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Preface

We would like to present, with great pleasure, the inaugural volume-11, Issue-3, March 2025, 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.

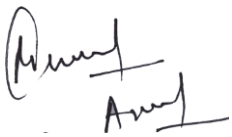
Environmental Research:

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.

Agriculture Research:

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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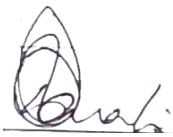

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Agricultural Sciences	
Soil Science	Plant Science
Animal Science	Agricultural Economics
Agricultural Chemistry	Basic biology concepts
Sustainable Natural Resource Utilisation	Management of the Environment
Agricultural Management Practices	Agricultural Technology
Natural Resources	Basic Horticulture
Food System	Irrigation and water management
Crop Production	
Cereals or Basic Grains: Oats, Wheat, Barley, Rye, Triticale, Corn, Sorghum, Millet, Quinoa and Amaranth	Oilseeds: Canola, Rapeseed, Flax, Sunflowers, Corn and Hempseed
Pulse Crops: Peas (all types), field beans, faba beans, lentils, soybeans, peanuts and chickpeas.	Hay and Silage (Forage crop) Production
Vegetable crops or Olericulture: Crops utilized fresh or whole (wholefood crop, no or limited processing, i.e., fresh cut salad); (Lettuce, Cabbage, Carrots, Potatoes, Tomatoes, Herbs, etc.)	Tree Fruit crops: apples, oranges, stone fruit (i.e., peaches, plums, cherries)
Tree Nut crops: Hazlenuts. walnuts, almonds, cashews, pecans	Berry crops: strawberries, blueberries, raspberries
Sugar crops: sugarcane. sugar beets, sorghum	Potatoes varieties and production.
Livestock Production	
Animal husbandry	Ranch
Camel	Yak
Pigs	Sheep
Goats	Poultry
Bees	Dogs
Exotic species	Chicken Growth
Aquaculture	
Fish farm	Shrimp farm
Freshwater prawn farm	Integrated Multi-Trophic Aquaculture
Milk Production (Dairy)	
Dairy goat	Dairy cow
Dairy Sheep	Water Buffalo
Moose milk	Dairy product
Forest Products and Forest management	
Forestry/Silviculture	Agroforestry
Silvopasture	Christmas tree cultivation
Maple syrup	Forestry Growth
Mechanical	
General Farm Machinery	Tillage equipment
Harvesting equipment	Processing equipment
Hay & Silage/Forage equipment	Milking equipment
Hand tools & activities	Stock handling & control equipment
Agricultural buildings	Storage

Agricultural Input Products	
Crop Protection Chemicals	Feed supplements
Chemical based (inorganic) fertilizers	Organic fertilizers
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Environmental health issues	Atmosphere and climate
Terrestric ecosystems	Aquatic ecosystems
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Table of Contents

Volume-11, Issue-3, March 2025

Common Pathogens Associated with Poultry Production in Awka South Local Government Area of Anambra State

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Abstract— *The worldwide poultry production is continually intensifying with progressively more challenges due to pathogens; hence it is crucial to ensure the bio-safety of the poultry farms and poultry products. This research was therefore designed to isolate and characterize the common pathogens associated with poultry production in Awka South Local Government Area of Anambra State. In addition, sensitivity tests were carried out to proffer solution to farmers in this area. Thirty fecal samples were collected from poultry farms in three towns in the study area using systematic random sampling. Standard microbiological techniques were employed to cultivate, isolate and characterize pathogens from each town. The study revealed the presence of various pathogenic bacteria including Salmonella spp, Escherichia coli, Shigella spp, Streptococcal spp and Staphylococcus aureus. The antimicrobial susceptibility testing of the isolated pathogens was conducted to determine their sensitivity to commonly used antibiotics. Some of these pathogens were resistant to common antibiotics often used by farmers indicating subnormal applications. The study therefore underscores the urgent need for the government to detail Extension Agents to Awka South Local Government Area of Anambra State in order to educate the poultry farmers on proper use of antibiotics. Again, since most of these isolates are zoonotic, caution should be applied in consumption of animal products from this area. The use of alternative bio security measures such as herbs are recommended in order to mitigate the imminent danger of using antibiotics.*

Keywords— Antibiotic Resistant, Bacterial Isolates, Pathogens, Zoonotic Disease.

I. INTRODUCTION

1.1 Background of the study:

Poultry production is a significant contributor to global food security, providing a vital source of protein; eggs and income for millions of people worldwide (Farrell, 2013). However, this industry faces challenges due to presence of various pathogens that can cause diseases in poultry (Sierżant *et al*., 2021). These diseases not only impact animal health and welfare but also lead to significant economic losses for farmers due to decreased productivity and mortality (Grzinic *et al.*, 2023). Pathogens can be introduced to a poultry flock via air, pests, people, water, feed, to mention but a few. The prevalence and impact of these pathogens is largely dependent upon the quality of environment and the health and welfare of the birds.

Pathogens such as *Salmonella spp.*, *Staphylococcus aureus*, *Escherichia coli,* and *Enterococcus spp* and *Campylobacter spp.* present a major concern for the poultry industry on a yearly basis due to their association with poultry-related foodborne illnesses. Crates use in transportation, poor environmental conditions, poor worker hygiene, and bird-to-bird pathogen transfer have all been identified as major preharvest contamination risk factors (Heyndrickx *et al.,* 2002; Bull *et al.,* 2006). During processing, poultry carcasses are primarily contaminated with pathogenic bacteria due to the leakage of fecal matter during major processing steps (Berrang *et al.,* 2001). Cross-contamination has also been identified as a major risk factor during processing (Rasschaert *et al.,* 2008). Intervention strategies are implemented at the pre harvest and postharvest levels to mitigate the risk of contamination of the poultry product by these pathogenic bacteria.

Globally, there is increased demand for antibiotic-free animal products, causing consumers to move towards the organic food market (Dimitri and Oberholtzer, 2009; Reisch *et al.,* 2013). This has impacted the poultry industry, where broiler meat harvested from alternative poultry farming production facilities, such as organic and free-range, have increased in demand (Van Loo *et al.,* 2011; Rothrock Jr. *et al.,* 2016). These types of operations are characterized by the lack of antibiotic use and the allowance of birds to access the outside environment. As such, birds are exposed to a less controlled environment, indicating an increased risk of microbial contamination of the birds.

Pathogens continue as a considerable threat to public health. Intensification of livestock production, especially poultry, facilitates diseases transmission by increasing population size and density (Feßler *et al*., 2011; Dhama *et al.,* 2014)

Studies by Timbermont *et al.* (2009) and Dolka *et al.* (2020) emphasized that poultry immunity, health and production are some of the factors that will challenge the future growth of the industry. Many foodborne and zoonotic diseases, emerging and re-emerging worldwide, are strictly linked with poultry farming. A foodborne disease outbreak is an incident during which at least two people contract the same illness from the same contaminated food or drink. Developing strategies to eliminate and control foodborne pathogens, while tackling the public health hazards linked to consuming foods with high antibiotic residues and the threat of antimicrobial resistance, remains as critical challenges for the industry.

Colibacillosis is the most common bacterial disease in poultry and it is caused by Avian Pathogenic *Escherichia coli* (APEC). It can be present among poultry of all ages. APEC is opportunistic in nature and it can grow rapidly in stress conditions. Initial exposure to APEC might occur in the hatchery from infected or contaminated eggs; however infections are commonly triggered by immunosuppressive diseases such as Infectious Bursal Disease, Mareks Disease, or Chicken Anemia. Colibacillosis is a major cause of morbidity, mortality, and economic loss for all types of poultry worldwide (Ramazani *et al.*, 2021).

Another common bacterium on poultry farms is *Salmonella*, which is pathogenic to both poultry and humans. Although the target habitat of Salmonella is the gastrointestinal tract, it is widely present in nature and makes a major microbial hazard in animal feed, as it can persist for long periods of time. Bacteria belonging to the *Salmonella* genus are responsible for a variety of acute and chronic diseases in poultry. Moreover, poultry flocks infected with this pathogen are the main zoonotic reservoir which can transmit infection through the food chain to humans, thus posing a serious health problem as well. Potential symptoms of *Salmonella* presence in birds include drowsiness, huddling together, poor growth, chalky white diarrhea, dehydration, reduced egg production, and increased mortality, among others. Salmonella symptoms, especially at subclinical level, can go unnoticed and not recognized as caused by *Salmonella* bacteria, while the birds' body is using its vital resources to fight off the bacteria instead of utilizing them for growth and productivity. This bacterium causes significant disturbances in chicken gut and it weakens overall bird health. *Salmonella* is the most reported cause of foodborne outbreaks in the European Union nearly one in three foodborne outbreaks in the EU in 2018 were caused by this bacterium. This is one of the main findings of the annual report on trends and sources of zoonoses published by the European Food Safety Authority (EFSA) and the European Centre for Disease Prevention and Control (ECDC). In 2020, EU Member States reported 3 166 foodborne outbreaks affecting 22,010 people. Over 56% of the outbreaks were linked to *Salmonella* (Ricke, 2021; Timbermont *et al.,* 2009).

In the United States, The Centre for Disease Control and Prevention (CDC) has cited about 1.35 million *Salmonella* infection cases and approximately 420 deaths in the United States every year.

Understanding the prevalence, distribution, and characteristics of these pathogens is essential for implementing effective disease control and prevention measures in poultry production systems. Therefore, studies focusing on the isolation and characterization of common pathogens associated with poultry production are crucial for enhancing biosecurity practices, improving animal welfare, and safeguarding public health.

1.2 Statement of Problem:

Limited research reports exist on how different pathogens affect the poultry production. However, poultry production faces numerous challenges, with disease outbreaks being a primary concern. Diseases not only lead to significant economic losses for poultry farmers but also pose potential threats to public health through the transmission of zoonotic pathogens.

The poultry industry is susceptible to various diseases that affect both layers and broilers, resulting in reduced production, increased mortality rates, and decreased profitability for farmers. However, there is a lack of comprehensive data on the prevalence and economic impact of these diseases in the local poultry population. Diseases affecting poultry can also have implications for human health, particularly if they are zoonotic in nature. The transmission of pathogens from infected birds to

humans through direct contact, consumption of contaminated poultry products, or environmental exposure represents a significant public health risk. However, the specific pathogens responsible for such risks and their prevalence in the local poultry population are not well-documented.

Effective disease management strategies rely on a thorough understanding of the pathogens present in the poultry population, their modes of transmission, and their susceptibility to available treatments. However, the lack of data on the specific pathogens affecting poultry hinders the development and implementation of targeted disease control measures.

Disease outbreaks in poultry farms can often be attributed to lapses in biosecurity practices and inadequate disease control measures. Without proper identification of the pathogens involved, it is challenging for poultry farmers to implement appropriate biosecurity protocols and preventive measures to mitigate disease spread effectively.

Sustainability and Food Sustainable poultry production is essential for ensuring food security and meeting the nutritional needs of the population. However, disease outbreaks can undermine the sustainability of poultry farming enterprises, leading to food shortages, increased prices, and reduced access to affordable protein sources for consumers.

This research is essential for informing targeted intervention strategies, improving biosecurity measures, safeguarding public health, and ensuring the sustainability and economic viability of the poultry industry in the region.

1.3 Objective of the Study:

The general objective of this study is to identify and characterize common pathogens associated with poultry production in Awka South Local Government Area of Anambra State with a view of drafting bio-security measures appropriate to farmers in this area.

1.4 Justification of the Study:

The study on isolating and characterizing common pathogens associated with poultry production is significant for several reasons. Understanding the prevalence and identity of pathogens affecting poultry is crucial for safeguarding public health. Many poultry pathogens have zoonotic potential, meaning they can be transmitted from animals to humans. By identifying these pathogens, we can implement measures to prevent human exposure and reduce the risk of disease outbreaks in both animal and human populations.

Knowledge of prevalent pathogens enables the development of effective disease management and control strategies. With accurate identification, targeted treatment protocols can be implemented to mitigate disease spread and minimize economic losses for poultry farmers. Additionally, understanding the antimicrobial susceptibility patterns of isolated pathogens can guide prudent antimicrobial use practices, thereby reducing the risk of antimicrobial resistance.

Disease outbreaks in poultry farms result in significant economic losses due to decreased productivity, increased mortality rates, and veterinary expenses. By identifying common pathogens and understanding their impact on poultry health, farmers can implement proactive measures to prevent disease transmission, reduce production losses, and enhance profitability. The study findings can inform the development and implementation of robust bio security measures tailored to the specific pathogens identified in the local poultry population. Improved bio security practices reduce the risk of disease introduction and transmission within and between poultry farms, thereby enhancing overall farm productivity and sustainability.

Sustainable poultry production is essential for ensuring long-term food security and environmental sustainability. By identifying and managing common pathogens, farmers can reduce reliance on prophylactic antimicrobials and promote environmentally friendly farming practices. Additionally, sustainable disease management strategies contribute to the resilience of the poultry industry in the face of emerging pathogens and changing environmental conditions.

Research findings on prevalent poultry pathogens can inform policy formulation and regulatory frameworks aimed at protecting public health, promoting animal welfare, and ensuring the sustainability of the poultry industry. Evidence-based policies can facilitate the adoption of best practices in disease management, bio security, and antimicrobial stewardship. The outcome of this study contributes to the advancement of disease management strategies, enhancement of bio security measures, promotion of sustainable farming practices, and formulation of evidence-based policies to support the poultry industry and protect public health.

II. MATERIALS AND METHODS

2.1 Study Area:

The study was carried out in Awka South Local Government Area Anambra State. Awka South Local Government Area lies in latitude 6°10'N and 6°15' N of the Equator and longitude 7°02'E and 7°07'E of the Greenwich Meridian. It has an average temperature of approximately 27°C, and an average humidity level of about 70 percent. The rainfall here is 2950mm per year. The local government comprised of 9 towns which are Amawbia, Awka, Ezinato, Isiagu, Mbaukwu, Nibo, Nise, Okpuno and Umuawulu with a population of about 270,300 and a density of 63.4sqm (Nigeria Media, 2023)

2.2 Sample Collection and Sampling Techniques:

Three towns were selected for this experiment using simple random sampling technique. One biggest farm was selected from each of the three towns (systematic random sampling) for this study. In each farm, 10 birds were sampled making it a total of 30 samples. A total number of thirty (30) freshly excreted feacal samples of chicken droppings were collected from the three towns in Awka South Local Government Area namely, Ify poultry farm Ifite Awka, KennyPassy Integrated farms Ltd Amawbia and Divine Favour poutlry farm Okpuno. They were properly labeled and transported with plastic test tubes to Amazing Grace Research laboratory, Nkwelle Izunaka in Oyi local government area of Anambra State, Nigeria for analysis. Each sample was prepared by making dilutions in distilled water.

2.3 Culture Media Preparation:

The media prepared were Salmonella Shigellar Agar, Macconkey Agar and Nutrient Agar for the isolation of bacteria respectively. They were prepared according to the manufacturer's instructions.

2.3.1 Salmonella-Shigellar (SS) Agar Test:

SSA is a selective and differential medium. It was used for the isolation, cultivation and differentiation of gram-negative enteric microorganisms. Differentiation of enteric microorganisms was achieved by the incorporation of lactose in the medium. Isolates were inoculated by streaking and incubated at 37°C for 24 hours. Organisms which ferment lactose produce acid which in the presence of the neutral red indicator, results in the formation of red or pink colonies. Lactose non-fermenters form colourless colonies.

2.3.2 MacConkey Agar Test:

MacConkey agar was the first solid differential media to be formulated which was developed in the 20th century by Alfred Theodore. MacConkey Agar is the earliest selective and differential medium for the cultivation of coliform organisms. It is used for the isolation and differentiation of non-fastidous gram-negative rods, particularly members of the family Enterobacteriaceae and the genius *Pseudomonas*. Isolates were in a dehydrated medium in 1000ml of distilled water. The isolated were heated to boiling to dissolve the medium completely. Then, it was sterilized by autoclaving at 121°C for 15 minutes. It was then cooled to 40 mins to 50mins. The isolates were mixed well before pouring into sterile plates.

2.3.3 Nutrient Agar Test:

Nutrient Agar is a basic culture medium commonly used for the culture of non-fastidous microorganisms, quality control and checking purity prior to biochemical testing. It is one of the most important and commonly used non-selective media for the routine cultivation of microorganisms. 28g of nutrient agar powder was suspended in 1L of distilled water; mixed and dissolved completely. It was sterilized by autoclaving at 121°C for 15 minutes. The liquid was poured into the Petri dish and wait for the medium to solidify. The agar was prepared in a clean environment to prevent any contamination. Once the agar solidifies, the agar is ready for use. The media forms light yellow colored clear to slightly opalescent gel on Petri plates after cooling.

2.4 Inoculation of the Media for Bacteria Isolation:

The test tubes containing the test samples were inoculated by streaking using a wire loop into both Macconkey and Salmonella Shigellar agar. 1ml of the test samples was transferred into a prepared Salmonella Shigellar Agar and Macconkey agar in culture plates and inoculated at 37°C for 24 hours (overnight). After 24 hours, the growth of organisms was checked. Fresh media was prepared, and then poured into the Petri dishes. Then, it was allowed to cool, it was subcultured with the aid of the wire loop. Then, it was incubated at 37°C for 24 hours (overnight) to get pure colonies.

2.5 Sensitivity Test:

Sensitivity test (the drug that can kill the organisms) was used to determine the actual active ingredient that can eradicate the organism. The various pure isolates were subjected to sensitivity test with commercial antibiotics discs using disc diffusion method. They were incubated at 37°C for 24 hours after which the zones of inhibitions were measured using a meter rule.

2.6 Characterization and Identification of the Isolates:

Different morphological and biochemical characteristics accompanied with colony characteristics on different selective medium were observed for the identification of isolates.

2.7 Biochemical Test:

The pure colonies were subjected to various biochemical tests for proper identification using methods as described by Chessburugh (2010). The biochemical tests carried out include Gram staining, Catalase test, Coagulase test, Oxidase test, Citrate utilization test, Indole test, Ureese test, Methyl red test, Voges proskauer test, Hydrogen sulphide test and Motility test.

2.7.1 Gram Staining:

This reaction differentiates the gram positive from the gram-negative bacteria due to differences in their cell wall structure. A drop of normal saline was placed on a clean Greece free slide, using a sterile wire loop, a smear of the culture was made on the slide and heat fixed. The fixed smear was flooded with crystal violet strains for 60 seconds, rinse with clean water and drained quickly before it was flooded with Lugols iodine for 60 seconds and was then washed off with distilled water. The slide was flooded with 95% ethanol which is a decolourizer for 5 secs. After that, the slide was washed using distilled water and then flooded with Safranin (counter stain) for 30 seconds and then washed off. The back of the slide was then cleaned and placed in a draining rack for the stained smear to air dry. The standard smear was then allowed to air dry. Then, a drop of oil immersion was added on the smear to prior to viewing under the microscope for examination.

2.7.2 Catalase Test:

Using a sterile dropping pipette, a drop of 3% hydrogen peroxide solution was placed on a slide and a colony of the test organism was added to the drop of hydrogen peroxide solution. Fermentation of oxygen bubbles which is an indicator for the presence of catalase was looked out for. This is done to differentiate between *Staphylococci* and *Streptococci*. Effervescence of gas indicates the presence of gram-positive organisms.

2.7.3 Coagulase Test:

For the analysis, a drop of sterile distilled water was added to both ends of the slide. A colony of the test organism was emulsified in each drop of water and a look full of plasma added to one of the suspension with thorough mixing. Change is observed within 10 seconds to determine the identity of the organism.

2.7.4 Oxidase Test:

Over-well isolated (pure culture) colonies of test bacteria from fresh culture; three drops of Kovac's oxidase reagent were added. Tilt the plate and shake it gently so that the colonies get exposed to oxygen. Observe for the formation of purple (deep blue) colour over the reagent moistened colonies and note the time requires for change in color for up to 60 secs. When the test is positive (+), there's development of purple to deep blue colour within 10-30 secs indicating a positive (+) oxidase test and development of purple to deep blue colour within 30-60 secs indicating a weak oxidase positive (+) reaction or delayed oxidase positive. When the test is negative (-), there is no development of purple to deep blue colour within 60 seconds and development of purple to deep blue colour after 60 seconds.

2.7.5 Citrate Utilization Test:

The test differentiates among bacteria by determining their ability to utilize citrate as their only source of carbon. Test organisms were inoculated on simmons citrate agar slants streaking gently and incubated at 30°C for 24 hours. A colour change in the agar from green to blue indicates a positive (+) reaction.

2.7.6 Indole Test:

This test is used to distinguish among members of the families of *Enterobacteriaceae* by testing their ability to degrade an essential alpha amino acid, tryptophan to produce Indole. The Isolates were inoculated in nutrient broth and incubated at 37°C for 24 hours. After incubation, few drops of Kovac's reagents were added to the tube and were shaken gently and allowed to stand. The pinkish and ring like color and the upper layer indicates Indole production in the tubes and if otherwise, no Indole production.

2.7.7 Urease Test:

Urease test is a biochemical test that detects the alkaline fermentation of urine (urea) with the resultant production of ammonia by microorganisms. It is performed by growing the test organisms on the agar medium containing the pH indicator phenol red. Positive (+) results show deep pink colour while no colour change is a negative (-) result.

2.7.8 Methyl Red Test:

Wire loops full of isolates under investigation were inoculated and incubated for 24 hours, three drops of methyl red solution was added and colour change observed. A colour change from light yellow to pink indicates a methyl red positive reaction, meaning that acid is produced, while no change in colour (colour remains yellow) indicated a methyl red negative (-) reaction.

2.7.9 Voges Proskauer Test (Vp):

Inoculate and voges proskauer broth tube with a pure culture of the test organism, then incubate for 24 hours at 35°C. at the end of this time measure 1ml of broth into a clean test tube. Add 0.6ml of 5% of naptol followed by 0.2 ml of 40% KOH. Shake the tube gently to expose the medium to atmospheric oxygen and allow the tube to remain undisturbed for 10-15 minutes. A positive VP test is development of a pink red colour at the surface within 15 minutes or more after the addition of the reagents. The rest should not be read after standing for over one hour because negative Voges-Proskauer cultures may produce a colourless colour, resulting in a false positive interpretation.

2.7.10 Hydrogen Sulphide:

Using a sterile rod, innoculate a well isolated colony from a fresh culture of the test bacterium, streak culture over the agar plate to get well isolated colonies and then incubate at 37°C for about 24 hours. Observe the colour of the developed colonies. Black colonies or colourless or coloured colonies with a black center will appear.

2.7.11 Motility Test:

With a sterile straight needle, touch a colony of a young (18-24 hours) culture growing up on an agar medium, then single stab down the center of the tube to about half the development of the medium, then incubate at 37°C and examine daily for up to 7 days. If it is motile, the organisms will spread out into the medium from the site of inoculation and if it's non motile, the organism remains in the site of inoculation.

2.8 Experimental Design:

This study was conducted using CRD (Complete Randomized Design), where the towns are the treatment and the poultry units within the towns are the replicates.

Given the formula,

$$Yij = \mu + Ti + Eij \quad (1)$$

where;

Yij= single observation made on jth observation and ith treatment

μ = Overall mean

Ti = Effect of treatment (i=1,2,.....n)

Eij= Random error.

2.9 Statistical Analysis:

Analysis of Variance (ANOVA) was carried out using SPSS version 17, and differences between the treatment means were separated using Duncans New Multiple Range Test.

III. RESULTS AND DISCUSSIONS

3.1 Characterization of pathogens:

Biochemical reaction patterns used include: colony morphology, gram reactions, catalase, oxidase, coagulase, citrate, indole, urease, methyl red, voges Proskauer, hydrogen sulphide and motility tests.

3.1.1 Isolated pathogens from Ifite town:

A total of four dominant isolates were obtained from 10 faecal samples collected from Ifite village in Awka South Local Government Area.

The biochemical tests carried out using isolates from Ifite town revealed the organism to be *E-coli*, Streptococcal *spp*, *Salmonella spp* and *Shigella spp.* (Table 1).

TABLE 1
BIOCHEMICAL REACTION OF THE ISOLATES FROM IFITE

Colony Morphology	Probable Organisms	Gram Reaction	Catalase	Coaguse	Oxidase	Citrate	Indole	Urease	Methl Red	VP	H2s	Motility
Pink Smooth	*E-coli*	-	+	-	-	-	+	-	+	-	-	+
White colonies in chains	*Streptococcal spp*	+	-	-	-	-	-	-	-	-	-	-
Dark coloured rods	*Salmonella*	-	+	-	+	-	-	-	+	+	+	+
Pink rod like colours	*Shigella*	-	+	-	-	-	+	-	+	-	-	-

3.1.2 Isolated pathogens in Okpuno town:

A total of five dominant isolates were obtained from 30 faecal samples collected from Okpuno in Awka South Local Government Area. The biochemical tests carried out using isolates from Okpuno town revealed the organism to be *E-coli*, Streptococcal *spp, Salmonella spp*, *Enterococcus spp* and *Shigella spp.* as presented in Table 2.

TABLE 2
BIOCHEMICAL REACTION OF THE ISOLATES FROM OKPUNO

Colony Morphology	Probable Organisms	Gram Reaction	Catalase	Coaguse	Oxidase	Citrate	Indole	Urease	Methl Red	VP	H2s	Motility
Dark coloured rods	Salmonella spp	-	+	-	+	-	-	-	+	-	+	+
Pink rods	*Shigella spp*	-	+	-	-	-	-	-	+	-	-	-
Pink smooth colonies	*E-coli*	-	+	-	-	-	+	-	+	-	-	+
Small white colonies in chain	*Streptococcal spp*	+	-	-	-	-	-	-	-	-	-	-
Colourless small colonies	*Enterococcus spp*	+	-	-	-	-	-	-	-	+	-	-

3.1.3 Isolated pathogens in Amawbia town:

A total of six dominant isolates were obtained from 30 faecal samples collected from Amawbia in Awka South Local Government Area.

Table 3 presents the biochemical tests carried out using isolates from Amawbia town. The organisms identified include *E-coli,* Streptococcal *spp, Salmonella spp, Staphylococcal spp, Enterococcus spp* an *Shigella spp.*

TABLE 3
BIOCHEMICAL REACTION PATTERN OF ISOLATES FROM AMAWBIA

Colony morphology	Probable Organisms	Gram rxn	Catalase	Coagulase	Oxidase	Citrate	Indole	Urease	Methyl red	Vp	H2s	Motility
Pink smooth colonies	*E-coli*	-	+	-	-	-	+	-	+	-	-	+
Dark colored rods	*Salmonella spp*	-	+	-	+	-	-	-	+	-	+	+
Pink rods	*Shigella spp*	-	+	-	-	-	+	-	+	-	-	-
Small white colonies in chain	*Streptococcal spp*	+	-	-	-	-	-	-	-	_	-	-
Clustered cocci	*Staphylococcal spp*	+	+	-	-	+	-	+	+	+	-	-
Colourless small colonies	*Enterococcus spp*	-	+	-	-	+	-	+	-	+	-	+

3.2 Common pathogens identified in poultry industries in Awka south local government area of Anambra state, Nigeria

The summary of the bacterial isolates identified in Ifite (T1), Okpuno (T2) and Amawbia (T3) are presented in Table 4.

TABLE 4
THE SUMMARY OF THE BACTERIAL ISOLATES IDENTIFIED IN IFITE (T1), OKPUNO (T2) AND AMAWBIA (T3)

Isolates	Ifite (T1)	Okpuno (T2)	Amawbia (T3)
E-Coli	+	+	+
Salmonella spp	+	+	+
Shigella spp	+	+	+
Streptococcal spp	+	+	+
Staphylococcal spp	_	_	+

In Amawbia, virtually all the bacterial isolates studied were present while *Staphloccoccal spp* was absent in Okpuno. So, *E.coli, Salmonella spp, Shigella spp and Streptococcal spp* are more endemic in Awka South Local Government Area.

3.3 Sensitivity test of the identified pathogens:

These isolates were subjected to sensitivity tests with commercially prepared antibiotics discs using disc diffusion methods.

3.3.1 Sensitivity test of the identified pathogens from ifite town:

These isolates were subjected to sensitivity tests with commercially prepared antibiotics discs using disc diffusion methods and the results showed that only *salmonella spp* were sensitive to Peflacine with inhibitor zone diameter of 17mm, Gentamicin with zone diameter diameter of 17mm and Nalidixic acid with zone diameter of 12mm.

3.3.2 Sensitivity test of the identified pathogens in Okpuno town:

These isolates were subjected to sensitivity tests with commercially prepared antibiotics discs using disc diffusion methods and the results showed that *Salmonella spp* were sensitive to Ofloxacin with zone diameter of 20mm, Ceporex (16mm), Nalidix acid (15mm), Pelacine (12mm), Ciprpflaxin (13mm) and Streptomycin (10mm).

Also, *shigella spp* were sensitive to Oflaxacin with zone of inhibitor of 17mm, Peflacine (12mm) and Ciproflaxin (10mm). Both *E-coli spp, strept spp and entercoccus spp* were all resistant to the antibiotics.

3.3.3 Sensitivity test of the identified pathogens in Amawbia town:

These isolates were subjected to sensitivity tests with commercially prepared antibiotics discs using disc diffusion methods and the results showed that only *streptococcal spp* were sensitive to Rifampin with inhibitor zone diameter of 20mm, Gentamicin (17mm), Streptomycin (15mm), Amoxicillin (20mm) and Ampiclox (20mm). Table 5 presents the summary of the sensitivity pattern of the isolates from the three towns studied

TABLE 5
SUMMARY OF THE SENSITIVITY PATTERN OF THE ISOLATES FROM THE THREE TOWNS STUDIED

Isolates	Ifite (T1)	Okpuno (T2)	Amawbia (T3)
E-Coli	Resistant	Resistant	Resistant
Salmonella spp	Resistant	Sensitive	Resistant
Shigella spp	Sensitive	Sensitive	Resistant
Streptococcal spp	Resistant	Resistant	Resistant
Staphylococcal spp	-	-	Sensitive
Enterococcus spp	-	Resistant	Resistant

3.4 Location effect on the number of bacteria isolates:

The effect of location on the number of the bacteria isolates obtained at poultry industries in Awka South Local Government Area of Anambra State, Nigeria is presented in Table 6.

TABLE 6
EFFECT OF LOCATION ON THE NUMBER OF BACTERIA ISOLATES

Isolates	Ifite (T1)	Okpuno (T2)	Amawbia (T3)
E-Coli	10	10	10
Salmonella Spp	10	10	10
Shigella Spp	10	10	10
Streptococcal Spp	10	10	10
Staphylococcal Spp	0.00b	0.00b	10.00a
Enterococcus Spp	0.00b	10.00a	10.00a
Key = + Indicates Presence of A Given Isolate While – Indicates Its Absence.			
Means Bearing Different Letter along with the Same Rows are Significantly Different (P<0.05)			

Amawbia poultry farms recorded the highest number of isolates followed by Okpuno and Ifite being the least.

IV. DISCUSSION

4.1 Escherichia Coli (*E. Coli*):

*Escherichia coli*is a common bacterium found in the intestines of warm-blooded animals, including poultry. While many *E. coli* strains are harmless, some can cause serious infections in animals and humans.

E. coli was identified in all three towns: Ifite, Okpuno, and Amawbia. Similar to findings by Akinpelu *et al.* (2023), who reported a high prevalence of *E. coli* in poultry farms in Southwestern Nigeria, our results confirm its ubiquitous presence in poultry environments. The researchers also highlighted that *E. coli* thrives in unhygienic conditions, which may explain its widespread occurrence in Awka South Local Government Area. *E. coli* infections can cause colibacillosis in poultry, leading to respiratory infections, reduced productivity, and increased mortality rates. Its presence suggests poor biosecurity measures. *E. coli* isolates from all towns showed resistance to antibiotics tested. Resistance could be due to the overuse of antibiotics in feed and treatment, as observed by Roth *et al. (*2019). Antibiotic-resistant *E. coli* strains pose challenges in managing infections in poultry. Antibiotic-resistant *E. coli* in poultry may transfer to humans via contaminated meat or direct contact, potentially causing foodborne illnesses or urinary tract infections (UTIs). This is consistent with findings by Kumar *et al.* (2019).

4.2 *Salmonella Spp.:*

Salmonella was present in all the towns studied, but exhibited varying antibiotic sensitivity. The frequency of its occurrence suggests that these locations might present favorable conditions for the survival and transmission of *Salmonella*, a common food borne pathogen in poultry environments. The prevalence aligns with findings by Ugbo *et al.* (2018), who reported high *Salmonella* presence in poultry farms in Eastern Nigeria. In contrast, our results showed variable sensitivity to antibiotics. *Salmonella* causes salmonellosis in poultry, leading to economic losses through poor growth and egg production. It also increases the cost of treatment when resistant strains emerge. Resistance patterns varied, with isolates from Okpuno being the most sensitive. Resistance in pathogenic bacteria can lead to recurrent disease outbreaks, increased mortality rates, and higher production costs for poultry farmers who need to invest in alternative treatments or adopt stricter management protocols. Misuse of antibiotics in poultry farming, as noted by Olovo *et al.* (2019), may contribute to this resistance. Additionally, resistant strains may persist in the environment and infect humans through direct contact or consumption of contaminated poultry products, thus posing a risk to public health. Drug-resistant *Salmonella* strains can cause severe foodborne outbreaks in humans. Contaminated poultry products are a major source of infection.

4.3 *Shigella Spp:*

The genus *Shigella* was first identified by Japanese microbiologist Kiyoshi Shiga in 1897 during an outbreak of dysentery. *Shigella* was found in Ifite and Okpuno but absent in Amawbia. *Shigella's* occurrence is less common in poultry compared to *Salmonella*, but our findings are consistent with studies like Odo *et al.* (2021), who identified *Shigella* in poultry litter in Nigeria. While *Shigella* primarily infects humans, its presence in poultry indicates possible contamination through water or feed, posing risks of zoonotic transmission. *Shigella* spp from Okpuno showed sensitivity to multiple antibiotics, suggesting recent introduction into the ecosystem. However, Ifite isolates showed limited sensitivity, likely due to previous exposure to antimicrobial agents. Antibiotic-resistant *Shigella* can lead to higher morbidity and mortality rates in poultry, necessitating frequent use of alternative treatments or combinations of antibiotics, which can increase production costs. *Shigella* causes dysentery in humans, with resistant strains exacerbating outbreaks. Zoonotic transfer is possible, especially in unsanitary conditions, as noted by Jenkins *et al.*, (2023).

4.4 *Streptococcal Spp.:*

Streptococcal species are a group of Gram-positive bacteria commonly found in the environment, including in the intestines of poultry. While many *Streptococcus* strains are non-pathogenic, certain species can cause diseases in poultry, such as septicemia, respiratory infections, and arthritis. *Streptococcal spp* were identified in all towns, with the highest sensitivity recorded in Amawbia. Our findings align with that of Odeyemi *et al.* (2019) who noted *Streptococcal spp* in poultry environments as opportunistic pathogens. These bacteria can cause infections such as septicemia in poultry, leading to reduced productivity. Resistant strains found in Ifite and Okpuno indicates prolonged antibiotic misuse in these towns. However, Amawbia isolates showed sensitivity to Rifampin and Amoxicillin, indicating less exposure to these drugs. The presence of antibiotic-resistant *Streptococcal spp*. in poultry farms poses a risk to human health, as these bacteria can cause infections that range from mild throat infections to severe conditions like meningitis and bacteremia. *Streptococcal* infections in humans, like pharyngitis or invasive diseases, could arise through zoonotic transmission, especially when handling infected birds without proper hygiene.

