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FOREWORD

I am pleased to put into the hands of readers Volume-6; Issue-1: Jan-Feb 2021 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
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Assessment of Caecal Microbiome in two Breeds of Rabbits Fed Fermented Cocoa Podhusk Meal

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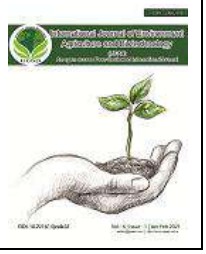

Effect of biochar and supplementary application of micronutrient on soil and growth of okra in Lafia, Nigeria

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Abstract— *The study was conducted at the Research farm faculty of Agriculture, Lafia-Shabu campus, Nasarawa State University Keffi. The objectives were to investigate the effect of Biochar and supplementary application of micronutrient on Soil and growth of Okra. Biochar and micronutrient at different rate were considered, Control (zero %), 4tons/ha Biochar, 16 tons /ha Biochar, 4 tons/ha Biochar + 1 litre / ha micronutrient, 16 tons/ha Biochar + ½litre/ha micronutrient, 4tons/ha Biochar + ½litre/ha micronutrient and 16tons/ha biocha+½litre/ha micronutrient. The experiments were laid out and the seven treatments arranged in a randomized complete block design (RCBD) replicated three times. Biochar was drill into the first 5cm of the soil. Micronutrient application was first done two (2) weeks after planting and this was followed weekly. All Soil samples collected were analyzed using standard laboratory soil analysis procedures. Data on growth parameters that were collected include, plant height, leaf area and stem girth. Data was analyzed using analysis of variance (ANOVA). The result showed that, there is no significant ($p < 0.05$) difference on the vegetative growth parameters of the plant. However, 16tons/ha Biochar increased significantly ($p < 0.05$) the percentage organic carbon, organic matter, cation exchange capacity, Nitrogen, and pH of the soil. This study demonstrated the potential of Biochar in improving the soil quality in the short term and growth parameters of the plant in the long term. Therefore 16 tons /ha Biochar is recommended.*

Keywords— ***micronutrient, Agriculture, ANOVA, Okra.***

I. INTRODUCTION

Okra, (*Hibiscus esculentus, L.*) belongs to the malvacea family. There are nine to ten species, out of which four are cultivated. There are five species in Africa, there are indigenous and two are alien having been introduced from Asia, one of these species, the West African Okra (A. *Caillei)*. Okra plays an important role in the diet by supplying carbohydrate, protein, fat, minerals and vitamins that are usually deficient in the staple food. Okra is basically low in calories and dry matter constituents which when consumed in a meal with basic starchy food makes the food more palatable (Savello *etal* 1982 and Uguru, M. I, 1996)

It is an important vegetable crop grown throughout the tropical and subtropical regions of Asia and Africa (Bisht and Bhat, 2006). Okra is believed to originate probable from south East Asia. It is popular in West Africa, Brazil, Philippian, Thailand, and India. It is distributed also to other Parts of the world by the Portuguese (ECHO, 2003).

In Africa (Nigeria), Okra is cultivated because of it high mucilage content which is used in thickening soup (Purse glove 1968, Wolfe *et al,* 1997). Fresh okra is high in vitamin A, B and C and calcium (NARP, 1993). Significant levels of carbohydrate, potassium, magnesium and other vitamins are also present in okra (Norman, 1992, Adeboye and Oputa 1996).

A mucilaginous preparation from the capsule can be used in plasma replacement or blood volume expander. The mucilage is also used to glace paper and also useful in confectionary (Markose and peter, 1990)

Contiguous use of agricultural land over several years creates on imbalance in the store of nutrients available. Also, increase in cropping density and introduction of high yielding varieties have caused considerable drain of nitrogen and crops showed a positive response to the addition of nitrogen in the soil (Ali *et al*, 2004). Despite the nutritional value of okra, its optimum yields (23t/ha) and quality have not been attended in tropical countries partly because of continued declined in soil fertility.

Serious depletion of soil fertility due to widening gap between nutrient removal and suppliers (Ramesh, 2008).Has affected crop productivity. The use of inorganic fertilizer alone has not been helpful because it promotes and increase degradation of plant nutrients (Sharma and mitral, 1991). The degradation according is brought about by loss of organic Mather which contently results in soil acidity, nutrient imbalance and low crop yields.

Heavy application of chemical inputs such as pesticide, insecticide and chemical fertilizers, greatly deteriorate the environment and also decrease production (Nishio, 1996). Tropical soil after receiving chemical fertilizers tend to be unproductive due to lack of proper amendments of organic malt or. With the growing environmental concerns the sole dependence on chemical input based agriculture is being replaced by integrated multi approach involving conjunctive use of both organic and on organic sources.

Micronutrients are equally important in plant nutrition as macronutrients, the simple occur in plants and soils in much lower concentrations. Plants grown in micronutrients deficient soils exhibit similar reductions in productivity as those grown in macronutrient deficient soils.

The micronutrients elements are Iron (Fe), Manganese (Mn), Copper (Cu), Zinc (Zn), Boron (Bo), Molybdenum (Mo) and Chloride (Cl). They are required by plants in small quantities, in contrast to macronutrients, which constitute relatively larger percentage of plant weight Fe, Mn, Cu, and Zn are Cations, while Mo, Bo and Cl are anions (Agbede, 2009).

According to Remesh, (2008).The use of organic manures particularly Biochar are the only option to improve the soil organic carbon for sustenance of soil quality and future productivity.

Hamer *et al*, (2004) identified that some microorganisms were able to live with Black carbon (BC) as the soil carbon(C) source and that Black carbon in soil may promote the rate of decomposition of labile carbon compound. Biochemist a charcoal substance produced from controlled, incomplete combustion of biomass in an oxygen- free environment. Biochar gives the soil its black colour and improves soil structure, aggregation, water infiltration and retention and nutrient storage capacities (Lehmann *et al*, 2003). One significant feature of Biochar is that it may increase stabilization of organic matter nutrient sources in the soil (Glaser *et al*, 2001) and reduce nutrient leaching losses (Lehmann *et al*, 2003) and hence improve nutrient retention.

Biochar is important as a soil conditioner and also help to spread and transform nutrients (Glaser *et al*, 2002, Lehmann *et al*, 2003). This therefore, makes it possible to modify N, P and S transformation in mineral soils. Besides, it has a high surface area that is highly porous and so has the ability to increase soil water holding capacity, cation exchange capacity (CEC) (Glaser *et al*, 2002, Liang *et al*, 2006).

The use of Biochar to meet the C and N requirements for crops would be inevitable in the years to come for sustainable improved agriculture since it generally retains nutrients in the soil for a long time (Lehmann and Rondon, 2005). It improves the soil physical and biological properties and efficient absorption of ammonium.

All plants require the same basic nutrient but plants differ in the way they respond to nutrient availability and used.

Many researchers have revealed the efficacy of Biochar on increasing growth, yield and essential oil of Okra and other crops in general. Essentially the use of chemical fertilizers alone or sole fertilizers may not be adequate enough to provide food needed to satisfy the needs of the populace. It is therefore expedient to properly combine the necessary soil amendment strategies to produce healthy food from an organic source with no environmental hazards. Soil nutrient and water resources need to be properly managed and conserved (Quansah, 1996).

To achieve this, several rates of micronutrient and Biochar were combined and investigated to ascertain the impact on crop yield and productivity. Therefore, this study was aimed to assess the effectiveness of application of Biochar on Growth and Development of okra, to compare the growth performance of okra on soil amended with Biochar at difference rate of application.

And to evaluate the effect of Biochar and micronutrient on the physiochemical properties of soil.

II. MATERIAL AND METHOD

The study was conducted at the faculty of Agriculture, Nasarawa State University Keffi, Shabu- Lafia Campus, Nasarawa State The location is situated at latitude 08°

33N,longitude 08^{O} 32E, with mean elevation of 181.53m above sea level . Rainfall usually starts from May – October and the average monthly rainfall figures ranges from 400 -350mm. The months of July and August usually records heavy rainfall. The daily maximum temperature ranges from 20.0ºC – 38.5ºC and daily minimum ranges from 18.7ºC – 28.2ºC. The months of February to early April are the months that have the highest maximum temperature while the lowest maximum temperature months are recorded in December and January because of the prevailing cold harmattan wind from the northern part of the country at this period. The relative humidity rises as from April to a maximum of about 75- 90 (NIMET, 2017). The soil is an oxilsol. It is deep porous brownish red and made up of kaolinite clay derived from sandy deposit of sand stone origin. The area is characterized by a sub-humid tropical climate with wet and dry season. The annual rainfall distribution is about 113mm. the mean annual temperature is 27ºC with minimum and maximum temperature of 24.80º C and 33º C respectively

Clemson spineless okra was used for the study, Seed were obtained from technisem co. Ltd, packed in France. Biochar was prepared and crushed in to granule forms at the experimental site. Foliar blend formulated micronutrient was used.

The experiments were laid out in a randomized complete block design (RCBD) comprising seven (7) treatments and (3) three replications. Each experimental plot was 25m^2 (5m x5m). The planting distance adopted was intra row 60 cm and inter row 45cm (60 cm x 45 cm)

Biochar from softwood prune branches were collected from the trees around and were prepared by pyrolysis method as described by Husk *et al*, (2010) using a local/traditional earth-mound oven (clay oven). The materials were cut and sun dried to reach water content of about 20% and then heated in the locally made clay oven. Pyrolysis time was ten days (then it became a wood Biochar).The produced Biochar requiring cooling in an air-tight pit. After the cooling, Biochar were crushed to a coarse powder for laboratory measurements; the Biochar were dry-sieved over 2mm sieved. For experiment, the Biochar were used without further treatment. Biochar characteristics were analyzed by the method as described by Husk *et al;* (2010).

The land was clear, deep ploughed and brought to a fine tilth by repeated harrowing on 10th April, 2018. The plots where prepared and Biochar was applied on 17th April, 2018 and by drilling to the depth of 10cm with the aid of a hoe.

After size reduction Biochar were drill in to the soil to a desirable depth of about 10-15cm and covered with top soil.

The seed were sown on 3th May, 2018. At planting three seeds were placed in each hole created with a hoe at a depth of 5cm and at a spacing of 60cm x 45cm.These were thinned to one plant per stand at 2 weeks after germination (WAG)

In order to keep the soil porous and also free form weeds, hand weeding and hoeing were done as an when necessary. Weeding was done manually with hoe. Weeds were controlled until harvesting was completed at least weed were controlled every two weeks.

To control pest and diseases, the recommended fungicide and insecticide like imi-force was applied at the vegetative stage. The necessary plant protection measures were taken up as per the recommended package. Flea beetle (*Podagrica spp*) the only pest that was noticed was controlled with imi-force.

Three plants from each treatment in each replication were randomly selected and tagged for record taking on growth parameters. Plant height was taking and record using a one meter rule from the base of the stem at soil level to the terminal bud of the plant of a fully opened leaf on the main shoot and the mean height was expressed in cm. Plant height was recorded from 5 weeks, to 10 weeks after planting.Plant leaf area was taken from the leaf of the tag plant and recorded using a meter tape.

Soil sampling were collected, air dried, sieved through a 2mm mesh and prepared for soil routine analysis.

Statistical analysis of the data was carried out using the General linear model of statistical package for social sciences SPSS for Randomized complete block design.

III. RESULT AND DISCUSSIONS

Soil sample was taken and analyzed before application of Biochar. The result explained the physiochemical properties of the soil before the amendment. Table.1 indicated that 16tons/ha biocha+1litre/micronutrient had the highest pH value of 6.71 and 4tons/ha biochar+1litre/ha micronutrient had the highest organic carbon, organic matter and potassium (K) 1.60, 1.85, and 0.64 as well as 4tons/ha Biochar+1/2litre/ha micronutrient with the highest Nitrogen % of 1.21 respectively.4tons/ha Biochar +½litre/ha micronutrient also had the highest CEC and Mg of 5.63 16tons/habiocha+½litre/ha micronutrient, had the highest percentage of sand, Sodium (Na) and Calcium (Ca), 83 %, 0.29 and 3.81

Table.2 showed the effect of wood Biochar on the soil physiochemical properties after harvest. The result

revealed that T2 (16tons/ha Biochar) had the highest organic carbon (1.00), organic matter (1.72), sodium (0.87), cat-ion exchange capacity (7.57) and pH value of 6.72 which is favorable for plant growth. While T4 and T6 (16tons/habiocha+½litre/ha micronutrient and 16tons/habiocha+1litre/ha micronutrient) follow with height percentage of organic carbon and organic matter, (0.84, 0.84) (1.44 and 1.44). T_0 (un-amended) had the low pH of 6.26 in salt solution, organic carbon, organic matter, and low CEC (cat-ion Exchange Capacity) of 6.45.

This result is in line with the finding that Biochar addition to soils has a lot of potentialsin Agriculture which include, liming of acid soils, addition of basic cat ions and micronutrients, improving water holding capacity and a gradual release of nutrients to the growing plant (Glaser *et al*, 2002.Laird *et al*, 2010. Soli *et al;* 2010,Vanzwieten *et al*, 2010) leached sandy soils typically have low soil pH values, poor buffering capacities, low cation exchange capacity (CEC), with values ranging from 2-8 cmol kg^{-1} and can have Aluminum(Al)toxicity (Novak *et al;*2009). The addition of Biochar to these highly leached, infertile soils gives an almost immediate increase in the availability of some basic cations (Glaser *et al;* 2002; Liang *et al;* 2006) as well as a significant improvement in crop yields, particularly where nutrient resources are in short supply (Lehmann and Rondon.2006).

Table: 1 Physio-Chemical Properties of the Soil before Biochar Amendment

	TREATMENTS						
Parameters	To	T1	T2	T3	T4	T5	T6
P^H in H_20	7.02	7.14	7.13	7.15	7.12	7.14	7.16
P^H in KCl	6.38	6.56	6.64	6.70	6.40	6.62	6.71
% O.C	0.92	0.94	1.00	1.60	0.98	1.03	1.00
% O.M	1.58	1.65	1.72	1.85	1.42	1.46	1.46
% N	0.41	0.24	0.16	0.35	0.34	1.21	0.45
Avail.P(ppm)	5.03	5.43	6.02	5.43	5.20	5.33	5.38
Na mol/kg	0.28	0.28	0.18	0.22	0.29	0.27	0.26
K mol/kg	0.31	0.56	0.49	0.64	1.44	0.33	1.52
Ca mol/kg	2.73	2.85	3.10	3.34	3.81	2.93	3.26
Mg mol/kg	1.29	1.53	2.25	2.62	1.67	2.70	2.30
E.Ameg/100g	1.00	0.83	0.29	0.61	0.85	0.52	0.25
C.E.C	5.33	5.44	5.45	5.62	5.47	5.63	5.42
Fe (ppm)	2.41	3.52	2.61	1.19	2.91	2.54	2.57
Zn (ppm)	1.26	1.23	1.22	1.23	1.32	1.43	1.24
Cu (ppm)	0.67	0.58	0.57	0.76	0.56	0.73	0.77
Mn (ppm)	1.14	1.05	1.10	0.63	1.20	1.32	1.12
% B.S	80.0	80.0	80.2	80.2	84.4	80.0	80.0
% Sand	78.2	76.5	79.1	79.3	83.0	75.3	76.2
% Silt	11.1	12.1	10.3	11.1	5.6	12.3	10.5
% clay	10.7	11.4	10.6	12.1	10.4	11.4	12.3
T.C	sand	sand	Sand	sand	sand	sand	Sand

O.C =organic carbon, O.M= organic matter, B.S= base saturation, CEC=cat ion exchange capacity

Table: 2 Physio-chemical Properties of the soil after biochar amendments.

	TREATMENTS						
Parameters	To	T1	T2	T3	T4	T5	T6
P^H in H_20	7.11	7.20	7.13	7.20	7.19	7.20	7.20
P^H in KCl	6.26	6.92	6.72	6.94	6.52	6.91	6.98
% O.C	0.70	0.62	1.00	0.80	0.84	70	0.84
% O.M	1.20	1.07	1.72	1.38	1.44	1.20	1.44
% N	0.07	0.07	0.14	0.14	0.28	1.14	0.35
Avail.P(ppm)	3.62	4.10	3.63	4.09	3.98	4.07	4.08
Na mol/kg	0.86	0.67	0.87	0.61	0.58	0.72	0.69
K mol/kg	0.13	0.10	0.18	0.23	0.34	0.28	1.30
Ca mol/kg	3.01	3.24	3.16	3.52	3.18	3.36	3.26
Mg mol/kg	1.34	1.56	1.26	1.70	1.63	1.58	1.46
E.Ameg/100g	1.17	2.00	1.00	0.67	0.83	0.67	0.33
C.E.C	6.45	6.51	7.57	6.73	6.53	6.61	6.04
Fe (ppm)	1.13	1.14	1.01	0.97	0.89	1.04	1.01
Zn (ppm)	3.10	3.03	2.98	2.87	3.11	2.90	2.87
Cu (ppm)	0.06	0.09	0.10	0.08	0.11	0.06	0.05
Mn (ppm)	0.03	0.06	0.08	0.10	0.04	0.06	0.07
% B.S	82.0	74.0	84.0	90.0	78.0	90.0	81.0
% Sand	90.6	90.5	90.0	90.6	90.0	90.0	90.2
% Silt	1.4	1.5	1.4	1.4	1.5	1.4	1.5
% clay	8.0	9.0	8.6	8.0	9.0	8.6	9.0
T.C	Sand	sand	Sand	sand	sand	Sand	Sand

O.C =organic carbon, O.M= organic matter, B.S= base saturation, CEC=cat ion exchange capacity

Effect of Wood Biochar and Supplementary Application of Micronutrient on plant Height (Okra)

After Biochar and Micronutrient application on the research area (soil) there was no significant ($p < 0.05$) increase of the plant height from the treatments at 5WAP,6WAP,7WAP,8WAP,9WAP and 10WAP respectively. Table: 3 However, the highest plant height (25.88cm) was obtained from the application of 0kg Biochar and 0 liter Micronutrient (control) at 10WAP. While the lowest plant height (22.58cm) was obtained from the application of 16tons/ha Biochar and 1litre/ha Micronutrient at 10WAP.this result is agree with the finding of Glaser *et al*,2002that, Biochar work better in the second and third year of application than it does in the first year of application

Effect of wood Biochar and supplementary of application of micronutrient on plant leaf area of okra

Table: 4 presents the effects wood Biochar and supplementary application of micronutrient on leaf area of okra. The result of the study revealed that there is no significant different in leaf areas of the treatment from 5WAP, 6WAP, 7WAP, 8WAP, 9WAP, and 10WAP.

The plant with the highest leaf area (29.96 cm and 29.02 cm was observed from the application of 16tons/ha Biochar+1/2litre/ha micronutrient and 0kg/ha Biochar+0litre/ha micronutrient then 16tons/ha Biochar had the lowest leaf area of 14.78cm at 5WAP. While at 8WAP 16tons/ha Biochar had the highest leaf area of 88.13cm then 4tons/ha Biochar+1/2Litre/ha micronutrient had the lowest leaf area of 1.23cm respectively.

Table 3. Effect of wood Biochar and supplementary application of micronutrient on plant height of okra

TREATMENT	5WAP	6WAP	7WAP	8WAP	9WAP	10WAP
Control	7.03	8.67	11.67	15.60	22.43	25.88
4tons/ha Biochar	6.29	8.24	10.55	11.88	19.88	21.60
16tons/ha Biochar	5.18	5.78	8.11	10.91	17.13	18.42
4tons/ha Biochar +1 litre/ha micronutrient	6.24	7.89	10.06	12.35	19.58	21.98
16tons/ha Biochar + $^{1}/_{2}$ litre/ha micronutrient	6.69	8.83	11.61	15.25	22.27	25.34
4tons/ha Biochar + $^{1}/_{2}$ litre/ha micronutrient	5.67	7.07	9.782	11.92	18.56	20.20
16tons/ha Biochar +1 litre/ha micronutrient	5.93	7.50	9.92	12.38	19.53	22.58
Means	6.16	7.72	10.25	12.03	19.93	22.27
Significant	0.564^{NS}	0.440^{NS}	0.734^{NS}	0.674^{NS}	0.953^{NS}	0.846^{NS}

WAP= week after planting

NS= not significant

Table: 4 Effect of wood Biochar and supplementary application of micronutrient on plant leaf area of okra (cm^2)

TREATMENT	5WAP	6WAP	7WAP	8WAP	9WAP	10WAP
Control	29.96	45.45	49.73	1.73	1.96	2.32
4tons/ha Biochar	26.12	34.84	43.70	1.32	1.56	1.91
16tons/ha Biochar	14.78	23.45	39.90	88.13	1.36	1.58
4tons/ha Biochar +1 litre/ha micronutrient	19.72	29.52	49.62	1.33	1.48	1.95
16tons/ha Biochar + $^{1}/_{2}$ litre/ha micronutrient	29.02	54.55	63.63	1.52	1.64	2.13
4tons/ha Biochar + $^{1}/_{2}$ litre/ha micronutrient	21.83	28.96	45.57	1.23	1.65	5.79
16tons/ha Biochar +1 litre/ha micronutrient	15.04	21.69	47.05	1.28	1.99	2.44
Means	22.72	34.69	48.53	1.33	1.65	2.59
Significant	0.886^{NS}	0.712^{NS}	0.979^{NS}	0.885^{NS}	0.917^{NS}	0.653^{NS}

The study shows that Biochar application increased soil pH on acidic soil and reduce leaching of organic carbon, organic matter, Nitrogen, Sodium, Potassium, Calcium and cation exchange capacity (CEC). Compared to un-amended treatment the study revealed not significant ($p < 0.05$) different on growth parameter (plant height, leaf area and yield of okra. This is in conformity with the study by (Lehmann, 2007) which said that fresh Biochar shows moderately low plant performance and cation retention properties relative to age Biochar.

Before sowing, the result of the soil analysis in table:1 revealed that the percentage of nitrogen, phosphorus, calcium and available K decreases at first among the treatments but there after increase significantly ($p < 0.05$) after harvesting especially on treatments which received the high rate of biochar.16tons/ha+½litre/ha micronutrient and 16 tons/ha+ 1litre/ha micronutrient had the highest organic carbon, organic matter and favorable pH for plant growth as shown in table: 2 compared to un-amended treatment (control) which has low organic carbon (0.70) organic matter (1.20), and CEC (6.45).After sowing to (un-amended soil) had lower pH (6.26) which is slightly acidic in alkaline solution compared with the Biochar applied treatment of pH (6.52, 6.72,) which is nearly neutral. Average pH range for plant growth (Agbede, 2009). The application of Biochar to soil changes their pH to a near neutral especially in an acidic soil. The result

also shown significant ($p < 0.05$) changes in soil cation exchange capacity and pH of the soil in question presented in Table :2 this agreed with (Lehmann *et al*, 2003) that soil cation exchange capacity (CEC) has to increase with the addition of Biochar. Biochar can be used as soil amendment to improve soil quality (Blackwell *et al*, 2009).

IV. CONCLUSION

Application of Biochar to soil has increased significantly ($p < 0.05$)the soil properties. This study revealed that Biochar addiction to the soil increased the soil N, P, K, Ca, Mg and soil pH it also played a major role in lowering E.C of the soil to enhance better nutrient availability as well as serving as a limiting agent which increased the pH from 6.26 to 6.72 which is ideal for okra production. 16tons/ha Biochar result on the increase in organic carbon, organic matter, cation Exchange capacity and favorable soil pH for Okra growth. It is therefore recommended that Biochar at 16tons/ha should be used or adopted to give better results for farmers. This recommendation corroborates with the fact that inorganic fertilizers are becoming too expensive to procure by small-scale farmers and also Biochar can stay for a long time in the soil.

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Determination of Heavy Metals Concentration in Soil around Sokoto Cement Company

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Abstract— *The main visible pollution generated by the cement industry corresponds to the dusts. The dusts can be emitted at every stage of the manufacturing process of the cement: extraction of the raw material, crushing, production. Eighty eight samples of soil were separately collected from the four directions of the company into polythene bags and brought to the laboratory for further analyses. The sample were digested using Aqua Regia Method, and the Hg, Zn, Cd, Cr, Ni, Pb and Cu contents were determined by (AAS). Data generated were subjected to Analysis of Variance (ANOVA) to depict the differences and the means were separated with the Duncan Multiple Range Test (DMRT), where significant differences occur. It was found that the concentration of each heavy metal decreased with increase in distance from the industry. It was further found that heavy metals contributed differently to the pollution status of the environment with the eastern part impacting most. The concentration of heavy metals in the soil is high. The result of the analyses showed that Hg, Zn, Cd and Cr were high in the collected from four directions while Pb was foundto be the least. Sokoto Cement campany was found to produce the wastes/ dust that pollutes the environment.*

Keywords— ***Heavy metals, Pollution, Dust, Cement and Soil.***

I. INTRODUCTION

Owing to increasingly international concerns on the soil contamination due to Cd, Cr and Pb (Williams and Robertson, 1999) there is need to probe into their effects. Cadmium, chromium and lead occur naturally in the environment and found in soil, plant, animals and human, typical as salts (Fleming and Parle, 1977). These metals can neither be destroyed nor degraded but can be converted to organic forms by biological action both in the environment and within living body (Eboget *etal.*, 2008). However, some heavy metals at low doses are essential micronutrients for plants but in high doses they may cause metabolic disorders and growth inhibition for most of the plant species (Claire *et al.*, 1991) Heavy metal contamination affects the biosphere in many places worldwide (Cunningham *etal.*, 1997; Meagher, 2000). Lead, chromium and cadmium are highly toxic pollutants which can be added to the environment through automobile exhaust (Adriano, 1986). Inhibition of germination and retardation of plant growth are commonly observed due to lead toxicity (Igbal and Siddiqui, 1992). Cadmium is a heavy metal with high toxicity and has an elimination half-life of 10-30 years (Mortvert *etal.*, 1981). Cadmium is very toxic to biological systems (Awofolu, 2005), the toxic effects of cadmium on growth and development of living organisms are well documented e.g. Servilla *et al.*, (2005). However the molecular mechanism responsible for the inhibition of plant growth by cadmium is still not completely understood (Mecharg, 1994). Some authors suggested that both Cd and Hg showed drastic effects at high concentrations and longer duration with regard to seedling growth and metabolism (Madejon et *al.*, 2002). Cadmium affects root metabolism, which shows sensitivity to Cd^{2+} toxicity by a reduction in lateral roots due to reductions in both new cell formation and cell

elongation in the extensions region of the root. Farooq *et al*. (2009) observed that lead (Pb) and cadmium (Cd) toxicity affect seed germination, root and shoot seedling length, root ration and dry biomass of *Albizia Lebbeck*. They further stated that the tolerance of *A. lebbeck* seedlings to lead and cadmium gradually decreased with increasing concentration of the elements. Moreover, lead toxicity has been implicated in the decline of the Roman Empire (WHO, 1972).Their foods and drinking water was presumed to have been contaminated from lead guttering, and lead lined channels (Patterson *et al*., 1987; Fergusson, 1990). The recent autopsies on the remain of some expedition members preserved in the permafrost showed very high level of Pb sufficient to cause of lead poisoning (Patterson *et al*., 1987; Fergusson, 1990). Furthermore, Pb toxicity can result in abdominal pain, convulsions, sleeplessness, headache, e.t.c (WHO, 1972).

As one of the consequences of heavy metals contaminations in the soil, water and air, plants are also contaminated. Heavy metals get accumulated in plants and crops cultivated in heavy metals contaminated areas and when consumed by man and other live-stocks, they accumulate and reach lethal levels quickly (Fleming and Parle, 1977).Cd and Cu become concentrated during bio transfer from soil surface layer to plants, herbivores and their predators (Hunter *etal*., 1989). High tissue content of Cu and Cd were found in small mammals feeding on contaminated plant materials. Thus within the ecosystem, the principal route of transfer responsible for the mobilization of toxic metals would appear to be from the soil to vegetation (Hunter *et al*., 1989).However, people are exposed to cadmium by intake of contaminated food or by inhalation of tobacco smoke or polluted air (Lee *et al*., 1999). High concentration of cadmium in the soil represents a potential threat to human health because it is incorporated in the food chain mainly by plants uptake (Alvarez-Ayuso, 2008). In human, cadmium can cause anemia, cardiovascular diseases, growth impairment, alopecia, learning disorders, poor appetite, e.t.c. (WHO, 1972).

II. MATERIALS AND METHODS

COLLECTION OF SAMPLES

Eighty eight samples of twigs *of* soil samples were collected in the vicinity of the factory (between the months of August-October,2011) at interval of 50m from the mill, that is at (11) different points (50 m, 100 m, 150 m, 200 m, 250 m, 300 m, 350 m, 400 m, 450 m, 500 m and 1km) from the mill, in which 1km served as Control point along each of the four cardinal points (i.e. East, West, South and North). The twigs of soil samples were collected and packed in clean polythene bags and taken to the laboratory for chemical analysis.

COLLECTION OF SOIL

Soil sample were collected from different points in each direction at 50m interval and 1km as control point by using measuring tape, hand glove and stainless steel soil Auger.The method of collecting the surface soil (the upper 3 cm layer) is according to the norm AFNOR X31B (AFNOR, 1999). The soil samples were packed in separately clean polythene bags and were taken to the laboratory for analysis.

DETERMINATION OF HEAVY METALS IN THE SOIL AND PLANT SAMPLES

The digested samples were separately analyzed for Cd, Cr, Pb, Ni, Cu, Zn and Hg by Atomic absorption spectrophotometeric method. The radiation lamp for each heavy metal to be analyzed was placed in a compartment in the AAS and a tiny pipe attached to the machine (Aspirator) was put inside bottle containing the sample and the concentration of the respective heavy metal was read off by the aid of computer system attached to the machine.

DATA ANALYSES

Data generated from the quantification of heavy metals were subjected to descriptive and inferential statistics. Their concentrations were compared with respect to location/site with the use of analysis of variance (ANOVA). The means were separated with the use of Duncan multiple range test (DMRT).The data obtained from the chemical analysis were also subjected to statistical treatment using correlation coefficient matrices. This was carried out in order to establish association and probable source of dispersion or pollution.

III. RESULTS

Table 1: Concentration of Cadmium (mg/l) in soil samples around Sokoto Cement Company

Distance (m)	North	South	East	West
50	31.11	26.68	49.72	45.28
100	30.68	26.01	50.11	44.99
150	32.01	21.22	49.22	41.28
200	33.14	19.98	48.98	42.01
250	29.22	20.01	47.66	41.68
300	30.01	18.68	48.14	41.72
350	28.88	14.46	48.01	39.21
400	28.68	15.01	47.77	38.99
450	27.62	12.22	46.62	40.01
500	26.44	10.22	47.01	37.98
1000	2.19	1.51	2.23	2.12

Table 2: Concentration of Chromium (mg/l) in soil samples around Sokoto Cement Company

Distance (m)	North	South	East	West
50	31.01	20.41	39.98	37.92
100	29.68	20.41	39.99	37.77
150	30.09	20.01	39.01	35.66
200	30.61	19.92	39.01	35.66
250	28.88	18.99	39.01	36.01
300	29.21	18.01	38.66	35.81
350	27.98	16.42	38.61	31.92
400	27.69	11.21	39.03	34.01
450	24.96	10.11	37.82	33.99
500	24.68	10.14	38.01	33.66
1000	1.11	1.09	2.03	1.32

Table 3: Concentration of Copper (mg/l) in soil samples around Sokoto Cement Company

Distance (m)	North	South	East	West
50	16.48	13.11	27.96	23.92
100	18.01	12.08	26.49	23.92
150	17.22	11.21	27.99	24.01
200	16.88	9.62	27.01	23.66
250	15.96	9.88	26.11	22.66
300	16.21	10.11	26.01	22.69
350	15.81	9.01	27.12	22.11
400	14.99	8.49	25.12	20.66
450	14.22	7.99	24.28	19.99

500	12.22	8.42	24.28	21.08
1000	1.03	1.01	1.03	1.02

Table 4: Concentration of Mercury (mg/l) in soil samples around Sokoto Cement Company

Distance (m)	North	South	East	West
50	43.24	27.01	59.26	55.48
100	41.91	26.42	59.48	54.99
150	41.88	20.18	58.92	54.64
200	38.46	18.08	59.02	55.01
250	31.23	17.21	58.41	53.66
300	34.01	16.92	56.68	53.76
350	29.99	16.11	57.33	49.98
400	31.21	15.68	58.14	51.04
450	26.01	14.54	55.96	49.64
500	26.66	14.54	56.01	50.50
1000	2.03	1.91	3.99	2.21

Table 5: Concentration of nickel (mg/l) in soil samples around Sokoto Cement Company

Distance (m)	North	South	East	West
50	31.22	18.98	49.68	45.69
100	33.04	17.04	50.41	45.98
150	30.48	12.22	50.01	45.66
200	30.78	12.68	49.71	45.01
250	26.11	11.92	49.01	44.21
300	25.76	8.48	48.48	43.98
350	24.92	6.42	48.71	39.21
400	24.08	2.66	48.62	38.88
450	21.41	5.82	46.46	37.68
500	19.21	2.42	47.01	39.01
1000	0.99	0.68	1.44	1.11

Table 6: Concentration of Lead (mg/l) in soil samples around Sokoto Cement Company

Distance (m)	North	South	East	West
50	11.22	9.08	22.08	17.99
100	12.13	9.21	22.66	18.22
150	10.99	9.06	21.96	17.08
200	11.01	9.01	21.81	15.66
250	10.61	9.01	21.01	16.01
300	10.48	9.01	20.69	17.21

350	9.99	8.98	20.02	14.28	
400	9.96	8.99	19.11	14.01	
450	9.61	9.01	18.98	13.99	
500	9.21	8.98	19.21	13.68	
1000	0.99	1.00	1.01	1.0	

Table 7: Concentration of Zinc (mg/l) in soil samples around Sokoto Cement Company

Distance (m)	North	South	East	West
50	37.88	26.08	58.08	47.99
100	36.98	25.92	53.06	48.61
150	36.04	20.01	53.68	47.92
200	37.01	24.02	53.68	44.66
250	30.48	19.68	52.99	45.31
300	29.72	18.79	50.01	45.66
350	30.01	16.01	49.66	43.96
400	29.61	14.99	48.98	40.01
450	30.01	15.01	49.81	44.03
500	26.44	13.09	49.01	39.86
1000	1.50	0.70	1.71	1.01

IV. DISCUSSION

This study shows a considerable abundance of the toxic metals on the soil around Sokoto Cement Company and its immediate environments.It was discovered that the concentrations of all the metals are higher in soil samples at various directions. The high concentration of the metals could be probably due to limited number of vegetation around the factory. The higher heavy metal concentrations observed in this study may be attributed to deposition of calcium oxide from Cement Company and calcium compounds in the soils of Sokoto. Brady and Weil (1999) reported that the neutral to alkaline soil pH observed in semi-arid soil such as that of Sokoto was due to low rainfall and alkaline compounds are not leached away, thus making soils of the region too alkaline. This is in support of the result of this study because the concentrations of cadmium (Cd), chromium (Cr), lead (Pb), nickel (Ni), copper (Cu), zinc (Zn) and mercury (Hg) in plant and soil samples are high.

Gbadebo and Bankole (2007) analysed potentially toxic metals in airborne cement dust around Sagamu, Southwestern Nigeria. The heavy metals investigated are As, Al, Ca, Cd, Pb, Co, and Zn. They reported elevated concentrations of these heavy metals and inducted them in the health problems among the inhabitants of the communities. Their observed confirmed favorably with the finding of this study. The result obtained in this study inducted potential environmental problems such as destruction of vegetation cover and environmental degradation (Topie, 1999; Hegazy,1996;). For example, Topie (1999) indicated that the cement industry was the main cause of destruction of vegetation cover and of environmental degradation in Solin and Kastela near Spilt, Croatia. Damages to coniferous trees by cement dust have been reported by Mandre and Tuulmets (1997).

Cement dust had a significant effect on the growth of some plant species compared with non-cement dusted plants. Toxic compounds such as fluoride, magnesium, lead, zinc, copper, beryllium, sulfuric acid and hydrochloric acid were found to be emitted by cement manufacturing plants (Andrej, 1987).This support the result of this study which showed that cadmium, chromium, copper, mercury, nickel, lead and zinc are available in soil around Sokoto Cement Company. Reduction in plant height, cover and number of leaves of *C. carandas* showed that the losses can generally be attributed to the cement dust which contained toxic metals. The results obtained are in close conformity with those reported by Stratmann and Van Haut (1966) who dusted plants with dust ranging from 1 to 48 g/m^2 day^{-1} and concluded that dust falling on the soil caused a shift in pH to the alkaline side. The findings of Prasad and Inamdar (1990) in *Vignamungo* (Black gram) also

confirmed the finding of this study. The cement dust kiln showed a reduction in chlorophyll content, protein, starch, yield and phytomass in ground nuts (*Arachis hypogaea L*.) (Liu et al., 1997) reported that cement dust decreased the yield of grape and rice in the vicinity of a cement factory in China. It has also been indicted in the reduction of crop yield.Plant response varies between species of a given genus and between varieties within a given species. Plants do not necessarily show similar susceptibility to different pollutants. Major variations in response to different species to air pollutants have been documented by Jacobson and Hill (1970). Studies of biochemical changes and pollution effects on the plant metabolism, i.e., reduction in chlorophyll and completely clogged stomates (Ahmed and Qadir, 1975) revealed that these parameters are important in regulating the productivity and also the number of flowers and seeds produced.

Cadmium, chromium, copper, mercury, nickel, lead and zinc that are prominent in cement dust were found to be higher in concentration in the samples analyzed. This indicates the extent to which the soil and vegetation were polluted by the cement dust from the Sokoto cement company. The concentrations of heavy metals are higher in eastern and western part followed by north and least was southern part of the company, the higher concentrations of heavy metals in eastern and western parts was attributed due to the loading of Cement and heavy trucks movement in the east and presence of quarry and production activities in western part of the Company. Ade-Ademilua and Umebese (2007) have pointed out that metal to metal association could cause an increase in concentration of other metal (absent in cement dust) in the polluted soil over time. Forstner (1995) also pointed out that 70% of metals contaminated soils involved two or more metals and the possibility of synergistic effects may be of considerable importance at some heavy metals contaminated sites.

The significant reductions in shoot length and total leaf area observed in the polluted plants have also been reported in the works of Singh and Rao (1981); Ayanbamiji (1996) and Igbal and shafoeg (1998). The reduction may be due to toxicity of heavy metals which Rout *etal*.(2000) stated that once inside the cells, the aluminium could inhibit root absorption and growth in both aluminium sensitive and tolerant plants. The significant decrease in vegetative growth of polluted plants may be an indication of a reduction in photosynthesis of polluted plants which could be explained on the bases of quantitative as well as qualitative changes in the incident light available for photosynthesis in cement encrusted leaves (Bohne,1963), interruption in gaseous through stomatal clogging (Darley *etal*.,1966), reduction in transpiration in terms of the absorption of minerals from soil and inhibition of intracellular processes (Singh and Rao,1981).

The cement dust caused the epidermal cells and stomata of the polluted leaves to become modified .The higher stomata frequency and index in the leaves of the polluted plants indicate morphological modification on the leaves. According to Ayanbamiji (1996), these may be adaptations for gaseous exchange and transpiration since the surfaces are covered by cement dust.Studies of the effects of cement-kiln dust deposited on the soils also raised questions. Some investigators reported no harmful effects of cement at levels from 1.5 to 7.5 g/m^2/day, while others reported that concentrations from 1.0 to 48 g/m^2/day caused shifts in the soil alkalinity which may be favorable to one crop but harmful to others (Lerman and Darley, 1975) thus confirming the result of this study whose reported higher concentrations of heavy metal in soil samples.Abdulrahman *et al.* (2007), on the analytical assessment of some trace metals in soils around the major industrial areas of Northwestern Nigeria and Sokoto Cement Company reported higher mean concentrations of heavy metals: Cd (0.10 ug/g); Cr(83.4 ug/g); Pb(486 ug/g) and Ni(22.9 ug/g). This study also showed higher concentration of heavy metals investigated, the mean values are Cd(16.9mg/l), Cr(15.2mg/l), Pb(8.30mg/l) and Ni(9.03mg/l) which are within the range reported by Abubakar *etal* (2006); Birnin Yauri and Argungu (2002).

Adejumo *et al*. (1994) showed that some toxic heavy metals like arsenic (AS), lead (Pb,) nickel (Ni), cobalt (Co), zinc (Zn), copper (Cu) and phosphorus (P) were significantly enriched in the neighbourhood of cement factories in Nigeria. This can lead to reduction of soil fertility, Adejumo *et al*., (1994); Sivakumar *et al.*, (1995), it has also been linked to the damage of dwellings (Wasserbauer *etal*., 1998). Deposit of cement dust on roofs can cause microbiological and chemical disintegration of the roofs. Wasserbauer *etal*. (1998) indicated that metabolic process of nitrifying bacteria (mainly *Nitrosomonas* and *Nitrobacter*) on cement dust which settle on roofs can cause chemical corrosion of the roofing materials thereby increasing porosity and weakening the strength of roofing materials.

On the basis of this study, it could be said that vegetation and soil around Sokoto Cement Company was found to be affected by cement dust, which might be due to the presence of different toxic pollutants in cement dust. The biological behavior of vegetation e.g.*A nilotica* was found to be highly affected due to the present of different heavy metals found in the twig of the plant. It is clear that the cement dust pollution is an operative ecological factor causing deterioration in the quality of our environment

(Shah *etal.* 1989). It is concluded that the presence of toxic pollutants in cement dust might be responsible for the reduction in vegetation and soil fertility. Traces of toxic metals such as chromium and copper are common in some varieties of Portland cement and are harmful to human beings and other living systems (Omar and Jasim, 1990). The heavy metals present in the cement dust can play an important role in disturbing the various metabolic processes of plant. The growth metabolic processes and yield of winter barley were found to be affected by the Duna cement and lime works (Borka, 1986), in an experiment of six kinds of dust which contained heavy metals introduced under the stand canopy. The dust was collected from zinc, cadmium, aluminum, iron plants, electric power station and the cement plant. The dust was introduced in concentrations of 100, 500, 1000, 2000 and 5000 t Km^{-2}. The experiment proved that the cement dust brought about changes in the ecosystem. A link between the forest ecosystem under the influence of heavy metals in dust and the effect of these changes on the growth of pine stands was also obtained.

The levels at which the toxic elements were found in soil sample around the factory in this study showed that they have exceeded regulatory standards, Cd (0.005ug g^{-1}); Cr (0.02ug g^{-1}); Pb (0.05ug g^{-1}) and Ni (0.5ug g^{-1}) WHO (1971). It showed that the cement plant is seriously polluting the environment and it confirmed the assertion of Bilen (2010) that cement production is of the main sources of environmental pollution. It also showed that control strategies for prevention of dust release by cement plants have not been implemented in the cement company. Fell *et al.* (2003) said that in advanced countries, control-strategies for the prevention of dust release by machinery enclosure, local exhaust ventilation, work automation and greater diligence in maintenance of machinery have been implemented, presumably leading to less dust exposure.

V. CONCLUSION

This study has shown that there is a significant emission of cement dust into the environment around Sokoto Cement Company, up to 1000m distances from the mill in the four cardinal directions. This dusts emitted are capable of posing a serious problem to the soil fertility even at a distance of 1000m away from the mill. This study has established that higher concentrations of heavy metals in soil samples (Cd, Cr, Cu, Hg, Pb, Ni and Zn) were obtained in different locations. It could be concluded that soil around Sokoto Cement Company were found to be affected by cement dust, which might be due to the presence of different toxic pollutants in cement dust. It was found that the concentrations of heavy metals were highest in eastern part followed by western then the northern part and the least was southern part of the company. It is clear that the cement dust pollution is an operative ecological factor causing deterioration in the quality of our environment (Shah *etal*., 1989).

I therefore recommend that to safeguard the quality of the soil around the company, the cement factory management should embark on regular/periodic monitoring of cement dust level in and around the factory environment, and should reduce the dust emission by the use of dust filters.

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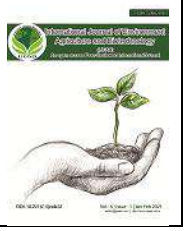

Effects of Cement dust on Vegetation around Sokoto Cement Company

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Abstract— *The heavy metal deposition around Sokoto Cement Company was investigated to the extent of pollution caused by this industry. Eighty eight samples of plants were separately collected from the four directions of the company into polythene bags and brought to the laboratory for further analyses. The sample were digested using Aqua Regia Method, and the Hg, Zn, Cd, Cr, Ni, Pb and Cu contents were determined by (AAS). Data generated were subjected to Analysis of Variance (ANOVA) to depict the differences and the means were separated with the Duncan Multiple Range Test (DMRT), where significant differences occur. It was found that the concentration of each heavy metal decreased with increase in distance from the industry. It was further found that heavy metals contributed differently to the pollution status of the environment with the eastern part impacting most. Mercury was found to be present in high concentrations in all the plant materials. Zn was the least except for the southern part where Pb was the least. Sokoto Cement campany was found to produce the wastes/ dust that pollutes the environment and acacia nilotica can be used as biomonitor.*

Keywords— ***Soil pollution, Vegetation, Heavy metals and Ecosystem.***

I. INTRODUCTION

Air pollutants responsible for vegetation injury and crop yield losses are causing increased concern in the environment (Fuji, 1973). Air pollution has become a major threat to the survival of plants in the industrial areas (Gupta and Mishra, 1994). Rapid industrialization and addition of the toxic substances to the environment are responsible for altering the ecosystem (Mudd and Kozlowski, 1975; Clayton and Clayton, 1982; Niragau and Davidson, 1986).The cement industry also plays a vital role in the imbalances of the environment and produces air pollution hazards (Stern, 1976). In comparison with gaseous air pollutants, many of which are readily recognized as being the cause of injury to various types of vegetation, relatively little is known and limited studies have been carried out on the effects of cement dust pollution on the growth of plants. Reduction in the number of flowers and yield of black gram (*Vigna mungo (L.)* Hepper) as observed by Prasad and Inamdar, (1990) were due to cement dust pollution. The effect of cement and stone dust on the stomatal clogging of *Iphoniagrantioides Boiss*, leaves were found by Abdullah & Iqbal, (1991). Lerman and Darle, (1975) observed a marked reduction in the growth of a popular tree *Carissa carandas L*. 1mile away from the cement plant. Stratmann and van Haut, (1966) dusted plants with quantities of dust ranging from 1 to 48 g/m^2 per day; dust falling on the soil caused a shift in pH to the alkaline side, which was unfavorable to oats but favorable to pasture grass. Darley *et al*., (1966) noted that plants were stunted and had few leaves in the heavily dusted portions of an alfalfa field downwind from a cement plant in California. Brandt and Rhoades, (1972) observed significant changes in the structure and composition of the seedling, shrub, sapling and tree strata

when they compared dusted and non-dusted forest communities in the vicinity of limestone quarries and processing plants.

Air pollution is a social disease, a disease generated primarily from the activities of man and adversely affecting his health and welfare (Gilette, 1984). Pollution stress can alter plant growth and quality and the effects are often extensive (Sagar *et al*., 1982). We are facing the fact that in relatively recent times, the total amount and complexity of toxic pollutants in the environment are increasing day by day. Karachi, the most industrialized and largest city in Pakistan, is suffering from heavy cement dust pollution originating from the cement industry. The cement dust is the source of particulate matter deposits on the buildings and plants. Despite these adverse effects, cement is widely used in building and construction works and a key ingredient in concrete products. It is a binding agent that holds sand and other aggregates together in a hard stone like mass.

II. MATERIALS AND METHODS

COLLECTION OF SAMPLES

Eighty eight samples of twigs *of Acacia nilotica* samples were collected in the vicinity of the factory (between the months of August-October,2011) at interval of 50m from the mill, that is at (11) different points (50 m, 100 m, 150 m, 200 m, 250 m, 300 m, 350 m, 400 m, 450 m, 500 m and 1km) from the mill, in which 1km served as Control point along each of the four cardinal points (i.e. East, West, South and North). The twigs of *A.nilotica* samples were collected and packed in clean polythene bags and taken to the laboratory for chemical analysis.

Acacia nilotica were choosen as plant sample because the species is found abundant in the study area and is quite tolerant to heat (>50^{o}C) and air dryness, It is also frost sensitive. These make the plant to withstand different period and accumulate heavy metals. Twigs of *A nilotica* were collected from different trees in each direction at 50m interval and 1km as control point by using measuring tape, hand glove and stainless steel pen-knife. The plant samples were packed in separately clean polythene bags and were brought to laboratory for analysis.

DIGESTION OF PLANT SAMPLES

Plant samples of *A. nilotica* were crushed and oven dried at 60^{o}C. One gram of each crushed sample was weighed into 125ml Erlenmeyer flask, which has been previously sterilized with acid, (Perchloric acid) and thoroughly rinsed with distilled water. The plant samples were subjected to wet digestion as follows; 4ml of perchloric acid, 25ml conc. HNO_3 and 2ml conc.H_2SO_4 were added in a fume hood into each sample contained in the flask. The contents were mixed and heated gently at medium heat on a hot plate under Perchloric acid fume hood until dense white fumes appear. The contents were heated strongly again for a minute and allowed to cool. Then 50ml distilled water was added and allowed to boil for half a minute on the same hot plate at medium heat.

The contents were allowed to cool and each solution was filtered using whatman No 42 filter paper into 100 ml Pyrex volumetric flask. (A.O.A.C., 1990). The digested samples were analyzed for the cadmium (Cd), chromium (Cr), lead (Pb), nickel (Ni), copper (Cu), zincs (Zn) and mercury (Hg) using Atomic Absorption Spectrophotometeric method (AAS).

III. METHODS

DETERMINATION OF HEAVY METALS IN PLANT SAMPLES

The digested samples were separately analyzed for Cd, Cr, Pb, Ni, Cu, Zn and Hg by Atomic absorption spectrophotometeric method. The radiation lamp for each heavy metal to be analyzed was placed in a compartment in the AAS and a tiny pipe attached to the machine (Aspirator) was put inside bottle containing the sample and the concentration of the respective heavy metal was read off by the aid of computer system attached to the machine.

DATA ANALYSES

Data generated from the quantification of heavy metals were subjected to descriptive and inferential statistics. Their concentrations were compared with respect to location/site with the use of analysis of variance (ANOVA). The means were separated with the use of Duncan multiple range test (DMRT).The data obtained from the chemical analysis were also subjected to statistical treatment using correlation coefficient matrices. This was carried out in order to establish association and probable source of dispersion or pollution

IV. RESULTS

Table 1: Concentration of Cadmium (mg/l) in plant samples around Sokoto cement Company

Distance (m)	North		South	East	West
50	7.98	7.46	8.66	8.39	
100	7.91	7.47	8.59	8.41	
150	7.72	7.01	8.61	8.28	
200	7.64	6.98	8.69	8.29	
250	7.72	6.99	8.63	8.21	
300	7.72	6.86	8.58	8.26	
350	7.64	5.01	8.56	8.18	
400	7.69	4.91	8.58	8.14	
450	7.41	4.88	8.41	8.16	
500	7.51	4.89	8.39	7.92	
1000	0.07	0.06	0.07	0.08	

Table 2: Concentration of Chromium (mg/l) in plant samples around Sokoto Cement Company

Distance (m)	North	South	East	West
50	3.86	2.21	5.82	5.02
100	3.82	2.08	5.79	5.01
150	4.01	1.92	5.83	4.82
200	3.46	1.71	5.88	4.51
250	3.01	1.68	5.46	4.49
300	3.21	1.56	5.48	4.48
350	2.98	0.92	5.56	4.41
400	2.86	1.02	5.32	4.01
450	2.48	1.21	4.98	4.09
500	2.29	0.99	4.66	4.28
1000	0.08	0.07	0.08	0.07

Table 3: Concentration of Copper (mg/l) in plant samples around Sokoto Cement Company

Distance (m)	North	South	East	West
50	1.08	0.71	3.08	1.72
100	1.10	0.88	2.06	1.84
150	1.06	0.62	2.18	1.48
200	1.09	0.61	2.68	1.26
250	1.09	0.64	2.32	1.31
300	1.02	0.59	2.01	1.46
350	1.04	0.51	2.11	1.21
400	1.02	0.41	2.22	1.19

450	0.78	0.41	1.89	1.21
500	0.68	0.32	1.68	1.11
1000	0.07	0.04	0.07	0.08

Table 4: Concentration of Mercury (mg/l) in plant samples around Sokoto Cement Company

Distance (m)	North	South	East	West
50	11.12	10.81	12.33	11.68
100	11.22	10.62	12.66	11.70
150	11.18	10.46	12.61	11.70
200	11.14	10.4	12.69	11.42
250	11.02	9.86	12.21	11.48
300	11.08	9.48	12.19	11.51
350	11.08	7.21	12.16	11.31
400	10.92	6.98	12.04	11.29
450	10.64	6.92	11.89	11.28
500	10.78	7.01	11.66	11.32
1000	2.21	2.10	2.35	2.24

Table 5: Concentration of Nickel (mg/l) in plant samples around Sokoto Cement Company

Distance (m)	North	South	East	West
50	2.38	1.99	10.62	7.01
100	2.39	2.09	10.01	6.21
150	2.41	1.99	9.86	5.89
200	2.21	2.09	9.66	5.86
250	2.26	2.01	9.01	4.92
300	2.38	2.08	8.08	2.64
350	2.18	1.97	8.19	3.08
400	2.09	1.98	8.16	2.98
450	2.01	1.92	7.18	2.89
500	2.16	1.88	7.16	2.48
1000	0.08	0.09	0.07	0.09

Table 6: Concentration of Lead (mg/l) in plant samples around Sokoto Cement Company

Distance (m)	North	South	East	West
50	2.61	2.07	3.08	3.02
100	2.62	2.09	3.08	2.98
150	2.62	1.88	3.11	2.99
200	2.51	1.89	3.09	2.92
250	2.53	1.86	3.06	2.91

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300	2.41	1.89	3.05	2.87
350	2.39	1.34	3.09	2.85
400	2.38	1.38	3.04	2.72
450	2.41	1.36	3.01	2.62
500	2.06	1.33	2.02	2.66
1000	0.08	0.07	0.04	0.06

Table 7: Concentration of Zinc (mg/l) in plant samples around Sokoto Cement Company

Distance (m)	North	South	East	West
50	0.53	0.42	1.31	1.04
100	0.48	0.36	1.31	1.06
150	0.56	0.31	1.29	0.98
200	0.54	0.29	1.24	0.99
250	0.43	0.26	1.16	1.01
300	0.39	0.22	1.19	0.92
350	0.39	0.17	1.18	0.89
400	0.41	0.16	1.09	0.99
450	0.29	0.19	1.02	0.88
500	0.32	0.18	1.08	0.86
1000	0.06	0.06	0.06	0.06

V. DISCUSSION

The analysis shows a considerable abundance of the toxic metals on the vegetation around Sokoto Cement Company and its immediate environments. It was discovered that the concentrations of all the metals are high in plant samples at various directions. The high concentration of the metals could be probably due to limited number of vegetation around the factory. The higher heavy metal concentrations observed in this study may be attributed to deposition of calcium oxide from Cement Company and calcium compounds in the soils of Sokoto. Brady and Weil, (1999) reported that the neutral to alkaline soil pH observed in semi-arid soil such as that of Sokoto was due to low rainfall and alkaline compounds are not leached away, thus making soils of the region too alkaline. This is in support of the result of this study because the concentrations of cadmium (Cd), chromium (Cr), lead (Pb), nickel (Ni), copper (Cu), zinc (Zn) and mercury (Hg) in plant samples is high.

Gbadebo and Bankole, (2007) analysed potentially toxic metals in airborne cement dust around Sagamu, Southwestern Nigeria. The heavy metals investigated are As, Al, Ca, Cd, Pb, Co, and Zn. They reported elevated concentrations of these heavy metals and inducted them in the health problems among the inhabitants of the communities. Their observed confirmed favorably with the finding of this study. The result obtained in this study inducted potential environmental problems such as destruction of vegetation cover and environmental degradation (Topie, 1999; Hegazy 1996; Raguotis, 1997). For example, Topie, (1999) indicated that the cement industry was the main cause of destruction of vegetation cover and of environmental degradation in Solin and Kastela near Spilt, Croatia. Damages to coniferous trees by cement dust have been reported by Mandre and Tuulmets, (1997).

Cement dust had a significant effect on the growth of some plant species compared with non-cement dusted plants. Toxic compounds such as fluoride, magnesium, lead, zinc, copper, beryllium, sulfuric acid and hydrochloric acid were found to be emitted by cement manufacturing plants (Andrej, 1987).This support the result of this study which showed that cadmium, chromium, copper, mercury, nickel, lead and zinc are available in soil around Sokoto Cement Company. Reduction in plant height, cover and number of leaves of *C. carandas* showed that the losses can generally

be attributed to the cement dust which contained toxic metals. The results obtained are in close conformity with those reported by Stratmann and Van Haut, (1966) who dusted plants with dust ranging from 1 to 48 g/m^2 day^{-1} and concluded that dust falling on the soil caused a shift in pH to the alkaline side. The findings of Prasad and Inamdar, (1990) in *Vignamungo* (Black gram) also confirmed the finding of this study. The cement dust kiln showed a reduction in chlorophyll content, protein, starch, yield and phytomass in ground nuts (*Arachis hypogaea L.*) (Prasad & Inamdar, (1990). Liu *et al*., (1997) reported that cement dust decreased the yield of grape and rice in the vicinity of a cement factory in China. It has also been indicted in the reduction of crop yield.

Plant response varies between species of a given genus and between varieties within a given species. Plants do not necessarily show similar susceptibility to different pollutants. Major variations in response to different species to air pollutants have been documented by Jacobson and Hill, (1970). Studies of biochemical changes and pollution effects on the plant metabolism, i.e., reduction in chlorophyll and completely clogged stomates (Ahmed and Qadir, 1975) revealed that these parameters are important in regulating the productivity and also the number of flowers and seeds produced.

Cadmium, chromium, copper, mercury, nickel, lead and zinc that are prominent in cement dust were found to be higher in concentration in the samples analyzed. This indicates the extent to which the soil and vegetation were polluted by the cement dust from the Sokoto cement company. The concentrations of heavy metals are higher in eastern and western part followed by north and least was southern part of the company, the higher concentrations of heavy metals in eastern and western parts was attributed due to the loading of Cement and heavy trucks movement in the east and presence of quarry and production activities in western part of the Company. Ade-Ademilua and Umebese (2007) have pointed out that metal to metal association could cause an increase in concentration of other metal (absent in cement dust) in the polluted soil over time. Forstner (1995) also pointed out that 70% of metals contaminated soils involved two or more metals and the possibility of synergistic effects may be of considerable importance at some heavy metals contaminated sites.

The significant reductions in shoot length and total leaf area observed in the polluted plants have also been reported in the works of Singh and Rao (1981); Ayanbamiji (1996) and Igbal and shafoeg (1998). The reduction may be due to toxicity of heavy metals which Rout *etal*., (2000) stated that once inside the cells, the aluminium could inhibit root absorption and growth in both aluminium sensitive and tolerant plants. The significant decrease in vegetative growth of polluted plants may be an indication of a reduction in photosynthesis of polluted plants which could be explained on the bases of quantitative as well as qualitative changes in the incident light available for photosynthesis in cement encrusted leaves (Bohne,1963), interruption in gaseous through stomatal clogging (Darley *etal*.,1966;Lerman, 1972), reduction in transpiration in terms of the absorption of minerals from soil and inhibition of intracellular processes (Singh and Rao,1981).

The cement dust caused the epidermal cells and stomata of the polluted leaves to become modified .The higher stomata frequency and index in the leaves of the polluted plants indicate morphological modification on the leaves. According to Ayanbamiji (1996), these may be adaptations for gaseous exchange and transpiration since the surfaces are covered by cement dust.

Studies of the effects of cement-kiln dust deposited on the soils also raised questions. Some investigators reported no harmful effects of cement at levels from 1.5 to 7.5 g/m^2/day, while others reported that concentrations from 1.0 to 48 g/m^2/day caused shifts in the soil alkalinity which may be favorable to one crop but harmful to others (Lerman and Darley, 1975) thus confirming the result of this study whose reported higher concentrations of heavy metal in soil samples.On the analytical assessment of some trace metals in soils around the major industrial areas of Northwestern Nigeria and Sokoto Cement Company reported higher mean concentrations of heavy metals: Cd (0.10 ug/g); Cr(83.4 ug/g); Pb(486 ug/g) and Ni(22.9 ug/g). This study also showed higher concentration of heavy metals investigated, the mean values are Cd(16.9mg/l), Cr(15.2mg/l), Pb(8.30mg/l) and Ni(9.03mg/l) which are within the range reported by Abubakar *etal*., (2006); Birnin Yauri and Argungu (2002).

Adejumo *et al*., (1994) showed that some toxic heavy metals like arsenic (AS), lead (Pb,) nickel (Ni), cobalt (Co), zinc (Zn), copper (Cu) and phosphorus (P) were significantly enriched in the neighbourhood of cement factories in Nigeria. This can lead to reduction of soil fertility, Adejumo *et al*., (1994), it has also been linked to the damage of dwellings (Perfettini *et al*., 1989. Deposit of cement dust on roofs can cause microbiological and chemical disintegration of the roofs. Wasserbauer *etal*., (1998) indicated that metabolic process of nitrifying bacteria (mainly *Nitrosomonas* and *Nitrobacter*) on cement dust which settle on roofs can cause chemical corrosion of the roofing materials thereby increasing porosity and weakening the strength of roofing materials.

On the basis of this study, it could be said that vegetation around Sokoto Cement Company was found to be affected by cement dust, which might be due to the presence of different toxic pollutants in cement dust. The biological behavior of vegetation e.g *A nilotica* was found to be highly affected due to the present of different heavy metals found in the twig of the plant. It is clear that the cement dust pollution is an operative ecological factor causing deterioration in the quality of our environment (Shah *etal.*, 1989). It is concluded that the presence of toxic pollutants in cement dust might be responsible for the reduction in vegetation and soil fertility. Traces of toxic metals such as chromium and copper are common in some varieties of Portland cement and are harmful to human beings and other living systems (Omar and Jasim, 1990). The heavy metals present in the cement dust can play an important role in disturbing the various metabolic processes of plant. The growth metabolic processes and yield of winter barley were found to be affected by the Duna cement and lime works (Borka, 1986), in an experiment of six kinds of dust which contained heavy metals introduced under the stand canopy. The dust was collected from zinc, cadmium, aluminum, iron plants, electric power station and the cement plant. The dust was introduced in concentrations of 100, 500, 1000, 2000 and 5000 t Km^{-2}. The experiment proved that the cement dust brought about changes in the ecosystem. A link between the forest ecosystem under the influence of heavy metals in dust and the effect of these changes on the growth of pine stands was also obtained.

The levels at which the toxic elements were found in both plants and soil sample around the factory in this study showed that they have exceeded regulatory standards, Cd (0.005ug g^{-1}); Cr (0.02ug g^{-1}); Pb (0.05ug g^{-1}) and Ni (0.5ug g^{-1}) Fell *et al.*, (2003). It showed that the cement plant is seriously polluting the environment and it confirmed the assertion of Bilen (2010) that cement production is of the main sources of environmental pollution. It also showed that control strategies for prevention of dust release by cement plants have not been implemented in the cement company. Fell *et al.*, (2003) said that in advanced countries, control-strategies for the prevention of dust release by machinery enclosure, local exhaust ventilation, work automation and greater diligence in maintenance of machinery have been implemented, presumably leading to less dust exposure.

VI. CONCLUSION

This research has shown that there is a significant emission of cement dust into the environment around Sokoto Cement Company, up to 1000m distances from the mill in the four cardinal directions. This dusts emitted are capable of posing a serious problem to vegetation and soil fertility even at a distance of 1000m away from the mill. This study has established that higher concentrations of heavy metals both in plant and soil samples (Cd, Cr, Cu, Hg, Pb, Ni and Zn) were obtained in different locations. It could be concluded that vegetation and soil around Sokoto Cement Company were found to be affected by cement dust, which might be due to the presence of different toxic pollutants in cement dust. The biological behavior of vegetation (*A nilotica)* was found to be highly affected due to the presence of different heavy metals found in the twigs of the plant. It was found that the concentrations of heavy metals were highest in eastern part followed by western then the northern part and the least was southern part of the company. It is clear that the cement dust pollution is an operative ecological factor causing deterioration in the quality of our environment (Shah *etal*., 1989). I therefore recommend that to safeguard the quality of vegetation around the company, the cement factory management should embark on regular/periodic monitoring of cement dust level in and around the factory environment, and should reduce the dust emission by the use of dust filters.

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Junkshop Industry as Waste Recycling Business: A Green Response towards Economic Sustainability and Social Responsibility

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Abstract**—** *This study was conducted to assess the junkshop industry in Cabanatuan City, Philippines. It aimed to determine the profile of junkshops, the materials they trade, and their compliance with legal requirements. Using descriptive qualitative research design with 20 randomly chosen participating junkshops as respondents, the researcher found out that junk shop operations are lucrative and can help support economic development as well as solve certain environmental issues. However, many of them were operating without registering with the concerned authorities. Aggressive involvement of the LGUs, by organizing junkshop operators, and close monitoring of junkshop operations are few from among the recommendations of the study.*

Keywords**— Junkshops operation, a requirement for junkshop operation, environmental benefits.**

I. INTRODUCTION

Junkshop business is a simple but lucrative business that many would not want to enter in the Philippines due to its "scrappy" nature as it deals with rejects and unclean or soiled materials that are usually smelly (Business Diary PH, 2018).

The junkshop business is very relevant these days when the campaign for environmental awareness, proper waste disposal, and recycling is most aggressive due to garbage's presumed contribution to the pollution that usually results in flooding in most areas especially prevalent in the Philippines.

According to Fridman (2016), the scrap industry does not get a lot of attention. According to him, although the industry is not glamorous, it can earn multi-million dollars and contribute to the recycling industry in a way. He implied that this far-from-being glamorous industry can earn a million dollars while at the same time contributes to the world's recycling industry.

Anlacan (2012) enumerated that scrap trading business requires the following to succeed: sufficient capital of at least two hundred thousand pesos (4,000 U$) for purchasing scarps and for space rentals; knowledge of and focus on the kind of scraps to trade like metal, paper, glass, and plastics; a suitable location on where to buy and store scraps; business registration and permits from the barangay or municipal hall, and Bureau of Internal Revenue registration for receipts; list `of good buyers to ensure the best offer for your scarps; good working relationship with at least two buyers for better leverage; knowledge of the going, buying and selling prices of scraps; proper equipment like weighing scale and delivery vehicle; and thorough knowledge of the business operation.

To start the business, the Filipino Entrepreneur (2010) stated that it would be best to have: potential clients; a walk-

in procedure to avoid pilferage; and daily accounting to determine what scrap needs to be maintained or replenished day by day. Like Anlacan, the Filipino Entrepreneur also believes that thorough knowledge about the trading process of scrap is necessary.

Junkshop owners and operators probably did not know that their business help in the reduction of trash builds–up in the community. It is a win-win solution that probably for both the owners and the scavengers. The scavengers "explore" their trash mounds, trash bags, and trash bins for anything recyclables they can sell to the junkshops where they get money for it. The junkshop operators who buy scraps provide an opportunity for the living of these scavengers, and the community without being mindful of it gets freed from more serious trash problem.

It is assumed that if only the number of those who indulge in the junkshop business would increase, dumped junks will not clog some areas, and thus, flooding would be prevented somehow.

This study presumes that if only many would indulge in the junkshop business, the more they could earn money while providing a source of livelihood to the scavengers as they help clean the community at the same time. However, since the job is dirty, only a few engage in it. Probably, if many would see and read about the potential of the business, many would go into it; hence this study.

II. STATEMENT OF THE PROBLEM

The study focused on scrap business in Cabanatuan City, Philippines as a green response to economic stability and social responsibility.

It specifically aimed to determine the following:

1. The profile of junkshops in Cabanatuan City in terms of :
   - 1.1 building structure;
   - 1.2 lot area;
   - 1.3 capital investment
2. The scrap materials in the junkshop in terms of:
   - 2.1 specifications; and
   - 2.2 buying and selling price
3. The operations of the junk shops in terms of registration with the authorized government agencies.

III. METHODOLOGY

This study used a descriptive survey design using a total enumeration of 20 junkshop owners as respondents. A descriptive survey design was employed because the study surveyed to describe the junkshop business as it existed at the time of the study. Total enumeration was used because there were only a limited number of junkshop operators in the city. Meanwhile; the primary tool used to gather data was a survey questionnaire triangulated by personal interview using an interview guide. For data analysis, descriptive statistics such as frequency, mean, and percentage were used.

IV. RESULTS AND DISCUSSION

The following were the findings of the study:

1. Profile of Junkshops in Cabanatuan City

Table 1 would show the detailed findings of the profile of the junkshops.

1.1 Building structure

It can be seen from Table 1 that in terms of building structure, wherein the junkshops surveyed were classified as to concrete or permanent and light or temporary, five (5) or 25 % were housed in concrete structures while 15 or 75 percent were housed in structures made of light materials.

It can be recalled that in 1978, the Philippine government had set an occupational safety standard in compliance with the constitutional mandate to safeguard the workers' social and economic wellbeing as well as their physical safety and health. According to OSH Standards (1989), this is considered a landmark in the Philippine labor and social legislation. Apparently, since most of the junkshops were made of light materials, it could be deduced that the junkshop industry in Cabanatuan City needs upgrading to ensure that they would not pose a threat to the safety of the workers and its adjoining structures.

Table 1. Profile of the Junkshops in Cabanatuan City

1.1 Building structure materials	*f*	*%*
Light	15	75
Concrete	5	25
	20	100%
1.2 Lot area		
< 1,000 sq.m.	13	65
1,001 - 2,000 sq.m.	5	25

2,001 - 3,000 sq.m.	2	10
	20	100%
1.3 Capital investment		
< Php 100,000	12	60
100,001 - 200,000 (2,000 – 4,000 U$)	4	20
200,001 - 300,000	2	10
300,001 - 400,000	2	10
> Php 400,001	0	-
	20	100%

1.2 Lot Area of the Junkshops

Table 1 further shows that the majority of the junkshops; comprising 13 or 65 % occupy a lot area of 1000 sq. m. and below. The second majority comprising five (5) or 25 % occupies an area that ranges from 1,021sqm to 2,020 sq. m. The rest, comprising only two (2) or 10% occupies a lot area between 2001 to 3000 sq. m.

The above figures imply that majority of the junkshops are not complying with the 2,000 sq. m. minimum lot area required for junkshops. This would mean that with the current situation of the junkshops, there would be a tendency for lesser movement between files of scraps and a tendency as well that in just a slight error an avalanche might happen and the workers may get buried alive beneath the scraps.

Another implication is that the situation would require a faster turnover of scrap materials from sellers to buyers so that there would be a larger space left inside the shop. This would decrease the chances of accidents but may require frequent transportation that in turn would also require an increased transportation budget.

1.3 Capital Investment

Evidently, the scrap business in Cabanatuan City is still in its infant stage considering that the capital investment of the majority, 12 or 60 % was only 100,000 pesos. Fewer, comprising four or 20%, were able to put up the minimum capital requirement of at least 200, 000 pesos. Only the remaining four (4) or 20 % were able to put capital investment above the minimum requirement.

The above figures suggest that most of the junkshop operators in the City were small players. Interviews revealed that most of those who are engaged in the business do not have the financial capacity to invest enough to operate in large scale transaction. This may also bear effect on the environment because it follows that when the capital to accommodate more scraps is limited, more scraps will tend to remain in the dumping sites and landfill around the communities, posing as eyesores and threats to the environment.

2. The scrap materials in the junkshop

2.1 Specifications

The study revealed as can be viewed from Table 2 that the scrap materials bought by the junkshops are categorized as plastics, glass, paper, and batteries.

The study also revealed that not all the 20 junkshops buy all the specified plastics namely HDPE (high-density polyethylene), PET (polyethylene terephthalate), LDPE (low-density polyethylene), PP (polypropylene), and HIPS (high impact polyethylene). HDPE includes soap holders, food trays and crates, motor oil containers, and the likes except for cosmetic bottles. PETS include bottles of mineral water, cosmetics, and soft drinks. LDPE includes clear plastic bags, crop protection sheets, ice cream container lids, and computer covers. PPs include wastebaskets, water basins, egg trays, bottle caps, and tube caps. HIPS include disposable drinking cups and disposable spoons, forks, and knives.

Table 2. Specifications of scraps accommodated in junkshops

Specifications	*n*	*%*	Buying Price/kg (Note: 1U$ = Php 50)
Plastics			
HDPE	18	90	Php 5.00 – 16.00 (10 – 32 cents U$)
PETS	12	60	
LDPE	4	20	
PP	9	45	
HIPS	8	40	
Glass			
Whole glass	20	100	Php 0.50 – 150.00 (0.01 – 3.00 U$)
Broken glass	20	100	-
Colored			Php 0.10 – 0.20
White			Php 0.50 – 1.00
Metals			
Aluminum	20	100	Php 30.00 – 45.00 (0.60 – 0.90 U$)
Scrap metals	20	100	Php 1.00 – 4.50
Copper	20	100	Php 70.00
Alloys	20	100	Php 30.00 – 45.00
Brass fender	20	100	-
Washers	20	100	-
GI Sheets	20	100	Php 0.25 – 1.00
Zinc	20	100	Php 8.00 – 15.00
Paper			
Assorted	20	100	Php 8.00 (16 cents U$)
Newspaper	20	100	Php 15.00
Cardboard	20	100	-
Magazine	20	100	Php 8.00
Office paper (white)	20	100	Php 11.00
Batteries			
1SNF	20		Php 50.00 – 70.00
1SMF	20		Php 150.00 - 400.00 (3.00 – 8.00 U$)
2SM	20		
3SM	20		
6SM	20		
2D	20		
4D	20		
8D	20		

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With the result, it could be said that if only all junkshops would buy all sorts of recyclable plastics as mentioned above, there would be no way plastics would litter the environment. Sadly, however, it was found out that not all junkshops in Cabanatuan City buy them. As can be gleaned in Table 2, only HDPE and PETS seemed to be most acceptable to buyers.

The glass bought by junkshop operators is classified as whole or broken. The broken ones are further classified as colored or white. Fortunately, all junkshops in Cabanatuan city buy all sorts of these.

Metals are also classified according to their rarity and it is very fortunate that these metals were not likely to be trashed somewhere as all can be bought by the junkshop and be recycled.

All kinds of papers, classified according to color and thickness, are also bought in the junkshops. In terms of batteries, however, it could be noted that only batteries of vehicles are accepted in junkshops.

With the above findings that show junkshop owners are buying almost all sorts of recyclable scraps, there is no doubt that they help in keeping and preserving the environment one way or the other.

2.2 Junkshop operators' selling price

It can also be viewed from Table 2 that the scraps bought by the junkshops get to be sold to bigger scrap traders, sometimes 100 percent higher than the cost they bought them. That is why, it can really be said that while these owners help clean the environment, they profit from the junks at the same time. Indeed, a junk shop operation can be a profitable business.

3. The operation of the junkshops in terms of government requirements

While there is a law requiring any business to get permission to operate from the local government unit (LGU), it is evident in Table 3 that the junkshop business in Cabanatuan City did not seem to be bothered by this requirement as only nine (9) of them are registered.

Business Registration with the LGU	n	%
With	9	45
Without	11	55
Total	20	100%

Further analysis revealed that 45 percent of registered shops were the larger ones; the smaller ones were not registered at all and operating without giving back in the form of fees to the LGU. When interviewed about why they did not register their business, the operators said that it was difficult to secure permits because of the building requirements. But they just went on with their business albeit in the absence of a permit, and ironically their attention was not even called by authorities for operating even without the necessary permit.

The result implies that while the junk shop business may be lucrative, only the operators and the sellers seem to profit from it. The government did not receive anything in return from them in the form of tax. This finding means that since these shops operate without LGU permission, they consequently do not pay dues; so while they earn, and somehow clean the surroundings of trash, they however do not contribute to the government coffer.

The above-mentioned findings also imply that these shops may pose a threat to the safety and well- being of the shop workers because they may be subject to danger of threats and abuses without the authorities knowing it. All these because they did not seek the permission of authorities who are supposed to ensure that they operate based on standards. There is also an indication of laxity among concerned authorities.

V. SUMMARY, CONCLUSIONS, AND RECOMMENDATIONS

Summary of findings

1. The study revealed that the majority of the junkshops in Cabanatuan City are housed in structures made of light materials, erected in a 20 to 1000-sq. m. areas of lot, and operate with only Php100,000 investment capital.

2. The junkshops buy almost all types of plastics, whole or broken glasses, metals, papers, and vehicle batteries. They buy junks by the kilogram, with paper as the cheapest (price ranging from 50 cents to Php15 per kg.) and with batteries and certain types of metal as most expensive (price ranging from 50 to 70 Php per kg) They sell the scarps they bought at 100 % over the price they bought them.

3. Only 45 % of junkshops in Cabanatuan City were reregistered. The rest are operating informally or without permits from the local government unit and other government agencies.

Conclusions

Based on the findings of the study, the following were thus concluded:

1. Junkshops operation in Cabanatuan City needs monitoring and control to ensure the safety of the owners, the workers, and the community they belong to.
2. Junkshop business is a lucrative endeavor that also helps in clearing the environment, preventing floods, and in preserving natural resources.
3. The communities are deprived of benefitting from taxes that the informal operators of junkshops failed to give to the LGUs by not registering.

Recommendations

Based on the findings and conclusions of the study the following are thus recommended:

1. The LGUs and other concerned authorities should pay more attention to the junkshop operations in the City through the following:
   a. Close monitoring to ensure that these junkshops meet the standard requirements in terms of building structure construction, prescribed lot area, and capital investment for proper taxation. This would ensure the safety of everyone involved in the business. This will further add dignity to the trade.
   b. Organizing the junkshop operators in Cabanatuan City that each member may police one another, and further ensure fair trade practice.

2. Because junkshop operation is a lucrative business that helps in maintaining cleanliness, preventing flood, and preserving natural resources, the following are suggested.
   a. Junkshop operation should be encouraged to practice corporate social responsibility (Mina, et.al., 2019).
   b. Junkshop owners should upgrade their strategies by offering on-call home cleaning services.
   c. Massive information campaigns should be conducted to encourage the communities to segregate waste and to call the services of junkshop owners in systematically disposing of their junk.
3. The LGU's should devise ways and means to ensure that junkshops in the city are operating legally and paying their tax dues regularly.

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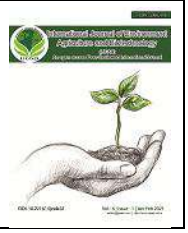

Water Quality in the Super-intensive Shrimp Ponds in Bac Lieu Province, Vietnam

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Abstract**—** *The study was conducted to assess the water quality in super-intensive shrimp ponds in Bac Lieu using water quality parameters such as pH, biochemical oxygen demand (BOD_5), and chemical oxygen demand (COD), total suspended solids (TSS), total nitrogen (TN), total phosphorus (TP), ammonium (NH_4^+-N), residual chlorine, total oil and grease and total coliform. Sixty water samples in super-intensive shrimp ponds were collected in 2019 Bac Lieu province. The results showed that the nutrients in the water samples include TN (0.54-36.12 mg/l), NH_4^+-N (0.016-1,246 mg/l) and TP (0.34-6.12 mg/l) did not exceed the limits of QCVN 40: 2011/BTNMT (column B). Residual chlorine (less than 0.5 mg/l), total oil and grease (<0.2 mg/l) and water pH (5.5-9.0) have not affected the water environment and the growth of shrimp. In water samples there were biodegradable organic substances (BOD_5/COD = 0.53> 0.4), BOD (4.43-55.19 mg/l) and TSS (6.1-320 mg/l) exceeding the limits of QCVN 40: 2011/BTNMT (column B) at some locations, while COD (10,21-90,12 mg/l) was below limit of QCVN 40: 2011/BTNMT (column B). Water in shrimp ponds is contaminated with microorganisms when the total coliform density ranged from 1,300 to 95,000 MPN/100ml and there were 40/60 locations having coliform density greater than 5,000 MPN/100ml. The current findings provide important information for shrimp pond management.*

Keywords**— Bac Lieu, nutrients, organic matters, super-intensive shrimp farming, microbial pollution.**

I. INTRODUCTION

As a coastal province in the Mekong Delta with an area of over 40,000 squared kilometers, Bac Lieu province has rich and diverse resources to develop marine economy, agriculture - fishery, trade, services and tourism. The strength of the province is agriculture - fishery, especially fisheries development (the key economic sector of Bac Lieu province). In recent years, the fisheries sector has had a strong development (accounting for 58% of the product structure of agriculture, nearly 21% of the economic structure of the province), especially shrimp production **(Current report on environmental status of Bac Lieu province in the period 2016 - 2020 and 2020)**. Many effective production models have been replicated such as intensive shrimp farming, semi-intensive, extensive and improved extensive farming, in which high-tech super-intensive shrimp farming has contributed to increase productivity and production of farmed shrimp. Total aquaculture production in 2016 was 304,400 tons, increasing to 365,000 tons in 2019. In the first 6 months of 2020, aquaculture production reached 103,680 tons, of which shrimp reached 49,349 tons, fish and other seafood was 54,331 tons. In 2019, the province has over 140,510 hectares of land for aquaculture, of which 1,001 hectares for super-intensive white shrimp farming **(Department of Agriculture and Rural Development of Bac Lieu province, 2020)**.

The main types of shrimp farming in Bac Lieu province are mainly industrial and semi-industrial shrimp farming, typically high-tech super-intensive shrimp farming in nethouses. Compared to traditional farming methods, this model increases shrimp production from 10 to 15 times, bringing the average yield to 25-70 tons/ha of water surface/crop **(Current report on environmental**

status of Bac Lieu province in the period 2016 - 2020 and 2020). However, the super-intensive shrimp farming methods also cause significant impacts and pressures on the environment if the wastewater generated from the ponds is not well managed. Wastewater in super-intensive shrimp ponds contains many pathogenic microorganisms such as Vibrio, Aeromonas, E.coli, Pseudomonas, Proteus, Staphylococcus, ... and many fungi and protozoa if not treated completely, then discharge directly into the receiving water source causing water pollution, epidemic spread and crop failure. In addition, at the end of the crop, a large amount of sludge in the pond, about 1,800,000 tons/crop without treatment, was discharged **(Department of Natural Resources and Environment of Bac Lieu Province, 2018)**. This bottom sludge contains a large amount of pollutants, excess food, animal excretion products, bleaching by rainwater polluting the natural water environment or the water in shrimp ponds.

Currently, there are a number of studies conducted to assess water quality in the areas of intensive shrimp farming, improved extensive farming or wastewater treatment methods of these ponds **(Phuong et al., 2007; Menezes et al., 2017; Giang and Quyen, 2018)**. However, the effluent water quality of super-intensive shrimp ponds in Bac Lieu has not been paid much attention. Therefore, this study was conducted to evaluate characteristics of the water environment in the super-intensive shrimp pond in Bac Lieu. The results could provide useful information on water quality so the effective measures to manage and treat could be proposed for sustainable development of super-intensive shrimp farming.

II. MATERIALS AND METHODS

2.1 Water sampling and analysis

Wastewater samples were collected at the same time in 2019 in 60 super-intensive shrimp ponds in key shrimp farming areas in Bac Lieu city, Gia Rai town, Dong Hai district and Hoa Binh province of Bac Lieu province **(Figure 1)**. Wastewater from 60 shrimp ponds was collected and denoted from VT1 to VT60. Wastewater samples, after being transported to the laboratory, were analyzed for pH (measured in the field), BOD_5, COD, TSS, total nitrogen, total phosphorus, ammonium, residual chlorine, total oil and grease, and total coliforms. Wastewater samples were collected and preserved according to TCVN 6663-1: 2011, TCVN 6663-3: 2016, TCVN 5999: 1995 and analyzed by specialized methods based on **APHA (1998)**.

Fig.1: The sampling areas in Bac Lieu province

2.2 Data processing

The water quality data were compared among the sampling locations and were also compared with QCVN 40: 2011/BTNMT-National technical regulation on industrial wastewater to assess water quality in farming areas.

III. RESULTS AND DISCUSSION

3.1 pH

Unfavorable pH values in water will disturb the ion regulation in shrimp **(Morgan and McMahon, 1982; Allan and Maguire, 1992)**. In the wastewater from the surveyed super-intensive shrimp ponds, the pH value fluctuated with a small amplitude of between 7.03 - 8.73 and an average of 7.7. At the positions VT59 and VT16 had the highest and lowest pH respectively (**Figure 2**). The pH value of the water was relatively similar to the pH value (7.4 to 7.8) in intensive shrimp ponds in coastal Quang Tri province **(Giang and Quyen, 2018)** and improved extensive shrimp ponds in Bac Lieu **(Anh et al, 2017)** with pH ranges between 7.2 - 8.1 (morning) and 8.3 - 8.7 (afternoon). However, in the effluent of intensive shrimp ponds in Brazil **(Menezes et al., 2017)** the mean pH value was relatively higher than that in the super-intensive shrimp farming area in this study (8.23 > 7.7).

The pH range in water in the survey ponds was from neutral to alkaline in nature and was within the allowable limits of QCVN 40: 2011/BTNMT, column B (5,5-9). In general, the pH values in these areas were suitable for the growth of aquatic animals, especially shrimp growth **(Boyd, 1998; Hai and Phuong, 2009)**.

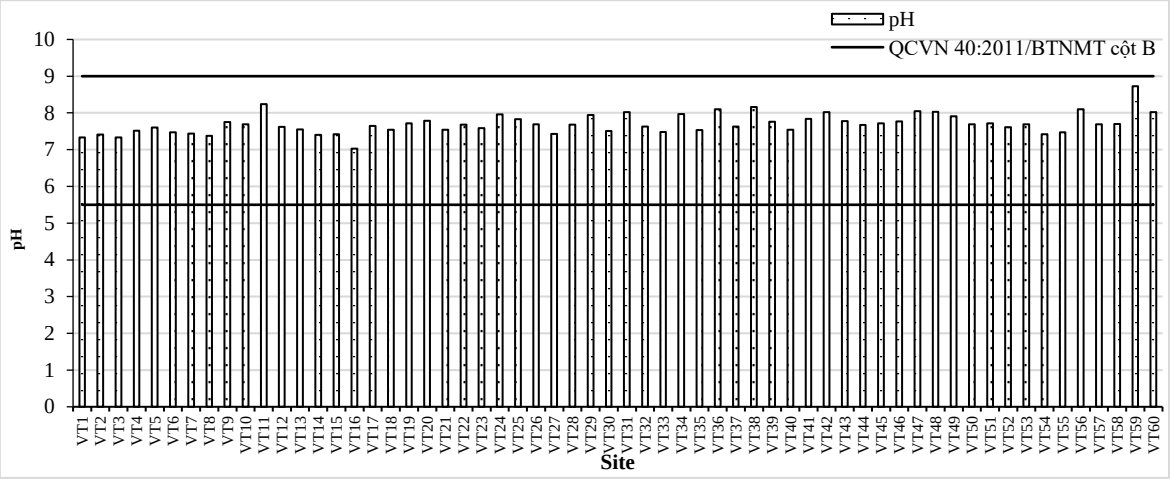

Fig.2: pH in the super-intensive shrimp ponds

3.2 Biochemical oxygen demand (BOD_5)

The biological oxygen demand is one of the parameters used to evaluate the pollution level of water bodies, reflecting the fast or slow rate of oxygen absorption in water of microorganisms **(Boyd, 1998)**. The average BOD at survey locations was 19.68 mg/L, ranging from 4.43 to 55.19 mg/l, the highest at VT10 and lowest in VT46. At most locations (except VT10 and VT17), BOD was below 50 mg/l and within the limit of QCVN 40: 2011/BTNMT, column B (**Figure 3**). According to **Boyd (1998)**, typical aquaculture ponds have BOD values of 5-20 mg/L and the higher BOD, the higher the level of organic matters. Water has a lot of organic matters due to accumulation of humus, excessive manure, excess food or the presence of a large number of algae. BOD in wastewater was relatively lower than the study of **Menezes et al. (2017)** in shrimp ponds in Brazil (19.68 <62.5 mg/l) but it was still consistent with the study of **Giang and Quyen (2018)** in shrimp ponds of coastal areas Quang Tri province with BOD ranging from 35 - 41.22 mg/l.

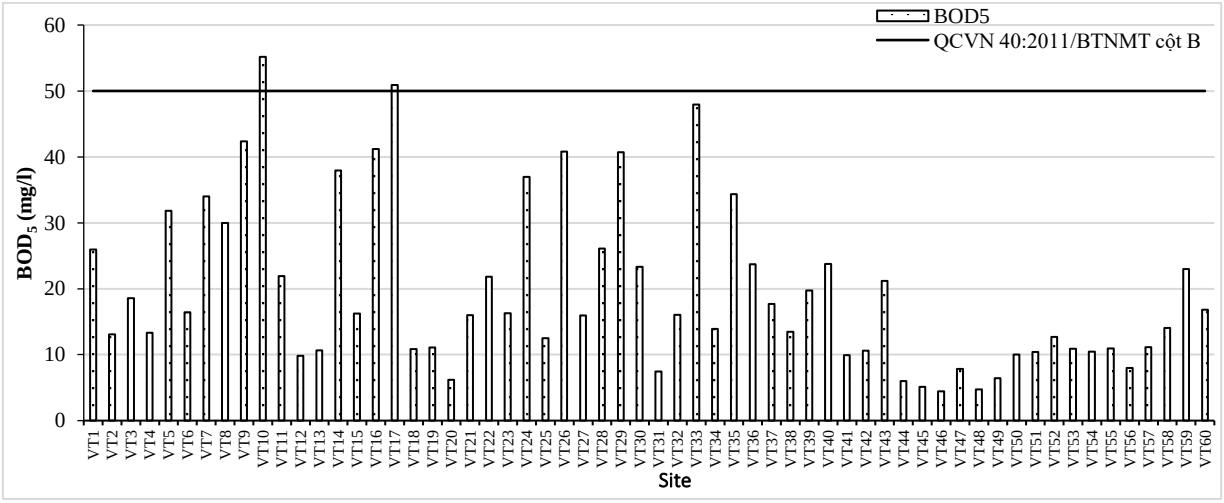

Fig.3: BOD_5 in the super-intensive shrimp ponds

The ratio of BOD/COD at the survey locations ranged from 0.35 to 0.66 mg/l and the average was 0.53 (**Figure 4**). Thus, wastewater in super-intensive shrimp ponds in Bac Lieu contains a lot of organic substances that are easily biodegradable when at most locations had BOD/COD ratios greater than 0.4 mg/l (except VT25, VT46 and VT49). However, this rate may change depending on the nature of the pollution source **(Thuong and Bach, 1999)**. The effects of the discharge of wastes from untreated shrimp ponds as well as the effect of

sedimentation from the sea contribute to the increase in the concentration of organic matters in the water.

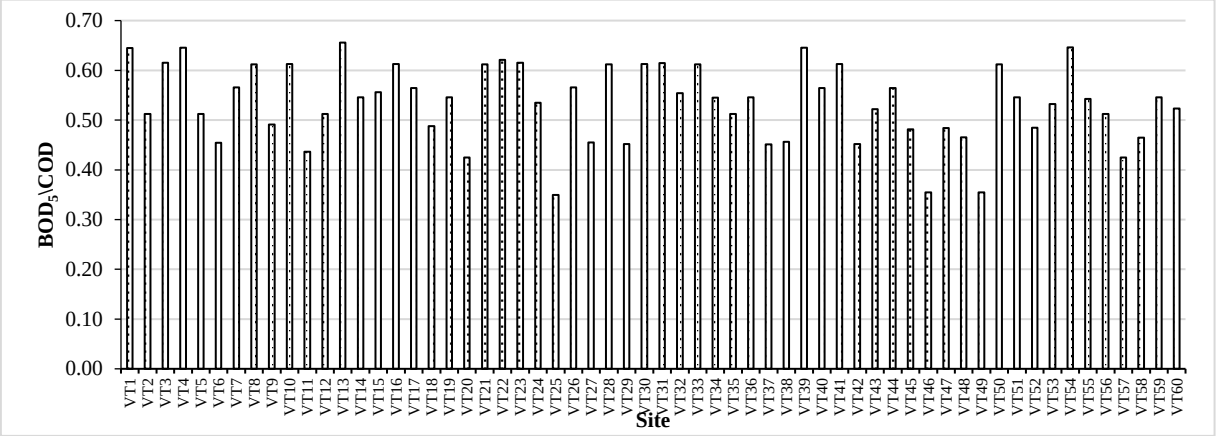

Fig.4: BOD/COD in the super-intensive shrimp ponds

3.3 Chemical oxygen demand (COD)

Chemical oxygen demand (COD) is an important measure of water pollution. The results showed that the difference in COD in wastewater in super-intensive shrimp ponds when the highest concentration was 90.12 mg/l (VT29), the lowest was 10.21 mg/l (VT48) and mean 36.5 mg/l (**Figure 5**). COD concentrations in wastewater from intensive shrimp ponds along the coast of Quang Tri (59.8 - 120.7 mg/l), Thua Thien Hue (130 - 200 mg/l) and Brazil (average 564 mg/l) were all higher than that found in the study area because water sources in surveyed ponds were mainly affected by shrimp farming activities **(Giang and Quyen, 2018; Ngan and Can, 2012; Menezes et al., 2017)**. In fact, the effects from the supply of food and fertilizers to different ponds lead to the difference in COD and BOD concentrations in water in the shrimp ponds **(Figure 3, Figure 4 and Figure 5)**.

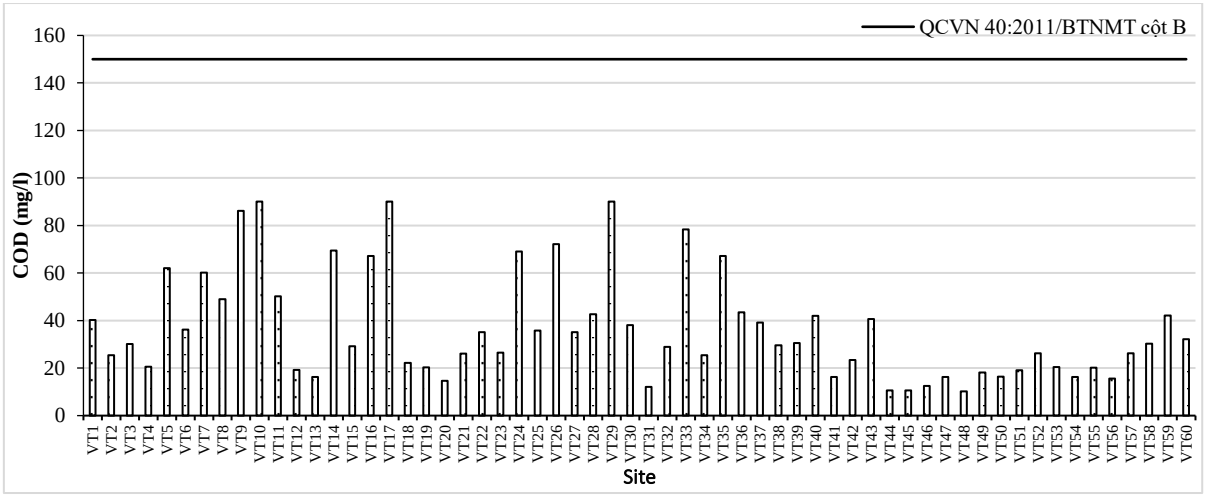

Fig.5: COD in the super-intensive shrimp ponds

In general, COD concentrations in the surveyed shrimp ponds were within the permitted limits of QCVN 40: 2011/BTNMT column B (150 mg/l). According to **Boyd (1998)**, COD is an indicator of the organic richness of pond water. COD of the pond water can range from 10 - 20 mg/L, usually ranges from 40-80 mg/L and COD suitable for aquaculture in the range of 15-20 mg/L. Thus, at the sites VT12, VT13, VT41, VT50, VT51, VT54 and VT56, COD valueswere more suitable for shrimp culture than other pond areas **(Figure 5)**.

3.4 Total suspended solids (TSS)

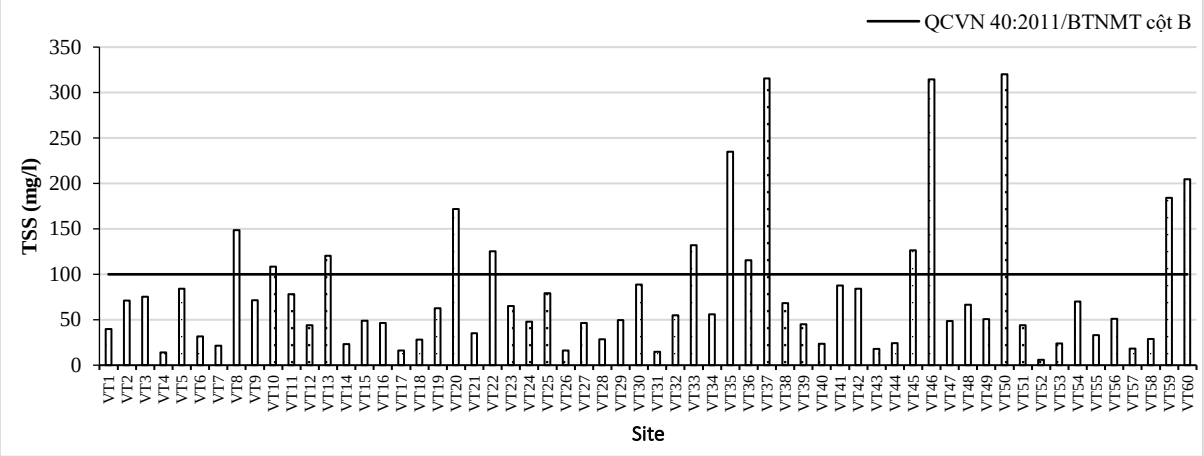

Fig.6: TSS in the super-intensive shrimp ponds

According to **Boyd (1998)**, total suspended solids (TSS) is very closely related to the particle size and the settling rate of suspended particles in water. Pollutants and microorganisms can cling to suspended matters further contributing to the spread of pollutants into the environment **(Ty et al., 2018)**. The TSS concentration in wastewater at the survey sites ranged from 6.1 to 320 mg/l, averaging 79.25 mg/l, the highest at VT50 and lowest in VT52 **(Figure 6)**. The results of the study were more variation than that in the intensive black tiger shrimp ponds in Quang Tri when the TSS concentration in this area ranged from 48.4 to 208.9 mg/l **(Giang and Quyen, 2018)**. The findings showed that 14/60 survey locations had TSS exceeded the permitted limit of the National Technical Regulation on industrial wastewater, column B (100mg/l) from 1.15 – 3.2 times. This proves that at these locations there was organic pollution.

3.5 Total nitrogen (TN)

The total nitrogen in wastewater were large difference between the survey locations. The lowestwas found at VT47 (0.54 mg/l), and the highest was at VT17 (36.12 mg/l) and the mean value was 6.21 mg/l **(Figure 7)**. At the locations of VT10, VT17, VT27, VT29 and VT35, the total nitrogen were quite high and were all greater than 20 mg/l due to the addition of fresh feed, leftovers, and decomposition of food. Compared to QCVN 40: 2011/BTNMT, the total nitrogen at all locations was below the allowable limit of industrial wastewater when discharged into water sources which is not used for domestic water supply purposes (column B, 40 mg/l). In intensive black tiger shrimp ponds in Soc Trang, the total nitrogen in the water ranged from 1.39 to 2.90 mg/L, with an average of 2.31 - 0.58 mg/L**(Phuong et al., 2007)** and TN in the water in the shrimp ponds in the coastal province of Quang Tri was from 10.25 to 18.42 mg/l **(Giang and Quyen, 2018)**. Thus, the results of TN measured in the shrimp ponds in the former studies were much higher than the current study posing a potential risks of eutrophication and water pollution in the farming areas.

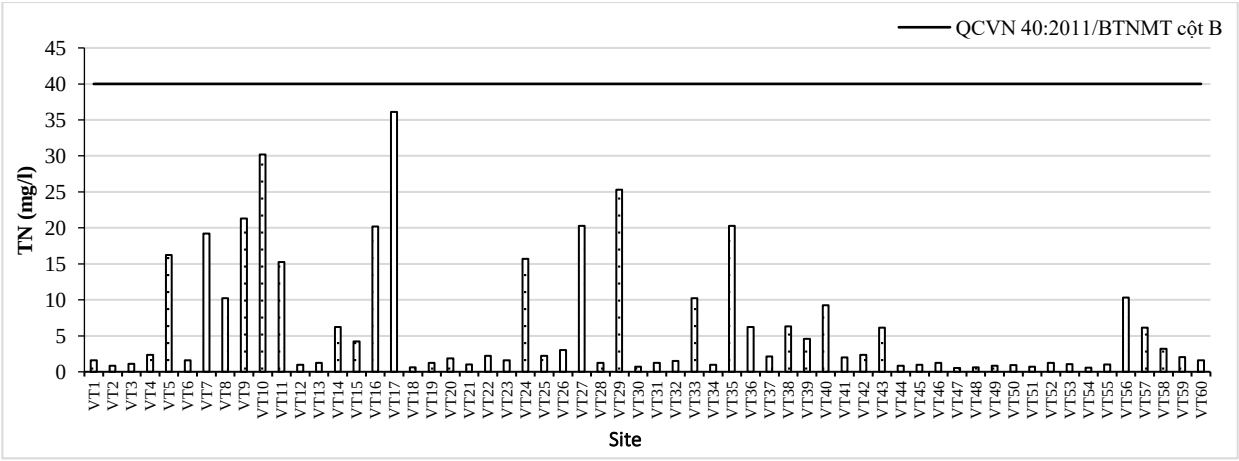

Fig.7: TN in the super-intensive shrimp ponds

3.6 Amoni (NH_4^+-N)

Ammonium values in the super-intensive shrimp ponds ranged from 0.016 to 1.246 mg/l, the highest was at VT7, the lowest was at VT19 and the average was 0.212 mg/l **(Figure 8)**. Ammonium values were lower than the maximum allowable limit of QCVN 40: 2011/BTNMT, column B at all sampling locations (10mg/l). However, the ammonium at most locations (37/60) was greater than 0.13 mg/l, not suitable for the growth of shrimp. However, this ammonium concentration has not greatly harmed the development of cultured shrimp, because in the pond pH was often at neutral values **(Figure 2)**, so ammonium cannot be toxic **(Dat et al., 2012)**. In the improved extensive shrimp ponds in Ca Mau, this concentration ranged from 0.06 - 0.1 mg/l **(Loc et al., 2008)** and in intensive shrimp ponds in Brazil was 0.07 mg/l **(Menezes et al., 2017)**. The results indicated that the water environment of super-intensive shrimp ponds was relatively high ammonium compared to other farming models due to the influence of excess feed, fertilizer and wastes of shrimp.

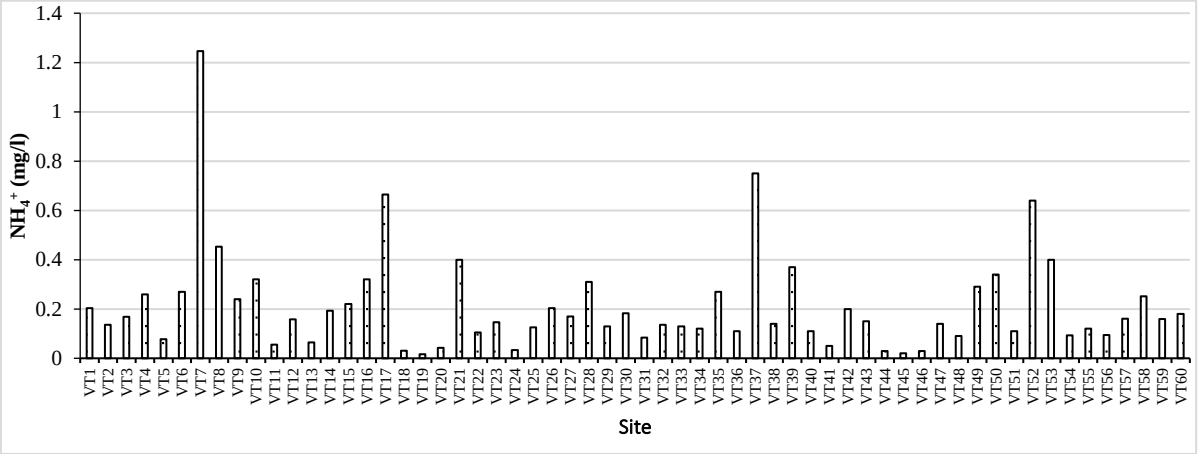

Fig.8: Amoni in the super-intensive shrimp ponds

3.7 Total phosphorus

In culture ponds, phosphorus is periodically supplied through fertilizers to maintain the desired density of phytoplankton. In feeding ponds, some phosphorus is also accumulated by decomposition of uneaten feed and animal manure **(Boyd, 1990)**. The analytical results showed that the total phosphorus fluctuated between the survey locations, reaching from 0.34 to 6.12 mg/l and the average was 1.27 mg/l **(Figure 9)**. At all locations (except VT44), the total phosphorus in wastewater was lower than QCVN 40: 2011/BTNMT column B (6 mg/l). The locations VT5, VT23, VT28, VT30, VT31 and VT44 had relatively high phosphorus and higher than the study in improved extensive shrimp ponds in Bac Lieu **(Anh et al., 2017)** with the phosphate in ponds reached from 0.1 to 1.5 mg/l and an average of 0.4 to 0.5 mg/l. In intensive tiger shrimp ponds in Soc Trang, this concentration ranged from 0.13 to 1.08 mg/l, with an average of 0.567 mg/l **(Phuong et al., 2007)**. Thus, the impact from the addition of food sources for super-intensive shrimp ponds contributes to increasing nutrient levels in the water environment. Therefore, when using this water source, it is necessary to have specific treatment measures for each use purpose.

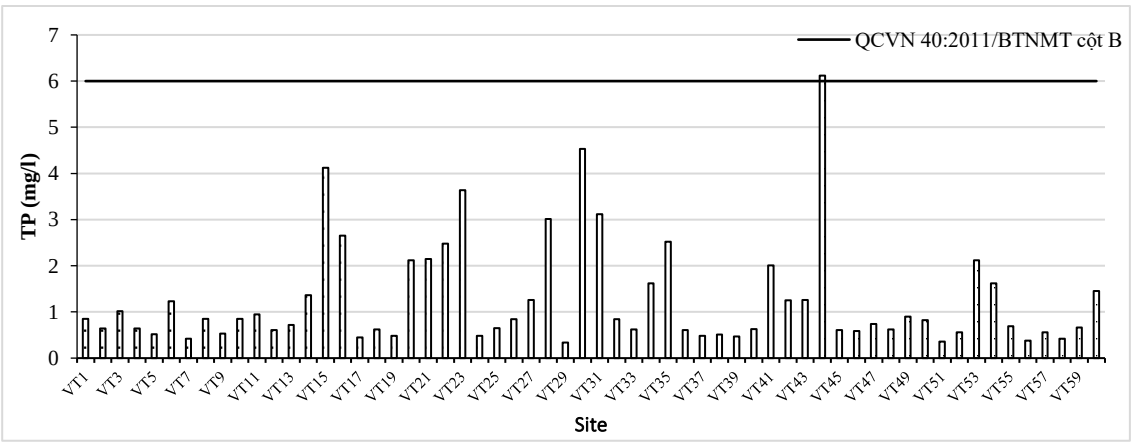

Fig.9: TP in the super-intensive shrimp ponds

3.8 Coliform

The density of coliform at the sampling sites was quite high, ranging from 1,300 - 95,000 MPN/100ml, the lowest at VT12, the highest at VT5 and an average of 16,355 MPN/100ml **(Figure 10)**. Coliform density in this area was relatively higher than the study of **Giang and Quyen (2018)** in coastal Quang Tri province and **Menezes et al. (2017)** in Bazil with the average density of coliform in intensive shrimp ponds were 1,900-3,000 MPN/100ml and 16,000 MPN/100 ml, respectively. The results presented that the water quality in shrimp ponds was showing signs of serious microbiological pollution when there were 40/60 locations exceeding the permitted limit of QCVN 40: 2011/BTNMT, column B (5,000 MPN/100ml) with an excess of 1.01 - 19 times. Waste generated from shrimp farming was the main cause of high density of coliforms in water because coliform is derived mainly from human and animal feces **(Bolstad and Swank, 1997; UNICEF, 2008)**. Therefore, it is necessary to strengthen the management of the pond, regularly apply lime and properly adjust the amount of daily food to limit disease spread in the pond.

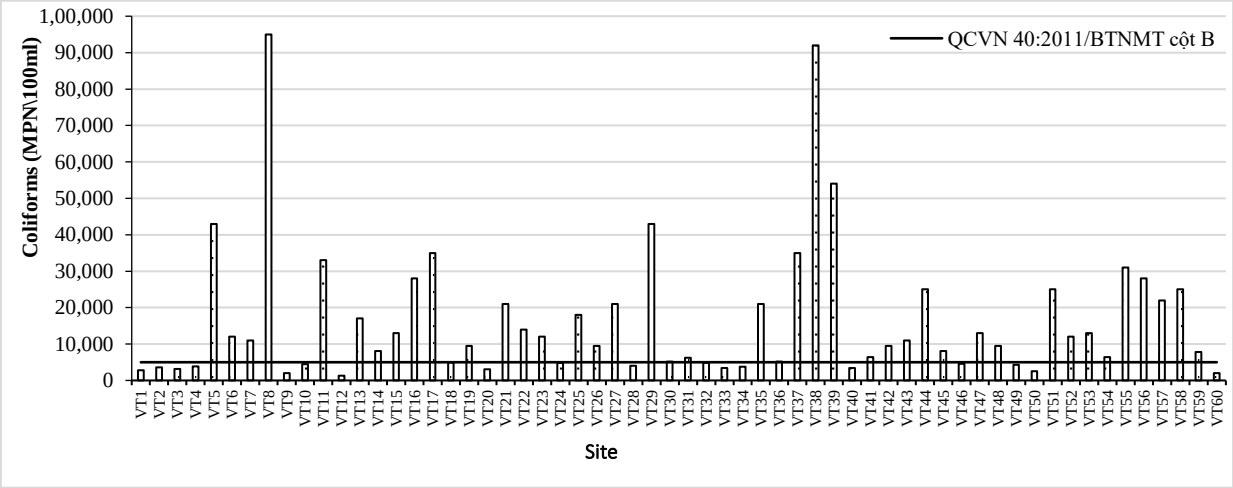

Fig.10: Coliforms in the super-intensive shrimp ponds

IV. CONCLUSION

In the wastewater from the super-intensive shrimp ponds, pH (5.5-9), COD (10.21-90.12 mg/l), total nitrogen (0.54 - 36.12 mg/l), ammonium (0.016-1.246 mg/l) and total phosphorus (0.34-6.12 mg/l) (except VT44) were all within the permissible limits of QCVN 40: 2011/BTNMT, column B. In all ponds, there were residual chlorine below 0.5 mg/l. However, in some ponds there were signs of organic and microbiological pollution since the BOD (4.43 - 55.19 mg/l), TSS (6.1-320 mg/l), total coliforms (1,300 - 95,000 MPN/100ml) in water were quite high, exceeding the allowable limit of QCVN 40: 2011/BTNMT, column B, especially at location VT10 and VT17. The average BOD/COD ratio was 0.53, which showed that the wastewater contained a lot of biodegradable organic matters. The use of fertilizers, excess feed and shrimp waste had a negative impact on the quality of the water source, so it is necessary to fine tune and control these activities.

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Effect of Nitrogen, Mulch and Gibberellic Acid on Quality of Multi-Purpose Pumpkin (*Cucurbita moschata* Duchesne) Fruits

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Abstract**—** *Emergence of multi-purpose pumpkin* ***(Cucurbita moschata Duchesne)*** *as an African indigenous vegetable is attracting great attention. This is due to its adaptation to a wide range of climates and high-yielding potential. Sub-optimal pre- and post-harvest factors have greatly contributed to low returns to the farmers through poor yields and high post-harvest loss of pumpkins. There is therefore need to determine the optimal pre- and post-harvest inputs which will provide nutritious pumpkin fruits to consumers. To contribute in solving this challenge, knowledge on the effect of nitrogen fertilizer, mulch and gibberellic acid on the fruit size, flesh thickness and firmness of multipurpose pumpkin (Cucurbita moschata Duchesne) is very important. A field experiment was therefore set at the Chuka University farm for two seasons between January 2019 and July 2020.It was arranged in a split-plot in randomized complete block design and replicated three times. Nitrogen (CAN) (0, 50, 100 and 150 kg N/ha), mulch (no mulch, black-painted and unpainted rice straws) and gibberellic acid (GA_3) (0 mg/L, 40 mg/L and 80 mg/L) were assigned to the main plots, split plots and sub-plots respectively. Data was collected fortnightly from the fourth week after emergence. Data values were subjected to analysis of variance using the SAS software and means separated using least significant difference. Application of N fertilizer was significant on fruit size (0.013) during the second season, flesh thickness during the first and the second season (0.002and 0.04respectively) and fruit firmness of 0.02 upper and 0.009 lower during the second season. Application of N at150kg N/ha and black-painted mulch resulted to fruit size of 2172 cm ^{2} and 2199 cm ^{2}respectivelyand flesh thickness of 3.387cm and 3.856cm respectively which was higher than the other treatments. The effect of GA_3 on fruit size, flesh thickness and firmness of multipurpose pumpkins was insignificant during both seasons. These results show that application of N at 150kg N/ha and black painted mulch would give the best fruit quality to the farmers while application of GA_3 may not necessarily result to beneficial returns.*

Keywords**— AIVs, Fruit size, Flesh thickness, Fruit firmness, Fruit set, Plant growth regulators.**

I. INTRODUCTION

Pumpkins are important horticultural crops worldwide. Pumpkin is a fruit-vegetable belonging to the Cucurbitaceae family together with gourds, melons and squashes. In Kenya, pumpkin production increased from 599 ha in 2015 to 681 ha in 2016, with a volume increase from 3580 metric tonnes to 4017 metric tonnes respectively (Horticulture Validated Report, 2015-16). In Kenya, pumpkin is regarded as a traditional vegetable that is grown in the high potential areas and the arid and semi-arid lands (Karanja et al., 2014). FAO (2005) reported that pumpkin has immense economic potential for use both as a food and as an industrial crop. Pumpkin is famous for its edible seeds, fruit and greens (Matsui et al., 1998).

Fertilizer affects the productivity of crops (Oloyede et al., 2013). Poor fruit setting, low crop yield and low nutritional quality result from inadequate levels of the primary nutrients namely: Nitrogen, Phosphorus and Potassium (Martinetti& Paganini, 2006; Liu et al., 2010).Application of N, P and K has been reported to increase the growth and productivity of pumpkin and crops generally (Johannes et al., 2003). Nitrogen (N) is by far the most critical plant growth element, yet soil testing for N is usually not practical due to its mobility in the soil. The use of chemical fertilizers as a supplemental source of nutrients has been on the increase in pumpkin production but they are not applied in balanced proportions by most farmers. Further, NPK fertilizer has been found to increase the leaf area, stem diameter, number of leaves and nutrient contents (N, P, K, Ca, Na and Mg) in the soil in pumpkin production (Okonwu& Mensah, 2012).

Mulches and particularly the clear form have been reported to enhance germination of direct-seeded pumpkins since they increase soil temperatures. The higher soil temperatures associated with mulches should also accelerate establishment of transplants and promote subsequent crop development thereby increasing yields and promoting crop maturity at harvest (Waterer, 2000). The exposed soil is exceptionally susceptible to the destructive effects of heavy rainfall, which causes the soil structure to break apart and intensifies the elimination of nutrients making mulching very necessary (Bucki&Siwek, 2019). Mulches of organic origin typically enter into a relationship with the soil, increasing the activity of the enzymes which break down plant residues (Sas-Paszt et al., 2014). Organic mulches with a high carbon to nitrogen ratio (such as sawdust) may cause temporary soil impoverishment in nitrogen due to the activity of microorganisms (Fang et al., 2007).

According to Yamaguch and Kamiya (2000), gibberellin (GA) hormone plays an essential role in many aspects of plant growth and development of pumpkins such as seed germination, stem elongation and flower development. Gibberellic acid (GA3) is an important PGR that affects plant growth and development by inducing metabolic activities and regulating nitrogen utilization (Sure et al., 2012). It also plays a significant role in seed germination, endosperm mobilization, stem elongation, leaf expansion, reducing the maturation time and increasing flower and fruit set and their composition (Roy &Nasiruddin, 2011). GA_3delays senescence, improves growth and development of chloroplasts and intensifies photosynthetic efficiency which could lead to increased yield (Yuan &Xu, 2001).

II. MATERIALS AND METHODS

2.1. Site and Experiment

The experiment was conducted at Chuka University Ndagani research farm. The farm lies at0^0 19' S, 37^0 38' E and 1535 m above sea level. The average annual temperature is 19.5 ºC (from 12.2 ºC and 23.2 ºC). The area experiences two rainy seasons with the long rainy season occurring in March through June and the short rainy season from October to December. The average annual rainfall is 1200 mm annually (http://en.climate-data.org). The soils are humicNitisols, deep, strongly weathered, well drained tropical soils with a clayey subsurface horizon made of angular, blocky structural elements that easily crumble into polyhedricipeds with shiny faces. The soil has a high cation exchange capacity (Koskey et al, 2017).

A three factor split-split block experiment embedded in a randomized complete block design (RCBD) with three replications was used. Individual plots in a block measured 2m x 2m separated from each other by 1 m. The three factors were four nitrogen rates, three mulch types and three gibberellic acid (GA) rates. Nitrogen occupied the main plot; mulch the split plots and gibberellic acid the subplots. The four nitrogen rates were 0, 50, 100 and 150 kg/ha. Nitrogen in CAN was applied in two split equal dosages for each rate, at three weeks post-emergence and at the beginning of flowering. Single fertilizers were used. The mulch factor included no mulch, black-painted rice straw and unpainted rice straw. Rice straw was easily available in a close proximity to experimental site and quantities required are easy to get. The black-painted dry rice straw and unpainted dry rice straw was placed on the respective split plots after land preparation. Painting of the rice straw was done by dipping them in a 200 L drum containing the black paint solution and afterwards spread out to air dry. The ingredients of the paint were noted based on the paint that was used. The mulch was uniformly spread to achieve 20 cm thickness. Planting holes were marked and opened during sowing.

Gibberellic acid rates were 0 mg/L, 40 mg/L and 80 mg/L. Gibberellic acid was dissolved in 50ml alcohol then the volume was made up to one liter stock solution by adding distilled water. The required concentration of spray solution was then prepared from stock solution by diluting with distilled water. A few drops of acceptable commercial sticker was added to solutions to facilitate the uptake of the GA. The gibberellic acid solution was sprayed to the plants with a hand sprayer of one liter capacity. Stock solution of lower rate was sprayed first followed by next higher rate. It was done once during the fourth week after emergence. To avoid drift, spraying was done on a calm morning.

