized)

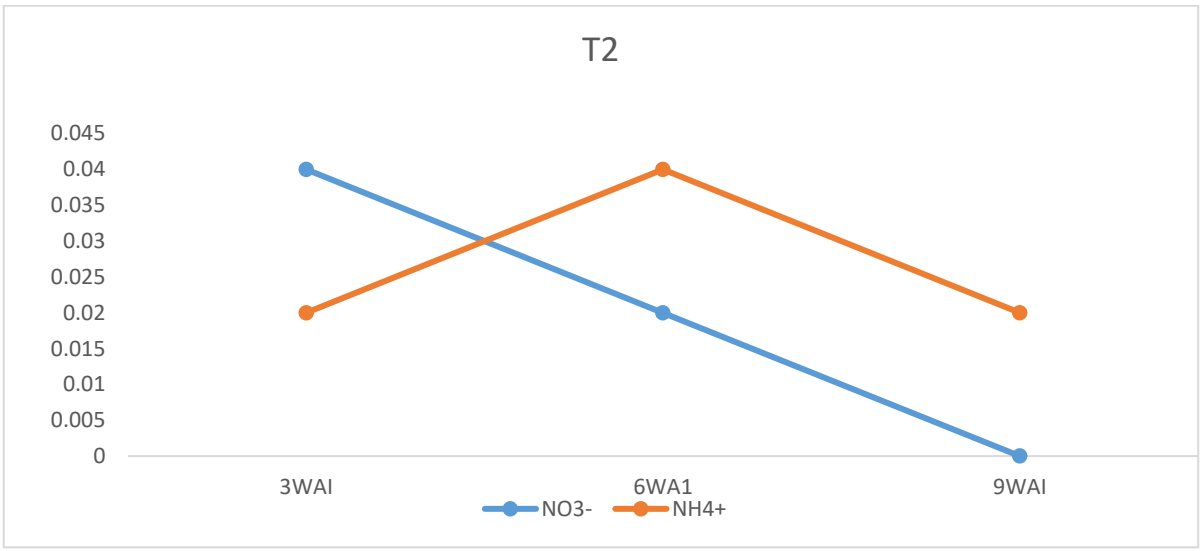

Fig.2: Effect of redox potential on nitrogen forms (reduced)

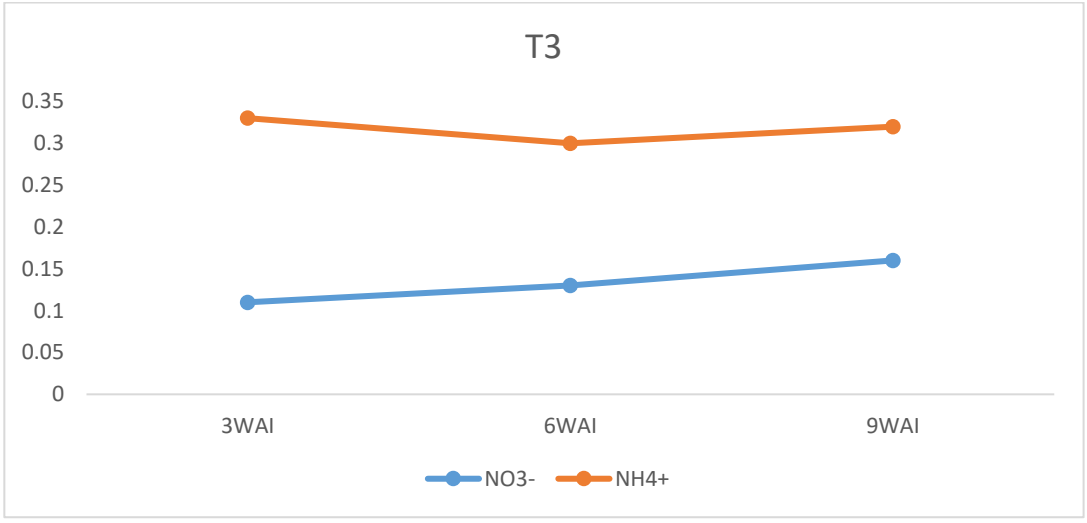

Fig.3: Effect of redox potential on nitrogen forms (moderately reduced)

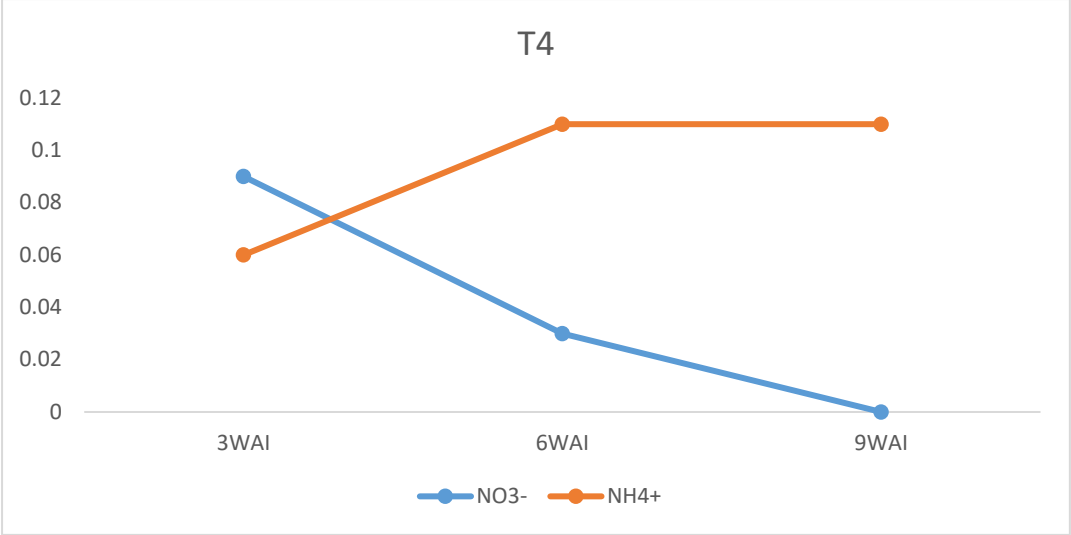

Fig.4: Effect of redox potential on nitrogen forms (reduced)

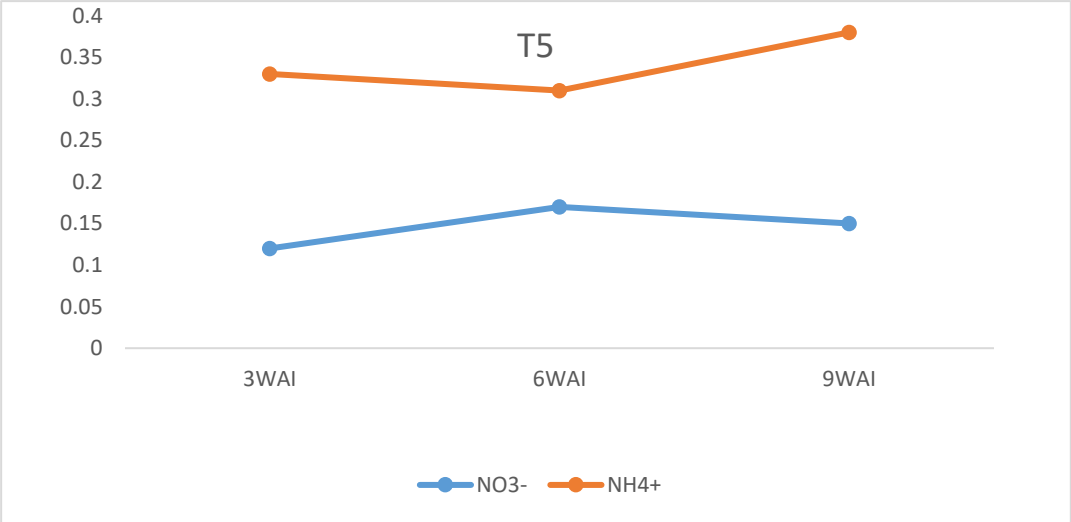

Fig.5: Effect of redox potential on nitrogen forms (moderately reduced)

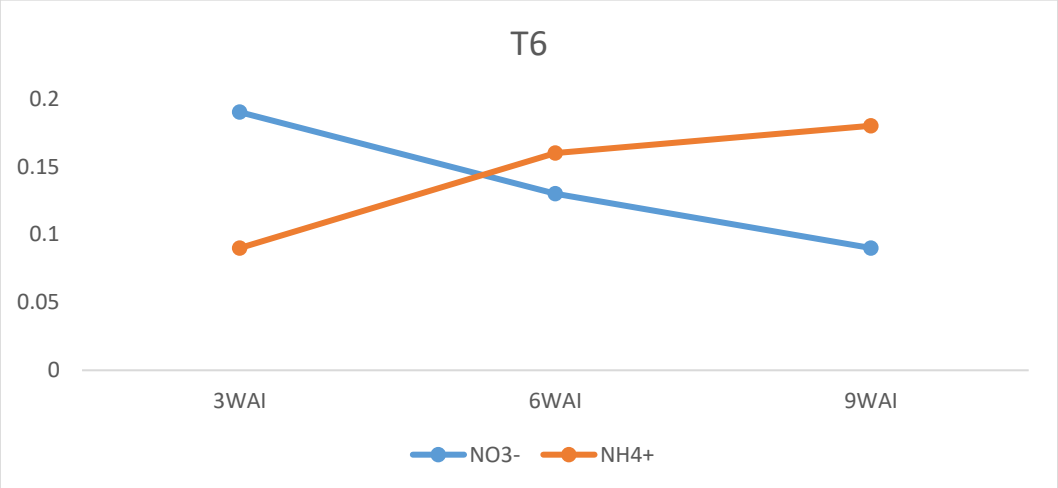

Fig.6: Effect of redox potential on nitrogen forms (reduced)