4.5 *Staphylococcal Spp.:*

Staphylococcal spp. is Gram-positive cocci known for their capacity to colonize various environments, including poultry. They are commonly associated with skin infections, respiratory diseases, and septicemia in poultry. *Staphylococcus* was identified only in Amawbia. This is consistent with the findings of Ezeh *et al*. (2023) and Islam *et al*. (2023), who found *Staphylococcus* to be less prevalent in rural poultry farms compared to urban areas. *Staphylococcus* causes conditions like bumblefoot and septicemia, affecting bird welfare and economic outputs. Sensitive to antibiotics like Ampiclox and Amoxicillin is indicating the possibility of effective treatment of the ailment caused by the pathogen in Amawbia. The occurrence of antibiotic-resistant *Staphylococcal spp*. in poultry farms could present significant challenges. Infected birds may exhibit prolonged illness, reduced growth rates, and increased mortality, leading to economic losses. *Staphylococcus aureus*, particularly drug-resistant strains (e.g., MRSA), poses significant risks to humans, especially farm workers. *Staphylococcus* infections in humans can range from mild skin infections to severe, life-threatening conditions, such as sepsis.

4.6 *Enterococcus Spp.:*

Enterococcus species are part of the normal gut flora in animals, including poultry, and can be found in various environmental sources. Although many *Enterococcus* strains are commensal, some can cause infections under certain conditions, particularly in immune compromised hosts. It was detected only in Okpuno and Amawbia. *Enterococcus spp* have been reported by Chukwu *et al*, (2022) in poultry farms with high antimicrobial use. *Enterococcus* infections in poultry, though rare, can complicate other diseases. Their presence suggests environmental contamination. Both towns showed high resistance, which is troubling since *Enterococcus* can act as a reservoir for resistance genes, as highlighted by Morgan *et al*.(2023). The resistance of *Enterococcus* to antibiotics can have significant implications for poultry farming, as these bacteria can act as reservoirs for antibiotic resistance genes that may transfer to other pathogenic organisms. This can make bacterial infections harder to treat, leading to increased disease incidence and economic losses.

Enterococcus spp, particularly vancomycin-resistant strains (VRE), pose serious health risks in clinical settings. The presence of multi-drug resistant *Enterococcus* in poultry poses a risk to human health, especially when considering the potential for zoonotic transmission. Humans can be exposed to these resistant strains through direct contact with poultry, handling contaminated poultry products, or consuming undercooked meat. Infections caused by resistant *Enterococcus* in humans can lead to conditions such as urinary tract infections, bacteremia, or endocarditis, which can be challenging to treat due to limited therapeutic options.

V. CONCLUSIONS

This study underscores the prevalence of bacterial pathogens in poultry industries in Awka South Loal Government of Anambra State. The major pathogens include *E-coli spp., Salmonella spp., Shigella spp., Streptococcal spp., Staphylococcal spp.* and *Enterococcus spp.* These pathogens pose significant economic losses to the poultry industry and public health risks due to their zoonotic potential.

Again, poultry farmers in this area are not lettered, and as such are not practicing proper management and bio-security measures. Equally, they use sub-lethal levels/ doses of antibiotics when confronting these pathogens. Hence, most of these antibiotics are resistant to commonly used antibiotics. The most resistant isolates identified were *E. coli spp., Salmonella spp., Shigella spp. and Streptococcal spp.* which are very common in the area.

The study therefore calls for close monitoring of antibiotic resistance in our environment and controlled use of antibiotics in poultry industries since these pathogens are zoonotic, and birds serve as the major source of protein in many homes. The use of the bird droppings for manure should be checked, because of the health hazard to the general populace when crops are consumed.

RECOMMENDATIONS

A multi-faceted approach is essential in combating the menace of bacterial pathogens in Awka south local government of Anambra state. Rigorous bio security measures, such as strict hygiene protocols, controlled access, and vaccination programs are pivotal in preventing disease outbreaks. Additionally, judicious antibiotic use and antimicrobial stewardship are crucial to combat antimicrobial resistance and maintain the efficacy of treatments.

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Effect of Organic Sources of Nutrient with or without Bio-stimulant {(*Kappaphycus alvarezii* (K Sap)} on Growth and Yield of Linseed

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Abstract— *An investigation titled "**Effect of organic sources of nutrient with or without bio-stimulant {Kappaphycus alvarezii (K Sap)} on growth and yield of linseed**" was carried out to assess the influence of different combinations of organic source nutrients, administered at various levels, on crop yield, and overall plant growth of the BLS-4 variety of linseed. During the Rabi season of 2023-24 at Organic Research Farm, Karguan ji, Department of Agronomy, Institute of Agricultural Sciences, Bundelkhand University, Jhansi, Uttar Pradesh. The experiment was laid in Randomized block design with 10 treatments and 3 replications with different combination of organic sources nutrients. In all 10 treatment viz T_0 (Absolute control); T_1 (10 ml K sap); T_2 (RDF [NPK: 30:15:20 kg/ha]); T_3 (RDF + 10 ml K sap); T_4 (RDF through Farmyard manure (FYM); T_5 (100% FYM + 10 ml K sap); T_6 (100% RDF through vermi-compost (VC); T_7 (100% Vermi-compost (VC) + 10 ml K sap); T_8 (50% VC + 50% FYM) and T_9 (50% VC + 50% FYM + 10 ml K sap) were evaluated. From the results it may be concluded that the treatment T_2 (RDF-NPK: 30:15:20 kg/ha) was found to be best in the terms of growth, and yield of linseed followed by T_4 (100% RDF through Farmyard manure (FYM) and T_9 (50% VC + 50% FYM + 10 ml K sap). The highest net return was also recorded in the T_4 (100% RDF through Farmyard manure (FYM) followed by T_9 (50% VC + 50% FYM + 10 ml K sap) and the highest B:C ratio was found in the same with 3.15 and 3.04 respectively. Therefore, for linseed cultivation using farmyard manure as organic source can be beneficial to get better grain yield and economic returns*.

Keywords— Linseed, Farmyard Manure, Kappaphycus alvarezii (K Sap), Benefit cost ratio.

I. INTRODUCTION

Oilseeds, which are the primary source of vegetable oils, are second only to food grains in importance to the Indian economy, making up around 10% of the country's cultivated land and the total value of its agricultural output. Based on the current level of fat and oil consumption (8.5 kg/capita/year) and the subsequent growth, the requirements for vegetable oilseed have been projected to be approximately 34 million tonnes by 2030 AD, of which 14 million tonnes is to be contributed by rapeseed - mustard to meet the annual domestic demand. Over the past three decades, the oilseeds sector has grown at a rate of 4.1% annually, outpacing the growth of livestock products and other agricultural products. This has made it one of the most dynamic sectors in the global agricultural sector. Linseed, also known as flax (*Linum usitatissimum* L.), is one of the oldest crops grown worldwide for its oil, fibre, and seed. Linseed stands out among oilseeds due to its ability to produce technical-grade vegetable oil and high-quality fibre, known for its strength and durability. With an oil content ranging from 35 to 45 percent, linseed is rich in omega-3 fatty acids, particularly alpha-linolenic acid (ALA). The presence of omega-3 fatty acids aids in reducing triglyceride levels in the blood, thereby lowering the risk of heart disease, and showing potential in combating rheumatoid arthritis. Linseed oil contains three times more omega-3 fatty acids than omega-6 fatty acids. Additionally, linseed seeds boast 36 percent protein, with 85 percent of it being digestible. The oil cake leftover after oil extraction is utilized as feed for dairy and fattening animals, contributing to milk and meat production. The linseed crop occupies an area of 1.82 lakhs hectare in the country yielding out 1.22 lakhs tones having an average productivity of 671 kg/ha for year 2019-20 (**Anonymous, 2020**). Linseed oil is the best vegetable oil for painting and varnishing because of its high iodine value, which is more than 180. It also gives the products good drying properties. As a result, the majority of linseed produced worldwide finds its application in

industry. Despite being categorised as an inedible oil, roughly 20% of the linseed produced in India is used for food, especially in states like Madhya Pradesh, Uttar Pradesh, Bihar, and Chhattisgarh. Uttar Pradesh is one of the important linseeds growing state of India, where linseed is being cultivated over 26.90 thousand hectares with a production of 17.89 thousand tones with productivity of 150 kg/ha (**Anonymous, 2021**).

Application of FYM along with inorganic fertilizers in soil leads to improve soil structure, water holding capacity and enhances system productivity. Organic sources of nutrients applied to the preceding crop benefits the succeeding crop to a great extent (**Hedge and Dwivedi, 1992**) and system productivity becomes more sustainable in nature. **Ismail *et al.*, (1994)** concluded that conservation tillage systems result significant and positive effects on several physical and chemical soil properties. Organics, in the context of agriculture, refer to plant and animal waste materials that serve as sources of nutrients for plants. These materials release their nutrients when they undergo decomposition. Vermi-compost has demonstrated remarkable capabilities as a growth enhancer for plants while also acting as a protective shield against pests and diseases (**Tiwari and Singh, 2021**). This makes it a valuable and multifaceted resource for improving soil health and supporting plant growth in agricultural and horticultural practices. Very recently the use of stimulant is gaining importance in crop production system including organic farming. Researching the effects of organic sources of nutrients, with or without bio-stimulant like *Kappaphycus alvarezii* (K Sap), on the growth and yield of linseed is crucial for several reasons. Firstly, organic farming practices are gaining momentum due to their potential to improve soil health, enhance nutrient availability, and promote sustainable agriculture. By exploring the efficacy of organic nutrient sources, farmers can reduce reliance on synthetic fertilizers, mitigating environmental pollution and minimizing health risks associated with chemical residues in food. Additionally, bio-stimulant derived from natural sources like *Kappaphycus alvarezii* have shown promising results in enhancing plant growth, stress tolerance, and nutrient uptake. Understanding their impact on linseed cultivation can contribute to optimizing organic farming techniques, improving yield, and ensuring the production of high-quality linseed crops.

II. MATERIALS AND METHODS

The goal of the current study entitled "**Effect of organic sources of nutrient with or without bio-stimulant {*Kappaphycus alvarezii* (K Sap)} on growth and yield of linseed** " was to assess the influence of different combination of organic nutrients with or without bio- stimulant, administered at various levels, on crop yield, and overall plant growth of the BLS-4 variety of linseed. The subsequent sections present details regarding the materials used and methodologies employed during the investigation, conduct in the *R*abi season of 2023 at the Organic Research Farm, karguaji, Department of Agronomy, Institute of Agricultural Sciences, Bundelkhand University, Jhansi. The experimental design employed was a Randomized Block Design (RBD) with three replications and ten treatments. The **Fisher and Yates, 1967** method was used to statistically analyse the data. The software used for analysis was OPSTAT. The linseed variety used was BLS-4. In the study, the height of randomly chosen plants from each plot was assessed in centimetres using a meter scale. Stem diameter was measured at last harvest stage using meter tape in random selected five plants. Additionally, the number of branches and leaves per plant, emerging from the main shoot, was counted, and the values were averaged. TSS was measured using refractometer. The details of treatments comprised of T_0 (Absolute control); T_1 (10 ml K sap); T_2 (RDF [NPK: 30:15:20 kg/ha]); T_3 (RDF + 10 ml K sap); T_4 (RDF through Farmyard manure (FYM); T_5 (100% FYM + 10 ml K sap); T_6 (100% RDF through vermi-compost (VC); T_7 (100% Vermi-compost (VC) + 10 ml K sap); T_8 (50% VC + 50% FYM) and T_9 (50% VC + 50% FYM + 10 ml K sap).

III. RESULTS AND DISCUSSION

There was significant difference recorded in plant height among different treatments of organic sources in linseed observed at 30, 60 and 90 DAS. The better height of plant (26.44, 44.80 and 69.55 cm) at 30, 60 and 90 days after sowing respectively was observed in T_2 (RDF [NPK: 30:15:20 kg/ha]) followed by T_4 (100% RDF through Farmyard manure (FYM) with 24.64, 43.38 and 68.06 cm recorded at 30, 60 and 90 days after sowing. T_0 (Absolute control) recorded lowest plant height (17.86, 30.54 and 49.71 cm) observed at 30, 60 and 90 days after sowing. Combining recommended dose of fertilizer (RDF) with Farmyard Manure (FYM) enhances linseed plant height due to synergistic effects. FYM enriches soil with organic matter, fostering microbial activity and improving nutrient availability. This aids in better root development and nutrient uptake, promoting vigorous growth. RDF provides essential minerals in balanced proportions, complementing FYM's organic benefits. The combined application ensures sustained nutrient release throughout the plant's growth stages, supporting steady and robust vertical development. Additionally, FYM enhances soil structure, aiding in water retention and aeration, further facilitating optimal plant growth. Consequently, linseed plants treated with RDF alongside FYM exhibit superior height compared to those receiving RDF alone, showcasing the advantages of integrated nutrient management for enhanced crop productivity. Reports were in close conformity with Kaushal and Umrao (2020); Singh *et al.*, (2021) in Linseed.

When comparing the fresh shoot weight per plant of linseed at 30, 60, and 90 DAS across the various treatments of organic sources, a notable and statistically significant difference was discovered. T_2 (RDF-NPK: 30:15:20 kg/ha) had the highest fresh shoot weight per plant (9.22, 56.60 and 159.67 grams) at 30, 60, and 90 days after sowing, respectively followed by T_4 (100% RDF through Farmyard manure (FYM) with 8.16, 56.46 and 154.82 grams at 30, 60, and 90 days after sowing. At 30, 60 and 90 days after sowing, the T_0 (absolute control) recorded the lowest fresh shoot weight per plant (3.01, 21.33 and 85.26 grams). Combining recommended dose of fertilizer (RDF) with Farmyard Manure (FYM) leads to the maximum fresh shoot weight per plant in linseed plants due to the synergistic enhancement of nutrient availability and soil health. FYM enriches the soil with organic matter, fostering microbial activity and nutrient retention, thereby promoting robust shoot growth. This facilitates the development of lush and vigorous shoots. RDF complements FYM by providing essential minerals in balanced proportions, further supporting shoot development and biomass accumulation. The integrated application ensures a sustained and balanced supply of nutrients throughout the growth stages, optimizing shoot production. Consequently, linseed plants treated with RDF alongside FYM exhibit the highest fresh shoot weight per plant compared to those receiving RDF alone, highlighting the advantages of integrated nutrient management for maximizing shoot growth and overall plant productivity. Findings were in close conformity with Jangid *et al.,* (2022); Rensang *et al.,* (2022) in Linseed.

When comparing the dry shoot weight per plant of linseed at 30, 60, and 90 DAS across the various treatments of organic sources, a notable and statistically significant difference was discovered. T_2 (RDF-NPK: 30:15:20 kg/ha) had the highest dry shoot weight per plant (3.39, 22.32 and 42.96 grams) at 30, 60, and 90 days after sowing, respectively followed by T_4 (100% RDF through Farmyard manure (FYM) with 2.99, 22.26 and 41.66 grams at 30, 60, and 90 days after sowing better over T_0 (absolute control) recorded the lowest dry shoot weight per plant (1.11, 8.42 and 22.93 grams).

When recommended dose of fertiliser (RDF) and Farmyard Manure (FYM) are combined, nutrient availability and soil health are synergistically enhanced, resulting in the maximum dry shoot weight per plant in linseed plants. By adding organic matter to the soil, FYM encourages microbial activity, nutrient retention, and strong shoot growth. This encourages the growth of lush, robust shoots. By supplying necessary minerals in balanced amounts, RDF enhances the growth of shoots and the accumulation of biomass. By providing a steady and balanced supply of nutrients throughout the growth stages, the integrated application maximises the production of shoots. Thus, when compared to plants treated with RDF alone, linseed plants treated with RDF plus FYM show the highest dry shoot weight per plant, demonstrating the benefits of integrated nutrient management for optimising shoot growth and overall plant productivity. Similar findings were reported by Janghel *et al.,* (2023) in Linseed.

When comparing the number of capsules per plant of linseed across the various treatments of organic sources, a notable and statistically significant difference was discovered. T_2 (RDF-NPK: 30:15:20 kg/ha) had the highest number of capsules per plant (65.77 capsules) followed by T_4 (100% RDF through Farmyard manure (FYM) with 62.30 capsules better over T_0 (absolute control) recorded the lowest number of capsules per plant (37.10 capsules).

When comparing the test weight of linseed across the various treatments of organic sources, a notable and statistically significant difference was discovered. T_2 (RDF [NPK: 30:15:20 kg/ha]) had the highest test weight (5.86 grams) followed by T_4 (100% RDF through Farmyard manure (FYM) with 5.71 grams better over T_0 (absolute control) recorded the lowest test weight (4.26 grams).

When comparing the seed yield per plant of linseed across the various treatments of organic sources, a notable and statistically significant difference was discovered. T_2 (RDF [NPK: 30:15:20 kg/ha]) had the highest seed yield per plant (3.15 g/plant) followed by T_4 (100% RDF through Farmyard manure (FYM) with 2.59 g/plant better over T_0 (absolute control) recorded the lowest seed yield per plant (0.93 g/plant).

When comparing the straw yield per plant of linseed across the various treatments of organic sources, a notable and statistically significant difference was discovered. T_2 (RDF [NPK: 30:15:20 kg/ha]) had the highest straw yield per plant (8.24 g/plant) followed by T_4 (100% RDF through Farmyard manure (FYM) with 7.75 g/plant better over T_0 (absolute control) recorded the lowest straw yield per plant (4.29 g/plant). Combining recommended dose of fertilizer (RDF) with Farmyard Manure (FYM) leads to better seed and straw yield in linseed due to synergistic effects on soil fertility and plant growth. FYM enriches the soil with organic matter, enhancing soil structure, microbial activity, and nutrient retention, fostering robust root systems and nutrient uptake. This promotes vigorous plant growth, resulting in increased biomass production and ultimately, higher straw yield. RDF supplements FYM by providing essential minerals, ensuring balanced nutrition critical for optimal plant development and reproductive success, thus leading to enhanced seed yield. The integrated approach provides a continuous and balanced supply of nutrients throughout the growth cycle, maximizing both seed and straw yield. Consequently, linseed plants treated with RDF alongside FYM exhibit superior overall yield compared to those receiving RDF alone, highlighting

the benefits of integrated nutrient management for maximizing productivity. Similar findings were earlier reported by Neware and Bobade (2018); Chaudhary and Rai (2021) in Linseed.

TABLE 1

EFFECT OF ORGANIC SOURCES OF NUTRIENTS WITH OR WITHOUT BIO-STIMULANT ON THE PLANT HEIGHT AND FRESH SHOOT WEIGHT OF THE LINSEED

Treatment Notation	Treatment details	Plant height (cm)			Fresh shoot weight per plant (g)		
		30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS
T_0	Absolute control	17.86	30.54	49.71	3.01	21.33	85.26
T_1	10 ml K sap	17.92	31.05	50.77	3.22	22.52	86.77
T_2	RDF [NPK: 30:15:20 kg/ha]	26.44	44.8	69.55	9.22	56.6	159.67
T_3	100% RDF + 10 ml K sap	21.1	34.15	50.52	7.04	43.17	120.65
T_4	100% RDF through Farmyard manure (FYM)	24.97	43.38	68.06	8.16	56.46	154.82
T_5	100% FYM + 10 ml K sap	19.4	32.78	53.51	5.11	32.43	114.18
T_6	100% RDF through vermi-compost (VC)	20.2	38.11	51.88	5.02	30.32	109.99
T_7	100% Vermi-compost (VC) + 10 ml K sap	21.42	34.46	56.57	5.14	34.52	100.94
T_8	50% VC + 50% FYM	19.7	35.91	53.17	4.72	28.21	96.62
T_9	50% VC + 50% FYM + 10 ml K sap	24.64	42.72	61.13	8.01	47.92	141.63
SE. m (±)		**0.33**	**0.53**	**0.65**	**0.13**	**0.45**	**0.49**
$CD_{0.05}$		**0.97**	**1.58**	**1.94**	**0.37**	**1.34**	**1.45**

TABLE 2

EFFECT OF ORGANIC SOURCES OF NUTRIENTS WITH OR WITHOUT BIO-STIMULANT ON THE DRY SHOOT WEIGHT, NUMBER OF CAPSULES PER PLANT OF THE LINSEED

Treatment Notation	Treatment details	Dry shoot weight per plant (g)			No of capsules per plant	Test weight (g)
		30 DAS	60 DAS	90 DAS		
T_0	Absolute control	1.11	8.42	22.93	37.1	4.26
T_1	10 ml K sap	1.32	9.01	23.66	38.22	4.54
T_2	RDF [NPK: 30:15:20 kg/ha]	3.39	22.32	42.96	65.77	5.86
T_3	100% RDF + 10 ml K sap	2.58	17.02	32.47	52.06	5.42
T_4	100% RDF through Farmyard manure (FYM)	2.99	22.26	41.66	62.3	5.71
T_5	100% FYM + 10 ml K sap	1.89	12.79	30.73	48.79	5.42
T_6	100% RDF through vermi-compost (VC)	1.84	11.95	29.6	56.42	5.32
T_7	100% Vermi-compost (VC) + 10 ml K sap	1.89	13.6	27.17	52.88	5.22
T_8	50% VC + 50% FYM	1.71	11.12	26.01	42.67	4.88
T_9	50% VC + 50% FYM + 10 ml K sap	2.94	18.89	38.14	59.84	5.68
SE. m (±)		**0.05**	**0.23**	**0.41**	**0.45**	**0.05**
$CD_{0.05}$		**0.15**	**0.7**	**1.21**	**1.33**	**0.16**

TABLE 3
EFFECT OF ORGANIC SOURCES OF NUTRIENTS WITH OR WITHOUT BIO-STIMULANT ON THE SEED AND STRAW YIELD PER PLANT OF THE LINSEED

Treatment Notation	Treatment details	Seed yield per plant (g)	Straw yield per plant (g)	Harvest index (%)
T_0	Absolute control	0.93	4.29	18.46
T_1	10 ml K sap	1.08	4.43	19.33
T_2	RDF [NPK: 30:15:20 kg/ha]	3.15	8.24	27.45
T_3	100% RDF + 10 ml K sap	2.19	5.36	29.96
T_4	100% RDF through Farmyard manure (FYM)	2.59	7.75	27.45
T_5	100% FYM + 10 ml K sap	1.76	5.41	25.8
T_6	100% RDF through vermi-compost (VC)	1.58	5.47	23.25
T_7	100% Vermi-compost (VC) + 10 ml K sap	1.34	5.46	18.92
T_8	50% VC + 50% FYM	0.95	4.66	18.36
T_9	50% VC + 50% FYM + 10 ml K sap	2.54	7.39	27.38
SE. m (±)		**0.07**	**0.09**	**0.06**
$CD_{0.05}$		**0.2**	**0.27**	**0.18**

When comparing the harvest index of linseed across the various treatments of organic sources, a notable and statistically significant difference was discovered. T_3 (100% RDF + 10 ml K sap) had the highest harvest index (29.96%) followed by T_2 (RDF [NPK: 30:15:20 %]) and T_4 (100% RDF through Farmyard manure (FYM) with 27.45% better over T_0 (absolute control) recorded the lowest harvest index (18.46%). Because of their synergistic effects on soil health and plant nutrition, combining recommended dose of fertiliser (RDF) with farmyard manure (FYM) improves the yield per hectare of linseed produced. By adding organic matter to the soil, FYM improves microbial activity, soil structure, and moisture retention—all of which help with nutrient availability and root development. This encourages rapid plant growth, which raises the potential yield due to increased biomass accumulation. RDF enhances seed yield by providing essential minerals in balanced amounts to support plant growth and reproduction, which is a supplement to FYM. By guaranteeing a consistent supply of nutrients throughout the growth cycle, the integrated approach maximizes total yield per hectare. Thus, linseed crops treated with RDF plus FYM have higher yields than those treated with RDF alone, demonstrating the advantages of integrated nutrient management. Reports were in close conformity with Neware and Bobade (2018); Chaudhary and Rai (2021); Jangid *et al.,* (2022) in Linseed.

IV. CONCLUSION

From the above experimental findings, it may be concluded that the treatment T_2 (RDF-NPK: 30:15:20 kg/ha) was found to be best in the terms of growth, and yield of linseed followed by T_4 (100% RDF through Farmyard manure (FYM) and T_9 (50% VC + 50% FYM + 10 ml K sap). While the highest net return was found in the T_4 (100% RDF through Farmyard manure (FYM) followed by T_9 (50% VC + 50% FYM + 10 ml K sap) and the highest B:C ratio was found in the same with 3.15 and 3.04 respectively. Therefore, for linseed cultivation using farmyard manure as organic source can be beneficial to get better grain yield and quality.

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A Comparative Performance Study using Dynamic Headspace Sampling and Sorbent Tube Methods of Broiler Litter Odour

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Abstract**—** *Broiler production emission is often obnoxious and little is known about complete speciation and levels of volatiles from poultry production due to challenges in selecting reliable sampling technique with less amendment to volatiles' characteristic. This study aimed to compare the performance between Tenax TA sorbent tube and dynamic headspace sampling methods to distinguish chemical and olfaction analyses of odorants in broiler litter emission using standard solutions. Standard solutions containing 13 volatile compounds at 50 ppmv, 100 ppmv and 250 ppmv were sampled using Tenax TA sorbent tube and dynamic headspace sampler. The samples were analysed using thermal desorption gas chromatography-mass spectrometry olfactometry system (TD-GC-MS/O), providing data of human olfactory for identification and description of odorants detected. The Tenax TA sorbent tube and dynamic headspace sampler have successfully determined all volatiles used at all three concentrations with the Tenax TA sorbent tube sampling exhibiting relevant multiplication of peak area of volatiles corresponding to concentration sample. However, the dynamic headspace sampler has shown lower relevant multiplication of peak area corresponding to concentration of standard solution. Hence, both methods can be compared directly for qualitative information (chemical and olfactory characteristic) than quantitative information. This is evident with the strong occurrence of volatile compounds elucidating at similar retention time using Tenax TA sorbent tube and dynamic headspace sampler using all three standard solutions under identical TD-GC-MS/O analysis. Fast and simplification in sampling technique is observed using dynamic headspace sampler compared to Tenax TA sorbent tube that it would be a better direct source sampling solution.*

Keywords— Broiler Litter, Odour, Sorbent Tube, Dynamic Headspace Sampling, TD-GC-MS/O.

I. INTRODUCTION

Demand for meat chicken (broiler) expands extensively with growing population and rise income globally (Miller *et al.* 2022). Broilers are usually grown on bedding material in ventilated tunnel sheds over 7 – 9 weeks. Though the intensive livestock practice ensures minimal nuisance generation to the surrounding condition, the facilities often become a target for odour complaints due to emerging urban encroachment in the rural environment (Powers *et al.* 2005). Odours from broiler shed are generally generated through aerobic and anaerobic microbial activities within the litter and from the animals (Rappert and Muller 2005; Dunlop *et al.* 2016). Occurrence of odour annoyance increase with accumulation of waste sources as litter over chicken growth period. This incident results in annoyance complains from the local residents living near the facility (Schiffman 1998; Modak *et al.* 2019; Dunlop and Atzeni 2020) and also personnel's working in the facility (Marinella *et al.* 2014). To abate odour in poultry production facilities, accurate sampling and characterisation of odours using reliable and representable techniques are essential to gain a clearer understanding of the emission nature (Schiffman 1998; Lacey et al 2004; Powers et al 2005; Conti et al 2020; Guo et al 2022) and to implement odour guidelines.

Sampling of odours from a matrix at ambient condition is often critical and challenging (Laura *et al.* 2013). Odorous emissions are combination of varieties of compounds with vast range of quality and quantity (Eva *et al.* 2011; Shicheng *et al.* 2010 and Lorenzo *et al.* 2022). Techniques of volatile collection mostly include solvent extraction, sorbent tubes and solid phase micro-extraction in which involves enrichment and collection of representative volatiles, especially those with low concentration, on a suitable medium prior to analysis (Eva *et al.* 2010). However, these techniques also may cause losses of volatile organic compounds as the sorbent materials tends to absorb water in high humidity circumstances and during multiple stages of stripping and loading of odours, that may change the characteristics of its components (Noelia et al 2010). Focusing of volatiles onto sorbent materials or cold trap offers adequate approach to obtain micrograms and nanograms per litre of concentrated

volatiles, resulting in the least amendments on the gas sample and sample matrix and interference of solvent. The desired volatiles are adsorbed on a sorbent material or cold trap at a preset flow rate prior to thermal desorption. Basic prerequisites are the choice of sorbents which are dependent on the affinity of desired chemical, appropriate breakthrough volume for all volatiles present in a gaseous sample, low water affinity, high recovery of volatiles during thermal desorption, low reactivity with volatiles adsorbed and the absence of the formation of artefacts (Pillonel *et al.* 2002 and Tholl *et al.* 2006). No one sorbent material is capable of adsorbing all kinds of volatiles and most sorbent material has affinity towards a particular volatile functionality (Munoz *et al.* 2002 and Ribes et al 2007). This aspect has led to the development and usage of multi-bed sorbent tubes that enables advanced levels of volatiles' focusing (Ribes *et al.* 2007). The most common sorbent materials available commercially include Tenax TA, Chromosorb carbon molecular sieves, graphitized carbon and multi-bedded sorbent materials such as Carbograph, Carbotrap C and Carbotrap/ Carbosieve (Chuandong *et al.* 2018).

Meanwhile, dynamic headspace sampling technique is used to separate volatiles above a liquid or solid prior to instrumental analysis (Curioni and Bosset 2002). It is a simple technique to collect and analyse volatiles with the least amount of deterioration and amendment of the chemicals (Bart 2001; Tholl *et al.* 2006; d'Acampora *et al.* 2008 and Hobbs *et al.* 2004) and significantly suitable for highly humid/wet samples. Dynamic headspace sampling strips volatiles of a sample matrix in a sample vessel using a continuous flow of a gas stream under non equilibrium conditions (Bianchi *et al.* 1989). Stripped volatiles are concentrated onto a sorbent material or cold trap prior to gas chromatography analysis. Two mode of dynamic headspace sampling volatiles extraction are open stripping and closed stripping (Capelli *et al.* 2013). In open system, problems related to increases in temperature and humidity or an accumulation of damaging vapours in the headspace that are eliminated by a constant air stream. Whereas, using closed-loop stripping, volatiles, especially analytes with low concentrations, are enriched onto sorbent materials during continuous circulation of the headspace air inside closed chambers with minimum air contaminants. Minor drawback of the headspace sampling techniques is related to the cleanliness of sampling apparatus, but this can be overcome with good laboratory practices to enhance the repeatability and reproducibility of the sampling method (Tholl *et al.* 2006).

Despite availability of several sampling methods for odorant analysis, identification of suitable method serving the objective of particular experimental analysis is essential. Suitability of a sampling technique depends on many factors including environmental aspects e.g ambient temperature, sampling site, costing of analysis, skill of staff and availability of compatible instrument and etc. Moreover, the selection and execution of particular experimental techniques should produce relevant output/result for optimum solution of events. Therefore, this study aims to compare the performance of between Tenax TA sorbent tune and dynamic headspace sampling for odour analysis of broiler litter emission. The broiler litter odour was represented using standard solutions containing 13 major odorants prepared based on literature reviews (Lacey *et al.* 2004; Steven *et al.* 2010 and Jiang *et al.* 2023) The sampling techniques were coupled to thermal desorption, gas chromatography – mass spectrometry and olfactory detection (TD-GC-MS/O) system for determine volatile organic compounds with odorant properties. The TD-GC-MS/O system used provides both instrumental and human sensory evaluation of isolated odorants. The study is expected to provide information on effective and suitable sampling technique selection for fast and reliably point source broiler litter odour analysis at ambient temperature. Olfactory analysis is required significantly in intense livestock facility to mitigated odour as the analysis provides clearer understanding of the emission nature. The TD-GC-MS/O technique is often used in the food, water, aroma and environmental studies to enable determination of volatiles with low threshold levels and offensive qualities responsible unpleasant odour (Kozicki 2022; Dang *et al.* 2022; Hong *et al.* 2021).

II. MATERIAL AND METHODS

Standard solutions consisting of thirteen pure odorous volatiles in broiler litter odour were prepared precisely in high performance liquid chromatography grade methanol (99%, Sigma Aldrich, Castle Hill) at 50, 100 and 250 parts per million of volume (ppmv). The solutions contained: acetone (99.5%, Sigma Aldrich, Australia), 2-butanone (99.5%, Supelco, US), 3-methyl-2-butanone (99.5%, Fluka, Europe), 2,3-butanedione (99.5%, Fluka, Europe), α-pinene (99%, Aldrich, Castle Hill), toluene (99.8%, Sigma Aldrich, US), dimethyl disulfide (99%, Aldrich, Castle Hill), 1-butanol (99%, Sigma Aldrich, US), limonene(97%, Sigma, US), 3-hydroxy-2-butanone (97%, Fluka, Europe), dimethyl trisulfide (98%, Aldrich, Castle Hill), acetic acid (99.7%, Sigma Aldrich, Castle Hill) and butyric acid (99%, Aldrich, Castle Hill).

2.1 Odour Sampling:

2.1.1 Dynamic headspace sampling:

Closed vessel dynamic dynamic headspace sampling was used to study volatiles. To ensure minimum occurrences of contamination, sampling vessels utilised for dynamic dynamic study were screened prior to use. Approximately 1 µL of respective standard solution was placed on the base of clean sampling vessel before instantly attaching to dynamic headspace

sampler for dynamic TD-GC-MS-O analysis. This dynamic headspace sampler was developed and reported by Pillai *et al.* (2010) and Sashikala and Richard (2023). The solution was purged through with helium (He) gas for a minute and the volatiles were concentrated on a general purpose graphitised carbon cold trap held at -10 °C for 3.5 min at a flow rate of 50 ml/min using a dynamic headspace sampler with 2 inlets attached directly to a thermal desorption unit (TDU) (Markes Unity, Markes International, UK)(Fig. 1). The cold trap was rapidly heated to 290°C approximately for 5 min at a rate of 20°C/s. The process desorbs the retrained volatiles on a gas chromatography column using a transfer line held at 140°C. The process was repeated using repeated using standard solutions of 100 and 250 ppmv concentrations, respectively.

FIGURE 1: Dynamic headspace sampler attached thermal desorption unit

2.1.2 Sorbent tube sampling:

In order to compare the application of dynamic headspace sampling method to the commonly used sorbent tubes, Tenax TA sorbent tubes (Fig. 2), packed with 2,6-diphenylene-oxide polymer resin were used. Tenax TA is a hydrophobic and weak sorbent material, which has been widely used to retrain and analyse volatiles from water, environmental air, soil, human breath, plants and commercial products. The sorbent material is packed in a stainless steel tube with a dimension of 6.35 mm (outer diameter) and 89 mm (length) to allow a surface area of 35 m^2/g attracting volatiles, commonly compounds with C_7 to C_{10}. Screened Tenax TA sorbent tube was loaded with 1 µL of 50 ppm standard solution using a calibration rig that were concurrently sealed with brass caps fitted with Teflon ferules prior to analysis. Volatile loaded tube was thermally desorbed at 275°C for 5 min retraining volatiles on a general purpose graphitised carbon cold trap held at -10 °C in the TDU. This cold trap was later subjected to a second stage thermal desorption at 290°C for 5 min at a rate of 20°C/s injecting volatiles on the gas chromatography column using a transfer line held at 140°C. The above process was repeated using standard solutions of 100 and 250 ppmv concentrations, respectively.