Soil analysis was done before plant establishment. It was done at the KALRO National laboratories Kabete. Testing was done to establish the nutrient contents which informed the effects of nitrogen applied in the experiment. The soil was sampled using a zigzag sampling design across the experimental field. A soil auger and plastic containers were used. Two different composite samples were prepared, taken from 0-15 cm and 16-30 cm respectively. The soil pH, total N, available P, K, Ca, Mg, organic carbon, and trace elements were assessed (Chang & Laird, 2002). .

2.2. Data Collection

Data was collected for two seasons (long and short rain seasons). Fruits with dry fruit stalk and hard skin were harvested from each experimental unit, counted, their size, (length and diameter was measured using a tape measure then translated using a ruler) and weight measured. Three fruits were randomly selected per treatment for flesh thickness data which was measured using a Vernier caliper.Fruit firmness was determined through a flesh penetration test which was carried out at room temperature. A penetrometer was used to give direct readings of fruit toughness in kilogram-force.

2.3. Data Analysis

Data values were subjected to analysis of variance to determine effects of the treatments using the SAS software version 9.3. Means separation was performed using the least significant difference (LSD) test at $\alpha = 0.05$.

III. RESULTS

3.1 Effects of Nitrogen Fertilizer on Fruit Size, Flesh Thickness, Upper and Lower Firmness

Nitrogen fertilizer had no significant effect ($P>0.05$) on fruit size during season one (S1)but was significant during season two (S2) ($P<0.05$) (Table 1). Application of 150kg N/ha produced the larger fruit sizes of 2172 cm^2 and 2199 cm^2 respectivelyduring S1 and S2 respectively. In both seasons, the fruit size increased with increase in N fertilizer up to 150kg N/ha. Non-application of N produced the lowest fruit sizes of 1513 cm^2 and 1643 cm^2 at S1 and S2 respectively.

N fertilizer had significant effect on the flesh thickness in both seasons ($P<0.05$). Application of 150kg N/ha produced the highest flesh thickness in both seasons; 3.387 cm and 3.856 cm respectively (Table 1). Flesh thickness increased with increase in N fertilizer up to 150kg N/ha during S1. Non-application of N produced flesh thickness of 3.752 cm during the S2. N fertilizer had no significant effect ($P>0.05$) on fruit firmness (upper and lower) during S1 but was significant during S2 ($P<0.05$) (Table 1). Application of 100kg N/ha produced the highest fruit firmness (upper and lower) of 39.63and 39.07, 38.58 and 38.03 respectively at S1 and S2 respectively. In both seasons, the lower fruit firmness increased with increase in N fertilizer up to 100kg N/ha. This was also the case with upper fruit firmness during S2. Non-application of N produced the least fruit firmness of 37.27 and 35.42 at S1 and S2 respectively.

Table 1: Effects of Nitrogen Fertilizer on Fruit Size, Flesh Thickness, Upper and Lower Firmness

Treatments	Fruit size (cm^2)		Flesh thickness (cm)		Upper Firmness		Lower Firmness	
	S1	**S2**	**S1**	**S2**	**S1**	**S2**	**S1**	**S2**
Control	1513	1643c	2.677b	3.752	38.77	36.66bc	37.27	35.42b
50	1870	1683c	2.837b	3.519	38.69	37.24b	37.46	36.24b
100	2013	1976b	3.191a	3.626	39.63	39.07a	38.58	38.03a
150	2172	2199a	3.387a	3.856	39.31	38.76a	38.24	37.76a
Significance	0.119	0.013*	0.002*	0.04*	0.533	0.02*	0.116	0.009*
LSD 5%	563.8	308.3	0.259	0.221	1.717	0.908	1.243	1.332

*Means followed by the same letters or no letters within a column are not significantly different according to the LSD Test at $P = 0.05$

3.2 Effects of Mulch on Fruit Size, Flesh Thickness, Upper and Lower Firmness

The effect of mulch on fruit size was not significant during S1 ($P>0.05$) but significant during S2 ($P<0.05$) (Table 2). Largest fruit sizes were produced when black-painted rice straws mulch was applied during both seasons at 1948 cm^2 and 2016 cm^2 for S1 and S2 respectively. The effect of mulch was insignificant during both seasons ($P>0.05$). According to the results, when black-painted rice straws mulch was applied, edible fruit flesh thickness was higher compared to the unpainted rice straws mulch or no mulch was applied. Results further showed that application of

mulch was only significant (P<0.05) on fruit upper firmness during S2. Fruit firmness was highest when black-painted rice straws mulch was applied. However, the difference in firmness was negligible with the application of unpainted rice straws mulch on both the lower and upper fruit firmness.

Table 2: Effects of Mulch on Fruit Size, Flesh Thickness, Upper and Lower Firmness

Treatments	Fruit size		Flesh thickness		Upper Firmness		Lower Firmness	
	S1	**S2**	**S1**	**S2**	**S1**	**S2**	**S1**	**S2**
Control	1812	1726c	3.053	3.522	38.55	36.82b	37.41	36.03
BL	1948	2016a	3.053	3.836	39.58	38.90a	38.25	37.69
BR	1917	1884b	2.963	3.706	39.16	38.08a	37.99	36.87
Significance	0.496	0.001*	0.546	0.605	0.923	0.001*	0.920	0.996
LSD 5%	250	131.8	0.198	0.264	0.822	0.904	0.961	1.639

BL- black-painted rice straws mulch, BR- unpainted rice straws mulch

3.3 Effects of Gibberellic Acid on Fruit Size, Flesh Thickness, Upper and Lower Firmness

Gibberellic acid had no significant effect on fruit size, flesh thickness, upper and lower firmness during both seasons (P>0.05). Application of 80 mg/L produced the larger fruit sizes of 1935 cm^2 and 1928 cm^2 respectively at S1 and S2 respectively. In S2, the fruit size increased with increase in GA_3 up to 80 mg/L. Results also showed that application of 40 mg/L of GA_3 resulted to 3.102 cm and 3.744 cm of flesh thickness which was highest compared to when 80 mg/L was applied and when no GA_3 was applied. In S2, both lower and upper fruit firmness increased with increase in GA_3 up to 80mg/L. The effect of GA_3 produced highest fruit firmness when GA_3 of 80mg/L was applied.

Table 3: Effects of Gibberellic Acid on Fruit Size, Flesh Thickness, Upper and Lower Firmness

Treatments	Fruit size		Flesh thickness		Upper Firmness		Lower Firmness	
	S1	**S2**	**S1**	**S2**	**S1**	**S2**	**S1**	**S2**
Control	1911	1825	2.929	3.614	38.99	37.56	37.89	36.82
40	1831	1872	3.102	3.744	38.90	37.89	37.78	36.66
80	1935	1928	3.038	3.706	39.41	38.36	37.99	37.11
Significance	0.717	0.188	0.240	0.312	0.200	0.396	0.152	0.706
LSD 5%	269.4	111.5	0.205	0.175	0.634	1.185	0.666	1.094

IV. DISCUSSION

In this study, quality traits of the multi-purpose pumpkins were enhanced by N fertilizer, mulch and GA_3 across both seasons. The optimum N rate of fertilizer was 150kg N/ha, black painted mulch produced the highest fruit qualities while 80 mg/l of GA_3 positively affected the fruit size, flesh thickness and fruit firmness of multipurpose pumpkins.

Fertilizers have been denoted as sources of plant nutrients that can be added to soil to supply its natural fertility (Oloyede et al., 2013).They are intended to supply plant needs directly rather than indirectly through modification of soil properties like the pH and structure. When appropriate fertilizers are added in the soils, the result is dramatic improvement in both quantity and quality of plant growth (Nahed & El-Aziz, 2007).According to Oluoch (2012), pumpkins tend to develop extensive root systems that efficiently explore the soil for water and available nutrients and thus, timely and appropriate applications of fertilizer can make a significant difference in the quality and quantity of fruit produced which the findings of this study corroborated with.

In this study, application of N fertilizers up to 150kg N/ha increased the fruits size and flesh thickness. The present findings are in agreement with the results of Kazemi (2014) which revealed an increase in fruits sizes of tomatoes using different combinations of fertilizers. This increment might be interpreted that plants during flowering and fruit setting stages need a high amount of nutrients to perform the biological operations like photosynthesis. However, application of N fertilizers

beyond 100kg N/kg reduced fruit firmness of the multi-purpose pumpkins. This shows that application of N fertilizer beyond 100kg N/kg makes the pumpkin fruit softer and of inferior handling quality.

Results showed that black painted mulch positively affected fruit size, flesh thickness and fruit firmness of multipurpose pumpkins. The results supports studies done by Lorenzo et al. (2001) and Ibarra-Jimenez et al. (2008) on mulching using black polythene showed higher cucumber yields in comparison with yields from plants cultivated in soil without mulching. Jodaugiene et al. (2010) denoted that as a result of the use of such mulches, increased presence of worms and their greater mass was observed positively affecting the quality of crops.

Results showed that largest fruit sizes were obtained when 80mg/Lof GA_3 was applied. The increase in fruit sizes might be attributed to increased cell division and cell elongation induced by the application of GA3 as also observed by Shah et al. (2006) & Roy &Nasiruddin (2011). Application of GA_3 beyond 40 mg/L reduced fruit flesh thickness. Upper and lower firmness were also highest when 80mg/L of GA_3 was applied. This could be attributed by the fact that GA_3 promotes DNA, RNA and protein synthesis resulting in increased biomass as denoted by Khan and Samiullah (2003).

V. CONCLUSIONS AND RECOMMENDATIONS

A number of studies that have been carried out on the multipurpose pumpkins are usually directed towards the effect of NPK fertilizers and different concentrations of gibberellic acid on the yield without evaluating the specific fertilizer component like N. whatever happens during growth and development of fruits will have a bearing on the fruit size, flesh thickness and fruit firmness. Additionally, the studies on quality parameters like firmness and flesh thickness of crops are not common. The main findings achieved and conclusions arrived at are:

- Nitrogen fertilizer influenced multi-purpose pumpkin fruit quality traits such as fruit size, flesh thickness and fruit firmness. N rate of 150 kg/ha significantly increased the traits and it is recommended for use by the farmers.
- Mulch also significantly affected positively fruit size and fruit firmness of multi-purpose pumpkin. Black painted rice straws produced higher quality traits compared to unpainted rice straws and therefore suitable for use by the farmers.
- Gibberellic acid did not significantly affect fruit size, flesh thickness and fruit firmness of multi-purpose pumpkin and therefore, the study recommends its use by the farmers for other traits other than those tested in this experiment.

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Phytochemical, Fluorescence and GC-MS Analysis of Methanolic Extract of *Sterculia foetida* L. Seeds

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Abstract— *The present work phytoconstituents of the seed powder of* Sterculia foetidaL. *extracted with 98% methanol. The extracted phytochemical compound subjected to qualitative analysis, quantitative analysis, fluorescence analysis and GC-MS analysis. The results of qualitative phytochemical screening confirm that the presence of tannins, phenols, steroids, cardiac glycoside and coumarin. The significant amount of carbohydrate, protein, lipid, tannin and total phenol estimated through quantitative analysis of phytochemicals. The seed powder with the picric acid exhibited fluorescent yellow during fluorescence analysis undertaken with short ultra violet light at 254 nm. There are 13 bioactive compounds were identified through GC-MS analysis of seed powder of S. foetida L. These various bioactive compounds possess a wide range of activities such as disease control, pest control and microbicidal effect.*

Keywords— ***Sterculia foetida, seed extracts, phytochemical screening, fluorescence analysis, GC-MS analysis.***

I. INTRODUCTION

The medicinal plants are widely used in traditional medicine to prevent and treat various diseases. The phytoconstituents present in the various part of the plant can be exhibit anti-cancer, anti-tumour, anti-diabetic, anti-spasmodic, anti-inflammatory, anti-oxidant and antibacterial actvities[23]. *Sterculia foetida* is commonly called as Wild Indian almond belonging to the family Sterculiaceae. The fruits, seeds and leaves of *S. foetida* have been conventionally known for its many therapeutic purpose[5]. A different variety of pharmacologically active compounds have been isolated from the leaves of *S. foetida* and these compounds from the leaves used as astringent, laxative, antifungal, anti-inflammatory and anti-ulcer medicines[26]. Nanadagopalan *et al*., (2015)[23] isolated twenty seven bioactive compounds from the methanolic extract of *S. urens* leaves. The important compounds present in the leaves extract were 3, 7, 11, 15-Tetramethyl-2-hexadecen-1-ol, sucrose, 2, 4-Dihydroxy2, 5-dimethyl-3(2H)-furan-3-one, 5(2H)-Oxazolone, 4-(phenylmethyl)-, 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-, Megastigmatrienone and 2-Methoxy-4-vinylphenol etc and these compounds could contribute the medicinal quality of the *S. foetida* leaves. *S. foetida* seeds are not toxic and edible to humans and animals[5]. The bioactive compounds sterculinine-I, sterculinine-II, and soyacerebroside-I were isolated from the seeds of *S. lychnophora*[34]. In view of that the present work aimed to identify the pharmacologically active biomolecules responsible for anti-cancer and antioxidant from the methanolic extract of *S. foetida* seed.

II. MATERIALS AND METHODS

2.1. Collection & processing of plant sample

Dried fruits of *Sterculia foetida* were collected from Kelambakkam-603103, Tamil Nadu, India. Seeds were identified and authenticated by Prof. P. Jayaraman, Director, Institute of Herbal Science, Plant Anatomy Research Centre (PARC), Chennai-600045. The seeds

were collected from dried fruits were grinded and sieved to make fine powder for further study.

2.2. Preparation of extracts

Two fifty grams (250 g) from seed powder was extracted with 250 mL of 98% methanol in Soxhlet apparatus for 24 hours. After the extraction the crude extract was filtered through a Whatman number 1 filter paper. Later, the crude extract was subjected to evaporation in a rotary vacuum evaporator for dryness. After the evaporation process completed the concentrated extract was collected and stored at 4°C for further analysis[9,31].

2.3. Qualitative analysis of phytochemicals

Methanolic extract of *S. foetida* seed powder was subjected to qualitative phytochemical analysis. The phytochemical analysis such as Ferric chloride test and Lead acetate test for tannins[28], Ferric chloride test and Gelatin test for phenols[28], Libermann-Buchard test and Salkowski test for sterols[28], Keller Killiani test for glycoside[28], Coumarin test for Coumarin[27] and Phlobatanin test for Phlobatanin[3] were performed.

2.4. Quantitative analysis of phytochemicals

The methanolic seed extract was subjected to quantitative analysis by spectrophotometer method. The extract was analyzed for Carbohydrate by Anthrone method[12], Protein by Lowry's method[20], Total Lipids[35], Total Phenol[2] and Total Tannin[2].

2.5. Fluorescence analysis

Fluorescence analysis is the most important parameter of pharmacognostical evaluation. This analysis was carried out as per the standard protocol[16,18,29]. In the present work, the seed powder was treated with different solvents and chemicals. The seed powder was subjected to fluorescence analysis in visible/daylight and UV light (254 nm).

2.6. GC-MS analysis

Aim of this analysis is to identify the pharmacologically active biomolecule (anticancer & antioxidant) present in the seed extract. The methanolic extract of *S. foetida* seed powder was subjected to GC-MS analysis on the instrument GC and MS JEOL GC mate equipped with secondary electron multiplier (Agilent Technologies 6890N Network GC system for gas chromatography). The column (HP5) was fused silica 50 m×0.25 mm I.D. The study conditions were 20 min. at 100°C, 235°C for column temperature at 3 minutes and 240°C for injector temperature, carrier gas was helium, and split ratio was 5:4. The 1 µl of the sample was evaporated in a split-less injector at 300°C and the run time was 22 min. The phytoconstituents of the extract was identified by Gas Chromatography coupled with Mass Spectrometry. The GC-MS spectrum was analyzed using the NIST08 library which has more than 62,000 patterns[4,25].

III. RESULTS AND DISCUSSION

3.1. Qualitative analysis of phytochemicals

The essential information regarding the phytochemical constituents is generally determined through qualitative phytochemical analysis of plant extracts. The qualitative analysis of the methanolic seed extract showed the presence of secondary metabolites such as tannins, phenols, steroids, cardiac glycoside and coumarin and this analysis also confirmed that the absence of the phytochemical phlobatanin (Table 1). Tannin is an important secondary metabolite found in many plant species with remarkable amounts when compare to other secondary metabolites. Tannin can be present in various parts of plants such as roots, sap, stem, bark, leaves, fruits and seeds. Many researchers proved that the pharmacological properties of plant based secondary metabolites. Vieira Pereira *et al*., 2015[33] reported that tannin is a pharmacologically active metabolites and it act as astringent and insecticidal agent. Phenol and phenolic compounds contain antimicrobial property, hence they are used to treat skin infection and wound[24]. Phenol prevent the enzymes that cause antioxidant, inflammation, immune enhancers, hormone modulators and anti-clotting[13]. Sterols present in the plant possess anti-inflammatory effects and might enhance immune function[7]. Glycosides are considered as a useful drugs in therapeutics and it display antitumor activity and antiviral activity against rhinovirus[22]. Researchers, Agarwal, 2000; Goodman & Gilman's, 2006; Jain and Himanshu Joshi 2012[1,8,15] proved that the anti-tumor, anti-cancer and anticoagulant activities of plant derived secondary metabolites coumarins. The above mentioned findings supported that the plant based secondary metabolites such as tannins, phenols, steroids, cardiac glycoside and coumarin have pharmacological properties.

Table 1: Phytochemicals present in the seed extracts of Sterculia foetida

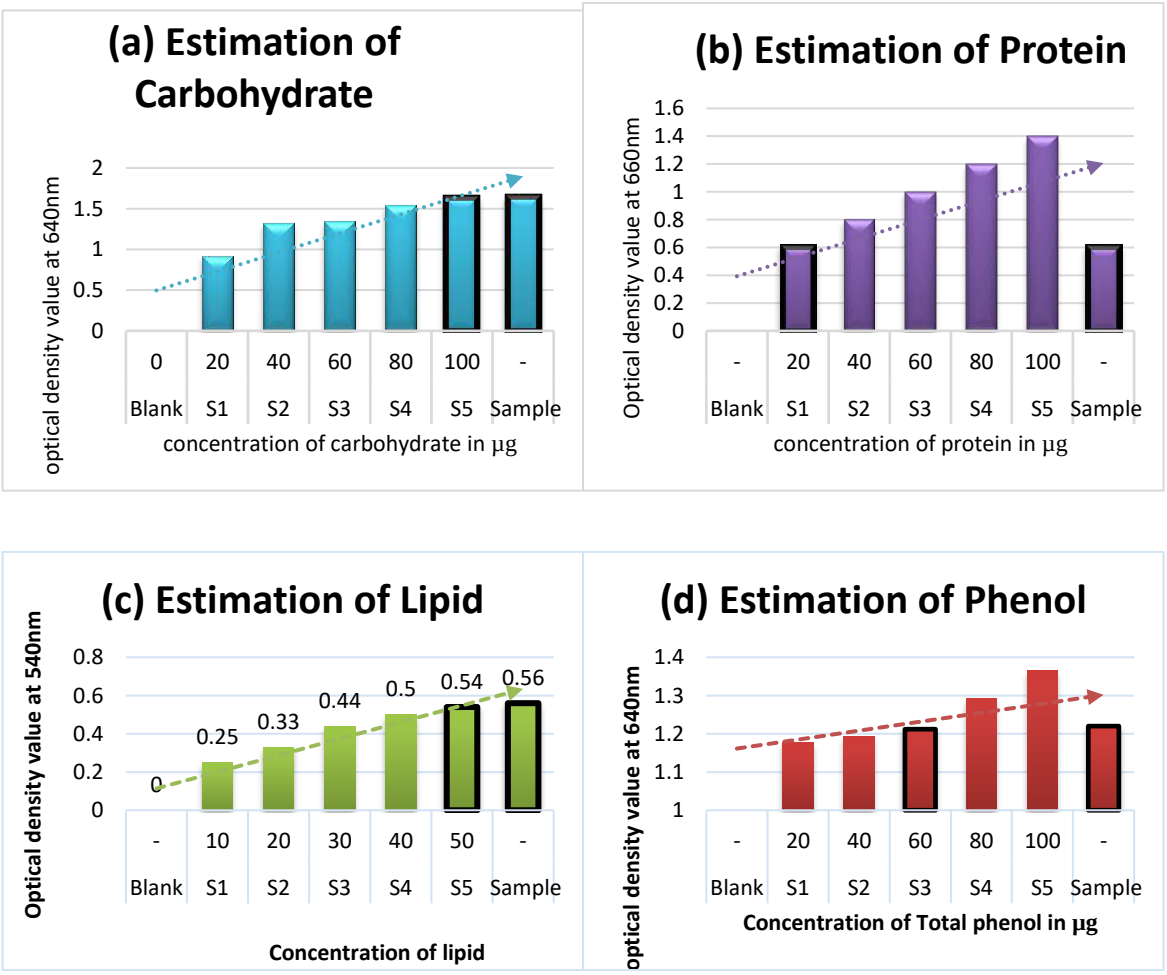
S. No.	Secondary metabolites	Results
1.	Tannins	
	Ferric chloride test	+
	Lead acetate test	+
2.	Phenols	
	Ferric chloride test	+
	Gelatin test	+

3.	Sterols	
	Libermann-Buchard test	+
	Salkowski test	+
4.	Glycoside	
	Keller Killiani test	+
5.	Coumarin	
	Coumarin test	+
6.	Phlobatanin	
	Phlobatanin test	-

+ = Presence; - = Absence

3.2. Quantitative analysis of phytochemicals

The amount of Carbohydrate, Protein, Lipid, Phenol, and Tannin present in the seed extract was obtained by plotting the Optical Density value for the standard test tubes. The optical density value for sample tube is compared with one of the same optical density value of the standard tube. The optical density reading of the sample tube is plotted on the graph and the concentration of the component is determined. The amount of carbohydrate present in the 2ml of seed sample is estimated to be 100µg as shown in the Fig. 1a. The amount of protein present in the 2ml of seed sample is estimated to be 20µg as shown in the Fig. 1b. The amount of lipid present in the 2ml of seed sample is estimated to be 50µg as shown in the Fig. 1c. The amount of phenol present in the 2ml of seed sample is estimated to be 60µg as shown in the Fig. 1d. The amount of tannin present in the 2ml of seed sample is estimated to be 60µg as shown in the Fig. 1e. The secondary metabolites like carbohydrate, protein and lipid have antioxidant, antimicrobial and antiviral properties[21]. According to the literatures, phenolic compounds are known to exhibit antioxidants, anticancer, anti-inflammatory, antimicrobial, anti-allergic and antifertility activity[14,30]. Tannin is one among the major active secondary metabolite found in wide variety of plants[11]. Tannin has been reported to possessing antiviral, antioxidant, antibacterial and antitumor activity[6,17]. In the present work confirm that there is a significant quantities of pharmacologically active secondary metabolites such as carbohydrate, protein, lipid, phenol, and tannin are present.



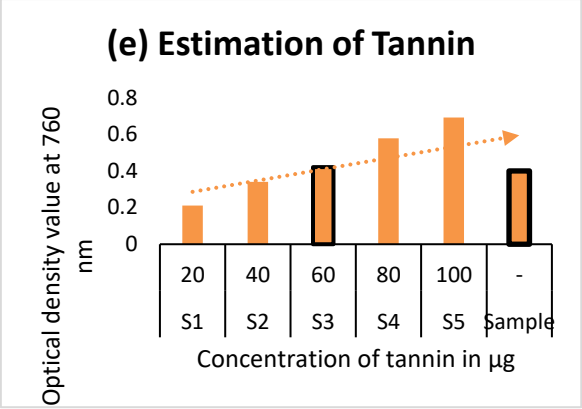


Fig 1 (a-e): The graphs showing results of quantitative analysis of methanolic extract of Sterculia foetida seed powder

3.3. Fluorescence analysis

The results of fluorescent analysis of dried seed powder of *S. foetida* with different chemical reagents are given in Table 2. The seed powder was treated with various solvents under visible and short UV light. Among the different solvents analyzed, picric acid showed characteristic coloration in both visible and UV light. Fluorescence analysis is a preliminary pharmacognostic parameter for determining various phytoconstituents present in the plant sample in short period of time[19]. The crude powder of plants shows different coloration when subjected to different chemical reagents at varied wavelength[10]. The researcher, VidyaKamble& Nikhil Gaikwad (2019)[32] reported that the secondary metabolites like coumarin, sapogenin and terpenoids show yellowish green fluorescence under short UV light. In the present work, the major bioactive metabolites present in the crude powder of *S. foetida* seed was found to be coumarin, phenols, tannins and sterols and this results could confirm that the pharmacognostic properties of *S. foetida* seed.

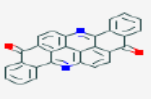
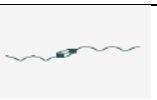
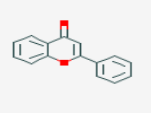
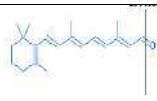
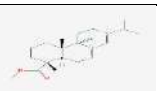
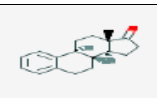
Table 2: Fluorescence analysis results of S. foetida seed powder

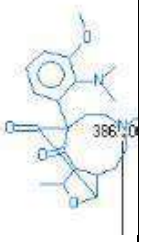
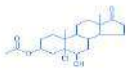
S. No	Treatment	Visible light	UV light (254 nm)
1.	Powder	Light brown	Blackish brown
2.	Powder+Water	Light brown	Blackish brown
3.	Powder+NaoH	Reddish brown	Dark reddish brown
4.	Powder+HCl	Brown	Dark brown
5.	Powder+Acetic acid	Light brown	Dark brown
6.	Powder+Picric acid	Yellow	Yellowish green
7.	Powder+Sulphuric acid	Reddish brown	Dark Reddish brown
8.	Powder+Nitric acid	Brown	Dark brown
9.	Powder+Iodine	Brown	Dark brown
10.	Powder+$Fecl_3$	Green	Dark green
12.	Powder+KOH	Brown	Dark brown
13.	Powder+Ammonia	Light brown	Dark brown
14.	Powder+Ethanol	Brown	Dark brown
15.	Powder+Alc.NaOH	Dark brown	Black

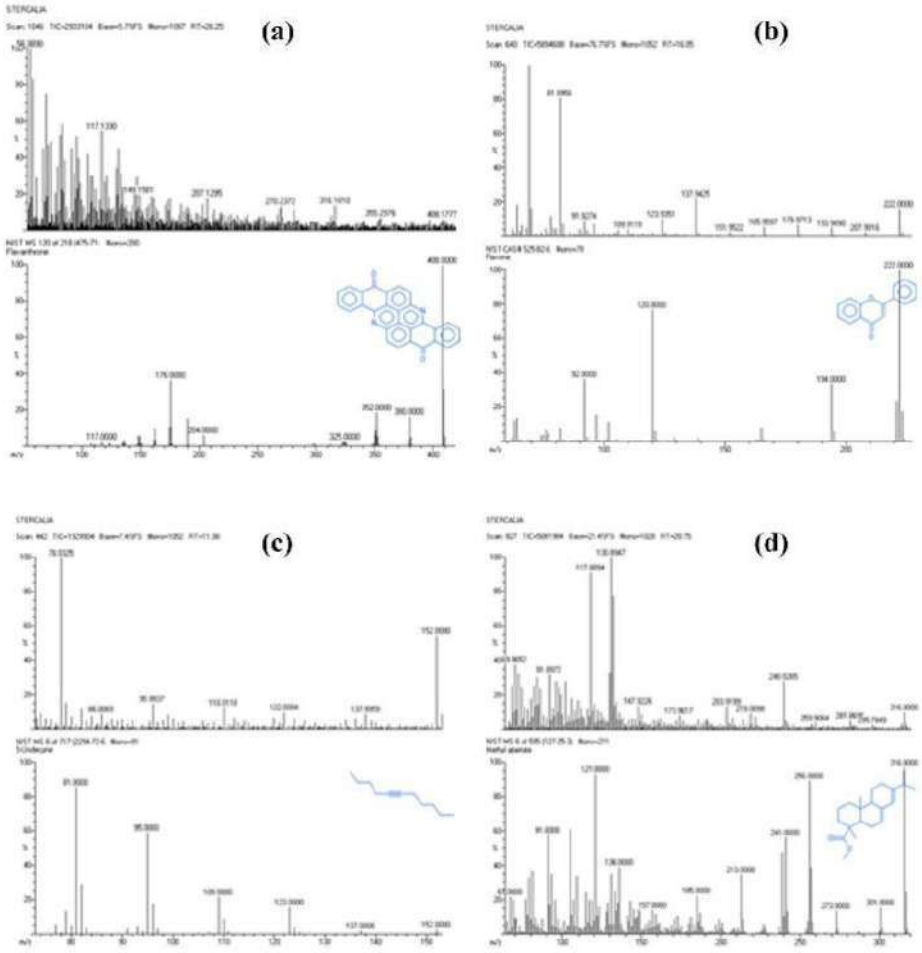
3.4. GC-MS analysis

The GC-MS analysis of methanolic extract of *S. foetida* seed powder divulge the presence of thirteen phytochemical compounds that could possess the pharmacological and microbicidal activity. The identification of the biomolecule was confirmed based on the retention time and molecular formula. The biologically active compounds with their Retention time (RT), Molecular formula, Molecular weight, Molecular structure and their Biological activity are presented in Table 3. The pharmacologically active major compounds present in the seeds were Flavanthrone & Flavone (Anticancer activity), 5-Undecyne & Methyl abietate (Antioxidant), 2-cyclohexen-3-ol-1-one,2(11-phenylundecanoyl) (Antidiuretic), Androstan-6-0l-17-one,3-acetoxy-5A-chloro (Vasodilator) and Testosterone Cypionate (Anti-inflammatory) apart from these compounds other major and minor compounds were also present. The GC-MS graph showing the compounds which showing anticancer (Figs. 2a & b) and antioxidant (Figs. 2c & d) activities are presented in Figs. 2 a-d. Similar to this study, twenty seven major phytochemical compounds were characterized through GC-MS analysis of the methanolic leaves extract of *S. urens* Roxb[23]. Asif Jafri *et al*., 2019[5] reported that there were thirty five bioactive compounds were characterized via GC-MS analysis of the ethanolic extract of *S. foetida* seed and they were confirmed further that among 35 bioactive compounds many of them possess pharmacological activity and these findings are similar to the present work.

Table 3: Phytoconstituents identified in the seed sample of Sterculia foetida using GC-MS

S. No	Retention time	Compound name	Molecular formula	Molecular weight	Structure	Activity
1	18.27	Oleic acid	$C_{18}H_{34}O_2$	282.468g/mol		Herbicide, Insecticide & Fungicide
2	17.53	10-Octadecenoic acid, methyl ester	$C_{18}H_{34}O_2$	282.468g/mol		Insecticide
3	26.25	Flavanthrone	$C_{28}H_{12}N_2O_2$	408.416g/mol		Anticancer
4	11.08	5-Undecyne	$C_{11}H_{20}$	152.281g/mol		Antioxidant
5	16.05	Flavone	$C_{15}H_{10}O_2$	222.243g/mol		Anticancer
6	16.6	Palmitic acid	$C_{16}H_{32}O_2$	256.43 g/mol		Herbicide
7	19.8	Retinal,9-cis	$C_{20}H_{28}O$	284.443g/mol		Cell differentiation & Embryonic development
8	20.75	Methyl abietate	$C_{21}H_{32}O_2$	316.485g/mol		Antioxidant
9	21.5	Estra-1,3,5,(10)-trien-17a-ol,3-methoxy-17-(2 methlallyl)	$C_{18}H_{22}O$	254.373g/mol		Antibacterial

10	22.15	2-cyclohexen-3-ol-1-one,2(11-phenylundecanoyl)	$C_6H_{10}O$	98.145 g/mol		Antidiuretic
11	22.15	3,9-Methano-10 H – furo[3,2-d] azonine-10,11-dione , 9-[2]dimethyl amine]-3-methoxyphenyl] decahydro-2,6-dimethyl-[2 R^X,3R^X,3a5^X,9R^X,10AR^X]	$C_{12}H_9NO$	183.21g/mol		Antibacterial & Antidote
12	22.73	Androstan-6-0l-17-one,3-acetoxy-5A-chloro	$C_{21}H_{32}N_2O$	328.5 g/mol		Vasodilator & Antimicrobial
13	23.78	Testosterone Cypionate	$C_{27}H_{40}O_3$	412.614g/mol		Anti-inflammatory



(a) Flavanthrone (b) Flavone (c) 5-Undecyne (d) Methyl abietate

Fig 2 (a-d): GC-MS graphs showing the compound possess anticancer and antioxidant activity

IV. CONCLUSION

On the basis of above findings, it can be concluded that the phytochemical screening and fluorescence analysis confirm that the methanolic extract of *Sterculia foetida* seed contains various pharmacologically active secondary metabolites. Furthermore, there are thirteen pharmacologically active biomolecules were identified through GC-MS study. Further studies are required to find out the efficacy of methanolic extract of *Sterculia foetida* seed as it may offer an effective alternative source against many diseases with less to no side effects.

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Dynamics of rubber cultivation in production systems in mountain zone of Côte d'Ivoire

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Abstract— *In Côte d'Ivoire, rubber cultivation is experiencing spectacular development. With land saturation in its traditional growing zones, rubber trees increasingly occupies new regions, including the west of the country. The objective of this study is to assess the place of rubber trees in the dynamics of production systems in the semi-mountainous region of western Côte d'Ivoire.*

This study was carried out through a survey carried out among nearly 402 rubber planters from the Tonkpi region and part of the Guemon region. A semi-open questionnaire was used to collect data, particularly those relating to the profile of the farmer and the relationships that prevail between rubber trees and other crops.

Data analyzes have shown that the total area of production systems is 2758.12 ha, or 6.86 ha per farmer, and that of rubber trees is 1268.69 ha, or 2.41 ha per farmer. The average age of rubber fields is 8 years in 2019. Rubber plots were established between 1990 and 2019, of which 84 pc between 2007 and 2015. Planters use perennial polyculture in 76.87 pc of cases. Rubber cultivation occupies 46 p.c. of cultivated areas with coffee (45.63 p.c.) as the dominant previous crop. The rubber tree is grown in combination in 67.87 p.c. of cultural situations. Food crops are mostly (86.83 p.c.) used in associations.

Rubber cultivation occupies half of the production system in western Côte d'Ivoire and the relations which prevail between rubber trees and the other components of this system are, on the one part, relations of substitution of rubber for perennial cultures (Coffee) and on the other part relations of association with food crops.

Keywords— ***Rubber, Production systems, Previous crop, Cultural association, Man, Côte d'Ivoire.***

I. INTRODUCTION

Since the independence of the Côte d'Ivoire in 1960, its agriculture has been dominated by the coffee-cocoa duo. The fluctuation in world prices of these two crops, coffee and cocoa, observed since the 1980s, has imposed on the State of Côte d'Ivoire a policy of diversification of export crops through the introduction of oil palm, coconut tree, cotton tree, cashew tree, sugar cane and rubber tree (Kéli, 2003). This latter speculation, initially developed by the large-scale farming sector, is now mainly carried out by small peasant farms (Ruf, 2009). First (1st) African producer of natural rubber since 1997, Côte d'Ivoire is now 6th in the world, with an estimated area of over 534,000 hectares for a production of 603,000 tons of dry rubber in 2017 (Kouassi, 2018).

Thanks to the improvement in the prices of natural rubber, the Côte d'Ivoire has defined a vast program of intensification and extension of rubber cultivation. This program, in support of smallholders, provided for the creation of 30,000 ha per year, with the objective of reaching a production of 600,000 tonnes of rubber by 2020 (Wahounou et al., 2013). But very quickly, the Côte

d'Ivoire was confronted with a land saturation in the traditional areas of rubber cultivation. This has resulted in a remarkable extension of village rubber growing to new geographical areas such as the West (Gnagne et al., 2016a), which until then was a coffee-growing region and where there are already land problems linked to occupation one third (1/3) of its area by mountains and boulders (ANADER, 2014 ; Keli, 2017).

The objective of this study is to determine the place of rubber trees in the dynamics of production systems in western Côte d'Ivoire.

II. RESEARCH PROCEDURE

The site of study

The study was carried out in four (04) departments of the Western Directorate of SAPH (Société Africaine de Plantations d'Hévéa) in the west of Côte d'Ivoire (Figure 1). These are the departments of Biankouma, Danané, Kouibly and Man. The coordinates of the study area are: 7 ° 13'0 "North latitude, 7 ° 40'60" West longitude and 329 m altitude (Anonymous, 2017). The climate there is tropical humid, characterized by a single-mode rainfall, with a long rainy season from March to October (CNRA-Man, 2017). The average annual rainfall from 2013 to 2017 was 1736.72 mm. (CNRA-Man, 2018). The vegetation is dominated by numerous fallows of *Chromolaena odorata* and *Alchornea cordifolia* and of coffee and oil palm plantations (ANADER, 2014; Mameri, 2019). The relief is marked by a set of mountain ranges. In this rocky set of 500 to 1000 m altitude and with steep slopes, the highest summit is Mount Tonkpi which rises to 1189 m altitude (ANADER, 2014). The soils are mainly ferralsols (WRB, 2004). Their cultural suitability is generally good, apart from any physical constraint, breastplate and boulders (Bahan, 2016). The indigenous populations are of the Yacouba (Dan) ethnic group and the Touras. Non-natives from the Center, the North of Côte d'Ivoire and the sub-region represent 30% of the population (Ehua, 2000). The economy is based primarily on cash crops (coffee, cocoa, rubber and oil palm). Finally, the main food crops in the region are rice, cassava and maize.

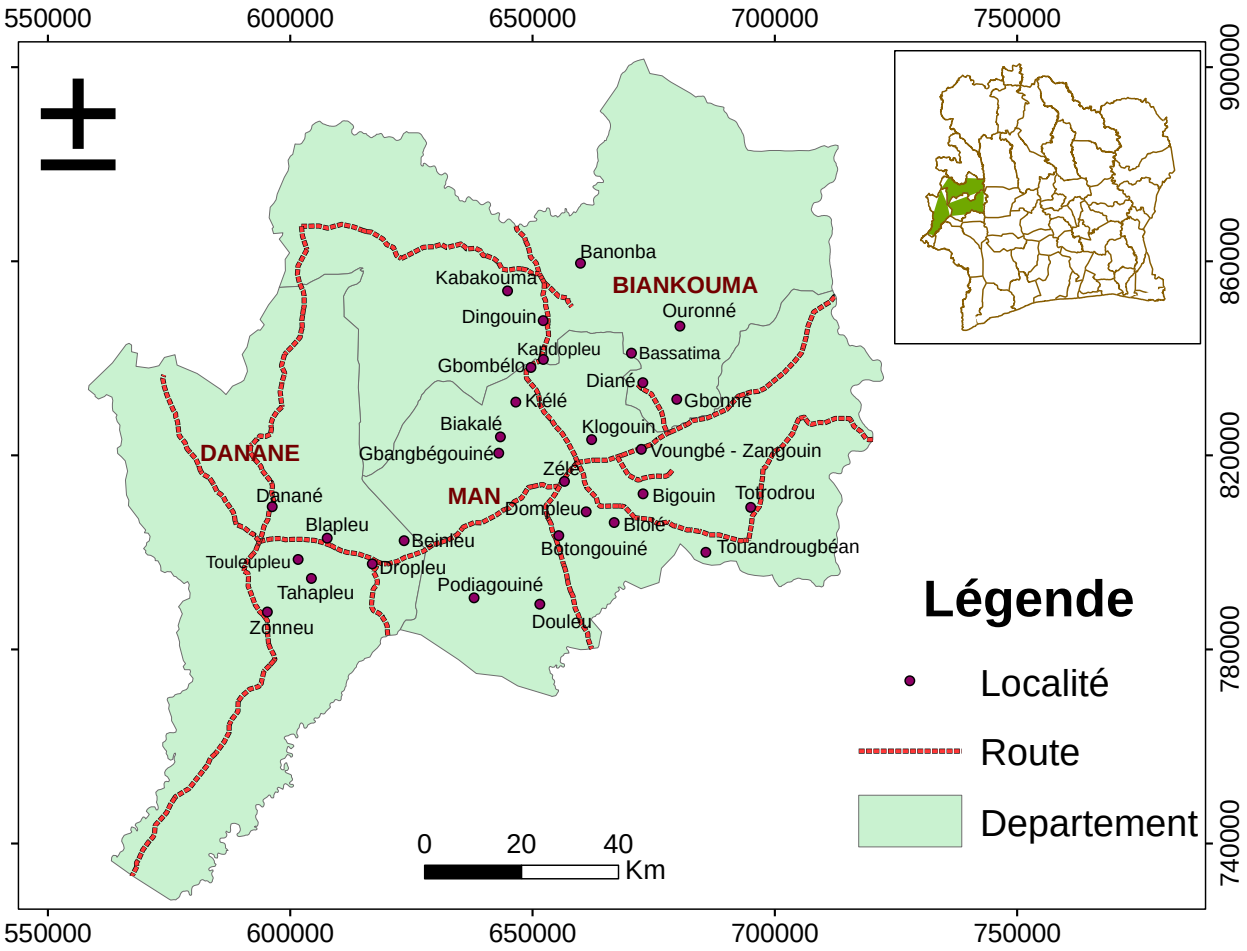

Fig.1: Survey area

Preliminary investigation

A preliminary survey was carried out with resource persons from research and supervisory structures, in order to constitute a database necessary for sampling rubber farms. This database includes the number of rubber planters, the areas of the plots, and the years of creation of rubber fields in the Tonkpi region.

Sampling

The sample was obtained following a random selection carried out among rubber growers in each department so that it is representative. The formula below, which is the one recommended for calculating the size of a finite population sample, allowed us to determine the sample size for a confidence level s = 95 pc (very often used level), t = 1.96 and the "p" proportion to be estimated is nearly 50 p.c.

$$n = \frac{1.96^2 N}{1.96^2 + (2e)^2(N-1)}$$

• N: Size of parent population

• n: Sample size for a parent population

• e: Margin of error that we give ourselves for the size that we want to estimate (for example we want to know the real proportion to within 5 p.c.)

Data collect

Data collection consisted of individual interviews with rubber growers and observations made in the fields. The data collected focused on the areas and the installation dynamics of rubber trees.

Data analysis

The data were entered on Excel 2010 software which was also used to determine the averages, frequencies and to produce the graphs. Descriptive statistics and crossover tables were performed on Stata SE 14 software.

III. RESULTS

The surveys focused on 402 rubber farmers, with 526 rubber farms, spread over 49 villages including 13 in the department of Biankouma, 11 in the department of Danané, 2 in Kouibly and 25 in the department of Man. The villages visited are grouped into 12 different sub-prefectures.

Place of rubber in production systems

Analysis of survey data revealed that 76.87 p.c. of planters are using perennial polyculture. This rate differs from department to department. It is 89.39 p.c. for Biankouma, 90.43 p.c. for Danané, 88.24 p.c. for Kouibly and 64.22 p.c. for Man. Food crops are practiced by 74.63 p.c. of respondents. The total area of the rubber growers' production systems surveyed is 2758.12 ha with an average of 6.85 ha per farmer (Table II). The most important production systems per farmer are those of Danané (8.61 ha) and the weakest are those of Man (6.02 ha).

The total area of rubber tree plantations in this production system is 1268.69 ha (Table I). These areas vary from 0.33 to 20 ha with an average of 2.41 ha. The largest areas are in the department of Danané (2.8 ha per farmer) and the smallest in Kouibly (1.29 ha per farmer). The majority (73.77 p.c.) of rubber growers have plots of less than 3 ha (Figure 2). This proportion, which varies from one department to another, is between 69.41 p.c. (Man) and 94.74 p.c. (Kouibly).

Rubber cultivation thus occupies 46 p.c. of the farmland in the semi-mountainous region of Côte d'Ivoire (Figure 3). This rubber tree occupancy rate is higher in Man (52.51 p.c.) and lower in Kouibly (23.49 p.c.).

Dynamics of the establishment of rubber plots

The prospected plots were established between 1990 and 2019. The first two fields were established in 1990 in Man and Biankouma. These creations were followed by two other plots set up in 1997 in Danané. The big wave of establishment took place between 2007 and 2015 with 84 p.c. of field creation (Figure 4). The average age of the fields is 8 years in 2019.

Table I : Statistics of rubber cultivation in production systems in the semi-mountainous west of Côte d'Ivoire (2019)

Département	Total rubber area	Totale exploitation area	Average area rubber	Average area exploitation	Ratio Hévéa/Expl (p.c.)
Biankouma	173,59	434,87	2,00	6,59	39,92
Danané	425,7	990,65	2,80	8,61	42,97
Kouibly	24,55	104,5	1,29	6,15	23,49
Man	644,85	1228,1	2,41	6,02	52,51
Total	1268,69	2758,12	2,41	6,86	46,00

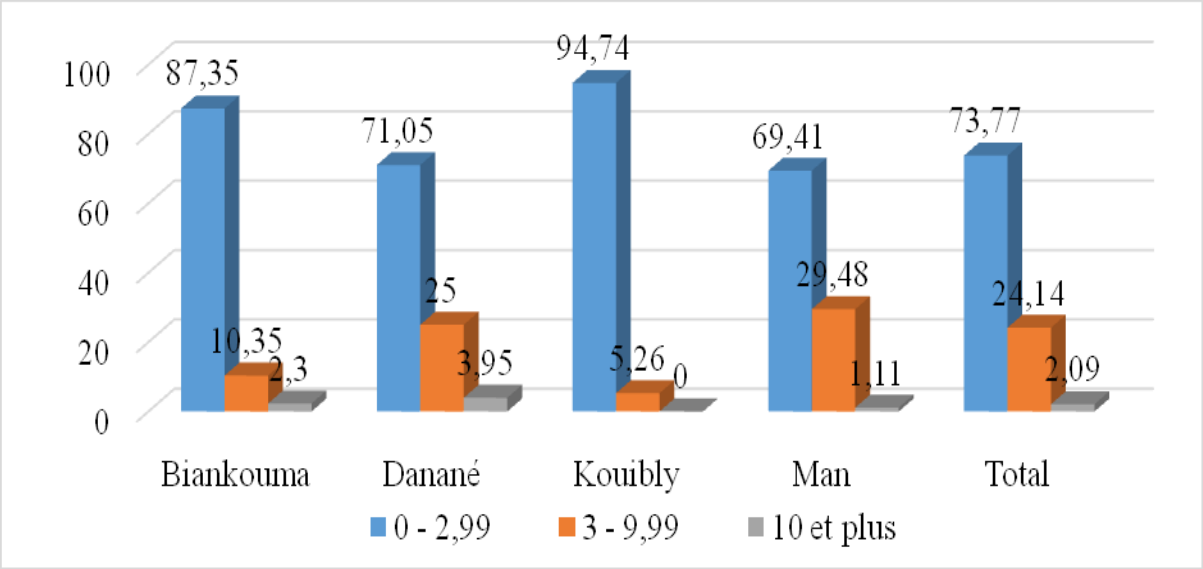

Fig.2: Surface area of rubber plots

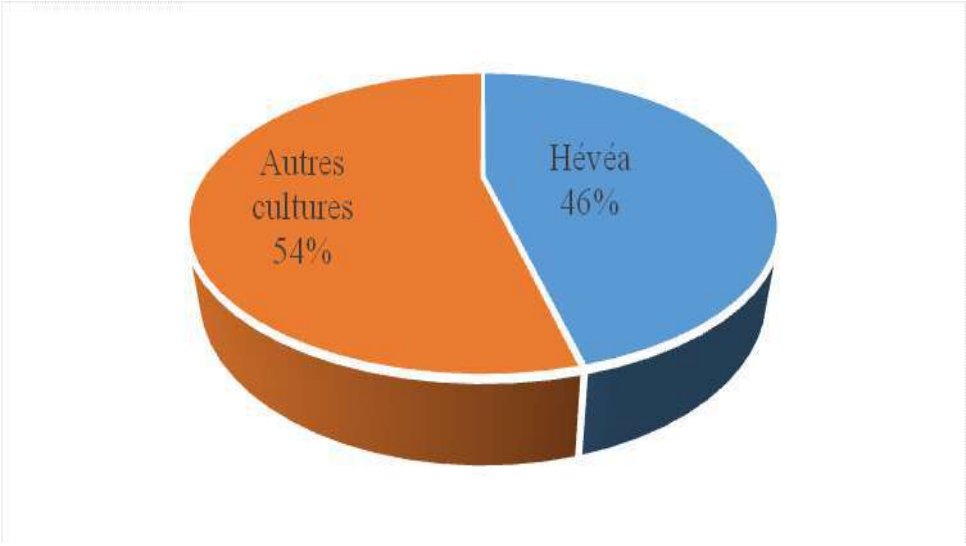

Fig.3: Place of rubber cultivation in the production system in the semi-mountainous west of Côte d'Ivoire

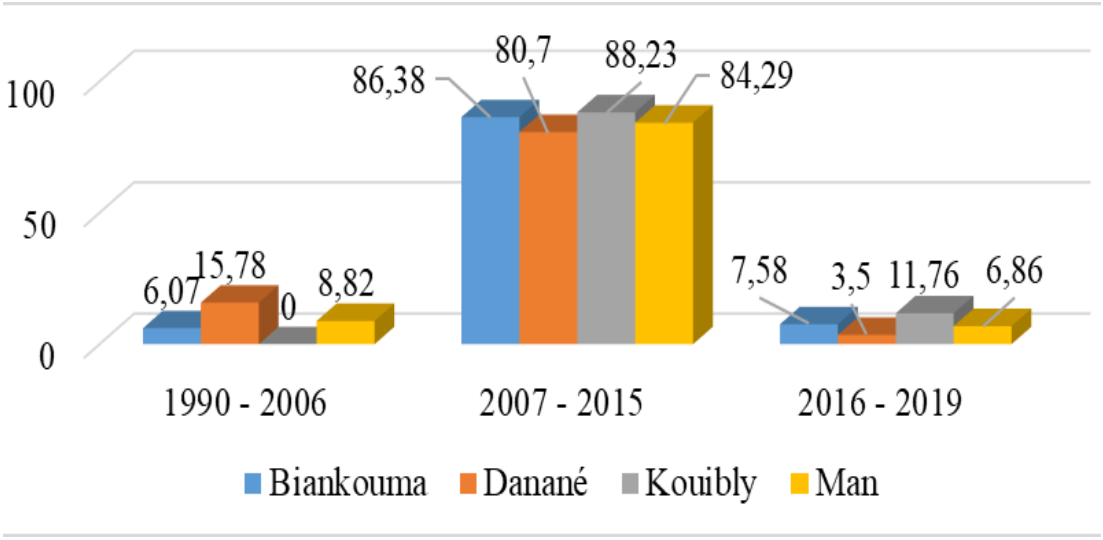

Fig.4: Installation period of rubber plots in the semi-mountainous west of Côte d'Ivoire (2019)

Previous crops of rubber

During the surveys, fallow, coffee, forest and crops were the four (04) previous crops observed. The previous dominant crop is coffee (45.63 p.c.) (Figure 5). However, the predominant crop precedent varies from one department to another. In Biankouma, fallow is the most important precedent (48.28 pc) followed by coffee with 39.08 pc In Danané and Man, coffee is the majority precedent with respective rates of 49.34 pc and 47.76 pc of sown fields. The department of Kouibly presents a completely different configuration with fallow as the previous culminating crop (36.84 p.c.), followed by forest (31.58 p.c.). Coffee and other perennial crops each represent 15.79 p.c. of previous crops in the Kouibly zone. Regarding the age of fallows, it varies from one (01) to ten (10) years with an average of 4 years.

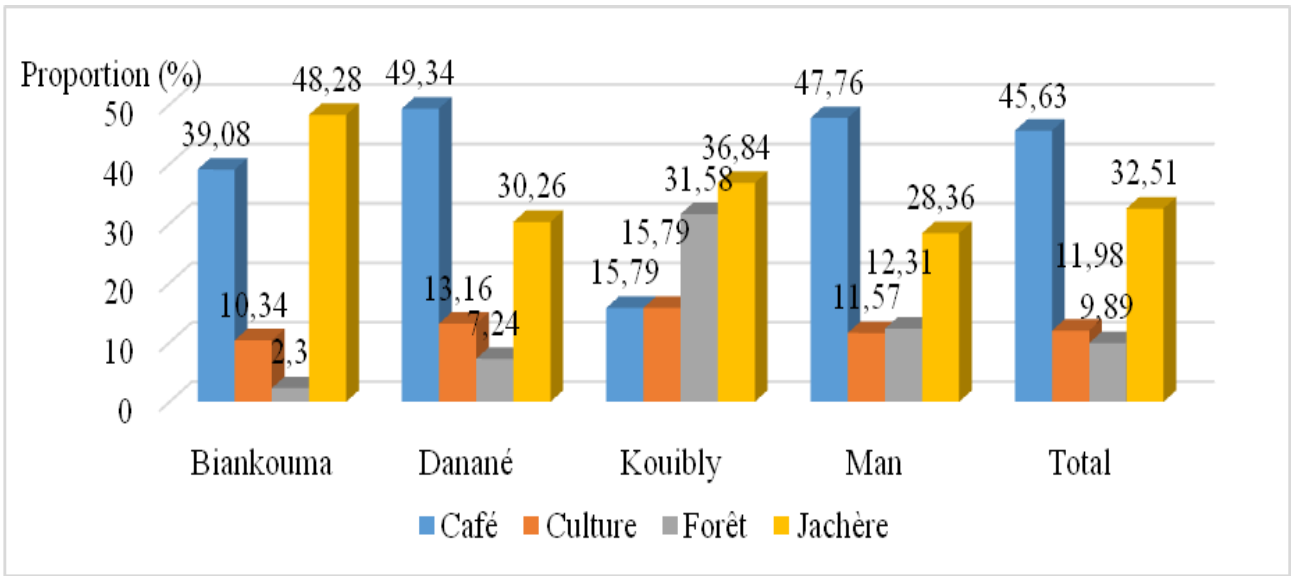

Fig.5: Previous crops of rubber plantations in the semi-mountainous west of Côte d'Ivoire (2019)

Crop associations involving rubber

The cultivation systems practiced relate, in 67.87 p.c. of cases, to associated cultures (Figure 5). The duration of associations with rubber varies from two (02) to ten (10) years, depending on speculation. Food crops (peanuts, rice, cassava, yam, beans, plantains and corn) are associated with rubber in 86.83 p.c. of cases. Export crops (Pineapple, Coffee, Cocoa and Cola) are associated with rubber trees in 7.56 p.c. of cases. Food-perennial combinations are associated with rubber trees in 5.6 p.c. of cases. The rubber

associations in the semi-mountainous West have involved eleven (11) speculations, including seven (7) food crops and five (5) exports (Figure 6). These associations involve culture in 45.10 p.c. of cases and a combination of cultures in 54.90 p.c. of associations. The crops most associated with rubber in western Côte d'Ivoire are maize (58.82 p.c.) and rice (49.86 p.c.). In associations with rubber trees, food crops are laid out in bulk in the rows of trees.

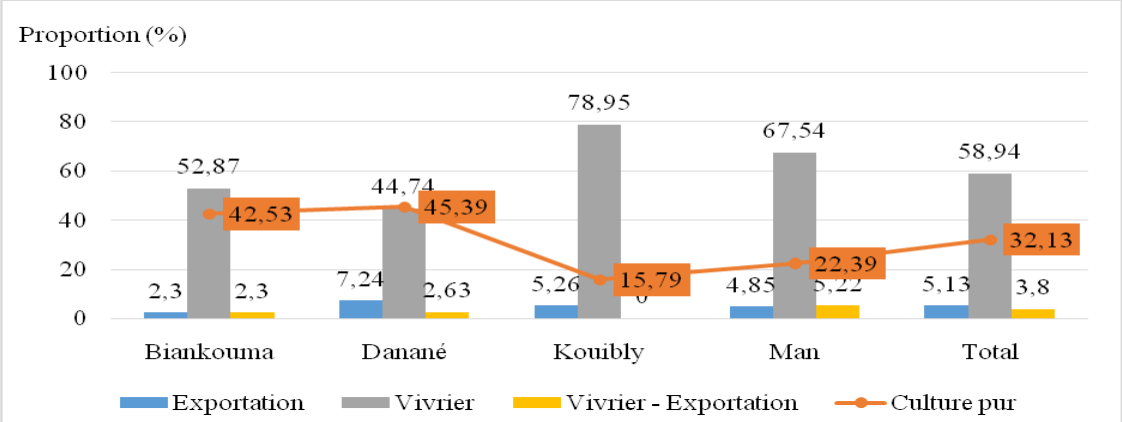

Fig.5: Associations with rubber

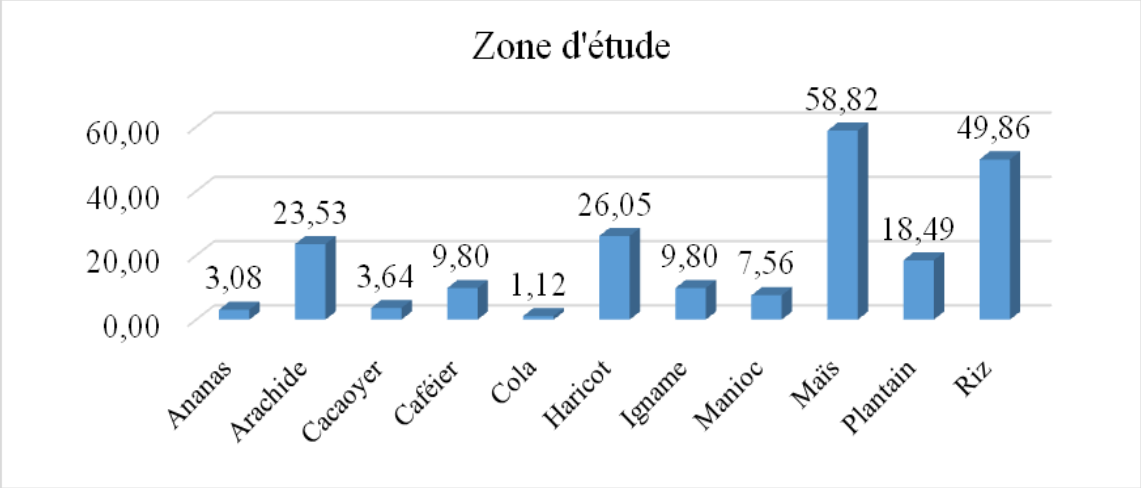

Fig.6: Crops associated with rubber

IV. DISCUSSION

Place of rubber in production systems

Surveys show that the orientation towards rubber cultivation does not exclude the presence of other perennial crops, notably coffee, cocoa, oil palm, cashew and cola. The total surface area of the rubber growers surveyed is 2758.12 ha, or 6.86 ha per farmer. This area is greater than that of Akiés planters (Attiés) which is 3.4 ha (Ruf, 2018). Farms with only rubber as an export crop represent 23.13 p.c. of cases. According to Ruf (2018), young planters are more interested in rubber cultivation as they easily overcome six years of unproductivity. This could justify the fact that some growers only engage in rubber cultivation.

The total rubber area surveyed is 1268.69 ha. These areas vary from 0.33 to 20 ha with an average of 2.41 ha. This average area per farmer is lower than that of the Songon sub-prefecture, which is 3.91 ha (Kouamé, 2014). The majority (73.77 p.c.) of rubber growers have plots of less than 3 ha. This finding could be explained by the presence of rock massifs, which occupy a third of the study area and which considerably limit the area of cultivable land. Population growth could also have had an impact on the size of the plots (Keli, 2009). According to Koua et al., (2018), The size of cocoa plantations in the departments of Abengourou, Divo and Soubré are increased by plantations of 2-5 ha (51 p.c.). These results show that perennial crops are still the business of "small planters" (ICCO, 2015). This low proportion of arable land is probably linked to the non-mechanization of Ivorian agriculture. The small size

of the orchards could also be explained by land saturation and the exhaustion of the country's forest reserves (Ruf and Allangba, 2001; FAO, 2007; Aka et al., 2013). Rubber cultivation occupies 46 p.c. of the farmland in the semi-mountainous region of Côte d'Ivoire. The highest rate was observed in Man (53.07 p.c.) and the lowest in Kouibly (20.54 p.c.). Coffee and cocoa occupy 26.44 pc and 15.26 pc respectively. ancient cultures. According to ICCO (2014), L'hévéa is emerging as a diversification crop again in 2017, despite the level of land saturation.

Dynamics of the establishment of rubber plots

The prospected plots were established between 1990 and 2019. Thus the age of the rubber orchard in the semi-mountainous region of Côte d'Ivoire is between 1 and 30 years. The average age of the fields is 8 years in 2019. On the other hand, the entry of rubber cultivation in Côte d'Ivoire dates from 1953 by agro-industrial companies through large farms (Losch, 1983; Canh, 1999; Hirsch 2002; Ruf 2013; Kouamé 2014). In 1978, rubber cultivation integrated family farming through small farms (Keli et al., 1997; Ruf, 2009). This shows that the cultivation of rubber trees in western Côte d'Ivoire is young. Given the operating life of 30 to 40 years, rubber trees could have a promising future in the west of the Ivory Coast.

Also, the first two fields were established in 1990 in Man and Biankouma. These creations were followed by two other plots set up in 1997 in Danané. According to Ruf (2009), the Ivory Coast owes the doubling of its production in 8 years to the village sector whose performance is approaching each year the production threshold of industrial plantations. Thus, the big wave of establishment is between 2007 and 2015 with 84 p.c. of fields created. According to Koulibaly (2016), rubber cultivation has increased dramatically with an increase of 479.38 p.c. in its recorded areas. This would be due to the price of the kilogram of rubber which increased from 2001 to 2010. It went from three hundred and seventy-five (375) FCFA to one thousand (1,000) FCFA. According to Akmel (2018), this state policy aimed to encourage actors, so that they become more involved in rubber production. This is corroborated by the increase in production to 603,000 tonnes in 2017 (Kouassi, 2018). Furthermore, the rationalization of income created by rubber cultivation due to the monthly payment of production could justify the massive investment of young people in rubber. Also, rubber farmers participate in the restoration of the green fabric.

Previous crops of rubber

The previous dominant crop is coffee with a proportion of 45.63 p.c. This shows that rubber trees are in the process of replacing coffee. The substitution of rubber for other export crops has also been revealed by surveys in the Dabou region in Côte d'Ivoire (Akmel, 2016). The phenomenon of substitution of rubber tree plantations for old cocoa or coffee trees is not new (Ruf, 2009). The development of rubber cultivation has taken place at the expense of other perennial crops in Songon and mainly of oil palm. Also, 12% of farmers replaced cocoa and coffee farms with rubber (Kouamé, 2014). According to Aguilar et al. (2003) cited by Akoua et al. (2018), the small size of cocoa plantations in the departments of Abengourou, Divo and Soubré would be linked to the abandonment and conversion of some old cocoa trees into palm oil plantations. and rubber (Ruf and Allangba, 2001; Kassin, 2009). Land blockades and the growing interest in rubber cultivation could account for this trend. However, the predominant crop precedent varies from one department to another. In Biankouma, fallow is the most important precedent (48.28 p.c.). The department of Kouibly presents fallow as the previous culminating crop (36.84 p.c.), followed by forest (31.58 p.c.) then coffee (15.79 p.c.). Regarding the age of fallows, it varies from one (01) to ten (10) years with an average of 4 years. The fallows are between 5 and more than 10 years old and belong to adults. One third of the mainly young farmers have no land reserves. The low percentage of the previous forest corroborates the observations on the decline of forest massifs in Côte d'Ivoire. Forest areas, which amounted to 15 million ha in the 1900s (SODEFOR, 1996) represented only 2 million ha in 2007 (FAO, 2007).

Crop associations involving rubber

The cultivation systems used relate, in 67.87 p.c. of cases, to associated cultures. This rate is lower than that of the Songon sub-prefecture which is 85.43 p.c. of farmers surveyed (Kouamé 2014). Cultural association could allow diversification of production and sources of income. However, according to the farmers surveyed, the cropping association in the semi-mountainous region of Côte d'Ivoire is motivated by the simultaneous maintenance of associated crops (48.61 pc) and the lack of land (44.44 pc). Benefits of this practice of combining annual crops with trees may include food security for households, income generated from the sale of both products, weed control and better use of cultivated resources (Balogoun et al., 2014). According to Kouamé (2014), To fill the land shortage, the populations practice the association of cultures. The duration of associations with rubber varies from two (02) to ten (10) years, depending on speculation. In fact, during the immaturity period of the rubber tree (6 years), producers form associations to ensure food security during this period, create sources of income and implement cultivation techniques likely to help the growth.

maintenance of the rubber tree. In addition, intercropping brings organic matter to the soil through the decomposition of crop residues. Monoculture with leguminous cover (often Pueraria) between the rows of rubber trees is a satisfactory practice from an agronomic point of view thanks to the fight against erosion and against weeds, supply of nitrogen and maintenance of humidity (IRRDB, 1996). The Hévéa brasiliensis species is actually cultivated in association with other domesticated species such as cover legumes (Pueraria phaseoloïdes, Centrosema pubescens, etc.), food and industrial species (Obouayeba et al., 2016). The crops set up on the whole of the young plot, accept a valuation of the land while guaranteeing a maintenance of the space beneficial to the development of the young rubber plants. The crops grown depend on the eating habits of the farm manager and his family. These are usually mixtures of species including plantain, corn, macabo, peanut, pistachio, yam and sweet potato. (Thierry, 2005). The rubberwood associations in the semi-mountainous West have involved eleven (11) speculations, including seven (7) food crops and five (5) exports. These associations involve culture in 45.10 p.c. of cases and a combination of cultures in 54.90 p.c. of associations. Associated food crops meet the self-consumption needs of growers and / or marketing (Kéli et al., 2006). Thus, food crops (peanuts, rice, cassava, yam, beans, plantains and corn) are associated with rubber in 86.83 p.c. of cases. In Songon, 48.27 p.c. of plots of food crops (maize, yam, vegetables) and mainly cassava were also planted with rubber in these two localities (Kouamé, 2014). The crops most associated with rubber in western Côte d'Ivoire are maize (58.82 p.c. of associations) and rice (49.86 p.c. of associations). This is corroborated by the fact that 73.25 p.c. practices the association with the objective of directly consuming the product of the harvest. Rice and corn therefore dominate the associations in Man. However, cassava, one of the staple foods in the region, is not associated with rubber, presumably for fear of the spread of root rot caused by the genus Fomes. Cassava is a vector of this disease. Therefore, the rubber-cassava combination has long been discouraged. However, recent work by the CNRA, led by Boko in 2012, has shown the possibility of leading such a cultural association. Cassava should be planted at a distance of at least 1.5 m from the rubber tree line. In addition, it was observed that the food crops associated with rubber trees were sown or planted in bulk, not respecting the technical recommendations of the research. In rubber associations, food crops are arranged in bulk in the rows of trees. Export crops (Pineapple, Coffee, Cocoa and Cola) are associated with rubber trees in 7.56 p.c. of cases. Food-perennial combinations are associated with rubber trees in 5.6 p.c. of cases. These associations are not permanent because they only last 5 years. Hevea is intended to replace associated cultivation as is the case in Brazil, where rubber-coffee associations only last about ten years (Penot & Ollivier, 2009). The rubber tree can be associated with fruit trees and forest species; cases of the Philippines, Malaysia, Thailand, Brazil; with tea; the case of China; rattan; cases of Malaysia and the Philippines; and the cocoa tree; cases of Côte d'Ivoire, Gabon, Brazil and Vietnam (Ruf et al., 2006; Penot & Ollivier, 2009).

V. CONCLUSION

The objective of the study was to assess the place of rubber trees in the dynamics of production systems in western Côte d'Ivoire. The study showed that 76.87 p.c. of planters are using perennial polyculture. The total surface area of the rubber growers' production systems surveyed is 2758.12 ha with an average of 6.85 ha per farmer and that of rubber trees is 1268.69 ha or 2.41 ha per farmer. Rubber cultivation, which thus occupies 46 pc of sown areas, could be considered as a substitute crop for old crops. The age of the rubber orchard is between 1 and 30 years with an average of 8 years in 2019. The great wave of he establishment is between 2007 and 2015 with 84 pc of field creation. Considering the juvenile age of rubber trees in the west, there could be a bright future.

In perspective, effective associations should be determined for optimal use of agrarian space. This study will overcome the problems of substituting rubber for other crops in the study area with a view to diversified and sustainable agriculture.

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Impacts of Dams on the Environment: A Review

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Abstract— *Dams are very important for generating electricity and providing drinking water. However, they have major negative impacts on water quality, aquatic ecology, land, terrestrial wildlife, vegetation, and air quality. Mitigation measures should be put in place to reduce these negative impacts on the environment. For example, to reduce the impact on water quality, sediment control drainage should be constructed. To control chemical pollution, containers should be provided for chemical waste. With respect to climate and GHG emissions, it is critical to clear vegetation prior to reservoir filling. Overall, dams negatively affect the physical and biological environment due to the various activities, during construction, filling the reservoir and during the operation of the reservoir and dams.*
Keywords—***Dams, environment, impacts on climate, reservoir, water quality.***

I. INTRODUCTION

Population growth and development in technology and constructions such as building large cities and building roads, bridges and dams have led to an imbalance in the ecosystem and a loss of the ecological balance (Huesemann and Huesemann, 2011). Day after day, the interest in the environment increases, and as the interest in it increases, contradictions abound between environmental officials and engineers who know that the construction of engineering projects, especially dams, causes a lot of damage (Gadgil and Guha, 1994). From other aspects, we know that the construction of engineering buildings or industrial projects contributes to the development and development of the country from all aspects, economically and socially (Tibaijuka, 2009). When planning the construction of any project, you should pay attention to the environmental problems and how this project will affect the community and the organisms, it is not necessary to pay attention only to the economic value (Richter *et al.*, 2010). Among the development projects, large dams are of great interest to the agencies and the World Bank (Ledec and Quintero, 2003). Environmentalists talked about the environmental and social impacts resulting from the construction of dam projects, such as population migration and the change in biodiversity, while the owners of hydroelectric projects talked that dams are a source of renewable energy (Basson, 2004). Dams produce many negative impacts on the environment and society, including during construction, and these end after the completion of construction, but the most serious impacts are in the operational phase, which lasts for thousands of years. It may also result from civil works, such as building roads, drilling, power transmission lines, and others. This paper summarizes the adverse of environmental impacts associated with dams during the reservoir impoundment, construction and operation, along with the typical kinds of mitigation measures.

II. ENVIRONMENTAL IMPACTS FOR DAMS

There are many potential impacts of dams on the physical and biological environment at the construction site, reservoir area and downs stream areas. These impacts were summarized in (Tables 1 and 2). We will discuss them in details as flow:

2.1. Impacts on Water Quality

Dams are being constructed due to the increasing demand for water and energy, however there are negative environmental impacts resulting from dams.

2.1.1. During Construction

The construction sites may be the origin of water pollution resulting from activities inappropriately managed and monitored, which may affect water quality downstream. Impacts typically observed in such circumstances concern the release of excessive sediment loads in the river stream, the release of chemicals (Skaggs *et al.,* 1994). Moreover, the distribution and cycling of chemical compounds in water bodies, such as dams, are closely related to water movement in the landscape and are influenced by processes in the hydrological and biological cycles (Lewis, 2002).

2.1.1.1. Excessive Sediment Release

Release of high sediment load in water may occur mainly during works in or close to river bed: diversion channel construction, excavation works at dam site, construction of protection dikes and coffer-dams, quarrying works, sand borrowing in river bed, creation of spoil areas too close to the river bank or with unstable slopes, etc. All these activities may have significant impacts on the sediment load discharged into the river (Wahlstrom, 2012). Most of these works will occur during the dry season, when the river flow is low. During the works, water infiltrated from the alluvial aquifer into the excavation will need to be pumped. Pumped water with heavy sediment load is frequently just discharge into the nearby stream generally consisting of low flow of very clean water drained from the superficial aquifers (Jean-Baptiste *et al.,* 2007).

The high sediment loads may reach the river at the beginning of the rainy season, when the first heavy storms wash out unstable slopes of spoils or bare soils in the construction sites or camps, or along the access roads. However, the impact at this period of the year is less detrimental because of the much higher flows in the river which carry already a high sediment content (Visser *et al.*, 2002 and Murakami, 1995).

2.1.1.2. Pollution from Hazardous Substances

During the construction of dams, a large amount of gasoline, lubricant products, and a large amount of explosives and chemicals (concrete topping, solvents, paints, thinners, acids) will be stored and processed at the construction site, and there is a risk of leakage or accidental leakage surroundings. This risk can be efficiently reduced by the implementation of preventive management procedures by the contractor: appropriate location of storage areas with a design complying with international good practices (bonded storage), collection and recycling of used oils, monitoring of all hazardous products with specific handling procedures and contingency plans (Webb *et al.,* 1995 and Basnyat *et al.*, 2000). Another source of pollution is represented by the batching plants, and particularly by the effluent from concrete truck cleaning which consist of wastewater with high pH and contaminants from the concrete additives. In case of direct release into the river, there may be severe effects on the downstream population which relies on the river for domestic and cattle water supply, washing and irrigation (Ellison, 2007).

2.1.1.3. Pollution from Domestic Wastewater

The operator's region and other temporary worker camps will probably accommodate few thousands of workers. Without appropriate sanitation system and wastewater treatment, the release of pathogens and Coliforms in the river may severely affect the population downstream (Jafarinejad, 2016).

2.1.1.4. Pollution from Solid Waste

During the construction of the dam, a very large amount of solid waste will be generated either from municipal or construction principle. Most of these waste, if not appropriately managed, may result in soil and water pollution, with possible detrimental impacts on the environment and on public health. Domestic waste will be produced by the worker camps (Chandrappa and Das, 2012; Tatsi and Zouboulis, 2002).

2.1.2. Impacts during Reservoir Impoundment

2.1.2.1. General Consideration

As the terrestrial area becomes flooded with rising water immediately following dam closure, several processes occur which have a dramatic effect on the impounded water quality. An eutrophication process will be initiated, where plants, starved of atmospheric oxygen die and start to decompose which, when combined with the decomposition of the organic matter from the top soil, depletes the dissolved oxygen of the reservoir water. If this deoxygenated water is not replaced quickly enough, then anoxic conditions will develop at the bottom of the reservoir (Spoor, 1990).

Hypoxia causes a reducing environment that changes insoluble compounds into soluble ones with the help of anaerobic bacteria with considerable unfavorable consequences: liberation of ammonia, metals such as iron, manganese, sometimes mercury. Additionally, Sulphate will be reduced to the highly acidic hydrogen supplied by bacteria which can corrode metal components of the turbines (Naja and Volesky, 2009). The riskiness of these changes is very harmful to both the aquatic life and the electro-mechanical equipment. The extent and severity of the anoxic region depends upon several parameters, the major being the quantity of organic matter flooded and the characteristics of the reservoir. Another consequence of the vegetation flooding is the release of nutrients such as nitrogen and phosphorus, which participate in the eutrophication process of the reservoir, with dramatic

consequences on water quality in the long term (Goldman, 1976 and Ashton *et al.*, 2001).

2.1.2.2. Flooded Biomass

The most active part of the biomass regarding the decay process and the consumption of dissolved oxygen is the soft biomass: fresh grasses, tree leaves, fruits and twigs. Hard biomass, represented by wood, decays very slowly in water, and if permanently flooded can stay for decades. Organic matter located in the first few centimeters of top soil contributes also quickly with the soft biomass to the fast decay process. The denser the vegetation cover flooded, the higher the degradation of the water during the first few years after impoundment (Zimmer, 2008).

2.1.2.3. Biomass Reduction

Some pre-impoundment measures can be taken in order to reduce as much as feasible the risk of water alteration. The most evident measure is the removal of the fresh vegetation by clearing. Two measures can be considered:

-Grassland, scrubland and fallow land located within the reservoir: burning of the vegetation during the last two or three months before impoundment, to avoid any significant regrowth of vegetation.

-Forested areas: these are small and localized areas mainly located in the upstream part of the reservoirs. Physical clearing with collection of wood by the local population and burning of the wood waste not collected.

Burning is advisable both in terms of water quality preservation and global warming prevention. Indeed, burning consumes atmospheric oxygen and releases carbon dioxide while decay consumes dissolved oxygen in water and, when oxygen is no more available in water (this happens very quickly), it turns to anaerobic process with the release of methane gas. This situation is more detrimental in terms of global warming, because methane is 25 times more damaging than carbon dioxide (Miranda, 2001and Coutant, 1999).

2.1.2.4. Floating Debris

If there vegetation cover, it is anticipated significant volumes of floating debris during the impoundment stage. Some facilities will be required at the spillway to collect and haul any major debris (tree) brought by the flood (Erikson, 1976).

2.1.2.5. Impacts on the Downstream Zone

As soon as the filling starts, some load of organic matter will be transported by the water and released downstream. The water will probably have a reduced dissolved oxygen content if not temporarily anoxic, and some fish kills could be observed downstream of the dam (Miller and Miller, 2007).

With an increased organic load in the downstream, the water may taste badly and could possibly be during few months incompatible with its use for domestic and animal drinking purposes. Typical mitigation measure is to provide the concerned villages with a water supply independent from the river water, generally hand pumps connected to the superficial alluvial aquifer (Baxter, 1977).

2.1.3. Impacts during Reservoir Operation

Forecasting of the most probable condition in the reservoir after impoundment and during the operation years is a perilous exercise. However, observation of phenomena in existing reservoirs all over the world and particularly in Africa has set up the basis for empirical modelling to assess mainly the nutrient and potential productivity in the future water body. Special criteria have thus been established to allow comparison and prevision, related mainly to the morphometry (shape) of the reservoir and to the water management (Gruner, 1963).

Predictions on the possible future characteristics of the Dams reservoirs are provided in the following:

2.1.3.1 Residence Time

The hydraulic residence time is related to the duration water is expected to stay in the reservoir. It is the ratio between the reservoir volume and the annual inflow. Indeed, the longer the water stay, the higher is the nutrient concentration and the risk of reservoir eutrophication. The faster the water is renewed in the reservoir, the lower is the risk of its quality degradation when compared with the inflow quality (Kunz *et al.*, 2011).

2.1.3.2. Shoreline Development

The reservoir shoreline development expresses the ratio between the reservoir perimeter and the circumference of a circle of an equivalent area. When a reservoir is highly dendritic, there is a risk of insufficient circulation and renewal of water in some isolated parts with local risk of eutrophication and aquatic vegetation development (Wetzel, 2001).

2.1.3.3. Temperatures and Reservoir Stratification

Temperatures prediction for reservoirs depend on geographical and meteorological criteria. The limited mean depth of the reservoirs means that a significant area of the reservoirs will consists of shallow waters which generally show higher temperatures (Loucks, 2017).

2.1.3.4. Electrical Conductivity and Salinity

Electrical conductivity or salinity should increase with time in the reservoirs, as a result of salt built-up resulting from evaporation. Could eventually pose a threat for irrigation (Mollema, 2016).

2.1.3.5. Nutrient Loading and primary Productivity

Since phosphor is generally the nutrient limiting factor for primary production, it is considered as the key criteria for anticipating on the eutrophication risk of a reservoir. Phosphor (P) is an important macronutrient. The productivity of aquatic systems decreases due to the scarcity or lack of bioavailability of the primary producers. Conversely, the addition of dissolved P to aquatic ecosystems often stimulates eutrophication, leading to blooms of algae, Phytoplankton or floating macrophysics on water surfaces (Carpenter *et al.*, 1998).

2.1.3.6. Reservoir Sedimentation

Rivers carry four different types of sediments downstream that allow formation of river banks such as deltas, silt, river drift phenomena, crescent-shaped lakes, dikes and coastal beaches. The construction of dams prevents the flow of these sediments to the downstream, which leads to the erosion of the riverbed from this sedimentary environment and the accumulated sediments in reservoirs or dams increase (McCully, 1996). The rate of precipitation varies between each dam and river and reservoirs put an end to water storage due to the exchange of storage space for sediments. Declining storage capacity results in a decrease in the capacity to produce hydropower, such as reduced availability of water for irrigation, and if left untreated, it may eventually lead to a failure in the dam and also in the river (Stevens, 2000).

2.1.4. Mitigation

2.1.4.1. Sediment Control

Drainage of construction sites and the use of sedimentation ponds in critical location inside or close to the river bed will be required from the concerned Contractors.

2.1.4.2. Pollution from Chemicals

Provide containers for all chemicals and chemical waste including fuel, engine oil and hydraulic fluids. Monitoring and recording of the quantities of used oils and used hydraulic fluids will also be required. These waste will constitute most of the hazardous waste produced on site. Contractors should have an option for recycling such as producing low grade diesel or using as an alternative fuel.

Obligate for contractors will also to prepare and implement a spill response plan including management of storage sites and equipment related to spill control (Kumari and Singh, 2018).

2.1.4.3. Pollution from Worker Camps

Worker camps must be equipped with a sanitation system in order to collect and treat all grey and black waters produced by the camps before discharge into the environment. Construction sites will be equipped with a sufficient number of toilets in order to control pollution discharge into the river streams.

Regular controls of faecal Coliforms must be carried out during construction in order to ensure treatment efficiency of the wastewater before discharge (Adams *et al.*, 2008).

2.1.4.4. Pollution from Solid Waste

The main contracting companies must develop action plan regarding solid waste management. This plan should cover (1) the management of domestic waste from the camps, (2) the management of non-dangerous construction waste and (3) the management of hazardous solid waste (Robles, 2010).

The necessary measures to treat polluted water (such as sewage treatment plants or enforcement of industrial regulations) may be needed to improve reservoir water quality. The selective forests within the reservoir area must be removed and disposed of before filling in order to avoid the decay of the submerged biomass that causes a decrease in the water quality.

2.2. Impacts on Aquatic Ecology

The closing of the rivers by the dams deeply modify the local aquatic ecology, which shift from river to lake habitat. The permanent floods inundate the surrounding wetlands, forests and other habitats in the river, and this leads to further disruption of the ecosystem that occurs along the banks of the river and the estuary, as the areas surrounding the banks of the river are characterized by being the richest in biodiversity (Ward *et al.*, 1998).

Dams suppress sediments that would naturally regenerate downstream ecosystems and because of this, some endemic species may or may not survive environmental changes. New species are likely to be dependent on adaptive habitats. However, dams have altered and made the main ecosystem. It adapts to this change, and it is clear from the construction of dams and reservoirs that they reduce the diversity of wildlife, either for better or for worse, and also leads to the loss of habitats for many organisms (Power *et al.*, 1996).

2.2.1. Impacts on Fish Biodiversity

The construction of dams negatively affects the aquatic organisms, especially fish, and when the dam is closed, the migratory fish are automatically destroyed due to inconvenience to spawning and they cannot reproduce, and this is a major reason for these fish switching from river water to lake water, and this makes sensitive fish vulnerable. Therefore, the number and types of fishes will change, which impact remarkable local fisheries. Aquatic living of EIPA mainly contained: (1) assessing the effects of the construction project on the planktonic animals and plants, benthic organisms, higher aquatic plants, fishes and other aquatic animals etc.; (2) focusing on evaluating the impact

on rare aquatic livings; (3) analyzing the effects on the habitats of fish spawning; (4) discussing the efficiency of conservation measures for aquatic livings and proposing further protection measures. Fish and other aquatic species cannot survive in artificial lakes; changes in downriver flow patterns adversely affect many species, and water quality deterioration in or below reservoirs (usually low oxygen levels; sometimes gas super-saturation) kill's fish and damages aquatic habitats. Freshwater mollusks, crustaceans, and other benthic organisms are even more sensitive to these changes than most fish species, due to their limited mobility (Bai *et al.*, 2011).

2.2.2. Mitigation

Management of water releases may be needed for the survival of certain fish species, in and below the reservoir. Fish hatcheries can be useful for maintaining populations of native species which can survive but not successfully reproduce within the reservoir. They are also often used for stocking the reservoir with economically desired species, although introducing non-native fish is often devastating to native species and not ecologically desirable. Fishing regulation is often essential to maintain viable populations of commercially valuable species, especially in the waters immediately below a dam where migratory fish species concentrate in high numbers and are unnaturally easy to catch.

2.3. Impacts on Land Use

2.3.1. Permanent Land Occupation

In the dam construction zone the permanent land occupation features comprise the dam and dyke structures and the power plant and switch yard. The land use at the different locations of the permanent land occupation (Beilfuss *et al.*, 2000).

Reservoirs may contribute to changes in Earth's climate. Hot climate reservoirs generate methane, which is one of the greenhouse gases at stratigraphic reservoirs, which are below the oxygen layers (meaning they lack oxygen), and this leads to the degradation of biomass through anaerobic processes. In some cases when water floods large basins and the size of the mass the vitality is high, it converts it into methane, so the potential pollution results are 3.5 times more than that of an oil-fired power plant (Roht-Arriaza, 2009).

2.3.2. Access Roads

When building dams, new roads are usually created to reach the dam, and this leads to changes in land use, especially if forests are removed to create these roads in this case, this will also lead to a new problem, which is the loss of biodiversity and the acceleration of erosion. Some projects do not need to construct new access roads to the reservoir and this goes beyond the environmental impacts (Patz *et al.*, 2000).

2.3.3. Mitigation

Obtainment the maximum use of the existing roads and paths to reach the dam site. Thus avoiding the need to create a specific access road that reduces the impact on land use.

2.4. Impact on Vegetation

2.4.1. Impacts during Construction

Dam construction, weed invasion, agricultural practices, and overgrazing are the main pressures that threaten riparian vegetation, and then influence the river Geomorphology and river ecosystem (Kondolf *et al.*, 2007).These activities affect the dynamic processes and environments of river, and lead to changes in vegetation succession in the riparian zone. Among these, river damming is essential to anticipate a barrier's long-term environmental influence and a dominant anthropological effect on river systems (la Cecilia *et al.*, 2016). As riparian vegetation is closely connected to discharge stochasticity, it can experience remarkable changes after the construction of artificial reservoirs (Tealdi *et al.*, 2011).

2.4.2. Impacts during Reservoir Impoundment

2.4.2.1. Floating and Aquatic Vegetation

Floating aquatic vegetation and aquatic weeds can rapidly proliferate in eutrophic reservoirs, causing problems such as (a) degraded habitat for most species of fish and other aquatic life, (b) improved breeding grounds for mosquitoes and other nuisance species and disease vectors, (c) impeded navigation and swimming, (d) clogging of electro-mechanical equipment at dams, and (e) increased water loss from some reservoirs (Ledec and Quintero, 2003).

2.4.2.2. Impacts during Operation Period

During the operation of the dam reservoir, seasonal fluctuations in the water level occur. At the end of the dry season, the water level will be at a specified height above sea level. With the first rains, the flood gates will be closed at a predetermined date to fill the reservoir to its maximum operational level during the rainy months. The rest of the year, the water level is gradually dropping. The area exposed between the maximum and minimum operating levels is known as the landing zone, and plants can develop in this zone. These plants grow naturally and consist of herbs that are prevalent in the area. Alternatively, these areas can be used for cultivation (Furey *et al.*, 2004).

2.4.3. Mitigation

- The avoidance and minimization of impacts on land use.

- With respect to the impact on vegetation from reservoir impoundment, the impact is unavoidable. Regarding the risk of aquatic weeds, a monitoring of aquatic vegetation in association with post impoundment monitoring of water quality is recommended and an action plan for the management of aquatic weeds should be established.

2.5. Impacts on Terrestrial Wildlife

2.5.1. Impacts during Construction Activities

2.5.1.1. Dust and Noise

Along paths, roads, and transmission lines, the source of noise and dust are mainly vehicles that transport materials, people, and equipment. Construction site noise and dust are generated by vehicles as well as earth moving equipment, lifting equipment, cutting and bombing equipment, various sirens and sirens. In the borrowing area, noise and dust are generated by vehicles, earth moving equipment, various sirens, sirens and the occasional detonation of explosives.

Roads, paths, and transportation lines intersect across land areas. In this type of habitat a number of mammals may be found, including sand fox, porcupine, rattlesnake, civet, porpoise, hedgehog, and wild cat. None of these mammals are endangered or of particular environmental importance. In the construction site of the dam and borrowing areas in riverine habitats, the wildlife is mainly composed of birds but there may be a pig, green monkey, crocodile and snake (Schexnayder and Ernzen1999; Ouren *et al.*, 2007).

2.5.2. Impacts during Reservoir Filling

2.5.2.1. Out-Migration

The inundation event have a number of complex impacts on the terrestrial fauna, which react in a number of ways, mainly out-migration and drowning. There is a timber and/or biomass clearance this could disturb many of the larger animals and cause them to leave the area. Even if these activities do not occur most large animals, and highly mobile small animals, will successfully leave the area as the water rises. It must be appreciated that the inundation event runs steadily through a 24-hour period, offering strictly nocturnal and strictly diurnal animals special problems. Sometimes the displaced animals may survive drowning, but usually they cannot be displaced and this causes a very rapid death rate. Even those animals which successfully establish themselves after displacement will temporarily at least, until population pressures decline through natural regulation processes, be subject with all other members of the species and its competitors, to higher rates of mortality (Barraclough and Ghimire,1995).

The ecological instability generated by the flooding of a large area and the consequent movement of large numbers of animals can have an impact on public health and on crop pests. If large numbers of rodents and carnivore are displaced the human health environment be disturbed. For similar reasons crop pests if became a more serious problem for period on fields close to the reservoir. This situation will require monitoring during, and immediately after, reservoir filling (Riley *et al.*, 2010).

2.5.2.2. Drowning Of Animals

Most of the more rapidly moving fauna have may be leave the inundation area during clearing of trees and biomass destruction (if these measures are taken).

Those which remain in the area or which return became trapped on the numerous temporary islands which form as inundation proceeds (due to the depth of the reservoir and the local topography), because it is not anticipated that permanent islands be created, animals trapped on islands will eventually drown unless they can swim to the shore. (Maltby, 2013).

Most of the animals that drown will be small flightless terrestrial mammals, immature and injured animals incapable of moving, and soil fauna. The bodies of drowned animals represent contribution to the total biomass decomposition.

2.5.3. Impacts during Operation

2.5.3.1. Reservoir Area

The impact on the wildlife is associated with the physical presence of the reservoir, which is a source of water available all year round, whereas for the baseline situation, the rivers are almost dry for several months of the year. The increased availability of water should be beneficial for some of the wildlife; however other factors need to be taken into consideration. The flooding of the reservoir; which destroy the Riverine habitat, cause the wildlife from these areas (sand fox, porcupine, ratel, civet, aardvark, hedgehog, wild cat, warthog) (Bartram and Ballance, 1996).

2.5.4. Mitigation

It is necessary to consider the prevailing attitudes of the developed world with respect to animals living in distant locations perceived as natural and unspoiled. It must be some measures, limited to the actual periods of rapid inundation, should be taken to provide safe retreats for the slow moving flood survivors.

For some dam projects, must be boat patrols are made during filling to capture stranded specimens and provide safe retreat and collection of slow moving survivors using tethered floating rafts.

The fleeing of the animals be the opportunity for the local population to hunt the fleeing animals and therefore the most effective means of protecting the fleeing animals will be to implement an effective means enforcing a ban on hunting.

2.6. Impacts on Air Quality

2.6.1. Dust and Air Emissions from Construction Activities

Most of the air pollution will originate from the fugitive dust resulting from traffic on the road and the earthworks and from the release of smoke from trucks and heavy equipment engines. Water spraying must be the primary protection measure against dust. Stabilization of spoil areas by herbaceous vegetation must be reduce the risk of fugitive dust during windy days during the dry season. Smoke emissions from engines can also be controlled by appropriate maintenance of engines. Additional sources of smoke result from clearing and burning of vegetation at the construction sites, at the early stages of the construction phase, and from burning of waste and refuse (Faber *et al.*, 2015).

2.6.2. Impacts on Climate

2.6.2.1. Greenhouse Gas Emissions and Contribution to Global Warming

Greenhouse gases, namely carbon dioxide and methane into the atmosphere, are released from reservoirs that flood forests and biomass sometimes slowly if the submerged organic matter decomposes or rapidly if forests are cut down and disposed of by burning before the reservoir is filled. It has been recognized that dam reservoirs throughout the world emit Greenhouse Gases (GHG) that contribute to global warming. The GHG emissions from different dams vary greatly because the emissions are dependent on many factors. Greenhouse gases emitted from dam reservoirs comprises methane (CH4). The Global Warming Potential (GWP) of methane is 25, i.e. one kilogram me of methane is equivalent to 25 kilograms of carbon dioxide.

2.6.2.2. Impacts on Micro-Climate

The impounding of the reservoir create a large water body that may influence the local climate. The annual water loss from the reservoir from evaporation is estimated using the average annual local value of Potential Evapotranspiration (PET). Because of the evaporation of water and the cooling effect of storing the water, the climate in the immediate vicinity of the reservoir is to have increase in humidity and temperature (Marsalek *et al.*, 2008).

2.6.3. Mitigation

The dust emissions from trucks transporting rock material using unpaved tracks should be minimized. The principal concern is with respect to dust emissions close to regions. However the other villages will be exposed to dust emissions. Means of controlling dust can be performed by regular water spraying to suppress the dust and/or to pave sections of the track. Because water spraying represents a number of organizational difficulties, the most suitable solution will be to pave the sections of tracks that are close to regions. With respect to climate and GHG emissions, the mitigation measure is to clear vegetation prior to reservoir filling. This mitigation is also applicable to problems of water quality.

III. TABLES

Table.1: Summary of Impacts – Physical Environment (Ledec and Quintero, 2003; Wahlstrom, 2012 and Baxter, 1977).

Activity	Dam sites and construction sites	Reservoir area	Downstream areas
Sediment, soils, land cover, seismicity			
Construction	Physical disturbance of top soils at construction site	Extraction of sand and gravel for dam construction.	Dumping of extracted soils and rock from the dam construction.
	Physical disturbance from access road construction and transmission line right of way		
	Potential for unauthorized dumping of hazardous waste. Similar level of risk for the three options		
Reservoir filling	Low risk of reservoir induced earthquake.	Change in land use	No predicted impacts
Reservoir operation	The impacts incurred during construction and filling.	Sediment deposition	Modification of river morphology, erosion of banks, deposition of sediment.

Table (1) Continue

Activity	Dam sites and construction sites	Reservoir area	Downstream areas
Groundwater			
Construction	Potential for contamination from unauthorised dumping of hazardous waste and leaks and spills from fuel and chemical storage and handling. This risk is approximately the same for all three options		
Reservoir filling	Potential for resurgences of groundwater causing water logging is some area.	Seepage of reservoir water into underlying aquifer.	Potential for resurgences of groundwater causing water logging in some area.
Reservoir operation	The impacts incurred during construction and filling are predicted		Groundwater may be contaminated occasionally with anoxic or eutrophic water from seepage of reservoir water. Will occur when anoxic or eutrophic conditions occur in the reservoir.
Air			
Construction	Emissions of dust, construction vehicle exhaust fumes and work camp power generation emissions.	No predicted significant impacts	Possible impacts from dispersion of dust from blasting
Reservoir filling	No predicted significant impacts	No predicted significant impacts	No predicted significant impacts
Reservoir operation	Greenhouse gas emissions from biodegradation of inundated vegetation		
	Greenhouse gas emissions from occasional anoxic conditions.		
	Creation of microclimate. The evaporation of water from the reservoir may cause a noticeable increase in the local climate.		
Hydrology			
Construction	Potential for contamination from unauthorised dumping of hazardous waste and leaks and spills from fuel and chemical storage and handling.	Potential for contamination from unauthorised dumping of hazardous waste.	Potential for contamination from unauthorised dumping of hazardous waste. Possible impacts from construction camp wastewater
Reservoir filling	Floating debris, rubbish from inundated villages and trees, branches and vegetation from inundated area.		Reduced flow of water and consequently reduced availability of drinking water for people cattle and reduced water for irrigated lands on the banks of the river.
Reservoir operation	Eutrophic and/or anoxic conditions from biodegradation of inundated vegetation		The water quality will be occasionally degraded when eutrophic and/or anoxic conditions occur in the reservoir
	Occasional anoxic or eutrophic conditions occur in the reservoir		
Noise			
Construction	Noise from construction work and traffic	Noise from vehicles of land clearing teams and dredging of construction material (sand and gravel)	Noise from dredging of construction material (sand and gravel)

Reservoir filling	No predicted significant impacts	No predicted significant impacts	No predicted significant impacts
Reservoir operation	Noise from turbines	No significant impacts predicted	No significant impacts predicted

Table.2: Summary of Impacts – Biological Environment (Ledec and Quintero, 2003; Wahlstrom, 2012 and Baxter, 1977).

Activity	Dam sites and construction sites	Reservoir area	Downstream areas
Aquatic ecology			
Construction	Loss of habitat for fish and aquatic life.	Physical disturbance of fish and aquatic life from the extraction of sand and gravel from the borrow areas some of which are located in the river bed.	Construction work may incur release of sediment in the river downstream of the dam construction site. The impact is expected to be of no significance
Reservoir filling	Increased fish population numbers in the new reservoir, however there will be a change in biodiversity		The dam may present a physical barrier for migration of fish
Reservoir operation	Risk of development of aquatic weeds and/or conditions favourable for disease vectors such as mosquitoes and vector snails of schistosomiasis		
Terrestrial vegetation			
Construction	Loss of natural vegetation at dam site and along access road and transmission line right of way	No significant impacts predicted	No significant impacts predicted
Reservoir filling	Loss of natural vegetation		No significant impacts predicted
Reservoir operation	No further negative impact compared to that of construction and reservoir filling. However the reservoir may represent a favourable habitat for introduction of new species		Reduced water for riparian vegetation, reduced numbers and biodiversity
Wildlife			
Construction	Loss of habitat will affect wildlife causing them to flee the area	No significant impacts predicted	No significant impacts predicted
	Possible hunting of wildlife by construction site workers. The crocodile (protected species) and or gazelle and antelope may be at risk from hunting		
Reservoir filling	Animals will be forced to flee the area and some animals will be drowned or stranded on islands		Influx of animals from the inundated area into the downstream areas
Reservoir operation	The area may attract birdlife to the area		No significant impacts predicted

IV. CONCLUSION

It concluded that, the most important impacts of dams are the effects on water and air quality, because they are the basic elements which affect directly on humans, animals and plants. The effectiveness on water quality due to excessive sediment release, pollution from hazardous

materials; household wastewater and solid waste. While the most dangerous impacts on air quality is the emission of greenhouse gases (carbon dioxide, methane).

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Profile of Progesteron, Estrogen and Pregnancy Level of Pasundan Cattle after Estrus Synchronization with Prostaglandin and Releasing Gonadothropin Hormone

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Abstract**—** *Twenty Pasundan cows were used in this study in order to determine the concentration of the hormones estrogen, progesterone, and the level of pregnancy. The cows were randomly divided into two groups of 10 each. The first group was synchronized with a prostaglandin double injection (Lutalyse TM, Upjohn, Kalmozoo USA, containing 25 mg of Dinoprost Tromethamin) at a dose of 5 ml/head intramuscularly 2 (two) times with an interval of 11 days. The second group was injected with prostaglandins (PGF2α) at a dose of 5 ml/head intramuscularly 2 (two) times with an interval of 11 days, but on the 9th day they were injected with gonadotropin realizing hormone (Fertagyl, Intervet Animal Health UK Ltd, Cambridge; which contained 100 µg Gonadorelin which is synthetic GnRH)), Cows in heat are artificially inseminated twice with an interval of 6 hours. The variables observed were concentrations of estrogen and progesterone during estrus and 2 months of pregnancy, service preconception, and calving rate. The data were processed with analysis of variance and descriptive. The results showed that cows that were injected with PGF2 α + GnRH, had an average plasma concentration of the hormone progesterone 2.68 ± 0.19 ng/ml and 26.65 ± 2.09 pg/ml estrogen, service preconception 1.0, a calving rate 90% higher than those injected with α PGF2 including progesterone. 2.68 ± 0.19 ng / ml and estrogen 26.65 ± 2.09 pg / ml, service preconception 1.2 and calving rate 90%. The results of the analysis of variance showed that the plasma concentrations of progesterone and estrogen when the cows were 60 days pregnant when they were injected with the PGF2α + GnRH combination had a very significant effect (P <0.01) higher than the control (PGF2α). It was concluded that the progesterone, estrogen profile, pregnancy rate results from estrus synchronization using a combination of PGF2 α and HnRH higher than single PGF2α.*

Keywords**— Progesterone, Estrogen, Pregnancy Level, Pasundan Cattle.**

I. INTRODUCTION

Pasundan cattle are livestock germplasm from West Java which has an important role in meeting food needs and welfare for society and the environment. In this case, the cow germplasm is the basic capital for the development of the livestock sub-sector because it can be engineered to form superior livestock seeds that are suitable for tropical conditions and socially and culturally acceptable to the community. The problem faced by many Pasundan cattle breeders is that the reproductive function of Pasundan heifers is not optimal, which is marked by late puberty and silent heat which causes the pregnancy rate to be not optimal. Many

factors affect the individual reproductive performance of Pasundan cattle which are often difficult to identify. The real conditions in the field are even in optimum conditions, the reproduction process of Pasundan cows can be imperfect due to the contribution of various influencing factors during the pregnancy process until the child is born safely. Understanding the relationship of various factors in influencing livestock fertility is of course essential to optimize the reproductive performance of each Pasundan female cow and livestock business. The development of the estrus synchronicity technique is one way to improve reproductive performance and productivity in Pasundan heifers. Synchronization of estrus is carried out by manipulating the viability of the corpus luteum (CL) using prostaglandin hormone preparations (PGF2α), or a combination of PGF2α and GnRH aimed at stimulating the development of dominant follicles resulting in ovulation.

Pregnancy is a series of processes of immunological and endocrinological changes to produce a child in the womb from fertilization to normal birth. In line with increasing gestational age, several conceptual hormones, especially estrogen and progesterone, experience an increase (Frastantie et al., 2019), which of course will be followed by changes in the mother's metabolism to support the success of the reproductive process (Pemayun, 2014; Geisert and Schmitt, 2002). In other words, the reproductive success of the parent to produce children in one reproductive cycle is influenced by various factors from the zygote, embryo, fetus to birth. Furthermore, Hafez and Hafez (2000) state that the reproduction process is closely related to the hormonal system mechanism, namely the relationship between hypothalamic-pituitary hormones, namely gonadotrophin-releasing hormone (GnRH), follicle-stimulating hormone (FSH), and luteinizing hormone (LH). Ovarian hormones (estrogen and progesterone) and uterine hormones (prostaglandins) Ovarian hormones that have a major role in reproduction are estrogen and progesterone.

The endocrine glands involved in the pregnancy phase are the corpus luteum, follicle, placenta, hypothalamus, and pituitary. The hypothalamus and pituitary are the regulatory glands, while those that play a major role are the corpus luteum that produces progesterone, the placenta that produces progesterone and estrogen and the follicles as a producer of estrogen Progesterone (P) is a key hormone that plays an important role in regulating the estrous cycle and maintaining a pregnancy (Gaja et al., 2013) while Estrogen is a steroid hormone produced by granulosa and theca cells from the de Graaf follicle in the ovary (Hardjopranjoto, 1995). The main function of the hormone estrogen is to stimulate sexual desire, stimulate the emergence of secondary sex characteristics, maintain the female udder system, and udder growth (Wodzicka-Tomaszewska et al., 1991).

Anderson (2003) states that during pregnancy, the growth and development of the uterus are affected by an increase in the concentration of the hormones progesterone and estrogen. Progesterone plays an important role in preparing the uterine environment for implantation and the increase in progesterone during pregnancy and plays a role in maintaining pregnancy. Progesterone, besides being produced by the corpus luteum at the beginning of pregnancy (Efendy et al, 2015). Furthermore, Ginther et al. (2010) stated that laboratory standards. Progesterone concentration in pregnant cows is maintained until near the end of pregnancy

To increase the efficiency of production and reproduction in cows, hormonal profile information is needed in the reproductive cycle (Katongole and Gombe, 2006). The results of previous studies reported that the concentrations of estrogen and progesterone had a high correlation with the number of corpus luteum in sheep (Sumaryadi and Manalu, 1995). Accurate information about reproductive hormones during pregnancy is important to study as the basic concept of the ovulation process, the corpus luteum regression cycle, the need for hormones for the manifestation of heat, pregnancy, and birth (Akusu et al., 2006). Many aspects of the reproductive appearance of Pasundan cattle have been investigated, but information on the profile of estrogen and progesterone in pregnant heifers has not been reported to date. Based on this, it is necessary to know the profile of progesterone, estrogen, and pregnancy rate in Pasundan cattle after being induced by exogenous hormones.

II. MATERIAL AND METHOD

The research material used was 20 Pasundan heifers aged 2.0 - 2.5 years with relatively the same weight and age, belonging to breeders who are members of the Rundayan Sawargi Group in Cibalong District, Garut Regency. All experimental cows were palpated rectally to determine their reproductive status and ensure that the cows were not pregnant. The selected animal is a healthy animal, not pregnant, and has never been a child. Experimental cows were divided into 2 groups of 10 each. The first group was synchronized with a prostaglandin double injection (Lutalyse

TM, Upjohn, Kalmozoo USA, containing 25 mg of Dinoprost Tromethamin), at a dose of 5 ml/head intramuscularly 2 (two) times with an interval of 11 days, to uniform physiological conditions of each cattle as control. The second group was injected with prostaglandins (PGF2α) at a dose of 5 ml/head intramuscularly 2 (two) times with an interval of 11 days, but on the 9th day they were injected with gonadotropin realizing hormone (Fertagyl, Intervet Animal Health UK Ltd, Cambridge; which contained 100 µg Gonadorelin which is synthetic GnRH)), at a dose of 2.5 ml/head intramuscularly to homogenize fertility conditions and increase fertility. Observation of estrus was carried out twice a day, namely in the morning (06.00 - 08.00 and 17.00 - 18.00) three days in a row after the last injection of PGF2α. Cows that are in heat are directly on IB 2 times with an interval of 6 hours after the first IB. Cows resulting from IB after 2 months do not show heat again, do rectal palpation by feeling the uterus to detect uterine enlargement that occurs during pregnancy (Jainudeen and Hafez, 2008). The variables observed were the concentration of estrogen, progesterone, service preconception, and conception rate. Blood sampling during pregnancy was done 3 (three) times during heat and at 60 days of gestation.

Blood samples for measuring the hormones estrogen and progesterone were taken from the jugular vein as much as 10 ml using a disposable syringe containing anticoagulant, then put into a test tube and placed in a flask filled with ice. Blood was left for 30 minutes then centrifuged at 2500 rpm for 15 minutes. The plasma formed is separated into an evendorf tube which will be used for the analysis of hormones and blood metabolites.

Estrogen. The concentration of estrogen in plasma was measured by KIT (Sigma Chemical Co., St Louis MO) using the Enzyme-Linked Immunosorbent Assay (ELISA) technique. Each Elisa plate was put in 25 µl of standard, sample, and control solution, then each was mixed with 200 µl of estradiol conjugate reagent in each well. Furthermore, incubation for 120 minutes at room temperature. The absorbance value was read on the ELISA reader after 10 minutes with a wavelength of 450 ± 10 nm.

Progesterone. The concentration of estrogen in plasma was measured by KIT (Sigma Chemical Co., St Louis, MO) using the Enzyme-Linked Immunosorbent Assay (ELISA) technique. On each Elisa plate, 25 µl of standard, sample, and control solution were inserted, then each was mixed with 200 µl of the progesterone conjugate reagent in each well. Furthermore, incubation for 120 minutes at room temperature. The absorbance value was read on an ELISA reader after 10 minutes with a wavelength of 450 ± 10 nm.

Data Analysis: The collected data were analyzed using analysis of variance.

III. RESULTS AND DISCUSSION

Estrogen and Progesterone Hormone Profiles

The concentrations of estrogen and progesterone hormones in Pasundan cattle induced by the combination of PGF2α with GnRH during heat, 60 days and 150 days of pregnancy are presented in Table 1.

Table 1. Profile of Estrogen and Progesterone Hormones in Pasundan Cows during Heat and 60 days of Pregnancy

Group	PGF2α		PGF2 α+GnRH	
Hormone Concentration	Estrus	Pregnant 60 days	Estrus	Pregnant 60 days
Estrogen (pg / ml)	28.83 ±2.29	22.74 ±2.50	32.76 ±2,00	26.43 ±1.32
Progesteron (ng/ml)	0.21±0.03	4.71±0.19	0.22 ±0.050	4.86 ± 0.48

The data in Table 1 shows that the mean concentrations of conceptus hormones, both estrogen, and progesterone at 60 days of gestation in Pasundan cattle, respectively synchronized with PGF2α + GnRH) were 26.43 ± 1.32 pg / ml and 4.86 ± 0.48 ng / ml, while in synchronization with (PGF2α) 22.74 ± 2.50 pg / ml and 4.71 ± 0.19 ng / ml, respectively. This is in line with Arimbawa et al. (2012) reported that the increase and decrease in progesterone levels are in line with the development of the corpus luteum. Based on the results of the analysis of variance, it shows that the

concentration of estrogen in the estrous phase, for treatment (PGF2α + GnRH) has a very significant effect (P <0.01) higher than PGF2α single. this suggests that synchronization using GnRH will increase the number of developing and mature follicles until the estrogen concentration increases and ovulation occurs. Furthermore, Nascimento et al. (2014) stated that the growth of follicles during the estrous cycle is controlled by the hormones FSH and LH, and these two hormones must be present if the growth and function (estrogen secretion) of the follicles are expected under normal conditions. The concentration of progesterone during exertion for both the PGF2α treatment and the combination of PGF2α and GnRH was not significantly different (P> 0.05), this indicates that the results of estrus synchronization using PGF2α alone and the combination of PGF2α and GnRH did not affect the progesterone concentration profile. GnRH injection on the 9th day before the 2nd injection of prostaglandin (PGF2α) (11th day), will stimulate the anterior pituitary to secrete gonadal hormones, namely FSH and LH. The FSH hormone can stimulate the growth of follicles and the maturation of ovarian follicles, while FSH together with LH induces the secretion of the hormone estrogen in large follicles. The first injection of PGF2α on day 0 has a role to regress CL, so that the level of the hormone progesterone will drop during the heat. Low levels of progesterone will have an impact on the increase in the FSH hormone which will stimulate the development of the follicles to maturity and in turn, will increase the concentration of estrogen and cause symptoms of heat in cows. This is in line with Hafez (2000) who stated that an increase in the number of follicles has a consequence of increasing levels of estrogen in the blood which will cause estrus in cows and spur ovulation due to its positive feedback effect on LH.

Based on Table 1 shows that in 60 days pregnant cows the concentration of progesterone has increased both in single PGF2α treatment and in the combination of PGF2α and GnRH. The results of the analysis of variance showed that the concentrations of progesterone and estrogen at the time of 60 days of pregnancy in cows that were injected with the combination of PGF2α and GnRH had a very significant effect (P <0.01) higher than single PGF2α. These conditions indicate that giving GnRH to Pasundan heifers can stimulate the formation of more and more active corpus luteum thereby increasing the secretion of progesterone. This result is in line with Cerri et al. (2009), who stated that giving GnRH can increase the number of follicles, corpus luteum, and placenta and cause an increase in secretion from glands that produce a pregnancy and mammogenic hormones such as estradiol and progesterone during pregnancy. Furthermore, Siregar (2006) states that with the active corpus luteum, progesterone secretion increases. Hafez (2000), states that progesterone levels are closely related to the amount of the corpus luteum. According to Anderson (2003), during pregnancy, the growth and development of the uterus are affected by an increase in the concentration of the hormones progesterone and estradiol. Siregar (2002) further states that the concentration of progesterone during the formation period of the corpus luteum is related to the number of corpus luteum, while the concentration of progesterone in mid-pregnancy is related to the number of children to be born.

Pregnancy Level (Service Perconception and Calving Rate)

Service preconception value (S/C) will affect Conception Rate (%). The results of the research regarding the S/C and CR values are presented in Table 2.

Table 2. Value of Service Perception and Conception Rate of Pasundan Heifers

Pregnancy Level	Hormone Induction	
	PGF2α	PGF2α+GnRH
Service per Conception (S/C)	1.20	1.0
Conception Rate (CR) (%)	90.0	100

The results of pregnancy examinations by palpation per rectally carried out on the 60th day after the first IB, showed that the calving rate for synchronizing the combination of PGF2α and GnRH was 100%, while for synchronization using PGF2α was 90%. This condition indicates that the use of GnRH can uniform ovulation in Pasundan heifers. This is in line with Efendy et al. (2015) stated that progesterone plays an important role in preparing the uterine environment for implantation and the increase in progesterone during pregnancy and plays a role in maintaining pregnancy. Progesterone, besides being produced by the corpus luteum at the beginning of pregnancy

The value of service preconception and conception rate for Pasundan cattle that were synchronized with estrus combination of PGF2α and GnRH was higher than that of PGF2α. This condition is in line with the picture that the concentration of estrogen and progesterone in the estrus synchronization of the combination PGF2α and GnRH is higher than the synchronization with single PGF2α. Based on the analysis of variance, it shows that the estrous synchronization treatment (PGF2α + GnRH) is significantly different (p> 0.05) higher than synchronization using single PGF2α on service preconception and calving rate. The results of this study indicate that the hormones estrogen and progesterone have a strong relationship and are directly proportional to service per conception and calving rate, meaning that estrogen and progesterone affect service per conception. This is in line with the statements of Hafez (2000) and Putro (2008) which state that the GnRH hormone functions to stimulate the hypofunction of LH and FSH which work together to stimulate follicles and corpus luteum formation. This condition is thought to have lower pregnancy rates in synchronization with (PGF2α) due to early embryonal death, due to hormonal imbalances, where the progesterone required for pregnancy has not been sufficiently produced by CL. Accordance with Willard et al. (2003), who stated that one of the main causes of early embryonic death which is the cause of the low conception rate is an insufficient luteal function which is indicated by low progesterone concentrations. The results of previous research Santosa et al. (2004) reported that generally, embryo mortality can occur before 60 days after IB, before the placenta is fully formed, including embryo death at the age of 0-7 days after IB (very early embryo). Putro (2008) further states that injecting GnRH 48 hours after injection of PGF2α for ovulation synchronization can improve the dynamics of follicular development, so that fertility will be better due to the influence of the GnRH hormone to stimulate follicular growth and the formation of the corpus luteum after ovulation.

The results in this study were higher than those of Chaikhun et al. (2010) who reported that NGOs that ovulated using PGF2α and GnRH resulted in pregnant buffalo pregnancy (15%) significantly lower than buffalo that had calved (42.9%). Furthermore, the report of Sianturi et al. (2012), who used the Ovsynch method to produce pregnancy (62.5%) in heifers. The difference in the results of the study with the previous researchers was thought to be due to the difference in the time to offer GnRH and the implementation of IB. In the ovsynch method, the implementation of IB was carried out 16-22 hours after the second GnRH (Ali and Fahmy, 2007), whereas in this study cows were indirect heat for 2 times with an interval of 6 hours after the first IB. On the other hand, the high pregnancy results of Pasundan cows in this study were probably due to livestock, and the condition of Pasundan cattle acceptors is quite good as indicated by the value of body conditioning scoring (BCS) 2.8 - 3.0 (for scoring 1-5).

IV. CONCLUSIONS

Induction of the combination of PGF2α and GnRH, has an average plasma concentration of the hormones progesterone 2.68 ± 0.19 ng/ml and estrogen 26.65 ± 2.09 pg/ml, service preconception 1.0, a calving rate 90% higher than those injected with single PGF2 α with progesterone mean of 2.68 ± 0.19 ng/ml and estrogen 26.65 ± 2.09 pg/ml, service preconception 1.2 and calving rate 90%. This success has yet to be proven by the rate at which childbirths occur.

It is recommended that the mother's nutritional adequacy during pregnancy will determine the success of giving birth so that the condition of the child is born and the mother is normal and healthy.

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Identification of The Potential of Degrading Carrageenan in Red Algae *Kappaphycus alvarezii* Symbiotic Bacteria

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Abstract— *Kappahycus alvarezii is a red alga contained large amount of bioactive material, such as carrageenan. Carrageenan is useful as a raw material for several industries and can be degraded by marine bacteria through breaking the linkages in polysaccharide carrageenan into oligosaccharide carrageenan. The aim of this study is identification of degrading carrageenan in K. alvarezii symbiotic bacteria. The results showed there was 14 isolate bacteria, and all of the isolates have clear zone on congo red staining activity. The isolate bacteria were 7 genera as K. alvarezii symbiotic bacteria, such as Labrenzia sp., Alteromonas sp., Vibrio sp., Celeribacter sp., Pseudoalteromonas sp., Phaeobacter sp. and Cobetia sp. Labrenzia sp., Alteromonas sp., Vibrio sp., Pseudoalteromonas sp., Phaeobacter sp. were recognized to have strong interactions with carrageenan in red algae, while the other Celeribacter sp. and Cobetia sp. have strong interactions with alginate in brown algae.*

Keywords— ***Kappahycus alvarezii, carrageenan, degrading bacteria.***

I. INTRODUCTION

Kappahycus alvarezii is red algae that generally has cylindric thallus, smooth surface, cartilaginous and consists of several types based on the color, such as green, yellowish green, gray, brown and red (Parenrengi *et al*., 2010). *K. alvarezii* was lived in tidal habitats, coral reef flats and attached to hard substrates (Erlania, 2013). *K. alvarezii* was contained large amount of carrageenan and used as stabilizer and gelling agent in processed meat, ice cream, chocolate, pudding, pet food, shampoo, toothpaste and cleaning products industrial (Hotchkiss *et al*., 2016; Barret, 2018).

In the ecosystem, bacteria were played an important role because of its ability to degrade organic matters to inorganic matters (Ginting *et al.*, 2019). The existence of symbiont bacteria was to protect their host and produce secondary metabolites (Funty, 2015). The use of secondary metabolites in algae, for example as bioactive materials (Nurhaedar, 2008). Carrageenan as a bioactive material in algae can be degraded by marine bacteria, especially gram-negative bacteria and produced enzymes to degrade carrageenans and it was useful for several industries (Chauhan & Saxena, 2016). Carrageenans degradation was a process to break the linkages in polysaccharide carrageenans to be oligosaccharide carrageenan with low molecular weight (Ghanbarzadeh *et al*., 2018). Several studies showed that *Pseudoalteromonas* sp. (Li *et al.*, 2013); *Tamlana* sp. was isolated from red algae *Hyalosiphonia caespitosa* (Sun *et al*., 2010) and *Cythopaga* sp. was isolated from red algae *Eucheuma gelutinue* (Mou *et al.*, 2004) have degrading carrageenan ability. Based on previous several studies, there is no *K. alvarezii* symbiotic bacteria in degrading carrageenan. Therefore, it is necessary to conduct research on the identification of degrading carrageenans in *K. alvarezii* symbiotic bacteria.

