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Preface

We would like to present, with great pleasure, the inaugural volume-8, Issue-7, July 2022, of a scholarly journal, *International Journal of Environmental & Agriculture Research*. This journal is part of the AD Publications series *in the field of Environmental & Agriculture Research Development*, and is devoted to the gamut of Environmental & Agriculture issues, from theoretical aspects to application-dependent studies and the validation of emerging technologies.

This journal was envisioned and founded to represent the growing needs of Environmental & Agriculture as an emerging and increasingly vital field, now widely recognized as an integral part of scientific and technical investigations. Its mission is to become a voice of the Environmental & Agriculture community, addressing researchers and practitioners in below areas.

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Environmental science and regulation, Ecotoxicology, Environmental health issues, Atmosphere and climate, Terrestric ecosystems, Aquatic ecosystems, Energy and environment, Marine research, Biodiversity, Pharmaceuticals in the environment, Genetically modified organisms, Biotechnology, Risk assessment, Environment society, Agricultural engineering, Animal science, Agronomy, including plant science, theoretical production ecology, horticulture, plant, breeding, plant fertilization, soil science and all field related to Environmental Research.

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Agriculture, Biological engineering, including genetic engineering, microbiology, Environmental impacts of agriculture, forestry, Food science, Husbandry, Irrigation and water management, Land use, Waste management and all fields related to Agriculture.

Each article in this issue provides an example of a concrete industrial application or a case study of the presented methodology to amplify the impact of the contribution. We are very thankful to everybody within that community who supported the idea of creating a new Research with *IJOEAR*. We are certain that this issue will be followed by many others, reporting new developments in the Environment and Agriculture Research Science field. This issue would not have been possible without the great support of the Reviewer, Editorial Board members and also with our Advisory Board Members, and we would like to express our sincere thanks to all of them. We would also like to express our gratitude to the editorial staff of AD Publications, who supported us at every stage of the project. It is our hope that this fine collection of articles will be a valuable resource for *IJOEAR* readers and will stimulate further research into the vibrant area of Environmental & Agriculture Research.

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Study of some Chemical Properties of Ultisols Soils Based on the Existence of Earth Worms

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Abstract—*The purpose of the study was to determine some of the chemical properties of the soil on Ultisols inhabited and uninhabited by earthworm populations.*

The research was carried out from April to June 2019, taking soil samples on mixed garden land in the Bukit Pinang Urban Village, Samarinda and soil analysis at the Soil Science Laboratory, Faculty of Agriculture, Mulawarman University, Samarinda, East Kalimantan Province.

The research activities are: field observations, determining the location of soil sampling, soil sampling, collection and calculation of earthworms, and analysis of soil chemical properties in the laboratory.

The data collected were: population density of earthworms, and some soil chemical properties: soil pH C-organic, N-total, P-available, and K-available.

The results showed that Ultisols inhabited by worm populations had several soil chemical properties (soil pH, C-organic, N-total, P-available and K-available) which were higher than Ultisols soils that were not inhabited by earthworms.

Keywords—Soil Chemical Properties, Earthworms, Ultisols.

I. INTRODUCTION

Soil is part of the natural body that covers the earth with a thin layer, is synthesized in the form of a profile from weathered rocks and minerals, and decomposes organic matter which then provides water and nutrients that are useful for plant growth. According to [1] that soil fertility provides an overview not only of the types of nutrients but also the amount of nutrients available in the soil. Earthworms are organisms that are indicators of soil fertility that play an important role in improving soil productivity.

Earthworms play a very important role in maintaining soil fertility physically, chemically and biologically. Physically, earthworms play a role in mixing coarse or fine organic matter between the top and bottom layers so that their distribution is more even. This activity also causes the formation of a stable and loose soil structure, better aeration, smoother water infiltration thereby reducing erosion [2] .

Earthworms are a group of macrofauna that play an important role in various physical, chemical or biological processes of soil [3]. Earthworms are one of the most important organisms in the soil because they can mix various layers of the soil and introduce carbon in the form of organic matter into the soil. It is in this mixing process that organic matter spreads throughout the soil and provides the nutrients that plants need. In addition, earthworms also spread microorganisms in their intestines which increase the biological population in the soil. These worms will improve the physical, chemical and biological properties of the soil which acts as a soil conditioner [4].

The presence of earthworms is an indicator of the health of a soil, because through the activities of these earthworms it can improve the physical and chemical properties of the soil [5]. Earthworm droppings contain nitrogen, phosphorus, potassium, magnesium and calcium which are essential for plant growth [6].

II. RESEARCH METHOD

2.1 Time and place

The research was carried out from April to June 2019, taking soil samples on mixed garden land in Bukit Pinang Urban Village, Samarinda and soil analysis at the Soil Science Laboratory, Faculty of Agriculture, Mulawarman University, Samarinda, East Kalimantan Province.

2.2 Materials and tools

The materials used are: soil samples and materials for soil analysis. The tools used are: hoes, machetes, shovels, sieves, tape measure, glass bottles, buckets, sacks, gloves, tweezers, cameras, laboratory equipment, and stationery.

2.3 Approach Method

The method used in this research is descriptive and comprehensive.

2.4 Research Implementation

The research implementation activities were as follows: (1) conducting field observations, (2) determining the location of soil sampling, namely 3 plots not found earthworms (a1, a2 and a3) and 3 plots found earthworms (b1, b2, and b3), (3) soil sampling (including plot making, sampling) (4) collection and calculation of earthworms, analysis of soil chemical properties in the laboratory.

2.5 Data retrieval

The data collected are: (1) population density of earthworms, (2) several chemical properties of the soil: soil pH C-organic, N-total, P-available, and K-available.

III. RESULTS AND DISCUSSION

3.1 General Condition of Research Site

The research location is a mixed garden area consisting of fruit trees such as lai, durian, banana, orange, salak, rambutan, manga, rambai, breadfruit, coconut, guava and manga; Some of the land is also planted with corn, taro, bamboo, rubber, cassava and part of the land is overgrown with weeds.

3.2 Earthworm Population

The results of observations of earthworm populations in predetermined sample plots are presented in detail in Table 1.

TABLE 1
EARTHWORM POPULATION STATUS

No	Soil Sample Location	Amount (earthworm)	Population Density (earthworm/cm$^{2)}$
1	There are no earthworms (a1)	0	0,00
2	There are no earthworms (a2)	0	0,00
3	There are no earthworms (a3)	0	0,00
4	There are earthworms (b1)	6	0,00192
5	There are earthworms (b2)	3	0,00120
6	There are earthworms (b3)	5	0,00160

Source : Primary Data Processed

The results of the study in Table 1 show that at the location of soil sampling (b1, b2, and b3) earthworms were found in a row, namely 6, 3, and tail or with a population density of consecutive 0,00192 earthworms cm^{-2}; 0,00120 earthworms cm^{-2}; and 0,00160 earthworms cm^{-2}.

3.3 Soil Chemical Properties

The results of the analysis of several soil properties in plots without and with earthworm populations in detail are presented in Table 2.

TABLE 2
SOME SOIL PROPERTIES IN PLOTS WITHOUT AND WITH EARTHWORM POPULATION

No	Soil Sampling Location	Soil pH	C-Organic (%)	N-Total (%)	P Available (ppm)	K Available (ppm)
1	There are no earthworms (a1)	5,20 (M)	0,45 (SR)	0,21 (S)	15,25 (R)	97,22 (ST)
2	There are no earthworms (a2)	5,50 (M)	0,51 (SR)	0,13 (R)	9,82 (SR)	77,78 (ST)
3	There are no earthworms (a3)	5,30 (M)	0,66 (SR)	0,23 (S)	1,64 (SR)	72,22 (ST)
4	There are earthworms (b1)	6,12 (AM)	4,98 (T)	0,38 (S)	23,45 (S)	116,67 (ST)
5	There are earthworms (b2)	5,92 (AM)	3,77 (T)	0,36 (S)	13,45 (R)	108,33 (ST)
6	There are earthworms (b3)	6,70 (N)	3,41 (T)	0,41 (S)	19,82 (S)	80,56 (ST)

Source: Primary Data Processed

Note: M = acid; AM = slightly acid; N = neutral; SR = very low; R = low; S = medium; T = high; dan ST = very high.

3.3.1 Soil pH

The results of the study in Table 2 show that the soil pH in the soil without earthworms (a1, a2, and a3) was 5.20; 5.50; and 5.30 (status classified as sour), while the plots containing earthworms (b1, b2, and b3) were 6.12; 5.92; and 6.70 (status classified as slightly sour to neutral). This situation indicates that the soil found in earthworms has a higher soil pH value than the soil that is not inhabited by earthworms. It was explained by [7] that earthworms play a role in mixing coarse or fine organic matter between the top and bottom layers so that their distribution is more even. Furthermore, it is stated by [8] that when organic matter is decomposed it will produce ions OH^- which can neutralize the ionic activity H+. Organic acids will also bind Al^{+++} and Fe^{++} which can form complex compounds (chelates), so that Al^{+++} and Fe^{++} not hydrolyzed again.

3.3.2 Content of C-Organic

The results in Table 2 show that the C-Organic content of the soil in the soil without earthworms (a1, a2, and a3) was 0.45%, respectively; 0.51%; and 0.66% (very low status), while in plots with earthworms (b1, b2, and b3) 4.98% respectively; 3.77%; and 3.41% (high status). The increased C-organic content was caused by earthworms being able to decompose and mix organic matter into the soil from plant remains that fell on the soil surface. It was stated by [9] that earthworms and other soil organisms play a major role in the destruction of organic matter. Added by [10] that the distribution of organic matter in influencing the earthworm population, because it is related to the source of nutrition, so that in soils that have low organic matter content, there is little or no earthworm population found.

3.3.3 Content of N-Total, P-Available and K-Available

The results in Table 2 show that the soil N-Total content in the soil without earthworms (a1, a2, and a3) was 0.21%, respectively; 0.13%; and 0.23% (low to moderate status), while in the soil that contained earthworms (b1, b2, and b3) that was 0.38%, respectively; 0.36%; and 0.41% (medium status).

The results in Table 2 show that the P-Available soil content in the soil without earthworms (a1, a2, and a3) is 15.25 ppm, respectively; 9.82 ppm; and 1.64 ppm (status classified as very low to low), while in the soil that contained earthworms (b1, b2, and b3), they were 23.45 ppm, respectively; 13.45 ppm and 19.82 ppm (low to moderate status).

The results in Table 2 show that the K-Available soil content in the soil without earthworms (a1, a2, and a3) is 97.22 ppm, respectively; 77.78 ppm; and 72.22 ppm (very high status), while the plots containing earthworms (b1, b2, and b3) were 116.67 ppm, respectively; 108.33 ppm and 80.56 ppm (status is very high). The results showed that the content of N-total, available P and available K in the soil that was inhabited by earthworms was higher than the content of N, P and K in the soil without an earthworm population. This is because earthworms can change the physical and chemical properties of the soil, facilitate the process of mineralization of organic matter, and stabilize the nutrient cycle. As stated by [1] that the activity of earthworms can increase inorganic N in the soil. [11] stated that the presence of earthworms causes accelerated mineralization of organic matter which causes C-organic and total N to increase, C/N ratio decreases, P-available and K-available content increases. Furthermore, it was stated [12] that the activity of earthworms increased the availability of soil

nutrients and increased the rate of nutrient cycling. All of these contribute to changes in the form of bound organic N, P and K to forms available to plants and shortening the nutrient supply period.

IV. CONCLUSIONS AND SUGGESTIONS

4.1 Conclusion

Based on the results of research and discussion, it can be concluded that Ultisols inhabited by worm populations have several soil chemical properties (soil pH, C-organic, N-total, P-available and K-available) which are higher than Ultisols soils that are not inhabited by earthworms.

4.2 Suggestion

It is necessary to carry out similar research on soils with different vegetation accompanied by observations of more complete physical and chemical properties as well as the number and types of earthworms.

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Effect of Avian Viscera Meal on the Carcas Quality and Histology of *Clarias gariepinus* Fingerlings

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Abstract—

Background and objectives: *the use of alternative animal protein sources in aquaculture feeds has become a research priority. This research was therefore conducted to investigate the Carcass quality (body composition) of Clarias gariepinus post-fingerlings fed avian viscera and to examine the effect of viscera meal on the histological characteristics of the experimental fish.*

Materials and Methods: *A total of ninety (90) Post-fingerlings fish of Clarias gariepinus were procured and transported in a 50-litre plastic container from the farm to Fisheries and Aquaculture Departmental farm complex in Ebonyi state University Abakaliki, CAS-campus. The feed (Aller-aqua) was procured from the dealers shop and was used to feed the experimental fish as the control diet. The avian viscera were bought from the popular Chicken market (New layout) at Abakaliki. The experimental design used was Completely Randomize Design (CRD). The experimental fish were randomly assigned to three (3) treatments and three replicates each. The samples (carcass) were analyzed chemically according to the official methods of analysis described by the Association of Official Analytical Chemist. Results were subjected to one way analysis of variance (ANOVA) using the Statistical package for social sciences (SPSS version 20) to determine the differences between the various treatments and control. Duncan Multiple Range Test was used to separate the difference among the means and the differences were considered significant at ($p<0.05$).Growth and food utilization data were collected for twenty four weeks. Values were expressed as means ± SE.*

Results: *In this study, although the different groups of experimental fish had similar initial mean weight and were fed with fresh, steamed avian viscera and formulated extruded diets, Clarias gariepinus showed significant different ($P<0.05$) in final weight gain, specific growth rate, feed intake, feed conversion ratio and protein efficiency ratio. Higher substitution of chicken viscera meal resulted in reduced growth performance, feed utilization efficiency and production. The histological results of the photomicrography of liver and heart of Clarias gariepinus post-fingerlings fed fresh avian visceral showed, normal hepatic architecture with central vein (CV) in liver. However there were no inflammation and no fatty change. For the heart, it indicated normal cardiac architecture with the three layers the endocardium (ED), Ectocardium (EC) and Myocardium (MYO). Where the liver of the fish fed steamed avian visceral showed moderate damage on the hepatic tissue with focal aggregate of inflammatory cell (FAIC) and clumping of the hepatocytes (CH). The result of heart of fish fed with steamed viscera shows mild effected cardiac tissue with mild distortion of the cardiac muscles (MDCM) at the endocardia region.*

Conclusion: *the study clearly demonstrated that chicken viscera has a good nutritive value for the growth of African catfish and could be incorporated or used whole to Clarias gariepinus as meal without adverse effects on growth, feed utilization and body protein content. The contribution of this work to the scientific knowledge is to reduce waste from the environment which has been a challenge to poultry farmers from the abattoirs. Therefore, it is recommended, that the use of chicken viscera could substantially reduce feed cost and increase profits.*

Keywords—alternative animal proteins, Clarias gariepinus, histology studies.

I. INTRODUCTION

Fisheries and Aquaculture remain important source of food, nutrition, income and livelihoods for hundreds of millions of people around the world. Fish continues to be one of the most traded food commodities world-wide with more than half of fish exports by value originating in developing countries (FAO, 2016). The most important fish species used in fish farming are: Carp, Tilapia, and Catfish (FAO, 2014). China provides 62% of the world's farmed fish. As of 2016, more than 50% of seafood was produced by aquaculture (FAO, 2016). *Clarias gariepinus* (African Catfish) is one of the most important tropical freshwater fish species for aquaculture because of its high fecundity rate, acceptance of a wide range of natural and artificial foods, fast growth, and tolerance to high stocking density and environmental extremes (Dada and Wonah, 2003).

Chicken viscera are considered among probable protein sources for enhancing fish if established by researchers. Viscera are the large organs inside the body: such as the heart, lungs, intestine and stomach. Research findings has revealed that certain chicken viscera organs such as heart contain over 80% protein of excellent quality while traditional fish meal normally contain 60 to 80% high quality protein. Nevertheless, the decrease in supply of stocks and increasing demand for aquaculture as well as degradation of natural fish populations has greatly increased the market price of fishmeal (Kristsanapuntu and Chaitanawisuti, 2015; Naylor *et al*., 2000; Tacon and Metian, 2008). Moreover, high level of fishmeal in aquatic feeds may cause a series of environmental problems due to the high content of phosphorus.

Therefore, the use of alternative animal protein sources in aquaculture feeds has become a research priority. In that way, some studies have evaluated some plant proteins as alternative sources of fishmeal in carnivorous diets because of their low price, consistent nutrient composition and supply (Amaya *et al.,* 2007). However, findings showed poorer digestibility and adverse effects on growth performance due to their high carbohydrate content and imbalanced amino acid composition (Lunger *et al*., 2007). Compared to plant-based proteins, chicken viscera is easily available at very low price throughout the year and have high protein value, low carbohydrate content, balanced amino acids profile, total digestible dry matter and lack of anti-nutritional factors (Goda *et al.,* 2007; Bhaska *et al.,* 2014). Hence, chicken viscera are considered a probable substitute for fish meal in diets for a number of fish species (Bhaska *et al.,* 2015 and Gupta *et al.,* 2013).

Recycling of poultry wastes into an acceptable source of animal protein in the catfish diet is a big challenge in the pursuit of sustained production. Nowadays, studies concerning the evaluation of poultry viscera meal in the *C. gariepinus* fingerlings diets are few. Therefore, efforts have long been directed to find alternate protein sources of good quality which are less expensive and readily available as substitutes for fish meal component in practical diets Shiba *et al*. (2010)**.** Fishmeal has been replaced by single animal protein sources such as maggot meal (Adewolu, 2001), black soldier fly pupae meal (St-Hilaire *et al.,* 2007), poultry by-product meal (Turker *et al.,* 2005), poultry viscera meal (Usman *et al.,* 2007), and feather meal (Hasan *et al.,* 1997). Most of these single animal protein sources were unable to completely replace fishmeal (Adewolu *et al*., 2010). The aim of this research was to investigate the Carcass quality (body composition) of *Clarias gariepinus* post-fingerlings fed avian viscera and to examine the effect of viscera meal on the histological characteristics of the experimental fish

II. MATERIAL AND METHODS

2.1 Study Area

The experiment was carried out in the Fisheries and Aquaculture Departmental farm, Ebonyi State University, Abakaliki. Ebonyi state is situated approximately within latitude 6°20'N and longitude 8°06'E in the derived savannah of south-Eastern part of Nigeria at an elevation of 117m above sea level. The rainfall pattern is bimodal (April-July and September-November) with a short spell in August referred to as August break and annual rainfall of about 1,800-2,000mm. The average temperature is between 25°C in January, 34°C in June and 30°C in November (Ude, 2011). It has a land mass of approximately 5935 square kilometers and it is bounded in the East by Cross River State, in the North by Benue State, West by Enugu State and South by Abia State (Nwakpu, 2004).

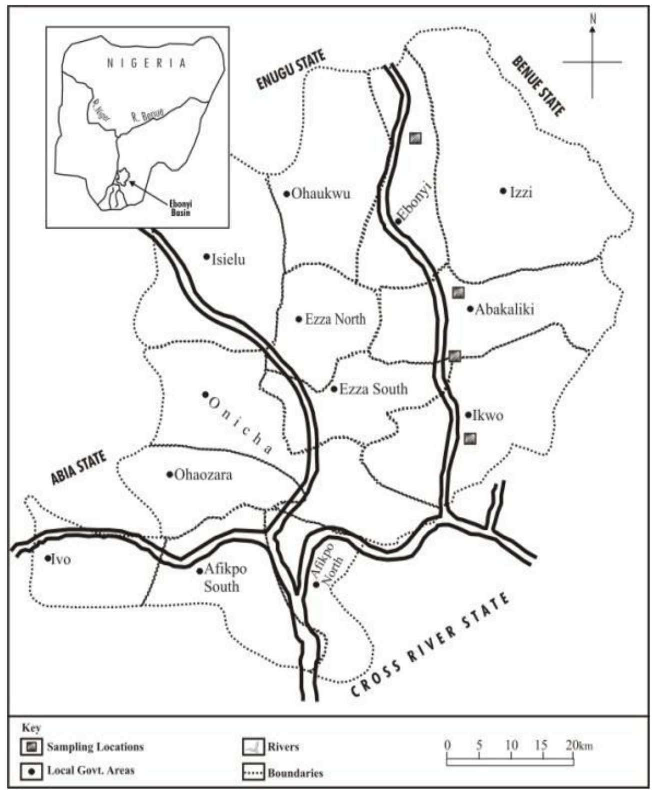

FIGURE 1: Map of Ebonyi showing Abakaliki

Source: Ude (2011)

2.2 Procurement of Experimental fish /Acclimation

The experimental fish was purchased from commercial fish farms (Regina Pacis fish farms), in Abakaliki. A total of ninety (90) Post-fingerlings fish of *Clarias gariepinus* were procured and transported in a 50-litre plastic container which was cut open at the top in well oxygenated clean water from the farm to Fisheries and Aquaculture Departmental farm complex in Ebonyi state University Abakaliki, CAS-campus. On arrival, the post-fingerlings were transferred gently into a large holding tank and allowed to stay in the tank for 14 days to acclimate. During this period of acclimatization, the post-fingerlings were fed two times daily using commercial feed. At the end of the acclimation period, the experimental fish were distributed randomly in groups of ten (10) fish into nine (9) concrete tanks each. A departmental sensitive electronic scale was used to determine the weight of the (10) fish that was assigned to each concrete tank. The avian viscera were washed and stored frozen at (20 ^{0}C). The by- products were thawed, then a portion was heated to steam and left to cool, while the other portion was the fresh one A total of ten (10) fish were weighed stocked in each of the experimental ponds measuring 1 m^3. Nine concrete ponds were cleaned very well with sponge and sodium chloride. They were filled with clean water. Every two weeks, the fish were weighed and their growth was determined. The quantity of feed was adjusted accordingly in line with the new weight. Each group was cut into tiny pieces) and was given to the experimental fish at 5% body weight daily for the period of 12 weeks. The experiment lasted for the period of 90 days (12 weeks).

2.3 Procurement of feed/preparation of avian viscera

The feed (Aller-aqua) was procured from the dealers shop and was used to feed the experimental fish as the control diet. The avian viscera were bought from the popular Chicken market (New layout) at Abakaliki, where large numbers of broiler bird are processed for human consumption on daily basis. These viscerals when gathered were processed (removal of the fecal waste) and preserved in deep freezer. The aim was to maintain freshness. The viscera collected were divided into parts; one part was used fresh to feed the fish while the second part was steamed before use. The viscera were chopped with sharp kitchen knife to reduce it to very small sizes to enable the experimental fish to pick them.

III. EXPERIMENTAL DESIGN

The experimental design used was Completely Randomize Design (CRD). The experimental fish were randomly assigned to three (3) treatments and three replicates each. A total of nine (9) concrete tanks were used with an area of one squared meter (1 m^3) each. The tanks were covered with netting material to prevent any predator from entering the tanks and 10 meters of the net were used. A hand net was also used during sampling to scoop the fish from the tanks into a bowl for weight determination. The fish in the experimental tanks were fed twice daily: between 8:00 and 8:30 a.m. and between 5:00 and 5:30 p.m. at 5% body weight per day.

3.1 Methods of Carcass Quality Analysis

The samples (carcass) were analyzed chemically according to the official methods of analysis described by the Association of Official Analytical Chemist (A.O.A.C, 2005). All analysis was carried out in duplicate.

3.2 Crude Protein Determination

The crude protein in the sample were determined by the routine semi-micro Kjeldahl, procedure/technique. This consists of three techniques of analysis namely Digestion, Distillation and Titration. Apparatus: Analytical Balance, Digestion tubes, Digestion Block Heaters, 50ml Burette, 5ml Pipette, 10ml Pipette, 10ml Measuring Cylinder, 100ml Beakers, Fume Cupboard. Reagents: Conc.H_2SO_4, 0.01NHCL, 40% (W/V) NaOH, 2% Boric Acid Solution, Methyl Red – Bromo cresol green mixed indicator, Kjeldahl Catalyst tablet.