FIGURE 2: Example of sorbent tube, Tenax TA

2.2 Separation and Identification of Volatiles:

Volatiles introduced on the gas chromatography (GC) column employing dynamic headspace sampler and Tenax TA sorbent tube techniques were analysed using a gas chromatography-mass spectrometry coupled to an olfactory detection port (GC-MS/O) (Agilent Technologies, USA and Gestrel, Germany) for chemical and sensory characterisation. Separations of volatiles collected were conducted using a polar HP-INNOWax column with dimension of 0.25 mm x 30 m x 0.25 µm (Agilent Technologies, USA) with He flowing at 1.6 ml/min. The initial oven temperature was set and held at 50°C for 2 min before being ramped at 5°C/min til 125°C for 10 min and finally at 10°C/min til 200°C for 2 min. The total run time of this program was 26.50 min. As the eluted compounds exited the GC column, a splitter separated the vapour at a ratio of 2:3 to a mass selective detector (MSD) (MSD 5975, Agilent Technologies, USA) and an olfactory detection port (ODP) (Gerstel, Germany).

The MSD functioned under electron impact mode at 70 eV, scanning m/z ranged from 35 to 500. Chemical identification of separated compounds was performed by comparing the mass spectra to the NIST02 library available in the GC-MS system. As no scientific instrument has the capability of interpreting perceived odorants in the way a human nose does, two screened human detectors with different sensitivities (i.e. butanol detection thresholds) were used for olfactory detection of the volatiles. The detection of volatiles were recorded the odour description and intensity of the compounds as low, mild, high and very high using scale system 1-4 on the ODP recorder software (Gerstel, Germany) (Fig. 3). The odorants were identified by matching total ion chromatogram obtained from the GC-MS with peaks obtained on the aromagram from the ODP to establish the key odorants being emitted from the litter.

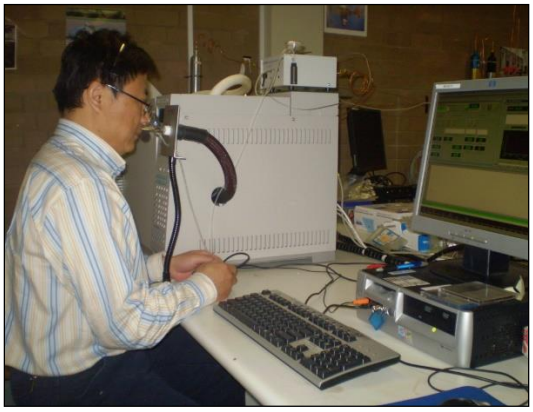


FIGURE 3: Operator at the odour detection port (ODP) using headset microphone and a control pad for intensity ranking

III. RESULTS AND DISCUSSION

A dynamic headspace sampler developed by Pillai *et al.* 2010 coupled to a TD-GC-MS/O was compared with Tenax TA sorbent tube for efficacy in chemical speciation, repeatability and reproducibility to monitor variations in volatiles at ambient environment. The assessment was anticipated to provide information on the sample extraction time in order to identify the breakthrough sampling time during dynamic headspace sampling which will be beneficial during analysis of real source of broiler litter. The validation of dynamic headspace sampler was compared with Tenax TA sorbent tube under identical GC conditions using standard solutions consisting of 13 common odorants reported in poultry facilities (Lacey *et al.* 2004; Steven *et al.* 2010 and Junyann *et al.* 2023). As a basic requirement to determine background noise, all Tenax TA sorbent tubes and dynamic headspace sampling vessels were screened thoroughly for contamination before use. The existence of carbon dioxide at retention time 2.18 min of Tenax TA and 2.44 min of dynamic headspace sampler were obtained.

During study, dynamic headspace samples were pre-concentrate on a general graphitised carbon cold trap placed in the thermal desorption unit. The introduction of analytes onto GC column occurred was in single stage thermal desorption procedure since dynamic headspace concentrates volatiles directly onto the cold trap. This process is anticipated to reduce deformation/amendment or losses of volatiles compounds due to multiple stages of thermal desorption. Elisa *et al.* (2023) has reported some compounds, in particular aldehydes and esters of carboxylic acids compounds such as propanal, hexanal and acetic acid and methyl ester were determined using non sorbent techniques, similar to dynamic headspace sampling. In addition, detection of these compounds that are characterised with low odour threshold limits are important for olfactory analysis (Elisa *et al.* 2023). Similarly, Junyaan and co-workers have noted Tenax TA may not the best sorbent of choice for substances with medium polarity or low carbon number (Junyaan *et al.* 2023), that this characteristic may be become a hinderance during real case sampling to collect complete volatiles available in a sample matrix, hence making dynamic headspace sampler a better choice instead.

The cold trap releases the trapped volatiles into the GC system. Volatiles were swept using purge gas onto the cold trap for 3.5 min. The pre-concentration time chosen showed sufficient extraction of the sample with post blank tests revealing high recovery of volatiles. Shorter extraction time employed on standard solution provided poor matching of volatiles identified by the mass-spectrometry library. Meanwhile, longer sample extraction with dynamic headspace sampling contributed to overloading of volatiles onto the cold trap and the GC system. This was visible with poorly separated ‘shark fin’ shaped volatile peaks noticed on the total ion chromatogram of GC. However, the extraction time is highly subjected to changes depending on the estimation of sample concentration, especially for environmental based samples.

Meanwhile for Tenax TA sorbent tube collection, volatiles trapped onto sorbent material were desorbed to be pre-concentrated onto the cold trap in thermal desorption unit where a second thermal desorption occurs to introduce analytes onto GC column. A temperature elevated flow path connection consisting of fused silica was used to link the thermal desorption unit and the GC-MS/O system. Increased temperature and appropriate carrier gas flow rate were required to transfer volatiles onto GC column while reducing the condensation of analytes along the transfer pathway. However, extreme precaution was practised during temperature setting at all levels during thermal desorption processes to prevent oxidation of volatiles attributed to exposure to high temperature.

Both sampling techniques were observed to be successful in discriminating all chemicals tested during validation stage at varying concentrations. Using the dynamic headspace, all volatiles were determined effectively at concentration as low as 50 ppmv except for acetone, 3-hydroxy-2-butanone and butanoic acid that required higher concentrations. Two important aspects owing to this circumstance were: a) further dilution with purge gas during sampling of volatiles onto the cold trap that reduce the concentration of substance and increase the detection limits in the GC system and b) the co-efficiency of a substance to volatilise from a condensed sample phase to the headspace gas phase that could have caused overlap of volatile separation. Meanwhile the Tenax TA sorbent tube method revealed all chemicals at concentration of 50 ppmv.

It is notable that the volume of samples carried onto the cold trap between sampling procedure differed attributed to varying sample pre-concentration technique though both techniques used 1 µL of standard solutions. Only partial amount of 1 µL was purged on cold trap within selected period using dynamic headspace sampler compared to Tenax TA sorbent tube that loaded complete 1 µL of standard solution. Despite owing to inadequacy to compare both techniques directly for chemical quantitative information, the capacity of dynamic headspace sampler to determine volatiles at low volume is highly notable. The extracting techniques are comparable directly for volatiles qualitative information. The occurrence of elucidating volatiles' retention time from the GC-MS system appeared to be consistent between the sampling methods, also presenting the reliability of the GC-MS system in responding to the chemicals tested. Fig 4 shows comparatively similar occurrence of odorants at retention times between the two sampling techniques used. Adequate repeatability between extraction methods was obtained. It is evident from the spectra that both dynamic headspace sampler and Tenax TA sorbent tubes were providing consistency volatiles retention time and abundance reflecting on the reliability of sampling and analysis system respectively (Fig. 5).

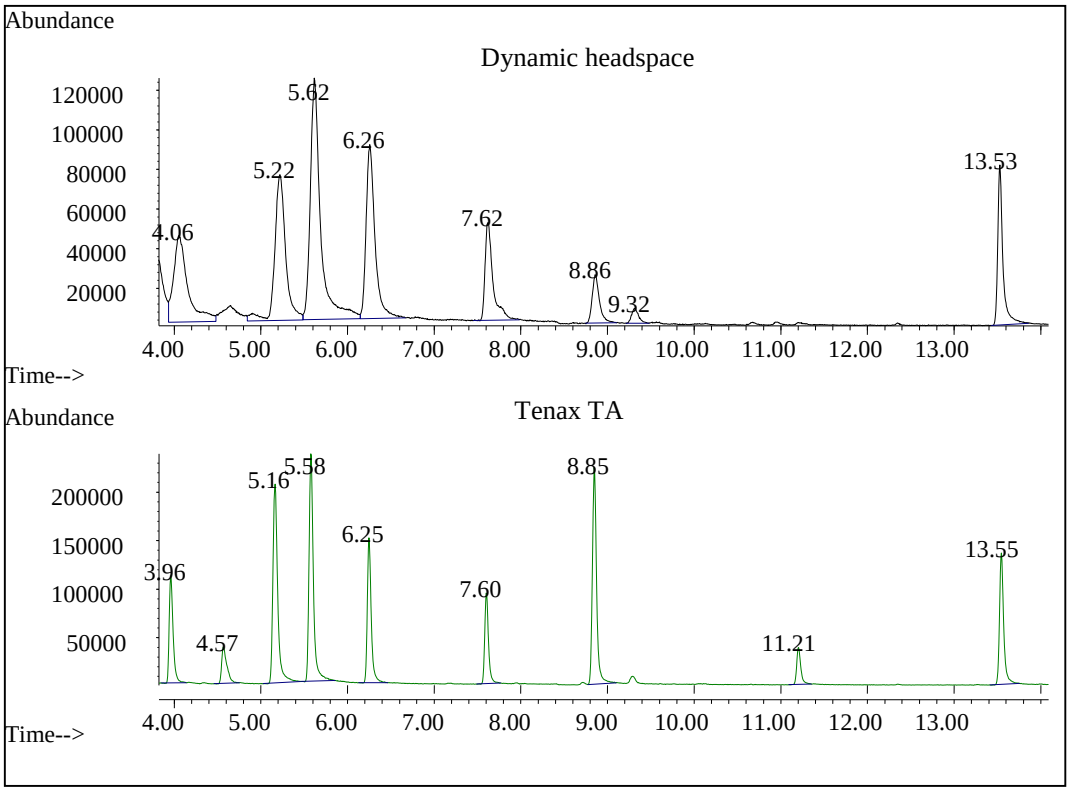


FIGURE 4: Standard solution with significant similarity of retention time of volatiles

Retention time (min): 4.06 =3-methyl-2-butanone, 4.57 = 2,3-butanedione, 5.22 = alpha pinene, 5.62 = toluene, 6.26 = dimethyl disulfide, 7.62 = 1-butanol, 8.86 =limonene, 11.21 = 3-hydroxy-2-butanone, 13.53 = dimethyl trisulfide

In addition, the GC-MS/O system responded efficiently in discriminating the variation in volatiles abundances (Fig. 6) and perceived odour intensity corresponding to differing standard solution concentrations. Good recovery of volatiles tested was revealed using Tenax TA sorbent tube sampling which could be due to complete thermal desorption of the sorbent tube and cold trap in thermal desorption unit. Tenax TA sorbent tube has tendency to exhibit good level of sample recovery, approximately 98% (Jacek *et al.* 2005). It is also visible from Fig. 7, which spectrums show relative multiplication of peak area of respective volatiles with increase in standard solution concentration. Unlike dynamic headspace sampler, spectrums exhibited smaller peak area compared to Tenax TA of respective standard solution concentration. Nevertheless, the dynamic headspace sampler was successful in detecting and exhibiting changes in standard solution concentration. Perhaps longer extraction time of volatiles needed to improve recovery using dynamic headspace sampler.

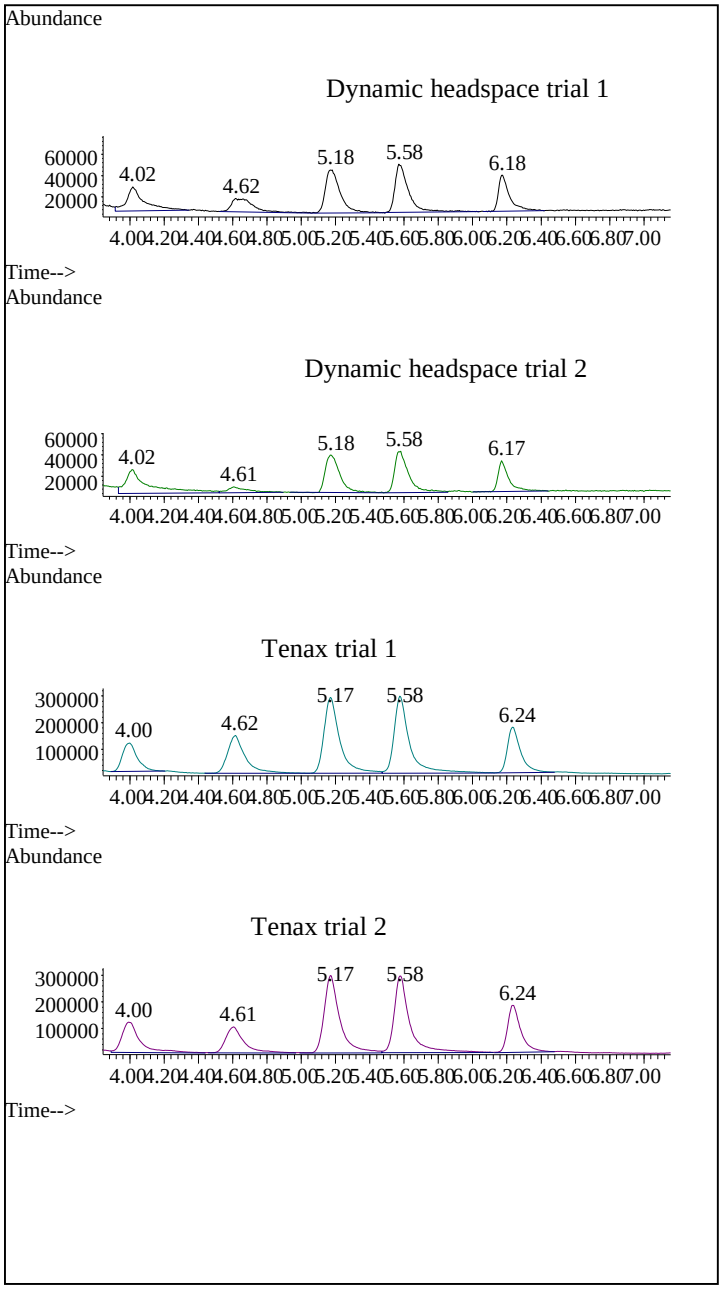
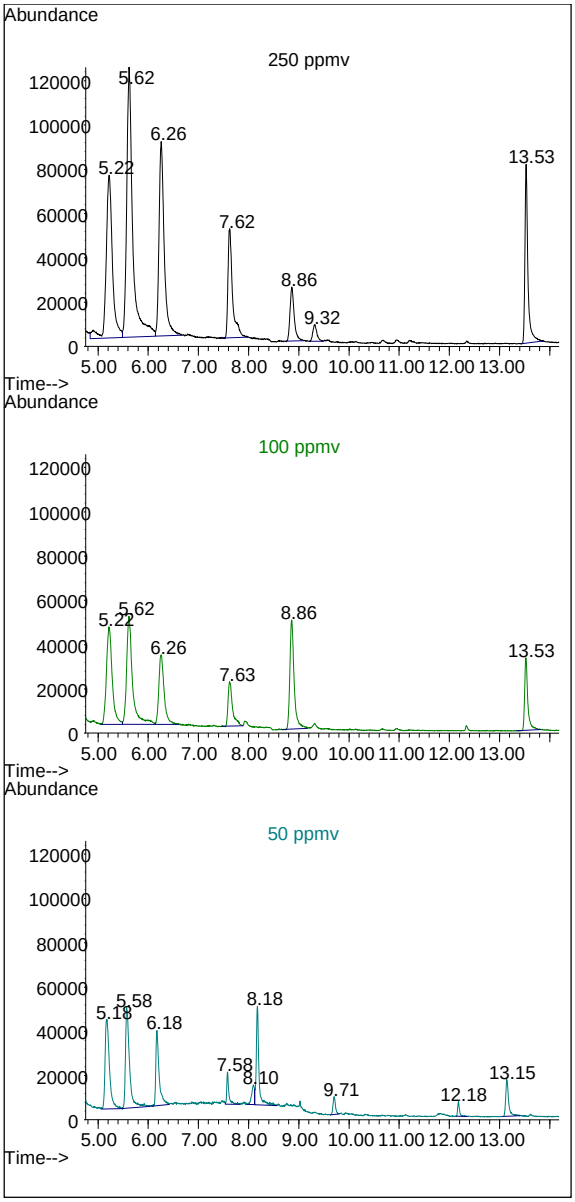
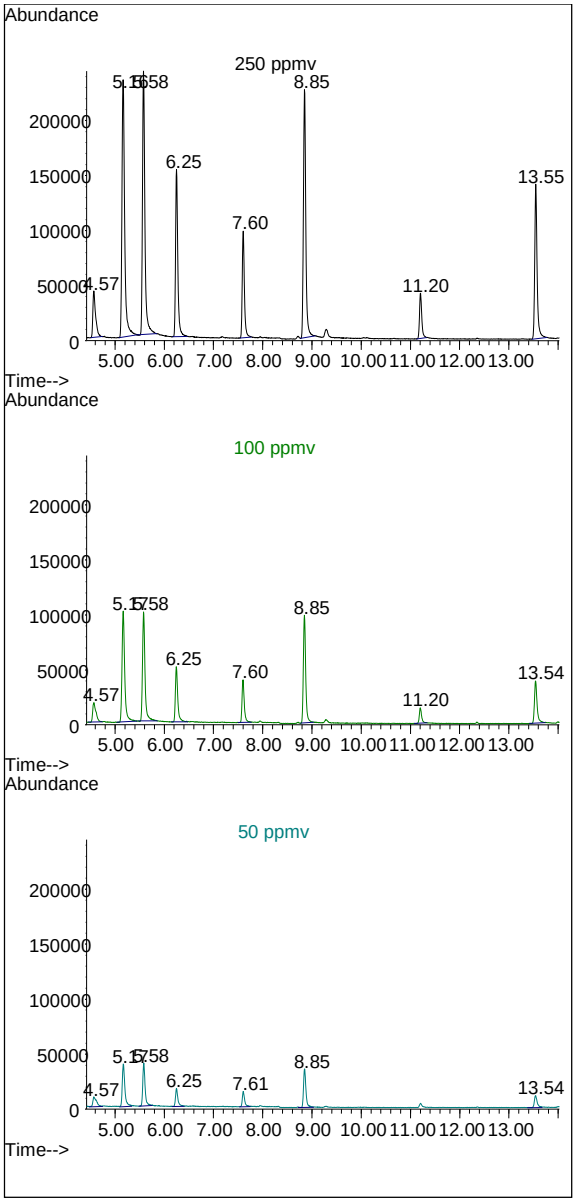


FIGURE 5: Good repeatability of sampling methods on volatiles' retention time and abundance obtained of 50 ppmv standard solutions





(a) Dynamic headspace **(b) Tenax TA**

FIGURE 6: TICs of standard solutions with significant differences exhibited with increases in standard solution concentrations

Focusing on the olfactory qualitative analysis obtained using GC-MS/O, the general trends observed for olfactometric analysis are confirmed between both sampling techniques. Procedure used to determine volatiles of potential odorants is shown in Fig. 7 and Fig. 8 for both sampling techniques. The total ion chromatograms were overlaid with olfactory responses (osmegram) to match odorants according to overlapping retention times. Comparison of chromatograms show relevant utilisation of method used to study volatiles using instrumental and human sensory evaluation which is important in drafting guideline and regulations as the effort may assist in odour assessment and abatement through determination and understanding of the key odorants within poultry odour that cause odour nuisance. The cause of those odorants needs to be identified in developing better odour reduction strategies. Panelists used have successfully determined compounds used in standard solution that is evident with precise overlay of olfactory note on compounds peak accurately. Difference in heights of peaks on osmegram represents intensity of the perceived smell as some compounds sustain low threshold limit. The higher the peak on osmegram, the stronger perceived smell of the particular compound.

Table 1 shows comparison of olfactory responses for standard solution via dynamic headspace sampling and sorbent tube using identical GC-MS/O parameters. Olfactometry characteristic of most volatiles were determined by both Tenax TA and dynamic headspace sampler. However, olfactory description for acetone at all concentrations were not determined by panelist using both sampling methods. Similar case for 2-butanone, 3-methyl-2-butanone 2,3-butanedione and 3-hydroxy-2-butanone using dynamic headspace sampler at 50 ppmv concentration. The olfactory responses produced for both sampling technique provided similar description of odorants but varied in terms of intensity attributed to varying abundance of respective chemicals sampled. During increase in concentration, olfactory description was noticed to change from pleasant to annoyance. This is observed for compounds with olfactory descriptive note as 2-butanone, 3-methyl-2-butanone, α pinene and toluene with pleasant olfactory note though at higher concentration. Meanwhile 2,3-butanedione and dimethyl disulfide, dimethyl trisulfide and butanoic acid produced offensive olfactory note even at lowest concentration tested as these compounds sustain lower threshold characteristics compared to other compounds.

Although intensity increases with concentration, an increase or decrease in concentration will not produce a corresponding proportional change in odour intensity as perceived by a human subject. Some odours containing offensive olfaction note can become intense at relatively low concentrations while the pleasant odours like lemon and pine require high concentration before considered intense. An odour with a strong intensity at low concentrations may cause odour problems even at low residual levels. Intensity is how a panellist perceives the magnitude (strength) of an odour once it is above its threshold (Sashikala and Richard 2022). This incident is evident on Figure 7 and Figure 8, 2,3-butanedione and butanoic acid at retention time between 4.5-5 min and 19-20 min respectively, with small signal intensity on total ion chromatogram produced high strength response on osmegram from panellist due to low threshold limits of the compound.

TABLE 1
SENSORY RESPONSES OF STANDARD SOLUTIONS OF DYNAMIC HEADSPACE AND TENAX TA

Compounds	Olfactory responses					
	50 ppmv		100 ppmv		250 ppmv	
acetone	**Dynamic**	**Tenax**	**Dynamic**	**Tenax**	**Dynamic**	**Tenax**
2-butanone		solvent	solvent	solvent	solvent	solvent
3-methyl-2-butanone		solvent	solvent	solvent	chemical	sweet solvent
2,3-butanedione		rotten cabbage	rotten cabbage	rotten vegetable	rotten vegetable	rotten vegetable
α pinene	smoky solvent	pinene	pine	pine	pine	pine
toluene	solvent	solvent	pleasant chemical	solvent	solvent	solvent
dimethyl disulfide	chemical	smoky	chemical	smoky	chemical	foul
1-butanol	smoke		solvent	alcohol	foul	foul
limonene	citrus	lemonish	solvent	alcohol	foul	lemonish
3-hydroxy-2-butanone		ash	musty	ash	musty	musty
dimethyl trisulfide	putrid, ferment	putrid, ferment	putrid, ferment	putrid, ferment	putrid, ferment	putrid, ferment
acetic acid	vinegar	foul	smoke	vinegar	vinegar	foul
butanoic acid	rancid	cheesy, rancid	rancid	cheesy, rancid	rancid	cheesy

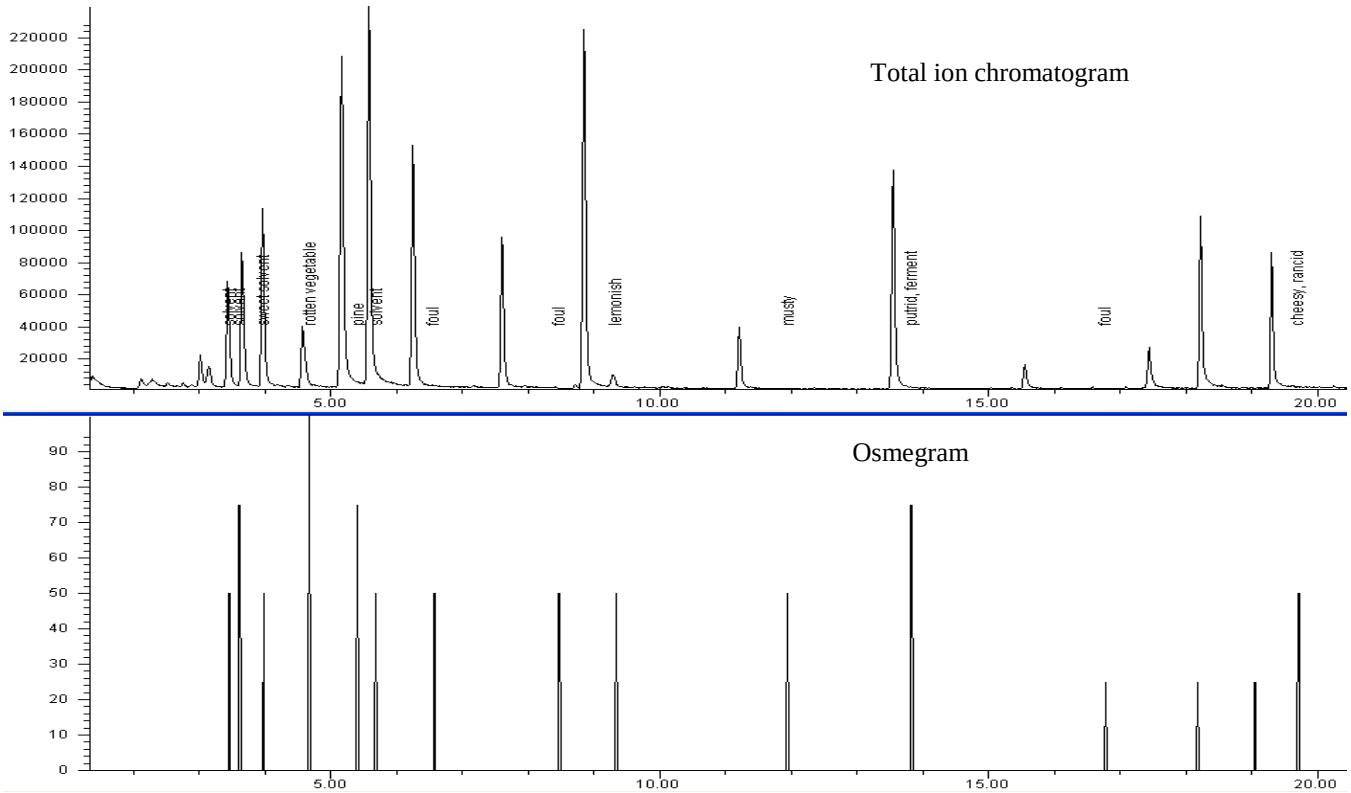


FIGURE 7: Comparison of TIC and osmegram identifying potential volatiles with odour properties of standard solution using Tenax TA sorbent tube method with descriptions of respective volatiles noted on TIC

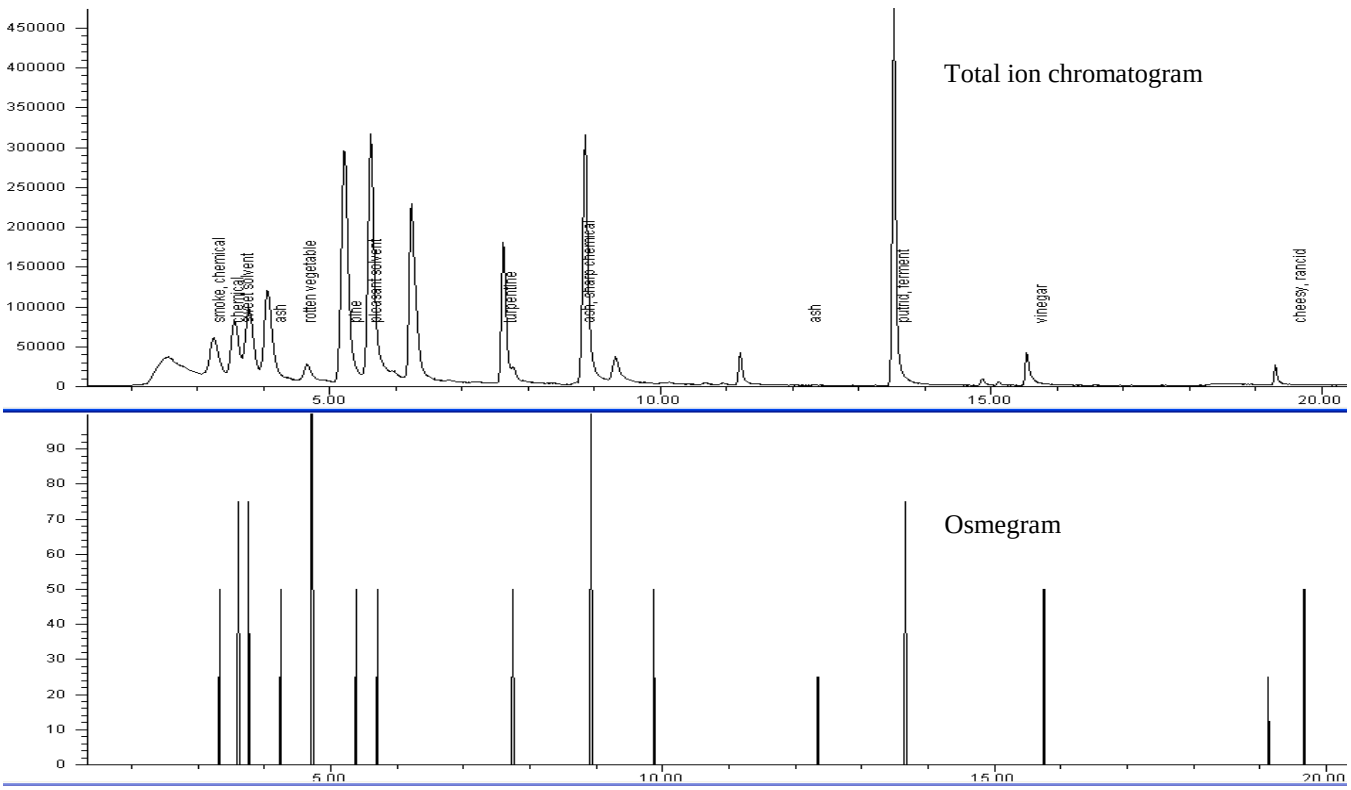


FIGURE 8: Comparison of TIC and osmegram identifying potential volatiles with odour characteristics of standard solution using dynamic headspace sampling with descriptions of respective volatiles noted on TIC

IV. CONCLUSION

The dynamic headspace sampler provided a simple and rapid platform to analyse volatiles during broiler litter standard solution. The main advantage of the dynamic headspace is the potential of the technique to collect volatiles with minimal sample preparation robust extraction and speciation of volatiles in original form besides reduction in the time required for volatiles collections prior to GC analysis. In addition, the capacity of dynamic headspace sampler to determined volatiles at lesser quantity used in Tenax TA sorbent tube is proven though quantitative analysis needs improvement. In comparison to Tenax TA sorbent tubes, dynamic headspace sampling able to exhibit relevant chemical and olfactory analysis using TD-GC-MS/O system at good repeatability comparatively with Tenax TA sorbent tube. In future, the dynamic headspace sampler can be utilised to study emissions from real samples with minimal preparation. The outcome using dynamic headspace sampling can be qualitatively beneficial and reliable as Tenax TA sorbent tube. In fact the engagement of both sampling methods for broiler litter samplers or other live samples can provide information of diverse range of chemicals in odour samples and assist in drafting of odour regulations and guidelines.

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Feeding Value of Dried Fermented and Unfermented Vegetable Based Agro By-Products (VBAP)

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Abstract— *Vegetable Based Agro By-Products (VBAP) can be used as an alternative feed ingredient amidst rising commercial feedstuff costs. VBAPs contains nutritional amounts needed by animal for growth and reproduction. The proximate analysis and mineral content of a number of vegetable based agro byproducts which includes; cabbage (Brassica oleracea var. capitate L) trimmings, carrot (Daucus carota L) tubers, chayote (Sechium edule) fruits, lettuce (Lactuca sativa L.) leaves, broccoli (Brassica oleracea L. var. Italica Plenck) trimmings, tomato (Solanum lycopersicum L.) fruits, Chinese cabbage/wombok (Brassica rapa subsp. pekinensis) trimmings and potato (Solanum tuberosum) tubers are presented in this research. Drying the VBAP and fermentation has been studied in these researches which are methods in prolonging the shelf life and easier integration to animal feeds. The study revealed that dried unfermented VBAP have lower moisture and NFE contents, while higher in DM, ash, CP, crude protein, crude fat content compared to dried fermented VBAP (p-values of less than 0.01). Mineral content such as calcium has lower amount in dried unfermented VBAP but has higher amount in phosphorus compared with fermented dried VBAP (p-values of less than 0.01). Therefore, dried unfermented VBAP has greater potential than dried fermented VBAP as animal feed. Though, complete potential of these alternative feeds and feed resources can be appreciated by intensive study.*

Keywords— Feeds, Fermentation, mineral content, proximate analysis, VBAP.

I. INTRODUCTION

Vegetable based agro by-products are unwanted to some farmers in cultivated areas and vegetable markets which considered as wastes, though, these by products can be considered as alternative sources of feed for animal production. Recycling of by-product as feedstuffs for animal production is an important factor in waste reduction. The by-product residues can be classified as fruit and vegetable by-products, oil industry by-products and grain- and legume-milling industry by-products. Use of by-products from agro-industry could lessen animal production cost and can resolve waste disposal and environmental pollution problem. Furthermore, production of feedstuffs can be supported with the use of fruit and vegetable by-products as feeds and feed sources (Yang et al., 2021).

Utilization of these byproducts give the needs to identify the nutritional content for feed formulation. Feed quality or nutritional value is an idea that involves many indicators like energy and protein content, chemical composition, consumption and digestibility (Kirilov et al., 2017). Vegetable wastes and by-products such as sugar beet leaves, potato vines, bottle gourd pulp, radish leaves, cabbage leaves, pumpkin oil meal, snow peas among others are good source of energy and crude protein (CP) particularly for ruminant animals which contains more than 20% CP. Other vegetable wastes hold 19% to 10% CP contents. On the other hand, vegetable waste such as sweetcorn husk, and cob, waste of jackfruit and carrot pulp contains a CP with less than 10% (Bakshi et al., 2015). Furthermore, fruit seed cakes of passion fruit, soursop and custard apple could be use as feed

for animals after industrial process. They contain high amount of dry matter with low moisture content which can become a better alternative for ruminant feedstuff that may lessen the expenses in animal production (Pinto et al., 2020).

Though, agricultural by products required preservation for longer shelf life and needs to improve the nutritional value. Fermentation plays important part in food preservation via development organic acid such as formic acid, lactic acid, propionic acid and acetic acid. It includes also the production of reutrin, ethanol, carbon dioxide, bacteriocins and diacetyl. Fermentation could improve food safety, remove toxic compounds, improve the food quality and nutritional value (Ray and Joshi, 2015). Acetic acid produced by LAB strains prevents spoilage and contributes to the aroma. The LAB producing thermostable amylases have potential fermentations of tubers, cereal, and root. The LAB acidifies foods that frequently exert proteolytic and lipolytic activities and produce amino acids upon further bioconversion that contributes to the aroma and flavor of fermented products. LAB could increase nutraceuticals content in fermented foods and increase the quantities of low-calorie polyols in order to lessen amount of sugar. Furthermore, LAB stains may prevent galactose accumulation and lactose intolerance by eliminating the lactose and galactose from fermented milks. Moreover, content of soy that results to intestinal cramps and flatulence can be removed through fermentation (Ray and Joshi, 2015).