II. MATERIAL AND METHODS

Kapphycus alvarezii AND CARRAGEENAN USED IN THIS STUDY

Algae *K. alvarezii* were collected from USA Marine Biological Institute, Kochi University, Japan and floated in Uranouchi Bay, Tosa, Kochi Prefecture, Japan for one week to collect the bacteria. While several commercial carrageenan was used in this study, κ-carrageenan and λ-carrageenan purchased from Wako.

ISOLATION OF *K. alvarezii* SYMBIOTIC-BACTERIA

Arificial water agar medium was made with adding 0.5% carrageenan and incubated with red algae *K. alvarezii* for 3 days. After 3 days, the artificial seawater bacteria were dropped around 0.1 ml in marine broth agar. Moreover, bacteria were incubated at 25°C for 2 days to get the pure bacteria cultured.

DEGRADING CARRAGEENAN SCREENING BY CONGO RED STAINING

Bacteria in marine broth agar medium was inoculated to marine broth medium and incubated at 25°C for 2 days. Congo red agar medium was made from 4 gr gar medium, 25 ml 0.5% carrageenan and 1 ml congo red. Bacteria colonies from marine broth medium was dropped into congo red agar medium for 0.1 ml. The bacteria were incubated at 25°C for 2 days to identify the clear zone. Formed clear zone was an indicator of the carrageenan degrading activity existence. Bacteria with clear zone was inoculated and analyzed by 16S rDNA.

16S rDNA ANALYSIS OF *K. alvarezii* SYMBIOTIC-BACTERIA

A colony of 14 isolate bacteria were used as a template for PCR. Isolate bacteria were amplified by using universal primer pr0R2 (5'-AGAGTTTGATCMTGGCTCAG-3') dan 534R (5'-ATTACCGCGGCTGCTGG-3'). PCR products were applied to agarose gel electrophoresis and purified using Wizard® SV Gel and PCR Clean-Up System (Promega). The purified DNA were sequenced by ABI PRISM® 3130 Genetic Analyzer (Applied Biosystems Japan) using BigDye® Terminator v3.1 and analyzed with BLAST on NCBI.

III. RESULT AND DISCUSSION

Bacteria isolation found 14 isolate bacteria with big size colonies. Congo red staining activity showed that there was clear zone in 14 isolate bacteria (Fig 1). Congo red was used for degrading carrageenans activity because congo red have strong interaction with polysaccharide which contained cellulose linked by β-1,4-glycosidic linkages (Teather & Wood, 1982). FAO (2003) was explained that red algae contain carrageenan and cellulose which insoluble in water and alkali. The clear zone was formed because of the reaction between *congo red* and β-1,4-glycosidic linkages in cellulose polymer (Missa *et al*., 2016).

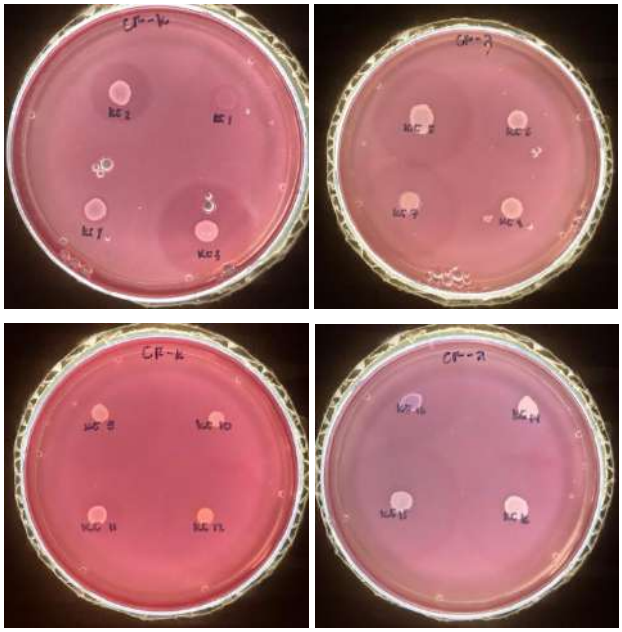

Fig 1: Congo red staining activity on 14 isolate bacteria

Electrophoresis showed that the measurement of bacteria DNA fragment was about 500 bp and it was compared to the marker 1 kbp DNA (Fig 2). The purification of DNA was measured by absorbance 260 nm and 280 nm. It showed that the absorbance of purified DNA was about 1.73-2.07 (Table 1). Thermo Fisher Scientific (2010) explained that the ratio of absorbance 260 nm and 280 nm was about 1.8 and it was "pure" for DNA purification.

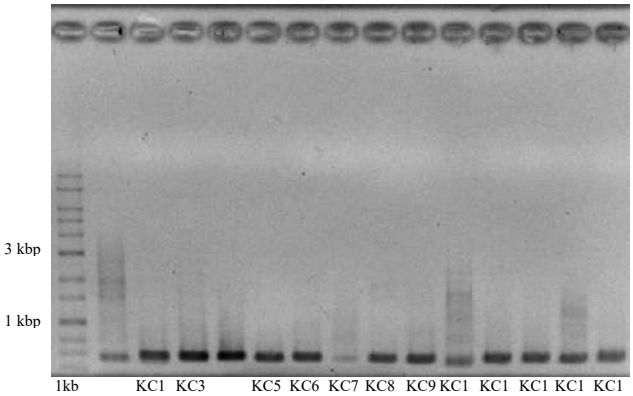

Fig 2. DNA fragment of K. alvarezii symbiotic bacteria

Table 1. Absorbance of K. alvarezii symbiotic bacteria DNA purification

Bacteria	$A_{260/280}$

Isolate 1	1.91
Isolate 2	1.95
Isolate 3	1.91
Isolate 4	2
Isolate 5	2.04
Isolate 6	1.9
Isolate 7	1.73
Isolate 8	1.93
Isolate 9	1.89
Isolate 10	1.76
Isolate 11	2.07
Isolate 12	1.97
Isolate 13	1.93
Isolate 14	1.82

16S rDNA showed that there were 7 genera of symbiotic bacteria in *K. alvarezii*, such as *Labrenzia* sp., *Alteromonas* sp., *Vibrio* sp., *Celeribacter* sp., *Pseudoalteromonas* sp., *Phaeobacter* sp. and *Cobetia* sp. (Table 2). Azizi *et al.*, (2018) found some bacteria was associated with 4 types of *K. alvarezii* and classified by 11 genera, such as *Alteromonas* sp., *Aestuariibacter* sp., *Idiomarina* sp., *Jejuia* sp., *Halomonas* sp., *Primoskyibacter* sp., *Pseudoalteromonas* sp., *Ruegeria* sp., *Terasakiella* sp., *Thalassospira* sp. and *Vibrio* sp. All of bacteria in this study was gram-negative bacteria and have carrageenan degrading ability by congo red staining activity. Chauhan & Saxena (2016) explained that carrageenase enzyme was only produced extracellularly by gram-negative bacteria.

Table 2. 16S rDNA of K. alvarezii symbiotic bacteria

***K. alvarezii* symbiotic bacteria**		***Identity***	**Reference**
Isolate 1	*Labrenzia* sp.	99.77%	*Labrenzia* sp. THAF35, Accession No. CP045380
Isolate 2	*Alteromonas* sp.	98.75%	*Alteromonas tagae*, Accession No. NR_043977
Isolate 3	*Alteromonas* sp.	99.58%	*Alteromonas tagae*, Accession No. NR_043977
Isolate 4	*Vibrio* sp.	99.60%	*Vibrio campbellii* MMRF1060, Accession No. MT307282
Isolate 5	*Alteromonas* sp.	100%	*Alteromonas macleodii* ROA033, Accession No. MT515801
Isolate 6	*Vibrio* sp.	99.80%	*Vibrio rotiferanus* AM7, Accession No. AP019798
Isolate 7	*Celeribacter* sp.	100%	*Celereibacter naphthalenivorans* EMB201, Accession No. NR_137260
Isolate 8	*Pseudoalteromonas* sp.	100%	*Pseudoalteromonas* sp. L10, Accession No. MN889153
Isolate 9	*Pseudoalteromonas* sp.	100%	*Pseudoalteromonas* sp. S4498, Accession No. MT514367
Isolate 10	*Phaeobacter* sp.	99.33%	Uncultured bacterium 5M23, Accession No. JF272132
Isolate 11	*Pseudoalteromonas* sp.	100%	*Pseudoalteromonas* sp. Md236, Accession No. AY461673
Isolate 12	*Vibrio* sp.	100%	*Vibrio campbellii* 1511126, Accession No. CP025953
Isolate 13	*Cobetia* sp.	100%	*Cobetia pacifica* GPM2, Accession No. CP047970
Isolate 14	*Alteromonas* sp.	100%	*Alteromonas macleodii* ROA033, Accession No. MT515801

The first *Labrenzia* sp. in red algae was *Labrenzia polysiphoniae* in red algae *Polysiphonia* sp. (Romanenko *et al*., 2019). *Alteromonas* sp. was found as *Kappahycus alvarezii* symbiotic bacteria and showed a pathogenetic. *Alteromonas* sp. was able to be pathogen agent that caused *ice-ice* symptoms (Syafitri *et al*., 2017). On the other hand,

Alteromonas sp. showed a potential to degrade some polysaccharide, such as alginate (Neumann *et al.*, 2015), ulvan (Koch *et al.*, 2019); agar (Wang *et al*., 2005); ι-karagenan (Barbeyron *et al.*, 2019) and κ-karagenan (Barbeyron *et al*., 1994). Araki *et al*., (1999) and Zhu & Ning (2016) found high activity of κ-carrageenase enzyme through *Vibrio* sp. purification. Moreover, *Pseudoalteromonas* sp. had an ability to utilize κ-carrageenan and ι-carrageenan for their energy source (Hettle *et al*., 2019). *Pseudoalteromonas* sp. was also degraded κ-carrageenan (Liu *et al*., 2011) and λ-carrageenan (Guibet *et al*., 2007). Furthermore, *Phaeobacter inhibens* was found in red algae *Tichocarpus crinitus* to degrade the carrageenan (Kalitnik *et al*., 2017). Based on the several studies, *Labrenzia* sp., *Alteromonas* sp., *Vibrio* sp., *Pseudoalteromonas* sp. and *Phaeobacter* sp. were recognized to have strong interactions with red algae through the utilization of red algae carrageenan. Whereas *Celeribacter* sp. and *Cobetia* sp. was found in brown algae through the utilization of brown algae alginate. Ihua *et al*. (2020) showed that *Celeribacter* sp. was found on brown algae thallus *Laminaria digitata* and Yagi *et al*. (2016) explained that *Cobetia* sp. was isolated from brown algae *Padina arborescens* with alginate degrading enzyme.

IV. CONCLUSION

In this study, we found 7 genera of *Kappahycus alvarezii* symbiotic bacteria, such as *Labrenzia* sp., *Alteromonas* sp., *Vibrio* sp., *Celeribacter* sp., *Pseudoalteromonas* sp., *Phaeobacter* sp. and *Cobetia* sp. All of the bacteria showed an activity on congo red staining based on the formed clear zone. The clear zone was indicated the carrageenan degrading activity.

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Yeast Extract: A Potential Substitute of Salt in Baked Crackers

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Abstract**—** *Yeast extract (YE) is a natural product that can be easily produced and has the advantage to be edible and to have a salty taste. It can be used as a food additive to give Umami taste, to intensify flavor and to give saltiness. YE can be produced naturally by autolysis, (a self lysis of the yeast cells) when incubated for four days at 45°C for the yeast cells to release their cytoplasmic content. YE is low in calories and fat, high in proteins, nucleotides and glutamic acid. It is used by food companies and restaurants as an aromatic ingredient in various applications such as soups, sauces, seasonings, snacks, ready meals and meats. The main objective of this study is to reduce the salt concentration and replace it by the edible YE in the salty crackers. In our experiment, salt concentration was reduced by 25%, 50%, 75% and 100% and replaced with YE. Physico-chemical properties of the product were investigated and sensory evaluation was performed by a set of 25 panelists. Triangle test and consumers' acceptance test were performed by comparing experimental sample against the control containing 100% salt. Obtained results supported the hypothesis; the taste of samples with 50% reduced salt showed no difference compared to those made using YE. The panelists gave the latter sample six points out of nine on hedonic scale. Our findings demonstrated that YE can be potentially used as a partial substitute of table salt.*

Keywords**— Yeast Extract, Salt, Monosodium Glutamate, Umami Taste, Crackers.**

I. INTRODUCTION

For many years and from a sensory perspective, salt had many desirable properties. Salt is a cheap ingredient that improves the sensory properties of every food that humans consume. It enhances the positive sensory attributes of foods even the unpalatable ones and makes them taste better. However, salt causes hypertension (Liem et al., 2011), water retention (Sunil et al., 2013), increased heart pressure (Lehnhardt & Kemper, 2011), edema and kidney failure (Trayes et al., 2013). Due to its availability in many processed foods, it is highly consumed. Several studies tried to decrease the salt concentration in food; the process was done gradually to be able to monitor the preservation of the salt effects on food (Henney, 2010).

Replacing salt with other substances represented a great interest for food producers. Yeast extract (YE) is one of the acceptable replacers of salt, as a natural product that can be produced easily (Taylor et al., 2018).

YE can be produced by different methods, mainly by enzymatic treatment, chemical treatment, sonication, plasmolysis or by thermolysis methods (Padmakumara, 2006). For the purpose of replacing salt with YE, this latter should be naturally produced in order to be edible and safe to consume. Autolysis is a fully natural method and gives an edible yeast extract, by only controlling two parameters, the temperature and the time of incubation (Padmakumara, 2006).

YE is a brown liquid that can be stored at -20°C for later usage. When spray dried, it gives a brown powder that shows a saltier taste and better effect in replacing salt. In order to evaluate the effect of saltiness of YE, it should be tested on a salty product such as crackers. Consumers buy

crackers as a salty snack, that is available in many flavors such as oregano and garlic, tomato and olives, basil and others (Mihalos, 2014).

Savory and salty snacks consumption has increased as a result of urbanization and modernization (Mihiranie et al., 2017). Crackers are popular as a healthy salty snack with a high potential to enhance their nutritional value by incorporating natural ingredients such as flavors(Mihiranie et al., 2017). People who cannot consume high amounts of salt seek alternative crackers that have the same salt taste but with less salt concentration.

The main objective of this study is to reduce the salt concentration of crackers and replace the reduced amount by YE. The commercially available crackers were used as a reference. All samples were prepared following the same recipe and under the same processing conditions. The sensory evaluation studies determined the acceptable level at which YE can replace salt.

In our study, the salt was decreased gradually and eventually replaced by dried YE. Different mixes were obtained by reducing salt concentration by 20%, 50%, 75% and 100%, and replacing the reduced amount with dried YE. Two sensory analysis tests were performed (the triangle test and the consumer's acceptance test) in order to evaluate the effect of YE on giving the same saltiness of the original crackers.

The physiochemical characteristics of YE were determined. Salinity, pH value, nitrogen amount, fat content, moisture content, density, amount of calories, nucleotides content, glutamic acid concentration and moisture content were all studied in order to fully characterize the YE.

II. MATERIALS AND METHODS

Preparation of Yeast Extract (YE)

The yeast strain *Saccharomyces cerevisiae* known as baker's yeast and used in many baked products, was subject to autolysis and then to spray drying before usage. The liquid yeast extract was used for the physiochemical studies, and the dried yeast extract was used for baking.

Autolysis of Yeasts

Autolysis is a self-digestion process that encourages the breakdown of cells by their endogenous enzymes. It is a natural process that can take place under specific conditions of pH, temperature or salt concentration (Padmakumara, 2006). For our purpose, autolysis has been initiated as described by Padmakumara. Dissolve 125g of dry yeast *Saccharomyces cerevisiae* in 500ml distilled water as shown in the Figure 1. Incubate for four days at 45°C then collect the supernatant (Figure 2) and centrifuge it for 15 minutes at 4000 rpm.

Fig.1: Dissolved yeast in distilled water prepared in our research lab

Fig.2: Yeast extract after 4 days of incubation, before separating the extract from the cell debris.

Spray Drying

Spray drying was used to produce a dry powder yeast extract from the liquid extract. The pre- prepared liquid

yeast extract was used. The process was conducted as described by Tanguler and Erten(Tangüler & Erten, 2009).

Characterization of Yeast Extract

Salinity of Yeast Extract

The electro conductivity meterfrom ORION 420APLUS meterwas used to measure the value of current that passes through the liquid yeast extract. Based on the flow of the current, the concentration of ions found in a solution can be evaluated and then used to assess the amount of salt present in different concentrations of liquid yeast extract. Results are recordedin microseimens (ms). The value obtained in ms is equal to milliohms; multiplied by 1000 to convert it tomicroomhs. To get the concentration in ppm, multiply the number by 0.64, and then divide it by 1000 to get the concentration in g/L.Concentrate 80 ml of liquid yeast extract by reducing water, using a water bath at 45°C. Measure salinity at 80, 60, 40 and 20ml and convert it from ms to g/L.

pH of Yeast Extract

The pH of the liquid yeast extract was measured using HannapH meter. Measure 30 ml of the yeast extract in a graduated cylinder.Put the cylinders in water bath at 45°C to concentrate the yeast extract into different volumes and measure the pH at each volume. Record the results (Table 1)

Table 1: Measured pH values of different concentrations of liquid YE.

Volume ml	pH			Average
Replicates	1	2	3	
30 ml (100%)	5.39	5.08	5.10	5.19±0.1
24 ml (80%)	5.37	5.37	5.38	5.37±0.005
18 ml (60%)	5.39	5.41	5.39	5.39±0.01
12 ml (40%)	5.33	5.40	5.33	5.35±0.04
6 ml (20%)	5.53	5.54	5.52	5.53±0.01

Determination of Fat by Soxhlet

Soxhlet is a process of continuous solid/liquid extraction. Powdered yeast extract previously prepared by spray drying, was placed in a thimble that allows the liquid to pass through. The Soxhlet apparatus FoodAlyt, model RS 40 was used to determine the fat content. The process was conducted as described in the FAO Manuals of Food Quality Control with some modifications. Weigh thimble + sample before extraction and record the values.Weigh the empty flasks where the solvent will be placed and record masses. Then measure 150 ml petroleum ether and introduce it into the flasks.Place the thimbles and flasks in the Soxhlet extraction machine and run it for 4 hours. Turn off the machine and let it cool completely.Allow petroleum ether to evaporate from the flasks using a water bath from J.P SELECTA, Spain.Measure the mass of the thimble and sample and the mass of the empty flasks after extraction. Calculate the fat amount extracted by subtracting the weight of the thimble after extraction from its weight before extraction.

Moisture Content

Use the moisture analyzer RADWAG of model number MA 210.R that measures the amount of moisture present in a sample of dried YE used in crackers baking.

Determination of Total Nitrogen Content by Kjenldahl

The kjendahl method is used to determine the nitrogen content in organic and inorganic samples. This procedure has high precision and simple application. It consists of three major steps.

The first step is a digestion process by acid at high temperature with the help of catalyst to speed up the reaction. The higher the temperature, the faster the reaction. The digestion procedures were performed using FoodAlyt SBS800 Kjendahl Digestion Unit. The process was done as stated by the FAO Manual for Food Quality Control. In a 250 ml Kjendhl test tube add 1 g of powdered yeast extract. Add some glass beads, 20 ml of sulfuric acid and one Kjendahl tablet (catalyst). Prepare three replicates and one blank flask that contains all of the above except the sample. The temperature was set at 420°C. Run the process for 4.5 hours or until the fumes disappear and the color of the sample turns transparent.

The second step is a process of distillation. After cooling the flasks of the Kjendahl, the FoodAlyt D4000 unit was used to perform the distillation with the following parameters: 50% NaOH, 4% boric acid and Methyl red-bromocresol as an indicator. Put each sample in the distillator for five minutes.

The third step is a process of titration. Direct titration takes place using 0.1M HCl , and the equivalence point is detected when color change occurs.The volumes of the used acid were recorded and the nitrogen percentage was calculated.

Determination of calories by bomb calorimeter

Bomb calorimeteris used to efficiently measure the heat released by combustion reactions. It is a sealed vessel that

contains a smaller container called a "bomb". This machine CAL3K-U calorimeter was used to determine the calories found in the powdered yeast extract. The procedure was performed as described by Mack and Black as follows:Weigh 0.5 g of powdered yeast extract and put it in the sample cup. Add 0.5 g benzoic acid to the sample.Tie the cotton rod around the iron rod above the sample cup, and make sure that the cotton rod is in touch with the benzoic acid tablet.

Tightly close the bomb and put it in the filling station to fill it with oxygen. Then put the bomb in the calorimeter and run it.Repeat the procedure to ensure three replicates.

The values are obtained in Joules and then converted into Kcal by dividing the value by 4.184.

Determination of Nucleotides Concentration

The determination of the concentration of the nucleotides 5'IMP (inosine 5'-monophosphate) and 5'GMP (guanosine 5'-monophosphate) has been carried out by spectrophotometric method. Pereira and his colleagues found that the best absorbance of IMP and GMP can be obtained at wavelength of 250 nm (Pereira et al., 2018). Therefore, the absorbance of IMP and GMP was measured at 250nm using Thermo Electron Corporation, model number Genesys 10-S in liquid yeast extract sample.

Two standard curves were plotted to calculate the exact amount of nucleotides found in our sample. Different concentrations of both nucleotides were prepared by dissolving different masses of both in distilled water, as shown in the table below:

Table 2: Masses of IMP and GMP used to prepare standard concentrations of both solutions.

Concentration	Mass of 5'IMP	Mass of 5'GMP
0.2 M	0.85 g	0.81 g
0.4 M	1.71 g	1.62 g
0.6 M	2.56 g	2.44 g
0.8 M	3.42 g	3.25 g
1 M	4.28 g	4.07 g

Then the absorbance of each concentration was measured at 250nm and the standard curves were plotted. According to these curves, the concentrations of nucleotides in our sample were determined.

Determination of Glucose Concentration

The refractometry method gives the total amount of glucose found in a sample. A protocol described by Laulloo and his colleagues (Laulloo & Cadet, 2003) was applied to measure the glucose amount in the liquid yeast extract, with some modifications, as follows:Use Hanna model number HI 96801 refractometer, then prepare standard solutions of glucose of concentrations 0.1, 0.3, 0.5, 0.7 and 0.9 g/ml.Measure the refractive index of each concentration three times, and plot a standard curve of concentration of glucose versus refractive index percentage. After that, measure the refractive index of the liquid yeast extract three times and plot it on the curve and calculate the concentration of glucose found in it.

Determination of Glutamic Acid Concentration

Earlier, Shah and his colleagues reported that amino acid reacts with acetylacetone-formaldehyde reagent to generate a yellow color product (Shah, 1992). They evaluated the effect of different parameters on this reaction to obtain maximum color intensity. As they stated in their protocol, the amount of glutamic acid was determined by first preparingacetylacetone-formaldehyde reagent solution by adding 7.8 ml acetylacetone to 15 ml formaldehyde 36% w/w. Then 100 mg of powdered yeast extract was weighed and dissolved in 100 ml distilled water in an Erlenmeyer flask and add to it 3 ml of the reagent.Put the Erlenmeyer flask in a boiling water bath.After 20 minutes, remove the Erlenmeyer from the water bath and measure and record the absorbance using at 415 nm.Prepare different concentrations of glutamic acid and repeat the same procedures to form a standard curve, measure the absorbance of the different concentrations at 450 nm and plot the standard curve.From the standard curve, calculate the amount of glutamic acid found in the powdered yeast extract.

Baking of Crackers

Recipe of Crackers

- Ingredients:
  1 cup all-purpose flour, 2 tablespoons unsalted melted butter and 7-8 tablespoons water.
- Method of preparation:
  1. Preheat the oven to 180°C
  2. In a medium bowl, mix together the flour, butter and water
  3. Add powdered yeast extract, flavor and salt according to conditions
  4. Turn the dough out onto a floured surface and roll out the dough as thin as possible (Figure 3)
  5. Cut into pieces and place the dough pieces onto a baking sheet
  6. Bake for 15 minutes not until crispy
  7. Allow the crackers to cool, serve at room temperature.

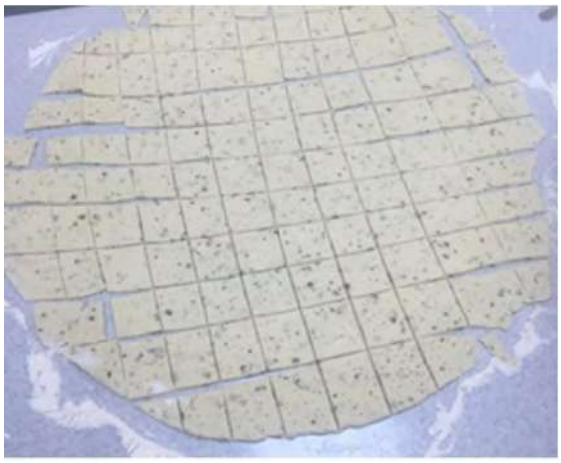

Fig.3: A sample of crackers dough cut into pieces.

The Modified Baking of Crackers

A. Determination of standard salt amount:
   Prepare different dough containing different amount of salt in one cup flour. The standard salt amount was determined by five panelists aged between 25-35, to be 7.5 g salt per one cup flour.
B. Determination of the flavor to be used:
   Prepare cracker dough as described above. Try different flavors like olive, tomato, basil, garlic, cinnamon and ginger. We decided to use the basil as a flavor based on a sensory preference test performed for 5 panelists.
C. Modified recipe:
   For the purpose of testing the salting effect of yeast extract, of different concentrations, we prepared five different dough (Table 5). The variables are the amount of salt and yeast extract added to each. All the dough included 2 g basil as a flavor.We substituted the amount of salt with powdered yeast extract as follows:

Table 3: The different concentrations of salt and YE used in each batch of crackers baking.

	Salt	Yeast extract
Control	100%	0%
Sample 1	75 %	25 %
Sample 2	50 %	50 %
Sample 3	25 %	75 %
Sample 4	0 %	100%

Sensory Evaluation

Werecruited panellists 25 males and females, aged between 18 and25 to conduct the sensory evaluation

Two tests were conducted:

- The first one is triangle test. Four different runs were made:
  - Control and sample 1
  - Control and sample 2
  - Control and sample 3
  - Control and sample 4

In each run, each panelist was subjected to a random set of three crackers, two different and one odd. Each panelist was asked to determine the odd sample and record his answer in a sheet.

Three coding system were used and each cracker was given a number of three digits randomly.

- The second test is the consumer acceptance test. The panelists were given one sample in each run and asked to evaluate different features of the cracker on a hedonic scale from 0 to 9.

III. RESULTS AND DISCUSSION

Despite its numerous potential effects, the usage of yeast extract in the food industry still limited. The main objective of this study was to shed the light over the potential saltiness of yeast extract, which goes far beyond using it as a simple food additive for enhancing the flavor or giving Umami taste. Being mainly an ingredient used in bakery and brewery, yeast has much more potential uses to be investigated. The hypothesis that YE can partially replace salt in salty crackers has been supported by our results.

Preparation of Yeast Extract

Autolysis

When 125g of dried yeast dissolved in 500 ml distelled water, a volume of 250 ml brown liquid extract is observed. The yeasts are continuously heated at 45°C and incubated for four days. According to the literature the most important condition for autolysis is the temperature (Luna-Solano et al., 2005). The optimal temperature determined by Padmakumara has been set at 45°C. Due to its convenience, this value has been also adopted to our experimental protocols. This process is enzyme driven where the catalytic activity is affected by temperature, at which it can reach its highest amplitude (Padmakumara, 2006). The endogenous enzymes come in contact with the cell wall, starting to break down the yeasts wall, allowing them to release all their content outside. In our experiment, we adopted the protocol applied by Zareiusing500g of baker's yeast (*Saccharomyces cerevisiae*) to 2L of distilled water.After four days, the extract was purified by centrifugation to allow the cell debris settle to the bottom. The supernatant or the extract

was then stored in a liquid form at -20°C until preparation of the yeast extract in powder form (Zarei et al., 2016).

Spray Drying

Spray drying is a commonly used protocol in food industry. In this process a solution of a desired compound is transferred into powder form using a heated air(Luna-Solano et al., 2005). The water in the solution is totally evaporated; this explains the increase in saltiness of the yeast extract after being spray dried. From a starting volume of 800ml of liquid yeast extract we were able to collect 10 g of powder that had more saltiness and more intenseflavor. The advantage of the spray drying is that we were able to work with a powder form of YE, that has an intense flavor of yeast, showed more saltiness, and has a longer shelf life like all powdered products. The limiting factor of this technique could be related to the need of large extract volume to be spray dried into powder.

Characterization of Yeast Extract

Salinity of yeast extract

Saltiness in the liquid yeast extract is measured by the electro conductivity (EC) meter. The salts are made of ions, and the EC measures the flowing electrical current between two metal plates (electrodes) in a liquid sample (Semat & Katz, 1958). The more dissolved salt in water, the stronger the current flow and the higher the EC. In our study we reported the EC of different concentrated samples of liquid yeast extract, and converted the EC to concentration of ions in g/L. When the yeast extract is concentrated from a volume of 100ml to 80ml, the EC recorded a value of 12.45ms/cm corresponding to 7.96g/L of salts. The higher salt concentration was recorded when the solution is concentrated to a volume of 40ml, where the EC recorded 17.9 ms/cm. However when the solution has been concentrated to 20ml, the EC could not record any flow of current in the first replicate, probably because the liquid became thick and many substances are broken or dissolved under heat effect. Golnabi recorded the EC of solutions with different concentrations of NaCl. The electrical conductivity of 0.025 mol/L NaCl is about 2.807 ms/cm. This value increases as concentration increases to reach a value of 37.242 ms/cm for 1 mol/L NaCl(Golnabi et al., 2009). When Golnabi ran the EC in 0.2 mol/L NaCl solution, a value of 18ms/cm was recorded. This value is equal to the one obtained in our experiment when we concentrated our YE solution to a value of 40 ml. We can sum up that 40 ml of our solution when contained approximately 0.2 mol/L NaCl. This is good evidence that the YE contains salty particles, and it is confirmed that when we concentrate the YE we can get a certain level of saltiness. However, heating the solution for a long period may damage some of the cell content and lead to significant difference in the flavor of the powder.

pH of Yeast Extract

The pH has been evaluated using a pH meter. The pH is a primary indicator about the acidity of a solution. The results showed that the pH increases from 5.19 to 5.53 as the volume of the yeast extract decreases from 30ml to 6ml. Zarei in his study found that the pH of the yeast extract was 6.29 (Zarei et al., 2016). This indicates that our product is slightly more acidic, so adding some sodium bicarbonate to the recipe tends to cover the acidic taste of the YE.

Determination of Fat by Soxhlet

Soxhlet extraction is one of the most commonly used methods for determination of total fat. It is associated with solvent extraction (Saunders, 1962). There are two ways to calculate the fat percentage by using the soxhlet. In the first method we used the mass of the flask were the extracted fat should be settled. The fat percentage was 3.96%. This percentage was higher than the calculated percentage using the second practice (3.4%); based on calculating the mass of the sample before and after fat extraction. Zarei tested the fat percentage of his powdered yeast extract that is produced by autolysis too, and found it to be 0.42%, lower than our results. This difference can be due to the autoclaving factor, where Zarei used autoclaving and fast cooling twice while preparing the YE. This might affected the action of the yeast cells, where they did not release all their content to the media. Despite this difference, both obtained values showed that YE is low in fat product.

Determination of Total Nitrogen by Kjendahl, Distillation and Titration

The first step is a process of digestion, where the entire nitrogen bonds are broken, and the organically bound nitrogen is converted into ammonium ions (NH_4^+). The Kjendahl tablets were used as a catalyst to speed up the digestion. The higher the temperature, the faster the digestion is, thus the temperature was set to 420°C (based on the manual of the machine). During digestion, foam appeared at the surface of the digest, and a clear solution indicates the completion of the digestion. After running the process for 4.5 hours, the sample is allowed to cool at room temperature.

The second step is a process of distillation. NaOH is used to convert ammonium ions (NH_4^+) into ammonia (NH_3). The final step is dedicated for the titration, where the sample is titrated against HCl, in order for the amount of nitrogen to be determined(Swoboda, 1922). Using the law of equivalence, at equivalence point, the number of moles

of nitrogen is equal to the number of moles of HCl, the concentration of nitrogen was determined to be 0.03192 M. Comparing our results to those of Zarei, who found that the percentage of total nitrogen is 9.2 %, we can conclude that our YE contain lower amount of nitrogen. We multiplied our results by a factor of 6.25 to calculate the amount of protein, and the value was 0.2g/100g. This value indicated that the YE contains an accepted amount of proteins

Determination of Nucleotides

The nucleotides 5'IMP (inosine 5'-monophosphate) and 5'GMP (guanosine 5'-monophosphate) are responsible of the intensification of naturalflavor and for the enhancement of Umami taste in the yeast extract(Pereira et al., 2018). The yeast contains about 7-12% RNA (Mutiu & Brandl, 2005). We followed the protocol of Pereira to determine the amount of these nucleotides by spectrophotometric methods. The previous records of the nucleotidesabsorbance varies within the range of 200-300nm, with a peak at 250nm (Pereira et al., 2018). The measured absorbance of the nucleotides in our yeast extract has been found to be 3.00 at 250 nm. By substituting the absorbance of the yeast extract in the equations of both curves (figure 3 and 4), we reported the value of 1.82M for 5'IMP and 1.38M for 5'GMP in our yeast extract. These results explained the intensified Umami taste of our YE, and showed that it may contain natural flavoring content that improves the taste of the food.

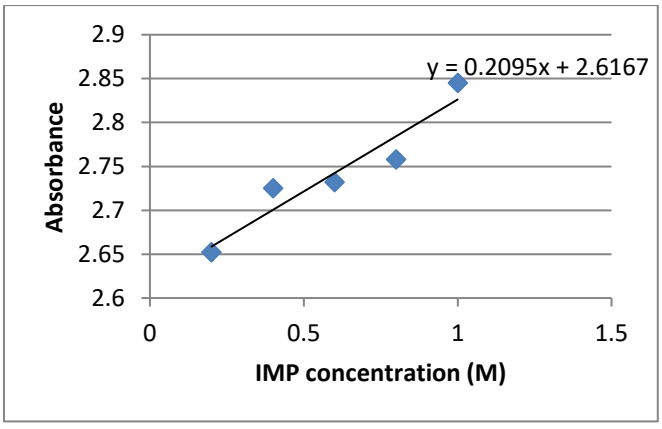

Fig.3: The standard curve determined by measuring the absorbance of different concentrations of IMP at 250nm.

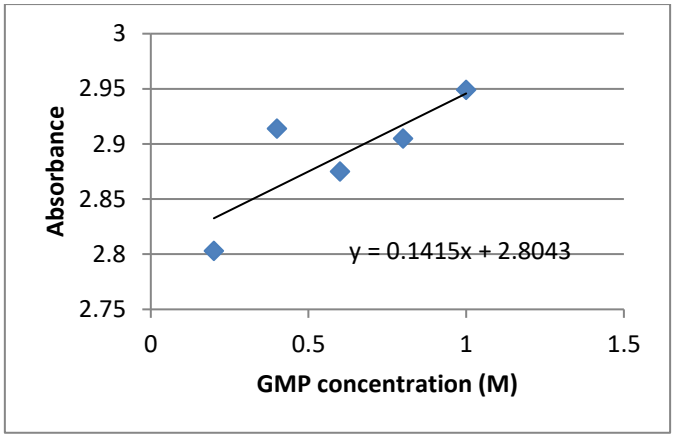

Fig.4: The standard curve determined by measuring the absorbance of different concentrations of GMP at 250nm.

Determination of Glucose by Refractometer

Refractometer is a method used to measure the Refractive Index of a particular substance. We prepared different concentrations of glucose (0.1-1g/ml) to draw the standard curve. The plot of the standard solution yielded a straight line with R^2 of 0.9771 (figure 5). This regressed line was used to determine the amount of sugar in our yeast extract, found to be is 1.4g/ml. This finding promotes the use of YE as a healthy product that is very low in glucose level.

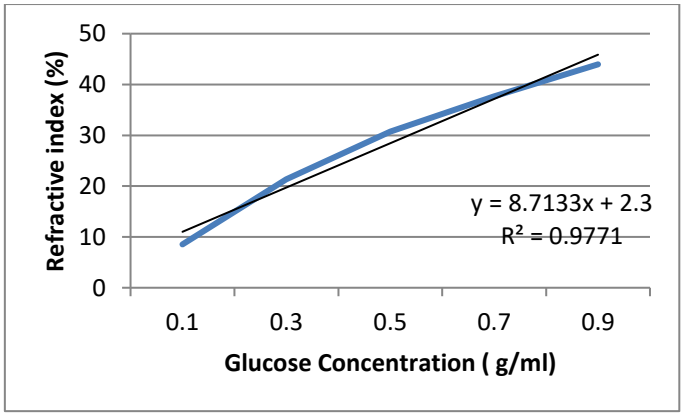

Fig.5: The standard curve was determined by measuring the refractive index of different glucose concentrations.

Determination of Glutamic Acid

Glutamic acid could be found naturally in different kinds of food like meat, poultry, cheese, milk and tomatoes (Kulkarni et al., 2005). Early it has reported that glutamic acid can react with the acetylacetone- formaldehyde reagent to give a yellow color(Shah, 1992). In his study, Shah and his colleagues determined the optimal conditions for that reaction and for obtaining the more intensified yellow color. A maximal value of glutamic acid absorbance was obtained at 415 nm using 3 ml of the

reagent, heated at 100°C in a boiling water bath for 20 minutes.

In our study we followed the protocol described by Shah, and we used his data to define the reaction parameters. At the end of the reaction, the absorbance of the dried yeast extract has been found to be 1.2383 at 415 nm. Based on the standard curve using acetylacetone- formaldehyde, the plot followed a regression line with R^2= 0.9696 (figure 6). The concentration of glutamic acid in our dried yeast extract was 0.689 g/ml. Therefore, our YE not only can be used to give Umami flavor, but also might be added to almost all foods as a flavor enhancer. This shows that our product could be a potential substitute of MSG in food industry.

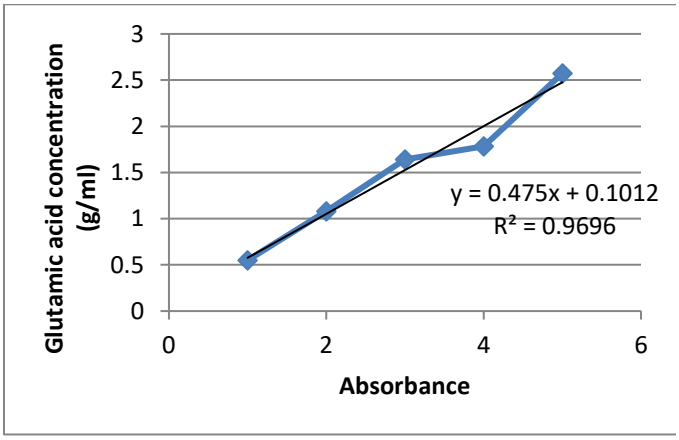

Fig.6: The standard curve determined by measuring the absorbance of different concentrations of Glutamic acid at 415 nm.

Sensory Analysis

Triangle Test

The first triangle test was conducted between the control and sample 1 (25% YE, 75% salt). Only seven panelists out of 25 were not able to identify the different sample, and the results showed that there is a significant difference between the control and sample 1. These results indicated that crackers samples containing a ratio of 1:3 (YE: Salt) were easily differentiated by panelists who were able to feel the difference in flavor.

The second run was conducted to compare the control with sample 2 (50% YE, 50% salt). Out of 25 panelists, 19 ones gave a wrong answer and could not identify the odd sample between the set and the difference was not significant. When the amount of YE was increased and substituted 50% of salt, its saltiness increased and the flavor was more intensified. Using this protocol, we could reduce the amount of salt in crackers to the half, and substitute it with a natural product, the YE, maintaining the same flavor of the product.

The third run was conducted between the control and sample 3 (3:1) (YE: Salt). The number of correct answers was 23. Only 2 panelists were not able to identify the different sample (control). The difference was significant between the control and this sample. So by substituting 75% of the initial salt amount by YE, the salty flavor was affected and the difference was apparent. The flavor of YE masked its saltiness, that's why the panelists were able to spot the odd cracker. This difference was on the level of flavor where the flavor of YE was apparent, not only on the level of saltiness.

The final run of the test was conducted between the control and the sample 4 (100% YE). Only one person was not able to identify the odd sample, while 24 panelists could recognize the different cracker. The difference was highly significant, and the two samples showed a huge difference between them. When salt is 100% substituted by YE the flavor and saltiness were totally different compared to the control.

Consumers' acceptance test

This test was conducted to evaluate how much consumers will like the edited crackers at the level of the flavor, saltiness and overall acceptability. The first test was held using sample 2 that is made up of 50% salt and 50% YE. The panelists were asked to score each trait on a hedonic scale from 1 (dislike extremely) to 9 (like extremely). At the level of saltiness, sample 3 scored 6.4 over 9, which is nearer to slightly like (on the scale). At the level of saltiness, it scored 6.48 over 9. The overall acceptability scored 6.28 over 9. These results are acceptable for a product for which, the salt content is reduced to the half.

The second test was conducted using sample 3 made up of 25% salt and 75% YE. The same hedonic scale was used as a reference. At the level of the saltiness, this sample scored 5.36 over 9 (neither like, nor dislike). Its saltiness scored 4.96 over 9, which are lower than sample 2. The overall acceptability was 5.48 over 9.

The numbers above are good evidence that reducing salt by half and replacing the reduced amount by YE gives a successful healthy salty cracker. The ratio of 2:2 (YE:Salt) gave a promising results that replacing salt by YE could be helpful in producing healthy products, while when the ratio was 3:1 (YE:Salt) the results showed that there is a significant difference between samples and thus the edited crackers were not helpful.

IV. CONCLUSION AND FUTURE STUDIES

Yeast extract proved its efficacy in replacing salt. Our results confirmed that consumers liked the crackers made up of 50% salt and 50% YE, and they showed no

significant difference with the control made up of 100% salt. Moreover, the crackers where 25% of the salt was replaced by YE showed a significant difference with the control. Therefore, YE can replace salt at a specific level and amount. The physiochemical properties of YE are interesting with very low amount of fat, glucose and calories; whereas the count of total nitrogen, nucleotides and glutamic acid were acceptable. The pH was acidic (5), and the liquid YE contained approximately 9g/L of salt. The presence of nucleotides and glutamic acid in the YE gave it the flavor enhancing property. In addition to that, the dried powdered YE gave a more salty flavor than the liquid YE; this can be explained by the higher concentration in active ingredients and a total evaporation of the water. Future studies should focus on using YE to replace salt not only for its salty taste, but also to intensify and enhance flavors of food when added. Also, the effect of YE in causing allergy to some people may have a great interest.

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Inhibitory Activity of Distilled water and Ethanol Extracts of *Moringa oleifera* Leaves Against *Enterobacter aerogenes*

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Abstract**—** *Enterobacter aerogenes is one of the bacteria that has an important role in converting histidine to histamine in fish. When consumed, histamine in fish can cause poisoning and even death. The growth of bacteria that cause damage to fish can be inhibited in several ways, including the addition of compounds that have the potential to act as bacteriostatic or even bactericide. One of the plants that has the potential as a source of bactericide compounds is drumstic tree (Moringa oleifera* Lamk.*). This study aimed to determine the effectiveness of several concentrations of Moringa leaves extract using distilled water and ethanol in the inhibition of Enterobacter aerogenes bacteria as a histamine producer. The results showed that distilled water and ethanol extracts of Moringa leaves had an effective inhibition against Enterobacter aerogenes. The distilled water extract at a 40% concentration produced the largest inhibition zone (14.73 cm) while that of the ethanol extract at a 40% concentration was 10.57 cm. The distilled water extract of Moringa leaves is suitable for use because it is cheap, practical, safe for consumption and does not leave any unsafe residue on food.*

Keywords**— *Distilled watert extract, Enterobacter aerogenes, Ethanol extract, Inhibitory activities, Moringa oleifera Leaves.***

I. INTRODUCTION

Enterobacter aerogenes is one of the bacteria present in the guts and gills of fish this bacterium has enzyme *histidine decarboxylase* capable of converting the amino acid histidine in fish into histamine in warm conditions [1]**.** Poisoning due to consuming foods containing high histamine are characterized by symptoms of a rash, nausea, vomiting and diarrhea and may even lead to death [2]. Histamine can also cause allergies in some people [3].

Histamine is a poisonous compound produced by several types of red meat fish, especially in the scromobidae family, due to the activity of bacteria and enzymes [4].

Among the histamine-forming bacteria such as using *Raoultella terrigena*, *Enterobacter* spp., *Microbacterium testaceum*, *Brevibacterium mcbrellneri; Micrococcus diversus; Staphylococcus* spp., the *Enterobacter* spp., have the greatest ability in forming histamine. Several types of the *Enterobacter* bacteria known to form histamine are *Enterobacter aerogenes*, *Enterobacter agglomerans*, *Enterobacter amnigenus*, *Enterobacter cloacae*, and *Enterobacter intermedium* [5].

The growth of bacteria that cause damage to fish can be inhibited in several ways, including the use of bactericide compounds. One of the plants that have the potential as a bactericide is Moringa [6]. *Moringa oleifera* or Moringa leaves contain bioactive compounds *saponins, alkaloids, phytosterols, tannins, phenolics* and *flavonoids* [7]. Moringa leaves have active compounds that act as antibacteria [8]. Moringa leaves extract can inhibit activities of several types of bacteria, such as *Streptococcus* sp., *Pseudomonas fluoroscens*, *Proteus mirabilis* and fungi *Aspergillus flavus* [9].

Moringa grows well in tropical and subtropical areas on all types of soil and its resists dry season with drought tolerance for up to 6 months [10]. Moringa leaves are easily be found throughout Indonesia. However, most Indonesians recognize Moringa leaves only as a vegetable dish that can be mixed with other types of vegetables [11]. So, it will be very useful if Moringa leaves can be used to inhibit the growth of bacteria, especially *Enterobacter aerogenes* as histamine-forming bacterium in fish.

This study, therefore aimed to determine the inhibitory activities of distilled water and ethanol extracts of *Moringa oleifera* leaves against bacteria *Enterobacter aerogenes*.

II. MATERIALS AND METHODS

2.1 Collection of moringa leaves samples

Moringa oleifera leaves (± 1 kg wet weight) were collected from Pinrang & Selayar, South Sulawesi Province, Indonesia. The collected leaves were then cleaned and washed under running water. Upon drained, the leaves were air-dried under shade (Fig. 1a) to avoid direct sunlight exposure. The dried samples were then mashed into a powder form (Fig. 1b), sieved with a commercial sieve and stored in a closed glass container until used.

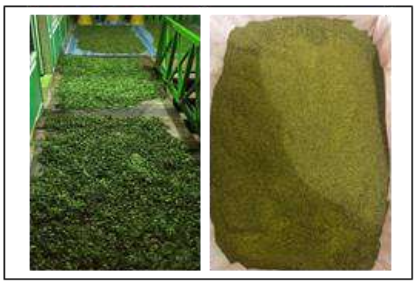

Fig 1. Air-drying process (a) Moringa leaves powder (b)

2.2 Preparation of moringa leaves estract

Extract of the Moringa leaves was prepared using two type of solvents, distilled water & ethanol. Extraction was performed by macerating (Fig. 2) 100 g of Moringa leaves powder in 500 mL of either distilled water or ethanol (1:5, w/v) for 48 h. The filtrate was then separated using Whatmann No. 1 filter paper. To recover solid extract, the water extract was lyophilized while the ethanol extract was dried using a vacuum rotary evaporator. The solid extract was stored in a capped vial until used.

Fig.2: Maceration process for 48 hours

2.3 Nutrient agar media preparation

Nutrient agar (NA) media was aseptically prepared by dissolving 4 g nutrient agar powder in 200 mL of distilled water, then heating it to a boil with regular stirring. The prepared NA media was then sterilized in an autoclave at a temperature of 121ºC for 15 min at a pressure of 1 atm [12].

While in warm conditions (40 - 45 ºC) the sterile media was poured into 15 mL sterile petri dishes and allowed to stand until the media solidified.

2.4 Bacterial rejuvenation

Enterobacter aerogenes was obtained from the Indonesian Culture Collection LIPI, Indonesia, code InaCC B865. The bacteria were grown on NA media, inoculated using a loop needle etched on NA media then incubated at 30ºC for 24 h.

2.5 Preparation of bacterial suspension

Preparation of the suspension test was carried out by taking a loopful of the isolate, suspend it in 2 mL 0.9% NaCl solution in a sterile test tube and then homogenizing it with a vortex. The turbidity of the suspension was then compared with that of the standard 0.5 McFarland (1.5 x 10^8 CFU/mL).

2.6 Preparation of test solution extract

The concentrations of the extract solution used in this study were 10, 20, 30, and 40%. These concentrations were made by thoroughly dissolving 25, 50, 75 and 100 mg, respectively, of the dry extract in 250 µL of DMSO. The negative control was 50 µL DMSO and the positive control was 10 µg /mL of Amoxicillin.

2.7 Inhibition test against *E. aerogenes*

The inhibition test was performed following the *Kirby-Bauer* disk diffusion method. Warm NA media was poured into the sterile petri disk and let to cool. Further, the bacterial suspension of 100 µL which was comparable to a standard suspension of 0.5 McFarland (1.5 x 10^8 CFU/mL) was added to the petri dishes. The bacterial suspension was spread evenly on the surface of the NA and let to stand for 5 min. Then, the paper discs pre-impregnated with test solution were placed on top of the NA containing bacteria

and incubated for 24 h at 37˚C. Upon completion of incubation, the inhibitory activity was determined by measuring diameter of the inhibitory zone.

2.8 Data analysis

Data were analyzed by one-way ANOVA using the statistical package SPSS 26 for windows to determine the antibacterial effect of *M. oleifera* extract against *E. aerogenes*. The difference in antibacterial activity among the test treatments was assessed using Duncan's Multiple Range Test with an adjusted p-value at 0.05.

III. RESULTS

The antibacterial activity of the distilled water and ethanol extract of Moringa leaves was determined by measuring the diameter of the formed inhibition zone.. The anti-bacterial activity is present if there is a clear zone around the disc paper [13]. Figure 3 shows clear areas around the discs indicating the anti-bacterial activity of the Moringa leaves extracts.

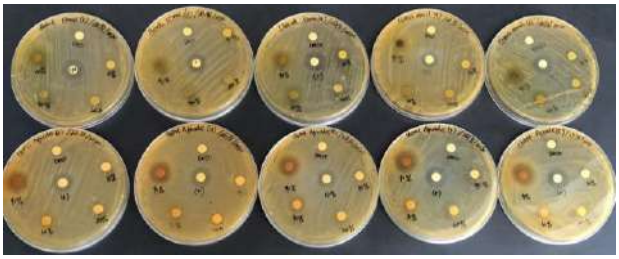

Fig 3: Inhibition zone of ethanol extract (upper row) & distilled water extract (bottom row)

Both the ethanol and water extracts of Moringa leaves showed positive results. The Inhibition zone diameter of Moringa leaves extracts is presented in Table 1. The 40% distilled water extract showed the largest diameter of inhibition, which was 14.73 mm, while the inhibition diameter of the positive control (Amoxicillin 10 µg/ml) was 13.98 mm and the 40% ethanol extract was 10.57 mm. This shows that the water extract of the Moringa leaves has an antibacterial ability comparable to the 10 µg/ml Amoxicillin antibiotic. The potential of an antimicrobial is estimated by comparing the growth inhibition zone to sensitive microorganisms from the inhibition of a concentration of a test solution to antibiotics [14].

Table 1. Diameter of Inhibitory Activities

Solvent Extract	Concentration (%)	Diameter of Inhibition (mm)
Etahonlic	10	7.01[a]
	20	7.17[a]
	30	8.17[a]
	40	10.57[b]
Distilled watert	10	7.90[ab]
	20	8.13[ab]
	30	9.56[ab]
	40	14.73[c]
Positive Control	Amoxicilin 10 µg/ml	13.98[c]
Negative Control	DMSO	0

The diameter of the zone of inhibition showed that the two extract at a 40% concentration had strong anti-bacterial activities in treating the *E. aerogenes*. The ability of the test material to inhibit the test bacteria is classified as very strong (>20 mm), strong (11-19 mm), moderate (5-10 mm) and weak (<5 mm) [15].

The results of ANOVA showed that the type of solvent and the concentration of the extract had significant effects on the activity of the *E. aerogenes*. The Duncan test showed that the distilled water extract of Moringa leaves at 40% showed a higher ($p<0.05$) inhibition activitiey against the *E. Aerogenes* compared to other concentrations of the water extract as well as to all concentrations of the ethanol extract tested. Previous study where three concentrations of [13] distilled water extract from *M. oleifera* had inhibitory diameters of 18, 19, 21 mm against *P. vulgaris* strain NCTC8196, while methanol and petroleum ether extracts did not show any effect on *P. vulgaris* bacteria [16]. *P. vulgaris* and *E. aerogenes* are of the same family.

The difference in the diameter of the inhibition zone between the distilled water and the ethanol extracts of Moringa leaves is caused by differences in the degree of polarity of each solvent. The polarity of distilled water is 9.0, while that of 96% ethanol is about 5.2. The principle of extraction is that polar substances only in polar solvents, and non-polar substances only dissolve in non-polar solvents [16]. The polarity of the solvent used determines the amount of the active substances because, in the extraction process, the principle of "like dissolves like" applies where the substance will only dissolve properly and get static if the solvent used has the same polarity level [17].

The phytochemical test results showed that the distilled water extract of Moringa leaves contained flavonoids and alkaloids. The ethanol, methanol, and ethyl acetate extracts of Moringa leaves contained alkaloid but they did not

contain flavonoids, whereas the n-hexane extract did not contain both alkaloids and flavonoids [18]. However, other study showed that the extract of Moringa leaves macerated in ethanol contained flavonoids and polyphenols [19]. The distilled water extract also contained steroids, triterpenoids, flavonoids, saponins, phenols, and tannins [12]. Nonetheless, both the distilled water and the ethanol extracts of Moringa leaves have the antibacterial ability. Still, the level of inhibition is different based on the solvent's degree of polarity.

The flavonoids, polyphenols, saponins, and tannins are active compounds in Moringa leaves with antibacterial properties [20]. According to [21], the flavonoid compounds in Moringa leaves extract have the same polarity as distilled water. Flavonoids are polyphenol group compounds widely distributed in plants in the form of glycosides that bind to sugar. Flavonoids are divided into several types, and each type has a different polarity depending on the number and position of the hydroxyl groups [21]. Polar solvents commonly used for flavonoid extraction are methanol, acetone, ethanol, water, and isopropanol.

Water as a solvent is polar, cheap, easy to obtain, stable, non-toxic, non-volatile, and flammable [22]. Water is also a recommended solvent for the food industry because it's leaves no residue in the extraction results so that the resulting product is safe for consumption [23].

According to [24], water is often referred to as the universal solvent because it dissolves many chemicals. Water is in a dynamic balance between the liquid and solid phases under standard pressure and temperature. In ionic form, water can be described as a hydrogen ion (H +) in association (bonding) with a hydroxide ion (OH-).

Ethanol is a volatile liquid commonly used as a solvent for most organic compounds. Ethanol, which is a semi-polar solvent, can dissolve both polar and non-polar compounds. Semi-polar solvents can induce polarity of non-polar solvent molecules. It acts as an intermediate solvent to mix non-polar and non-polar solvents. Ethanol has a high selectivity for reactions [25]. Therefore, selection of solvent must consider several factors, including selectivity, ability to extract, toxicity, ease of evaporation, and solvent price [25].

IV. CONCLUSION

The present study demonstrated that both the distilled water and the ethanol extracts of the Moringa leaves exhibited an antibacterial activity against *E. aerogenes*. At high concentration (40%), the antibacterial activity of the distilled water extract was more powerful than that of the ethanol extract. Nevertheless, both extracts were similar in their anti *E. aerogenes* activity at the concentrations of up to 30%. Since distilled water is considered to be safer than ethanol, it is recommended to use water for extraction of anti *E. aerogenes* from the Moringa leaves, especially if the extract is intended to be used to preserve food products. However, further study using different types of pathogenic and/or spoilage bacteria is warranted.

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Utilization of Mesquite Pods for Feeding Kids Grazing the Natural Ranges of Halaib Region

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Abstract— *This study was amid to investigate animal response to replacement 15% of concentrate feed mixture (CFM) with ground mesquite pods without and with 4% Polyethylene glycol (PEG) 4000 in invitro and invivo experiments. In vitro rumen gas production values at 24h were 58.67, 55.0 and 61.67 ml/400mg for CFM1, CFM2 (15% mesquite pods) and CFM3(15% mesquite pods + 4%PEG4000) respectively. Dry matter digestibility (DMD %) was 63.58, 64.50 and 66.65% for CFM1, CFM2 and CFM3, respectively. In vivo experiment was done using 15 growing goat kids (10.49±1.29 Kg) which were randomly divided into three groups. Group one (G1) was fed on concentrate feed mixture (CFM) and pasture (desert grass, Panicumtidrgium), group two (G2) was fed on CFM with 15% ground mesquite pods and pasture and group three (G3) was fed on with 15% ground mesquite pods plus 4% PEG 4000. Using PEG 4000 in G3 resulted in increasing intake from pasture by 19.45 and 30.51% from pasture intake in G1 and G2, respectively. Digestibility coefficients were very close to each other's. Values of NDF and ADF digestibility tended to increase in mesquite pods groups (G2 and G3) compared to control group (G1). Also, TDN% values were 74.46, 77.69 and 77.68% for G1, G2, and G3, respectively. Using PEG 4000 (G3) with mesquite pods had the best values over all groups in average daily gain (60.13 g/day),body weight gain (9.02 Kg) and final body weight (19.5 Kg).Serum parameters were under normal range of healthy goats. In general, using mesquite pods decreased feed cost per day by about 21% from control diet and PEG group as well.*

Keywords— ***Mesquite pods, Polyethylene glycol4000, Kids, digestibility, body weight, Shrub encroachment.***

I. INTRODUCTION

Despite their robust growth and their high ability to adapt to harsh conditions in a wide range of environments around the world, invasive mesquite trees (*Prosopisspp*) represent a tangible threat to the sustainability of natural resources in rangelands and cultivated lands in many countries of the world. Mesquite (*Prosiopsjoulifrolra*) is an evergreen, nitrogen-fixing and xerophyte tree with many advantages like, fast growth, drought tolerance, Sand dune stabilization, sources of feeds (leaves and pods), offering shelters for wildlife, fire woods and coals. But also has disadvantages like highly competitive growth with other plant species (by allelopathic effects) which reduce the plant biodiversity, consume water resources in arid and semi-arid regions, leaves are less to unpalatable (except for camels) due to thorns and high anti- nutritional factors (like tannins) in the leaves, meanwhile dry matter intake maybe reduced when animals fed on diet consisted of 20% or more of mesquite

pods and pods maybe toxic for livestock if it was consumed up to 50% of diet (Sawal et al., 2004 , Mahgoub et al., 2005; Shackleton et al., 2014; and Patnaik et al 2017). From Central America mesquite trees are invading many places in the world as well as Africa, Asia and Australia (Shackleton et al., 2014 and Patnaik et al 2017).

Several studies reported that shrub encroachment like mesquite in some Arab countriessuch Sudan, Egypt, Saudi Arabia, Somalia, Oman, and Yemen which resulted in negative impact on rangeland heath and productivity. Mesquite encroachment had negativity impacted the rangelands condition in southeastern Egypt, whereas areas with high mesquite cover were associated with lower *Panicumturgidum* coverage (Mohamed, et al., 2015; Mohamed, 2019). The area of lands that were invaded by Mesquite in Tokar Delta in eastern Sudan was estimated at 297 thousand acres out of the total area of Tokar Delta, which is estimated at 406 thousand acres. In a recent study (Nzumira, 2014) to assess the changes occurring in the vegetation cover of mesquite trees and the extent of their invasion of the Gash delta lands in eastern Sudan, it was found that the mesquite trees covered an area of 142 thousand hectares of the total area of the Gash Delta, which is estimated at about 372 thousand hectares in the period between year 1979 and 2013.

Many efforts were done in infected areas around the world to control mesquite trees widespread using physical (by firing), mechanical (by removing), chemical (pesticides) and biological (by introducing insects) control (Patnaik et al 2017 and Zimmermann 1991) but all these methods have many disadvantages that make them unsustainable approach for examples mechanical methods are laborious and expensive, chemical method can be source of environmental pollution and affect non target organisms and finally biological method by introducing new organism into new habitat it can turned itself into new bio-invasion (like what was happened in Austuralia).In south eastern part of Egypt (Halaib and Shalateen region) our team reported increasing in mesquite trees populations in from 2002 to 2012 by using high resolution satellite imagery (Mohamed et al., 2015). According to Makkar et al., (1990) mesquite pods have high nutritive value (77%TDN and 11-17 CP%). Unfortunately the main source of widespread of seeds is range animals (cows, camels, sheep and goats) which feed on mesquite pods and excrete seeds in feces which can grow easily after that (Sawal et al., 2004 and Shackleton et al., 2014). But, collecting and grinding (so destroying seed germination ability) of mesquite pods that can break the life cycle of mesquite trees and reduce future widespread by sustainable method. Also usingcollected mesquite pods as animal feed can reduce feed cost (Sawal et al., 2004) and reducing unemployment that maybe will be source of income and enhance livelihood of poor farmers in arid regions.

Experience has shown that the mesquite extermination programs achieve temporary success, as soon as it fades away and the mesquite trees re-spread again, so presenting an integrated management program for mesquite trees in the Arab world is of importance in achieving sustainable development for the Arab countries that have been negatively affected by the invasion of mesquite and those that exhibit the spread of mesquite in those countries. This program should include a mixture between sustainable control methods and the economic adaptation of mesquite trees to become a source of additional income for farmers and herders.So, this study is aimed to investigate the responses of growing goats' kids when feeding on ground mesquite pods as part of concentrate portion of diet using in vitro and in vivo tools.

II. MATERIALS AND METHODS

Study Location:

This study was carried out at Shalateen Research Station - Desert Research Center (DRC), Ministry of Agriculture and Land Reclamation, at RasHadraba valley- Halaib region- Red Sea Governorate, (about 1400 km southeast of Cairo), Egypt.

The region is located at longitudes (36˚, 52`, 676" & 36˚, 45`, 002") and latitudes (21˚, 59`, 795"& 22˚, 59`, 992"). It is classified as an arid region with average ambient air temperatures are 35°C and 22°C, and relative humidity values are 37% and 43% for the summer and winter seasons, respectively. The average annual precipitation is about 58.5 mm, and mostly as erratic showers in November and December. Water resources are scarcity and available only too nomadic inhabitants and their animals from shallow wells and desalination Seawater. Thus, agricultural activities are absent and livestock (sheep, goats and camels) grazing on rangelands is considered the only option of livelihood for the farmers(Askar et al., 2014;Nassar, 2020).According to Badawy (2005),Allam et al. (2007) and Raef (2012)desert grass (Panicumturgidum) is the main dominant pasture grass with abundance (95.9% and 97.7%) and plant density (0.455 and 0.296 plant/m^2) for wet and dry seasons, respectively. The dry biomass yield of *P. turgidum* was 1.46 Ton/feddan

(4200 m^2) in the wet season and 1.25 Ton/ feddan in the dry season, respectively.

Mesquite pods collection and Preparation

Air dried full mature mesquite pods were collected from around mesquite trees – Halaib and Shalateen region - by hands and stored under indirect sun cured and well ventilated shade. Then ground into fine powder and well mixed with other feed ingredients to make three different combinations of concentrate feed mixtures as shown in Table(1):

Table.1: Composition of concentrate feed mixtures (kg/100 kg)

Ingredient composition	CFM1	CFM2	CFM3
Yellow corn	42	41	41
Undecorticated cotton	30	30	30
Wheat bran (coarse)	24.5	10.5	10.5
Mesquite pods	0	15	15
lime stone	2	2	2
Common salt	1	1	1
Trace minerals &	0.5	0.5	0.5
+ PEG 4000	0	0	4

- CFM1: Concentrate feed mixture

- CFM2: Concentrate feed mixture included 15% crushed mesquite pods

- CFM3: Concentrate feed mixture included15% crushed mesquite pods + 4% PEG 4000

In vitro experiments

In vitro gas production was carried out according to Menke and Steingass (1988). Two healthy adult fistulatedBarki rams were fed on good quality Egyptian clover hay (60%) and concentrate feed mixture (40%) which were used as a source of rumen liquor which. Finely ground(Approximately 400mg, air dried) concentrates samples (CFM1, CFM2 and CFM3) were accurately weighted into glass bottle then incubated with buffered rumen liquor for 48h and incubated anaerobically at 39°C. Total gas production was measured at 24 and 48h. Then pH was measured immediately using digital pH meter. Ammonia and, total volatile fatty acids (TVF's),in vitro dry matter (DM), neutral detergent fiber(NDF) and acid detergent fiber (ADF) digestibility were determined.

Organic matter digestibility (OMD) , Metabolisable energy (ME) and total digestible nutrients (TDN) were calculated from the amount of gas produced at 24hrs of incubation with the components of feeds from crude protein (CP), ether extract (EE) and total ash (TA) according Menke and Steingass (1988) for concentrate samples.

- OMD% = 9+ 0.9991GP+ 0.0595CP(g/kg)+ 0.0181TA(g/kg)

- ME(MJ/kgDM) = 1.06 + 0.1570GP+ 0.0084CP(g/kg)+ 0.022EE(g/kg)- 0.0081TA(g/kg)

- TDN (%) = [ME (MCal/kg DM) + 0.45] / 0.0445309 (NRC, 1989)

In vivo experiment

Fifteen growing male Blady goat kids (10.49 ± 1.29 Kg) were used in the feeding trail for five months divided on randomly basis into three groups (five animals per each group). Before commencing the trial all animal were drenching against internal parasites, animals fed on CFM by 3% of live body weight according NRC (1981) and allow daily to graze desert grass (*Panicumturgidum*). Animals were weighted biweekly and concentrate feeds were adjusted according animal body weight. Drinking water was available three times per day. Mineral blocks were freely available.

Digestibility trials and determine pasture dry matter intake:

Fecal collection bags were used to quantitative collection of feces from each animal daily for five days. Fecal bags were emptied daily weighted and 10% samples were taken and dried at 65° C for a constant weight.The pasture intake and digestibility were determined using the acid insoluble ash (AIA) method as internal marker (Schneider and Flatt (1975). Using the following equations:

- Marker in pasture = Marker in feces – Marker in concentrate diet.

[Total marker in range intake per day]

- Estimated DMI (gm/day) = ----------

[Concentration of marker in range dry basis]

Analytical procedures

Proximate analysis for dry matter (DM), crude protein (CP), crude fiber (CF), ether extract (EE) were determined according to the official methods (AOAC, 1990). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were estimated according to Van Soest and Robertson (1985). Acid insoluble ash (AIA) was analyzed according to Van Keulen and Young (1977). Total volatile fatty acids (TVFA's) were by steam distillation determined according to Annison (1954), Ammonia was determined concentration was carried out by a modified Nessler's method modified by

Szumacher-Strabelet al., (2002). Total tannins (TT) were determined gravimetrically with copper acetate method according to Balbaa (1986). Condensed tannins (CT) were determined by spectrophotometer according to Makkar*et al*. (2007). TT and CT were determined only for mesquite pods.

Blood samples were collected from the jugular vein before morning feeding and were allowed to stand at room temperature for 1h then centrifuged at 3000 rpm for 20 min to get serum then and stored at -20 °C for further analysis of blood metabolites using colorimetric methods as follows: Total protein and albumin were measured according to Gornal et al. (1949) and Doumas et al. (1971), respectively and globulin was determined by the difference (TP-Alb). Blood urea nitrogen (BUN) was determined according to Fawcett and Soctt (1960). Blood serum creatinine was determined according to Bartles et al. (1972) and Larsen (1972).

Statistical analysis

Data Differences among treatments were significantly checked using one way analysis of variance (ANOVA) and Duncan's new multiple range test (Duncan, 1955) was used to compare between means. The relationships between in vitro parameters were checked for simple correlation. The General Linear Model (GLM) of SAS (1996) was applied.

III. RESULTS AND DISCUSSIONS

Chemical compositions of experimental feeds:

Chemical composition of ground mesquite pods (*Prosopisjuliflora*), concentrate feed mixtures and desert grass (*Panicumturgidum*) are shown in Table (2). Mesquite pods chemical composition of CP,CF and EE was 18.8, 20.39 and 4.07%, respectively. Which is comparable with Sawal et al (2004) who reported that the range of CP (11.99 – 16.5%), CF (16.9- 30.77%), EE (1.3 – 4.26). Differences in Mesquite pods chemical composition maybe due to different environmental factors (soil type, water viability, temperature) and plant varieties. In current study replacing wheat bran in concentrate feed mixtures with mesquite pods at 15% didn't make differences on chemical compositions (Table 2) and the concentrate combinations are iso-caloric (18.46, 18.92 and 18.89 MJ/Kg DM) andiso-nitrogenous CP (17.42, 17.79 and 17.39%) for CFM1, CFM2 and CFM3, respectively.

Table.2: Chemical composition of experimental feeds (%, DM basis)

Item	Mesquite Pods	CFM1	CFM2	CFM3	Desert grass
DM	91.56	91.28	91.88	91.53	37.96
OM	95.17	92.52	93.56	93.34	92.74
Ash	4.83	7.48	6.44	6.66	7.26
CP	18.80	17.42	17.79	17.39	7.72
CF	20.39	7.05	8.43	7.84	33.58
EE	4.07	4.98	6.15	6.28	3.54
NFE	51.92	63.07	61.18	61.81	47.91
NDF	48.9	34.61	36.83	35.82	74.78
ADF	33.68	15.9	18.32	17.11	42.37
GE (MJ/ Kg DM)*	18.82	18.46	18.92	18.89	17.57

* Mesquite pods (*Prosopisjuliflora*)

*CFM1: Concentrate feed mixture

* CFM2: Concentrate feed mixture included 15% ground mesquite pods

*CFM3: Concentrate feed mixture included15% ground mesquite pods + 4% PEG 4000

*Desert grass (*Panicumturgidum*)

* GE = 0.0176 OM + 0.0064 CP + 0.0214 EE according to SCA (1990)

* GE (MJ/KgDM) = 0.0176 OM (g/kg) + 0.0064 CP(g/kg) + 0.0214 EE(g/kg) according to SCA (1990)

In vitro experiment

In vitro gas production parameters are shown in Table (3) and correlation coefficient of gas production parameters are shown in Table (4). In general, CFM2 with 15% ground mesquite pods had the lowest values in all gas production parameters estimated and calculated and this can be explained by the presence total tannins (TT) and condensed tannins (CT) in mesquite pods (1.64 and 0.83%,

respectively). But, with little small effects on DMD% being 63.58, 64.50 and 66.65% and on NDF% as 45.32, 42.92 and 44.61% for CFM1, CFM2 and CFM3, respectively(Table 3).

Polyethylene glycol (a synthetic polymer) has higher affinity to tannins more than protein. It disrupts tannin–protein complexes and increases protein availability for rumen microflora and then to the host animal. The PEG has positive effects like increase feed intake, digestibility, and live weight gain in animals fed on tannin-rich diets. On other hand PEG had negative effects like very expensive especially in developing countries and it may reduce the effect of tannin on decreasing methane production and internal parasites (Makkar et al., 1995; Kabasa et al., 2000; Ben Salem et al., 2005, Bhatta et al., 2009 and Ghandour et al., 2014).

Using PEG 4000 as feed additives in CFM3 enhanced total gas production in 24 and 48h by 12.13 and 8.11% of CFM2, respectively. But this addition just increased DMD% with 3.33% over mesquite pods without PEG (CFM2). In the same direction Guerrero et al., (2012) found that in in vitro study that PEG supplementation of Acacia constricta shrub with low CT(0.3%) that increased significantly gas production from 137 to 207 ml 24h, but with small little increase in DMD 54.6 to 57.9% non-significant. This results agreed with Bayssa et al., (2016) who reported very high values of phenolic compounds in mesquite pods for example TT was 14.1% and CT was 7.2% and they found increasing in OMD% (from 48 to 57.8%), ME from (6.2 to 9.9 MJ/Kg) and TVFA (from 0.63 to 0.92 mmol/L) in mesquite pods and mesquite pods treated with 2% PEG solution, respectively. On contrary Makkar et al., (1990) reported values of mesquite pods had TT (2.92%) and CT (0.68%) (whichsimilar to current study) and not detected value of Protein–Perceptible phenolics (was), not detected Protein – precipitation capacity, Degree of polymerisation (DP) was 2.33, that indicated low activity of mesquite pods tannins. In the same trend Hanafy et al., (2015) reported that in vitro gas production (24h) values of *Acacia sligna*shrub that had high TT (6.66%) and CT (4.69%) were 22.0 and 17.8 ml/200mg for acacia without and with 4%PEG 4000, respectively.

The values of ADFD% (Table 3) were 55.13, 46.98 and 59.01% for CFM1, CFM2, CFM3, respectively. Decreasing ADFD% in CFM2 may be explained by the lowest rumen microbe fermentation as reflected the lowest gas production at 24 and 48h and TVFA's concentration (table 3). That supported by strong correlation coefficient (with highly statistically significance) between ADFD% and TG24, TG48, pH (Table 4). Santos et al., (2015) reported low ADFD% being 42.2 to 33.4% when replacing mesquite pods from total diet by 0 to 15 in sheep (in vivo). Obeidat and Shdaifat (2013) also reported decreasing in ADFD% with linearity increasing of mesquite pods in lactatingAwasssi ewes' diet from 0, 12.5 and 25%.

Table.3: Rumen in vitro gas production and nutritive value of different concentrate feed mixtures.

Item	CFM1	CFM1	CFM1	±SE
TG24, ml	58.67^{b}	55.00^{c}	61.67^{a}	1.10
TG48, ml	75.00^{b}	74.00^{b}	80.00^{a}	0.97
pH	6.48^{b}	6.56^{a}	6.41^{b}	0.02
NH3-N, (mg/100 ml)	24.78	20.70	21.60	1.32
TVFA's (m.equiv./100 ml)	4.096	3.421	3.570	0.22
DMD%	63.58^{b}	64.50^{ab}	66.65^{a}	0.61
OMD%	79.33^{a}	75.70^{b}	82.17^{a}	1.03
NDFD%	45.32	42.92	44.61	0.80
ADFD%	55.13^{b}	46.98^{c}	59.01^{a}	1.77
ME, (MJ/DM kg)	12.22^{b}	12.02^{b}	13.05^{a}	0.17
TDN%	75.71^{b}	74.63^{b}	80.12^{a}	0.92

*CFM1: Concentrate feed mixture

* CFM2: Concentrate feed mixture included 15% ground mesquite pods

*CFM3: Concentrate feed mixture included15% ground mesquite pods + 4% PEG 4000

ME = 1.06 + 0.1570GP+ 0.0084CP+ 0.022EE- 0.0081TA

OMD% = 9+ 0.9991GP+ 0.0595CP+ 0.0181TA

TDN (%) = [ME (MCal/kg DM) + 0.45] / 0.0445309

In vivo experiment

Feed intake

Inclusion ground mesquite pods by 15% from concentrate diet and replaced mainly from wheat bran (Table 1, 2) that resulted in iso-caloric (18.46, 18.92 and 18.89 MJ/Kg DM) and iso-nitrogenous CP (17.42, 17. 79 and 17.39%) for, CFM1, CFM2 and CFM3, respectively (Table 2). Dry matter intake from concentrate, forage and total intake are shown in Table (5). Average dry matter intake from concentrate feed mixtures (CFM) were 27.38, 27.56 and 27.46 g/kg BW for G1, G2 and G3 groups, respectively. Without any adverse effects of mesquite pods and prove that mesquite pods was palatable at this level of replacement.

Average dry matter intake from forage, desert grass (*Panicumturgidum*), was 233.14, 213.38 and 278.48 g/day for G1, G2 and G3 groups, respectively. Using PEG 4000 in G3 resulted in increasing intake from pasture (by 19.45 and 30.51% from pasture intake in G1 and G2, respectively). That may be explained by presence of some phenolic compounds like total phenolics, flavonoids and tannins in desert grass as reported by Farag et al., (2016) and Al-Rowaily et al., (2019) but with low concentrations. Also Khan et al., (2009) reported total oxalate reached 4.14% in *Panicumturgidum*. This compound may be decrease feed intake by unpleasant taste (bitter and/or astringent taste). However, Ghandour (2014) reported enhancing DMI in sheep fed on plant rich tannins (Acicia hay) by using PEG 4000.

Table 4: Correlation coefficients of Gas production parameters

Item	TG24	TG48	pH	NH3	VFA	ME	TDN	DMD	OMD	NDFD	ADFD
TG24	1.00	0.86 **	-0.93 ***	0.45 ns	0.45 Ns	0.94 ***	0.94 ***	0.35 Ns	0.99 ****	0.41 Ns	0.90 **
TG48		1.000	-0.84 **	0.13 ns	0.14 Ns	0.94 ***	0.94 ***	0.61 ns	0.85 **	0.27 Ns	0.92 ***
pH			1.00	-0.54 ns	-0.54 Ns	-0.88 **	-0.88 **	-0.50 ns	-0.93 ***	-0.31 Ns	-0.91 ***
NH3				1.00	0.99 ****	0.24 ns	0.24 Ns	-0.29 ns	0.46 ns	0.68 ns	0.35 ns
VFA					1.00	0.25 ns	0.24 Ns	-0.29 ns	0.46 ns	0.68 *	0.35 ns
ME						1.00	0.999 ****	0.52 ns	0.93 ***	0.28 Ns	0.90 ***
TDN							1.00	0.53 Ns	0.93 ***	0.29 Ns	0.90 ***
DMD								1.00	0.34 ns	-0.49 Ns	0.53 ns
OMD									1.00	0.42 Ns	0.89 *
NDFD										1.00	0.34 ns
ADFD											1.00

Average total DMI was 733.69, 708.43 and 822.72 g/day for G1, G2 and G3, respectively. Calculated mesquite pods percentage from total diet in current study were 14.05% from G2 and 14.15 % from G3 that level didn't induce negative effects on feed intake which started to decrease feed intake may be at 20% inclusion as reported by Obeidatand Shdaifat (2013) who also reported that replacement mesquite pods in lactating Awasssi ewes' diet from 0 to 12.5% didn't affect DMI but when increasing the percentage of replacement to 25% then TDMI decreased by 2.36 and 2.6 % of 0 to 12.5% mesquite pods replacement groups, respectively. Similar finding was observed for OM, CP and fibre fraction (NDF and ADF) intakes, it can notice that, kids in G3 group had higher intake from OM, CP, NDF and ADF than other groups, but, this increase was insignificantly.

Table.5: Effect of treatments on feed intake by kids during digestibility trial.

Item	G1	G2	G3	±SE
No of animal	5	5	5	-
Body weight,kg	18.28	17.96	19.82	0.70
Dry matter intake,				
Concentrate intake,				
g/day	500.56	495.06	544.24	19.17
g/Kg BW	27.38	27.56	27.46	0.02
Forage intake,				
g/ day	233.14	213.38	278.48	21.96
g/Kg BW	12.82	12.62	14.28	1.22
Total DM intake				
g/day	733.69	708.43	822.72	27.97
g/Kg BW	40.21	40.18	41.74	1.22
Total OM intake,				
g / day	680.45	661.04	763.01	25.81
g/Kg BW	37.29	37.49	38.71	0.11
Total CP intake,				
g / day	105.21	104.57	116.17	3.58
g/Kg BW	5.76	5.88	5.88	0.09
Total NDF intake,				
g / day	347.59	341.89	403.19	17.18
g/Kg BW	19.07	19.59	20.52	0.92
Total ADF intake,				
g / day	178.36	181.12	211.11	9.52
g/Kg BW	9.79	10.40	10.75	0.52

*G1: Concentrate feed mixture + Pasture

* G2: Concentrate feed mixture included 15% ground mesquite pods+ Pasture

*G3: Concentrate feed mixture included 15% ground mesquite pods + 4% PEG 4000+ Pasture

Nutrients digestibility and nutritive value

Nutrients digestibility and nutritive values are shown in the Table (6) most digestibility values closely similar in the three groups and the values statistically non-significant. But all values tended to increase in mesquite pods groups (G2 and G3) compared to control group (G1). This pattern was reflected on TDN% values being 74.46, 77.69 and 77.68% for G1, G2, and G3, respectively. That indicate using mesquite pods at this level didn't have adverse effects on animal digestibility and nutritive value. In the same trend Obeidat et al., (2008) found that similarity in DM, OM, CP, NDF and ADF among Awassi lambs groups with 0, 10 and 20% mesquite replacement. Obeidat and Shdaifat (2013) also reported that replacement mesquite pods in lactating Awasssi ewes' diet from 0 to 12.5% there were no effects on DM, OM, CP and NDFD% digestibility except decreasing in ADFD% in mesquite (12.5%) group. But when using increasing mesquite pods to 25% from the diet all digestibility parameters were dropped.

Table.6: Nutrients digestibility and nutritive values of the experimental diets

Item	G1	G2	G3	±SE
Digestibility, %				
DM	69.60	71.78	70.71	1.02
OM	77.13	78.11	78.88	0.75
CP	71.16	73.62	71.86	0.95
CF	68.54	72.77	69.51	1.32
EE	75.70	79.60	80.33	0.92
NFE	79.03	79.55	81.07	1.00
NDF	53.64	57.65	57.10	1.46
ADF	42.10	46.81	45.95	1.68
Nutritive value, %				
TDN	74.64	77.69	77.68	0.87
DCP	10.25	10.88	10.19	0.25

*G1: Concentrate feed mixture + Pasture

* G2: Concentrate feed mixture included 15% ground mesquite pods+ Pasture

*G3: Concentrate feed mixture included + 4% PEG 4000+ Pasture

Body weight changes

Body weight changes through feeding period are illustrated in Fig. (1). It can be observed from the figure that goats consumed about one month as adaptation period (which means no changes in ADG). Same resulted was obtained by Mahgoub et al., (2005) who reported goats fed on 0, 10, 20 and 30% mesquite pods needed about one month to start gaining weight.

Initial, final body weight and average daily gain are shown in Table (7). Using mesquite pods (G2) had very close results of control group of final body weight, body weight gain and average daily gain as 18.6 and 18.5Kg; 7.96 and 8.16 Kg and 53.10 and 54.4g/day for G1 and G2, respectively. Meanwhile using PEG 4000 (G3) had the best values over all groups in final body weight (19.5 Kg), body weight gain (9.02 Kg) and average daily gain (60.13g/day) which reflects high TDMI (Table 5) high TDN% (Table 6). Shamseldein et al., (2013) reported that ADG of Sudanese Nubian goats kids was decreased by increasing mesquite pods gradually in the diet from 0, 10, 20 to 30% to be 96.33, 97.55, 91.22 and 73.06 g/day, respectively.

Feed conversion and economic evaluation

Feed conversionratios were 10.87, 10.77 and 9.60 DMI/kgBW for G1, G2 and G3, respectively (Table 7). That in agreement with Mahgoub et al., (2005) when goats fed on 0, 10, 20 and 30% mesquite pods for two months FCR values were 10.78, 10.76, 6.56 and 13.86 DMI/kgBW, respectively.

Economic evaluation indicators data are shown in Table (7). Using mesquite pods (G2) decreased the feed cost per day and cost of BWG per Kg dropped by 21.3% and 23.16% from control group (G1) and this due to low cost of mesquite pods. Meanwhile high price of PEG 4000 (G3) resulted in approximately equalization of feed cost per day and cost of BWG per Kg in G1 and G3. Sawal (2004) reported when replacing wheat bran with mesquite pods reduced feed cost of lamb feeding. Economic efficiency was the highest G2 (2.1) then G3 (1.7) and G1(1.6).That maybe support and encourage researchers to find out affordable alternative of imported PEG 4000 like bentonite clay (Ghandour 2014 and Hanafy et al 2014).

Table.7: Body weight changes, feed conversion and economic efficiency of goats fed on experimental diets.

Item	G1	G2	G3	±SE
Body weight changes :				
Initial BW, Kg	10.64	10.34	10.48	0.33
Final BW, Kg	18.6	18.5	19.5	0.65
Total gain, Kg	7.96	8.16	9.02	0.55
Average daily gain, g	53.10	54.40	60.13	3.65
Feed conversion ratio :				
Feed conversion ratio, Kg DMI/Kg BW	10.87	10.77	9.60	0.78
FCR (kg TDN/Kg BW)	8.23	8.30	7.45	0.62
FCR (kg DCP/Kg BW)	1.11	1.14	0.98	0.10
Economic evaluating indicators :				
Feed cost/day * Egyptian Pounds	2.02	1.59	2.1	
Total feed cost	303	238.5	315	
Cost of one Kg BW gain	38.04	29.23	34.92	
Price of total gain	477.6	489.6	541.2	
Economic efficiency	1.6	2.1	1.7	

Economic efficiency (price of total body gain / cost of total feed consumed)

* feed cost/day is for concentrate and forage assumed to equal zero according to Nasser (2020) Economic indicators of feeds were calculated in Egyptian pound (EP) based on the price of the year 2020. The price per ton was assigned as follows; Ground mesquite pods 1000 EP; CFM 5000 EP; PEG 30000 EP, The price of kg live body weight at purchase or selling was 60 EP.

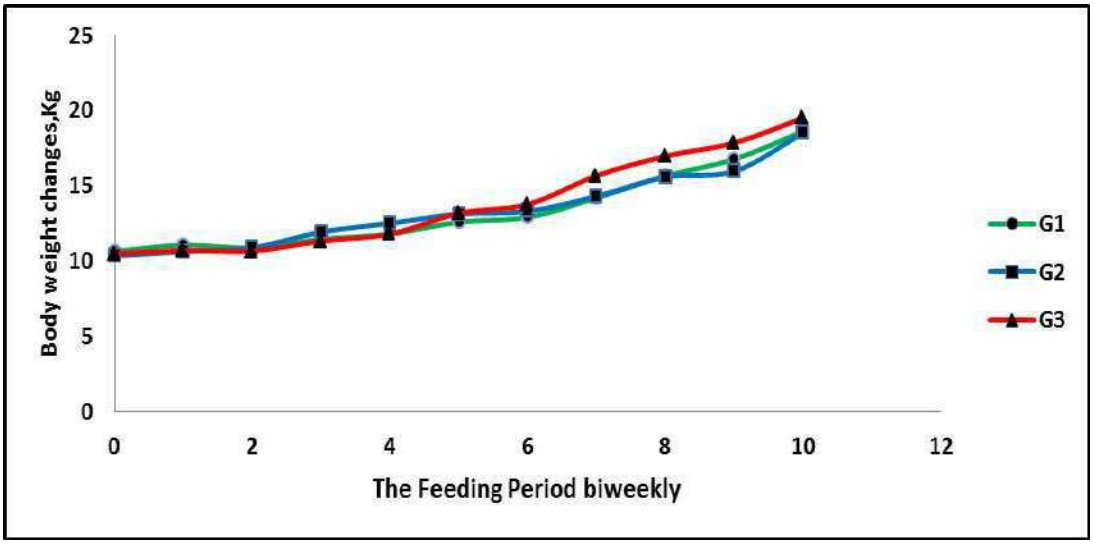

Fig.1: Body weight changes of growing goats during the feeding period

Some blood metabolites

Blood serum metabolites values of experimental goats are shown Table (8). All blood parameters were involved in the normal range of goats according to Jackson and Cockcroft (2002).That reflects safety level of mesquite pods were used in current study without any toxicity indicators in the blood serum parameters. In the same trend Chharan g*et al*., (2020) reported that blood serum protein was 8.02 and 8.10 g/dl and serum glucose was 72.00 and 69.75 mg/dl in growing goats fed on control diet or diet with 20% mesquite pods for105

days, respectively. Cook *et al*, (2008) reported closely results of serum metabolites like creatinine, blood urea nitrogen (BUN) Aspartate transaminase (AST) gamma glutamyl transferase (GGT) among goats fed on mesquite pods at 0, 30, 90% of the diet but for short period (14 days).

Table.8: Blood serum metabolites of goats fed the experimental diets.

Item	G1	G2	G3	SE	Normal Range*
TP (g/dl)	6.74	6.50	7.01	0.23	(6.0-7.9)
Al (g/dl)	3.42	3.28	3.41	0.15	(2.9–4.3)
Glb (g/dl)	3.32	3.21	3.61	0.27	--
Al/Gl ratio	1.22	1.26	1.23	0.15	--
Creat	0.92	0.88	0.75	0.10	(0.6–1.6)
BUN(mg/dl)	15.22	16.89	15.92	0.66	(12–26)
Glucose (mg/dl)	66.19	69.42	68.75	2.22	(50- 75)

*G1: Concentrate feed mixture + Pasture

* G2: Concentrate feed mixture included 15% ground mesquite pods+ Pasture

*G3: Concentrate feed mixture included15% ground mesquite pods + 4% PEG 4000+ Pasture

IV. CONCLUSION

It can be concluded that replacing wheat bran with mesquite pods at level 15% of concentre diet did not have any adverse effects on growing goat kids and it can reduce feed cost and increase economic efficiency of goat feeding under study conditions. There is need for further studies about how to reduce negative animals' responses to increase levels of mesquite pods up to 20% or more. Also there is a need to find out affordable alternative of PEG 4000 when using mesquite pods.These results could encourage small holders in desert areas to feed low cost Mesquite pods for goats, sheep and camels and reduce invasion of Mesquite trees at the same time.

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Bioeconomic Analysis of Indian Scad (*Decapterus ruselli*) in the Bone bay Waters of South Sulawesi

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Abstract**—** *The purpose of this research is to analyze the optimal level of production and the efforts to utilize Indian ScadFish in Bone Bay Waters. This research was conducted from July to September 2021. The method used was descriptive quantitative using a questionnaire tool. The sampling method used was the random sampling method with a total of 10 fishing Indian Scad business units. The data analysis used was standardizing fishing gear, estimating biological and economic parameters and estimating dynamic bioeconomic parameters.*

The results showed that generally the level of utilization of Indian Scad fish in the purse seine fishing gear had fluctuatedvalue. The actual production conditions of the use of Indian Scadfish were higher when compared to the production in the Bioeconomic management regime, which is52,543.67 tons while the maximum sustainable value recommended in the regime MSY is 62,889.11, MEY is284,661.66 tons.

Keywords**— Bioeconomics, Maximum Sustainable Yield (MSY), Maximum Economic Yield (MEY).**

I. PRELIMINARY

Indian Scad is the largest contributor to the number of fish catches in South Sulawesi. Apart from being consumed by the community, this fish is also used as bait for tuna fisheries companies and is exported frozen. In the last five years, there has been a decline in the catch of Indian Scad fish in South Sulawesi due to increased exploitation of these fish (Umar et al., 2013).

The waters of Flores and Bone Bay, South Sulawesi, are one of the dominant Indian Scad fish producers (> 50%) in South Sulawesi (Fisheries Statistics, 2017). Purse seine is the dominant tool used to catch Indian Scad fish and several areas that are caught in Bone Bay waters include Bone, Sinjai and Palopo districts.

The potential area for Indian Scad fish is caught using fishing gear between the Payang and Purse Seine. The high demand for Indian Scad fish can further increase the exploitation of these fish resources. As a result, there will be a decline in population growth that continues with extinction (Sugiyono, 2015).

Stock assessment in every scientific study of fisheries is to determine the productivity of fisheries resource, the effect of fishing on resources and the impact of changing fishing patterns. Estimation of stock abundance is essential for evaluating the size of management units, and for estimating ratesof exploitation caused by fishing (Gulland, 1983).

Some of the problems that occur due to the intensive use of Indian Scad fish (*Decapterus spp*) are that the catch has fluctuated every year, the fishing time is getting longer, the fishing area is getting farther away and the fishermen no longer choose their catch, for example,Indian Scad fish which is still small in size. The basis for resource management is how to utilize resources to produce high economic benefits for business actors, but their sustainability is maintained (Fauzi and Anna, 2010).

In an open-access system, every user feels entitled to exploit resources on a large scale (over-exploitation). As a result, resources are under pressure and become overfishing and eventually resource depletion occurs, while every user often engages in unfair competition for increasgly limited resources.

Biological aspects are also commonly used to evaluate fish resources, but without neglecting the economic aspects. Meanwhile, fishermen as business actors and resource users are oriented towards economic principles by prioritizing the maximum profit without paying attention to sustainability. For this reason, an analysis of the utilization rate of Catch Per Unit Effort (CPUE) is needed, the determination of the Level of Sustainable Potential, Optimal Effort, and the level of utilization of Indian Scad fish resources, where this information is very much needed in sustainable fisheries management (Sangaji, et al. 2017).

The fact shows that fishermen as business actors and resource users are oriented towards economic principles by prioritizing the maximum profit possible to meet individual needs without paying attention to sustainability.This research is focused on cases of high fishing effort so that overfishing and exploitation are indicated or a reduction in the availability of fish stocks, causing depletion of the Indian Scad fish resources in Bone Bay waters. This study aims to analyze the optimal level of production and the efforts to use the Indian Scad fish in Bone Bay Waters.

II. RESEARCH METHODS

A. Time and Place

This research was conducted for 3 months July - September 2020. The location of this research was Palopo City, Bone Regency and Sinjai Regency, Indonesia. The sample in this study was Indian Scad fish. This research was conducted in Bone Bay by taking locations in 3 (three) districts that serve as fishing bases, namely Palopo City, Bone Regency and Sinjai Regency, South Sulawesi Province. The three locations were location points that represent the waters of Bone Bay.

B. Type of Research

This research used quantitative methods. Quantitative is the process of finding knowledge using data in the form of numbers as a means of finding information about what is the research problem (Sugiyono, 2013). This research was conducted with a survey method, namely by conducting field observations and direct interviews with respondents and using a questionnaire as a data collection tool. Then the data that had been obtained was analyzed quantitatively.

C. Sampling Method

The research method is a method used by researchers to obtain data and information on various matters relating to the problem to be studied. Population is a collection of all elements in the population where the sample is taken while the sample is a part of the population (Welhelmus, 2007).

This study used a random sampling method in which respondents or samples were randomly selected by taking the research location as a fishing base, namely TPI Pontap Palopo City, TPI Lonrae Bone Regency and TPI Lappa Sinjai Regency with 10 fishing business each, bringing a total of 30 business units as a sample. From the use of fishing gear, the 3 research locations represent the main fishing gear of Bone Bay, namely: TPI Pontap Palopo City with Bagan dominant fishing gear, TPI Lonrae Bone Regency with Purse Seine fishing gear, and TPI Lappa Sinjai Regency with dominant Purse Seine fishing gear.

D. Data Sources

Sources of data used in this study were primary and secondary data, including:

1. Primary data is data obtained directly from observations in the field. Primary data collection was carried out by structured interviews using a list of questions (questionnaire) supported by direct observation of fishermen's activities.
2. Secondary data is data obtained from books, records, and research or documents collected to support primary data. Secondary data collection was obtained from the Office of Marine Affairs and Fisheries, District Offices, Village Offices and BPS. The data collected includes geographic conditions and area administration, population conditions, fishery facilities and infrastructure, Indian Scad fishing effort data and

Indian Scad fish production data for the last 10 years (2010-2019).

E. Data Collection Techniques

The research data were obtained in the following stages:

1. Field observations to determine the general condition of the research locations.
2. Questionnaire

   The questionnaire is a data collection technique by providing a set of written questions to respondents to be answered. The data taken using a questionnaire was quantitative.
3. Literature study, which was collecting data by studying documentation, reading literature or research results that are considered relevant to the research theme.

F. Data Analysis

Most of the data analysis carried out was quantitative analysis according to the model approach used, namely the dynamic bioeconomic model and the value of depreciation and degradation of Indian Scad fish with the following steps

1) Compiling production and effort data (input and effort) in the form of time series (time series), the data used in this study were 2010-2019 data.
2) Standardization of fishing gear.
3) Estimating biological parameters including water carrying capacity constants (K), natural growth constants (r), technological parameters (q).
4) Estimating economic parameters in the form of price per kg and production costs per trip.
5) Performing parameter estimation
6) Dynamic bioeconomics.
7) Calculating the optimal value based on a predefined formula. This step can be done using the help of Excel software to facilitate analysis and to create charts.
8) Calculating the depreciation and degradation value.

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 differentunits, 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. Estimation of Economic Parameters

Economic parameters include the estimated input costs, the estimated price of capture output and the cut rates of resources.

The cost of catching or estimating the cost of input is obtained from primary data, which is then made the yearly real fishing cost data series using the formula : (Najamuddin, 2014)

$$c = \sum c_i / n_1$$

Where :

c = Average fishing costs (IDR) per year

c_i = Catching cost per capture attempt of respondent i

n_1 = Number of respondents

The output price estimate is obtained from primary data, which is then made a data series of real sales prices in the year using the formula:

(Clark *et al*., 1992)

$$p = \sum p_i / n_2$$

Where:

p = Average catch price per kg

p_i = Average price during the i season

n_2 = Number of seasons (peak, regular, famine)

The cut-off rate parameter (d) uses the equation (Clark, 1985) :

$$d = \ln (1+i)$$

Where :

i = investment interest rate – inflation rate

d = resource cut rate

5. 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):

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

$$Y^* = rX^* (1 - X^*/K)$$

$$E^* = Y^*/qX^*$$

Where :

K : Environmental carrying capacity

c : Operating costs for catching the Indian Scad fish

p : the price of Indian Scad fish per kilogram

r : fish growth rate

q : catching power coefficient and Indian Scad fishing gear

σ : resource cut rate

As a comparison, the MSY, MEY and Open Access potentials were calculated. The calculation of the Maximum Sustainable Yield (MSY) model uses the following equation:

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

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

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

Where :

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

Y_{MSY} : The catch in MSY condition

X_{MSY} : Estimating optimal stock of MSY conditions

With the assumption that the demand curve is perfectly elastic, the fishery resource rent based on the Maximum Economic Yield (MEY) value is obtained using the following equation:

$$E_{MEY} = \frac{r}{2q}\left(1 - \frac{c}{pqK}\right)$$

$$Y_{MEY} = \frac{rK}{4}\left(1 + \frac{c}{pqK}\right)\left(1 - \frac{c}{pqK}\right)$$

$$X_{MEY} = \frac{K}{2}\left(1 + \frac{c}{pqK}\right)$$

Where :

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

Y_{MEY} : The catch in MEY conditions

X_{MEY} : Estimating optimal stock of MEY conditions

III. RESULTS AND DISCUSSION

A. Graph of CPUE value development for Indian Scad fish in Gulf Waters 2010-2019

Can be seen in Fig 3.1

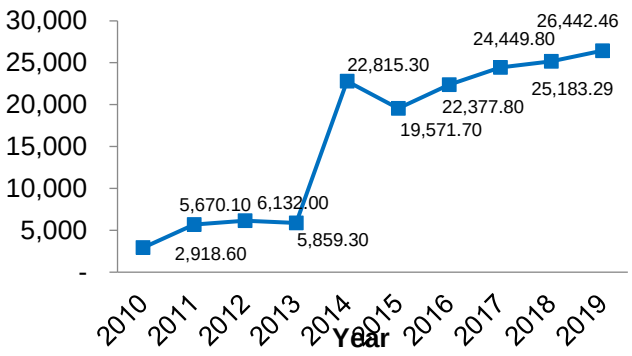

Fig.3.1: CPUE Development of Indian Scad fish Resources in the Waters of Bone Bay, South Sulawesi Province

In Figure 4.1, it can be seen that the development of the CPUE of Indian Scad fish resources in the waters of the Bay of Bone from year to year tends to fluidize or there was a tendency for a fluorination pattern. A significant increase occurred from 2013 to 2014 and decreased in 2015. According to Alamsyah (2012), one of the characteristics of overfishing is the fluctuating or erratic fishing chart in time units and decreased production. This meant that the decrease in CPUE also indicated that the fish resource was overfishing.

B. Relationship Catch per Unit Effort (CPUE) and Effort

The relationship between Catch per Unit Effort (CPUE) and Effort can be described through the graph and the equation of the trendline, namely y = α + βx. The relationship between CPUE and Indian Scad fish Effort in this study can be seen in

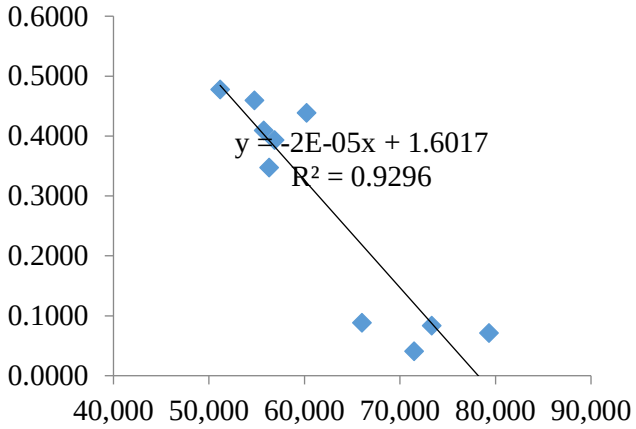

Fig.3.2: Relationship between CPUE and Effort for Indian Scad fish in Bone Bay Waters, 2010-2019.

In Figure 4.12, it can be seen that the relationship between CPUE and Indian Scad fish Effort is in the equation y = -2E-05x + 1.6017, from this equation the intercept value (α) is 1.6017 and the slope (β) value is -0.00002x. From this equation, it can be seen that the relationship between CPUE and Effort showed a negative relationship, Anna (2010) says that a negative relationship is a relationship when an increase in a variable will cause a decrease in another variable and vice versa an increase in one variable will cause a decrease in other variables. This meant that an increase in fishing activity (effort) would decrease the productivity of the catch (CPUE). The coefficient of determination (R2) is 0.993 or 99.3%. This meant that the variation or rise and fall of CPUE by 99.3% was due to the rise and fall of the effort value, while the remaining 0.7% was caused by other variables not discussed in the model.

C. Estimation of Biological Parameters

From the value obtained, it was then entered into the biological parameter equation so that the constant rate of fish growth, fishing coefficient and constant water carrying capacity can be estimated.

The estimation results of the three parameters presented in table 2 were useful for determining the level of sustainable productions such as maximum sustainable yield (MSY), maximum economic yield (MEY). These values can be seen in table 2.

Table 2. Estimation Result of Indian Scad fish Biological Parameters

No	Biological Parameters	Estimation Results	Unit
1	Rate Constant Natural growth of fish (r)	0.53533	ton per tahun
2	Fishing coefficient (q)	0.75124	ton per unit
3	Water carrying capacity constant (K)	330.64575	ton per year

Source: Data from analysis, processed in 2021

Biological parameters were one of the factors that greatly affected the continuity of life, especially the Indian Scad fish. Because if one of the variables of the biological parameters, for example, the carrying capacity of the environment, was not following the needs, this would have an impact on the growth rate of the Indian Scad fish.

Based on the data obtained as presented in Table 2. The constant rate of fish growth (r) was 0.54 which means that the fish would grow naturally without any disturbance from natural phenomena with a coefficient of 0.54 tonnes per year.

The catch coefficient (q) was 0.7512, the catching ability coefficient value was influenced by the number of fishing gears available and the availability of the Indian Scad fish.

The constant carrying capacity of waters (K) was 330.6458, this indicated that the aquatic environment supports the production of Indian Scad fish by 330.6458 tons per year from its biological aspects, including food abundance, population growth and fish size.

D. Estimation of Economic Parameters

1. Estimated Input Costs

The costs of using the Indian Scad fish in the waters of Bone Bay consisted of fixed costs and variable costs. Fixed costs were costs that were not used up in one fishing operation (trip). Fixed costs consisted of depreciation costs for fishing tools such as boats, Purse Seine fishing tools, machines and other supporter tools. Meanwhile, variable costs were costs that were used up for one capture (Trip).

The economic theory of fisheries states that in open access fisheries where fishing costs are assumed to be proportional to fishing effort, the business will continue to increase even though the income per business unit decreases and ultimately the income will decrease until it is equal to the costs incurred (Gordon in Kar and Chakraborty, 2011).

The cost of catching or estimating the cost of input is obtained from primary data which is then generated for cost data series

its annual real catch by the equation:

$$c = \sum c_i / n_1$$

Where :

c = Average fishing costs (IDR) per year

c_i = Catching cost per capture attempt of respondent i

n_1 = Number of respondents

The overall estimation results of the crab input costs in this study can be seen in Table 3.

Table 3. Analysis of Indian Scad fish Business in Bone Bay Waters

No	Kabupaten	Penerimaan	Total Biaya	R/C Ratio
1	Sinjai	1,596,000,000	750,558,567	2.13
2	Bone	2,300,400,000	896,953,528	2.56
3	Palopo	568,200,000	327,123,391	1.74

The Purse Seine Fishing Gear

Source: Data from analysis, processed in 2021

From the results of the data analysis, the value C is Rp. 65,821,182.84.

2. Estimated Output Prices

Apart from the fishing cost component required in this analysis, price component data was also needed. The price component that would be used in the analysis was the average price obtained from primary data or through direct interviews with fishermen and also secondary data from the Fisheries Service of Sinjai Regency, Palopo Regency and Bone Regency with time series from 2010 to 2019.

One of the problems in determining prices was the existence of abnormal price movements due to the monetary crisis and the inflation rate. To overcome the abnormality of this movement, adjustments were made by converting the nominal prices obtained into real prices (Adnan, 2010).

The output price estimate was obtained from primary data, which was then made the annual real sales price data series using the formula:

$$p = \sum p_i / n_2$$

Where :

p = Average catch price per kg

p_i = Average price during the season -i

n_2= Number of seasons (peak, regular, famine)

Table.4: Average Real Prices of Indian Scad fish in the Waters of Bone Bay, South Sulawesi Province (Rp / Kg)

No.	Season	Price
1	Musim Puncak	18,750
2	Musim Biasa	15,000
3	Musim Paceklik	12,500
	total	46,250
	p	15,417

Source: Primary data that has been processed, 2021

Based on Table 4, it can be estimated that the real price value or the p-value = IDR 15,417.

3. Estimated Discount Rate Rate (Resource Cut)

The parameter discount rate or resource discount rate refers to the investment interest rate and the inflation rate. The prevailing interest rate was 10.80 and the inflation rate was 3.58%. To get the value of the cut-rate of resources estimated by the equation, d = ln (1 + i) where i = the investment interest rate minus the inflation rate, so that the value of the resource cut rate is d = ln (1+ (10.80% - 3.58%)) = 2.10657. dValue = 2.10657

E. Bioeconomic Analysis of Indian Scad fish Utilization

The bioeconomic analysis of the use of Indian Scad fish in this study was estimated into 2 (two) management regime models, namely the Maximum Sustainable Yield (MSY) management regime, the Maximum Economic Yield (MEY) management regime.The two management regime models can be determined using analytical solving tools. through the Excell program. The results of the bioeconomic optimization analysis of each Indian Scad fish management regime in this study are briefly presented

Table 5. Results of Analysis of Bioeconomic Optimization of Indian Scad fish Utilization.

Model	Effort (Unit)	Yield (Y) (Ton)	Biomass (X) (Ton)
Bioekonomi	75,191.23	52,543.67	36,547.88
MSY	126,545.56	62,889.11	125,778.23
MEY	356,295.16	284,661.66	128,619.67
Aktual	62,506.41	16,142.04	

Source: Data from analysis, processed in 2021

Production in the Bioeconomic management regime was 52,543.67, while the maximum recommended sustainability in the MSY regime was62,889.11 tonnes, MEY was 284,661.66 tonnes. Biomass in bioeconomics was 36,547.88 tonnes while in actual conditions the production was 16,142.04 tonnes.

IV. CONCLUSION

The actual production of Indian Scad fish from the calculation results is greater than the production value based on bioeconomic calculations. This means that efforts have been made to optimize the actual catch of the Indian Scad fishing production which has reached technical efficiency and the level of Indian Scad fish in general on the purse seine fishing gear has fluctuating or fluctuating values.

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Seaweed Cultivationn Business Development Strategy *(Gracillaria sp)* Through Spores Culture in Takalar Regency

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Abstract—*This study aims to calculate income and determine alternative strategies for seaweed cultivation business development (Gracillaria sp) through spores culture in Takalar Regency. Using revenue, IE, SWOT, and QSPM formula analysis methods. The results showed that farmers in Sanrobone subdistrict earned a higher income of Rp.3,609,100 compared to Mappakasunggu District which earned revenues of Rp.2,234,500. While the total score of values in the internal matrix is 2.92 and the external matrix is 1.31. The combination of the two values indicates the position of the business lies in cell VIII or growth strategy. The right strategy is to use quality seeds to increase export value, optimize the region as a productive area, and provide stock of spore seeds for export needs. These three strategies can be implemented simultaneously, because they support each other.*

Keywords—***seaweed, gracillaria sp, spores culture, IE, SWOT, QSPM.***

I. INTRODUCTION

Gracillaria sp seaweed utilization is so widely characterized by the growing market demand. However, the increasing need for seaweed production is not offset by a promising increase in production. During the period 2015 to 2018, there was a significant decrease in production, namely from 103.995 million tons in 2015 to 74.131 million tons in 2018 (DKP Takalar, 2019). The decrease in production in the seaweed cultivation business Gracillaria sp will affect the amount of income received by seaweed farmers.

Cultivation of Gracilaria sp through spores culture is one of the best solutions to increase the amount of production in seaweed cultivation efforts that are beneficial for seaweed growers for industrial-scale development that requires large amounts of seeds, timely, flexibility of storage time and does not depend on natural conditions. The use of spores as a source of seeds has been successfully carried out in several countries, including Japan and Korea. In addition, several studies on sporingspores have been conducted, among others, factors that influence the sporulation of Gracillaria cornea (Alberto and Robledo, 1999) and the paste of Gracilariagigas spores in several subsrats (Lideman et al., 2016).

Currently, some seaweed farmers in Takalar district use seeds from spores culture, where the growth of seaweed spores culture cultivated is very significant because the differences that local seeds produce with seeds from cultures are very different. Local seaweed has a certain season and location, compared to seaweed spores culture cultivators are very tolerant of the seasons and locations.

Based on the description described above about seaweed cultivation business that is still low quality but high market demand and the potential of water in Takalar

Regency of South Sulawesi Province is very large, the need to be multiplication of seedlings through spores culture to improve the results of seaweed cultivation efforts. Therefore, a strategy is needed for the development of gracilaria sp cultivation business so that seaweed production in the coming year is better, so that the income of seaweed farmers will increase.

II. RESEARCH METHODS

The research was conducted in Takalar Regency in two sub-districts. This research was conducted from September 2019 to March 2020. Primary data is data obtained directly from sources studied through interviews and surrvey (questionnaires) with seaweed farmers. The method of selecting respondents at the research site was carried out purposively with the consideration that in the sub-district is one of the production centers gracilaria sp that uses spore seeds. This study used three analyses namely revenue analysis, IE analysis, SWOT analysis, and QSPM analysis.

III. RESULTS AND DISCUSSION

3.1 Seaweed Grower Income

Income is the result of receipts reduced by the total costs incurred during the seaweed cultivation process Gracilaria sp. The purpose of the calculation of income is to find out how much net income from seaweed cultivation business through spores culture. The higher the income received by seaweed farmers, the higher the amount of goods consumed both in terms of quality and quantity of goods to be consumed.

One of the things to note in running a business is the cost of production. Where the cost of production is the total cost of all costs used from production preparation to marketing. In table 1, the total income obtained by seaweed farmers in Takalar Regency is presented as follows :

Tabel 1. Average Income in seaweed cultivation business Gracillaria sp in Takalar Regency

No	Description	District	
		Sanrobone	Mappakasunggu
1	Acceptance	8.370.000	6.540.000
2	Total Cost	4.760.900	4.305.500
Average Income (RP)		**3.609.100**	**2.234.500**

Source : *Primary data after processing, 2020*

From the table above, it is seen that the income received by seaweed farmers Gracilariasp in Sanrobone District amounted to Rp. 3.609.100 per production. While the revenue received by seaweed farmers in Mappakasunggu District amounted to Rp. 2,234,500 per production. Average income of seaweed cultivation Gracilariasp that uses spore seeds when compared to seaweed cultivation Gracilariasp in the pond has a relatively small income difference or the average amount of income received is almost the same. This is in accordance with the research of Riskiyani (2019), where the average income received by seaweed farmers in Takalar Regency amounted to Rp. 1,229,452.

3.2 Seaweed Cultivation Business Development Strategy

3.2.1 Identification of Internal and External Factors

According to Zain (2012) in planning the strategy should analyze the external environment (opportunities and threats) to know the various possible opportunities and threats. The problem of the strategy to be monitored must be determined because this problem may affect future efforts. After completing the analysis of external strategic factors, it should also analyze the internal strategy factors (strengths and weaknesses). The internal and external factors of seaweed cultivation business Gracillaria sp in Takalar regency as follows :

Table 2. Internal and external factors

Internal Factors	External Factors
STRENGHTS	**OPPORTUNITIES**
Seaweed cultivation area	Seaweed cultivation investors
Seaweed cultivation business experience	Market demand is still high
Quality seeds	Increase the value of exports
Effective use of capital	Government assistance
WEAKNESSES	**THREATS**
Land use is not optimal	Level of competition with similar businesses
The number of cultivators is still low	Fluctuating prices
Limitations of spore culture seeds	The influence of the seasons
Presence of pests and diseases	

Source : *Primary data after processing, 2020*

3.2.2. Business position based on IE matrix

3.2.2.1 IFE Matrix

Identification of internal factors in the form of strengths and weaknesses affect the development of seaweed cultivation business Gracillaria sp in Takalar Regency. The result of identification of strengths and weaknesses as an internal strategy factor, is then given weight and rating for each factor so that the total score is obtained. The results of internal factor analysis obtained the number of selisishnya as follows :

Table 2. IFE Matrix

Internal Factors Strategy	Weights	Rating	Valeur
Strengths			
Seaweed cultivation area	0,19	4,00	0,76
Seaweed cultivation business experience	0,13	2,67	0,34
Quality seeds	0,19	4,00	0,76
Effective use of capital	0,11	2,33	0,26
Amount	**0,62**		**2,12**
Weaknesses			
Land use is not optimal	0,08	1,67	0,13
The number of cultivators is still low	0,10	2,00	0,19
Limitations of spore culture seeds	0,13	2,67	0,34
Presence of pests and diseases	0,08	1,67	0,13
Amount	**0,38**		**0,79**
Total	**1,00**		**2,92**

Source : *Primary data after processing, 2020*

Based on the results of the analysis from the table above obtained the number of strength scores with a value of 2.12 and the number of weakness scores with a value of 0.79 in seaweed cultivation business Gracillaria sp. So that obtained a total score of 2.92 which shows the internal factor is above the average value of 2.5 which indicates the internal position of seaweed cultivation business Gracillaria sp strong enough that seaweed cultivation business has the ability above average in utilizing strengths and anticipating internal weaknesses.

3.2.2.2 EFE Matrix

Identification of external factors is carried out to determine the opportunities and threats faced by seaweed growers Gracillaria sp. Identification results are collected to analyze external factors. Furthermore, it is given weight and rating for each factor so that the total score is obtained. The results of internal factor analysis obtained the number of selisishnya as follows:

Table 3. EFE Matrix

External Strategy Factors	Weights	Rating	Valeur
Opportunities			
Seaweed cultivation investors	0,14	3,00	0,42
Market demand is still high	0,19	4,00	0,75
Increase the value of exports	0,19	4,00	0,75
Government assistance	0,13	2,67	0,33
Amount	**0,64**		**2,26**
Threats			
Level of competition with similar businesses	0,14	4	0,42
Fluctuating prices	0,14	4	0,42
The influence of the seasons	0,08	3	0,13
Amount	**0,36**		**0,97**
Total	**1,00**		**1,31**

Source : *Primary data after processing, 2020*

Based on the table above obtained the number of opportunities gracillaria sp seaweed cultivation business with a value of 2.26 and the number of scores against the threat of seaweed cultivation with a value of 0.97. So the total score obtained from the analysis is 1.31. This value indicates that the company's external position is not strong, namely the seaweed cultivation business Gracillaria sp does not have the ability above average in utilizing opportunities and anticipating external threats.