3.3 Crude Fat or Ether Extract Determination

Apparatus: Soxhlet apparatus and accessories, oven, desiccators and analytical balance. Reagents: Petroleum spirit or Ether (40^0C – 60^0C b.pt). **Determination:** 1gm of each dried sample was weighed into fat free extraction thimble and plug lightly with cotton wool. The thimble was placed in the extractor and fitted up with reflux condenser and a 250ml soxhlet flask which has been previously dried in the oven, cooled in the desiccators and weighed. The soxhlet flask is then filled to 3/4 of its volume with petroleum ether (b.pt. 40 ºC to 60 ºC), and the soxhlet flask. Extractor plus condenser set was placed on the heater. The heater was put on for six hours with constant running water from the tap for condensation of ether vapor. The set is constantly watched for ether leaks and the heat source is adjusted appropriately for the ether to boil gently. The Ether is left to siphon over several times say over at least 10 to 12 times until it is short of siphoning. It is after this is noticed that any ether content of the extractor is carefully drained into the ether stock bottle. The thimble containing sample is then removed and dried on a clock glass on the bench top. The extractor, flask and condenser are replaced and the distillation continues until the flask is practically dry. The flask which now contains the fat or oil is detached, its exterior cleaned and dried to a constant weight in the oven. If the initial weight of dry sox let flask is Wo and the final weight of oven dried flask + oil/fat is W1, percentage fat/oil is obtained by the formula:

$$\frac{W1 - Wo \times 100}{Wt.\ of\ Sample\ 1}$$

3.4 Dry Matter and Moisture Determination

Apparatus: Oven, crucibles, desiccators and balance. Reagents: Silica gel, grease. Determination: 2g of the sample was weighed into a previously weighed crucible. The crucible plus sample taken was then transferred into the oven set at 100C to dry to a constant weight for 24 hours overnight. At the end of the 24 hours, the crucible plus sample was removed from the oven and transferred to desiccators, cooled for ten minutes and weighed.

If the weight of empty crucible is W_O

Weight of crucible plus sample is W_1

Weight of crucible plus oven-dried sample W_3

$$(\%\ DM)\ \%\ Dry\ Matter = \frac{W3 - WO}{W1 - Wo} \times \frac{100}{1}$$

$$\%\ Moisture = \frac{W1 - W3}{W1 - Wo} \times \frac{100}{1}$$

Or % Moisture = 100 – % DM.

3.5 Determination of Ash

Apparatus: Porcelain Crucibles, desiccators, Analytical Balances and a Furnace. **Determination:** 2.0gm of the sample were weighed into a porcelain crucible. This was transferred into the muffle furnace set at 550°C and left for about 4hours. About this time it had turned to white ash. The crucible and its content were cooled to about 100°C in air, then room temperature in desiccators and weighed. This was done in duplicate. The percentage ash was calculated from the formula below:

Ash content = wt. of ash x 100 original wt. of sample

3.6 Fibre Determination

Apparatus: Heating mantle, crucibles, furnace, sieve cloth, fibre flask, funnel, analytical weighing balance, a desiccators. Reagents: 0.255N H_2SO_4, 0.313N NaOH and Acetone. **Determination**: 2.0gmof the sample was accurately into the fibre flask and 100ml of 0.255N H2SO4 added. The mixture was heated under reflux for 1 hour with the heating mantle. The hot mixture was filtered through a fibre sieve cloth. The filtrate obtained was thrown off and the residue was returned to the fibre flask to which 100ml of (0.313N NaOH) was added and heated under reflux for another 1 hour. The mixture was filtered through a fibre sieve cloth and 10ml of acetone added to dissolve any organic constituent. The residue was washed with about 50ml hot water on the sieve cloth before it was finally transferred into the crucible. The crucible and the residue were oven-dried at 105°C overnight to drive off moisture. The oven-dried crucible containing the residue was cooled in desiccators and later weighed to obtain the weight W1. The crucible with weight W1 was transferred to the muffle furnace for Ashing at 55^0C for 4 hours. The crucible containing white or grey ash (free of carbonaceous material) was cooled in the desiccators and weight to obtain W2. The difference W1 – W2 gives the weight of fibre. The percentage fibre was obtained by the formula:

$$\% \text{ Fibre} = \frac{W1 - W2 \times 100}{\text{Wt of sample}}$$

3.7 Nitrogen-Free Extract (NFE) or Carbohydrate by Difference Determination

The NFE determined by difference. This was done by subtracting SUM of (Moisture % + % Crude Protein + % Ether Extract + % Crude Fibre + % Ash) from 100 i.e. (100 – (% M + % CP + % EE + % CF + % Ash) **7**

3.8 Measurement/ monitoring of Water Quality Parameters

TABLE 1
THE WATER QUALITY WERE DETERMINED USING THE METHOD OF AMERICA PUBLIC HEALTH ASSOCIATION

S/N	Parameters	Methods
1	Oxygen mg/l	Dip and Read oxygen meter
2	PH	Dip and Read pH meter
3	Temperature (°C)	Dip and Read thermometer
4	Nitrite (mg/l)	Titration
5	Nitrate (mg/l)	Titration

Source: (APHA, 2000)

3.9 Measurement of Temperature

Temperature was determined by dipping the bulb of mercury in glass measured in degree centigrade dry bulb thermometer for 3 - 5 minutes, to allow the mercury movement stabilizes. An average of three readings gave the temperature for each sample.

3.10 Determination of Dissolved Oxygen

Dissolved oxygen was determined by titration methods. Water testing kit for dissolved oxygen (Fresh Innovative Multitec water analysis test kit) produced by NIFFR, New Bussa, Niger State, Nigeria was used to titrate with the indicator chemicals and matching the color changes with reference papers.

3.11 Determination of Hydrogen Ion Concentration (pH)

Hydrogen ion concentration of the water parameter was determined using titration method with (P^H) water Testing kit (Fresh Innovative Multitec water analysis test kit) produced by NIFFR, New Bussa, Niger State, Nigeria by titrating with indicator chemicals and matching color changes with reference paper.

3.12 Determination of Ammonia, Nitrate and Nitrite

Ammonia, nitrate and nitrite were determined by the use of Ammonia Water Testing Kit (Kochi-682024), produced by Nice Chemicals pvt. L t d P.B N0 2217, Manimala, Road, Edapply, India, through titration methods with the indicator chemicals and matching color changes with reference paper.

3.13 Determination of Total Hardness

Hardness was determined by the use of Water Testing Kit (Fresh Innovative Multitec water analysis test kit) produced by NIFFR, New Bussa, Niger State, Nigeria through titration methods with the indicator Chemicals and matching color changes with reference paper.

3.14 Determination of Water Quality Parameters

Water quality parameters including temperature, dissolved oxygen, pH, nitrite and nitrate were monitored during the study to ensure that their optimal ranges are maintained throughout the experimental period. The dissolved oxygen level (DO) was measured in milligram per litre (mg/l) using dip and read oxygen meter. The meter was dipped in water and allowed for about 20-30 seconds for the reading to stabilize before it was read and recorded. The dip and read pH meter was employed in monitoring water pH throughout the experimental period. This was done by dipping the meter in the water for 10-15 seconds before the value was read and recorded. The water temperature was monitored with dip and read thermometer. The mercury in bulb thermometer was used. The thermometer was dipped in water for about 2-3 minutes for the mercury to stabilize before it was read and recorded. Nitrate and nitrite were among the water parameters monitored during the study, using titration method and comparing the changes in color with the color chart provided in test kits.

3.15 Measurement of Fish growth parameters/Growth Indices

Fish were weighed biweekly and data recorded. From the growth data obtained, specific growth rate (SGR), food conversion ratio (FCR) Protein efficiency ratio (PER) and percentage body weight (BWG) were calculated as follows, according to Ogunji *et al.* (2008c).

FCR=food fed/live weight gain

SGR= $(\text{In } W_2\text{-In } W_1/T_2\text{-}T_1) \text{ x } 100$

PER=weight gain/crude protein fed

BWG= $(\%) = (W_2\text{-}W_1/W_1) \times 100$

Where:

W_2= Final weight of fish

W_1= Initial weight of fish

T_1 and T_2= time (day)

TABLE 2
DETERMINATION OF GROWTH PERFORMANCE AND NUTRIENT UTILIZATION INDICES

S/N	Parameter	Methods
1	Mean Weight Gain	WF-WI/N
2	Relative Growth Rate	Weight Gain x100%/Initial body weight
3	Specific Growth Rate	InW2-InWI x100%/T
4	Feed Conversion Ratio	Feed Intake/Fish Weight Gain
5	Protein Efficiency Ratio	Weight Gain /Protein Intake

3.16 Histopathology Analysis

Mouth part of sacrificed fish was dissected open to collect the liver, and heart for histological analysis. The organs collected were fixed in 10% formalin to avoid autolysis and preserve cells in conditions identical to that during life.

Procedure

Histological method of processing tissue: this involves various ways of preparing, cutting, staining, and examination slides for histological report.

Stages in tissue processing:

1. Fixation: the tissue was fixed in 10% formal saline in a container with light fitting lids for 3 day to prevent autolysis; improve staining quality and to aid optical differentiation of cell.
2. Dehydration: The tissue was dehydrated to remove water that is not miscible with xylene and wax using different grades of alcohol ranging from 50% –absolute Alcohol for 30mins each
3. Clearing /Dealcoholization: the dehydrated tissue was cleared by removing the alcohol from the tissue by immersing it through 3 changes of xylene for 30mins each.
4. Wax impregnation/ infiltration: The cleared tissue was impregnated and infiltrated to remove the clearing agent (xylene) in the hot oven temp of 60^{0c} by passing it through three changes of molten paraffin was in a hot air oven for 30mins.
5. Embedding: The infiltrated tissue was buried or embedded with molten paraffin wax in an embedded mould and allowed to solidify.
6. Mounting on wooding block: The paraffin block of tissue was attached to a wooding block with the aid of a hot spatula held in between wood block and paraffin wax, the spatula melts the wax which solidifies when spatula was removed.
7. Microtome: the block of tissues was sectioned using rotary microtome, it was trimmed to obtain the cutting surface of the tissue at 15 micron and was sectioned at 5micron, and dry in hot plate for staining.

3.17 Haematoxylin/Eosin (H/E) Method of Staining

Procedure:

- Dewax in xylene for 30mins
- Remove xylene by raising in absolute alcohol ,90%, 70% and 50% alcohol for two seconds each.
- Wash in 2 changes of water.
- Stain in haematoxlyin for 20mins
- Wash in water
- Differenciate in 1% acid
- Blue in tap water, wash in water.
- Counter stain in Eosin for 5mins.
- Wash in water
- Dry and Clear in xylene.
- Mount in D.P.X and dry for micrograph and interpretation.

This procedure was conducted at Federal Teaching Hospital (FETHA ii) Abakaliki Ebonyi State, Nigeria.

3.18 Histology of the Liver and Heart

The liver is made up of hepatocytes that were not arranged systematically, but were arranged in layers and were demarcated by endothelium. The result of this study also revealed that the histology of the liver of the control groups showed normal

hepatic architecture with central vein and nucleus well positioned. However, there were no histological inflammation and fatty change observed (plate 5). The histology of the liver of fish fed with fresh and steamed avian viscera are presented in the plates 5 and 6 respectively. The histological abnormalities include moderate damage on the hepatic tissues with focal aggregates of inflammatory cells and clumping of hepatocytes. The heart of fish (*C. gariepinus*) is consisted of endocardium, ectocardium and myocardium with intra and intercellular veins well arranged. The histology of the heart of the control groups of fish in this investigation demonstrated the same. Meanwhile, the result obtained in the treatment groups (plate 6) showed mild to moderate distortion of cardiac muscles at the endocardia region and tissue degeneration.

3.19 Statistical Analysis

Results were subjected to one way analysis of variance (ANOVA) using the Statistical package for social sciences (SPSS version 20) to determine the difference between the various treatments and control. Duncan Multiple Range Test was used to separate the difference among the means and the differences were considered significant at ($p<0.05$).Growth and food utilization data were collected for twenty four weeks. Values were expressed as means ± SE.

IV. RESULTS

TABLE 3
PROXIMATE COMPOSITION OF AVIAN VISCERA

Sample	Commercial feed (Aller-aqua)	Fresh avian viscera (FAV)	Steamed avian viscera (SAV)
%CP	45	58.92	63.64
%CFAT	12	5.23	7.94
%CFIBRE	2.6	0.00	0.04
%ASH	6	12.51	13.29
%M	-	8.36	7.08
%NFE	2.6	14.98	8.01
%Phosphorus	1	-	-
% Calcium	0.8	-	-
% Sodium	0.6	-	-

TABLE 4
PROXIMATE COMPOSITION OF THE COMMERCIAL FEED (ALLER AQUA)

Parameter	Value (%)
Crude protein	45
Crude fat	12
Nitrogen free extract	2.6
Ash	6
Phosphorus	1
Calcium	0.8
Sodium	

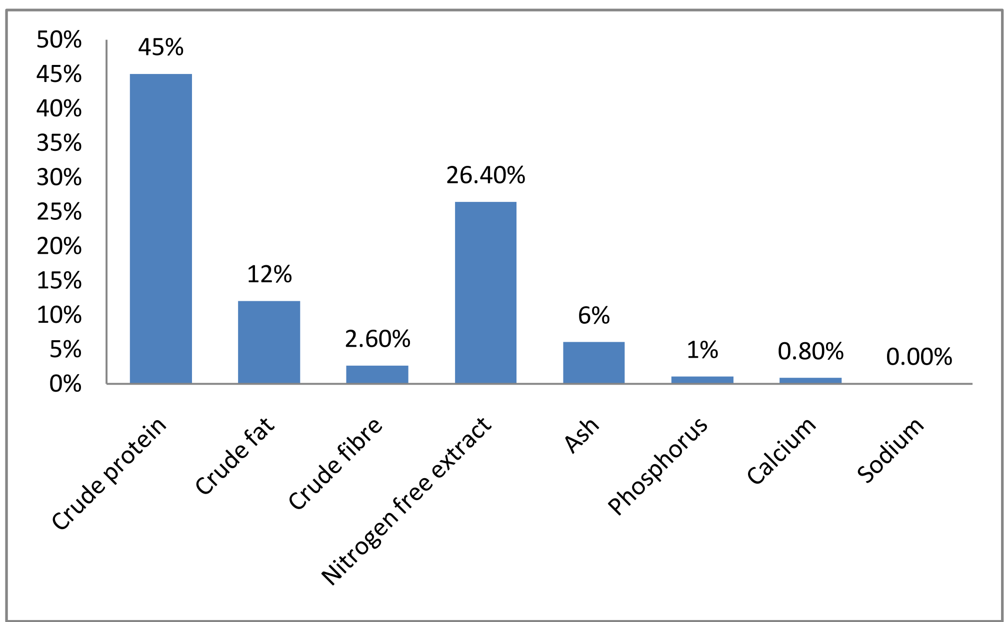

FIGURE 1: Proximate composition of control diet (Aller aqua)

TABLE 5
WATER QUALITY RESULTS MONITORED DURING THE EXPERIMENTAL PERIOD

Water quality parameters	Values(T_1)	T_2
Dissolve oxygen (mg/l)	4.80±1.70	4.80± 1.70
pH	6.80±2.38	6.80±3.40
Temperature (^{0}C)	28.90±8.75	28.90± 8.75
Nitrate (mg/l)	2.07±0.21	2.05± 0.11
Nitrite (mg/l)	2.18±0.28	2.16± 0.41

TABLE 6
THE INITIAL GROWTH PARAMETERS AND NUTRIENT UTILIZATION OF C. GARIEPINUS POST- FINGERLINGS TREATMENTS

Growth indices	Tr_1 (FAV)	Tr_2 (SAV)	Tr_3 ctrl (FEXD)
No of fish stocked	30	30	30
Total initial wt (g)	99.30±0.89	99.30±0.26	99.30±0.52
Mean initial wt (g)	3.31	3.31	3.31
Mean initial Lt (cm)	6.15	6.15	6.15
Feed intake (g)	61.16±0.27	16.72±0.09	384.94±1.16
Total final wt (g)	1223.16±0.55	334.28±0.58	7698.71±0.58
Total wt gain (g)	40.14±0.06	23.21±0.07	292.29±0.01
Mean final Lt (cm)	18.93±0.06	14.19±0.02	31.01±0.003
FCR	0.50±0.05	1.05±0.003	0.05±0.46
SGR %	9.44±0.003	2.73±0.003	63.86±0.003
RGR	1736.13±2.79	404.96±0.91	7652.98±12.83
PER	0.3444±0.10	0.2449±0.09	0.8364±0.16
Survival Rate %	93.33±9.69	46.67±4..97	86.67±7.59
No of survival	28	14	26

Where FCR- Food Conversion Ratio; SGR- Specific Growth Rate; RGR- Relative Growth Rate; PER- Protein Efficiency Ratio; FAV- Fresh Avian Viscera; SAV- Steamed Avian Viscera

TABLE 7
RESULT OF CARCASS ANALYSIS OF THE EXPERIMENTAL FISH

Parameters	T_1 (FAV)	T_2 (SAV)	Control (FEXD)	Initial
% CP	60.23±0.004^a	61.23±1.22^a	64.38±3.39^a	40.58±0.01^b
% CFAT	6.73±0.03^b	7.72±0.07^a	7.96±0.21^a	7.44±0.01^a
% CFIBRE	0.03±0.01^a	0.02±0.004^a	0.02±0.03^a	0.00±0.00^a
% ASH	12.96±0.03^a	12.92±0.20^a	12.98±0.07^a	12.19±0.01^b
% M	7.79±0.05^b	7.13±0.05^a	6.83±0.43^a	7.09±0.01^a
% NFE	12.28±0.05^a	10.98±1.44^a	7.84±3.08^a	32.71±0.03^b

Means on a row with same superscript are not significantly different but means with different superscript are significantly different. Mean separation by Duncan' Multiple Range Test at 5% level of significance (P<0.05).

4.1 Proximate Composition of Avian Viscera

The proximate composition of the experimental fish fed fresh avian viscera, steamed and commercial extruded feed (Aller aqua) are shown in Tables 3. The crude protein (CP) content were 58.92 and 63.64% for fresh and steam while formulated extruded feed (Aller aqua) suggesting that Avian viscera (AV) is a good source of protein for the fish particularly the *Clarias gariepinus* because it meets the protein requirement of catfish post fingerlings. The crude fat recorded 5.23 and 7.94% for fresh. The crude fibre recorded 0.03, and 0.04% among the treatments and the control respectively. Ash and ether extracts also had the following records (T_1)12.59, (T_2)13.29, (T_3)12.91 and (initial) 12.19% while nitrogen free extract is having 14.98, 9.99, 8.01% respectively.

4.2 Water Quality Parameters

The mean values (± SD) of the water quality parameters measured in the stagnant water concrete pond during the experimental period are presented in Table 5. There were no significant differences between temperature, dissolve oxygen, pH, nitrate and nitrite in all treatments (p>0.05). The results is indicating the mean value of dissolve oxygen 4.80±1.70, pH 6.80±2.38 temperature 28.90±8.75, nitrate 2.07±0.21 and nitrite 2.18±0.28.The result obtained in all the parameters tested did not differ significantly (P>0.05) compared with control.

4.3 The Initial Growth Performance Recorded With Commercial Diet

The best growth performance was recorded with commercial extruded feed. The fish fed in treatment one (T_1) fresh Avian viscera was recorded as second with the mean weight gain of (40.14±0.06g) and the least in the growth performance was obtained in fish in treatment (T_2) fed with steamed viscera. The control had the mean weight gain of (292.29±0.01g) while the least weight gain of (23.21±0.07) was seen in T_2 where the Avian viscera was steamed before using it to feed the experimental fish. The result showed that there was significant difference (p<0.05) between treatments. The relative growth rate (RGR) was observed to be highest in the fish fed commercial extruded with the relative growth rate of (7652.98±12.83), followed by the fish fed fresh viscera (T_1) with (1736.13±2.79). Specific growth rate (SGR) was highest in control with (63.86±0.003) where the fish was fed with commercial feed (Aller Aqua), followed by (T_1) and (T_2) with the specific growth rate of (9.44±0.003) and (2.73±0.003) respectively with significant difference among the treatments.

Food conversion ratio (FCR) was observed more efficient in control diet with (0.05±0.46), Followed by (T_1) (0.05±0.05 and the least FCR was seen in T_2 with (1.05±0.003) with variations showing significant difference (p<0.05) among the treatments.

Protein efficiency ratio (PER) was shown to be higher from the result (0.8364±0.16) in control; the fish fed commercial extruded diet. This was followed by the fish in T_1 (0.3444± 0.10) in fresh viscera while the least was recorded in T_2 (steamed

viscera) with protein efficiency ratio of (0.2449±0.09). The variations among treatments showed significant difference ($p<0.05$).

4.4 The Overall performance of *Clarias gariepinus* Post-Fingerlings Fed Avian Viscera

The mean weight gain obtained during first month of feeding post-fingerlings of *Clarias gariepinus* with experimental diets gave various values. These values did not differ significantly ($P>0.05$) among treatments. The total weights of 68.43±8.69 to 173.44±121.0.1 g were T_2 and T_3 respectively. The average body weights of mortality were 8.21±1.29 (T_2) to 18.00±12.54 g in T_3. 50% body weight gain for T_1 and T_3 were 3.42±0.43 to 8.68±6.05 g, total length for T_1 and T_3 82.05±6.94 to 117.38±31.90 cm and the average length were 9.69±1.06 to 12.15±3.31 cm in T_1 and T_3. The various mean weight values obtained during the experiment appear to have increased across the growth indices within the first month although it was not sufficient to induce significant changes among the treatments.

Furthermore, the growth performance obtained in the second month of the experiment of post-fingerlings of *Clarias gariepinus* showed a significant difference across the treatments. The total weight values of 102.80±28.31 in T_2 to 769.71±303.12 g T_3 differ significantly ($P<0.05$) within treatments. Number of fish mortality was 7.50±0.00 (T_2) to 9.70±0.00 but did not differ significantly ($P>0.05$) within the treatments. The average body weight of 15.66 ±2.98 in T_2 to 80.03±31.53 g in T_3 was significantly ($P<0.05$) different from T_1 and T_2. 50% body weight gain from 5.63±1.14 in T_2 to 38.49±15.16 g and T_3 was significantly ($P<0.05$) different in other treatments. Total length of 105.24±11.52 in T_2 to 232.69±24.66 cm showed a significant difference ($P<0.05$) across the treatments, and average length of 14.48±1.66 cm in T_2 to 24.14±2.57 cm and T_3 showed significant difference ($P<0.05$) from the other treatments. Overall mean weight values obtained during the second month of the experiment appear to have increased more in T_3 and the numbers of significant values are higher in T_3.

In the third month, the mean values of the growth performance obtained from the experiment of post-fingerlings of *Clarias gariepinus* were fed fresh and steamed avian viscera, total weight of 176.42±27.20 in T_2 to 1553.63±683.78 g in T_3 differ significantly ($P<0.05$) from T_1 and T_2. The average body weight values recorded 24.83±3.55 (T_2) to 168.83±85.82 g while T_3 differ significantly ($P>0.05$) T_1 and T_2. 50% body weight gain 8.97±1.36 in T_2 to 77.68±34.19 cm and T_3 differs significantly ($P<0.05$) from other treatments. The total length of 30.24±15.12 in T_3 to 214.99±36.77 cm in T_1 but does not differs significantly ($P<0.05$) across the treatments. The average length of 118.85±3.83 in T_2 to 31.55±2.49 cm while T_3 was significantly ($P<0.05$) different from other treatments. The various mean weight values obtained during third month indicated that these parameters experienced increases particularly in T_3.

4.5 The Carcass Quality (Body Composition) of experimental fish

Carcass composition of fish in the feeding trial was summarized in table 7. The crude protein was the highest in control and was not significantly different for the other treatments. Crude fibre was the least in the treatment. The carcass Ash content of the treatment were significantly different ($p>0.05$) in fresh avian viscera (T_1) and steamed avian viscera (T_2). Fats (Lipid) are an important source of dietary energy for fish which have limited ability to utilize dietary carbohydrate for energy. When all the required essential nutrients are available in the diet, *Clarias gariepinus* will grow and survive well regardless of the lipid source. However, *Clarias gariepinus* are able to utilize plant based lipids sources better than animal based lipid sources in term of growth and carcass analysis.

The proximate analysis of body composition of the experimental diet, fresh avian viscera, steamed and commercial extruded feed (Aller aqua) are shown in Table 7. The crude protein (CP) content was 60.23% for the fresh viscera and 61.23% steamed. while formulated extruded feed (Aller aqua) suggesting that Avian viscera (AV) is a good source of protein for the fish particularly the *Clarias gariepinus* because it meets the protein requirement of catfish post fingerlings. The crude fat recorded 6.73% for the fresh viscera and 7.72% for the steamed viscera meal. The crude fibre recorded 0.03, for fresh avian viscera meal, and 0.02% for the steamed avian viscera, Ash also had the following records (T_1, FAV) 12.51, (T_2, SAV), 12.92, while Moisture is having 7.79 (FAV) and 7.013% (SAV) respectively. The Nitrogen fixation extract recorded the value of 12.28 (FAV) and 10.98% for steamed avian viscera respectively.