Great challenge in feed security is preservation because agricultural by products are highly perishable. To preserve these products, drying is an important technique used. Drying of food by products is appropriate way for extending shelf life and storage. Moreover, drying favors easy and economical packaging and transport, reduction of greenhouse gas, smaller space for storage and lesser environmental weight (Noori et al., 2022). Drying are used for the long-term storage to preserve raw materials where in, some grains need atleast 9% moisture content depending on the grain and climate (Lyda and Kyosung, 2018). Methods of drying raw materials includes convection/air drying, this is characterized by a method simultaneous movement of mass and heat. Other method is freeze-drying which is the most conventional technique of drying fruits, vegetables and LAB, since the resulting product is of good quality, less effect on nutritional value, aroma, texture and color (Janiszewska-Turak et al., 2021). Other drying methods used for agricultural products, meat products and medicinal plants includes solar drying, ultrasound drying, microwave drying, spray drying, fluidized bed drying and vacuum drying (Noori et al., 2022).

Recent researches disclosed that drying agricultural by products is important for preservation and significant result of fermentation improved the nutritional value of agricultural byproducts, though published data for the nutritive value of VBAP is limited.

This study aims to determine the chemical composition and mineral content of locally available VBAP and comparing the value of dried unfermented and fermented VBAP.

II. MATERIALS AND METHODS

2.1 VBAP Collection:

Collection of VBAP were conducted at vegetable farms around Benguet province and La Trinidad Vegetable Trading Post which includes; cabbage (*Brassica oleracea var. capitate L*) trimmings, carrot (*Daucus carota L*) tubers, chayote (*Sechium edule*), lettuce (*Lactuca sativa L.*), broccoli (*Brassica oleracea* L. *var. Italica Plenck*), tomato (*Solanum lycopersicum* L.) fruits, Chinese cabbage/wombok (*Brassica rapa subsp. pekinensis*) and potato (*Solanum tuberosum*) tubers.

2.2 Fermentation procedure of VBAP:

VBAP samples were chopped at approximately 3 cm in size and weighed. Molasses was mixed at the chopped VBAP at 15% rate based from the weight of the VBAP. Mixture of VBAP and molasses were filled-in and compacted firmly in the empty high-density polyethylene plastic jar that were installed with airlock fermenter. After filling, containers were tightly sealed using waterproof aluminum foil tape to avoid oxygen from entering inside the container. Containers filled with VBAP were stored within 20 days in a cool dark area. After fermentation, Samples were subjected to oven drying at 70 degrees Celsius within 48 hours.

2.3 Proximate analysis:

Oven dried Unfermented and fermented VBAP undergo proximate and mineral analysis. Nutrient contents such as crude protein (CP), crude fat, crude fiber (CF), moisture, Dry Matter (DM), Nitrogen Free Extract (NFE) and ash following the AOAC International guidelines (2005) content and mineral contents such as calcium and potassium were analyzed at Lipa Quality Control Center Inc. (LQCCI) North, Gov F Halili Ave, Barangay Turo, Bocaue, Bulacan.

2.4 Experimental Design and Statistical Analysis:

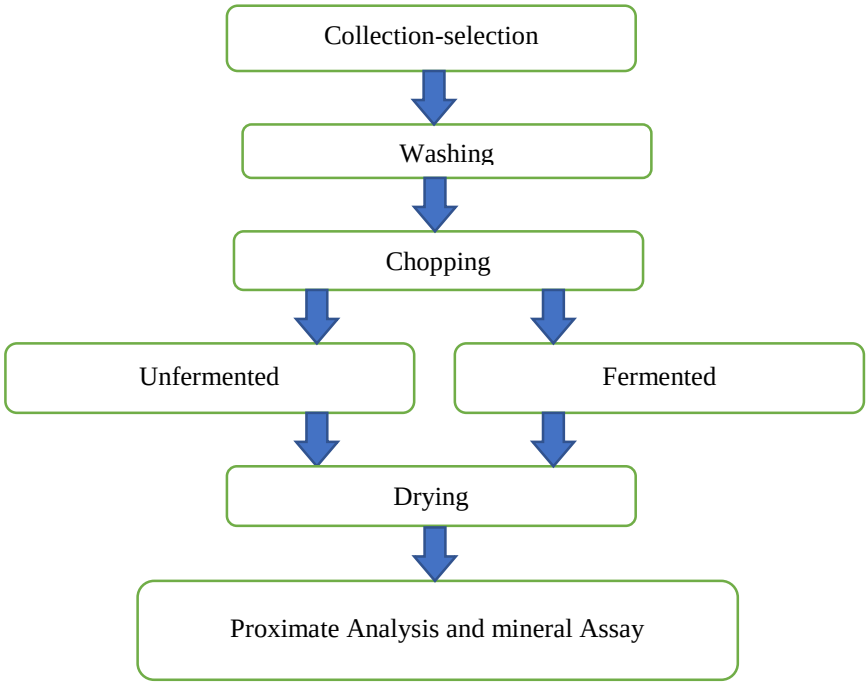

FIGURE 1: Research diagram of Study 2

This study was conducted in an 8x2 factorial in CRD replicated 3 times with different unfermented VBAP as Factor A namely: cabbage trimmings, carrot tubers, sayote, lettuce, broccoli, tomato fruits, Chinese cabbage/woombook and potato tubers and fermented VBAP as Factor B (with 15% molasses). Data was analyzed using SPSS ver 21. Analysis of variance (ANOVA) and statistically significant parameters will be compared using Least Significant Difference (LSD).

III. RESULTS AND DISCUSSION

3.1 Proximate Analysis Content of Dried Unfermented and Fermented VBAP:

Table 1 presents the results on the proximate analysis content of dried VPAB with computed p-values of less than 0.01. Result indicates that broccoli has higher moisture, ash and CP content after fermentation but has lower amount of DM, crude fiber, crude fat and NFE after fermentation compared with unfermented broccoli. Unfermented cabbage has lower moisture, ash, crude fiber and NFE content but higher DM, CP and crude fat and NFE compared with fermented cabbage. In carrot, unfermented one contains greater amount of moisture, crude fiber and crude fat content while lesser amount on DM, ash, CP, and NFE compared with the fermented one. Unfermented chayote has higher amount of moisture, CP, crude fiber and crude fat content while lower amount on DM, ash and NFE compared with fermented. Unfermented Chinese cabbage has lower moisture, crude fat and NFE content and while higher DM, ash, CP and crude fiber than fermented Chinese cabbage. Meanwhile, unfermented lettuce has lower moisture and NFE content but higher DM, ash, CP, crude fiber and crude fat content than fermented. I addition, unfermented potato has higher DM, ash, CP and NFE content but lower moisture, crude fiber and crude fat content compared with fermented potato. Lastly, unfermented tomato has higher moisture, CP, crude fiber, and crude fat content while lower content on DM, ash and NFE compared with fermented tomato.

On the other hand, unfermented tomato, fermented Chinese cabbage and fermented cabbage contained the highest amount of moisture while unfermented potato and unfermented lettuce has the lowest moisture content. VBAP with highest dry matter content are unfermented lettuce, unfermented potato and unfermented broccoli while lowest in unfermented tomato and fermented carrot. Unfermented lettuce followed by unfermented Chinese cabbage and fermented Chinese cabbage contains the highest amount of ash while unfermented and fermented potato has the lowest ash content. In CP content, unfermented Chinese cabbage, unfermented cabbage and unfermented lettuce contains the highest amount while lowest amount of CP in fermented chayote and unfermented carrot. Furthermore, unfermented broccoli, unfermented lettuce and fermented broccoli contained the highest amount of crude fiber while unfermented potato and fermented chayote have the lowest crude fiber content. VBAP with highest crude fat content are unfermented lettuce, unfermented tomato and unfermented broccoli while fermented potato

and unfermented potato has the lowest crude fat content. Lastly, VBAP with highest NFE content are unfermented potato, fermented potato and fermented chayote while lowest NFE content are unfermented Chinese cabbage and unfermented lettuce.

Unfermented carrot contains 14.31% amount of moisture higher than 8.78% moisture content of carrot powder stated by Gazalli et al. (2013). Unfermented chayote has 10.71% moisture content which is little higher than the result of the study of Islam, et, al., (2018) with 10.18% amount of moisture of oven dried chayote. Ashraf, (2015) stated that unfermented carrot peels contain 7.46%, while fermented carrot peels has 26.7%.

Result from the study of Gazzali et al. (2013) presented lower content on ash (5.05%), protein (6.16%) and crude fat (2.43%) while higher amount on crude fiber (24.66%) of carrot powder. Meanwhile, Islam et al. (2018) reported lower amount on ash (0.21%), crude protein (0.48%), crude fiber (0.32%) and crude fat (0.06%) of chayote compared with the result of this study.

Findings of Sun et al. (2017) showed lower content on ash (4.0%), crude protein (8.65%) but higher crude fat (11.31%) of dried unfermented potato. On the other hand, Ashraf (2015) discussed higher content on ash (11.2%) and crude fiber (17%) crude fat (3.84%) of unfermented carrot peels while lower content on CP (4.47%) and lower content on ash (7.8%), CP (7.81%) and crude fat (1.54%) though higher crude fiber (12.75%) of fermented carrot peels compared with the findings in this study.

TABLE 1
PROXIMATE ANALYSIS CONTENT (%) OF DRIED UNFERMENTED AND FERMENTED VBAP

		Broccoli	Cabbage	Carrot	Chayote	Chinese Cabbage	Lettuce	Potato	Tomato
Moisture	un	5.52	10.51	14.31	10.71	6.66	4.6	4.8	14.66
	f	9.82	10.77	10.71	9.61	11.67	9.86	6.85	8.4
DM	un	94.48	89.49	85.69	89.29	93.34	95.4	95.2	85.34
	f	90.18	89.23	89.29	90.39	88.31	90.14	93.15	91.6
Ash	un	11.83	10.51	10.88	7.74	16.63	24.57	6.03	9.91
	f	11.92	12.01	11.44	9.65	13.91	12.88	5.96	10.28
CP	un	13.73	20.73	10.15	10.83	28.27	18.98	11.84	18.3
	f	17.95	18.74	11.42	10.24	16.23	17.39	10.67	12.04
Crude Fiber	un	14.33	10.39	8.82	8.72	12.52	14.06	2.63	11.38
	f	13.21	10.72	7.11	2.38	7.09	8.78	6.97	7.95
Crude Fat	un	4.76	3.48	2.63	3.07	3.03	5.88	1.26	4.82
	f	3.82	2.96	2.08	2.48	3.87	3.73	1.28	3.85
NFE	un	49.84	44.38	53.21	58.93	32.89	31.92	73.45	40.94
	f	43.29	44.8	57.25	65.64	47.21	47.35	68.26	57.48

Note- un - unfermented; f - fermented

3.2 Calcium (Ca) and phosphorus (P) Content of Dried Unfermented and Fermented VBAP:

Table 2 shows the result of calcium and phosphorus content of dried unfermented and fermented VBAP with computed p-values of less than 0.01. Unfermented broccoli, unfermented Chinese cabbage and unfermented lettuce have higher calcium contents while unfermented cabbage, unfermented carrot, unfermented chayote, unfermented potato and unfermented tomato have lower calcium content than fermented ones. Moreover, unfermented broccoli, unfermented chayote and unfermented potato have lower phosphorus content while higher content on unfermented cabbage, unfermented carrot, unfermented Chinese cabbage, unfermented lettuce and unfermented tomato compared to fermented counterparts.

Meanwhile, unfermented broccoli had the highest calcium content followed by unfermented Chinese cabbage and unfermented lettuce respectively. On the other hand, unfermented chayote and unfermented tomato had the lowest calcium contents with values of 0.42% and 0.41%, respectively. Furthermore, unfermented Chinese cabbage had the highest phosphorus content with a value of 0.91%. This was followed by unfermented cabbage and unfermented lettuce with values of 0.56% and 0.49%, respectively. On the other hand, unfermented chayote and unfermented potato had the lowest phosphorus contents with values of 0.27% and 0.23%, respectively.

Sun, et, al. (2017) reported lower phosphorus (0.098) content of unfermented potato and fermented potato with 0.056% phosphorus compared with the finding in this study.

TABLE 2
CALCIUM AND PHOSPHORUS CONTENT OF DRIED UNFERMENTED AND FERMENTED VBAP

VBAP	Ca		p	
	un	f	un	f
Broccoli	1.66	0.86	0.33	0.39
Cabbage	0.72	1	0.56	0.51
Carrot	0.45	0.83	0.48	0.43
Chayote	0.42	0.84	0.27	0.28
Chinese Cabbage	1.25	1.11	0.91	0.42
Lettuce	1.14	1.14	0.49	0.39
Potato	0.24	0.49	0.23	0.29
Tomato	0.41	0.73	0.45	0.31

IV. CONCLUSION

In conclusion, VBAP contains nutrient needed by the animals as revealed in the gathered data results and can be used as alternative feeds for animal production. Likewise, dried unfermented VBAP appears to have higher potential as animal feed compared with dried fermented VBAP as exposed in the result of proximate analysis which includes moisture content, dry matter, ash, crude protein, crude fiber, crude fat, nitrogen free extract and analysis of mineral content such as calcium and phosphorus. Nevertheless, problems of insufficient records of published study on VBAP were observed. Further exploration of VBAP on fermentation, practical application and nutrient analysis of VBAP is hereby recommended.

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Chemical and Sensory Properties of Complementary Foods Formulated from Blends of Maize (*Zea mays*), African Yam Bean (*Sphenostylis stenocarpa*), Groundnuts (*Arachis hypogea*) and Crayfish (*Procambarus crarkii*) Flour

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Abstract—

Background: *The use of indigenous foods in the formulation of complementary food is advocated for sustainability and improved food security.*

Objectives: *The study investigated the chemical composition and sensory properties of complementary foods made from maize, African yam bean (AYB), groundnut, and crayfish flour blends.*

Methodology: *Whole maize, AYB, groundnuts, and crayfish were made into flour using standard procedures. The blends were formulated in ratios of maize: African yam bean: groundnut: crayfish flour (80:10:5:5; 70:15:10:5; 60:20:15:5; 50:25:20:5) and made as complementary foods. A commercial maize-based complementary food served as the control. The samples were evaluated for chemical and sensory properties using standard methods. Data collected were analyzed with IBM Statistical Product for Service Solutions software (version 21) and presented as descriptive statistics (frequencies, percentages, means, standard deviation). The means were compared and separated using analysis of variance and Duncan's multiple range test.*

Results: *The complementary food contained 55.75% to 65.32% moisture, 1.43% to 2.05% fat, 0.23% to 1.84% crude fiber, 1.43% to 2.05% ash, 12.97% to 18.48% protein, 11.77% to 28.33% carbohydrate, and 127.9 Kcal to 178.07 Kcal energy. There was also calcium (25.95 mg to 28.30 mg), iron (1.81 mg to 1.96 mg), potassium (2.68 mg to 5.4 mg), sodium (0.71 mg to 1.24 mg), and magnesium (1.35 mg to 1.94 mg). Beta-carotene (4.22 to 11.60 mg), thiamin (0.42 mg to 1.05 mg), riboflavin (1.80 mg to 2.85 mg), niacin (0.73 mg to 1.01 mg), vitamin C (3.10 to 30.17 mg), tannin (0.01 to 0.39 mg), phytate (0.01 to 1.43 mg), saponin (0.12 to 1.18 mg), flavonoids (0.09 to 1.13 mg), and phenol (0.01 to 0.14 mg) were also present. Samples 60:20:15:5, 70:15:10:5, 50:25:20:5, and 80:10:5:5 were superior in taste (6.80), appearance (6.25), mouthfeel (6.10), and color (6.70), respectively.*

Conclusion: *The complementary foods had improved protein, fat, fiber, and ash contents and comparable mineral and vitamin contents with the control. The general acceptability scores ranged from 6.13 to 6.23 on a nine-point scale.*

Keywords: Chemical; sensory; complementary foods; indigenous flour blends; crayfish.

I. INTRODUCTION

Undernutrition constitutes a significant nutritional challenge in impoverished communities with limited access to affordable and diverse food sources. These communities rely primarily on staple foods with little or no protein sources due to economic

constraints. As a result, malnutrition particularly protein-energy malnutrition and micronutrient deficiencies—not only affects growth and development but also leads to nutritional vulnerability, significantly impacting learning outcomes. Nutrient deficiencies can hinder cognitive development and academic performance [1]. When growth failure, increased morbidity and mortality, and impaired learning occur, the overall well-being and future prospects of the community are severely compromised. The root causes of malnutrition are poverty, ignorance, and inadequate care. Many impoverished communities have access to indigenous food sources, but due to a lack of nutritional knowledge, they fail to utilize these natural resources efficiently. The situation is further aggravated by market globalization, which introduces foreign, expensive foods that are neither accessible nor affordable. The desire for these costly imported foods often results in inappropriate dietary choices, leading to undernutrition, poor development, stunted growth, and increased mortality.

Complementary foods are introduced to children from six months onward to supplement breast milk and support growth. These transitional foods help infants adapt to family meals. The recommended period for complementary feeding is between 6 and 23 months, alongside continued breastfeeding, to ease the transition from liquid to solid foods. However, this phase is highly vulnerable to malnutrition as the infant's diet shifts from sterile breast milk with immune factors to foods that may be prepared, stored, and served in unhygienic conditions. Additionally, the introduction of insufficient or inappropriate complementary foods, either too early or too frequently, exacerbates the risk of malnutrition. Proper nutrition during early childhood is crucial for optimal growth and development. The first 1,000 days of life are critical for ensuring long-term health, cognitive development, and disease prevention. Prolonged malnutrition can lead to impaired intellectual performance, reduced work capacity, diminished reproductive potential, and an increased risk of chronic diseases [2]. The primary causes of malnutrition in children include inadequate breastfeeding and complementary feeding practices, combined with high rates of diarrhea and acute respiratory infections. Historically, food choices were location-specific, but with advancements in science, technology, and globalization, a variety of food options from different origins have become available. However, the selection of these foods depends on economic capacity, nutritional awareness, and food trends influenced by advertising and marketing. Caregivers with financial means and adequate nutritional knowledge can provide diverse and nutrient-rich foods to their infants. Conversely, low-income caregivers, swayed by advertising, may insist on purchasing expensive foods, leading to inadequate feeding practices that heighten the risk of malnutrition and infections.

Ensuring the availability of affordable, nutrient-dense local foods is essential to combating malnutrition. Many indigenous food sources are rich in nutrients, but since they primarily consist of staples, combining different food groups can enhance their nutritional value and sustainability. Maize is a widely cultivated cereal, accessible in both rural and urban communities. It is rich in carbohydrates and serves various purposes, including meal preparation, oil production, fermentation, alcohol distillation, animal feeds, and biofuel production [3]. Groundnuts, a widely grown legume in the tropics, are an excellent protein source. Due to their high oil content, they are sometimes classified as oil seeds. They contain a variety of essential nutrients that can complement maize-based diets. To further diversify and improve nutrient intake, the addition of African yam bean (AYB), a legume that grows naturally but is at risk of extinction due to underutilization, is recommended for complementary food formulation. AYB is highly nutritious, containing substantial amounts of protein, starch, and calcium [4]. Its amino acid profile will support early childhood development. Additionally, crayfish, a readily available animal protein source, is widely consumed globally for its rich nutrient content [3]. The combination of these indigenous foods in complementary feeding could enhance nutrient intake, promote food sustainability, and improve overall child health.

II. MATERIALS AND METHODS

2.1 Study Design:

An experimental research design was adopted for this study.

2.2 Study Area:

The study was conducted at Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria. The institution houses the Department of Human Nutrition and Dietetics, and the research was carried out in the Food and Analytical Laboratory of the College of Applied Food Sciences and Tourism.

2.3 Source, Identification, and Processing of Samples:

2.3.1 Source of Crops:

Maize, AYB, groundnuts, and crayfish were purchased from different stalls at Ubani Main Market, Umuahia, Abia State, Nigeria.

2.3.2 Sample Identification:

The maize, African yam bean, and groundnuts were identified as *Zea mays*, *Sphenostylis stenocarpa*, and *Arachis hypogaea*, respectively, by Dr. Onyeonagu Chike C., a Crop Scientist in the Department of Agronomy (CCSS). Crayfish was identified as *Procambarus crarkii* by Dr. Reginald Nosike in the College of Animal Science and Animal Production, Michael Okpara University of Agriculture, Umudike.

2.3.3 Processing of Maize, AYB, Groundnuts, and Crayfish into Flours:

The maize grains were sorted to remove foreign materials, washed, and soaked in tap water for 24 hours. The soaked maize was rinsed, drained using a colander, sun-dried for 12 hours, and further dried to a constant weight in a digital food dehydrator (Excalibur® 3926TCDB) for 36 hours at 150°F. The dried maize seeds were milled into fine flour using a mechanical grinder (St Donkey powder crusher - Leshan Dongchaun) with a 5mm sieve as described by [5].

The AYB flour was prepared as described by [6]. The seeds were sorted to remove foreign materials, washed, and drained in a colander. They were toasted in a saucepan for 45 minutes over a gas burner at 191°C, then milled into fine flour using a mechanical grinder with a 5mm sieve.

Groundnuts were sorted, washed, drained, and sun-dried for 24 hours. The dried nuts were toasted at 191°C, dehulled, milled using a hand milling machine (Corona), and defatted with hot water. The defatted cake was oven-dried at 50°C for 24 hours in a Surgifield laboratory oven, then milled and sieved through a 2.5mm sieve to obtain fine flour.

Crayfish flour was prepared as described by [7]. A kilogram of crayfish was sorted to remove foreign materials, dried for 12 hours in a laboratory oven at 70°C with occasional stirring at 30-minute intervals to ensure uniform drying. The dried crayfish was winnowed and milled using a hammer mill with a 500-micron mesh sieve.

2.4 Formulation of Flour Blends:

To enhance protein content and improve amino acid balance, the flours were blended in the following ratios:

- 80:10:5:5 (Maize:AYB:Groundnut:Crayfish)
- 70:15:10:5
- 60:20:15:5
- 50:25:20:5
  A commercially available maize-based complementary food (Cerealac, 100%) was used as a control.

2.5 Preparation of Complementary Foods (Gruels):

Each blend (200g) was mixed with 200ml of clean water to achieve a smooth consistency. The mixture was placed in a saucepan over a gas burner, and 300ml of boiled hot water was added with continuous stirring. An additional 100ml of hot water was sprinkled over the gruel and allowed to cook for 2 minutes before chemical evaluation.

2.6 Chemical Evaluation and Sensory Analysis:

Proximate composition (moisture, ash, protein, fat, fiber) was analyzed using AOAC [8] methods. Carbohydrate content was determined by difference. Mineral composition, vitamins, and antinutrients were also analyzed using AOAC methods.

2.7 Statistical Analysis:

Data were analyzed using IBM SPSS version 21. Results were presented as means and standard deviation, with statistical comparisons made using ANOVA and Duncan's multiple range test at a 5% significance level ($p < 0.05$).

III. RESULTS

The proximate composition of the complementary gruel ranged as follows: moisture content (55.75% to 65.32%), crude protein (12.97% to 18.48%), fat (1.43% to 2.05%), crude fiber (0.23% to 1.84%), ash (1.29% to 3.02%), carbohydrate (11.77% to 28.33%), energy content (127.91Kcal to 178.07Kcal), and dry matter (34.68% to 44.25%) (Table 1).

TABLE 1
DRY MATTER, PROXIMATE, AND ENERGY COMPOSITION OF COMPLEMENTARY FOODS

Sample	Dry matter (%)	Moisture content (%)	Crude protein (%)	Fat (%)	Crude fiber (%)	Ash (%)	Carbohydrate (%)	Energy (Kcal)
MAGC1	$34.68^{c} \pm 0.27$	$65.32^{a} \pm 0.27$	$16.61^{d} \pm 0.01$	$1.71^{d} \pm 0.01$	$1.72^{b} \pm 0.02$	$2.88^{c} \pm 0.01$	$11.77^{d} \pm 0.27$	$127.91^{c} \pm 0.41$
MAGC2	$44.66^{b} \pm 0.11$	$57.35^{d} \pm 0.11$	$17.40^{c} \pm 0.01$	$1.77^{c} \pm 0.01$	$1.76^{b} \pm 0.01$	$2.92^{bc} \pm 0.01$	$18.81^{b} \pm 0.08$	$160.75^{b} \pm 0.24$
MAGC3	$39.68^{d} \pm 0.13$	$60.32^{b} \pm 0.13$	$17.84^{b} \pm 0.01$	$1.98^{b} \pm 0.01$	$1.81^{a} \pm 0.01$	$2.96^{b} \pm 0.02$	$15.10^{bc} \pm 0.09$	$149.56^{d} \pm 0.44$
MAGC4	$40.33^{bc} \pm 0.11$	$59.68^{bc} \pm 0.11$	$18.48^{a} \pm 0.01$	$2.05^{a} \pm 0.03$	$1.84^{b} \pm 0.01$	$3.02^{a} \pm 0.01$	$14.94^{c} \pm 0.18$	$152.11^{bc} \pm 0.40$
CC	$44.25^{a} \pm 0.03$	$55.7^{c} \pm 0.13$	$12.97^{e} \pm 0.01$	$1.43^{c} \pm 0.01$	$0.23^{c} \pm 0.03$	$1.29^{d} \pm 0.01$	$28.33^{a} \pm 0.17$	$1.78^{a} \pm 0.61$

Values are mean ± standard deviation of duplicate samples determinations. $^{a-e}$Means with similar superscripts within the same column are not significantly different (p>0.05).MAGC1= 80% maize: 10% African yam bean:5% groundnut:5% crayfish, MAGC2 = 70% maize:15% African yam bean:10% groundnut:5% crayfish, MAGC3 = 60% maize:20% African yam bean:15% groundnut:5% crayfish, MAGC4 = 50% maize:25% African yam bean:20% groundnut:5% crayfish,CC = control cerealac.

MAGC4 had the highest calcium (28.30 mg), iron (1.96 mg), potassium (5.40 mg), and sodium (1.24 mg), while MAGC1 had the lowest calcium (25.95 mg), iron (1.81 mg), and magnesium (1.35 mg). The commercial control sample had the lowest sodium content (Table 2).

TABLE 2
MINERAL COMPOSITION OF COMPLEMENTARY FOODS

Sample	Calcium (mg/100g)	Iron (mg/100g)	Potassium (mg/100g)	Sodium (mg/100g)	Magnesium (mg/100g)
MAGC1	$25.95^{e} \pm 0.02$	$1.81^{d} \pm 0.01$	$3.59^{d} \pm 0.03$	$0.73^{d} \pm 0.03$	$1.35^{e} \pm 0.03$
MAGC2	$27.31^{c} \pm 0.01$	$1.87^{b} \pm 0.03$	$4.09^{c} \pm 0.03$	$0.86^{c} \pm 0.03$	$1.49^{d} \pm 0.01$
MAGC3	$27.93^{b} \pm 0.02$	$1.92^{ab} \pm 0.02$	$4.82^{b} \pm 0.03$	$0.97^{b} \pm 0.03$	$1.72^{c} \pm 0.03$
MAGC4	$28.30^{a} \pm 0.01$	$1.96^{a} \pm 0.01$	$5.40^{a} \pm 0.01$	$1.24^{a} \pm 0.01$	$1.87^{b} \pm 0.01$
CC	$26.72^{d} \pm 0.01$	$1.85^{c} \pm 0.02$	$2.68^{e} \pm 0.01$	$0.71^{d} \pm 0.03$	$1.94^{a} \pm 0.03$

Values are mean ± standard deviation of duplicate samples determinations. $^{a-e}$ Means with similar superscripts within the same column are not significantly different (p>0.05).MAGC1 = 80% maize: 10% African yam bean: 5% groundnut: 5% crayfish, MAGC2 = 70% maize:15% African yam bean: 10% groundnut: 5% crayfish, MAGC3 = 60% maize: 20% African yam bean:15% groundnut: 5% crayfish, MAGC4 = 50% maize: 25% African yam bean: 20% groundnut: 5% crayfish, CC = control Cerealac.

The vitamin content of the complementary foods compared favorably with the commercial control sample (Table 3). The formulated samples contained more beta-carotene (4.09 mg – 11.61 mg), riboflavin (2.66 mg to 2.85 mg), and vitamin C (14.52 mg to 30.19 mg) than the control, which was higher in thiamin (1.05 mg) and niacin (1.01 mg).

TABLE 3
VITAMIN COMPOSITION OF COMPLEMENTARY FOODS

Sample	Beta-carotene (mg/100g)	Thiamin (mg/100g)	Riboflavin (mg/100g)	Niacin (mg/100g)	Vitamin C (mg/100g)
MAGC1	$4.90^{d} \pm 0.01$	$0.42^{d} \pm 0.01$	$2.85^{a} \pm 0.01$	$0.73^{c} \pm 0.02$	$14.52^{d} \pm 0.01$
MAGC2	$7.43^{c} \pm 0.03$	$0.46^{d} \pm 0.02$	$2.81^{a} \pm 0.01$	$0.77^{c} \pm 0.01$	$25.98^{c} \pm 0.01$
MAGC3	$9.31^{b} \pm 0.01$	$0.51^{c} \pm 0.01$	$2.72^{b} \pm 0.02$	$0.87^{b} \pm 0.03$	$27.65^{b} \pm 0.01$
MAGC4	$11.61^{a} \pm 0.01$	$0.62^{b} \pm 0.01$	$2.66^{c} \pm 0.01$	0.91 ± 0.01	$30.19^{a} \pm 0.02$
CC	$4.22^{e} \pm 0.01$	$1.05^{a} \pm 0.01$	$1.80^{d} \pm 0.02$	$1.01^{a} \pm 0.01$	$3.10^{e} \pm 0.01$

Values are mean ± standard deviation of duplicate samples determinations. $^{a-e}$ Means with similar superscripts within the same column are not significantly different (p>0.05).MAGC1 = 80% maize: 10% African yam bean: 5% groundnut: 5% crayfish, MAGC2 = 70% maize:15% African yam bean: 10% groundnut: 5% crayfish, MAGC3 = 60% maize: 20% African yam bean:15% groundnut: 5% crayfish, MAGC4 = 50% maize: 25% African yam bean: 20% groundnut: 5% crayfish, CC = control Cerealac

The complementary foods had higher levels of antinutrients (Table 4). MAGC4 had the highest tannin (0.39 mg), phytate (1.43 mg), flavonoids (1.13 mg), and phenol (0.14 mg), while MAGC1 contained the highest saponin levels. The commercial control had the lowest antinutrient content.

TABLE 4
ANTINUTRIENT COMPOSITION OF COMPLEMENTARY FOODS

Sample	Tannin (mg/100g)	Phytate (mg/100g)	Saponin (mg/100g)	Flavonoids (mg/100g)	Phenol (mg/100g)
MAGC1	0.32^{c}± 0.01	1.33^{c}±0.03	0.18^{a}± 0.01	1.03^{c}± 0.01	0.11^{c}± 0.02
MAGC2	0.22^{d}± 0.03	1.40^{b}± 0.01	0.15^{b}± 0.01	1.07^{b}± 0.01	0.12^{b}± 0.01
MAGC3	0.35^{b}± 0.01	1.41^{b}± 0.01	0.14^{b}± 0.01	1.11^{ab}± 0.01	0.12^{b}± 0.01
MAGC4	0.39^{a}± 0.01	1.43^{a}± 0.01	0.12^{c}± 0.01	1.13^{a}± 0.02	0.14^{a}± 0.01
CC	0.01^{e}±0.01	0.01^{d}± 0.00	0.00^{d}± 0.00	0.09^{d}± 0.01	0.01^{d}± 0.01

Values are mean ± standard deviation of duplicate samples determinations. $^{a-e}$ Means with similar superscripts within the same column are not significantly different (p>0.05).MAGC1 = 80% maize: 10% African yam bean: 5% groundnut: 5% crayfish, MAGC2 = 70% maize:15% African yam bean: 10% groundnut: 5% crayfish, MAGC3 = 60% maize: 20% African yam bean:15% groundnut: 5% crayfish, MAGC4 = 50% maize: 25% African yam bean: 20% groundnut: 5% crayfish, CC = control Cerealac.

TABLE 5
SENSORY PROPERTIES OF COMPLEMENTARY FOODS

Sample	Taste	Appearance	Mouthfeel	Color	General acceptability
MAGC1	6.25^{d}± 1.48	5.85^{c}±1.95	5.85^{d}±1.84	6.70^{b}±1.30	6.16^{c}± 1.37
MAGC2	6.25^{d}± 1.86	6.25^{b}± 1.65	5.90^{c}± 1.77	6.10^{e}± 1.48	6.13^{d}± 1.27
MAGC3	6.80^{b}± 1.70	5.70^{d}±1.72	5.85^{d}± 1.79	6.55^{c}±1.28	6.23^{b}± 1.19
MAGC4	6.55^{c}± 1.90	5.80^{cd}± 1.82	6.10^{b}± 1.37	6.25^{d}± 1.83	6.23^{b}± 1.19
CC	8.55^{a}± 0.51	8.00^{a}±1.08	8.00^{a}± 1.21	8.15^{a}± 0.88	8.18^{a}± 0.66

Values are mean ± standard deviation of duplicate samples determinations. $^{a-e}$ Means with similar superscripts within the same column are not significantly different (p>0.05).MAGC1 = 80% maize: 10% African yam bean: 5% groundnut: 5% crayfish, MAGC2 = 70% maize:15% African yam bean: 10% groundnut: 5% crayfish, MAGC3 = 60% maize: 20% African yam bean:15% groundnut: 5% crayfish, MAGC4 = 50% maize: 25% African yam bean: 20% groundnut: 5% crayfish, CC = control Cerealac.

IV. DISCUSSION

The complementary foods were analyzed on a wet basis. The moisture content (55.75% to 65.32%) was significantly different ($P<0.05$) and higher than the control. However, both the formulated blends and the control had superior moisture levels compared to malted rice, soybean, and pumpkin pulp complementary foods (8%-10.3%) [9], yet lower than values (64.32% to 83.70%) reported in complementary foods given in Giginyu, Kano, Nigeria [10]. High moisture content can lower nutrient density and shorten shelf life.

The protein content (16.31% to 18.48%) was significantly higher ($P<0.05$) than in the commercial maize-based complementary food (12.97%) but lower than the 20.49% to 41.07% reported in malted rice, soybean, and pumpkin pulp flour [9]. Protein is essential for growth and development, particularly in catch-up growth [11].

The fat content (1.71% to 2.05%) was higher than the control (1.43%) and comparable to nutrient-rich complementary foods (1.88% to 9.0%) from local sources [12]. Higher fat content enhances energy density. The ash content (2.88% to 3.02%) was significantly higher than the control (1.29%) and similar to nutrient-rich complementary foods (2.30% to 4.32%) [12]. Ash content is associated with mineral composition.

Crude fiber content was lower than the 1.85% reported by Onwuchekwa [9]. Carbohydrate levels were lower than the control and prior reports [9,12]. Carbohydrates are essential for energy, and complementary foods should be formulated with adequate levels. The dry matter content of the formulated complementary foods was comparable to the commercial product. Dry matter content is important in regulating metabolic function and preventing weight loss [13,14].