IV. DISCUSSION

The results from these research has been able to show that redox status of the soil can affect chemical properties of the soil as well as N-forms. The pH of T1 which is oxidized soil reduced across the trial period. (3WAI, 6WAI and 9WAI). It went from 5.03 to 5.00 i.e. it became more acidic. Reduced soil T2, T4 and T6 increase in pH from 5.10 to 5.18, 4.82 to 5.74, 5.68 to 6.05 at 9WAI, T3 and T5 which is moderately reduced soil there had an increased in pH also. Reduction of pH of oxidized soil T1 from 5.03 to 5.00 at 9WAI could be due to nitrification process. In oxidized soil nitrification process progresses and there is conversion of ammonia to nitrite and from nitrite to nitrate. This process is induce by nitrosomonas and nitrobacter respectively. The first step which involves the oxidation of ammonia to nitrite releases four molecules of hydrogen ion into the system and hydrogen ion is an acid forming ion. Hence, the net effect is a reduction in pH or acidification of the soil.

$$2NH_4^+ + 3O_2 \xrightarrow{\text{nitrosomonas}} 2NO^-_2 + 2H_2O + 4H^+ \text{ (oxidation)}$$

There was an increase in the pH of reduced soils and this could be attributed to the fact that the nitrification process is hindered since majority of the organism that are responsible for nitrification are aerobic in nature when the environment becomes reduced they cannot perform or function very well or can even die out. Also, reduction process according to Ojanuga, (1996) consumes hydrogen ion,since hydrogen ion is used up, the environment becomes less acidic. This process of hydrogen ion consumption is common in denitrification process where twelve (12) molecules of hydrogen ion is consumed to produce atmospheric nitrogen and six molecules of water.

$$2NO_3^- + 10e^- + 12H^+ \longrightarrow N_2 + 6H_2O \text{ (Denitrification)}$$

There is continuous reduction of nitrogen in oxidized soil and this can be attributed to the loss of nitrate through leaching as the major form of nitrogen in oxidize soil is nitrate (Powlson and Addiscott, 2005). In moderately reduced soil there was reduction in nitrate formation and later increased at 9WAI this could be attributed to the fact that both nitrate and ammonia are present in the soil. Ammonia content was highest under reduced condition because there is accumulation of ammonia in the system resulting from a halt in nitrification process, this causes a build-up of ammonia in the system as nitrate formation from NH_4^+ is hindered. Also under reduced conditions many other processes can lead to accumulation of ammonia, one of such is hydrolysis of urea in the presence of urease to form ammonium and carbon dioxide (Guillermina and Kenneth, 2004). The net effect of all this is a build-up of ammonia in reduced soil. This could account for the high ammonia content in reduced soils (T2, T4 and T6).

$$(NH_2)_2CO + H_2O \xrightarrow{\text{urease}} CO_2 + 2NH_3$$

V. CONCLUSION

Nitrate was found to be more in redox potential greater than 300 compared to other levels of oxidation or reduction while ammonium was more in reduced condition < -100. It was concluded that the fate and forms of nitrogen in the soil environment is also a function of the oxidation- reduction status of the soil.

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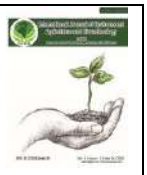

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Metallic Percolations and Environmental Impacts of Spent Lubesat Local Auto-Mechanic Workshops

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Abstract— *The illusion of herbs and grasses atmost mechanic workshops was given the concern to know the effects of mechanic activities on the environment using the Epe Central mechanic village as a case study. Soil and water samples from the environment were analysed for Copper (Cu) and Lead (Pb) with the view to investigating the extent of percolations and the consequential effects of the heavy metals from spent lube on the surrounding water and soils. The concentrations of Lead (Pb) and Copper Cu recorded at the soil surface were 2049.64 ppm and 153.22 ppm respectively. At depths between 7.8 to 30.1 meters (25 to 100 ft), the concentrations profile estimated for Copper metals reduced drastically to 59.98 ppm in a linear relationship defined with the depths as* $Y_{(x)}$*=-1.03064*x+175.758 while Lead concentration reduced to 342.35 ppm. The concentrations of Copperwere0.63ppm in well water and 0.36ppm in the borehole water sources. Likewise, the Lead evaluated was 0.13 ppm in well water and 0.04 ppm in the bore-hole water sources respectively.These values were however higher than evaluated values at distant locations. Mechanic activities at the location are believed to be responsible for biotic degradations. It is anticipated that the heavy metals in the spent oil percolate into the soil as leachates and runoff which may prime the pollution of underground water in a long run.*

Keywords— ***Spent oil, Leachates, Concentration, Bio-toxic, Heavy metal, Hazardous, Percolation*1.0**

I. INTRODUCTION

Soils are the major sink for heavy metals and organic contaminants released into the environment either in the short run or at a distant time in life. Organic components may get degraded by microbial actions into compounds of lesser molecular weights thereby remediating the soil.Soils may become contaminated by the accumulation of heavy metals and metalloidswhich may be obtained through emissions from the rapidly expanding industrial wastes and bye products, mine tailings, disposal of high metal wastes, leaded gasoline and paints (Khan et al., 2008; Raymond and Felix 2011).The presence of a pollutant within any medium of interest has a consequential effect on the biotic system. In addition to physical transport process effects, complex biological and chemical transformations could take place. The types and mechanisms of transformation that a pollutant will undergo in deciding its ultimate fate rest on a combination of factors (Ewing and Lin 1991; Fang and Hong 1999). These vary from the medium of discharge and environmental conditions, nature of the soil and quantities ofthe pollutants. Therefore, the absorption of heavy metals in soils has been an issue of great interest in the past few years not only to ecologists, environmentalists and farmers but tobiologists as well (Grzebisz and Ciesla 2001).

Assessment of the environmental risk due to soil pollution is also of importance to both agricultural and non-agricultural sectors as heavy metals are potentially harmful to plants and human health in the secondary linkage. The presence of pollutants in soils for a very long time enters the food chain in significantly elevated amounts (Lacatusu, 1998). Some heavy metals are useful as micro-elements which are essential to plant metabolic activities in trace amounts, their non-biodegradability leads to their

accumulation and persistence in soils at levels that may be harmful to the environment and public health (Fang & Hong, 1999; Lenntech, 2002; Hakan, 2006). It is not only when the heavy metals are present in bioavailable forms and at excessive levels that they have the potential to become toxic to plants, essential nutrients may also be shielded from being available.The consequence is the evasive nature of the locations where the heavy metals abound as plants cease to thrive or exist after a long time(Nagajyoti et al., 2010)

Spent Engine Oil used oil that had lost its properties due to abrasion, temperature,and denaturation and cannot be used as such in machinery unless reclaimed. Copper is the third most used metal in the world as they are used as alloys with other metalloids [Martinez and Motto].Most commonly found at contaminated sites are lead (Pb), chromium (Cr), arsenic (As), zinc (Zn), cadmium (Cd), copper (Cu), mercury (Hg), and nickel (Ni) [Adriano, 2003; VCI, 2011] Though Copper is an essential micronutrient required in the growth of both plants and animals.as micro-element,Cu's interaction with the environment is complex (Raymond and Felix 2011)

Lead is a metal belonging to group IV with a high melting point 327.4°C, and a boiling point of 1725°C.It is a naturally occurring, bluish-gray metal usually found as a mineral combined with other elements, such as sulphur (i.e., PbS, $PbSO_4$), or oxygen ($PbCO_3$) and ranges from 10 to 30 mg kg^{-1} in the earth's crust [USDHHS]. The mean Pb concentration for surface soils worldwide averages 32 mg kg−1 and ranges from 10 to 67 mg kg−1 (Kabata and Pendians, 2001). The toxicities and environmental effects of organolead compounds are particularly noteworthy because of the former widespread use in metal alloys, distribution of tetraethyllead as a gasoline additive and incursion in automotive batteries.

A common practice in many artisan and mechanic workshops is the indiscriminate disposal of the spent engine oil into drains and in the open spaces around workshops. Spent lube has dark brown to a black colour looks and it is harmful to the soil environment. This is anticipated as it contains a mixture of different chemicals including low to high molecular weight (C15-C21) compounds, lubricants, additives and decomposition products and heavy metals which are harmful to the soil and human health (Duffus, 2002). These characteristics may lead to Soils contaminated with used lubricant, more so as oil is capable of displacing air and water being non-miscible, it may lead to changing soil consistency and chemical aggregate compositions.

Lubricants or engine oil is used in automobiles which include motor vehicles, bikes, electric generators and heavy-duty turbines while the disposal of the spent oil is not yet under any regulations for appropriate disposal or recycling in Nigeria. This study aims at investigatingthe effects of contamination of heavy metals on the soil and water sources around the aged automobile workshops with due consideration to Copper and Lead metals accumulation.