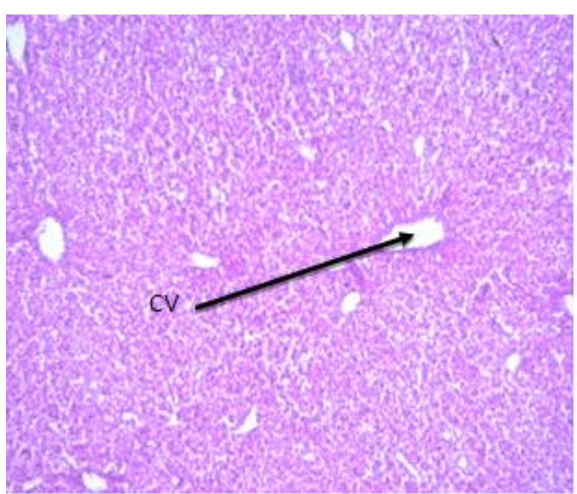

Plate 1: Photomicrograph of Avian Liver (AL) cross section of liver (X150 haematoxylin/eosin (H/E) shows normal hepatic architecture with normal central vein (CV)

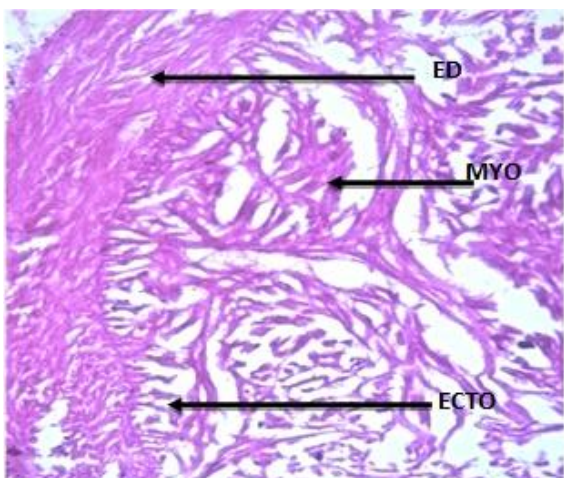

Plate 2: Photomicrograph of Avian histology (AH) control section of heart (X150) haematoxylin/eosin (H/E) showing normal cardiac architecture with the three layers shown endocardium (ED), Ectocardium (ECTO) and Myocardium (MYO)

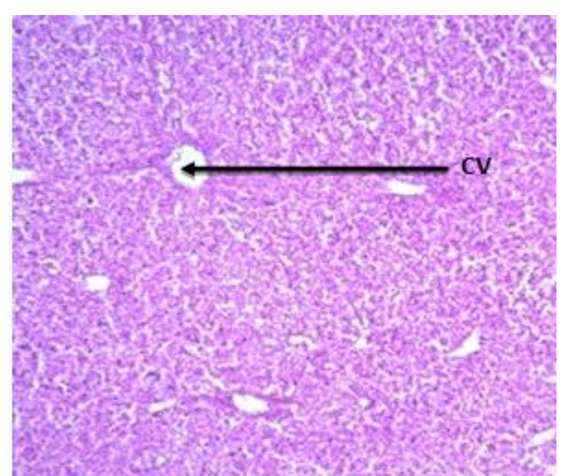

Plate 3: Photomicrograph cross section of liver (X150) fed with fresh viscera shows normal hepatic architecture with central vain (CV). However there are no inflammation and no fatty change .The overall feature appears normal

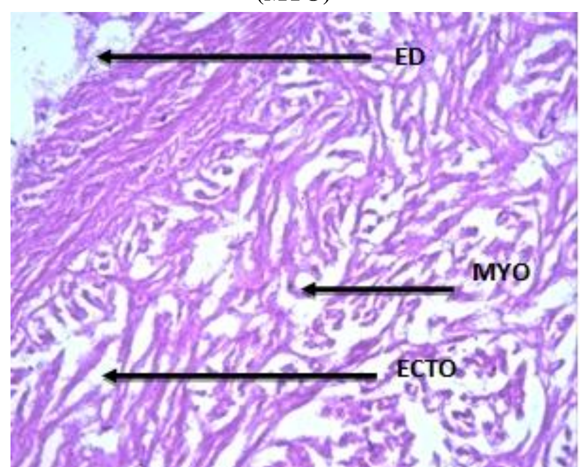

Plate 4: Photomicrograph section of heart (X150) haematoxylin/eosin (H/E) shows normal cardiac architecture with the three layers the endocardium (ED), Ectocardium (ECTO) and Myocardium (MYO)

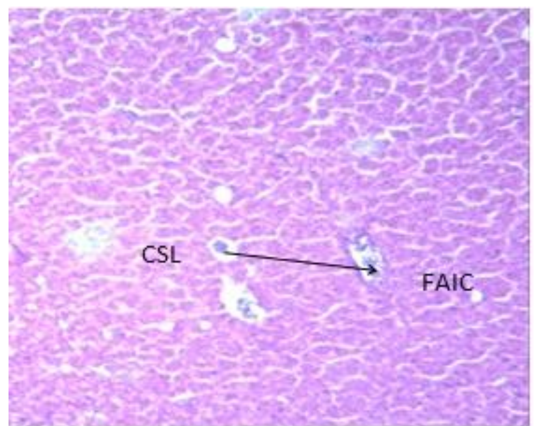

Plate 5: Photomicrograph of cross section of the Liver (CSL) fed with steamed viscera (X150) showing moderate damage on the hepatic tissue with focal aggregate of inflammatory cell (FAIC) and clumping of the hepatocytes (CH)

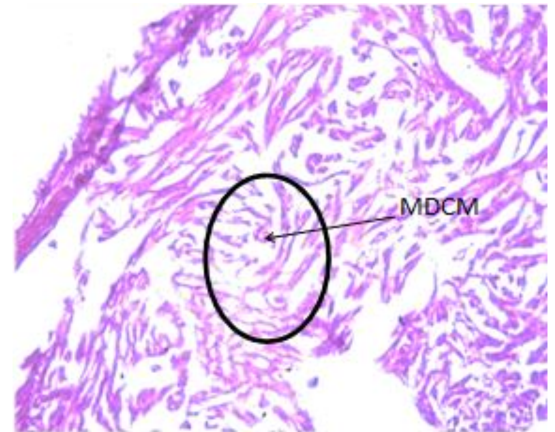

Plate 6: Photomicrograph of cross section of heart (CSH) feed with steamed viscera (X150) haematoxylin/eosin (H/E) shows mild effected cardiac tissue with mild distortion of the cardiac muscles (MDCM) at the endocardia region

4.6 The histological report on the effects of avian viscera meal on the characteristics of liver and heart of *clarias gariepinus* post-fingerlings

The control section of liver (X150) shows normal hepatic architecture with normal central vain (CV) plate 1. Photomicrograph of Avian histology (AH) control section of heart (X150) heart shows normal cardiac architecture with the three layers shown endocardium (ED), Ectocardium (EC) and Myocardium (MYO) plate 2.. The liver of fish fed with fresh viscera shows normal hepatic architecture with central vain (CV). However there are no inflammation and no fatty change. The overall feature appears normal in (plate 3). The liver of fish fed with steamed viscera shows moderate damage on the hepatic tissue with focal aggregate of inflammatory cell (FAIC) and clumping of the hepatocytes (CH) (plate 5). The heart of fish fed with fresh viscera shows normal cardiac architecture with the three layers the endocardium (ED), Ectocardium (EC) and Myocardium (MYO) in (plate 4) The result of heart of fish fed with steamed viscera shows mild effected cardiac tissue with mild distortion of the cardiac muscles (MDCM) at the endocardia region (plate 6).

V. DISCUSSION

According to the result of the present study, weight gain was observed in all fish in the treatment groups at the end of the experiment. The growth of fish was represented as a function of initial mean weight, feed intake, total final weight, total weight gain, mean weight gain, feed conversion ratio and specific growth rate. The lower the values of food conversion ratio the higher the feed efficiency. Fish were being fed with chicken intestine at 5% body weight, the highest percentage of weight gain was observed.

5.1 Effect of water quality on the experimental fish

The water quality parameters like Temperature, Dissolve oxygen, pH, nitrate and nitrite in all treatments were monitored. And the results existed within the permissible limit for fish culture as recommended by environmental pollution agency (EPA) for the survival and culturing of aquatic organisms. Therefore no effect of the water parameters was attributed to the fish mortality and there was no significant difference ($p<0.05$) among the treatments on the water quality.

Clarias gariepinus can support temperature as low as 6 °C and as high as 50 °C. However, a temperature of 28-30 °C is considered optimal for the growth of this species (Ikeogu *et al*., 2020). The water temperature of 28.9 °C recorded in this study is optimal for the growth of *C. gariepinus*. Similarly, dissolved oxygen levels and pH recorded during the experiment were not a limiting factor for fish growth. Indeed, this species can live in low oxygen conditions water for a long time (Ikeogu *et al*., 2020) and tolerate a wide range of adverse environmental conditions (Légendre & Proteau, 1996).

5.2 Effect of fresh and steamed avian viscera on growth performance of *Clarias gariepinus* post-fingerlings

In this study, although the different groups of experimental fish had similar initial mean weight and were fed with fresh, steamed avian viscera and formulated extruded diets, *Clarias gariepinus* showed significant different in final weight gain, specific growth rate, feed intake, feed conversion ratio and protein efficiency ratio. Higher substitution of chicken viscera meal resulted in reduced growth performance, feed utilization efficiency and production. Different results were reported by Studies of Takagi *et al*. (2000), Davis and Arnold (2000) that, by using good quality of high protein value poultry by-product (PBP), it could be possible to replace even 100% of fish meal without compromising on the fish growth. Sealey and Hardey (2011) reported that complete replacement of Fishmeal (FM) with PBP could be possible in the feed formulation by using good quality poultry by-products (PBP) without any addition of amino acids, fed on diets containing graded poultry viscera meal. The result of this work is in agreement with report of Olaniyi and Amusan (2016) who revealed the growth performance of *Clarias gariepinus* fed varying inclusion levels of chicken intestine. Weight gain was observed in all fish at the end of the experiment. The FMW- final mean weight; MWG- mean weight gain; SGR- specific growth rate: FCR- feed conversion ratio, MFI- mean feed intake; PER- protein efficiency ratio; PI- protein intake; were all significantly different ($P<0.05$). The poor growth performance on steamed avian visceral may be due to the presence of undigested grain and their by-products from the poultry diet, and the imbalanced amino acid content of the meal without fishmeal and it opined with reports of Gupta, *et al.,* 2013 who reported higher substitution levels of chicken viscera meal resulted in reduced growth performance, feed utilization efficiency and production of *C. gariepinus* fingerlings. Similar results were reported for *Carassius auratus gibelio* (Yang, *et al.,* 2006 and Hu *et al.,* 2008), *Clarias gariepinus* (Goda *et al.,* 2007), *Clarias batrachus* (Giri, *et al.,*2010; Gupta, *et al.,* 2013 and Giri, *et al.,*2000), grass carp *Ctenopharyn godonidella* fry (Tabinda and Butt 2012), *Cirrhinus mirigala* (Tabinda *et al.,* 2013), *Pelodiscus sinensis* (Sun, *et al.,*2014)and *Lutjanus guttatus* (Hernandez, *et al.,* 2014) fed on diets containing graded poultry viscera meal.. The survival rate of fish fed fresh avian visceral during the

experiment was greater than 92%, indicating that fish has grown in good experimental conditions. (Goda *et al.,* 2007) pointed out that due to the ash and carbohydrate content in Chicken viscera meal (CVM), by producing a faster gut transit rate, resulting in increased feed intake, associated with poor growth performance and feed efficiency is also in affirmation with the result of this work. Same results have been reported by (Yamamoto *et al.,* 2002) who found that higher feed intake and lower feed efficiency in juvenile rainbow trout can be attributable to higher quantity of ash in diets. Also, feed intake and growth performance decreasing trends were found in *Clarias gariepinus* (Goda *et al.,* 2007 and Abdel-Warith *et al.,* 2001), *Clarias batracus* (Giri, *et al.,*2010 and Hu *et al.,* 2008) and *Anabas testudineus* (Bhaskar *et al.,*2015) at high inclusion levels of poultry viscera meal.

5.3 Effects of avian viscera meal on the histological characteristics of liver and heart of *Clarias gariepinus* post-fingerlings

The histological results of the photomicrography of liver and heart of *Clarias gariepinus* post-fingerlings fed fresh avian visceral showed, normal hepatic architecture with central vein (CV) in liver. However there were no inflammation and no fatty change. The overall feature appears normal. For the heart, it indicated normal cardiac architecture with the three layers the endocardium (ED), Ectocardium (EC) and Myocardium (MYO). Where the liver of the fish fed steamed avian visceral showed moderate damage on the hepatic tissue with focal aggregate of inflammatory cell (FAIC) and clumping of the hepatocytes (CH) (plate 1). The result of heart of fish fed with steamed viscera shows mild effected cardiac tissue with mild distortion of the cardiac muscles (MDCM) at the endocardia region (plate 2). The results are in conformity with report of Abdel-Warith *et al.* (2001) who reported accumulation of lipid in the liver histology of African catfish fed the higher level Poultry Bye-product Meal (PBM) diets could be related to a dietary imbalance between saturated and unsaturated fatty acids, as a consequence of the high ratio of saturated fatty acids present in this ingredient.

VI. CONCLUSION

The present study clearly demonstrated that chicken viscera has a good nutritive value for the growth of African catfish (*Clarias gariepinus*). It could be incorporated or used whole to *Clarias gariepinus* as meal without adverse effects on growth, feed utilization and body protein content. Chicken viscera are available in Abakaliki and can be obtained throughout the year. Therefore, the use of chicken viscera can substantially reduce feed cost and increased profits. This might permit the development of that fish culture in Abakaliki. However, water quality determination is also an important environmental factor that influence significantly on the fish welfare. The liver of the fish fed steamed avian viscera showed moderate damage on the hepatic tissue with focal aggregate of inflammatory cell (FAIC) and clumping of the hepatocytes. The heart of fish fed with steamed viscera showed mild affectation on cardiac tissue with mild distortion of the cardiac muscles (MDCM) at the endocardia region. Therefore, based on the result obtained from the experiment, it is hereby recommended that chicken intestine meal can be included in the diet or fed whole to *Clarias gariepinus* without any adverse effect. The result of histology recommends that famers could use fresh avian viscera for their fish because their organs were not affected. Further studies on the utilization of avian viscera by *Clarias gariepinus* to achieve optimal growth and maximization of profit are recommended. Extending this research by the use of other fish species is also advised. The contribution of this work to the scientific knowledge is to reduce waste from the environment which has been a challenge to poultry farmers from the abattoirs. Farmers are advised to feed their fish with avian viscera because it is healthy.

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Biochemical Strategy of Drought Resistance of Dry Habitat Plants of Georgia

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Abstract**—*****The climatic cataclysms taking place in different corners of the world are clear confirmation of the climate negative change in our planet. Especially disturbing is the temperature rise, accompanied by hot waves, forest fires, intensive melting of the ice cover, and other undesirable aftereffects. Under these conditions chances of dying of many plant species significantly increases. Drought resistant plants have the highest potential of adaptation to increased temperature and water deficiency. Thus, knowledge of their biology will be especially important in deserted regions restoration. The nonspecific mechanisms of resistance, especially antioxidant system, are concerned as one of the leading in plants drought resistance. The presented study aimed the comparative study of the indices of antioxidant system of drought resistant species - Astragalus microcephalus Willd. (Astracantha microcephala (Willd.) Podlech) - goat's thorn, Theucrium polium L. – felty germander, Euphorbia seguieriana Neck. - spurge, Capparis spinosa L. – caper bush, Paliurus spina-christi Mill. – Christ's thorn, growing in different arid habitats of the East Georgia (Iagludja and Kvernaqi hills). The defence mechanisms of the antioxidant system appeared to be partially different in one and the same species of various habitats, as well as in different species of the same habitats. Activation of phenolic substances and anthocyanins synthesis against extreme conditions of both habitats (water deficiency, high temperature and intensive irradiation) was common for all tested species. Additionally, activation of peroxidase in Kvernaqi species and intensive accumulation of soluble carbohydrates in Iagludja plants was mentioned.*

Keywords—antioxidants, drought resistance, Georgia.

I. INTRODUCTION

The latest scientific documentations demonstrating evident negative changes all over the world has been presented in the preliminary report of the world meteorological organization on the climate global situation in 2021. Especially disturbing is the temperature rise, accompanied by hot waves, forest fires, intensive melting of the ice cover, and other undesirable aftereffects. According to data last seven years were regarded as the hottest through the whole history of climate observation (WHO report, 2021).

Climate warming significantly rises the risk of plants dying off under the increased stresses (Allen et al., 2015; Overpeck and Udall, 2010; McDowell et al., 2008). Presumably the area of distribution of many plant species will change. The migration rate will depend on species features, competition, climate conditions, etc. (Garamvolgyi and Hufnagel, 2013) Drought resistant species will have the highest potential of adaptation to increased temperatures and accompanying water deficiency. Thus, the knowledge of their biology will be very important in the restoration of deserted areas; moreover, most of them are used in medicine

Drought resistant plants possess evolutionary developed physiological and biochemical mechanisms of stability against water deficiency and high temperature. The nonspecific mechanisms of resistance are regarded as one of the principle under stress conditions; the antioxidant system is of special importance among them (Li, and Liu, 2016; Laxa et al., 2019).

Characteristics of the antioxidant system of drought resistant plants of the arid territories of Georgia are practically unexplored.

The presented work aimed comparative studying of some characteristics of drought resistant species growing at different arid habitats of east Georgia (Yagludja and Kvernaqi hills). The study may be regarded as the continuation of previous year's investigations (Badridze et al., 2021).

Content of ascorbic acid, tocopherol, carotenoids, anthocyanins, soluble phenols, proline, total proteins and soluble carbohydrates, as well as the activity of catalase, peroxidase, nitrate reductase and the total antioxidant activity in percents of inhibition have been studied in leaves of experimental plants,

II. MATERIALS AND METHODS

2.1 Research area

Experimental species were collected in July of 2020-21, at two different arid habitats of East Georgia – Iagludja hill (Gardabani municipality) and Kvernaqi hill (Kaspi municipality).

Iagludja hill is situated in Kvemo Kartli (lower Kartli), on the north-east of Marneuli plane, near the city Rustavi. The climate here is continental, with mild winter and hot, dry summer. The mean annual temperature is 12°-13^oC; in the coldest month – January the mean temperature is 0.3^o-0^oC. Especially hot is July and August. Absolute minimal temperature is minus 20°-25^oC and maximal – 40°-41^oC. The mean annual amount of precipitations is 350-500mm (Kordzakhia and Djavakhishvili, 1971).

Most part of Marneuli municipality soils are degraded to various degrees; this is clear from the worsening of their physical and mechanical, chemical and microbiological properties and fertility decrease. Degradation of a plant cover resulted in a formation of clay-rich, low-humic, calcareous grey-brown soils (Official web-page of Marneuli municipality).

Kvernaqi hill is situated in Shida Kartli (inner Kartli), near the city Kaspi. Climate here is transitional from subtropical to humid. The winter is moderately cold, summer is dry and hot. The mean annual temperature of air is 11.4^oC, in January - minus 0.5^oC, in August – 23^oC. The absolute minimal temperature is minus 27^oC, and absolute maximal – 40^oC. Annual amount of precipitations makes 450mm. On the south slope of Kvernaqi hill (where the material was collected) the radiation is intensive in summer; that is why the air temperature here is higher compared to the northern slope. In spite of comparatively high precipitations in Kvernaqi, compared to central regions of Shida Kartli plane, their efficiency here is low; because of it the soil is dryer. Kvernaqi hill is constructed of Neogenic conglomerates, sandy-gravel shales. In Kotsakhura gorge, where the material was picked, soils are alluvial, here and there rocky and gravel (Kordzakhia and Djavakhishvili, 1971; Ukleba, 2018).

2.2 Experimental plants

Middle age, mature, healthy leaves were collected at least from 5 different individuals of each experimental species: *Astragalus microcephalus* Willd. (*Astracantha microcephala* (Willd.) Podlech) - goat's thorn, *Theucrium polium* L. – felty germander, *Euphorbia seguieriana* Neck. - spurge, *Capparis spinosa* L. – caper bush, *Paliurus spina* Mill. – Christ's thorn. Material was taken at 530-395m above sea level; in fruit bearing phase, during the hottest period for these locations (38°-40^oC). Analyses were performed both on raw and dry material, with 3-fold repetitions.

2.3 Biochemical assays

2.3.1 Antioxidant enzymes assay

Peroxidase activity was determined spectrophotometrically: optical density of the products of guaiacol oxidation was measured at the wave length of 470nm by the spectrophotometer (SPEKOL 11, KARL ZEISS, Germany) (Ermakov, 1987).

Catalase activity was studied gasometricaly: volume of the oxygen released in the process of reaction between hydrogen peroxide and enzyme was measured (Pleshkov, 1985).

2.3.2 Nitrate reductase assay

Method of determining the nitrate reductase activity was based on measurement of nitrites amount, which were formed as a result of nitrate reductase reaction with the infiltrated nitrates (Ermakov, 1987).

2.3.3 Ascorbic acid

A titration method was used to measure the content of ascorbic acid in plant material. 2 g of fresh leaves were mashed in 15 ml of 2% hydrochloric acid and 10 ml of 2% metaphosphoric acid, and filtered. One ml of the filtrate was added to 25 ml of distilled water and titrated with a 0.001 M solution of dichlorphenolindophenole (Ermakov, 1987).

2.3.4 Tocopherol

Two grams of ground leaves were extracted with 20-25ml of pure ethanol (three-fold). The combined extract was mixed with 20 ml of 60% potassium hydroxide, and saponificated on water bath for 2h. Tocopherol was extracted from the obtained hydrolyzate using diethyl-ether (3-fold extraction). The combined extract was washed with distilled water until a complete removal of alkaline residuals. Water was removed with Na_2SO_4; the obtained solution was evaporated on the water bath, cooled, mixed with alcohol-nitric acid (1 ml of concentrated HNO_3:5ml of 96° alcohol), and boiled during 3 min till the color became dark red. Extinction of the extract was measured at 470nm by the spectrophotometer (SPEKOL 11, KARL ZEISS, Germany) (Filippovich et al., 1982).

2.3.5 Anthocyanins

100mg of grinded leaves were added with 20 ml of 96% acidified (with 1% HCl) ethanol (99:1). After 24h retention in dark the optical density at 540nm was measured (spectrophotometer SPEKOL 11, KARL ZEISS, Germany) (Ermakov, 1987).

2.3.6 Plastid pigments

Chlorophyls and carotenoids were determined spectrophotometricaly. Fresh leaves (100-200mg) were mashed with sand and $CaCO_3$ and washed with ethanol. Optical density of the filtrate was measured (spectrophotometer SPEKOL 11, KARL ZEISS, Germany). Concentration of chlorophylls a and b, also carotenoides was calculated by the formula of Wintermanns (Gavrilenko et al., 1975).

2.3.7 Total phenols

A 0.5 g of fresh leaves was boiled in 80% ethanol for 15 min. After centrifugation the supernatant was saved, and residues of leaves were mashed in 60% ethanol and boiled for 10 min. Obtained extract was added to the first supernatant and evaporated. The sediment was dissolved in distilled water. One ml of the received solution was added with the Folin-Ciocalteu reagent and optical density was measured at 765 nm. The chlorogenic acid served as control (Ferraris et al., 1987).

2.3.8 Total protein assay

Content of proteins was determined after Lowry (1951).

2.3.9 Proline

0.5 g of dry leaves were mashed in 10ml of 3% sulphosalicylic acid and filtered. 2 ml of the filtrate was added to 2 ml of acid ninhydrin and 2 ml of ice acetic acid. After 1 h exposition on a water bath the extract was cooled and added with 4 ml of toluene and divided in a separating funnel. Optical density of upper layer was measured on a spectrophotometer (SPEKOL 11, KARL ZEISS, Germany) at 520 nm (Bates et al., 1973).

2.3.10 Soluble carbohydrates

Content of soluble carbohydrates was tested with anthrone reagent (Turkina and Sokolova, 1971). To 100mg of air-dry leaf material was added 96° alcohol for extraction (3-fold). The total amount of the obtained extract was evaporated on a water bath and dissolved in 5ml of distilled water. To 0.5ml of the tested water extract was added 2ml of anthrone reagent and heated in a water bath for 10min. After this procedure the test-tubes were placed in a cold water bath and 15min later the optical density of the solution was measured at 620nm with a spectrophotometer (SPECOL 11, KARL ZEISS, Germany).