The calcium content (25.95 mg to 28.30 mg) was comparable to the control (26.72 mg) but higher than malted rice, soybean, and pumpkin pulp complementary food (0.50 mg to 1.02 mg) [9]. However, it was lower than locally sourced nutrient-dense

complementary foods (156.50 mg to 500.00 mg) [12]. Iron levels (1.81 mg to 1.96 mg/100g) were low compared to Kilimanjaro complementary foods (2.48 mg to 22.86 mg) [15] but comparable to the control (1.82 mg). Iron deficiency in infancy is linked to developmental challenges, emphasizing the need for iron-rich complementary foods [16].

The potassium content was higher than the control but significantly lower than the recommended daily intake (600 mg to 1100 mg/day) for children [17]. This suggests the necessity of additional dietary sources. Sodium content was within safe limits, aligning with the American Heart Association's recommendation of no more than 2,300 mg/day [18] and lower than reported sodium levels (>210 mg/170 g) in some infant foods [19]. Magnesium content was within acceptable ranges and is vital for physiological functions [20].

The antinutrient composition of the study maize was within the permissible level and lower than 10.41 to 11.77 tannin, and 64.74 to 69.98 phytate reported for maize-pea-anchote-made complementary foods [23]. This could be attributed to the differences in crops used. The health benefits of antinutrients at lower levels have been underscored [24, 25].

The sensory scores of the complementary foods, although lower than the commercial maize-based control, are understandable and acceptable as time, market orientation, and information are required for introduction and acceptance of new products. Factors such as cross-functional teams, solid upfront homework prior to product development, enhanced market orientation, quality of execution, and early product definition were listed as ingredients of projects that were both on time and fast to market [26].

V. CONCLUSION

The chemical composition of the maize-AYB-groundnut-crayfish blended complementary foods compared favorably with the commercial maize-based control. Its nutrient profile varied and is expected to contribute to growth and development. The maize-AYB-groundnut-crayfish blended complementary foods will enhance nutrient intake, provide a locally available and affordable option, and support sustainable food security, as the base ingredients were locally sourced.

The crude protein, fat, fiber, ash, potassium, sodium, beta-carotene, riboflavin, and vitamin C contents of the study samples were superior to those of the commercial maize-based control. Additionally, calcium and iron levels in samples MAGC2, MAGC3, and MAGC4 were higher than in the maize-based control, although the latter had greater energy and dry matter content. Overall, the formulated products compared favorably in several key nutrients and have the potential to improve nutrient intake, diversify local food options, and enhance food security.

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A Review on Dry and Wet Spell Probability Analysis for Agricultural Crop Planning by using Markov Chain Model

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Abstract— *Climate change and variability pose significant challenges to global agriculture, particularly in regions reliant on rainfed farming systems. Erratic rainfall patterns, prolonged dry spells, and extreme weather events disrupt traditional cropping practices. Indian agriculture is predominantly influenced by south-west monsoon rainfall (June to September), The southwest monsoon season accounts for 60 to 90 percent of India's yearly rainfall, which is critical to the country's agricultural economy. Variations in rainfall distribution patterns are the primary cause of the country's increased likelihood of experiencing a drought-like condition. To successfully plan and manage agricultural ecosystems, it is necessary to understand the sequences of dry and wet spells, as well as the advent and withdrawal of the rainy season. The purpose of this review is to present the predicted odds of a dry and rainy spell by various researchers using the same model. From these reviews the estimated probabilities of dry and wet spell are not same per different researchers because the uneven distribution and erratic nature of rainfall in different regions. And the estimated date of advent and withdrawal of rainy season is also not same for results obtained from different researches due to the inconsistency of rainfall and the methods used by different researchers.*

Keywords— Crop planning, Dry and wet spell, Markov Chain Probability Model, Onset and withdrawal, Rainfall.

I. INTRODUCTION

In a predominantly agricultural system, natural rainfall is the primary source of water for agricultural sector. Agricultural crop output during the rainy season is determined by the regional and temporal distribution of rainfall, as well as its intensity and duration. But with alteration in world's climate, temperature and rainfall will increases in some places and in other places this situation is inverse. Due to these climatic changes as almost, all crops are season-dependent as well as rainfall-dependent. Climate change is exerting profound effects on global agriculture, disrupting traditional cropping patterns and posing significant challenges to food security. The intricate interplay of increasing temperatures, altered rainfall patterns, and increased frequency of extreme weather events has far-reaching consequences for crop production systems worldwide. Changes in precipitation patterns, including shifts in timing and intensity, significantly impact water availability for crops. Irregular rainfall distribution can lead to droughts or waterlogging, both of which adversely affect crop growth. Regions experiencing increased aridity face challenges in maintaining adequate soil moisture for optimal plant growth. Agricultural productivity in rainfed areas remains low and unstable due to weather anomalies, with major crops affected by monsoon delays, variations in spatial and temporal aberrations, and breaks in the monsoon causing prolonged dry spells and being responsible for early, mid, and terminal drought. These circumstances call for attention of agricultural scientists and planners can develop contingent measures to save the rainfed crops from varied monsoon aberrations.

A scientific approach to agriculture is crucial for optimizing a region's rainfall patterns and maintaining consistent crop output levels. Analyzing dry and rainy spells helps build a crop plan for rainfed areas. Using scientific forecasting to analyze wet and dry spells can help farmers improve their crops and economic conditions (Bora et al., 2022). Knowing when dry and wet seasons occur is crucial for agricultural planning and farming operations since it has a considerable influence on crop output

and, as a result, rural populations' lives. The success or failure of any crop, especially in rainfed areas, is influenced not only by total rainfall received during the crop season, but also by how it is distributed during the critical phonologic time of crop growth. The concept of forecasting the likelihood of dry and wet periods, as well as the possibility of consecutive 2/3 days of dry and wet spells based on a threshold amount of rainfall, is very useful for crop planning and agricultural operations. Understanding the occurrence of dry spells and wet spells is critical for mitigating the negative consequences of dry spells during sensitive crop growth phases. The probable behavior of rainfall was studied by many researchers (Kumar et al., 2007); (Chakravorthy and Mandal.,2008); (Chand et al., 2011); and (Jakhar et al., 2011).

The probability concept is commonly used to highlight the significance of dry and wet spells when planning weather-sensitive agricultural activities. (Shrivastav et al., 2004). The purpose of estimating probability with respect to a given amount of rainfall is tremendously helpful for agricultural planning (Subash et al., 2009). Markov chain model has been found suitable to describe the long-term frequency behavior of wet or dry spells. (Victor and Sastri 1979). A Markov Chain Model considers less than 20 mm of rainfall in a week to be a dry week, and 20 mm or more as a wet week. Pandarinath (1991), Dash and Senapati (1992). The Markov chain probability model predicts whether rain will fall on a given week based on the previous week's weather conditions. (Manikandan et al., 2017). A Markov chain process's probability relationship must incorporate the conditional probabilities that the process will transition from any state at period (t) to any subsequence state at period (t+1). As a result, the relation $P(Xt+1 = J / Xt = i)$ represents the conditional probability of transitioning from state i to state j at time t+1. The likelihood of a week being dry or wet is determined by initial probability, however in conditional probability, if a particular period i is wet or dry, the possibility of the (i+k)th period being wet is predicted and expressed as wet/wet or wet/dry. The threshold limits of 10 mm and 20 m of rainfall were chosen as crucial for various agricultural planning purposes.

The weekly analysis of rainfall is particularly significant in agricultural planning, and the week period has been deemed the optimal length of time. (Reddy et al., 2008). In general, a Markov process describes only step-by-step dependence, called a first order process. The erratic nature of rainfall has been the primary reason of droughts in India. Drought causes significant yield reductions both for rainfed and irrigated crops. On the area basis, the dry spell analysis would assist in formulation of contingency plan against drought. Knowing the average monthly, seasonal, and yearly rainfall is beneficial in comprehending the overall picture of a certain location, but weekly rainfall data analysis provides more relevant and accurate information for rainfall-based crop planning (Tiwari et al., 1992). Another factor essential for crop planning is the forward and backward accumulation of rainfall, which determines the start and withdrawal of the effective monsoon. The onset and withdrawal features of the monsoon heavily influence the success of rainfed agriculture. The late beginning of the monsoon slows agricultural sowing, resulting in poor yields. Similarly, early removal of rainfall reduces yield owing to severe moisture stress, particularly when kharif crops are in crucial growth phases of grain formation and development (Dixit et al., 2005).

II. MATERIAL AND METHODS

Agriculture in rainfed areas remains a gamble, with rainfed farmers facing a number of concerns such as unpredictable weather, a lack of timely inputs, and loans, all of which contribute to poor and inconsistent production and profitability. The crop production in rainfed region carries inherent hazards since rain is unpredictable in terms of timing and quantity. The principal source of water for rainfed crops is rain, a major portion of which is received during the south-west monsoon period. The monsoon period is beset with breaks of rain in almost all parts of the country Normally, there are at least four important aberrations in rainfall behavior:

i) Early commencement of the rains, or significantly delayed monsoon,

ii) Intermittent “breaks” during the cropping season,

iii) Variation in spatial and/or temporal aberrations,

iv) Early cessation of rainfall or sustained wet periods over a prolonged time.

Keeping all of these aspects in mind, the current review was conducted.

2.1 Computation of dry and rainy spells with the Markov chain probability model:

The Markov chain probability model predicts rainfall based on prior weeks. The week was either rainy or dry. Rainfall quantity is solely used to determine the existence or absence of rain. In the first order Markov chain, the probability of an event occurring on a single day is determined by the previous day's conditions and is not affected by subsequent days' occurrences. The model predicts the likelihood of experiencing a dry or rainy spell during a regular meteorological week. Calculating conditional

probabilities can reveal if a dry spell is followed by a rainy spell, and vice versa. The computation of initial and conditional probability is shown below.

Initial Probability of Dry and Wet Weeks:

$$P(D) = F(D)/N \quad (1)$$

$$P(W) = F(W)/N \quad (2)$$

Where,

P(D) =Probability of occurrence of dry week, P(W) = Probability of occurrence of wet week,

F(D) =Frequency of occurrence of dry week, F(W) =Frequency of occurrence of wet week,

N = Total number of years.

Conditional Probability of Dry and Wet Weeks:

The following formulas are used to compute the conditional probability of a dry or wet week preceding another dry or wet week.

$$P(D/D) = F(DD)/ F(D) \quad (3)$$

$$P(W/W) = F(WW)/ F(W) \quad (4)$$

$$P(W/D) = 1-P(D/D) \quad (5)$$

$$P(D/W) = 1-P(W/W) \quad (6)$$

Where,

P(D/D) = Probability of a week being dry preceded by another dry week,

P(W/W) = Probability of a week being wet preceded by another wet week,

F(D/D) = Frequency of dry week preceded by another dry week,

F(W/W) = Frequency of a wet week preceded by another wet week,

P(W/D) = Probability of a wet week preceded by a dry week, and

P(D/W) = Probability of a dry week preceded by a wet week.

2.2 Consecutive Probability of Dry and Wet Weeks:

The following formulas are used to calculate the chance of two or three consecutive dry and wet weeks.

$$P(2D) = P(DW1) \times P(DDW2) \quad (7)$$

$$P(3D) = P(DW1) \times P(DDW2) \times P(DDW3) \quad (8)$$

$$P(2W) = P(WW1) \times P(WWW2) \quad (9)$$

$$P(3W) = P(WW1) \times P(WWW2) \times P(WWW3) \quad (10)$$

Where,

P(2D) = Probability of two consecutive dry weeks starting with the week,

P(DW1) = Probability of the first week being dry,

P(DDW2) = Probability of the second week being dry, given the preceding week being dry,

P(3D) = Probability of three consecutive dry weeks starting with the week,

P(DDW3) = Probability of the third week being dry, given the preceding week dry,

P(2W) = Probability of two consecutive wet weeks starting with the week,

P(WW1) = Probability of the first week being wet,

P(WWW2) = Probability of the second week being wet, given the preceding week being wet,

P(3W) = Probability of three consecutive wet weeks starting with the week, and

P(WWW3) = Probability of the third week being wet, given the preceding week wet.

2.3 Thresholds of rainfall for Crop planning using Initial and conditional probabilities:

For agricultural planning, many researchers used the Markov Chain model, with threshold limits of 10, 20, 40, and 80mm/week. These threshold levels were deemed adequate for crop activities such as soil preparation (10mm), crop planting or sowing (20mm), and fertilizer and/or weeding (40mm). According to Reddy, if a given week 'i' of a given year received more than 20mm/week at more than 50% (W/W) threshold level, then week 'i' is the right time for planting. If weeding/fertilizer application is to be carried out in week 'i' then the week should have at least 75% (W/W) probability at 40mm/week. If the interest is when we should not apply fertilizer/pesticides, then one can use the probability estimate at 80mm/week. If fertilizer and/or insecticides/pesticides are applied on week 'i' then W should not exceed 25% probability level at 80mm/week. Rajendram and Sivasami applied the same threshold limits to estimate. If weeding/fertilizer application is to be carried out in week 'i' then the week should have at least 75% (W/W) probability at 40mm/week. If the interest is when we should not apply fertilizer/pesticides, then one can use the probability estimate at 80mm/week. If fertilizer and/or insecticides/pesticides are applied on week 'i' then W should not exceed 25% probability level at 80mm/week. Rajendram and Sivasami applied the same threshold limits to estimate.

2.4 Computation method for the onset and withdrawal of rainy season:

The onset and withdrawal of the rainy season were calculated from weekly rainfall data using forward and backward accumulation methods (Kothari et al. 2009). Each year was divided into 52 standard meteorological weeks (SMWs). The first SMW of each year runs from January 1st to 7th, and the 52nd SMW runs from December 24th to 31st. Weekly rainfall was totaled by forward accumulation (20+21+…+52 weeks) until 75 mm was reached. The monsoon season begins when 75 mm of rainfall accumulates, and the rainy season ends after backward accumulation (48+47+46+…+30 weeks). The percent probability (P) of each rank was computed by sorting them in increasing order and picking the highest rank assigned for that week. The following Weibull's formula was used to calculate percent probability (Robert et al., 1971).

$$P = \frac{m}{N+1} \times 100 \quad (11)$$

Where, m = Rank number, N = Number of years of data used.

Table 1 compares the chance of dry and wet weeks using the Markov chain probability model, as well as the onset and withdrawal of the effective monsoon based on forward and backward rainfall accumulation.

A review on the likelihood of dry and wet weeks using the Markov chain probability model, as well as the onset and withdrawal of the effective monsoon using forward and backward rainfall accumulation

TABLE 1
THE PARAMETERS AND METHODS USED IN DIFFERENT PROBABILITY MODEL

Parameters	Onset and withdrawal and of rainy season	Initial, conditional and consecutive probability of wet and dry spell.	Mean weekly rainfall
Relevant Methods	Forward, backward accumulation and Weibull's formula	Markov Chain Probability Model	Statistical analysis

TABLE 2
COMPARATIVE ANALYSIS OF RAINY SEASON ONSET, WITHDRAWAL, AND PROBABILITY STUDIES

Sr. No	Obtained Results	Author
1.	Rainy season started effectively from 24th SMW (11^{th} – 17^{th} June) and remained active up to 45^{th} SMW (5^{th} – 11^{th} November). Except for the 25 th-27 th SMW and the 44 th-48 th SMW, the likelihood of a rainy week was greater than 35%. The probability of a dry week was also higher. More than 75% of the monsoon season was preceded by another dry week on the 20 th SMW, 24 th-26 th SMW, 29^{th} SMW, 44^{th} SMW, and 47^{th}-48^{th} SMW. The average weekly rainfall was > 40 mm during the 36 th-41 st SMW and less than 20 mm during the 20 th, 25 th-27 th, and 44 th-48 th SMW.	Reddy et al., (2008)
2.	Onset of effective monsoon started effectively from 25th SMW (18^{th} –24^{th} June) and remained active up to 39th SMW (24th–30th September). The chance of existences of initial and conditional probability was > 50% on the 26^{th} SMW at the 10 mm per week threshold limit and 28^{th} week at 20 mm per week threshold limit, hence land preparation & sowing for planting could be undertaken in 26^{th} and 28^{th} SMW respectively. Initial and conditional probabilities at 20 mm threshold limit per week disclosed that, supplementary irrigation and moisture conservation practice need to be practiced between 38th and 40th week for short duration crops.	Admasu et al., (2014)
3.	The analysis revealed that the likelihood of a dry week increases from the 1-14 week, as well as from the 41^{st} to the $52^{nd.\ The}$ chance of a dry week in these weeks' ranges from 41.67% to 100%. The probability of a wet week increases from week 17^{th} to week 40^{th}. The chance of a wet week in these weeks' ranges from 66.67% to 100%. The weeks 1^{st} to 4^{th} and 43^{rd} to 52^{nd} of the year remain under stress on average, with 50% to 95.83% possibilities of two consecutive dry weeks and the monsoon started in week 23^{rd} (4th to 10th of June). Hence, week 25th (18th to 24th June) was an appropriate period to begin wet soil preparation for planting short duration rice varieties. Pre-monsoon season begins in week 14^{th} (2nd April to 8th April). Summer maize (rain fed) can be sown starting in week 14^{th}. Week 15th (9th April to 15th April) was an opportune period to begin wet soil preparation for producing long-duration rice varieties.	Dabral et al., (2014)
4.	The monsoon season began on the 25th SMW (18th-24th June) and continued until the 39th SMW (24th-30th September). Probability of existence of wet week was ≥ 40% except during 24^{th} SMW. And mean weekly rainfall was > 40 mm during 26^{th} –39^{th} SMW and found to be ≥ 20 mm during entire rainy season (1th June–30^{th} September) including 24^{th} and 25^{th} SMW.	Singh et al., (2014).
5.	The initial, conditional and consecutive dry and wet week probabilities specified that chances of incidence of a week getting wet was high during from 24^{th} week onwards upto 40^{th} week. Chances of occurrence of wet week of more than 50% at the beginning of Kharif season designated that summer ploughing and initial seed bed preparations, affording to the findings should be taken up in the 20^{th} – 22^{nd} SMW (14^{th} May – 3^{rd} June) and sowing operations can be taken up since 23^{rd} SMW (4^{th} – 10^{th} June). The probability of receiving initial and conditional probability was more than 50 per cent on 35^{th} week with threshold limit of 20 mm per week. Hence, results revealed that, the land preparation for sowing or planting could be undertaken in 35^{th} SMW respectively for the main rainy season crop cultivation for the study area.	Punitha et al., (2017)
6.	The monsoon season began on the 24th SMW (11-17 June) and will continue until the 46th SMW (12-18 November). The probability of a wet week occurring was greater than 30% at the start of the Kharif season, representing that summer ploughing and primary seed bed preparations were carried out between the 20^{th} and 22^{nd} SMW (14th May - 3rd June), and sowing activities could begin on the 23rd SMW (4^{th} - 10^{th} June). At the rabi season, from the 37^{th} to the 49^{th} week, the chances of occurrence of a wet week were more than 50%. The large number of consecutive wet weeks recommended that all agricultural operations, such as planting/sowing underneath rainfed conditions, could be accomplished effectively at that time.	Manikandan et al., (2017)

7.	The monsoon began on the 24th standard meteorological week (11-17 June) and lasted until the 43rd SMW (22-28 October), for a total of 20 weeks. Initial, conditional, and consecutive dry and wet week probabilities revealed that the likelihood of a week being wet was high from the 24th week forward until the 40th week. The probability of a wet week occurring greater than 50% at the start of the Kharif season recommends that summer ploughing and first seed bed preparations should be completed between the 20th and 22nd SMW (14th May - 3rd June), and sowing activities can begin as early as the 23rd SMW (4th - 10th June).	Ray et al., (2018)
8.	The initial rainfall probability P (W) of receiving 20 mm of rainfall each week was more than 30% during the 22nd SMW, hence field preparation should take place during this time period. The initial and conditional likelihood of wet week followed by wet week P (W/W) of receiving 20 mm rainfall was more than 50% in the 23rd SMW, indicating that this week was better suited for crop sowing. During the 44th to 46th SMW (October 29th to November 18th), the likelihood of receiving 10 mm of rainfall each week was greater than 30%. This season was ideal for land preparation and rabi crop planting in study area, where the monsoon started effectively from 24 SMW (12 June to 15 June) and lasts till 41 SMW (9 October to 13 October) indicated a good monsoon rain in the region for approximately 17 weeks (from the 24th to the 41th SMW).	Dugal et al., (2018)
9.	The average date of start of the effective monsoon in the research region was between June 25th and July 16th, hence this time should be considered for planting chosen kharif crops in contingency crop planning. More than 50% of dry weeks were observed from SMW 24th to 29th, 31st to 34th, and 40th to 42nd in the crop growth period, while more than 50% of wet weeks were observed from SMW 23rd, 30th, and 35th to 39th SMW, prompting contingency crop planning for the study area.	Joshi et al., (2012)
10.	The monsoon started effectively from 24th SMW (11-17th June) and remained active up to 43rd SMW (22-28th October). The likelihood of a wet week was more than 35% during the 23-24th SMW (4th–17th June), and average weekly rainfall varied from 27.4 to 41.9 mm; this rain may be used for summer ploughing and initial seed bed preparations. The results of this research were used to plan agricultural activities and mitigate dry spells in the study region.	Pawar et al., (2019)
11.	The mean regional onset, cessation and duration of the rainy season were week 15th (9th – 15th April), week 42nd (15th – 21st October) and 29 weeks (203 days) respectively. The initial and conditional probabilities of wet spell week were more than 50% from the week 22nd for all the stations at 8.4mm per week threshold. The study concludes that land preparation for crop planting should commence from week 20th - 22nd whereas crop irrigation farming should start after week 42nd in the study region.	Tyubee and Iwan (2019)
12.	According to the analysis, the rainy season began and ended in the study area on the 26th and 40th SMW, respectively. The initial likelihood of rainy weeks (25th - 34th SMW) and the conditional probability of wet week followed by another wet week in the Saurashtra area ranged from 47.6 to 66.7% and 20.0% to 64.3%, respectively. The results suggested that land preparation and kharif crop sowing should take place between the 24th and 26th SMW. The sowing of dryland crops in the rabi season may be finished between the 40th and 41st SMW, as the following weeks have a lower chance of receiving adequate rainfall.	Hirapara et al., (2020)
13.	Data on the beginning and withdrawal of rainy season revealed that the monsoon began effectively from the 23rd SMW (04th to 10th June) and continued active until the 42nd SMW (15th to 21st October). hence, 23rd to 25th SMW was ideal time for sowing of selected kharif crops and 38th to 40th SMW is ideal time for sowing of selected rabi crops in the contingency crop planning of the study area. More than 50% of dry weeks occurred on the 23rd, 29th, 35th, and 37th to 42nd SMWs throughout the crop growth period, whereas more than 50% of wet weeks occurred on the 23rd to 26th, 28th, 30th to 38th, and 40th SMWs. Thus, chances of rainwater harvesting were observed more and the harvested water at the end of kharif season were used as protective irrigation in rabi season.	Sonawane et al., (2021)

14.	The rainy season lasts 161 days (from May 21st to October 28th), which was onset and withdrawal. The rainfall total was 883 mm. The yearly effective rainfall was 47% of the normal annual rainfall. During the conclusion of the rainy season, the chances of having two or three consecutive dry weeks range from 0 to 50% and 0 to 45%. During weeks 21-35 and 38-39, there is a greater than 75% likelihood of a rainy week. Additionally, from the 21st to 38th SMW, the likelihood of P (ww) exceeds 75%. During this time, there is no need for additional water supply, allowing excess to be stored for the rest of the season.	Bora et al., (2022)
15.	From the 1st to the 32nd SMW, the chance of a continuous dry week was 75-100%. The possibility of a dry week followed by another dry week increased to the 32nd standard week, while the probability of a dry week followed by a wet week increased to the 31st standard week, ranging from 75 to 100%. During the 37th to 45th weeks, the probability of a wet week followed by another rainy week ranged from 43.8 to 68%. A examination of successive dry and wet spells revealed that two consecutive dry weeks were 55 to 97.5% likely to occur within the first 32 weeks of the year. In the first 32nd week of the year, the probability of three consecutive dry weeks ranged from 32.6 to 92.6%. Consecutive dry weeks indicate a need for more irrigation and effective moisture management measures. In contrast, successive rainy calendar weeks imply an excess of surplus water available for rainwater collecting, highlighting the importance of adequate soil erosion prevention methods.	Nagaraju and Nagarajan (2022)

III. CONCLUSION

The study concluded that, many researchers worked on Markov Chain Model for estimating the dry and wet week probabilities of rainfall for crop planning. From the above reviews the estimated dry and wet week probabilities of rainfall were different by using same model. Most of the researchers consider 10mm, 20mm & 40mm threshold limit for weekly rainfall but the main cause for the variation of the estimated probabilities of dry and wet week is uneven distribution of rainfall in different regions. Whereas, the onset and withdrawal of rainy season determine the success of rainfed agriculture. From the above reviews, the monsoon started and remains active effectively in between 15st SMW up to 46th SMW respectively for different study areas based on the rainfall behaviour. Based on the results, many researchers had made crop planning as well as contingency crop planning and measures for the study region.

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High Protein, Low Carbohydrate, High Non-Trans Fat, and Decitabine for Survival-ITP and LGC Leukemia

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Abstract—

Introduction: *Therapy of TP53, mutation of MSD/AML with Niclosamide plus Azacitidine, is the end-stage phase of ITP/LGC Leukemia. The Constant therapy is unbroken with CHOP or R-CHOP as the second line therapy, plus nutrition HPLCHF, and decitabine ever since the early phase which should be given. Aims: No transfusion should be held since this early phase. Cytokine storm is the inducer of this advancement. Hypothesis: High Protein Low Carbohydrate High Fat (HPLCHF) and decitabine without transfusion should be the winner for survival ITP/LGC. Liquid therapy and aspirin are the first line symptomatic during the cytokine storm phase. Two dead cases of Large Granular Chronic Leukemia support this study.*

Method: *Hybrid My library and ChatGPT based, with academic search engine using Science Direct and EBSCOHost MEDLINE full text using keywords ITP/MDS/AML, and therapy, and case reports.*

Result: *Variables drugs is recorded such as pro-apoptotic agent Venetoclax a Bcl2- inhibitor, Fostamatinib a spleen tyrosine Kinase inhibitor, decitabine/azacitidine hypomethylation agent, CHOP (chemotherapy with prednisone) and splenectomy are given in the thrombocytopenia last moment, with MOD end-stage mortality less than 5 days due Multi/X/Total Antibiotic Resistance. Low carbohydrate, high-fat, high protein should be given in this early prediction low albumin level of severe thrombocytopenia in Dengue Lymphoma Malignant Fever (DFLM).*

Discussion: *Pathogenesis ITP, MDS, AML therapy in all phases, and the recorded mutation genetic/epigenetic, and immune landscape of TP53 and other mutant AML & higher risk MDS treated with azacitidine/decitabine.*

Conclusion: *HPLCHF nutrition and decitabine ever since the early phase should be given to support the body to cure themselves.*

Keywords— ITP; Splenectomy; Prednisone; Low carbohydrate; often Protein nutrition; Decitabine.

I. INTRODUCTION

Anemia Immune Thrombocytopenia (ITP) a.k.a. Previously Idiopathic Thrombocytopenia Purpura progressed to MDS/AML or is a Life-threatening situation “bridge therapy” to surgery or invasive procedure,[1] splenectomy and or chemotherapy. ITP due to Defective Peripheral Immune Response often has high prevalence causes of this disease in our everyday practice.[1] ITP turns to MDS which increases bone marrow increasing of CD 34-blast, and CD16-granulocytes,[2] then progresses to AML. Germline genetic (and epigenetic) have been recorded in MDS and AML traditional outpatients.[3] AML is an aggressive hematological malignancy with a higher incidence in older people.[4] Clonal hematopoiesis (Ch) is a common premalignant state in the blood and confers an increased risk of blood cancers and all-cause mortality.[5] Variables drugs are recorded such as pro-

apoptotic agent Venetoclax a Bcl2- inhibitor,[4,6,7] Fistumatinib a spleen tyrosine Kinase inhibitor,[8] decitabine/azacitidine hypomethylation agent,[7,9,10] due to R-loop dysregulation and resultant genomic instability in the disease progression of MDS & AML,[10] a therapy strategy for Myeloid cancers.[10] Erythropoietin agents [11] and/or with RBC transfusion,[12] which reported as a cause of first hospitalization due the decreasing hemoglobin which drops common everyday Quality of Life (QL).[11] Daratumumab in refractory autoimmune cytopenia.[13] Plasmapheresis (plasma exchange) for inflammation cytokine as the pathogenesis of MDS is yet to be fully established.[12,14]

Novel Anti-CD38 monoclonal antibody for treating ITP,[15] Notable treatment advances have been made for patients with MDS/ neoplasm to live longer and better for what will likely remain a largely incurable disease,[16]describe the failure to complete recovery. This study brings to parsimony and calm reaction therapy like in fluidic only therapy on DHF in 16.000 platelet count is usually successful.(Case Report)

II. METHOD

Review and case report of therapy in all phases of ITP and LGC using hybrid recommendation of My library Google Scholar and Academic Search engine i.e., ScienceDirect and EBSCOHost MEDLINE full text, and ChatGPT. This hybrid Search used keywords ITP/MDS/ATP and therapy. Bayesian network and analysis are being used in including and excluding the references.

III. RESULTS

This study also recorded GvHDs therapy which does not respond to standard Systemic steroids which is given Ruxolitinib (JAK1 inhibitor for MS)[17] and Fostamatinib for curing thrombocytopenia got successful tapering and discontinuation of corticosteroid while maintaining platelet count above 50,000/uL.[18] Avatrombopag (a thrombopoietin receptor agonist) plus fostamatinib combination with multifactorial ITP is also recorded.[19] Patients with thrombocytopenia usually do not experience serious bleeding until their platelet count is very low.

TABLE 1
RECORDED THERAPY IN ITP/MDS/AML, PHARMACOLOGY FUNCTION, PROGNOSIS

Studies, years	Phase	Agents/Surgery	Pharmacology function combat	Prognosis
[1]Gonzalez-Lopez, 2023	Refractory ITP	Chemotherapy/Splenectomy	Splenomegaly	MOD sepsis
[18]Goel R, 2024	ITP	Standard Systemic Steroid (SSS) + Fostamatinib	Antiinflammation	Successful tapering
[17]Teshima T, 2024	Acute GvHD	Ruxolitinib as MS inhibitor	JAK1 inhibitor	Respond to SSS again
[20]He Q, 2024	cGv-HD	SHR0302 + prednisone	JAK1 Inhibitor	1st line Rx/
[18]Goel R & [8]Gonzalez-Lopez 2024	ITP	Fostamatinib	Spleen TKI	High efficacy rate
[3]Banaszaky LG, 2024	MDS/AML	Driven Germline genetic/epigenetic	Traditional out patients	Genetic counseling
[15]Chen Y, 2024	ITP	Anti-CD38	Anti-cancer monoclonal antibody	Boosted platelet level
[13]Hu U, 2024	Refractory autoimmune cytopenia	Daratumumab	Anti-CD38 monoclonal antibody	Effective Rx/
[5]Waarts MR, 2024	Premalignant state	CRISPR/Cas9	Editing	Identify genetic dependencies in mutant HSPCs
[4]Salamero O, 2020 [6]Wang D, 2024 [7]Kobayashi, 2024	AML	Venetoclax pro-apoptotic agent	A Bcl2-inhibitor	Phase I oke; Inhibit entering mitotic phase; Complete remission

[9]Enjeti A, 2024	Genomic instability ITP/MDS/AML	Decitabine/azacitidine hypomethylation agent,	Gene silencing	The most MDS Rx/
[11]Battaglia MR, 2024	Hemoglobin drops until disturb everyday QL common living	EPO agents RBC transfusion	Fit for everyday living	LR-MDS std Rx/ in the future
[12]Topping J, 2024	Cytokine storm (CS) pre- MDS	Plasmapheresis	Decreasing inflammation cytokine	For Dx/ and Px/: CS to first RBC transfusion
[21]Gangat N, 2024	Essensial thrombocytemia in JAK2 and CALK-mutated	Aspirin	Anti-inflammation Anti-cytokine storm	with a lower pregnancy loss
[10]Zhang F, 2024	Splicing factors are frequently mutated in pts. with MDS&AML	induce to anemia and ITP progress to MDS/AML	Rx/ strategy for Myeloid Ca with mutations in SRSF2	R-loop disruption by PRAP activation
[22]Rathje, 2024	GvHD acute & chronic	Anti-T-Lymphocyte Globulin (ATLG)	Inflammation and immune reaction	Lower rates of severe cGvHD
[23]Salma RS, 2018	Non-Hodgkin Lymphoma high prevalence	CHOP and R-CHOP	Responses	Still a challenge (partial remission)
[24]Zeidan AM, 2024	TP53 mutant AML and higher risk MDS	Azacitidine	Hypomethylation agents	Integrated Rx/
[25]Maslah N, 2024	MSD/AML	Niclosamide plus Azacitidine	TP53 mutation + hypomethylation agents	Niclosamide is an anthelmintic
[26]Kammerer, 2021	Cancer	HPLCHF	Decrease body fat	Lifestyle diet

Cancer cells, MDS, and ITP consume glucose at a higher rate and induce insulin resistance. Anemia and hypoglycemia are found in these thrombocytopenia patients. Dextrose infusions on the day or first hospitalization due to Quality of Life (QL) to get transfusion is also a common symptomatic treatment, where the next reaction is reported. Stress and its treatments can cause the body to release various hormones that increase blood sugar. This can weaken the immune system, and the fear of hypoglycemia makes the patients drink glucose by feeling the drop of QL. Cauda pancreas pancreatectomy with splenectomy has been the case reported. Glucose can be supplied from gluconeogenesis, protein synthesis disrupts from many viral infections, and could not do apoptosis. Protein should be supported by external supply the whole day for everyday QL. High-fat diet without saying how high the fat is, refers to the popular diet ketogenic which is 75% fat, 20 % protein, and 5% carbohydrate. HPLCHF is a lifestyle diet to drop bodyweight successfully. Also, the well-known trans fats which is the worst for health, e.g. shortening and margarine.

Therapy of TP53 mutation of MSD/AML with Niclosamide plus Azacitidine,[25] is the end-stage phase of ITP/LGC. The Constant therapy is unbroken with CHOP or R-CHOP[23] as the second line therapy, plus nutrition HPLCHF and decitabine ever since the early phase, which should be given. Supportive care in lower-risk MDS with anemia ITP is also rapidly evolving.[11]

IV. DISCUSSION

Immune Thrombocytopenia (ITP) a.k.a. previous well-known Idiopathic Thrombocytopenia Purpura, is an Autoimmune bleeding/without bleeding or fibrous disorder characterized by immoderate reticuloendothelial platelet (Mononuclear Phagocytic System) destruction & inadequate compensatory platelet production.[27] In all phases, ITP needs specific metabolism so that becomes MDS (Myelodysplastic Syndrome)[2,12] or myelofibrosis.[28] In the next advanced progress when the body cannot recover, MDS transforms to AML.[1,2,27]

The aims of this study record the success and the failure of therapy in finding references in association with complete recovery, not refractory ITP/MDS/AML. This study discusses each therapy as follows:

4.1 Transfusion, like Graft versus Host Disease (GvHDs):

To suppress the immune system reaction, steroids are needed. The reaction needs immunosuppressive drugs, CHOPs, antifungal, antiviral, and antibiotics. Thrombocyte donors attack recipients' cells, also RBC, etc. The etiology of GvHDs is the cytokine storm reaction. The mutated TP53 and other mutations, block apoptosis, and decrease JAK1 & 2, a group of kinases expressed on many cells, surfaces that mediate cytokine signaling.[20,29] One-third of patients with Acute GvHD are resistant to standard systemic steroids, and need second-line approach such as JAK1 and 2 inhibitors,[17] anti-T Lymphocyte globulin.[22] Aspirin therapy is associated with a lower pregnancy loss in both JAK2- and CALK-mutated essential thrombocytemia.[21] Complete response ITP are recorded with prednisolone.[30]

4.2 Luspatercept & ESA (erythropoietin stimulating agents)[11]:

This EPO agent is better than Transfusion and is given since the patients need to be hospitalized for the drop of hemoglobin which decreases Quality of Life. Luspatercept in these with SF3B1 mutations has an EPO level < 500 U/L.