3.2.2.3 IE Matrix

The results of the internal matrix evaluation are further combined with the results of the evaluation of the external matrix that produces the IE matrix, so that the business position can be mapped to make it easier to formulate alternative business development strategies. The strategic positioning of the IE matrix is based on the results of the total IFE values weighted on the x-axis and the total EFE values on the y-axis (Setyaningsih, 2012). The results of IE matrix analysis on seaweed cultivation business in Takalar Regency are as follows :

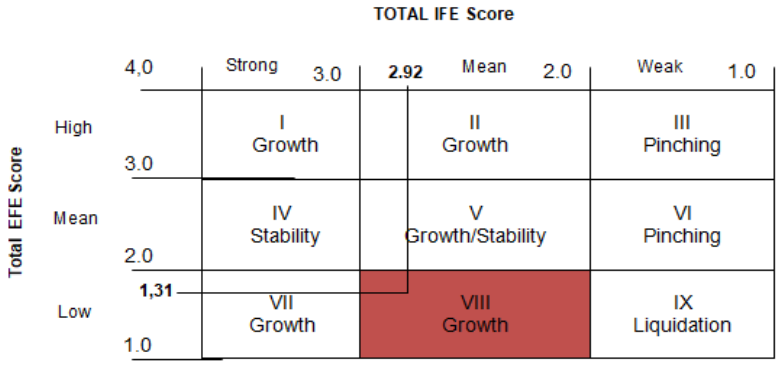

Fig.1: IE Matrix

3.2.3 SWOT Analysis

SWOT can describe how the opportunities and threats from the external environment of gracillaria sp seaweed cultivation business are anticipated with its strengths and weaknesses. This matrix can produce four sets of strategies, each of which is as follows:

Table 4. SWOT analysis matrix

INTERNAL **EXTERNAL**	**Strengths** a. Seaweed cultivation area b. Long business experience c. Quality seeds d. Effective use of capital	**Weaknesses** a. Land use is not optimal b. The number of Gracilariasp cultivators is still low c. Limitations of spore culture seeds d. Presence of pests and diseases
Opportunities a. Seaweed cultivation investors b. Market demand is still high c. Increase the value of exports d. Government assistance	**Strategy S >< O** 1. Designing the development of gracillariasp cultivation area (S1,O1) 2. Optimizing your experience to facilitate seaweed marketing and production (S2,O2) 3. Use of quality seeds to increase export value (S3,O3) 4. Improving business efficiency through optimization of government assistance utilization (S4,O4)	**Strategy W >< O** 8. Building land alignment zoning in cooperation with the government (W1,O4) 9. Building business certainty in cooperation with various parties (W2,O1) 10. Provision of spore culture seed stock for export needs (W3,O3) 11. Conducting pest and disease control for market needs (W4,O2)
Ancaman (Threats) a. Level of competition with similar businesses b. Fluctuating prices c. The influence of the seasons	**Strategy S >< T** 5. Optimizing the region as a productive area(S1,S4,T1) 6. Diversifying products so that prices can be controlled (S2,T2) 7. Maintaining the quality of the use of spore culture seeds to minimize the impact of the changing seasons (S3,T3)	**Strategy W >< T** 12. Utilizing land for the development of cultivation business Gracillariasp(W1,W3,O1) 13. Business partner development (W2,T3) 14. Providing training on how to cultivate good and business management (W4,T2)

Source : *Primary data after processing, 2020*

3.2.4 QSPM Analysis

Quantitative Strategic Planning Matrix (QSPM) is a tool that allows strategists to evaluate alternative strategies objectively based on important external and internal success factors previously identified. The matrix objectively demonstrates the best alternative strategies. QSPM uses inputs from SWOT matrix analysis to obtain the information needed to build a QSPM matrix. The MATRIX QSPM (Quantitave Strategies Planning Matrix) seaweed cultivation business Gracillaria sp in Takalar Regency as follows :

Table 5. QSPM analysis matrix

Alternative Strategies	Linkages	Weights	Ranking
Strategy S-O			
Designing the development of gracillariasp cultivation area	S1,O1	0,85	**IV**
Optimizing your experience to facilitate seaweed marketing and production	S2,O2	0,64	**VII**
Use of quality seeds to increase export value	S3,O3	1,14	**I**
Improving business efficiency through optimization of government assistance utilization	S4,O4	0,37	**XI**

Strategy S-T			
Optimizing the region as a productive area	S1,S4,T1	1,02	**II**
Diversifying products so that prices can be controlled	S2,T2	0,55	**VIII**
Maintaining the quality of the use of spore culture seeds to minimize the impact of the changing seasons	S3,T3	0,54	**IX**
Strategy W-O			
Building land alignment zoning in cooperation with the government	W1,O4	0,34	**XII**
Building business certainty in cooperation with various parties	W2,O1	0,38	**X**
Provision of spore culture seed stock for export needs	W3,O3	0,96	**III**
Conducting pest and disease control for market needs	W4,O2	0,81	**V**
Strategy W-T			
Utilizing land for the development of cultivation business Gracillariasp	W1,W3,O1	0,70	**VI**
Business partner development	W2,T3	0,18	**XIII**
Providing training on how to cultivate good and business management	W4,T2	0,64	**VII**

Source : *Primary data after processing, 2020*

Based on the results of the analysis from the table above shows that the most appropriate strategies for business development are the improvement of quality seeds to increase the value of exports (score 1.20), provision of spore culture seed stocks for export needs (score 1.05), and optimize the region as a productive area (0.95). These three strategies can be implemented simultaneously because they support each other.

IV. CONCLUSION

The results showed that farmers in Sanrobone subdistrict earned a higher income of Rp.3,609,100 compared to Mappakasunggu District which earned revenues of Rp.2,234,500. While the total score of values in the internal matrix is 2.92 and the external matrix is 1.31. The combination of the two values indicates the position of the business lies in cell VIII or growth strategy. The right strategy is to use quality seeds to increase export value, optimize the region as a productive area, and provide stock of spore seeds for export needs. These three strategies can be implemented simultaneously, because they support each other.

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Traditional Knowledge of açaí (Euterpe precatoria Mart. - Arecaceae) usage in the Sustainable Development Reserve - RDS Piagaçu Purus – Amazonas - Brazil

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Abstract*— *The historical reciprocity among traditional populations and the usage of biodiversity in natural environments have outlined the Amazon as one of the richest and most complex socio-ecological systems on the planet. Conservation Units (CU's) are effective strategies to protect biodiversity, although the simple creation does not guarantee its efficiency. Conservation depends on the integration between ecological and social aspects of the forest peoples and the management of institutions involved with the use and protection of biodiversity and environment. In this context, Non-Timber Forest Products (NFTPs) are important elements in the culture and subsistence of traditional populations and can represent significant sources of income. Specifically, the açaí palm (Euterpe precatoria) is one of the species that stands out in the Amazon, with high cultural and economic values. Given the importance of the species, this research was carried out using traditional knowledge associated with this palm tree, along with residents of three communities of the RDS Piagaçu Purus, to identify and evaluate the uses of parts of the plant of greatest interest to local communities. Within a two-months period, in 2006, information on the diversity of uses and knowledge of the species were collected. It was applied the method of qualitative-quantitative exploratory research, surveying primary and secondary information. The results showed the great potential of açaí usage, especially the fruit, which is consumed in the form of wine and the root, in popular medicine, indicating that the species is a key resource for families subsistence besides its high economic and social potential.

Keyword*— *Biodiversity, Ethnobotany, Palm Trees, Conservation Units, Riverside Communities.

I. INTRODUCTION

Planted and native forests offer diversified, high-quality timber and non-timber forest products to the market, with the possibility of developing other products that, when valued, can improve the quality traditional populations life (LAY, 2008).

Traditional populations are local populations, whose knowledge comes from wisdom about the elements of nature and its dynamics, which are used as a means of survival. The indigenous peoples, quilombolas, riverside dwellers, artisanal fishermen and many other local rural communities, have territoriality in the context of

contemporary urban society (SILVA PIMENTEL and RIBEIRO, 2016).

The historical reciprocity between traditional populations and the use of timber and non-timber products in the natural environment outlined the Amazon, as one of the richest and most complex socio-ecological systems on the planet. Therefore, the conservation of this biome depends on the integration between the ecological and the social aspects of the inhabitants, management of sustainability-concerned institutions, protection of biodiversity and the ancestral knowledge, regarding the use of Non-Timber Forest Products - NTFP's.

NTFP's are a cultural element of great importance for traditional populations and can be considered a significant source of income for them. According to Takeda (2015), the exploitation of forest resources has always played a relevant role in society and the economy, being more intense in tropical countries, which encourage the generations of jobs and income, promoting the development of rural infrastructure and the provision of social services, among others.

In addition to the ecological and economic importance of NTFPs, the literature has increasingly demonstrated the nutritional richness of plants present in the eating habits of traditional populations. Some authors, having theorized and analyzed eating habits of globalized society, show the unawareness and mischaracterization of traditional and regional cuisine with the increase in consumption of industrialized food, which has impacted the nutritional status of urban populations (CERDA, 2014). Other authors also highlight that there are few works that emphasize the plant diversity consumed by Amazonian populations. However, it is remarkable the contribution in a diet that plants, especially wild and cultivated, represent in terms of micronutrients and macronutrients (DUFOUR et al., 2016). In fact, the Amazonian species used in cooking have greater visibility, as is the case of açaí, which has nutritional properties that put the specie in the market spotlight.

The national and international recognition of açaí, as a source of antioxidants makes it the target of investments and research, which grow every year and also makes it a reference within natural resources for new products and technologies, bringing forth the importance of antioxidant compounds that are present in a large part of the Brazilian flora (SOZO et al., 2016).

The açaí tree is native to the Amazon region of Brazil, being one of several species of plants that make up the traditional set of forest products grown in the Amazon estuary floodplain. At the height of the canopy, purple fruits grow in bunches and hand-harvested by collector-farmers.

Açaí is one of the most important forest products in Brazil (MATOS et al, 2017; NOBRE, 2017). Data from 2017 indicate that 93.1% of Brazilian açaí production comes from the species *Euterpe oleraceae* ("açaí-do-Pará"), naturally restricted to the eastern Amazon (IBGE, 2018). But the supply of açaí by the species *E. precatoria* has been growing exponentially year by year (IBGE, 2018). Intriguingly enough, until 2018 the species was not considered a source of açaí pulp by Brazilian legislation, which recognized açaí pulp only as the one extracted from *E. oleraceae* (BRAZIL, 2018). Brazil produces about 220,000 tons of açaí of extractive origin. From those, about 50,000 tons came from *E. precatoria*, and the activity generates an income of just over US$ 150 millions/year (IBGE 2018).

In Brazil, three species of açaí palms are well known: *E. oleracea* - native of Pará and Amapá states, which are responsible for most of its commercial production; *E. precatoria* - native of Amazonas; and, *E. edulis* - native of the Atlantic Forest, which was much exploited in an unsustainable way for palm heart extraction. The açaí chain involves extractivists, producers, intermediaries, processing industries and artisanal açaí beaters (people who process açaí in and artisanal way), being of crucial importance to generate income to an expressive group of families of small producers (TAVARES and HOMMA, 2015).

The Conservation Units (UCs) are important açaí extraction areas. In 2000, the Federal Government created the National System of Conservation Units (NSCU), which together with The Program for Protected Areas of the Amazon (Programa Áreas Protegidas da Amazonia - ARPA), were responsible for the strengthening and protection of about 60 million hectares in 117 UCs in the Amazon (BRASIL, 2000; MMA, 2019).

This study was developed in order to collect and analyze information on the social and ecological aspects of the use and commercialization of natural resources by riverine communities in the central Amazon, specifically the açaí (*Euterpe precatoria*). The objective was to generate basic scientific knowledge in the extractive development by the social organization of residents in the State Reserve of Sustainable Development Piagaçu-Purus (RDS-PP), municipality of Beruri in the state of Amazonas.

II. MATERIAL AND METHOD AREA OF STUDY

This study took place in the months of November 2006 and February 2007, in two riverside communities located in areas of *terra-firme* forests of Lake Ayapuá (Pinheiros and Uixi) and one of Lake Uauaçu in the lower course of the Purus River, in the Piagaçu-Purus State Reserve of Sustainable Development (RDS-PP), Municipality of

Beruri, State of Amazonas, Brazil. (Figure 1). In these communities, açaí extractivism is one of the main income source practices of the communities.

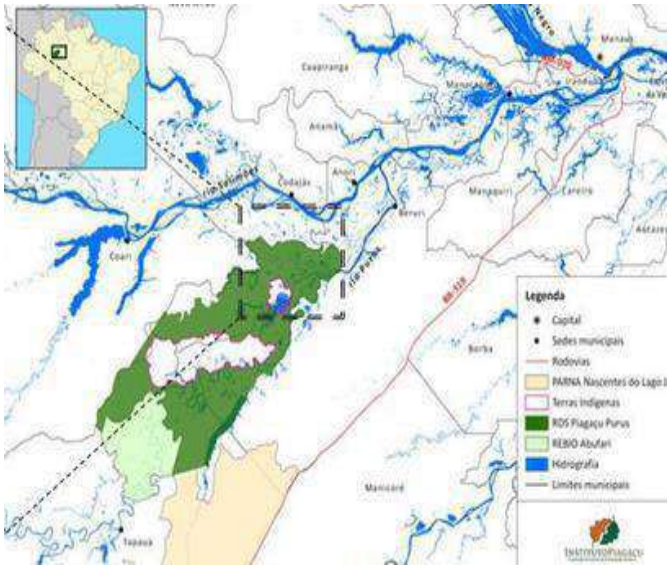

Fig.1: Location of the Piagaçu-Purus Sustainable Development Reserve in the state of Amazonas, Brazil- Highlighted in green.

Source: Inst. Piagaçu (2009).

2.1 Data collection

The communities in Lake Ayapuá are called Divino Espírito Santo (Pines) and Nossa Sra. do Livramento (Uixí), and there is also São João do Uauçu, in Lake Uauaçu.

For the collection and analysis of information on the social and ecological aspects of the use and commercialization of natural resources in those communities, it was developed forms and conducted interviews to cover the aspects (1) general information; (2) use of plant species; and (3) commercialization of products.

2.2 Sampling areas establishment

The phytosociological data of occurrence and distribution pattern of "açaizais", in the community areas, were obtained through a floristic inventory in 60 sample plots, with 20 x 20 meters each, totaling 24,000 m^2 delimited and inventoried.

Twenty sample plots were installed, randomly distributed, in 8 hunting spots in each community. They were distant from each other in approximately half an hour, traveling by boat and far from the headquarters.

In the center of each plot was set sub-plots of 5 x 5m, for the survey of natural regeneration, as used by Marangon *et al*., (2008), in a semidecidual seasonal forest fragment, and also by Machado (2018), in which was studied the forest dynamics of areas in natural regeneration with different degrees of disturbance. The center of each plot was identified with a 1.20-meter-high wooden stake.

A main trail, with an extension of 100 meters, was opened in the central part of each secondary forests (capoeira) in each community. The secondary forests are the result of a natural process of vegetation regeneration. The plots were installed on the sides of the main trail, on the distance of 20 meter, to minimize the effect of the trail's edge on the vegetation structure in the studied plot.

The total height of each tree was measured with a 5 meters long pole, with identification of each meter.

III. RESULTS AND DISCUSSION

Literature lists the species of seeded plants that grow in the Amazon forest at an elevation lower than or equal to 1,000m, excluding savannas and dry forests. The data informs the occurrence of 10,674 species in the Brazilian Amazon and 14,003 species in Pan-Amazon (CARDOSO et al., 2017).

Among the results, about 300 species native to the Amazon or introduced, have already been catalogued by the National Institute of Amazonian Research (INPA), as well as their nutritional, medicinal, herbal and cosmetic purposes. These surveys represent less than 3% of known species contained in the Brazilian Amazon (REVILLA, 2001).

In the three communities, 66 species occurred in secondary forests. The açaí, occurring in a frequency of 50% in the 15 and 30-year-old secondary forests (Table 1). This frequency is only lower than that of the Brazil nut (*Bertholletia excelsa* Humn. & Bonpl.) with 62.9% of citations. Among all 66 species, 28 (42%) are used in food.

Mostly mentioned in literature, it was found palm trees such as bacaba (*Oenocarpus bacaba*) (32.3%), tucumã (*Astrocaryum aculeatum*) (30.6%) and babaçu (*Attalea speciosa*) (30.6%). Meanwhile, the number of medicinal use species was 26 (39%), including the Brazil nut, açaí, tucumã, babaçu, and so forth. Açaí has different uses, ranging from medicinal, reported in 46% of the interviews, to handicrafts in only 2%.

Table 1. Different aged species on secondary forests (capoeiras) and their extractivist usages by communities

N	Common Name	Scientific name	Citation frequency (%)	Age of capoeiras		Usages
				15 years	30 years	
1	Castanha	*Bertholletia excelsa*	62,9	X	X	Food, medicine, coal, canoe caulking and dyeing
2	Açaí	*Euterpe precatoria*	50	X	X	Food and medicine
3	Bacaba	*Oenocarpus bacaba*	32,3	X	X	Food
4	Tucumã	*Astrocaryum aculeatum*	30,6	X	X	Food and medicine
5	Babaçu	*Attalea speciosa*	30,6	X	X	Food, medicine and construction
6	Palha branca	*Attalea attaleoides*	24,2	X	X	Construction and handcraft
7	Uixi	*Endopleura uchi*	19,4			Food and medicine
8	Taperebá	*Spondias mombin*	17,7		X	Food and medicine
9	Maracujá do mato	*Passiflora sp.*	14,5			Food
10	Dema	*Croton sp.*	12,9	X	X	Construction
11	Envira	*Cochlospermum sp.*	12,9	X	X	Construction, handicraft, reed and birds cage.
12	Urucuri	*Attalea excelsa*	9,7			Food
13	Unha de gato	*Uncaria guianensis*	8,1			Medicine
14	Piquiá	*Caryocar villosum*	6,5			Food
15	Lacre	*Vismia sp.*	6,5	X	X	Construction and medicine
16	Cacau jacaré	*Herrania sp.*	6,5			Food
17	Quebra pedra	*Phyllanthus spp.*	4,8			Medicine
18	Capeba	*Pothomorphe peltate*	4,8			Medicine
19	Caiauê	*Elaeis oleifera*	4,8			Animal feed and vegetable garden
20	Carapanauba	*Aspidosperma sp.*	4,8		X	Medicine
21	Ingazinho	*Inga marginate*	4,8			Food
22	Pitomba	*Talisia esculenta*	4,8	X		Food
23	Caferana	*Picrolemma sprucei*	4,8	X		Medicine
24	Ingá de macaco	*Pithecelobium cochleatum*	4,8		X	Food
25	Timbó	*Derris sp.*	3,2			Medicine and fishing
26	Cacau	*Theobroma guianensis*	3,2	X	X	Food
27	Sucuuba	*Himatanthus sp.*	3,2	X	X	Medicine
28	Bacuri	*Rheedia sp.*	3,2			Food
29	Apuruí	*Alibertia edulis*	3,2			Food

30	Embaúba	*Cecropia spp.*	3,2			Medicine
31	Batatão	*Operculina alata*	3,2			Medicine
32	Goiaba de anta	*Eschweilera truncate*	3,2	X	X	Food and canoe caulking
33	Marupá	*Simarouba amara*	1,6	X	X	Construction
34	Algodorana		1,6			Construction
35	Pé de Jabuti	*Astronium le-cointei*	1,6	X	X	Food
36	Araçá	*Eugenia sp.*	1,6			Food
37	Surucuzinho		1,6			Construction
38	Surucucumirá	*Spathelia excelsa*	1,6			Construction
39	Jatobá	*Hymenaea sp.*	1,6			Medicine construction, fishing equipment and panniers bindings (tree bark and trunk).
40	Mari	*Poraqueiba sericea*	1,6			Food
41	Chichuá	*Maytenus guyanensis*	1,6			Medicine
42	Capurana	*Não identificada*	1,6			Medicine
43	Muirapuama	*Ptychopetalum olacoides*	1,6			Medicine
44	Sorva	*Couma macrocarpa*	1,6			Food, canoe repairing
45	Saracura	*Ampelozizyphus amazonicus*	1,6			Medicine
46	Caramurí	*Ecclinusa guianensis*	1,6			Food
47	Ingá	*Inga edulis*	1,6			Food
48	Abiu	*Pouteria caimito*	1,6			Food
49	Japecanga	*Smilax spp.*	1,6			Medicine
50	Tintarana	*Elvasia calophyllea*	1,6			Construction
51	Cupuí	*Theobroma subincanum*	1,6		X	Food
52	Patauá	*Oenocarpus bataua*	1,6			Food
53	Cipó Titica	*Heteropsis flexuosa*	1,6			Broom production, panniers, panniers binding
54	Mata-mata	*Eschweilera coriacea*	1,6	X	X	Construction
55	Cupiuba	*Goupia glabra*	1,6	X	X	Construction
56	Vassourinha	*Scoparia dulcis*	1,6			Medicine
57	Cebola brava		1,6			Medicine
58	No name		1,6			Canine drug
59	Abiurana	*Pouteria sp.*	1,6			Construction
60	João Mole	*Neea sp.*	1,6			Construction
61	Urtiga	*Urticaceae*	1,6			Slimming effect
62	Canapú	*Physalis angulate*	1,6			Food and medicine

63	Muirapiranga	*Brosimum paraense*	1,6			Construction
64	Arumã	*Ischnosiphon sp.*	1,6			Panniers crafting
65	Mururé	*Brosimum sp.*	1,6			Medicine
66	Embaúba branca	*Cecropia sp.*	1,6			Medicine

X – occurrence in secondary vegetation area (capoeira).

Among the species mentioned by residents and communites, many are the medicinal plants and used in or out of combinations with other species to cure diseases, especially the açaí roots (*E. precatoria*). Besides that, all residents interviewed consumed açaí wine as a part of their diet.

Considering the nutritional value, açaí became the most collected Non-Timber Forest Product in Brazil, yielding the largest NTFP revenue in the Brazilian Amazon (IBGE, 2017), having several possibilities for the use and exploitation of the açaí tree (NOGUEIRA, 1989; SHANLEY et al.1998).

Açaí is an important and traditional fruit present in the diet of riverine populations and commonly consumed along with cassava flour and sources of protein, such as fish and shrimp. The State Department of Agriculture of Pará (SAGRI) listed the by-products that can be obtained from the pulp and seeds, informing that, the pulp represents 15% of the total weight of the fruit and can be used in the manufacture of ice cream, liquor, jam, nectar, jelly, among others (SAGRI, 2011).

Similarly, to other species and products, the management of açaí is a complex activity, given that it consists of several stages, ranging from community organization to manufacturers training, as well as special post-harvest care of the fruits and market notions.

Thus, in order to a community to be successful in the management of açaí, it is necessary to have a group of interested families, training the harvesters to climb the palm tree safely, providing good hygiene conditions for the fruits handling soon after harvest, good outlet conditions (by vehicle, boat, railroad branch and others) and the right destination or buyer for the production (WADT et al. 2004; ROCHA & VIANA, 2004).

However, to facilitate the work of the harvesters and without climbing the palm, the INPA in Manaus, Am, has developed tools for the collection of clusters of 20 species of palm trees in the Amazon. The tools have simple technology for easy handling and were made with materials easily found on the market. In order to allow collections of tall palm trees, mechanisms were developed for support, stability and balance of the stem. People or companies interested in acquiring PALMHASTE, can contact COETI / INPA, by e-mail ceti@inpa.gov.br.

3.1 Açaí population structure in a secondary forest (capoeira)

E. precatoria plants with DBH (Chest Height Diameter) equal to or less than 5 cm (seedlings), occurred in a greater proportion in relation to adults. The number of seedlings and young plants was 5 times greater than the number of adults. These results coincide with those of Rocha (2004) in forests known as lowland and upland "baixio / terra-firme".

However, in a secondary forest with canopy above 30 m in the Amazon, in the municipality of Manaquiri, Castro (2000) found 232 fertile individuals on one hectare. Interviewees from the three communities reported 66 species as ones stemming from secondary forest. The açaí had the frequency of 50% in secondary forest aged 15 and 30 years (Table 1).

Of the ten types of fruit most consumed in the communities, six was from palm trees. Among these, four was used to prepare a thick drink known as "vinho" (wine), which can be consumed immediately or a day after preparation. Cassava flour and sugar may be added. These data show the importance of açaí both as a food and income source for rural Amazonian communities (SACRAMENTO et al., 2015).

Among the species mentioned by residents and community members, many are medicinal plants and are used in combination or not with other species to cure diseases, especially the açaí root (*E. precatoria*). All residents interviewed consumed açaí wine as part of their diet.

3.2 The evolution of açaí production in the Northern Region of Brazil

The production of most NTFP is concentrated in the northern region, with an emphasis on the açaí (93.1%). In 2015, some extractivist products presented positive variation productions when compared to that obtained in 2014. Açaí in this case was the most expressive in absolute values due to the growing demand for the product, increasing 9% when compared to the previous year (IBGE, 2016).

NTFP's in 2015 generated just over R$ 298 million in the state of Amazonas. The production of açaí was approximately 39% of the total value produced, with approximately R$ 116 million (IBGE, 2016)

In the state of Amazonas, the NTFP's segment receives support from the Institute of Sustainable Agricultural and forestry development of the state of Amazonas (IDAM), along with organizations, associations and cooperatives of extractivists. In 2018, 3,435 family farmers/rural producers were said to be assisted in good management practices: extraction, collection, storage, processing and marketing, in activities related to Brazil nuts, açaí, rubber, piassava, as well as andiroba and copaíba oils, with the production of approximately 25,000 tons of extractive products (IDAM, 2018).

3.3 Marketing

The extractivism practiced in secondary forest, by the inhabitants of the communities, also serves for the commercialization of products such as straw, fruits and others.

Açaí used by residents of these communities, 16% of respondents sell the fruit or pulp. The commercialization of the fruit is carried out sporadically and by few families, thus being considered of little importance. Açai is transported by regional boats to the municipalities of Codajás and Beruri.

In the communities of RDS Piagaçu-Purus, the sale of açaí was concentrated in a smaller number of community members, according to the seasonal variation. However, market pressures could cause a super exploitation above the rate of the resources renewal, but this does not happen because the traditional populations of the Amazon culturally produce for their own subsistence.

Thus, the use and trade of these resources is fundamental for the families' subsistence and is also associated with the commercial relations network which presents barter as the main base.

The barter transaction in the 3 communities continues to have striking characteristics, such as nonmonetary commercialization in the exploitation relationship between salespeople and customers.

The commercialization relies on the merchant known as "*regatão*", which means river trader, who does not have fixed customers or that does not require exclusivity, even if some community members deliver their whole production to this marketing agent. Therefore, Sacramento et al. (2015) regard it important to highlight, in this context, the relevance of açaí as a source of income for rural Amazonian communities.

IV. CONCLUSION

The diagnosis with residents of traditional communities of RDS Piagaçu Purus, in the state of Amazonas, shows that the species of açaí (*E. precatoria*) is especially important as food, medicine and a source of economic income for the families of the Conservation Units.

The use of açaí fruit, mainly consumed in the form of wine, and the roots in popular medicine, indicates that the species is a fundamental natural resource in the subsistence of families and has high potential for local economic development.

A determining factor for the production of açaí, in general, is the availability of young male labor, since the collection of the fruits depends directly on the climbing of the palm stem, to reach the brunches and with the use of equipment craft called "*peconha*", which depends on skill and physical effort, but with the risk of accident.

To improve the açai fruit collection process, it is necessary to invest in regular training in harvesting and to encourage the purchase of safety equipment.

Alternatively, mechanization of the harvest is indicated, in areas planted or not, with motorized artifacts or with collection equipment, which can prevent the climb of the palm stem, to harvest the fruits with less risk of accidents.

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LED night lighting improving mini-budding technology in *Hevea brasiliensis*

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Abstract**—** *Mini-budding, exploiting seedlings of about 20 days, is a major technology for planting material propagation of rubber tree in China. The first pair leaves should be kept on rootstocks for assimilation. Manually axillary bud picking is needed to promote scion bud germination, but it is difficult and labor intensive. In this study, topping was carried out on the position below the first pair leaves and the subsequent plants were subjected to LED night lighting. The plant growth and the plant survival rate were improved by night lighting. The scion growth of the 1st flush was not comparable to that of plants with leaves on rootstocks. The high amount of chlorophyll content in the leaf might be response for this. The plant survival rate of the treatment was similar with that of plants with leaves on rootstocks. Thus, applying LED night lighting to the plants without leaves on rootstocks improved mini-budding technology by avoiding axillary bud picking.*

Keywords**— grafting, nursery, photoperiod, vegetative propagation, rubber tree.**

I. INTRODUCTION

The Para rubber tree (*Hevea brasiliensis* Willd. Ex. A. de. Juss.Müll-Arg.) is preferred over alternative sources of natural rubber worldwide due to the quality and quantity of latex produced. The planting materials of rubber trees are vegetatively propagated by bud grafting (budding is the colloquial term). The combined plants include clonal scions and hetero-rootstocks. After grafted, the rootstock can affect the initiated growth of scion. The carbohydrate for germination of grafted bud (scion) is directly provided by the rootstock. The leaves of germinating shoots could not assimilate until at the stage of mid light green period (Dong et al., 1986).Therefore, the early growth of grafted shoot is mainly controlled by the rootstock. In general, the larger size of rootstock, the more carbohydrate stored in the rootstock, and the stronger of germinating shoot.

There are several types of bud grafting according to the size of rootstocks (Priyadashan, 2017). Mini-budding(Figure 1), developed in the 1980s in China by Huang (Huang, 1989), is carried out when the seedling rootstocks are as young as 2-3 weeks old. The diameter and height of seedling rootstock (graft position) is less than 5 mm and 30 cm, respectively. One month after grafting, topping is carried out on the position just above

the first pair leaves. The axillary buds above the grafted bud are removed to eliminate apical dominance. Using mini-budding, planting materials could be produced as fast as 6 months after sowing seeds in the seedbed during August–September. Mini-budding is preferred in China because it reduces cost of production by decreasing the time and nursery area for production of grafted planting materials.

As the rootstocks for mini-budding are quite small and could not supply sufficient carbohydrate for the shoot (scion) grow, at least one or two leaves must be kept on the grafted root stocks for assimilation (Figure 1-C). However, manually axillary budpicking is difficult and labor intensive. Axillary bud picking is practiced twice or thrice sometimesbefore axillary buds are totally picked. Therefore, it is necessary to improve topping and subsequent axillary bud picking for mini-budding plants.

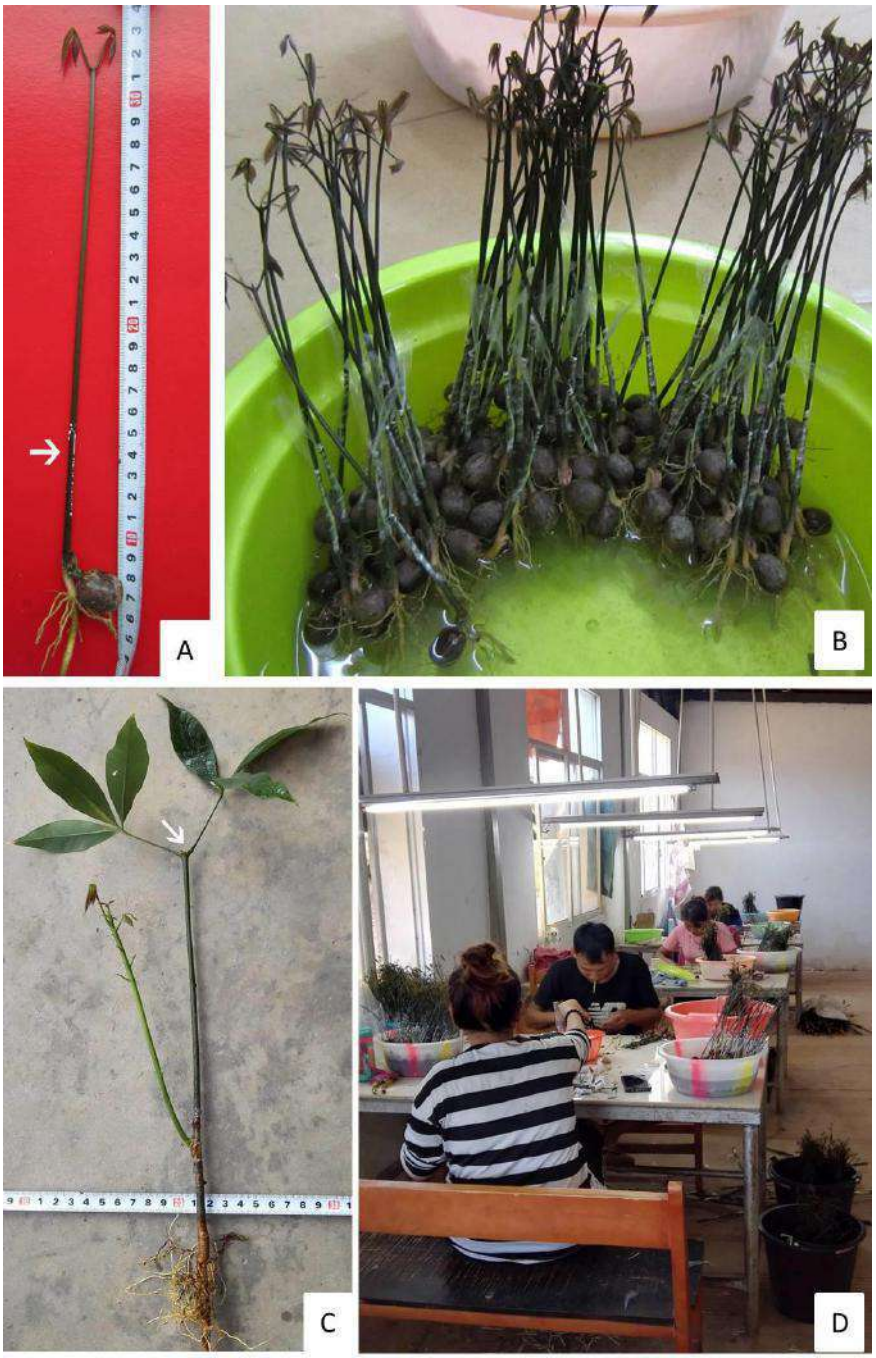

Fig. 1: Mini-budding profile. (A),Seedlings suitable for mini-budding. Arrow: graft position. (B),Graft finished. (C), Scion shoot germination. Arrow: axially buds have been picked. (D), Grafting in a room.

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Light is a key environmental factor regulating plant growth by affecting photosynthesis. Extended photoperiod can improve plant growth by synthesizing more carbohydrate. Supplemental lighting during the night extends the photoperiod and thus promotes plant growth. Light-emitting diode (LED) lamps are the preferred source for supplemental lighting due to their low operating temperature, durability and low cost (Singh et al., 2015). Supplemental night lighting has been widely used in the production of crops and other plants to promote growth (Fukuda et al. 2000, 2004; Okushima et al., 2012; Zhou et al., 2013; Kweon et al., 2016; Tewolde et al., 2016).

In this study, the min-budding rootstock plants were cut off on the position just below the first pair leaves (the first flush) when the plants grew to the stationary phase of the second flush. The grafted plants were then subjected to overnight LED lighting. The effect of LED night lighting on the growth of mini-budding plants was investigated.

II. MATERIALS AND METHODS

Plant materials

Hevea seeds of clone GT1 were sowed in August, 2019. Mini-budding was carried out with scion clone CATAS 7-33-97 in September. The grafted plants were planted in polybags which were placed in an arched shed with sun shade cloth. Topping was carried out in November, 2019, when the grafted rootstock plants grew to the second flush.

The grafted plants were cut off on the position just below the first pair leaves. Two treatments were carried out and each replicated thrice. Treatment one (designated as I-LED) was subjected to LED lamp night lighting. LEDs were hung 0.5 m above the plants. The lamp spectrum was red and blue combined with a photosynthetic photon flux density (PPFD) of 200 μmol m^{-2} s^{-1} measured at 10 cm from the LED lamp. Supplemental lighting was applied during the night from 19:00 to 06:00.Treatment two (designated as II – no LED) was subjected to no LED night lighting. The plants obtained by conventional mini-budding were used as a control.

Plant growth measurements

The plant height and stem (scion) diameter were monitored in May, July, September and November, 2020. The flush number and flush length were measured when the experiment was finished in November, 2020. The stem diameter was measured at 5 cm above the shoot germinating position. The percentage of plant survival (scion shoots that successfully grew to the stationary phase of second flush) was calculated.

Chlorophyll content analysis

After cutting off, the green bark of the rootstock stems was analyzed for chlorophyll content. The green bark was peeled from the stems and the fresh weight of bark was measured. Approximately 0.1 g of bark tissue was ground and incubated in 20 ml of a mixture of acetone, ethanol and deionized water (4.5:4.5:1) in the dark for 24 h at 25°C. The absorptions of chlorophyll a andchlorophyll b were measured at 647 and 664 nm, respectively, using a spectrophotometer (TU-1810s, PERSEE, China). The chlorophyll content was calculated according to the methods of Wellburn(1994).The total amount of chlorophyll of green bark was calculated as chlorophyll content multiplying fresh weight.The chlorophyll content of the first pair leaves kept on the rootstock was also analyzed. The fresh weight of the two trifoliate leaves (without petioles) was measured. The total amount of chlorophyll of leaf was calculated as chlorophyll content multiplying fresh weight.

Statistics analysis

The statistical analyses were performed using SPSS 22.0 software. Significance at the 0.05 level was determined using Tukey's multiple range test.

III. RESULTS AND DISCUSSION

The diameter of seedling rootstocks was measured when mini-budding was carried out. The mean diameter was 3.51 mm at the position of 3 cm above the root collar. After cutting off on the position below the first pair leaves, the grafted plants were grown under the condition with or without supplemental LED night lighting. The plant height and stem diameter were monitored after growing 6, 8, 10 and 12 months. The plants of I- LED exhibited larger

stem diameter and higher plant height than II- no LED (Figure 2, 3), showing that supplemental LED night lighting promoted plant growth.The plants of I- LED grew more flushes (mean 5.5) than that of II- no LED (mean 4.6), which indicated that the rhythmic growth has been accelerated. The mean flush length of plants of I- LED was significantly longer than that of II- no LED (Figure 4). The 1st,2nd, 3rd, 4th and 5th flush length between I- LED and II- no LED were compared, respectively. The 3rd flush length of I- LED was significantly longer than that of II- no LED, but the other flushes showed similar flush length (figure 4). The 1st flush length of control plants (obtained from conventional mini-budding)was 14.35 cm, significantly longer than that of I- LED (6.67 cm) and II- no LED (5.43 cm). The stem diameter of control plants was also larger than that of I- LED when the shoots finished the first flush growth.This indicated that more carbohydrate has been assimilated by plants with leaves kept on rootstocks. Even though the grafted plants without leaves grew under night lighting, the carbohydrate they assimilated was not comparable to that assimilated by the leaves in control plants.

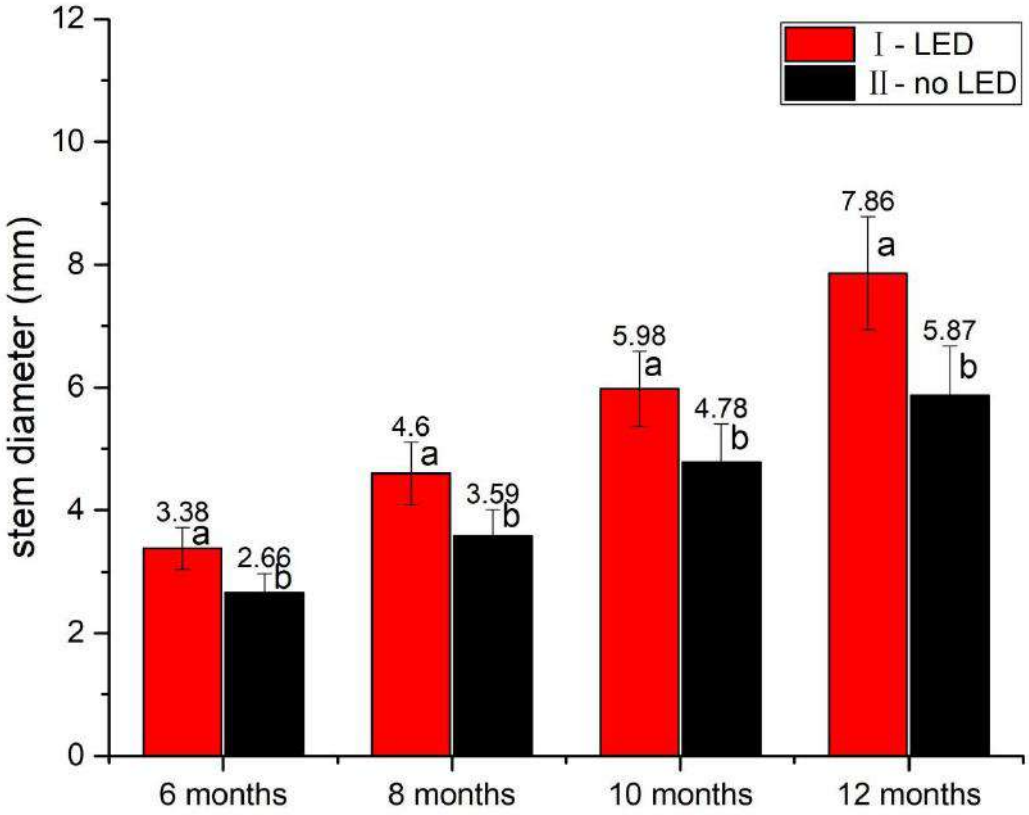

Fig. 2: Comparison of stem diameter between I– LED and II– no LED.

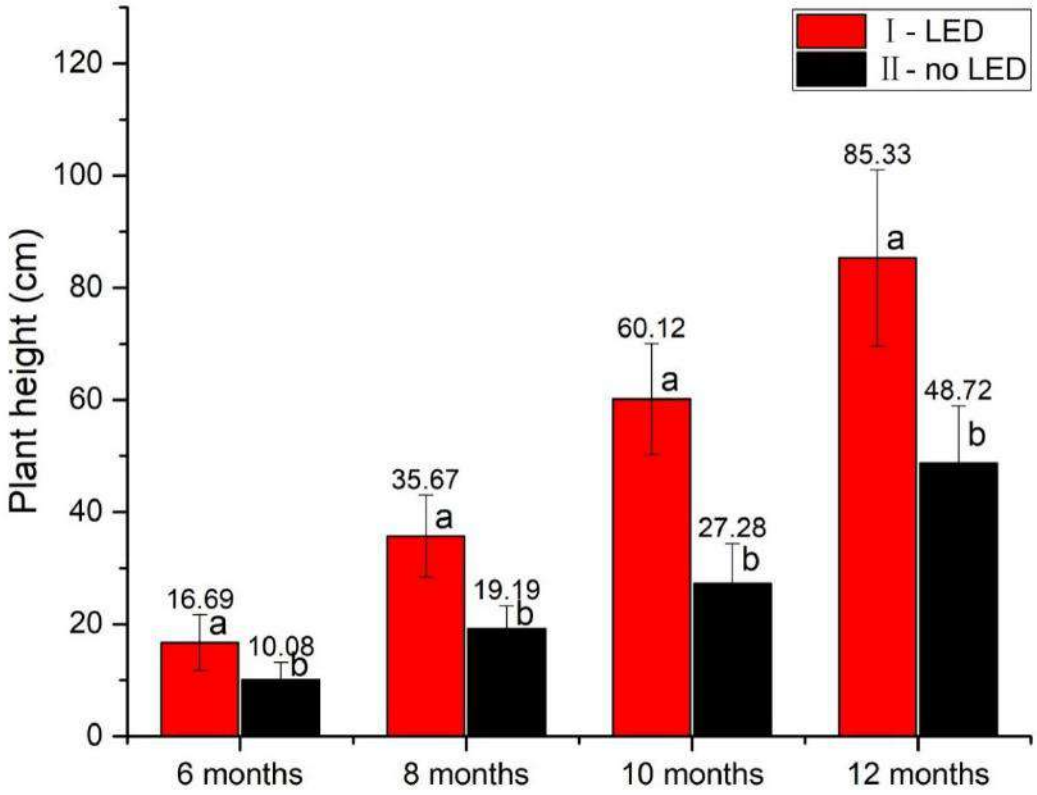

Fig. 3: Comparison of plant height between I– LED and II– no LED.

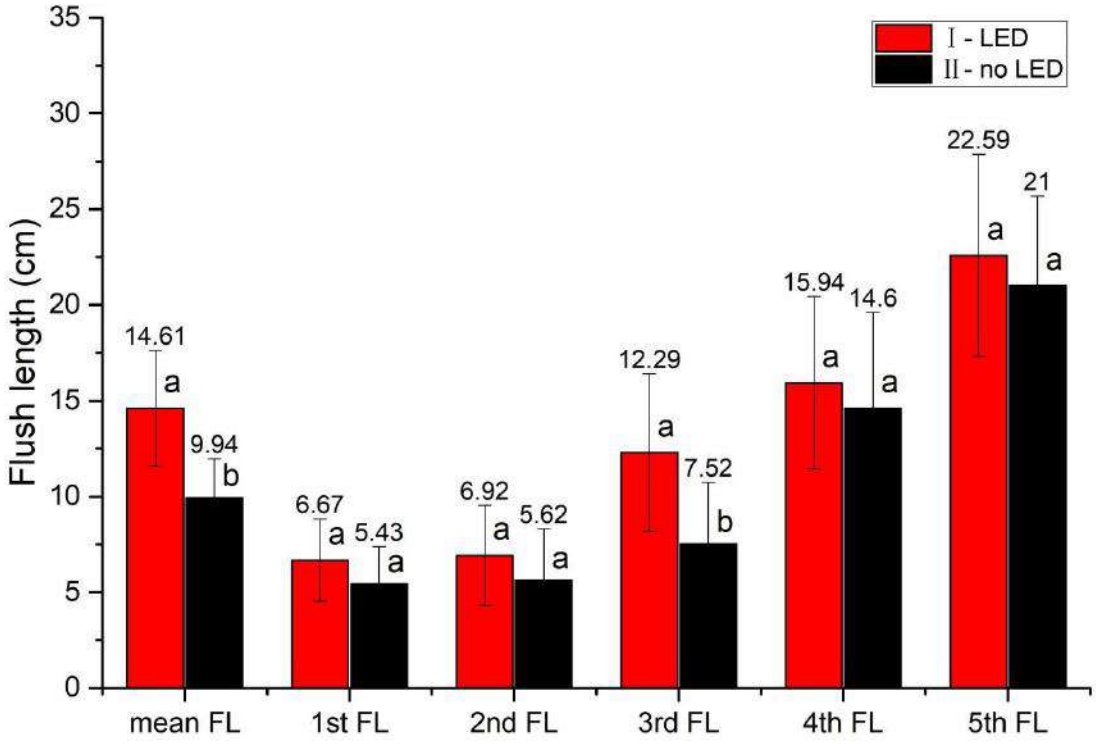

Fig. 4: Comparison of flush length (FL) between I– LED and II– no LED.The earliest germinated flush was designated as the first flush.

The plant survival rate is an important index for evaluating seedling cultivation technology because it affects the production cost. The plant survival rate of I- LED and II- no LED was92.3% and 45.4%, respectively. There was significant difference between I- LED and II- no LED. This implied that the plants of I- LED could assimilate during night lighting. Therefore, more carbohydrate had been synthesized, which was then utilized for shoot growth. The percentage of plant survival of I- LED (92.3%) was comparable with that of control plants (93.4%).This indicated thatthe mini-budding plants without leaves on rootstock could provide proper carbohydrate for normal shoot growth when grew under supplemental LED night lighting.

The chlorophyll content in the green bark tissue of young rootstock was 1.77 mg g^{-1} FW (fresh weight), which suggested that the green bark could photosynthesize. The chlorophyll content in the leaves was much higher (3.62 mg g^{-1} FW). The fresh weight of leaf was 3 times of that of green bark. The total chlorophyll amount in the bark tissue and the leaves was 0.97 and 5.86 mg per plant, respectively. The total chlorophyll amount in the leaves was about 6times of that in the bark tissue. Therefore, the plants with leaves on rootstock can assimilate much more carbohydrate than plants ofI- LED during daytime. According to Wang et al (2010)the net photosynthetic rate of CATAS 7-33-97 under PPFD 200 μmol m^{-2} $s^{-1}$was about 3~5 μmol m^{-2} s^{-1}, and the dark respiration rate was about 1.75 μmol m^{-2} s^{-1}. It indicated that the amount of carbohydrate of I – LED synthesized during night lighting could not compensate for the carbohydrate gap during daytime between I- LED and control plants. This probably was thereason why control plants were stronger than plants of I- LED.

The size of mini-budding plants is smaller, compared to that of green and brown budding. The plant density of mini-budding plants without leaves on rootstocks during night lighting period wouldbe greater. Therefore, applying LED night lighting will not cost too much for power energy. Axillary bud picking is labor intensive and the mean time is 45 second per one plant. However, topping with a scissor on the position below the first pair leaves only spends 1 second per plant. There is no procedure of axillary bud picking. Therefore, the labor cost will reduce greatly.

IV. CONCLUSION

Mini-budding is a prevalent technology for vegetative propagation of rubber tree in China. The first pair leaves should be kept on rootstocks for assimilation when topping is conducted, but the axillary bud picking which is labor intensive must be carried out. Mini-budding plants without leaves on the rootstock exhibited extremely poor growth and low plant survival rate. LED night lighting could improve the growth as well as plant survival rate of mini-budding plants without leaves on the rootstock. Therefore, applying LED night lighting to the mini-budding plants without leaves on the rootstock improved mini-budding technology by avoiding axillary bud picking.

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Trophic status and phytoplankton diversity of two dam ponds in Eastern Cameroon (Central Africa)

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Abstract**—** *A study aimed to determine the level of water pollution and phytoplankton diversity of two dam ponds (Ngaikada and Kpokolota) in Bertoua city was conducted from March 2016 to April 2017 using a monthly sampling frequency. Water samples were collected at surface directly using a 1L polyethylene vials and at 1.5 m depth using a 6L Van Dorn bottle. Physicochemicals analyzes were carried out according to the standard methods of APHA and Rodier, while the harvesting of phytoplankton organisms was done by direct sampling and analyzed by the Utermôhl method. The results of the physicochemicals analyzes reveal no significant difference (P > 0.05*) from surface to depth, high temperature (26.18 ± 1.40°C; 26.29 ± 1.01°C), low transparency (48.57 ± 17.15 cm; 51.43 ± 11.51 cm), high levels of orthophosphates (4.05 ± 3.38 mg/L; 4.15 ± 3.52 mg/L) and oxidability (8.49 ± 6.40 mg/L; 8.10 ± 6.68 mg/L) and high levels of chlorophyll 'a' (33.65 ± 24.66 µg/L; 43.67 ± 22.97 µg/L) respectively for Ngaikada and Kpokolota ponds. These characteristics classify these water bodies as hypereutrophics. Biological analyzes have shown that these ponds are quite diversified with 136 and 143 species identified in Kpokolota and Ngaikada respectively. The specific richness, the abundance and the density of phytoplankton organisms recorded at the surface were significantly higher (P < 0.05*) than those at 1.5 m depth. Rehabilitation processes such as control of nitrogen compound flows by purifying waste water from plantations and households, cutting aquatic plants and cleaning mud from ponds should be quickly put in place for a resumption of aquaculture activities.*

Keywords**— Anthropogenic, Bertoua, hypereutrophics, phytoplankton, ponds, rehabilitation.**

I. INTRODUCTION

In recent decades, rapid urbanization transformed human ecosystem in a disorderly manner, causing a disruption of some major ecological balances with disastrous consequences on the environment and strong repercussions on hydrosystems. These anthropogenic pressures lead from year to year to an increase in the emission of pollutants of all kinds, most of which reach the aquatic ecosystems that constitute the major biospher receptacle (**Colas *et al.*, 2014**). Eutrophication has become a growing phenomenon in most developing countries due to the excess of nutrients (nitrogen and phosphorus) released into the environment that are transported to hydrosystems without prior treatment (Kengne Tenkeu *et al.*, 2020). This eutrophication phenomenon undermines the ecological integrity of ponds making fish farming difficult, or even impossible, yet it covers huge animal protein needs by reducing the massive outflow of foreign exchange (**Agadjihouèdé *et al.*, 2011**). Ponds contain many

phytoplankton organisms that are primary producers at the base of the food chain of the freshwater food web. These phytoplankton organisms are considered the first biological community to respond to anthropogenic pressures and are the most direct indicator of nutrient concentrations in the water column of all quality biological elements (**Solimini *et al.*, 2006**).

In Cameroon, the environmental profile of fish farming ponds shows multiple and complex problems, including the degradation of water quality and biodiversity (**Kramkimel *et al.*, 2004; Mikolasek *et al.*, 2009; Efole Ewoukem *et al.*, 2017**). Studies based on phytoplankton biodiversity have focused mainly on large metropolitan areas (**Kemka *et al.*, 2006; Ebang Menye *et al.*, 2012**), neglecting some regional capitals such as the city of Bertoua, which has experienced a rapid population growth that has led to an increase in animal protein needs and consequently a proliferation of ponds that are strongly impacted by human activities making fish farming impractical. Very little data are available on water quality and planktonic fauna in these ponds. The aim of this work is therefore to study the physicochemical quality of water and phytoplankton dynamics of two dam ponds (Ngaikada and Kpokolota) in the city of Bertoua in order to propose measures to rehabilitate these ponds for resumption of fish farming activities. To achieve this objective, it will act to: (1) analyze the physicochemical quality of pond waters in order to determine their trophy status; (2) identify and count phytoplankton organisms in the different pond strata; (3) establish the links between the physicochemical quality of water and phytoplankton abundance.

II. MATERIAL AND METHODS

1.Description of the study site

The city of Bertoua is located in the Department of Lom-and-Djérem, Eastern Cameroon Region (Figure 1). This locality is a vast peneplain whose altitude varies between 400 and 900 m. The temperature is high throughout the year and oscillates between 18 and 30°C. Precipitation is relatively abundant (1500 to 2000 mm of rainfall per year) and its climate is subtropical with two seasons (**Olivry, 1986; Sighomnou, 2004**).

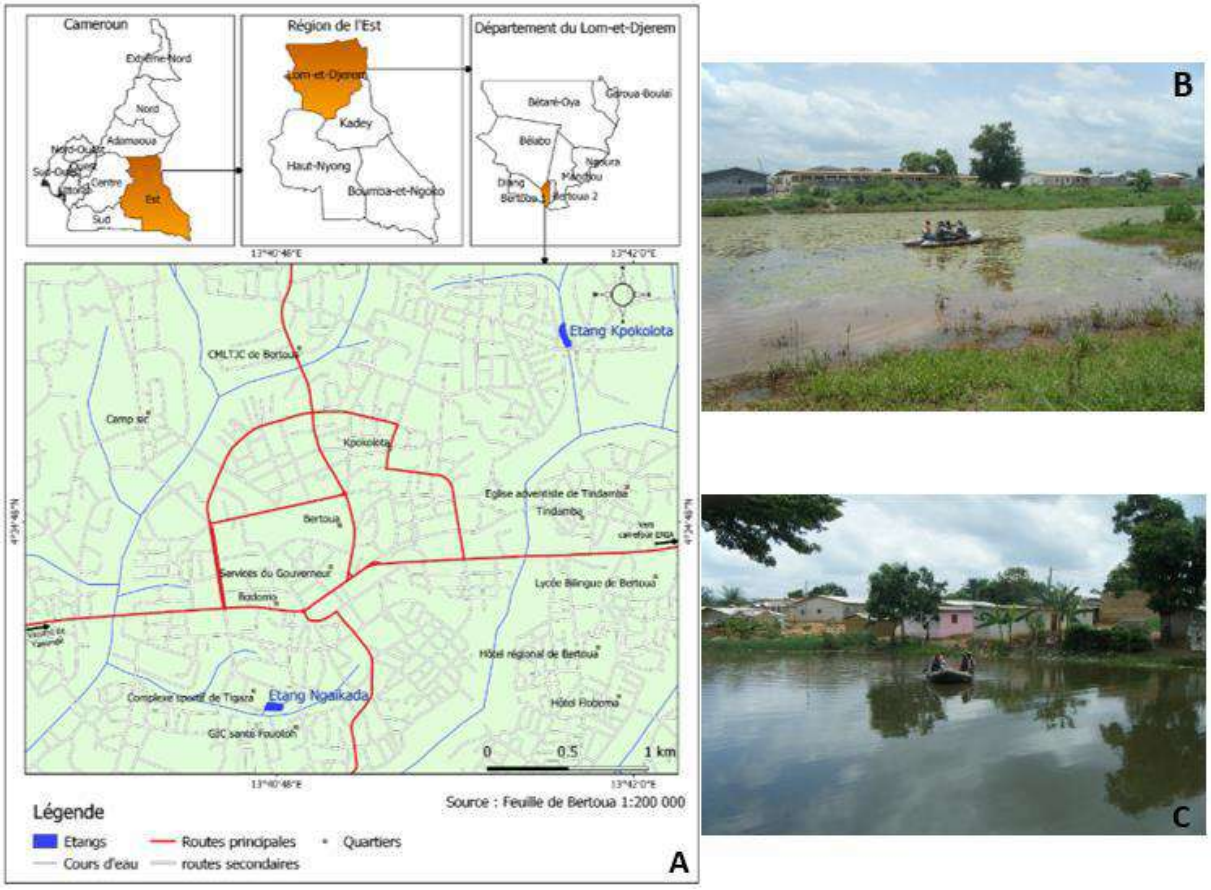

Fig.1: Geographical location of the two study ponds in Bertoua: A -General situation; B- Ngaikada Pond; C - Kpokolota Pond.

1.1. Ngaikada Pond

Ngaikada Pond is a wild, unmaintained and highly anthropized dam pond in an advanced state of decay with a fairly pronounced siltation. The geographical coordinates of this body of water are: 04°34'175" North latitude, 013°40'759" East longitude with an altitude of 655 m (Figure 1). This pond has an average depth of about 1.5 m, a perimeter of 420 m, an area of 10800 m^2 and a water volume of about 10692 m^3. The water from this pond is used by the local population for agriculture and for watering animals. This pond is mainly characterized by a strong vegetation consisting of plants and higher plants (*Nymphaea lotus, Pistia stratiotes*...), that cover the surface of the pond making navigation difficult. The main sources of pollution come from dead plants, numerous dwellings and plantations located in the watershed.

1.2. Kpokolota Pond

Kpokolota Pond is a very poorly maintained, wild and highly anthropophized dam pond. Its geographical coordinates are: 04°35'432" North latitude, 013°41'770" East longitude and an altitude of 654 m (Figure 1). The average depth is about 151.43 m, with a perimeter of 630 m, an area of 14300 m^2 and a water volume of about 21450 m^3. The main sources of pollution come from household wastewater located in the catchment area, from garbage dumps near the pond and especially from bottom-less latrines whose waste is dumped directly into the pond.

2. Sampling

The movements on the ponds were made using an inflatable Zodiac MR II. Sampling was carried out from March 2016 to April 2017 during a monthly frequency with surface and 1.5 m depth sampling for physicochemistry and biology.

2.1. Physicochemical analysis

Samples for surface physicochemical analyzes were collected directly at the surface using polyethylene vials, while at 1.5 m depth, these samples were collected using a 6 L Van Dorn bottle. Some parameters were measured directly in the field. In fact, the temperature was measured using a mercury column thermometer graduated to $1/100^{th}$ of a degree, the transparency (Zs) measured using a 30 cm diameter black and white Secchi disc, the depth measured using a weighted and graduated string, the percentage of dissolved oxygen saturation using a HACH HQ14d Oxymeter, electrical conductivity and Total Dissolved Solids (TDS) using a HANNA Hi 99300 portable TDS/Conductimeter. Other parameters such as turbidity, suspended solids (SS) and nutrients (NO_3^-, NO_2^-, NH_4^+, and PO_4^{3-}) were measured in the laboratory using the colorimetric method with the HACH/DR 2010 spectrophotometer; oxidizable materials were measured by volumetric method and chlorophyll 'a' content measured using the Lorenzen colorimetric method. These analyzes were carried out according to the technical and recommendations of **APHA (1998)** and **Rodier *et al.* (2009)**.

The stoichiometric ratio[N]/[P] (nitrogen concentration/phosphorus concentration) was calculated for each pond and compared to the **Redfield *et al.* (1963)** standard ratio value ([N]/[P] = 16) according to the following formulas:

$$[N] = [NO_3^-] + [NO_2^-] + [NH_4^+]$$

$$[P] = [PO_4^{3-}]$$

Nitrogen, phosphorus or the two elements will be limiting if the ratio[N]/[P] is less than, greater than or equal to 16, respectively. The principal component analysis (PCA) will be used to establish the abiotic typology of sampling stations based on all environmental parameters measured at the surface and at depth. The Trophic State Index (TSI) of **Carlson (1977)** is a mathematical model based on three variables: transparency (t), chlorophyll 'a' (chl 'a') and orthophosphates (PO_4^{3-}) calculated from the following three equations:

$$TSI\ (t) = 60 - 14{,}41 \times \ln (t)$$

$$TSI\ (chl\ 'a') = 30{,}6 + 9{,}81 \times \ln (chl\ 'a')$$

$$TSI\ (PO_4^{3-}) = 4{,}15 + 14{,}42 \times \ln (PO_4^{3-})$$

The average of the indices from the three variables indicates a precise position of the trophic status of the ponds. It is calculated as follows:

$$TSI = [TSI\ (t) + TSI\ (chl\ 'a') + TSI\ (PO_4^{3-})] / 3$$

The Carlson scale used to determine the trophic level of each pond is a set of indices ranging from 0 to 100 (Table I).

Table 1 Limit values of the Trophic State Index (TSI) of Carlson (1977).

TSI	Trophic state
[0-40[	Oligotrophic
[40 -50[	Mesotrophic
[50 -70[	Eutrophic
[70-100]	Hypereutrophic

2.2. Biological analysis

Phytoplankton organism was collected by direct sampling at the surface and using a Van Dorn bottle at depth and then transferred to clean, transparent 500 ml glass vials and fixed with 2.5 ml of a Lugol solution. After 48 hours of sedimentation, the supernatant was gently removed and the sub-sample of approximately 15 ml denser was preserved.

After homogenization, 1 ml of the sub-sample was pipetted and observed in a Sedgewick-Rafter counting cell with an inverted microscope (Olympus CK2). The count was duplicated to minimize the risk of error and the identification of at least 400 individuals per sample was recommended for an accuracy of +/- 95% (**APHA, 1998**). Due to the richness of some samples in particles and organisms, a dilution to $1/10^{th}$ or $1/20^{th}$ with distilled water was essential to facilitate enumeration. The count was carried out using an OLYMPUS CK2 inverted microscope with enlargement of 400x, with scans from left to right of the surface of the counting cell with alternating transects. Taxa have been identified through the specialized literature of: **Bourrelly (1985 & 1990)**; **Branco & Senna (1991)**, as well as books and publications on phytoplankton taxonomy from **Couté & Iltis (1981)**; **Kemka (2000)** and **Couté & Perrette (2011)**.

The density was calculated using the formula:D= Ni x S x 1000/ (v x s) with D = density (ind/L); S = area of the counting cell (1000 mm^2); Ni = number of individuals counted; 1000 = conversion factor in liters; s = area of the total counted field and v = volume of sedimented sample (5 ml). The transformation of chlorophyll 'a' (chl 'a') contents into carbon (C) following the works of **Reimann *et al.*, (1982)**, **Dessery *et al.***, (1984) and **Aleya & Devaux (1989)** was done by the formula: C = [chlorophyll 'a'] x 30. Since carbon represents 12% of the fresh weight of a cell (**Hamilton & Holm-Hansen, 1967**; **Pridmore & Hewitt, 1984**), the phytoplankton biomass was calculated using the formula given below:

$$\text{Biomasse } (\mu g/L) = \frac{C \times 100}{12} = [\text{chl } 'a'] \times 250$$

With C = phytoplankton carbon mass and 250 = Conversion coefficient. The Shannon and Weaver (1963) diversity index has been used to highlight the overall stands diversity and their degree of organization (**Tonkin *et al.*, 2013**). The Piélou regularity index (E) was used to reflects the quality of organization of the stands and varies from 0 to 1. It is close to 1 when all species tend to have the same abundance and close to 0 when one or a few species dominate the stand (**Dajoz, 2000**).

III. RESULTS

1. Physicals parameters

The transparency values recorded in Ngaikada pond (A) fluctuate at the surface from 20 cm to 80 cm with an average of 48.57 ± 17.15 cm and the depth values range from 70 cm to 130 cm with an average of 98.93 ± 21.23 cm (Figure 2A). In Kpokolota pond (B), the transparency varies at the surface from 30 cm to 65 cm with an average of 51.43 ± 11.51 cm and the depth values oscillates from 130 cm to 170 cm with an average of 151.43 ± 11.17 cm (Figure 2B). The student t-test shows a significant difference ($P < 0.05$*) between the different depths of the Ngaikada and Kpokolota Ponds.

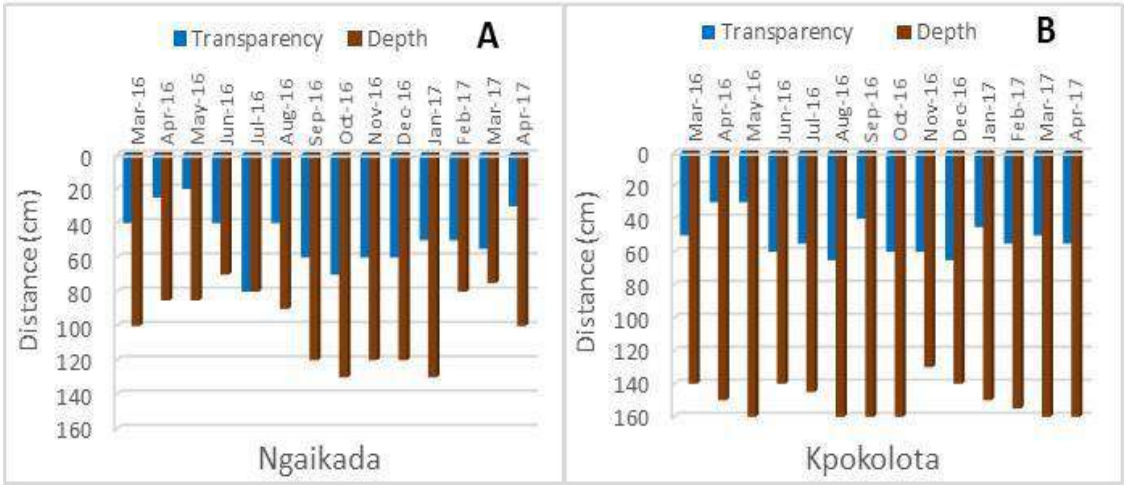

Fig.2: Spatiotemporal variations of the transparency and the depth in Ngaikada (A) and Kpokolota (B) ponds.

Water temperatures were relatively high in the ponds studied. In Ngaikada pond, it varies at the surface from 24°C to 30°C with an average of 26.57 ± 1.6°C and at depth from 24°C to 28°C with an average of 25.79 ± 1.19°C. In Kpokolota pond, the surface temperature fluctuates from 25°C to 29°C with an average of 26.43 ± 1.16°C and at depth from 24°C to 27°C with an average of 26.14 ± 0.86°C (Figure 3A). The suspended solids values recorded in Ngaikada pond range at the surface from 1 mg/L to 173 mg/L with an average of 27.07 ± 44.37 mg/L and at depth these values range from 1 mg/L to 235 mg/L with an average of 53.43 ± 63.55 mg/L. In Kpokolota pond, suspended solids values at the surface range from 4 mg/L to 117 mg/L with an average of 28.07 ± 29.63 mg/L and at

depth these values range from 4 mg/L to 230 mg/L with an average of 54.29 ± 75.72 mg/L (Figure 3B). In Ngaikada pond, Total Dissolved Solids (TDS) values fluctuate at the surface from 25 mg/L to 51 mg/L with an average of 35.5 ± 7.5 mg/L and at depth from 25 mg/L to 52 mg/L with an average of 35.86 ± 7.61 mg/L. These TDS values range in Kpokolota pond at the surface from 28 mg/L to 52 mg/L with an average of 35 ± 7.27 mg/L and at depth from 28 mg/L to 51 mg/L with an average of 35.71 ± 6.63 mg/L (Figure 3C). Turbidity varies in Ngaikada pond at the surface from 2 FTU to 173 FTU with an average of 38.43 ± 46.37 FTU and at depth these values fluctuate from 5 FTU to 258 FTU with an average of 52.18 ± 69.06 FTU. In Kpokolota pond, Turbidity fluctuates at the surface from 8 FTU to 84 FTU with an average of 36.64 ± 22.27 FTU and at depth these values vary from 0 FTU to 97 FTU with an average of 39.79 ± 27.9 FTU (Figure 3D).

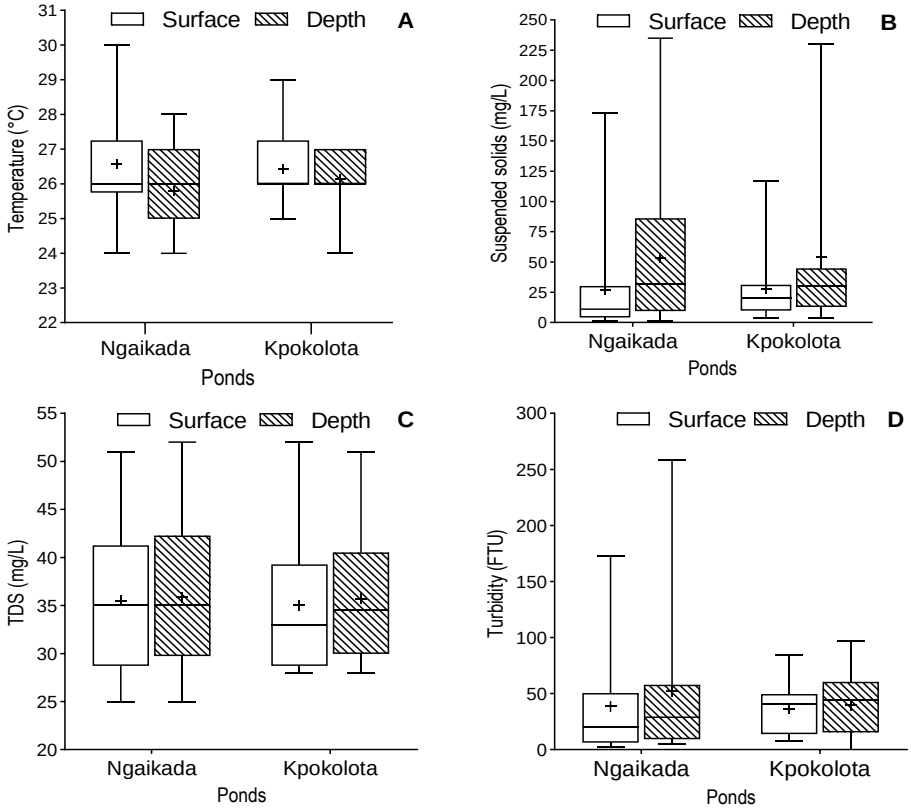

Fig.3: Spatial variations of temperature (A), Suspended Solids (B), TDS (C) and turbidity (E) in Ngaikada and Kpokolota ponds.

2. Chemicals parameters

The potential Hydrogen (pH) values obtained in Ngaikada pond range at the surface from 5.31 to 7.46 with an average of 6.4 ± 0.71 and at depth, these values range from 5.22 to 7.54 with an average of 6.28 ± 0.68. In Kpokolota pond, these pH values range at the surface from 5.16 to 7.51 with an average of 6.41 ± 0.7 and at depth from 5.13 to 7.5 with an average of 6.37 ± 0.64 (Figure 4A). Electrical conductivity values vary in Ngaikada pond from 48 µS/cm to 103 µS/cm at the surface with an average of 69.74 ± 15.46 µS/cm and at depth from 49 µS/cm to 104 µS/cm with an average of 69.74 ± 15.76 µS/cm. These electrical conductivity values fluctuate in Kpokolota pond at the surface from 53.6 µS/cm to 103 µS/cm with an average of 68.64 ± 14.66 µS/cm and at depth from 54.8 µS/cm to 103 µS/cm with an average of 71.2 ± 14.23 µS/cm (Figure 4B). Dissolved oxygen (O_2) levels in Ngaikada pond at the surface range from 33.9% to 83.9% with an average of 58.81 ± 12.32% and at depth from 34.4% to 70.7% with an average of 54.64 ± 10.13%. In Kpokolota pond, surface oxygen levels range from 35.3% to 84.7% with an average of 60.09 ± 11.12% and at depth from 36% to 69.4% with an average of 56.62 ± 8.45% (Figure 4C). Dissolved carbon dioxide (CO_2) levels in Ngaikada pond range from 0.66 mg/L to 10.56 mg/L at the surface with an average of 3.32 ± 2.95 mg/L and at depth from 0.66 mg/L to 7.04 mg/L with an average of 3.25 ± 2.13 mg/L. These CO_2levels change in Kpokolota pond from 0.76 mg/L to 15.84 mg/L at the surface with an average of 3.95 ± 4.34 mg/L and at depth from 0.66 mg/L to 15.84 mg/L with an average of 3.8 ± 5.02 mg/L (Figure 4D).

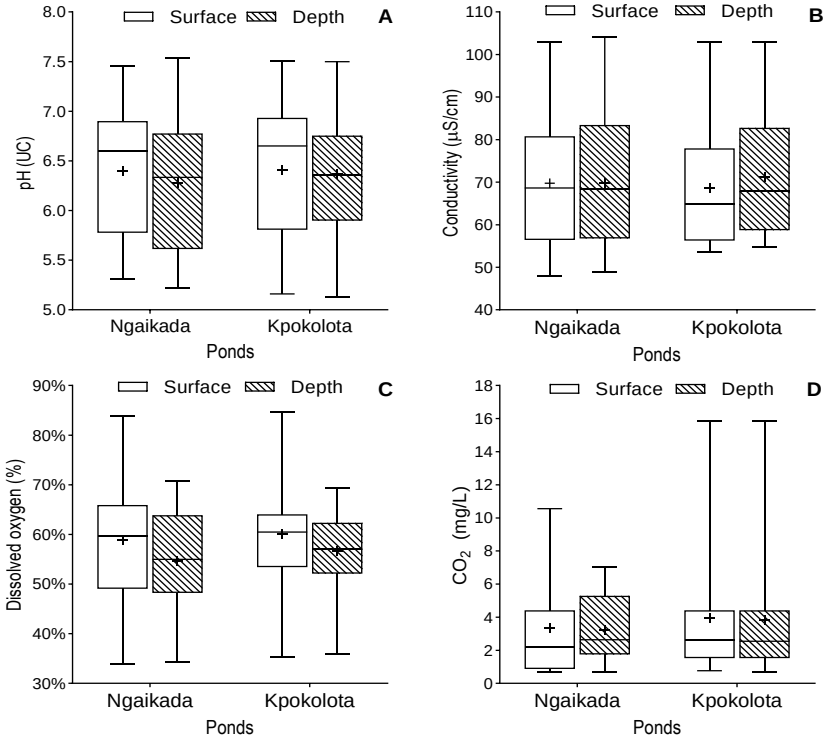

Fig.4: Spatial variations of potential Hydrogen (A), conductivity (B), dissolved oxygen (C) and dissolved carbon dioxide (D) in Ngaikada and Kpokolota ponds.

Nitrate (NO_3^-) contents in Ngaikada pond range at the surface from 0 mg/L to 4.1 mg/L with an average of 1.81 ± 1.13 mg/L and at depth from 0 mg/L to 5.5 mg/L with an average of 1.8 ± 1.63 mg/L. These nitrates content change in Kpokolota pond from 0 mg/L to 1.7 mg/L at the surface with an average of 0.78 ± 0.43 mg/L and at depth from 0 mg/L to 1.7 mg/L with an average of 0.66 ± 0.48 mg/L (Figure 5A).

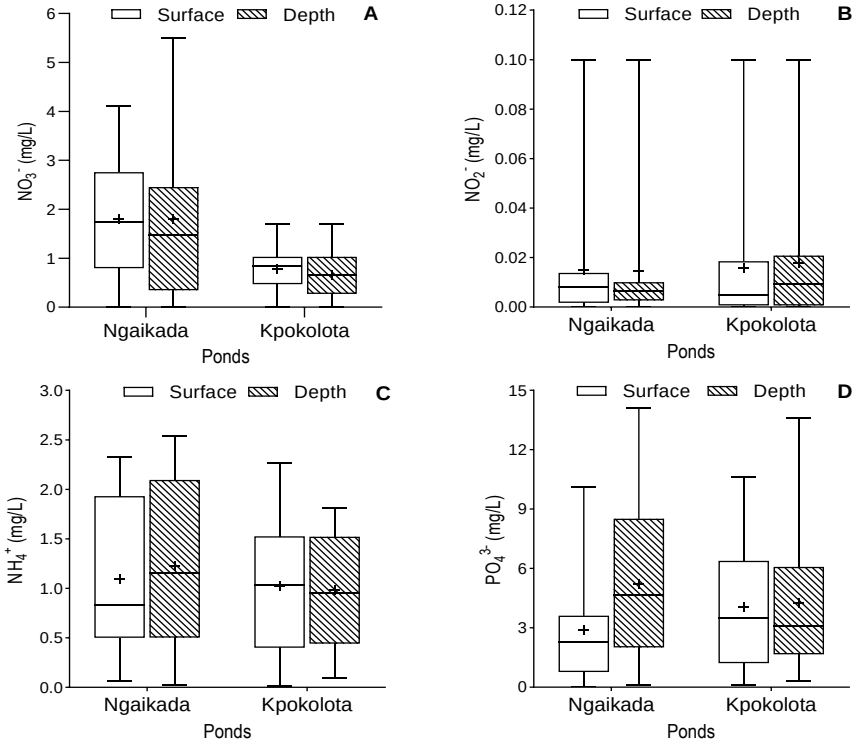

Fig.5: Spatial variations of nitrate (A), nitrite (B), ammoniacal nitrogen (C) and orthophosphate (D) in Ngaikada and Kpokolota ponds.

Nitrite (NO_2^-) contents fluctuate in Ngaikada pond from 0 mg/L to 0.1 mg/L at the surface with an average of 0.01 ± 0.03 mg/L and at depth from 0 mg/L to 0.1 mg/L with an average of 0.01 ± 0.03 mg/L. These nitrates levels change in Kpokolota pond from 0 mg/L to 0.1 mg/L at the surface with an average of 0.02 ± 0.03 mg/L and at depth from 0 mg/L to 0.1 mg/L with an average of 0.02 ± 0.03 mg/L (Figure 5B). Ammoniacal nitrogen (NH_4^+) levels fluctuate in Ngaikada pond from 0.06 mg/L to 2.23 mg/L at the surface with an average of 1.09 ± 0.76 mg/L and at depth from 0.02 mg/L to 2.54 mg/L with an average of 1.23 ± 0.82 mg/L. These ammoniacal levels change in Kpokolota pond from 0.01 mg/L to 2.27 mg/L at the surface with an average of 1.03 ± 0.72 mg/L and at depth from 0.09 mg/L to 1.81 mg/L with an average of 0.981 ± 0.6 mg/L (Figure 5C). Orthophosphate (PO_4^{3-}) levels fluctuate in Ngaikada pond from 0 mg/L to 10.1 mg/L at the surface with an average of 2.88 ± 2.77 mg/L and at depth from 0.1 mg/L to 14.1 mg/L with an average of 5.23 ± 3.98 mg/L. These orthophosphate levels change in Kpokolota pond from 0.1 mg/L to 1.6 mg/L at the surface with an average of 4.05 ± 3.33 mg/L and at depth from 0.3 mg/L to 13.6 mg/L with an average of 4.25 ± 3.71 mg/L (Figure 5D).

Oxydability levels in Ngaikada pond range from 0.59 mg/L to 22.91 mg/L at the surface with an average of 9.24 ± 7.15 mg/L and from 0.19 mg/L to 20.34 mg/L at depth with an average of 7.74 ± 5.64 mg/L. These oxydability levels change in Kpokolota pond from 0.4 mg/L to 21.33 mg/L at the surface with an average of 7.76 ± 6.63 mg/L and at depth from 0.2 mg/L to 24.12 mg/L with an average of 8.44 ± 7.03 mg/L (Figure 6A). The chlorophyll 'a' (chl 'a') contents in Ngaikada Pond range from 0.6 µg/L to 80.3 µg/L at the surface with an average of 30.1 ± 24.88 µg/L and from 0.6 µg/L to 96.2 µg/L at depth with an average of 37.19 ± 24.44 µg/L. These chl 'a' level change in Kpokolota pond at the surface from 3.1 mg/L to 83.1 mg/L with an average of 37.79 ± 19.45 mg/L and at depth from 13 mg/L to 108.3 mg/L with an average of 49.56 ± 26.49 mg/L (Figure 6B). The stoichiometric ratio of dissolved inorganic nitrogen to orthophosphate in Ngaikada pond at the surface range from 0 to 9.61 with an average of 1.75 ± 2.36 and at depth from 0.15 to 13.8 with an average of 2.04 ± 3.94. This ratio fluctuates in Kpokolota pond at the surface from 0.07 to 31.71 with an average of 3.03 ± 8.31 and at depth from 0.09 to 2.46 with an average of 0.72 ± 0.64 (Figure 6C).

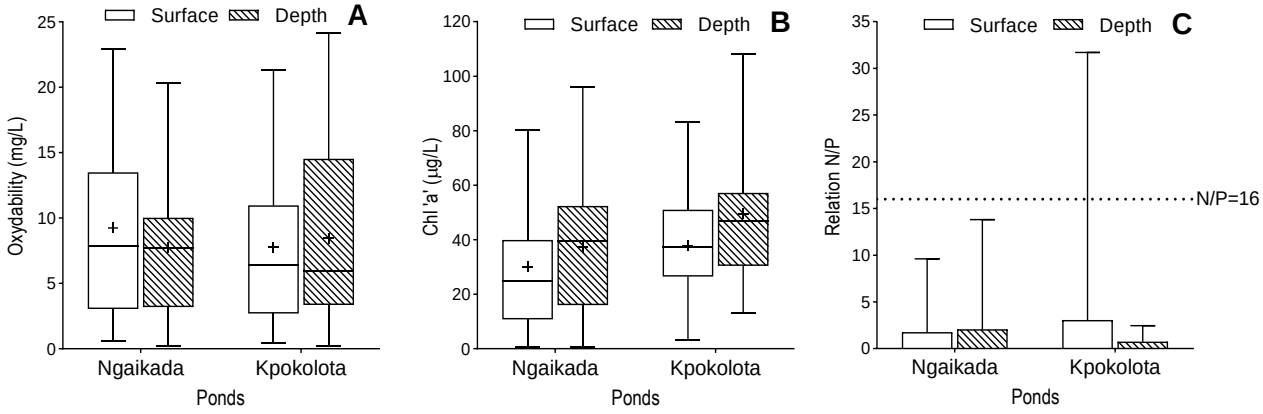

Fig.6: Spatial variations of oxydability (A), Chlorophyll 'a' (B) and N/P ratio (C) in Ngaikada and Kpokolota ponds.

3. Abiotic typology of sampling stations

The Principal Component Analysis (PCA) of the ponds is provided by the first two factor axes F1 (55.85%) and F2 (33.87%) which together account for 89.72% of the total inertia. The factor map shows a distribution of the four stations in relation to their physicochemicals characteristics. Two main groups emerge in this factorial design (Figure 6):

- Group I, whose F2 axe discriminates in positive coordinates between the Ngaikada surface (Ngai surf) and Kpokolota surface (Kpo surf) stations, characterized by good transparency of water, good oxygenation and high temperatures.

- Group II, whose F2 axe discriminates in its negative part, the Ngaikada depth (Ngai depth) and Kpokolota depth (Kpo depth) stations characterized by turbid water, rich in nutrients, with high chlorophyll 'a' contents and strong mineralization of water.

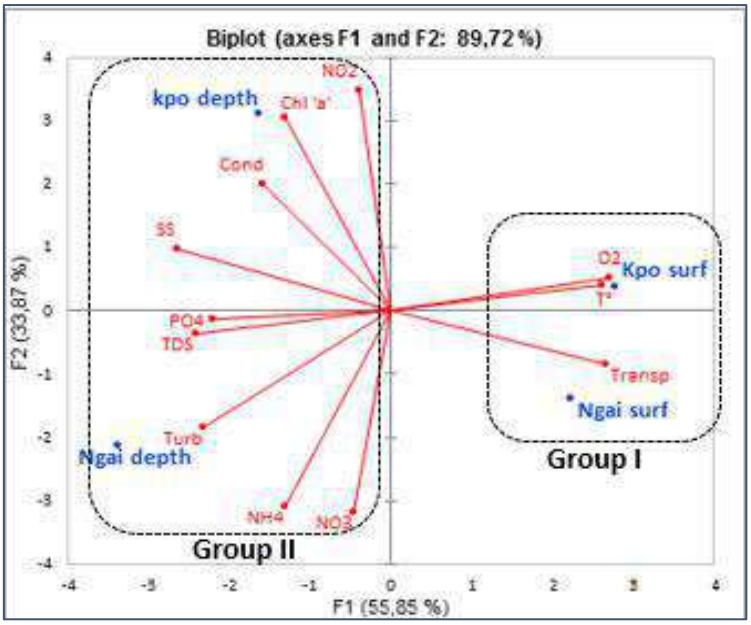

Fig.7: Principal Component Analysis (PCA) performed on the environmental variables of the station's studies. Chl 'a': Chlorophyll 'a', Cond: Electrical conductivity, SS: Suspended Solids, NH4: Ammonium, NO2: Nitrite, NO3: Nitrate, O2: Dissolved Oxygen, PO4: Orthophosphate, T°: Temperature, TDS: Total Dissolved Solid, Trans: Transparency and Turb: Turbidity.

4. Trophic level of the ponds studied

Ngaikada pond shown a TSI (t) = 70.28, TSI (PO_4^{3-}) = 123.94 and TSI (chl 'a') = 65.09. The Trophic State Index (TSI) of Ngaikada pond was 86.44. Kpokolota pond presented a TSI (t) = 69.7, TSI (PO_4^{3-}) = 124.27 and TSI (chl 'a') = 67.65. The Trophic State Index (TSI) of Kpokolota pond was 87.21.

5. Distribution of phytoplankton taxonomic units in ponds

During this study, 143 species of phytoplankton were identified in Ngaikada pond belonging to 62 genus, 54 families, 28 orders and 9 classes while in Kpokolota pond, the diversity was 136 species of phytoplankton grouped into 54 genus, 49 families, 28 orders and 14 classes (Tableau 2).

Table 2 Taxonomic units of phytoplankton in ponds

Ponds	Classes	Orders	Families	Genus	Species
Ngaikada	9	28	54	62	143
Kpokolota	14	28	49	54	136

6. Phytoplankton structure of the ponds

The specific richness in Ngaikada pond at the surface varied from 5 species to 26 species with an average of 15 ± 6 species and at depth from 5 species to 21 species with an average of 11 ± 4 species. In Kpokolota Pond, this diversity evolved at the surface from 11 species to 20 species for an average of about 15 ± 3 species and at depth from 7 species to 16 species with an average of about 11 ± 3 species. The specific richness of surface in Ngaikada (P=0.03*) and Kpokolota (P=0.001**) ponds were significantly higher than those at depth (Figure 8A). The abundance in Ngaikada pond at the surface varies from 40 ind. to 431 ind. with an average of 236 ± 125 ind. and at depth from 21 ind. to 285 ind. with an average of 120 ± 77 ind.. In Kpokolota Pond, this surface abundance fluctuates from 61 ind. to 701 ind. with an average of 317 ± 169 ind. and at depth from 28 ind. to 330 ind. with an average of 178 ± 80 ind.. The phytoplankton abundances of surface in Ngaikada (P=0.006**) and Kpokolota (P=0.009**) ponds are significantly higher than those at depth (Figure 8B). The phytoplankton density in Ngaikada pond at the surface ranges from 4000 ind./L to 43100 ind./L with an average of 23600 ± 12500 ind./L and at depth from 2100 ind./L to 28500 ind./L with an average of 12000 ± 7700 ind./L. In Kpokolota Pond, surface density fluctuates from 6100 ind./L to 70100 ind./L with an average of 31700 ± 16900 ind./L and at depth from 2800 ind./L to 33000 ind./L with an average of 17800 ± 8000 ind./L. The phytoplankton densities of surface in Ngaikada (P=0.006**) and Kpokolota (P=0.009**) ponds are significantly higher than those at depth (Figure 8C). The phytoplankton biomass in Ngaikada Pond at the surface ranges from 18 µgC/L to 2409 µgC/L with an average of 903 ± 746.4 µgC/L and at depth from 18 µgC/L to 2886 µgC/L with an average of 1115.79 ± 733.25 µgC/L. In Kpokolota Pond, this biomass changes

at the surface from 93 µgC/L to 2493 µgC/L with an average of 1133.57 ± 583.54 µgC/L and at depth from 390 µgC/L to 3249 µgC/L with an average of 1486.71 ± 794.72 µgC/L (Figure 8D).

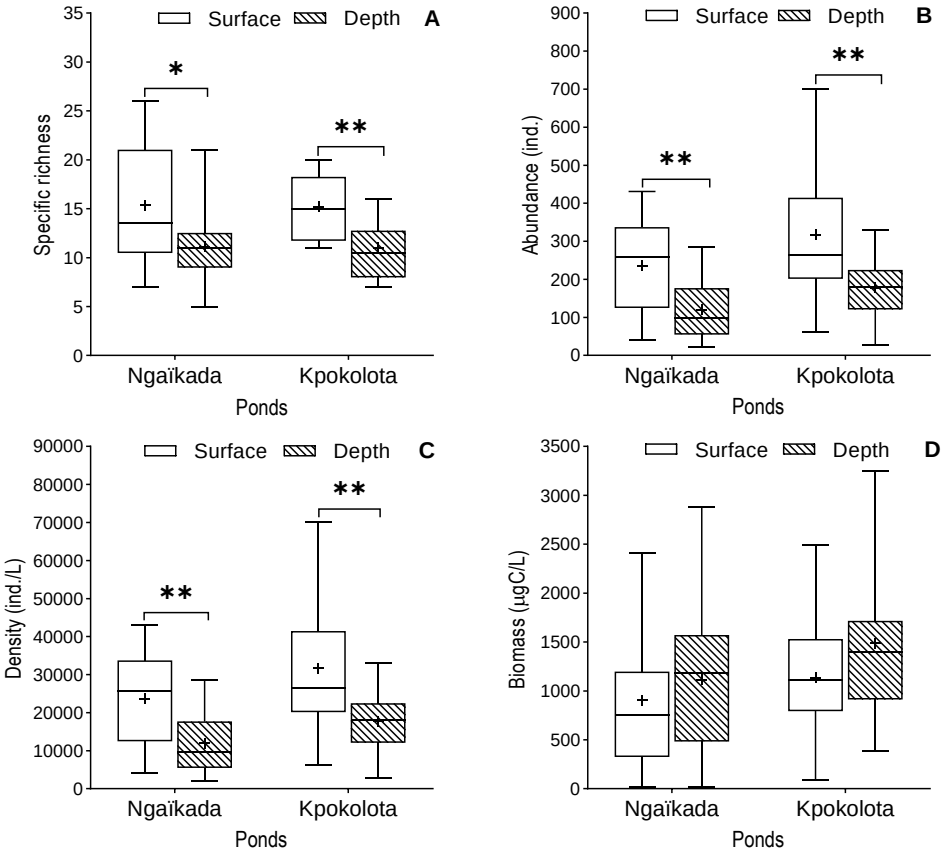

Fig.8: Spatial variations of specific richness (A), abundance (B), density (C) and biomass (D) in Ngaikada and Kpokolota ponds.

The diversity index of Shannon and Weaver (1963) varied in the Ngaikada pond at the surface from 2.23 bits/ind. to 4.12 bits/ind. with an average of 3.42 ± 0.53 bits/ind. (H'max = 3.83 bits/ind.) and at depth from 2.26 bits/ind. to 4.05 bits/ind. with an average of 3.05 ± 0.49 bits/ind. (H'max = 3.39 bits/ind.).

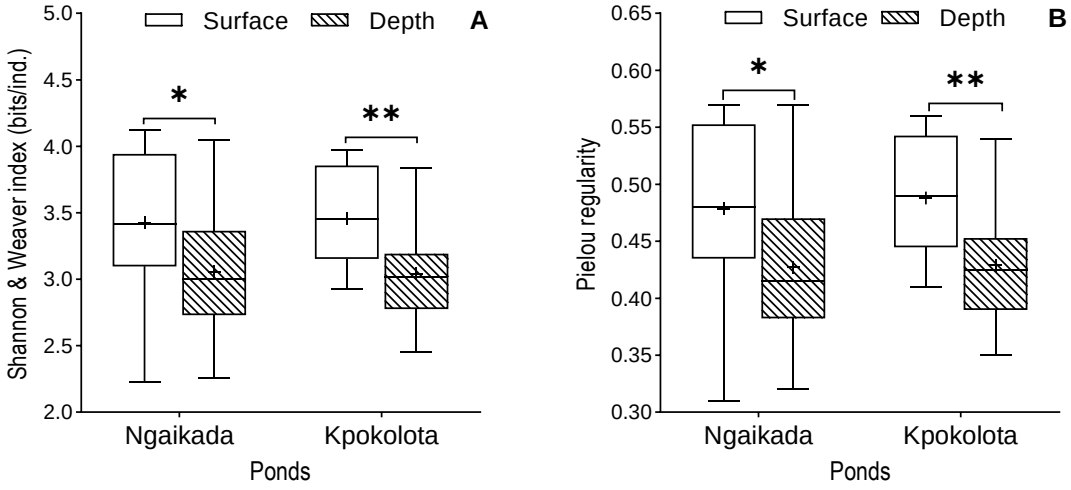

Fig.9: Spatial variations of Shannon and Weaver diversity index (A) and Piélou regularity (B) in Ngaikada and Kpokolota ponds.

This index varies in Kpokolota pond at the surface from 2.93 bits/ind. to 3.97 bits/ind. with an average of 3.46 ± 0.36 bits/ind. (H'max = 3.89 bits/ind.) and at depth from 2.45 bits/ind. to 3.84 bits/ind. with an average of 3.04 ± 0.34 bits/ind. (H'max = 3.41 bits/ind.). The diversity index of surface in Ngaikada (P=0.003*) and Kpokolota (P=0.005**) ponds are significantly higher than those at depth (Figure 9A). The Piélou regularity index varies in Ngaikada pond from 0.31 to 0.57 at the surface with an average of 0.48 ± 0.07 and at depth from 0.32 to 0.57 with an average of 0.43 ± 0.06. In Kpokolota Pond, this regularity fluctuates at the surface from 0.41 to 0.56 with an average of 0.49 ± 0.05 and at depth from 0.35 to 0.54 with an average of 0.43 ± 0.04. The Piélou regularity of surface in Ngaikada (P=0.003*) and Kpokolota (P=0.004**) ponds are significantly higher than those at depth (Figure 9B).

IV. DISCUSSION

1. Physicochemical variables

The low transparency values obtained in the ponds studied can be attributed to the action of winds combined with their shallow depths. Winds cause turbulence that, coupled with the shallow depth of the ponds, constantly suspend particles matter and phytoplankton that reduce the transparency of the pond water (**Cunha *et al.*, 2019**). In addition, these ponds are dam ponds, which are therefore located on the bed of the watercourses and subject to sedimentary inputs carried by the tributaries from the catchment area. The temperature average values recorded in Ngaikada (26.18°C) and Kpokolota (26.29°C) ponds are relatively high and depend strongly on the amount of sunlight and the degree of mineralization of the organic matter that produces energy that can increase the water temperature. In this connection, **Antalé (2012)** affirms that the temperature of surface waters depends closely on the amount of sunshine and exchanges with the atmosphere. The high values of suspended solid well above the threshold (25 mg/L) are due to untimely and irregular rainfall in the study area that transported particular matter into the ponds by erosion. High levels of suspended solids have resulted in high turbidity values with averages above the 35 mg/L threshold. **Al-Aubadi*et al.* (2019)** point out that rainfall favors the erosion of mineral and organic particles from the catchment area, which are transported by runoff water to the ponds where they cause the mix of water, increasing the suspended solids content of the environment and consequently its transparency and turbidity. The electrical conductivity and Total Dissolved Solids have been elevated in ponds, reflecting high anthropogenic activity and high organic pollution (Kengne Tenkeu *et al.*, 2020). The high average of electrical conductivity values in ponds would be justified by leaching from agricultural land and the input of domestic wastewater into the ponds. On this subject, **Zébazé Togouet *et al* (2007)** confirm that the supply of wastewater to the aquatic environment increases its content of ionizable salts and consequently its electrical conductivity. The percentage of dissolved oxygen saturation was high in the pond, characteristic of a strong photosynthetic activity of algae and aquatic plants that release oxygen into the environment (**Ramade, 2005**). Dissolved carbon dioxide evolves in the opposite of oxygenation and the observed fluctuations in levels are undoubtedly linked to CO_2 using for photosynthesis and to the activity of aerobic bacteria that degrade fermentable organic matter, consuming dissolved oxygen while releasing carbon dioxide. In this regard, **Boyd (2020)** points out that in water, breathing has the effect of reducing O_2 while increasing CO_2. Nutrient levels were high and sometimes well above the standards prescribed for fish farm ponds, reflecting overall richness organic matter in the ponds. The high levels of nitrogen elements in ponds are due to non-native inputs of organic matter and nitrogenous metabolic waste from human activity, mainly from agricultural activities in the pond catchment area (**Moss *et al.*, 2013**) or from residential areas (**Wang *et al.*, 2014**). The high levels of ammonium are explained by the significant decomposition of organic matter accompanied by a high consumption of dissolved oxygen, favoring its production by ammonification. High levels of orthophosphates show advanced trophic status of the ponds and would come from runoff from the watershed. **Dunnette (1992)** rightly argues that the phosphorus content of a biotope is a predictor of the degree of eutrophication of its waters. The high levels of chlorophyll 'a' reflect a high phytoplankton biomass, reflecting high photosynthetic activity and therefore high productivity (**Lu *et al.*, 2016**).

The ponds studied show a homogeneous physicochemical quality of water from surface to depth ($P > 0.05$), making it possible to recommend a single sampling for future studies. In the ponds studied and compared to **Redfield's (1963)** standard ratio, only one N/P ratio data is greater than 16 in Kpokolota Pond (31.71) at the surface showing a limitation of eutrophication by phosphorus at that month. However, the overall surveys are all lower than the Redfield ratio (N/P = 16), showing that nitrogen is the limiting factor of eutrophication in these two ponds. The ponds studied with a low transparency and a high level of orthophosphates and chlorophyll 'a', have high TSI values in Ngaikada (86.44) and Kpokolota (87.21) allowing to characterize these two ponds as being hypereutrophic. The hypereutrophic state of the ponds can explain the abandonment of fish farming activities and could lead to their disappearance in the near future. The measures to be

implemented for the rehabilitation and the management of eutrophication in these ponds should therefore focus on mowing aquatic plants, cleaning pond mud and controlling the flow of nitrogen compounds, which appear to be the factor whose absence or low content would limit algal growth. However, other factors such as light intensity, transparency or water temperature also control the increase in algal biomass (**Beck and Hall, 2018**).

2. Phytoplankton structure

Taxonomic analysis of phytoplankton in Bertoua ponds identified 143 species in Ngaikada and 136 species in Kpokolota. The diversity observed in the ponds is higher than 103 taxa obtained by **Kemka (2000)** in Yaounde municipal lake, but smaller than the 152 taxa obtained by **Djiogo Kingfack (2007)** in Mefou dam lake, 237 taxa obtained by **Ebang Menye *et al.* (2012)** in Mfoundi rivers and 162 taxa obtained by **Koda (2015)** in CAPFORT fish farm in Mbalmayo. This relatively high taxonomic richness recorded in the Bertoua ponds compared to other studies is due to the strong anthropization of the catchment area of the ponds with organic and mineral materials containing high levels of nutrients that promote the rapid and continuous growth of algae and aquatic plants. These organic pollutants, which have a high nitrogen and phosphorus load, offer favourable conditions for the development of different phytoplankton species. In this regard, **Aboim *et al.* (2020)** point out that the low water volume associated with high nutrient levels is favourable to the development of phytoplankton organisms. Spatial variations of phytoplankton abundances and densities are logical responses to changes and physicochemical conditions in the environment. The relatively low taxonomic richness compared to other studies is due to the hypereutrophic character of the ponds studied because, productivity is maximal in all groups of living beings at the eutrophic stage (**Zébazé Togouet, 2008**). The higher abundances and densities of phytoplankton at the surface than at depth ($P < 0.05$) are believed to the high temperatures and high light intensities offered by solar energy for phytoplankton photosynthesis. **Azhikodan and Yokoyama (2016)** confirm these observations by stating that light intensity is the main environmental factor likely to vary with depth and probably plays a very important role in the observed taxonomic differences between the surface and depth of water bodies. The differences of abundance and densities lower in Ngaikada than in Kpokolota ($P < 0.05$) are due to the high presence of aquatic plants on the surface of Ngaikada pond, which prevents better light penetration, an essential condition for photosynthesis. The high phytoplankton biomass is due to high nutrient concentration in the ponds absorbed by phytoplankton organisms and reflect high photosynthetic activity and consequently high primary productivity in ponds (**Lu *et al.*, 2016**). The high phytoplankton biomass recorded in Kpokolota than in Ngaikada ($P < 0.05$) is due to the high levels of chlorophyll 'a' and nutrients recorded in this pond that promoted phytoplankton growth.

The Shannon and Weaver Diversity Index is higher at the surface than at depth in Ngaikada ($P < 0.05$*) and Kpokolota ($P < 0.01$**) Ponds. This high diversity of surface organisms could be explained by the high light intensities in this stratum in both ponds, which provides essential conditions for photosynthesis, and playing an important role in the observed differences of diversity (**Azhikodan and Yokoyama, 2016**). The regularity of Piélou is low in the ponds, but shallower in depth than on the surface in the Ngaikada ($P < 0.05$*) and Kpokolota ($P < 0.01$**) ponds. This low regularity shows populations that are not in equilibrium, due to the strong dominance of a small group of species to the detriment of the others (**Djego *et al.*, 2012**) mainly represented in the Ngaikada pond by *Pinnularia gibba* and *Stauroneis phoenicenteron*species and in the Kpokolota pond by *Azpeitia africana* and *phacus orbicularis* species. Some species such as *Azpeitia africana*, *Microcystis aeruginosa*, *Surirella capronii*, *Rhizolenia* sp., *Cymatopleura solea* and *Closterium aciculare* are positively correlated with ammoniacal nitrogen. Nitrate are correlated with the species *Rhizolenia* sp. and *Trachelomonas hispida*. The taxa *Trachelomonas armata*, *Volvox dissipathrix*, *Phacus orbicularis* and *Pleurotaenium trabecula* are positively correlated with nitrite while *Pinnularia cardinalis*, *Pleurotaenium subcornulatum* and *Pleurotaenium trabecula* are positively correlated with orthophosphate in the pond's studies. The preference of some taxa for various nutrients is due to the fact that nutrients promote phytoplankton growth. For this purpose, **Lapointe *et al.* (2004)** claims that nutrient inputs from urban or agricultural effluents are a cause of the great richness and phytoplankton abundance of some water bodies.

V. CONCLUSION

The results of this study show that the Ngaikada and Kpokolota ponds are both hypereutrophic with nitrogen as the limiting factor of eutrophication. These ponds have a homogeneous physicochemical quality of water column from the surface to the depth, allowing a single sampling point to be recommended for future studies. specific richness, abundance and density are higher at the surface than at depth in all ponds, mainly due to the ever-increasing light intensity in the upper layers of the ponds. These ponds are quite diversified, but not in balance because of the dominance of a small group of species. The correlations

observed between some organisms and nutrients support the idea that these nutrients are responsible for phytoplankton growth. The hypereutrophication of these ponds due to strong anthropization is incompatible with fish farming and restoration measures must be quickly put in place at the risk of seeing them disappear in the very near future. These rehabilitation measures will have to focus on controlling the flow of nitrogen compounds, mowing aquatic plants and cleaning the mud from ponds.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest regarding the publication of this paper.

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Effects of feeding ration incorporating *Piliostigma thonningii* (schum.) pods on growth and gastrointestinal parasites in West African Dwarf goats of Burkina Faso

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Abstract— *The objective of this study was to determine the availability and the use of Piliostigma thonningii pods through individual interviews using semi-structured questionnaires, and to evaluate the effect of diet incorporating these pods on growth and parasitic status of goats. The trial consisted in feeding diets to 12 adult West African dwarf goats, divided into three batches of four animals each. Batches 1 and 2 received a diet with 40% Piliostigma thonningii pods, and bamboo charcoal as a zootechnical additive (1g/kg PV) in the diet of batch 2. For batch 3, the animals received a diet without the pods, proportionally replaced by cotton seed cake. The experiment lasted for 78 days including two weeks of adaptation. The Mac-Master technique was used for identification and enumeration of strongyle eggs and coccidia oocysts. The results showed a higher frequency of the use of pods in shredded form (38.18%) compared to crushed (25.45%) and whole (20.00%). The availability of pods was confirmed by 57.1% of the respondents. The ADG obtained with the batches fed diet incorporating the pods were 16.39±4.10 and 24.01±5.97 g/d for batches 1 and 2 respectively, significantly lower than those obtained with the animals in batch 3 that received the cotton seed cake (31.39±5.80 g/d). The level of EPG recorded in animals from batch 1 (50±100) was significantly lower than those obtained with animals from batches 2 and lot 3 (500±455 and 925±736 respectively). The use of Piliostigma thonningii pods could be of great use in goat intensive production improvement programs.*

Keywords— ***Pods, Piliostigma thonningii, Bamboo charcoal, ADG, EPG.***

I. INTRODUCTION

Livestock farming offers many advantages for people in developing countries. First of all, it allows to get a more nutritional and diversified diet, especially in terms of animal proteins. In addition, livestock farming is an important source of income with the sale of animal products such as milk, eggs or wool. Finally, it provides manure, traction services, insurance functions and social status [1]; [2]. Thus, most rural households in Africa combine agriculture with livestock production.

In Burkina Faso, the livestock sector is one of the main sources of rural households' income. It contributes for improving their living standard and consequently to poverty reduction of rural population. Even if this contribution to household income varies significantly depending on the region [3]; [4]. Livestock production

remains the primary source of access to basic social services for 38.8% of the population living in rural area. The domestic ruminant stock of the country was estimated to 9.8 M cattle and 24,5 million (M) of small ruminants [5]. The number of livestock is increasing, especially that of goats, which has grown from 10.64 M in 2005 to 13.89 M heads in 2014 [5]. Goats are one of the most important sources of meat in rural areas where it is not common to slaughter cattle during social and ritual events [6].

In spite of all these advantages, livestock production is facing food deficit due to the reduction of rangelands resulting to demographic pressure, extensive cropping, bush fires, uncontrolled exploitation and the erratic of rainfall. In addition, the mode of management practiced, essentially based on the exploitation of natural pasture, results in gastrointestinal parasitosis, which is a major constraint to the development of small ruminant productions [7]. Losses induced by digestive pathologies result in mortality and reduced performance [8]. The poor management of crop residues, the high cost of Agro-Industrial By-products and conventional anthelmintics limit livestock production [9]; [10]. All these factors impact negatively animal production systems, which requires an improvement.

Regarding these food and health constraints, the intensification of animal production and the use of browse species with anthelmintic properties are proving to be an alternative to address feed shortage and gastrointestinal parasites. Many authors have already mentioned the relevance of tree fodder and their rational use in animal feed to address physical and economical inaccessibility of agro-industrial by-products [11]; [12]; [13] and [14]. Tree fodder are a significant source of nitrogen during the dry season, when this element is the main limiting factor for animal production [15]. Also, improving goat production must necessarily involve optimizing the use of ligneous fodder, which are an appreciable source of locally available forage and have a significant nitrogen content during the dry season ([9]; [15]. Among browse species, *Piliostigma thonningii* is one of those which pods are used in ruminant nutrition [16] and has anthelmintic properties [17]. Feeding tree fodder, as supplement or as a sole feed, generally results in improved production and health of ruminant animals [18]. The objective of this experiment was therefore to evaluate the effects of feeding diets including pods of *Piliostigma thonningii* on growth rate and gastrointestinal parasites in goats in the sub-humid zone of Burkina Faso, for intensive goat production.

II. MATERIALS AND METHODS

2.1. Study area

The experiment was conducted on farm, in Kiri, a village located at 15 km of Bobo-Dioulasso the second biggest town of Burkina Faso, at about W 4°19' longitude and N 11°19' latitude. The mean altitude is roughly 420 m above sea level. Soils of the study area are mostly hydromorphic on ancient cuirass and are therefore an asset for agricultural activities [19]. The average temperature is 27°C with minima of 20°C in December and maxima of 35°C in April. Compared to the other parts of the country rainfall at Kiri even erratic is relatively abundant. It varies between 900 and 1200 mm per year [19] with an average of 995.15 mm. Relative humidity varies between 21% (January to February) and 82% (August).

2.2. Experimental animals, feeds and management

The animals used were growing males and females of the West African dwarf goats breed, 6 months of age and with a mean initial body weight (bw) of 10.50 kg (sd=0.69), bought from farmers around the research station. Before starting of the experiment, animals were vaccinated against pasteurellosis, trypanosomosis and foot and plague of small ruminants.

The feeds used were crushed pods of *Piliostigma thonningii*; rice straw, maize bran, cotton seed cake and molasses. Cotton seed cake was used in the control group because it is the most available protein supplement in the region. The pods were collected manually from the trees from December to March 2018 and the other feed were purchased from local factories. The pods were sun dried and crushed. Rice straw was hand chopped into pieces of approximately 10 cm before being treated with molasse. The treatment of the rice straw consisted in spreading, and spraying with diluted molasses using a sprayer. The dilution was 250 ml of water per 600 g of chopped rice straw following [20]. The bamboo charcoal is obtained by pyrolysis, i.e. by complete carbonization of traditional woody materials then crushed.

The animals were housed in separated individual pens (1.5 m x 1.5 m) and were fed on cotton seed cake or *Piliostigma thonningii* at 07:00 h and 14:00 h, and on rice straw and maize bran at 10:00 h and 16:00 h. The animals had free access to water and a commercial mineral lick block containing 6% P, 12% Ca, 63% NaCl, 2% Mg, 15% cement as a binding agent, and Fe (2 g/kg), Cu (1.5 g/kg), Mg (0.75 g/kg), Co (0.03 g/kg), Zn (0.9 g/kg) and I (0.1 g/kg).

2.3. Experimental design

A total of 12 adult West African dwarf goats were used for the experiment. They were divided into three batches of 4

animals each. Three treatments (diets) were allocated to the animals. They were fed on the same amount of maize bran and hay from rice straw (Table 1). The treatments were three supplements: cottonseed cake (CSKD), pods of *Piliostigma thonningii* without bamboo treatment (PTD), and pods of *Piliostigma thonningii* with bamboo treatment (PTCD). The molasses-treated rice straw and maize bran were the staple food. Cottonseed cake and pods were used as feed supplements. The addition of bamboo charcoal was done to explore its effects on the improvement of the digestibility. It was incorporated at a dose of 1g/kg PV of the animal. Water and lick stone were administered ad libitum.

Table 1. Centesimal composition of the diet (%)

Feed ingredients	**Treatment diets**		
	PTD	**PTCD**	**CSKD (control)**
Molasses	5	5	5
Rice straw	20	20	20
Cotton seed cake	-	-	40
Piliostgima thonningii	40	40	-
Maize bran	35	35	35
Bamboo charcoal (g/kg PV)		1	
Mineral lick block		*ad libitum*	
Total	100	100	100

PTD= Piliostigma thonningii Diet; PTCD= Piliostigma thonningii and Charcoal Diet ; CSKD= Cotton seed Cake Diet

2.4. Measurements and chemical analysis

The animals were weighed at the beginning of the experiment and at seven days intervals, always in the morning (06:00 h) before offering the feed, using a 50±0,01 kg electronic scale. Data were used to calculate initial average weights (IW), final average weights (FW), average daily gain (ADG) and feed consumption index (CI). The Average Daily Gain (ADG) obtained according to the formula: ADG (g) = (FW-IW) / (ND), where IW= initial weight, FW= final weight and ND= duration in days. The Consumption Index (CI): was calculated according to the formula: CI= DFC (g)/ADG(g), with DFC = daily food consumption obtained by the difference between the amount of food distributed and the refusal and ADG defined above. The amounts of feeds offered were measured daily and representative samples were taken every week and pooled to monthly samples. Sub-samples of the monthly samples of feed were taken for chemical analysis. The faecal samples were also collected every month directly from the rectum of each animal. Each sample was identified and kept in a cooler containing cooling blocks until conducting the analysis. The parasite EPG (eggs per g faeces) were determined using the modified technique of McMaster [21]. Eggs of nematodes, cestodes and oocystes of coccidia were identified and counted according to [22]. For the survey, sixty-one (61) farmers were interviewed using a semi-structured question guide with the focus to collect information on the availability and the use of patterns of *Piliostigma thonningii*. The sub-samples of feeds offered were analyzed for DM, CP, OM and Ash. The DM (967.03), CP (988.05), and Ash (942.05) were analyzed according to the standard methods of AOAC [23]. The metabolic energy (ME) of the feeds was estimated using: ME (MJ)=0.82 x 19.3 x DOM (kg) derived from the equations given by Devendra and McLeroy (1982): DE (MJ)=19.3 x DOM (kg) and ME (MJ)=0.82 x DE (MJ).

2.5. Statistical analysis

The EXCEL 2013 spreadsheet was used to develop the database and XLSTAT software Version 2015 .5. 01.

22537 was used for statistical analysis of data. The EPG data were submitted logarithmic transformation (log10 x + 1) to approximate normal distribution. For data from trial and EPG recording, the Fisher's test (LSD) was used to separate the averages where analysis of variance reveals significant differences between treatments at the threshold of probability p<5%. For the survey data, a descriptive analysis was conducted using of the SPSS software to determine the percentages.

III. RESULTS

3.1. Measurements and chemical analysis

The nutrient contents of the feeds used are presented in the Table 2.

Table 2. Chemical composition of the experimental feeds

	DM (%)	g/kg DM		
		CP	OM	Ash
Cotton seed cake	93,15	373,7	922,3	77,7
Pods of *P. thonningii*	94,49	86,9	915,0	85,0
Maize bran	94,95	126,0	911,7	88,3
Rice straw	92,44	59,0	831,0	169,0
Molasses rice straw	90,42	50,9	837,9	162,1
Molasse	77,23	35,4	877,0	123,0

DM= Dry Matter ; CP= Crude Protein; OM= Organic matter

The CP contents of the feeds used differed (Table 2). Cotton seed cake had the highest CP content (373,7 g/kg DM) and the molasse, the lowest (35,4 g/kg DM). The CP contents of the pods of *Piliostigma thonningii* (86,9 g/kg DM) was higher than that of rice straw.

3.2. Feed intake, growth performance and feed conversion ratio

Feed intake, goats Daily Weight Gain (DWG), absolute weight gain and Feed Conversion Ratio (FCR) are shown in Table 3 below.

Table 3. Initial and final weight, average daily gain and feed conversion ratio (LS means and SE)

	Diets		
	PTD	PTCD	CSKD
Total DM (g/day)	304,08±72,93a	309,6±61,1a	371,75±92,63b
Total DM (g/kg $W^{0,75}$)	52,13±66,10	53,08±55,98	63,73±83,96
Initial weight (kg)	9,11±0,52	9,62±0,64	10,56±1,62
Final weight (kg)	10,5±1,14	11,49±1	13,03±1,91
Live weight gain (kg)	1,38±0,77a	1,87±0,52a	2,43±0,78b
Average daily gain (g)	16,39±4,10a	24,01± 5,79a	31,39±5,80 b
Feed conversion ratio			
kg DM/kg BWG	18,06±9,32a	10,83±2,43b	10,3±4b

a,b,c Means within row with different superscripts are significantly different (P<0.05).

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DM intake ranged from 304.08±72.93 g to 371.75±92.63 g/animal/d. The highest value 371.75±92.63 g/d was recorded from animals fed control diet and was significantly different from that of animals fed diets with *Piliostigma thonningii*. The animals offered the control diet gained significantly more weight and had a higher ADG (2.43 kg and 31.39 g/day, respectively) than the animals fed pods of *Piliostigma thonningii*. (Table 3). Among the *Piliostigma thonningii* diets, the ADG was not significantly different ($p>0.05$). The Average Daily Gain (ADG), overall, ranged from 8.01 to 41.73 g/day/animal. The FCR expressed as kg DM per kg BWG, ranged from 10.3 to 18.06. No significant difference was obtained between the diet with *Piliostigma thonningii* treated with bamboo charcoal and the control diet.

3.3. Availability and use of *Piliostigma thonningii* pods

Figure 1 provides information on the availability of *Piliostigma thonningii* pods. The results show that 57% of the farmers confirmed that the pods are fairly available. The cumulative citation frequency of pod availability was 77%.

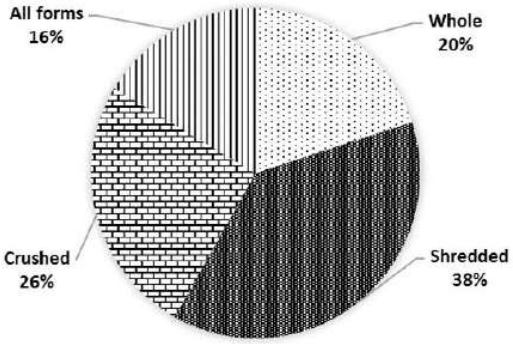

Fig.1: frequency of citation of the availability of Piliostigma thonningii pods.

Figure 2 shows the frequency of citation of the forms in which *Piliostigma thonningii* pods are used by farmers.

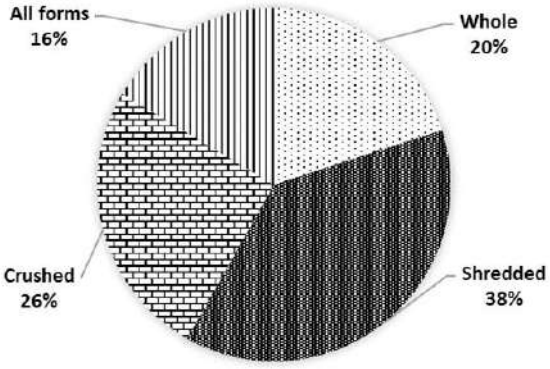

Fig.2: Frequency of citation of Piliostigma thonningii pod usage patterns

The frequency of citation of the use of pods in crushed form is the highest (38,18%). That of the whole pods was the lowest (20%).

The Figure 3 shows the evolution of the EPG of the strongyles.

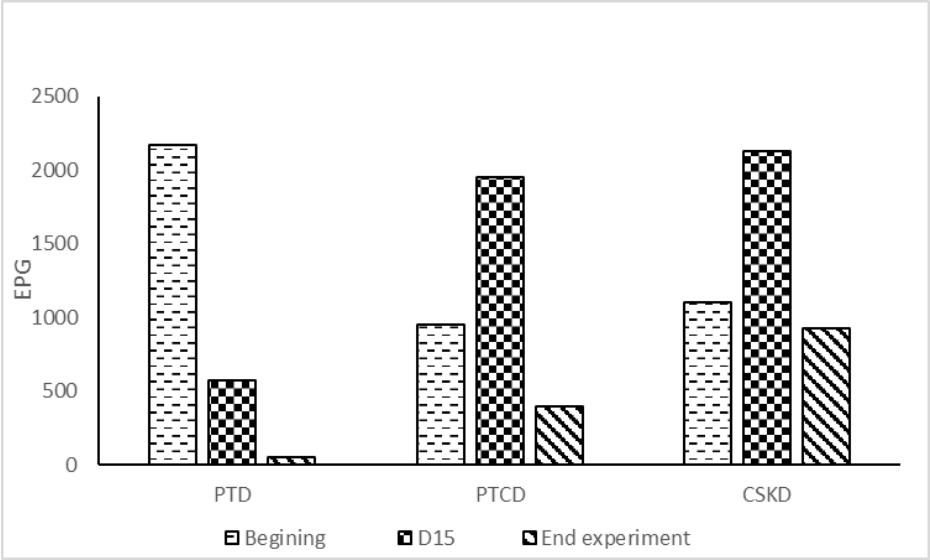

Fig.3: Level and evolution of EPG of strongyles

An overall slight increase in was observed between the period of the beginning of the experiment and the end of the adaptation period, followed by a significant decrease at the end of the trial (78 days after).

The level and evolution of the EPG of the coccidia are presented in table 4.

Table 4. level and evolution of the EPG of the coccidia

Treatment diets	Mean EPG		
	D0	D15	D78
PTD	525±1050 a	129255±12503b	625±299c
PTCD	400±735a	17225±20090b	1025±741b
CSKD	0a	21625±27010b	975±1031b

a,b,c Means within rows with different superscripts are significantly different (P<0.05);

D0= Day 0 (beginning); D15= 15th Day , D78= 78th Day

The results show an increase in EPGs of coccidia between the beginning of the experiment and 15 days after. Between the 15th day and the end of the experiment, a decrease in EPGs has been noted. However, between the beginning and the end, a slight increase was observed.