2.3.11 Nitrates

After the water-extraction of 500g of plant material (homogenized for 30min at room temperature), it was filtered. Hydrogene peroxide was added to 10ml of the filtrate and evaporated. disulphophenolic acid was added to the obtained sediment and optical density was determined at 410nm (SPEKOL 11, KARL ZEISS, Germany) (Danilova, 1963; Pleshkov, 1985).

2.3.12 Total antioxidant activity

This index was measured by modified method using diphenyl-picryl-hydrazyl (DPPH) (Koleva *et al.,* 2002). 200 mg of experimental powder was extracted with 96° ethanol (two-fold). The obtained extract was evaporated on a water bath and the sediment was dissolved in 10ml of water-alcohol mixture. The 0.01ml of the received solution was added with 4ml of 40μM DPPH solution and after 30 minutes of incubation in the dark, the optical density was measured at 515nm by the spectrophotometer (SPEKOL 11, KARL ZEISS, Germany). The percent of inhibition was calculated.

2.3.13 Statistical processing of data

One way ANOVA and Tukey's multiple comparison tests were used to test differences between the means. All calculations were performed using statistical software Sigma Plot 14.5.

III. RESULTS AND DISCUSSION

As it was mentioned above, the experimental plants were collected in July, which is the hottest and driest month of the studied habitats. Also the fact that the last seven years are regarded as the hottest through the whole history of climate observation must be taken into account (WHO report, 2021). Thus, the tested species had to survive under combined stress of high temperature, water deficiency and intensive irradiation simultaneously.

3.1 Nitrate reductase and nitrates

Nitrate reductase (EC 1.6.6.1.) plays an essencial role in plant's physiological and metabolic condition as it is a key enzyme in synthesis of amino acids, proteins, chlorophylls and other nitrogen-containing substances; it may serve as a marker of drought stress as well (Ananthi, Vijayaraghavan, 2012; McCarthy et al., 2017; Sinay, Suripatty, 2019). It is established that the activity of nitrate reductase decreases under the water deficiency; which is associated with photosynthesis inhibition (Kapoor et al., 2020). Moreover, as the nitrate reductase is the substrate-dependent enzyme, and its activity is regulated by the concentration of nitrates and ammonium, the decline of enzyme's activity may be caused by reduction of nitrates in leaves (Nicodemus et al. 2008; Dias et al., 2011). As the absorbtion of water and minerals, among them of nitrates, is reduced during the drought, this will be reflected on enzyme's activity as well (Correia *et al.,* 2005; Kapoor et al., 2020)

According to the experimental results it is clear that the one and the same species of two habitats showed statistically different activity of nitrate reductase ($p \leq 0.002$); indices of goat's thorn and felty germander growing at Iagludja were respectively 3.6 and 1.6-times higher, compared to Kvernaqi results ($p \leq 0.002$). In other tested species on the contrary – Kvernaqi data of nitrate reductase activity were higher: in caper bush – 2-times, in spurge – 2.5 times, and in Christ's thorn – 1.5-times (Fig. 1).

Although the activity of nitrate reductase has been associated with nitrates content in leaves (Nicodemus et al. 2008; Dias et al., 2011), in our experimental plants this correlation was not clear. Generally, the content of nitrates in Kvernaqi species was higher that of Iagludja ones ($p<0,05$) (Fig. 5). Moreover, in Iagludja experimental species content of nitrates was low and statistically similar, while the same species of Kvernaqi statistically differed by this index ($p<0,05$) (Fig. 1).

If we consider the content of chlorophylls as the indicator of photosynthetic activity, there may be assumed some relationship between nitrate reductase activity and chlorophylls content in leaves; as the decrease of the enzyme's activity is associated with photosynthesis inhibition under the water deficiency (Kapoor et al., 2020).

Comparision of nitrate reductase activity and chlorophylls content in tested plants revealed the coincidence of the decrease of these two indices (Fig. 1, 2).

As the nitrate reductase gives some idea on the general physiological condition of a plant, according to the obtained results it may be concluded that Iagludja conditions were more stressful for experimental plants, than Kvernaqi. Equally low data of nitrates in Iagludja species is demonstration of this (Fig. 1).

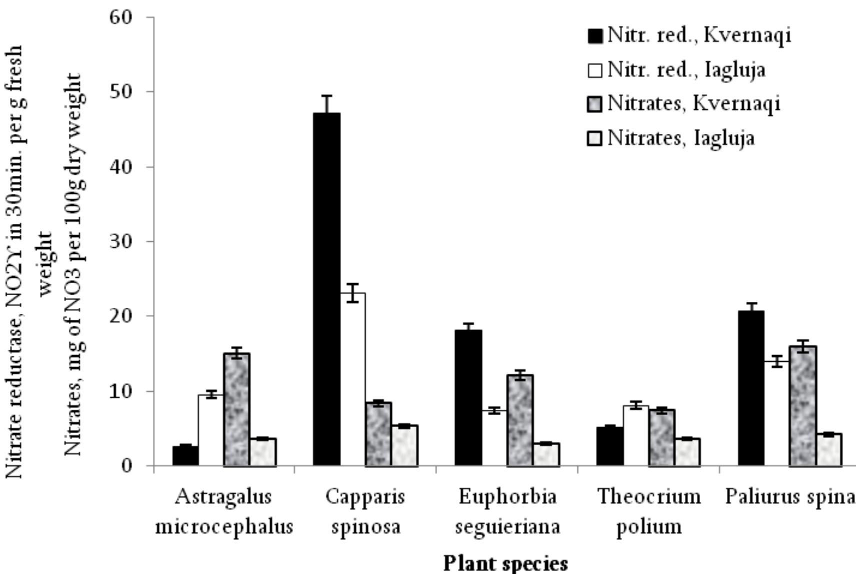

FIGURE 1: Nitrate reductase activity and content of nitrates in leaves of Kvernaqi and Iagluja (East Georgia) plants

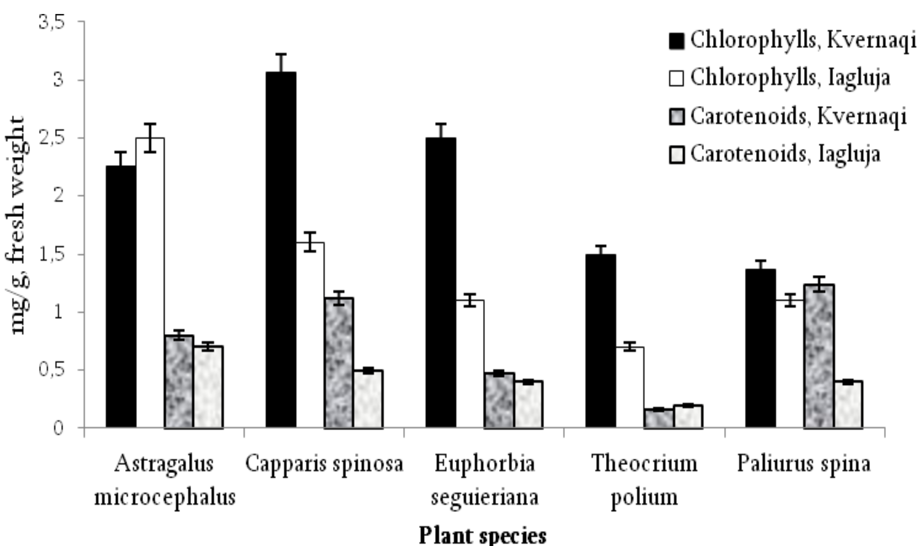

FIGURE 2: Content of chlorophylls and carotenoids in leaves of Kvernaqi and Iagluja (East Georgia) plants

3.2 Catalase and peroxidase

Generally the high activity of antioxidant enzymes – catalases and peroxidases has been mentioned in drought resistant plants (Laxa et al., 2019; Kapoor et al., 2020). Although the high specificity of the activity is characteristic for enzymatic antioxidants – they affect particular type of radicals, with specific cellular and organ localization (Chupakhina et al., 2011). Catalases of the photosynthesizing tissue mainly are localized in peroxisomes (CAT, EC 1.11.1.6), where they distruct the hydrohen peroxide produced during photorespiration (Noctor et al., 2014); Moreover, proliferation of peroxisomes and diffusion additional hydrogen peroxide from the cytosol may take place under stress, which is actively neutralized by catalases as well (Lopez-Huertas et al., 2000); Althogh, in some plants decrease of catalases activity is possible, which is regared as a demonstration of a significant role of peroxidases and ascorbate-glutathione cycle, as of principal oxygen-scavengers. (Harinasut et al., 2003).

So called classic, or non-specific peroxidases (EC 1.11.1.7) are multifunctional enzymes, which like catalases, neutralize the active forms of oxygen. For this purpose they use different reducing agents, often phenolic substances. They are concentrated mainly in a vacuole and cytosol (Kolupaev and Kokorev, 2019).

In studied species the activity of catalase was generally low (Fig. 3). Comparison of results of one and the same species of two habitats revealed their statistical identity (p>0,05).

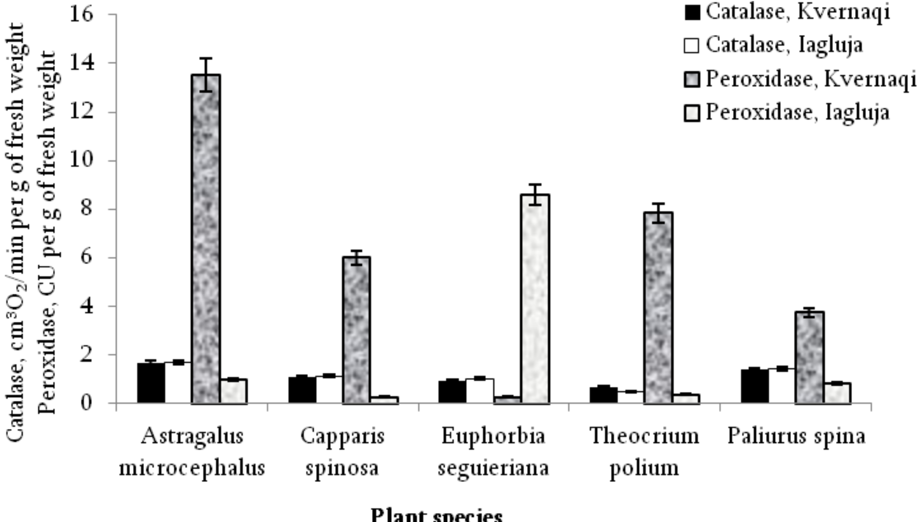

FIGURE 3: Activity of catalase and peroxidase in leaves of Kvernaqi and Iagluja (East Georgia) plants

Among Kvernaqi plants statistically similar activity of catalase was found in goat's thorn and Christ's thorn (p=0,09), caper bush and Christ's thorn (p=0,4), caper bush and spurge (p=0,2). Among Iaglidja species statistically similar activity of catalase was revealed in caper bush, spurge and Christ's thorn (p = 0,5) (Fig. 3).

The peroxidase activity of same species from different habitats was statistically different (P≤0,001). Generally the peroxidase activity of Kvernaqi experimental species was significantly higher, compared to Iagludja data (except spurge) (Fig. 3): in goat's thorn leaves it was 14-times higher, in caper bush – 26-times, in felty germander – 22-times, and in Christ's thorn – 4.5-times higher

While comparing peroxidase activity of different species, among Iagludja plants spurge and Christ's thorn results were similar (p=0,5), in other species there was statistical difference (p<0,05) (Fig. 3). In Kvernaqi plants all data on peroxidase activity were statistically diverse (p<0,01) (Fig. 3).

Analyzing the experimental results it may be supposed that low catalase activity in plants of both habitats indicates to the low concentration of hydrogen peroxide in leaves. Since catalase has a low affinity to hydrogen peroxide, compared to peroxidase, it is active under high concentrations of the substrate (Mhamdi et al., 2010; Chakraborty et al., 2016).

Decreased activity of catalase may be caused by its inactivation because of intensive radiation as well (Chupakhina et al., 2011).

High activity of peroxidase in experimental plants of Kvernaqi hill may be explained if we take into account the fact that peroxidases are active in those systems, where the primary transformation of derivatives of hydrohen peroxide – organic peroxides takes place by means of appropriate reducing agents (Mhamdi et al., 2010).

High peroxidase activity of spurge individuals at Iagludja, as exception, may be linked with the diverse from other species C4 way of CO2 assimilation in this species.

Thus, more extreme conditions of Iagludja hill compared to Kvernaqi, cause diverse biochemical changes in experimental species.

It may be supposed that the enzymatic antioxidant system of Iagludja experimental plants is not leading in adaptation to the intensive irradiation, high temperature and water deficiency conditions of this habitat; while under Kvernaqi conditions peroxidase was distinguished from this point of view.

3.3 Chlorophylls and carodinoids

It is known that water deficiency, together with intensive radiation and high temperature inhibits photosynthesis. One of the reasons is chloroplasts damage and chlorophylls decrease, regarded as some kind of protective reaction towards stresses (Ommen et al., 1999; Herbinger et al., 2002; Ma et al., 2020).

Comparative study of chlorophylls and carotenoids in one and the same species of the studied habitats has revealed the statistical similarity of chlorophylls in leaves of goat's thorn felty germander and Christ's thorn (p=0,4, p=0,2 and p=0,7 respectively), while in other species it was different (p<0,05). Moreover, Kvernaqi data were higher than Iagludja ones: in caper bush - 1,9-times, in spurge – 2,3-times (Fig. 2).

The between-species comparison of results revealed statistical similarity of chlorophylls in spurge and goat's thorn (p=0,6), caper bush and spurge (p=0,1), felty germander and Christ's thorn (p=0,5) growing at Kvernaqi hill; while among Iagludja species chlorophylls content of leaves was statistically similar in all tested species (p<0,05), except goat's thorn (Fig. 2).

It must be mentioned that in spite of stressful conditions in leaves of both habitat plants content of chlorophylls was not low; which indicates to reliable protection of the photosynthetic apparatus (Fig. 2).

As for carotenoids, between-habitat differences were revealed in caper bush (p=0,003) and Christ's thorn (p=0,006) individuals (Fig. 2).

Between-species comparison cleared statistical similarity of carotenoids in caper bush and Christ's thorn leaves (p=0,4) growing at Kvernaqi; while at Iagludja statistically same were indices of goat's thorn and caper bush (p=0,06), as well as of all other species (p>0,05) (Fig. 2).

3.4 Ascorbic acid and tocopherol

Ascorbic acid is regarded as one of the leading substances for protection of the photosynthetic apparatus against the oxidative stress (Venkatesh, et al., 2014). Under the intensive irradiation, as well as drought, content of ascorbic acid in plant increases (Yang et al., 2008). High content of the substance is one of the features of plant stress-resistance (Singh et al., 2012).

In a number of plants the increase of tocopherol, together with ascorbic acid, has been indicated as one of the primary reactions against drought or intensive radiation stress (Abbasi et al., 2007; Giacomelli et al., 2007).

Among one and the same species of different habitats the content of ascorbic acid was statistically similar in leaves of spurge and felty germander (p=0,1 and p=0,8 respectively), while in other species it was different (p<0,05); in particular, Kvernaqi indices were higher compared to Iagludja: in caper bush by 28%, in spurge – by 6%, in Christ's thorn – by 66,5%. In leaves of goat's thorn in contrary – it was by 22,5% lower (Fig. 4).

According to obtained results it may be supposed that in leaves of caper bush, spurge and Christ's thorn, growing in Kvernaqi conditions, ascorbate system is one of the leading mechanisms in leaves protection against stress; while under Iagludja conditions this mechanism was clearly expressed only in caper bush and spurge leaves.

It must be mentioned that chloroplast membrane contains high amount of α-tocopherol, which protects it against photo oxidation. By well-known ascorbate-tocopherol cycle the oxidized tocopherol is reduced by means ascorbic acid (Mullineaux et al., 2006; Gill, Tuteja, 2010).

Among the same species of studied habitats content of tocopherol was statistical identical only in goat's thorn leaves (p=0,109) (Fig. 4); in other experimental species Iagludja data were higher compared to Kvernaqi ones (p<0,05): in caper bush - 9-times, in spurge - 2,8-times, in felty germander – 2 times; in Christ's thorn – in contrary Kvernaqi index was 2,5-times higher (Fig. 4); According to results it may be supposed that increase of the tocopherol synthesis in leaves of caper bush, spurge and felty germander is one of the stress-protective mechanisms under Iagludja conditions, while in Kvernaqi this mechanism was essential only in Christ's thorn (Fig. 4).

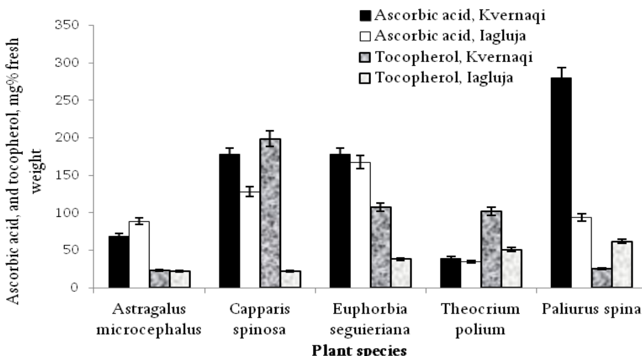

FIGURE 4: Content of ascorbic acid and tocopherol in leaves of Kvernaqi and Iagluja (East Georgia) plants

3.5 Soluble phenols and anthocyanins

Among the low-molecular antioxidans phenolic substances are distinguished by the active interaction with free radicals. Accordingly, their role in the defence of cell membrane against the oxidative stress is very high (Winkel-Shirley, 2002; Cesar, Fraga, 2010). Some papers deal with the phenolics accumulation in plant cell under water deficiency (Sharma et al., 2019).

It must be mentioned that generally high content of phenols was revealed in plants of both habitats; although in same species of the two habitats statistical similar results were received only in goat's thorn and felty germander (p=0,08) (Fig. 5). As for other species, Kvernaqi results of spurge and Christ's thorn were 2-times higher compared to Iagludja data (p<0,05), while in caper bush in contrary – Iagludja results were 2-times higher (Fig. 5).

According to results it is clear that high content of phenolics in both habitats is one of the leading stress-protective mechanisms in tested species.

One of the groups of phenolic substances – anthocyanins, which are partially concentrated in leaf epidermis, plays a role of light reflector, protecting chlorophyll from excess radiation (Gould et al., 2018). It has been established that accumulation of anthocyanins in vegetative tissues increases under various stresses (drought, intensive radiation, etc.) (Kovinich et al.; 2015; Gould et al., 2018; Kamjad et al., 2021). It is supposed that they take part in diminution of cell osmotic potential and thus retain water and turgor in the cell (Chalker-Scott, 2002).

High content of anthocyanins was mentioned in experimental plants of both studied habitats. Although, while comparing data of one and the same species growing at different locations, it is evident that in Kvernaqi individuals anthocyanins were higher, compared to Iagludja (p≤0.001); in particular results of goat's thorn were 3-times higher, that of caper bush and Christ's thorn – 2-times, and of spurge – 1,5-times higher. (Fig. 5).

According to experimental results it may be assumed that accumulation of anthocyanins, especially in Kvernaqi plants demonstrates the leading stress-protective role of these substances.

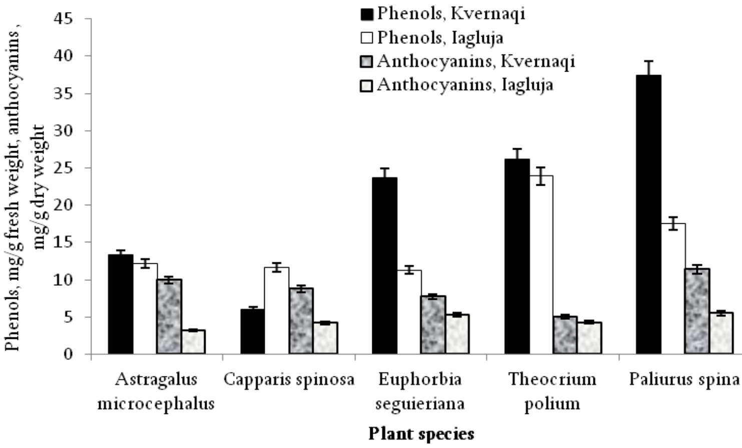

FIGURE 5: Content of polyphenols and anthocyanins in leaves of Kvernaqi and Iagluja (East Georgia) plants

3.6 Proline, total proteins and soluble carbohydrates

Other metabolites may also take part in plant protection against oxidative stress, together with antioxidants. These substances (amino acids, soluble carbohydrates, proteins) - so called osmoprotectants, are not antioxidants, but reveal similar properties and reliably protect lipo-protein components of membranes against damage (Szabados, Savory, 2010; Meng, 2014; Iqbal et al., 2020).

Many authors point to a positive role of amino acid proline in drought-resistance of plant (Kaur, Asthir, 2015; Ashraf et al., 2018). It holds water in the cell and retains its turgor (Kartashov, 2013; Joseph et al., 2015).

Individuals of one and the same species, growing at different habitats had statistically different content of proline in leaves ($p \leq 0.01$); moreover, in Iagludja species the content of amino acid was lower compared to Kvernaqi: in goat's thorn – 2,7-times lower, in caper bush – 2,5-times, in spurge – 3,3-times, and in Christ's thorn – 5,6-times lower. Only data of felty germander coincided in both habitats ($p=0,3$) (Fig. 6). Generally the content of proline in plants of both habitats was not high. Exception was Christ's thorn; especially Kvernaqi individuals of this plant were distinguished with high content of proline in leaves. Thus, it may be concluded that the stress-protective function of proline was not principal in tested plants, except Christ's thorn.

Adaptation of plants to unfavorable conditions accounts for the qualitative and quantitative changes of the composition of cell proteins (Parida et al., 2007; Mohammadkhani and Heidari, 2008). It is well-known that the synthesis of so called stress-proteins – dehydrins is activated under stress; they reveal osmolyt-type activity and take part in the stabilization of membrane proteins and cell osmotic regulation; thus protecting the cell structures against the oxidative stress (Iqbal et al., 2020; Mohammadkhani and Heidari, 2008).

Content of total proteins, like proline, was higher in Kvernaqi plants compared to the same species of Iagludja ($p \leq 0.03$): in goat's thorn it was 2,5-times higher, in caper bush – 3,2-times, in spurge – 2,2-times, and in Christ's thorn – 1,6-times higher. Again, the exception was felty germander. Proteins content in this species appeared to be higher in Iadludja individuals by 30%, compared to Kvernaqi data (Fig. 6).

It has been established that decrease of the amount of total proteins takes place under various stresses. This is explained by the outflow of nitrogenous substances from the leaf and their synthesis decrease (Sorkheh et al., 2012). At the same time the inhibition of photosynthesis and proteins proteolysis may become the reason of total proteins decline (Mohammadkhani and Heidari, 2008; Taiz and Zeiger, 2016).

Significant decrease of total proteins in Iagludja experimental species may be explained by the decline of nitrates and nitrate reductase activity in their leaves (Fig. 1). The increase of total proteins content in felty germander leaves under Iagludja conditions, which we consider to be more extreme than Kvernaqi, may be one of the individual mechanisms of protection against the severe stress.

Many authors have mentioned the accumulation of soluble carbohydrates in plant cells in response to various stresses (Prado et al., 2000; Finkelstein and Gibson, 2001; Mohammadkhani and Heidari, 2008). These substances decrease the water potential of cells and support the water retention. At the same time they protect proteins from denaturation and account for membrane stability (Couee et al., 2006; Laxa et al., 2019)

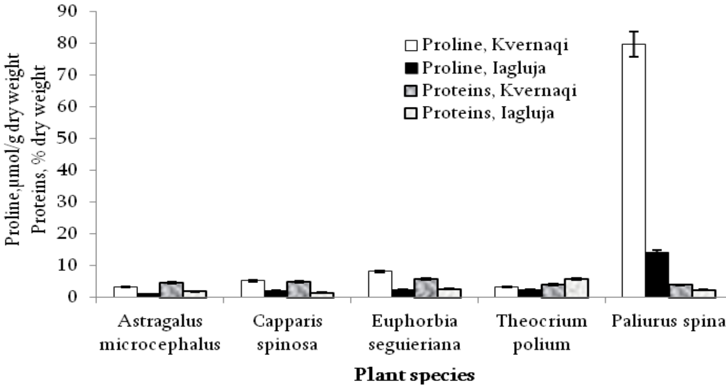

FIGURE 6: Content of proline and total proteins in leaves of Kvernaqi and Iagluja (East Georgia) plants

Evidently higher concentration of soluble carbohydrates was established in Iagludja experimental plants, compared to the same species of Kvernaqi hills ($p \leq 0.001$) (Fig. 7). The results were 6-times higher in goat's thorn, 20-times higher in caper bush, 5-times – in spurge and 1,2-times higher in Christ's thorn. It is clear that accumulation of such a big amount of soluble carbohydrates under the severe conditions of Iadlugja hill is indication to the leading stress-protective function of these substances, while in Kvernaqi hill the protective mechanism of carbohydrates was evident only in Christ's thorn (Fig. 7).