4.3 Lenalidomide:

Lenalidomide, is T Immunosuppressive therapy in del 5q syndrome, has both efficacy & durability of response.[11] Lenalidomide is an immunomodulator.

4.4 Imetelstat:

Telomerase inhibitor is though in the phase III trial support, may become a standard option[11]. Imetelstat binds to the telomerase RNA component with high affinity, directly inhibiting telomerase activity.

4.5 Bone Marrow Transplantation:

Bone Marrow Transplantation using ATLG (anti-T Lymphocyte Globulin) depletion.

4.6 Supportive[11,31]:

The gut microbiome in ITP affects health through human metabolism, immune modulation, and maintaining physiological,[27] while older people who cannot make protein anymore have a higher incidence of progressing to AML.[4]

4.7 Ruxolitinib[32] and Fistamatinib[8]:

Ruxolitinib, inhibits JAK1 & 2, Belumosudil inhibits ROCK2, Ibrutinib inhibits BTK in Systemic Sclerosis (SSc) save for treatment of chronic GvHD (cGvHD)[29] Ruxolitimib in JAK1 inhibitor-naïve myelofibrosis.[28] Gilteritinib with/without Venetoclax improved outcomes when sequenced early for salvage.[33] JAK1 Inhibitor SHRO302 + prednisone for first-line treatment of cGvHD may have less effect on hematopoiesis.[20] Rx/ Fostamatinib in Thrombocytopenia give successful tapering & discontinuation of corticosteroids while maintaining platelet count above 50,000/uL.[17]

4.8 Selinexor[28]:

Selinexor causes cell cycle arrest and apoptosis in cancer cells. Selinexor is a selective inhibitor of nuclear export, an oral drug that prevents access to the nucleo-cytoplasmic transport of nuclear protein in JAK/STAT and non-JAK/STAT pathway. Selinexor induces nuclear localization of tumor suppressor proteins (incl. p53), be a route to the selective induction of apoptosis, and inhibits the performance of DNA damage repair protein.[34]

V. LIMITATION

The integrated genetic, epigenetic, and immune landscape of TP53 mutant AML and higher-risk MDS give medical care with azacitidine, has been talked of in Therapeutic Advance in Hematology, based on TP53 mutation MDS and AML,[24] which is Rx/ Niclosamide + Azacitidine has successfully totally recovered the sensitivity of TP53-mutated cells to hypomethylating agents. It's targeting TP53-mutated MDS/AML cells.[25] Inflammation reaction and immune reaction induce anemia, and ITP progresses to MDS/AML.[12] Inflammation storm and immune reaction in GvHD-free and relapse-free survival,[22] has not been elaborated specifically. Splicing factors are frequently mutated in ITP patients which induces anemia and MDS/AML progression.[10] Anti-CD38 monoclonal antibody has been used as a prognostic marker in leukemia: Daratumumab is effective in refractory cases.[13] This study has been successful in the use of hypomethylation agents in all phases, but still has limitations in building deductive and inductive protein production for RBC, platelet, and antibody/anti-cytokine storm.

VI. CONCLUSION

HPLCHF nutrition and decitabine ever since the early phase should be given to support the body to cure themselves.

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CONFLICT OF INTEREST

The author declares no conflict of interest in any insights.

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Efficacy of Organic Amendments and Bio-Agents for Management of Chickpea Wilt in Field Conditions

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Abstract— *Chickpea (Cicer arietinum L.) is the second most important crop in the world after dry bean. It belongs to the family Fabaceae. A large number of diseases have been reported on chickpea among them wilt is caused by Fusarium oxysporum f. sp. ciceri. The evaluation of organic amendments viz., neem cake, castor cake and mustard cake and two bio-agent viz., Trichoderma viride and T. harzianum were selected for individual as well as with combination in field conditions. Per cent disease incidence was recorded at 60, 90 DAS and at harvest. After 60 DAS, minimum PDI (09.01%) was recorded in neem cake (500 kg ha^{-1}) + T. viride (2.5 kg ha^{-1}) which was followed by neem cake (500 kg ha^{-1}) + T. harzianum (2.5 kg ha^{-1}) (13.09%). The same results were observed after 90 days of sowing and at harvest. In yield, the maximum yield (1407 kg ha^{-1}) was noted in neem cake (500 kg ha^{-1}) + T. viride (2.5 kg ha^{-1}), whereas minimum yield (867 kg ha^{-1}) in T. harzianum (2.5 kg ha^{-1}).*

Keywords— Chickpea, wilt, organic amendments, bio-agents and per cent disease incidence.

I. INTRODUCTION

Pulses are recognized as one of the most important sources of edible vegetable proteins, which are taken in the form of dal. Besides being a rich source of protein, they maintain soil fertility through biological nitrogen fixation in soil and thus play a vital role in furthering sustainable agriculture (Kannaiyan, 1999). Among the pulse crops, Chickpea (*Cicer arietinum* L.) is the second most important crop in the world after dry bean. It belongs to the family *Fabaceae*. Chickpea seed contains 17-24 per cent of protein, 61.2 per cent carbohydrates, 9.8 per cent moisture (Smartt, 1976) and essential amino acids like isoleucine, leusine, lysine, phenylalanine and valine (Karim and Fattah, 2006).

Global production of pulses is 60 MT. Globally India ranked first in pulses production. Area, production and productivity of chickpea in India are 31.03 million hectares, 27.69 MT and 892 kg ha^{-1} respectively. Among the states of India, Madhya Pradesh ranked first in area and production of chickpea followed by Maharashtra, Rajasthan and Gujarat (Anon., 2022a). In Gujarat, chickpea was grown in 11016 hectares, producing 21014 MT with an average productivity of 1908 kg ha^{-1}, which is high as compared to national average productivity (Anon., 2022b).

A large number of diseases have been reported on chickpea *viz*. fusarium wilt [*Fusarium oxysporum* Schlechtend.: Fr. f. sp. *ciceri* (Padwick) T. Matuo & K. Sato], black root rot [*Fusarium solani* (Mart.) Sacc.], dry root rot *[Macrophomina phaseolina* (Tassi) Goidanich], wet root rot [*Rhizoctonia solani* Kuhn] and collar rot [*Sclerotium rolfsii* Sacc.] are of considerable importance (Nene *et al*., 1981). Among them, chickpea wilt complex is considered the most important, devastating and challenging one, being responsible for seed rot, seedling blight, root rot and mature plant wilt, culminating in 60-70 per cent yield loss (Tewari and Mukhopadhyay, 2001) The disease complex observed first in the history caused by *Fusarium* and

Meloidogyne species on cotton (Atkinson, 1892). Wilt complex caused by several soil-borne pathogens is the major yield reducing malady.

Chickpea wilt caused by *Fusarium oxysporum* f. sp. *ciceri* was first reported from India by Butler (1918). The mystery of the chickpea wilt complex was solved by Nene *et al.* (1979) in India and also developed multiple disease resistance screening techniques and disease-resistant varieties. The wilting pathogens are seed-borne (Haware *et al*., 1978; Pande *et al*., 2007) as well as soil-borne (Jimenez-Fernndez *et al.*, 2011) in nature. The mycelium and chlamydospores can survive in seed and soil, and also infected crop residues, roots and stem tissue buried in the soil more than the six years in absence of host plant (Haware *et al*., 1986).

II. MATERIAL AND METHODS

The field experiment was conducted during *Rabi* 2023-24 at Agronomy Instructional Farm, S. D. Agricultural University, S. K. Nagar for the management of wilt complex of chickpea through bio-agents and organic amendments individual as well as combination. The three organic amendments *viz.*, neem cake, castor cake, mustard cake and two bio-agents *viz. T. viride*, *T. harzianum* were selected for individual as well as with combination in field conditions as below.

TABLE 1
DETAILS OF EXPERIMENT

Sr. No.	Content	Descriptions
1	Experimental site	Agronomy Instructional Farm, SDAU, Sardarkrushinagar
2	Season	2023-24 (*Rabi*)
3	Design	Randomized Block Design
4	Variety	GJG 3
5	Spacing	45 × 10 cm
6	Treatments	12 (Twelve)
7	Replications	3 (Three)
8	Plot size	Gross: 4.5 × 5.0 m

III. OBSERVATIONS RECORDED:

3.1 Disease incidence:

Percent disease incidence of wilt was calculated from each plot after 60, 90 days of sowing and at harvest by using following formula,

$$\text{Per cent Disease Incidence (PDI)} = \frac{\text{Total no. of infected plants}}{\text{Total no. of plants}} \times 100$$

IV. RESULT AND DISCUSSION

The three organic amendments *viz*., neem cake, castor cake and mustard cake and two bio-agent *viz.*, *T. viride* and *T. harzianum* were selected for individual as well as with combination in field conditions. Observations on disease incidence was taken at 60, 90 days after sowing (DAS) and at harvest (Table 2).

TABLE 2

EFFICACY OF ORGANIC AMENDMENTS AND BIO-AGENT FOR MANAGEMENT OF CHICKPEA WILT IN FIELD CONDITIONS

Tr. No.	Treatment	Disease Incidence (%)			Yield (Kg ha^{-1})
		60 DAS	90 DAS	At harvest	
T_1	Neem cake (500 kg ha^{-1})	26.00^{bcd} (19.22)	29.36^{cd} (24.04)	33.81^{bc} (30.96)	1063^{bc}
T_2	Castor cake (500 kg ha^{-1})	29.72^{bc} (24.58)	33.56^{bc} (30.55)	38.38^{b} (38.54)	943^{cd}
T_3	Mustard cake (500 kg ha^{-1})	29.90^{b} (24.84)	33.71^{bc} (30.79)	38.61^{b} (38.94)	937^{cd}
T_4	*T. viride* (2.5 kg ha^{-1})	29.61^{bc} (24.41	33.30^{bcd} (30.14)	38.05^{b} (37.98)	987^{cd}
T_5	*T. harzianum* (2.5 kg ha^{-1})	30.00^{b} (25.00)	33.90^{b} (31.10)	38.72^{b} (39.13)	867^{d}
T_6	Neem cake (500 kg ha^{-1}) + *T. viride* (2.5 kg ha^{-1})	17.47^{f} (09.01)	20.35^{g} (12.10)	23.54^{f} (15.96)	1407^{a}
T_7	Neem cake (500 kg ha^{-1}) + *T. harzianum* (2.5 kg ha^{-1})	21.21^{e} (13.09)	24.52^{f} (17.22)	28.34^{e} (22.54)	1220^{b}
T_8	Castor cake (500 kg ha^{-1}) + *T. viride* (2.5 kg ha^{-1})	21.55^{e} (13.49)	24.76^{ef} (17.54)	28.56^{de} (22.86)	1217^{b}
T_9	Castor cake (500 kg ha^{-1}) + *T. harzianum* (2.5 kg ha^{-1})	21.86^{e} (13.86)	24.93^{ef} (17.77)	28.78^{cde} (23.18)	1197^{b}
T_{10}	Mustard cake (500 kg ha^{-1}) + *T. viride* (2.5 kg ha^{-1})	25.54^{d} (18.59)	28.91^{def} (23.37)	33.48^{bcd} (30.42)	1117^{bc}
T_{11}	Mustard cake (500 kg ha^{-1}) + *T. harzianum* (2.5 kg ha^{-1})	25.86^{cd} (19.03)	29.09^{de} (23.63)	33.65^{bcd} (30.7)	1100^{bc}
T_{12}	Control	33.76^{a} (30.88)	38.17^{a} (38.19)	43.52^{a} (47.42)	630^{e}
	S. Em.±	1.24	1.39	1.61	57.92
	C. D. at 5%	3.63	4.07	4.71	169.88
	C. V. %	8.23	8.14	8.2	9.49

Figures in parentheses are retransformed values of arcsine transformed values.

Treatment means with the common letter(s) are not significant by Duncan's New Multiple Range Test at 5% level of significance

All the treatments applied before sowing

All treatments were highly effective as compare to control. Among the all treatments, combination of treatments was highly effective than individual treatments. All treatments were highly effective as compare to control. Among the all treatments, combination of treatments was highly effective than individual treatments. After the 60 days of sowing the minimum disease incidence (09.01%) was observed in treatments of neem cake (500 kg ha^{-1}) + *T. viride* (2.5 kg ha^{-1}) which was followed by neem cake (500 kg ha^{-1}) + *T. harzianum* (2.5 kg ha^{-1}) (13.09%) and maximum disease incidence (25.00%) was observed in *T. harzianum* (2.5 kg ha^{-1}) alone. After 90 DAS, minimum PDI (12.10%) was recorded in neem cake (500 kg ha^{-1}) + *T. viride* (2.5 kg ha^{-1}) which was followed by neem cake (500 kg ha^{-1}) + *T. harzianum* (2.5 kg ha^{-1}) (17.22%). At harvest, minimum PDI (17.96%) was recorded in neem cake (500 kg ha^{-1}) + *T. viride* (2.5 kg ha^{-1}) which was followed by neem cake (500 kg ha^{-1}) + *T. harzianum* (2.5 kg ha^{-1}) (22.54%). Maximum yield (1407 kg ha^{-1}) was noted in treatment neem cake + *T. viride* which was followed by neem cake + *T. harzianum* (1220 kg ha^{-1}), castor cake + *T. viride* (1217 kg ha^{-1}) and castor cake + *T. harzianum* (1197 kg ha^{-1}) whereas minimum in *T. harzianum* (867 kg ha^{-1}).

These results are also confirmity with Barnwal (2009) showed that minimum wilt incidence, maximum yield and maximum ICBR recorded when seed priming with *T. viride* + soil application of neem cake. Same result confirmed by Mahanthi *et al.* (2019), Kumari *et al.* (2020) and Shekhawat *et al*. (2021).

V. CONCLUSION

The study demonstrated that integrating organic amendments with bio-agents significantly reduces chickpea wilt incidence and enhances crop yield. Among the tested treatments, neem cake + *T. viride* was the most effective, achieving the lowest disease incidence and the highest yield under field conditions. This suggests that the combination of organic amendments and bio-control agents can serve as a sustainable and eco-friendly approach to managing chickpea wilt complex.

Furthermore, these treatments not only improve plant health but also contribute to soil fertility by fostering beneficial microbial activity. Given the increasing concern over chemical pesticides and soil degradation, adopting such integrated disease management strategies can play a vital role in sustainable chickpea production. Future research should explore the long-term effects of these treatments on soil microbial diversity and crop productivity across different agro-climatic regions.

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Exploring Ecosystem Protection Role in Advancing Ecotourism; Case of Volcanoes National Park, Rwanda

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Abstract— *This study assessed the contribution of ecosystem protection to developing ecotourism in Rwanda despite the persistent illegal human activities. Volcanoes National Park was the study area. Specifically, the research (1) found out the contribution of ecosystem protection to the development of ecotourism in that park, (2) the faced challenges, and (3) the strategies to overcome them. Descriptive research design and stratified and purposive random sampling techniques were applied. The literature review and the survey conducted with 404 respondents, who were randomly selected from the population of 27,221 residents of Kinigi Sector and 25 RDB staff, indicated that through the use of ecosystem protection strategies including the established zone areas, and local community involvement, the VNP's revenues, visitors, and ecotourism activities increased. Human illegal activities such as hunting, illegal farming, and cutting trees were among the challenges facing ecosystem protection in the VNP. Due to RDB's mobilization on the advantages of ecosystem protection, serious punishments, and different benefits they earn from ecotourism projects in and around the VNP, many residents stopped doing such illegal activities. As a result, the mountain gorillas' number increased. It was concluded that proper implementation of ecosystem protection strategies is a good way to ensure ecosystem protection and ecotourism development. It was recommended that RDB should increase the benefits earned by local communities from ecosystem protection. Local leaders should work with RDB for awareness of ecosystem protection, while local communities should feel concerned about fighting all illegal activities threatening ecosystem protection and sustainable development in the VNP.*

Keywords— Ecosystem, ecosystem protection, ecotourism.

I. INTRODUCTION

Ecotourism is a form of tourism that aims at decreasing its effect on the environment, is ecologically found, and avoids the negative impacts of many large-scale tourism developments undertaken in places that have not earlier been developed (Rahman et al., 2022). According to (Baloch et al., 2023), ecotourism was developed in the period of environmental movement in the 1970s and 1980s in the western society in Europe. The development of environmental concern went with an emerging dissatisfaction with mass tourism and led to increased demand for nature-based experiences of an alternative nature. In the same period, undeveloped countries including Asian and African countries started realizing that nature-based tourism provides a way of getting foreign exchange and gives a less damaging use of wealth than substitutes like accommodation and farming. In the 1980s, many of those countries such as Costa Rica had recognized ecotourism development as a good strategy to attain both conservation and development goals (Gu et al., 2023).

Based on its great importance in both ecosystem conservation and tourism growth, governments of many countries have undertaken strong strategies to build sustainable ecotourism development. In India for example, the country benefits from having several various wildlife species, including elephants, rhinoceroses, and tigers, and also many well-managed protected areas whereby, the possible ecotourism benefits are enormous (Zeng et al., 2023).

According to Yoon and Lee (2023), the benefits of ecotourism growth have indeed been recognized where it is considered as a good business providing direct and indirect incomes not only to the governments but also to residents. In India for example, the local people living in the surrounding areas of the Periyar tiger reserve in Kerala, earn lots of benefits from ecotourism activities performed there. Furthermore, well-organized and performed activities have resulted in the increase of revenues for

the park management. The residents actively participate in tourism-based activities through various ecotourism development projects.

In South Africa, ecotourism has contributed to sustainability and improvement of the country's rich biodiversity and economic growth. In that country, tourism is the fourth largest source of foreign exchange earnings and ecotourism plays more significant roles by encouraging visitors while promoting and supporting the country's ecosystem. As South Africa has several natural ecosystem habitats including water bodies, forests, and parks, ecotourism is a way for this country to earn more from various ecotourism activities performed by both domestic and international visitors (Ochieng et al., 2023).

Muriithi (2023), indicated that in Kenya, ecotourism has weakly developed from an intangible idea to a more niche-driven market that pulls both loyal and new visitors. While some tourism destination sites mix traditional tourism with ecotourism activities, there are so many destinations that promote themselves as exclusive eco-tourism sites. According to Yang et al., (2023), not only is the huge support for ecotourism in Kenya given by the government and people, but also by companies dedicated to conserving Kenya's natural resources and enabling local communities. For example, Basecamp Foundation is a non-profit organization working with local communities and partner organizations to establish and implement sustainable ecotourism destination sites in Kenya and other developing countries, accentuating the positive efforts of tourism on the natural, social, and economic environment.

In the case of Rwanda, ecotourism contributes more to environmental protection and ecosystem conservation, and too much effort has been used by the Rwandan Government together with other different ecotourism stakeholders such as Karisoke Research Centre for the development of this new form of tourism (Jones et al., 2023). With plastic bag bans, intensive animal conservation including resurgence of the endangered mountain gorilla population and the reintroduction of both lions and rhinos to complete the big five animal species in Akagera National Park, restoration and establishment of Gishwati-Mukura Natural forests as the fourth national park, and the increase of eco-lodges, Rwanda is changing the face of ecotourism in a great way (Sabuhoro et al., 2023).

In addition, the Rwandan Government is highly promoting ecotourism whereby, environmentally friendly and luxury lodges including the unparalleled Bisate Lodge and Ruzizi Tented Lodge were built. For the budgetary tourists and travelers, new accommodation sites such as the Kitabi EcoCenter in Nyungwe National Park and Cyuza's Island on Lake Burera motivate visitors to live as sustainably as possible, promoting solar energy, local food options, and composting latrines (Gubić et al., 2023).

Through various eco-tourism projects around the Volcanoes National Park, local communities understand better the value of gorilla tourism, and they directly benefit from its revenues whereby, via RDB's tourism revenue sharing program, health centers, schools, electricity, water supply, and other tourism infrastructure were built and developed in the surrounding areas of the Volcanoes National Park (Mbale et al., 2023). Thus, despite the availability of more studies conducted by different researchers such as (Fennell, et al., 2023), on ecotourism and ecosystems, there was still a need to research how ecosystem protection contributes to the development of ecotourism in Rwanda.

II. LITERATURE REVIEW

2.1 Definition of key terms:

- **Ecosystem:** This is a geographic area where plants, animals, and other organisms, as well as weather and landscape, work together to form a bubble of life (Carius & Job, 2019).
- **Ecosystem protection:** This is a comprehensive plan that seeks to preserve all plant and animal species in a geographic area through the management of natural resources (Galbany et al., 2017).
- **Ecotourism:** According to The International Ecotourism Society (TIES), ecotourism is defined as responsible travel to natural areas that conserve the environment, sustains the well-being of the local people, and involves interpretation and education (Go et al., 2016). According to Ohe (2021), ecotourism is an alternative tourism, that involves visiting natural areas to learn, study, or carry out activities environmentally friendly, that is tourism based on the nature experience, which enables the economic and social development of local communities.
- **Ecotourism development:** Anderson & Juma (2018), defined ecotourism development as a development that is based on the uniqueness and condition of existing areas through development by empowering communities to develop socially and improve social status in society.

2.2 Contribution of ecosystem protection to the development of ecotourism:

Petit & Seetaram (2019), indicated that as a form of tourism that involves responsible travels to natural areas that conserve the environment, sustains the well-being of the local people, and involves interpretation and education, the development level of ecotourism depends mostly on how ecosystems' natural habitats such as parks, wetlands, natural forests, hills, and mountains are well protected.

According to Uwayo et al., (2020), ecosystem protection influences the development of ecotourism at the destination sites whereby, most ecotourism activities are directly based on the services provided by ecosystems. These recreational values offered by ecosystems are among the main cultural services that nature provides to humankind, along with spiritual, aesthetic, and educational values. Almost every experience an ecotourism site visitors get is an interaction with the ecosystem. They may just perform different eco-tourism activities such as mountain hiking, nature walking, birdwatching, and game viewing in well-protected ecotourism sites like parks, forests, and marshlands.

Uwayo et al., (2020), further indicated that ecosystem protection attracts more investments in ecotourism projects at ecotourism sites. In Uganda for example, with the development of ecotourism activities like gorilla and chimpanzee tracking, birding, forest or nature walks, and cultural trails, the country has taken significant steps that may regarded as a shift from traditional tourism to responsible travel to ecologically sensitive areas. A tangible example of an ecotourism project created due to ecosystem protection in Uganda is the Kibale Association for Rural and Economic Development (KAFRED, a community-based organization managed and run by the local community. It was initiated with the main theme of protecting Bigodi Wetland (adjacent to Kibale Forest National Park) while advancing health, education, and economic growth in the wider local community. 100% of employees are from the community and the participatory planning of the organization includes members and representatives of homesteads neighboring the swamp.

The project has contributed towards the conservation of the wetland and the park at large and raising the socio-economic standards of the people through setting up a tourist reception; construction of the wetland boardwalks; advancing ecosystem conservation practices in the area's primary schools including Bigodi, Busabura, Busiriba, and Kiyoima; training local guides; formation and supporting women groups like the Bigodi the women's groups, and Enyange drama actors. All this has improved the image of the area and created ecosystem conservation awareness amongst the local people.

In Rwanda, construction of different ecotourism projects due to ecosystem protection has also taken place where for example, after the government took the responsibility of managing wetlands by prohibiting all unauthorized activities, the swamp of Nyandungu in Kigali City has been prepared and turned into an attracting Eco-Tourism Park "Nyandungu Urban Eco-Tourism Park", a project which is currently managed by QA Venue Solutions, a Pan-African venue solutions provider in the event venue, entertainment and hospitality industry in South Africa and Rwanda. According to Munanura et al., (2018), the project of Nyandungu wetland restoration and creation of an eco-tourism park saw the planting of 17,000 trees made up of 55 indigenous species. The 121-hectare park features a medicinal garden, a Pope's Garden, five catchment ponds, three recreational ponds, an Information Centre, a restaurant as well as 10 km of walkways and bike lanes.

Other ecotourism projects created due to ecosystem protection in Rwanda include Buhanga Eco Park, a small forest covering about 31 hectares in Northern Province, at Nyakinama, near IPRC Musanze, which is always termed as sacred because it is a place where enthrone and initiations for Kingship was done. There is also Nyungwe Nziza Ecolodge situated very close to the Nyungwe Forest National Park, and One&Only Gorilla Nest around the Volcanoes National Park (Roe et al., 2020).

2.3 The challenges facing ecosystem protection at ecotourism sites:

To decide on ecosystem protection measures to undertake, it is first of all very important for all stakeholders to have a common understanding of the challenges facing ecosystem services in their natural habitats. Illegal activities such as overexploitation of natural resources, poaching, hunting, deforestation, bushfires, pollution, logging, illegal grazing, mining, and agriculture performed by some local people especially, those who live in the surrounding areas of ecotourism sites such as national parks, swamps, mountains, and natural forests are among the major challenges (Lemieux, 2014).

Globally, the most vulnerable wild animal species that are about to completely disappear on the earth due to humans' illegal activities if no serious conservation measures are undertaken include: elephants for ivory and tigers for their skins and bones, buffaloes for meat, and rhinoceroses for their horns (Marcot et al., 2015). Additionally, those illegal activities may result in climate change that can cause some disasters such as droughts, severe diseases, landslides, and desertification which negatively affect ecosystem stability and people's lives (Mekonen et al., 2017).

2.3.1 Ecosystem protection in developed countries:

Rich nations do not participate too much in ecosystem protection compared to the remaining countries of the world where for example, only 19% of countries in North and Central America are considered to use strong ecosystem protection methods contrary to the percentage of African countries which made up four of the five top-performing megafauna conservation nations, with Botswana, Namibia, Tanzania and Zimbabwe taking the first places. Oppositely, the USA occupies the 19th place out of the first twenty ecosystem protection leading countries worldwide while, nearly one-quarter of countries in Asia and Europe are considered as the weakest megafauna protectors (Roe et al., 2020).

2.3.2 Ecosystem protection in both developing and least developed countries:

At present, both developing and least developed nations are the areas where, ecosystem resources, including animal and plant species, are terribly threatened by human illegal activities and also where various conservation measures have been undertaken. The used programs include Community-Based Conservation (CBC) which refers to ecosystem conservation efforts that involve rural residents as the most fundamentals of ecosystem protection policies whose key elements are that they play a big role in resource planning and management as the first earners of economic benefits from ecosystem utilization (DeCaro & Stokes, 2008). In Nepal for example, the success in ecosystem conservation has been achieved through the introduction of a protected area network and the assistance of residents in the adjacent areas (Paudel et al., 2020).

Local communities' involvement in ecosystem protection was also given more priority in Zanzibar where local people living around Jozan-Chwaka Bay National Park have become highly motivated to participate in the conservation of this park thanks to the benefits they earn from the park's revenues that are equitably shared between the regional government and residents. The gained benefits range from direct employment, and social capital development to ecosystem services (Carius & Job, 2019).

Ecosystem protection, primarily through shared tourism revenues, contributes to all 17 United Nations Sustainable Development Goals (SDGs) in different ways for example, benefit sharing in different countries like Uganda where 25% of the national parks' revenues in Western Uganda is given to the surrounding local communities, brought solutions to the problem of human-wildlife conflicts, improved communities' awareness on wildlife conservation and weakened pressures on ecosystems (Locke et al., 2019). Between 1995 and 1998, people living in surrounding areas of these parks spent a total of US $83000 of tourism revenues to construct different infrastructure including 21 schools, four clinics, one bridge, and one road (Spenceley et al., 2019).

2.3.3 Ecosystem Protection in the Volcanoes National Park:

Volcanoes National Park was established in Rwanda and also shared by Congo in 1929 by the Belgian colonialists, it was later named Albert National Park when it was managed and controlled by the Belgian authorities of then, protecting its ecosystem species including the rare endangered mountain gorillas was severely challenged by poaching and other illegal activities mostly performed by people from local communities surrounding the park during the colonial period (Galbany et al., 2017). The situation continued even after the independence when the park was split into two, one part for Congo and another one (the current Volcanoes National Park) for Rwanda (Verweijen & Marijnen, 2018).

It was in 1967 when the conservation work in the Volcanoes National Park was ratified by an American zoologist, called Dian Fossey, who was researching the rare endangered mountain gorillas in Congo. But, because of political issues and insecurity in Congo, Fossey decided to leave the country for Rwanda, where she created the today Karisoke Research Center located in the area between Bisoke and Karisimbi mountains (Ronald et al., 2020).

To deal with the problem of poaching in the Volcanoes National Park, Fossey organized a campaign on the conservation of mountain gorillas in 1985 where she took the initiative to accumulate the resources required to fight poaching in the park. In addition to poaching, the liberation war which existed in Rwanda in the 1990s where the Volcanoes National Park was used as a fighting ground, severely threatened the protection of ecosystem species in the park where, up to the1999, lots of animal species were killed and eco-systems demolished (Robbins et al., 2005).

After restoring peace in the whole country, the Rwandan Government has undertaken some ecosystem conservation strategies including the "Revenue Sharing Scheme", which was adopted in 2005, where 2019 with the rise of the price of gorilla permits from 750U$ to 1500U$ in 2018, local people living near the park are given 10% of all parking revenue (Sabuhoro et al., 2020).

According to Kyrylov et al., (2020), ecotourism development on the other side faces several challenges including the following:

Difficulty in preserving the ecotourism resources due to lack of local people awareness and in some cases, tourists; inadequate and lack of skilled, interested, and understanding personnel (especially public officers) involved in ecotourism activities and ecotourism interpretative services hence the problem of difficulty in securing a budget for ecotourism development (Petit & Seetaram, 2019).

Furthermore, there are no clear norms and implementation guidelines for ecotourism in national wildlife reserves in most countries and other areas of interest due to a significantly low number of eco-tourists thus a few programs have been produced and most travel agencies are mindless of its operation; and government intervention which poses a problem as regards the control and management of national parks, wildlife and game reserves and other resources, and how to include local communities in ecotourism activities. For example; the damaging of ecotourism resources in a bid to promote industrialization and urban development in most destination countries (Petit & Seetaram, 2019).

2.4 Strategies to overcome the challenges facing ecosystem protection at ecotourism sites:

Carius & Job (2019), indicated that based on their various roles in life existence on the globe where they provide habitats to wild plants and animals, promote various food chains and food webs, control essential ecological processes, and promote lives, as well as involving in the recycling of nutrients between biotic and abiotic components, awareness of the strategies to address the challenges to ecosystems protection is very important, and some of these strategies are the following:

2.4.1 Implementation of anti-poaching policies:

To deal with human illegal activities such as poaching as a threat to ecosystem protection, two different categories of anti-poaching policies might be applied by governments to ensure maximum conservation of wildlife species in an ecosystem. The first category involves fighting poachers, either by direct contact or by setting some physical barriers such as fences that can stop them from having easy access to the habitats. The second category is about organizing anti-poaching campaigns aiming to weaken the illegal trade of wildlife products (Nguyen et al., 2016).

In Rwanda, to deal with the problem of poaching, the parks' management has undertaken different approaches and the main one has been to make local people become anti-poaching patrol members where they significantly participate in anti-poaching patrols planning and decision-making. The parks' management further works in close collaboration with community organizations at the village level to encourage and support the implementation of anti-poaching laws, recognizing that preserving park resources has lots of benefits such as community living style improvement and human-wildlife conflict minimization. Another important anti-poaching approach is RDB's Revenue sharing program through which 10% of the park's revenues is offered to the local communities living in the areas adjacent to the park (Munanura et al., 2019).

2.4.2 Establishment of buffer zone areas:

Roe et al., (2020), argued that the fact that human activities such as construction, agriculture, bushfires, and animal husbandry as also natural disasters such as erosion, volcanic eruption, and earthquakes are among the major challenges to ecosystem protection, establishment of buffer zone areas, which according to (Ohe, 2021), are peripheral to a national park or equivalent reserve, where restrictions are placed upon resource use or special development measures are undertaken to enhance the conservation values of the area can be a good ecosystem protection strategy, whereby not only do those zones serve as transitional areas of connectivity outside protected areas from which people can derive material or economic benefit, but they also enlarge wildlife habitat, reduce edge effects and enhance ecosystem services.

In Rwanda for example, to ensure maximum conservation of wetlands, the habitats of various aquatic wildlife species, the government through Rwanda Environment Authority (REMA) has taken responsibility for managing swamps and other marshland areas, by prohibiting all illegal activities in those areas (Locke et al., 2019). According to Carius & Job, (2019), the Article 429 in the Environmental Organic Law and the Land Law under which wetlands are currently being managed, indicates the distance required from the banks of rivers and shores of lakes, and respect of reserved areas, where the article says that any person who carries out agricultural activities without complying with a distance of ten (10) meters required from the banks of rivers and the distance of fifty (50) meters from the shores of lakes; animal breeding activities requiring swamp-based agricultural works without complying with a distance of ten (10) meters required from the banks of rivers, and a distance of fifty (50) meters from the shores of lakes; who erects animal sheds without complying with a distance of sixty (60) meters required from the banks of rivers, a distance of two hundred (200) meters from the shore of lakes; who develops fish ponds

and rising fish species without authorization; who erects in a wetland area, whether urban or rural, a building, a market, a dump, a cemetery and any other building that may have a negative impact on that area without complying with a distance of twenty (20) meters required from the banks of swamp; who carries out any other activity other than research activity in reserved wetlands area;

Shall be liable to a term of imprisonment of two (2) months but less than six (6) months and a fine of five hundred thousand (500,000) to five million (5,000,000) Rwandan francs or one of these penalties. The convict may be ordered to rehabilitate the damaged area.

Concerning the Volcanoes National Park, a habitat of the rare endangered mountain gorillas, the Rwandan Government has a five-year project of US$ 225 million to expand the park from 13,000 hectares to 23,000 hectares. Of the new 10,000 hectares, park activities will expand into an estimated 3,740 hectares and an estimated 6,260 hectares will be used to create a buffer zone between the national park and its fauna and flora and the surrounding human communities. The buffer zone will be used for agriculture, particularly agroforestry, which will benefit the park and the humans together. It will also reduce human-wildlife conflicts by 80 percent, which will contribute more to the development of ecotourism in that volcanic region (Munanura et al, 2018).