II. MATERIALS AND METHODS

2.1 Sampling Map and Site

The investigation was conducted in a central Automobile workshop at Mechanic Village in Epe opposite the Federal Road Safety Commission Epe. Epe Local Government Lagos State, Nigeria. Epe is located in Lagos State, an African megacity which is located in southwestern Nigeria on the West Coast of Africa, within latitudes 518 6 o 23'N and 6o41'N and longitudes 2o42'E and 3o42'E. The sampling area Map is presented in Figure 1:

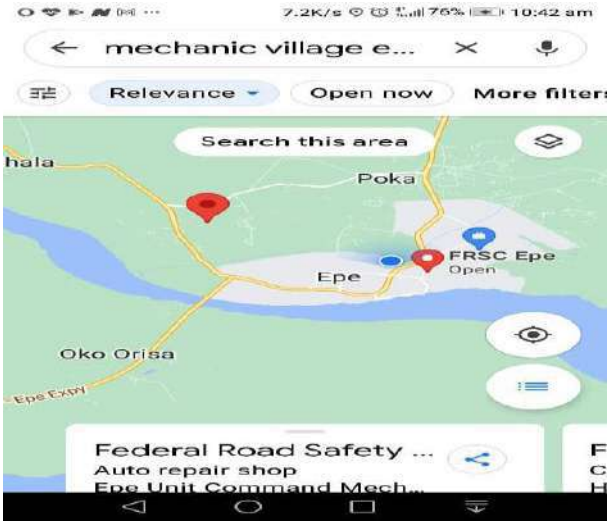

Fig.1: Sampling Map and site of Investigation.

Two metals of interest were lead (Pb) and copper (Cu) as contaminants from machine abrasion and lube constituents. They were investigated in the water and soil samples at the sites and the surroundings.

2.2 Sample Collection and analyses

Soil samples from the study site were collected into a pre-cleaned polythene bag using a stainless Van-ven grab, air-dried and then sieved with a 200 mm mesh screen. Soil samples at the top surface and at different depths of 25, 50, 75 and 100 cm of soil column were obtained to determine the extent of percolation of the heavy metals at the workshop site.

2.2.1 Soil Analysis: Nitric-peroxide acid digestion was carried out using 5ml of 65% nitric acid and 5 ml of 30%

hydrogen peroxide added to 1g each of sample in a 100ml conical flask placed on a hot plate for about forty-five minutes until the solid dissolved and the volume of contents was reduced to about 5 ml. The content of the flask was then filtered through a 0.45 µMillipore membrane filter paper, transferred quantitatively to a 50 ml volumetric flask by adding distilled water to make up to mark and analyzed for copper and Lead heavy metals using Atomic Absorption Spectrophotometer (AAS) model Buck Scientific 210 GVP (Mester and Sturgeon, 2003).

2.2.2 Water Analysis:

Samples of water from different wells and boreholes of water sources in locations around the mechanic village water samples were collected into plastic specimen bottles and labelled. 5 ml of concentrated nitric acid was added to 100 ml of water sample and evaporated to 25 ml. The concentrate was transferred to a 50 ml flask and diluted to the required volume with distilled water. Metal contents were determined using Atomic Absorption Spectrophotometer to determine the presence of copper, and Lead in the samples (Olowu et al., 2019). The assays were repeated for the respective samples and the average values were recorded.

III. RESULTS AND DISCUSSIONS

The physical appraisal of the environment gave distinct effects of the usage and impacts of the spent engine oil disposed of indiscriminately. This observation was registered at different sites of mechanic workshops and this is evident as patches around the respective site as shown in Plate1:

Fig 2: Environmental effects of used lubricating oil on soil patches.

At designated places of maintenance ladened with automobile repair and used engines, the places are devoid of plant growth and appeared as dark spots. Obscuration of biomes at the indicated points is inferential of the pollutants.

The water sources around the Mechanic village mainly wells and boreholes have depthsin the range of 5 and 30 mdepth respectively. The concentrations of copper and lead in the water samples were found to be in the range of 0.63 ±0.05 (ppm) of Copper from the well water sources and 0.13 ±0.03 (ppm) of Lead. The United State Environmental Protection Agency (USEPA) sets the maximum contamination level for copper and lead in water to be 1.3(mg/l or ppm) and 0.01(mg/l or ppm) respectively.

The presence of these heavy metals at the different depths showed that there are possibilities of leaching /percolation of the heavy metals into the soil by different mechanisms or it may be characteristics of the soil origin. The heavy metals at all the depths dug decreased gradually as the depths increasedfrom topsoil toother subsurface layers and aretrapped within a range of 0-25 cm inthe soil.

The analysis revealed that the soil samples contaminated with the lead (Pb)andCopper (Cu)were pronounced at the soil surfacesevident from the pictorial feature of the environment as shown in Fig 1. This may be responsible for the clear absence of plants in some areas. All the contaminated areas were devoid of biomass and shrubs. However, the concentration decreased in the soil as the distance/depth of the soilcolumn increased. Lead concentrations distribution as the different depths of the soil profile is shown in Fig. 2:

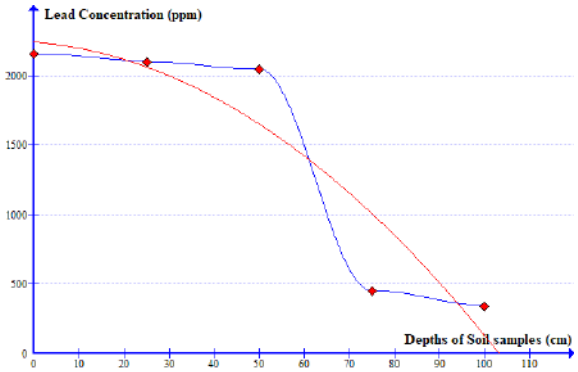

Fig.3: Concentration distribution of Lead in an oil-polluted mechanic workshop

The regression R^2 obtained was 0.8527, the pattern of the concentration distribution was quadratic and was of the form described in equation 2:

$$Y_{(x)}=-0.186192*x\text{^}2-2.46816*x+2238.93 \quad \ldots 2$$

The major part of the metallic content was engaged at the topsoil but may be admissible to further relocation by run-offs especially into the neighbouring water bodiesand soil when rainfall which may lead to increased concentration. The degree of metallic distribution in the soils environment depends on the metal species and its reactive status. gradient of percolations of Lead (Pb) and Copper

(Cu) differs such that Copper was consistently reducing while a sharp decline having with Square (R^2) regression of 0.9377

A substantial decrease with increasing soil depth was observed as the concentration changed drastically from 2040.57 ± 0.5 ppm at 30 cm to 475.40 ± 0.5 ppm at about 75 cm depth. This may however depend on the type of soil and its consistency. The level of heavy metal pollution in areas where there are automobile workshops is higher than in those that do not have automobile impacts.

The effects on the environment water sources as investigated by the analysis of the metals showed that the concentration of Lead was 0.13 ± 0.01 ppm in the well water and 0.04 ± 0.01 ppmin water obtained from a borehole which was in the average depthsof 25ft and 70 ft for the well and borehole sources respectively.

After the test carried out on the well water and borehole within the automobile workshop the concentrations of copper as the soil depth increased is found to follow a decreasing trend displayed in Figure 3:

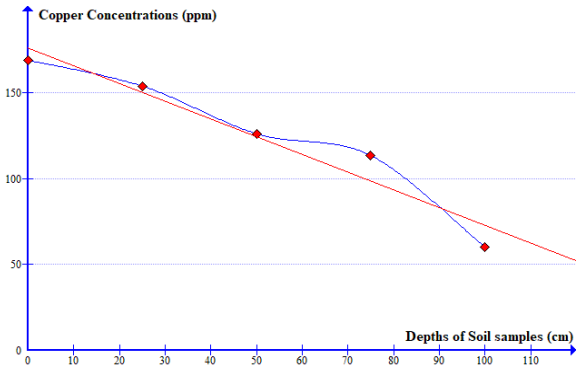

Fig.4: Concentration gradient of copper as the depth of soil decreased

The trend of degradation was close to linearity and was clearly described in Equation (2) with a Regression Square (R^2) of 0.9377.

$$Y(x)=-1.03064*x+175.758 \quad \ldots.3$$

This implied a progressive decrease in concentration as the depth of soil increased. The Copper concentration in the well water sample was determined to be 0.63mg/l and 0.36mg/l in borehole water sourced around the mechanic workshop location relatively at about 70±5 ftdepth. There was a sharp decrease and the two sources did not show any alarm above their threshold values. It is observed that as the depth increases, the concentrations of the metal decrease. This implies that if the depth of the well and the borehole are increased, the concentration of the heavy metals may reduce far below their threshold and safer for consumption. The United State Environmental Protection Agency (USEPA) sets the maximum contamination level for copper and lead in water to be 1.3(mg/l or ppm) and 0.01(mg/l or ppm) respectively.

The experiment carried out on the soil samples shows that Copper is 0.63mg/l in well water and 0.36mg/l in the borehole. Lead is 0.13mg/l in well water and 0.04mg/l in borehole. The well and borehole have a depth of 5 and30 meters respectively.It is noteworthy that concentrations of the metals in all the samples analyzed are lower than the permissible limits set by WHO and FEPA which implies that the water sources are fit and safe for human consumption as the depth is enhanced irrespective of the super facial disruption.

IV. CONCLUSIONS

Heavy metals in the spent oil percolate into the soil at different degrees as leachates which depends on the solubility of the metal species and its interactions with other chemical species in the soil.Spent oil-contaminated soil aggregates to form black patches in the environment. Underground water in the environment presently was revealed to have Cu and Pb as heavy metals sampled below the WHO and FEPA thresholds and so are safe at radial distances over30 meters to the automobile workshop and witha recommended depth of30 meters. The Spent lubricant and greases in the soil increased soil bulk density and consequently decreased the water holding capacity and aeration propensity. The medium of communication of heavy metals toxicity with the bio component of the environment could be traced to the leaching of the elements which may depend on the soil characteristics such as soil porosity and soil types. Direct transport of the elements from the highly concentrated regions of the workshops especially during heavy rainfalls and the consequential runoff to the water bodies such as wells, and boreholes streams is another means of communication and contamination.