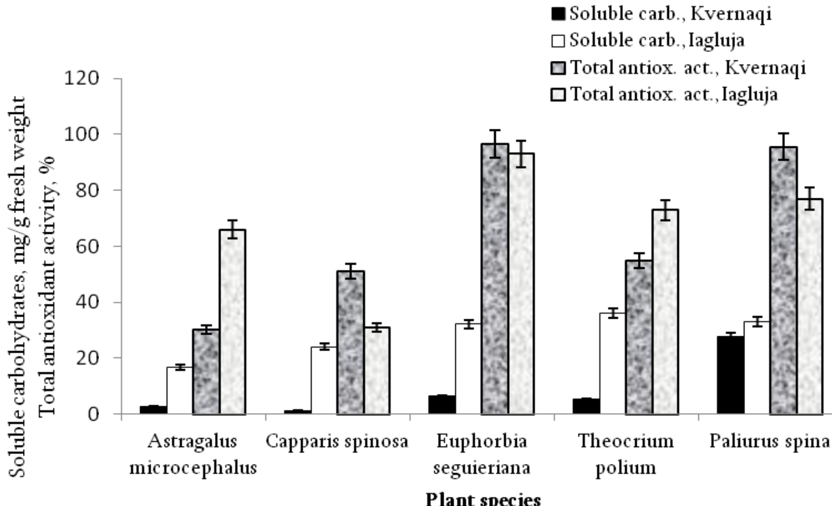

FIGURE 7: Content of soluble carbohydrates and total antioxidant activity in leaves of Kvernaqi and Iagluja (East Georgia) plants

3.7 Total antioxidant activity

Is a significant characteristic which may be used as a criterion for the evaluation of plant's stress-resistance.

The antioxidant activity of the leaves of one and the same plant species appeared to be different by habitats (except the spurge) (Fig. 7). In goat's thorn and felty germander the Iagludja data prevailed over Kvernaqi ones (2 and 1,3-times respectively), while in caper bush and Christ's thorn in contrary – the Kvernaqi results were higher (1,8 and 1,2-times respectively) (Fig. 7).

According to obtained results the antioxidant activity of caper bush leaves from Iagludja may be regarded as low, that of goat's thorn – moderate, and of spurge, felty germander and Christ's thorn - high (Luzia and Jorge, 2014). Among Kvernaqi species the low antioxidant activity had goat's thorn, moderate – caper bush and felty germander; the high antioxidant activity was revealed in spurge and Christ's thorn (Fig. 7).

Soil conditions are one of the important factors for normal development of plant. It has been established that the soil microflora significantly accounts for plant's stress-resistance by means of suitable mechanisms, and facilitates their existence under stress conditions (Bardi and Malusa, 2012; Hossain et al., 2022). Accordingly it may be supposed that local soil conditions play a significant role in experimental plants' adaptation to stress-conditions of the studied habitats as well. By our opinion, this fact must be taken into account in further observation.

IV. CONCLUSIONS

1. Inspite of similarity of climatic conditions of Kvernaqi and Iagludja hills, obtained results let assume that Iagludja conditions are more extreme for plants; which may be linked with soil conditions as well.
2. The antioxidant defence mechanisms of experimental species appeared to be partly diverse following habitats.
3. It is evident that stress-adaptive mechanisms have specific peculiarities; athough these mechanisms are determined by those factors which plant has to adapt.
4. Activation of the phenol-anthocyanin antioxidant defence mechanism against the stress conditions of both studied habitats was common in all tested plants.
5. Together with the phenol-anthocyanin mechanism of stress-protection, activation of peroxidase was expressed in all tested species of Kvernaqi hill; while in Iagludja accumulation of soluble carbohydrates was evident.

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Feather Meal as Enhancer of Protein in Starter Broiler Birds (A Case Study in Ishiagu, Ebonyi State)

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Abstract**—***The study was conducted with one hundred and forty-four (144) day old "Sayyed" broiler birds to determine the effect of replacing fishmeal with feather meal on the growth performance and cost benefit analysis of starter broiler birds. The birds were randomly distributed into four treatment groups, each comprising of three replicate per treatment with twelve birds per replicate laid out in a completely randomized design (CRD). Four isocaloric and isonitrogenous diets were formulated with inclusion of the feather meal to replace fishmeal at the rate of 0%, 25%, 50% and 75% corresponding to T1, T2, T3 and T4 respectively. Feed and water were given ad-libitum and relevant drugs and vaccination were duly administered as at when due. Proximate composition of feather meal and the experimental diet were carried out. Data obtained from proximate analysis showed that the feather meal had a crude protein of 79.50%, ether extract of 3.98%, ash content of 4.47%, crude fiber of 1.30% and nitrogen free extract of 1.54%. Growth performance was significantly (P<0.05) influenced across the treatments with control having a superior (P<0.05) value for final body weight, average daily weight gain and feed conversion ratio with values of 765.41g, 25.50g and 2.28 respectively. Cost benefit analysis showed that profit obtained and cost benefit ratio were superior in treatments 4 (75%) with values of #645.43 and 1.63 respectively. Thus, it can be concluded that feather meal up to the inclusion levels of 75% in the diet of starter broilers to replace fishmeal is viable, without any negative impact on the final weight of the birds and leads also to better profit at the short and long run.*

Keywords— Feather meal, fishmeal, starter broilers, growth performance, cost benefit analysis.

I. INTRODUCTION

The significant role played by poultry in the provision of animal protein required by man has made the poultry industry to occupy a prominent position in animal production (Olabode *et al*., 2020). In line with this indispensable position of poultry in protein supply in the daily protein intake, great emphasis is continually placed on research to ensure continued substance of the poultry industry (Ojewola and Annah, 2011). A major challenge is the increasing price of conventional feedstuff with resultant effect of shortage in animal protein and high cost of poultry production and hence poor animal protein intake among Nigerians. Thus, the low level of animal protein intake by Nigerians has generated concerns as it affects both physical and mental development in Nigerian youths and labour force. Among the ways of tackling this 'hydra' headed problem is focusing on the production of animals with higher and faster growth rate, of which one is the poultry birds. According to Olabode *et al*. (2020) poultry birds are the quickest source of meat as it matures very quickly as compared to other livestock produced in the country.

Feather meal is a by-product of processing poultry; it is made from poultry feathers by partially grinding them under elevated heat and pressure and then grinding and drying. Although total nitrogen levels are fairly high (up to 12%), the bioavailability of this nitrogen may be low. Feather meal is used in formulated animal feed and in organic fertilizer (Crawshaw, 2019). Feather meal is made through a process called 'rendering'. Steam pressure cookers with temperatures over 140°C (284°F) are used to "cook" and sterilize the feathers. This partially hydrolyses the proteins, which denatures them. It is then dried, cooled and ground into a powder for use as a nitrogen source for animal feed (mostly ruminants) or as an organic soil amendment. Feathers represents 3-7% weight of the live bird, therefore producing a considerable mass of protein (Soni *et al*., 2019).

II. MATERIALS AND METHOD

The experiment was conducted at the poultry unit of the Animal Production Department, Federal College of Agriculture, Ishiagu, Ebonyi State. The feathers were sourced from Artisan market Enugu in Enugu state where there is surplus heap of feather lying as waste from processed chickens. The feathers were adequately washed and boiled at 140-145°C for about fifty minutes. It was later dried in the sun and ground in the hammer mill using a 1.5mm screen mesh and then incorporated into the diets of the birds at the levels of 0%, 25%, 50% and 75% corresponding to treatments 1, 2, 3 and 4 respectively.

TABLE 1
EXPERIMENTAL DIET FOR STARTER BROILERS FED DIFFERENT LEVELS OF FEATHER MEAL TO REPLACE FISHMEAL

	Treatments			
Ingredients	**T1**	**T2**	**T3**	**T4**
Maize	53.5	53.5	53.5	53.5
Wheat offal	9.5	9.5	9.5	9.5
Soya bean meal	10	10	10	10
Groundnut cake	17.15	17.15	17.15	17.15
Fishmeal	3	2.25	1.25	0.75
Feather meal	0	0.75	1.75	2.25
Bone-meal	2	2	2	2
Limestone	1.5	1.5	1.5	1.5
Bloodmeal	2.5	2.5	2.5	2.5
Methionine	0.2	0.2	0.2	0.2
Lysine	0.1	0.1	0.1	0.1
Starter premix	0.3	0.3	0.3	0.3
Salt	0.25	0.25	0.25	0.25
Total	**100**	**100**	**100**	**100**
Calculated Analysis				
Crude protein (%)	23.4	23.58	23.76	23.94
Met. Energy (Kcal/kg)	2850.74	2846.16	2841.59	2832.02
Crude fiber (%)	3.68	3.59	3.53	3.44
Ether extract (%)	4.08	3.98	3.87	3.68

TABLE 2
PROXIMATE COMPOSITION OF FEATHER MEAL

Components	% Composition
Dry matter	90.79
Moisture	9.21
Crude protein	79.5
Crude fiber	1.3
Ether extract	3.98
Ash	4.47
Nitrogen free extract	1.54

The completely randomized design (CRD) was used. One hundred and forty-four (144) day old “Sayyed” broiler chicks were used for the research work. Each treatment had thirty-six birds with three replicates consisting of twelve birds each. Feed and water were given *ad-libitum* and vaccinations were given as at when due according to standard practices. The initial weight of the birds was taken at the beginning of the study and then subsequently on a weekly basis. Feed intake was also recorded

as the difference between the quantity of feed given the previous day and the quantity that was left the next day. Feed conversion ratio was obtained as the ratio of feed intake divided by the body weight gain. Data collected were subjected to analysis of variance (ANOVA) according to procedure. Significantly different means were separated according to the method of Duncan multiple range test. Proximate analysis of feather meal was carried out using the standard procedure of AOAC (2015). Cost benefit analysis was calculated using the following formulas;

- **Cost of bird =** Amount expended or spent on purchase of bird
- **Cost per kg of feed** = Cost of feed/25kg
- **Cost of feed consumed** = Total feed intake x cost per kg of feed/1000
- **Other cost**
- **Total cost of production**
- **Revenue** = Average final Weight of birds x cost per kg of current market price of 1kg meat of broiler/1000
- **Benefit/Profit** = Revenue – cost of production
- **Cost benefit ratio** = Cost of production/Benefit

III. RESULTS AND DISCUSSION

The performance characteristics of starter broilers fed replacement levels of feather meal was presented in table 3. Dietary effects showed no significant (P>0.05) difference across the treatments studied for average daily feed intake. Value of 90g was observed in treatment 1, which did not differ (P>0.05) from the values of 90.72g, 90.99g and 92.06g obtained for birds in treatments 2, 3 and 4 respectively. The progressive increase obtained numerically in the values of daily feed intake could be due to the low level of energy obtained from control treatment to treatment 4. Oluyemi and Robert (2007) had earlier reported that birds eat to satisfy their energy requirement; the lower the energy in the diet, the higher the consumption rate. This work contradicts the report of Madubuike *et al.* (2009) who observed an increase in the feed intake of broiler birds when feather meal was included in their diets. Also, Caires *et al.* (2010) observed superior (P<0.05) feed consumption in control which was closely followed by those of birds in treatments fortified with feather meal to replace fishmeal at 2.5, 7.5 and 7.5% respectively.

TABLE 3
PERFORMANCE CHARACTERISTICS AND COST BENEFIT ANALYSIS OF STARTER BROILER BIRDS FED FEATHER MEAL AS REPLACEMENT FOR FISHMEAL

	T1	T2	T3	T4	SEM
Parameters	0%	-25%	-50%	-75%	-
Initial body weight (g)	230	230	230	230	-
Final body weight(g)	965.41[a]	960.75[a]	949.19[b]	938.25[c]	11.08
Average daily feed intake (g)	90	90.72	90.99	92.06	4.67
Average daily weight gain(g)	35.02[a]	34.76[b]	34.21[b]	33.72[c]	0.92
Feed conversion ratio	2.57[bc]	2.61[b]	2.66[b]	2.73[a]	0.03
Cost of day-old chick (#)	450	450	450	450	-
Cost per kg of feed (#)	169.46[a]	156.34[b]	142.80[c]	131.68[d]	9.36
Cost of feed consumed (#)	320.28[a]	297.85[b]	272.86[c]	254.57[d]	4.92
Other expenses (#)	350	350	350	350	-
Total cost of production (#)	1120.28[a]	1097.85[b]	1072.86[b]	1054.57[b]	20.33
Revenue (#)	1700	1700	1700	1700	-
Benefit/Profit (#)	579.72[d]	602.15[c]	627.14[b]	645.43[a]	14.8
Cost benefit ratio	1.93[a]	1.82[b]	1.71[c]	1.63[d]	0.04

Results obtained for average daily weight gain revealed that birds in control had a higher ($P<0.05$) value of 35.02g which differ from those of T2 (34.76g) and T3 (34.21g), while 33.72g obtained in treatment 4 had the least value for average daily weight gain for starter broiler birds. The lower level of weight gain obtained in treatments with inclusion level of feather meal could be related to amino acid imbalance and the relatively low digestibility and biological value associated with the feather meal. The results obtained in this study agrees with the observations of Xavier *et al*. (2011) who observed reduced weight gain in starter broiler birds fed diet supplemented with feather meal to replace fishmeal. Effect of diets on feed conversion ratio were significantly ($P<0.05$) influenced. Higher ($P<0.05$) value of 2.73 was observed in treatment 4, which was significantly ($P<0.05$) different from those obtained in treatments 2 (2.61) and 3 (2.66). The lowest value of 2.57 which also represents the most viable treatment was seen in treatment 1. This suggest that the inclusion of feather meal in the diets of starter broiler birds to replace fishmeal could not sustain the increase in weight in relation to the quantity and quality of feed consumed by the birds. This agrees with the report of Ahaotu and Ekenyem (2009) who observed higher feed conversion ratio value in treatments fortified with feather meal to replace fishmeal up to 100% level of replacement. Cost benefit analysis values for benefit/profit and cost benefit ratio were significantly ($P<0.05$) better at the inclusion level of 75% corresponding to treatment 4, which was closely followed by those in treatment 3 (50%) with values of #645.43 and #627.14 and 1.63 and 1.71 for cost benefit ratio respectively. The least value of #579.72 and 1.93 for benefit and cost benefit ratio was reported in treatment 1. This was similar to the work carried out by Olabode *et al*. (2017) who observed better and cost effectiveness in treatments fortified with an alternative source of protein to fishmeal in starter broiler birds.

IV. CONCLUSION

Results obtained from the experiment showed that feather meal up to the level of 75% at starter phase can be used conveniently to replace fishmeal in the diet of broiler birds, without any negative impact on the performance of the birds and also more and better profit can be achieve especially at 75%.

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Growth Response and Cost Benefit Analysis of Starter Broiler Birds Fed Supplemental Levels of Black Plum Leaf Meal (A case study in Ishiagu, Ivo local government area of Ebonyi state).

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Abstract**—***A research work was carried out to determine the influence of Black plum leaf meal on the growth performance and cost benefit analysis of starter broiler birds. Ninety-six (96) day old 'Sayeed' broiler birds were used for the research work. The birds were brooded for a week after which they were randomly distributed into four treatment group of twenty-four each been replicated three times with eight birds per replicate. Four different diets were formulated such that the black plum leaf meal was incorporated into the diets at the rate of 2.00%, 4.00% and 6.00% respectively, while treatment 1 served as the control with 0% level of Black plum leaf meal. Feed and water were given ad-libitum throughout the experimental period of twenty-one days. Proximate composition of the Black plum leaf meal was carried out. Birds on control diet was superior to birds in other treatments in terms of final body weight, body weight gain and feed conversion ratio, which was followed closely by those of birds in treatment 3 (4%bplm). Thus, birds in treatment 1 (control) had better performance among the treatments under review. Thus, black plum leaf meal can be added into the diet of starter broiler birds up-to the level of 6% without any detrimental effect on the bird's performance. Result for cost benefit analysis showed that birds in treatment 3 had the highest (P<0.05) profit of #528.54 when compared to the other treatments under review.*

Keywords— Growth performance, cost benefit analysis, starter broiler birds, Black plum leaf meal, proximate composition.

I. INTRODUCTION

With the current emphasis on improvement of livestock production in Nigeria, foliage plants have found an application without compromising nutritional standard (Ekenyem *et al*., 2003). The inclusion of leaves in the diet of poultry birds is becoming adaptable due to its availability and phytochemical constituents responsible for medicinal or organoleptic properties of the plant (Ugwu *et al*., 2013). It is a known fact that profitable livestock enterprise depends on the availability and affordability of feedstuff. With increased interest in foliage plants as feed ingredient, several plants have been assessed with respect to their effectiveness in yielding positive results in terms of growth and performance in poultry. Some of these plants include *Napoleon imperialis, Ipomea asorfolia, Moringa oleifera, Azadirachta indica, Ipomea purpurea* etc (Adeyina *et al*., 2014). One of such foliage plants is the *Vitex doniana*. *Vitex doniana* is among plants whose leaves has potentials for improving animal productive performance. It is an indigenous tropical plant distributed across tropical and sub-Saharan Africa coastal savannas and savanna woodland. The tree is none domesticated, but it is found at the centre of West African villages. The bark, leaves and roots of the plants are used in ethno-medicine for the management and treatment of numerous disorders such as microbial infection, cancer, rheumatism, hypertension and inflammatory diseases (Atawodi, 2005). The back of the stem is aromatic and serves as blood tonic. It has also been reported that the extract of *Vitex doniana* plant lowered blood pressure.

II. MATERIALS AND METHODS

The research work was conducted at the poultry unit of the Animal Production Department, Federal College of Agriculture, Ishiagu, Ebonyi State. The black plum leaves that was used for the experiment was sourced from Ishiagu town and environment all within Ebonyi state. The black plum leaves were obtained fresh, washed and then air dried for about seven days after which it was sun-dried to get a cripsy-like leafy material. The cripsy leaves were then turn to powder by means of grinding and then incorporated into the diets of the birds at the levels of 0%, 2.00%, 4.00% and 6.00% respectively.

TABLE 1
PROXIMATE COMPOSITION OF BLACK PLUM LEAF MEAL

Components	% Composition
Dry matter	89.65
Moisture	10.35
Crude protein	10.7
Crude fiber	7.68
Ether extract	2.67
Ash	8.92
Nitrogen free extract	59.68
Metabolizable energy (Kcal/kg)	2690.25

The completely randomized design (CRD) was used. Ninety-six day old “Sayeed” broiler chicks were used for the research work. Each treatment had twenty-four birds with three replicates consisting of eight birds each. Feed and water were given *ad-libitum* and vaccinations were given as at when due according to standard practices.The initial weight of the birds was taken at the beginning of the study and then subsequently on a weekly basis. Feed intake was also recorded as the difference between the quantity of feed given the previous day and the quantity that was left the next day. Feed conversion ratio was obtained as the ratio of feed intake divided by the body weight gain. Data collected were subjected to analysis of variance (ANOVA) according to procedure. Significantly different means were separated according to the method of Duncan multiple range test. Proximate analysis of Black plum leaf meal was carried out using the standard procedure of AOAC (2005). Cost benefit analysis was calculated using the following formulas;

- **Cost of bird =** Amount expended or spent on purchase of bird
- **Cost per kg of feed** = Cost of feed/25kg
- **Cost of feed consumed** = Total feed intake x cost per kg of feed/1000
- **Other cost**
- **Total cost of production**
- **Revenue** = Average final Weight of birds x cost per kg of current market price of 1kg meat of broiler/1000
- **Benefit/Profit** = Revenue – cost of production
- **Cost benefit ratio** = Cost of production/Benefit

TABLE 2
EXPERIMENTAL DIET FOR STARTER BROILERS FED SUPPLEMENTAL LEVELS OF BLACK PLUM LEAF MEAL

	Treatments			
Ingredients	**T1**	**T2**	**T3**	**T4**
Maize	52	51	50	49
Wheat offal	7.25	6.75	6.75	6.25
Soya bean meal	6.6	6.6	6.1	5.6
Full fat soya	5	5	5	5
Groundnut cake	17.5	17	16.5	16.5
Fish meal (72%)	3.5	3.5	3.5	3.5
Blood meal	3.5	3.5	3.5	3.5
Black plum leaf meal	0	2	4	6
Limestone	1.5	1.5	1.5	1.5
Bonemeal	2	2	2	2
Methionine	0.35	0.35	0.35	0.35
Lysine	0.2	0.2	0.2	0.2
Starter premix	0.35	0.35	0.35	0.35
Salt	0.25	0.25	0.25	0.25
Total	100	100	100	100
Calculated value				
Crude protein (CP) (%)	23.5	23.34	23.02	22.85
MEnergy (Kcal/kg)	2872.5	2868.1	2863.86	2860.12
Crude fiber (%)	3.47	3.53	3.61	3.66
Ether extract (%)	4.55	4.49	4.46	4.44
Calcium (%)	1.25	1.27	1.29	1.29
Phosphorus (%)	0.48	0.49	0.49	0.49
Methionine (%)	0.72	0.72	0.72	0.72
Lysine (%)	1.3	1.3	1.3	1.3

III. RESULTS AND DISCUSSION

The proximate analysis showed that the black plum leaf meal (bplm) had a low crude protein value of 10.70%, high ash content of 8.92%, low ether extract value of 2.67% and a moderate level of crude fiber (7.68%). The result of growth performance and cost benefit analysis of starter broiler bird is revealed in table 3. Superior ($P<0.05$) value of 987.50g for final body weight was seen in treatment 1 (control) which did not differ ($P>0.05$) from those of birds in treatments 2 and 3 with values of 979.17g and 983.33g, while the least value of 904.17g was obtained in treatment 4 respectively. The decrease in weight of birds on *Vitex doniana* leaf meal-based diets suggest that the bioactive substance in the black plum leaf meal could not support increase in body weight to a greater extent as compared with the control. Also, it could be due to the inability of the birds at the starter phase to utilize to the fullest some of the growth promoting substances like vitamins, micro-minerals and the phytochemicals contained in the *Vitex doniana* leaf meal. The results obtained in the present study disagrees with the report of Nnamani *et al.* (2007) who observed higher weight in birds fed the *Vitex doniana* leaf meal diets. Feed intake value was highest ($P<0.05$) in treatment 2 (4%bplm) with a value of 1391.67g. But the lowest value of 1325.10g was observed in treatment 4. Birds in treatment 1 and 3 had feed intake values of 1366.68g and 1358.28g respectively. Result for feed conversion ratio showed that birds in treatment 1 had the lowest feed conversion ratio of 1.81, which happens to be the best performed treatment which was followed closely by birds in treatment 3 (1.85). Birds in treatments 2 and 4 had similar ($P>0.05$) values of 1.92 and 1.96 respectively. This result differed from those reported by Adeyina *et al.* (2017) who observed better performance in birds fed *Vitex doniana* leaf-based diet. This showed that the constituents of phytochemical

may not have contributed to the beneficial properties of *Vitex doniana* in affecting feed improvement. Data obtained for cost benefit analysis showed that there was significant difference (P<0.05) in the values obtained across the treatment groups for cost of kg of feed, total expenses and net profit. While that of cost of day-old chicks, other expenses and income from sales of birds did not differ (P>0.05). The highest (P<0.05) value for profit was obtained in treatment 3 (#528.54), while the least value of #403.17 was obtained in treatment 4. Treatment 2 had value of #496.77 which differ (P<0.05) from those of #485.35 obtained in treatment 1 respectively.

TABLE 3
GROWTH PERFORMANCE AND COST BENEFIT ANALYSIS OF STARTER BROILER BIRDS FED DIFFERENT LEVELS OF BLACK PLUM LEAF MEAL (BPLM)

Parameters	T1 (0.00%Bplm)	T2 (2.00%Bplm)	T3 (4.00%Bplm)	T4 (6.00%Bplm)	SEM
Initial body weight (g)	233.33	254.17	250	229.17	-
Final body weight (g)	987.50[a]	979.17[a]	983.33[a]	904.17[b]	13.47
Body weight gain (g)	754.17[a]	725.00[c]	733.33[b]	675.00[d]	8.79
Feed intake (g)	1366.68[b]	1391.67[a]	1358.28[b]	1325.10[c]	7.31
Daily feed intake (g)	65.08[a]	66.27[a]	64.68[a]	63.10[b]	0.5
Daily bodyweight gain (g)	35.91[a]	34.52[a]	34.92[a]	32.14[b]	0.53
Feed conversion ratio	1.81[b]	1.92[a]	1.85[b]	1.96[a]	0.03
Cost of birds at day old (₦)	560	560	560	560	-
Cost of Kg of feed (₦)	279.62[a]	255.62[b]	244.02[c]	237.22[d]	4.89
Cost of feed consumed (₦)	382.15[a]	355.74[b]	331.45[c]	314.34[d]	7.74
Other cost (₦)	350	350	350	350	-
Total cost of production (₦)	1292.15[a]	1265.74[b]	1241.45[c]	1224.34[d]	7.73
Revenue (₦)	1777.50[a]	1762.51[c]	1769.99[b]	1627.51[d]	18.7
Benefit/profit (₦)	485.35[c]	496.77[b]	528.54[a]	403.17[d]	13.98
Cost benefit ratio	2.66[b]	2.55[c]	2.35[d]	3.04[a]	0.08

[abcd]Means on the same row with different superscripts are significantly (p<0.05) different.