IV. DISCUSSION

4.1. Chemical composition

Apart from cotton seed cake, the CP contents of the ingredients analyzed are relatively low, compared to those reported by [16] and [24] who found values ranged between 10.53 and 9.51% DM for the pods of and *Piliostigma thonningii* and *Piliostigma reticulatum*, respectively. Values founded in the present investigation are higher than those obtained by [25] with *Pilisotigma. reticulatum* pods (5.06% MS). These variations would be related to the species, climate, soil types, state of maturity and shelf life. The CP content of the corn bran used (12.60% of DM) is lower than the levels found by [26] and [27] which obtained values of 13.28 and 17.49% of DM, respectively. However, the CP content of maize bran recorded was higher than that of [28]. Moreover, it is above the limiting level (6 to 8% of DM) below which appetite and forage intake are depressed [29].

4.2. Feed intake, growth performance and feed conversion ratio

The results showed variations between the mean intake values obtained from the three groups of animals. The high value obtained with the animals fed on control ration, could be attributed to the high CP content of the cotton seed cake contained in the diet (CSKD). The slight increase in feed intake value even non-significant obtained with animals fed on ration incorporating *Piliostigma thonningii* and bamboo charcoal could be related to the action of the charcoal. The charcoal would be able to complex the secondary compounds and thus increase the digestibility of the constituents. This result could be due to an improvement in the digestibility of the ration having incorporated bamboo charcoal. Values found herein are similar to those of [25] with substitution of cotton seed cake by *Piliostigma reticulatum* pods and recorded 390.83; 386.35; 384.25g and 362.48g/d with rations incorporating the crushed pods at 20%, 50%, 30% and 0%, respectively. However, our results are higher than 278.5 g; 125.6 g/day, obtained respectively with sheeps and goats by [30]. Our values are lower than 449.5 g/d and 549.2 g/d reported by [31] who worked on animals subjected to rations incorporating 20% *Piliostigma reticulatum* pods. According to [32], an increase in the level of nitrogen through supplementation in a ration stimulates rumen function and consequently leads to an increase in the level of intake in sheep. Another important factor such as protein quality (solubility) could explain the higher intake recorded with the control group.

The ADG of animals fed diets incorporating *Piliostigma thonningii* pods do not differ significantly, but are significantly lower than those of the control group. The results obtained are in line with those of [25] with the partial substitution of crushed *Piliostigma reticulatum*

pods and reported ADG of 13.20 to 22.06 g/d with Maradi goats and that of 25g/d reported by [24] with Djallonké sheep led to pasture and receiving as a supplement of the *Piliostigma reticulatum* pods associated with maize bran. However, they are lower than those obtained by [31] using Maradi goats fed with densified feed (50.9 to 78.3g/d). Similarly, they are also lower than those obtained by [16] who, with growing sheep, obtained ADG values of 78 g, 61 g and 56 g respectively for animals supplemented with Mucuna meal, *Piliostigma thonningii* pods and cotton cake. These differences could be related to animal species, initial average weight and age of the animals, the study environment and the composition of the rations used.

The weight gain observed with rations incorporating *Piliostigma thonningii* pods is explained by the fact that the condensed tannins would allow an increase in the flow of assimilable proteins and animate acids in the intestine by protecting dietary proteins from degradation in the rumen. According to Min and [33], at moderate doses (20 to 40 g/kg dry matter), condensed tannins bind to proteins, prevent their degradation in the rumen and thus increase the intake of amino acids in the small intestine, thus improving the nutritional status of the animal. In addition, *Piliostigma thonningii* pods with a CP content of 8.99% DM would allow a better digestion or even a high ingestion. This level of weight gain recorded with rations incorporating *Piliostigma thonningii* pods is online with [16].

The Feed Conversion Ratio (FCR) translates the efficiency of the transformation of the feed into meat and therefore into weight gain. The best FCR was obtained with animals in the CSKD group (10.3±4) and was significantly higher than those in PTD and PTCD groups ($p<0.05$). The values obtained with our work are comparable to those of [25] who recorded 12.76±3.72; 8.42±3.89; 7.65±6.23 and 4.17±4.91, with Maradi goats fed diets incorporating different proportions of *Piliostigma reticulatum* pods. Our values are similar to those of [31] who obtained values ranging from 7.1 to 10.6.

4.3. Availability and use of *Piliostigma thonningii* pods

The results of our study showed that *Piliostigma thonningii* pods are available. Several factors can influence this availability: demand, the seasonal production period [28] and infestation [16]. Indeed, the consumption of tree fodder becomes more important when herbaceous forage becomes scarce and especially low in nitrogen, hence its higher proportion in the hot dry season found by [34]. The availability of *Piliostigma thonningii* pods found during the surveys could indicate the renewed interest in tree fodder. This would also be an indicator of the difficulties in accessing available herbaceous pasture or their drastic reduction due to several factors mainly anthropogenic. The surveys have shown that pods are most commonly used in crushed form. Grinding and crushing are practiced to reduce the size of the pods to make them easier to grip, because the pods contain a certain lignin content which makes the pod rigid and therefore reduces its ingestion. The low use of whole pods would be due to the extensive nature of the farms. The relatively low level of treatment reveals the extensive character of these farms because in these types of breeding, complementation is insignificant or absent and the methods which make it possible to increase the ingestion are not practiced.

4.4. Parasites infestation

Strongyles eggs decreased significantly towards the end of the experiment (78 days after the start of the experiment) for all animals, but at different rates. The lowest levels at the end of the treatment were observed with animals on the PTD ration. In our study goats were not bleached prior to the trial and parasite species were not isolated and identified. The decrease in EPG could be due to the direct or indirect anthelmintic effect of pods on the strongyles. The direct effect could be explained by the action of tannins on the cuticle of the strongyles or by disrupting nutrition and reproduction. The indirect effect by the improvement of the immune system due to the protein contribution [35]. In the context of this study, if we refer to the interpretation grid or severity scales of [36] based on OPGs, we could say that at the beginning, the infestations were high (> 1,000 OPGs), average (500 - 1,000 OPGs) for lot 1, lot 2 and lot 3 respectively, although the animals were not de-infested at the beginning of the trial. At the end of the trial, lot 1, lot 2 and lot 3 showed low and medium infestation respectively.

The lowest value coccidia EPG was found in the faeces of goats fed with PTD without charcoal treatment. The difference between the number of coccidia oocysts in the faeces of goats fed on diets incorporating *Piliostigma thonningii* pods, and that of goats fed on concentrate support the presence of anti-nutritional factors in tree pods able to control the development of gastrointestinal parasites [37]. This could be explained by the fact that the tree fodder used has anthelmintic properties. It appears that the incorporation of the pods allows to reduce the level of excretion of coccidia eggs. The presence of gastrointestinal parasites in animals can result in a requirement for extra nutrients to repair or replace damaged tissue and express immunity. The significantly lower number of coccidian oocysts in goats fed diet incorporating *Piliostigma thonningii* pods without charcoal treatment could be due to some feed characteristics of pods, regarding the low level of CP content of the diet compare to that of others. The

EPG obtained in our experiment, were similar to 1123±467 reported by [38] in sheep.

V. CONCLUSION

Finaly, the results indicate that *Piliostigma thonningii* pods have a potential value and can be adequately used to address the negative effects of feed scarcity on the performance of goats. It would appear as an alternative that could help in parasite control and animal feeding; but still needs further and longer investigation. The results obtained should make it possible to create a craze for its use by breeders in view of the ever-increasing difficulties in animal feeding. Livestock farmers can easily protect wild trees and collect pods on the fields to prevent their disappearance and to increase the availability of feed for animals. The results will enable the technical and research services to consider strategies for the management of locally available tree fodder resources for sustainable exploitation. Thus, we can suggest to farmers the incorporation of *Piliostigma thonningii* pods in animal diets as a protein supplement. Specific treatments such as grinding and addition of bamboo charcoal can improve their nutritional value.

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Phenotypical characterization of cassava (*Manihot Esculenta* Crantz) accessions in Cameroon's mono-modal rainforest zone

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Abstract— *One hundred and five (105) accessions of cassava collected in the South West and Littoral Cameroon regions were evaluated on a morphological and epidemiological basis. The objective was to study the phenotypic diversity of accessions and its structure based on variables selected among the cassava (Manihot esculentaCrantz) descriptors. Descriptive analysis showed significant phenotypic differences for the traits. Principal component analysis confirmed this important morphological variability. The hierarchical ascending classification made it possible to structure the accessions into 6 and 4 groups. In Ekona, group I consists of accessions with high incidence (IN = 54.03 ± 20.41), group II contains high height (H = 2.01 ± 0.41m), group IV consists of accessions with high severity (SE = 40.44 ± 11.68), petiole length (LP = 39.66 ± 0.03), lobe length (LL = 19.00 ± 0.2 cm) and lobe number (NL = 7.66 ± 0.2).In Njombé, group I consists of accessions with a high incidence (IN = 59.31 ± 20.38), lobe length (LL = 18.08 ± 1.00 cm) and considerable lobe number (NL = 7.44 ± 0.2). These groups offer a wide range of broodstock choices for the creation of improved cassava varieties with interesting potential and adapted to these agro-ecological zones.*

Keywords— ***Manihot esculenta, Accession, Cameroon, cassava, morphological diversity.***

I. INTRODUCTION

Cameroon is a country whose economy remains heavily dependent on its agricultural sector, which employs nearly 68% of the working population and accounts for a significant share of its GDP and about 15% of public budget resources. Cassava (*Manihot esculenta* Crantz) is a staple food crop and contributes to food security in Cameroon. World cassava production is estimated at more than 268.28 Mt. In Cameroon, national production is estimated at 5.83Mt [1]. Its importance is attested not only by the enthusiasm of the population for its cultivation but also by the diversity of its use in both food and industry. Local and improved varieties of cassava are cultivated in all the agro-ecological zones of the country as combined crops or often as pure crops, depending on the objectives set by the farmer [2]. The ease of cultivation of this plant, its adaptability to different soil types and its tolerance to drought have contributed to the expansion of its cultivation [3]. This plant is cultivated for its starchy tubers, which provide food for more than 500

million people worldwide, particularly in tropical countries [4]. World production is estimated at more than 268.28 Mt. In Cameroon, national production is estimated at 5.83Mt [1]. Despite these somewhat laudatory figures, much remains to be done due to the fact that the phenotypic diversity of the cassava varieties grown in Cameroon is poorly known and therefore little valued on the one hand, and on the other hand due to the susceptibility of cassava to diseases and pests. It is very important to have a perfect knowledge of the phenotypic diversity of cassava accessions in some production areas in Cameroon.

The exploitation of this genetic diversity is of particular importance for maintaining and improving the productivity of this species in developing crops. Numerous procedures have been developed to quantify and analyses the existing genetic diversity. Any varietal improvement programme requires more and more information about the structuring and spatial distribution of the diversity to be conserved. This goes through the process of characterization in order to determine the real varietal potential. Phenotypic identification has already been used in genotypic and taxonomic classification [5]. In the absence of molecular markers, the use of morphological descriptors remains the most widely used method to study variety diversity. Farmers' varieties, the basis of ex situ collections, are recognized as reservoirs of genes useful for the improvement of the plants used [6]. A good knowledge of the forms of this traditional varietal diversity is necessary because it constitutes the reservoir of genetic variability. The analysis of morphological descriptors makes it possible to reveal the diversity as it is perceived and selected by local farmers and the main stakeholders in the management of varietal diversity [7]. It is the most practical approach for phenotypic differentiation provided it is combined with a statistical tool that estimates the variation related to the experimental environment [8]. Morphological descriptors have made it possible to describe and classify thousands of forms in maize ([9]; [10]; [11]; [12]; [13]. The existence of significant variability for most agro-morphological traits in Cameroon has been demonstrated on several crops such as *Dioscoreadumetorum* [14]. The study of the diversity of these genetic resources is essential to create new varieties with interesting characteristics, adapted to climatic variations and resistant to diseases. The objective of this study is to assess the morphological diversity of 105 cassava accessions originating from several localities in the South-West and Littoral regions of Cameroon. The production of roots and tubers, especially cassava, is hampered by various constraints. In Cameroon, diseases and pests are among the main constraints to production. The most common diseases of cassava are: cassava mosaic, bacterial blight, anthracnose, bud necrosis and root rot, brown streak disease. In the south-western regions (Ekona) people tend to attribute less importance to cassava cultivation due to the unsuitability of the varieties present to the climatic conditions.

II. MATERIEL AND METHODS

2.1. Study sites

The study took place in 2014/2015 and 2015/2016 in the stations of the Agricultural Research Institutes for Development (IRAD) of Njombé and Ekona respectively and had the following characteristics: Ekona is 16 km from Buea and 10 km from Muyuka on the No8 Buea-Kumba road axis in Cameroon with an altitude of 400 m and covers an area of 55,000 km2. The IRAD station is located at 06° 30' 00 latitude N and 08° 30' 00 longitude East.

Njombé is located 80 km from Douala, the economic capital of Cameroon with an altitude of 80 m; latitude 4° 35' North and 9° 39' East. The climate is equatorial-Guinean, with relative humidity between 32 and 100 percent and an average of 72 percent. The average rainfall is 2086 mm with approximately 161 rainy days per year. Temperatures vary between 22.1 and 32.2°C with an average of 27.1°C. The relief is a plain, the slope is between 0 and 16%, the soil is volcanic, brown and eutrophic.

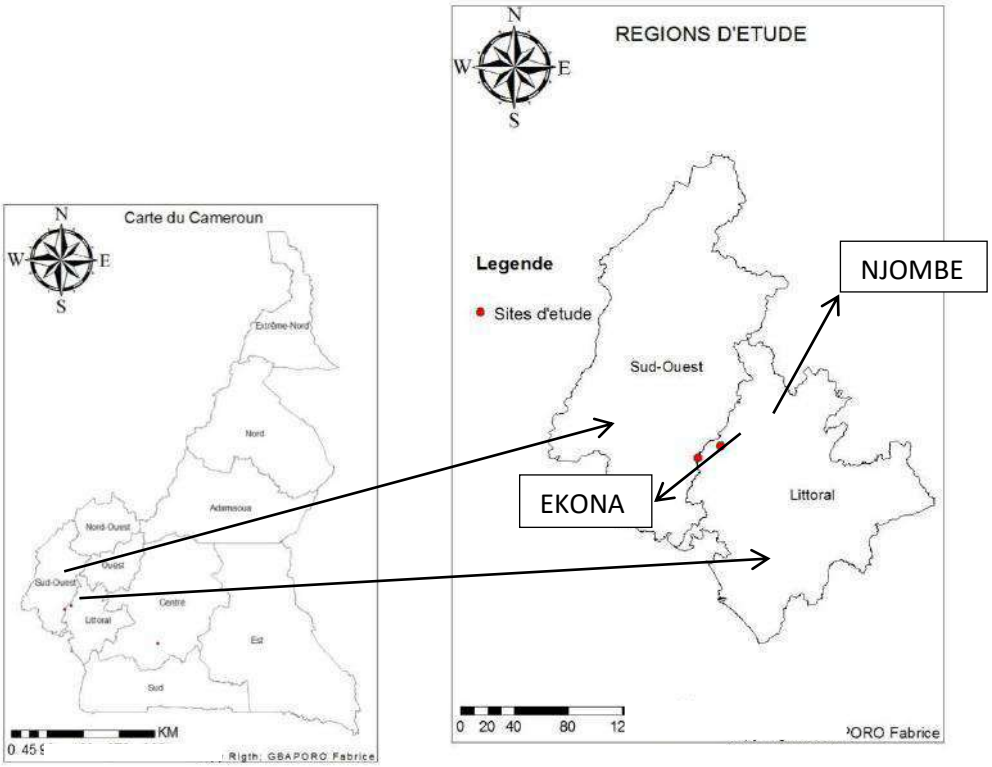

Fig. 1. Sites of study

2.2. Experimental design

The experimental design was non-statistical and consisted of a block in which the varieties were arranged in rows with 30 plants per row per variety. The distance between lines was 1 m and the distance between bunches in the same line was 0.50 m. The sowing was carried out with a 20 cm cutting with 6 knots per bunch. Regular weeding was carried out during the vegetative phase of the crop.

2.3. Data collection

Measurements were made on all 30 plants per variety. Five characteristics selected from the descriptors of cassava (Manusset) were retained. These were plant height (H), number of lobes (NL), length of lobe (LL), length of petiole (LP), color of petiole (CP), number of branches (NR) and two epidermal indices namely incidence (IN) and severity (SE).

2.4. Statistical analysis

Multivariate analysis : The principal component analyses were done using SPAD V55 software. The phylogenetic tree was constructed using the R software and the Ward D method was used to calculate the Euclidean distance. The SAS V9 software for the ANONA.

III. RESULTS AND DISCUSSION

3.1. Results

3.1.1. Descriptive analysis of morphological characteristics

Descriptive statistics revealed a wide variation in morphological traits (LL, LP, RA, LL, NL) among cassava varieties.

The mean, minimum, maximum values of the morphological variables are grouped in Table I and II. In Ekona, large differences between minima and maxima were observed for important morphological traits such as petiole length (LP), lobe length (LL), branching (RA) and very large differences between maxima and minima of the epidemiological variables. On average, accessions have a severity of 36.18 ± 16.1; an incidence of 66.12 ± 3.02; a petiole length of 32.42 ± 8.59 and a lobe length of 18.07± 2.40 (Table 1).

At Njombé, large differences are also observed between minima and maxima for morphological traits such as lobe number (NL), lobe length (LL), petiole length (LP) and very large differences between minima and maxima in epidermal indices. On average, a severity of 29.59 ± 17.44; incidence of 49.81 ± 11.61; petiole length of 20.32 ± 6.94; LL (15.44 ± 3.16) (Table 2).

Table 1. Minimum, maximum, mean values of morphological characteristics and two Ekona epidermal indices.

Variable wording	Minimum	Maximum	Mean± standard deviation
SE	0.000	60.000	36.18 ± 16.1
IN	0.000	100.000	66.12 ±3.02
LP	19,000	48.000	32.4 ± 8.59
LL	14.000	23.000	18.07±2.4
NL	6.000	9.000	7.47 ± 0.73
RA	0.000	4.000	2.73 ± 0.78
H	0.800	2.42	1.71 ±0.36

SE: severity; IN: incidence; LP: petiole length; LL: lobe length; NL: number of lobes; RA: branching; H: height.

Table 2. Minimum, maximum, mean values of morphological characteristics and two epidermal indices of Njombé.

Variable wording	Minimum	Maximum	Mean± standard deviation
SE	0.000	80.000	29.59 ± 17.44
IN	0.000	100.000	49.81 ±5.02
LP	3.000	37.000	20.32 ± 6.94
LL	9.000	26.000	15.44±3.16
NL	3.000	9.000	6.01 ± 1.52
RA	0.000	4.000	2.51 ± 1.52
H	1.100	3.400	2.26 ±0.58

SE: severity; IN: incidence; LP: petiole length; LL: lobe length; NL: number of lobes; RA: branching; H: height.

3

.1.2. Structuring morphological diversity

The correlation matrix of the different characteristics studied are reported in Tables 3 and 4. Significant correlations (≥ 0.50) were obtained between several pairs of variables. The correlation coefficient index greater than 6 for specific pairs of traits indicates that there is a statistically acceptable factorial solution. We observe positive and perfect correlations between severity and incidence (r = 0.70), between lobe length and petiole length (r = 0.80) and between petiole length and number of lobes (r = 0.62) (Table 3). Similarly, in Ekona correlations were observed between height and severity (r = 0.79), incidence and severity (r = 0.79), height and incidence (r = 0.68) and length and number of lobes (r = 0.62) (Table 4)

Table 3. Correlation matrix between the variables measured in the Njombé collection

	SE	IN	H	LL	LP	RA	NL
SE	1,00						
IN	**0,70***	1,00					
H	0,09	-0,09	1,00				
LL	0,14	0,26	0,10	1,00			
LP	0,26	0,28	0,17	**0,80***	1,00		
RA	-0,15	-0,06	-0,13	-0,12	-0,22	1,00	
NL	0,22	0,20	0,14	0,53	**0,62***	-0,24	1,00

SE: severity; IN: incidence; LP: petiole length; LL: lobe length; NL: number of lobes; RA: branching; H: height; * significant correlation

Table 4. Correlation matrix between the variables measured in the Ekonacollection

	SE	IN	H	LP	LL	NL	RA
SE	1,00						
IN	**0,79***	1,00					
H	**0,79***	**0,68***	1,00				
LP	0,41	0,28	0,51	1,00			
LL	0,01	-0,04	-0,05	0,58	1,00		
NL	0,45	0,32	0,26	**0,62***	0,35	1,00	
RA	0,31	0,55	0,43	0,06	-0,09	-0,06	1,00

SE: severity; IN: incidence; LP: petiole length; LL: lobe length; NL: number of lobes; RA: branching; H: height; * significant correlation

3.1.3. Principal Component Analysis

Tables 5 provide an estimate of the variability represented by each axis. Two (02) axes with an eigenvalue greater than 1 were obtained at Ekona and three (03) were obtained at Njombé. These axes explain 72.40% and 74.37% of the variance present in the variables in Ekona and Njombé respectively. The variance accumulation test confirms that these different axes are relevant and will be used to describe the total variability of the accessions.

In Ekona, the first axis describes 46.96% of the variation. This component is defined by severity (SE), incidence (IN), height (H) and petiole length (LP). The second component (axis 2) describes 25.44% of the variable. It is defined by petiole length and lobe length. All these variables are negatively correlated to the axes (Fig.2).

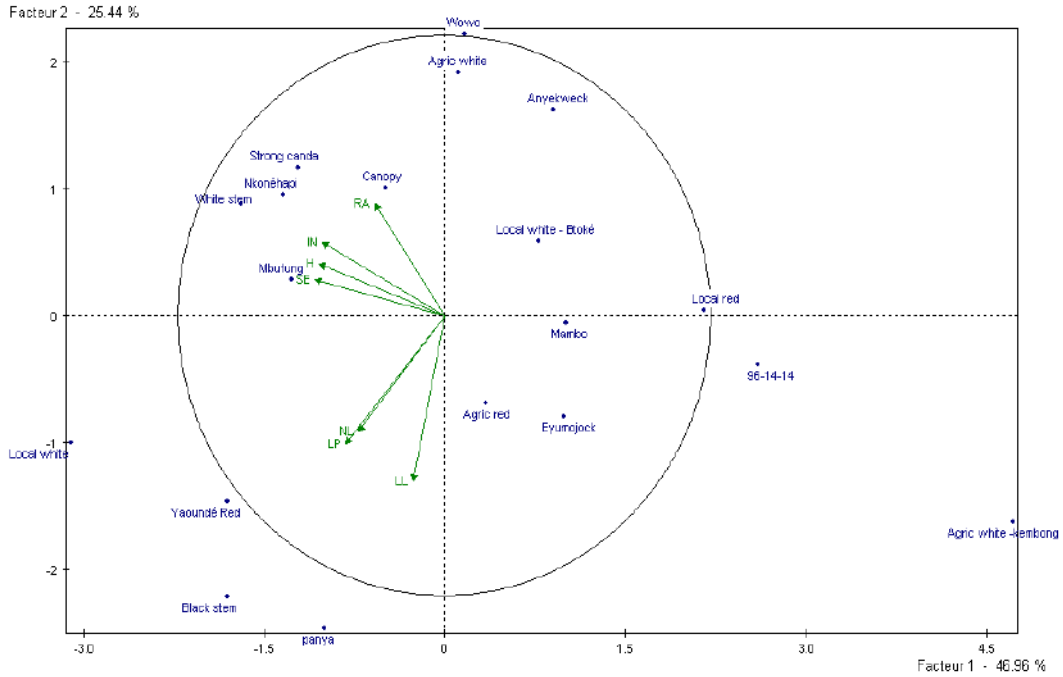

Fig.2. Distribution of variables on axes 1 and 2 of the main component

In Njombé, axis 1 describes 39.20 % of the total variable, it is defined by the number of lobe (NL), the length of the lobe (LL), the length of the petiole (LP). Axis 2 describes 20.19% of the total variable and is defined by severity (SE) and incidence (IN). The third component describes 14.97% of the variable and is defined by plant height (H) and number of branches (NR). All of these variables are negatively correlated to the axes except for the third component where branching is positively correlated to axis 3 (Fig. 3).

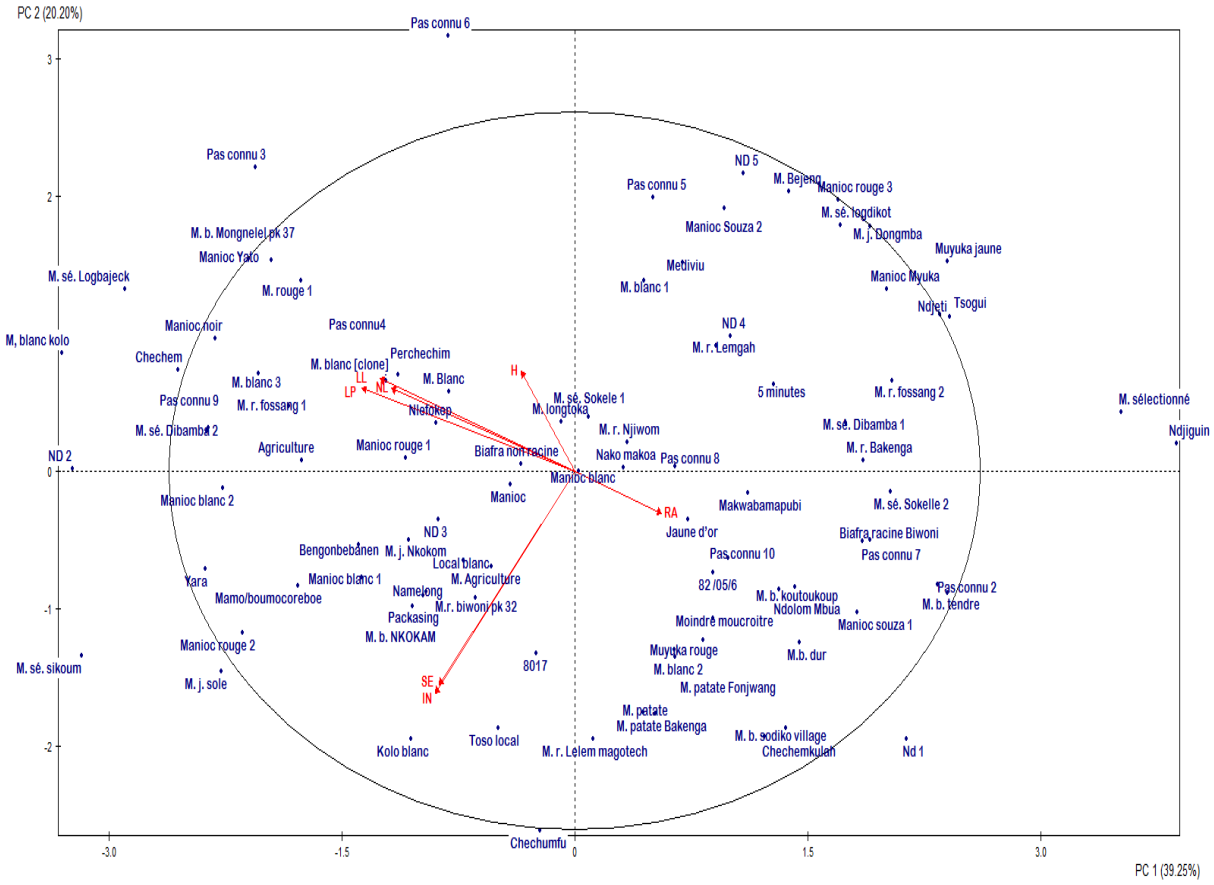

Fig.3. Distribution of variables on axes 1 and 2 of the main component

Table V. Own vectors and percentage of variation expressed by the axes from the characteristics analysed in Ekona and Njombé

Main components	**Ekona**		**Djombe**		
	Axe1	Axe 2	Axe 1	Axe 2	Axe 3
0wn variance	3.287	1.7807	2.7441	1.413	1.047
Total variance	46.96	25.44	39.2	20.19	14.97
Cumulative variance	46.96	72.4	39.2	59.4	74.37
SE	**-0.88***	0.17	-0.55	**-0.71***	-0.24
IN	**-0.84***	0.35	-0.57	**-0.73***	0.04
H	**-0.85***	0.25	-0.22	0.33	**-0.68***
LP	**-0.68***	**-0.61***	**-0.79***	0.31	-0.33
LL	-0.22	**-0.78***	**-0.87***	0.28	0.18
NL	-0.59	0.55	0.35	-0.14	**0.61***
NR	-0.47	0.53	**-0.75***	0.28	0.06

3.1.4. Diversity Analysis by Ascending Hierarchical Classification

The dendrograms obtained show a clear separation of accessions and structure the diversity into four (4) groups in Ekona and six (6) groups in njombé (Fig.1 and 2 respectively).

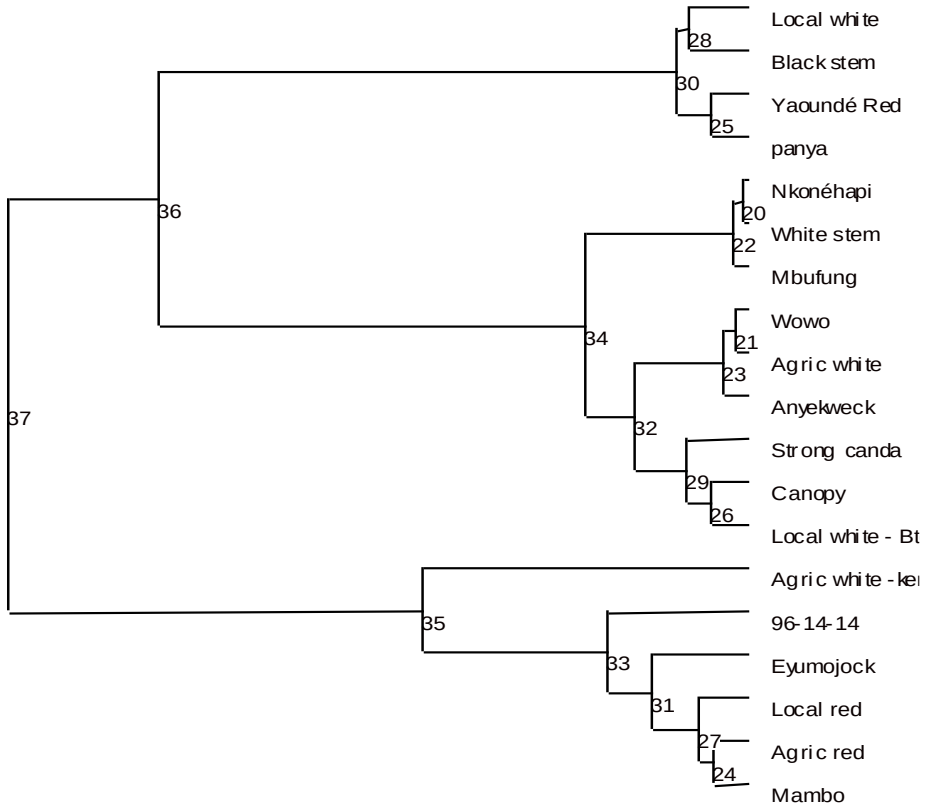

Fig.4.Ascending hierarchical classification (AHC) of the 19 cassava accessions according to the aggregation criterion of weighted averages

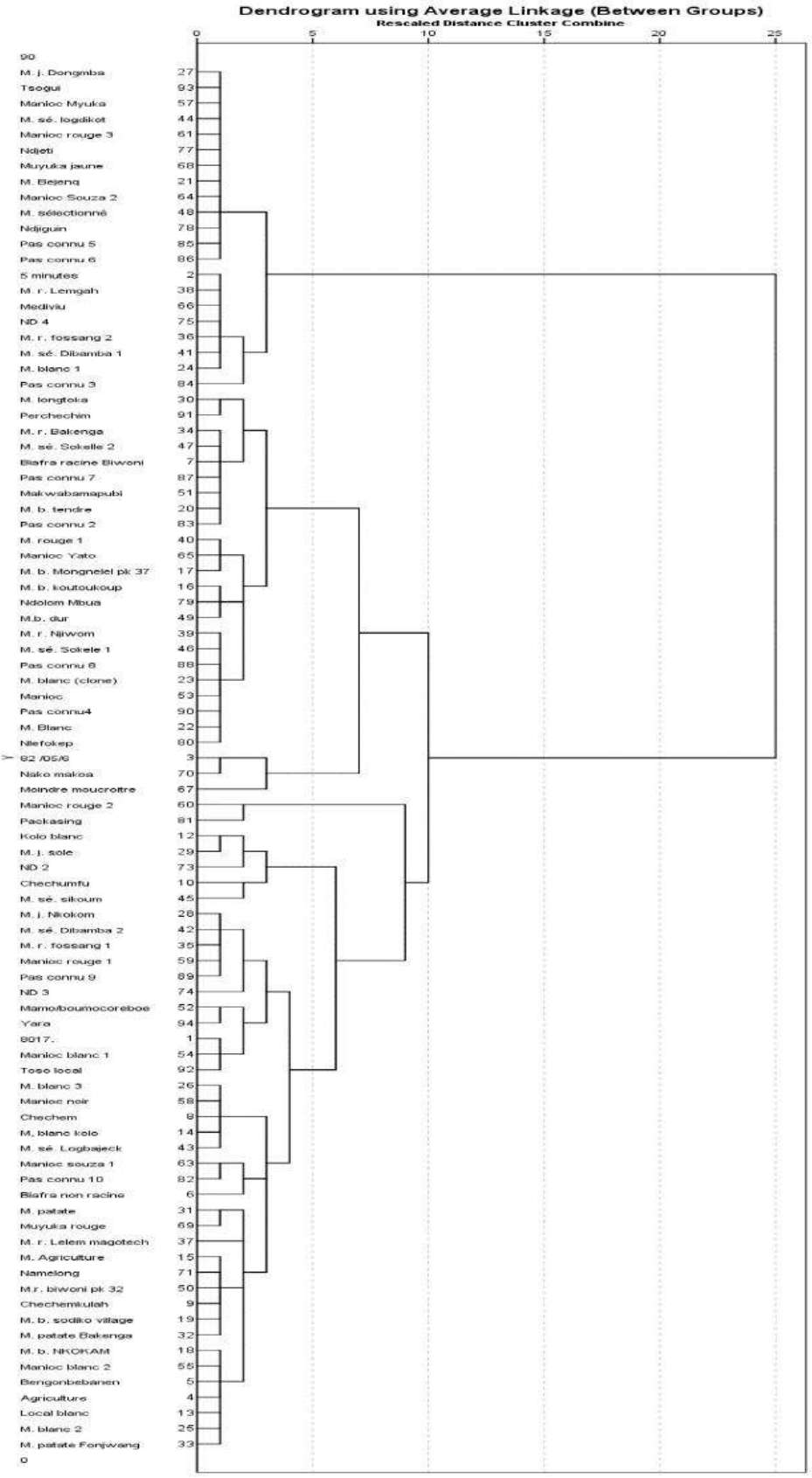

Fig.5: Ascending hierarchical classification (AHC) of the 96 cassava accessions according to the aggregation criterion of weighted averages

3.14.1. Cluster obtained from AHC

The family tree divides the varieties into several groups. In Ekona, group I consists of accessions with high incidence (IN = 54.03 ± 20.41), group II contains accessions with high height (H = 2.01 ± 0.41m), group IV consists of accessions with high severity (SE = 40.44 ± 11.68), petiole length (LP = 39.66 ± 0.03), lobe length (LL = 19.00 ± 0.2) and lobe number (NL = 7.66 ± 0.2). Whereas in Njombé, group I consists of accessions with a high incidence (IN = 59.31 ± 20.38), lobe length (LL = 18.08 ± 1.00) and considerable lobe number (NL = 7.44 ± 0.2). Group II consists of the significantly branched accessions (RA = 5,22 ± 0,2). Group V consists of the high height accessions (H = 2.28 ± 0.60) and Group VI consists of the high severity accessions (41.30 ± 12.53) (Tables 6 and 7).

Table 6: Main characteristics of the different groups formed by CAH at Ekona

Variables	GI	GII	GIII	GIV
SE	35.8 ± 12.63	00 ± 00	22 ± 5.7	40.44 ± 11.68
IN	54.03 ± 20.41	00 ± 00	20.03 ±6.17	49.84 ± 17.49
H	1.71± 0.34	2.01± 0.41	1.85 ± 0.37	1.70 ± 0.30
LP	32.27 ± 1.04	27.5 ± 0.06	21.2 ± 0.02	39.66 ± 0.03
LL	17.74 ± 0.4	16.5 ± 0.05	12.50 ± 0.01	19.00 ± 0.22
NL	7.45 ± 0.3	7.00 ± 0.01	6.5 ± 0.11	7.66 ± 0.2

Table 7: Main characteristics of the different groups formed by CAH at Njombe

Variables	GI	GII	GIII	GIV	GV	GVI
SE	32.91±9.89	24.41± 5.62	39.08±17.83	23.30±7.19	37.47±15.98	**41.30±12.53**
IN	**59.31±20.38**	20.58±6.73	21.65±8.85	37.65±10.99	57.66±22.01	42.38±11.1
H	2.26±0.52	1.98±0.33	2.01±0.50	2.15±0.25	**2.28±0.60**	2.20±0.58
LL	**18.08±1.00**	16.07±1.02	12.5±0.22	14.53±0.1	17.94±0.03	13.75±0.34
NL	**7.44±0.2**	6.55±0.01	5.5±0.04	4.70±0.01	6.4±0.15	6.5±0.01
RA	4.71±0,23	**5.22±0.2**	2.5±0.2	3.34±0.02	2.4±0.01	1.00±0.00

3.1.4.2. Number of varieties obtained per Clusters

The percentage of each group is high in group I (58.89%) followed by group IV (21.05%) and relatively low at around 10.52% for groups II and III of the Ekona varieties, while in Njombé 40.89% was recorded in group I followed by 24.41% and 25.58% for groups II and IV respectively. Small numbers were obtained in groups III, V and VI with values of 3.48%, 5.81% and 2.32% (Table 8).

Table VIII. Percentage distribution of genotype groups (clusters) according to sites

Ekona	GI	GII	GIII	GIV
Effective (%)	57.89	10.52	10.52	21.05

Njombé	GI	GII	GIII	GIV	GV	GVI
Effective (%)	40.69	24.41	3.48	25.58	5.81	2.32

IV. DISCUSSION

Principal Component Analysis (PCA) is a statistical method commonly used in population genetics to identify structure in the distribution of genetic variables, across geographic location and ethnic knowledge [15]. The aim is to assess the importance of each variable in the relationship to the total variable between genotypes. This method offers possibilities to exclude the less important traits in each study group and simultaneously retain the most salient traits to clarify diversity [16].

Knowledge of genetic variability is essential in varietal selection. Highlighting this genetic variability is the first essential step in the description of genetic resources [17].

In this study, lobe length, lobe number, branching, height, incidence and severity are the traits or variables.

The analysis of the agro-morphological diversity of local accessions of cassava grown in certain regions of Cameroon revealed significant differences between the traits analysed, indicating strong phenotypic heterogeneity among the 105 accessions. This morphological and epidermal diversity was structured into 04 groups in Ekona and 06 groups in Njombé.

These different groups taken separately have distinctive and similar characteristics such as plant height, number of lobes, severity and incidence. These results are similar to those of [18].Who, however, was working on maize accessions. This structuring of morphological diversity shows that in cassava, morphological differentiation is most often based on agronomic traits ([8]; [19].).

Peasant phenotypic selection based on perceptible traits could explain the contribution of these variables to structuring variability. According to [20]., vegetative characteristics such as plant height and number of branches are the main characteristics used by farmers to identify varieties. In addition to these characteristics, the epidemiological factor also plays an important role in structuring morphological diversity. The evaluation of the genetic diversity of the germplasm is not only necessary but also constitutes a prerequisite in the hybridization programme and helps to promote the efficiency of the genetic variables used ([20]; [21]. To promote the efficiency of a crop, it is important to obtain information on genetic diversity and kinship through correlations between the variables highlighted. This is because the assessment of genetic diversity is essential not only for crop improvement but also for stewardship and germplasm conservation. In this study, the focus on incidence and severity will now enable us to control varieties that are resistant and susceptible to the flagship cassava disease, which is the mosaic in the different zones investigated in Cameroon.

V. CONCLUSION

Preliminary results on the diversity and morphological structuring of cassava in the single-mode rainforest zone of Cameroon clearly show that there is a high phenotypic diversity within cassava cultivars, via the descriptors used. Several distinct groups have been identified and each group has clear traits of specificity for which it differs from the others. The accessions analysed show variation for all the traits used, especially those related to phenology and architecture (branching). The accessions are grouped according to their geographical origin. This genetic variability observed between the groups resulting from the hierarchical classification indicates that those with interesting characteristics such as mosaic resistance, a large number of lobes will serve as a source of broodstock in programmes aimed at improving cassava productivity. However, it is important to combine morphological characteristics with molecular techniques, such as microsatellites, to better characterize accessions within the different groups.

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Why is China's the Belt One Road Initiative successful? — Lessons from the Cooperation with the Five Central Asia Countries

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Abstract— *This paper investigates Why is China can gain great success in capacity cooperation with the countries involving in the Belt One Road Initiative. This paper uses the cooperation with the five Central Asian countries, Kazakhstan, Kyrgyzstan, Tajikistan, Turkmenistan and Uzbekistan, as examples to find out the potential factors. Through analyzing the complementarity of long-term strategies of these five countries and China, and the supplementary policies for countries involving in Belt One Road Initiative, this paper finds that cost-effective technologies and excellent skills in infrastructure construction are in line with the needs of the five countries in their long-term development plans. Combining with supplementary policies like the 'five connectivity' and support from international institutions like AIIB and ADB, the benefit and security of the two groups China and countries involving in Belt One Road Initiative are highly guaranteed. Moreover, China has no tendency in geopolitical target in those areas and after all projects are finished, cooperation through public-private partnership can eliminate the concern about being control by China in infrastructure and energy sectors.*

Keywords— Capacity cooperation, the Belt One Road Initiative, Strategy complementarity, Country safety and international cooperation.

I. INTRODUCTION

Since the Belt and Road initiative was put forward in 2013, more than 100 countries and international organizations around the world have actively responded to support this initiative. Over the past five years, from top-level design to pragmatic advancement, the Belt and Road development has covered smoothly from specific locations to regions. Among all the plans and projects, cross-border direct investment is a key area of economic cooperation between China and countries related to the Belt and Road initiative. The Vision and Actions for Promoting the Joint Construction of the Silk Road Economic Belt and the 21st Century Maritime Silk Road issued by China in 2016 and the Belt and Road International Cooperation Summit Conference in 2017 all listed investment cooperation as core topic for the Belt and Road construction. Located in the core area of the Eurasian continent, the five Central Asian countries are the key land

linkage of the Silk Road Economic Belt. They are also the primary area for China to seek economic cooperation from the vast Eurasian continent. They play an important role in connecting China to Europe and West Asia. It is also regarded as the area with the greatest potential for economic and trade investment in the Silk Road Economic Belt. In early January 2017, China successively celebrated the 25th anniversary of the establishment of diplomatic relations with the five friendly neighbors in Central Asia. In the past 25 years, with the rapid improvement of the economic and trade cooperation environment between China and Central Asian countries, China's direct investment in Central Asian countries has developed rapidly and the scale has continued to expand. For a long time, the five Central Asian countries have formed a resource-export-oriented open economy, and their economic development is highly dependent on foreign countries. The countries' economic growth is overly dependent on the comparative advantage of a single factor formed by their natural resources. Therefore, expanding the attraction of foreign investment is important to promote their national economic development. As the largest developing country, China is in the mid-stage of industrialization and has the dual advantages of high-end technology and labor costs. It has a complete industrial system, and its equipment manufacturing level is at the mid-end of the world's industrial chain. These advantages make it extremely cost-effective as a choice for economic partner. However, the five Central Asian countries have different levels of economic development. For this reason, they all had different domestic investment environments and widespread problems such as insufficient infrastructure construction and industrial investment. With the acceleration of the urbanization process in developing countries, the demand of the improvement of infrastructure and production capacity has become a practical factor to meet the gap between the supply and demand.

Faced with such large national differences, how did China gain the support of countries along the Belt and Road initiative to achieve win-win result for both parties and even multiple parties? This paper combs the long-term development strategies of the five Central Asian countries-Kazakhstan, Kyrgyzstan, Tajikistan, Turkmenistan and Uzbekistan in detail, and combines them with the country-specific plan conducted by China for fulfilling the promises of the Belt and Road initiative, which will help us understand the reason why the Belt and Road Initiative has gained widely support from the countries.

II. THE LONG-TERM DEVELOPMENT STRATEGIES OF THE FIVE CENTRAL ASIAN COUNTRIES AND THE COMPLEMENTARITY OF THE BELT AND ROAD INITIATIVE

2.1 Kazakhstan's long-term development plans and key development areas

Kazakhstan-2030 issued by the President of Kazakhstan, Nazarbayev proposed a grand blueprint 'to be one of the top 50 countries in the world by 2030' for Kazakhstan's social and economic development. The development strategy is divided into 3 stages for the above purpose, and focused on the improvement of the following five key areas: (1) national security and social stability; (2) high-level foreign investment; (3) national health, education and welfare; (4) energy resources; (5) infrastructure construction. At the beginning of the planning and design, Kazakhstan took into account the 30-year active cycle of a generation, especially the iterative factors of the country's resource stock and new energy expected 30 years later. The first long-term plan set the time node in 2030. However, due to the outbreak of the financial crisis in 2008, Kazakhstan is expected to misjudge the economic cycle in its long-term planning, and the unexpected financial crisis will bring new challenges to Kazakhstan's economic development. Planning cannot effectively guide the country's long-term economic development. Under this circumstance, in 2013, Kazakhstan drafted Kazakhstan-2050, which made major adjustments to the original State of the Union address. In addition to some strategic objectives, the original five areas of emphasis were adjusted significantly in details. In order to give more information about the needs of Kazakhstan and the relationship between Kazakhstan and China, Table 1 give more details about projects signed by these two nations after 2000.

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Table 1: Summary of important bilateral agreements between China and Kazakhstan

Country	Specific Agreement (year)
Kazakhstan	China-Kazakhstan Framework Agreement on Comprehensive Cooperation in the Oil and Gas Field (2004) China-Kazakhstan Economic and Trade Cooperation Agreement (2004) China-Kazakhstan Framework Agreement on Establishing China-Kazakhstan Khorgos International Border Cooperation Center (2004) China-Kazakhstan Economic Cooperation Development Plan (2006) China-Kazakhstan Cooperation Plan in the Field of Non-Resource Economy (2007) China-Kazakhstan Medium and Long-term Economic and Trade Cooperation Development Plan (to 2020) (2013) China-Kazakhstan Pipeline Export Crude Oil Uniform Pipeline Transportation Fee Calculation Method and Agreement on the Method of Distribution of Pipeline Transportation Fee Income for Each Stage Owner (2014) Framework Agreement for the Construction of Large Diameter Steel Pipe Plant in Kazakhstan (2014) China-Kazakhstan Framework Agreement on Strengthening Cooperation in Production Capacity and Investment (2015) China-Kazakhstan Memorandum of Understanding on Promoting SME Cooperation (2016) Kazakhstan and China Sign a Series of Cooperation Agreements (2016) Memorandum of Understanding on Strengthening Production Capacity and Investment Cooperation (2016) Memorandum of Cooperation between CRRC Corporation Limited and Kazakhstan Railway Joint Stock Company (2016) Memorandum of Understanding on Strengthening Cooperation in the Field of Transportation Infrastructure by the Ministry of Commerce of the People's Republic of China and the Ministry of Investment and Development of the Republic of Kazakhstan (2017) Joint Statement between the People's Republic of China and the Republic of Kazakhstan (2017) Loan Agreement for the Kalbatta-Makapsagai and Melk-Brubaital Highway Renovation Project (2017) Protocol on Phytosanitary Requirements for Barley Exported from Kazakhstan to China (2018) Protocol on Phytosanitary Requirements for Exported Corn from Kazakhstan to China (2018) Petroleum Contract Extension and Deepening Cooperation Agreement in the Oil and Gas Field (2018) Agreement between China National Petroleum Corporation and the Ministry of Energy of Kazakhstan on The Extension of Oil Contracts and Deepening Cooperation in The Oil and Gas Field (2018) Memorandum of Understanding on E-commerce Cooperation between the Ministry of Commerce of the People's Republic of China and the Ministry of National Economy of the Republic of Kazakhstan (2018) EPC Contract for Zanatas 100MW Wind Power Project (2018) General Contracting Contract for the Project of 5 Cascade Hydropower Stations on the Upper Reaches of the Tantentaike River (2018) Memorandum of Understanding on the List of Key Projects of the 15th Round of China-Kazakhstan Capacity and Investment Cooperation by the Ministry of Investment and Development of China and Kazakhstan (2018) Cooperation in the Fields of Industrialization, Investment, Innovation, Digitalization, Agriculture and Energy (2018)

2.2 Kyrgyzstan's long-term development plan and key development areas

Prior to 2010, Kyrgyzstan's Country Development Strategy (CDS) mainly emphasized through sustainable economic development, job creation, high sustainable income, high universality of social services, and high quality in a healthy, higher standard of living with friendly environment and the improvement of people's living standards. Key development areas include energy, minerals, agriculture, processing, construction, tourism, transportation infrastructure, telecommunications, and technological innovation and development. In early December 2012, President Atambayev of Kyrgyzstan announced the Kyrgyzstan 2013-2017 Development Strategy (KDS) at the first conference of the National Council for Stable Economic Development, which is the five-year plan for economic development, aiming to attract domestic and foreign investors to the greatest extent, with special emphasis on support for minerals, energy, tourism, agriculture, transportation, finance, and environmentally friendly technologies. The agricultural industry, energy, mining, transportation and communications, tourism and service industries are regarded as strategic sectors that promote economic development. In October 2017, the newly-appointed Kyrgyz President Sorumby Zhenbekov drafted the latest long-term development plan-Sustainable Development Strategy 2018-2040 (hereinafter Kyrgyzstan 2040). The plan took 2017-2023 as the first stage, clarifying the goals, scope of government work, special tasks and projects for the next five years, and regards political stability as a guarantee for achieving long-term strategic goals. The main goal of Kyrgyzstan 2040 emphasized the establishment of a peaceful and prosperous Kyrgyzstan for the people. In terms of economy, Kyrgyzstan hoped to improve the economic welfare of the people by providing decent jobs and stable income. Specifically, it included creating high-productivity jobs, decent jobs, stable incomes, and increasing the export potential of labor-intensive jobs, and help governments to transform from the status of an absolute service provider to the status of a manager by issuing policies, standards and work frameworks, etc., and provide individuals with more personal training and protect the rights of consumers, specifically by improving the national health and education level, more accessible and high-quality medical insurance system, popularizing high-quality educational opportunities, etc. All these actions are regarded as the main driving force for social development. In terms of national security, Kyrgyzstan aimed to create a safe and stable social environment for every citizen, so that citizens can live in a peaceful and secure environment, and the rights and freedoms ensured include the construction of Reliable laws and methods for enforcing the environment, protecting nature, environmental resources, etc. serve as the stability of Kyrgyzstan's society and environmental security. To sum up, the sectors of agriculture, light industry, agro-processing industry, and tourism, which provide the most jobs in Kyrgyzstan, have become the key of sustainable development of the national economy. In order to give more information about the needs of Kyrgyzstan and the relationship between Kyrgyzstan and China, Table 2 give more details about projects signed by these two nations after 2000.

Table 2: Summary of important bilateral agreements between China and Kyrgyzstan

Country	Specific Agreement (year)
Kyrgyzstan	China-Kyrgyzstan Energy Cooperation Agreement (2000) China-Kyrgyzstan Agreement for Avoiding Double Taxing (2000) China-Kyrgyzstan Cooperation Program from 2004 to 2014 (2004) China Banking Regulatory Commission and the State Banking Regulatory Cooperation Agreement The Cooperation Plan of the Ministry of Foreign Affairs of China and Kyrgyzstan in 2007 (2006) Joint Declaration of the People's Republic of China and the Kyrgyz Republic on the Establishment of Strategic Partnership (2013) Joint Declaration of the People's Republic of China and the Kyrgyz Republic on Further Deepening the Strategic Partnership (2014) Strategic Cooperation Agreement between Yanqi County and the Kyrgyz Republic to Build the Silk

	Road Economic Belt (2014) China-Kyrgyzstan RMB Currency Swap Agreement (2015) China-Kyrgyzstan Cooperation Framework Agreement on Bilateral Economic and Technical Assistance Projects (2015) Investment Agreement between the Kyrgyz Government and the China-Kyrgyzstan Gas Pipeline Company (2015) Contract for the Survey and Design of Kyrgyzstan's 50 MW Balekchi Wind Farm (2017) The Asia Star Agricultural Industry Cooperation Zone Signs a Memorandum of Understanding on Economic and Trade Cooperation (2017) Memorandum of the Ministry of Commerce of the People's Republic of China and the Investment Promotion and Protection Agency of the Kyrgyz Republic on the Construction of Economic and Trade Cooperation Zones (2018) CNAS-KAC Accreditation Cooperation Memorandum of Understanding (2018)

2.3 Tajikistan's long-term development plan and key development areas

In 2005, to provide an ideal platform for the country's further development, the Presidential Palace of Tajikistan drafted the National Development Strategy (NDS) to ensure the country's long-term and orderly development and match the country's Millennium Development Goals (MDGs). All regions, departments, and regional conceptual frameworks, policies, projects, plans, and activities of all government agencies related to national development are carried out under the contents of the NDS. Combining this plan with the requirements of the national Poverty Reduction Policy, Tajikistan will focus on three areas between 2007 and 2015. They are:(1) reform the administrative department, establish more transparent, clear-cut powers and responsibilities, and Anti-corruption government; (2) through a freer economic environment, strengthen property rights and legal efficiency, and adopt Public-Private Partnership to develop the private sector and attract investment; (3) effectively tap the potential of human resources in improving the quantity and quality of social services for the poor and provide the masses with great opportunities to participate into the process of social construction and strengthen social cooperation to achieve the Millennium Development Goals.

On December 1, 2016, the Parliament of Tajikistan approved the National Development Strategy 2030 (hereinafter Tajikistan 2030). Combining the Tajikistan 2030 with the Sustainable Development Goals (SDGs) of Tajikistan at the 70th United Nations General Assembly in 2015, Tajikistan's work centers in the next 15 years are: (1) education; (2) health; (3) employment; (4) reducing inequality, fighting corruption; (5) food safety and nutrition; (6) competent government; (7) social welfare; (8) prevention of potential conflicts; (9) Energy security, environmental protection, and (10) population management. The Tajikistan government will prevent or reduce economic vulnerabilities and enhance industrialization and efficiency of energy use. Moreover, it promised to provide a better environment for innovation or integrate new technologies into the development of society and economy. The Tajikistan 2030 combined with the 10 work centers, will ultimately achieve the five ultimate goals of ensuring energy security, achieving economic transformation, ensuring food safety and high-level national health, and expanding employment. In order to give more information about the needs of Tajikistan and the relationship between Tajikistan and China, Table 3 give more details about projects signed by these two nations after 2000.

Table 3: Summary of important bilateral agreements between China and Tajikistan

Country	Specific Agreement (year)
Tajikistan	Agreement on Economic and Technical Cooperation between the Government of the People's Republic of China and the Government of the Republic of Tajikistan (2001) Agreement on Avoiding Double Taxing and Preventing Tax Evasion on Income and Property of China and Tajikistan (2000) China and Tajikistan Government on Energy Cooperation Agreement (2002) The People's Republic of China and the Republic of Tajikistan Treaty of Good-Neighborliness, Friendship and Cooperation (2007) Joint Statement of the People's Republic of China and the Republic of Tajikistan on Further Development of Good-neighborly, Friendly and Cooperative Relations (2008) The Agreement between the Governments of China and Tajikistan on Strengthening Cooperation between Xinjiang, China and Tajikistan (2010) Joint Declaration of the People's Republic of China and the Republic of Tajikistan (2012) Agreement on the basic principles of joint venture cooperation (2014) China-Tajikistan Huaxin Cement Investment Agreement (2014) Agreement on the Establishment of China-Tajikistan Gas Pipeline Co., Ltd. (2014) Agricultural Cooperation Agreement (2014) China-Tajikistan 3 billion RMB Swap Agreement (2015) China Electrician and Tajikistan Kajie Coal Mine EPC Project Cooperation Agreement (2015) Agreement on Sino-Tajikistan Joint Venture Establishment of Fertilizer Production Enterprise (2016) Tripartite Cooperation Agreement on Hydropower Projects in Tajikistan (2016) State-owned and Private Partnership Agreement (2016) Agreement between the Ministry of Commerce of the People's Republic of China and the Ministry of Economic Development and Trade of the Republic of Tajikistan on Strengthening Cooperation in the Field of Infrastructure (2017) China-Tajikistan Joint Statement on Establishing a Comprehensive Strategic Partnership (2017) 500kV Transmission and Transformation Project Foreign Aid Preferential Loan Agreement (2017) Memorandum of Understanding on the Establishment of the China-Tajikistan Science and Technology Cooperation Committee (2017) Agreement between the Ministry of Commerce of the People's Republic of China and the Ministry of Economic Development and Trade of the Republic of Tajikistan on Strengthening Cooperation in the Field of Infrastructure (2017) China-Tajikistan Cooperation Planning Outline (2017) The Export-Import Bank of China and Tajikistan 500 kV Transmission and Transformation Project Foreign Aid Preferential Loan Agreement (2017) The General Contract Agreement between CITIC Heavy Industries and Tajikistan's Million Ton Cement Plant (2018)

2.4 Turkmenistan's long-term development plan and key development areas

Turkmenistan promulgated the Political, Economic and Cultural Development Strategy of Turkmenistan to 2020 (hereinafter Turkmenistan Strategy) in order to achieve the economic development level, living standard and social security of developed countries. In addition, Turkmenistan Strategy will continue to increase GDP per capita and increase the investment environment and attractiveness, which together constituted the center of

long-term development at this stage. In addition, Turkmenistan Strategy regarded the development of the nine economic sectors of the oil and natural gas industry, the chemical industry, the textile industry, the building materials industry, agriculture, transportation, communications, and the social sector, as the cornerstone of achieving goals. However, the financial crisis exposed the fragility of Turkmenistan's economy—product production was too concentrated in certain places and the consumption market was single. Therefore, after 2008, a key to the country's policy was to achieve economic diversification. Increasing investment in infrastructure, especially in inter-provincial and remote areas, had become the key to economic diversification. In addition, the improvement of monetary and credit policies and the banking system are important supplements to ensure the realization of Turkmenistan's various tasks. In August 2016, Turkmenistan released the latest national long-term development plan—Turkmenistan's National Social and Economic Development Plan from 2018 to 2024. This plan further emphasized the importance of knowledge and innovation, and the diversification of sectors. In order to give more information about the needs of Turkmenistan and the relationship between Turkmenistan and China, Table 4 give more details about projects signed by these two nations after 2000.

Table 4: Summary of important bilateral agreements between China and Turkmenistan

Country	Specific Agreement (year)
Turkmenistan	Agreement on Economic and Technical Cooperation on China's Provision of Gratuitous Aid to Turkmenistan (2005) Agreement on Economic and Technical Cooperation between the Government of China and the Government of Turkmenistan (2005) Framework Agreement for the Chinese Government to Provide Concessional Loans to the Turkmenistan Government (2005) Agreement on Cooperation in the Field of Oil and Gas (2005) China-Turkmenistan Joint Statement (2006) On the Implementation of the Turkmenistan-China Gas Pipeline Construction Project and Turkmenistan's General Agreement on the Sale of Natural Gas to China (2006) Framework Agreement for China to Provide Concessional Loans to Turkmenistan (2006) China-Turkmenistan Economic and Technical Cooperation Agreement (2006) China-Turkmenistan Joint Statement on Further Consolidating and Developing Friendly Cooperative Relations (2007) Joint Statement of the People's Republic of China and Turkmenistan (2008) Agreement on the Establishment of the China-Turkmenistan Intergovernmental Cooperation Committee (2008) Agreement on Avoiding Double Taxing and Preventing Tax Evasion by the Government of China and Turkmenistan (2009) China-Turkmenistan Government Economic and Trade Cooperation Agreement (2011) China-Turkmenistan Joint Declaration on Establishing Strategic Partnership (2013) China-Turkmenistan Treaty of Friendship and Cooperation (2014) Memorandum of Understanding on Basic Principles of Cooperation in the Field of Transportation by the Ministry of Economic Development of China and Turkmenistan (2014) The Treaty of Friendship and Cooperation between the People's Republic of China and Turkmenistan (2015) Minutes of the Fifth Meeting of the Energy Cooperation Sub-Committee of China and Turkmenistan (2016) China-Turkmenistan US$4.1 Billion Loan-for-Natural Gas Agreement (2017) The Agreement between the Chinese Government and the Government of Turkmenistan on Avoiding Double Taxing and Preventing Tax Evasion on Income (2) (2018)

2.5 Uzbekistan's long-term development plan and key development areas

An earlier long-term economic plan of Uzbekistan was called Building the Future: Uzbekistan's Unique Market Economy Transformation Road (hereinafter UUMETR), which was proposed by the President of Uzbekistan in 1993. The UUMETR emphasized the investment of domestic resources and foreign capital in limited sectors to realize the transformation and upgrading of economic structure. Although the 2008 financial crisis swept the global economy, compared with Uzbekistan's neighbors, conservative macroeconomic management policies had protected Uzbekistan from greater negative shocks. In general, Uzbekistan's overall public policy experienced a major turning point only in 2007. Therefore, the last important national priority areas in 2006 were taken as the representative of the early long-term policies. In February 2017, Uzbekistan approved the 2017-2021 new five-year plan. In addition to improving existing problems, Uzbekistan further provided explanations and future solutions for all aspects of social and economic issues. In terms of economic development, Uzbekistan will focus on five areas: departments, legal systems, economic development, social progress, and national security. Among them, the overall requirements of the economic sector include basically strengthening macro-stability and maintaining high economic growth rates, enhancing economic competitiveness through deepening economic structural reforms and diversifying pillar industries, modernizing and strengthening agricultural sector development, deepening institutional and structural reforms, and reducing government intervenes, and introducing stricter policies to protect intellectual property rights and private property rights. Moreover, Uzbekistan still emphasized the development of transportation and communication infrastructure, development and utilization of renewable energy, tourism, agriculture and light industry. In order to give more information about the needs of Uzbekistan and the relationship between Uzbekistan and China, Table 5 give more details about projects signed by these two nations after 2000.

Table 5: Summary of important bilateral agreements between China and Uzbekistan

Country	Specific Agreement (year)
Uzbekistan	China-Uzbekistan Joint Statement on Further Developing and Deepening the Friendly and Cooperative Partnership (2004) China-Uzbekistan Government Economic and Technical Cooperation Agreement (2004) Framework Agreement for the Government of the People's Republic of China to Provide Concessional Loans to the Government of the Republic of Uzbekistan (2004) Memorandum of Understanding on Expanding Economic, Trade, Investment and Financial Cooperation between the Governments of China and Uzbekistan (2004) China-Uzbekistan Friendship and Cooperation Partnership Treaty (2005) China-Uzbekistan Agreement on Mutual Customs Assistance (2005) China-Uzbekistan Government Joint Communiqué (2007) China-Uzbekistan Governmental Non-Resource and High-tech Cooperation Plan (2010) Framework Agreement on Natural Gas Purchase and Sale (2010) Memorandum of Understanding on Expanding Cooperation between the Chinese and the Government of Uzbekistan in the Field of Natural Gas (2010) Agreement on the Establishment of an Intergovernmental Cooperation Committee (2011) Decision on Approving the Treaty of Amity and Cooperation between China and Uzbekistan (2014) China-Uzbekistan Gas Pipeline D Line Inter-Enterprise Agreement (2014) Muballek Natural Gas Chemical Plant Cooperation Memorandum (2014) Joint Declaration of the People's Republic of China and the Republic of Uzbekistan (2014) China-Uzbekistan Economic and Technical Cooperation Agreement Bank Account Handling Rules (2015)

	Agreement on International Road Transport between the Government of the People's Republic of China and the Government of the Republic of Uzbekistan (2017) Memorandum of Understanding on Infrastructure Construction Projects in Uzbekistan (2017) Joint Statement between the People's Republic of China and the Republic of Uzbekistan (2017) Agreement on Economic and Technical Cooperation between the Government of the People's Republic of China and the Government of the Republic of Uzbekistan (2017) The Cooperation Agreement Between Hytera and Uzbek Electrical Equipment Co., Ltd. Memorandum of Understanding on Strategic Cooperation in Thermal Power Stations (2018)

III. STRATEGIC DOCKING OF PRODUCTION CAPACITY COOPERATION UNDER CHINA'S ONE BELT ONE ROAD INITIATIVE

3.1 Policy docking under the Belt and Road Initiative

3.11 Belt and Road Initiative

The Belt and Road Initiative itself provides strong cooperation basis for China and the Central Asian countries. The Belt and Road Initiative was proposed by China's President Xi Jinping in 2013. He mentioned that the five Central Asian countries are currently facing very similar problems: these countries lack sufficient self-owned funds, technology, professionals and infrastructure. However, huge geographical advantages, energy advantages and labor advantages are also outstanding. These conditions provide strong cooperation basis for China and the five Central Asian countries. The Belt and Road initiative is based on the principles of mutual consultation, construction and sharing. It aims to promote economic globalization and cultural diversification, and helps countries realize interconnection and inclusiveness, mutual learning and mutual benefit. Starting from China's own development experience, it contributes Chinese wisdom to solve world development problems and becomes maintenance and advancement. The Belt and Road initiative provides countries along the route with unparalleled development experience of China. As the largest developing country and the second largest economy in the world, China has strong and highly cost-effective infrastructure construction capabilities. Moreover, the technological accumulation of renewable energy such as photovoltaic energy, hydropower, and electric energy is fully in line with the needs of the long-term development of the Central Asia countries. In terms of industrial capacities, and the rapid and stable economic development provides China with stable and excellent capacity output strength, which is in line with the current needs of the five Central Asian countries.

3.12 Long-term plans in China: Made in China 2025 and Guidelines on Promoting International Cooperation in Capacity and Equipment Manufacturing

The Guidelines on Promoting International Cooperation in Capacity and Equipment Manufacturing (hereinafter Guidelines) emphasizes accelerating the pace of international co-operation in railway construction, vigorously developing overseas electricity power projects and improving the level of international cooperation in the light industry and textile industry, improving international competitiveness of communication industry, promoting the export of construction machinery and other manufacturing industries to cover more the global business network, etc., which almost completely meets the technology and resource needs of the five Central Asian countries in terms of infrastructure construction, industry, agriculture and energy exploitation, etc. Moreover, the long-term plan Made in China 2025 also reflects China's determination to become a strong manufacturing country in the future. The ten major areas and five major projects (Table 6) emphasized are also a strong guarantee for countries' long-term cooperation in these fields with China. Briefly, the Guidelines provide a policy basis for the current capacity cooperation between China and the five Central Asian countries, while the Made in China 2025 guarantees the long-term benefits of cooperation with China in related fields.

Table 6: Ten key areas and five major projects of Made in China 2025

Ten key areas	Five major projects
1.Information Technology Industry	1. Manufacturing Innovation Center Construction Project
2. Advanced CNC Machine Tools and Robots	
3. Aerospace Equipment	2. Intelligent Manufacturing Engineering
4. Marine Engineering Equipment and High-Tech Ships	
5. Advanced Rail Transit Equipment	3. Projects on Strengthening Basic Industries
6. Energy Saving and New Energy Vehicles	
7. Electricity power equipment	4. Green Manufacturing Engineering
8. Agricultural Equipment	
9. New Materials	5. Advanced Equipment Innovation Project
10.Biomedicine and High-Performance Medical Equipment	

Source: Autor's collection from Made in China 2025 and Guidelines on Promoting International Cooperation in Capacity and Equipment Manufacturing

3.2 Supplementary Policies for China-Central Asia Capacity Cooperation

China has also established a multi-dimensional cooperation guarantee mechanism in promoting the Belt and Road Initiative. The 13th Five-Year Plan for Economic and Social Development of the People's Republic of China (the 13th Five-Year Plan) listed the Belt and Road Initiative as core content and main tasks, which is a symbol the basic perfection of the top-level design and planning in national level. Since then, the Belt and Road Initiative has interpreted the essence of win-win with the Five Connectivity: (1) policy connectivity;(2) infrastructure connectivity;(3) trade connectivity;(4) finance connectivity; (5) people connectivity.

Policy connectivity. The Regional Comprehensive Economic Partnership Agreement (RCEP), the Rome Declaration and other cross-regional bilateral policy consensus have laid the foundation for political, economic and trade mutual trust at the macro level for countries and regions joining in the Belt and Road Initiative. Under the guidance of these macro policies, China and the countries related to the Belt and Road Initiative signed cooperation, memorandums of understanding, and multi-country joint statements such as China-Japan-Thailand Trilateral Cooperation in the Eastern Economic Corridor of Thailand, Lanmen-Mekong Cooperation, China-Pakistan Economic Corridor (CPEC) , the Dushanbe Declaration, the Joint Statement on E-commerce between China and the 75 WTO countries and so on to reach cooperation consensus. On the premise of adhering to mutual benefit and win-win results, using the Belt and Road Initiative to realize the organic connection of bilateral and multinational economic and trade cooperation, to further promote the establishment of political mutual trust and strategic partnerships. Emphasizing on complementary advantages and achieve win-win economics and trade is the essence of the Belt and Road.

Infrastructure connectivity. China attaches great importance to the standards for road construction in the Belt and Road Initiative. The Belt and Road Transport Alliance established in 2016 paid particular attention to exchanges and cooperation with Central Asian countries in the field of road construction. In 2017, the 13th National Development and Reform Commission included 13 Various departments and units have jointly established the Belt and Road Initiative Public-Private Partnership (PPP) working mechanism to strengthen cooperation with countries involved in the Belt and Road Initiative in the field of infrastructure construction and to actively promote the PPP model to achieve proper and rapid implementation of infrastructure projects in those countries. Since most of the road infrastructure in Central Asian countries uniformly used the standards of the former Soviet Union, after the collapse of the Soviet Union, the Central Asian countries were unable to repair and maintain the existing road system. Due to problems such as inefficient

supervision and rent-seeking corruption, coupled with long cycles in road investment, transit roads usually pass through remote and rural areas, which are typical low-return, high-risk projects, especially that the five Central Asian countries are typical inland country which always has very complex terrain. Therefore, in the field of infrastructure connectivity, China's infrastructure construction has actually helped Central Asian countries to narrow the development gap between regions. Through PPP, BOT, etc., the main benefits in the future will definitely be transferred to the cooperative countries. The concept of infrastructure connectivity has undoubtedly greatly compensated for the crux of the resource allocation of Central Asian countries, enabling these countries to locate limited resources in the economic development and balance regional development.

Trade connectivity. Economic and trade cooperation is one of the key contents of the Belt and Road Initiative, and its foundation and leading role have injected strong impetus into the realization of the Five Connectivity. China has implemented many measures in order to ensure the smooth flow of trade, universities, win-win and development. First, building a fair multilateral trading platform. Through the establishment of platforms such as China-ASEAN Expo, China-South Asia Expo, China-CEEC Investment and Trade Expo, China-Asia-Europe Expo, Canton Fair, and China International Import Expo, China has continued to reduce trade barriers, improve policies to promote imports and exports, achieve economic complementarity, and maximize economic and trade potential. Second, promoting free trade agreement negotiations. Speeding up the construction of free trade zones with countries with Central Asian countries is an important measure to promote trade facilitation with the Belt and Road Initiative countries and to deepen economic and trade cooperation on a larger scale and at a higher level. Third, building economic and trade industrial cooperation zones. Overseas economic and trade cooperation zones have always been an important carrier for China to promote international cooperation in production capacity and equipment manufacturing and are an important part of China's adherence to the principle of openness and transparency as well as the promotion of trade liberalization. Finally, help countries related to the Belt and Road Initiative to strengthen capacity building. China is an important participant in the United Nations 2030 Agenda for Sustainable Development so that it will provide a large number of research and training places domestically and send senior economic and trade experts to support trade and investment promotion arrangements proposed by the WTO and the United Nations. Although China and the five Central Asian countries have not signed a free trade agreement, the success of the China-Kazakhstan Khorgos-Gate of the East Special Economic Zone and China's continuous efforts to promote the establishment and construction of the China-Central Asian Free Trade Zone will further guarantee the implementation of the concept of trade connectivity between China and Central Asia.

Finance connectivity. The shortage of funds is a major obstacle for Central Asian countries to achieve long-term planning. In addition to government-level cooperation and assistance, China has also established a more complete mechanism to increase mutual trust in politics and economy. At the end of 2014, the China Administration of Foreign Exchange, China Investment Corporation and other departments and units jointly funded the establishment of the Silk Road Fund, with the first phase of registered capital reaching 10 billion US dollars. To end of 2016, the China Development Bank had loaned more than US$1,100 to countries involved in the Road and Belt Initiative, supporting over 600 projects. At the same time, 603 capacity improvement and equipment manufacturing projects are funded by the Export-Import Bank. ICBC established 123 branches in 18 countries involved in the Road and Belt Initiative and provided those countries more than 22 billion US dollars loans. In addition, the establishment and mechanism of the Asian Infrastructure Investment Bank (AIIB) meets the capital and institutional requirements for the bilateral cooperation. AIIB is a government multilateral development institution with a capital scale of US$100 billion. At present, the number of member countries has increased to more than 70, surpassing the membership of the Asian Development Bank (ADB). While providing funds for infrastructure construction in Asia, the multinational board of directors has well protected the legal rights of member states, and

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the institutional arrangements have effectively reduced moral and economic risks. Since the use of funds meets the demands of member states and is driven by China's interests, the establishment of AIIB has effectively responded to the misinterpreted Chinese interest theory in the West. AIIB has used its own actions to prove that the AIIB is not China's AIIB. At present, except Turkmenistan, all other Central Asian countries are the members of AIIB. To the end of 2017, AIIB's total investment reached 7.5 billion U.S. dollars, with a total of 1.73 billion U.S. dollars in loans and 35 projects were approved. The two projects passed in Central Asia are both in Tajikistan and are energy and transportation projects. In contract, only one project in China has been approved.

People connectivity. The friendship between the nations lies in the closeness of the people, and the closeness of the people lies in the communication of hearts. People's hearts are the biggest political and most fundamental basis for cooperation. The misinterpretation of the Belt and Road Initiative by the Western media tends to incite the people and create pressure on public opinion. This proves that the ultimate vision of the Belt and Road Initiative determines that it cannot fulfill without the long-term support of average people. The cause of people connectivity aims to build up the spirit of ownership of all parties and promote the development of relationships. It is an important practice for building a community with a shared future for mankind. On the cultural level, China has established Confucius Institutes in Central Asia, co-hosted cultural year activities, and provided government scholarships to study in China. In humanitarian aspects, Chinese governments has provided more than 2 billion RMB in emergency food aid and donated 1 billion US dollars to the South-South Cooperation Assistance Fund and provided 1 billion US dollars in aid to relevant international organizations, benefiting countries in many important fields such as food, daily use, medical care, and education. In addition to activities in the fields of culture and education, China also provided assistance to the Central Asian countries in the fields of military, infrastructure construction, and people's livelihood (Table 7). Action to implement the Belt and Road Initiative not only requires economic cooperation, but also requires sincere exchanges and people's resonance to provide the most solid foundation for promoting economic and trade cooperation between China and Central Asian countries.

Table 7: Incomplete statistics of China's aid to the five Central Asian countries

Country	Content
Kazakhstan	In 2013, China assisted Kazakhstan in building the Eurasian Canal; In 2014, China Meteorological Administration provided technical assistance to Kazakhstan; In 2015, the Health and Family Planning Commission of ShanXi Province and Kazakhstan signed a framework agreement to assist in the construction of the ShanXi Village hospital; In 2015, China provided Kazakhstan with free military technical assistance for tractors and loading platforms; In 2016, China National Petroleum Corporation completed the construction of the Astana National Dance Academy and Ballet Theater in Kazakhstan; In 2017, China-Kazakhstan completed the handover of the 1 MW solar power plant and the 5 MW wind power plant aided by China; In 2018, China completed the aid construction of the Kazakh Junior Military Academy's electronic classroom; To the end of 2017, China had provided US$43 billion in aid to Kazakhstan.
Kyrgyzstan	In 2013, China assisted Kyrgyzstan in the construction of the Issyk-Kul road restoration project; In 2014, China provided Kyrgyzstan with US$16 million in military assistance; In 2014, China completed the aid construction of the new medical building project of the Kyrgyz National Hospital; In 2015, the foreign aid project China-Kyrgyzstan Modern Agricultural Technology Joint Research and Demonstration Center was approved by the Ministry of Science and Technology; In 2015, China completed the aid of 267 tractors in Kyrgyzstan; In 2017, China aided the construction of Osh Hospital in Kyrgyzstan;

	In 2018, China assisted in the construction of the asphalt pavement restoration project in the capital of Kyrgyzstan; In 2018, China assisted Kyrgyzstan's irrigation system renovation project; In 2019, China assisted Kyrgyzstan with 30 tons of rice, 20 tons of soybeans, 10 tons of corn, 5 tons of beef, RMB 2 billion of interest-free loans and five years of free railway-related technical support.
Tajikistan	In 2013, China assisted in the construction of the Yawan-Wahdat railway project in Tajikistan; In 2013, China assisted Tajikistan with 20 million RMB of police supplies; In 2015, China provided 10 million RMB in material assistance to Tajikistan; In 2016, China assisted in the construction of four border checkpoints and a military training center in Tajikistan; In 2016, China completed the construction assistance project of the office building of the Tajikistan Anti-Narcotic Branch Office; In 2016, China completed the construction of two schools in Vakhdat City and Rudaki Region; In 2016, China completed the Dushanbe No. 2 thermal power station aid project; In 2018, China completed the aid construction of road restoration projects in Kurganqiube and Kulyab cities in Takhatlon Prefecture; In 2018, China provided US$6 billion in assistance for the construction of the 400-capable gas pipeline in Tajikistan for the Central Asia-China Gas Pipeline; In 2018, China assisted in the construction of 3 schools in Tajikistan; In 2018, China helped build the Tajikistan government parliament complex with 1.5 billion RMB free of charge.
Turkmenistan	In 2013, China South Locomotive Co., Ltd. assisted Turkmenistan passenger locomotive and accessories; In 2013, Migan Village Water Plant Project, a component of China Natural Gas Transportation Corporation; In 2014, China initiated a telecommunications completion project for components in Turkmenistan.
Uzbekistan	In 2013, China provided Uzbekistan Customs with a US$4.4 million mobile inspection and inspection equipment; In 2015, China Railway Tunnel funded rural schools in Uzbekistan; In 2015, China Shanghai Pharmaceuticals assisted Uzbekistan with 80 million RMB in medical equipment; In 2015, China completed the assistance of the new electrified railway project in the Malakand to Karshi section of Uzbekistan's national railway; In 2016, China assisted the restoration project of historical and cultural relics in Kwarazmian Prefecture, Uzbekistan; In 2017, China assisted Uzbekistan's Hydrometeorological Administration to provide meteorological-related technologies and equipment; In 2017, China's Huawei assisted in the construction of the Smart Classroom project of 59 Middle School in Tashkent, Uzbekistan; In 2017, China completed the aid project for the Uzbekistan dredger; In 2018, China completed the aid project for teaching equipment in Uzbekistan; In 2018, China completed assistance to the Uzbekistan Children's Medical Center and the Tashkent Children's Hospital for medical equipment projects; In 2018, China funded Uzbekistan's water supply project (TA-9481 UZB).

IV. CONCLUSION

The success of China's cooperation with the five countries in Central Asia is not accidental. With the help of top-level strategies and various safeguards, bilateral interests have been greatly protected. In addition, because China often uses Public-Private Partnership and other

methods to participate in the construction of the host country and provide diversified preferential loans, the host country's doubts about China's penetration have been relieved. Judging from the cooperation experience of China and the five Central Asian countries, this article finds that the following points may be the reasons why China has achieved great achievements with the countries participating in the Belt and Road Initiative:

1. Complementary strategies. Before China and the five Central Asian countries reached capacity cooperation, they had a good relationship foundation. They established diplomatic relations with the five Central Asian countries for more than 25 years. Both sides have understood each other's strategic needs from previous cooperation. Therefore, by combining the national macro-level development strategy and the country-specific strategy, China and the five Central Asian countries have achieved complementarity from the development strategy level. This complementarity is not deliberate, but the inevitability of the different development stages of the two groups. Therefore, the top-level design and difference in the development stage determine that the cooperation between the five Central Asian countries and China is more balanced and more cost-effective compared to cooperation with advanced countries in many aspects.

2. Safeguard measures. In addition to the bilateral cooperation between China and the five Central Asian countries, the multilateral international institutions that the five Central Asian countries participate in can provide additional guarantees for the cooperation between the five Central Asian countries and China. For example, the AIIB and ADB mentioned in Finance Connectivity can greatly protect the rights and interests of relatively disadvantaged countries. These institutions can also protect the resources and security of Central Asian countries from being plundered.

3.Nothing about geopolitics. China's participation in the cooperation of the five Central Asian countries does not design political and geopolitical goals, although the increase in economic influence will inevitably bring political and geopolitical influence. However, from top-level design to specific strategic planning, there is not much military cooperation between China and the five Central Asian countries, and there has never been a tendency to participate in geopolitical conflicts for China and no actual conflict cases involving China.

4. Central Asian country will finally control all the projects. The cooperation between China and the five Central Asian countries usually adopts Public-Private Partnership, BOT and other similar cooperation methods, which ensures that the infrastructure and other similar assets within the projects are owned by Central Asian countries.Therefore, there is no need to worry about China's influence on Central Asian countries by controlling infrastructure, basic energy supply, etc. In this process, there will inevitably be technical, financial, and training support and Central Asian countries can not only improve their material base, but also achieve breakthroughs in their own technologies through the cooperation with China.

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Study of Some Parameters Influencing the *post mortem* PH of Carcass's muscles of Dromedaries Slaughtered at the Touggourt Slaughterhouse. Algeria

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Abstract— *This work aims to study Biological and abiological factors influencing the postmortems hydrogen potential (pH) of four muscles, of twenty dromedary's carcasses slaughtered at the slaughterhouse in Touggourt municipality, Algeria. The temperature and pH were measured at one hour and a half after slaughtering, skinning and evisceration of carcasses. Electronic thermometer and electronic pH meter were used with under-implementation of breeder's know-how and knowledge of veterinarian. The 4 muscles are respectively: Cleido- mastoidien, Semi membranosus, Triceps Brachii and Psoas major. The dromedaries studied are from two populations : The Sahraoui and the Targui. The sample is composed of males and females between 1 and 7 years of age, coming from three types of livestock farming (Intensive, Semi-intensive and Extensive), and before slaughtering the animals were in different housing periods. The statistical analysis is carried out by the SPSS (statistical software) in two "2" steps: the pH overage varies according to the muscle's type, with a highest values recorded in the Triceps Brachii (6.99 ≤ pH ≤ 7.14), then the Cleido-mastoidian with a pH of 6.98 to 6.84 and for the Semi membranosus and Psoas major which are the closest, with a pH respectively of 6.82 to 6.62 and 6.80 to 6.65. Regarding parameters linked to the animal, the age affects the pH with a strong negative correlation (r = -0.99; p> 0.0001). The carcass weight has a negative relationship (r = -0.98; p> 0.0003). Then sex, while the race is not related to pH. For the parameters controlled by humans, the fasting time is at the origin of the increase in post-mortem pH values, corresponding to a positive correlation (r 0.74; p>0.0007). Also, the mode of livestock farming affects this parameter.*

Keywords— ***dromedary, muscle, post mortem pH, slaughterhouse, Touggourt.***

I. INTRODUCTION

The dromedary (*Camelus dromedarius*) is a species of domestic mammal from the family of camelids and the genus Camelus (Charnot, 1959). It is an animal which its morphology, physiology and particular behavior allow it to adapt better than any other breeding animal to the desert conditions.

In Algeria, the dromedary plays a primordial social and economical role, it has been associated with life forms in arid and semi-arid pastoral areas. It meets the multiple needs of the population by providing milk, meat and serving as a means transportation and for agricultural work as well (Siboukeur, 2007).

According to M.A.D.R, in 2013, the headcount in Algeria is estimated at 344,015 head. The dromedary, thanks to its high carcass yield, its meat is appreciated and consumed on a large scale in Algerian Sahara (Faye et *al*, 2013).

Picard et *al*, (2002), report that meat quality varies according to not only biological factors for both antemortem and post-mortem. According to Boudjellal et *al*, (2008), acidification of the muscle is considered as major determinant of meat quality. Benaissa et *al*, (2014)

obtaine that the evolution of post-mortem pH is characterized by a rapid drop during the first eight hours after slaughter, followed by a slowdown.

As defined by Hocquette et *al*, (2012), the post-*mortem* pH is influenced by biological factors linked to the animal (species, race, age, sex and type of muscle) and factors controlled by humans that could be from animal's stress before and after slaughter, feeding and livestock farming mode.

The purpose of this study is the influence assessment of some parameters, whether related or not to the animal, on post-mortem pH of four types of carcass's muscle from dromedaries that have been slaughtered at the slaughterhouse of Touggourt municipality.

II. MATERIAL AND METHODS

2. 1. Presentation of Touggourt municipal slaughterhouse

The Touggourt slaughterhouse is one of the red meat supplier (beef, sheep, goat and camel) in southern Algeria. It covers an area of 23363 square meters. It contines an animals rest area and two slaughter and evisceration rooms with two offices one for the veterinarian and the other for butchers reception and a changing room (Agricultural Services Directorate 2018).

2.2. Experimental methodology

To study parameters influencing the *post mortem* pH of dromedaries muscles, we used 4 types of muscles from young animals (1 to 4 years old) and young adults (5 to 7 years old) from two populations , The Sahrawi and The Targui, the most slaughtered in the study region. These animals are slaughtered according to the Muslim rite "ENAHR" to be marketed. The four muscles studied are:

- Neck muscle : *Cleido Mastoidien* (CM)
- Thigh muscle : *Semi membranosus* (SM)
- Shoulder muscle : *Triceps Brachii* (TB)
- Back muscle : *Psoas major* (PM)

Cleido Mastoidien & *Semi membranosus* are two muscles often used as indicator muscles for assessing the tenderness of red meat for beef and sheep (KLONT et *al*, 1998 et VERGARA et *al*, 1999). *Triceps Brachii* & *Psoas major* are indicators of red meat organoleptic quality assessment (Kamoun, 1995).

Identification of parameters related to the animals

The determination of the animal's race or more precisely its population, aims to characterize and compare the morphology of 20 dromedaries from two populations (the Sahrawi 14 animals and the Targui 6 animals). For this, we called in veterinarians. This choice is not fortuitous because the Sahrawi population corresponds to the Northerns Sahara race and Targuis correspond to Tamanrasset's Population (Ouled Laid, 2008). Direct observation of animal's reproductive system was used to determine the studied animals sex. While the age of these dromedaries has been assessed by veterinarians based on the animal's dentition. Knowing that, the presence of incisors on the upper jaw and canines on both jaws is the species feature. For the carcass weight, in absence of an appropriate weighting tool, the weight was determined after the carcass cutting by summing different separated parts which comprises nine pieces: The neck, the two shoulders, the thoracic part, the right ribs, the left ribs, the lumbar part and the two thighs. These weighings were carried out using an electronic "Crane Scale Cap" with a maximum capacity of 150 kg.

Identification of parameters controlled by humans

To determine the breeding system for these camels we used the information given by camel drivers. So 3 types of camel farming system were deduced: extensive, intensive and semi-intensive. To determine the duration of fasting for the dromedaries studied, we appealed to the breeders or owners of these animals.

Location of muscles studied and measurement of their temperature and pH

The 4 muscles studied are located after skinning, evisceration and cutting of the carcasses. The intramuscular temperature of the 4 muscles is expressed in degrees Celsius. It is directly read using an electronic thermometer "Digital Thermometer", equipped with a penetrating probe allowing the measurement of the temperature at the heart of the muscle. Three measurements are made for each muscle and the temperature recorded is an average of three readings taken. Post mortem pH is the focus of our study. The measurement of this parameter is carried out by a direct method, using an electronic pH meter 'pH Meter 8000', equipped with a penetrating probe allowing a pH measurement at the muscle heart. The reported pH value is an average of three readings for each muscle.

Data analysis and processing

Analysis and processing of the measured data is carried out by the SPSS (statistical software) in two 2 steps:

First step, served to rearrange the measured pH values in tables to facilitate the calculation of the average for each muscle. these measurments are taken from 20 animals according to the parameters studied (breed, sex, age, breeding method, warm carcass's weight, fasting time and temperature).

Second step, at this stage we applied different graphical forms like figures to present the results in a clearer way which helps to show variations in pH according to the studied parameters.

III. RESULTS

3.1. Influence of parameters related to animal on *post-mortem* pH

Influence of the animal breed

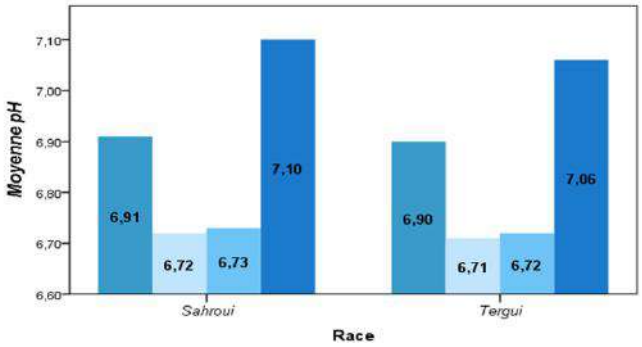

Fig 1 : post-mortem pH of the four muscles of both races : Sahraoui and Targui

To assess the average pH value recorded for each muscle, we calculate the average pH measured on all the muscles studied of the same type, from different carcasses and from the same population. The average pH values recorded for the 4 muscles studied, from animals belonging to both populations, were all close to neutrality. The maximum averages were recorded on the TB muscle for both breeds with 7.10 and 7.06 respectively for the Sahrawi and the Targui. While the minimum values were taken for the SM muscle for the two animal races (6.72 for the Sahrawi and 6.71 for the Targui). A slight decrease in this parameter for the 4 muscles was noted in the Targui population. The pH follows the same profile for both populations, this parameter was influenced by the muscle type more than the breed (Figure 1).

Influence of the animal's sex

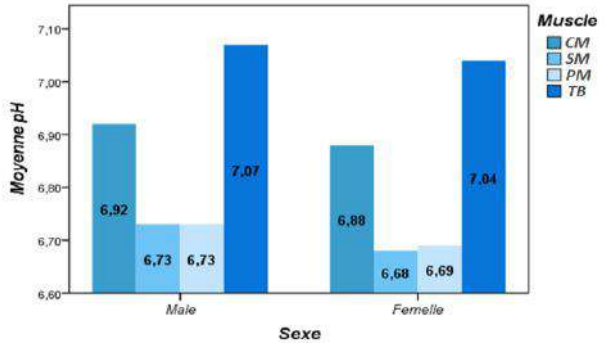

Fig 2: The four muscles post-mortem pH according to sex ;Males and Females

To assess the average pH value recorded for each muscle, we calculated the average of the pH measured on all the muscles studied. These muscles are from the same type of different analyzed carcasses and from the same sex without taking into account the animal's race. The post-mortem pH of all muscles studied were close to neutral; values of this parameter vary between 7.07 for male's TB and 6.68 for female's SM. A difference between the pH values recorded for the same muscle but coming from different sexes ; the highest values are measured on muscles obtained from male animals (Figure 2).

Influence of animal's age

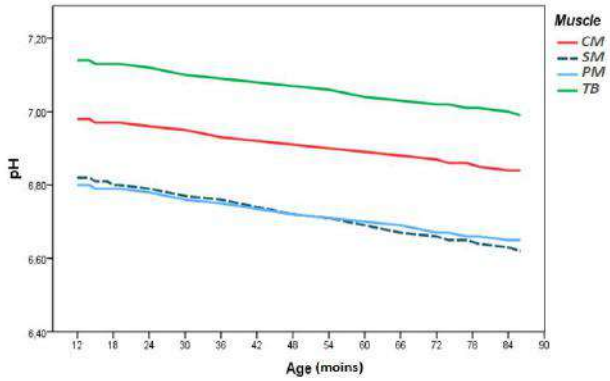

Fig 3 : Influence of animal's age on the post-mortem pH of the four muscles.

The average pH value calculation recorded for each muscle, is obtained by calculating the pH average measured on all muscles of the same type, studied from different carcasses from animals of the same age category without taking into account the animal race.

The pH follows the same profile for the 4 types of muscles studied. A decline in this parameter and with the same amplitude is recorded with the animal's age increase (Figure 3). This indicates that there is a mechanism affecting the muscles or more precisely affecting the muscle fibers in the same way.

Influence of carcasses weight

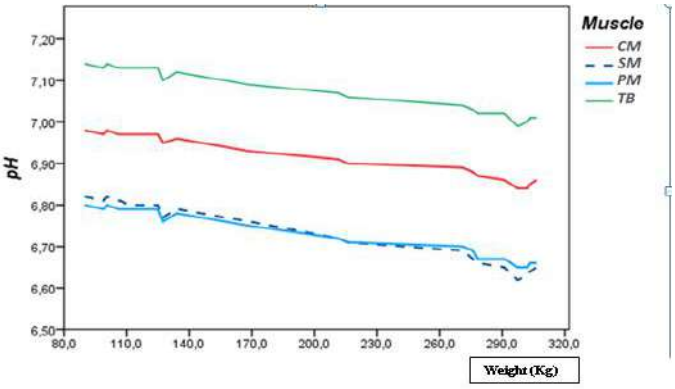

Fig 4: Influence of carcass weight on the four muscles post-mortem pH.

The average pH calculation recorded for each muscle, is obtained by calculating the average of the pH measured on all same type of studied muscles from different carcasses, taking into account their weight and neglecting the animal race and the age category. All curves present the same shape for all muscles. A fall in this parameter is noted with

the increase in carcass weight for the four muscles types (Figure 4).

3.2. Variations of *post-mortem* pH according to parameters controlled by humans

Influence of livestock farming mode

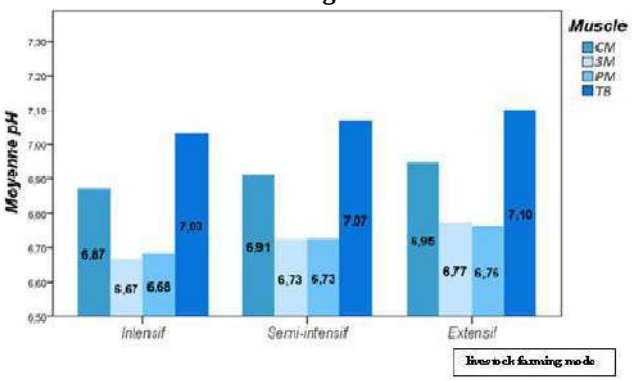

Fig 5 : Influence of livestock farming mode on post-mortem pH of the four muscles.

The average pH evaluation recorded for each muscle, is obtained by calculating an average of the pH measured on all muscles that have same type. And whome are coming from animals of a same breeding type, without considering neither the breed, the age nor the sex of the animal. Variations in post-mortem pH between the 4 types of muscle and for the same muscle as well, are recorded depending on the farming method. Values of this parameter vary between 7.10 to 6.67 recorded respectively on the muscles;TB (extensive) and SM (intensive). The lowest values are noted in dromedaries from intensive breeding, while the highest ones are recorded from extensive livestock farming (Figure 5).

Influence of fasting time

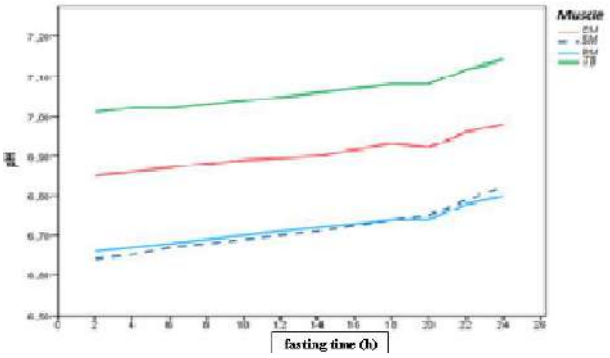

Fig 6: Influence of the fasting time on the post-mortem pH of the four muscles.

Considering the animal's fasting time and neglecting its race and regardless of its age, carcasses are used in order to obtaine the average pH recorded for each muscle. The average pH measured is calculated of all muscles having the same type of carcasses. A variation in pH is noted between the muscles according to dromedaries fasting duration. pH values increased linearly with fasting time up to 20 hours, after this duration they are significantly higher. Therefore, more animal's fasting time before the butchery is longer more the possibility of acquiring a meat with pH close to 7 is bigger (Face 6).

Influence of carcass's temperature

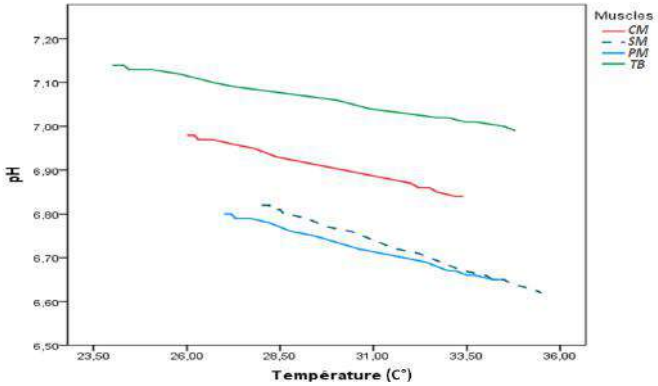

Fig 7 : Relationship between temperature and post-mortem pH of the four muscles.

The average value of pH recorded for each muscle, is obtained by calculating the measured pH average on all the muscles of the same type of different carcasses, taking into account the muscle temperature and neglecting the animal's race and age category. For all muscles, a higher post-mortem temperature is accompanied by a lower pH value p. m. Knowing that sampled temperatures vary between a maximum of 36 ° C and a minimum of 24 ° C. This variability could be explained by muscle's exposure to the air after carcasses cutting and its richness in fat (Figure 7).

3.3. Correlation analysis

Table 1 : The correlation matrix between the studied parameters

	Race	Sex	Age	PC	ME	TMJ	TC
Race	**1**						
Sex	**-0,190**	**1**					
Age	**0,003**	**0,701**	**1**				

CW	**0,016**	**0,659**	**0,989**	**1**			
LFM	**-0,150**	**-0,484**	**-0,456**	**-0,532**	**1**		
FT	**-0,187**	**-0,424**	**-0,751**	**-0,798**	**0,589**	**1**	
CT	**-0,004**	**0,702**	**1,000**	**0,988**	**-0,457**	**-0,747**	**1**

Significance level ***p<0,05*** ; **CW** : Carcass weight ; **CT** : Carcass temperature ; **FT** : Fasting time ; **LFM** : Livestock farming mode.

Carcass weight is correlated with most parameters, mainly the age (r = 0.989; p> 0.0001), and the carcass temperature (r = 0.988; p> 0.0002). On the other hand, an inverse relationship between the weight and the fasting time (r = -0.798; p> 0.0009), While the fasting time and the farming method, their correlation is positive (r = 0.589; p> 0.006) (Table I).

IV. DISCUSSION

The results of this study show a variation in post-mortem pH depending on the muscle type. According to ASHMORE and DOESE (1971) this acidification is related to the type of skeletal fibers constituting each muscle, because several characteristics like morphological, physiological and biochemical differentiate these fibers. JOO in 2017 reports that for cattles the percentage of fibers and their diameters differs depending on the muscle type. The muscle type derives from the relative proportions in composition of different fiber types, this influences muscle metabolism. Knowing that all the muscles studied presented a post-mortem pH near to neutral.

Analysis of the race effect on pH p. m indicates that there is no significant effect (r = -0.006, p <0.982). Our results coincide with those obtained by several authors, who confirm that there are few comparisons between races concerning the pH post mortem evolution. A lot of experiments involving many races led to the conclusion that racial differences in this field is weak (Menissier et *al*, 1982). Regarding the effect of sex on this parameter, the variance analysis shows a significant difference in pH according to sex (α = 0.05; p> 0.001). Our results are consistent with those of Martin and Freeden (1974) on cattles carcasses. They report that after slaughter, the rate of pH drop in muscles is slower for males than females of cattles. Similar results were previously announced by Benaissa et *al*, (2014) and BENAISSA in 2016 on dromedary muscles of the Sahrawi population of 1 hour after slaughter at the Ouargla slaughterhouse and by Bendall in 1973, on cattles muscles. According to Seideman and Crouse (1986), the male animal's muscles have a higher percentage of slow oxidative fibers and less rapid glycolytic fibers. Also Didier Micol et *al*, (1993) report that generally, for the same muscles type, fibers diameters for males are greater than those for females. Regarding to the age, according to Pearson's correlation, there is a negative relationship between pH and age with a very strong significant effect (r = -0.998; p> 0.0001). KADIM et *al*, (2006) point that the pH vary depending on the animal's age where generally young tend to produce meat with a higher pH more than older ones due to the lower glycogen level. For carcass weight, our results consist with those obtained by Hopkins et *al*, (2007), on beef and Kadim et *al*, (2008), on dromedary. They report that a faster drop in pH is noted with carcass weight increase. Unlike the study of Przybylski et *al*, (2015), on pork muscles, shows that an increase in carcass weight does not influence the meat's pH. In addition, about the temperature influence on *post-mortem* pH, Molette et *al*, (2003) note on turkey meat (at 40, 20 and 4°C after 6 hours) that the rate of pH drop is higher in the pectoral muscle subjected to the highest temperature (40 °C). Our results also show a negative relationship between these two parameters, according to Pearson's correlation r = -0.998 with p <0.05. There is also a relationship between the post-mortem pH of the 4 muscles studied and the livestock farming method (r = 0.456 and p <0.05). Berri, (2015), Lebret and Faure, (2015) and Lebret and PICARD, (2015) report that animal husbandry condition influence muscle properties, in particular the level of energy reserves (glycogen) and *post-mortem* metabolism. Also, Priolo et *al*, (2001), announced this influence in a study carried out on cattles raised on grass and others fed with a ration based on concentrated nutriment. Micol et *al*, (2010) add to the farming method the physical activity's factors at pasture. For food deprivation before animail's slaughter, Nijdam et *al*, (2005) and Terlouw et *al*, (2015), report that it could decrease the glycogen levels and influence the pH, but the results vary depending of the muscles. Therefore, in order to preserve muscles reserves which are metabolized into lactic acid during the *post-mortem* transformation of muscle into meat; time and conditions of fasting must be optimized for each species. A positive relation ship between the fasting time and the *post mortem* pH (r 0.745 with p<0.05) is noted in our study as well.

V. CONCLUSION

At one hour and a half after slaughter, all post mortem pH averages measured on four muscles : *Cleido-mastoidien*, *Semi membranosus*, *Triceps Brachii* and *Psoas major*, located in different parts of the carcass: neck, thigh, shoulder and back respectively, are close to neutrality ($7.14 \geq pH \geq 6.62$). For a temperature varying from 35.5 °C to 24 °C. The *post-mortem* pH of first hours (initial pH) was influenced by :

The muscle type (the *Triceps Brachii* pH is the highest, followed by the *Cleido-mastoid's* one, for the *Semi membranosus* and the *Psoas major* have close values).

Second, the age that has a negative correlation which is highly significant ($r = -0.99$; $p > 0.0001$).

The carcass weight having a negative relationship ($r = -0.98$; $p > 0.0003$).

The sex, pH values are higher for males than females.

The race, the highest pH values have been recorded for animals from the Sahrawi population than the Targui one.

For parameters controlled by humans;

The fasting time, the extension of its duration is at the origin of an increase in the pH value p. m, corresponding to a positive correlation ($r = 0.74$; $p > 0.0007$).

The livestock farming method, low pH values for intensive breeding and higher values for semi-intensive and extensive breeding. *post mortem* pH is an important parameter for the analysis of a number of meat quality characteristics. Therefore, it is necessary to generate the best pH decline estimation for each animal and choose the initial pH to obtain its ultimate optimum.

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Assessment of farmers' vulnerability to climate variability and extreme events: Evidence from dry Kitui, Kenya

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Abstract**—** *Climate variability and extreme events are some of the most pressing environmental challenges occurring in the contemporary world. Farming communities in Sub-Saharan Africa, particularly in arid and semi-arid areas are more vulnerable to climate vulnerability and extremesdue to high dependence on rain-fed agriculture. There is little understanding of factors influencing farmers' vulnerability to climate variability and extremes at household level in arid and semi-arid parts of Kenya. This study analyzed farmers' vulnerability to climate variability and extremes in arid and semi-arid parts of Kitui County, Kenya. The study utilized data collected from 341 households from Yuku, Kaveta, Kauwi and Kasaini sub-locations located in four different agro-ecological zones. Descriptive survey was used and purposive sampling method applied in identifying the sub-locations of study. Proportionate sampling was used to select the number of households to be interviewed in each sub-location and systematic sampling used to select the households which formed the units of analysis. Vulnerability analysis was based on indices constructed from carefully selected indicators for exposure, sensitivity, and adaptive capacity. The indicators were weighted using Equal Weight Analysis. Vulnerability indices indicated that Yuku sub-location (arid) was the most vulnerable to climate variability and extreme events (1.487) followed by transitional zone from semi-arid to semi-humid- Kauwi (0.214), semi-arid- Kasaini (0.085) and semi-humid- Kaveta sub-location (-0.530). Development and policy measures should be focused towards improving the adaptive capacity of the rural farming households, while keeping the post-disaster emergency relief measures in place for zones with higher biophysical vulnerability to climate variability and extremes.*

Keywords**— Exposure, sensitivity, adaptive capacity, and agro-ecological zones.**

I. INTRODUCTION

Natural climate variability and extremes have been identified as major challenges facing human livelihoods globally. Anthropogenic climate change has added a complex new dimension to this challenge. Studies have shown that natural climatic variability, compounded with human induced climate change will adversely affect millions of livelihoods around the world (LVBC, 2011). Worldwide, the livelihoods of 2.5 billion people depend on rain-fed agriculture (FAO, 2016). These small-scale farmers, herders, beekeepers, fishers and forest-dependent communities generate more than half of the global agricultural production and are particularly at risk from climate variability and extremes that destroy harvests, equipment, supplies, livestock, seeds, crops and stored food.

In Africa, climate is warmer than it was 100 years ago and model-based predictions of future human-induced climate change for the continent clearly suggest that this warming will continue and, in most scenarios, accelerate (Christensen *et al*., 2007). Observational records by Hulme *et al*. (2001)

showed that during the 20th century, Africa warmed at a rate of about 0.05°C per decade. Rural households in Sub Sahara Africa (SSA), predominantly those in arid and semi-arid regions are likely to be more exposed and vulnerable to impacts of climate variability and extremes, a situation aggravated by limited knowledge regarding the implementation and effectiveness of current measures taken up to reduce exposure and vulnerability (Spear *et al*., 2015).

In Kenya, the impact of climate change has been more pronounced in the Arid and Semi-Arid Lands (ASALs) which supports 25% of the country's total human population that relies on nearly 75% livestock and crop production. The agricultural sector is a key contributor to Kenya's economic growth. The sector is estimated to contribute at least 25% of the country's GDP (Republic of Kenya, 2005). The productivity of this sector has conversely been compromised by the effects of climate change and variability. In recent years, Eastern Africa (Kenya included), has been known to be prone to climate variability. Anomalous strong rainfall events seem to have increased (Van Oldenborgh *et al*., 2008). The temperatures in these regions have also been increasing considerably due to the changing climate. The situation is exacerbated by increasing climate induced extreme events such as floods, droughts, pests, diseases among others which have resulted in a reduction in the economic activities practiced in such regions (Zoellick, 2009).

Kitui County is one of the ASALs counties in Kenya. A large part of the county receives erratic and unreliable rainfall with most of the areas being generally hot and dry leading to high rate of evaporation (Khisa *et al*., 2014). The county has been experiencing a reduction in food production because of its vulnerability to changing and erratic rainfall pattern which has adversely affected the agricultural sector. Variations in climatic conditions experienced in the county are thought to be responsible for crop failure and changes in food production. Agriculture dependent households particularly, have been and continue to be vulnerable to climate variability and extremes (Khisa *et al*., 2014).

Contemporary research efforts on vulnerability to climate variability and extremes have focused on regional and national assessments of the potential impacts and adaptive capacity of farmers. The current study focused on household level analysis of farmers' vulnerability to climate variability and extremes in different agro-ecological zones.

II. MATERIALS AND METHODS

2.1 The study area

The study area consisted of Yuku, Kaveta, Kauwi and Kasaini sub-locations in Kitui County which represented arid, semi-humid, transition from semi-arid to semi-humid and semi-arid zones respectively (Figure 1). Kitui County is located between longitudes 37º45´ and 39º0´ East and Latitudes 0º3.7´ and 3º0´ South (GoK, 2009b). The county lies between 400m to 1,830m above sea level and generally slopes from west to east. The climate of the area is semi-arid with very erratic and unreliable rainfall.The temperatures range from a minimum of 14-22° centigrade to a maximum of 26-34° centigrade. Rainfall is bimodal distributed within two seasons yearly and varies from 500-1050mm with about 40% reliability.The soil types range from sedimentary rocks, red sandy soils, to clay black cotton soils which are generally low in fertility.

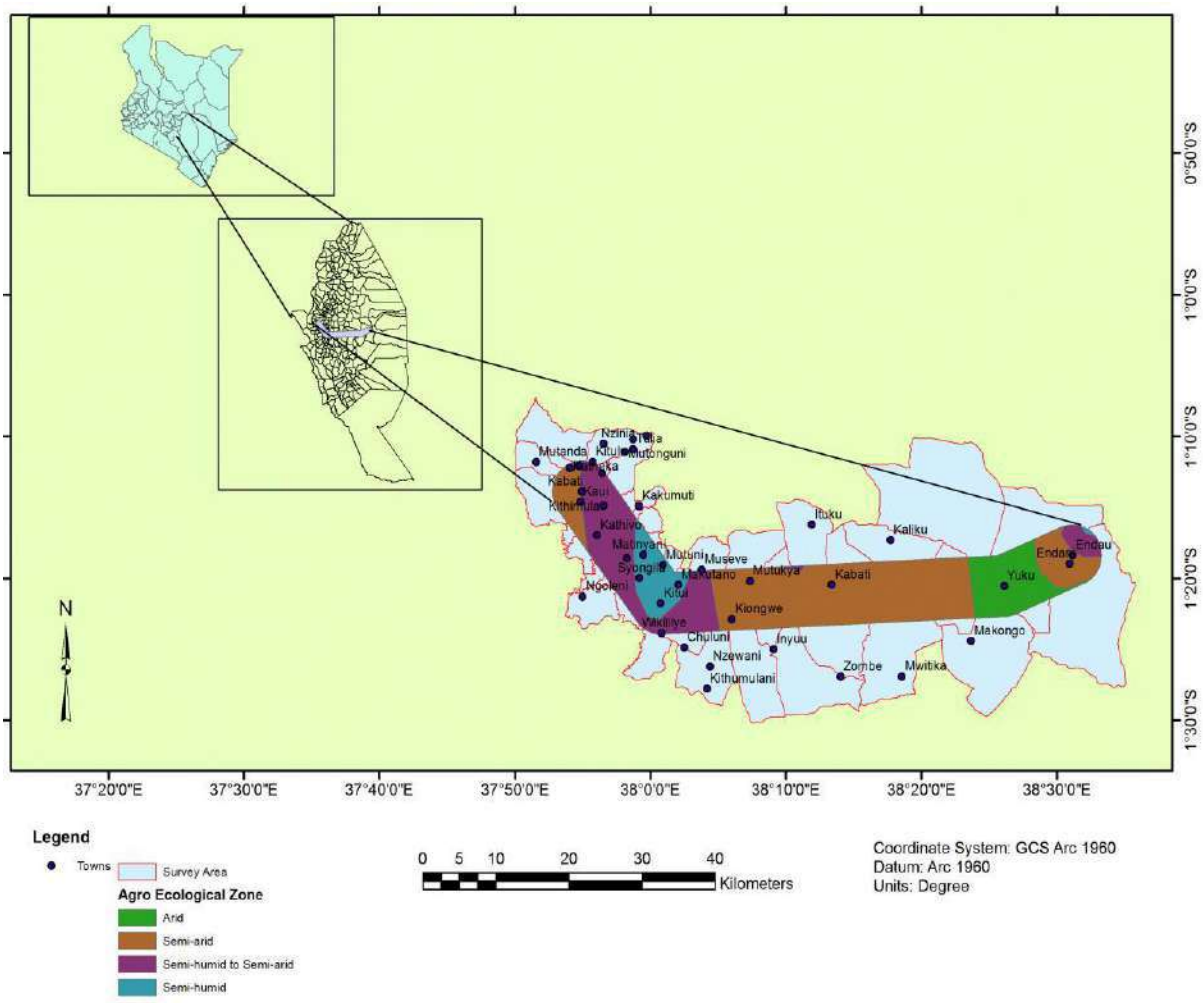

Fig.1: Map of the Study Area

(Source: ILRI GIS database)

2.2 Research design, data collection and data analysis

This study used descriptive survey design. Purposive sampling was used to select the sub-locations while simple random sampling was used to select the households. Households were selected as the main units of analysis because major decisions about adaptation to climate induced stresses and livelihood processes are taken at that level. For this study, four sub-locations were selected namely; Yuku, Kaveta, Kauwi and Kasaini which represented arid, semi-humid, transition from semi-arid to semi-humid and semi-arid zones of Kitui County, respectively. The aim of selecting the four sub-locations was to gain insights into differences in farmers' vulnerability to climate variability and extremes as influenced by the different agro-ecological zones. The sample size for the study was 341 households (Yuku= 39, Kaveta= 104, Kauwi= 160 and Kasaini= 38). Primary data was collected through household interviews and personal observation while secondary data was obtained from desktop studies. Statistical Package for Social Sciences (SPSS version 20) was used to analyze the quantitative data.

2.3 Choosing vulnerability indicators

The steps in constructing the vulnerability index comprised of the selection of indicators for exposure, sensitivity and adaptive capacity, assignment of weights to the indicators and finally aggregating them to form vulnerability index.The selection of suitable indicators for exposure, sensitivity and adaptive capacity was done based on theories that provided insight into the nature and causes of vulnerability complemented by personal observations.

2.4.1 Exposure

The number of extreme climate related natural disasters were taken as indicators of exposure. These included floods, droughts, storms/strong winds, livestock diseases, wild forest fires, community inter-border conflicts and human wildlife conflicts and the data was collected through household survey. It was hypothesized that the higher the number of climate related disasters, the higher the exposure of the agro-ecological zone to climate variability and extremesthus an increasing functional relationship (↑).

2.4.2 Sensitivity

Sensitivity was indicated by the degree to which climate-related stimuli adversely affected land, water resources and household income structure. For the purpose of this study, human fatalities, livestock fatalities, share of natural and non-natural based income, water quantity and property damages (land, houses, roads, trees and crop) due to climate related disasters over a period of ten years were the sub-indicators of sensitivity. This was in line with Luni *et al.* (2012) who while working on vulnerability of rural households to climate change and extremes in the Mid-Hills of Nepal used deaths of family members, loss of properties (land, livestock, and crop) due to climate related disasters and income structure as determinants of household sensitivity. It was hypothesized that higher livelihood impacts of climate related disasters, higher share of natural resource based income, and increased frequency of water sources drying up increases sensitivity thus an increasing functional relationship with vulnerability (↑). On the converse, higher share of non-natural resource based remunerative income sources will reduce the sensitivity thus a decreasing functional relationship with vulnerability (↓).

2.4.3 Adaptive capacity

For this study, indicators for adaptive capacity were based on the DFID sustainable livelihoods framework, where adaptive capacity is taken to be an emergent property of human, social, natural, physical and financial assets possessed by the households (DFID, 1999; Ellis, 2000). Any indicator with a positive relationship with adaptive capacity results to a decreasing functional relationship (↓) with vulnerability while indicators that reduce adaptive capacity ultimately increase vulnerability (↑).

2.5 Construction of vulnerability index

Having selected the indicators of different components of vulnerability and their functional relationship with vulnerability identified, the UNDP's Human Development Index (HDI) (UNDP, 2006) was followed to normalize them. This was done in order to obtain comparable figures free from the units and scales of the indicators. Variables with a ↑ functional relationship with vulnerability were normalized using the formula:

$$X_{ij}=\frac{X_{ij\_Min_{i(X_{ij})}}}{Max_{i(X_{ij})}-Min_{i(X_{ij})}}$$

Where; $X_{ij}$is the value of the indicator j corresponding to region i

$Min_{i(X_{ij})}$is the smallest value of the indicator in all the study areas and $Max_{i(X_{ij})}$is the largest value of the same indicator.On the other hand, normalized score for variables with a ↓ functional relationship with vulnerability were computed using the formula:

$$Y_{ij}=\frac{Max_{i(X_{ij})}-X_{ij}}{Max_{i(X_{ij})}-Min_{i(X_{ij})}}$$

Equal weights were given to all variablesandsimple average of all the normalized scores used to construct the vulnerability index by using the formula:

$VI=\frac{\sum_j X_{ij}+\sum_j Y_{ij}}{K}$where K is the number of used indicators.Vulnerability index of each agro-ecological zone was calculated as:V = E + S –AC, where, V is the vulnerability index, E is exposure index, S is sensitivity index and AC is adaptive capacity index (Ndungu *et al.*, 2015).

For the purpose of this study, the above-namedformula was modified and expressed as: V = PI-AC, where, PI is potential impact index (E+S) and AC is adaptive capacity index.

The vulnerability indices were then used to rank the different agro-ecological zones in terms of vulnerability. A zone with highest index was said to be most relatively vulnerable and was assigned the rank 1while the zone with next highest index was given rank 2 and so on. Negative value of the index did not imply that the agro-ecological zone was not vulnerable at all rather; it meant that the study site was comparatively less vulnerable.

III. RESULTS AND DISCUSSION

3.1 Exposure of farmers to climate variability and extremes in the study area

Close analysis of the results indicated that, number of floods, droughts and livestock diseases over a period of ten years was highest in Yuku (at 1.03, 7.56 and 4.92 respectively) followed by Kauwi (0.72, 6.69 and 3.41), Kasaini (0.37, 4.79 and 2.45) and Kaveta at 0.08, 4.46 and 1.84. There was a

statistically significant difference in the mean values for the number of floods, wild/ forest fires, community inter-border conflicts and incidences of human-wildlife conflictacross the four sub-locations($p<0.05$; Table1).