2.4.3 Tourism revenue sharing programs :

According to Spenceley et al., (2019), tourism revenue sharing (TRS) has become a widespread policy intervention in Africa and elsewhere charismatic populations of wildlife remain. It aims to balance the disadvantages people encounter living next to protected areas while fostering improved conservation behaviors. With this program, local communities living in surrounding areas of protected areas such as parks, benefit from enjoying the revenues from tourism activities taking place in those areas, where for example in Rwanda since 2018, local communities living around the four national parks have been given by Rwanda Development Board (RDB) gives 10% of the parks' through tourism revenue sharing program introduced in 2005. Before 2018, the money given to those people was 5%. The same program is also applied in Uganda, where 20% of protected area entrance fees are shared with local governments to benefit communities adjacent to national parks (Munanura et al., 2019).

III. METHODOLOGY

3.1 Study area description:

This research was conducted in the Volcanoes National Park; one of the four national parks of Rwanda, which is found along 1^0 21'-1^0 35' South and 29^0 22'-29^0 44' East in North-Western Rwanda. It consists of 160 km^2 of rainforest together with five of the eight volcanoes in the Virunga Mountains, namely Karisimbi, Bisoke, Muhabura, Gahinga, and Sabyinyo. This park is a home to the rare endangered mountain gorillas; which are only found in three countries worldwide namely Rwanda, the Democratic Republic of Congo (DRC) in the Virunga National Park, and Uganda in Mgahinga and Bwindi Impenetrable National Parks (Tuyisingize et al., 2023).

The Volcanoes National Park was the first national park to be established in Rwanda in 1925 for safeguarding the rare endangered mountain gorillas, other primates such as golden monkeys together with other different natural resources accommodated by the park from poachers. Concerning weather conditions, due to the highest elevation of the volcanoes where Karisimbi Mountain is 4,507 meters of altitudes, the park is always wet and cold. Temperature is almost 15^0 C/59^0 F in the year and it is about 7^0 C/45^0 F during the night (Sabuhoro et al., 2023).

Regarding vegetation, the Volcanoes National Park has rainforests, thickets, swamps, marshes, meadows, alpine forests, and grasslands located in various altitudinal ranges. These include a rain forest in the ranges between 2400 and 2500m, a bamboo forest, and the Arundinaria alpha between 2500 to 32000m. Volcanoes National Park is adjacent to the Virunga National Park in the Democratic Republic of Congo (DRC) and Mgahinga Gorilla National Park in Uganda. The area adjacent to the park is made up of four districts: Burera, Musanze, Nyabihu, and Rubavu. This zone has the highest population densities in the country (500 to 1,041 inhabitants per km^2). Agriculture is the main economic activity in that area (Nsengimana et al., 2023).

Regarding tourism, although gorilla trekking is the dominant ecotourism activity in the Volcanoes National Park, visitors perform other different ecotourism activities such as golden monkey trekking, nature walk to Dian Fossey Tomb and Ngezi Lake, mountain biking, and visiting Buhanga Eco-Park (Hansen et al., 2023).

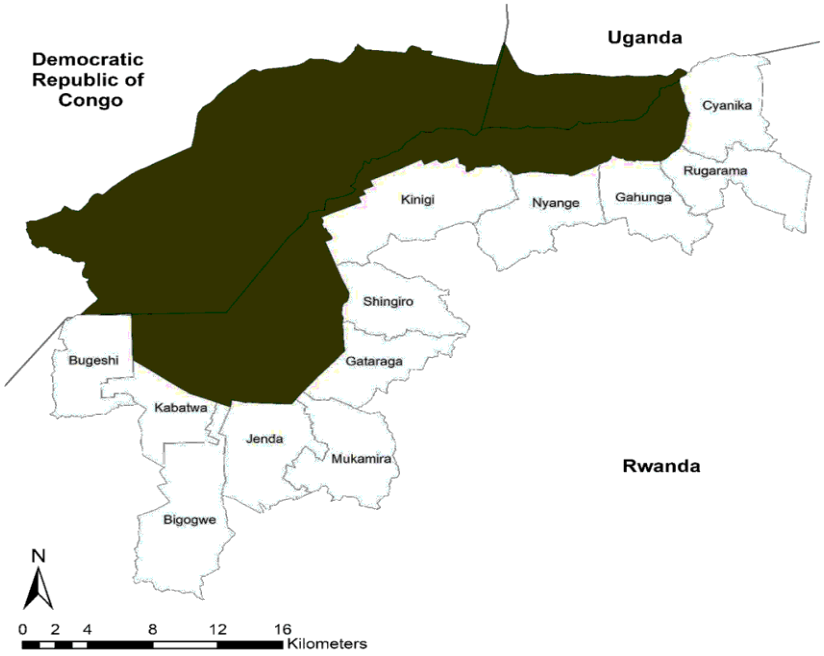

FIGURE 1: A map showing the different districts adjacent to VNP, Rwanda
Source: ***www.researchgate.net***

3.2 Sampling Techniques and Data Collection:

Kinigi Sector, according to the National Population Census of 2012 (Uwayo et al., 2020), is inhabited by 27,221 residents dispersed in five cells namely: Bisate, Kampanga, Kaguhu, Nyonirima and Nyabigoma together with the Volcanoes National Park Headquarters with 25 staff, are the two places where primary data were collected. The total number of the respondents was 404 people; a sum of 379 residents of Kinigi Sector and 25 employees of RDB at the Volcanoes National Park Headquarters in Kinigi. These were randomly selected from a study population of 27,246 people using the (Krejcie and Morgan 1970) formula. However, the formula was applied to 27,221 residents of Kinigi Sector, while for the 25 employees of RDB, all of them were surveyed based on the fact that their number was very small.

Thus, the following is the applied formula:

$$s= \frac{x^2NP(1-P)}{d^2(N-1)+x^2P(1-P)} \quad (1)$$

Where,

s = required sample size

X^2=the table value of chi-square for 1 degree of freedom at the desired confidence level (3.841).

N= Population size =27246

P=the population proportion (assumed to be 0.50 since this would provide the maximum sample size).

d= the degree of accuracy expressed as a proportion (0.50).

Therefore, the sample size on the side of Kinigi Sector residents was calculated as follows:

$$s= \frac{x^2NP(1-P)}{d^2(N-1)+x^2P(1-P)} \quad (2)$$

$$s= \frac{3.841X27246X0.5(1-0.5\ (1-0.5)}{(0.05)^2(27246-1)+3.841X0.5(1-0.5)} \quad (3)$$

$$s= \frac{26162.9717}{68.115+0.96025} \quad (4)$$

$$s= \frac{26162.9717}{69.075225} \quad (5)$$

$$s= 378.78 \approx 379 \quad (6)$$

So, the total number of respondents in this study was 404 people; a sum of 379 residents of Kinigi Sector and 25 RDB staff.

Therefore, to obtain the required number of respondents on the side of Kinigi Sector residents (379 respondents), the researcher used stratified random sampling techniques, while for the 25 RDB staff, a purposive sampling technique was applied. The former were surveyed using questionnaires, while face-to-face interview was used for the latter.

3.3 Data Analysis and Presentation:

After collecting primary data, the researcher took enough time to organize and analyze them well to easily draw conclusions and recommendations. The resulting quantitative figures were entered into Excel and transferred to the Statistical Package for Social Sciences (SPSS) for analysis. Analysis and interpretation were carefully done based on the research objectives.

IV. RESULTS AND DISCUSSIONS

4.1 Contribution of ecosystem protection to the development of ecotourism in the VNP:

During the survey, the respondents were asked to indicate how protecting the ecosystem contributes to the development of ecotourism development in the Volcanoes National Park. Their responses are provided in Table 1.

TABLE 1
CONTRIBUTION OF ECOSYSTEM PROTECTION TO ECOTOURISM DEVELOPMENT IN THE VNP

Contribution	Frequency	Percentage %
Increase in the park's revenue	102	25.30%
Increase in ecotourism activities	100	24.70%
Ecotourism marketing through "Gorilla Naming Ceremony"	99	24.50%
Increase in the number of park visitors	103	25.50%
Total	**404**	**100%**

Source: Primary data, 2024

Table 1 presents the data about the contribution of ecosystem protection in the Volcanoes National Park. Of 404 respondents who were surveyed, 25.3% responded that it contributes to the increase of the park's tourism revenue, where for example RDB staff through the interview revealed that "revenue from gorilla trekking in the Volcanoes National Park grew by 25% in 2018 to $ 19.2M from 2017". Other 24.7% said that through ecosystem protection, ecotourism activities such as gorilla and golden monkey trekking, nature walking, and volcano climbing have increased. 24.5% said that ecosystem protection, which has caused the increase of gorillas' numbers in the Volcanoes National Park, contributes to ecotourism marketing through the "Gorilla Naming Ceremony", while the remaining 25.5% confirmed that the number of park visitors has increased, which was strongly affirmed by the interviewed 25 RDB staff that "in 2019, the park was visited by over 17,249 tourists".

4.2 The challenges facing ecosystem protection in the Volcanoes National Park:

Despite the huge contribution of ecosystem protection plays to the development of ecotourism development in the Volcanoes National Park, it faces several challenges as shown by the data presented in Table 2:

TABLE 2
CHALLENGES FACING ECOSYSTEM PROTECTION IN THE VOLCANOES NATIONAL PARK

Challenges	Frequency	Percentage %
Poaching	105	26%
Hunting	101	25%
Illegal farming	98	24.30%
Cutting trees	100	24.70%
Total	**404**	**100%**

Source: Primary data, 2024

Table 2 presents the data about the challenges facing ecosystem protection in the Volcanoes National Park. The question on this issue was addressed to all 404 respondents whereby, all of them confirmed that human's illegal activities such as poaching (26%), hunting (25%), and illegal farming (24.3%), and the remaining 24.7 % who highlighted cutting trees (including bamboos; the favorite food for mountain gorillas) as the serious challenges in that national park. More interestingly, many of the residents of the Kinigi Sector who were surveyed, confirmed that sometimes they have performed some of these illegal activities, because of different reasons including those presented in Table 4 below. However, they mentioned some factors influencing local people to stop doing such illegal activities as are mentioned in Table 3:

TABLE 3
FACTORS INFLUENCING LOCAL PEOPLE TO STOP DOING ILLEGAL ACTIVITIES THREATENING ECOSYSTEM PROTECTION IN THE VNP

People stop doing illegal activities in the VNP because of:	Frequency	Percentage %
RDB's mobilization on the advantages of ecosystem protection	134	33.20%
Serious punishments from the government	137	33.90%
Different benefits earned from ecotourism projects created in and around the Volcanoes National Park	133	32.90%
Total	**404**	**100%**

Source: Primary data, 2024

Table 3 presents the data about the factors influencing residents of the sectors surrounding the Volcanoes National Park including the Kinigi Sector, to stop doing illegal activities threatening ecosystem protection in that park. On this issue, 33.2% of the respondents revealed that they stopped doing such illegal activities because of RDB's mobilization through the "Tourism Revenue Sharing Program" on the advantages of ecosystem protection, other 33.9% said that it is because of serious punishment of the government, while the remaining 32.9% confirmed that they were motivated by the different benefits they earn from ecotourism projects including eco-lodge hotels such as One&Only Gorilla's Nest, Bisate Lodge, and Tiloreza Volcanoes Eco-lodge created in that volcanic region and from which they get jobs.

TABLE 4
CAUSES OF ILLEGAL ACTIVITIES PERFORMED BY LOCAL PEOPLE IN THE VOLCANOES NATIONAL PARK

People do illegal activities threatening ecosystem protection in the VNP because of:	Frequency	Percentage %
Poverty	104	25.70%
Culture	106	26.30%
Ignorance	99	24.50%
Influence of others	95	23.50%
Total	**404**	**100%**

Source: Primary data, 2024

Table 4 presents the data about the reasons why some local people do illegal activities (the challenges to ecosystem protection and the development of ecotourism in the Volcanoes National Park). Of 404 people who were surveyed, 25.7% revealed that it is because of poverty, 26.3% said that it is because of culture, where for example they do hunting as an inheritance from their ancestors who were also hunters, other 24.5% confirmed that they do such illegal activities because they do not have any knowledge about the importance of ecosystem protection (ignorance), while the remaining 23.5% said that it is because of the influences of others. However, the respondents clarified what the Rwandan Government through the Rwanda Development Board (RDB) does to overcome these challenges as is clarified by the data presented in the section below.

4.3 The strategies to overcome the challenges facing ecosystem protection in the VNP:

The results from the survey indicated the strategies used by the Rwanda Development Board (RDB) to overcome the challenges facing ecosystem protection in the Volcanoes National Park. Those strategies are presented in Table 5.

TABLE 5
THE STRATEGIES USED BY RDB TO OVERCOME THE CHALLENGES FACING ECOSYSTEM PROTECTION IN THE VNP

The strategies used are:	Frequency	Percentage%
Implementation of anti-poaching policies	132	32.60%
Establishment of buffer zone areas and expanding the park's surface	137	34%
Community involvement through the Tourism Revenue Sharing Program	135	33.40%
Total	**404**	**100%**

Source: Primary data, 2024

Table 5 presents the data about the strategies used by the Rwanda Development Board (RDB) to overcome the challenges facing ecosystem protection in the Volcanoes National Park. Of 404 respondents who were surveyed, 32.6 % highlighted the implementation of anti-poaching policies as the used strategies, another 34% revealed that RDB has established a buffer zone area whereby, the 25 RDB staff through interviews revealed that to ensure a bright future for mountain gorillas and other park's ecosystem resources "there is a project of expanding the park's surface by approximately 23%, increasing its size by 37.4 square kilometers (3,740 hectares)", while the remaining 33.4% responded that RDB through its "Tourism Revenue Sharing Program" uses community involvement to deal with these illegal challenges.

TABLE 6
THE ROLE OF LOCAL COMMUNITIES IN THE DEVELOPMENT OF ECOTOURISM IN THE VOLCANIC REGION

Local communities' roles:	Frequency	Percentage %
Participating in the protection of ecotourism sites including the Volcanoes National Park	133	32.90%
Creation of different ecotourism projects	137	33.90%
Providing services to ecotourism site visitors	134	33.20%
Total	**404**	**100%**

Source: Primary data, 2024

Table 6 presents the data about the role played by local communities in the development of ecotourism in the volcanic region, precisely in the Kinigi Sector. Of 404 respondents who were surveyed, 32.9% confirmed that local communities participate in the protection of the VNP either as park rangers or as Irondo Personnel, other 33.9% said that they create different ecotourism projects, which respond to the needs and preferences of eco-tourists, while the remaining 33.2% revealed that they contribute through provision of services to ecotourism sites visitors.

TABLE 7
ECOTOURISM ACTIVITIES PERFORMED BY TOURISTS IN THE VOLCANOES NATIONAL PAK

Ecotourism activities in the VNP:	Frequency	Percentage %
Volcanoes climbing	139	34.40%
Nature walking	131	32.40%
Trekking both mountain gorillas and golden monkeys	134	33.20%
Total	**404**	**100%**

Source: Primary data, 2024

Table 7 presents the data about ecotourism activities performed by tourists in the VNP. Of 404 respondents who were surveyed, 34.4% highlighted climbing volcanoes including Bisoke and Kalisimbi volcanoes as the performed activities, other 32.4% said that tourists do nature walking when they example visit Ngezi Lake or Dian Fossey Tomb, while the remaining 33.2% pointed out trekking both mountain gorillas and golden monkeys as the performed activities.

TABLE 8
INDICATORS OF ECOSYSTEM PROTECTION IN THE VOLCANOES NATIONAL PARK

Indicators:	Frequency	Percentage %
Increase in the mountain gorillas' number	141	35%
Reduction of poaching and other illegal activities in the park	128	31.60%
Reduction of human-wildlife conflict cases	135	33.40%
Total	**404**	**100%**

Source: Primary data, 2024

Table 8 presents the data about the indicators of ecosystem protection in the Volcanoes National Park. On this issue, 35% of the respondents highlighted the increase in the mountain gorillas' number whereby, according to the information given by 25 RDB staff through interviews, the currently around 450 in approximately 1,000 mountain gorillas left in the three countries worldwide namely: Rwanda, Uganda and the Democratic Republic of Congo (DRC). Other 31.6% said that there has been a reduction of the rate poaching cases and other illegal activities, while the remaining 33.4% confirmed that the cases of human-wildlife conflicts, which most of the times take place when animals quit the park and come in local communities to destroy local people's properties including crops and livestock, have reduced, whereby through interview, the 25 RDB staff confirmed that "only 3 cases have been recorded since the beginning of this year 2022".

V. CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion:

This study aimed to assess the contribution of ecosystem protection to the development of ecotourism in Rwanda where the Volcanoes National Park was the study area. The findings indicated that protecting the ecosystem has contributed more to the development of ecotourism in the Volcanoes National Park where, there has been an increase in the park's revenue and visitors, ecotourism activities, and marketing through the "Gorilla Naming Ceremony".

The study further clarified that human's illegal activities such as hunting, poaching, illegal farming, and cutting trees. However, due to RDB's mobilization on the advantages of ecosystem protection, serious punishments of the government, and the different benefits they earn from ecotourism projects created in and around the Volcanoes National Park, many residents have stopped doing such illegal activities, especially that they use to do those activities just because of poverty, lack of knowledge about the importance of ecosystem protection, culture, and influences of others.

Other strategies used by the Rwanda Development Board (RDB) to overcome the challenges facing ecosystem protection in the Volcanoes National Park according to the research findings include the implementation of anti-poaching policies, establishment of buffer zone area, and expanding the park's surface, and involving local communities through Tourism Revenue Sharing Program. As a result, the number of mountain gorillas' number has increased; poaching and other illegal activities together with human-wildlife conflicts were reduced. However, the fact that ecotourism development is one of the key priorities of the Rwandan Government which can be easily achieved through ecosystem protection, while this one still faces several challenges especially, humans illegal activities, despite the implementation of RDB's tourism revenue-sharing program, other research on "the contribution of ecotourism development to the improvement of local communities' lifestyles are still needed.

5.2 Recommendations:

To completely end the challenges facing ecosystem protection for ecotourism development in the Volcanoes National Park, the Rwanda Development Board (RDB) was recommended to increase the benefits earned by local communities from ecosystem protection. Local leaders were advised to work with RDB by educating local communities about the benefits of ecosystem protections, while local communities as the most beneficiaries of the park's revenue, were recommended to undertake a leading role in fighting all illegal activities threatening ecosystem protection and ecotourism development in the Volcanoes National Park.

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Growth Performance and Carcass Characteristics of Growing Rabbits Fed Diets Containing Sweet Potato Tubermeal Supplemented with *Centrosema Pubenscens* Leaves

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Abstract— *The study was carried out to determine the growth performance and carcass characteristics of growing rabbits fed diets containing waste sweet potato tuber meal supplemented with Centrosema pubenscens leaves. Sun-dried sweet potato waste tuber meal supplemented with Centrosema pubenscens leaves were used at various levels of 0%, 15%, 20% and 25% to determine the best replacement that would give optimum performance of growing rabbits. 16 growing rabbits of 3 months (12 weeks) of age were used for this experiment. They were divided into four treatment groups of 4 rabbits each. Each treatment was subdivided into two replicates of two rabbits each. Each replicate was housed in a cage. Each group was randomly assigned to experimental diet in a completely randomized design (CRD). Parameters measured were initial body weight, final body weight, body weight gain, feed intake, Feed conversion ratio (FCR), daily body weight gain, cost of production, internal organ weight, cut parts weight, dressed weight and percent dressed weight. Data collected were analyzed using one way Analysis of variance (ANOVA). In terms of daily feed intake, TMT 4 had the highest feed intake of 91.66g while TMT 3 had the lowest feed intake of (64.66g). The growing rabbits on TMT 4 (25% PWM) recorded significantly (P< 0.05) higher body weight gain than the other TMT groups while those in TMT 2 and TMT 3 recorded significantly low body weight gain. The growing rabbit in TMT 4 recorded the best feed conversion ratio of 4.45 which was significantly (P<0.05) better than the control TMT 2 and TMT 3 respectively. The significantly better feed conversion ratio for the rabbits in TMT 4 may be attributed to higher feed intake and high body weight gain of the rabbits. The values obtained for visceral organs (internal organs) varied (P < 0.05) with sweet potato waste meal supplemented with Centrosema pubenscens leaves inclusion in their diets. TMT 2 and TMT 1 (control) have the highest percent liver which was significantly (P <0.05) higher than other TMT groups. TMT 4 recorded the lowest kidney percent. However, heart, lungs and spleen of the growing rabbits did not show any significant difference (P > 0.05). The results of cut parts and organs obtained did not follow a definite pattern that can be attributed to treatment effects. This showed that the supplementation of sweet potato waste meal with Centrosema pubenscens leaves did not affect the development of certain body organs. The result of the carcass characteristics showed that the evaluated were significantly (P<0.05) influenced by dietary treatments. The live weights and eviscerated weight were highest in rabbits fed with 25% sweet potato waste meal supplemented with Centrosema pubenscens leaves. The cost/kg diet showed that the cheapest diet was diet 4 (₦212.48) while the costliest was the control diet (Diet 1). In terms of cost of production, the lowest cost was TMT 4 (25%SPTM) (₦886.04) while the costliest was TMT 1 (control) (₦3,471.44). The result of the study showed that sweet potato waste meal supplemented with Centrosema pubenscens leaves could be used up to 25% in the diets of growing rabbits without affecting body weight gain, feed intake and feed conversion ratio.*

Keywords— Growth performance, carcass characteristics, rabbits, sweet potato, centrosema leaves.

I. INTRODUCTION

Rabbits (*Oryctolagus cuniculus*) descended from wild rabbits found in the Mediterranean Countries and was introduced into England in the late Eleventh to early Twelfth Century. The Nigerian wild hares which are mostly grayish brown in colour are still very common.

The prolific nature of rabbits coupled with its short gestation period and generation interval, makes it the animal of choice for multiplication and a short way of increasing animal protein intake (Uchewa, Orogwu, & Nwakpu, 2014). Domestic rabbits are ubiquitous, providing protein, fibre, research models, and companionship.

Rabbit production presents a promising avenue for food security and income generation in developing countries, particularly due to its low capital requirement, rapid reproduction, and efficient feed conversion. However, feed cost often contributes significantly to rabbit production expenses, especially when relying on conventional commercial feed. Exploring readily available and cheaper alternative feed sources is crucial for sustainable and profitable rabbit farming (Akinmutimi, & Osuagwu, 2008).

The utilization of alternative feed resources for livestock production has gained significant attention due to the increasing demand for animal products and the need for sustainable agricultural practices. In rabbit husbandry, the quest for cost-effective and nutritionally balanced feed sources has led to the exploration of unconventional ingredients such as waste potato tuber meal supplemented with plant materials like *Centrocema pubens*.

II. MATERIALS AND METHODS

2.1 Experimental site:

This research was conducted at the Rabbitry unit of the Teaching and Research farm of Michael Okpara University of Agriculture, Umudike located on latitude 05°29'N and longitude 07°33'E. Umudike is on an elevation of 122 m above sea level and located in tropical rain-forest zone of Nigeria, which is characterized by annual rainfall of about 2177 mm; monthly ambient temperature ranges of 22-33°C and relative humidity of 50-95 **%** depending on the season.

2.2 Experimental animal and management:

A total of fourteen (14) Rabbit were purchased from reputable Rabbit Farms. Two weeks to the arrival of the Rabbit, the Rabbit Hutches were cleaned, disinfected and allowed to dry. On arrival, anti-stress preparations were administered to enable the rabbits recover from transportation stress. The Rabbits were isolated for a week before separating into Research treatments and replications. After Isolation, the rabbits were randomly assigned to Five (5) treatment with Two (2) replicates of two rabbit each and one (1) control. Antibiotics drugs were administered in water at relevant periods as a prophylactic measure. The experiment lasted for 9 weeks.

2.3 Procurement and processing of potato waste meal *Centrosema pubenscens* LEAVES AND other feed ingredients

The sweet potato wastes were collected from potato dealers at Ahia-eke market in Abia State. They were dried under the sun for one week before it was milled to sweet potato waste meal and bagged for use and also *C. pubescens* leaves were harvested from the school environment and wilted under room temperature. Other feed ingredients like palm kernel meal, maize, wheat offal, premix, salt, methionine, soybean meal, lysine, fish meal; bone meal were procured from Jocan livestock service, Umuahia.

2.4 Anti-Nutrition Determinations:

The test materials fresh and dried potato waste meal waste meal and *C. pubescens* leaves were analyzed for the anti-nutritional contents such as tannin, oxalate, phytic acid, saponins, alkaloids and flavonoids. Total oxalate was determined according to Association of Official Analytical Chemist (AOAC, 2005). Phytic acid was determined according to Maga (1982). Saponin was determined according to Brunner (1984). Tannin was determined using the spectrometric method of AOAC (2005). Alkaloids was determined according to Henry (1993) and Allen (1992) method. Flavonoids was determined according to spectrophotometric methods of Allen (1979). Phytates was estimated as phytic acid using Maga method (1982).

The anti-nutritional factors were analyzed for the presence of flavonoid, tannic acid, saponin and alkaloid with value ranging from 0.65 to 6.48%. Other anti-nutrients determined were cyanide, polyphenols, phytate and oxalate in *C. pubescens* leaves.

2.5 Chemical analysis of feed ingredients:

All the processed feed ingredients; sweet potato waste meal, palm kernel cake, wheat offals, soybean meal, fishmeal, *C. pubescens* leaves were subjected to proximate analysis according to (AOAC 1995) to determine their nutrient composition and gross energy. All analysis was based on 100% dry matter. This was done to use the value obtained to determine the nutrients

composition of experimental diets that were formulated from them. The components that were determined include dry matter (DM), crude protein (CP), Ether extracts (EE) and nitrogen free extract (NFE).

2.6 Experimental growing rabbit diets:

For growing rabbit, a control (Diet 1) based on maize as the major source of energy was formulated. Three other diets were formulated such that diet 2, 3 and 4 contained 15%, 20% and 25% of waste sweet potato tuber meal in the control diet (Table 1). The other dietary ingredients were varied in order to provide the required protein and energy for the growing rabbits. The following diets were produced and tested in the feeding trial.

TABLE 1
EXPERIMENTAL DIETS FOR GROWING RABBITS CONTAINING SWEET POTATO WASTE TUBER MEAL

Ingredients (%)	Diet 1 (Control)	Diet 2 15% (SPTM)	Diet 3 20% (SPTM)	Diet 4 25% (SPTM)
Maize	40	34	32	30
Waste potato tuber meal	0	6	8	10
Soybean meal	5	5	5	5
Palm kernel cake	13	13	13	13
Fish meal	3	3	3	3
Wheat offal	35	35	35	35
Bone meal	3	3	3	3
Premix	0.25	0.25	0.25	0.25
Lysine	0.25	0.25	0.25	0.25
Methionine	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25
Centrosema pubenscens	0	0	0	0
Total	**100**	**100**	**100**	**100**
Nutrient composition of the rabbit diets				
Crude protein (%)	16.95	16.67	16.57	16.48
Crude fibre (%)	5.27	5.25	5.25	5.2
Ether extract (%)	8.65	8.3	7.92	7.55
Ash (%)	2.28	2.29	2.3	2.25
Phosphorus (%)	1.63	1.6	1.57	1.54
Calcium (%)	1.65	1.64	1.64	1.64
ME Kcal/kg	3132.39	3113.54	3094.65	3079.23

Premix supplied per type kg diet: Vit. A, 10,000 IU; Vit. D 2,000,000 IU; Vit. E, 2,300 mg; Vit. K3 2,000 mg; Vit. B, 3,000 mg; Vit. B2, 6,000 mg; Niacin, 5,000 mg; Calcium, 800 mg; Panthotenate, 10,000 mg; Vit. B6, 5,000 mg; Vit B12, 250 mg; Folic acid, 100 mg; Biotin, 50 mg; Choline chloride, 40,000 mg; Selenium, 120 mg and Anti oxidant, 12,000 mg

2.7 Data collection:

Data was collected at both starter and finisher stages. The following parameters were measured on each strain:

2.7.1 Growth performance parameters :

The parameters measured are as follows:

Initial weight of the growing rabbit was taken at the beginning of the feeding trial and mean weight was taken on weekly basis. Feed intake was taken daily.

Daily feed intake = Feed offered – leftover

$$\text{Daily weight gain} = \frac{\text{Body weight change}}{\text{Number of days of feeding trial}}$$

$$\text{Feed conversion} = \frac{\text{Average daily feed intake}}{\text{Average daily weight gain}}$$

2.8 Carcass Analysis:

At the end of feeding trial, 2 rabbits per replicate was randomly selected, weighed and then starved overnight (24 hours) but water was provided. The fasted weight was recorded in the morning before slaughter. They were then slaughtered, dressed and weighed individually. Carcass analysis was carried out as described by (Blasco, et al, 1993). The animals were thoroughly bled by hanging head down through the hind legs on nail. Furs were removed by roasting to get the dressed weight. Then the carcass was dissected and the internal organs evacuated (to get the eviscerated weight). The carcass was then dissembled into wholesale cuts as described by (Akinmutimi and Anakebe, 2008) and each primal part (thighs, ribs, neck, forelimbs, hind limbs and back and loin) was weighed using a digital scale. The organ weights (lungs, stomach, heart, kidney, liver and intestine) were also taken. The cut-up parts and organs weight recorded were expressed as percentage of the dressed weight.

2.8.1 Organs and other visceral components:

The organs and other visceral components such as lungs, liver, kidneys, heart, intestine, caecum, and abdominal fat were removed from individual carcasses in all the treatment groups and weighed using electronic sensitive scale and were expressed as percentage (%) of the live weight.

2.8.2 Laboratory analysis:

The proximate analysis of the experimental diets was carried out at the Animal Science Department Laboratory to determine the dry matter, crude protein, nitrogen free extract, ether extract, and crude fibre using the procedure of AOAC (1995).

2.8.3 Statistical analysis:

The data collected was subjected to Analysis of Variance (ANOVA) in a Completely Randomized Design (CRD) as described by Steel and Torrie using Statistical Package for the Social Sciences (SPSS, 2003) version 23.0. Significantly different means was separated using Duncan's New Multiple Range Test (Duncan, 1955).

2.9 Experimental design

The experimental design was completely randomized design (CRD). The design model was:

$$Y_{ij} = \mu + T_i + e_{ij}$$

Where:

Y_{ij} = observation

μ = mean

T_i = effects of treatment

e_{ij} = error means

III. RESULTS AND DISCUSSION

3.1 Anti-nutrient composition of fresh (raw) and sun dried sweet potato waste tuber meal:

The result of the anti-nutrients of fresh and sun dried sweet potato waste tuber meal were shown in (Table 1a).

TABLE 1 (a)

ANTI-NUTRIENT COMPOSITION OF FRESH (RAW) AND SUNDRIED WASTE SWEET POTATO TUBERMEAL

Parameters	Fresh (Raw) Waste sweet potato tuber meal	Sundried waste sweet potato tuber meal
Tannin (mg/g)	1.62	1.6
Saponin (mg/g)	0.05	0.05
Alkaloids (mg/g)	4.86	4.75
Oxalate (mg/g)	0.45	0.45
Phytate (mg/g)	0.07	0.07
Flavonoid (mg/g)	1.06	1

The values of tannins (1.62 mg/g), saponin (0.05 mg/g), alkaloid (4.86 mg/g), oxalate (0.45 mg/g), phytate (0.07 mg/g) and flavonoid (1.06 mg/g) recorded in this study fall within the range reported by Akinmutimi (2004), Ameh (2010). Sun drying of sweet potato waste tuber meal helped to reduce the tannin, alkaloids and flavonoid as stated in (Table 1a). Sun drying of sweet potato waste meal was not effective for reducing saponin, oxalate and phytate content as stated in (Table 1a).

TABLE 1 (b)

PROXIMATE COMPOSITION OF FRESH (RAW) AND SUNDRIED SWEET POTATO WASTE TUBER MEAL

Parameters	Fresh (Raw) sweet potato waste tuber meal	Sundried sweet potato waste tuber meal
Dry matter (%)	61.25	88.75
Moisture (%)	38.75	11.25
Ash/Mineral (%)	4.6	6.26
Crude protein (%)	3.28	4.95
Ether extract (%)	0.6	0.82
Crude fibre (%)	1.17	2.64
Nitrogen Free Extract (%)	51.6	74.06
Metabolizable Energy (Kcal/kg)	2068.6	3013.59

The proximate composition of fresh and sundried sweet potato waste tuber meal is presented in (Table 1b). All the values obtained for crude protein, crude fat, moisture content, ash, nitrogen free extract and metabolizable energy fall within the range reported by Aduku (1993), Oyenuga (1968), Osuagwu (2006), Akinmutimi and Anakebe (2008), Ameh (2010), Solomon et al (2015), AOAC (2005), Ola and Oba (2004) and Anyegbu et al., (2021). Sun drying of the sweet potato waste tuber meal was effective for increasing the dry mater, ash/mineral, crude protein, ether extract, crude fibre, nitrogen free extract and metabolizable energy (Kcal/Kg) content of the test ingredients (Table 1b).

TABLE 1 (c)

PROXIMATE COMPOSITION OF FRESH *CENTROSEMA PUBENSCENS* LEAVES

Parameters	Fresh (*Centrosema pubenscens*)
Dry matter (%)	84.26
Moisture (%)	15.74
Ash/Mineral (%)	3.39
Crude protein (%)	23.08
Ether extract (%)	0.35
Crude fibre (%)	25.6
Nitrogen free extract (%)	31.84
Metabolizable energy (Kcal/kg)	3181.88

The proximate composition of ***Centrosema pubenscens*** leaves is shown in (Table 1c). The values obtained for crude protein, crude fibre, moisture content, ash and metabolizable energy fall within the ranges obtained by Aduku (1993), Obua et al (2012), Osakwe and Ekwe (2007), Adebayo et al (2019), Bamigboye and Oluwarinde (2017), Mecha and Adegbola (1980), Ikhimieya and Olaguiju (1996).

TABLE 2
PROXIMATE COMPOSITION OF THE EXPERIMENTAL GROWING RABBIT DIETS

Parameters	T_1 Control	T_2 15% SPTM	T_3 20% SPTM	T_4 25% SPTM	SEM
Dry matter (%)	91.86	91.79	91.76	91.71	0.03
Moisture (%)	8.14	8.21	8.24	8.29	0.03
Ash/Mineral (%)	6.24	7.85	8.56	9.51	0.68
Crude protein (%)	15.95	15.5	15.3	15	0.19
Ether extract (%)	4.05	3.86	3.82	3.75	0.64
Crude fibre (%)	8.28	8.94	9.65	10.39	0.45
Nitrogen Free Extract (%)	57.34	55.64	54.43	53.06	0.9
Metabolizable Energy (Kcal/kg)	2843.78	27843.78	2761.35	2728.43	2366.5

TABLE 3
PERFORMANCE OF THE GROWING RABBITS FED DIETS CONTAINING WASTE SWEET POTATO TUBER MEAL SUPPLEMENTED WITH *CENTROSEMA* LEAVES

Parameters	T_1 Control	T_2 15% SPTM	T_3 20% SPTM	T_4 25% SPTM	SEM
Initial body weight (kg)	0.7	0.6	0.87	0.55	0.07
Final Body weight (kg)	1.55^b	1.33^c	1.40^c	1.70^a	0.08
Body weight gain (kg)	0.85^b	0.73^b	0.53^c	1.15^a	0.12
Daily body weight gain (kg)	15.18^b	13.0^b	9.46^c	20.5^a	2.31
Daily feed intake (g)	83.58^b	72.00^c	64.66^c	91.66^a	5.99
Feed Conversion Ratio (FCR)	5.48^b	5.52^b	6.83^a	4.45^c	0.48

^{abc} ***means on the same row with different superscripts are significantly (P < 0.05) different***

The performance of growing rabbits fed sweet potato waste meal supplemented with ***Centrosema pubenscens*** leaves in their diets is shown in (Table 3).