Note:

* Bplm = Black plum leaf meal
* A kg of broiler meat cost #1800

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Climate Change, Ultraviolet-B Radiation and Effects on Plants: A Review

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***Abstract*—*Climate change induces variations in environmental conditions and severely affects agricultural crop productivity and yield. The gradual depletion of the stratospheric ozone layer in the atmosphere has led to an increase in solar ultraviolet-B radiation (UV-B) reaching the earth's surface. Relatively little information exists on the effects of UV-B radiation on field crops. The aim of this review is to summarize the results of recent studies on the interaction between climate change, in particular the increase in UV-B radiation, and crops, in terms of yield, stress damage, defense and quality. Adaptive mechanisms, such as the increased production of secondary metabolites in leaf tissues under enhanced UV-B radiation, are also described.*

Keywords— Agriculture, secondary metabolites, stress damage, defense.

I. INTRODUCTION

Climate change has occurred mainly due to fossil fuel burning and rise in concentration of harmful greenhouse gases (GHGs) in the atmosphere during post-industrialization era. It induced variation in environmental conditions severely affect agricultural crop productivity and yield. Gradual depletion of stratospheric ozone layer in atmosphere has lead to increase in solar ultraviolet-B radiation (UV-B) that reaches earth surface. Relatively little information exists on the effects of UV-B radiation on field crops. The aim of this review is to summarize the results of recent studies on the interaction between climate change, in particular the increase of UV-B radiation, and crops, in terms of yield, stress damage and defence and quality.

1.1 Climate change

The climate on Earth has always followed natural cycles linked to the variation of solar insolation resulting from changes in some parameters of the Earth's orbit. By now it is incontrovertible that the climatic changes that are currently occurring are different and more dramatic than those that have marked the history of our planet: the greater concentration of greenhouse gases in the atmosphere and the rise in temperature and in consequent sea levels have never been so rapid and had such far-reaching consequences. Extreme climatic events, droughts, storms and catastrophic floods are and will be increasingly intense and frequent. As evidenced by periodic reports from the UN's IPCC (Intergovernmental Panel on Climate Change), the decade 2010-2019 was the warmest on record since reliable and regular records have been kept.

Starting from the 1980s, each subsequent decade has been increasingly hot, with increasingly frequent climatic events.

Human activities are primarily responsible for this problem, with a 147% increase in greenhouse gases in the atmosphere, such as CO_2, compared to pre-industrial levels. Therefore, studies on climate change play an increasingly central role in multidisciplinary scientific research involving researchers from various cultural backgrounds: agronomists, foresters, ecologists, botanists, zoologists, naturalists, geologists, engineers, even physicians and sociologists.

Climate change, recognized all over the world by the scientific community, is already having a strong impact on the world population due to changes in the yields, qualitative characteristics and water requirements of agricultural crops [1,29].

There are two elements to consider when it comes to climate change: the greenhouse effect and the thinning of the ozone layer.

While the former can be considered a normal phenomenon for regulating the temperature of our planet, the problem arises when, with the increase of so-called "greenhouse gases" such as CO_2, CH_4 and N_2O, in the atmosphere, an increasing amount of thermal energy from the sun raises seasonal temperatures.

The temperature of the planet has always reported risen in modern times, starting from 1880 until today, rising by about 1.5° C over the period, a trend that shows no signs of stopping.

The ozone layer is fundamental for the survival of every living species, since it creates a "shield" in the atmosphere against harmful radiation from the sun.

Its gradual decrease in thickness is mainly due to the release into the atmosphere of chlorofluorocarbons (CFCs) [54].

The thinning phenomenon is currently present at the South Pole and is expanding at a rate of 5% every 10 years. This represents a huge risk for survival, since the vanishing of the "shield" effect allows an ever increasing quantity of ultraviolet rays of the types B (UV-B) and C (UV-C) to enter into the atmosphere [35].

Most of our knowledge on the effects of UV-B radiation on plants derives from studies on economically important crops, most of which have emerged as sensitive to UV-B rays. Sensitivity differs between cultivars of the same species [30].

1.2 UV rays and higher plants

Plants are sessile autotrophic organisms that must constantly adapt to changes in the surrounding environment. Plant adaptation to UV rays has been of particular interest to researchers in recent years.

UV rays represent a range of electromagnetic radiation with a well-defined wavelength, divided into 3 specific regions: UV-A, with a wavelength ranging from 315 nm to 400 nm, UV-B, with a wavelength from 280nm to 315 nm, and UV-C, with a wavelength from 200 nm to 280 nm [27].

The global changes in the chemical composition of the atmosphere, with a substantial reduction of the protective ozone layer, have led to an ever more worrying increase in solar radiation on the earth, in particular UV-B rays [2].

Currently, the levels of UV-B reaching the earth during the harvest season are roughly between 2 and 12 kJ m^{-2} per day at the earth's surface, with a 6% to 14% increase in UV-B radiation compared to levels recorded before 1980 [3].

Under normal conditions, only rays included in the UV-A spectrum and a small portion of UV-B rays (0.5%) reach the earth's surface. The changing composition of the atmosphere however is allowing an ever greater passage of the latter rays. Although they are a minor component of sunlight, they may potentially cause biological damage that is a cause for concern, due to the high energy emitted (3.94 - 4.43 eV) [4].

Numerous studies have already shown that the increase in UV-B rays has significant effects on the yield and on the morphological, physiological and biochemical processes of many crops.

Radiative stress has been classified as an abiotic stress.

These notes will briefly examine the various effects found in some herbaceous crops, and the defense mechanisms developed to counteract damage from UV-B rays.

1.3 UVB rays and damage

1.3.1 UV-B and effects on yield

The deleterious effects of enhanced UV-B radiation vary markedly within and among species [36,37].

Zeng et al. [38] showed for winter wheat that the supplemental UV-B can cause a decrease in yield of winter wheat of up to 24% with an 11.4% increase in UV-B.

One species that has been studied extensively is soybean (*Glycine max*). Two soybean cultivars grown with enhanced UVB radiation showed conflicting sensitivities. One of the Essex cultivars showed substantial reductions (20-25%) in yields, while another, the Williams cultivar, was not affected by the increase in UV-B radiation [31].

UV-B rays interfere with the photomorphogenic development of plants and give rise to numerous regulatory effects on the morphology, development, physiology and biochemical composition of plants [5].

II. UV-B RAYS AND MORPHOLOGICAL EFFECTS

The symptoms most visible to the naked eye, referring to stress from overexposure to UV-B rays, are certainly morphological in nature. The appearance of the plant can often differ appreciably from the reference ideotype. Changes in the color and shape of leaves have been observed in several species. The initial appearance of yellow-brown punctate areas, which may be a symptom of chlorosis, necrosis or desiccation of the leaves, may be followed, with prolonged exposure to UV-B rays, , by an involution of the leaves with a classic cupped shape, resulting in a decrease in leaf volume, decrease in the number of stomata, smaller stomata and an increase in axillary branching. Prolonged exposure, after the appearance of the aforementioned symptoms, leads to the drying and fall of the leaf.

The presence of chlorotic and necrotic patches has been attributed to the decrease in the chlorophyll content in the leaves further to exposure to UV-B rays [6].

Chlorotic and necrotic symptoms are not exclusive to UV-B radiation, in various studies they have also been found in plants subjected to both nutritional and mineral deficiencies, in particular sodium, potassium, magnesium, iron, manganese, copper, chlorine and nitrogen [7], and exposed to environmental pollutants.

Among the morphological effects that were found, size reduction was highlighted in various plant species after exposure to UVB rays, for example in the cucumber, [8] after two weeks of exposure to UV-B rays, the stem of the plant had reached only 68% of its normal development in length compared to that of plants not subjected to any radiation treatment.

The decrease in elongation growth was not limited to plant height only; a decrease in the elongation of the petioles was also found.

The effects of UV-B radiation were particularly pronounced for younger leaves, with a decrease in growth of more than 40% compared to the control sample.

Like other visible effects on a morphological level, the length of the hypocotyl decreased in various model species, such as *Arabidopsis thaliana* [38], with a reduction in fresh weight, reduction in root growth and decrease in cell wall thickness.

Furthermore, many studies have reported that UV-B radiation can delay or postpone the development phases of alpine plants and the beginning of flowering of other plants [32,33]. A delay in growth is seen as an advantage in some particular situations [34].

III. UV-B RAYS AND EFFECTS ON PHOTOSYNTHESIS

When it comes to plant metabolism, the main reference process is certainly chlorophyll photosynthesis, one of the metabolic processes most sensitive to the effects of UV-B rays.

Various researches have shown that the photosynthetic apparatus is the main target of damage from UV-B rays, since it is responsible for receiving the light signal to transform it into energy and organic substance useful for the plant [2,12,14,26, 27,28].

There are various negative effects on the photosynthesis process, depending on species and cultivars, experimental conditions of growth, state of growth of the plant, the relationship between PAR and dosage of UV-B rays and finally interaction with other environmental stress factors. (e.g. cold, drought, mineral availability) [2].

The most immediate effects include: damage to proteins and photosynthetic pigments leading directly to an inhibition of the process itself [2], decreasing plant yield in terms of both growth and production. damage to the thylakoid membranes, damage to photosystem II, which can extend to photosystem I [9], reduction in CO_2 fixation, decrease in dry weight, starch and chlorophyll contents [5].

Indirectly, these cascading effects lead to other problems in the plant, such as: the reduction in gas exchange efficiency due to the closure of the stomata; the change in thickness and anatomy of the leaf, which directly modifies foliage, already mentioned among the morphological effects; this also has an effect on the photosynthetic rate and plant yield [39].

The cause of the damage to the photosynthesis process is to be found in the proteins and pigments that are directly related to this process.

Since proteins in plants can absorb UV light, as can pigments, both can be easily damaged.

Marwood and Greenberg [10] reported that exposure to UV-B rays of different wavelengths leads to a selective destruction of chlorophyll, in particular chlorophyll a, with regard to both its biosynthesis and precursors.

Also, Swarna et al. [11] reported that UV-B rays affect the photochemistry of PS II with an attested loss of functionality of 68%; this inhibition is closely related to the extent of lipid peroxidation of thylakoid membranes, studied in maize leaves.

Oxygen evolving complex (OEC) also appears to be sensitive to UV-B rays [12]. Damage to the PS II reaction center occurs mainly in the water oxidizing manganese cluster (Mn), which causes the electron transport chain to be inactivated.

Rubisco (ribulsio-1,5-bisphosphate carboxylase/oxygenase), one of the most abundant enzymes present in the leaves, is particularly vulnerable to exposure to UV-B rays. Increased UV-B radiation causes reductions in both Rubisco activity and content in many crop fields, such as rapeseed (Brassica napus L cv. Topas) or the pea plant (Pisum sativum L., cv. Greenfeast) [13,55,15].

However, the effects of UV-B rays cannot always be seen as negative. Many studies have reported that UV-B radiation can delay or postpone the development phases of alpine plants and the beginning of flowering of other plants, such as Ursiniaanthemoides L., Senecio elegans L., Gladiolus carneus Delaroche and corn (Zea mays L.) [32,33].

IV. UV-B RAYS AND THEIR EFFECTS ON BIOCHEMICAL BEHAVIOR

Finally, the biochemical behavior of plants is influenced both positively and negatively by the activity of UV-B rays.

The biochemical effects of UV-B include the accumulation of flavonoids in the epidermis of the plant organism, providing a shield to protect the plant [16,17].

At low doses of fluency, UV-B stimulates some genes responsible for the production of flavonoids and phenolic compounds [18].

High doses of UV-B radiation produce damage to biomolecules, generating reactive oxygen species (ROS), which can cause the oxidation of lipids and proteins, damage to DNA [19] and enhancement of lipid peroxidation [17,20]. To lessen the impact of ROS, the plant produces antioxidants such as ascorbic acid and alpha-tocopherol [19,20] and antioxidant enzymes such as superoxide dismutase, and ascorbic acid peroxidase, glutathione reductase, and guaiacol peroxidase [16,20,21].

It is interesting to focus on ROS production in plants.

Oxidative stress in plants is the result of an imbalance in the accumulation and removal, within the plant tissues, of oxidizing compounds such as free radicals and reactive oxygen species, which include hydrogen peroxide (H_2O_2), superoxide anion (O_{2-}), hydroxyl radical (OH) and other highly oxidizing molecules. These molecules target membrane lipids, proteins and nucleic acids with their oxidizing action, leading to an accumulation of H_2O_2 in the affected tissues.

The most serious effects can determine more or fewer point mutations, and macroscopically damage the DNA itself by altering the chemical structure of the nitrogenous bases and, by interrupting transcription, influencing gene regulation models, reducing protein synthesis [22,23].

Oxidative damage to DNA is therefore capable of having a profound impact on cell growth and development, which can have serious consequences for the entire organism.

The most common form of protection against reactive oxygen species is that which uses enzymes responsible for the conversion of ROS into less reactive and toxic products for the cell. In the past these ROSs were considered as only harmful to cells, now it is recognized that redox regulation involving oxidative stress products is also an important factor in modulating cellular activity [24].

The accumulation of hydrogen peroxide in plant tissues acts as a signal molecule for the activation of many plant genes, including those defending the plant, programmed cell death, apoptosis and genes for the biogenesis of peroxisomes, very important organelles for the detoxification of toxic molecules [25].

V. CONCLUSIONS

Solar UV-B radiation and its potential effect on global agriculture is a major concern for the future. UV-B radiation has very important photobiological effects, from various points of view. This leads to the assertion that rising UVB radiation will have deleterious effects on chlorophyll photosynthesis and crop productivity on a large geographical scale.

It has been ascertained that UV-B radiation seriously damages the photosynthetic apparatus and membrane of plants, alters protein content and enzymatic activity, damages DNA, and transforms the chemistry of the leaves.

Morphological damage, such as stunted plant growth, discoloration of leaves or reduction of vegetative biomass, must also be taken into consideration. UV-B radiation may directly contain some characteristics of cell division and key growth, which must retard the growth rate of plants. This growth retardation has been recognized as one of the UV-B radiation protection mechanisms.

In the future it will be important to develop study methodologies not only to estimate the effects of exposure to radiation and other abiotic and non-abiotic stresses. This is because environmental stresses act in isolation, but often act in synergy with other similar conditions, such as drought and extreme heat.

Further studies seek to understand the responses of plants to UV-B radiation and other variables of climate change, in particular temperature, ozone, drought conditions and mineral treatments.

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Optimized Supplementation Ratio of Wheat Flour and African Yam Bean Flour for Best Possible Bread Specific Volume and Crumb Hardness in Nigeria

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Abstract— *An optimized substitution mix ratio for of Wheat flour and African Yam Bean flours (AYB) was developed in this study. Wheat flour was substituted with African Yam Bean flours at different mix ratios of 90% to 100% of wheat flour and 0% to 10% of AYB flour. The experiment was conducted in I-Optimal mixture design by Design-Expert Software version 12. The dough Composite was prepared in different mix ratios according to the design matrix and subsequently baked under the same conditions and analysed for the following loaf quality attributes: Loaf Specific Volume and Bread Crumb Hardness as response variables. The objective functions were to maximize Loaf Specific Volume and minimize Bread Crumb Hardness to obtain the most acceptable product to consumers. Predictive models for the response variables were developed with the coefficient of determination (R^2) of 0.7500 for Loaf Specific Volume (LSV) and 0.8734 for Bread Crumb Hardness (BCH) at 95% confidence interval (CI). The predicted optimal substitution ratio was obtained as 96.833% Wheat flour and 3.167% AYB flour to yield Loaf Specific Volume was 2.106cm^3/g, Bread Crumb Hardness was 24.778N. With this result, it is inferred that substituting up to 3.167% of AYB flour into wheat dough formulation would optimise the LSV and BCH of the Wheat-AYB bread at the resulting mix ratio of 96.833:3.167. The benefit of the results of this study to bread industries is the reduction in the cost of bread by consequent reduction in the quantity of imported wheat flour utilization.*

Keywords— Bread, wheat, African Yam Bean, Flour, Dough, Specific Volume, Hardness.

I. INTRODUCTION

Bread is the most widely consumed food by all social classes around the world. It represents about 80% of the baked products sub-sector in Nigeria (KPMG, 2016). However, bread is relatively expensive in tropical countries where wheat is imported for bakery industries (Eleazu et. al, 2014). This is because wheat is a temperate crop that will not do well under tropical conditions as a result of unfavourable soil and climatic conditions (Abdelghafor, 2011).Whole-wheat grain consists of the entire grain, and, unlike refined grains, they still contain bran and germ, which are rich in dietary fibre and micronutrients (Kyro and Tjonneland, 2016).Whole-grain foods are associated with reduced risk of several chronic diet-related diseases and it contains a lot of compounds with reputed health benefits (Dalton *et al.*, 2012).

The bread industry commands a large market share in Nigeria. According to KPMG (2016), the bread industry is worth ₦122.1 billion and about 72% market share is allocated to the small and medium-sized bakeries. Majority of the bread consumed in Nigeria are baked by small and medium scale bakeries and come in different sizes that suit the consumer's need, whose standard could be compromised by inadequate quality control. There is a large market for bread in Nigeria with population of over 200 million people with an estimated national population growth rate of 5.7% per annum, and an average economic growth rate of 3.5% per annum in the past 5 years with all social classes consuming it as a staple food.

Nigeria, being a tropical country in sub-Saharan Africa, does not have the agronomic variables that support profitable and economical production of wheat. This circumstance causes bakeries to depend highly on imported wheat for bread and other

confectionaries. Foreign Agricultural Service (FAS) Lagos under the United States Department of Agriculture forecasts that wheat imports in Marketing Year (MY) 2019/20 is estimated to be 5.6 million metric tons, which is up by almost 4% compared to MY 2018/19. The rise in wheat consumption is attributable to the population growth of about 2.54% (2015-20), with Nigerians increasingly consuming greater amounts of wheat-flour based products (USDA-FAS, 2019). Successive governments in Nigeria, in a bid to reduce the increasing cost of wheat importation, enacted wheat control policies ranging from a complete ban in the years 1987 to 1991, via composite flour approach, which included 5% cassava inclusion from 2007 to 2010, 10% cassava inclusion from 1979 to 2007 and up to 40% cassava inclusion in 2012 (Ohimain, 2015). These policies have not been successful due to the undesired physical quality of wheat-cassava bread.

Though Wheat gluten plays a vital role in the quality and structure of baked bread, a small fraction of the flour matrix can be replaced with other non-wheat flour without affecting its final bread quality.

The use of local and available flours which could produce similar characteristics as gluten or support the available gluten strands during fermentation, proofing and baking would make the applicability of composite flour technology successful. Shittu *et al.* (2007) defined composite flours as either binary or ternary mixtures of flours from some other crops with or without wheat flour. The reason for considering composite flours in various food products has been to exploit the economic advantage of reducing or even eliminating the cost of imported wheat in bakery and pastry products by partial or complete use of domestically grown products instead of wheat.

African yam bean (AYB) (*Sphenostylis sternocarpa L.*) is a drought-tolerant legume crop grown primarily as a food in West Africa, especially southern Nigeria. It grows well even on acidic and highly leached sandy soils. It is the second biggest and one of the most economically important within the legume family. Despite its abundant yielding potentials, the crop is still largely under-exploited and under-utilized (IITA, 2010). It is expected that its successful inclusion in bakery products will enhance the value and importance to the crop.

In spite of huge investments made by the Federal Government in the cassava bread initiative over a period of 34 years, the project has not achieved (Punch Newspaper, December 19, 2016). According to Eduardo *et al.* (Eduardo *et al*., 2013) commercial production of wheat-cassava bread is still difficult due to the impaired bread structure. Consequently, research has been in progress to enhance the specific volume and texture of wheat-cassava bread by adding bread enhancement substances such as hydrocolloids, enzymes, and emulsifiers (Shittu *et al.,* 2009; Serventi, 2020; Eduardo *et al*., 2014). *Shittu et al., (2007)* has shown that bread with improved specific volume and crumb softness can be made from composite cassava-wheat flour with added xanthan gum. But very little has been done by utilizing this native and underutilized crop of Nigerian origin to improve the quality of wheat-cassava bread. Also, utilizing a non-gluten protein may help compensate for some lost protein by substituting more fraction of wheat flour and therefore can help in proper shaping of the bread structure and texture (Ziobro *et al*., 2016). The specific volume of a loaf is a good indicator of bread quality. It represents the ability of gluten strands to retain enough gas released during fermentation and dough proofing. Higher gas retention ability will lead to a higher specific volume (Nada and Hasan, (2015); (Franco *et al*., 2020). It has been reported in the literature as a definite measure of loaf size (Shittu *et al.*, 20017; Purna *et al*., 2011). It is also used by the Standard Organization of Nigeria (SON) as a measure of standard bread quality (NIS, 2004). It is the ratio of loaf volume (cm^3) to loaf weigh (g). The objective of this paper therefore to develop an optimized substitution mix ratio of wheat flour and AYB flour production of an acceptable bread quality.

II. MATERIALS AND METHODS

2.1 Materials

A creamy variegated African Yam Bean (AYB) seed (*Sphenostylis stenocarpa*) and Commercial grade hard wheat grain were purchased at a local commodity market in Kubwa, Federal Capital Territory Abuja in Nigeria. Dough preparation and bread baking were done in the food Science Department of Federal University of Technology, Abeokuta in Nigeria.

The flour of the two crops was prepared as shown in the process flow charts of Figure 1.

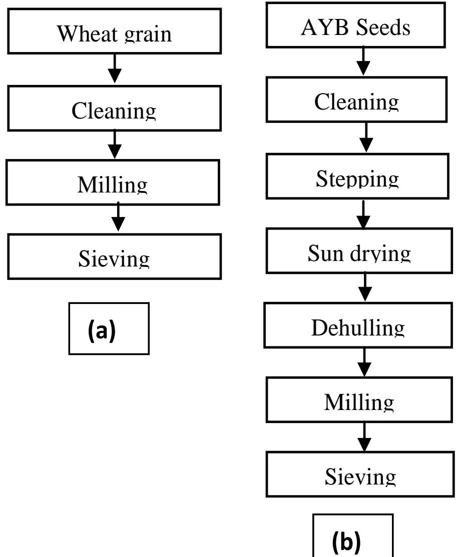

FIGURE 1: Process Flow process for the flours production: (a) Wheat, (b) AYB

2.2 Methods

The process flow chat for the Bread baking was shown in Figure 2. The composite flour was mixed intimately to attain uniform distribution of flour particle for a period of 10 minutes, while kneading was done manually for 7 minutes to develop the gluten structure of the dough when hydrated. Dough proofing was done in a proofing chamber set to 37°C for 30min between 78–80% Relative Humidity before baking in a preheated oven at about 220°C for 20 minutes.

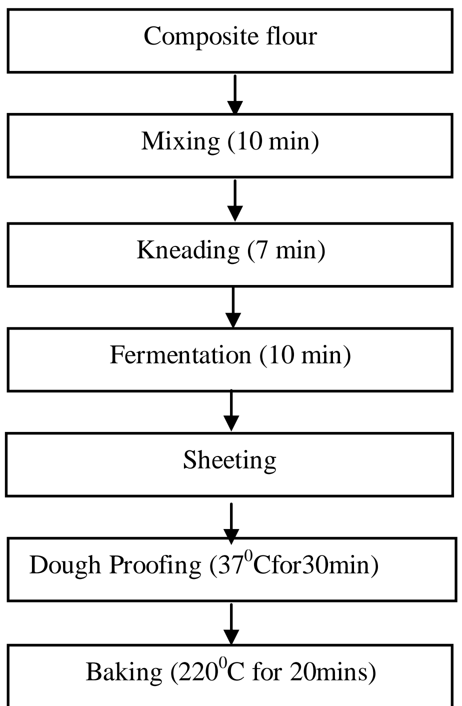

FIGURE 2: Process Flow chart for bread production

2.3 Modeling and Optimization

The experiment was designed in I-Optimal, 2-Mixture design using Design Expert version 12.0BL2 (2017-2015). The design matrix is shown in Table 1.