It is evident that farmers in Yuku (arid) and Kauwi (transitional zone from semi-arid to semi-humid) experienced higher number of climate related natural disasters compared to those in semi-arid and semi-humid zones. The high number of disasters highlighted the zones' exposure to climate variability and extremes due to property and infrastructural damages.This could be attributed to high number of floods, droughts, strong winds, wild/ forest fires, community inter-border conflicts, incidences of human-wildlife conflicts and livestock diseases. The situation was exacerbated by the fact that thearid and transitional zones had shallow soils with low water retention capacity and thus prone to disasters such as floods and droughts.Conversely, farmers in Kaveta (semi-humid) and Kasaini (semi-arid) experienced comparatively lower incidences of the disasters thus reducing their exposure to extreme events. The soils in semi-humid and semi-arid zones are predominantly loam and cotton in nature and thus less prone to disasters such as floods and droughts. Moreover, farmers in Kaveta and Kasaini had better access to extension services and weather information and thus better adapted against the disasters.

Similar findings by Ndungu *et al*. (2015) while working in Mid-hills of Himachal Pradesh in India indicated that increase in natural disasters such as droughts, floods among others lead to property destruction and subsequent exposure of farmers to hazards. In addition, findings by Luni*et al*. (2012) indicated that the number of natural disasters experienced in a locality is a salient component to determine the overall exposure of the locality.

Table 1: Mean values for Indicators of Exposure in the study area

Indicators	**Aggregate (n= 341)**	**Yuku (n=39)**	**Kaveta (n=104)**	**Kauwi (n=160)**	**Kasaini (n=38)**	**P-Value**
Floods	0.41 (1.16)	1.03 (2.07)	0.08 (0.34)	0.71 (1.16)	0.37 (1.65)	0.00**
Droughts	5.90 (9.80)	7.56 (5.39)	4.46 (4.70)	6.69 (13.32)	4.79 (3.97)	0.18
Storms/strong winds	3.82 (8.07)	6.49 (6.76)	2.87 (5.18)	4.11 (10.21)	2.50 (4.10)	0.02**
Wild/forest fires	0.15 (1.40)	0.92 (3.72)	0.11 (0.99)	0.03 (0.25)	0.00 (0.00)	0.00****
Livestock diseases	3.00 (7.74)	4.92 (5.82)	1.84 (3.42)	3.41 (10.35)	2.45 (3.78)	0.14
Community inter-border conflicts	0.62 (2.22)	2.90 (4.72)	0.66 (2.00)	0.16 (0.99)	0.13 (0.81)	0.00****
Human-wildlife conflicts	0.42 (2.04)	3.08 (5.10)	0.04 (0.24)	0.05 (0.22)	0.26 (1.62)	0.00****
Total disasters	14.32 (32.41)	26.90 (71.90)	10.06 (10.24)	14.89 (27.59)	10.50 (10.10)	0.00**

Note: Figures in parenthesis indicate standard deviation

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

3.2.1 Sensitivity of farmers to climate variability and extreme events in the study area

Scrutiny of the results indicated that mean values for human fatalities, livestock fatalities, number of houses damaged, roads and productive land damaged as a result of the climate related natural disasters over a period of ten years were statistically significant ($p<0.05$) across the four sub-locations (Table 2). The results revealed that livelihood damage caused by climate related natural disasters under study was highest in Yuku and lowest in Kaveta sub-location. This could be explained by proximity of Yuku sub-location to Transnational park and Kitui South conservation area thus increasing human-wildlife conflicts. Moreover, neighboring pastoral communities often experience severe droughts leading to invasion into the area in search for pasture and water. This eventually raises tension between the communities thus increasing the incidences of community inter-border conflicts. Further, higher sensitivity of farmers to climate variability and extremes in Yuku can be explained by high incidences of drought and short but very intensive

and destructive rainfall events that are common in thearea thereby contributing to more damages in the arid zone.

The share of natural resource-based income was highest in Yuku followed by Kasaini, Kauwi and lowest in Kaveta sub-location. On the contrary, share of non-natural resource-based income was highest in Kaveta sub-location and lowest in Yuku sub-county. Higher share of natural resource-based income compared to non-natural resource-based income in Yuku and Kasaini could be attributed to the nature of livelihoods in the two zones which were predominantly based on natural-resource based activities notably agriculture, livestock, sand harvesting, handicraft and forestry. The education levels in the two areas were lower compared to Kaveta and Kauwi, thus making it difficult for people from these areas to secure professional jobs out of the usual natural based activities. Moreover, Yuku and Kasaini are quite far from the county headquarters where most non-natural income generating opportunities are found. Conversely, the higher ratio of non-natural resource-based income to natural resource-based income in Kaveta and Kauwi sub-locations could be explained by the nature of livelihoods in the areas. Income generating activities in these areas are primarily non-natural resources based and includeformal employment, non-skilled off farm employment and other trainings. Kaveta and Kauwi are near the County headquarters where formal and non-formal off farm opportunities are available. In addition, education levels in Kaveta and Kauwi were relatively high making it easier for residents to acquire skilled jobs in the County headquarters or even outside the County. Increase in share of non-natural resource-based income had a negative functional relationship with sensitivity while increase in share of natural resource-based income increased farmers' sensitivity as such resources are highly dependent on climate.

Table 2: Mean values for fatalities, damages and income indicators of sensitivity in the study area

Indicators	**Aggregate (n= 341)**	**Yuku (n=39)**	**Kaveta (n=104)**	**Kauwi (n=160)**	**Kasaini (n=38)**	**P-Value**
Human fatalities	5.28(71.64)	45.18(209.94)	0.19(0.78)	0.09(0.45)	0.05(0.32)	0.00**
Livestock fatalities	27.16 (208.42)	204.44 (593.00)	2.24 (4.76)	5.56 (10.23)	4.39 (8.08)	0.00**
Number of houses damaged	0.36 (1.39)	1.87 (3.50)	0.14 (0.53)	0.21 (0.59)	0.03 (0.16)	0.00**
Roads damaged	0.12 (1.10)	0.85 (3.17)	0.03 (0.22)	0.00 (0.00)	0.00 (0.00)	0.00**
Productive land damaged	0.98 (3.71)	4.03 (9.82)	0.28 (0.76)	0.81 (1.71)	0.36 (1.07)	0.00**
Share of total natural resource-based income	47325.20 (98017.40)	60909.00(52706.00)	40054.90 (54726.30)	46828.20 (125985.50)	54471.10(92137.90)	0.68
Share of total non-natural resource-based income	130169.80 (475022.90)	33423.10(58392.80)	164466.30(633050.40)	138441.30(337962.20)	62418.40(100181.70)	0.36

Note: Figures in parenthesis indicate standard deviation

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

Vulnerability of water resources to climate variability was indicated the number of times the water sources had dried up over a period of ten years as indicated in Table 3. The results indicated that the number of times water sources had dried up over a period of ten years were highest in Yuku sub-location and lowest in Kaveta sub-location. (Table 3). This could be attributed by severe waters scarcity in Yuku forcing farmers to frequent their visits to the water sources in search for household and livestock water. Farmers revealed that the water sources dried up twice every year after every rainy season. On the contrary, farmers in Kaveta had alternative water sources such as piped water and thus, they did not visit the water sources frequently. Moreover, Kaveta being semi-humid, the water sources had adequate water and hardly dried up except during rare prolonged droughts.

The study concluded that income structure and impacts of climate related disasters on livelihoods and water quality influenced the overall sensitivity index. The study also

revealed that high share of non-natural based income assists to decrease the overall household sensitivity, while higher share of natural resource-based income makes the household more sensitive to climate change and extremes.

The current trend of results is in consonance with findings of Collier *et al*. (2008) and Davis *et al*. (2007) who reported that off farm income is stable, reliable and less climate sensitive. Similarly Ndungu*et al*. (2015) established a positive relationship between effects of extreme events on physical properties, livestock and land, and sensitivity of the communities to the changing climate.

Table 3: Mean values for indicators of water quantity in water sources in the study areas

Indicators	**Aggregate (n= 341)**	**Yuku (n=39)**	**Kaveta (n=104)**	**Kauwi (n=160)**	**Kasaini (n=38)**	**P-Value**
Number of times the nearest river had dried up (over a period of 10 years)	5.23 (4.59)	5.44 (4.79)	4.31 (4.48)	5.76 (4.53)	5.32 (4.68)	0.04**
Number of times the nearest borehole had dried up (over a period of 10 years)	1.41 (3.37)	2.85 (4.72)	1.53 (3.25)	1.08 (3.04)	1.05 (3.11)	0.03**
Number of times nearest shallow well dried up (over a period of 10 years)	3.45 (4.40)	4.72 (4.85)	2.47 (3.96)	3.69 (4.46)	3.82 (4.49)	0.03**
Number of times nearest spring dried up (over a period of 10 years)	1.95 (3.98	3.87 (5.38)	1.43 (3.45)	1.91 (3.86)	1.58 (3.70)	0.01**
Number of times nearest earth/sand dams dried up (over a period of 10 years)	3.03 (4.35)	4.82 (4.50)	1.73 (3.41)	3.55 (4.65)	2.53 (4.28)	0.00**
Number of times water pans dried up (over a period of10 years)	2.62 (4.90)	4.67 (8.79)	1.99 (3.94)	2.65 (4.23)	2.16 (395)	0.03**
Number of times other sources dried up (over a period of 10 years)	1.21 (3.40)	4.00 (6.10)	1.02 (2.86)	0.87 (2.76)	0.26 (1.62)	0.00**

Note: Figures in parenthesis indicate standard deviation

**indicate significant at 5% level of significance

3.2.2: Potential impact indices in the study area

Potential index was calculated as the sum of exposure index and sensitivity index. The results indicated that exposure to climate variability and extreme was highest in Yuku (exposure index=1.000) and least in Kaveta sub-location (exposure index=0.057; Table 4). The high exposure index in Yuku sub-location could be attributed to high incidences of climate related natural disasters such as floods, droughts, strong winds, forest fires and community inter-border conflicts. On the contrary, low exposure index in Kaveta could be explained by minimal occurrences of the climate related disasters over a period of ten years. The mean value for total disasters was higher in Kauwi compared to Kasaini sub-location as indicated in Table 4.5 thereby resulting to higher exposure in Kauwi sub-location.

Similarly, Yuku sub-location had the highest sensitivity index (0.853) while Kaveta sub-location had the lowest sensitivity index 0.106 (Table 4). High sensitivity in Yuku sub-location could be explained by highmean values for sensitivity indicators particularly human and livestock fatalities as well as houses, productive land and roads damaged by extreme climatic events. The mean value for non-natural resource-based income was lowest in Yuku significantly contributing to the high sensitivity index. Conversely, Low sensitivity in Kaveta sub-location could be attributed to lesser incidences of the sensitivity indicators coupled with highest mean values for non-natural resource-based income which as hypothesized reduced sensitivity of the area to climate variability and extremes. Potential impact index was highest in Yuku, followed by Kauwi, Kasaini and Kaveta sub-location. Despite Kauwi performing better in sensitivity index than Kasaini, its exposure index was comparatively high resulting to higher potential impact index in the sub-location compared to Kasaini (Table 4).

Table 4: Potential impact indices in the study area

		Sub-locations			
	Indicators	**Yuku**	**Kaveta**	**Kauwi**	**Kasaini**
Indicator variables for exposure	Floods	1.000	0.000	0.660	0.310
	Droughts	1.000	0.000	0.720	0.110
	Strong winds	1.000	0.090	0.400	0.000
	Wild/forest fires	1.000	0.120	0.030	0.000
	Livestock diseases	1.000	0.000	0.510	0.200
	Community inter-border conflicts	1.000	0.190	0.010	0.000
	Human-wildlife conflict	1.000	0.000	0.010	0.050
	Sub-total scores	**7.00**	**0.40**	**2.34**	**0.67**
	Exposure Indices	**1.000**	**0.057**	**0.334**	**0.096**
Indicator variables for sensitivity	Human fatalities	1.000	0.003	0.001	0.000
	Livestock fatalities	1.000	0.000	0.016	0.010
	Houses damaged	1.000	0.060	0.098	0.000
	Roads damaged	1.000	0.035	0.000	0.000
	Productive land damaged	1.000	0.021	0.141	0.000
	Natural resource income	1.000	0.000	0.324	0.691
	Non-natural resource income	0.000	1.00	0.199	0.779
	Rivers dried up	0.779	0.000	1.000	0.697
	Boreholes dried up	1.000	0.267	0.017	0.000
	Shallow wells dried up	1.000	0.000	0.542	0.600
	Springs dried up	1.000	0.000	0.197	0.061
	Earth/sand dams dried up	1.000	0.000	0.589	0.259
	Subtotal scores	**10.779**	**1.386**	**2.88**	**3.15**
	Sensitivity indices	**0.853**	**0.106**	**0.240**	**0.315**
	Potential impact indices	**1.853**	**0.163**	**0.574**	**0.411**
	Rank	**1**	**4**	**2**	**3**

3.3.1 Adaptive capacity of farmers to climate variability and extremes in the study area

Examination of results on mean values of adaptive capacity indicators revealed that Kaveta (semi-humid) had the highest asset possession while Kasaini (semi-arid) had the least asset possession among the study sub-locations. Based on results analyzed and presented in Table 5, Kaveta ranked best in three of the asset categories (human, financial and social assets) and second-best in physical assets, thereby scoring the highest in overall adaptive capacity index. Yuku ranked last in terms of human, financial and social assets; first in natural assets and faired averagely in physical assets thus, ranked second in terms of overall adaptive capacity index. On the other hand, Kauwi and Kasaini ranked third and fourth, respectively in terms of overall adaptive capacity index (Table 5).

Based on the results, the primary policy focus in the arid zones particularly Yuku, should be to increase their access to

financial assets and improve human assets which could go a long way in improving other assets categories including social, physical and natural assets. Financial assets enable households to make investment in education and the savings can be used as capital for investments like buying good quality land or buying necessary inputs for cash crop cultivation such as cotton and the emerging "Ndengu (green gram) revolution". However, financial asset in Yuku was found to be very limited contrary to Kaveta due to the remoteness of the area, long distances to the market and fewer opportunities that generate cash income. Development of infrastructure that creates employment opportunities for cash income generation in the area is thus recommended. In addition, educational qualification among interviewed household heads was very low in the arid- Yuku sub-location and this could have negative consequences on their livelihoods. Consequently, low educational qualification could have hindered them from attaining the skills required to make more productive use of the available natural and physical resources. Policies should be geared towards improving the literacy rate of the community, and also towards providing trainings and vocational education for capacity building and skills development, so that they can diversify their livelihoods to more remunerative sources.

The current trend of results is in line with findings of Agnes *et al*. (2017) who established that smallholder farmers in Busia County, Kenya had low financial/economic adaptive capacity, moderate social and institutional knowledge and consequently, diminished informed farming related decision making capacity. The low financial and economic resource was explained by overreliance on climate sensitive rain- fed agriculture largely affected by erratic rainfall in Busia County. Therefore, the low financial and economic capacity among the smallholder farmers in Busia County reflected their limited ability to deal with and adapt to climate change effects. This affected smallholder farmers' ability to plan, prepare for, facilitate and implement adaptation measures. Similarly, Simotwo *et al*. (2018) reported that education levels, dependency ratio and farm sizes had positively significant association with farmers' adaptive capacity in Trans-Mara East, Kenya. The authors further indicated that there was a positive relationship between individual's marital status and diversity of livelihood streams and their adaptive capacity.

Table 5: Mean values for Indicators of Adaptive Capacity in the study area

	Indicators	Aggregate (n= 341)	Yuku (n=39)	Kaveta (n=104)	Kauwi (n=160)	Kasaini (n=38)	P-Value
Physical asset	Number of extension services (last 1 year)	0.53 (1.48)	0.59 (1.09)	0.95 (2.35)	0.33 (0.81)	0.18 (0.46)	0.00***
	Number of sources of timely weather information	1.23 (1.00)	1.18 (0.89)	1.47 (1.23)	1.09 (0 85)	1.24 (1.03)	0.03**
	Distance to nearest motorable road(KM)	1.51 (6.27)	2.53 (3. 32)	2.28 (10.90)	0.98 (1.90)	0.62 (0.88)	0.22
	Distance in Km to the nearest market	2.79 (3.24)	4.83 (7.36)	2.35 (1.58)	2.53 (2.32)	3.00 (2.37)	0.00**
	Distance to nearest Water source(KM)	1.49 (2.41)	4.23 (5.53)	1.21 (1.20)	1.10 (1.30)	1.04 (1.22)	0.00**
	Distance to nearest health facility(KM)	3.51 (3.75)	8.31 (7.44)	2.62 (2.01)	2.92 (2.59)	3.50 (2.08)	0.00***
Human asset	Highest number of formal schooling years	12.43 (4.42)	10.85 (3.98)	13.07 (4.74)	12.40 (4.18)	12.42 (4.68)	0.02**
	Number of persons in the family with salaried jobs	0.64 (1.07)	0.44 (0.85)	0.91 (1.31)	0.50 (0.85)	0.66 (1.26)	0.01**

	Trainings/vocational courses in the family	0.57 (0.97)	0.44 (0.85)	0.83 (1.19)	0.45 (0.85)	0.50 (0.76)	0.01**
	Farming experience by household head	25.63 (16.55)	19.28 (12.20)	26.59 (15.35)	26.42 (17.64)	26.16 (17.99)	0.04**
Natural asset	Size of productive land (acres)	4.35 (5.71)	8.72 (10.83)	2.34 (2.77)	4.72 (5.09)	3.80 (3.67)	0.00**
	Size of unproductive land (acres)	1.20 (4.30)	6.00 (10.70)	0.62 (1.78)	0.56 (1.82)	0.57 (1.21)	0.00**
	Small livestock	5.74 (8.81)	8.56 (11.17)	2.50 (3.12)	7.13 (10.40)	5.84 (6.75)	0.00**
	Large livestock	2.42 (3.50)	6.15 (6.85)	1.26 (1.74)	2.32 (2.74)	2.13 (2.22)	0.00**
Financial asset	Gross household income /month	20513.80 (53271.60)	13095.50(19727.90)	26453.40 (68048.00)	19583.50 (52978.30)	15944.70 (24359.50)	0.51
	Household savings /month	2161.60 (7275.60)	843.80(2516.00)	2933.00 (9593.90)	2121.40(6440.50)	1592.10(6532.50)	0.45
	Total annual earnings from livelihood strategies	165687.3 (415236.4)	127039.30(115022.8)	189789.10 (477960.40)	149416.10(344170.2)	208534.40(648955.60)	0.72
Social asset	CBO memberships (number)	8.06 (38.62)	2.57 (4.00)	17.67 (64.50)	2.79 (4.41)	3.82 (6.89)	0.21
	Cooperative society memberships (number)	7.82 (3819.31)	1.00 (0.00)	4.16 (14.15)	1.70(5240.59)	3.71 (364.80)	0.69
	Credit facilities accessed /last 5 years	1414.86 (9482.25)	1282.26 (6561.2)	1951.94 (8895.1)	1896.63(11175.1)	1789.84 (4866.5)	0.84

Note: Figures in parenthesis indicate standard deviation

**indicate significant at 5% level of significance

3.3.2 Adaptive capacity indices in the study area

Examination of the results established that Kaveta had the highest adaptive capacity index (0.693)followed by Yuku sub-location (0.366; Table 6). Despite Kaveta sub-location having highest indices for physical assets, human assets and majority of financial and social assets, the sub-location had lowest values for natural assets. Yuku sub-location ranked second as regards adaptive capacity index owing to the high values for natural assets such as size of land, size of productive land and number of small and large bullocks owned. However, Yuku had the lowest human, physical, financial and social assets.

Table 6: Adaptive capacity indices in the study area

Indicator variables for adaptive capacity	Sub-locations	Yuku	Kaveta	Kauwi	Kasaini
Physical assets	Gadgets owned	0.026	1.000	0.322	0.000
	Reliable weather information sources	0.237	1.000	0.000	0.395
	Nearest market	1.000	0.000	0.073	0.262
Human assets	Schooling years	0.000	1.000	0.698	0.707
	Family salaried jobs	0.000	1.000	0.128	0.468
	Trainings/vocational in family	0.000	1.000	0.026	0.154
	Farming experience	0.000	1.000	0.977	0.941
Natural assets					
	Productive land	1.000	0.000	0.373	0.229
	Unproductive land	1.000	0.011	0.000	0.002
	Small livestock	1.000	0.000	0.764	0.551
	Large livestock	1.000	0.000	0.217	0.178
Financial assets	Gross income	0.000	1.000	0.486	0.213
	Household savings	0.000	1.000	0.612	0.358
	Earnings from livelihood strategies	0.000	0.770	0.275	1.000
Social assets	Access to extension services	0.532	1.000	0.195	0.000
	CBO memberships	0.000	1.000	0.015	0.088
	Access to credit facilities	0.424	1.000	0.9552	0.000
Sum of scores		6.219	11.781	6.112	5.546
Adaptive capacity index		0.366	0.693	0.360	0.326
Rank		2	1	3	4

3.4 Overall vulnerability indices in the study area

The results in Table 7 indicated that among the selected study sites, Yuku sub-location ranked the most vulnerable to climate variability and extremes (1.487) while Kaveta sub-location was the least (-0.530). Despite Kauwi having relatively higher adaptive capacity and lower sensitivity index compared to Kasaini, it still ranks the second most vulnerable sub-location owing to its high exposure index. In spite of having lower adaptive capacity than Kauwi sus-location, Kasaini sub-location ranked better in overall vulnerability as it faced less exposure. The two least vulnerable sub-locations have the least exposure to climate variability and extremes. However, higher sensitivity coupled with low adaptive capacity results to higher vulnerability in Kasaini compared to Kaveta.

The results are in consonance with findings of Luni*et al.* (2012) who established that the most vulnerable households are always the ones with the lowest adaptive capacity, highest exposure and sensitivity indices irrespective of the locality. Thus, improving the adaptive capacity of these vulnerable households will also invariably reduce their sensitivity and finally decreases their overall vulnerability. Agro-ecological zones' comparison of vulnerability showed that despite having higher adaptive capacity, such capacity

may not be fully realized in the face of higher exposure and sensitivity (Ndungu*et al.*,2015).

Table 7: Vulnerability Indices in the study area

Sub-locations	Exposure index	Sensitivity index	Potential impact index	Adaptive capacity index	Vulnerability index	Rank
Yuku	1.00	0.853	1.853	0.366	1.487	1
Kaveta	0.057	0.106	0.163	0.693	-0.530	4
Kauwi	0.334	0.240	0.574	0.360	0.214	2
Kasaini	0.096	0.315	0.411	0.326	0.085	3

IV. CONCLUSION AND POLICY AND RECOMMENDATIONS

The present study established that farmers in arid agro-ecological zone were the most vulnerable to climate variability and extremes while those in semi-humid zone were the least vulnerable. In addition, the study revealed that biophysical elements determining exposure to climate variability and extremes like temperature, rainfall and climate related natural disasters were beyond the immediate influence of the policy makers. Therefore, amongst the three components of vulnerability, adaptive capacity was found to have direct policy implications. Further, improving the adaptive capacity also had indirect implications on improving the sensitivity of the farmers. For this reason, enhancing adaptive capacity through creating opportunities for off-farm income, improving infrastructure for community development, provision of post-disaster relief measures and establishment of early warning systems would go a long way in reducing vulnerability to climate variability and extremes in Kitui County.

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Investigating Pesticide Use and Management in King Orange (*Citrus nobilis*) Farming in Vung Liem District, Vinh Long Province, Vietnam

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Abstract— *The study was conducted to evaluate the current situation of pesticide use on the model of growing king orange (Citrus nobilis) in Hieu Nghia commune, Vung Liem district, Vinh Long province, Vietnam thereby assessing the potential effects of the pesticide use on health and the environment by direct interviewing 60 farmers growing king orange. Information on the potential effects of the pesticide use is retrieved from available scientific databases of chemical toxicity. The results showed that 100% of the households use pesticides during cultivating king orange in which 60% of the farmers using chemical pesticides, 36.7% using biological ingredients, 36.7% and 3.3% using pesticides with unknown sources. The interview results showed that the households used 6 pesticides with toxic group I; 53 pesticides with toxic group II; 22 pesticides with toxic group III and 40 pesticides with toxic group IV. There were 60% of the households used pesticide dosage according to the instructions on the package; 26.7% of the households used more than recommended dosage and 13.3% households used less than recommended dosage. The number of the farmers with clear understanding of the harmful effects of plant protection chemicals accounted for 5.0%, with knowledge accounting for 48.3%, with little knowledge of 31.7% and ignorance at 15%. Regarding the methods of handling pesticides packages after use, there were 45% of the farmers treated the wastes by disposing of in the fields, 30% selling to vendors and 25% burning the wastes. With the current knowledge and practice of using and managing pesticides, the risk to the environment and the health of the people is very serious. Health, environment and agriculture agencies should take measures to support people in protecting health, managing wastes generated from pesticides use to reduce risks to health and the environment.*

Keywords— ***king orange, active ingredients, environment, health, Vung Liem, Vinh Long.***

I. INTRODUCTION

The Mekong River Delta (Mekong Delta) has about 307,000 ha of fruit trees, giving an annual yield of about 3.5 million tons of fruit; of which over 120,000 hectares were planted with special crops such as durian, Hoa Loc mango, Lo Ren milk fruit, Nam Roi pomelo, green skin pomelo, king orange, rambutan. Vinh Long is located between Tien River and Hau River, and also belongs to the arterial traffic route connecting the Southeast region with the Mekong Delta, so most of the province's urban districts are next to the large waterway - land transport system, relatively close distance, on average 18 km and the city furthest from the center of the province only about 45 km. The potential for developing peri-urban agriculture is huge, equivalent to about 60% of the province's agricultural land. King orange (Citrus nobilis) is one of the main fruit trees of our country, grown in Vinh Long, Hau Giang and Soc Trang provinces. Particularly in Vinh Long, king oranges have been grown for a long time due to high economic efficiency. In recent years, many households have converted their economically inefficient rice land to cultivated king oranges. The area of cultivated

king oranges in Vinh Long province as of 2018 was 9,804 hectares, with an output of 105,022 tons/year, concentrated in Tra On, Tam Binh, Vung Liem districts. Vinh Long province has nearly 8,000 hectares of cultivated king oranges. This is a specialty fruit tree with high economic efficiency, planned by the provincial agriculture sector into specialized farming areas. Hieu Nghia Commune, Vung Liem District, Vinh Long Province is considered a golden land for growing king oranges because the soil is quite suitable, the tree grows well, and the yield is high. The total area of king oranges on field land in Hieu Nghia commune (Vung Liem) is now more than 371 ha, accounting for the most out of 502 ha of cultivated oranges in 9 communes with king orange in the district's field land (Department of Agriculture and Rural Development in Vung Liem district, 2019).

Recently, in addition to farming techniques, farmers need to manage pests and diseases on king orange to protect the yield and quality of king orange. Pests and diseases often cause very heavy harm, losing commercial value of the fruit and reducing yield. Thus, it reduces profits for growers of king orange. Currently, farmers who grow king orange tend to use more plant protection chemicals. There have been many studies on the use of pesticides on rice cultivation (Toan, 2011) and vegetable cultivation (Nga and Viet, 2010; Chau et al., 2019) and showed the uses of pesticides can pollute water sources (Binh, 2008; Hoai et al., 2011; Nhan, 2018). Former study by Cong et al. (2008) showed that the presence of pesticides in water can affect fish and even human health (Margni et al., 2002). Up to now, very little information on the current status of pesticide use on the king orange model has been reported. The study was conducted to investigate the current status of pesticide use on the king orange growing model in Hieu Nghia commune, Vung Liem district, Vinh Long province, thereby assessing potential impacts on the environment and health. of people cultivating king oranges.

II. MATERIALS AND METHODS

Data in this study were collected through field surveys and interviews with 60 king orange farmers in Vung Liem district, Vinh Long province. The questionnaire was designed in a semi-structured form to collect information about pesticides types, dosage, frequency, and understanding of the harmful effects of pesticide uses, and methods of handling the pesticide packages after use. Potential effects of pesticides on the environment and health are assessed through the type of pesticides used, the dosage, frequency, and method of treatment of the pesticide wastes after use. The effects of pesticides were also assessed using the toxicological information of the active substance found to the organism. On the basis of analysis of the current situation, a number of recommendations will be proposed to minimize the harmful effects of pesticides use on the environment and humans.

III. RESULTS AND DISCUSSION

3.1 General information of the interviewees

Results of interviewing 60 households of king orange farming showed that 73.35% of whom were male and 26.7% were female were of working age of 30-50 years old. There were 100% of the main labor force in this household is male. These men are the ones directly involved in the cultivation and use of plant protection pesticides. The education levels of people interviewed with primary, secondary, high-school and college degrees were 56.7%, 25%, 15%, and 3.3%, respectively. No interviewee was illiterate or had a university degree. The number of people residing in the study area for more than 5 years accounted for 93.3% while the number of people residing under 5 years was 6.7%. Through the interview results, it showed that 33.3% of the households have cultivated land area over 5000-10,000 m^2; 26.7% of the households have cultivated land area from 1,000-5,000 m^2. According to the interview results, 100% of the households have main income source from growing king oranges. In addition, local people earn additional income from other occupations, for example 65% grow vegetables (65%), livestock (17%), freelance (10%), and civil servants and small businesses (8.3%). The number of interviewees who have experience growing king oranges for more than 10 years, 5-10 years, less than 5 years and just started growing king oranges were 18.3%, 46.7%, 21.7% and 13.3%, respectively.

3.2 Current status of pesticide use in cultivation of king orange farming model

The results showed that cultivating oranges have to use pesticides continuously to maintain the yield and quality of king orange. If farmers do not use pesticides, the tree would be susceptible to disease, the fruit would significantly fall, grow slowly, the orange appearance would not be beautiful and thus, influencing productivity and profitability. There were 60% of the households using chemical pesticides to prevent and treat pests and diseases for king orange trees and 36.7% of the households use biological pesticides. The study results also showed that 60% of the households used pesticide doses according to the instructions; 26.7% of the households used more than the dosage specified in the instructions for using pesticides and 13.3% of the households used less than the dosage

specified in the instructions for using pesticides. The number of sprays was from 2 to 6 times per crop. The results of the study showed that there were currently 120 commercial names of pesticides with 73 active ingredients used. There were 6 types of pesticides belonging to group I; 53 pesticides of toxic group II; 22 pesticides in toxic group III and 40 pesticides in toxic group IV. The following information regarding toxicity of active ingredients found in the king orange farming model could indicate that the pesticides residuals could have huge impact on the environment and human health.

3.2.1 Acephate

Acephate has moderate acute toxicity in mammals. Evidence of carcinogenicity is limited to liver tumors that occurred in rats fed high doses of acephate over several months, and U.S. EPA considers acephate to be a possible human carcinogen (U.S.EPA, 2012). Humans and animals absorb acephate into the body quickly when it is eaten, breathed in, or gets on the skin. In animal studies, acephate was absorbed into the blood and went to skin, liver, kidneys, and heart.In humans and other mammals, about three quarters of the acephate moves through the body unchanged. Some is broken down into smaller chemicals, and a very small amount converts to methamidophos. In rats, most acephate was cleared through the urine, with small amounts cleared through the feces or exhaled breath. Acephate clears the body quickly; less than one percent of the acephate remained in rats three days after eating it.

3.2.2 Chlorpyrifos Ethyl

Chlorpyrifos is an organophosphate pesticide used on crops, animals, and buildings, and in other settings, to kill a number of pests, including insects and worms. It acts on the nervous systems of insects by inhibiting the acetylcholinesterase enzyme. Chlorpyrifos was patented in 1966 by Dow Chemical Company. Chlorpyrifos is considered moderately hazardous to humans by the World Health Organization based on its acute toxicity. Exposure surpassing recommended levels has been linked to neurological effects, persistent developmental disorders, and autoimmune disorders. Exposure during pregnancy may harm the mental development of children, and most home uses of chlorpyrifos were banned in the U.S. in 2001.In agriculture, it is one of the most widely used organophosphate insecticides in the United States, and before being phased out for residential use it was one of the most used residential insecticides. For acute effects, the World Health Organization classifies chlorpyrifos as Class II: moderately hazardous. The oral LD50 in experimental animals is 32 to 1000 mg/kg. The dermal LD50 in rats is greater than 2000 mg/kg and 1000 to 2000 mg/kg in rabbits. The 4-hour inhalation LC50 for chlorpyrifos in rats is greater than 200 mg/m3. Acute poisoning results mainly from interference with the acetylcholine neurotransmission pathway, leading to a range of neuromuscular symptoms. Relatively mild poisoning can result in eye watering, increased saliva and sweating, nausea and headache. Intermediate exposure may lead to muscle spasms or weakness, vomiting or diarrhea and impaired vision. Symptoms of severe poisoning include seizures, unconsciousness, paralysis, and suffocation from lung failure.

Among freshwater aquatic organisms, crustaceans and insects appear to be more sensitive to acute exposure than are fish. Aquatic insects and animals appear to absorb chlorpyrifos directly from water rather than ingesting it with their diet or through sediment exposure. Acute exposure to chlorpyrifos can be toxic to bees, with an oral LD50 of 360 ng/bee and a contact LD50 of 70 ng/bee. Risk assessments have primarily considered acute exposure, but more recently researchers have begun to investigate the effects of chronic, low-level exposure through residue in pollen and components of bee hives. A review of US studies, several European countries, Brazil and India found chlorpyrifos in nearly 15% of hive pollen samples and just over 20% of honey samples. Because of its high toxicity and prevalence in pollen and honey, bees are considered to have higher risk from chlorpyrifos exposure via their diet than from many other pesticides.

3.2.3 Diazinon

Overall, diazinon is fairly low in toxicity when inhaled, ingested or when it gets on the skin, so minor exposures are not likely to cause severe symptoms. However, small exposures to diazinon can cause mild skin or eye irritation. Diazinon generates high acute toxicity to a wide variety of aquatic organisms, leading to a wide range of sublethal biochemical effects, damage to specific target organs and tissues, and adverse ecological impacts. The toxicity of diazinon has extensively studied in fish and crustaceans, and it has been reported to be moderately toxic to early life stages of zebrafish. The 96 h LC50 values ranging from 0.32 to 1.53 mg/L for larval and from 2.2 to 10.3 mg/L for adult of several fish have already been recorded.

3.2.4 Abamectin

Toxicity of abamectin is oral but there are some contact activities. Intoxication manifestations include mydriasis, vomiting, tremor, seizure, partial ptosis, confusion, and coma. Mild intoxications manifest symptoms such as nausea, vomiting, diarrhea, and weakness.As all macrocyclic lactones, abamectin acts as

agonist of the GABA (gamma-aminobutyric acid) neurotransmitter in nerve cells and also binds to glutamate-gated chloride channels in nerve and muscle cells of invertebrates. In both cases it blocks the transmission of neuronal signals of the parasites, which are paralyzed and expelled out of the body, or they starve. It also affects the reproduction of some parasites by diminishing oviposition or inducing an abnormal oogenesis. In mammals the GABA receptors occur only in the central nervous system (CNS), i.e., in the brain and the spinal cord. But mammals have a so-called blood-brain barrier that prevents microscopic objects and large molecules to get into the brain. Consequently, macrocyclic lactones are much less toxic to mammals than to parasites without such a barrier, which allows quite high safety margins for use on livestock and pets. A notable exception to this is dog breeds that carry the MDR-1 gene defect.

3.2.5 Trichlorfon

Trichlorfon is moderately to highly toxic to birds, and highly toxic to fish and aquatic invertebrates. Toxicity varies considerably depending on temperature, pH and water hardness. As a general rule, toxicity increases with temperature and pH. Trichlorfon is a synthetic organochlorophosphate compound and organophosphate acetylcholinesterase inhibitor that is used as a pesticide. It is characterized as a white crystalline or colorless to yellow or pink waxy solid, and exposure occurs by inhalation, ingestion, or contact. Marketed since the 1950s, trichlorfon is used to protect field and fruit crops, to control forest insects, and to control internal and external parasites in domestic and farm animals. Trichlorfon, formulated as metrifonate, has also been used for the pharmacological treatment of Schistosoma haematobium in millions of patients. The compound is under investigation as a treatment for Alzheimer's disease. A review of environmental levels and human exposure concentrates on the significance of residues detected in crops, the milk of treated cows, and other food items as possible sources of exposure for the general public. Noting that detected levels are far below the established acceptable daily intake, the report concludes that trichlorfon does not constitute a health hazard for the general population. Concerning effects on environmental organisms, the report cites evidence that trichlorfon is moderately toxic for fish and birds, and moderately to highly toxic for aquatic arthropods, supporting the conclusion that this insecticide should never be sprayed over water bodies or streams.

3.2.6 Fenitrothion

The data provided by Sumitomo outlined the toxicology (acute, irritation and sensitization and sub-acute to chronic), mutagenicity and ecotoxicological profile ofthe fenitrothion technical material.Acute toxicity was low in Rats, Rabbits, Guinea Pigs, in both males and females in general toxicity and neurotoxicity terms, as shown by the LD_{50}, LC_{50}, NOELs and NOAELs. The Hershberger assay in Rats showed no anti-androgenicity or androgenicity. Fenitrothion is unlikely to pose a carcinogenic risk to humans. Fenitrothion was not genotoxic in a range of studies in vitro and in vivo. Fenitrothion is not persistent in soil and leaching is not significant. Therefore there is negligible risk to following crops or of groundwater contamination. Volatilization is a significant dissipative process in the environment although, once in the vapourphase, fenitrothion is short-lived. Transport to surface water via spray drift poses a risk to aquatic species, although the duration of exposure is brief because fenitrothion dissipates in microbially active natural water systems with a half-life ofless than one week. The compound will also tend to migrate to sediment. Health risks for avian and mammalian species following the consumption of treated vegetation, grain and contaminated insects are likely to be low. The low long-term risk to insectivorous species and birds grazing on treated grassland is not likely to arise due to:(i) the high acute toxicity of fenitrothion to insects, preventing residue build-up in this food source; (ii) the generally low persistence of fenitrothion in the environment; and(iii) the rapid decline of residues in species forming the diet. Aquatic organisms (fish and invertebrates) are potentially at risk, especially in the event of overspray to static water bodies. However buffer zones appropriate to eachcrop and monitoring wind direction to prevent spray drift should ensure that aqueous concentrations remain below the environmentally acceptable concentrations even inthe event of multiple applications. Although fenitrothion is fat-soluble, the rates of biotransformation and excretion of metabolites largely mitigate bioconcentration. Label warnings are intended to minimise the risks to fish. The risks to algal species, earthworms, soil micro-organisms and sewage bacteria

are considered to be low, even in worst-case scenarios, without taking into consideration the rapid dissipation processes that occur in the environment. Fenitrothion is extremely toxic to honeybees and highly toxic to non-target arthropods. However, it does not have growth inhibitory activity and the effects of treatment are relatively short-lived. Most beneficial insect populations would recover quite rapidly. Label warnings are intended to minimise the risks to honeybees.

Soil microbiological processes are generally unaffected by use of fenitrothion on agricultural land. It is used in agriculture and for public health use. An

acceptable daily intake (ADI) of 0-0.005 mg/kg body weight was established in2000. The IPCS hazard classification of fenitrothion is: moderately hazardous, class II.

3.2.7 Mancozeb

Mancozeb (MCZ) is one of the most used fungicides worldwide, despite its ancient use and toxicity demonstrated in different taxa. Its primary mechanism of toxicity is via skin contact, leading to contact dermatitis and dermal sensitization. Mancozeb has also been shown to have teratogenic and reproductive effects. Mancozeb exposure also alters the reproductive and endocrine structures, leading to decreased fertility. The potential of mancozeb to induce chromosomal damage in somatic cells has been investigated in four micronucleus tests in rats and mice and two cytogenetics studies in rats and mice. The toxicity profiles for lethal and sublethal effects were performed. At all exposure times, MCZ was more toxic to embryos, for instance, NOEC 504 h were 0.01 and 0.05 mg MCZ/L for embryos and larvae, respectively. Thus, embryo sensitivity was 5-fold higher than larvae. A Teratogenic Index of 14 indicated the significant teratogenic potential of this fungicide. Among sublethal effects, embryos exhibited a wide range of abnormalities with high incidence.

3.2.8 Glyphosate

Glyphosate is a widely used herbicide. Glyphosate is one of the most widely used herbicides with applications in agriculture, forestry, industrial weed control, lawn, garden, and aquatic environments. Sites with the largest glyphosate use include soybeans, field corn, pasture and hay. It can cause gastrointestinal corrosive symptoms and due to the surfactant, that is present a severe metabolic acidosis, hyperkaliemia and cardiovascular collapse can occur. The toxicity of glyphosate is largely due to the surfactant and other co-formulants. Glyphosate is low in toxicity to rats when ingested. The acute oral LD50 in rats is greater than 4320 mg/kg. Glyphosate is low in toxicity to rabbits when applied to the skin. The acute dermal LD50 in rabbits is greater than 2 g/kg. Glyphosate is low in toxicity for eye irritation and very low in toxicity for dermal irritation. In studies with glyphosate manufacturing use products, researchers observed mild eye irritation in rabbits that cleared in seven days. Glyphosate is very low in toxicity to rats when inhaled. The acute inhalation LC50 in rats is greater than 4.43 mg/L based on a 4-hour, nose-only inhalation study.

3.2.9 Zinc Phosphide

Zinc phosphide has been used widely as a rodenticide. Upon ingestion, it gets converted to phosphine gas in the body, which is subsequently absorbed into the bloodstream through the stomach and the intestines and gets captured by the liver and the lungs. Phosphine gas produces various metabolic and nonmetabolic toxic effects. Clinical symptoms are circulatory collapse, hypotension, shock symptoms, myocarditis, pericarditis, acute pulmonary edema, and congestive heart failure.

3.3Current status of pesticide packaging and bottles management after use in the study area

The interviewing results showed that there were 45% of the households that treated pesticide bottles and packaging after use by immediately discarding in the fields. As previously discussed, the pesticides are high toxic therefore the indiscriminate disposal of pesticide packaging and bottles after use in the garden could result in several negative consequences, for example, directly affecting the health of users and the whole community and environmental pollution. There were 30% of the households handled pesticide bottle and packaging after use by selling, whereas there were 25% of the households treated pesticide bottles and packaging after use by burning. The collection and handling of pesticides bottles and packaging after use were improper. Several households bury or burn the bottles and packaging of pesticides after use at the same time with domestic wastes. This could lead to seriously affect the air, soil, water environment and especially the health of the people. The indiscriminate discharge of pesticides in the environment could pose a great threat to surrounding environment and human health. More than 65% of the respondents confirmed that they discard the packaging and bottles of pesticides after use right at the places where the pesticides were sprayed. This behavior is actually causing the environment in rural areas to degrade rapidly. In addition, the pesticides were abused that could lead to the accumulation of pesticides in the agricultural products. The study also found that there were 60% of the households uses pesticide dosage according to the instructions on the package or bottles; 26.7% of the households used more than recommended dosage and there were 13.3% the households used less than recommended dosage. The number of people with clear understanding of the harmful effects of plant protection chemicals accounted for 5.0%, with knowledge accounting for 48.3%, with little knowledge of 31.7% and with ignorance at 15%.

The results of the interviews showed that the households accessing information on pesticides use via television and newspapers accounted for 11.7% and 15% respectively. Accessing to information through agricultural extension officers accounted for 33.3% and through

pesticides dealers occupied 40%. Besides, the rate of people accessing information through cooperatives in coordination with local authorities was 40.5%. Thus, there are many information channels for people to learn about the use of pesticides. However, the majority of people use pesticides in the wrong way, at the wrong time. The purchase of pesticides is mainly based on advertising information from many small agents, so the origin and quality were not ensured. The use of labor protection in the pesticides spraying process is too sketchy, only using a mask during the spraying process. Up to 30% of interviewed people still eat and drink while spraying pesticides. The results implied that the use of pesticides by king orange growing farmers is highly risk to health and environment.

IV. CONCLUSION

The findings of the present study showed that king orange growing model uses pesticides continuously from planting to harvest. The king orange farmers have used 6 pesticides with toxic group I; 53 pesticides with toxic group II; 22 pesticides with toxic group III and 40 pesticides with toxic group IV. There were 60% of the households uses pesticide dosage according to the instructions on the package or bottles; 26.7% of the households used more than recommended dosage and there were 13.3% the households used less than recommended dosage. The number of people with clear understanding of the harmful effects of plant protection chemicals accounted for 5.0%, with knowledge accounting for 48.3%, with little knowledge of 31.7% and ignorance at 15%. Information on pesticides was accessed from television, newspapers, extension workers, plant protection agents, and the coordination between local authorities and cooperatives. Bottles and packages after use were treated by directly discharged at the fields (45%), sold to the vendors (30%), and burned (25%). People only used masks when spraying pesticides and up to 30% of people interviewed eat and drink during the spraying process. The findings demonstrated that the current knowledge and practice of using and managing pesticides is not good. The risks to the environment and the health of the people caused by the pesticides are very serious. Local authorities need to take measures to raise qualifications and awareness of safe and sustainable use of pesticides in the study area.

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Determinants of Level of Accessibility to Microfinance among Loan beneficiaries of some Commercial Crop Production Farmers in Niger state, Nigeria

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Abstract**—** *The study analyzed the determinants of some selected commercial crop farmers' accessibility to microfinance services in Niger state, Nigeria. Multistage random sampling technique was used in selecting respondents who were beneficiaries of Microfinance Institutions (MFIs) spread across the 3 agricultural zones in the state; from which primary data were collected using questionnaires. A total of 185 crop farmers who are beneficiaries of Microfinance Institutions (MFIs) were used in the study. Method of data analysis used was means, frequencies, percentages, and double hurdle analysis. The result revealed that gender, age, education, household size, income, farm size and farming experience are the socio-economic variables influencing commercial crop farmers' access to MFIs. The accessibility of microfinance to crop farmers was found to be determined by household and loan characteristic of the farmers. It showed that there was a significant difference in the total income, farm capital, land size, household size and education level between the two groups of farmers but no significant difference in their age, marital status, farming experience and output level. It was also found out that age, farm size, income, education and household size were factors that significantly affect access to credit. Equally, age, farm size, marital status, cost of loan, education level and farming experience was found to have significant influence on loan size. The study also found that majority of the loan beneficiaries [70%] borrowed above ₦100,000.00, the average loan borrowed was ₦145,166.67 at an average interest rate of 15.16% for 10months. Based on the findings of this study, group borrowings should be encouraged by the farmers. In other to facilitate credit access, crop farmers should form groups or cooperatives because financial institution will prefer lending credit to groups than individuals. It is therefore recommended that government policies can capitalize on the socio-economic variables in this study as veritable tools to encouraging accessibility to MFIs.*

Keywords**— Accessibility, Microfinance Institutions (MFIs), Crop farmers, Credit.**

I. INTRODUCTION

Access to finance is a necessity when it comes to investing in economic activities so as to ensure production and growth (Nosiro, 2010). However, lack of access to credit facilities is almost universally indicated as a key problem for small and micro enterprises. In most cases, even where credit is available mainly through banks, the entrepreneurs may lack freedom of choice because the banks' lending conditions may force the purchase of heavy, immovable equipment that can serve as collateral for the financial institution. Access to affordable agricultural credit enables farmers, who constitute the majority of the population in most developing countries, to adopt new technology and take advantage of new

economic opportunities to increase production and income (Gyau and Oduol, 2016).

Despite the recent growth in the microfinance sector, advancing loans and credit to farmers to increase crop production is still a challenge (Tenaw and Islam, 2009). Miller (2011) reports that in order for microfinance organizations to venture into crop agriculture, it is important to understand the context of crop agriculture and their potential role in it. Indeed, agricultural microfinance is not business as usual but requires a different approach from that typically applied in many microfinance organizations. The agricultural sector is characterized by generally much lower returns on capital, slower velocity of capital, higher uncontrolled risks and less understanding of finance and business (Miller, 2011). Also, although it is argued that improved productivity and output levels will be achieved through the introduction of new production technology, credit is a prerequisite to gain access to such technology particularly for the small-scale farmers in Africa with little or no capital of their own. Therefore, microfinance is very critical in increasing crop production. In realization of the enormous potentials of small and medium enterprises as an engine room of economic development and grassroots empowerment, Microfinance are granted to farmers for arable crop cultivation, roots crops cultivation, animal husbandry, poultry farming, fish farming and processing and marketing of agricultural products.

The problem is, there are many obstacles impeding the contribution of microfinance to food and cash crops production. These are the quantity and volume of credit, credit access, high transaction costs, and limited knowledge of Microfinance and inadequate management of information system necessary for Microfinance to achieve positive impacts on agricultural production in the study area. For this reason farmers rely on the costly source of accessing financial services especially through informal sources at higher costs and difficult loan terms and repayment, thus necessitating the research to find out to what extend this institutions have contributed to crop production and find out the factors militating against the achievement of farmers goals in the study area.

In the light of the above, this study tends to answer the following research questions:

i. What are the socioeconomic characteristics of the crop farmers who borrowed from Microfinance and those who did not in the study area?
ii. What are the factors determining the accessibility to microfinance
iii. What are the factors affecting the farmers' level of accessibility to microfinance in the study area?

Justification

Rural financial services is about providing financial services-secure savings, credit, financial transactions, money transfer services for remittance and insurance-in rural areas. The ability of rural households to make long term investments to ensure time-patterned income flow is shaped by an economy's financial services. Despite the rapid development of financial services, a majority of smallholders around the world especially third world countries remain without access to financial services that they need to improve their livelihoods. Despite the significant demand for financial services in rural areas, institutions offering financial services-such as Banks, credit unions, cooperatives, Microfinance Institutions (MFIs) or insurance companies-are typically reluctant to serve in rural areas due to precarious nature of agricultural production. As a result, the majority of poor households are bereft of financial access to the formal financial system. (Inter-American Development Bank, 2001).

Microfinance does not just have a positive impact on poverty but on agricultural productivity. Despite Nigeria's abundant agricultural resources and oil wealth, poverty is still a challenge in the country (IFAD, 2009). Agricultural productivity is very low in Nigeria. This is because about 90 percent of Nigeria's food is produced by small scale farmers who cultivates small plots of land and depend on rainfall rather than on irrigation. Neglect of rural infrastructure affects the profitability of agricultural production. The neglect of rural roads impedes the marketing of agricultural commodities, prevent farmers from selling their produce at reasonable prices and leads to spoilage. Limited accessibility to credit cuts small scale farmers off from sources of inputs, equipment and new technology and this keeps yields low (IFAD, 2009).

II. METHODOLOGY

There are thirty-two (32) Microfinance Banks established in the state (as at the time of this research) but only nineteen (19) have commenced full financial operations. The list of crop farmers (those cultivating rice, maize, yams and other crop for commercial purpose from the three distinctive

zones) using micro credits was obtained from the nineteen operational Microfinance Banks in the study area. This list represents the sample frame for the micro credit beneficiaries. Ten (10) percent of the beneficiaries from each of the nineteen (19) microfinance bank was estimated and randomly selected. This gave a sample size of one hundred and eighty five (185) microfinance beneficiary farmers. Primary data was used for the study. Primary data was collected through administration of structured questionnaire. A cross-sectional data from a farm survey of crop farmers for 2014 growing season was used. The data collected included demographic information, such as age, educational level, and farm size, amount of credit, crops grown, labour, fertiliser, output levels and years of experience in farming. Also information on marital status, family size, land ownership, income, cost of borrowing, extension contacts (number of visits) and distance to microfinance banks was collected. The analytical tools that were used to achieve the objectives are; descriptive statistics and double hurdle analysis.

Model specifications

The Double Hurdle model is a parametric generalization of the Probit model, in which the causes and extent of access to credit are determined by two separate stochastic processes given as:

Observed loan size: Y = d.Y**..... ..... (1)

Loan participation: W = α'Z + u (u ϵ N(0,1)) ....(2)

d = 1 if W > 0 and 0 otherwise.

Loan size equation: Y* = β'X + v (v ϵ N(0, δ2) .....(3)

Y** = Y* if Y* > 0 and 0 otherwise.

Where

W is defined whether the households decide to take out credit,

Y* is latent variable showing farmers' loan amount obtained,

Y is the observed dependent variables (the amount of money the farmer obtained),

Z is a vector of variables explaining the credit participation decision,

X is a vector of variables determining on the credit amount,

u and v are the corresponding error terms assumed to be independent and distributed as u ϵ N(0,1) and v ϵN(0,δ2).

This model was solved in one procedure in Strata.The log likelihood of the Double Hurdle model is given as:

Log (L) =

$$\sum_0 in\left[1-\phi\left(z_i^{'}\propto\right)\phi\left(\frac{x_i^{'}\beta}{\delta}\right)\right]+\sum_+ in\left[\phi\left(z_i^{'}\propto\right)\frac{1}{\delta}\phi\left(\frac{y-x_i^{'}\beta}{\delta}\right)\right]$$

Where

Log (L) = Accessibility to microfinance (i.e. loan size) (Naira)

X_1 = age of the farmer (years)

X_2 = land size (hectares)

X_3 = ownership of land (1, owned, 0 otherwise)

X_4 = marital statuses (1 if married, 0 otherwise)

X_5 = farm income of the respondent in 2014(Naira)

X_6 = cost of borrowing from microfinance (Naira)

X_7 = education level (Years)

X_8 = distance/ outreach to microfinance (Km)

X_9 = farming experience (Years)

X_{10} = family size of the respondents (No)

X_{11} = extension contact (No of time)

Yi = whether farmers access to credit (takes the value of 1 if the farmers take credit, 0 for otherwise).

Z and *X*= is the vector of farmers characteristics

β and α= is the vector of parameters

μ and ε = the error term N (0, 1)

Property based determinants are land size, livestock, and other assets. Determinants of borrowing tested in this study include age, educational level, marital status, family size, land ownership, income, cost of borrowing, farming experience, extension contacts (number of visits) and distance to microfinance banks.

III. RESULTS AND DISCUSSIONS

Loan characteristics of the farmers

The loan characteristics of the borrower farmers in the study area showed average loan size of ₦145, 166.67. The interest rates charged per year on the borrower farmers was 15.16%, while the average duration of the loans was about 9.69

months. This implies that shorter loans were given to the borrower farmers for agricultural production.

Table 1 Characteristics of loan received by borrower farmers in the study area

Variables	Mean	S.D	T-test
Average loan size(₦)	145166.67	53539.26	2.71***
Interest rates(%p.a)	15.16	7.612	1.99**
Loan duration(mth)	9.69	2.909	3.033***

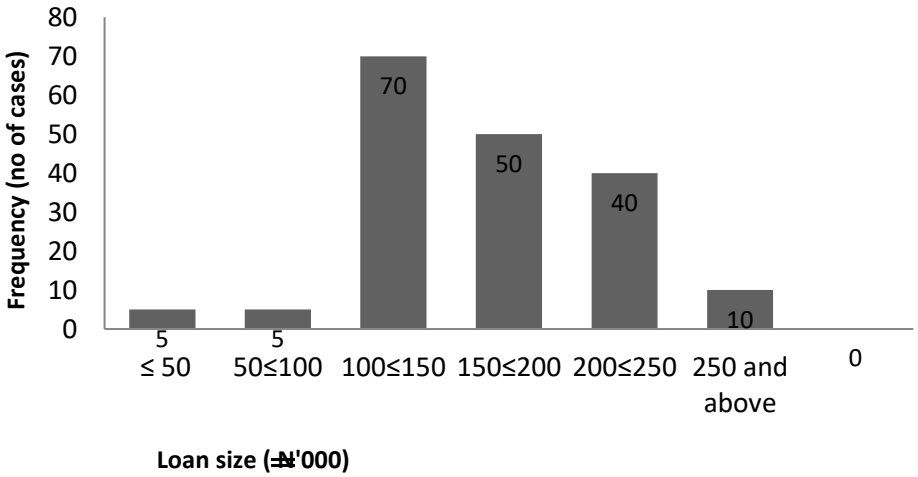

Fig.1 Distribution of Loan amount received by Borrower Farmers

Figure 1 shows that most of the borrower farmers have an average loan of ₦ 145,166.67. Majority (70) of the farmers borrowed above ₦100, 000.00. Only ten farmers in the sample borrowed more than ₦250, 000.00, just five of the farmers borrowed least amounts (below ₦50, 000.00). Overall, the credit supplied by the formal financial institutions in the study area is rather limited.

Determinants of Access to Credit by Crop Farmers

Following the results of the Probit model, access to credit was positively related to the age, farm size, income, and education level of the farmers. The coefficients in table 2 show that the probability of individual farmers' access to credit is positively affected by age, farm size, income, education level of the farmers and negatively related to farming experience, land ownership and household size. These results revealed that variables with positive signs indicate that their higher values increase the chances that the farmers have to access credit and vice versa.

Age and farm size was found to be statistically significant and having positive influence on the probability to access credit. This implies that the chances of the farmers in accessing credit increases with age.

Farm size also plays a crucial role in farming decisions and was considered as an important variable in determining both access to microcredit and size of loan applied for. Households with small farm lands may not need to borrow to finance their production or may only need small loans. However households with large farm lands may need more loans. Furthermore, households with large farm lands may be wealthier or better-off in the community and this can influence their access to credit. Lenders are also more likely to give bigger loans to farmers with large farms compared to those with small farms. This finding is in consistence with Diagne (1999), who noted that farm size was a significant determinant of access to informal credit and the loan size. And also the finding of Okurut (2006), who noted that household with more land are more likely to have an interest

to expand production and a higher probability of borrowing. Land can also be used as collateral for the loan.

Income of the farmers was found to be a significant factor with a positive influence on the probability to access to microfinance. Household income plays a role in the decision-making of the household regarding whether to seek loan for farming or not. As observed by Dodson (1997), demand for agricultural credit over the short term is influenced by income level and the need to replace capital stock. In rural communities, economic status, such as household income, plays a major role in participation in projects and access to resources. Hence the income of the household is hypothesised to influence both loan access and size. Poorer households may be considered as risky borrowers which can affect their loan access and amount borrowed.

Education level of the farmers was found to be a significant factor with a positive influence on the probability to access to microfinance.The level of education attained by a farmer not only increases his/her farm productivity but also enhances ability to understand and evaluate new production technologies (Ezeh, 2007).

Household size is another important household characteristic which influences many household decisions. Household size was found to have a negative influence on probability to access credit but a significant factor in accessing credit. This implies that household size decreases the probability to access to microcredit. Evidence supported that household size was negatively associated with access to microcredit (Lawal et al, 2009). People with large family size are less likely to accept microcredit (Lawal et al, 2009).However, large family size of 9 and above is most likely to spend more of the microloan in financing consumption and other basic needs as such stands less chance to access microcredit (Akram *et al*,2008).

Farming experience and land ownership was found to have negative influence on the decision to access to microfinance. This might probably be because of non-acceptability of rural lands by most financial institutions due to its traditional ownership systems.

Table 2 Factors Affecting Access to Credit by Crop Farmers

Variables	Coefficients	S.D	Z test
Age	0.0507	0.0166	3.04***
Farm size	0.2867	0.0403	7.11***
Farming experience	-0.0110	0.0156	-0.71
Income	1.07E-06	6.43E-07	1.66*
Education	0.2969	0.0638	4.65***
H/hold size	-0.0842	0.0274	-3.07***
Land ownership	-0.0378	0.1563	-0.24
Constant	-3.1331	0.7013	-4.47***
Log likelihood	-192.4287		
LR $chi^2(7)$	114.21		
Prob chi^2	0.0000		
Pseudo R^2	0.2288		

Determinants of loan amount/size

Following the results in table 3, the determinants of the loan amount/size as calculated in the double hurdle models showed that loan amount/size has positive coefficient and was related to marital status, farm size, education, cost of borrowing, farming experience, house hold size, distance to the microfinance bank and extension contacts. The coefficients also showed the probability of borrower farmer loan size is negatively related to age of the borrower and income level. Age was found to be statistically significant and having negative influence on loan amount/size.The negative coefficients of age imply that the chances of the farmers in accessing credit and its size decreases with age. It also means that old age tends to reduce the probability of accessing microfinance credit and loan size. It infers that younger farmers stand better chance than older farmers in accessing microfinance. This is however in agreement with Adeyemi (2008) who showed that older farmers stand less chance of accessing microfinance.This result is also consistent with the findings of Sebopetji and Belete (2009).

Farm size was found to be positively correlated with loan size and was statistically significant at 1% level. This finding was in consistence with Diagne (1999), who noted that farm size was a significant determinant of access to informal credit and the loan size. Land ownership had a positive influence amount borrowed but not statistically significant in

the amount/size of the loan. This is so because land in rural areas especially land held under custom, generally lacks formal documentation. Mobuogwu (2013) noted that since such lands lack documentation, securing loan with such (as collateral) becomes problematic, as banking institutions require titles for land to be eligible as collateral. In the same vein, it was noted that under many customs, rural dwellers have only possessory rights to the land they occupy. As a result, the consent of the family or village head is needed to transact with or alienate such land. Moreover, under many cultures in Nigeria, women are excluded from inheritance, despite women representing 50 percent of the agricultural labor force and farming constituting the principal business in rural areas. As such, 50 percent of the agricultural labor force is often deprived of the assets needed to obtain loans (FOA, 2011).

Marital status has a positive influence on loan amount/size and was found to be statistically significant at 1% level. This could be as a result and perception that marriage confers responsibility and some degree of trustworthiness which could be a strong weapon particularly when it comes to loan repayment.

Cost of borrowing from the microfinance had a positive influence on the loan size and was also significantly related to the loan amount. This implies that the more the volume of the loan acquired by the borrower the high the cost of obtaining it. This also is a determinant of loan size.

Education was found to be statistically significant at 5% level and also having a positive influence on the loan amount/size. The implication is that it may be deliberate policy of MFIs to issue microcredit to literate clientele. Education is perhaps supposed to impact positively on farmers' access to credit and other resources and even in their usage. Adereti (2005) confirms that education is an essential tool in accessing and using farm resources efficiently.

Years of farming experience of the borrower farmers was noted to be positively and significantly related to loan amount/size. The years of farming experience of the household head is believed to influence both access to loan and the size of loan. This is because older farmers with years of farming experience are expected to be knowledgeable about farming and the various sources of credit. They are also expected to have better credit management skills and credibility with lenders (Anang et.al,2015).

Distance to the microfinance bank has a positive influence on the loan amount. In the findings of Pedrosa and Do (2008) noted that long distances between clients and microfinance offices limits access to basic financial services and thus a major barrier to development.

Household size was found to have a positive influence on loan size. Access to microcredit and the amount of loan borrowed are hypothesised to be influenced by the size of the farming household as it determines the household labour supply which is important for agricultural production. Households with limited labour supply may need to borrow to augment their labour supply while households with excess labour may not face such liquidity constraints. Household size can therefore ease the liquidity constraints of the household, thus influencing the decision to borrow as well as the loan amount.

Extension contacts were found to have a positive influence on the loan amount/size but not statistically significant in the amount/size of the loan acquired. This indicates that extension service delivery enhances accessibility to microcredit. The result is expected because extension agents are important source of information for many rural farmers. Extension agents also help to link farmers' groups to credit sources. Thus extension contact is expected to positively impact access to microcredit. The result agrees with Sanusi and Adedeji (2010) who reported a positively significant relationship between extension contact and access to formal credit in Rwanda. Efforts to improve access to agricultural microcredit to smallholder farmers must therefore take into consideration the improvement of extension service delivery to farmers.

Table 3 Determinant of Loan Size

Loan size	Co-efficient	T value
Age	-1032.382(463.473)	-2.23***
Farm size	3669.716(947.855)	3.87***
Land ownership	7.144(4313.064)	0.001
Marital status	44985.230(13137.070)	3.42***
Income		
Cost of borrowing	-0.020(0.127)	-1.63
Education	4.407(0.175)	25.07***
Distance to bank	3806.714(1871.141)	2.03**
Farming experience	199.013(154.433)	1.29

Household size		
Extension contact	792.147(386.378)	2.05**
-Constant	611.824(787.543)	0.78
	1133.284(1020.318)	1.11
	-26994.89(25997.89)	-1.04

Figures outside the parentheses_co-efficient, figures inside the parentheses_standard deviation, ***Significant at 1% **Significant at 5%

IV. CONCLUSION

It was observed from the study that age, farm size, income, education level and household size were factors that significantly affect access to credit. And equally, age, farm size, marital status, cost of borrowing, education level and farming experience had a significant influence on the loan size. The findings of the study indicates that there is high interest rates charged by the microfinance and the volume of loans availed to the farmers was too low for any meaningful crop production. The distance to most of these microfinance banks, coupled with lot of bureaucracy the farmers undergoes before obtaining loan and untimely disbursement makes the farmer want to go without the loan and sometimes divert it to other use other than crop production.

Based on the findings of the study and conclusion drawn, these policy recommendations are made. Banking policies for agricultural credit are still business oriented rather than directed towards development. So, it is imperative on the part of Federal Government to outline some policies and programmes aimed at larger national interest rather than individual and personal gains. Thus, the Central Bank of Nigeria (CBN) through credit policies should make efforts to simplify the borrowing procedure in the terms of time-lag, acceptance of security, documentation and disbursement of loan. On the other hand, credit facility by microfinance banks should be provided on time, otherwise the delay in the completion procedure for taking loans will occur and the farmers will not get maximum profit regarding their plans. Finally, acquisition and recovery process for credit should be simple to give benefits to maximum number of farmers.

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Epidemiology and Determinants of Soil-transmitted Helminthiases among Selected Primary School Children in Tuguegarao City, Cagayan, Philippines: A Cross-sectional Study

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Abstract— *Soil-transmitted Helminth (STH) infections are one of the top causes of morbidity among primary school children. This study was aimed to assess the prevalence, infection rate, and determinants of STH infection among primary school children aged 7-10 years old in Barangay Balzain, Tuguegarao City. Thirty-two Grade 1 and 2 pupils from Balzain East Elementary School were selected through purposive random sampling. Direct fecal smear testing of stool samples elucidated the prevalence of ascariasis and trichuriasis in primary school children. Data such as socio-demographic, water source, environmental sanitation, personal hygiene and health status were collected to better understand the correlation of these factors with STH infection. STH infection was found to be more prevalent [25% (95% CI: 6.02-43.98)] than Grade 1 pupils [16.67% (95% CI: -4.42-37.76)]. The relative prevalence rate was 21.88% (95% CI: 7.56-36.20), which has a qualitative interpretation of a good environmental sanitation behavior. Number of siblings was positively correlated ($p<0.05$) with STH infection. Through a multiple logistic regression analysis, it was found out that a family with one additional child increases the probability of STH infection by about a factor of 4.2192 ($p<0.05$). All of these findings indicate further that a good sanitation is associated with a reduced risk of transmission of STH to primary school children. Identifying factors that contribute to the dynamics of STH transmission in vulnerable groups can help to plan for effective prevention strategies.*

Keywords— ***Soil-transmitted Helminthiases, Environmental Sanitation, School Children, personal hygiene.***

I. INTRODUCTION

Soil-transmitted helminthiases are among the most common infections worldwide, and the global disease burden caused by common STHs, such as *Ascaris lumbricoides*, *Trichuris trichiura*, and Hookworm, is estimated to be about 39 million disability-adjusted life years (DALY) (WHO, 2002), which pose a serious concern. According to WHO (2013), 24% of the world's population or roughly 1.5 billion people worldwide are infected with STH infections. The WHO (2003) also noted that such infections affect the poorest and the most deprived communities, especially the communities of the tropical region.

A lot of factors are deemed to be contributory in the prevalence of such infection, and these factors are said to be predominant in tropical countries (WHO, 2003). The Philippines is one of the countries at risk of having a high prevalence of STH infection. One of the possible reasons that can be attributed to such is that a lot of areas in the Philippines are prone to flooding. Also, it has been known

that STH infection is a risk brought about by flooding vis-à-vis poor sanitation (Yodmani *et al.*, 1982). Primary school children living in such environment are especially vulnerable to STH infections, such as Ascariasis and Trichuriasis, since the agents of the latter infections are said to have increased activity in potentially infective environment and lack of appropriate sanitary behavior (Lander *et al.*, 2012).

Socio-economic, behavioral, and environmental factors are always a part of the health system. These factors directly or indirectly affect the infestation of STH. In the same way, the prevalence of STH infection can also become a key determinant of a country's health and hygiene status (Mukherjee *et al.*, 2013).

In this study, the researchers hoped to generate an idea about the prevalence and infection rates of STH infection among primary school children of Barangay Balzain, Tuguegarao City, as well as to correlate this with the socio-economic variables that could precipitate the process or spread of STH infection.

II. METHODOLOGY

This is a descriptive cross-sectional study that dealt on determining the prevalence of soil-transmitted helminth infection among the primary school children of Barangay Balzain, Tuguegarao City, and on finding the determinants of the prevalence of STH infection. A total of 32 respondents were included in this study through purposive sampling technique. The respondents were all from Barangay Balzain, Tuguegarao City, and were all studying at Balzain East Elementary School. Out of the 32 selected pupils, 12 pupils were in Grade I Level, while 20 pupils were in Grade II Level. The researchers used descriptive survey methods in gathering the data regarding the pupil's profile.

This study was approved by the review panels of School of Health Sciences of St. Paul University Philippines, Tuguegarao City, Cagayan, Philippines. Also, prior to the conduct of the study, permission was obtained from the head of school division and school principal of Balzain East Elementary School in Tuguegarao City concerning the conduct of interviews and health education to the pupils. Upon approval, health education to the students was conducted. Informed consent was obtained prior to the conduct of the interview and sample collection. The questionnaires were then distributed to the respondents and retrieved after. This was followed by the interview of the school children and their parents. After the interview, fecal sampling was done.

A semi-structured questionnaire was used to collect information on the demographic, socioeconomic and environmental background, personal hygiene and practices as well as health status of the school children under study. Both parents and the pupils were interviewed to ensure complete gathering of information. The interviews included personal hygiene of the children and house cleanliness, availability and usage of toilets, water source, wearing shoes/slippers when outside the house, and hand hygiene behavior.

The collection of fecal samples was done according to the procedures in the Philippine Textbook of Medical Parasitology (2nd Edition). Prior stool collection, a specimen container was provided to each pupil. The pupils together with their parents were taught how to do the proper fecal sampling. Each specimen container was labeled with the name of the pupil. After specimen collection, the fecal samples were brought to St. Paul University Philippines Microscopy Laboratory for microscopic examination. Direct fecal smear technique was used for observation and identification of STH eggs. Presence of STH eggs was identified and verified with the assistance of parasitology experts.

Descriptive statistical tools (frequency, cumulative frequency, and percentages) were used to characterize the study population. Pearson's Correlation Coefficient was used to test the associations of the prevalence of STH infection with demographic, socio-economic, environmental, and behavioral factors. The level of significance of 0.05 was used for all the statistical tests. The variables that showed association with p-value $\leq$ 0.20 in the univariate analysis were used to develop a multivariate logistic regression model.

III. RESULTS AND DISCUSSION

3.1 Prevalence and Infection Rate of Soil-transmitted Helminthiases

The prevalence of soil-transmitted helminth infection for Grade levels 1 and 2 pupils is shown in Table 1. A total of 7 pupils were infected with soil-transmitted helminths. Grade 2 pupils have a higher prevalence rate of STH infection with 25.00% (95% CI: 6.02-43.98) compared to that of the Grade 1 pupils with a rate of 16.67% (95% CI: -4.42-37.76). The relative prevalence rate of STH infection among the selected primary school children is 21.88% (95% CI: 7.56-36.20). This prevalence rate has a qualitative interpretation of "good" sanitation status. This is despite the fact that Barangay Balzain is a flood-prone barangay.

Table 1. The Frequency Distribution and Prevalence Rate of Soil-Transmitted Helminth (STH) infections among children in Balzain East Elementary School.

Parameter (Grade level)	Total number of subjects (N)	Number of infected subjects	Prevalence Rate (95% Confidence interval)
I	12	2	16.67% (-4.42,37.76)
II	20	5	25.00% (6.02,43.98)
Total	32	7	21.88% (7.56,36.20)

Table 2. The Infection Rate of Soil-Transmitted Helminths among Primary School Children of Balzain East Elementary School, Tuguegarao City.

Parasite	Number of Parasite (N=32)	Infection Rate (%)
Ascaris lumbricoides	**6**	**18.75**
Trichuris trichiura	**1**	**3.13**
Hookworm	**0**	**0**

In this study, *Ascaris lumbricoides* and *Trichuris trichiura* were observed to be present in the stool samples [Table 2]. *Ascaris lumbricoides* has a higher infection rate (18.75%) than *Trichuris trichiura* (3.13%). The preponderance of ascariasis is supported by the literature authored by Bethony et al (2006), in which he stated that ascariasis is the most prevalent (25%) helminthiasis worldwide. Also, it is the most prevalent STH infection in children of tropical and developing countries, and in regions where hygiene is poor (Werkman *et al.*, 2020).

In this present study, there were no hookworms found in the stool samples examined. This finding is unusual because in other parts of the world, ascariasis and hookworm infection are more common than trichuriasis (Anuar, Salleh, and Moktar, 2014). This finding may be attributed to the age range (Ages 7 to 10) of the study population and may also be due to the potential resistance of T. *trichiura* to most of antihelminthic drugs (Anuar, Salleh, and Moktar, 2014).

The absence of hookworm infection in this study may be due to the fact that the study population was, on the average, younger in age (Belyhun *et al.*, 2010). According to Haldeman and his group (2020), hookworm infection has a higher rate among adults. Also, based on the survey and interviews conducted by the present researchers, it was noted that only a few parents had occupations that are in line with agriculture. In fact, in this study, majority of the primary caretakers of the primary school children were businessmen (28%). This factor could have affected the presence of hookworm infection since a higher prevalence rate of hookworm infection is seen in family heads whose occupation is in line with agriculture (Alemu *et al.*, 2011)

3.2 Determinants of Soil-transmitted Helminthiases

Among the categorical variables, only one variable showed significant correlation with the prevalence of STH infection. The number of siblings had a marked positive correlation with the prevalence of STH infections. All other factors had a negligible correlation.

The result of multiple logistic regression analysis of STH infection and number of siblings revealed that a family with one additional sibling increases the probability of being infected by about a factor of 4.2192. This implies further that in every one child increase in the family, the odds of STH infection increase approximately 4 times. This finding coincides with the results of several studies. In the study of Zang et al (2013), both poverty and the number of siblings were found to be significantly and positively correlated with STH infections as well as reinfections, and that a family with one additional sibling increases the probability of being infected by about 8%. In the study of Nasr et al (2013), it was reported that a large family size (tantamount to more children) is a key factor that is significantly associated with STH infection among children.

Nasr et al (2013) also reported that large families were at higher odds of ascariasis compared to children from smaller families. This finding is parallel to the results obtained by the present researchers, that is, a higher prevalence rate of ascariasis was observed in this study. This may be attributed to a possible presence of infection among other family members as well as to the horizontal spread or the focal transmission of infection among family members in the vicinity of the home (Nasr *et al.*, 2013). Moreover, increased number of household members and overcrowded conditions are associated with a higher frequency of parasitic infections (Ostan *et al.*, 2007; Maia *et al.*, 2009). The results of the present study concur with these findings.

IV. CONCLUSION

Despite a number of limitations, the result of this study significantly revealed that a good sanitation and smaller

family size were associated with a reduced risk of transmission of STH infection to humans. Therefore, access to improved sanitation as well as health education should be prioritized alongside preventive chemotherapy to achieve a durable reduction of the burden of STH infections.

V. RECOMMENDATIONS

The procedural method used in this study for the stool examination was direct fecal smear. For future researchers, it is recommended that methods with higher accuracy, such as Kato Katz technique, be utilized. Also, it is recommended that the sample size be increased in order to achieve a lower margin of error. We also recommend to compare the prevalence of STH infection between a flood-prone area and a non-flood-prone area.

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Genotoxic Effect of Automobile Exhaust Exposure among Motorized Tricycle Drivers in Tuguegarao City, Cagayan, Philippines using Micronucleus Assay: A Retrospective Cohort Study

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Abstract— *Motorized tricycle drivers are among the populations of the transport industry who are very much prone to the effects of hazardous substances found in automobile exhausts. This research was aimed to determine the risk factors of cytogenetic damage brought about by automobile exhaust exposure among motorized tricycle drivers of Tuguegarao City. In this study, a total enumeration of motorized tricycle drivers from of Pengue Ruyu, Tuguegarao City was done. Out of 52 tricycle drivers, only 51 participants were included in the study. Most of the tricycle drivers of Pengue Ruyu were primarily middle-aged drivers; substantially exposed to automobile exhaust; cigarette smokers and alcoholic drinkers; non-users of protective masks; had no vitamin supplementation; and had routes that involved driving across Barangay Tanza. A high incidence of genotoxicity [76.47% (95% CI: 64.83% to 88.11%)] among the study participants was observed. Among the categorical variables, only automobile exhaust exposure and vitamin supplementation showed significant correlation ($p<0.05$) with the development of cytogenetic damage. Automobile exhaust exposure was found to be a risk factor, while intake of vitamins as a protective factor. Statistical analysis showed that tricycle drivers substantially exposed to combustion products in automobile exhaust had a significantly ($p<0.05$) higher mean frequency of micronuclei and were 2.13 (90% CI: 1.12 to 4.08) times more likely to develop cytogenetic damage than those tricycle drivers who have low confidence of exhaust exposure. Tricycle drivers who have vitamin supplementation tend to have a significantly ($p<0.05$) lower mean frequency of micronuclei and reduce their risk of developing cytogenetic damage by 44% (90% CI: 4% to 55%) than those who do not take any vitamins at all. Wearing protective masks could be a protective factor as it may reduce the risk of developing cytogenetic damage by 46% (90% CI: -46% to 72%) but was found to be statistically insignificant. Identifying factors that contribute to the pathogenesis of genotoxicity in vulnerable and susceptible groups can help to plan for effective prevention strategies.*

Keywords— ***Genotoxicity, Automobile Exhaust Exposure, Micronucleus Assay***

I. INTRODUCTION

All life on earth needs to have a clean air to breathe. This is so because when the air becomes unhealthy, a plethora of complications may arise and may affect not only the ecological wealth of the country but most especially the health of its people (WHO, 2008).

Based on the four-year national total suspended particulate (TSP) monitoring from 2003-2007, the Philippines pollution level has improved from 144ug/Ncum in 2003 to 97ug/Ncum in 2007 (DENR EMB, 2009). Though there has been a stabilizing trend observed in the air quality status of the Philippines, the value still exceeded the standards set by the World Health Organization, which is at 90ug/Ncum (DENR EMB, 2009). The degradation of air quality of the Philippines could be attributed to three sources (DENR EMB, 2009). These pollution sources are stationary, mobile and area sources. Among these sources, large proportion is pointed to mobile sources (65% of all pollution sources), as indicated in the National Emission Inventory in 2006. According to DENR EMB (2009), majority of the regions in the country attributed the degrading air quality to the transport sector, which is reported as the major source of air pollution in the Philippines.

Moreover, air pollution levels are tightly linked to climate and topography. Air pollution episodes can be particularly troublesome if the affected city is located in a valley surrounded by mountains, wherein a nocturnal inversion layer traps dangerous concentrations of pollutants in the cool air at the base of the valley that sometimes results to dense smog over urban areas (WHO, 2008). This can be observed in Tuguegarao City, which has been dubbed as the Tricycle City of Cagayan Valley Region (Domingo, 2013).

At least 12, 000 tricycles wander around Tuguegarao City (Domingo, 2013). The increasing numbers of Tricycles make Tuguegarao City one of the most polluted cities in terms of air quality. In fact, the air pollution level in Tuguegarao city has exceeded the air quality standards set by the WHO. Based on the National Air Quality Status Report (2003-2004) of DENR-EMB (2005), air pollution level of Barangay Tanza, Tuguegarao City has reached 198 ug/Ncum in 2004 from only 59 ug/Ncum in 2003. In fact, the maximum TSP level reached by Barangay Tanza in 2004 was 346 ug/Ncum (DENR-EMB, 2005).

Motorized Tricycle drivers, because of their work, are exposed to complex mixture of hazardous substances coming from automobile exhaust. The majority of situations involve exposures to low dose for a long period of time. This may involve the individual's entire lifetime and result in small increments in health risks (Franco et al., 2008). One way of studying the effects of automobile exhaust exposure on a population, especially the tricycle drivers, is to conduct a monitoring study by which damages to the DNA or to the chromosome can be identified (Pant and Rao, 2010).

Occupational exposure to hazardous substances puts a great public health concern. This is such since low doses of carcinogenic substances and mixtures, to which workers are exposed, can produce genotoxic effects (Maluf and Erdtmann, 2000). Genotoxic effects, in the form of cytogenetic damage, may be manifested by the presence of Micronuclei (MN), which are fragments or whole chromosomes that did not reach spindle poles during mitosis and remained encapsulated at telophase in a separate nucleus (Fenech *et al.*, 2003). Micronucleus assay for exfoliated buccal mucosal cells have been used to evaluate cytogenetic damage (Maluf and Erdtmann, 2000). Several studies using Micronucleus assay have shown that smoking, alcohol, ionizing radiation, duration of exposure, and some drugs have an additive effect on the induction of cytogenetic damage.

Therefore, to add further knowledge to the genetic risk related to automobile exhaust exposure, this study will use Micronucleus (MN) as a biomarker in occupationally exposed motorized tricycle drivers. With this, the present researchers have come out with the research purpose, which is threefold: a.) to determine the risk factors of the genotoxic effects of automobile exhaust among motorized tricycle drivers of Tuguegarao City, b.) to examine relationships between the profile of the subjects and the cytogenetic damage brought about by automobile exhaust exposure using Micronucleus Assay, and c.) to determine the extent of genotoxicity among tricycle drivers.

II. METHODOLOGY

The research design used in this study was a retrospective cohort study design. This study examined exposure to suspected risk factors in relation to genotoxicity. In this study, a total enumeration of the Motorized Tricycle drivers of Pengue Ruyu, Tuguegarao City was done. Only those who satisfied the inclusion criteria were included in the study. The total number of tricycle drivers of Pengue Ruyu was 52. However, only 51 participants were included in the study because one of the tricycle drivers did not satisfy the inclusion criteria. The subjects were conveniently grouped based on a questionnaire that includes items about age, occupational exposure, smoking habit, consumption of alcohol, and use of protective masks, as well as the presence of micronuclei in the exfoliated buccal mucosa. The health and occupational histories of the subjects were also collected. All study participants were consented and were informed about the objective of the study. Inclusion criteria include: 1. A tricycle driver of Pengue Ruyu, Tuguegarao City, 2. Male,

3. Drives across Barangay Tanza, 4. Drives a motorized tricycle at least thrice a week, 5. Has not been exposed to

x-rays seven days before the sampling, 6. Has not taken any antibiotics for three months prior the sampling, 7. Has not recently taken a vaccination, and 8. No viral illnesses.

The main instruments that the researchers used in this study were modified structured questionnaires, history-taking, and microscopy. The questionnaires used in this study comprised important variables as indicated in the protocol set by Thomas et al (2009). The questions were written in a closed-ended manner and were relatively narrow. For the Micronucleus assay, a light compound microscope was used.

Health education was first executed. Informed consent was obtained prior to the conduct of interview and specimen collection. Interview of the study participants was then done. After all the necessary preliminaries, the Buccal Micronucleus assay was performed according to the protocol of Thomas et al (2009) with slight modifications.

Before buccal cell collection, the study participants were asked to rinse their mouth twice thoroughly with 100 ml of water to remove unwanted debris. For each specimen, a 30-ml container containing 10 ml of Carnoy's fixative was prepared. Gently but firmly, a wooden spatula was swabbed 10 times against the inside of the cheek wall from the middle and gradually increasing in span. The spatula was then placed into the fixative container.

Buccal cells from the fixing solution were harvested for slide preparation. Samples were centrifuged for 10 min at 3200 rpm at room temperature. The pellet was resuspended on 300 µl Carnoy's solution. The resulting solution was subjected to vortex mixer to increase the number of single cells in suspension. In duplicate, 100 µl of cell suspension was placed onto a clean, dry and appropriately labeled microscope slide using a micropipettor. Cells were heat-fixed and were subjected to the following schedule: 2 min in 95% ethanol; 2 min in 75% ethanol; 2 min in 50% ethanol; and 2 min in distilled water.

Finally, cells were stained using Giemsa's stain. Using light microscope, the number of micronuclei in at least 1,000 differentiated cells were determined. A minimum of 1, 000 cells possessing intact nuclei and cell boundaries were counted in each slide. The following criteria given by Tolbert et al. (1991) were considered to designate an extranuclear body as micronucleus: Rounded smooth perimeter suggestive of a membrane; Less than a third the diameter of the associated nucleus, but large enough to discern shape and color; Staining intensity similar to that of the nucleus; Texture similar to that of nucleus; Same focal plane as nucleus; and Absence of overlap with, or bridge to, the nucleus.

The number of micronuclei scored per slide was recorded. To ensure the certainty of MN scored per slide, an Inter-rater reliability technique was used.

The statistical analysis of the data obtained was done through Microsoft Excel. The mean values and standard deviation were computed for the age and MN scores. To detect a significant trend and association between variables, Fisher's exact test was performed. To establish a significant difference in the mean values between variables, Student's t-test for independent means was used. Also, to establish a significant difference between the proportions of those who have low confidence and substantial exhaust exposure, z-test for independent proportion was used. A p-value of less than 0.05 was considered significant. To determine the relative risks, risk ratios were computed. The confidence interval of each risk ratio was set at 90%.

III. RESULTS AND DISCUSSION

3.1 Profile of the Study Participants

The profile of the respondents consists of three factors that are necessary in the study: (1) Personal Factor, (2) Lifestyle Factors, and (3) Occupational Factors. The personal factor consists of the respondents' age only. The lifestyle factors consist of the respondents' smoking history, alcohol consumption history, and medical history. Lastly, the occupational factors consist of the respondents' number of years in service as a tricycle driver, regularity of driving across Tanza, duration of work, as well as use of protective masks.

A total of 52 tricycle drivers of Pengue Ruyu were asked to participate in the study. However, one of the tricycle drivers, who is a female, was excluded for not satisfying the inclusion criteria set by the researchers.

Majority (80%) of the respondents had substantial exposure to automobile exhaust, while only 20% had low confidence of exposure. This suggests that more tricycle drivers of Pengue Ruyu were exposed to automobile exhaust and were prone to having genotoxic effects.

Table 1. The Mean Age of the Tricycle Drivers of Pengue Ruyu, Tuguegarao City (N=51).

	N	Mean Age	Standard deviation
Sample Population	**51**	**44.37**	**11.95**
Low Confidence of Exposure	**10**	**38.63**	**13.22**
Substantial Exposure	**41**	**46.32**	**10.96**

The mean age of the tricycle drivers of Pengue Ruyu is illustrated in Table 1. The arithmetic mean age of the sample population (N=51) is 44.37 years old. Meanwhile, the mean age of tricycle drivers with low confidence of automobile exhaust exposure is 38.63 years old, while the mean age of tricycle drivers with substantial exposure is 46.32 years old. The result implies that the tricycle drivers of Pengue Ruyu who had low confidence of exposure were relatively younger than the tricycle drivers with substantial exposure as well as the sample population in general. Nevertheless, the tricycle drivers of Pengue Ruyu who had substantial exposure to automobile exhaust were relatively older than the sample population.

Majority of the respondents (67%) were smokers, while only 33% were non-smokers. Thus, more tricycle drivers of Pengue Ruyu were exposed to cigarette smoke and, as such, were prone to have genotoxic effects.

Eighty four percent of the respondents were alcoholic beverage consumers, while only 16% were alcoholic beverage abstinents. This proportion implies further that more tricycle drivers of Pengue Ruyu were exposed to noxious substances of alcoholic beverages and hence were prone to having genotoxic effects.

In this study, only a small proportion (18%) of the sample population had a positive history of respiratory diseases (such as asthma, chronic cough, and pneumonia), while 82% denied any history of respiratory diseases.

It was observed that only 31% of the study participants had a habit of taking vitamins. A relatively lower proportion of tricycle drivers of Pengue Ruyu had a habit of taking vitamins, which may serve as antioxidants and offer protection from further occurrence of genotoxicity.

Thirty five percent of the respondents had been in the transport industry as tricycle drivers for more than 15 years, while 25% of the respondents had been driving tricycles for about 11 to 15 years. On the other hand, 20% and 18% of the respondents had been in the transport service for about 1 to 5 years and 6 to 10 years, respectively. This just shows that more tricycle drivers of Pengue Ruyu had been exposed to automobile exhaust for a long time already, which posed a higher health risk to the tricycle drivers.

Most of the respondents (69%) had a duration of driving around the city of about 11 to 15 hours. On the other hand, only 31% of the respondents claimed a duration of driving of 6 to 10 hours.

Also, most of the tricycle drivers of Pengue Ruyu, Tuguegarao City had consistently and substantially been exposed to automobile exhaust. As such, most of the tricycle drivers were more likely at risk of the genotoxic effects of automobile exhaust.

In the National Air Quality Status Report of DENR-EMB (2005), Barangay Tanza has reached an air pollution level of 198 ug/Ncum in 2004, which is far higher than the standards of 90 ug/Ncum by the WHO. As such, the higher frequency of driving across Barangay Tanza indeed posed a higher chance of exposure to complex mixture of hazardous substances coming from automobile exhaust. Majority of the study participants (65%) “always” drive across Barangay Tanza, while only 35% claimed that they “sometimes” drive across Barangay Tanza.

In this study, a very small proportion (8%) of the sample population used protective mask while driving. This means that a high proportion of tricycle drivers were very much exposed to automobile exhaust and were prone to having genotoxic effects.

Based on the profile of the study participants, most of the motorized tricycle drivers of Pengue Ruyu, Tuguegarao City were middle-aged, were substantially exposed to automobile exhaust, were cigarette smokers, were alcoholic drinkers, were non-users of protective masks, had no history of any respiratory diseases, had no habit of taking vitamins, and always drive across Tanza.

3.2 Extent of the Genotoxicity of Automobile Exhaust

The photomicrograph of micronucleated buccal cells collected from the sample was shown in Fig. 1. The presence of micronuclei in buccal cells indicates a positive result.

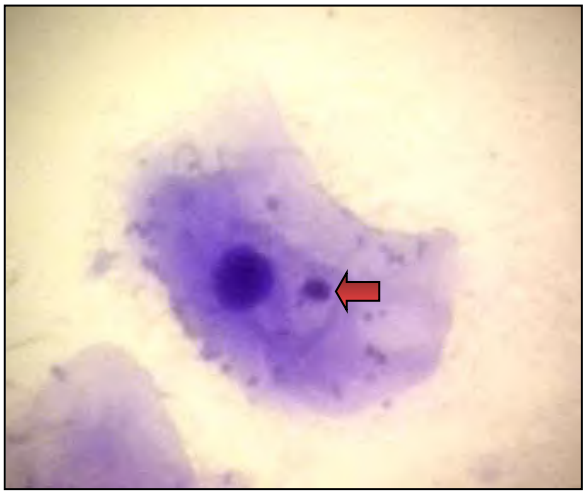

Fig.1: Photomicrograph of Giemsa-stained Micronucleus (arrow) in Human Buccal Cells (under High Power Objective).

Table 2. The Incidence Rate of Genotoxicity among Tricycle Drivers of Pengue Ruyu, Tuguegarao City (N=51).

	Tricycle drivers (N)	Percentage (%)	95% Confidence Interval	
			Lower	Upper
Positive MN	39	76.47	64.83	88.11
Negative MN	12	23.53	11.89	35.17

The incidence rate of genotoxicity among tricycle drivers of Pengue Ruyu is shown in Table 2. The table illustrates that 76.47% of the study participants tested positive for micronuclei. The high incidence of genotoxicity among the tricycle drivers could be attributed to several factors. One of these factors would be lower socioeconomic position (SEP). Samet et al (2000) indicated that groups with lower socioeconomic position (SEP) may receive higher exposure to air pollution. As such, people with low socioeconomic status may become more susceptible to the health effects of air pollution (Samet et al., 2000), and to cytogenetic damage brought about by automobile exhaust exposure.

Another factor that may have increased the incidence of genotoxicity among the tricycle drivers of Pengue Ruyu is the pollution level in Tuguegarao City. DENR EMB (2009) reported that Tuguegarao City exceeded the standards set by the WHO. In the study of Sree Devi et al (2009), a significant increase in the mean frequency of chromosomal aberrations was observed in traffic policemen exposed to polluted air. Also, they stated that vehicular exhaust, smoking, age, and duration of exposure had a cumulative effect with respect to the chromosomal aberrations in exposed traffic policemen. In the present study, majority of the tricycle drivers of Pengue Ruyu were middle-age men, had substantial exposure to automobile exhaust, and were cigarette smokers.

The length of exposure to urban air pollution could also be an attributable factor. The study conducted by Knudsen et al (1999) suggested that long-term exposure to urban air pollution (with traffic as the main contributor) induces chromosome damage in human somatic cells. In the present study, more than half of the population of the tricycle drivers of Pengue Ruyu had been exposed to urban air pollution for more than 10 years already.

Table 3. The incidence rate of genotoxicity among tricycle drivers of Pengue Ruyu who had low confidence of exposure and had substantial exposure to automobile exhaust.

	Low Confidence of Exposure			**Substantial Exposure**		
	f	**%**	**95% C.I.**	**f**	**%**	**95% C.I.**
Positive MN	**4**	**40**	**9.64 to 70.36**	**35**	**85.37**	**74.55 to 96.18**
Negative MN	**6**	**60**	**29.64 to 90.36**	**6**	**14.63**	**3.82 to 25.45**
Total	**10**	**100**	**--**	**41**	**100**	**--**

Table 3 illustrates the incidence rate of genotoxicity among tricycle drivers of Pengue Ruyu who had low confidence of exposure and had substantial exposure to automobile exhaust. The incidence of genotoxicity in tricycle drivers who were substantially exposed to automobile exhaust [85.37% (95% CI: 74.55%– 96.18%)] is higher than that of the incidence in tricycle drivers with low confidence of exposure [40% (95% CI: 9.64%–70.36%)]. This finding coincides with the results obtained by Anwar and Kamal (1988). The elevated cytogenetic damage may be attributed to exposure to pollutants from automobile exhaust (Anwar and Kamal, 1988).

3.2 Risk Factors

The present study also assessed the risk factors of genotoxicity among tricycle drivers of Pengue Ruyu, Tuguegarao City.

Table 4. Fisher's exact test statistic values

Independent Variables	**Dependent Variables**	**p-value**	**Qualitative Interpretation**
Automobile Exhaust Exposure	Presence of MN	0.0065*	CORRELATED
Age distribution	Presence of MN	0.9359	NO CORRELATION
Smoking Habit	Presence of MN	1.0000	NO CORRELATION
Alcohol consumption	Presence of MN	1.0000	NO CORRELATION
History of Respiratory Disease	Presence of MN	1.0000	NO CORRELATION
Vitamin	Presence	0.0334*	CORRELATED

intake	of MN		
Regularity of driving across Tanza	Presence of MN	0.7324	NO CORRELATION
Duration of work	Presence of MN	0.2961	NO CORRELATION
Protective Mask use	Presence of MN	0.2320	NO CORRELATION

**Significant association at p<0.05.

Table 5. The Mean Frequency of Micronuclei (MN) among Study Groups.

	N	**Number of Micronuclei per 1000 intact cells (MN±SD)**	p-value
Low confidence of Exposure	10	1.20±1.81	0.0138*
Substantial Exhaust Exposure	41	4.61±4.10	
With vitamins	16	2.00±2.61	0.0232*
Without vitamins	35	4.60±4.06	
Protective mask users	4	0.5±0.58	0.0793
Protective mask non-users	47	4.02±3.89	
Alcohol consumers	43	3.98±4.07	0.3241
Alcohol abstinents	8	2.5±2.20	
Smokers	33	4.54±4.09	0.2802
Non-Smokers	18	3.33±3.12	
With Respiratory disease	9	3.89±4.62	0.9005
Without Respiratory disease	42	3.71±3.74	
Always drive across Tanza	33	4.39±3.91	0.2778
Sometimes drive across Tanza	18	3.11±4.11	

*Significant association at p<0.05.

Table 4 reveals that among the categorical variables, only two variables, the automobile exhaust exposure and the vitamin intake, showed significant correlation with the presence of micronuclei.

Automobile exhaust exposure was found to be correlated with the presence of micronuclei. It was observed that the incidence of genotoxicity among tricycle drivers who were substantially exposed to automobile exhaust is lower compared to that of the incidence of genotoxicity among those with low confidence of exposure. Also, as shown in Table 5, the difference between the mean frequency of micronuclei in those with low confidence of exhaust exposure and in those with substantial exhaust exposure was established to be significantly different (p<0.05). It was found out that the mean frequency of micronuclei in tricycle drivers who were substantially exposed to automobile exhaust is significantly higher than those with low confidence of exhaust exposure.

In this study, the tricycle drivers who were substantially exposed to the combustion products were those tricycle drivers who had been in the transport industry for more than 5 years, while those who had low confidence of exposure were those tricycle drivers who had been in the transport industry for 5 years and below. The above findings indicate that tricycle drivers substantially exposed to combustion products in automobile exhaust exhibit a significantly higher mean frequency of micronuclei compared to tricycle drivers who had low confidence of exposure. The higher mean frequency of micronuclei among the substantially exposed group may be an evidence of the clastogenic and/or aneugenic effects of the combustion products in automobile exhaust.

Another variable which was found to be statistically correlated with the presence of micronuclei is the intake of vitamins. It was observed that the incidence of genotoxicity among tricycle drivers who were taking vitamins is significantly lower than that of the incidence in tricycle drivers who had not taken any vitamins at all.

Table 5 shows also that the mean frequency of micronuclei in those who had taken vitamins and in those who had not taken any vitamins at all were significantly different (p<0.05). It was found out that tricycle drivers who were taking vitamins tend to have a significantly lower mean frequency of micronuclei when compared to those who had not taken any vitamins at all. This implies further that those tricycle drivers who had not taken vitamins were more susceptible in developing cytogenetic damage due to automobile exhaust exposure.

In a study conducted by Sia Su and Kayali (2008) regarding blood Vitamin C levels of the motorized tricycle drivers, it was found out through correlation analysis that majority (79.6%) of drivers had low blood vitamin C levels. They reported that workplace and vitamin C supplementations (p<0.5) were significantly related to the blood vitamin C levels of the motorized tricycle drivers. In the present study, the lower rates of vitamin supplementation and the workplace of the tricycle drivers

could be factors that have increased the susceptibility of the tricycle drivers to cytogenetic damage.

Table 6. Relative Risk

Exposure	Outcome	Risk Ratio	90% Confidence Interval	
			Lower Limit	Upper Limit
Automobile Exhaust Exposure	Presence of MN	2.13*	1.12	4.08
Intake of Vitamins	Presence of MN	0.66**	0.45	0.96
Protective Mask use	Presence of MN	0.64	0.28	1.46
Respiratory Disease	Presence of MN	1.02	0.74	1.42
Smoking Habit	Presence of MN	1.00	0.76	1.31

*Risk factor

**Protective factor

In Table 6, the computed risk ratios of the different categorical variables were shown. Automobile exhaust exposure was found to be a risk factor, while intake of vitamins was found to be a protective factor.

Those tricycle drivers who were substantially exposed to automobile exhaust are 2.13 times more likely to develop cytogenetic damage than those tricycle drivers who had low confidence of exhaust exposure. Moreover, 90% of the time, when tricycle drivers were to be exposed at a higher exposure rate, the risk of developing cytogenetic damage may rise up to 4.08 times.

Meanwhile, those tricycle drivers who were taking vitamins reduce their risk of developing cytogenetic damage by 44%. Also, when tricycle drivers are to take vitamins more frequently, the risk of developing cytogenetic damage may be reduced up to 55%; however, when tricycle drivers are to take vitamins less frequently, the risk of developing cytogenetic damage may be reduced up to 4% only.

The usage of protective mask is supposed to be a protective factor since it has a risk ratio of 0.64. However, it is not statistically significant. Nonetheless, it is noteworthy as it may reduce the risk of developing cytogenetic damage by 72%.

The results of this study significantly revealed that lesser exposure to automobile exhaust as well as more frequent intake of vitamins were associated with a reduced risk of developing cytogenetic damage among workers of the transport industry, especially among motorized tricycle drivers. Therefore, access to improved air sanitation as well as health education should be prioritized alongside preventive chemotherapy such as vitamins to achieve a durable reduction of the effects of automobile exhaust exposure among the motorized tricycle drivers.

IV. CONCLUSION

In the light of the analysis of data and the findings of the study, the following conclusions were drawn:

1. The respondents were primarily middle-aged tricycle drivers whose route involves driving across Barangay Tanza. They were substantially exposed to automobile exhaust, majority of which do not use protective masks while driving. A significant proportion had no medical history of respiratory disease. Majority of the respondents were cigarette smokers and alcoholic beverage drinkers. Only a small proportion took vitamin supplements.
2. There is a high incidence of genotoxicity among the motorized tricycle drivers of Pengue-Ruyu, Tuguegarao City.
3. Among the variables, only automobile exhaust exposure and vitamin supplementation showed a significant correlation ($p<0.05$) in the development of cytogenetic damage. Automobile exhaust exposure was found to be a risk factor, while intake of vitamins as a protective factor.
4. There is no significant difference in the cytogenetic damage between those who use protective masks during driving and those who do not.
5. There is a significant difference in the cytogenetic damage between those who had a low-risk of exposure and high-risk of exposure to automobile exhaust.

V. RECOMMENDATIONS

To the Future Researchers, we recommend a larger sample size to achieve a lower margin of error. We would like also to recommend to the future researchers the use of other DNA-specific stains such as Feulgen stain so as to reduce the incidence of possible errors in the identification of micronucleus. Other procedural methods can also be used for the examination of the cytogenetic damage such as comet assay and Ames test.

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Elemental Composition and Proximate Analysis of Shea Butter Sold in Swali Market, Yenegoa, Nigeria

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Abstract**—** *This study evaluates the elemental and proximate composition of Shea butter sold in Swali market Yenagoa, Bayelsa State, Nigeria. Proximate analysis was carried out using standard method of AOAC (2005) while elemental analysis was determined using Atomic Absorption Spectrophotometer and Flame photometer. The proximate values were: Moisture (5.220±0.113 %), Crude fibre (0.111±0.001%), Crude Protein (42.316±0.000%), Crude fat (97.000±0.000%), Ash content (0.084±0.006%), Carbohydrate (34.096±0.011%). Results of the elemental analysis (mg/100g) showed that Sodium, Potassium, Iron, Magnesium, Copper, Calcium, Zinc and Manganese contents of the Shea butter were: 836.83±0.101, 45.81±000, 0.61±0.111, 2.56±0.111, 0.18±0.000, 31.31±0.113, 0.30±0.101 and 0.23±0.113 respectively. The elemental and proximate values indicated that Shea butter has nutritive components comparable to other popular nutritious food substances. Hence, its consumption for edible and non- edible purposes should be promoted.*

Keywords**— Shea butter, Swali market, crude protein, moisture, Yenagoa.**

I. INTRODUCTION

The African Shea tree (*Vitellaria paradoxa)* formerly known as (*Butyrospernum parkli)* is a tree of the Sapotaceae family, it is the only species in genus *Vitellaria* and is indigenous to Africa (Byakagaba *et al*., 2011). The English word "Shea" comes from "s'*i"* the tree name in the Bambara language of Mali. It is known by many local names e.g "*kade*" or "*kadanya*" in Hausa language, "*karate*" in Wolof language of Senegal (Goreja, 2004), "*ori*" in some parts of West Africa and many others.The Shea fruit consists of a thin, tart, nutritious pulp that surrounds a relatively large, oil-rich seed from which Shea butter is extracted. The Shea tree is a traditional African food plant, it has been claimed to have potential to improve nutrition, boost food supply in "annual hungry season" (Masters *et al*., 2010) foster rural development and support sustainable land care (NRC, 2006). Shea butter is a fat extracted from the nut of the African Shea tree (*V.paradoxa)*. It is usually yellow in colour when raw with refined, unrefined and ultra refined Shea butter being ivory or white in colour (Alfred, 2002) The Shea tree belongs to *Sapotaceae* family and was first named by the German botanist Carl Gaertner as *V. paradoxa*. In 1961, the species already described by Gaertner was renamed as *Butyrospermum parkli* (Maranz *et al*., 2003), with the epithet "*parkli*" referring to Mungo Park who was the great Scot's explorer introducing Shea butter to Europe (Goreja, 2004).

The trees grow wild across a 5000 km wide belt of savanna including West African Countries of Senegal, Mali, Cote d' Ivoire, Burkina Faso, Togo, Ghana, Benin Republic, Nigeria, Niger, Cameroon and further east in Uganda, Sudan and Ethiopia (Byakagaba *et al*., 2011; Chalfin, 2004; Goreja, 2004). This savanna belt is also called "Shea belt" among traders (Ferris *et al*., 2001). Among these countries Ghana and Burkina Faso are the main Shea nut exporters (Matt, 2009).

The Shea Tree starts bearing its fruits when it is 10 to 15 years old, full production is attained when the tree is about 20 to 30 years old. It then produces nuts for up to 200 years. The fruit resembles large plums and takes 4 to 6 months to ripen. The average yield is 15 to 20 kilogram of fresh fruits per tree, with optimum yield up to 45 kilogram. Each kilogram of fruit gives approximately 400 grams of dry seeds (Maranz *et al*., 2003). Fat and oil is considered to be a basic requirement for the human health and well being and Shea butter is a poly saturated fatty acid joined together in groups of three forming a molecule called triglycerides. When fatty acid is taken into the body, it is converted into glucose and stored in the body as energy. It is an essential study with aids to know the health benefits of Shea butter sold in Swali market, Bayelsa State, Nigeria. Shea butter is oil rich in fat extracted from the nuts of the African Shea tree. It exists as solid at room temperature. It is usually yellowish in colour when raw, with unrefined, refined and ultra Shea butter being ivory or white in colour (Lovett and Haq, 2000). Shea butter is a triglyceride (fat) derived mainly from stearic acid and oleic acid. Shea butter is edible in most cases and used in food preparation in some African countries

Traditionally, the extraction of Shea butter has been done at the village level, where Shea butter is sold in local markets. In recent years, the dried kernels have been exported to processing countries in Europe, Japan and Asia where Shea butter is extracted in large - scale industrial plants (Lovett *et al*., 2000). Traditional extraction has been usually done by boiling water and skimming off the released oil while commercial one is conducted by pressing or solvent extraction with further refining and deodorizing of Shea butter (Israel, 2014). However, with the increased interest in naturally derived products, organic Shea butter production is preferred and thus efforts have been made to industrially produce Shea butter by following the traditional extraction methods. The Shea butter obtained from the traditional extraction procedure not including a refining stage is called “unrefined Shea butter”. Either at the village or industrial level, Shea butter is extracted from dried Shea kernels.

It is divided into two uses primarily: Medicinal use and industrial use of Shea butter. Shea butter is sometimes used as a base for medicinal ointment. Some of the isolated chemical constituents are reported to have ant-inflammatory, emollient and humectants properties. The butter has been used as a sun-blocking lotion and some of its components have limited capacity to absorb ultraviolet radiation (Israel, 2014). It is mainly used in the cosmetics industry for skin and hair related products (lip moisturizer, skin moisturizers, creams and hair conditioners for dry and brittle hair (Israel, 2014). It is also used by soap makers typically in small amounts (5-7% of the oils in the recipe), because it has plenty of unsaponifiables, and higher amounts results in softer soaps that have less cleaning abilitpries. The economic importance of Shea butter includes: moisturizes dry skin, treats acne and blemishes through its healing properties attributed to the presence of fatty acid and plant sterols such oleic, palmitic, stearic and linoleic acids. It also reduces skin inflammation, the presence of cinnamic acid that exhibits anti – inflammatory process (Israel, 2014). It has also been reportedly used as an anti – ageing and anti free radical agents. Provides relief to itchy and peeling skin, reduces stretch marks during pregnancy. Helps soothes skin and baby diaper rash. Excellent lips care during harmattan. Repair damaged hair and prevents dandruff. It soothes and helps to soften the hair. Soothes dry and itchy skin, serves as natural hair conditioners because of the presence of vitamin A and E to the end of the hair. Used in the treatment of rheumatism and arthritis (Moharram *et al*., 2006).

II. MATERIALS AND METHODS

Sample collection

Shea butter was procured from traders at Swali market Bayelsa state, Nigeria on 12th April, 2018 from five different stores.

Chemicals and Reagents

The reagents used in the current study include Sulphuric acid, Sodium hydroxide, Petroleum ether, Nitric acid and Ethanol. All reagents are of Analytical grades.

Apparatus and Equipment

Petri dishes, Crucibles, Conical flask, Beakers, Hot plate, Burette, Round bottom flask, Soxhlet apparatus, Vacuum oven, Furnace, Water bath, Measuring cylinders and 100ml volumetric flask, AAS, Flame photometer.

Sample Preparation

A container was washed, cleaned and sun dried, a composite mixture of the Shea butter was blended together in an electric blending machine (Qlink QBL-20L330) and the homogeneous mixture was stored in a clean glass container with lid and stored at room temperature for further analysis

Determination of Moisture Content

Three Petri dishes were cleaned with ethanol, dried, labeled A, B & C and pre-weighed using an analytical weighing balance. 2g of Shea butter was weighed in each Petri dish.

The sample was dried in the vacuum oven at a temperature of 50^{0}C for 3 hours, cooled in desiccators and weighed. The drying and weighing was repeated twice until constant weight was achieved. The moisture content was achieved following the method of AOAC (2005).

$$\%\ Moisture\ content = \frac{weight\ of\ fresh\ sample - weight\ of\ dry\ sample}{weight\ of\ fresh\ sample} \times \frac{100}{1}$$

Determination of Ash Content

Three crucibles were cleaned with ethanol, dried, labeled A, B & C and pre-weighed using an analytical weighing balance. 2g of Shea butter was weighed in each crucible. The samples were dried in the furnace at a temperature of 650^{0}C for 4 hours, cooled in desiccators and weighed (AOAC, 2005).

$$\%\ Ash\ Content = \frac{Mass\ of crucible + fresh\ sample - Mass\ of\ crucible =}{mass\ of\ crucible + fresh\ sample} \times \frac{100}{1}$$

Determination of Crude protein

1g of Shea butter was weighed into a conical flask, 15ml of H_2SO_4 was added and heated using a hot plate inside a fume cupboard for 3 minutes until a reddish brown colour was achieved. 50ml of deionized of water was added to the digest. The mixture was filtered using a whatmann filter paper no 14 and a funnel into a 100ml volumetric flask and filled to mark with distilled water.

20ml of digested Shea butter was pipette into a conical flask, 5 drops of phenolpthalin indicator was added to give a reddish pale colour. Then NaOH was titrated against the digested Shea butter till end point was reached.

The conversion factor of 6.25 was used to calculate the amount of protein contained in the Shea butter (AOAC, 2005)

$$\%\ Crude\ Protein = \left(\frac{Titrant\ value \times 1.401 \times N\ of\ base \times 6.25 \times Vol\ of\ NaOH}{Mass\ of\ Sheabutter\ digested}\right) \times 100$$

Determination of carbohydrate

Carbohydrates were determined by difference using the method in AOAC (2005)

$$Carbohydrates = [100 - (Moisture\ content - Crude\ lipids - Crude\ protein)]$$

Determination of Crude Fat

5g of Shea butter was extracted with petroleum ether solvent using soxhlet apparatus heated with a water bath for 4 hours. The crude fat extracted was concentrated in a water bath. The percentage crude oil content was then determined gravimetrically (AOAC, 2005).

Determination of Crude fibre

The residue of Shea butter and petroleum ether in the thimble of the soxhlet apparatus were collected in a pre-weighed beaker dried in a vacuum oven at 500C and kept in a desiccators to cool and weigh.

1 g of Shea butter was weighed into a 30ml digestion tube, 10ml of concentrated sulphuric acid (H_2SO_4), 5ml concentrated nitric acid (HNO_3) and 5ml of per chloric acid ($HClO_4$) was added. The sample was digested in a Gallenkamp Hot plate set at 300^{0}C.The digestion was continued until the solution was colourless this was to ensured the removal of all traces of nitric acid (HNO_3).

The samples were allowed to cool and 20ml of deionised water was added with gentle swirling, the solution was filtered using a Whatman filter paper No.42 followed by dilution to the mark with deionized water in a 50ml volumetric flask. The digested Shea butter was analyzed for the selected mineral elements concentration using Buck 211 Atomic Absorption Spectrophotometer and UV/V Spectrophotometer.

Determination of selected metals

Sodium (Na), Magnesium (Mg), Potassium (K), Copper (Cu), Manganese (Mn), Iron (Fe), Calcium (Ca), Zinc (Zn) was determined using Buck Scientific 200 AAS (AOAC, 2005)

III. RESULTS AND DISCUSSION

Proximate of composition Shea butter

Table 1: Proximate analysis for Shea butter sold in Swali market Yenagoa, Nigeria.

Parameters	Mean±SE
Moisture Content	5.220±0.113
Ash Content	0.084±0.006
Crude protein	42.316±0.000
Crude Fat	97.000±0.000

Carbohydrate	34.096±0.011
Crude Fiber	0.111±0.000

Table 2: Elemental analysis for Shea butter sold in Swali market Yenagoa, Nigeria.

Parameters	Mean±SE
Na	836.83±0.101
K	45.81±0.000
Mg	2.56±0.111
Ca	31.31±0.113
Cu	0.18±0.000
Zn	0.30±0.101
Fe	0.61±0.100
Mn	0.23±0.103

IV. DISCUSSION

The results showed that the carbohydrate content ranged between 30 and 40%. According to Pearson (1990), values of total carbohydrates in the range of 40-60% are for edible, domesticated and wild fruits. Carbohydrates are very vital in nutrition because they are good sources of energy (Anhwange *et al*., 2004). This therefore justifies the promotion of consumption and commercialization of Shea butter sold in Swali market Bayelsa state Nigeria.

The crude fibre content is beneficial in the diet of man because it plays an important role in decreasing many disorders such as constipation, diabetes, cardiovascular diseases and obesity. The value of crude fibre 0.111 when compared to other saturated fats is acceptable W.H.O (2007).

Protein is a large macromolecule consisting of a long chain amino acids held together by peptide linkages. The concentration of protein in the Shea butter is more than sufficient for consumption as advised by W.H.O (2003).

The crude fat (lipid) content of Shea butter has a concentration of 97.000±0.000%. Fat plays a very important role in nutrition and health. They are the second major sources of energy and antioxidant (Arayne *et al*., 2005).

As represented in Table 2, Shea butter contains very high concentration of Sodium (Na) (836.830±0.101 *mg/100g*) this makes it useful in the management of high blood pressure. Shea butter contains average amount of Potassium (K) (45.810±0.000 *mg/100g*) which is very good at managing Osteoporosis and kidney stone. The presence of Calcium and Magnesium in the Shea butter sample makes it useful in blood coagulation (clotting), the strengthening of the bones and teeth and in relieving anxiety (Bhutta *et al*., 2000). The values obtained for Copper, Zinc, Iron, and Manganese were within acceptable ranges.

The results show that consumption of Shea butter sold in Swali market is safe and has very good health benefits.

V. CONCLUSION

The findings of this study showed that Shea butter has adequate nutritional and health benefits. Hence, its consumption should be promoted among communities. Additionally, previous studies on physicochemical characteristics of Shea butter have shown that it has a very high industrial viability and as such; could be used as a raw material for cosmetics, soap and food processing industries.

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Investigation of Cowpea Variety and Storage Methods on Cowpea Beettle Infestation

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Abstract**—** *Investigation of effect of cowpea variety and storage methods on cowpea beetle (callosobuchus maculates) control was carried out with the main aim of providing suitable, safe and affordable methods of storing various varieties of cowpea devoid of infestation. Three varieties of cowpea which are White, Brown and Black varieties were used for this research. Various storage methods which include Plastics, Polyethylene, Hessian bags and Aluminum Bins of 10 kg capacity each was used in storing the cowpea. Twenty (20) cowpea beetle were introduced into the stored cowpea. Storage chemicals (phostoxine and Atelic dust) were introduced into the various stored cowpea at the same time of introducing the cowpea beetle. For each treatment there was a control and each was replicated three times. The storage was observed for a period of 90 days. The data generated were analyzed using MINITAB statistical software in analysis of variance (ANOVA).The result showed thatthe control treatment was generally less effective than the phostoxine and atelic dust at 90 days of storage and infestation of the cowpea. From tables 1, 2 and 3 all the storage methods were effective against the insect with significantly varying degree of efficiencies. Cowpea beetle mortality was significantly affected on the white variety more especially on the polyethylene storage and plastic containers with 95% and 100% mortality respectively. The Atelic showed mortality rate of 85 % for both polyethylene and plastic containers while the control treatment has a value of 65 % and 70 % for both the polyethylene and plastic containers respectively. The Aluminum bin showed 80% mortality on phostoxine combination, 75% for the atelic and 50 % for the control treatment.The mortality on the Black variety was significantly high after the 90 days infestation and storage for all the treatments and storage methods except for the Hessian bags that indicated 65 % for phostoxine, 60 % for atelic and 0 % for the control treatment. The black variety showed less susceptibility to cowpea infestation when stored in polyethylene bag and airtight plastic container which had 100 % mortality each.*

Keyword**— Cowpea, storage insect, varieties, infestation, mortality rate.**

I. INTRODUCTION

Cowpea (*vignaunguiculata*) (L) walp is a warm weather crop that is well adapted to drier region of the tropics like Nigeria where other food legumes do not thrive well. It is one of the most economically and nutritionally important indigenous African grain legumes produced throughout the tropical and subtropical areas of the world (Golob et. al., 1999). Nigeria is its largest producer and consumer, accounting for about 45 percent of its world production (Degri, 2008), while Africa account for about 75% (Brternburg et.al., 1995). Cowpea seed pods are consumed in fresh form as green vegetables in some African countries, while the rest of the cowpea plant serves as a nutritious fodder for livestock and as a source of cash income when sold to farmers who use them as livestock

feed. Cowpea seeds are also a rich source of minerals and obtains (Adeduntan et.al., 1998). Cowpea is sometimes called poor man meat or vegetable meat due to its high protein content. Cowpea grain contain 23.4% protein, 1.8 % fat and 60.3 % carbohydrates and a good source of vitamins and phosphorus (Adediran and Akinneye. 2004).

Despitethe great value of cowpea particularly in Nigeria, their availability and utilization have been impaired due to the seed damage by insect pest particularly the larvae of cowpea beetle (*callosobuchusmaculatus)* (Ofuya and Lale, 2001). Attack by insect pest species begins in the field and continues in storage causing substantial damage to store grain legumes as the pest rapidly increase. It has been reported that both quantitative and qualitative losses arising from physical, chemical and biological factors e. g fungi, rodent, birds and insect occur during storage of grains (Emeasor*et.al*., 2007). Up to 100% *Callusobruchus maculatus*infestation of cowpea can occur after three to six months storage (Maina, 2011).