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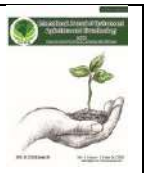

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Effect of leaf phenology on the growth of rubber buddings

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Abstract— *Rubber scion growth is the key step of rubber budding propagation. However, there is still a lack of systematic studies investigating leaf phenology effect on rubber scion growth. In this study, leaf phenology, stem diameter, plant height and the chlorophyll parameters were observed at six periods. The results showed that leaf-unfolding stage was the rapid growth and regulation stage of plant height and stem diameter, and light green leaf stage was the sensitive to environmental temperature. Taken together, leaf phenology affects the growth speed of rubber buddings, and distinguishing the differences in phenological effects helps to regulate the phenological process to raise rubber buddings.*

Keywords— ***Hevea brasiliensis, growth rate, leaf phenology, buddings.***

I. INTRODUCTION

The natural rubber self-sufficiency rate has gradually declined from about 50% in the 1990s to about 13.7% in 2020, and the self-sufficiency rate has been less than 20% for seven consecutive years, making China a major importer of natural rubber. Insufficient natural rubber self-sufficiency rate affects China strategic security to a certain extent. The implementation opinions of the Ministry of Agriculture and Rural Affairs of the Chinese Ministry of Agriculture and Rural Affairs on implementing the deployment of the Party Central Committee and the State Council in 2022 to comprehensively promote the key tasks of rural revitalization, namely Nongfa [2022] No. 1, pointed out that it is necessary to speed up the renovation of old rubber plantations and promote the standardized production of rubber plantations. As a result, the demand for rubber seedlings has risen sharply in the short term. Rubber propagation in China is mainly by the bud-grafting method and this technique requires an appropriately sized scion at the period of transferring budded plants out of the nursery. The speed of scion growth is related to the change of phenological process, which directly affects the speed of transferring budded plants out of the nursery. Leaf phenology is related to bud-grafting survival rate, disease control, and rubber tapping[1]. Ambient temperature[2], light[3-4], water and nutrients[5-6] affect the phenological process. At various developmental stages of leaf phenology, there are fluctuations in leaf color and shape, accompanied with the changes of leaf SPAD[7], leaf nutrients[8], leaf photosynthesis[9], the laticifer number[10] , leaf protein expression[11] and leaf gene expression[12]. However, the detailed changes of rubber budding growth index was still not clear, which make it difficult to precisely regulate the propagation process. Here we investigated growth index of buddings at different leaf phenology to further extend current knowledge of rubber budding propagation and to further regulate the growth of rubber buddings by the process of leaf phenology.

II. MATERIAL AND METHODS

The experiment was conducted from November 2018 to October 2020 at the nursery base of natural rubber of Rubber Research Institute of Chinese Academy of Tropical Agricultural Sciences, Danzhou City, Hainan Province, China. Clone GT1 seeds were sown in sand bed for germination and about 20-25 days later the GT1 seedlings were transplanted in polybags(8cm width*33cm length) for seedling nursery. Leaf phenology was investigated

from July 7 to September 30, which was best growth period for rubber seedling with 9mm average rainfall daily and good temperature(24.6℃-32.6℃). At different leaf phenology of new leaf whorl(Figure1), stem diameter was measured with vernier caliper (0.01 mm) and plant height were recorded with plastic ruler (0.1 mm). A Portable Chlorophyll Meter(CY-YL04, China) was used for detecting chlorophyll content(SPAD value), nitrogen content, leaf temperature and leaf humidity of plants during 9:30-10:30. Each leaf phenology of new leaf whorl 100 plants and 30 plants were investigated for the growth index(stem diameter and plant height) and for the chlorophyll parameters, respectively. Correlation heatmap analysis among the growth index and the chlorophyll parameters was evaluated on Tutools platform(http://www.cloudtutu.com), a free online data analysis website. Statistical analyses were performed with data processing system (DPS) statistical software package version 16.5 using student's t-test and one-way ANOVA followed by the Duncan's Multiple Range Test (SSR) to evaluate significant difference among different leaf phenology of seedlings at P<0.05. All data were shown in the mean ± SE.

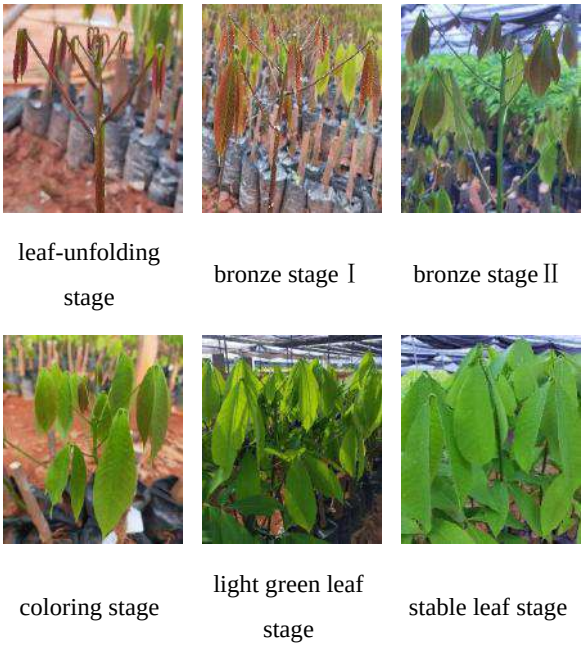

Fig.1: Leaf phenology of rubber buddings

Notes: Leaf-unfolding stage, bronze stageI, bronze stageII, coloring stage, light green leaf stage, stable leaf stage took 2 days, 2 days, 1 days, 2 days and 3 days, respectively.

III. RESULT AND DISCUSSION

Effect of leaf phenology on plant height

As shown in Figure2A, plant height of buddings new leaf whorl at bronze stageI, bronze stageII, coloring stage, light green leaf stage, and stable leaf stage were higher 40.36%($P < 0.01$), 53.38%($P < 0.01$), 60.56%($P < 0.01$),64.68%($P < 0.01$), and 66.47%($P < 0.01$) than that at leaf-unfolding stage, respectively. Plant height of buddings at bronze stageII, coloring stage, light green leaf stage, and stable leaf stage were higher 9.28%($P < 0.01$), 14.40%($P < 0.01$), 17.33%($P < 0.01$), 18.60%($P < 0.01$) than that at bronze stageI, respectively. Plant height of buddings at light green leaf stage and stable leaf stage were higher 7.37%($P < 0.05$) and 8.53%($P < 0.01$) than that at bronze stageII, respectively. There was no significant difference in plant height of buddings between bronze stageIIand coloring stage, among coloring stage, light green leaf stage, and stable leaf stage, respectively. These results indicate that leaf-unfolding stage is the rapid growth and regulation stage of plant height for buddings.

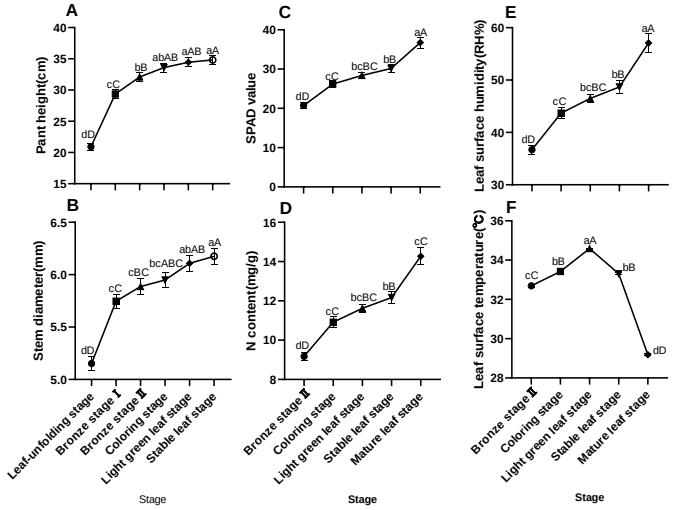

Fig.2 Comparison on plant height (A), scion stem diameter(B), SPAD value(C), N content(D) , leaf surface humidity(E) and leaf surface temperature(F) in rubber seedlings at different leaf phenology

Effect of leaf phenology on stem diameter

As shown in Figure2B, stem diameter of buddings new leaf whorl at bronze stageI, bronze stageII, coloring stage, light green leaf stage, and stable leaf stage were more 11.53%($P < 0.05$), 14.27%($P < 0.01$),15.46%($P < 0.01$), 18.54%($P < 0.01$), and 19.86%($P < 0.01$)than that at leaf-unfolding stage, respectively. Stem diameter of buddings new leaf whorl at light green leaf stage and stable leaf stage were more 6.29%($P < 0.01$) and 7.47%($P < 0.01$) than that at bronze stageI, respectively. Stem diameter of buddings new leaf whorl at light green leaf stage and stable leaf stage were more 3.74%($P < 0.05$) and 4.89%(P

< 0.01) than that at bronze stageII. Stem diameter of buddings s new leaf whorl at stable leaf stage were more 3.81%(P < 0.05) than that at coloring stage. These results indicate that leaf-unfolding stage is the rapid growth and regulation stage of stem diameter for seedlings.