TABLE 1
THE DESIGN MATRIX OF THE EXPERIMENT

Run	Component 1 A: Wheat %	Component 2 A: AYB %	Response 1 Crumb Hardness N	Response 2 Specific Volume cm^3/g
1	100	0		
2	95	5		
3	95	5		
4	90	10		
5	100	0		
6	90	10		
7	97	3		
8	95	5		
9	95	5		

Equation 1 represents the model used in the optimization study to express the behaviour of the system responses which are the crumb hardness (N) and specific volume (cm^3/g), each as a function of the mix ratios (Wheat **:** AYB).

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \varepsilon \quad (1)$$

Where, β_0 is the offset term or model constant; β_1, β_2, are the linear or first order terms, is the random error term that allows uncertainties between the experimental and predicted values.

Therefore, by using this design, a total of nine (9) experimental runs were conducted. The experiments were performed in random order to avoid systematic error.

The acceptability of the models depended on the p-value of the analysis of variance and the value of the correlation coefficient (R^2) as shown in Equation 3.

The goodness of fit of the model, which was estimated by the coefficient of determination (R^2), was obtained from Equation 3.

$$R^2 = 1 - \frac{\sum_{i=1}^{n}\left(Y_{i,pre} - Y_{i,exp}\right)^2}{\sum_{i=1}^{n}\left(Y_{i,exp} - Y_m\right)^2} \quad (3)$$

Where $Y_{i,pre}$ is predicted value of the response variable, $Y_{i,exp}$ is the expected (experimental) value of the same variable, n is the number of experiments and Y_m is he mean value f the variable.

I-optimal Mixture design (custom) method of Design Expert (version12, 2007-2015, Stat-Ease Co., USA) was used for the modelling and optimization of response variables using experimental data. Substitution levels of the mix components ranged from 90% to 100% for wheat flour and from 0% to 10% for AYB flour. Proofing and baking periods were constant for all the experimental samples. Baker's ingredients percentages by the total mass of flour used in this study is shown in Table 2.

TABLE 2
PROPORTION OF INGREDIENTS USED FOR ALL FORMULATION

Ingredient	Baker's Percentage	Composition
Composite flour	100%	151.6 (g)
Water	62%	94 (ml)
Yeast	3%	4.5 (g)
Salt	2%	3.0 (g)
Sugar	4%	6.1 (g)
Margarine	3%	4.5 (g)

(Source: NIS-470, 2004)

2.4 Determination of Loaf Specific Volume

The weights (g) of baked bread samples were taken after sufficiently cooling in desiccators by using a digital balance (0.01 g accuracy). The bread loaf volume (cm^3) was determined using a modified standard seed displacement method (AACC, 2000). Millet seeds were used for volume displacement experiment. The container used for measurements was a pan of 14.9cm × 9.3cm × 7.8cm. The loaf was placed in the container of known volume (1080.846 cm^3) into which millet seeds were poured until the container was filled to the brim and levelled off by a straight-edged baton moving in the direction of the container's length axis.

The volume of seed displaced poured into the container subtracted from the volume of the container gives the loaf volume (cm^3) as shown in Eqn. (4).

$$Loaf volume\ (cm^3) = vol. of container(cm^3) - vol. of poured grain(cm^3) \tag{4}$$

The loaf specific volume was calculated by dividing volume (cm^3) by loaf weight (g) taken 24 hrs after baking as expressed in Eqn.(5).

$$Loaf Specific Volume, LSV\,(\text{cm}^3/g) = \frac{Loaf volume\ (\text{cm}^3)}{Loaf weight\ (g)} \tag{5}$$

The minimum acceptable specific loaf volume for whole wheat bread as specified by Standard Organization of Nigeria (SON) is 2.0 cm^3/g (NIS, 2004).

2.5 Bread Crumb Hardness

The textural analysis was carried out to determine crumb softness/hardness. The textural analysis of the bread crumb was done using a Perten Instrument (TVT-300XPM) by double-cycle compression Texture Profiles Analysis (TPA) method. The bread loaf was sliced into a cube of 25mm thickness using a stainless kitchen bread knife. The extreme sides (back) of each loaf were discarded. For the TPA a 25mm-diameter probe was inserted into the prepared sample cube at 40% strain, speed of 2mm/s and trigger force of 5g (Dvorakova et al., 2012). The TPA data were used to determine the crumb hardness.

III. RESULTS AND DISCUSSION

3.1 Bread Crumb Hardness

In this study, Bread Crumb Hardness (BCH) ranged from 22.35 to 34.09 N. The crumb with 100% wheat flour and 0% AYB has the least BCH which is not within the optimization objectives. This result can be explained by the fact that more gluten strands contained in wheat are available for raising the dough during fermentation/proofing which, will eventually form a light textured and less compact crumb (Nwatu *et. al.*, 2020). BCH was at maximum when the mix proportion of wheat flour and AYB flour was 90% and 10% respectively. This can also be interpreted as a result of insufficient gluten strand available for rising, therefore forming a more compact and impaired bread structure (Nwatu *et. al.*, 2020).

Models were developed to predict each of the studied response variables (Bread Crumb hardness and Loaf Specific Volume) for any selected mix ratio with coefficient of determination (R^2) of 0.8734. The analysis of variance (ANOVA) table for BCH data is presented in table 3. The **Model F-value** of 48.31 implies that the model is significant. There is only a 0.02% chance that an F-value this large could occur due to noise. **P-values** less than 0.0500 indicates that model terms are significant. In this case the linear terms of Wheat (A) and AYB (B) in the model are significant. P-Values greater than 0.1000 indicate the model terms are not significant.

TABLE 3
ANOVA TABLE FOR BREAD CRUMB HARDNESS

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	206.74	1	206.74	48.31	0.0002	significant
Linear Mixture	206.74	1	206.74	48.31	0.0002	
Residual	29.95	7	4.28			
Cor Total	236.70	8				

The predictive equation for BCH in terms of actual factors for the bread specific volume is presented in Eqn. 6.

$$BCH\ (N) = 0.2106 * Wheat\ (\%) + 1.385 * AYB\ (\%) \tag{6}$$

The coefficient of determination (R^2) of 0.8734 represents a good fit at p<0.01, which indicates the suitability of the model to predict the response with a good degree of accuracy. The response plots are shown in Figure 3(a,b,c).

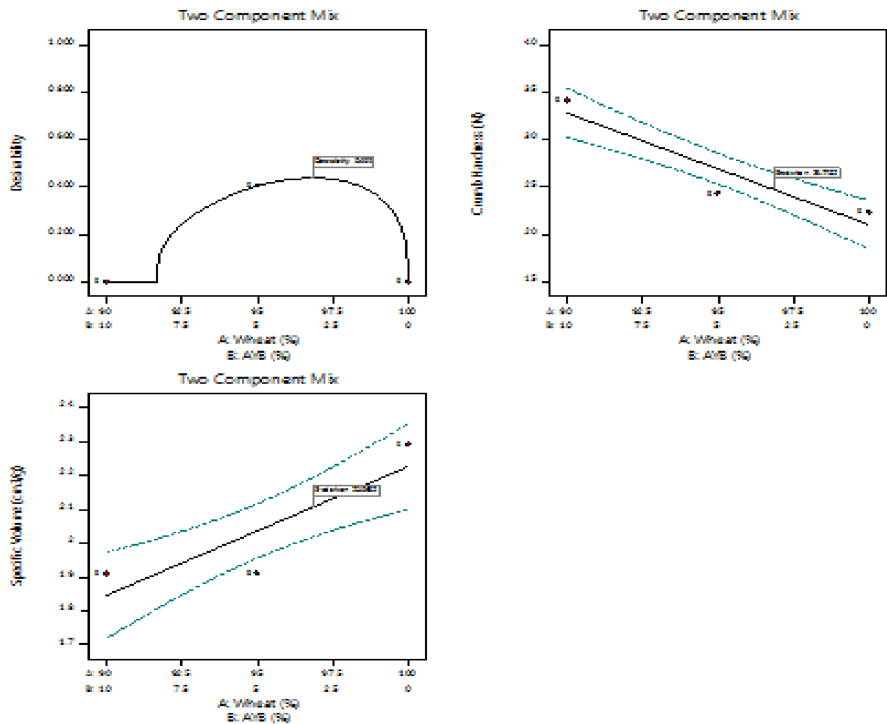

FIGURE 3: Graph of two component mix: Desirability, Bread Crumb Hardness and Specific volume vs wheat-AYB mix ratios.

To demonstrate how well the models can predict the response variables, the observed data were plotted against the model predicted data as shown in Fig. 4.

The predictive model was tested for validity by plotting the predicted value against the observed data. If the scatter points lie closely along the trend line the model is considered adequately valid to predict the data.

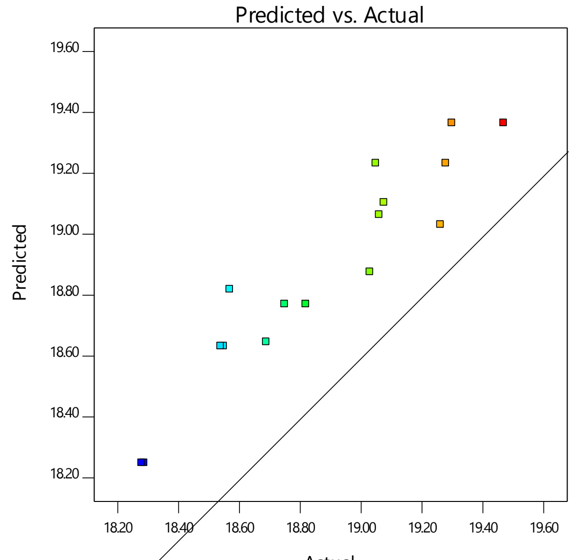

FIGURE 4: Graph of predicted against observed data for Crumb hardness

From Figure 4, the points lie very closely to the trend line showing reasonable agreement between the predicted and observed data. This shows that the prediction of BCH from the mix components percentage, by the model, was good. The summary of solution of the optimization process is presented in Table 4.

TABLE 4
SUMMARY OF THE OPTIMIZATION PROCESS

Number	Wheat	AYB	Crumb Hardness	Specific Volume*	Desirability	Desirability (w/o Intervals)	
1	96.833	3.167	24.778	2.106	0.439	0.506	Selected

From Table 4, the optimal solution was located at the mixture ratio of 96.833% Wheat flour and 3.167 % AYB flour , which gave a Bread Crumb Hardness of 24.778 N.

3.2 Loaf Specific Volume (LSV)

In this study, loaf specific volume (LSV) ranged from 1.89 to 2.31cm^3/g. The lowest value of LSV occurred when the mixture was 90% wheat and 10% AYB while the maximum occurs at the mixture of 100% wheat flour and 0% AYB for the same reason as in crumb hardness. A loaf specific volume for whole-wheat bread should not be less than 2.0cm^3/g (NIS, 2004). Ngozi (2014) reported specific volume which ranged from 2.16 to 3.51cm^3/g for bread made by substituting parts of wheat flour with whole-wheat flour. Malomo *et al.,* (2011) reported a specific volume of bread that ranged from 2.08 to 3.39cm^3/g with 5% breadnut flour substitution level and 95% wheat bread significantly different from others. The result of the ANOVA for LSV is presented in Table 5.

TABLE 5
ANOVA TABLE FOR LSV

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	0.2166	1	0.2166	21.00	0.0025	significant
Linear Mixture	0.2166	1	0.2166	21.00	0.0025	
Residual	0.0722	7	0.0103			
Lack of Fit	0.0722	1	0.0722			
Pure Error	0.0000	6	0.0000			
Cor Total	0.2888	8				

P-value less than 0.0025 indicates that model terms are significant. The predictive equation of LSV in terms of the mixture ratios of the two flours is given in Eqn. (4).

Specific volume=0.02227*wheat-0.015733*AYB 4

The coefficient of determination (R^2) is 0.7500 and F-value is 21.00 at 95% confidence interval, which is an indication of a good fit. The coefficients of the Equation 4 show that LSV was negatively affected by the AYB flour component. From Table 3, the optimal solution of the optimization occurs at the mix ratio of 96.833% of wheat and 3.16% AYB for specific volume of 2.106 cm^3/g

The validation plot of predicted against observed values of LSV is presented in Figure 5. The points lie very close to the trend line showing good agreement between the predicted and observed data. This shows that the prediction of LSV was very good.

This trend as explained earlier shows a very good prediction given that the entire points lie almost on the trend line.

IV. CONCLUSION

Response Surface Methodology (Mixture Design) algorithm was used for optimization of the wheat-AYB substitution ratio in this study. The optimised composite flour of Wheat and AYB was developed for improved loaf specific volume and bread crumb hardness. The objective function was to obtain the most suitable substitution ratio that will maximize Loaf Specific Volume and minimize Bread Crumb Hardness by minimizing %wheat flour component and maximizing %AYB flour component. Based on the composite desirability index of 0.428, the predicted optimal composite mix formulation for enhanced quality of composite bread were 95.328% of wheat flour and 4.672 AYB flour respectively. At this formulation, the predicted loaf specific volume was 2.049cm^3/g and bread crumb hardness was 26.545N, whereas the coefficient of determination (R^2) for loaf specific volume was 0.7500, and that for bread crumb hardness was 0.8734. The predicted optimal loaf specific volume complies with Standard Organization of Nigeria (SON) standard on the quality specifications given on the loaf specific volume of whole-wheat bread as 2.0cm^3/g minimum. Increasing the substitution level of AYB flour and consequently reducing the wheat flour impacts the quality of the composite bread physically by reducing loaf specific volume and increasing crumb hardness. This agrees with Yaver and Bilgiçli (2018) that Cereal-Legume Flour Blend (CLFB) in bread formulations lowered the volume in Commercial Bread (CB) and increased the hardness of bread.

This study proves that an acceptable physical attributes of bread can be achieved by supplementing wheat with AYB flour up to 4.672%, based on Nigerian standard. It can be more in other countries based on their standards.

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Microbia in Plant Growth Promoting Rhizobacteria Bamboo, Reed Grass and Banana

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***Abstract*—*Plant Growth Promoting Rhizocbacteria (PGPR) is a type of bacteria that lives around plant roots. These bacteria live in colonies covering the roots of plants so as to provide benefits for plants. The purpose of this study was to determine the types of bacteria found in PGPR bamboo roots, reed roots and banana roots. The research was conducted in the Plant Diseases Pests laboratory, Faculty of Agriculture, Mulawarman University. Isolation of PGPR bacteria was carried out by taking samples from the three PGPR solution materials. Then 2 ml of each PGPR sample was taken and grown on Nutrient Agar (NA) media by the scatter method. From each PGPR made in 4 (four) petri dishes, in order to obtain as many as 12 isolates of PGPR bacteria capable of growing on the media. Some of the genera included in the PGPR are Pseudomonas, Serratia, Azotobacter, Azospirillum, Acetobacter, Burkholderia, Enterobacter, Rhizobium, Erwinia, Flavobacterium and Bacillus. Each rhizobacteria isolate has an important role in controlling pathogen attack and triggering growth. Bacterial analysis is used as a parameter to determine the effectiveness and potential contained in these bacteria.*

***Keywords*—PGPR, bamboo roots, reed roots, banana roots, types of bacteria.**

I. INTRODUCTION

*Plant Growth Promoting Rhizocbacteria (*PGPR) is a type of bacteria that lives around plant roots. These bacteria live in colonies covering the roots of plants so as to provide benefits for plants. Some of the genera included in the PGPR are: Pseudomonas, Serratia, Azotobacter, Azospirillum, Acetobacter, Burkholderia, Enterobacter, Rhizobium, Erwinia, Flavobacterium and Bacillus [1]. Each rhizobacteria isolate has an important role in controlling pathogen attack and triggering growth. PGPR bacteria can be inoculated from various plant roots such as bamboo roots, reeds and banana roots. It is necessary to analyze the type and number of bacteria contained in the PGPR isolate used before being applied to cultivated plants.

Based on the results of research that has been carried out, the application of PGPR biological agents that have an effect in delaying the incubation period, it is necessary to analyze the type and number of bacteria contained in the PGPR isolate used before being applied to cultivated plants and suppress the intensity of the attack so that the severity of the disease is not too high. Bacterial analysis is used as a parameter to determine the effectiveness and potential contained in these bacteria. PGPR has properties as a bioprotectant that can protect plants from pathogen attacks [2].

II. RESEARCH METHOD

2.1 Place and time

This research was conducted at the Laboratory of Pests and Plant Diseases, Faculty of Agriculture, University of MulawarmanSamarinda from February 2022 to May 2022.

2.2 Materials and tools

The materials used are PGPR bamboo roots, reed roots and banana roots that have been fermented in liquid form. Aquades, alcohol, spirit, and Nutrient Agar (NA) media. The tools used for research in the laboratory are petri dishes, test tubes, ultra violet lamps, pipettes, measuring cups, autoclaves, laminar air flow cabinets (LAFC), microwaves, 250 ml media bottles,

Bunsen lamps, ose needles, lighters, plastic, plastic wrapping, sprayer, scissors, aluminum foil, cotton, sterile tissue, measuring cup (vol. 100 ml), and label.

2.3 Research Activities

The research activities carried out were: preparation, observation, taking PGRP on (bamboo roots, reed roots and banana weevil roots), fermentation, isolation on nutrient agar media, and analysis in the laboratory.

2.4 Data collection

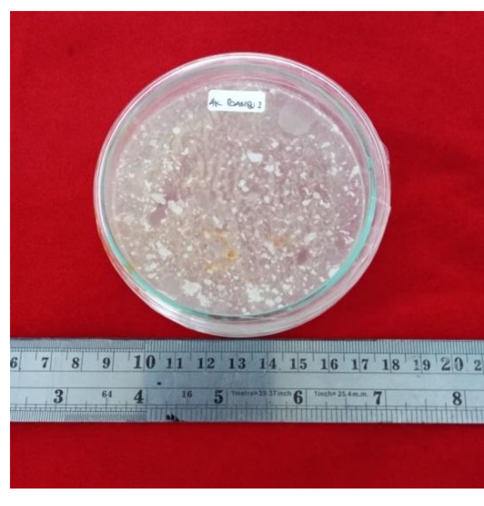
The data collected were: the shape of the colony, the shape of the edge of the colony, the size of the colony, and the color of the colony, gram-positive and negative observations and the microscopic shape of the PGPR bacteria.

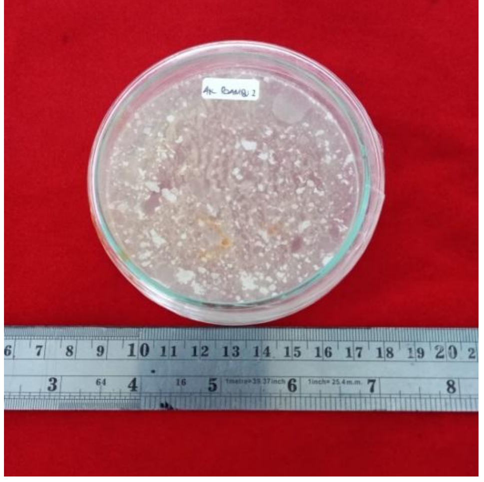
III. RESULTS AND DISCUSSION

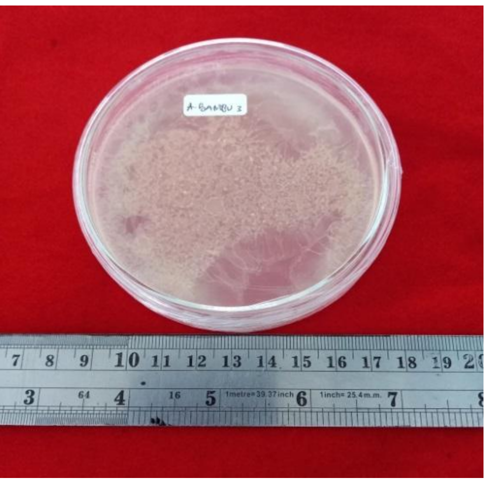
Plant Growth Promoting Rhizocbacteria(PGPR) obtained from several types of plant roots such as bamboo roots, reed roots and banana hump roots. The three ingredients are fermented for 8 days with a mixture of several ingredients such as brown sugar, shrimp paste, bran, and coconut water. Isolation of PGPR bacteria was carried out by taking samples from the three PGPR solution materials. Then 2 ml of each PGPR sample was taken and grown on Nutrient Agar (NA) media by the scatter method. From each PGPR made in 4 (four) petri dishes, in order to obtain as many as 12 isolates of PGPR bacteria capable of growing on the media. According to [3] that bacterial isolation is taking or removing microbes from their natural environment and growing them as pure cultures in artificial media.

3.1 PGPR bacterial colony morphology on bamboo roots

Morphological observations made on PGPR bacteria on bamboo roots are presented in Figure 1.







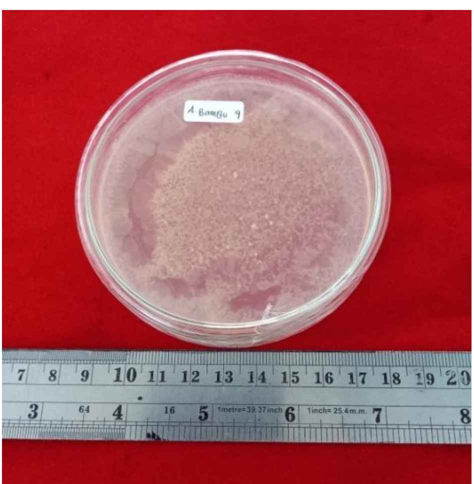


FIGURE 1. PGPR Isolation Results on Bamboo Roots

Figure 1a shows that bacteria originating from bamboo roots have irregular rounded colonies with jagged edges. The surface of the colony is wavy. Colony size was obtained from the smallest to the largest size, which ranged from 1.0 mm to 3.0 mm, the color of the isolates was mostly yellowish white. Figure 1b has a jagged colony edge that is thick and thin. Colony size was obtained from sizes ranging from 1.0 mm to 3.0 mm. Some isolates were white and most of the isolates were yellowish white. Then in Figure 1c shows the shape of the colony is wavy and thick, the edges of the colony are jagged and milky white and measuring 1 mm to 4 mm. Figure 1d shows the shape of the colonies that are wavy and thick, the edges of the colonies are jagged and yellowish white and measuring 1.0 mm to 3.0 mm. In detail the results of observations can be seen in Table 1.

TABLE 1
OBSERVATIONS ON THE MORPHOLOGY OF BAMBOO ROOT PGPR BACTERIA

No	Isolat	Colony Form	Colony edge shape	Colony size	Colony color	Gram	Cell Shape	Genus
1	AB 1	Irregular round	jagged	2,0 mm	Yellowish white	+	Basil, cocci	*Sporoformes, sp*
2	AB 2	Irregular round	jagged	1,0 mm	White, white yellowish	+	Basil, cocci	*Basillus. sp*
3	AB 3	Wavy, thick	jagged	3,0 mm	milky white	+	Basil, cocci	*Streptococcus Faecalis*
4	AB 4	Wavy, thin	jagged	3,0 mm	Yellowish white	-	Basil, cocci	*Pseudomonas sp.*

Microscopically with 1000 times magnification using an Olympus CX23 microscope, gram staining results were obtained from several different colonies showing the type of PGPR bacteria in bamboo roots presented in Figure 2.