Majority of farmers in Northern Nigeria and some other countries, including the Sudan, (Baribusta et. al, 2010)use local or indigenous storage facilities to forestall the menace of these insect pest they use storage insecticide where available and affordable like the banned and highly restricted lindens (gammalin A) and the acceptable are like Aluminum or Atelic EC for storing their legume grains against cowpea beets, termites, rats and disease pathogens (Degri,2007).

Some local plants have been studied to show they have effect against the activity of insect pest. They include; *NeemAzadiracta* (A.juss), Nicotine (*Nicotinia*spp), pyrethrum *chrysantheumceneraefolium*), Rotenme (*Derriselliptica*) (C.P.F, 1987). Sadim apple "Locally name Usher" (Calotropis*procera* (J.), Sesame (*Sesamumindicum* L.), Garlic (*AllumSativum* L.) and (Lantana Camara), (Mueller et. al., 1995). They were all found to lower fecundity per female and adult emergence (Singh et'al, 1996). But the availability and side effects of these are also a major concern to farmers. Hermetic storage technology has emerged as a potent alternative to other method of storage that protects commodities from insect and molds have been developed and applied and they abound in type and the PICS (Purdue Improved Cowpea Storage) which was founded by the Bill and Melinda Gates foundation, is just one of these. The goal of the project is to have 50 % of farm-stored cowpea in hermetic storage without insecticide in west and central Africa (Murdock et al., 2003). This is still on-going.

From the forgoing, some methods of cowpea beetle control abound but not without so many limitations, they are not cheap and some are also hazardous to health. Application of storage chemicals are sometimes not done properly by the local farmer which can lead to food poisoning. Larger quantity of cowpea are sold off immediately after harvest by the local farmers because of lack of adequate storage methods and fear of infestation by cowpea beetles thereby selling at a lower price compared to cost of production. This makes the produce scarce after the period of harvest.

Thisresearch was carried out to investigate the effect of variety and storage methods on the control of the cowpea beetle *Callosobruchus maculates (f) (coleopteran: Bruchide)* on stored cowpea. Effect of various storage methods on the control of cowpea beetle was also investigated as well as the variety that responds well to the various storage methods.

II. MATERIALS AND METHODS

2.1 Sample collection and preparation

The following materials were used for the research, three varieties of cowpea: white variety (Kanannado), brown (Ife brown) and black (Akidi) variety. Insect pest cowpea beetle *callosobruchusmaculates*, was used as the insect pest, which were introduced to each treatment at same level. The seed scanner also known as dianophoscope was used to scan the cowpea seed in order to detect the effect of insect damage from each treatment. The storage methods used in this research are polyethylene (hermetic), storage bins which are made of aluminum, plastic containers and hessian bags. The storage chemicals that were used are phostoxin and atelic dust. These chemicals were chosen because they are mostly used by farmers in Bauchi State and in the wrong proportion and application. All the experimental materials were purchased from a local grain market in Bauchi State, Nigeria.

2.2 Methods

2.2.1 Cleaning and Determination of Moisture content

The purchased cowpea was cleaned to remove debris and all other foreign materials, this was done by hand picking, sorting and using winnower.Moisture content of each of the cowpea variety were determined using standard methods as used by Abodenyi et. al., 2018. This was to ensure that the sample were at the safe storage moisture content to minimize spoilage during storage period.

2.2.2 Experimental procedures

2 kg of each variety were put in nine Polyethylene bags, the first three had phostoxine tablets introduced into it, and the next three had the atelic dust of 2 gm introduced into them, the last three served as control with no treatment. Each of the storage samples had Twenty (20) cowpea beetles introduced into them. These methods were repeated for the Aluminum storage bins, the Plastic containers and the Hessian bags for each variety. After introduction of the storage pest, the samples were agitated for one minute each to allow even spread of the pest and storage chemical (Ebiamadon et al., 2011)

The experimental set up were laid out in a completely randomized design with three replicates kept in the post-harvest laboratory of the department of agricultural bio-environmental engineering of federal polytechnic, Bauchi, Nigeria at 31± 2 ^{o}C and a relative humidity of 65±5 for a period of 90 days

2.3 Data Collection and Statistical Analysis

The rate of infestation was determined for each variety after 90 days of infestation with the pest, the following data were collected.

1. Number of live and dead insects: this was counted manually and recorded from each treatment.
2. Percentage damage grains. The number of grains with holes and grains without roles in all the treatments in each variety: this was done by pouring the seed on a seed scanner to detect the damage seeds in each treatment, and manually counting the number of grains with holes and those without holes. The holes on the grain was used as an indicator of damage. Percentage grain damage was determined using the following formula.

$$percentage\ damage\ (\%) = \frac{number\ of\ damage\ grains}{total\ number\ of\ grain\ sampled}\ X100$$

Minitab statistical software was used in the analysis of variance (ANOVA) to determine the variation in results of all the experiments under the various independent variables and their interaction at 95% level. Descriptive statistics such as percentage was also used in presenting the data.

III. RESULTS

The results obtained are as presented in the tables below for the three varieties of cowpea

Table 1: Mean Effect of Cowpea Beetle Mortality on White Variety at 90 Days after Infestation

	Treatments								
Storage methods	Phostoxine			Atelic			Control		
	Number of live beetles	Number of dead beetles	Percentage mortality (%)	Number of live beetles	Number of dead beetles	Percentage mortality (%)	Number of live beetles	Number of dead beetles	Percentage mortality (%)
Polyethylene Bags	1	19	95	3	17	85	7	13	65
Aluminum Bins	4	16	80	5	15	75	10	10	50
Hessian Bags	7	13	65	10	10	50	20	0	0
Plastic containers	0	20	100	3	17	85	6	14	70

Table 2: Mean Effect of Cowpea Beetle Mortality on Brown Variety at 90 Days after Infestation

	Treatments								
Storage Methods	Phostoxine			Atelic			Control		
	Number beetles	Number dead beet	Percentage mortality (%)	Number beetles	Number o beetles	Percentage mortality (%)	Number beetles	Number o beetles	Percentage mortality (%)
Polyethylene Bags	3	17	85	5	15	75	9	11	55
Aluminum Bins									
Hessian Bags	6	14	70	6	14	70	15	5	25
Plastic containers	9	11	55	11	9	45	20	0	0
	3	17	85	4	16	80	9	11	55

Table 3: Mean Effect of Cowpea Beetle Mortality on Black Variety at 90 Days after Infestation

	Treatments								
Storage methods	Phostoxine			Atelic			Control		
	Number of live beetles	Number of dead beetles	Percentage mortality (%)	Number of live beetles	Number of dead beetles	Percentage mortality (%)	Number of live beetles	Number of dead beetles	Percentage mortality (%)
Polyethylene Bags	0	20	100	1	19	95	7	13	65
Aluminum Bins	3	17	85	5	15	75	11	9	45
Hessian Bags	7	13	65	8	12	60	20	0	0
Plastic containers	0	20	100	2	18	90	7	13	65

Table 4: Mean Percentage (%) of Damaged Cowpea at 90 Days after Infestation

	Treatments								
Storage methods	White variety			Brown variety			Black variety		
	Phostoxine	Atelic	Control	Phostoxine	Atelic	Control	Phostoxine	Atelic	Control
Polyethylene Bags	10	12	40	15	15	50	8	10	30
Aluminum	40	50	60	50	55	60	30	40	55
Bins	50	60	90	60	60	90	40	50	70
Hessian Bags	9	10	35	12	20	40	8	10	30
Plastic containers									

3.1 Discussion

3.1.1 Cowpea Beetle Mortality on the various varieties of cowpea

The control treatment was generally less effective than the phostoxine and atelic dust at 90 days of storage and infestation of the cowpea. From tables 1, 2 and 3 all the storage methods were effective against the insect with significantly varying degree of efficiencies. Cowpea beetle mortality was significantly affected on the white variety more especially on the polyethylene storage and plastic containers with 95% and 100% mortality respectively. The Atelic showed mortality rate of 85 % for both polyethylene and plastic containers while the control treatment has a value of 65 % and 70 % for both the polyethylene and plastic containers respectively.The Aluminum bin showed 80% mortality on phostoxine combination, 75% for the atelic and 50 % for the control treatment. The reduction of oxygen during the 90 days of storage after the infestation reduced the insect count drastically especially in the polyethylene bags and the plastic containers. This cannot be said of the Hessian bags because they are porous and allowed the thriving of the storage pest in all the treatments. This result agrees with the findings of (Ebiamadon et al., 2011) which researched the effectiveness of different botanical pesticides on control of *C. maculatus*at 30 and 90 days of infestation.

The mortality of cowpea beetle on the Brown variety, cowpea beetle mortality was significantly affected by the storage chemicals and the storage methods. Polyethylene together with phostoxine and plastic containers showed high mortality of 85 %. Atelic with polyethylene and plastic containers has mortality rate of 75 % and 80 % respectively. The control treatment indicated mortality of 55 %, this result agrees with PICS project (Villers, et al., 2008) which used the Hermetic storage methods by keeping away oxygen from the pest they were able to record 50 % mortality. The Hessian bags showed 0 % mortality for the control treatment.

Cowpea beetle mortality on the Black variety was significantly high after the 90 days infestation and storage for all the treatments and storage methods except for the Hessian bags that indicated 65 % for phostoxine, 60 % for atelic and 0 % for the control treatment.

3.1.2 Cowpea Damage at 90 Days after Infestation

Table four shows the degree of damage on the three varieties of cowpea after 90 days of infestation. The Hessian bag recorded the highest percentage of damage on all the storage methods and treatments for the three varieties. This can be attributed to the fact that the Hessian bag is porous that allowed intake of oxygen that allowed the survival of the storage pest. The White and black variety recorded less damage form the beetle from all the storage methods and treatments, this could be as a result of the high protein content of Brown beans, storage pest tends to feast more on highly protein food (AOAC, 2010).

IV. CONCLUSION

From the above results, it can be concluded that the black variety is less susceptible to cowpea infestation when stored in a polyethylene bag as well as in an airtight plastic container.

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Rating the Potential Landscape Changes through the Visual Resource Inventory in North Metn Lebanon

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Abstract— *North Metn is known for its miscellaneous landscapes varying from mountainous, coastal, agricultural, natural, rural, urban and industrial. However its land-cover, land-use and landscape structures are highly affected by manmade and natural means of transformation throughout a short period of time. Since the assessment of these changes is essential for any landscape management in the district, the Visual Resource Inventory (VRI) was selected for that purpose. VRI provides the basic visual resource data for future landscape planning purposes in the evaluation area. The results were analyzed for the district's visual potential based on several criteria. It is essentially used to rate the landscape for its Scenic Quality and Sensitivity Level within Distance Zones delineated. North Metn is distinguished by its valuable scenic quality and panoramic landscape views taking into account the users Landscape Importance and the particular Viewing Locations threatened by drastic changes, eighty-two Viewpoints (VP) were selected to be assessed using the VRI methods. Each VP was decomposed by its Landscape Elements in the Foreground, Middle and Background Extent taking into consideration its forms, textures, lines and colors. Each VP Scenic Quality (SQ) was rated for its Integrity, Vividness and Harmony while the View Importance (VI) was rated for its VP, Viewed Landscape and Viewer Concern. Later on, the SQ and the VI ratings will provide rankings ranging from Very high Scenic Inventory Value (SIV) rate to a Very Low SIV rate. North Metn's landscape was classified within three landscape classes depending on each rate.*

Keywords— ***Landscape character, landscape elements, Scenic inventory value, Scenic quality rating, view importance rating and viewpoints.***

I. INTRODUCTION

Around the world, rates of land change surge increased greatly over the next 20–50 years, as human populations continue to grow and migrate (Alig et al. 2004; Theobald 2005) Hence, Human social and economic activities drive land change at all scales, and may enhance or hinder the movement of materials via wind, water, and biological and social vectors, sometimes in surprising ways that cut across scales (Kareiva et al, 2007; Peters et al., 2008. Urbanization, in particular, alters both biotic and abiotic ecosystem properties within surrounding, and even at great distances from urban areas (Grimm et al. 2008). Besides the Mediterranean being one of the most prominent and vulnerable climate change hotspots (Giorgi F. 2006, Kim GU et al, 2019), other factors were eminent in the recent Mediterranean region's characterization such as: land abandonment, tourism development, population concentration along the coast, the construction of extended transportation networks (Burke & Thornes, 1998) and the accelerated socio-economic changes (Arianoutsou M., 2001). Thus, this region is witnessing landscape changes because of the dynamic equilibrium between humans and the Mediterranean environment resulting in a remarkably rich landscape. (Arianoutsou M., 2001)

Landscape means an area, as perceived by people, whose character is the result of the action and interaction of natural and/or human factors; (European Landscape Convention, 2000) Therefore, any study related to

landscapes is often considered subjective especially when it comes to Visual Resources which The Bureau of Land Management (BLM) in the United States (US) has defined as: the visible physical features of a landscape (e.g., land, water, vegetation, animals, structures, and other features). And since all land has inherent visual values that warrant different levels of management (BLM, 2011), they have been developing the Visual Resource Inventory (VRI). It develops, refines and implements visual analysis and management systems. It provides a tool for assessing the visual qualities of the landscape in objective terms (BLM, 2016b). The VRI is implemented by governments, environmental ministries, consultants and NGO's throughout the years for future sustainable planning purposes and it is updated thoroughly.

Lebanon's main landscape visual resources were determined by topography which has always played a decisive role in the history of its settlements and still is to date: It has granted Lebanon its magnificent landscapes (mountains, valleys, cirques, plains, etc.), enriching its potentials and its quality of life (SDATL, 2005). However, intensive urban sprawl, increasing road networks, lack of urban planning, agricultural intensification, and carelessness of natural, cultural and visual resources, political actions, wars and land abandonment are widespread landscape change processes. Its capital Beirut and its surroundings have an average urban growth rate of 1.8 km2 per year and it continues to increase along the coastal zone and the nearby mountain, greatly reducing the green space and the rural character of these areas. (Faour G., 2014) North Metn is one of the casas combining the coastal zones and the mountainous areas, forming a large variety of landscape to study.

North Metn is a casa near Beirut, enclosing 100 localities under 52 municipalities. It is a living example of the anthropogenic changes that a landscape could face. Therefore, in order to understand the impact of the changes, the VRI should be implemented as a basic guide in order to evaluate the status of any future planning to achieve sustainable development plans. Thus, throughout this paper, this inventory's methodology will be detailed and the results will be analyzed within specific viewpoints after classifying the North Metn's landscape.

1. Materials and methods

The VRI has proved to be an efficient way in quantifying the landscape in order to achieve maximum sustainability, therefore, it was developed specifically to meet requirements and designed to work for many types of landscapes and visual settings. It considers, for instance, the context of a park area's visual setting and provides a framework for understanding and protecting the scenic values within that context (National Park Services, 2016). Thus, it helps in recognizing the need to develop a comprehensive approach to assess scenic values of landscapes and their surrounding areas, to develop strategies to protect them as a resource for future generations and to evaluate the scenic resources under its jurisdiction and to develop management objectives for those resources (Meyer and Sullivan, 2016).

The inventory process is a systematic description of the landscape characters, visual elements, scenic quality, sensitivity level and importance to visitor experience and interpretive goals for important views inside and outside studied areas. (BLM, 2016b). It takes into consideration the detailed forms and descriptive views of a landscape with the visual receptors and the view amenity. Hence visual assessment of the landscape using these tools establishes identifiable, consistent qualities that can be described and measured (Meyer and Sullivan, 2016).

Therefore, after all the transformation in the landscapes of North Metn, accomplishing several goals in protecting, managing should be executed and Visual Resource Inventory is one of the newest updated methodologies to help in this matter.

The inventory consists of choosing viewpoints based on different criteria. Then, it starts with a scenic quality evaluation, sensitivity level analysis, and a delineation of distance zones. Based on these three factors, the regions of North Metn are placed into one of four visual resource inventory classes. These inventory classes represent the relative value of the visual resources. Classes I and II being the most valued, Class III representing a moderate value, and Class IV being of least value (BLM, 2011). Usually, the implementation options and the detail of the inventory will vary with the visual character of the landscapes being inventoried.

The components of landform, land cover including urban areas, infrastructure and water as per the New Zealand Landscape Classification Guide helped in recognizing that landscapes surround and are experienced from a multiple of geometrical perspectives that can be obtained from movement and exploration (Brabyn, 2006). Therefore, the landscape of North Metn was classified as coastal, urban, rural and mountainous after multiple field visits throughout 2019's summer and 2020's winter, (Fig. 1).

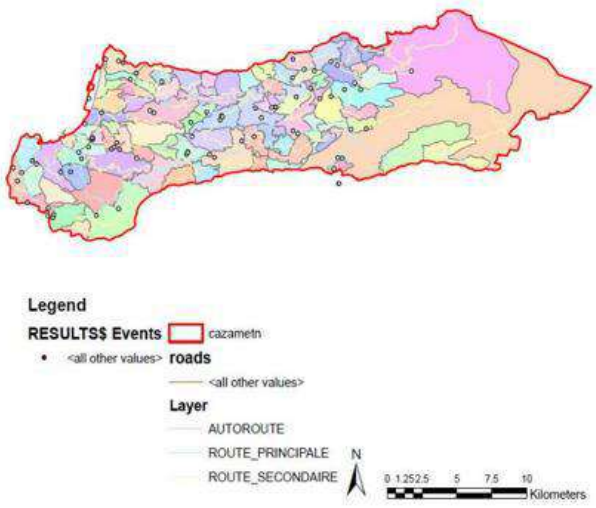

Fig. 1: Map of viewpoints distribution in North Metn

Choosing the right viewpoints was the key to the inventory based on the points considering a human's normal field of vision and panoramic view, locations which represent a range of near-, middle- and long-distance views, views from both public and private viewpoints and views from sensitive receptors within designated landscapes.

During field visits, the altitude, distance from view, GPS coordinates of each viewpoint was registered. And each viewpoint had its unit name; for example, the region of Bikfaya's unit name was: BKFA19_1 where 19 shows the year of the photo taken and the 1 stands for the 1st viewpoint taken for this region.

Besides, the description of the weather, observer position and view direction were also stated and taken into consideration. Later on, a Form of Landscape Description & Scenic Quality and a form of View Importance were filled as described in the Table 1 (Meyer and Sullivan, 2016).

Table 1: Scenic Inventory Value Factors

SCENIC QUALITY FACTOR													**VIEW IMPORTANCE FACTOR**													
Landscape character integrity				Vividness				Visual harmony							Viewpoint importance				Viewed landscape importance				Viewer concern						
Landscape character elements	Elements quality & condition	Inconsistent elements	**Integrity total**	Focal points	Forms/lines	Colors	**Vividness total**	Spatial relationship	Scale	Color	**Harmony total**	**Total**	**SQR**		Publicity	Facilitites and management	Interpretive services	Viewpoint total	Publicity	Specially designated areas	Interpretive themes	Viewed landscape total	Daily visitation	View duration	Viewer sensitivity	Viewer concern total	**View importance total**	**VIR**	**SIV**

The Landscape Description and Scenic Quality form takes into consideration the View type, character type, landscape elements and Design elements based on Landscape Character Integrity, Vividness and Visual Harmony in order to obtain the Scenic Quality Rating (SQR) The SQR is defined as the value of the viewed landscape based on its perceived visual attractiveness, as determined by the aesthetic composition of the visual elements. The Rating goes from A with the highest scenic quality to E with the lowest (BLM, 2016c).

Whereas the View importance of the viewed landscape, viewpoints and the viewer take into consideration the Viewed landscape Importance, the Viewpoint Importance and the Viewer Concern in order to have the View Importance Rating (VIR) going from 1 as the highest View Importance to 5 as the lowest (BLM, 2016c).

Eventually, the Scenic Quality Rating and the View Importance Rating will give the Scenic Inventory Value (SIV) for future landscape planning and environmental purposes in North Metn.

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Table 2: SIV Rating Matrix

Scenic Quality	View Importance Rating				
	1	2	3	4	5
A	VH	VH	VH	H	M
B	VH	VH	H	M	L
C	H	H	M	L	L
D	H	M	L	VL	VL
E	M	L	VL	VL	VL

The Scenic Inventory Value (SIV) is the combination of SQR and VIR into a single measure. The SIV is derived using a matrix as seen in TABLE 2 to arrive at one of five SIV going from (VH) very high to (VL) very low.

II. RESULTS AND DISCUSSIONS

After the implementation of the VRI in North Metn after collecting all the data needed, a summarizing table was created as seen in TABLE 3 below, showing in the end column the SIV of each landscape.

The actual data was then imported into GIS as shape files in order to materialize the SIVs throughout North Metn and to help in analyzing the data within its context. The main two factors studied were the altitude and the Land use.

At the end, the topography of North Metn do play a decisive role in the creation of its landscapes and in the distribution of its land use. Both remaining key factors in comprehending the scenic quality of North Metn leading the way in understanding the human intervention's impact.

Table 3: Scenic Inventory Value results

VP	UNIT NAME	SQR	VIR	SIV
1	ABMZ19_1	**C**	**3**	**M**
2	ABMZ19_2	**D**	**4**	**VL**
3	AEQ19_1	**C**	**3**	**H**
4	AEQCH19_1	**C**	**3**	**M**
5	ANT19_1	**D**	**3**	**L**
6	AS19_1	**B**	**3**	**H**
7	ATCH19_1	**B**	**4**	**M**
8	AZBD19_1	**B**	**2**	**VH**
9	BAY-MT20_1	**D**	**3**	**L**
10	BAY-MT20_2	**C**	**3**	**M**
11	BBMNA19_1	**B**	**2**	**VH**
12	BDT19_1	**B**	**3**	**H**
13	BDT19_2	**B**	**3**	**H**
14	BKFA19_1	**B**	**2**	**VH**
15	BKFA19_1	**B**	**3**	**H**
16	BKFA19_2	**B**	**3**	**H**
17	BMK19_1	**C**	**1**	**H**
18	BMNA19_1	**B**	**2**	**VH**
19	BMNA19_2	**D**	**4**	**VL**
20	BR19_1	**D**	**3**	**L**
21	BSK-AEA19_1	**A**	**2**	**VH**
22	BSKTA19_1	**B**	**4**	**M**
23	BSLM19_1	**B**	**2**	**VH**
24	BTGN19_1	**A**	**2**	**VH**
25	BZDN19_1	**B**	**2**	**VH**
26	BZDN19_2	**A**	**2**	**VH**
27	BZDN19_3	**B**	**2**	**VH**
28	CHWA19_1	**C**	**3**	**M**
29	DBMRN20_1	**E**	**2**	**L**
30	DCH19_1	**B**	**3**	**H**
31	DH-A19_1	**D**	**3**	**L**
32	DHCH19_1	**A**	**2**	**VH**
33	DHCH19_2	**A**	**2**	**VH**
34	DH-Z19_1	**D**	**3**	**L**
35	DLB19_1	**B**	**2**	**VH**
36	DLB19_2	**B**	**3**	**H**
37	DM19_1	**C**	**3**	**M**
38	DO19_1	**B**	**2**	**VH**
39	ECH19_1	**D**	**4**	**VL**
40	ENM19_1	**D**	**3**	**L**
41	ENM19_2	**D**	**4**	**VL**
42	ENM19_3	**B**	**3**	**H**
43	ENM19_4	**C**	**3**	**M**
44	FNR19_1	**D**	**3**	**L**
45	HMLY19_1	**B**	**3**	**H**
46	HT19_1	**D**	**3**	**L**
47	JAR19_1	**B**	**2**	**VH**
48	K_TR19_1	**C**	**4**	**L**
49	KFAQ19_1	**B**	**2**	**VH**
50	KHNCH19_1	**C**	**2**	**H**
51	MC-B19_1	**B**	**2**	**VH**
52	MC-D19_2	**C**	**3**	**M**

53	MCHKH19_1	**C**	**3**	**M**
54	MCHKH19_2	**B**	**2**	**VH**
55	MCHR19_1	**B**	**3**	**H**
56	M-DB19_1	**C**	**3**	**M**
57	MK19_1	**D**	**4**	**VL**
58	MKMNSR19_1	**C**	**3**	**M**
59	MNSR19_1	**C**	**4**	**L**
60	MNSR19_2	**C**	**3**	**M**
61	MRJ19_1	**B**	**4**	**M**
62	MRJ19_2	**C**	**3**	**M**
63	MS-ELH19_1	**B**	**3**	**H**
64	MS-ELH19_2	**C**	**4**	**L**
65	MZK19_1	**D**	**4**	**VL**
66	NEM19_1	**E**	**4**	**VL**
67	NENM19_1	**C**	**3**	**M**
68	NNM19_1	**C**	**4**	**L**
69	NNM19_2	**C**	**3**	**M**
70	R19_1	**B**	**2**	**VH**
71	ROU19_1	**E**	**5**	**VL**
72	SEF19_1	**D**	**3**	**L**
73	SEF20_2	**D**	**3**	**L**
74	SEF-BH19_1	**E**	**5**	**VL**
75	TR-JDSF19_1	**C**	**4**	**L**
76	ZATR19_1	**E**	**5**	**VL**
77	ZBGH19_1	**B**	**2**	**VH**
78	ZGHN19_1	**B**	**3**	**H**
79	ZKH19_1	**B**	**3**	**H**
80	ZKH19_2	**C**	**4**	**L**
81	ZKH19_3	**C**	**3**	**M**
82	ZKT19_1	**D**	**3**	**L**

The maps below in Fig. 2 & 3 show the viewpoints in relation with the altitude and the land use respectively.

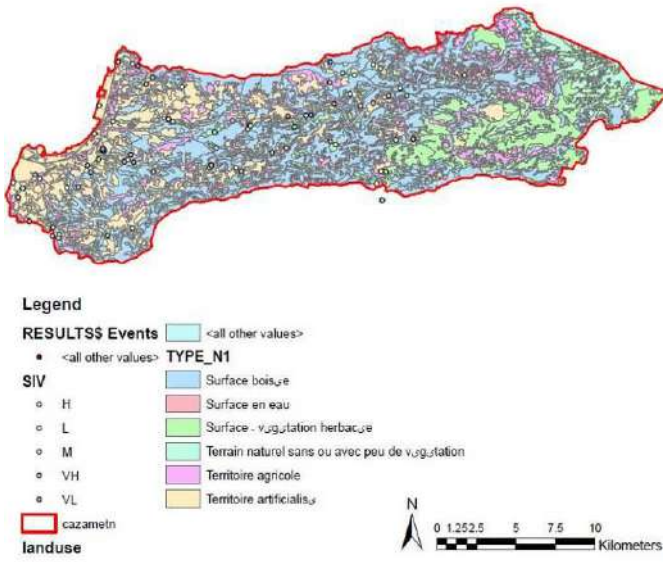

Fig. 2: Map of Viewpoints within North Metn Land use

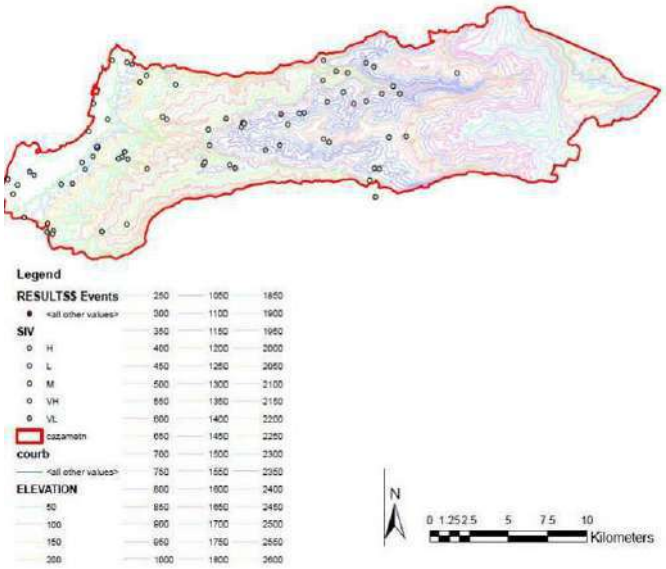

Fig. 3: Map of Viewpoints within North Metn altitudes

After analyzing the data, it's identifiable that the dominating landscape on altitudes from the sea level to 600m are wooded areas and artificial anthropogenic areas. On the other hand, all wooded area, vegetation area, agricultural area and water areas were dominating for area within the 600 and 1000 meters limitation whereas, all of the landscapes above 1000m were selected water areas, vegetation areas, natural areas with few vegetation and agricultural areas. Thus, the importance of sectioning the scenic quality analysis within these ranges of altitude and land use in order to classify the landscape for future protection, restoration and sustainable management proposals. Thus, now the SIV results obtained will be distributed into 3 sections based on the altitude as

mentioned before. And based on each SIV results, the landscape will be classified based on the Manual of BLM created for VRI. Classes range from Class I to Class IV as mentioned before.

As for the SIV results of the regions between 15 to 600 meters, (fig. 4 & 5), it is clear that the SIV is always between very low and medium with a combination of 64%. This result puts it in the class IV objective for providing for management activities which require major modifications if the existing character of the landscape. The level of change to the characteristic landscape can be high. Looking further into landscapes within these altitudes, the urban residential landscape characters and cement blocks are dominant (photo 1 & 2).

Photo 1: East North Metn Coastal landscape

Photo 2: North Metn urban landscape

These changes that this landscape has witness dominated the view and is now the major focus of the viewer attention. Therefore every attempt of future proposals should minimize the impact of these activities through careful location, minimal disturbance and repeating the basic elements (BLM, 2011).

As for the SIV results for the regions between 620 to 1000 meters are clearly recognized for a 71% of medium to very high SIV while 29% of the rest are between very low to low (fig. 6 & 7). This shows the importance of the scenic views for the receptors where noticeably the variety of the existing landscapes and the low impact of the changes occurring (photos 3 & 4). This puts it under the class III objective to retain the existing character of the landscape. The level of change of the characteristic landscape should be moderate while management activities may attract attention but should not dominate the view of the casual observer. Thus, the basic elements found in the natural feature of the characteristic landscape

Photo 3: Rural landscapes of North Metn

Photo 4: Rural and mountainous landscapes of North Metn

should be used in future interventions (BLM, 2011).

At the end, the SIV results of the regions going from a thousand meters and above has a percentage of 87% from medium to very high SIVs while only 13% rates as low (fig. 8 & 9). These landscapes are unique and shows the true cultural identity of the Lebanese mountains (Photos 5 & 6).

This landscape classifies as class II objective of retaining the existing character of the landscape whereas the level of change to the characteristic should be low. The human future activities could be seen but should not attract the attention of the casual observer. Any changes must repeat the basic elements of form, line, color and texture found in the predominant natural features of the characteristic landscape (BLM, 2011).

Photo 5: Mountainous landscapes of North Metn

Photo 6: Highest mountain's peak in Lebanon, Sannine

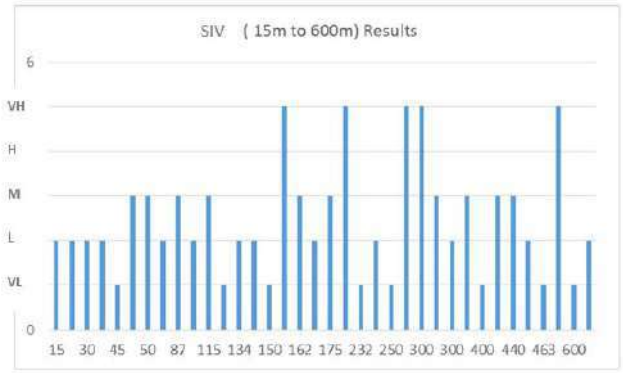

Fig. 4: SIV results of altitudes between 15m to 600m

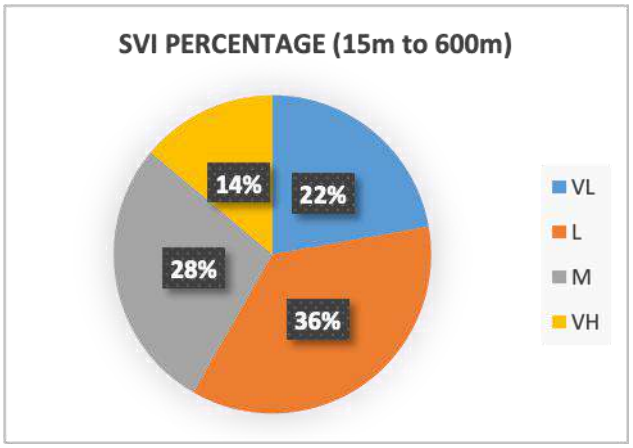

Fig. 5: SIV percentage of altitudes between 15m to 600m

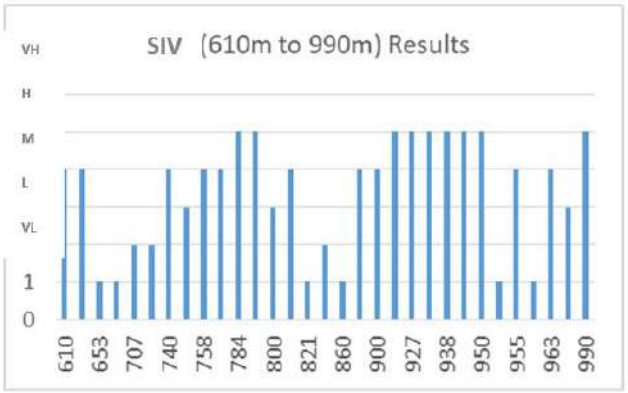

Fig. 6: SIV results of altitudes between 610m to 990m

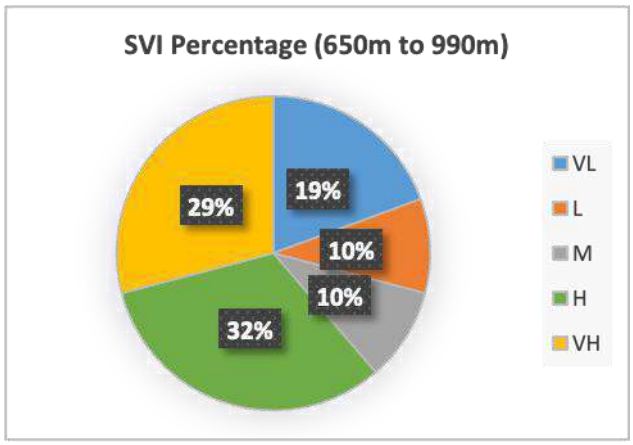

Fig. 7: SIV percentage of altitudes between 610m to 990m

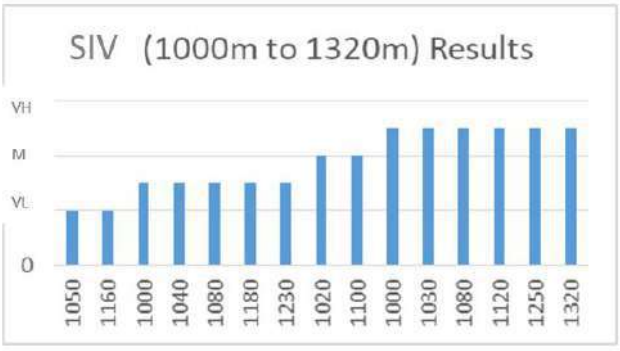

Fig. 8: SIV results of altitudes over 1000m

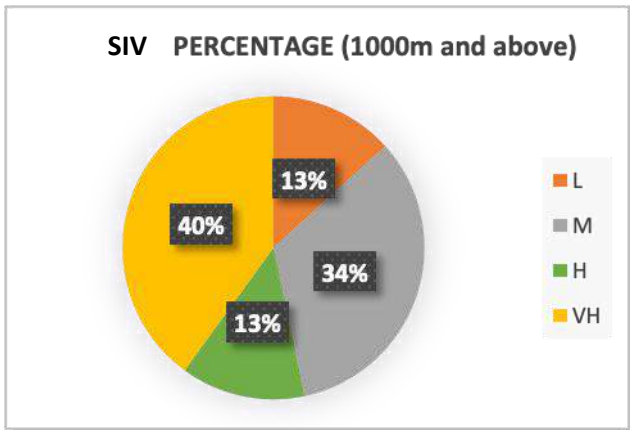

Fig. 5: SIV percentage of altitudes over 1000m

The descriptive information gathered in the VRI process and the scenic quality and view importance results can be used in the development of plans for the management of scenic resources.

III. CONCLUSION

North Metn is facing a lot of changes and concerns arising from everyday chaotic constructions on the coastal landscapes as much as on the mountainous landscapes. Even though, some changes have irreversible impacts, finding the solutions to preserve the existing cultural and patrimonial landscape and to restore the scenic quality landscapes should be the focus. By studying all the landscape characters and scenic values of North Metn, the process of preservation and restoration should be at ease in every future proposal and intervention.

Added to that, historic and cultural resources are essential to the scenic experience of the visitors and the users and the VRI incorporates historic, cultural, and other values in a measure of view importance, in addition to scenic quality.

Thus, Visual Resource Inventory is the solution to achieve objectives of reserving the cultural and historical landscapes, restoring damaged and transforming landscapes as managing and planning to new potential sustainable strategic land use. Hopefully, it would be used in all the remaining casa in Lebanon to achieve sustainable development and landscape protection and management all over the country.

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Transfusion Transmissible Infections among Voluntary Blood Donors in Port-Harcourt Metropolis, Rivers State, Nigeria

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Abstract**—** *A survey of Blood Transfusion Transmissible Infections (TTIs) among voluntary blood donors of different age groups was conducted between March 2017 and June 2018in Port-Harcourt metropolis, Rivers State, Nigeria. A total of 200 voluntary blood donors consisting of 151(75.5%) males and 49(24.5%) females participated in the study. All the blood donors were within the ages of 18 to 65 years. Blood samples were taken from the veins of blood donors. The blood samples were screened in the laboratory for Venereal Diseases (VD), Hepatitis B surface antigen (HBsAg), Hepatitis C Virus (HCV) and Human Immuno-deficiency Virus (HIV) 1 and 2 using test Kits. Of the 200 blood donors involved, none was positive for HIV 1 and 2 infections. Only 2(1%) participants were positive for VDRL infections. A total of 8(4.0%) of the study participants were positive for HBsAg while 1(0.5%) was positive for HCV. The prevalence of TTIs was higher in females 6(3.0%) than in males 5(2.5%), butthere was no significant differences in prevalence among genders (P> 0.05). Blood is still one of the main sources of transmission of Hepatitis B Virus (HBV), HCV and VD. Availability of safe blood for transfusion is necessary for the recuperation of the recipient. This can be achieved by vigorous screening of donors and donated bloods.*

Keywords**—** ***Transfusion, Infections, Blood, Donors, Port-Harcourt, Metropolis.***

I. INTRODUCTION

Blood transfusion worldwide is currently facing interesting challenges. A myriad of TTIs,HIV, HBV, HCV andVD, have provoked a greatly heightened emphasis on safety with inescapable implications for the complexity and cost of providing a transfusion service. The discovery that HIV, HBV and HCV could be transmitted through transfusion has heralded a new era in blood transfusion practice worldwide with emphasis on two fundamental objectives, safety and protection of human life(Diwan & Mathur, 2012; WHO, 2012).

There are estimated 33 million people living with HIV/AIDS in the world and of these 23 million are in sub-Saharan Africa. Blood transfusion accounts for 5-10% of the HIV infections in sub-Saharan Africa (Okoroiwu *et al*., 2018). Similarly, 12.5% of patients who received blood transfusion are at risk of post-transfusion hepatitis(Fasola & Otegbayo, 2002).People in developing countries still face the risk of acquiring these infections because mandatory screening test are not carried out. Many countries still lack a national blood transfusion service despite some recent improvement in this important area. Human Immunodeficiency Virus, HBV, HCV and VD are of great concern as transfusion-

transmissible infections because of their prolonged viraemia and carrier or latent state. They also cause fatal, acute, chronic and life-threatening disorders. These viral infections have emerged a global public health problem and a significant cause of mortality and morbidity in Nigeria and many parts of the world(Ejele *et al*., 2005).

The demand for blood is high and far outweighs its supply. Transfusion requirements and increasing demand for blood drives paid donors to donate more often than the required 12 weeks minimum interval which further complicates it. A significant number of the paid donor population has also been reported to be anaemic and further donation by such individuals without considering their baseline haemoglobin investigation could be harmful(Benedict *et al*., 2012)..Best practice as recommended by the World Health Organization ought to be put in place to be able to curb some of these issues.

There is a need to strictly regulate blood donation practices locally and the activities of those involved in blood banking. There is also a need to encourage non-remunerated voluntary blood donation practice which remains the gold standard in blood donation. However, voluntary donation is less than 25 % in Rivers State, Nigeria(Okoh *et al*., 2014) as a whole whereas in other States the rate is high. The aim of this study was to investigate TTIs among voluntary blood donors in Port-Harcourt Rivers State, Southern Nigeria.

II. MATERIALS AND METHODS

2.1 Study Area

This study was conducted in Port Harcourt.Port-Harcourt is a metropolitan city in the heart of the oil and gas industry in the Niger Delta region of Nigeria with a high influx of migrant workers from all parts of the world. The metropolis is an industrial centre with large number of multinational firms as well as other industrial concerns, particularly business related to the petroleum industry. It is the chief oil-refining city in Nigeria and has two main oil refineries that process around 210,000 barrels of crude oil daily. It is a port town that lies along the Bonny River (an eastern tributary of the River Niger), 66 km upstream from the Gulf of Guinea, with a population of 6,144,673 (Encyclopaedia Britannica). However, the city is characterized by low level of environmental sanitation, poor housing, lack of potable water and improper management of wastes especially in the indigenous core areas characterized by high density and low/middle income populations (Ayotamuno& Gobo, 2004).

2.2 Study Design

The study was a cross sectional survey of transfusion-transmissible infections among voluntary blood donors of different age groups in Port-Harcourt metropolis. The research was conducted between March 2017 and June 2018.

2.3 Study Population

A total of two hundred 200 voluntary non-remunerated blood donors who consented, were recruited in the study. The blood specimens were collected over a period of six months.

2.4 Ethical approval

Ethical approval for this study was obtained from the ethical committee of the Ministry of Health, Government of Rivers State, Nigeria (MH/PRS/391/VOL.2/705).

2.5 Consent

Informed consent of the study participants were obtained after due sensitization. Donors' Confidentiality was assured.

2.6 Criteria for selection of participants

2.6.1 ***Inclusion criteria:*** included in the study were apparent healthy donors between 15 to 65 years of age, with no history of long-term medication use, with no history of blood transfusion within the last 3 months preceding the study, haemoglobin concentration ≥12.5 g/dL prior to donation, willingness to give oral informed consent after counselling and non-menstruating or breast-feeding women.

2.6.2*Exclusion criteria*: excluded from the study were all blood donors who did not meet the inclusion criteria.

2.7 Demographic Data of Participants

The socio-demographic data of the blood donors, especially the age andgender were recorded.

2.8 Blood Samples Collection

Prior to collection of blood samples from the donors, a pre-blood donation sample was collected from each donor and sent to the Department of Pathology, River State University Teaching Hospital, Port-Harcourt for screening. Only donors who passed the inclusion criteria were bled, to avoid wastage of blood pints.Blood samples for the diagnosis of HBV, HIV, HCV and VD infection were collected from all qualified study participants. The upper arm area of each donor was thoroughly cleaned and sterilized using cotton wool soaked in methylated spirit. Five millilitres of venous blood were obtained from the ante-cubital vein. About 2.5 ml of the blood was introduced into blood specimen bottle containing anticoagulant Ethylene Diamine Tetra-Acetic Acid (EDTA). The remaining 2.5 ml was transferred into plain tubes without anticoagulant according to WHO standard method. The blood was allowed to clot and

afterwards centrifuged. The serum samples were then aliquoted into a vial which was labelled and stored at -20°C prior to testing. All blood donations were drawn manually and none was automated in this study. None of the blood donations were separated into components.

2.8.1 Examination of Blood Samples for Venereal Disease Research Laboratory (VDRL), HBV and HCV

The donor blood samples were screened for VDRL, Hepatitis B antigen (HBsAg) and HCV. Testing for HBV and HCV were based on chromatographic methods using test kits according to the manufacturers' instructions. All the reactive samples were repeated in duplicate before labelling them seropositive or seronegative. The VDRL test for antibodies to *Treponema pallidum* was conducted using the DiaSpot® Rapid Detection Testing kits (ACON one-step insert rapid chromatographic immunoassay test strips). The test was performed according to the manufacturer's instructions.

2.8.2 Examination of Blood Samples for HIV 1 and 2

HIV test was done according to the national testing algorithm. Using the stored serum samples earlier collected from the participants, HIV screening was done with two kits as recommended by the World Health Organization(WHO, 2015). Determine® HIV-1/2 (5 Abbott Laboratories, IL, USA) was used for the test, while Uni-Gold® HIV-1/2 (Trinity Biotech, Dublin, Ireland) was used for the confirmatory test.The test was carried out by applying 50 µL of the serum to the Sample Pad (marked by the arrow symbol) of the Test Unit and allowed to lay flat in the bench for 20 minutes, after which the result was read and interpreted based on the presence or absence of two pink/red lines (Control line and Ab line). The Control line appears in the Control Area and the Ab line appears in the Lower Test Area of the Test Unit. The intensity of the Ab and Control lines may vary, however, any visible pink/red colour in both the Control and Lower Test Areas, regardless of intensity, is considered reactive. A Reactive test result means that HIV-1 and/or HIV-2 antibodies have been detected in the sample. The test result is therefore interpreted as preliminary positive for HIV-1 and/or HIV-2 antibodies.Since the serum sample was used for the test, the Chase Buffer was not added. As part of quality assurance measures, the test initiated within 2 hours after removing the protective foil cover from each Test Unit and results were not read after 30 minutes of adding the serum sample.

2.9 Data Analysis

The data collected was analysed using the Statistical Package for Social Sciences (SPSS) version 2.3. All the categorical variables were summarised percentages and used to evaluate descriptive statistics. Significant levels were measured at 95% CI with the levels of significance set at 5% probability value ($P > 0.05$).

III. RESULTS

Of the 200 participants examined, none tested positive for HIV 1 &2. A total of 8(4.0%) were positive for HBSAg. Only 1(0.5%) was positive for HCV while 2(1.0%) were positive for VDRL. The prevalence of TTIs was higher in females 6(3.0%) than in males 5(2.5%). There was no significant differences in prevalence among genders ($P > 0.05$) [Table 1].

Table 1: Prevalence of TTIs among the study participants in Port Harcourt metropolis

TTIs	Number tested	Seropositive		
		Positive males (%)	Positive females (%)	Total (%)
HIV	200	0 (0)	0 (0)	0 (0)
VDRL	200	0 (0)	2 (1.0)	2 (1)
HBsAg	200	5 (2.5)	3 (1.5)	8 (4)
HCV	200	0 (0)	1 (0.5)	1 (0.5)
Total	200	5 (2.5)	6 (3.0)	11 (5.5)

Of the 11 positive cases of other transfusion transmissible infections 9(81.82%) were in the age group 15 – 25 years and 2(18.18%) were in the age group 26 – 35 years. None in the age groups between 36 and 65 years had any blood transfusion transmissible infections. Also, of the 11 positive cases, 2(18.18%) had syphilis (VDRL), 8(72.73%) had HBV, and 1(9.09%) had HCV infection. HBV infection was the predominant blood transfusion transmissible infection, followed by syphilis and HCV infection among the blood donors (Table 2).

Table 2: Prevalence of TTIs among the different age groups of study participants in Port Harcourt metropolis

Age groups (Years)	Number tested (%)	Transfusion transmissible infections				
		HIV (%)	VDRL (%)	HBSAg (%)	HCV (%)	Total (%)
15-25	64 (32.0)	0 (0)	2 (3.13)	6 (9.38)	1 (1.56)	9 (14.06)
26-35	86 (43.0)	0 (0)	0 (0)	2 (25.0)	0 (0)	2 (2.38)
36-45	38 (19.0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
46-55	9 (4.5)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
56-65	3 (1.5)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Total	200	0 (0)	2 (1)	8 (4)	1 (0.5)	11

IV. DISCUSSION

In his study, the prevalence of HIV was found to be 0.0% which means that none of the blood donors had antibodies to human immunodeficiency virus detected by the testing method used. A similar study by Tiwari *et al*. (2008)reported 0.084% and 0.054% prevalence of HIV among voluntary non-remunerated blood donors using rapid test kits. This shows that the blood donors in the present study area were safe. But a contrary report by Dada *et al*., (2015)reveals that some of the apparently healthy blood donors in their study, were seropositive for HIV.For VDRL, the prevalence was found to be 1% in the present study, which compares favourably with another study(Ahmad *et al*., 2019).

A total of 4.0% were positive for HBsAg. Prevalence of HBV among blood donors differs. Another study carried out in South Eastern Nigeria shows that among healthy blood donors, 2.7% were seropositive of HBsAg(Dada*et al*., 2015). The major route of HBV transmission is parenteral and it is most infective among blood-borne viruses in chronic carrier state and is associated with chronic liver disease, cirrhosis and Hepatocellular carcinoma.

For Hepatitis C, the observed prevalence in this study was 0.5%. This agrees with an earlier observation by Narayanasamy *et al*. (2015)which reported a similar prevalence of HCV in their study in India. Transmission of HCV is primarily through blood exposure and majority of the infected persons progress to chronic and chance of cirrhosis and hepatocellular carcinoma is more as compared to HBV. This study has once more reaffirmed that blood is one of the main sources of transmission of HBV, HCV and VD, therefore donor selection is of paramount importance (Houghton, 2009).

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Contribution of traditional Date palm (*Phoenix sylvestris*) agroforestry in income generation and livelihood improvements: A case of Jashore district, Bangladesh

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Abstract— *Products and services provided by the agroforestry production systems support the basic needs and enhance the livelihood of millions of farmers throughout the world. The benefit of the traditional agroforestry systems and their livelihood improvement strategies are, however, often not systematically addresses in Bangladesh. Thus, the objective of the study was to assess the impact of traditional agroforestry on income generation and livelihood capital development of the rural farmers in Bangladesh. The study selected one of the most common and popular Date palms (Phoenix sylvestris) agroforestry cases practiced in the Jashore region of Bangladesh. The findings indicated that the most common important feature of the Date palm-based agroforestry system was to generate income and diversified products for the rural farmers. In addition, the physical, financial and natural capital developments were a strong indication of the livelihood developments of farmers. However, the social and human capital development was not satisfactory and this urges an immediate intervention from the government and regional levels. The scientific training on agroforestry and local conflict resolution mechanism process from the government levels would be boosted up the income generation and livelihood development of the farmers in the study area. Therefore, the study recommends to minimize constraints of the agroforestry production systems through training and also enhance social relationship through government official interference would be very effective to make the Date palm agroforestry a more sustainable land-use practice in Bangladesh.*

Keywords— ***Agroforestry, Date palm, Benefit-Cost, Livelihood capitals, Bangladesh.***

I. INTRODUCTION

With rapid population growth and a significant increase in wealth, put pressure to convert natural forests into commercial agriculture, industrial and infrastructure used in the world. This pressure also increases the demand for food, timber, fuel, fodder, fiber and other tree products, and also puts additional pressure on the existing forests. On the contrary, these forest ecosystems are expected to provide a diverse array of environmental services (Roshetko, 2013). Agroforestry practice can be a solution to the rising population of the developing world as it helps to utilize every piece of land sustainably. Agroforestry systems that rural people have developed with limited resources (e.g., land) to meet their household needs are a key opening to advance the sustainable use of forest products and services. Agroforestry is an integral part of the rural livelihood systems for centuries and plays a key role in providing household food and energy security, income and employment generation, investment opportunities and environmental protection in Bangladesh (Miah *et al*, 2002).

In Bangladesh, the rural farmers adopted a number of traditional agroforestry systems on their farm and sustain their livelihood since time immemorial. Wild Date palm

(*Phoenix sylvestris)* based traditional agroforestry is one of the common and popular systems practiced in the greater Jashore region of Bangladesh (Abedin *et al*, 1997). Alone with Date palm there are only a few traditional agroforestry systems are practiced in different agroecological zones in Bangladesh, and due to variation in soil, climate and rainfall these traditional agroforestry systems are found in specific locations mainly. The farmers manage the Date palm tree mainly for sap production with which sugar-based secondary goods are manufactured. The sap is either used fresh as a drink or after some sort of processing as molasses (Chowdhury *et al*, 2008). In Jashore area, farmers are cultivating different vegetables and cereal crops in association with Date palm trees and sustain their livelihood with higher farm outputs (Islam *et al*, 2013; Islam, 2019). The Date palm tree required minimum management effort and farmers get tapped juice from the tree every year and the production cycle runs for more than 20 years.

The Date palm-based agroforestry systems have the capacity to enhanced farmers' income which has already been documented by the researchers (Miah *et al*, 2002; Hajong*et al*, 2016). However, the livelihood improvement of the traditional agroforestry and its contribution to different asset development of the rural farmers have not been addressed thoroughly. Only some previous study has sporadically calculated income generation of the Date palm trees in Bangladesh and as a traditional agroforestry system, the productivity of the system has not assessed properly. Therefore, the objectives of the study were to assess the impacts of Date palm-based agroforestry systems on the income generation and livelihood improvements of rural farmers of Jashore region of Bangladesh.

Conceptual and Theoretical frameworks: Most of the livelihood concepts have a focus on the household as the most appropriate social group for the investigation of livelihood. Several agencies (e.g., CARE, UNDP, FAO) have adopted livelihood approaches and make use of livelihood frameworks. This study used the DFID Sustainable Livelihood framework as a point of reference. The DFID's sustainable livelihood framework looks at the basic dynamics of livelihoods and how people are represented on a set of capital/assets as a basis for their livelihoods (Carney, 1998; Hussein and Nelson, 1998). In the DFID's sustainable livelihood framework these assets are represented by – Human capital (skill, knowledge, capacity, labor ability, good health), Social capital (relationship of trust and reciprocity, networks, membership of groups), Physical capital (basic infrastructure, transport, shelter, communications), Natural capital (land, forest, water, wildlife, biodiversity) and Financial capital (monetary resources-savings, credit, remittances).

The traditional agroforestry systems in Bangladesh have been contributing to the livelihoods of the rural farmers mainly by contributing to the development of livelihood assets/capital. The assets are the livelihood building blocks and a range of assets are needed to attain positive livelihood outcomes (Islam and Sato, 2012a&2013; Warner, 2003). Improvement in all the five capitals could be termed as strong improvement, while improvement in only some of the capitals that compensate for any decline in other capitals could be termed as weak or poor improvement of livelihoods (Das, 2009). This study analyses the base assets of the participants and explores their access to livelihood assets in the context of the traditional agroforestry systems (Shahabaz, 2009). So, the livelihoods of the rural farmers have been evaluated in this study with the help of DFID's livelihood framework.

II. METHODOLOGY

Description of the Date Palm agroforestry systems

The Date palm-based traditional agroforestry systems are very popular and common practices in the greater Jashore region of Bangladesh. The Date palm tree is an evergreen and commercial species that has been practicing in this region since time immemorial (Abedin and Quddus, 1990; Hajong*et al*, 2016). Usually, the Date palm trees are growing in the boundary or inside the cropland in a scattered manner (Figure 2), and different crops are grown in association with the trees. As a deep-rooted and straight canopy bearing tree species, the Date palm has occurred minimum competition with associated crops in terms of water, light and nutrients (Nair and Graetz, 2004; Nair and Latt, 1997). The Date palm tree provided juices in the winter season and the juice is used to make molasses (locally called *Khejurgur*). Molasses is an important source of sugar and a good substitute for sugarcane sugar in Bangladesh. The farmers sell juice and sugar to the market and also collected firewood, timber and fruits periodically from the Date palm trees. The Date palm is easily germinated through seeds and established in the soil even in poor-quality soil. The soil (sandy loam) and climate (moderately higher temperature) of the greater Jashore region are favorable for growing Date palm tree (Hajong*et al*, 2016; Dalibard, 2007). The study observed that rural farmers were cultivated different winter vegetables (e.g., cabbage, cauliflower, radish, turnip, green pea, carrot, Indian spinach, etc.) and cereal crops like rice and mustard in association with Date palm trees in the Jashore district of Bangladesh. The Date palm-based agroforestry systems could be continuing up to 30 years

and the young tree produce juice after 5-years of tree plantation.

Study Location: The study was conducted in Barinagar, Panchbaria and Khajura, 3 villages under the Jashore district of Bangladesh. The villages were belonging to the High Ganges River Floodplain Agroecological zones (Figure 1) and the soil of the Jashore region is calcareous dark grey to the brown floodplain and the average rainfall is 1640 mm per annum.

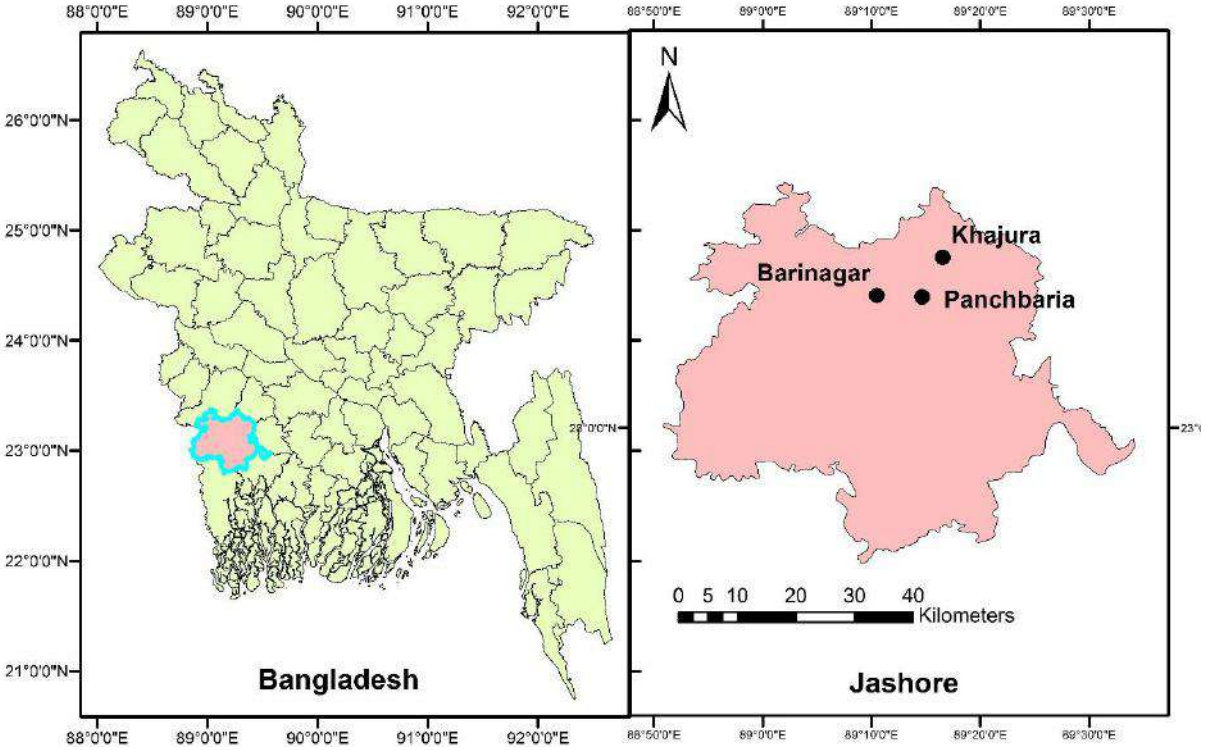

Fig.1: Study area map showing Bangladesh (left) and Jashore (right) district

The soil and climate of the Jashore region are good for vegetable cultivation as the soil is predominantly sandy loam. The farmers grow different winter and summer vegetables like (Cabbage, cauliflower, radish, etc.) and cereal and pulse crops (rice, pulses) in association with Date palm trees.

Sampling and Data Collection

Prior to data collection, the study was collected traditional agroforestry farmers' information with an informal discussion with the local people of the communities and from the respective agricultural offices. A pilot survey of 5 farmers was conducted in the Jashore region and then the interview questionnaire was adjusted and finalize for data collection. The primary data of this study was collected through household interviews and focus group discussions. For the farmers' household survey, the study selected 60 farmers randomly from the three villages, where 20 farmers represent from each village. The selected farmers were practiced traditional agroforestry on their own farms for more than 5 years. The interview was carried out with the help of a semi-structured questionnaire, the questionnaire consists of farmers socioeconomic information, their agroforestry income, improvement of five livelihood capitals through agroforestry systems and the problems faced by the farmers in the community. Interviews were held in the day time with the help of two enumerators and in the native Bengali language. In addition to the household interview, the study also conducted focus group discussions and in each of the villages we took two focus group discussions. The farmers were informed about the focus group discussion and their perceptions were recorded accordingly. Repeated conservation with farmers was made in each common and decision-making question to ensure the validity of the information. Finally, the collected data were verified, cross-checked and validated in the study, the whole data collection period was conducted between 2019 and 2020 time period.

Data analysis

The Date palm-based agroforestry provided diversified outputs from crops and tree products. Crops were cultivated at a different time of the whole year and Date palm tree provided Juice during the winter season of (Late November to February) Bangladesh. The study collected

all crops yield grown in association with Date palm tree and converted it to hectare basis. Like crop yield, all firewood, fruits, juice, molasses and other non-timber products income were calculated on the year per hectare basis. Finally, all of the products' income were calculated with their prevailing average market price in Bangladesh Taka (1 USD ≈ 84 Taka). The study also calculated the Benefit-Cost Ratio (BCR) of the Date palm-based traditional agroforestry system, which indicates the rate of return per unit cost, was calculated using the following formula, BCR = $\frac{\sum_{t=0}^{n}\frac{B_t}{(1+r)}}{\sum_{t=0}^{n}\frac{C_t}{(1+r)}}$ here, B_t= gross benefit in i^{th}year, C_t= total cost in i^{th}year, t= number of year and i= interest (discount) rate (assuming 11% interest rate). Finally, the procured data were tabulated and analyzed using Microsoft Excel.

III. RESULTS AND DISCUSSIONS

Demographic Characteristics of the Farmers

The study found that the variation of farmer households' size among the three villages was very insignificant and their average size was more than 6 (Table 1). The mean ages of the participants were 46 years (Table 1). The male and female ratio among the participant households was almost the same among the three villages. In the case of the literacy rate, the farmers from Khajura possess a slightly higher (60%) rate (Table 1) than the literacy rate of the overall civil district (Jashore) 56.5% (BBS, 2013). The majority of participants' religion was Islam and the rest was Hinduism. The average landholding of households was 0.63 ha (Table 1) and the households' main sources of income are dominantly agriculture (Table 1).

Table 1: Demographic features of the respondent

Characteristics	Khajura	Barinagar	Panchbaria
Age (Mean)	46.8	43.65	48.2
Household size (Mean)	6.6	6.1	6.35
Male: Female ratio	49:51	50:50	49:51
Literacy rate	60%	45%	55%
Per household landholding (ha) (Mean)	0.72	0.56	0.60
Distribution of households by religion			
- Muslim	90%	85%	85%
- Hindu	10%	15%	15%
- Others	0%	0%	0%
Households main sources of income			
- Agriculture	60%	65%	70%
- Wage labor	20%	15%	15%
- Business	10%	15%	10%
- Unemployment	0%	5%	5%
- Remittances	5%	0%	0%
- Others	5%	0%	0%

Economic Return from Date Palm Agroforestry

Economic outcomes from the tree-crop based agroforestry systems play a key role in the household income in rural farmers of Bangladesh. The traditional agroforestry system based on date palm was executed to improve the livelihood of the local community. The economic analysis showed that the outputs of traditional agroforestry mainly depend on the crops' income (Table 2). The Date palm traditional agroforestry needs an intensive labor cost that was considered the main costs of the production systems. However, the date palm tree can regenerate easily through seeds and farmers did not need to pay a higher amount of money to buy and establish the Date palm tree in their farms. The whole production costs and the average yield of the production systems are presented in Table 2.

Table 2: Economic returns from the Date Palm based agroforestry practices

Items	Different cost/income
Tree establishment and new seedlings costs	5917
Land preparation costs	12900
Vegetables cultivation costs	29250
Seasonal crops cultivation costs	15217
Labor costs	33000
Fertilizers costs	8075
Manure costs	6200
Insecticides and pesticides costs	5883
Weeding and irrigation costs	6825
Harvesting of crops and vegetables costs	14167
Other management costs	5750
Income from date timber*	29333
Income from date palm juice	12733
Income from date palm molasses/Ghur	43583
Thinning tree and firewood income	13308
Fodder income	6200
Vegetables income	125167
Other seasonal crops income	71167
Total Gross Income	301492
Total Production Cost	143183
Net Income	**158308**
Benefit Cost Ratio (BCR)	**2.11**

It was evident that the farmers were cultivated different vegetables and cereal crops in association with Date palm trees and earned a significant income (126267 and 71167 Taka) respectively (Table 2). Date palm tree provided fresh juice in the winter season and farmers reported that they tapped tree twice a week and, on average, they collected 4-liter juice from one tree per tap. On average, they collected more than 120 liters of juice from a tree in the whole winter season. The farmers produced molasses from the juice in simple burning/heating techniques, and on average 100-liter juice produce 16 kg molasses locally. The local price of 1 kg molasses (*Ghur*) has more than 170 Taka and the farmers can profit more than 2200 Taka from the molasses per Date palm tree in a year. Therefore, the income from molasses was an additional income from the agroforestry farmers. In addition to the timber, firewood and juice income, the date tree leaves are used to produce some local mat and sold in the local market. The benefit-cost ratio (BCR) is a common indicator of economic analysis as it takes into account both costs and returns of both components. The BCR value of the date palm-based agroforestry showed 2.11 which is quite satisfactory in respect of the regions.

The study pointed out that the resilience of agroforestry farmers was strengthened through the locally adopted Date palm species in their agricultural lands which provide farmers a valuable source of income. The production systems outputs like timber, firewood, juice, molasses and other non-timber products were the backup against income risks in case of crop failure (Avelino *et al*, 2011; Branca*et al*,2011). The outcomes of the agroforestry systems also revealed that the farmers get a continuous source of income throughout the year and the benefit-cost ratio of the systems was much higher than the general agriculture practices of the country (Islam, 2019).

Livelihood Improvements

The rural poor farmers have practiced Date palm-based traditional agroforestry system in order to support their daily living. After involvement in the program, they were

able to build up several types of livelihood capitals and this study examines some important variables of these capitals (DFID, 2001; Islam *et al*, 2012a&2012b).

A few training sessions and workshops (28%) were conducted by the local NGOs and GOs in order to familiarize the scientific training on agroforestry (Table 2). But it is not enough to train farmers in an effective fashion as the Date palm-based agroforestry would require specific knowledge to manage the systems scientifically. Most of the farmers were thus used traditional knowledge to manage their production systems which they learned from their parents and grandparents. The literacy rate among farmers appeared to be improved slowly (53.5%) among the participants (Table 2). The available labor of the participants' families had decreased and it occurred due to the consciousness of education and migration to the capital city and suburbs for better livelihood, mentioned by the respondents. Food sufficiency of the household members throughout the year was found to be quite positive and improving after involving in the traditional agroforestry system. Nevertheless, poor populations living in rural areas normally have limited access to health services. In Bangladesh, the government health program tends to have better coverage in urban areas (Islam *et al*, 2013). The participants were found to be capable to manage their family healthcare systems and visited the local clinic/hospital for the treatments. The income from the agroforestry systems was able to accommodate the farmers to visits the private clinic or local hospital, mentioned by the participants. So, the human capital of the farmers' waw improved but not at a good level, three is ample of scope to improve further.

There is much debate about what is exactly meant by the term 'social capital' (DFID, 2001). Social capital refers to a network of mutual support that exists within and between households, extended family and communities, which people can utilize to gain, for example, loans, child care, food, accommodation and information about employment and opportunities (Dersham and Gzirishvili, 1998; Moser, 1998). In addition, social capital is a valuable and critical resource for poor people, especially during times of crisis and socioeconomic change (Islam *et al*, 2013). The study found out that the agroforestry farmers were able to build a strong relationship with other agroforestry farmers in the community, however, their social relationship with the elite and leaders of the community was negative. In addition, the farmers' relationship with agriculture officers were moderate. Date palm farmers have developed the social relationship and trust in themselves, however, the traditional agroforestry systems have a long history and acceptable production systems in the Jashore region, Bangladesh. Although social relationship and networks are attributes of an individual in a social context (Sobel, 2002), the establishment of social assets depends on the institution, attitudes and values that govern interactions among participants and contribute to economic and social development (World Bank, 2002), and the overall social assets of the agroforestry farmers have been in improving trends (Table 2).

Natural capital is the term used for the natural stocks from which resources flow and from which services, useful for livelihoods, are derived (DFID, 2001). It refers to environmental assets such as land and common property resources or free (open access) natural resources such as forests, water, or grazing land (Islam *et al*, 2013). The land is an important natural capital and the respondents were categorized as either landless (having 0 to 0.2 ha land), marginal (0.2 to 0.6 ha), small (0.61 to 1.0 ha), medium (1.0 to 2.0 ha) or large (>2.0 ha) farmers according to their farmland area (Iqbal 2007). The participants were small farmers as they had less (0.62 ha) household land (Table 2). The study found out the farmers' perceptions towards conserving the Date palm trees were very positive and every farmer has maintained a good (around 90 trees per ha) number of trees in their farmland. In addition, farmers' awareness to preserve and maintain green cover in their homestead area was also high and they planted fruit trees in their homestead. On average each farmer has 17 tree species in their homestead, which provide diversified foods and nutritional sources for their family members. Therefore, the development of their natural capital was improving and the traditional agroforestry systems tents to improve the situation gradually.

Table 2. State of major characteristics of the participants in the community.

Parameters	Status	Trends
Farmers Literacy rate	53.5%	Improving slowly
Children literacy rate	82%	Sharply improving
Involvement in social organizations	>9	Increasing
NGOs and GOs	>21	Increasing
Micro-credit and easy loan facilities	Good	Increase Microcredit/easy

		loan
Farmers received scientific training on agroforestry	28%	Not satisfactory
Farmers social relationship with other stakeholders of the community	Moderate	Gradually improving
Household infrastructure and physical assets	Tin-wall and Roof, shift from mud-wall and sun-grass roof	Increasing physical assets
Road infrastructure to farm and markets	Brick and bitumen seal	Improving
Livestock small (e.g., chicken)	3.8	Increasing
Livestock big (e.g., cow, goat)	3.2	Slightly improving
Available of labor in the households	1.6	Slightly decreasing
Alternative market facilities to sell agroforestry products	Two	Not changing
Food sufficiency of the household members throughout the year	11 months	Increasing
Annual expenditure	94.5%	Slightly improving
Local Clinic and Hospital facilities for farmers	2.0	not changing but small clinic increasing in the village market area
Tree stock in household premises	16.5	Improving
Tree stock in AF system	90	Not changing
Alternative livelihood options	Exist	Increasing

Physical capital is comprised of the basic infrastructure and producer goods needed to support livelihoods. It includes assets such as housing, the tools and equipment that people own, rent, or use and the public infrastructure that they have access to (DFID, 2001). Housing is normally one of the most important assets for poor rural households as it is used both for shelter and reproductive purposes and for productive or income-generating purposes (renting out of rooms or using the space as a workshop area) (Moser, 1998). Nowadays, houses made using brick walls and tin roofs represent the standard house structure in the rural areas of Bangladesh (Islam *et al*, 2013). The majority (>80%) of the participants' houses were made of mud-walls with sun-grass roofs but some participants (about 20%) did have tin-wall and tin-roofed houses. On the contrary, local road infrastructure was gradually improved and mud-roads had been replaced by brick and bitumen sealed roads (Table 2). Participants were able to buy small and big livestock with the money they earned from the date-palm based traditional agroforestry program. Moreover, few respondents stated that they bought smartphones and television with the profit from the traditional agroforestry system. Farmers received a good amount of money after selling molasses in the winter seasons and able to buy some small and big livestock and often repaired their house structure. Therefore, the development of the physical asset of the traditional agroforestry farmers was a good sign to improve their livelihood.

Financial capital denotes the financial resources that participants use to achieve their livelihood objectives (DFID, 2001). Income from the sale of labor was often one of the most important assets for the rural farmers. There are two main sources of financial capital: available stock (e.g., cash, bank deposit, or liquid assets) and regular flow of money (e.g., remittance, pension, and sale labor) (Islam *et al*, 2013). The participants have been involved in different organizations to get loans and technical assistance to manage the Date palm-based traditional agroforestry program, and the study observed that farmers get loan facilities and micro-credit with the easy condition. The total tree and crop outputs are the strong financial assets of the farmers and farmers can invest these assets in their human, physical and other assets as well. The study found that the proper management of the agroforestry

systems would strongly build farmers' financial capital (Table 2).

The study also assesses the associated problems of the Date palm-based agroforestry systems that the farmers faced in the community. Most of the farmers (more than 90%) mentioned that lack of updated information and assistance from the government officials (e.g., agriculture officers) were the main problems in their community. One of the important problems mentioned by the farmers was the marketing systems, lack of alternative market facilities and intermediary's intervention would hinder the farmers' income from selling their agroforestry products. Besides, these problems, the rural farmers have faced other minor problems like the unavailability of fertilizers and pesticides at the village levels. The study summarized that these associated problems have hindered the profitability of the systems and affecting their livelihood.

IV. CONCLUSION

As a tree-crop-based production system, traditional Date palm agroforestry has numerous benefits that can contribute to rural farmers in light of the household income generation and livelihood improvements. The results of the study summarized that the Date palm agroforestry has to augment farmers' total household income in maximizing the benefit-cost ratio of the farm. Farmers received continuous income generation throughout the year the Date palm outputs also act as a backup for farmers in case of crop failure due to adverse climatic conditions. The study also concluded that the impact of the Date palm agroforestry has strongly improved the financial, physical and natural capital development of the rural farmers, however, the social and human capital development was not satisfactory. More emphasis needs to be placed on how traditional agroforestry could improve the farmers' human and social capital in the community. In addition, the rural farmers also faced some constraint which would hamper the productivity and outcomes of the traditional agroforestry system. There is now a current need to build on the existing social and human capital development of the farmers by providing them training on scientific agroforestry production and improve their relationship with agriculture officials in order to promote a more effective agroforestry system in the Jashore region of Bangladesh. Finally, the study can argue that the traditional agroforestry could be a more sustainable land-use system if the government and related stakeholders would provide their mutual support to the rural farmers and dissolve their production problem in a sustainable way.

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Comparative analysis between KINEROS2 and SWAT for hydrological modeling: A case study from Tleta Watershed in Morocco

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Abstract—*Hydrological models are very useful tools for simulating the effect of natural processes and management practices on soil and water resources at the watershed level. However, the applicability of a model relies on the accuracy to simulate measured data. Thus, the aim of this study is a comparative review of the results of two recent studies carried out on the tleta watershed, to evaluate the performance of two models, namely KINEROS2 (Kinematic Runoff and erosion), coupled with AGWA (Automated Geospatial Watershed Assessment), and SWAT (Soil and Water Assessment Tool), to predict runoff and sediment yields deposited at Ibn Batouta dam at the outlet of tleta watershed. The comparative analysis between simulated and observed value of runoff and sediment yield was performed using two statistical criteria. The results showed that these two models were able to simulate runoff during the calibration period, with (R^2=0.98 and NSE=0.96) for runoff K2, and (R^2=0.92 and NSE=0.89) for SWAT. A slight reduction in agreement between sediment yields was observed for SWAT (R^2=0.84 and NSE=0.74) and a better accuracy was noted for K2 (R^2=0.99 and NSE=0.97).*

Keywords— ***Water Erosion, Hydrological Modeling, KINEROS2, SWAT, runoff, sediment yield, Tleta Watershed, Morocco.***

I. INTRODUCTION

The primary concern of water resource management is the appropriate study and planning, that's why hydrological models are used to understand the functioning of watersheds [2]. Indeed, hydrological models address water flows as well as the flow of solids, solutes, and pollutants. Furthermore, several environmental problems require model coupling to water and energy or biomass flows [3]. The comparison of the performance of different models according to the processes they describe, require different types of data and parameters [4]. Also, problems often arise during the validation processes of simulation options, spatial discretization, and in the course of expressing hydrological phenomena. Models are categorized as Global models (ORCHIDEE [5], GR2M [6]), Semi-distributed models (SWAT [7], TOPMODEL [8], WASA [9]), and Distributed models (STREAM [10], KINEROS2 [11], ANSWERS [12].

The hydrological modelling approach is based on the degree of model complexity for the representation of the real hydrological system [13]. The use of several model parameters increases the forecast uncertainty, and requires a complicated calibration procedure to reduce the risk. Robust and uncalibrated models, on the other hand, produce excellent results and are applied to uncalibrated areas [14]. However, they lack the capability to represent in detail all hydrological processes and their complete spatial and temporal distribution. By comparing a complex and a simple model with and without calibration, [15] concluded that, in a semi-arid area, a complex model requires as much as simple model a longer calibration procedure to generate results. GIS and remote sensing are tools that highlight environmental degradation due to soil

water erosion by integrating physical variables and human activities [16, 17].

In this paper, we will compare the performance of two hydrological models in the Tleta watershed. Firstly, KINEROS2 [11], which is a physics-based model, whose equations describe the physical phenomenon (mass conservation, energy, etc.). KINEROS2 addresses surface erosion and the process of runoff and erosion in arid and semi-arid zones. This model was used for modeling water and solid flow in numerous pieces of researches [18-21]. It was also extensively used in Mexico (San Pedro) and Arizona, where it has been developed and validated [22-24]. KINEROS2 was likewise applied in the Mediterranean region [25], coupled with AGWA in Northern Moroccan[26], and in Africa sub-Sahara in Mali [27]. Secondly, SWAT, operating on a daily time-step basis [28], has proven its effectiveness over the years in several studies [29-31]. Its applicability in the Northern Moroccan context was tested by [32]. The model delineates the watershed using DEM and hydrographic networks. SWAT involves an extensive database (multi-source, multi-disciplinary and spatio-temporal). It is highly parameterized, which makes the calibration phase very complicated and time-consuming. The simulation results of the model will be derived from a study conducted by [32].

The main objective of this paper is to assess the performance of KINEROS2 and SWAT models under Moroccan conditions and compare the results of two modeling studies (Choukri et al.,2019; El Harche et al., 2020) carried out in the Tleta watershed in northern Morocco. Although these two hydrological models have demonstrated good performance in the assessment and the management of water resources, one needs to examine which of the models can be adapted in the Moroccan context.

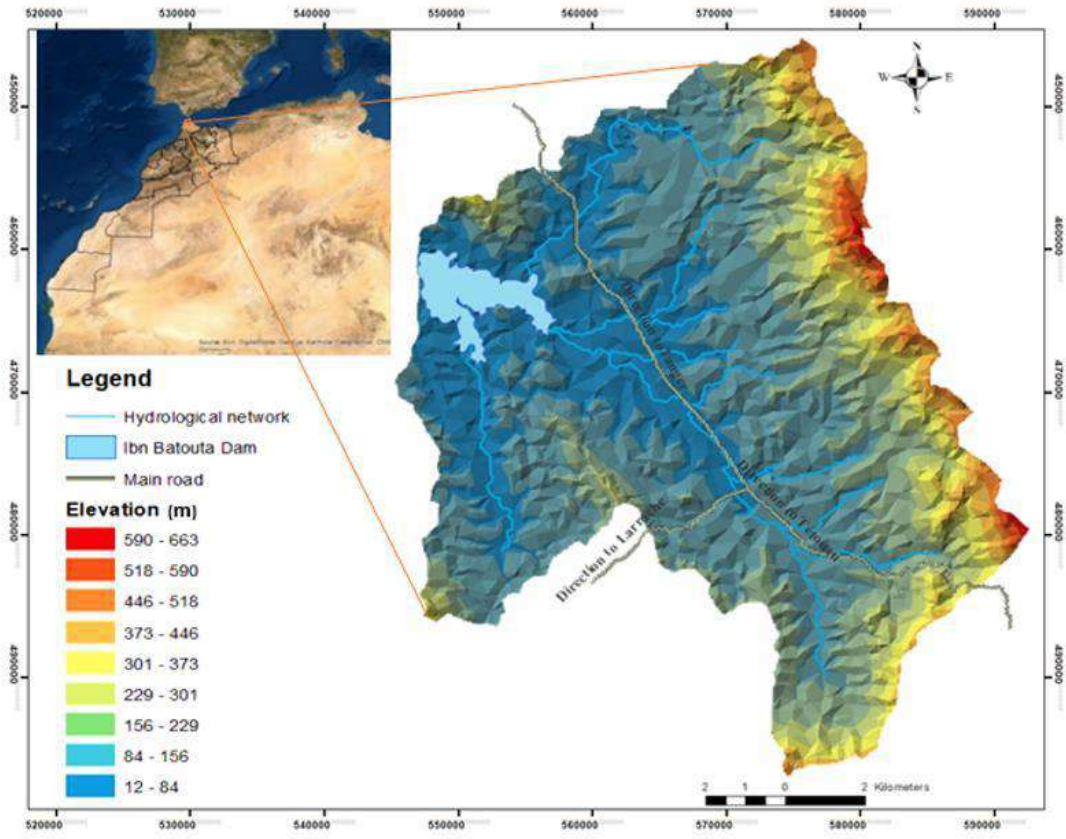

Fig.1: Geographic location and elevation of Tleta watershed

II. MATERIAL AND METHODS

1. Study site

The Tleta watershed, covering an area of 17 700 ha, is located in the Western Rif, Morocco (Figure 1). Nearby both the Atlantic Ocean and the Mediterranean Sea, the site is subjected to a dual moderate influence. Furthermore, the climate of the Tleta watershed is sub-humid with contrasted seasons, which are dry from May to September and humid from October to March [33]. The coldest month is January, with a mean temperature that reaches 8 °C. While August is the hottest month with mean temperature attaining 35 °C [34]. The Ibn Batouta dam, built with an initial storage capacity of 45 Mm³ in 1977, is now reduced

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to 30 Mm^3 due to annual siltation, which represents 1% of the volume of the dam [34]. Soil redistribution process is mainly controlled by topography; thus, an appropriate geometric representation of the topography is necessary to represent this process. Terrain altitude in Tleta watershed varies between 12 and 663 m.

KINEROS2 (K2) needs four sets of input data to operate, which are the digital elevation model (DEM), soils, land cover and precipitations. SWAT model similarly need these input data to describe the catchment area in terms of hydrological and geometric parameters and precipitation.

The data are summarised in Table 1, which also contains the description of erosion and runoff data used for comparison with the results obtained from the two models.

Table 1. Input dataset used to run modeling and simulations

Type	Source	Description
DEM	Spot	Digital elevation model resolution 20 m
Soils	Inypsa (1987)	Soil classes/ Soil characteristics at 1/50000
Land cover	Classified Landsat image	Land cover classes (2009, 30 m)
Rainfall	ABHL (Loukkos Hydraulic Basin Agency)	Daily rainfall, Ibn Batouta station and Saboun station at 5 min (1980-2010)
Erosion		Daily sediment yield, Ibn Batouta station (1980-2010)
Runoff		Daily runoff, Ibn Batouta station (1980-2010)

The land cover classification was processed using Landsat image (30 m). Eight classes of land cover units (Figure 2) were distinguished: Forestland (A), Woodland (B), Grassland (C), Agricultural land (D), Matorral (E), outcrop (F), Urban area (G), Water Body represented by the Ibn Batouta dam (H) [34].

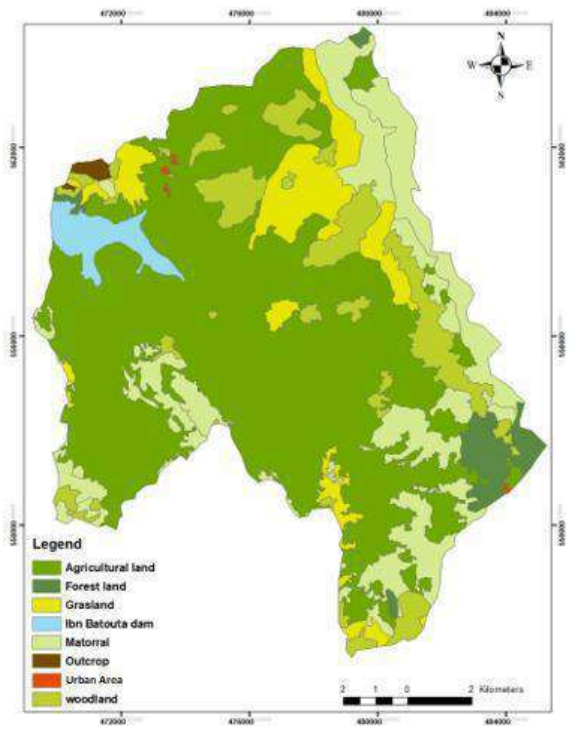


Fig.2: Map of land cover and use

Soil texture was derived from the study by [35] at a scale of 1:50000 (Figure 3). Overall, the soils of Tleta watershed belong to two soil type categories: Clay soils, which dominate the entire study area, and sandy loam clay soils that are located northwest, towards the watershed outlet.

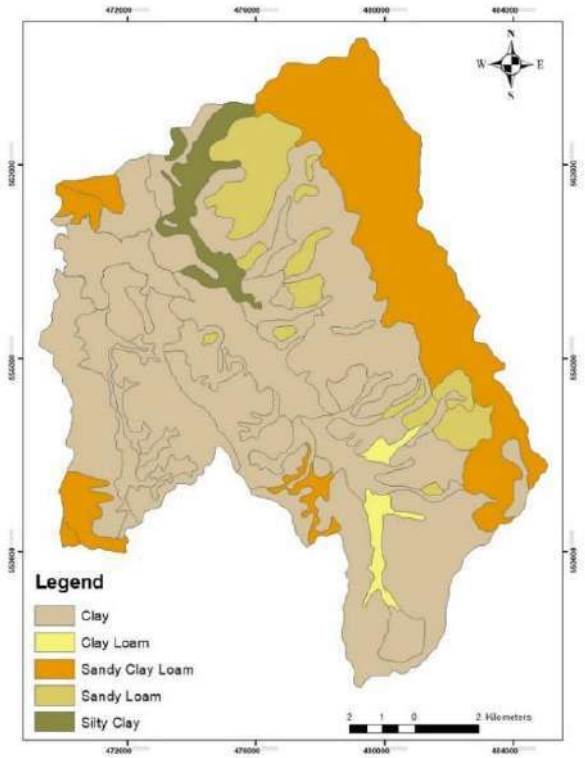


Fig.3: Map of soil texture

The rainfall database consists of a long series (1974-2018) of data recorded at existing stations within the Loukkos river basin (Table 2). The precipitation parameters considered are total precipitation (mm), maximum intensity (mm/h) and duration of rainfall (hours). Annual rainfall in the Tleta watershed is about 709 mm/year (of which 86% falls between October and March). The climate type is Mediterranean sub-humid, with an average temperature of 18 °C.

Table 2. Meteorological data used

Name	Latitude N	Longitude W	Measures
Béni Harchane	35,531	-5,720	Daily
Ibn Battouta	35,645	-5,733	Daily
Kalaya	35,673	-5,747	Daily
Romane	35,704	-5,656	Daily
Aéroport-Tanger	35,726	-5,906	Daily

2. Models overview

The Kinematic Runoff and Erosion Model (KINEROS2) is a physical model that describes the processes of surface runoff, interception, dynamic infiltration and erosion in watersheds, mainly characterized by land-based flow as a function of topography, precipitation, soil and land cover properties [36]. Developed by Environmental Protection Agency (EPA), the Agricultural Research Service of the United States Department of Agriculture (USDA) and the University of Arizona, the tool is designed to provide qualitative estimates of runoff and erosion in relation to landscape changes. It has been developed above all to ensure that configuration procedures are simple, direct, transparent, and repeatable; which is compatible with the available GIS data layers and useful for the evaluation and development of future scenarios [37]. In this model, the watershed is represented by subdividing the area into a cascade of one-dimensional surface flows and channel elements using topographic information, in order to allow for a good understanding of watershed response to land-use changes and land cover management. The model is highly dependent on spatially distributed data; thus, the allocation of appropriate parameters takes time and complicates the compilation processes. KINEROS2, that is an updated version of KINEROS [38] has been described in details by [11]. The computation of Hortonian runoff on plans and channels is based on physical and mathematical equations as follows [11]:

$$q(x,t) = \frac{\partial h}{\partial t} + \frac{\partial Q}{\partial x}$$

$$q_c(x,t) = \frac{\partial A}{\partial t} + \frac{\partial Q}{\partial x}$$

Where, h is the water height per width unit, Q the flow unit, A the wet section, t is the time, x the distance and q(x, t) the net inflow.

The equation of infiltrability (fc) is calculated as a function of the hydraulic saturation conductivity (Ks), the capillary drive element (G), the porosity (φ), the pore size distribution index (λ), and the accumulated infiltrated water (i).

$$f_{c=} Ks\left[1 + \frac{\propto}{e^{(\alpha 1/\beta)} - 1}\right]$$

Where α is a soil-dependent parameter, taken as 0.85 and β combining the effects of effective capillary (G), surface water height (hw) and unit storage capacity (Δθ) = (θs-θi), as in following equation.

$$\beta = (G + hw) \times (\Delta\theta)$$

The dynamic mass balance equation for erosion is giving by the following formula:

$$\frac{\partial(AC_s)}{\partial t} + \frac{\partial(QC_s)}{\partial x} - e(x,t) = q_c(x,t)$$

Where A is the cross-sectional area of flow, Cs is the local sediment concentration, Q is the water discharge, e is the surface erosion or deposition rate and q_c is the rate of lateral sediment in flow for channels.

Soil and Water Assessment Tool (SWAT), developed by the USDA agricultural research service is a conceptual agrohydrological, semi-empirical, physically based model and distributed at a daily time step [7, 39]. SWAT model is adapted to arid environments, which allow for the consideration of infiltration into river bed, as well as low flows and dynamic vegetation growth [40]. Coupled with GIS, it makes it possible to manage raster and vector data, which facilitate and automate the preparation of input data. Output files are converted to ASCII format with their structure. The model calculates for each cell, the flows and direction of water accumulation according to topography portrayed by digital elevation model. The computation grid is the Hydrologic Response Unit (HRU), which is a spatial combination of soil type, land cover and slope class in each sub-basin. The volume of surface runoff is predicted using the soil conservation service (SCS) curve number (CN). Erosion and sediment yield are estimated for each HRU using MUSLE model [41].

$$Q_{surf} = \frac{(R_{day} - I_a)^2}{(R_{day} - I_a + S)}$$

$$S = 25.4\left(\frac{1000}{CN} - 1\right)$$

Where Qsurf is the accumulated runoff, R is the daily rainfall (mm), I is the surface storage, interception and infiltration prior to runoff (mm), and S is the retention parameter.

$$Sed = 11.8 * (V_t * Q_{max})^{0,56} * K * LS * C * P$$

Where Sed is the sediment flow per day (t), Vt is the surface runoff volume (m^3), Q_{max} is the peak flow rate (m^3/s), K is the soil erodibility factor, LS is the topographic factor, C is the land cover and management factor and P is the erosion control practices factor.

In table 3 we summarized the characteristics of the two models and highlighted their advantages and limitations.

Table 3. Comparison between KINEROS2 and SWAT models

Model	KINEROS2	SWAT
Suitability	- Agricultural and urbanized basins - Small Watershed	- Agricultural basins - Cultivation practices
Surface runoff	- Hortonian flow - Kinematics equation	- SCS equation
Lateral flows	- None	- Percolation, water balance
Simulation of chemical components	- None	- N, P, pesticides, carbon
Type	- Physics	- Empirical
Spatial scale	- Distributed	- Semi-distributed
Time scale	- Event	- Continuous
Representation of the watershed	- Plans and streams, 1D	- Sub-basins, HRUs, groundwater, waterway
Operations	- Describe the processes of interception, infiltration, runoff, erosion - Used to determine the impact of developments in the watershed	- Predict the effects of land management on water - Predict the effects of sediment and chemical resources on agricultural yields in large river basins
Input	- Topography (DEM) - Soil type - Land cover - Precipitation data	- Topography (DEM) - Soil type - Land cover - Meteorological data (temperature, humidity, wind and precipitation)
Output	- Runoff m^3 - Sediment yield kg/ha - Infiltration m^3/ha - Peak flow rate m^3/s - Maximum sediment flow rate kg/s	- Precipitation (mm) - Evapotranspiration (mm) - Percolation (mm) - Surface runoff (mm) - Transmission losses (mm) - Water yield (mm) - Sediment yields (t/ha)
Advantages	- Addresses linear erosion coupled to GWA - Reduced time of use - Simple, straightforward parameter setting routine - Estimates runoff and erosion/landscape change - Useful for running scenarios	- Simulates nutrient, sediment and pesticide transfers to the drainage system and to aquifers

Limits	- Model parameters based on look-up tables (FAO database) - Needs local calibration for accuracy	- Requires large database (spatio-temporal, multi-source and multi-disciplinary) - The model is highly parameterized; thus, the calibration phase is very complex and time-consuming

KINEROS2 (Figure 4): The first step is the delineation of watershed limits. Once the boundaries are defined, the watershed is subdivided into model elements. Streams are used to define surface flow paths, and thus to control the complexity of landscape representation. The partition of the watershed into plans and channels preserves the spatial variability of the catchment's components.

This partition taking into account the relief, the hydrographic network and the spatial data series available at the global level to derive the necessary parameters for model elements [24]. The description of channel geometry is crucial for efficient water flow. The surface elements are represented by rectangular flat surfaces. The transition from the real-world watershed to the mapping scale

complies with all the geometric characteristics of the terrain (relief, micro-topography and slope). One of the particularities of K2 is that it can represent two distinct soil layers per plan. The channels are represented by two trapezoidal sections representing the main section and the minor river bed with their parameters. K2 will be coupled in this study with AGWA (Automated Geospatial Watershed Assessment), a GIS interface designed to facilitate watershed water management and analysis. AGWA tool also allows for spatial visualization and comparison of model results, and thus makes it possible to assess hydrological impacts associated with landscape changes. The use of GIS provides means of linking model results with other spatial information [37].

SWAT (Figure 4): As described previously, the first step before running the model is the delineation of the watershed from the extraction of the hydrographic network and the second step is discretization. During this phase, SWAT model delineates homogeneous hydrologic response units (HRUs) on the basis of the structure of the hydrographic network from which the sub-basins are extracted. The integration of watershed delineation and discretization is achieved by overlying the three-shape file of information, namely soil type, land cover and slope data. Once imported, the distribution of HRUs in the watershed is carried out. The SWAT-HRUs command allows us to specify the criteria for distribution. A single or multiple HRUs can be selected for each sub-basin based on soil type, land cover and dominant slope class. The construction of these units triggers the creation of SWAT view interface. This is the basis for entering the last necessary data that are climate data and agricultural practices. SWAT model is based primarily on MUSLE method, a modified version of USLE [42] [41], which calculates erosion caused by rainfall and runoff [43] and predicts average erosion as a function of rain energy. Finally, water and sediment transfer, as well as surface runoff, are predicted based on the study of [44-46]. The period selected for model initiation is 1983-2010 according to the available data.

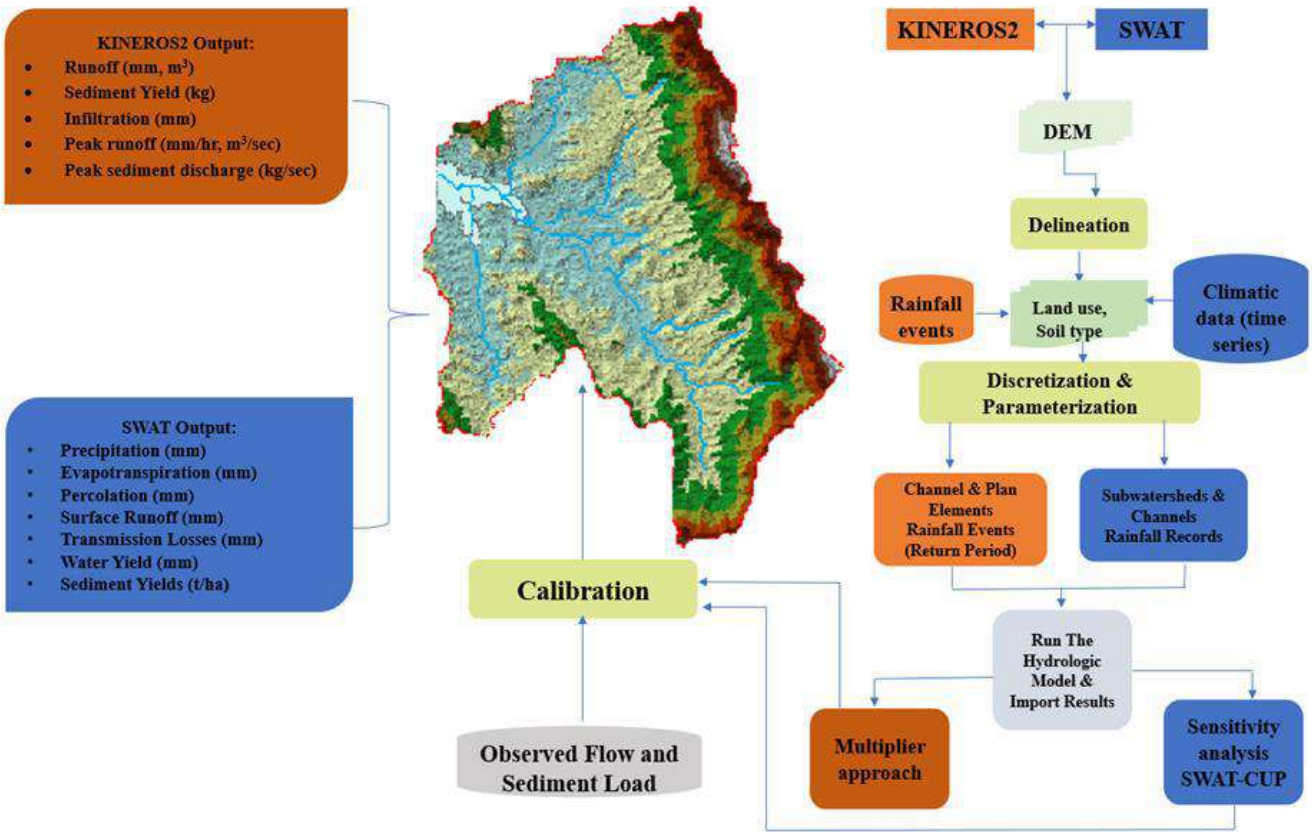


Fig.4: Operating diagram of KINEROS2 and SWAT models [1]

For **KINEROS2**, the calibration phase requires the availability of observed data. It is essential in this case to adapt some parameters, such soils, which influence strongly the simulations. The events selected for KINEROS2 calibration and validation depend on the data available. Rainfall events were used with a magnitude of small event (15 mm), medium event (21 mm), two medium large event (27 mm, 29 mm) and large event (37 mm) to evaluate the effect of rain quantity on the hydrological response of the watershed (Table 4).

The execution of the model is performed on basis of pervious field measurements in the Tleta watershed (Table 4). To calibrate K2, it is recommended to use multipliers [13, 37]. Hydraulic conductivity at saturation (MK), net effective capillarity (MG) and Manning coefficient (Mn) are the most sensitive parameters of the model [47, 48]. In our case, we will rely on Ks field measurements. The remaining parameters will be estimated using table references of the K2 original manual and documentation [38]. Model input parameters are derived directly from these data using optimized search tables provided with the tool to facilitate parameterization and calibration of K2 [37]. Data required by AGWA include elevation, land cover and soil type, in addition to rainfall data [48].

Table 4. Selected events for K2 simulations

	Rainfall	V (m³)	I (mm/h)	Rainfall (mm)	Qs (Kg/s)
Calibration	24/12/2009	30 249	33	24	527
	03/02/1998	840 672	29	29	7 982
	28/03/2004	113 184	29	21	281
Validation	22/04/2003	254 880	27	27	1 544
	30/11/2012	375 839	33	28	3 101
	06/01/2010	2 533 247	36	37	29 519
	19/11/1999	3 455	15	15	9.91

For **SWAT** model calibration and validation, the period has been respectively decided to span over 1983-1996 and 1997-2010, to take into account initial environmental

conditions [32]. All the required data are extracted from the database of [34]. Other weather data were acquired from Tangier airport and the Loukkos Hydraulic Basin Agency (ABHL) authorities. Parameter sensitivity analysis was based on changes between output variables and model parameters [49]. The sensitivity analysis was performed using SWAT_CUP program [50]. This tool identifies sensitive parameters that are related to runoff and infiltration (CN2), the interaction between river flow and underground compartment (RCHRG_DP, GWQMN, REVAPMN, GW_DELAY) and the evapotranspiration calculation factor (ESCO). The indicators elected to examine the performance of the model are the coefficient of determination (R^2), the Nash-Sutcliffe coefficient (NSE) and the bias (%PBIAS). The most sensitive parameters for sediment transport are slope (SLOPE), slope length (SLSUBBSN), Manning roughness coefficient (OV_N) and river sediment transport adjustment factor (PRF). A calibration simulation was run to adjust flows, sediments and nutrients according to the known approach [51]. Bathymetric measurements have been used to determine the sediment fluxes used during the calibration of SWAT model [32].

3. Comparison analysis

To evaluate the performance of hydrological forecasts, several criteria are involved, namely two statistical criteria (NSE) and (R^2) for both models and (%Pbias) for SWAT model only [32].

$$NSE=1-\frac{\sum i(Q_{m,i}-Q_s)i^2}{\sum i(Q_{m,i}-Q_m)i^2}$$

$$R^2=\frac{[\sum i(Q_{m,i}-\bar{Q}_m)(Q_{s,i}-\bar{Q}_s)]^2}{\sum i(Q_{m,i}-\bar{Q}_m)^2 \sum i(Q_{s,i}-\bar{Q}_s)^2}$$

$$\%Pbias=\left[\frac{\sum_{n=1}^{n}.(Q_{,i}-Q_s)*100}{\sum_{n=1}^{n}.(Q_i)}\right]$$

Where Q is a variable of runoff and sediment yield, m and s are the measured and simulated variables and i is the measured or simulated data.

III. RESULTS AND DISCUSSIONS

K2 calibration was performed using the multiplier of Ks parameter M(Ks). The model relies on SCS method to calculate runoff [52]. It approaches runoff module based on Horton overland flow that occurs when rainfall exceeds infiltration capacity. The transport of solids is solved using finite difference techniques [53]. Runoff and sediment yield simulations were carried out using five storm events in order to compare the effect of rainfall duration and intensity on the basin hydrology as well as on the load conveyed towards the dam. The results in (Table 5), and (Figure 5 and 6) showed that there is a good agreement between observed and simulated values for the selected floods and the parameters adopted, although K2 seems to slightly underestimate runoff and to overestimate sometimes sediments yields. This is probably due to errors during model calibration phase. The implementation of KINEROS2 model offers the possibility to simulate variations in runoff at Tleta watershed as a function of rainfall amount and land cover.

Table 5. K2 observed Vs simulated results at Ibn Batouta [26]

	Events	Rainfall mm	Runoff (m^3)		NSE	R^2	Sediments (kg/s)		NSE	R^2
			Observed	Simulated			Observed	Simulated		
Calibration	24/12/2009	24	30 249	41 492	0.71	0.99	527	407	0.72	0.99
	03/02/1998	29	840 672	511 961			7 982	4 734		
	28/03/2004	21	113 184	52 524			281	695		
Validation	22/04/2003	27	254 880	300 984	0.98	0.99	1 544	2 785	0.97	0.99
	30/11/2012	28	389 664	477 692			3 291	5 130		
	06/01/2010	37	2 533 247	2 275 739			29 519	26 003		
	19/11/1999	15	3 455	586			9.91	15		

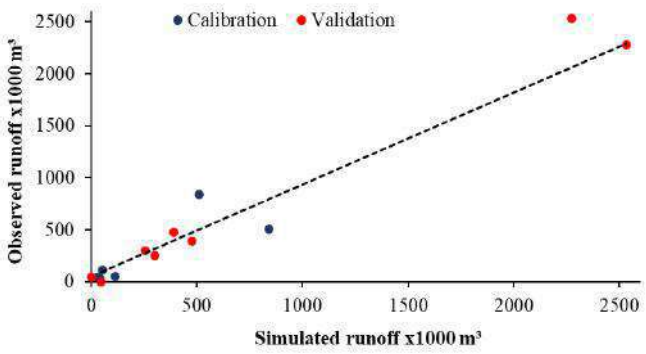


Fig.5: Observed and simulated runoff at Tleta watershed [26]

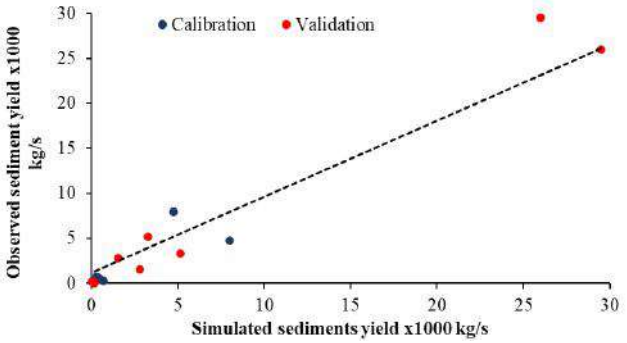


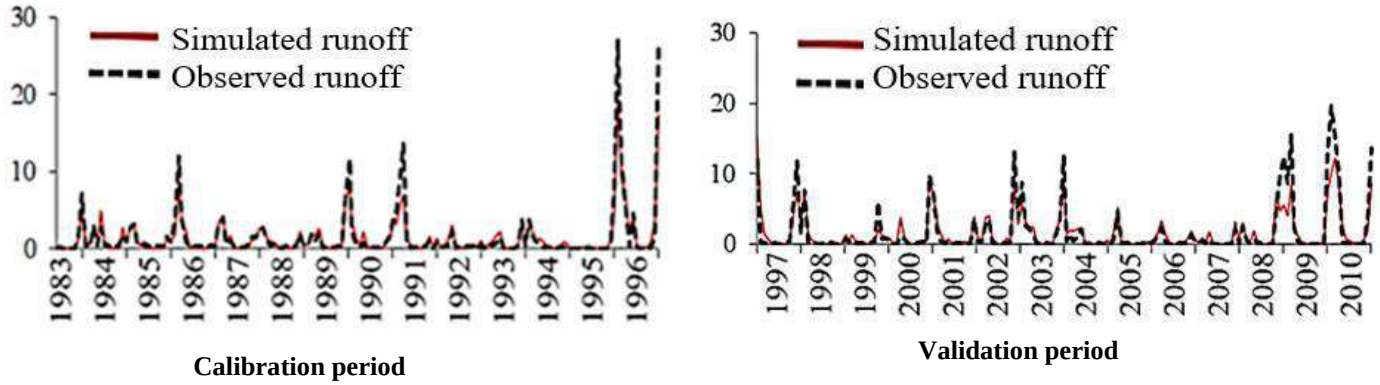
Fig.6: Observed and simulated sediment yields at Tleta watershed [26]

For SWAT model simulation, a good agreement has been achieved between simulated and observed runoff and sediment yields for monthly than for daily simulations (Table 6). The statistical validation criteria of the model also confirmed this agreement for both calibration (1983-1996) and validation period (1997-2010). The evaluation criteria used for the calibration of sediment load conveyed towards the outlet of the watershed are those of [54]. The results achieved are also satisfactory for the calibration and the validation periods, as it is shown in (Figure 7 and 8).

The assessment results attained by [32] allowed to highlight the capability of the model to estimate erosion parameters. Indeed, SWAT model seems to slightly underestimate daily runoff and erosion, and this is due possibly to errors during model calibration. The model appears to be more robust in the monthly forecasts than in the daily forecasts.

Table 6. SWAT daily and monthly simulations at Ibn Batouta dam [32]

Component	Time step	R^2		NSE		PBIAS (%)	
		Calibration	Validation	Calibration	Validation	Calibration	Validation
Runoff	Daily	**0.55**	**0.47**	**0.52**	**0.43**	**-2**	**-2**
	Monthly	**0.92**	**0.84**	**0.89**	**0.81**	**-3**	**-3**
Erosion	Daily	**0.56**	**0.40**	**0.40**	**-0.01**	**-10**	**-37**
	Monthly	**0.84**	**0.70**	**0.74**	**0.52**	**-10**	**-37**



Calibration period **Validation period**

Fig.7: Observed and simulated SWAT monthly runoff (m3/s) [32]

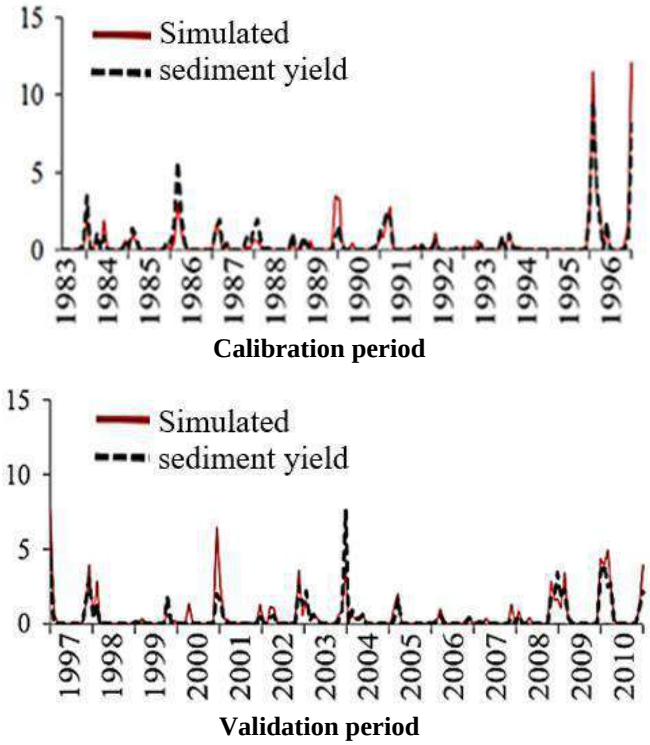


Fig.8: Observed and simulated SWAT monthly sediments yields (10^3tons) [32]

The Nash-Sutcliffe Simulation (NSE) and the coefficient of determination (R^2) for K2 and SWAT models were satisfactory (>0.5) when comparing observed with simulated data [55]. Both models appeared very stable, as (R^2=0.98 and NSE=0.99) for runoff K2 estimation and (R^2= 0.92 and NSE=0.89) for monthly SWAT results. A slight reduction in agreement between sediment yield was observed for SWAT (R^2=0.84 and NSE=0.74) and a better accuracy is noted for K2 (R^2=0.99 and NSE=0.97). Our findings are supported by [56], who demonstrated that SWAT model performance for the sediment yield simulation in Algeria with a R^2 of 0.76 and NSE of 0.75. A study by [57] analyzed the use of Nash as a goodness-of-fit measure for daily runoff simulation with SWAT, demonstrated that the monthly NSE corresponding with five studied versions of simulation model was 0.90.

From these results it can be concluded that the K2 model, coupled with AGWA, requires a small set of parameters, which is a great advantage for simple, fast and practical use. The results attained remain close to the field reality, which makes AGWA-K2 a functional coupling for integrated soil and water resource management in conjunction with sustainable use at the level of Tleta watershed.

The SWAT model is flexible and capable of using biogeographic information to simulate erosion parameters, but it is necessary to bear in mind that the setting of parameters affects infiltration/runoff partition, which requires a fine-tuning before [32]. Last but not least, the SWAT model appears to be more robust with monthly than with daily data (Table 6).

IV. CONCLUSION

In this study, the accuracy of KINEROS2 and SWAT models to simulate runoff and sediment yields was compared. It is concluded that the two models require specific measures for simulations and can simulate runoff and sediment yields in Tleta watershed.

Observed data from Tleta watershed were used for this comparison. To evaluate the performance of each model, the calibration was performed against the observed data. SWAT simulations were for the period 1983-2010. Even the events selected for K2 were included in the same period. The comparison of the simulated sediment yields during the calibration and validation periods leads to the conclusion that Kineros2 predictions are slightly better than Swat, with higher R2 and NSE values.

Based on the results, Kineros2 can be recommended for hydrological and sediment yield simulations. Indeed, the model has simulated the runoff and sediments more precisely than SWAT on a daily scale for the Tleta watershed. Swat includes many parameters to represent cycles, loss and transport by erosion. Calibrating these parameters in this model can be tedious and a lengthy process. In K2, most of the parameters are automatically generated from GIS data integrated in AGWA, or other information and relatively easy to adjust with appropriate instructions. It is also possible to couple SWAT with AGWA, this will minimize errors and time during the simulations. We suggest coupling the model with AGWA tool and then comparing the results obtained.

It should be noted that SWAT model addresses sheet and gully erosion compared to Kineros2 model who treats surface erosion, this type of erosion causes less damage and siltation of the dam.

Finally, to resume, the differences between the two models play a crucial role in their performance. This study revealed discrepancies between the simulation and operation processes of the two models. But the results confirmed their ability to infer the impact of water erosion on the entire watershed and select risk areas for future soil conservation planning.

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Effect of differential expression of pro-region on the transglutaminase productivity in pichia pastoris

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Abstract— *Transglutaminase (TG) induces protein cross-linking by catalyzing the reaction of acyl transfer. Mature sequence of TG is inactive when express without its pro-region. Since pro-region is critical for inhibiting the TG's action and for correctly folding it extracellularly, the production is either poor or forming inclusion bodies without its pro-region. One of the fundamental steps for higher yield to date is to increase the transcriptional level of the recombinant gene by generating multicopies that could only be accomplished by cloning the concatemers. Here, co-expressing strain was successfully generated by incorporating pro-region into ribosomal DNA (rDNA) sites to achieve different copies. The maximum enzyme activity was up to 3.9u/ml compared to the wild type that was only 2.1u/ml in shake flasks being inducted for 96hrs.This research work provides an important strategy for its pro-region to take advantage of the degree of transglutaminase folding.*

Keywords— ***Pichia pastoris, protein folding, pro-peptide, transglutaminase.***

I. INTRODUCTION

Transglutaminase (EC 2.3.2.13) also called protein-glutamine-gammaglutamyl-transferase belonging to the class of transferase that catalyzes acyl-transfer between residues of glutamine and a wide range of primary amine residues[1]. The reaction leads to stable, insoluble product complexes of macromolecules[2]. The cross-link reaction leads to the creation of an intra- and inter-molecular isopeptide bond that leads to the polymerization of proteins[3].

TGases, present in vertebrates, invertebrates, molluscs, plants and microorganisms, are considered to be widely distributed in nature[4,5].They have myriad functionalities and involved in most of the physiological functions, such aswound healing, epidermal keratinization, blood clotting, plant programmed cell death and photosynthetic complex chloroplast stabilization. In addition, TGases are also widely used in the food industry, biochemical and biomedical research, tissue engineering, and in the development of leather and textiles[6–10].In early TG studies, calcium dependent mammalian transglutaminases were used to assist in protein polymerization but due to the high-cost, poor reaction kinetics and instability of animal origin TGases, triggered the scientist to look for cheaper TG sources. TG from streptomyces mobaraensis was first identified in 1989 by the researchers of Ajinomoto Co., Inc and currently, majority of the industries used microbial transglutaminase[11,12].

Several proteins, proteases in particular, are expressed as inactive precursors that are triggered by the removal of the respective protein pro-region. It is understood that the pro-region functions as a protein transporter and inhibitor of the protein concerned[13–15]. MTG produce extracellularly as inactive zymogen and the N-terminal pro-region consisting of 45 amino acids must be removed by exo- or endogenous proteases for fully functional enzyme[16]. Producing recombinant proteins in engineering strains is troublesome for their activation and downstream processing. Keeping in view the activation problem, pichia pastoris is a perfect host with integrated kex2-endpeptipase and capable of separately generating pro- and mature region without needing any in vitro protease.MTG activity reached 1.83U/mL in flasks, suggesting that mature enzymes could be promoted and activated by the pro-peptide sequence[17]. However, the processing and manufacturing of MTG acquired so far is not enoughto reach the industry demand.Therefore, to boost the enzyme activity of MTG, a rising demand for MTG is urgently required.

Pichia pastoris carries repeated ribosomal DNA (rDNA) sequence separated by non-transcribed intergenic spacer (NTS)[18]. It could be used as the site of recombination to increase the target genecopy number. TG expression in recombinant host is problematic without its pro-region, having dual role: assisting the folding of TGase into active conformation and preventing its activation[14,19]. The higher-yield pro-peptide strategy will be used in this research work, providing the importance of the pro-region for TG.

In order to boost the expression and enzyme activity, the genes for pro- and TG were optimized according to codon bias of pichia pastoris in view of the stable mRNA secondary structures and GC ratio. Two different sites at chromosome were selected for pro- and TG gene insertion. The pro-region inserted at ribosomal DNA repeated sites (rDNA)and TG at the mutant histidinol dehydrogenase site (His4). Both of the genes were regulated by AOX1 promoter. By optimization of fermentation conditions, a high co-expression of pro-peptide and MTG was obtained compare to wild type.

II. MATERIALS AND METHODS

2.1. General Strains and Vectors

Strains and vectors are described in Table.1 shown below. The E. coli was cultured in Luria-Bertani (LB) medium at 37 °C, whereas the P. pastoris was cultured in buffered methanol-complex medium (BMMY) at different temperature and pH.

Table 1. Strains and plasmids used in this study

Strains	Genotype	References
E. Coli JM109	endA1, recA1, gyrA96, thi, hsdR17 (rk–, mk+), relA1, supE44, Δ(lac-proAB), [F′ traD36, proAB, laqIqZΔM15]	Invitrogen
P. Pastoris GS115	his4, host strain	Invitrogen
PPIC9K	Description	Invitrogen
pET-22b(+)	Description	Invitrogen
pPIC9K-Pro	pPIC9K vector carrying 135bp of pro region, regulated under AOX1 promoter	This study
pPIC9K-TG	pPIC9K vector carrying 1135bp of mature TG, regulated under AOX1 promoter	This study
GS115-pro-rDNA	Pro-region integrated at rDNA site of GS115	This Study
GS115(PrDNA/TGhIS4)	Mature sequence of TG integrated at HIS4 site and transferred to GS115-pro-rDNA	This study

2.2. Construction of wild type Recombinant plasmid

The *mtg* gene was selected from the cDNA of S. mobaraensis (accession no. Y18315). The PCR was performed to amplify mTG by using primer TG-F (5’-AGAGAGGCTGAAGCTGACAATGGCGCGGGGGAAG AG-3’) as a forward primer and primer TG-R (5’-GAATTCTACGTAtcaGTGGTGGTGGTGGTGGTGCG-3’) as a reverse primer from the plasmid pET-22b(+). The resultant gene was then cloned into pPIC9K vector by one step cloning. The recombinant vector was transformed into *E.*

coli JM109, sequenced to ensure base sequence was not mutated.