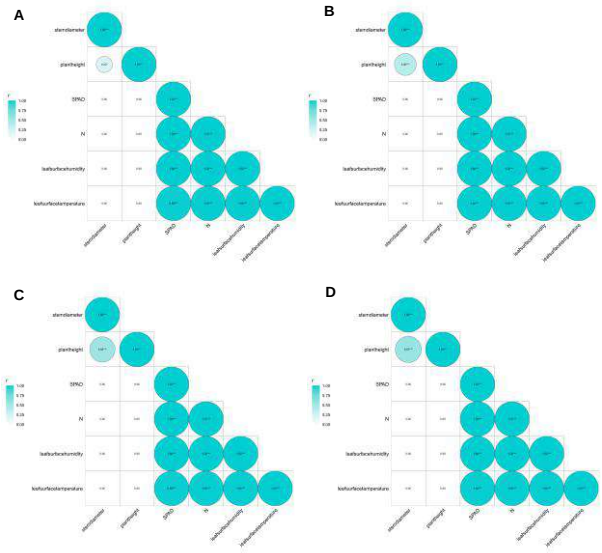

Fig.3 Correlation analysis among stem diameter, plant height, SPAD value, N content, leaf surface humidity and leaf surface temperature at bronze stageII(A), coloring stage(B), light green leaf stage(C), and stable leaf stage(D).

Effect of leaf phenology on the chlorophyll parameters

As shown in Figure2C, SPAD value, N content and leaf surface humidity of buddings new leaf whorl showed a same and upward trend with leaf phenology. SPAD value of seedlings new leaf whorl at coloring stage, light green leaf stage, stable leaf stage and mature leaf stage were more 26.46%(P < 0.01), 36.99%(P < 0.01),45.58%(P < 0.01), and 77.40%(P < 0.01) than that at bronze stageII, respectively. SPAD value of buddings new leaf whorl at stable leaf stage and mature leaf stage were more 15.12%(P < 0.01) and 40.28%(P < 0.01) than that at coloring stage, respectively. SPAD value of buddings new leaf whorl at mature leaf stage was more 29.50%(P < 0.01) and 21.86%(P < 0.01) than that at light green leaf stage and at stable leaf stage, respectively. There was no significant difference in SPAD value of buddings between coloring stage and light green leaf stage, between light green leaf stage and stable leaf stage, respectively.

As shown in Figure2D, N content of buddings new leaf whorl at coloring stage, light green leaf stage, stable leaf stage and mature leaf stage were more 18.95%(P < 0.01), 26.58%(P < 0.01), 32.57%(P < 0.01), and 55.45%(P < 0.01) than that at bronze stageII, respectively. N content of buddings new leaf whorl at stable leaf stage and mature leaf stage were more 11.45%(P < 0.01) and 30.68%(P < 0.01) than that at coloring stage, respectively. N content of buddings new leaf whorl at mature leaf stage was more 22.81%(P < 0.01) and 17.26%(P < 0.01) than that at light green leaf stage and at stable leaf stage, respectively. There was no significant difference in N content of buddings between coloring stage and light green leaf stage, between light green leaf stage and stable leaf stage, respectively.

As shown in Figure2E, leaf surface humidity of buddings new leaf whorl at coloring stage, light green leaf stage, stable leaf stage and mature leaf stage were more 18.95%(P < 0.01), 26.58%(P < 0.01), 32.57%(P < 0.01), and 55.45%(P < 0.01) than that at bronze stageII, respectively. Leaf surface humidity of buddings new leaf whorl at stable leaf stage and mature leaf stage were more 11.45%(P < 0.01) and 30.68%(P < 0.01) than that at coloring stage, respectively. Leaf surface humidity of buddings new leaf whorl at mature leaf stage was more 22.81%(P < 0.01) and 17.26%(P < 0.01) than that at light green leaf stage and at stable leaf stage, respectively. There was no significant difference in Leaf surface humidity of buddings between coloring stage and light green leaf stage, between light green leaf stage and stable leaf stage, respectively.

As shown in Figure2F, leaf surface temperature of buddings new leaf whorl at coloring stage, light green leaf stage, stable leaf stage and mature leaf stage were more 2.24%(P < 0.01), more 5.82%(P < 0.01), more 1.89%(P < 0.01) and less 10.73%(P < 0.01) than that at bronze stageII, respectively. Leaf surface temperature of buddings new leaf whorl at light green leaf stage and mature leaf stage were more 3.50% (P < 0.01) and 12.68% (P < 0.01) than that at coloring stage, respectively. Leaf surface temperature of buddings new leaf whorl at stable leaf stage and mature leaf stage were less 3.71% (P < 0.01) and 15.63% (P < 0.01) than that at light green leaf stage, respectively. Leaf surface temperature of buddings new leaf whorl at mature leaf stage were less 12.38% (P < 0.01) than that at stable leaf stage. Leaf surface temperature of buddings new leaf whorl at light green leaf stage showed a rapidly increase in comparison with other leaf stages. These results indicate that light green leaf is the sensitive stage to environmental temperature for seedlings, and as well stable leaf stage and mature leaf stage are the start of proper time and the best time to transplant the buddings out of the nursery to the field.

Correlation analysis between the growth index and the chlorophyll parameters at different leaf stage

As shown in Figure3A-D, there was a significant positive correlation between plant height and stem diameter at bronze stageII($r=0.22$, $p<0.05$), coloring stage($r=0.40$, $p<0.01$), light green leaf stage($r=0.54$, $p<0.01$), stable leaf stage($r=0.57$, $p<0.01$), respectively. There was a significant positive correlation($r=1$, $p<0.01$) among SPAD value, N content, leaf surface humidity and leaf surface temperature at different leaf stages. Plant height had no significant correlation with chlorophyll parameters(SPAD value, N content, leaf surface humidity and leaf surface temperature) . Stem diameter had no significant correlation with chlorophyll parameters(SPAD value, N content, leaf surface humidity and leaf surface temperature) . These results indicate that there is no significant correlation between growth index(plant height and stem diameter) and chlorophyll parameters(SPAD value, N content, leaf surface humidity and leaf surface temperature) for rubber buddings at different leaf stages, which might be related with the different methods of rubber buddings propagation[13-14].

IV. CONCLUSION

For rubber buddings propagation, leaf-unfolding stage was the rapid growth and regulation stage of plant height and stem diameter, light green leaf stage was the sensitive to environmental temperature, and mature leaf stage with high nutrient accumulation and low metabolism was the best time to transplant the buddings to the field.

ACKNOWLEDGEMENTS

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AUTHORS' CONTRIBUTION

X.H. Chen conceived the experiments, R. Wang and X.H. Chen conducted the experiments, R. Wang and X.H. Chen analyzed the data and drafted the manuscript, R. Wang , X.H. Chen, and J. Wang discussed the results and finalized the manuscript.

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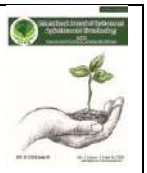

Peer Reviewed

Analysis on 8 × 8 diallel crosses of Cucumber (*Cucumissativus* L.) for potential yield improvement at Can Tho, VietNam

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Abstract— *Combining ability analysis provides useful information for the selection of parents, in the breeding program of cucumber at Can tho, VietNam, as well as information related to the nature and extent of the gene activities involved. Cucumberimprovement involves strategies to enhance yieldpotential and quality of cucumber. Targeting the improvement of new varieties for breeding in cucumber plants, combining abilities and genetic parameters for 12 traits estimated from the full diallel analysis technique 8×8. The results showed that the differences due to general combining ability (GCA) and specific combining ability (SCA) capabilities are significant for most of these important traits.It indicates the importance of both plus- and non-plus gene activity. GCA variances are more intense than SCA variances for all hybrid materials studied showing the advantages of linear effects in their genetics. P1 (PhungTuong) appears as the best general combination for early flowers; P3 (MaLai) has a high primary branch number; P3(MaLai);P4 (TL 00L);P5(TayNinh) P6(BinhPhu) and P7(TQ) have the best combining ability for use in the breeding program to improve the number of fruits per plant. P4 (TL 00L) gives the length of the fruits. The SCA effect as well as the reciprocal effect also have implications for most important combinations in different hybrid combinations such as: the crossing P1xP6, P2xP3, P2xP4,P2xP5 and P2xP7for fruit weight/plant. The length of the fruit forP1x P6 and P2 x P7,these selected crossing were associated with the agronomic performance of cucumber varieties in future.*

Keywords— ***Cucumber, diallel, GCA, SCA, genetic parameters.***

I. INTRODUCTION

There is a big gap between demand and vegetable supply in the Mekong Delta, VietNam. Therefore, considerations improving crop yields and the production of is desirable and important to minimize future demand. A breeding program provides the opportunity to produce high-yielding cultivars of outstanding quality. However, the development of breeding program needs information about the nature of the gene-controlled gene activity of the gene on economic traits and other important traits.Knowledge of the genetic architecture of the characters is improving which is essential to apply the proper breeding process. Such knowledge leads to plant breeders developing new commercial varieties of cucumber. Begna (2021) emphasizes that information about the change due to genetic differences and the relationship between different quantitative characteristics is fundamentally significant in the crop improvement program. In spite of its considerable morphological variability, cucumber showed a narrow genetic base (Staub et al. 1997), which restricts development of new cucumber cultivars by cross-breeding. Being a largely consumed vegetable cucumber had great scope to improve the production productivity to meet the requirement by adoption of improved varieties/hybrids (Singh et al., 2016, Pandey et

al., 2016).In a breeding program, breed selectors often face problems in the selection of desired parents. The popular approach of choosing parents on the basis of performance does not necessarily lead to the best results in the hybrid program (Labroo et al., 2021).Combine more reliable possibility studies because they provide useful information for the selection of parents about the performance of hybrids and shed light on the nature and extent of the different types of gene action involved in the expression of quantitative characteristics.Significant general (GCA) and specific (SCA) combining ability variances were obtained in all the traits implying that both the additive and non-additive gene effects operated in the genetic expression of the traits(ChikezieOnuoraEne et al.,2018).Diallel cross-analysis provides estimates of the genetic parameters involved in combining abilities as well as a quick overall picture of the parent's dominant relationship studied using the first generation of hybrids (F1) with or without reciprocity(Kundua et al 2021). Combining ability is useful in successful prediction of genetic capability of parental lines and crosses (Singh vet al., 2011, Singh vet al., 2013)The GCA of the yield and yield component traits of the cultivars showed that 'Zeina' had negative GCA effect in all the traits except days to male and female flower initiation .Diallel analysis involving parents provides additional information such as the presence or absence of epigenetic, average levels of dominance, distribution of dominant genes, and recession in parents (Zongo et al., 2019).The genetic data of 8 lines subjected to selection represent both genomic variations and their usefulness in cucumber breeding improvement.Here, diallel cross-analysis with 8x8 cucumber lines and found that general combining ability (GCA) and specific combining ability (SCA) for yield and components yield. The data from our 8 parents revealed general combining ability related to breeding efforts, and these selected crossing were associated with the agronomic performance of cucumber varieties and harbored many reportedly important genes.