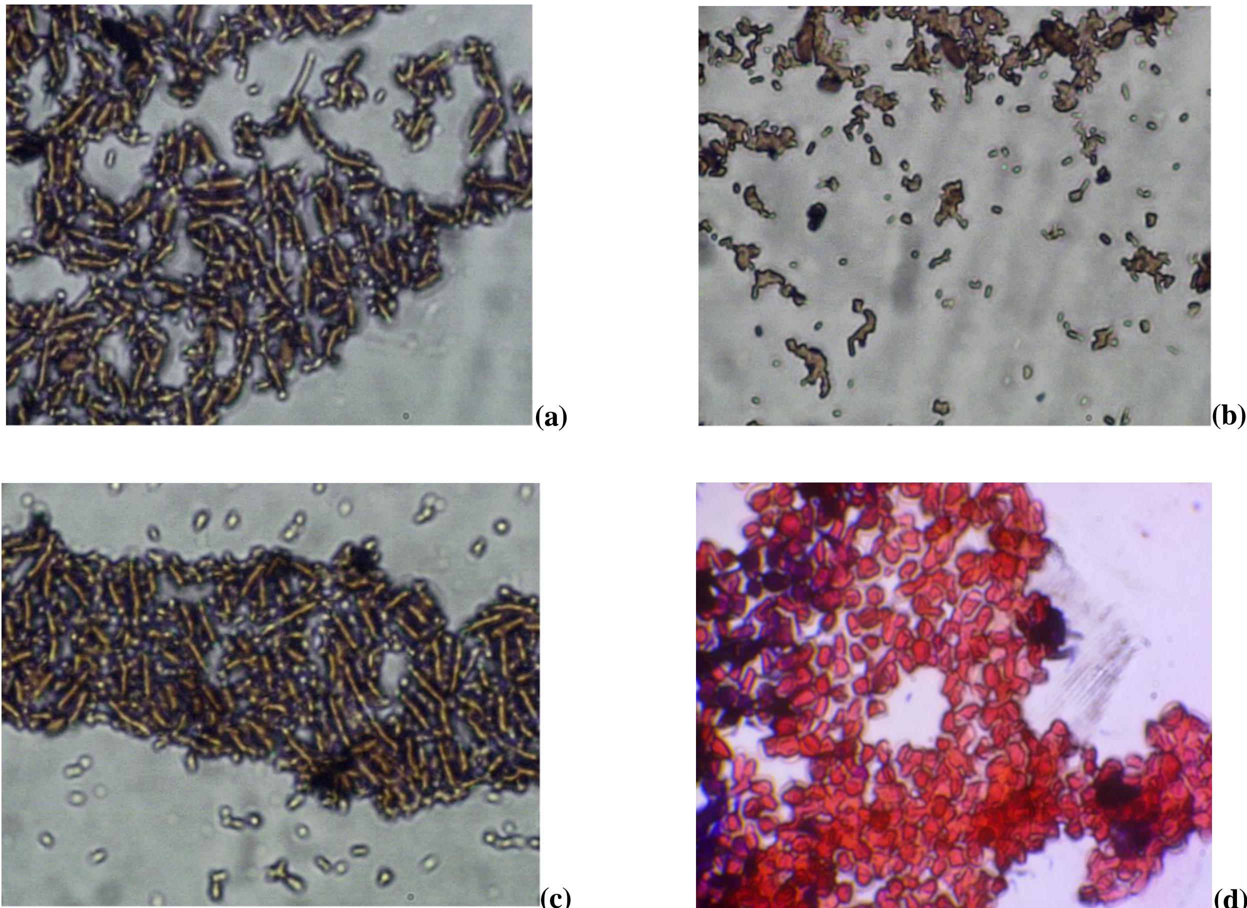


FIGURE 2: Gram Staining of Bamboo Root PGPR Bacteria Using a Microscope

On microscopic observation in Figure 2a. showed that the PGPR bacteria of bamboo roots showed a purple color so that it could be said that the bacteria were gram-positive, with the shape of a bacillus. In Figure 2b. shows that the bacteria is purple, which means the bacteria are gram positive with a cocci shape. Next in Figure 2c. shows the purple colored bacteria means Gram Positive, and the shape of a bacillus, while in Figure 2d. it is seen that the bacteria is red which means that the bacteria is Gram Negative while the shape of the bacteria looks like a bacillus.

Based on the above observations that PGPR found on bamboo roots, it is in accordance with the identification [4] that the bacteria are Bacilliaceae bacteria. The results of observations of the shape of the colony where the growth of bacteria on the Nutrient Agar media was yellowish white in color. according to research [5] that in bamboo roots found bacteria Enterobactericeae (*Escherichia coli*, *Salmonella*, *Shigella*), *Pseudomonas*, and There are also bamboo rhizosphere PGPR bacteria that do not have mucus/gram positive, such as *Bacillus, Enterococcus*. This is also corroborated by the statement [6] that several rhizobacteria genera that act as PGPR are: *Pseudomonas, Enterobacter, Bacillus, Azospirilum, Azotobacter, Burkholderi*a and *Serratia* and it is also stated that PGPR plays a role in the process of plant growth.

3.2 PGPR bacterial colony morphology on reed root

Morphological observations made on PGPR bacteria on the roots of reed are presented in Figure 3.

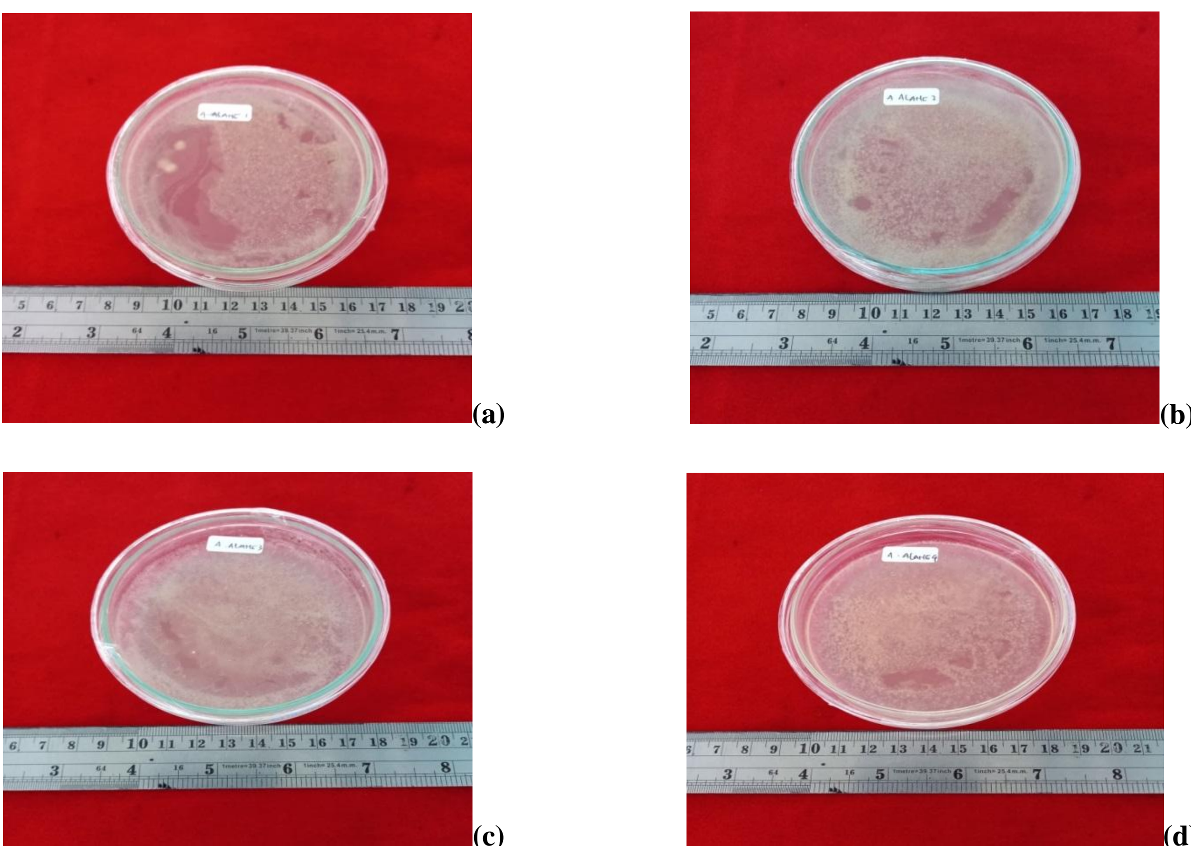


FIGURE 3. PGPR Isolation Results on reed Roots

The results showed that the isolates in Figure 3a were wavy, thick and thin, round and some were irregularly rounded, and the edges of the colonies were flat and jagged. The color of the isolate is milky white and yellowish white. Figure 3b Colony shape irregular round, thick, jagged colony edges. The color of the isolate was yellowish white and the average colony size was 1.0 mm. Figure 3c shows the shape of a round, flat colony. Colony size obtained ranged from 1.0 mm to 3.0 mm. The isolates were wavy and thin, the edges of the colonies were jagged. The color of the isolate was yellowish white and the average colony size was 1.0 mm. then in the 3d image shows the shape of the colony is round and thin, the edges of the colony are jagged. The color of the isolate was yellowish white and the average colony size was 1.0 mm. All isolates were in the form of jagged colony edges and some were yellowish white and almost all isolates were gram negative with bacilli and cocci cell shapes. In detail the results of observations can be seen in Table 2.

TABLE 2
PGPR BACTERIAL MORPHOLOGICAL OBSERVATION RESULTS ON REED ROOTS

No	Isolat	Colony Form	Colony edge shape	Colony size	Colony color	Gram	Cell Shape	Genus
1	AA 1	Irregular round, Thin	Flat, jagged	3,0 mm	Milk white, yellowish white	+	Basil, Cocci	*Sporoformessp.*
2	AA 2	Irregular round, thick	jagged	1,0 mm	Yellowish white	-	Basil, Cocci	*Pseudomonassp.*
3	AA 3	Round, Wavy, Thin	jagged	1,0 mm	Yellowish white	-	Basil, Cocci	*Klebsiella. Sp*
4	AA 4	Round, Thin	jagged	1,0 mm	Yellowish white	-	Basil, Cocci	*Klebsiella. sp*

Microscopically with 1000 times magnification using an Olympus CX23 microscope, gram staining results were obtained from several different colonies by showing the type of PGPR bacteria on the roots of the Imperata, which is presented in Figure. 4.

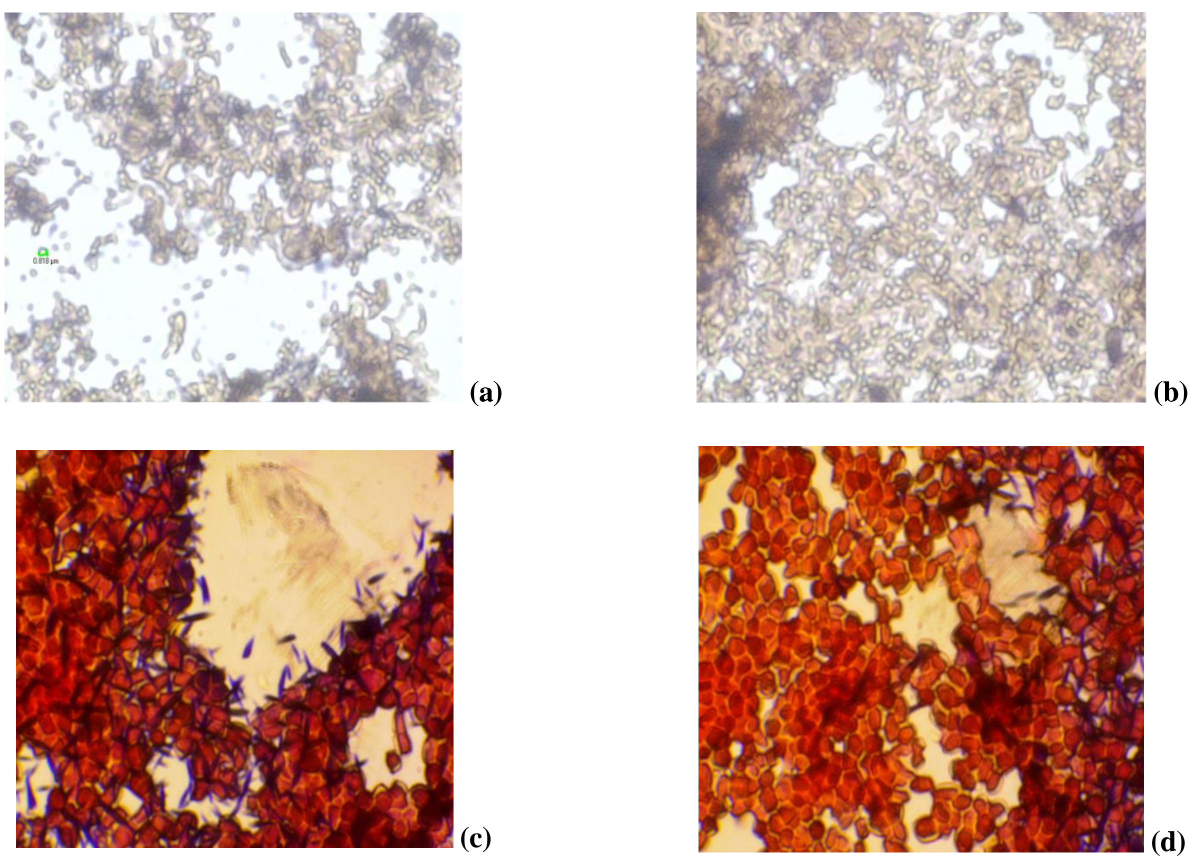


FIGURE 4: Gram staining of PGPR bacteria on the roots of reed, using a microscope (magnification 1000 X)

On microscopic observation in Figure 4a. showed that the PGPR of reed roots showed a red color so that it could be said that the bacteria were gram negative, with bacilli and cocci shape. In Figure 4b. shows that the bacteria is red, which means the bacteria are gram negative with the shape of bacilli and cocci. Next in Figure 4c. shows the bacteria in bright red color means Gram negative, and the shape is bacillus and cocci, while in Figure 4d. it is seen that the bacteria is red which means that the bacteria is Gram Negative while the shape of the bacteria looks like a cocci.

Based on the above observations, the PGPR found in the roots of the weeds is in accordance with the identification [7] that the bacteria are Bacilliaceae bacteria. The results of observations of the shape of the colony where the growth of bacteria on Nutrient Agar media was yellowish white, the periphery of the colony was irregularly rounded, the surface was round, irregular and wavy, while microscopically it showed that Gram negative bacteria were in the form of bacilli and cocci, it

could be predicted that the bacteria were *Azotobacter*, and *Pseudomonas* sp. This is also in accordance with research [8] which stated that the *Azotobacter, Pseudomonas* sp. which plays a role in promoting plant growth.

3.3 PGPR bacterial colony morphology on banana hump roots

Morphological observations made on PGPR bacteria on banana hump roots are presented in Fig 5.

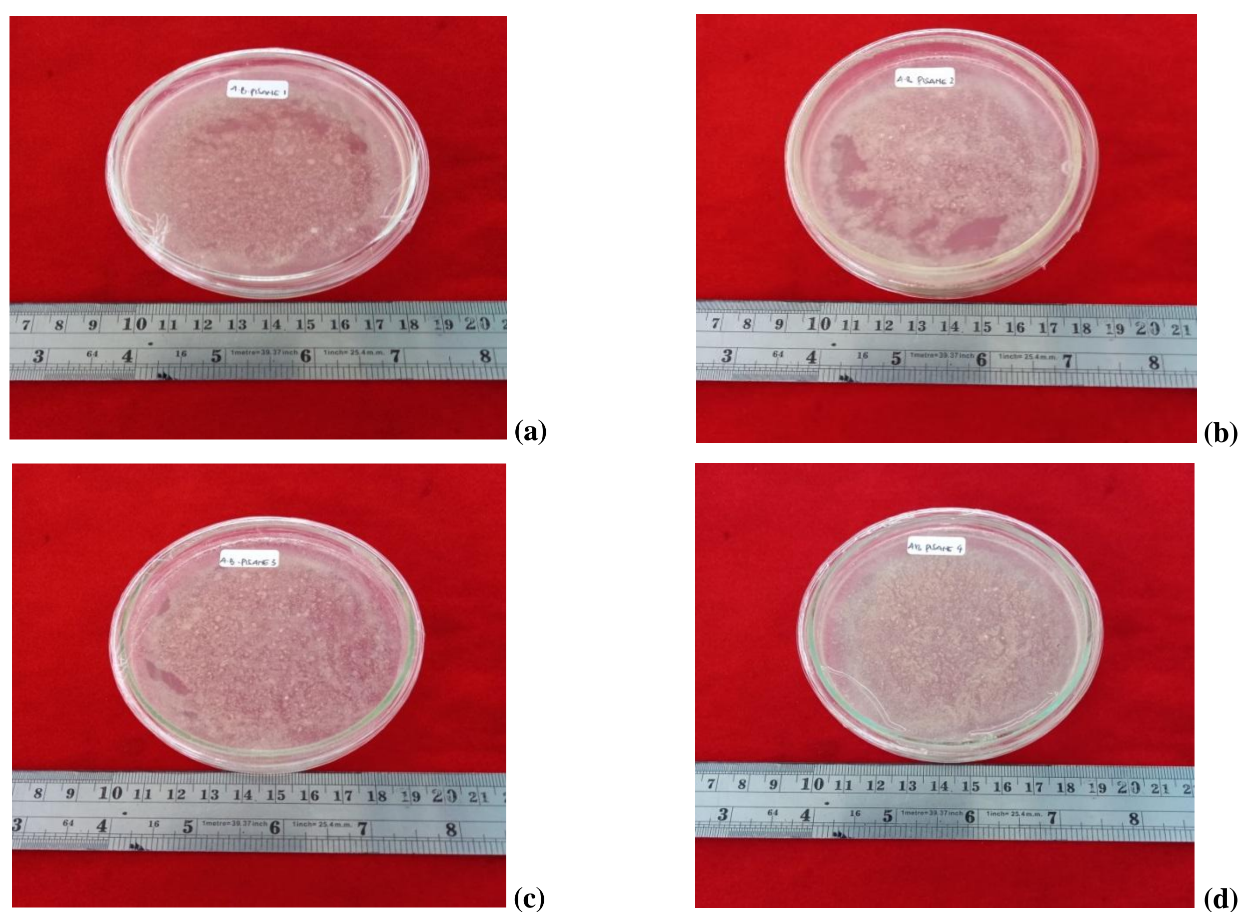


FIGURE 5. PGPR Isolation Results on Banana Hump Roots

From the results of the isolation of banana humo root PGPR, it shows that in Figures 5a, 5c, and 5d all isolates have irregular and thin round colonies, almost all isolates have jagged colony edges, the size of the colonies obtained ranged from 1.0 mm to 3.0 mm. In Figure 5b, the shape of the colonies is wavy and irregularly round, the edges of the colonies are jagged, the isolate is yellowish white and the size ranges from 1.0 mm to 2.0 mm. In detail the results of observations can be seen in Table 3.

TABLE 3
PGPR BACTERIAL MORPHOLOGICAL OBSERVATIONS ON BANANA HUMP ROOTS

No	Isolat	Colony Form	Colony edge shape	Colony size	Colony color	Gram	Cell Shape	Genus
1	AP 1	Irregular round, thick, thin	Jagged	2,0 mm	Yellowish white	-	Basil	*Klebsiella. sp*
2	AP 2	Irregular round, thin	Jagged	3,0 mm	Yellowish white	-	Basil	*Pseudomonas sp.*
3	AP 3	Irregular round, thin	Jagged	1,0 mm	White, Yellowish white	-	Basil	*Pseudomonas sp.*
4	AP 4	Irregular round, thin	Jagged	1,0 mm	Yellowish white	-	Basil	*Pseudomonas sp.*

Microscopically with 1000 times magnification using an Olympus CX23 microscope, gram staining results were obtained from several different colonies by showing the type of PGPR bacteria on the banana hump root which is presented in Figure 6.

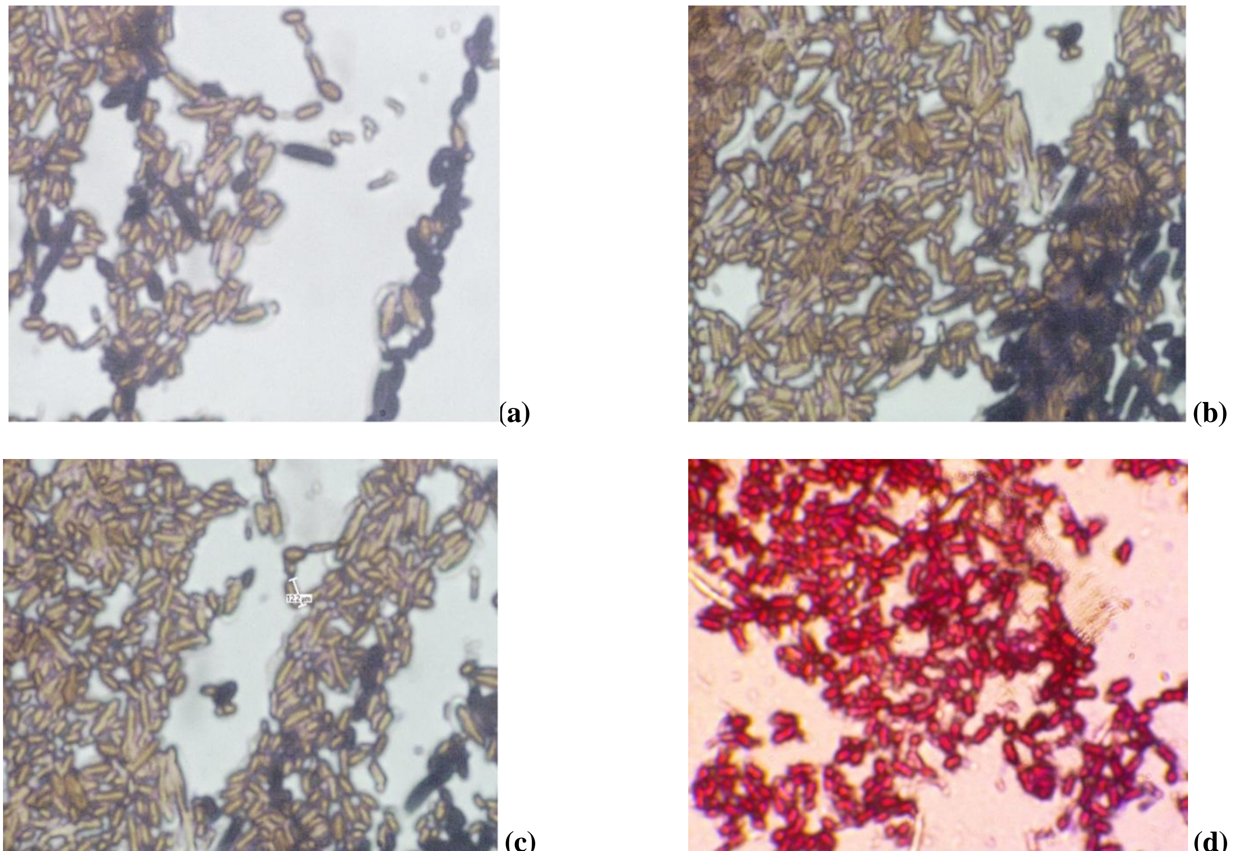


FIGURE 6. PGPR Bacterial Gram Stain Banana Hump Root using a Microscope (Magnification 1000 X)

On microscopic observation in Figure 6a. showed that the banana root PGPR bacteria showed a reddish purple color so that it could be said that the bacteria were gram-positive, with the shape of a bacillus. In Figure 6b. shows that the bacteria is purple, which means the bacteria are gram positive with the shape of bacilli and cocci. Next in Figure 6c. showing the purple colored bacteria means they are Gram Positive, and the shape is bacillus, while in Figure 6d. it is seen that the bacteria is red which means that the bacteria is Gram Negative while the shape of the bacteria looks like a bacillus.

Based on the above observations, the PGPR found in banana roots is in accordance with the identification [9] that the bacteria are bacteria from the Bacilliaceae family. The results of observations of the shape of the colony where the growth of bacteria on Nutrient Agar media was yellowish white, the periphery of the colony was round, jagged, the surface was round, irregular and wavy, while microscopically it showed that the bacteria were Gram positive and negative and were in the form of bacilli and cocci. This is also in accordance with research [10] which states that bacillus bacteria are found in banana roots.

Observations on the morphological characteristics of bacterial colonies need to be carried out, in order to facilitate the process of identifying the type of bacteria. This is in accordance with the statement [11] which states that gram-positive bacteria in Gram staining are purple due to the violet-iodine crystal dye complex being maintained even though the acetone-alcohol bleach solution is given, while gram-negative bacteria are red because the complex is soluble when the bleaching solution is given. acetone alcohol so that it takes on a red color of safranin. The difference in color between gram-positive and gram-negative bacteria indicates that there are differences in cell wall structure between the two types of bacteria. Gram-positive bacteria have a cell wall structure with a thick peptidoglycan content, while gram-negative bacteria have a cell wall structure with a high lipid content. According to [11] that based on the morphological characteristics of bacterial colonies and

pure cultures, the identification process of the types of microorganisms can be carried out, but to obtain perfect identification results it must be continued with biochemical tests.

IV. CONCLUSIONS

Based on the results of the study, it can be concluded that the results of the identification of bacteria in PGPR of bamboo roots, alang-alang roots and banana roots microscopically showed that the bacteria were Gram Positive and Negative and in the form of bacilli and cocci. The bacteria found were dominated by basillieae bacteria. To obtain perfect identification results, it is necessary to carry out further research with biochemical tests.

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