2.3. Media Preparation for Subsequent procedures

(a) YPD Media (g/L): Dissolved 20g peptone and 10g yeast extract in 900mL while 20g glucose in 100mL separately. For solid media, 2% agar was added

(b) BMMY (g/L): 10g yeast extract, 20g peptone, 100mM potassium phosphate pH 6.0, 1.34% YNB, 4 x 10-5% biotin, 0.5% methanol were prepared and sterilized.

(c) MD solid medium (g / L): 1.34% YNB, 4 x 10-5 % and biotin 2% dextrose were the recipes used.

2.4. Pichia GS115 competent cell preparation

Pichia pastoris GS115 strain was streaked on YPD plate to attain the colonies. After the colonies grew, a single colony was picked and inoculated into 50mL YPD medium, and cultured at 30°C for 24h. About 0.5 mL of the bacterial solution was taken from the culture and inoculate it into another 50mL of fresh YPD medium. The media was cultured at OD600 1.3~1.5 at 30°C. The culture was centrifuged at 1500×g and 4°C for 5min. The cells were resuspended in 8mL YTB, 180ul DTT and kept it at room temp for 30min. After, centrifuged it at 1500×g and 4°C for 5min. The cells were resuspended and washed in 50mL(1M sorbitol) at 1500×g and 4°C for 5min. This step was repeated three times. Finally, the cells were resuspended by adding 1.0mL of 1M sorbitol and aliquot of 100ul added in each tube for subsequent transformation.

2.5. Construction of GS115-Pro-rDNA expression cassette

The pro-region sequence was copied with forward primer (P1) and reverse primer (P2) and fused to the 3'AOX1 end of S. cerevisiae α-mating factor signal sequence. The rDNA sequence was isolated from pichia genome with the forward primer (P3) and reverse primer (P4). The rDNA sequence fused to the 3'end of the 3'AOX1 terminator.The earlier prepared Pichia GS115 competent cells were taken out and thaw them on ice. The recombinant plasmid GS115-Pro were linearized by digesting with SpeI restriction enzyme and transformed into competent cell, kept it for 15min on ice. The samples were then transformed to e-cups followed the electro-transformation condition 2000Ω, 25μF, 1500V. Immediately added 1mL of 1M sorbitol solution after the completion of the transformation and incubate it for 1~2hr at 30°C. Centrifuged the transformed culture at 5000rpm for 3min. The cells were resuspended and poured on YPD agar plate and incubate it at 30°C for 2-3 days.

Table.2 PCR primers used in this study

Primers	Sequence direction (5' → 3')
P1	gataacggtgctggtgaagaaacta
P2	tgctggtccatcttttagagctcca
P3	tagttaggttaccgtttttcctaatatttaagg
P4	gactggtggttgactgttggtggaag

2.6. Screening and Identification of Positive recombinants

PCR was used to verify positive recombinants of the engineered strain. Eight colonies from the YPD plate were randomly picked, and 5AOX and 3AOX primers were used for colony PCR. Agarose gel electrophoresis was used to verify that the correct colony number was selected.

2.7. Construction of the co-expression cassette

The recombinant vector GS115-Pro successfully transformed to pichia genome to form GS115-Pro-rDNA. The GS115-Pro-rDNA strain was used as a host strain for the recombinant vector GS115-TG. As mentioned earlier that the TG gene was isolated from the cDNA of S. mobaraensis was fused to the 3'AOX1 end of S. cerevisiae α-mating factor signal sequence. The competent cells were made in the same way from the host train GS115-Pro-rDNA. To linearize the GS115-TG-his4 plasmid for transformation, the SalI restriction enzyme was used. The co-expression strain GS115(PrDNA/TGhIS4) was successfully engineered and cultured on MD plate for 1~2 days at 30°C. Clones with higher growth were selected on YPD with 1-1.5ug concentration of G418. The successful clones from YPD plate were cultured in YPD medium for 24hr at 30°C and induce in BMMY media for expression. In the supernatant, the TG was confirmed by colorimetric hydroxamate based method that gives the color of burgundy by treatment with substrates.

2.8. GS115(PrDNA/TGhIS4) engineered strain expression in shake flask

The strain with higher activity was added into 50mL YPD for overnight culture. The overnight culture was centrifuged at 1500xg for 5min at 4°C when the OD reached 5~6. The overnight culture was harvested in 50mL BMMY media with different pH and temperature conditions. 0.5% methanol was added routinely 24hr for about 96hr course of shake flask fermentation. Samples were taken every 24hr for analysis and enzyme activity was measured by colorimetric hydroxamate method described below.

2.9. Enzyme assays and definition of units

TGase activity was measured by the colorimetric hydroxamate procedure. Enzyme solution (50ul) was mixed with 500 ul of reagent A (50mM MES, 100mM NH2OH, 10mM glutathione (reduced form), and 30mM Nα-CBZ-GLN-GLY, pH 6.0, and incubated at 37° C for 10 min. The reaction was stopped by adding 500ul of reagent B(a,3N-HCl; b, 12%TCA; c, 5%FeCl3.6H2O;a: b:c=1:1:1), and the resulting red color was measured at 525 nm.

2.10. Data statistical analysis

Statistical data analyzing was performed with the aid of IBM-SPSS-20 software (SPSS Inc., Chicago, USA). The significant changes among the tested samples were estimated with ANOVA by means of Duncan's test on a confidence level of 95% ($p \leq 0.05$). All experiments were accomplished in triplicate and the data were expressed as mean ± standard deviation.

III. RESULTS AND DISCUSSION

3.1 Constructing the co-expressing strains for Pro- and TG

Two different vectors pPIC9K-Pro and pPIC9K-TG were constructed for expression of Pro- and TG genes, respectively (Fig.1). Both of the genes were inserted separately into pPIC9k vector, fused with the S. cerevisiae α-mating factor signal sequence and regulated under AOX1 promoter. The pro and TG genes could be attached to rDNA and HIS4 loci of GS115 when vectors successfully transformed to the host strain.

In order to generate the co-expressing strain, the vector pPIC9k-Pro were first transformed to GS115 to make GS115-pro-rDNA host strain for TG.Successful clones of GS115-pro-rDNA were selected on yeast dextrose peptone (YPD) plate. The clone with pro-gene insertion were used as a host strain for pPIC9K-TG to form GS115(PrDNA/TGhIS4) co-expressing strain. The co-expressing strain GS115(PrDNA/TGhIS4) contains pro and TG genes were analyzed through PCR to verify the insertion (Fig.2a, and 2b).

Multi-copy integration is anticipated to occur by single homologous recombination during integration, resulting in tandem integrated copies of the vector[20].The copy number of genes for TG expression is an important factor. In pichia pastoris, TG with 3 copy numbers recorded the best activity of 1.41U/ml[21].Therefore, the co-expression strain was selected on YPD with two concentration of G418 (contains 100 ug/ml, 150ug/ml) to ensure the successful transformant and appropriate copy number selection for TG expression. Strategy for multi-site integration of pro-region via rDNA and selection of an appropriate copy of TG produced 3.9u/ml in the shake flask, indicating the role of pro-region in proper folding of more enzyme extracellularly (Fig 3a and 3b).

3.2. Effect of pH, Temp and different induction parameters on the TG activity

The effects of three temperatures (20°C, 25°C and 30°C)and four pH values (5.0, 6.0, 7.0 and 8.0) on TGproduction were tested in shake flask experiments using a single copy of the engineering strain GS115(PrDNA/TGhIS4). The clones were inoculated in 50mL YPD for 24hr and the overnight cultures were transferred to 50mL BMMY for 96hr induction. The samples were collected every 24hr and activity was measured by colorimetric hydroxamate-based method.

From the activity analysis, it appears that suitable environment for transglutaminase production is low temperature and alkaline environment from pH 6.5~7.5 as shown in (Fig 4a, 4b and 4c). The production of transglutaminases at a lower pH is difficult to enable activation and requires regulation of activation enzymes such as dispase I with exchange buffer.Lower temperatures are ideal for foreign proteins to reduce degradation, given theimportance of proteases. The finding in this research work may be due to the poor activity at low temperatures of extracellular protease enzymes.Higher temperatures are also problematic for the successful folding of foreign proteins[22]. Here, thesecretion of TG at temp 30°C is nearly the same as 25°C, given the role of pro-region to enhance folding of TG amid higher temperature.

Three different induction parameters were chosen to see if different methanol intensities affect the engineering strain with pro-region higher integration at rDNA.In the shake flasks experiment, three different concentration, 0.5%, 1% and 1.5% of methanol were conducted. The product did not affect with methanol concentration from 0.5% to 1% but declined at higher concentration of 1.5%, suggesting the accumulation of methanol which is toxic for the cells.

According to the data from shake flasks experiments, the recombinant strain expressed at higher level with low temperatures and higher pH values. However, the production at high temperature was nearly the same as at lower temperature compare to the wild type which showed little activity. The optimum extracellular production conditions for the engineering strain to produce the TG enzyme were therefore calculated at 20°C and pH 7 at shake-flask level with 0.5% methanol induction.

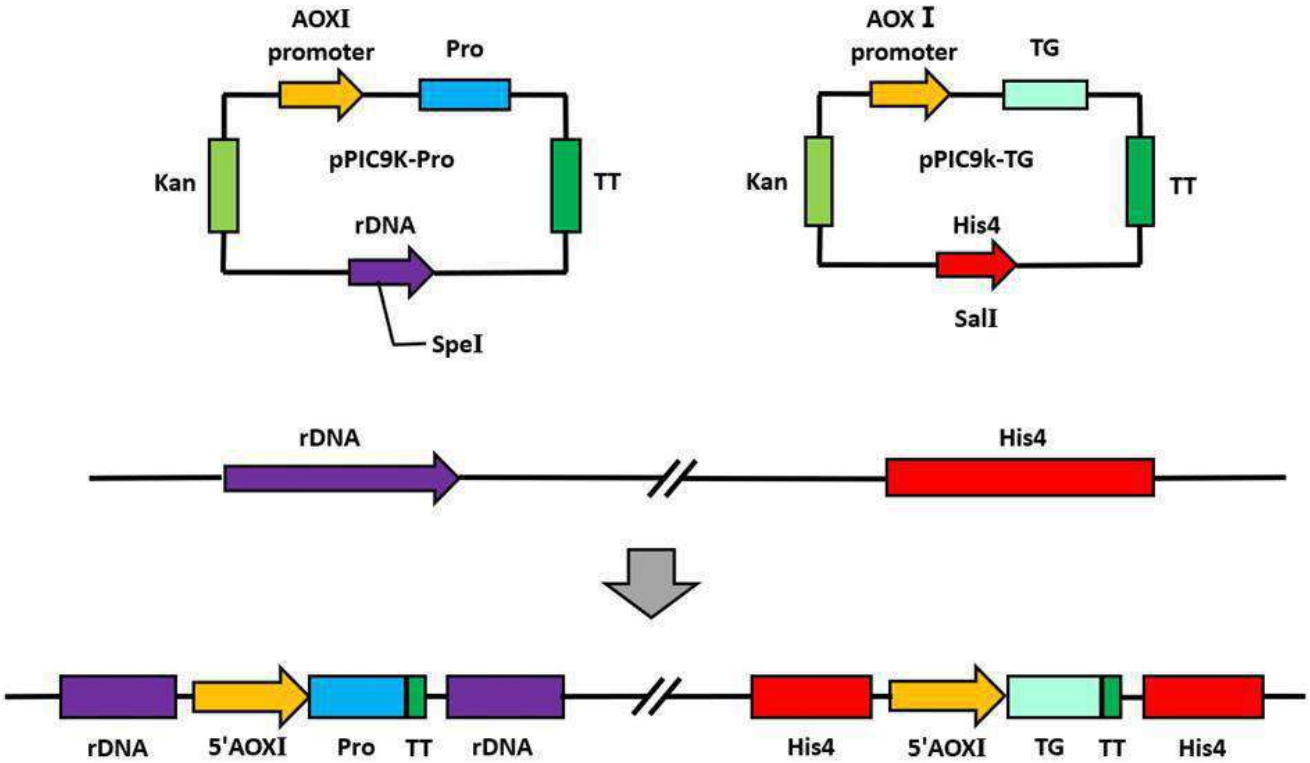

Fig.1 Construction of recombinant plasmids for TG expression

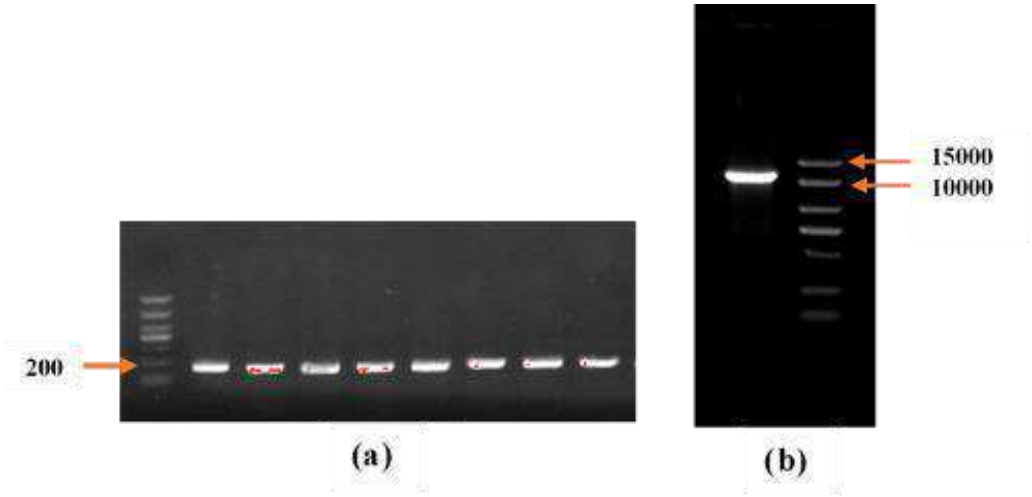

Fig. 2 Colony PCR from the host strain GS115-prorDNA showing pro region having 135bp (a). The TG gene having 1135bp from co-expressing strain GS115(PrDNA/TGhIS4) (b).

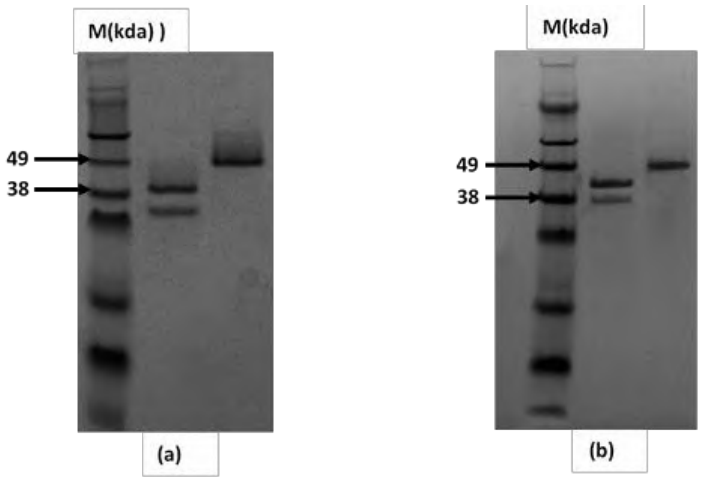

Fig. 3 The SDS analysis of wild type and co-expressing engineered strain. Both of the expression were treated with and without dispase I. (a) Wild type TG, lane 1 is treated with dispase showing 40kda while lane 2 is without any dispase addition shows 55kda. (b) Co-expressed GS115(PrDNA/TGhIS4) strain, lane 1 is treated with dispase showing 40kda while lane 2 is without any dispase addition shows 55kda. Here, the engineered strain shows big bands suggesting more of the proteins secreted in the extracellular matrix

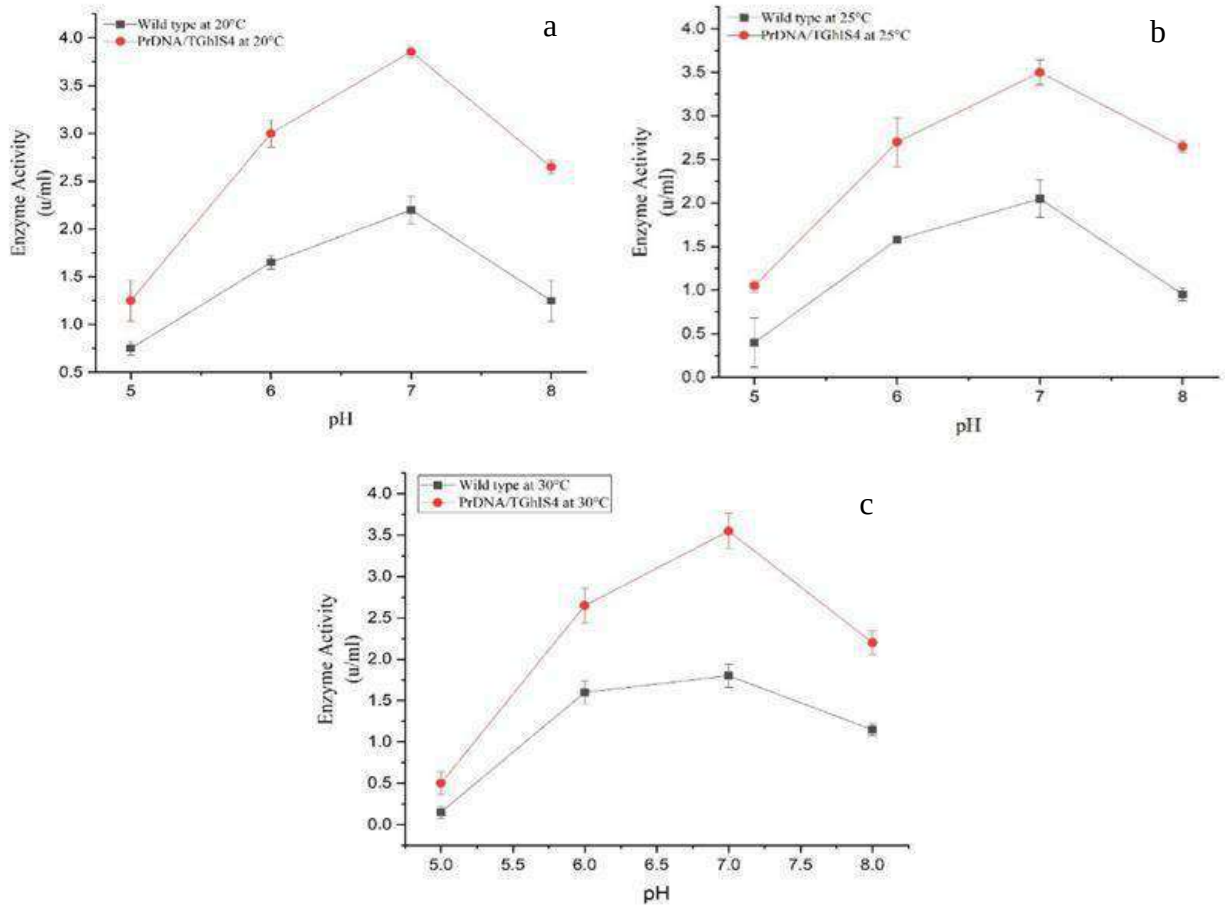

Fig. 4: Activity analysis of wild type TG and GS115(PrDNA/TGhIS4) engineered strain at three different temperatures (a)20°C, (b)25°C, and (c) 30°Cand four different pH values.

IV. CONCLUSION

The co-expression strain GS115(PrDNA/TGhIS4) was successfully engineered by integrating the pro-region at non-transcribed ribosomal rDNA of pichia pastoris followed by TG incorporation into his4 site. The expression of pro-region with uncountable copies at rDNA sites secreted more folded extracellular proteins. However, for industrial applications, the output of this study work is minimal, and needs to be improved.It offered an insight for potential pro-region enhancement and secretion investigation.Co-expression of pro-region and mTG is a challenging task. These findings could be applied and expanded by harnessing the pro-region role for the folding of "difficult-to-express" recombinant co-express pro-transglutaminase.

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VI.

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Performance and Nutrient Utilization of Pullet Chicks Fed Diets Containing *Prosopis* Africana Seed Coat Meal Treated with Polyzyme

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Abstract**—** *The study was conducted to evaluate the effect of prosopis africana seed coat meal (PASCM) on the performance and nutrient utilization of three hundred (300) Nera brown day-old pullet chicks that were fed for 56 days. The birds were randomly allotted to 5 experimental diets with 3 replications of 20 birds each. The diets were formulated with the inclusion of PASCM at 0, 15, 20, 25 and 30% levels, respectively and the data collected were subjected to analysis of Variance in a completely randomized design. Results obtained showed that the 0%(control diet) had the highest feed intake of 31.94g/bird/day. While the best FCR (3.09) and highest final weight gain (608.33g/bird) were obtained in 0% PASCM inclusion level. Also birds on 0% PASCM inclusion level had the highest value of weight gain (10.33g/bird) and best protein efficiency ratio (0.64). Mortality was not observed. The digestible CP and EE were highest (P<0.05) in 0% PASCM inclusion but did not differ significantly (P > 0.05) from 15% and 20% inclusion levels. It was therefore concluded that 20% PASCM inclusion level could be adopted for optimum growth performance and digestibility in pullet chicks' diets.*

Keywords**— Pullet Chicks, Performance, Nutrient Utilization and PASCM.**

I. INTRODUCTION

Inadequate supplies of feedstuffs at economic prices continue to limit the production of animal protein in Nigeria. This is because the cost of animal feed accounts for 60% and 70% of the cost of production in poultry enterprises in Nigeria. Nutritionist and other professionals therefore, strive to reduce this cost to maximize profit [1,2].

This high cost of feed ingredients has scared some farmers from poultry business [3]. The conventional feed like maize continues to be expensive. Maize constitutes the main component of energy diet in poultry production in Nigeria, suggesting that any increase in the price of maize may increase the price of animal products. Therefore, there is the need to find an alternative feed resource which can replace maize [4, 5] in the diets of pulletchicks. The use of agricultural by-products and kitchen wastes like maize bran, rice bran and *Prosopis africana* seed coat meal (PASCM) etc. as feed resources can be achieved in poultry diet after careful study. This will help to reduce the competition for maize and increase animal protein at a relatively lower cost and improve net profit [6, 7,8, 9].

The availability of PASCM and its free acquisition brings it into focus as a replacement for maize in poultry nutrition. PASCM is high in crude fibre and low in energy compared

to maize diet but can be used to replace maize as energy source [10, 11] in pullet chick diets with some exogenous enzymes (e.g polyzme) fortification [12, 13, 14]. This study was sought to provide alternative feedstuffs to address the global feed crisis with the use of PASCM without affecting the performance and nutrient digestibility in pullet chicks.

II. MATERIALS AND METHODS

Experimental Site

This study was conducted at the poultry unit of Ohagwu farm,Ochodu Ukpa Igede, Oju Local Government Area of Benue State, Nigeria. Oju Local Government Area lies between Latitude $6^{0}51^{1}$ north and Longitude $8^{0}25^{1}$ east in the Southern Guinea Zone of Nigeria, with a climate that has two distinct seasons. The wet season covers mid-March to mid-November, while dry season starts in late November to early March in which high temperature is experienced between February and April. Oju Local Government Area has an annual rainfall ranging from 1200 mm to1500 mm. The temperatures are generally very high during the day, particularly in March and April with a mean daily temperature of $26^{O}C$, and daily minimum temperature of $16^{0}C$ to $21^{O}C$ and maximum daily temperature of $31^{O}C$ to $37^{0}C$ in dry and wet seasons. The relative humidity ranges from 42% to 75% depending on the time of the day and season of the year [15].

Test ingredient

*Prosopis africana*seed coat meal (PASCM) was sourced from women in Oju Local Government Area that produced food condiment (Okpehe or Dawadawa) from *prosopis africana* seeds.

Experimental Birds and Management

A total of 300 Nera brown day-old pullet chicks were randomly divided in five groups in a complete randomized design with each treatment having three replicates containing twenty birds per replicate. Five experimental diets were formulated from a mixture of maize, *Prosopis africana* seed coat meal soybean meal, rice bran, blood meal, bone meal, palm oil and vitamin/mineral/premix as shown in Table 1. They were intensively managed in deep litter system throughout the experimental period. Feed and water were given *ad libitum*. Record of initial weight, final weight, body weight gain and feed intake were taken while feed conversion ratio, protein intake and protein efficiency ratio were estimated.

Dietary treatment

The PASCM was sundried for 10 days and milled. It was then incorporated into 5 diets at 0, 15, 20, 25 and 30% levels as replacement for maize (Table 1).

Digestibility trials

At the 7^{th} week of the experiment, 2 pullet chicks from each replicate were randomly selected and managed in clean disinfected metabolic cages. They were allowed 3 days of acclimatization and four (4) days of feacal collection. A known weight of feed was given daily. The feaces voided each day per treatment per replicate were weighed fresh and oven dried to a constant weight, ground and used for proximate analysis as outlined in A.OA.C [16].

Statistical analysis

The data obtained were subjected to one way analysis of variance (ANOVA) and in a completely randomized design using the procedure outlined in the Minitab [17]. Where significant difference between treatment means occurred, they were separated using Minitab [17] software.

III. RESULTS AND DISCUSSION

Pullet Chick Performance

Table 2 showed the effect of PASCM treated with polyzyme on the performance of pullet chicks. Average final weight, average weight gain, average feed intake and feed conversion ratio were observed to be significantly different ($P < 0.05$) among treatment groups but there was no discernable pattern. The average final weight (463.33 – 608.33g/bird) obtained in this study are comparable with the value (580.00g/bird) reported by [7] as growth performance of pullet chicks from 0 – 8 weeks in the tropics.However, protein intake and protein efficiency ratio were not significantly difference ($P > 0.05$) among the treatments.

Birds placed on 0% (control) PASCM inclusion level recorded the highest feed intake (31.94g/bird/day). This result disagrees with the reports of [18, 19] who obtained higher feed intake (38.72 – 57.86g/bird/day) in which the fibre content of feed was increased when non-conventional feedstuffs were used. The decrease in feed intake observed in the PASCM based diets may be attributed toinherent anti-nutritional factors in PASCM. [20, 21, 22] have reportedsome anti-nutritional factors like non-starch polysaccharides (NSP) and phytotoxins in*Prosopis africana* products that effect performance.[23] had earlier suggested the use of exogenous enzymes in the nutrition of poultry when fibrous feed materials were included in the diet. The best Feed Conversion Ratio (FCR) was recorded in0%

PASCM inclusion level, though polyzyme was included in the diets, the ability of the chicks to utilize the fibre content of PASCM was less efficiency beyond 20% level. [24, 19] reported that higher levels of fibre in the diets have negative effect on protein intake and this was evident in the 25% and 30% PASCM inclusion levels.

Nutrient Digestibility

The result of nutrient digestibility as shown in Table 3 indicated significant difference ($P < 0.05$) in the digestible dry matter (DM), crude protein (CP), ether extract (EE) and nitrogen-free extract (NFE). However, crude fibre (CF) did not show significant difference ($P < 0.05$) among the treatments. This may be due to inclusion of polyzyme in PASCM based diets. [12, 14,19,23, 25] have reported increased apparent nutrient digestibility of high fibre diets in monogastric (e.g. Poultry) diets when exogenous enzymes were included in the diets.

IV. CONCLUSION

The non-significant difference in treatment 0, 15 and 20% in the final weight, weight gain, feed intake, feed conversion ratio, protein intake and protein efficiency ratio suggests that upto 20% of PASCM inclusion level in the diets of pullet chicks could be adopted for optimum growth performance and nutrient digestibility.

Table1. Ingredients and dietary Composition of Pullet Chick Diets

Experimental diets					
Ingredients	**0%**	**15%**	**20%**	**25%**	**30%**
Maize	51.45	43.73	41.16	38.58	36.02
PASCM	-	7.72	10.29	12.86	15.44
Sobean meal	31.00	31.00	31.00	31.00	31.00
Rice bran	9.00	9.00	9.00	9.00	9.00
Palm oil	1.00	1.00	1.00	1.00	1.00
Blood meal	2.00	2.00	2.00	2.00	2.00
Bone meal	3.00	3.00	3.00	3.00	3.00
Limestone	1.50	1.50	1.50	1.50	1.50
Vit./Min/permit	0.25	0.25	0.25	0.25	0.25
Salt (Nacl)	0.30	0.30	0.30	0.30	0.30
Enzymes	-	+	+	+	+
Total	100.00	100.00	100.00	100.00	100.00
Determined nutrients					
Dry matter	88.37	88.34	86.30	87.47	86.82
Crude protein	22.77	22.45	23.31	22.84	223.35
Crude fibre	5.68	6.79	6.39	7.13	6.73
Ether extract	4.69	5.29	5.39	4.78	5.55
Ash	10.44	9.53	9.388.98	8.75	8.61
Nitrogen-free Extract (NFE)	56.43	56.16	55.30	56.49	55.77
Metabolizable Energy (kcal//kg)	3226.11	3253.35	3262.75	3238.13	3301.90

PASCM = *Prosopis africana* seed coat meal

Table 2: Effect of Prosopis africana Seed Coat Meal on the Performance of Pullet Chicks

	PASCM ICLUSION					
Parameters	0%	15%	20%	25%	30%	P.V.
Initial weight	29.45	29.58	29.98	29.10	29.87	0.83
Final weight	608.33^{a}	558.33^{a}	560.00^{a}	478.33^{b}	463.33^{b}	0.01
Weight gain(g/bird/day)	10.34^{a}	9.44^{a}	9.46^{a}	8.02^{b}	7.74^{b}	0.01
Feed intake(g/bird/day)	31.94^{a}	31.34^{a}	30.74^{ab}	30.42^{ab}	28.88^{b}	0.03
Feed conversion ratio	3.09^{b}	3.37^{ab}	3.25^{ab}	3.79^{a}	3.74^{a}	0.04
Protein intake (g/bird/day)	6.64	6.39	6.23	6.14	5.79	0.68
Protein efficiency ratio	0.64	0.68	0.66	0.77	0.75	0.30

a.b means within each row with different superscripts are significantly different (P<0.05), NS = Not significantly different (P > 0.05).

Table 3: Effect of Prosopis africana Seed Coat Mealon the Nutrient Digestibility

	PASCM INCLUSION					
Digestible	0%	15%	20%	25%	30%	SEM
Dry matter	74.12 ^{a}	71.40 ^{b}	74.28 ^{a}	70.16 ^{bc}	68.63 ^{c}	0.00
Crude protein	70.56 ^{a}	68.33 ^{ab}	69.65 ^{a}	64.52 ^{b}	67.99 ^{ab}	0.05
Crude fibre	67.17	64.90	67.20	63.49	62.90	0.19
Ether extract	81.03 ^{a}	79.57^{a}	78.54 ^{a}	73.18 ^{bc}	74.21 ^{b}	0.01
Nitrogen free Extract	70.71^{a}	68.03^{ab}	70.59^{a}	66.75^{bc}	64.19^{c}	0.00

a.b.c: Means in the same row with different superscripts differed significantly (P<0.05). SEM = Standard Error of Mean.

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Metabolic Engineering of Microorganisms to Increase Production of Violacein

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Abstract— *Violacein, an indole derivative, is a violent pigment which is extracted from the bacteria. It is considered to be an important aromatic compound as it exhibits essential antiparasitic, antimicrobial and antitumoral characteristics. One of the most eminent derivatives that is being induced by the biosynthetic pathway of violacein is deoxyviolacein. However, it is produced in an insignificant amount. By expunging the VioD protein from the violacein pathway, deoxyviolacein can be generated which is devoid of a hydroxyl group. Another derivative that was produced in addition to deoxyviolacein is oxyviolacein, generated by the derivative of tryptophan i.e. 5-hydroxytryptophan. In this review, our main focus is on different engineered microorganisms in increasing the production of the violacein. On undergoing genetic analysis and determining the basic mechanism of violacein production showed that, violacein is formed by the condensation of 2 tryptophan molecules in presence of vioABCD gene cluster. However, later on, the presence of another gene vioE was revealed to be involved in violacein biosynthesis and a new pathway was suggested. McClean reported the involvement of quorum sensing mechanism via AHL's in violacein biosynthesis. Then using the above information and using violacein gene cluster vioABCDE, the violacein was produced in C. violaceum, Pseudoalteromonas sp. 520P1, V. natriegens, C. glutamicum, E. coli, Y. lipolytica and D. violaceinigra. Then the amount of violacein was increased by subjecting it to either batch or fed-batch fermentation. Then after its production, its anti-microbial activity was determined against Staphylococcus species. Also, its anti-cancerous activity was also determined on resistant leukemia cells.*

Keywords— ***Violacein, Deoxyviolacein, Oxyviolacein, VioABCD.***

I. INTRODUCTION

Metabolic engineering refers to the optimization of genetic and regulatory processes that occurs within the cell in order to increase the production of a specific substance in the cells. It includes series of biochemical reactions together with the enzymes to convert the raw materials into that specific substance and increase its amount. Metabolic engineering specifically deals with creating a mathematical model of these pathways, calculating its yield and blocking the path that will constrain the production of our desired substance (Yang, Bennett, & San, 1998). In this review, our main focus is on different engineered microorganisms in increasing the production of the violacein.

Violacein, an indole derivative, is a violent pigment which is extracted from the bacteria. It is considered to be an important aromatic compound as it exhibits essential antiparasitic, antimicrobial and antitumoral characteristics (Durán et al., 2007). Being a bisindole, it is produced by a vast genus of bacterial strains which includes: *Microbulbifer sp*., Pseudoalteromonas, Collimonas and Duganella etc. These strains are phylogenetically varied and thus, are isolated from different locales like the rhizosphere of olive groves (Aranda, Montes-Borrego, & Landa, 2011), within glaciers (Avguštin, Bertok, & Avguštin, 2013) and even from sea sponges surfaces. Among the genus of violacein-producing strains, Chromobacterium is the best one i.e. Chromobacterium violaceum (Durán & Menck, 2001). Violacein as a secondary metabolite is associated with the production of biofilm. Quorum sensing mechanisms modulate its production within C. violaceum and other bacterial strains (McClean et al., 1997). Due to its facile visualization, its production has become an effective criterion of quorum sensing substances and their inhibitors (Burt, Ojo-Fakunle, Woertman, & Veldhuizen, 2014).

1. OXYVIOLACEIN AND DEOXYVIOLACEIN

Despite grabbing most of the attention, violacein is not only the substance produced within the bacterial hosts by proteins i.e. VioA, VioB, VioC, VioD and VioE. One of the most eminent derivatives that is being induced by the biosynthetic pathway of violacein is deoxyviolacein. However, deoxyviolacein, in comparison to violacein, is produced in an insignificant amount in *Janthinobacterium lividum* (Rodrigues et al., 2012).

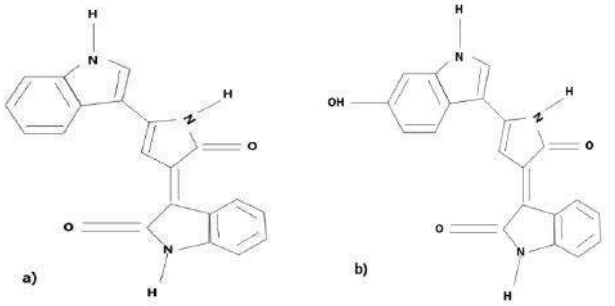

Fig.1: Chemical structures of a) deoxyviolacein and b) violacein

Compatible results were also depicted in research with *Duganella sp*. B2 where also deoxyviolacein was produced in low concentration as compared to violacein which was evaluated by HPLC (Jiang et al., 2012). Crude violacein extracts that are procured from the natural bacterial strains contain around ~ 10-20% of deoxyviolacein with 85% mostly violacein on the basis of HPLC evaluation.

By expunging the VioD protein from the violacein pathway, deoxyviolacein can be generated which is devoid of a hydroxyl group. A recent study by Xing group divulged the elevated production and characterization of deoxyviolacein using the chimeric Citrobacter freundii having a plasmid with VioD gene knocked out of it. Their research disclosed that deoxyviolacein showed comparatively better photostability as compared to the violacein under tests with either UV or natural light. However, both were proved to be toxic when are exposed to the 24h toxicity tests with HepG2 cell lines. However, deoxyviolacein impact was dose-independent as compared to violacein i.e. dose-dependent. The variance between violacein and deoxyviolacein was more conspicuous when the viability of HepG2 cell lines was determined after 48h (Jiang et al., 2012).

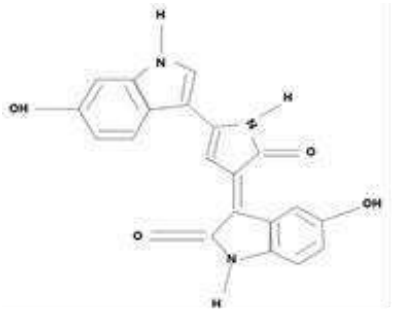

Fig.2: Chemical structure of oxyviolacein.

Another derivative that was produced in addition to deoxyviolacein is oxyviolacein, generated by the derivative of tryptophan i.e. 5-hydroxytryptophan (Sánchez, Braña, Méndez, & Salas, 2006). In oxyviolacein, there is an extra hydroxyl group as compared to deoxyviolacein that lacks the hydroxyl group. This presence of extra hydroxyl group has elevated its efficiency against human pathogens like Staphylococcus aureus that was reduced in deoxyviolacein as it is devoid of its hydroxyl group (H. Wang et al., 2012).

2. GENETIC ANALYSIS AND THE BASIC MECHANISM OF VIOLACEIN BIOSYNTHESIS

An intermediate of glycolysis i.e. phosphoenolpyruvate (PEP) and an intermediate of Hexose monophosphate pathway (HMP) i.e. erythrose-4-phosphate (E4P) together initiates the biosynthesis of the aromatic amino acids that leads through various steps to produce chorismate that lead to a branch that commences with anthranilate that finally leads to tryptophan pathway. The defective mutants in the HMP pathway can produce an enhanced mount of E4P that act as a limiting substance in the biosynthesis of violacein (Ikeda & Katsumata, 1999).

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Previous studies on the violacein biosynthetic pathway claimed 5-hydroxy-L-tryptophan to be the precursor in violacein synthesis (Hoshino & Ogasawara, 1990). However, it was revealed later that two trp molecules generate all the C, N and H atoms while molecular O_2 generates the O atoms of the violacein (Antonio, Haun, & Pilli, 1994) (Momen & Hoshino, 2000). It is now perceptible that 2 trp molecules form a fundamental structure of violacein which is accompanied by the indole ring oxygenation of the intermediate. Like in *E. coli*, anthranilate biosynthesis initiates the biosynthesis of tryptophan which is encrypted by various genes i.e. *trpA, trpB, trpC, trpD, trpE, trpF and trpG*. However, unlike *E. coli*, they are not categorized into an operon. Instead, they concoct clusters with genes not involved in tryptophan biosynthesis. August and his co-workers cloned and sequenced the complete operon of violacein biosynthesis. It was disclosed that *VioA, VioB, VioC* and *VioD* genes constitute the violacein biosynthetic operon genes as shown in figure 3 and table 1.

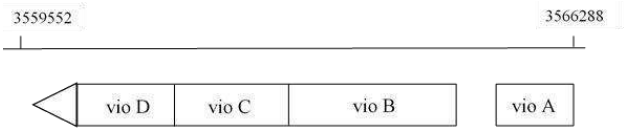

Fig.3: Structural gene operon of violacein biosynthesis

After cloning and sequencing the operon for biosynthesis of violacein, these scientist designed a model biosynthetic pathway showing the imputed activities of every gene product in this pathway (figure 4) (August et al., 2000).

Table 1: Major ORF's (open reading frames) for violacein metabolism and expression (de Vasconcelos et al., 2003)

ORF name	ORF number	ORF product name
vioA	CV3274	Tryptophan-2-monooygenase
vioB	CV3273	Polyketide synthase
vioC	CV3272	Monooxygenase
vioD	CV3271	Hydroxylase
trpA	CV2761	Tryptophan synthase, alpha subunit
trpB	CV2762	Tryptophan synthase, beta subunit
trpC	CV2712	Indole-3-glycerol phosphate synthase
trpD	CV2173	Anthranilate phosphoribosyltransferase
trpE	CV2179	Anthranilate synthase component-I
trpF	CV2763	Phosphoribosylanthranilate isomerase

The vioA, vioC and vioD gene products showed similarity with the nucleotide-dependent monooxygenases. Research on violacein biosynthesis suggested that while the hydroxylation of one tryptophan molecule is catalyzed by vioD gene, the oxidative deamination of the second trp molecule is catalyzed by vioA gene and similarly, intermediate oxidation of violacein is catalyzed by vioC. Similarly, vioB protein was found to be compatible with polyketide synthase, an enzyme that exhibits an amazing activity i.e. its capability to catalyze non-ribosomal peptidal bonds and in the biosynthesis of violacein, it mediates the condensation of 2 trp derivative molecules that are important for pigment production (Antônio & Creczynski-Pasa, 2004).

3. VIOLACEIN AND DEOXYVIOLACEIN BIOSYNTHESIS IN *C. VIOLACEUM*

Till now, it was believed that in violacein biosynthesis, only four genes (vioABCD) were involved (August et al., 2000) (Brady, Chao, Handelsman, & Clardy, 2001).

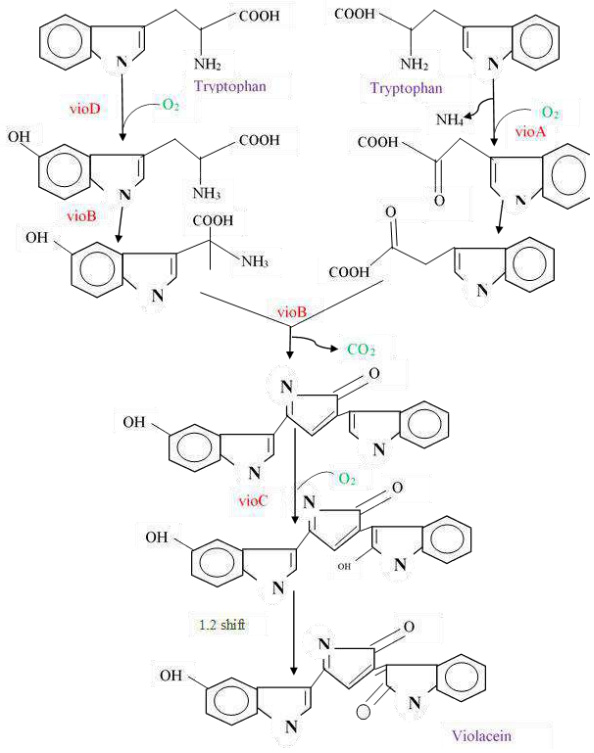

Fig.4: Violacein biosynthetic pathway suggested by August et al. 2000.

However, whole-genome sequencing of Chromobacterium violaceum in 2003 disclosed the existence of the 5th gene in the gene operon of violacein (de Vasconcelos et al., 2003). Thus, until the role of this 5th gene was not revealed, the complete violacein biosynthetic path couldn't be identified (Sánchez et al., 2006) (Balibar & Walsh, 2006). It was revealed that the

product of 5th gene, vioE protein catalyzes the protodeoxyviolacenic acid formation from an unidentified intermediate, compound X (Asamizu, Kato, Igarashi, & Onaka, 2007) (figure 5). Thus, it was evident that vioE proved to be requisite for the formation of violacein structure. Recent studies have reported the comprehensive structure and reaction process of the VioE protein. The five violacein biosynthetic enzymes i.e. VioABCDE were encoded by 5 genes arranged in a single operon i.e. vioABCDE (Hirano, Asamizu, Onaka, Shiro, & Nagano, 2008) (Ryan, Balibar, Turo, Walsh, & Drennan, 2008). These 5 enzymes VioABCDE were cloned and expressed under invitro conditions by Balibar and Carl who reported L-tryptophan as a precursor of violacein instead of 5-hydroxy-L-tryptophan. The pathway model suggested for violacein and deoxyviolacein biosynthesis is shown in figure 5.

Oxidation of tryptophan is catalyzed by VioA. The coupling of 2 IPA imine molecules to generate an unidentified intermediate X is the responsibility of VioB. Protodeoxyviolaceinic acid is produced by the newly discovered enzyme, VioE. Inevitably, oxygenation of indole rings at 2 and 5 positions to form violacein is catalyzed by VioC and VioD respectively. Oxygenation of indole ring at position 2 by VioC from protodeoxyviolaceinic acid generates deoxyviolacein, the by-product of violacein (Hirano et al., 2008).

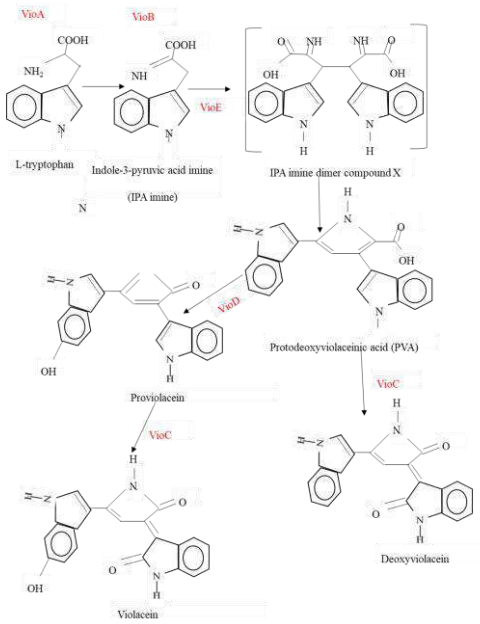

Fig.5: Violacein and deoxyviolacein biosynthesis pathway in C. violaceum

3.1 QUORUM SENSING MODULATION OF SECONDARY METABOLITES

Quorum sensing processes via autoinducer molecules that the bacteria secrete modulates the production of violacein in *C. violaceum*. Quorum sensing is a mechanism that bacteria used in order to communicate with each other by releasing signaling substances (Waters & Bassler, 2005) (Williams, Winzer, Chan, & Camara, 2007). This quorum sensing mechanism regulates the production of many secondary metabolites like pigments, toxins, antibiotics, bioluminescence and biofilm formation.

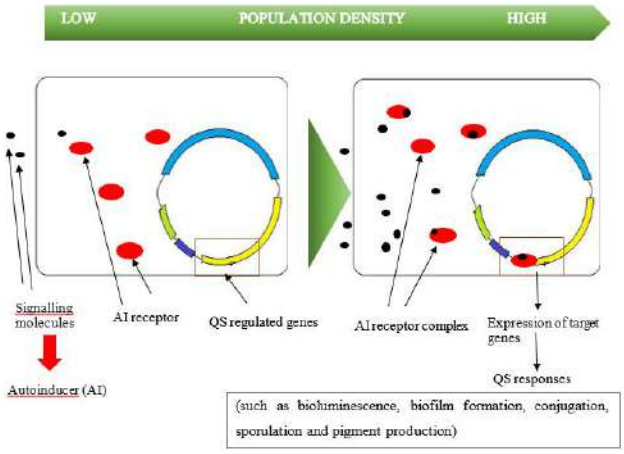

Fig.6: Quorum sensing mechanism

3.2 QUORUM SENSING IN VIOLACEIN BIOSYNTHESIS

It was reported by McClean that quorum sensing via AHLs i.e. N-acyl homoserine lactones regulates the production of violacein in C. violaceum (figure: 7).

It was suggested that the cumulated AHL forms a complex by binding with receptor proteins which then activate the expression of gene cluster by interacting with transcriptional regulator site of violacein operon, which eventually leads to the synthesis of violacein. This alleged transcriptional promoter site is present in the intervening area between gene vioA and its upstream adjoining protein gene. There is little evidence for the location of quorum sensing regulatory sites (McClean et al., 1997).

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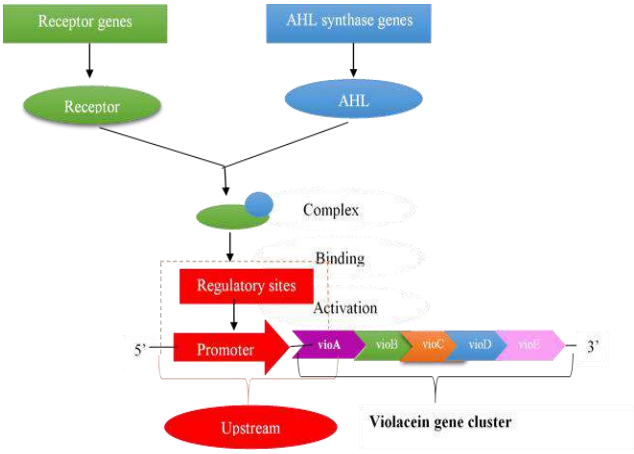

Fig.7: Quorum sensing model of regulation of violacein gene regulation in C. violaceum.

4. VIOLACEIN PRODUCTION AND REGULATION IN PSEUDOALTEROMONAS SP. 520P1

Zhang conducted an experiment to demonstrate the production and regulation of violacein by Pseudoalteromonas sp. 520P1 under quorum sensing systems via AHLs. This 520P1 strain is a gram-negative bacteria which was sequestered from the seawater of Cap Muroto in Japan (Yada et al., 2008). However, this strain showed violacein production under static culture circumstances only. However, previous studies showed that the 520P1 strain can produce violacein under the regulation of the quorum sensing process through AHLs (Y. Wang et al., 2008). In this research, they characterized and cloned the gene cluster of violacein and its upstream region using a fosmid library. It was used to create stable libraries using complex genomes (Huang et al., 2009) and sequestering genes (Schloss et al., 2010) from the genomic DNA. It contained ~ 13,000 clones developed from the 520P1 strain's genomic DNA. 5 clones were isolated containing violacein gene clusters. Thus, 5 ORF's cluster i.e. vioABCDE of 7383 total lengths for the biosynthesis of violacein was obtained. A highly conserved sequence was determined in 520P1 strain at ~ 200bp upstream of gene cluster having promoter sequences i.e. -10 and -35 box. ~700bp downstream and ~1500bp upstream are sequences that encodes for 2 putative proteins (figure: 8).

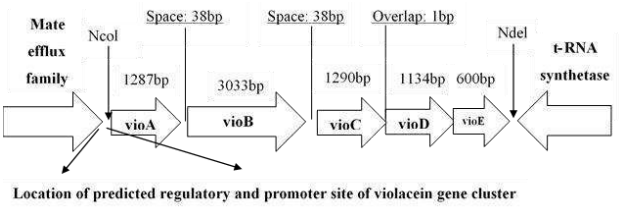

Fig.8: Schematic diagram of Pseudoalteromonas sp. 520P1 strain gene cluster

Heterologous expression of gene cluster was accomplished using a recombinant pET vector in *E. coli* to analyze its ability to produce violacein (figure: 9). Successful violacein gene cluster expression of 520P1 strain in *E. coli* was confirmed by the emergence of violet colonies of recombinant *E. coli*.

However, the violet color indicating violacein production appears only when recombinant *E. coli* were incubated in absence of IPTG inducer at 50 rpm at 20°C. HPLC and UV-VIS spectrum analysis confirmed violent pigment produced by recombinant *E. coli* to be identical to violacein. Normally, 520P1 strain produced the mixture of violacein and deoxy violacein, but, elution profile in HPLC analysis indicated a single peak showing the production of the only violacein in recombinant *E. coli* (ZHANG, 2010).

5. HETEROLOGOUS BIOSYNTHESIS OF VIOLACEIN IN VIBRIO NATRIEGENS

5.1 C. VIOLACEUM BIOSYNTHETIC PATHWAY CONSTRUCTION IN V. NATRIEGENS

Ellis et al in 2019 conducted a research to determine the feedstock flexibility and heterologous natural product (violacein) production in *V. natriegens* using the gene cluster of C. violaceum. *V. natriegens* only encodes for the precursor of tryptophan, thus, can't encodes the violacein biosynthesis (Lee et al., 2019).

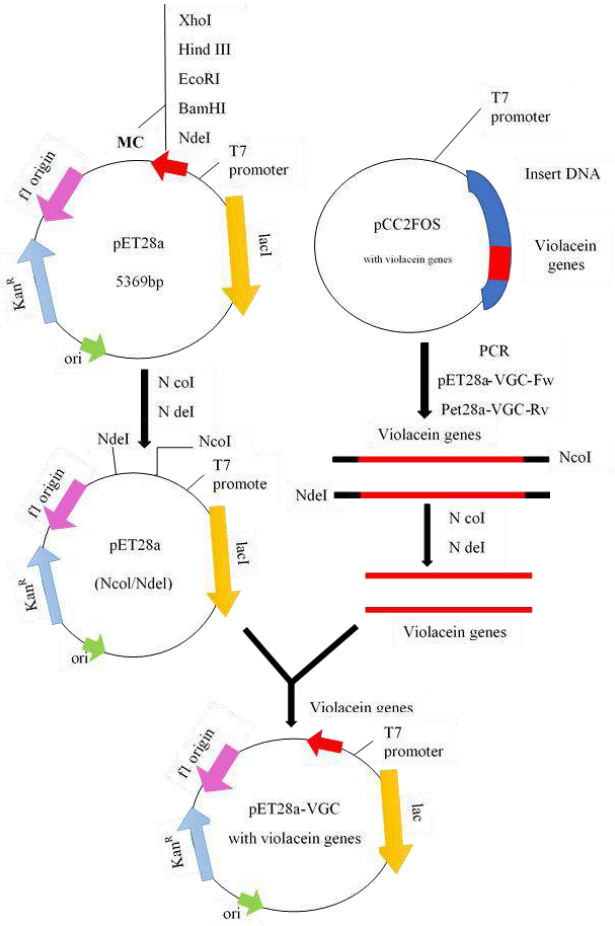

Fig.9: Schematic diagram of recombinant vector construction containing violacein gene cluster

Tryptophan is produced from basically two pathways in *V. natriegens* i.e. in glycolysis form phosphoenolpyruvate (PEP) and in pentose phosphate pathway from erythrose-4-phosphate (E4P) (figure:10).

Table 2: Reported heterologous genes for constructing violacein synthetic pathway in V. natriegens (Kim et al., 2016) (M. E. Lee et al., 2013)

Gene	Gene ID	Protein
vioA	24947400	L-tryptophan synthase
vioB	24945600	Iminophenyl-pyruvate dimer synthase
vioC	24948167	Violacein synthase
vioD	24947515	Tryptophan hydroxylase
vioE	24949508	Protodeoxyviolaceinate monooxygenase

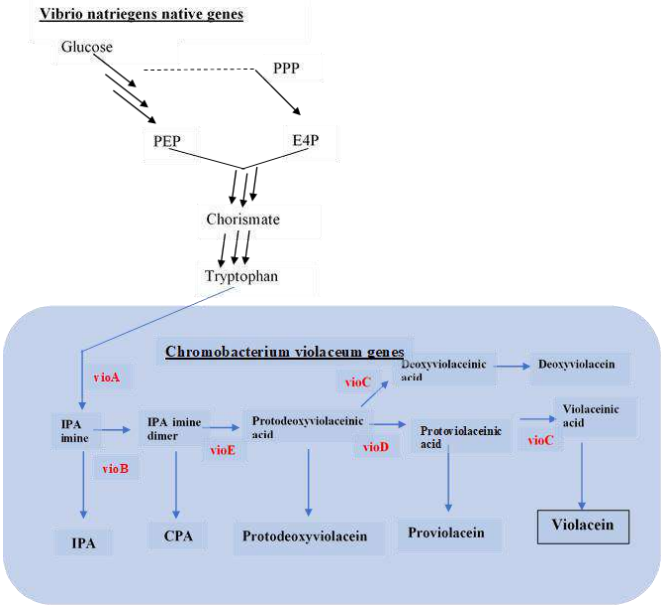

Fig.10: Violacein biosynthetic natural and heterologous pathways in V. nitrogens. Plasmid (pVio) map or violacein biosynthesis

As described above, there is the involvement of 5 enzymes for the conversion of tryptophan to violacein. It was also disclosed that the violacein biosynthetic pathway consists of various opportunities to convert intermediates non-enzymatically to terminal co-products, limiting the overall yield as seen in figure10. Genes, gene ID and enzyme names (Jiang, Wang, Zhang, Lou, & Xing, 2010) (Dantas, Volpe, Durán, & Ferreira, 2012) are listed in the table 2. For the production of violacein, a plasmid (pVio) was constructed which contained violacein pathway gene cluster (vioABCDE) from C. violaceum ATCC 12472 (figure: 11).

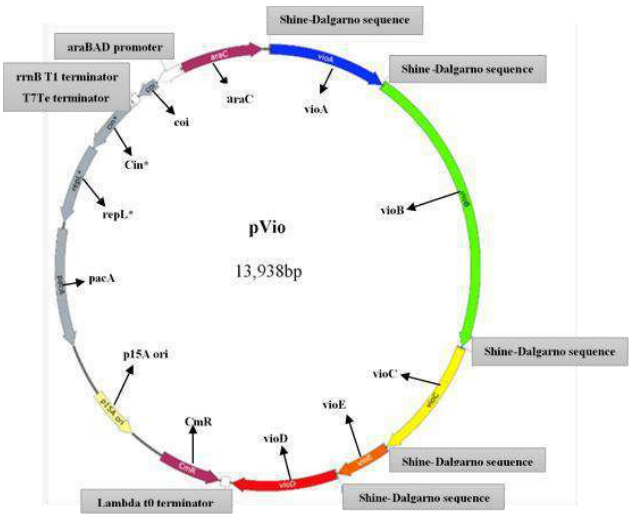

Fig.11: Plasmid (pVio) map or violacein biosynthesis

5.2 FEEDSTOCK FLEXIBILITY AND HETEROLOGOUS PRODUCTION OF VIOLACEIN BIOSYNTHESIS

V. natriegens having plasmid (pVio) was then tested in both minimal media and LBv2 rich media consisting of various carbon sources required for the biosynthesis of violacein and deoxyviolacein (by-product). UV-HPLC analysis showed that in rich media, *V. natriegens* produced 13.1 ± 0.9 mg/l and 24.9 ± 3.1 mg/l of violacein and deoxyviolacein respectively. It was observed that a higher amount of violacein is produced in mannitol followed by glucose, fructose and N-acetyl-glucosamine when absorbance is determined at OD_{600}. A similar amount of violacein is produced in both LBv2 rich media and minimal media with 15.5 mg/l mannitol. However, the deoxyviolacein to violacein ratio was very interesting. Rich media produced 1.9X more deoxyviolacein than violacein but, more violacein is produced as compared to deoxyviolacein i.e. by 4.2X in minimal media with different carbon sources. Another significant finding was the exclusion of minimal media + arabinose for violacein biosynthesis because the findings will most likely to be confuted due to induced arabinose synthesis by phagemid-elements present in pVio plasmid. Violacein production by transformant containing pVio plasmid is comparatively less as compared to other carbon sources due to the synthesis of these proteins or lysis of *V. natriengens* due to activation of prophages (Ellis et al., 2019).

6. VIOLACEIN HYPER-PRODUCTION FROM ENGINEERED CORYNEBACTERIUM GLUTAMICUM

Because of the numerous advantages of Corynebacterium glutamicum as a microbial cell factory, it is basically identified as safes as compared to *E. coli*. *C. glutamicum* has dominated the fermentation processes of industrial-scale to synthesize various amino acids and other products for food, animal feed, cosmetics and health (Pühler, Kalinowski, & Tauch, 2008). One of the well-developed hyper-producer of L-tryptophan is ATCC 21850 strain of C. glutamicum. Since L-trp is the direct precursor of crude violacein biosynthesis, this strain has provided an attractive platform. For violacein biosynthesis, L-tryptophan producing and wild type strains i.e. ATCC 13032 of *C. glutamicum* has been engineered as novel carcass (Heery, Fitzpatrick, & Dunican, 1994). Expression vectors of *E. coli*/ *C. glutamicum* shuttle (pEC-XK99E) (Jakoby, Krämer, & Burkovski, 1999) have provided a plasmid backbone for the construction of functional plasmids. For the construction of plasmid, *E. coli* DH5α was used using golden-gate DNA assembly process (Hillson, Rosengarten, & Keasling, 2012) and this plasmid was then transformed into C. glutamicum strains i.e. ATCC 21850 or ATCC 13032 (Tauch et al., 2002). As a metabolic engineering carcass, ATCC 21850 strain is used as a novel host as it produced 162.98 mg/l of L-tryptophan. In ATCC 21850 strain having key promoter, heterologous vio operon from *C. violaceum* was hyper-expressed to produce 532 mg/l of violacein. Keeping the violacein toxicity in consideration, inducible promoters were used while expression of vio operon and 629 mg/l of violacein was synthesized under batch culture conditions. The compressed RBS of vio genes were exchanged with whole strong C. glutamicum because of vio operon's economical coding nature. And synthetic operon was developed by assembling extended expression units. Using this techniques, 1116 mg/l of violacein was synthesized under batch culture conditions. Fermentation temperature, culture composition, induction time and induction concentration was studied to optimize the fermentation process and consequently, in 3L bioreactor, 47 mg/l/h of productivity and 5436 mg/l of titer was achieved (Sun, Zhao, Xiong, Zhang, & Bi, 2016).

7. INCREASED PRODUCTION OF CRUDE VIOLACEIN FROM *E. COLI*

7.1 VIA GLUCOSE ENGINEERED WITH INTERACTIVE CONTROL TRYPTOPHAN AND VIOLACEIN SYNTHETIC PATHWAY

Fang and his co-workers conducted an experiment to engineer *E. coli* to increase the production of crude violacein by controlling trp and violacein synthetic pathways via glucose. Firstly, strains were generated that have a multivariate module for differed throughputs of trp. This was achieved by overexpression of 2 vital genes from the upstream trp metabolic pathway i.e. $trpE^{fbr}$ /trpD genes along with conjugational knockout of 3 genes i.e. pheA/tnaA/trpR genes (fig. 12).

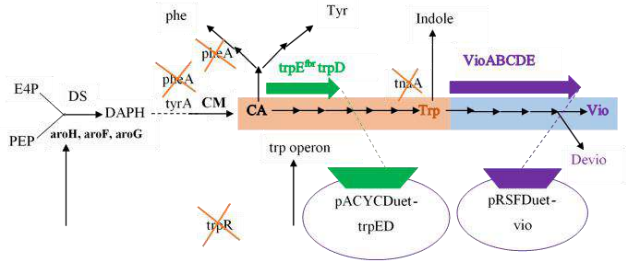

Fig.12: Plasmid construction and trp accumulation gene knockdown

To achieve this, trp metabolic pathway was thoroughly studied in C. glutamicum and *E. coli*. Pathway initiates with E4P and PEP condensation to form chorismate which is accompanied by trp synthesis via trp branch

pathway. There is also the presence of trp branches and phenylalanine which force chorismate to produce tyrosine and phenylalanine. Trp repression, attenuation and feedback inhibition controlled the flow of trp metabolic pathway (Ikeda, 2006). Hence, there are 2 possible ways of generation of trp-accumulating strains i.e. either by improving metabolic pathway flux increasing key enzyme's activity in the metabolic biosynthetic pathway of chorismate and trp operon or by deletion of genes associated with trp accretion inhibition from the chromosome by repressing trp degradation (tnaA), chorismate competition to produce aromatic amino acids (pheA) and trp operon transcription (trpR) (Zhao et al., 2011) (Gu, Yang, Kang, Wang, & Qi, 2012).

After the generation of trp biosynthetic pathway, the violacein synthetic gene cluster was instigated downstream. Maximum production of crude violacein directly via glucose was achieved in a cultural flask with a titer of 0.6 ± 0.01 g/l in *E. coli* B2/pED + pVio that was 3.98X more than control B1/pVio devoid of trp pathway upregulation i.e. 0.153 ± 0.005 g/l after collaborating these 2 pathways. The highest crude violacein productivity i.e. 36 mg/h/l and titer 1.75 g/l was evinced by recombinant *E. coli* B2/pED + pVio, which was 4.48X more than *C. freundii* (pCom10vio) (Xiao et al., 2011) when subjected to the same cultural conditions devoid of the addition of trp, using C-source glucose (glucose yield = 0.116 g-violacein/g-glucose and glucose consumption= 15.1 g/l) in a 5L bioreactor batch fermentation (Fang et al., 2015).

7.2 VIA GLUCOSE BY OVEREXPRESSING RATE-LIMITING VITAL ENZYMES

For the production of crude violacein using the economic industrial source of carbon, Zhou with his co-workers initiated the biosynthetic pathway of violacein in *E. coli* strain B8/pTRPH1, on which work had done in the previous study as described above to engineer this strain metabolically for trp accumulation via glucose. By using glucose as a carbon source in a medium, they were able to produce a higher amount of crude violacein of capacity 0.25 g/l/OD_{600}. They disclosed VioE enzyme to be the rate-limiting step biosynthesis of violacein by further over-expression of all of the 5 genes i.e. vioABCDE that were associated with the biosynthetic pathways of violacein. In a 5L bioreactor undergoing fed-batch fermentation, the crude violacein productivity 98.7 mg/l/h along with the titer of 4.45 g/l was produced using the optimal *E. coli* strain i.e. B8/pTRPH_1-pVio-VioE. It was revealed that this strain had produced the highest amount of crude violacein productivity and titer so far (Zhou, Fang, Li, Zhang, & Xing, 2018).

8. VIOLACEIN BIOSYNTHESIS BY ENGINEERING OLEAGINOUS YEAST YARROWIA LIPOLYTICA

In the food industry, *Y. lipolytica*, as a host, is extensively espoused for the production of β-ionone (Czajka et al., 2018), β-carotenoids (Larroude et al., 2018) (Gao et al., 2017) and citric acid (Fickers et al., 2005). It was found that both C. violacein and Y. lipolytica were collected from the marine surroundings that had high GC contents i.e. about 65%. Scientists argue that due to the GRAS status of the *Y. lipolytica*, it can provide a novel platform for the biosynthesis of violacein. In this study, the extraction of the violacein from yeast culture was optimized in order to improve the purity and recovery ratio of violacein from the culture by keeping in consideration incubation time, using cell wall degrading enzymes as the cell wall of Y. lipolytica is composed of thick polymer i.e. chitin having galactose and mannose (Liu, Ding, Sun, Boussetta, & Vorobiev, 2016), mechanical shear stress choice i.e. using glass beads and vortex (Jones et al., 2015) and organic solvent's variations i.e. methanol or ethyl acetate (Xu, Rizzoni, Sul, & Stephanopoulos, 2017). The quantitative relation between the microplate reader method and HPLC proved to be equivalent to measuring the production of violacein from the yeast culture. By using the extraction protocol, maximum production of violacein and deoxyviolacein was obtained in shake flasks i.e. 70.04 mg/l and 5.28 mg/l respectively. At 60 C/N ration with the incorporation of 10g/l of $CaCO_3$ in order to optimize the pH of the media, the purity of violacein reached 86.92% (Tong, Zhou, Zhang, & Xu, 2019).

9. VIOLACEIN BIOSYNTHESIS BY DUGANELLA VIOLACEINIGRA

In this study, Choi and his collaborators isolated a violacein biosynthetic new strain that was identified to be the relative of D. violaceinigra YIM 31327 on the basis of a phylogenetic analysis by using FAME (fatty acid methyl ester) analysis, vioA and gyrB gene sequences and 16S rRNA sequencing. Along with its isolation and identification, within the last few years, cloning of vioABCDE genes and its heterogeneous expression and fermentation for the biosynthesis of violacein had been reported (Rodrigues et al., 2012) (Rodrigues et al., 2013). This newly isolated strain had been identified as D. violaceinigra NI28 strain. Though the phylogenetic analysis showed similarity between these 2 strains, N128 strain showed a different phenotype than YIM 31327 strain, as it was able to grow 25% faster than YIM 31327 on nutrient media and was able to produce 45X more violacein at a higher rate (Li et al., 2004) (Kämpfer, Wellner, Lohse, Martin, & Lodders, 2012). N128 was

disclosed to be the best producer of violacein when was compared with *J. lividum* and other violacein producers. For example, at 24h, N128 strain produced a yield of 6.0 mg/OD of crude violacein, which was 2X more than other strains of D. violaceinigra (Choi, Kim, Lyuck, Kim, & Mitchell, 2015).

10. ANTI-MICROBIAL IMPACT OF VIOLACEIN ON STAPHYLOCOCCUS SPECIE

After high-level production of violacein as discussed above, Choi et al scrutinized the antibacterial activity of crude violacein on drug-tolerant Staphylococcus aureus. It was divulged that the minimum concentration of crude violacein to undergo inhibitory effect on S. aureus was 1.8 µM. However, 96% loss in the initial population of S. aureus was achieved by adding 30 µM of the crude violacein (Choi et al., 2015).

Similarly, in another study conducted by Dodou et al, the anti-microbial activity of violacein was determined on *S. epidermidis* and its symbiotic impact on the antibiotics. Excellent anti-microbial effect of violacein was revealed on both non-biofilm and biofilm-forming strains of S. epidermis i.e. ATCC 12228 and ATCC 35984 respectively. In short time period of exposure, both the bactericidal (for both strains, MBC = 20 µg/ml) and bacteriostatic effects (MIC = 10 µg/ml for ATCC 12228 and MIC = 20 µg/ml for ATCC 35984) were observed. After exposure to 2-3h, the bactericidal concentration of violacein led to the death of *S. epidermidis*. In addition, the violacein symbiotically optimized the action of various antimicrobial types on S. epidermidis strain ATCC 35984 (545%; n = 6) and strain ATCC 12228 (818%; n = 9), thus decreases the MIC up to 16X of these respective antibiotics (Dodou et al., 2017).

11. ANTI-CANCEROUS ACTIVITY INDUCED BY VIOLACEIN IN THE RESISTANT LEUKEMIA CELLS

Generally, it is recognized that the cancerous processes are intimately linked with the various modes of PCD (programmed cell death). But the problem is that there is not well-known PCD mechanism that is involved in the chemoprevention of cancer and it can differ between types of tumor cells involved and kinds of chemo-preventive agents. Thus, according to pharmacologists, it is quite vital to describe the candidate's cellular specificity along with its bypass dysfunctional tumoral signaling pathway's capability to provide insensitivity to the death stimulus during the initial steps of drug development. While studying the violacein's cytotoxic effect, it was disclosed that the death that was induced in the leukemia progenitor cells i.e. $CD34^+$/c-Kit^+/P-glycoprotein$^+$/$MRP1^+$ TF1 was not mediated by autophagy or apoptosis, as this compound did not significantly affect the biomarkers of both kinds of cell death. Working mechanisms of violacein were clarified by performing kinome profiling that used peptide arrays that determined the elaborated descriptions of activities of the cellular kinase. Activation of PDK, PKA and AKT along with the inhibition of DAPK1 and calpain carried out the pro-death activity of violacein that is accompanied the structural changes that are caused by Golgi apparatus collapse and ER stress, that lead to cellular quietus. The results declared that kinome reprogramming was induced by violacein that overcame death signaling affliction of tolerant leukemia cells (Queiroz et al., 2012).

II. CONCLUSION

This review is basically based on the study of the production of violacein and its derivatives from metabolically engineered microorganisms. Violacein being a secondary metabolite is found to have a high range of biological activities like anti-microbial activities and anti-cancerous activities. Due to these biological activities, scientists have developed an increased emphasis to study this compound and increasing the production via both wild-type strains and recombinant microbial strains. As discussed in this review, the production of violacein and its characterization can't be achieved without its struggles and obstacles and there is still more work that can be done. This, in particular, is based on the mode of action of violacein that needs to be studied in more in detail. The current tendency in the molecular genetic field has basically helped the researchers to genetically engineered the bacterial host that can undergo the overproduction of the violacein within the fermentation. A supplementary scheme in order to enhance the production abilities of genetically engineered strain should be kept in consideration including all the aspects of gene expression, optimization of bioprocessing and downstream processing of violacein and its derivative. Generally, bioprocess optimization for heterologous product formation entails the incrementation of metabolic abilities of engineered hot for the desired compound or product. The engineered hot of violacein and deoxyviolacein possess a high flux trp pathway enciphered in their genome that acts as a strong initiating point to obtain various high-value trp-bed therapeutic. In the future, violacein and its derivative will become readily accessible for clinical studies and the scientific community.

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Isolation and Characterization of Phylloplane Bacteria from Papaya Plant for the Biocontrol of post-harvest Diseases in Papaya

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Abstract**—** *Papaya is one of the most significant crops cultivated in tropical and subtropical countries all over the world. Post-harvest diseases are the major threat to papaya fruit yield leading to huge losses. This study focuses on controlling the fungal pathogens of papaya fruit by isolating the disease control bacteria from the phylloplane of the papaya plant and screening them for antagonism towards the pathogens. The fungal pathogens chosen for this study were Colletotrichum, Fusarium and Rhizopus. The three bacterial isolates showing the maximum diameter for the zone of inhibition against these pathogens were selected for morphological and biochemical characterization. In studies, the isolates were found to be Bacillus and Pseudomonas. Consortium study was conducted between Bacillus and Pseudomonas which showed more efficiency in controlling the growth of fungal pathogens when combined. Fruit assay was then performed to establish these bacterial isolates as biocontrol agents. Papaya fruits were inoculated with fungal pathogens and fungal pathogens along with bacterial isolates. It was observed that the papayas inoculated with bacterial isolates showed a better shelf life than those without. The present study reports the biocontrol ability of the bacteria which can be used as disease control agents.*

Keyword**— Biocontrol, papaya, post-harvest diseases, phylloplane, fungal pathogens.**

I. INTRODUCTION

Papaya, (*Carica papaya*), also called papaw or pawpaw, is a tropical and sub-tropical fruit that is classified under various plant families, including Passifloraceae, Cucurbitaceae, Bixaceae, and Papayaceae. Currently, it is placed under Caricaceae, a plant family incorporating species in four genera, *Carica*, *Cylicomorpha*, *Jacaratia* and *Jarilla* [1].

An additional aspect of the mechanisms of the pesticides through which they reduce plant growth is exhibited [2]. Thus, there is a need for new solutions to plant disease problems that provide effective control while minimizing negative consequences for human health and the environment [3]. Biological control, using microorganisms to suppress plant disease has offered a powerful alternative to the use of synthetic chemicals [4].

The production of phytopathogen inhibitor compounds by the biocontrol agents and their biocontrol potential was evaluated by measuring the production of these compounds, hydrolytic enzymes (amylases, lipases, proteases, and chitinases) and phosphate solubilisation [5]. It has been proven that induced resistance as an alternative for the control of postharvest diseases in fruit is effective in both the laboratory and a few cases in the field [6]. Biocontrol of diseases in plants is a difficult subject for understanding because these diseases mostly occur in the non-static environment such as the interface of the plant root and the aerial parts of plants [7].

II. REVIEW OF LITERATURE

Papaya is majorly cultivated in the region of tropics and sub-tropics. According to a report in 2004, this fruit was

produced over 6.8 million tonnes (Mt) worldwide, which is about 389,990 Ha [8]. Fungal plant pathogens are known to cause considerable post-harvest loss of fruit and vegetables [9]. Papaya is susceptible to more than a dozen fungal pathogens like Phytophthora rot (*Phytophthora palmivora*) root and fruit rot, anthracnose (*Collectricum gloerosporioides*), powdery mildew (*Oidium caricae*), Rhizopus rot (*Rhizopus stolonifer*) and black spot (*Asperisporium caricae*) are, however, the more important fungal pathogens [10]. Anthracnose, caused by *Colletotrichum gloeosporioides* (Penz.), primarily affects papaya fruit and is an important postharvest disease in most tropical and subtropical regions [11].

The widespread use of pesticides in agricultural settings, public health, commerce, and individual households throughout the world is an indication of the importance of these compounds [12]. The sources of these chemicals are houses, factories, water bodies etc which finds their applications in public spaces; home, garden, and lawn use and occupational association [13]. Due to the interest in public safety concerns, the exploitation of the integrated pest management aspect in being worked on [14].

Biocontrol microbes/micro-organisms are cellular or non-cellular entities, capable of replication or of transferring genetic material. The list of biocontrol agents included in CIB for registration is many [15]. The dual activity of *Pseudomonas* BCAs (i.e. direct antagonism of phytopathogens and induction of disease resistance in the host plant) further highlights their potential as plant protection products (PPPs) [16]. In current times, the focus is aimed at understanding, how *Pseudomonas* strains to act as efficient biological control agents. This approach of understanding the mechanism is helping the development of novel strains with enhanced modified traits for its increased biocontrol efficacy [17].

Elicitors, as a part of integrated pest management (IPM) approach, are usually used to induce resistance against postharvest diseases [18]. It has been proven that induced resistance as an alternative for the control of postharvest diseases in fruit is effective in both the laboratory and a few cases in the field [6]. The results of various studies confirmed the potential use of some essential oils for protection of fruits and vegetables against postharvest pathogens and for increasing the shelf life of plant products [19].

III. MATERIALS & METHODS

3.1. Isolation and screening of bacteria

Potent bacterial biocontrol agents were obtained from phylloplane of papaya.

5 different phylloplane samples were procured from the papaya growing fields in Bangalore.

Isolation of phylloplane bacteria was carried out by the leaf imprint method [20]. These plates were then incubated at 37˚C. The isolates obtained were maintained on nutrient agar plates.

Fig. I. Leaf Imprint method

3.2. Procurement of potent papaya fungal pathogens

Fungal pathogens were isolated from diseased papaya fruits which were collected from vegetable and fruit markets. The pathogens were isolated by direct plating on Potato Dextrose Agar. The plates were incubated at room temperature.

The following are the pathogens that were isolated from the diseased papaya.

1) *Colletotrichum* sp.
2) *Fusarium* (type 1)
3) *Fusarium* (type 2)
4) *Rhizopus*
5) *Penicillium*

3.3. Dual assay of phylloplane bacteria against papaya fungal pathogens

To test the antagonistic potential of each isolate, the pathogen and bacteria were inoculated 3 cm apart on potato dextrose agar plates. Fungal growth on each plate was observed and the zone of inhibition, if present, was determined. The treatments were replicated in triplicates for statistical validation. Results were expressed in terms of percentage inhibition which was calculated as per the given formula.

$$\% \text{ inhibition} = \frac{\text{Diameteroftheinhibitionzone}}{\text{Totaldiameter}} \text{ x } 100$$

3.4. Morphological characterization of the isolates

Morphological characteristics like cell and spore morphology, motility; growth characteristics (growth in the presence of NaCl 7%) were investigated [21]. Gram staining was performed by standard procedures.

3.5. Biochemical characterization of the isolates

Various biochemical tests were conducted based on Bergey's manual of systematic bacteriology and Manual of Microbiology Methods [22].

3.6. Evaluation of Individual and Consortium biocontrol potential

The individual biocontrol agents, as well as the consortium of biocontrol agents, were co-inoculated into tubes containing potato dextrose broth to evaluate and compare the potential of biocontrol capacity. This was conducted between the obtained isolates. The tubes were then kept for incubation at room temperature for a week. At the end of the incubation period, dry weight analysis was performed by filtering out the fungi on a filter paper and subjecting it to hot air oven mediated drying at 60℃ for 1 hour [23]. The dry weight of the fungi was taken and inhibition % was calculated.

3.7. Preliminary bioassay to evaluate disease control ability of the isolates

INVITRO BIOASSAY

Disease control potential of the isolates was checked on papaya fruits in vitro. Fruits were spot inoculated [24] and subjected to various treatments. The treatments were replicated in triplicates for statistical validation.

Table I. Various treatments with its abbreviations

ABBREVIATION	TREATMENT
T1	CONTROL
T2	*RHIZOPUS*
T3	IS-6 + *RHIZOPUS*
T4	IS-7 + *RHIZOPUS*
T5	*COLLETOTRICHUM*
T6	IS-6 + *COLLETOTRICHUM*
T7	IS-7 + *COLLETOTRICHUM*
T8	*FUSARIUM*
T9	IS-6 + *FUSARIUM*
T10	IS-7 + *FUSARIUM*

IV. RESULT & DISCUSSION

4.1. Dual Plate Assay

The bacterial isolates obtained from the phylloplane samples were subjected to screening using a dual plate assay method. 3 different isolates numbered IS1, IS6 and IS7 exhibited a good percentage of inhibition against the fungal pathogens and hence were chosen for further studies.

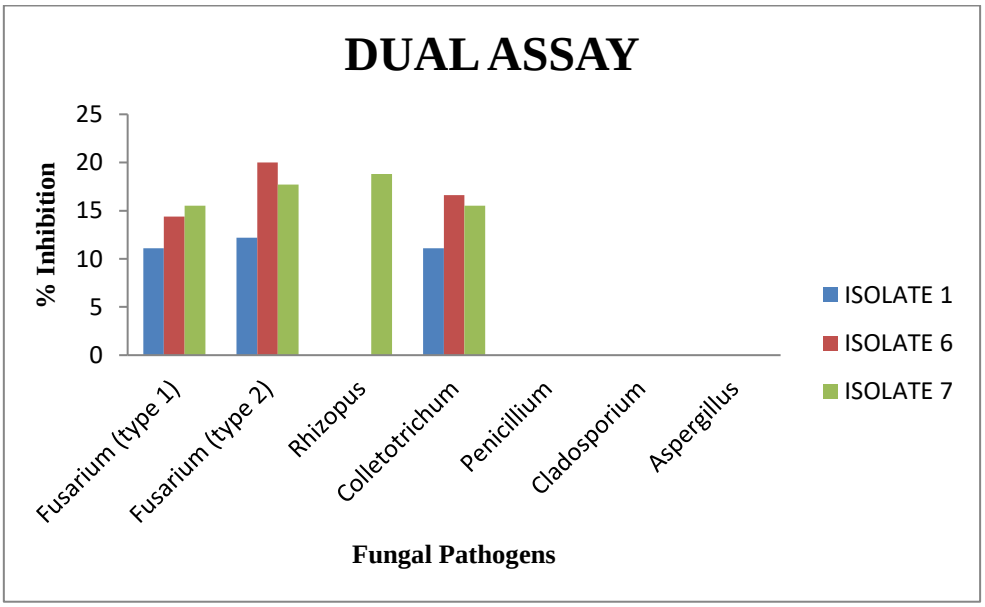

Fig. II. Dual assay of phylloplane bacteria against papaya fungal pathogens

4.2. Morphological and Biochemical characterization

Isolate 1 was found to be gram-positive rods; Isolate 6 was identified as gram-positive, spore-forming rods. The hanging drop method confirmed it to be motile rods. It was able to degrade starch and casein. It showed positive for catalase test, negative for citrate and gelatin liquefaction. It was able to grow well on a nutrient agar plate containing 7% NaCl. Isolate 7 was identified based on its colony colour on nutrient agar, its gram character, its inability to ferment sugars tested, MR, VP, catalase, oxidase, citrate and gelatin liquefaction tests.

Thus, IS6 was identified as *Bacillus* and IS7 was identified as *Pseudomonas*.

4.3. Evaluation of Individual and Consortium biocontrol potential

The consortia of IS6 and IS7 showed higher disease control potential in papaya fruit against the pathogens *Fusarium* (type 2) and *Colletotrichum*.

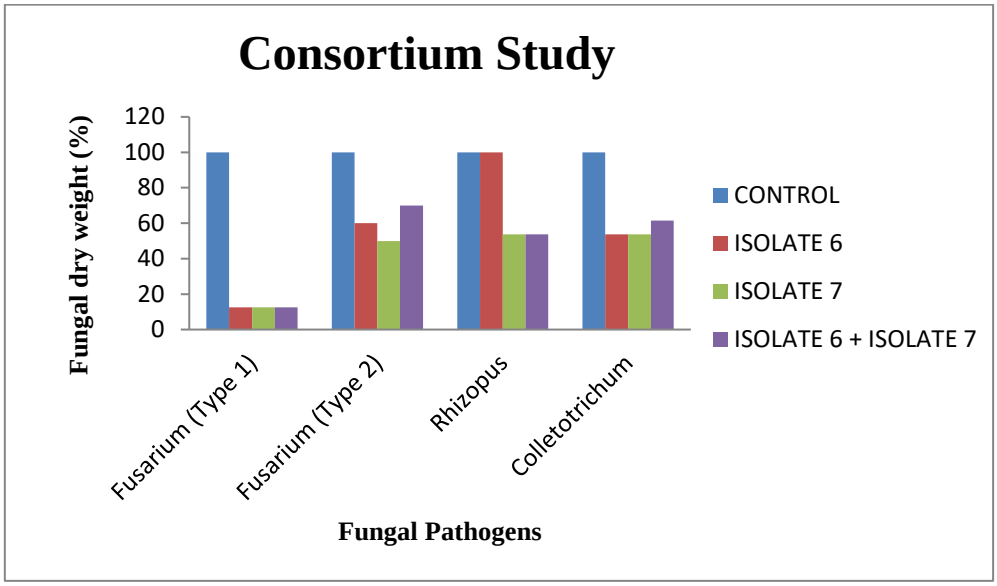

Fig. III. Evaluation of Individual and Consortium biocontrol potential

4.4. Invitro bioassay to determine the disease control potential of the isolates

Fruit assay conducted to assess the shelf life of the papaya fruits and disease control potential of the isolates under in vitro conditions showed that fruits treated with the isolates and the pathogen exhibited better shelf life and appeared fresh in comparison with the control and only pathogen inoculated fruit. The reason for the same could be bacterial-fungal antagonism where the disease control bacterial isolates might produce antifungal metabolites or modify the environment such that the fungal pathogens are unable to grow. Fruits co-inoculated with IS – 7 and the pathogens showed the maximum freshness. This shows the significant biocontrol ability of post-harvest diseases of the phylloplane bacterial isolates.

Table II. Invitro bioassay to determine the disease control potential of the isolates

TREATMENTS	INFECTION PERCENTAGE (%)
T1	22.2 ± 1.0
T2	100 ± 1.2
T3	100 ± 1.2
T4	11.1 ± 0.7
T5	100 ± 1.2
T6	0
T7	0
T8	100 ± 1.2
T9	11.1 ± 0.7
T10	0

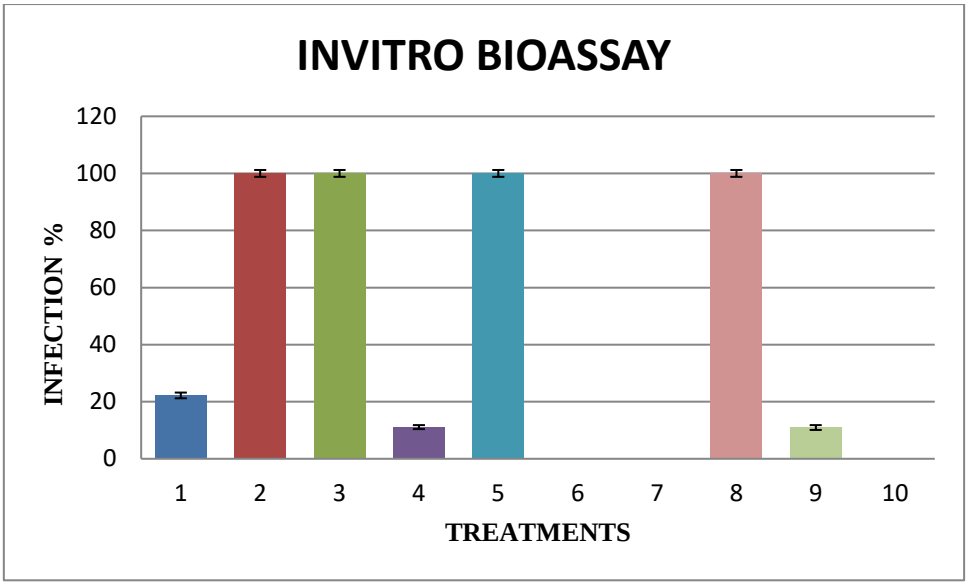

Fig. IV. Invitro bioassay to determine the disease control potential of the isolates

Fig. V. Control (papaya fruits without any treatment)

Fig. VI. Papaya fruits inoculated with Rhizopus

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Fig. VII. Papaya fruits inoculated with Rhizopus and Isolate 6

Fig. VIII. Papaya fruits inoculated with Rhizopus and Isolate 7

Fig. IX. Control (papaya fruits with any treatment)

Fig. X. Papaya fruits inoculated with Colletotrichum

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Fig. XI. Papaya fruits inoculated with Colletotrichum and Isolate 6

Fig. XII. Papayas fruits inoculated with Colletotrichum and Isolate 7

Fig. XIII. Control (papaya fruits with any treatment)

Fig. XIV. Papaya fruits inoculated with Fusarium

Fig. XV. Papaya fruits inoculated with Fusarium and Isolate 6

Fig. XVII. Papaya fruits inoculated with Fusarium and Isolate 7

V. CONCLUSION

From the studies conducted it is observed that bacterial isolates from the phylloplane have the ability to control fungal pathogen growth in papaya fruits. Out of 20 isolates studied, it can be concluded that IS 6 and IS 7 had maximum inhibitory activity and increased shelf life of the papaya fruits. These two isolates were found to be Gram-positive rods with endospores and Gram-negative rods, respectively. The maximum inhibition was seen against *Fusarium*. By the Dual assay test, it was revealed that maximum antagonistic ability was revealed by IS – 6 and IS – 7. The papaya fruits treated with IS – 6 and are – 7 showed better shelf life and appeared fresh. The isolates were morphologically and biochemically characterized and identified as *Bacillus* and *Pseudomonas* respectively. Thus it can be concluded that *Bacillus* and *Pseudomonas* obtained from the phylloplane of the papaya plant act as potential biocontrol agents against various post-harvest diseases of papaya.

VI. FUTURE SCOPE

- Pathogenicity testing of the potential biocontrol agents
- Mode of action of the biocontrol agents
- Formulation studies
- Field studies
- Extension of post-harvest biocontrol potential to other fruits and vegetables

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Assessment of Caecal Microbiome in two Breeds of Rabbits Fed Fermented Cocoa Podhusk Meal

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Abstract**—** *The study was conducted to determine the effect of inclusion of fermented cocoa pod husk meal in feed of rabbits on the microbial flora in the caecum.Sixty 5 weeks (35 days) old grower rabbits comprising 2 breeds (thirty New Zealand White and thirty Chinchilla) were used for the study. They were randomly distributed into six treatments of 10 rabbits per group and fed diets containing fermented cocoa pod husk meal (FCPHM) at 0%, 12.5% and 25% inclusion levels for a period of 8 weeks. Contents of the caecumof experimental rabbits were collected at the end of the feeding trial for analysis of bacterial counts/load, identification and characterization of the bacteria organisms. The results showed that the different breeds of rabbit and varying inclusion levels of FCPHM had significant influence (P<0.05) on the bacterial and coliform counts in the caecum. The average caecal bacterial and coliform population increased as the inclusion levels of FCPHM increased with rabbit fed control diet having 56.88 × 10^2cfu/ml; 7.38 × 10^2cfu/ml; those fed 12.5% FCPH having 87.82 × 10^2cfu/ml; 10.63 × 10^2cfu/ml and those fed 25% FCPHM having 101.63 × 10^2cfu/ml; 15.25 × 10^2cfu/ml bacteria and coliform counts respectively. The breed effect also showed that New Zealand White had higher bacterial and coliform counts of 93.67 × 10^2cfu/ml and 13.58 × 10^2cfu/ml when compared to Chinchilla with 70.58 ×10^2cfu/ml and 8.56 × 10^2cfu/ml.Only Salmonella and Bacillus species of bacteria were commonly isolated from the rabbits fed the three dietary treatments. It can be concluded that the different breeds of rabbit and inclusion of FCPHM in their diets significantly altered the composition and population of caecal microbiota.*

Keywords**— *Caecum, Cocoa pod husk, Microbial flora, Rabbits.***

I. INTRODUCTION

In Nigeria presently the demand for animal protein is far higher than the supply, thus for quick increased supply of animal protein and products, it is necessary that animals with short generation intervals be reared. One of the domestic animals with short generation interval considered in this study is the domestic rabbit (*Oryctolaguscuniculus*).

In terms of commercial production, the rabbit excels other livestock animals like cattle, sheep and goat and ranks close to broiler chicken in terms of growth rate, feed conversion efficiency and meat quantity(Adegbola*et al*., 1986). Nutritionally, rabbit meat is considered the healthiest meat option containing a higher protein (20-21%) and lower fat content (10-11%) when compared with meat from other species (Ajayi*et al*., 2007). Furthermore, Janieri (2003) had reported that rabbit meat has the cholesterol value of 169mg/100g (dry matter basis) when compared with beef (200mg), chicken (220mg), and low sodium content. Consequently, rabbit meat has been listed in United State

Department of Agriculture (USDA) as an approved meat source for hypertensive patients.

However, as reported by Ozor and Madukwe (2005) nutrition and housing are some of the constraining factors in the adoption of improved rabbit technologies by small-scale farmers, with similar observations being made by Oseni, *et al*. (2008) in western Nigeria.Therefore, the resurgence of interest in rabbit production in Nigeria calls for research into alternative sources of energy and protein yielding ingredients to replace or supplement the expensive conventional cereal grains and legumes. The prices of such conventional protein and energy feed ingredients such as maize, rice, sorghum, ground nut, soybean have escalated over time that it is becoming uneconomical to use them in rabbit feed (Esonu*et al*., 2004; Oduguwa*et al*., 2004).Animal nutritionists have therefore advocated for the use of agro-industrial by-products as unconventional feedstuffs because they are cheaper and available in large quantities in producing countries.

Several crops and their by-products have potential as possible alternatives for livestock feed industry. One such crop is cocoa, a very abundant crop in tropical regions of Africa and its by-products have been successfully used as alternative feedstuff in livestock production (Makinde et al., 2019). Cocoa pod husk, cocoa bean shell and cocoa bean meal form over 70% (w/w) of a whole matured fruit of cocoa (*Theobroma cacao* L.), and these are the major agro-industrial by-products from cocoa processing industries and are usually considered as "waste" and left to rot on the cocoa plantation.

Diet plays an important role in modulating gut microbiome by providing food substrates forgut microorganisms (Conlon and Bird, 2014; Kim *et al*., 2015). Furthermore, several studies demonstrated that a close relationship exists between gut microfloraand health of host. The role of indigenous microorganisms includesboth a protection against pathogens (the barrier effect) and a strong implication in thedevelopment and maturation of digestive mucosa immunity. Also the maintenance of guthealth is complex and relies on a delicate balance between the mucosa (including theabsorptive epithelium and the digestive immune system), the commensal microflora andenvironmental factors including diet(Fortun-Lamothe and Boullier, 2004).

Hence this study was carried out to determine the effect of inclusion of CPH meal in diets on caecal microbiome and inadvertently the health status of two breeds of rabbits.

II. MATERIALS AND METHOD

Experimental site

The feeding trial of the experiment was carried out at the Livestock section (Rabbit unit) of the Teaching and Research Farm and laboratory analysis in Microbiology Laboratory of the Department of Animal Production and Health both of The Federal University of Technology, Akure, Nigeria.

Collection and Fermentation of Cocoa Pod Husk

The cocoa pod husks (CPH) were collected from cocoa plantations in Idanre and Ondo towns, Nigeria. The pods were cleaned with sterile water, chopped, sun-dried, milled and analyzed for proximate composition. The milled cocoa pod husk (CPH) was then subjected to solid state fermentation using *Rhizopusstolonifer* to reduce the theobromine and fibre contents prior to its usage. The fermentation process was carried out by dissolving ten (10) grams of urea in 100 litres of water which was used to moisten the CPH meal. One litre of the prepared inoculums of the starter culture of *Rhizopusstolonifer*was used to inoculate the urea treated CPH meal and kept in a tray incubating chamber to initiate the fermentation process. The fermentation of the cocoa pod husk meal was terminated on the 14^{th} day followed by sun drying of the substrates for 5 - 7 days to inactivate the microorganisms. The dried CPH meal was subsequently kept in air-tight plastic container while a sample was taken for post-fermentation proximate analysis.

Experimental Animals and Arrangement

Sixty 5 weeks (35 days) old grower rabbits comprising thirty New Zealand White breed and thirty Chinchilla breed were purchased from a reputable farm in Ogun State, weighed individually and grouped into treatments after balancing for weights and penned individually in their hutches using completely randomized design for eight experimental weeks. There were ten replicates per treatment with one rabbit per replicate. Weekly weights and feed intake of each rabbit were measured.

Experimental Diets

Three experimental diets were formulated to meet the nutritional requirements of the grower rabbits in which fermented cocoa pod husk meal (FCPHM) was incorporated into the feed as the test ingredient at varied levels of 0.0, 12.5 and 25.0% which were designated as Diet I (control), Diet II and Diet III respectively. The animals were provided feed and water *ad libitum* throughout the eight-week experimental period. The gross composition of the experimental diets for the rabbits is presented in Table 1.

Table 1: Gross composition of the experimental diets for rabbits.

Ingredients	Diet I	Diet II	Diet III
	0.0%	12.5%	25.0%
Maize	18.40	21.10	19.40
GNC	8.70	7.80	4.60
PKC	25.50	10.80	7.40
SBM	4.50	4.30	3.10
Wheat offal	6.90	3.40	0.40
GNH	34.20	38.50	38.50
FCPH	**0.00**	**12.50**	**25.00**
Vegetable Oil	0.80	0.80	1.10
Lysine	0.10	0.10	0.10
Methionine	0.10	0.10	0.10
Limestone	0.40	0.30	0.10
Premix	0.20	0.15	0.10
Salt	0.20	0.15	0.10
Total	100.00	100.00	100.00
Calculated Composition			
Dry Matter, DM (%)	90.15	89.87	89.29
Metabolisable Energy, ME (Kcal/kg)	2505.89	2506.44	2501.95
Crude Protein, CP (%)	15.92	15.95	15.68
Crude Fiber, CF (%)	15.64	15.05	15.04
Calcium, Ca (%)	0.94	0.93	0.82
Av. Phosphorus, P (%)	0.69	0.64	0.59
Lysine (%)	0.87	0.88	0.79
Methionine (%)	0.60	0.59	0.59
Analysed Composition			
Dry Matter, DM (%)	88.30	88.90	87.86
Crude Protein, CP (%)	16.38	16.47	16.01
Crude Fiber, CF (%)	15.62	15.07	14.95
Ash (%)	4.51	7.33	7.18
Ether Extract (%)	6.32	5.14	5.06
Nitrogen-Free Extracts (%)	45.47	44.89	44.66

SBM = Soybean meal, PKC = Palm kernel cake, GNC = Groundnut cake, GNH =Groundnut husk, FCPH = Fermented cocoa pod husk.

Sample Collection

Two rabbits were taken from each dietary treatment groupand humanely slaughtered. The animals were dissected and the gastrointestinal tract was located, then was removed. Samples of the caecal content for microbial analysis were collected in a sterile manner from approximately 3 cm from the ileocaecal junction. The following analysis were carried out on the samples collected.

Microbiological Analysis

Bacterial isolation and determination of total viable counts

A portion of each sample (1g) taken from the caecum of experimental rabbits was added into test tubes containing sterile distilled water (9ml) and was thoroughly mixed to serve as stock. Four fold serial dilutions ($10^{-1}$to 10^{40}) of the stock was done using 1ml stock homogenate and 9 mls sterile distilled water in order to obtain discrete colonies (Moshood*et al.*, 2012). The media (Nutrient Agar) used was prepared from commercially dehydrated products and reconstituted according to the manufacturer's directives, sterilized and allowed to cool. 1ml each of the serially diluted sample was dropped at the centre of a Petri-dish followed by pouring of the nutrient agar using the pour plate method as described by Mumtaz*et al.* (1986). It was allowed to solidify for some minutes and then incubated at 37 °C for 24 hours. The colonies that emerged were counted and calculation for the colony forming units were expressed as log cfu/ml using the formula as described by Rukayya*et al.* (2016).

Identification and characterization of bacterial isolates

The bacterial colonies that developed on the nutrient agar plates were sub-cultured by streaking on freshly prepared nutrient agar plates and MacConkey agar plates until pure colonies were obtained according to the conventional procedure as highlighted by (Fawole and Oso, 2001).

Then isolates were characterized and identified based on their morphological and cultural characteristics including shape, size, pigmentation, elevation and marginal characteristics of the colony and Gram staining. Then a series of biochemical reactions which include oxidase test, catalase test and coagulase test were done. Sugar fermentation assay and indomethyl red tests were also carried out as stated by Olutiola*et al.* (1999).

Statistical Analysis

All data collected were subjected to two – way analysis of variance (ANOVA) using Statistical Package for Social Sciences (SPSS, version 23) and where significant differences were found, the means were separated using Duncan Multiple Range Test of the same statistical package.

Table 2: Bacterial and Coliform counts isolated from caecum of New Zealand White and Chinchilla rabbits fed diets containing FCPHM

Factors		Total Bacterial Count ($\times10^2$cfu/ml)	Total Coliform Count ($\times10^2$cfu/ml)
Breed	NZW	93.67[a]	13.58[a]
	CHL	70.58[b]	8.56[b]
	SEM	7.18	1.15
	P-value	0.036	0.006
FCPHM	Control	56.88[b]	7.38[b]
Inclusion levels	12.5%	87.82[a]	10.63[b]
	25%	101.63[a]	15.25[a]
	SEM	8.80	1.41
	P-value	0.006	0.003
Interaction Effect (Breed X FCPHM Inclusion Level)			
NZW	Control	55.25	9.25
	12.5%	101.25	15.50
	25%	124.50	16.00
CHL	Control	58.50	5.50

12.5%	74.50	5.75
25%	78.75	14.50
SEM	12.44	1.99
P-value	0.168	0.129

CHL=Chinchilla; NZW=New Zealand White, FCPHM=Fermented Cocoa Pod Husk Meal; cfu = Colony formed unit.

III. RESULTS

Bacterial and Coliform Counts

Tables 2 shows the total viable bacterial and coliform counts obtained from samples in the ceacum of the New Zealand White and Chinchilla rabbits fed the different experimental diets. It revealed that the breed effect had significant (P<0.05) influence on the bacteria and coliform (P<0.01) counts. The chinchilla rabbit had fewer bacterial (70.58 × 10^2cfu/mi) and coliform (8.58 × 10^2cfu/ml) counts than the New Zealand White rabbits. The dietary inclusions of FCPHM also had significant effect (P<0.05) on the bacterial and coliform counts and the least count was recorded in the control group (56.88 × 10^2cfu/ml) followed by the group fed diet containing 12.5% FCPHM (87.88 × 102 cfu/ml) and then the 25% FCPHM group (101× 10^2cfu/ml) . The interaction effect between the breed and varying dietary treatment factors had no significant effect (P>0.05) on both bacterial and coliform counts.

Percentage distribution of bacteria organisms isolated from caecum of experimental rabbits

The varying diets significantly (P<0.05) affected the abundance and richness of certain types of bacteria detected in the experimental rabbits.

Detailed percentages of the bacteria organisms isolated from the caecum of the Chinchilla rabbits fed the different levels of dietary FCPHM is shown in Figures 1A-C. Only*Salmonella spp.* and *Bacillus cereus* were commonly isolated from the rabbits fed the three dietary treatments while *Aerobacter cloacae, Enterobacter spp*and *Klebsiellaspp*were further isolated from the control group; *Aerobacter cloacae, Enterobacter spp* and *Bacillus subtilis* were isolated from the 12.5% FCPHM group while*Streptococcus faecalis, Proteus spp, Bacillus subtilis* and *Klebsiellaspp* were further isolated from the 25% FCPHM group.

Figures 2A-Cshows that *Klebsiellaspp*, *Salmonella typhi* and *Bacillus cereus* were present in the caecum of the New Zealand White rabbits in all the treatment groups. *Staphylococcus epidermidis, Enterobacter spp* and *Bacillus subtilis* were also found in rabbits fed the control diet and the 12.5% FCPHM supplemented diet while*Proteus spp* and *Staphylococcus epidermidis* were further isolated from those fed 25%FCPHM supplemented diet.

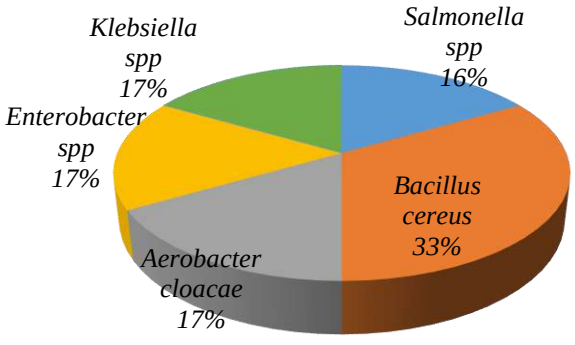

Fig. 1A : Percentage distribution of bacteria isolated from caecum of Chinchilla rabbits fed with control diet

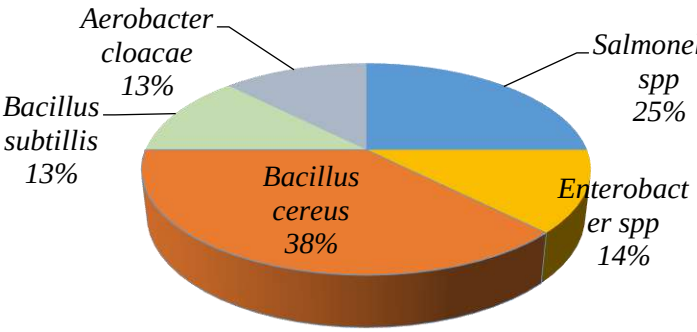

Fig. 1B : Percentage distribution of bacteria isolated from caecum of Chinchilla rabbits fed diet containing 12.5% FCPHM

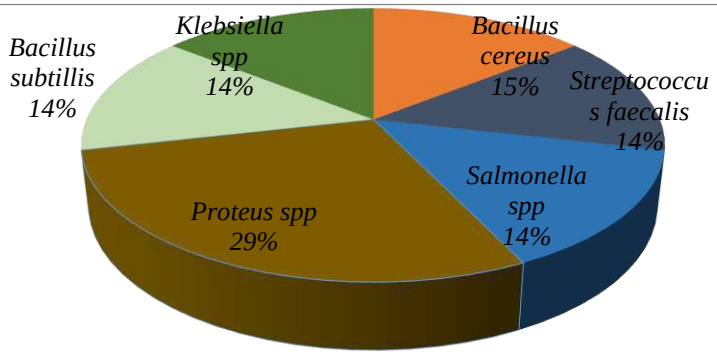

Fig. 1C : Percentage distribution of bacteria isolated from caecum of Chinchilla rabbits fed diet containing 25% FCPHM

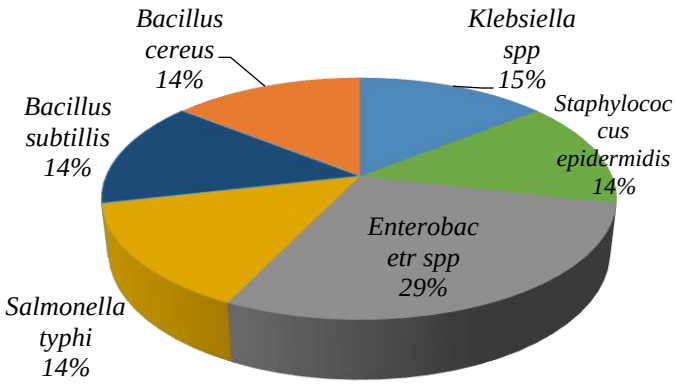

Fig. 2A : Percentage distribution of bacteria isolated from caecum of Newzealand Rabbits fed control diet

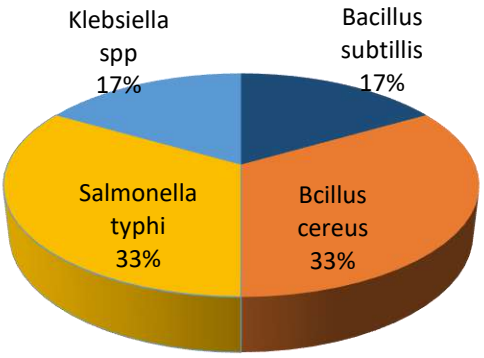

Fig. 2B : Percentage distribution of bacteria isolated from caecum of Newzealand Rabbits fed diet containing 12.5% FCPHM

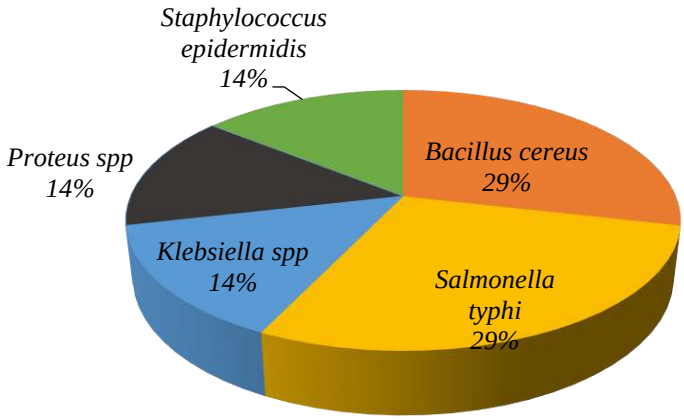

Fig.2C: Percentage distribution of bacteria isolated from caecum of Newzealand Rabbits fed diet containing 25% FCPHM

IV. DISCUSSION

The rabbit enteric microbiota plays a key role in maintaining rabbit health, including helping to digest forage-based diet and aiding in immune system regulation and development (Kylie, 2016).

This present study revealed the presence of a wide variety of bacteria in the caecum of the experimental rabbits.Earlier speculationhas been made that rabbit caeca contain a large proportion of undescribed bacterial species (Michelland*et al*., 2010) and this suggested that the composition of these novel species may vary between individuals which was further buttressed by the report of North *et al*. (2019).In this study for the overall bacterial communities, the bulk of the bacterial population across breeds and diets were observed to be *Bacillus*, *Salmonella*,*Klebsiella*, *Enterobacter*, *Staphyloccusspp*, *Aerobacter*and *Proteus.* However, a high percentage of reads across samples were assigned to *Bacillus species* and *Salmonella* organisms which suggests that the richness of certain bacteria was significantly affected by diet as opined by Zhu *et al*. (2015).

It was noted that the bacteria *Lactobacillus* genus was not isolated from the gut of experimental rabbits which is in line with report by Fortun-Lamothe and Boullier (2004) that the absence of the genus *Lactobacillus* in the rabbit flora is original. Also Zhu *et al*. (2015) reiterated the absence of the genus *Lactobacillus* in the rabbit flora as unique and being in accordance with previous data obtained by Yu and Tsen (1993) with culture-based methods. Penney *et al*. (1986) previously hypothesized that this is due to highly acidic environment in the GIT of adult rabbits.

Also, this present study revealed the absence of *Escherichia coli* in the gut of adult rabbits and this is similar to reports of

large-scale studies that the cultivable fraction of rabbit digestive microbiota in healthy adults is characterizedby the absence or low density of *Escherichiacoli* (Yu andTsen 1993; Pupo*et al*. 1997). Fortun-Lamothe and Boullier (2004) also made similar observations that *Escherichia coli* reached a maximum level at the 2nd or 3rd week of life and then decreased to be *residual*or absent after weaning in the gut of rabbits.

It was further discovered in this present study that the microbial abundance varied with the different nutritional treatments and breeds of rabbits. This may be due to the fact that an important fraction of the diets enters the caecumas substrates for microbial fermentation and a change indiet composition can modify the nature of the digestato be fermented in the caecum and, consequently, canaffect microbiota composition and activity as suggested by Jehl andGidenne(1996). Previously, a study by BogoneVantus*et al*. (2014) reported that microbes present in the gastrointestinal tract are a direct function of the nutrition of the rabbits. This suggests that the FCPHM inclusion in the diets of the experimental rabbits aided the proliferation of different types of bacteria organisms. This is further buttressed by the work of Michelland*et al*. (2011) which demonstrated that the bacterial communities of therabbit caecum change and adapt rapidly to reach a newequilibrium in response to nutrition.However, in a particular study, the impact of dietary composition on gut microbiota was not reported (Massip*et al*. 2012).

Also, in line with this present study where a variety of bacterial organisms was found in the caecum of the experimental rabbits, Combes *et al.* (2013) asserted that an abundant bacterial community is present throughout the caecum-colon and in the hard and soft faeces (10^{10} – 10^{12} bacteria/bag) of rabbits.

The diverse and abundant bacterial population reported in the caecum of experimental rabbits probably as a result of inclusion of FCPHM in diet at varying levels may influence their general performance and health. This is because studies havesuggested that the composition and the activity of the caecal microbiota could have a strong influence on health, because of its role in nutrition, pathogenesis and immune function as manifested by hydrolysis of plant fibers and cell walls by bacterial enzymes, which is not possible by host animal digestive enzymes as opined by Gibson and Roberfroid (1995).

The effect of breed significantly influenced the total counts of bacteria isolated from the experimental rabbits. The effect of the varying dietary treatments were also pronounced on the bacterial and coliform counts whereas the combined effects of breed and dietary treatments were not pronounced on the bacterial and coliform counts.

The bacteria and coliform counts in caecum of New Zealand White rabbits were found to be more than that of Chinchilla rabbits which can be attributed to breed effect, while the rabbits fed with varying levels of FCPHM were found to have higher counts of bacteria compared with those fed the control diet without FCPHM. The result also showed that the higher the level of inclusion of FCPHM the higher the bacteria and coliform counts recorded. This may be as a result of the highfibre content in the diet with the highestinclusion level of FCPH meal which is in line with reports of previous studies that in animals fed a high-fiber diet, bacteria were found in the highest abundance compared with animals fed diets with lower fiber content (Gidenne and Bellier 2000; Gidenne and Fortun-Lamothe 2002). Similarly, Gidenne*et al*. (2004) suggested that the total bacterial biomass production was 3-fold higher for rabbits fed a high fiber/starch ratio. Though, Zhu *et al*. (2015) suggested that an unbalanced diet withexcessive fiber or starch reduces microbial richness and diversity. Earlier studies had also reported that dietary starch/fibre was long thought to be a factor that predisposed rabbits to the development of undesirable microbiota (Cheeke and Patton. 1980).

The low coliform bacteria counts relative to the total bacteria counts ratio in the FCPH meal fed rabbits suggested that the caecum was predominantly colonized by non-pathogenic bacteria and toxic substances of pathogens were inhibited by gut beneficial bacteria as earlier hypothesized by Phuoc and Jamikorn (2017). It may also be due to the fact that acetic acid produced by resident bacteriaduring fermentation process is able to penetrate into bacterial cytoplasm resulting in a reduced internal bacterial pH and collapse of the electrochemical proton gradient which leads to bacteriostasis and death of susceptible bacteria such as caecal coliforms (Eklund, 1989).

Overall, this large bacterial community in the gut influences the overall health status of the rabbits as well as imparts on the digestion process in the animals. This is ascertainedbecause short-chain fatty acids (acetate, butyrate, and propionate) which are metabolic end products from bacteria such as those present in the gut of the experimental rabbits are pivotal in several host physiological functions, such as nutrient acquisition, immunity, cell signalling, proliferation control, and pathogen protection as reported earlier by Tremaroli and Bäckhed(2012).

V. CONCLUSION

The inclusion of fermented cocoa pod husk meal (FCPHM) in feed of New Zealand and Chinchilla breeds of rabbit enhanced number of gut beneficial bacteria populations which could improve caecal fermentation and ultimately lead to better gut health and growth performance. In addition, caecal coliform population were reduced in the experimental rabbits. Also, the varying diets caused the proliferation of different types of bacteria in the gut.

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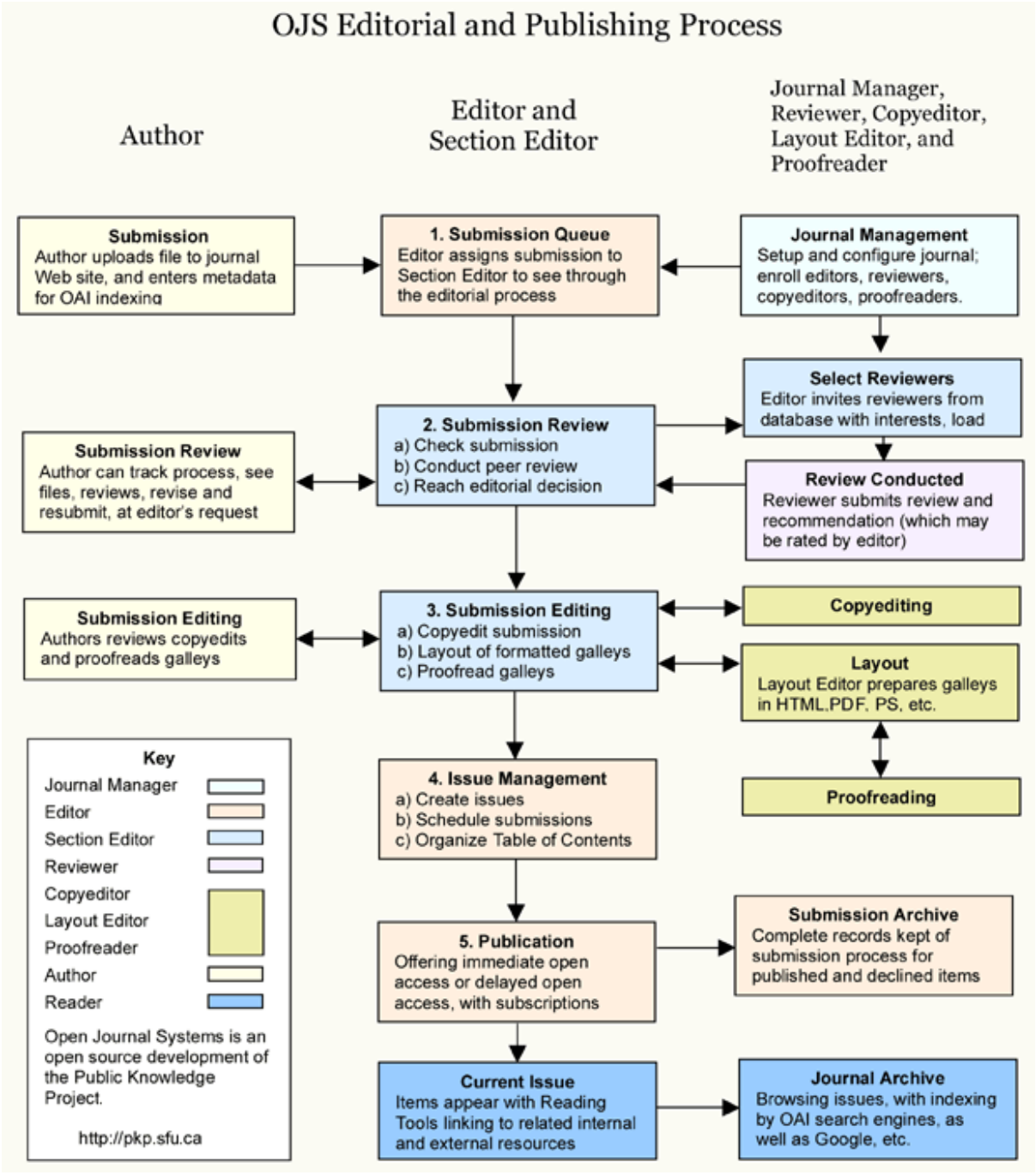

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