II. MATERIAL AND METHODS

2.1. Material : The experiment was conducted with eight genotypes of cucumbers include: Phuong Tuong, DaiLoan, Malai, Thai (00L) TayNinh, BinhPhu, TQ, and the Philippines selected as parents (marked P1, P2, P3, P4, P5, P6, P7, and P8, respectively). Eight parents were growed with planted F1 for comparison and evaluation.

2.2. The experiment was designed in a completely random block with three repetitions. There are nine plants for each repetition. The distance is (30 x40cm) . A good drainage system has been maintained for draining rainwater and irrigation water. In order for the plant to grow and grow better, combine the balance of manure (well decomposed cow manure 10 ton / ha) and fertilizer (Urea 150 kg / ha; TSP 175 kg/ha; MP 150 kg/ha; lime 100 kg/ha;).

Fig.1: The experiment was conducted with eight genotypes and F1 of cucumbers at center vegetable crop of High Agricultural Technology Research Institute for Mekong delta (HATRI)

2.3. Collect data for quantity calculations

Days to first male flower opening :days to first female flower opening, the length of the main plant (cm), the number of primary branches per plant, the date of the first harvest of the fruit, the time of harvest, the number of fruits per plant , the average weight of the single fruit (g), the yield of the fruit per plant (kg), length of fruit (cm), Fruit diameter(cm) . For every parameters, data were collected three times and average was calculated.

2.4Analysis of variance (ANOVA) All the quantitative data taken were subjected to ANOVA. The total variances of each character were partitioned in two block, genotype and error differences. The differences within the classes of effects were tested by F-test.

2.5***Combining ability***

The statistical analysis of data for 8 × 8 diallel crosses including reciprocals were done as quoted in Fasahat et al. (2016). The diallel progenies (n2 =64) were portioned into parents (P), crosses (F1), parent vs crosses (P vs. F1), direct crosses (f), reciprocal crosses (l) and direct vs. reciprocal items. The parents, crosses, direct cross, reciprocal crosses items tested the significant differences among the parents, crosses, direct crosses and reciprocal crosses, respectively. For the genetic analysis of diallel population, data were subjected to Griffing's approaches. (1956) method 1, Model 2 (Random effects model) was used for combining ability analysis for each of the trait.

III. RESULTS AND DISCUSSION

3.1.Mean performance of cucumber genotypes

Variance Analysis (ANOVA) shows very significant differences between parents for 12 traits (Table 1).

The day of first male flowers

The duration of first male flowerslied between15.00 and 30 day (Table 1). Maximumharvest duration (30days) was recorded in P4(TL-00L) whichwas statistically at with 7 other genotypes while, itwas minimum (15.00 days) in P1 (PhungTuong).

The duration of first female flowers

The duration of first female flowers between 25.6.00 and 37.5 day (Table 1). Maximumharvest duration (37.5days) was recorded in P4(TL-00L) whichwas statistically at with 7 other genotypes while, itwas minimum (25.60 days) in P1.

The number of primary branches

The number of primary branchesbetween 3 and 7 (Table 1). Maximum number of primary branches / plant (7) was recorded in P 3(MaLai) and P4(TL-00L) which was statistically at with 6 other genotypes while, it was minimum (3) in P6(BinhPhu).

Harvest duration

The duration between first and final fruit harvest lied between 21.00 and 40 day (Table 1). Maximumharvest duration (40 days) was recorded in P4(TL-00L) whichwas statistically at with 7 other genotypes while, itwas minimum (21.00 days) in P7(TQ) .

Number of fruits per plant

Observations recorded for this trait ranged from 6.25 to 9.2(Table 1). The genotype, P 7 (TQ) producedmaximum number of fruits per plant(9.2) whereas, minimum number of marke table fruits(6.25.) were produced inP1(PhungTuong) .

Single fruit weight

Single fruit weight varied from 115 to 265 gram (Table 1). The genotype P 2(DaiLoan)recordedmaximum single fruit weight (265 gram).Minimum single fruit weight (115 gram)was recorded in P 7 (TQ).

Fruit weight/plant

Fruit weight / plant varied from 750 to 1568 gram (Table 1). The genotype P4(TL-00L) recorded maximum fruit weight/ plant (1568 gram). Minimum fruit weight/plant (750 gram) was recorded in P 1 (PhungTuong).

Fruit length

The observations recorded on fruit length ranged from 13.0 to 21.2 cm(Table 1). The genotype in P4(TL-00L) had longest fruit (21.2cm) among all the studied genotypes,which was statistically at par with that of P 6 (BinhPhu) (17.0cm), whereas, the genotype P 7(TQ) had minimumfruit length (13.0cm).

Fruit diameter

Significant variations were observed among all the genotypes for this character and it varied from 11-to12.6cm (Table 1). Fruits of F7 (TQ) had maximum fruitdiameter (12.6cm).

Yield per ha

Yield per plant varied significantly in all thegenotypes and it ranged from 14.50 to 33.10ton.ha (Table 1).Maximum per plant yield (33.10ton/ha) was recorded inP7 , whereas, it was minimum in(P5).

Table 1 Mean performance of cucumber genotypes for different yield and yield components of 8 parents.

Parents	The duration of first male flowers(days)	The duration of first female flowers(Days)	The number of primary branches	The first harvest time(days)	Harvest time(days)	The length of the plant(cm)	Number of fruit/ **plant**	**Single fruit weight (g)**	**fruit weight / plant**	The length of the fruit **(cm)**	**Fruit diameter** (cm)	**Yield (Ton/ ha)**
P1	**15h**	**25.6c**	**5c**	**40b**	**26c**	**175c**	**6.25**	**121c**	**750d**	14.0c	12.4c	16.50c
P2	**21e**	**37.0a**	**6b**	**45b**	**33b**	**197b**	**7.45c**	**265a**	**1250c**	16.1b	13.6b	15.85d
P3	**20f**	**31.0d**	**7a**	**55a**	**37b**	**190b**	**8.5b**	**132**	**1425b**	13.0d	11.0d	22.04b
P4	**30a**	**37.5a**	**7a**	**49b**	**40a**	**206a**	**7.6c**	**255a**	**1568a**	21.2a	12.0c	32.10a
P5	**29b**	**37.6a**	**5c**	**45b**	**34b**	**195b**	**6.5d**	**120c**	**1427b**	13.5d	12.3c	14.50e
P6	**18g**	**25.0c**	**3d**	**51a**	**39a**	**215a**	**8.4b**	**172b**	**1234c**	17.0b	12.6c	31.10a
P7	**28c**	**26.5b**	**6b**	**57a**	**21d**	**196b**	**9.2a**	**115d**	**1542a**	13.0d	15.6a	33.10a
P8	**25d**	**36.0a**	**5c**	**45b**	**30b**	**222a**	**7.6c**	**174b**	**1447b**	13.7d	12.4c	30.20a
CV%	**1.12**	**2.36**	**0.23**	**0.12**	**1.25**	**1.36**	**4.25**	**5.36**	**10.23**	7.36	5.2	7.63

3.2. General combining ability effects(GCA)

The results of GCA effect for 12 different characters of eight parental lines are presented in the Table 2a, 2b.

The number of first male flowers

Negative GCA effect is preferable for days to flowering as it indicates the earliness of the parents. The positiveGCA effect is preferable for many days to flower because it indicates the time of parental flowering. Very important (1.45*) for P7 (TQ)and the highest positive GCA effect (1.52*) was recorded for parents fromTL(00L) for several days to the first male flowers (Table 1a). P6 (BinhPhu) showed a significantly but negative effect. On the other hand, P1 and P6 show very significant and negative GCA effects (-3.10** and - 3.41**). Therefore, P1 and P6 (DaiLoan) are the best GCA to promote early flowering in cucumbers.

The number of first female flowers

The number of first female flowers, P4 (TL (00L) has a very significant GCA effect (-1.71*) and a negative GCA effect (37.5 days), while P1(PhungTuong) has a very significant GCA effect (-1.23*) but negative (25.6 days). The GCA effect of P2 parents, P3 and P4 for this parameter is negligible. Therefore, P1 is the best general combination(Table 2a). P7 (TQ) provides a very significant positive GCA effect (1.43*) for many days to give the first female flower (26.5days).On the other hand, P1 show very significant and negative GCA effects (-3.33**) and the best GCA to promote early flowering in cucumbers.

The number of primary branches

The number of primary branches of cucumbers affects the yield of the fruit. The best GCA for parents to use hybrid materials is P2, P4, P7 and P8 ,the GCA effect for other parents is negligible. The P4((T.L 00L) would be a better option for the number of primary branches . In breeding programs for the number of primary branches per plant and P2 will be a better option for the number of branches. P4 is very important (0.60).

Harvest time: P1 and P 8 showed GCA (-1.87*; -1.47* respecitive) and the negative and average GCA effect of parents was lower (26-30days) for this trait. On the other hand P6 (BinhPhu) showed for positive (1.30*) and P3(MaLai) give GCA (1.21*) can be selected as the best GCA for enhancing fruiting . (Table 2a).

The length of the plantrecords the cucumber for negative general combining ability on P1, P2, P3, and P7 varieties. The P1 (for the shortest length in table 2a .P2(DaiLoan)

shows that the GCA effect is very important (-13.42**) followed by P1(PhungTuong) with GCA (- 10.61 **).

Table 2a. General combining ability effect the biological characteristics of the 8 genotypes of cucumbers

No	Lines	The duration of first male flowers (days)	The durationof first female flowers (Days)	The number of primary branches	The first harvest time(days)	Harvest time(days)	The length of the plant(cm)
P1	PhungTuong	-3,10**	-3,33**	-0,14ns	-1,43*	-1,87*	-10,61**
P2	DaiLoan	1,37*	-0,40ns	0,10ns	-1,35*	0,08ns	-13,42**
P3	MaLai	0,89ns	-0,15ns	-0,64ns	1,31*	1,21*	-3,12*
P4	TL(00L)	1,52*	-1,71*	0,60ns	-0,15ns	0,91ns	3,49*
P5	TayNinh	0,33ns	-0,33ns	-0,39ns	-0,12ns	-0,13	5,67*
P6	BinhPhu	-3,41**	-0,65ns	-0,14ns	0,50ns	1,30*	9,65**
P7	TQ	1,45*	1,43*	0,36ns	0,56ns	-0,03ns	-0,67ns
P8	Philippine	0,99ns	0,96	0,23ns	0,93ns	-1,47*	9,02**

*Significant at 5% level of probability; **Significant at 1% level of probability. Ns: non-significant .

Fruit/ Plant

Other parents show negligible values generalcombining ability for characteristics (Table 2b).

Single fruit weight

Estimates of the GCA effect on average single fruit weight of plant (255g) show that P4 has the highest positive value significantly (16.17**) followed by P3(Malai) with GCA (14.75**) and P1 with GCA (13.67**) (Table 2b). Other parents showed significant negative effects on this trait such as P5, P6, P7 and P8 . Therefore, P4 is a good general combining ability for use in passing to improve single fruit weight on theplant . P4 has a significantly positive SCA effect (16.17**) on fruit yield (255g) (Table 2b).

Considering fruit/ plant. The five parents showed positives as P3(MaLai) ; P4(TL00-L) ;P5(TayNinh) , P6(BinhPhu) and P7(TQ) give goods for GCA and positive and other parents showed negative GCA effects. Therefore, 5 parents P3,P4,P5, P6 and P7 have the best general combining ability for use in the breeding program to improve the number of fruits per plant in cucumbers as it has a higher average value along with a significantly higher and higher GCA. (Table 2b).

The length of the fruit:

The length of the fruit: Other parents show negligible or negative values for this trait. Therefore, P4 is the combination with the best GCA for use in the breeding program to improve the length of the fruit. P1 and P6 parents can be seen as alternative parents to this trait (Table 2b). For P4 has significance (2.43*) and the highest positive GCA effect on length of fruit (21.2 mm) followed by P6 (2.27*) (Table 2b).

Fruit diameter:

Parents negatively impact this trait. With the exception of the P7 which has a high GCA, (1.46**). Next comes the DaiLoanand Philippine varieties (however, these two varieties are not of high significance).

The yield

Four parents showed positive effects on yield /ha P3, P4, P6 and P7 and 4 parent significantly negatively impacted this trait. Therefore, P4 is a parent with a good GCA to use in hybridization to improve fruit/plant carrying capacity per plantand total yield. Very significant (2.43**) and the highest positive GCA effect on ton/ha yields (32.1ton/ha) was found in P4, followed by P6 (2.28**) and P7 (1.70**) (Table 2b). Other parents showed significant negative effects on this trait.

Table 2b. general combining ability effects the yield and component of yield in 8 genotypes of cucumbers

numbers	**parents**	Fruit/ **plant**	**Single fruit weight (g)**	**fruit weight / plant**	The length of the fruit **(cm)**	**Fruit diameter** (cm)	**Yield Ton/ha)**
P1	PhungTuong	-0,41 ns	13,67**	-2,65*	-2,13**	-0,82 ns	-2,41**
P2	DaiLoan	-0,33 ns	2,67*	-36,5**	-1,46*	0,64 ns	-1,47*
P3	MaLai	0,07 ns	14,79**	59,68**	1,40*	-0,35 ns	1,41*
P4	TL(00L)	-0,05 ns	16,17**	30,53**	2,43**	-0,65 ns	2,43**
P5	TayNinh	-0,08 ns	-10,89**	10,68**	-0,64 ns	-0,05 ns	-0,65ns
P6	BinhPhu	0,33 ns	-3,76*	74,25**	2,27**	-0,24 ns	2,28**
P7	TQ	0,31 ns	-21,14**	90,31**	1,69*	1,46*	1,70*
P8	Philippine	0,18 ns	-11,52**	-53,31**	-3,56**	0,02 ns	-3,56**

*Significant at 5% level of probability; **Significant at 1% level of probability. Ns: non-significant.

3.3. Specific combining ability effects(SCA)

The SCA effect of 10 F1 combinations for the 12 traits studied is presented in Table 3a, 3b. Of the 10 F1s, six show negative SCA values (Table 3a) Based on the SCA effect, the P1 x P3 cross-combination; P1xP6; P2XP4 and P3XP5 were found to be superior to the duration of first male flower.

For the flowering time female indicates that there are significant differences to this combination and that at least 40% of the hybrids flower earlier than the means of their parents. Among hybrid pairs, the four combinations show very significant negative SCA values with the largest negative values in the combinations P1 x P4 (-0.29), P2 x P4 (-3.53**), P2 x P5 (-0.47), P2 x P7 (-0.25), P3XP5(-1.47*) and are the best specific P5xP8 (-0.54) combinations for early flowering. While the combinations of P1 x P2 (3.08**) and P1 x P6 (2.58**) showed a significantly positive SCA effect that produced flowering later than their average value. The most significant and positive SCA effects for this trait are expressed by P4 x P6 (3.02**). Other combinations that showed significant and positive SCA effects were P3 x P5 (2.59**), P4 x P5 (2.48**) and P2 x P3 (1.78*). The P2 x P5 cross-combination showed the highest and negative SCA effect (-2.94**).

The first day of fruit harvest is well recorded on the P1xP4 complex (2.35*); P2xP7(3.06**) P3xP5(6.18**) and P5xP6(2.0*). Among the combinations, P23x P5 (6.18**) showed the most significant and positive SCA effect on this character,

The length of the plant

Out of 10 F1, 4 give positive and 6 show negative SCA values. So there is little scope for characteristic changes in this crossed. Only four pairs of crossedshow significant SCA values, P1 x P3 crossed combinations show significant positive values (5.25*), P2xP3 (1.52) ; P5xP6 (12.99**) and P5xP8(15.56**), on the other hand, hybrid pairs give negative individual coordination capability values. Therefore, the hybrid pair shows that the negative SCA value is the best pair of hybrids to improve the length characteristics of the combination has a negative SCA value is best to improve this characteristic. Of the cross combinations, about 60% of F1 show a negative SCA value for this trait (Table 3a). This indicates that these F1s produce a shorter length of the plant than the average parent better.

Table 3a. Specific combining ability effects10 cross of hybrids on the biological characteristics of cucumbers

No	Crosses	The duration of first male flowers (days)	The duration of first female flowers (Days)	The number of primary branches	The first harvest time(days)	Harvest time(days)	The length of the vine (cm)
1	P1XP3	2,70**	3,08**	-0,98	-1,0n	1,13*	5,25**
2	P1xP4	-0,83	-0,29ns	0,26 ns	2,31*	-1,36*	-1,31*
3	P1xP6	3,66**	2,58**	1,01*	-0,68 ns	4,00**	-6,61**
4	P2xP3	-1,68*	1,84*	-0,23 ns	-5,68**	-1,11*	1,52*
5	P2xP4	3,16**	-3,53**	-0,48 ns	0,13n	3,18**	-10,10**
6	P2xP5	-2,12**	-0,47ns	1,06*	-1,125*	1,43*	-5,87**
7	P2xP7	0,75ns	-0,25ns	-0,73	3,06**	1,38*	-7,52**
8	P3xP5	2,35**	-1,41*	-0,23	6,18**	2,94**	-5,73**
9	P5xP6	-2,70**	0,08ns	-0,61n	2,0*	0,21ns	12,99**
10	P5xP8	-2,25**	-0,54ns		-0,43n	0,13 ns	15,56**

*Significant at 5% level of probability; **Significant at 1% level of probability. Ns: non-significant.

Number of fruits

The P2 x P6 combination can be considered the best specific combination for strengthening the number of fruit of cucumbers. Of the 10 F1s, most of the 10 combinations for their own combined abilities showed positive and statistically significant (Table 3b).

Single fruit weight

Specific combining ability effects showed positive and statistically significant excep P2xP3 and P2XP4 . The cross-combination of P1 x P3;P1 x P4; P1xP6; P2 x P5; P2 x P7; P3 x P5 P5 x P6 and P5XP8 exhibit significant and positive SCA effects, where P3 x P5 provides maximum value (52.51**) for single fruit weight.Other combinations show significant or negligible SCA effects. Therefore, the P2 x P3 is the next best specific combination of P2 x P4.(Table 3b).

Fruit weight/ plant

Out of 10 F1, 5give positive (P1XP6; P2xP3;P2xP4;P2xP5 and P2xP7 and 5 show negative SCA values remain .

The length of the fruit

Length of the fruit: All combinations show that the SCA effect is very statistically significant . The most significant and positive SCA effect for this trait is provided by P1x P6 (5.71**) P2 x P7 (5.62**) followed by P5x P8 (5.47**).

Fruit diameter

Therefore, the hybrid crosses P5 x P8(1.03*) is the best specific combination for this trait whose large diameter is undesirable. Of the 10 pairs of F1, three combinations showed negative SCA values indicating that the hybrids gave the fruit a smaller diameterthan the average parent. The lowest positive significant SCA value obtained from P1 x P6 (-1.33*) (Table 3b).

The yield : Highest significant and positive SCA effects were observed in yield .Of the 10 F1s, most of the 10 combinations for their specific combining ability effects showed positive and statistically significant (table 3b)

Table 3b. Specific combining ability effects10 cross of hybrids on the yield and component of yield of cucumbers

No	Crosses	Fruit/ plant	Single fruit weight (g)	fruit weight / plant	The length of the fruit (cm)	Fruit diameter (cm)	Yield Ton/ha)
1	P1XP3	-0,73 ns	25,43**	-81,12**	3,84*	0,73 ns	3,57**
2	P1XP4	0,19 ns	12,07**	-82,87**	2,45*	0,83 ns	2,44**
3	P1XP6	-0,38 ns	42,62**	42,0**	5,71**	-1,33*	4,75**
4	P2xP3	-0,89 ns	-5,04*	18,6**	3,38*	-0,28 ns	3,45**
5	P2xP4	-0,62 ns	-51,92**	25,06**	2,24*	0,66 ns	2,07**
6	P2xP5	0,32 ns	13,14**	24,43**	3,78*	0,91 ns	4,85**
7	P2xP7	0,83 ns	30,39**	10,98**	5,62**	0,08 ns	4,31**
8	P3xP5	0,51 ns	52,51**	-40,87**	2,82*	-0,03 ns	2,73*
9	P5XP6	0,65 ns	13,07**	-23,61**	3,98*	0,55 ns	3,21**
10	P5XP8	-0,69 ns	19,67**	-24,63**	5,47**	1,03*	4,44**

*Significant at 5% level of probability; **Significant at 1% level of probability. Ns: non-significant.

IV. DISCUSSION

We concluded that the genotype P1 was best with respect to earliness, P4 was found to be seed length of fruit and fruit weight/ plant. P7 had maximum number of fruit /plant and yield ton/ ha. Further selection can be carried out in these genotypes or these may be subjected to future breeding programmes for exploitation of hybrid vigour in cucumber(table 1) . Variance Analysis (ANOVA) shows very significant differences between parents and hybrids for all hybrid pairs studied (Table 2a, 2b,3a,3b). For GCA :Anlysis for yield the parents good for GCA such as (P3, P4, P6 and P7). The significant mean square due to general combining ability (GCA) and specific combining ability (SCA) for all the characters indicated that both additive and non-additive gene actions played dominant role for the expression of these characters.The higher magnitude of SCA variance than that of GCA variance for only one character out of 12 characters studied indicated the predominance of the non-additive gene effects for that character.The average of the reciprocal pair effect of 12 traits is higher than the SCA, indicating that the reciprocal effect also exists in cucumbers. In cucumbers (Golabadi et al., 2017), and in the gourd Quamruzzaman et al. (2020) found non-additive gene effects for the first female attrition as well as the number of female flowers per stem, branches per plant in the cucumber. The Specific combining ability effects signify the the role of non-compounding gene activity in the expression of traits. It indicates theSCA ,specifically leading to the highest performance of some specific hybrid combinations. That's why it involves a specific hybrid being successful. High SCA effects can arise not only in hybrid combinations involving highly coordinated substances but also in hybrids associated with low combinations. Therefore, in practice, some combinations with low individual coordination capabilities should also be contained in the breeding program such as Average fruit weight/ plant on P1xP6, P2xP3, P2xP4,P2xP5 and P2xP7 hybrid crosses.

V. CONLUSION

The ability to coordinate in relation to 8 x 8 full diallel hybrids indicates both plus and non-cumulative gene activity in the characteristic expression of different traits. The P4 genotype is the best general combination for promoting flowering. The P4 genotype is great for boosting productivity as well as most other important characters, namely, the average single fruit weight and fruit yield per plant. P4 is best for female flowers to grow. P6 and P7 are varieties that have good general coordination capabilities for use in passing to improve the fruit /plant. P1(PhungTuong) and P2(DaiLoan) are the best general coordination to promote early flowering in cucumbers. Significant SCA effects have been shown for early flowering in pairs of P1xP2 and P3xP5 hybrids. Average fruit weight/ plant on P1xP6, P2xP3, P2xP4,P2xP5 and P2xP7 hybrid crosses. Significant reciprocal effects are also observed in most important characteristics namely, the date to the first male and female flowers (highly desirable), the number of fruits per

plant, the average singlefruit weight and fruit yield. Reciprocal hybrid pairs can be used to improve the desired characteristics in future breeding processes. Further selection can be carried out in these genotypes or these may be subjected to future breeding programmes for exploitation of hybrid vigour in cucumber.

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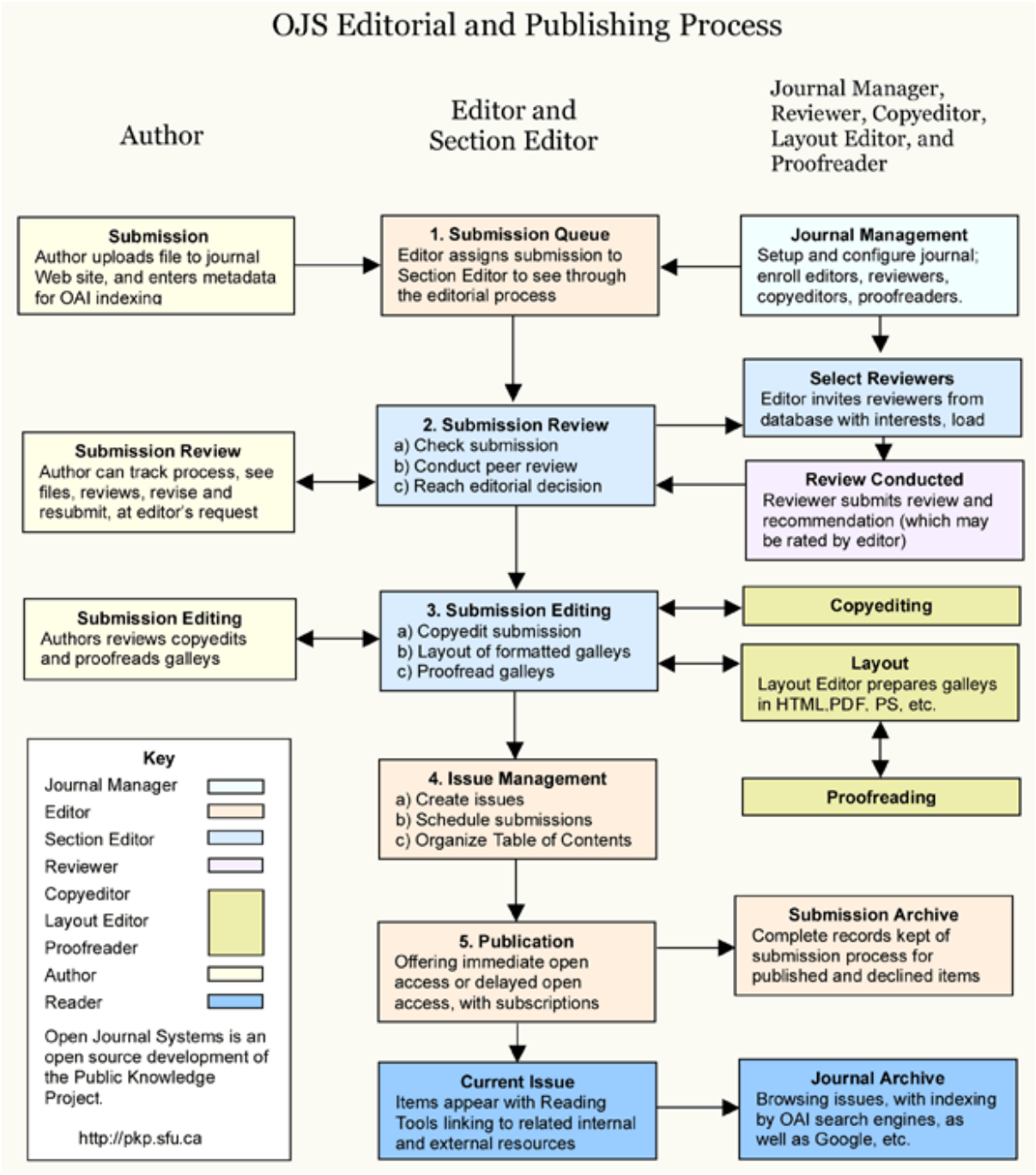

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