3.2 Feed intake:

The daily feed intake of the experimental growing rabbit were 83.58g, 72.00g, 64.66g and 91.66g for treatment 1 (control), TMT 2, TMT 3 and TMT 4 respectively. Significant differences ($P < 0.05$) existed among the various TMT groups. Increasing the dietary inclusion of sweet potato waste tuber meal supplemented with ***Centrosema pubenscens*** from 15% to 25% did not significantly ($P > 0.05$) increase the body weight gain though the rabbits, from treatment 4 had consumed significantly ($P < 0.05$) more feed than those in the control treatment TMT 2 and TMT 3. Daily feed intake did not differ among the treatments, however TMT 4 had the highest daily feed intake of 91.66g and TMT 3 had lowest feed intake of (64.66g), this may be as a result of palatability of the experimental diet at 25% inclusion.

3.3 Body weight gain:

The body weight gain of the growing rabbits fed sweet potato waste tuber meal supplemented with ***Centrosema pubenscens*** leaves were 0.85g, 0.73g, 0.53g and 1.15g for TMT 1 (Control), TMT 2, TMT 3, and TMT 4 respectively. Significant differences ($P < 0.05$) existed among the various TMT groups in their body weight gain. The growing rabbits in TMT 4 (25%) was fed with sweet potato waste tuber meal supplemented with ***Centrosema*** leaves recorded significantly ($P < 0.05$) higher body weight gain more than those on the control diet, TMT 2 and TMT 3. The growing rabbits in TMT 2 and those in TMT 3 recorded significantly ($P < 0.05$) low body weight gain.

3.4 Feed Conversion Ratio (FCR):

The feed conversion ratio of the experimental growing rabbits were 5.48, 5.52, 6.83, 4.45 for the control (TMT 1), TMT 2, TMT 3, and TMT 4 respectively. Significant difference ($P < 0.05$) existed among the various TMT groups. The growing rabbits in TMT 4 recorded the best feed conversion ratio of 4.45, which was significantly ($P < 0.05$) better than the control TMT 2 and TMT 3 respectively. The significantly better feed conversion ratio for the rabbit in TMT 4 may be attributed to higher feed intake and higher weight gain of the rabbits.

3.5 Percentage of cut parts weights of growing rabbits fed sweet potato waste tuber meal supplemented with *Centrosema pubenscens* leaves in their diets

TABLE 4
PERCENTAGE OF CUT PARTS WEIGHTS OF THE GROWING RABBITS FED EXPERIMENTAL DIETS CONTAINING SWEET POTATO WASTE TUBER MEAL SUPPLEMENTED WITH *CENTROCEMA PUBENSCENS* LEAVES

Parameters	T_1 Control	T_2 15% SPTM	T_3 20% SPTM	T_4 25% SPTM	SEM
Live weight (kg)	1.65^{c}	1.05^{b}	0.90^{ab}	1.62^{b}	0.19
Dead weight (%)	97^{b}	95.2^{a}	94.4^{ab}	95.7^{c}	0.54
Defurred weight (%)	90.9^{c}	90.5^{b}	88.9^{a}	74.1^{ab}	4.02
Fur (%)	1.45^{a}	1.24^{c}	1.56^{ab}	1.54^{a}	0.07
Head (%)	9.7^{a}	10.9^{b}	9.67^{ab}	9.38^{b}	0.33
Drum Stick (%)	3.2^{a}	8.57^{b}	3.11^{a}	2.41^{b}	1.42
Thigh (%)	9.69	3.05	10.2	9.32	1.68
Hind (Fore) (%)	188^{b}	1.43^{ab}	1.67^{a}	1.4^{b}	46.62
Ribs (%)	9.1^{a}	7.14^{c}	9.8^{a}	8.5^{c}	0.56
Back cut (%)	18.1^{ab}	1.64^{c}	14^{c}	16.2^{a}	3.71
Shoulder (%)	6.24^{a}	5.90^{a}	5.8^{a}	7.5^{c}	0.39
Hind (Leg) (%)	1.45^{a}	1.62^{b}	1.8^{c}	1.3^{c}	0.1

^{abc} means on the same row with different superscripts are significantly ($P < 0.05$) different

The results of the effect of the graded levels of sweet potato waste tuber meal supplemented with *Centrocema pubenscens* leaves on the carcass characteristics, major cuts and organs of the growing rabbits were shown in Table 4. The results showed that carcass characteristics evaluated were significantly ($P < 0.05$) influenced by dietary treatments. The live weight was highest in rabbits fed diet with 25% SPWM supplemented with *Centrocema pubenscens* leaves in their diets. This result was not unexpected since the average daily weight gain of rabbits in this treatment was comparatively higher than those in other dietary groups.

The cut parts (fore limbs, hind limbs, thigh and head) varied ($P < 0.05$) across treatments (Table 4). There were significant differences ($P < 0.05$) on head and thigh weights of rabbits, however, the highest head (10.9%) and thigh (10.2%) was recorded for rabbits in (TMT 2 and TMT 3) respectively. The highest fore limb (188%), back cut (18.1%) was recorded on TMT 1 (control).

3.6 Percentage of internal organs of experimental rabbits fed sweet potato waste tuber meal supplemented with *Centrosema pubenscens* leaves in their diets

TABLE 5
INTERNAL ORGAN WEIGHTS OF THE GROWING RABBITS FED EXPERIMENTAL DIETS CONTAINING SWEET POTATO WASTE TUBER MEAL SUPPLEMENTED WITH *CENTROSEMA PUBENSCENS* LEAVES

Parameters	T_1 Control	T_2 15% SPTM	T_3 20% SPTM	T_4 25% SPTM	SEM
Dressed weight (kg)	1.00^{a}	0.53^{b}	0.80^{a}	1.20^{b}	0.14
Heart (%)	0.3^{a}	0.6^{b}	0.25^{b}	0.42^{ab}	0.07
Liver (%)	4^{a}	4.7^{b}	2.1^{b}	3.7^{c}	0.54
Spleen (%)	0.1^{a}	0.2^{b}	0.1^{ab}	0.1^{a}	0.02
Lung (%)	1.1^{b}	1.32^{ab}	1.5^{c}	1.3^{a}	0.81
Kidney (%)	1.2^{c}	1.5^{a}	0.9^{ab}	0.8^{b}	0.15
Bile (%)	0.1^{a}	0.2^{b}	0.3^{c}	0.1^{b}	0.04
Abdominal fat (%)	0.2^{a}	0.2^{c}	0.3^{b}	0.8^{ab}	0.14
Large intestine (%)	9.9^{a}	22.1^{c}	4.1^{b}	1.1^{ab}	4.64
Small intestine (%)	20.2^{a}	21.1^{c}	9.8^{b}	14.5^{b}	2.64

^{abc} means within the same row with different superscripts are significantly (P < 0.05) different.
SEM = Standard Error Mean

The values obtained for visceral organs (internal organs) in Table 5 varied ($P < 0.05$) with sweet potato waste tuber meal supplemented with *Centrocema pubenscens* inclusion in their diets. TMT 1 (Control) and TMT 2 had the highest percentage on liver significantly ($P < 0.05$) higher than other TMT groups. TMT 4 recorded the lowest kidney percent (0.8%). However, heart, lungs and spleen of the growing rabbits did not show any significant differences ($P > 0.05$). The results of cut parts and organs obtained did not follow a definite pattern that can be attributed to treatment effect. This shows that the supplementation of sweet potato waste meal with *Centrocema pubenscens* leaves did not affect the development of certain body organs

3.7 Cost Benefit Analysis of Experimental Growing Rabbits

TABLE 6
COST BENEFIT ANALYSIS OF EXPERIMENTAL GROWING RABBITS FED WITH SWEET POTATO WASTE TUBER MEAL LEAF MEAL DIET CONTAINING *CENTROCEMA PUBENSCENS* LEAVES

Parameters	T_1 Control	T_2 15% SPTM	T_3 20% SPTM	T_4 25% SPTM
Cost/kg diet (₦)	370.88	327.68	356.48	212.48
Cost of feed consumed (₦)	4,636	4,096.00	4,456.00	2,656.00
Cost/kg weight gain (₦)	26,491.43	11,702.86	11,882.67	4,283.87
Cost of production (₦)	3,471.44	2907.74	1,226.26	886.04

The economics of production of the experimental diets was shown in (Table 6). The cost/kg diet were ₦370.88, ₦327.68, ₦356.48, ₦212.48 for the control diet, diet 2, diet 3 and diet 4 respectively. The cheapest diet was diet 4, while the costliest was the control diet. In term of the cost of production, the cost of production ₦/growing rabbit was lowest for those on diet 4 (25% SPTM) (₦886.04) while those on diet 1 (control) (₦3,471.44) was the costliest.

IV. CONCLUSION

The result of the study showed that sun-drying of sweet potato waste tuber meal (*Ipomea batatas*) was not effective for removing the anti-nutrients in the dried samples. Sundried potato waste meal supplemented with *Centrosema pubenscens* leaves could be used up to 25% in the diet of growing rabbits without affecting body weight gain, feed intake and feed

conversion ratio. Based on the result of the study it was therefore recommended that sundried potato waste tuber meal supplemented with *Centrocema pubenscens* leaf meal could be used up to 25% in the diet of growing rabbits for optimum performance.

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Dietary Supplementation of Diets Containing Sun-Dried Sweet Potato (*Ipomea Batatas*) Waste Meal with Yeast Additive on the Growth Performance of Starter Broiler Chicks

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Abstract— *The study was conducted to determine the dietary supplementation of sun-dried sweet potato (Ipomea batatas) waste meal with yeast additive on the growth performance of starter broiler chicks. The sundried sweet potato waste meal supplemented with yeast was used to replace yellow maize at various levels of 0%, 10%, 15% and 20%, respectively for diets 1, 2, 3 and 4 to determine the best replacement that would give optimal performance in starter broiler chicks. The result of the proximate composition of sundried sweet potato waste meal showed that it contained 88.75% dry matter, 11.25% moisture, 6.28% ash, 4.95% crude protein, 0.82% Ether Extract, 2.64% crude fibre, 74.06%, Nitrogen free extract (NFE), and 3013 Kcal/Kg metabolizable energy. One hundred (100) day-old Anak starter broiler chicks (Ross broiler chicks) were used for the experiment. They were divided into four (4) treatment groups of 24 broiler chicks each. Each treatment was subdivided into three replicates of 8 chicks per replicate. Each starter broiler diet was fed to a group of 24 chicks using completely randomized design (CRD) for 4 weeks. Feed and water were given to the chicks ad-libitum. Parameters measured included initial body weight, final body weight, body weight gain, feed intake, feed conversion ratio (FCR) and economics of production. Data collected were analysed using one way Analysis of Variance (ANOVA). In the starter broiler phase, the starter broiler chicks on 20% sweet potato waste meal supplemented with yeast compared favourably with the control ($P < 0.05$) in terms of feed intake, body weight gain and feed conversion ratio and significantly ($P < 0.05$) performed better than those on other diets. The cost of production of the starter broiler chicks was lowest for those fed the control diet (Diet 1), N156.59, while the costliest was the starter broiler chicks on diet 3 (15% PWM) (₦295.45). The cost/kg diet was cheapest for diet 1 (Control) (₦560.07) and the costliest diet was diet 2 (10% PWM) (₦625.66). It was therefore concluded that sundried sweet potato waste meal (Ipomea batata) could be used in the diets of starter broilers chicks up to 20% without affecting the body weight gain, feed intake and feed conversion.*

Keywords— Dietary supplementation, sweet potato, yeast, growth performance, broiler chicks.

I. INTRODUCTION

The broiler chicken is a domesticated bird mainly kept for meat production. It is a quick growing monogastric animal and the returns on investment is very high compared to other livestock (Omole *et al.,* 2006). Feed, which is one of the major problems that affect broiler chickens production constitutes about 60 – 70 percent of the total cost of production (Omole *et al*, 2006). This is due to the competition between man, animals and industries for conventional feedstuffs that can meet up with the nutritional requirements of broiler chickens and at the same time, reduce the cost of the feed, thereby reducing the cost of broiler chickens production in 'Nigeria (Olerede *et al.,* 2002).

Great emphasis has continued to be placed on research into the use of alternative sources of basal energy feedstuffs in broiler chicken production moreso, with the ever increasing price of maize, which has continued to be the main energy source in livestock feeds (Ojewola, 2005). Agro by-products and wastes have been identified as alternative feedstuffs and can form a major source of energy in animal feeds (Adejumo, 2006). One of such agro-by-products that can be harnessed is sweet potato wastes. Sweet potato is a staple food in Nigeria with the annual yield of 12,245 kg/ha of which some fractional percentage

constitutes the wastes (FAO, 1989). The sweet potato wastes are a good source of quality carbohydrate. Sweet potato waste contains about 5.36 percent crude protein and metabolizable energy of about 3,180 Kcal/kg (Oyenuga, 1968; Jansen, 1989 and Osuagwu, 2006). This research was carried out to evaluate the dietary supplementation of diets containing sundried sweet potato (*Ipomea batatas*) waste meal with yeast additive on the growth performance of starter broiler chicks.

II. MATERIALS AND METHODS

2.1 Experimental Site:

The study was conducted at the poultry unit of the Teaching and Research farm of Michael Okpara University of Agriculture, Umudike, Abia State. The site is located on latitude 05° 29N and longitude 07° 33E at an attitude of 123m above sea level. Umudike has an annual rainfall of 2177mm, with maximum and minimum daily temperature of 27°-36°C and 20°-26°C, respectively. It has a relative humidity of 57-91% (NRCRI, 2010).

2.2 Procurement and Processing of Test Materials and Feed Ingredients:

The sun-dried sweet potato waste meal was collected from sweet potato dealers at Imo land. Orie Ugba market, Ubani Market, Arochukwu steel and other traders within Umuahia North and Umuahia South Local Government Areas and their environs in Abia State. After drying the sweet potato wastes, it was grounded or milled and bagged. The experimental diets were formulated to contain varying levels of sun-dried sweet potato wastes as T_1(0%), T_2(10%), T_3(15%) and T_4(20%), respectively. The experiment was divided into four treatments groups comprising twenty-four (24) birds per treatment and three replicates of 8 birds each. The percentage composition of experimental diet is shown in the (Table 1) below:

TABLE 1
EXPERIMENTAL STARTER BROILER CHICKS DIETS

Ingredients	T_1(0%)	T_2 (10%)	T_3 (15%)	T_4 (20%)
Maize	55	49.5	46.75	44
Sweet potato waste meal	0	5.5	8.25	11
Soyabean meal	30	30	30	30
Palm kernel cake	4.3	4.3	4.3	4.30-
Wheat offal	4	4	4	4
Fishmeal	3	3	3	3
Bone meal	3	3	3	3
Common salt	0.25	0.25	0.25	0.25
Vitamin/Mineral Premix	0.25	25	25	25
DL-Methionine	0.1	0.1	0.1	0.1
Lysine	0.1	0.1	0.1	0.1
TOTAL	100	100	100	100
Crude protein (%)	22.99	22.73	22.61	22.48
ME (Kcal/kg)	2887.15	2875.05	2869	2862.95

TABLE 2
PROXIMATE ANALYSIS OF SUN-DRIED SWEET POTATO WASTE MEA

Parameters	Sweet potato waste meal
Dry matter (%)	88.75
Moisture (%)	11.25
Ash/mineral (%)	6.28
Crude protein (%)	4.95
Ether extract (%)	0.82
Crude fibre extract (%)	2.64
Nitrogen Free Extract (%)	74.06
Metabolizable Energy (kcal/kg)	3013.59

2.3 Sources of Enzyme

The Baker's yeast (*Saccharomyces cerevisiae*) used for this study was purchased from De-Meck International supermarket, Umuahia, Abia State.

2.4 Anti-nutrient Determination of Sweet Potato *(Ipomea batatas)* Waste Meal:

The test materials fresh and sundried sweet potato waste meal were analysed for the anti-nutrient contents such as oxalate, phytic acid, saponins, tannins, alkaloids and flavonoids. Total oxalate were determined according to Association of Official Analytical Chemist (AOAC, 2005). Phytic acid was determined according to Maga (1982). Saponin was determined according to Brunner (1984), Tannins was determined using the spectrophotometric method of AOAC (2005). Alkaloids determination by Henry (1993) and Allen (1992) method was used. Flavonoids was determined according to spectrophotometric method of Allen (1979). Phytate was estimated as phytic acid by Maga method (1982).

2.5 Management of Experimental Broiler Chicks (birds):

One hundred-day old chicks of Anak strain (Ross broiler birds) were procured from Ibadan hatchery which was used for the experiment. The environment was cleaned and disinfected three days before the arrival of the birds. The feeders and drinkers were washed and disinfected. The electrical appliance that supplied heat was properly checked and switched on few hours before the arrival of the birds. Lantern was also provided as a source of heat. Wood shavings were used as litter materials. The litters were changed periodically after every one week.

Food intake and weight gain were determined on a weekly basis. Medications and vaccinations programme were carried out as at when due. At the first day, the chicks were vaccinated using Newcastle Disease vaccine intra-ocula (NCDV-i/o) through the eyes. Multivitamins drugs was administered for one week as anti-stress.

Gumboro vaccine was administered at the 10^{th} day while Lasota vaccine was given at the 28^{th} day against Newcastle disease. Also, Amprolium was given against coccidiosis disease and multivitamin was given in subsequent days.

2.6 Diets:

Four diets were formulated (Table 1), all the diets contained maize, soyabean meal, palm kernel cake, wheat offal, fish meal, bone meal, common salt, vitamin/mineral premix, DL- Methionine, Lysine. Sweet potato waste meal was used to replace maize meal at (0%), (10%), (15%) and (20%), in diets 1, 2, 3 and 4, respectively.

2.7 Data Collection:

Initial body weight, final body weight, body weight gain, feed intake, feed conversion ratio, cost of production.

2.8 Economics of Production Parameters:

Economics of the experimental diets were calculated using the following parameters:

a. Cost/kg of diet = total cost of producing 1 kg of feed

b. Cost of feed consumed = Cost/kg x total feed consumed

c. $\text{Cost/Kg Weight Gain} = \frac{\text{Cost of feed consumed}}{\text{Body weight Gain}}$

d. Cost of production = Feed conversion ratio x cost of feed/Kg

e. Revenue = Price of 1Kg meat x Weight Gain

f. Gross Margin = Revenue – Cost of Production

g. $\text{Return on Investment} = \frac{\text{Gross Margin}}{\text{Cost of production}} \times 100$

2.9 Data Analysis:

Data collected was subjected to Analysis of Variance (ANOVA) as outlined by Steel and Torrie (1980) in a Completely Randomized Design at 5% level of significance.

2.10 Experimental Design:

The design of this study is a Completely Randomized Design.

$$Y_{ij} = \mu + T_1 + e_{ij}$$

Where:

Y_{ij} = Individual observation

μ = Population mean

T_i = Treatment effect

e_{ij} = Random error which is independently identically normally distributed with zero mean and constant variance.

III. RESULTS AND DISCUSSION

3.1 Anti-nutrient and proximate composition of fresh (raw) and sun-dried sweet potato waste meal:

The result of the anti-nutrients of raw (fresh) and sun-dried sweet potato waste meal were shown in (Table 3).

TABLE 3
ANTI-NUTRIENTS OF RAW (FRESH) AND SUN-DRIED SWEET POTATO WASTE MEAL

Parameters	Sweet potato waste meal	Sweet potato waste meal
Tannin (mg/g)	1.62	1.6
Saponin (mg/g)	0.05	0.05
Alkaloids (mg/g)	4.86	4.75
Oxalate (mg/g)	0.45	0.45
Phytate (mg/g)	0.07	0.07
Flavonoid (mg/g)	1.06	1

Anti-nutritional factors are substances that when present in animal feed hinders or reduces the availability of one or more nutrient. In other instances, they form complexes with some of these nutrients thus making it impossible for them to be digested and or degraded. Phytate lowers absorption and impairs solubility of protein. Saponin decreases performance and growth rate. The values of tannin (1.62 mg/g), saponin (0.05 mg/g), alkaloids (4.86 mg/g), oxalate (0.45 mg/g), phytate (0.07 mg/g) and flavonoid (1.06 mg/g) recorded in this study fall within the range reported by Akinmutimi (2008), Ameh (2010.

TABLE 4
PROXIMATE COMPOSITION OF FRESH (RAW) AND SUN-DRIED SWEET POTATO WASTE MEAL

Parameters	Sweet potato waste meal	Sweet potato waste meal
Dry matter (%)	61.25	88.75
Moisture (%)	38.75	11.25
Ash/mineral (%)	4.6	6.28
Crude protein (%)	3.28	4.95
Ether extract (%)	0.6	0.82
Crude fibre (%)	1.17	2.64
Nitrogen Free Extract (%)	51.6	74.06
Metabolizable Energy (kcal/kg)	2068.66	3013.59

The proximate composition of fresh (raw) and sun-dried sweet potato waste meal is presented in Table 5. All the values obtained for crude protein, crude fat, moisture content, ash, nitrogen free extract, and metabolizable energy fall within the range reported by Aduku (1993), Oyenuga (1968), Osuagwu (2006), Akinmutimi and Anakebe (2008), Ameh (2010), AOAC (2005).

TABLE 5
PROXIMATE COMPOSITION OF EXPERIMENTAL BROILER STARTER DIETS

Parameters	T_1 0% Pwm	T_2 10% Pwm	T_2 15% Pwm	T_4 20% Pwm
Dry matter (%)	91.28	91.06	90.92	90.87
Moisture (%)	8.72	8.94	9.08	9.13
Ash/mineral (%)	8.26	8.7	8.98	9.26
Crude protein (%)	23.75	23.41	22.96	22.53
Ether extract (%)	4.92	4.88	4.85	4.85
Crude fibre (%)	7.95	8	8.08	8.15
Nitrogen Free Extract (%)	46.4	46.07	46.05	46.08
Metabolizable Energy (kcal/kg)	2800	2850	2875	2806

3.2 Growth performance of starter broiler chicks fed diets containing sweet potato waste meal supplemented with yeast (*Saccharomyces cerevisie*):

TABLE 6
GROWTH PERFORMANCE OF STARTER BROILER CHICKS FED DIETS CONTAINING SWEET POTATO WASTE MEAL SUPPLEMENTED WITH YEAST (*SACCHAROMYCES CEREVISIE*)

Parameter	Diet 1 (Control)	Diet 2 (10% Pwm)	Diet 3 (15% Pwm)	Diet 4 (20% Pwm)	SEM
Initial Body weight (g)	158	167.87	159.09	161.59	4.4
Final Body weight (g)	507.25^{c}	531.99^{b}	558.21^{ab}	574.88^{a}	67.08
Body weight Gain (g)	348.79^{c}	367.12^{b}	399.12^{ab}	413.30^{a}	65.1
Daily Body weight Gain (g)	12.46^{c}	13.11^{b}	14.25^{ab}	14.76^{a}	1.58
Daily feed intake (g)	83.71^{c}	84.22^{ab}	84.42^{ab}	84.76^{a}	2.84
Feed Conversion Ratio (FCR)	6.71^{c}	6.42^{b}	5.92^{ab}	5.74^{a}	1.22

^{abc} mean within the same row with different superscript are significantly (P < 0.05) different SEM: Standard Error Mean, Pwm = Potato waste meal

3.2.1 Feed intake:

The feed intake of the experimental starter broiler groups were 83.71, 84.22, 84.42 and 84.76g for the control, treatment 2, 3, and 4 respectively. Significant differences (P < 0.05) existed among the groups in their feed intakes. The starter broiler chicks in treatment 4 (20% Pwm) supplemented with yeast recorded the highest feed intake which was significantly (P < 0.05) higher than those in other groups. The feed intake of those in treatment 2 and 3 were similar and significantly (P < 0.05) higher than those on the control diet (Treatment 1). The increase in feed intake by the starter broiler chicks in treatment 4, 3, and 2 could be as the result of yeast (probiotic) additive in their diets. According to (Shim et al., 2012), the improvement in performance and productivity of poultry due to the use of probiotics in feed has been attributed to increased feed intake and improved efficiency. Afsharmanesh and Sadaghi, (2010) reported that probiotics increased feed intake without significant improvement in feed conversion ratio (FCR).

3.2.2 Body weight gain:

The body weight gain of the experimental starter broiler chicks fed the sweet potato waste meal supplemented with yeast in their diets were 348.79, 367.12, 399.12 and 413.30g for the control, treatment 2, 3, and 4 respectively. Significant difference s (P < 0.05) among the groups in their body weight gain. The starter broiler chicks in treatment 4 (20% Pwm) supplemented with yeast recorded the highest body weight gain which was significantly (P < 0.05) higher than those on the control diet and other

treatment groups. It appeared that the body weight gain of the starter broiler chicks increased with increased level of sweet potato waste meal supplemented with yeast in their diets. The increased body weight could be as the result of yeast additive in their diets.

3.2.3 Feed conversion ratio (FCR):

The feed conversion ratio of the experimental starter broiler chicks fed diets containing sweet potato waste meal supplemented with yeast probiotic in (Table 6). Significant differences ($P < 0.05$) existed among the starter broiler chicks groups in their feed conversion ratio. The starter chicks on 20% sweet potato waste meal supplemented with yeast recorded the best feed conversion ratio of 5.74 which was significantly ($P < 0.05$) better than those on the control diet and those on treatment 2 (10% Pwm), and treatment 3 (15% Pwm) supplemented with enzyme.

3.3 Economics of Production of Experimental Starter Broiler Chicks:

TABLE 7
ECONOMICS OF PRODUCTION OF EXPERIMENTAL STARTER BROILER CHICKS FED DIETS CONTAINING SWEET POTATO WASTE MEAL SUPPLEMENTED WITH YEAST.

Parameter	Diet 1 (Control)	Diet 2 (10% Pwm)	Diet 3 (15% Pwm)	Diet 4 (20% Pwm)	SEM
Cost/Kg Diet (₦)	560.07^{c}	625.66^{a}	618.45^{ab}	611.25^{ab}	5.26
Cost of feed consumed (₦)	840.11	813.36	865.83	855.75	11.3
Cost/Kg Weight Gain (₦)	842.16	937.18	986.62	953.26	19.6
Cost of production (₦)/bird	156.59^{c}	167.36^{b}	295.45^{a}	291.67^{ab}	6.4

The economics of production of the experimental diets are shown in table 7. The cost/kg diet were ₦560.07, ₦625.66, ₦618.45, ₦611.25 for the control diet, diet 2, diet 3 and diet 4 respectively. Significant differences ($P < 0.05$) existed among the different groups. The cheapest diet was diet 1 (control) while the costliest was diet 2 (10% PWM). In terms of the cost of production, the cost of production/₦/bird was lowest for those on Diet 1 (control) (₦156.59) while those on diet 3 (15% PWM) was the costliest.

IV. CONCLUSION

The result of the study demonstrated that sun-drying of sweet potato waste was not quite effective for eliminating the anti-nutrients in sweet potato tubers because there were still traces of anti-nutrients in the dried samples. Sun-dried sweet potato waste meal supplemented with yeast could be used in the diets of starter broiler chicks up to 20% without affecting the body weight gain, feed intake and feed conversion ratio.

RECOMMENDATION

Based on the result of this study, it was therefore recommended that sun-dried sweet potato waste meal supplemented with yeast could be used up to 20% to replace maize in the starter broiler chicks' diet for optimum performance.

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A Study on Rural Biomass Energy Scenarios in Haor Ecosystem

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Abstract— *The study was conducted in the Project area of LIFCHASA in village Purbo Tethulia under Mohanganj upazila of Netrakona which lies in AEZ 2. A total of 102 households were interviewed using a pre-tested questionnaire to identify the farmers' socio-economic condition, bio-diversity, total biomass production and utilization. Data for the study were obtained through personal interviewing. Four categories of farmer viz. landless, marginal and small, medium and large were included in the study. There were 462 households in the village consisting of 2245 persons with average of 4.86 family-1. In the study area 23 tree, 27 field crop and vegetable and 25 weed species were observed. Biomass of field crops, vegetable and weed was estimated through harvesting methods. Biomass production was unequally proportionate among landless, marginal and small, medium and large farmers. The total biomass estimated in the village was 10.04 t farm-1 year-1 and the total utilization was found 14.55 t farm-1 year-1. The bio-energy was found 111.02 GJ farm-1 year-1 where 54.71 GJ farm-1 year-1 bio-energy consumed as fuel. The relationships between the variables were positively significant with the family size, farm size, annual income and problem faced on biomass production and utilization versus total biomass production and utilization.*

Keywords— Biomass energy, Haor ecosystem, biomass production and its utilization, source, biomass pattern, Haor Ecosystem.

I. INTRODUCTION

Biomass is biological material from living, or recently dead organisms, most often referring to plant or plant derived materials. As a renewable energy source, biomass can either be used directly or indirectly-once or converted into another type of energy product such as bio-fuel. Biomass is the overall plant matter created by photosynthesis. It constitutes man's oldest and most fundamental source of renewable energy. It includes new plant growth, plant residues and wastes. The available forms are: wood, short duration trees, herbaceous plants; residues such as agricultural and forest residues e.g., straw, husks, bagasse, cobs, forest or wood based materials like bark, sawdust, roots and even animal droppings, waste comprising garbage, night soil, sewage solids and industrial refuse (Sharma, 1985).

In Bangladesh, biomass is generally used as food, feed, fuel, fodder, organic manure, building material and as the raw material for cottage industries including ploughs, fishing requirement, bullocks, carts and country boats (ESCAP, 1989). The primary production of living biomass on the earth surface is around 172 × 109 t (dry) year-1 of all the biomass resources, the greatest quantity of which is known as phytomass is formed by plant (Aziz and Jalal, 1982).

In the haor area, biomass plays an important role. Crop grains, pulse, vegetable, spices, etc. are used as human food, bran, straw, leaves, weed, etc. are used for feed, the best portion of straw is used for fodder and the rest is used as fuel or bio-fertilizer/organic manure or for housing materials, etc. About 90% of the fruits, vegetable, fodder and biomass fuel requirement are met by home gardens (Hossain and Bari, 1996). Davidson (1984) observed that over half of the fruits, vegetable and spices grown in home gardens are sold to meet family expense.

In Bangladesh, the forest productivity is very low. It is only 0.5-2.5 m2 ha-1yr-1 in both plantation and natural forests (ADB, 1993). It cannot meet the country demand. According to FAO (1982), in Bangladesh 48% of saw and veneer logs and upto

70% of fuel and 90% bamboos come from villages. Extraction rate from village forests is 8.9% and increment rate is 5% (FAO, 1982). At present the dominant source of biomass in the rural areas is field crops, vegetable, trees, livestock etc. from village forests, roadside and homestead plantation. Home gardens along with marginal waste lands and non-forest lands supply more than 80% of fuel wood in Bangladesh (Gujral, 1990). If not properly managed this reserve will be diminished in the near future. So the village forest resources need to make more productive.

Biomass is closely linked to biodiversity which is dependent on mankind or any living species that lives on earth. The mass of living organisms is as biomass which described population in unit area or a volume of the earth surface. It is more précised that the amount of living matter incorporated into a circulatory system of plants (producer), animals (consumer) and micro-organisms (decomposer) in unit area or volume of habitat. Biomass, therefore, can be trees, grasses, crops, aquatic flora and fauna, agricultural residues, fish, etc. (Hossain and Hossain, 1985). Around the homestead area different varieties of tree including crops, shrubs, herbs and aerial plants are grown in nature. Small poultry and livestock production is also a part of home production system.

According to FAO (1986), home gardens are one of the most elaborate systems of indigenous agro-forestry, found most often in tropical and subtropical areas where subsistence land use systems predominate. According to Linda (1990), the high diversity of plant species in village home-gardens assures continuous production of fruits and vegetable, fuel woods, timber and medicinal and cash crops. Michon et al. (1983) noted that the ecological value that they represent in items of genetic diversity and preservation of species in areas when original forest resources have been largely depleted.

Biomass is the principal source of domestic energy in the country. It has been the primary source of energy for cooking, heating and other basic needs since pre-historic time (Sarhandi, 1985). About half of the population in the present world use biomass for cooking at a rate of 1.3 to 2.5 m3 capita-1 yr-1. In Bangladesh contribution of biomass to meet the total energy demand is nearly 63% (Islam, 1991), as compared to only 37% commercial resources. In rural areas the share of biomass is overwhelming.

Biomass resource is now being depleted very quickly due to population pressure and intensive cropping with the use of high input high output technology. Actually basic information on biomass production and utilization at the community and national level are very scarce and limited. It needs to initiate micro level studies in Bangladesh to generate information and suggest policy to address the existing situations of biomass production and utilization by adopting appropriate techniques and technologies in haor farming systems and other means. Therefore, it is needed to investigate about biomass production and their utilization in haor areas of Bangladesh. With the above discussion, the present study was undertaken into micro level, concentrated on rural biomass energy scenarios in haor ecosystem.

The general objective of the study is to assess the biomass production and its utilization in haor ecosystem. The specific objectives of the study were:

a) To identify the source of biomass energy in the study area.

b) To assess the biomass utilization pattern in the haor ecosystem.

c) To identify ways and means of increasing the biomass energy fuel.

II. METHODOLOGY

This chapter describes the materials used and methods followed in the experiment. The Research Site situated at Purbo Tethulia village in Mohanganj Upazila of Netrakona District. The Research Site was a representative of flood prone *haor* area which covers 932793 ha i.e. about 6.5% area of Bangladesh.

2.1 Study Area:

2.1.1 Location and climate:

The study was conducted in the village Purbo Tethulia belonging to the Mohanganj Upazila in Netrakona district. Netrakona is situated in the northern part of Bangladesh. The upazila is situated at 29 km east from Netrakona headquarters. The village is located 9 km north-east of Mohanganj Upazila. The village lies in AEZ 21 which is Sylhet Basin.

2.1.2 Socio-economic condition:

There are 462 families in the village consisting of 2245 persons. The male is 1145 and female is 1100 persons. The average family member is 4.86. The literacy rate is 35.6 % in the village. The main source of income of the village is agriculture and fishing.

2.1.3 Soil:

The land topography is mainly low land with non-calcareous dark gray floodplain soils. Soils are medium in organic matter content and acidic in reaction. General fertility level is high with high CEC (Cation Exchange Capacity) but P and K status is low. The experimental field was clayey in textured soil having P^H value of 5.61.

2.2 Population and Sampling Procedure:

The village farmers (usually head of the farm) constituted the population for this study. The population constituted 462 households. Among the population, 102 households were selected for sample that follows the random sampling method.

2.3 Preparation of Survey Schedule:

In order to collect relevant information, a survey schedule or a questionnaire was prepared. The schedule was carefully designed keeping the objectives of the study in view. It contained both open and close form of questions. The schedule was prepared in Bengali. Before finalizing the schedule, it was pretested with 15 households for judging the suitability of schedule and necessary correction and modification were done accordingly.

2.4 Collection of Data:

Data for the study were collected through personal interview during July 2012 to June 2013. Before starting collection of data, the Researcher made an advanced appointment' with the people of LIFCHASA Project area in Purbo Tethulia village by the help of the Scientific Officer (LIFCHASA Project). The Researcher explained the purpose of the study and requested necessary help and co-operation in collecting data from the respondents. They provided information from their memory. In order to minimize the response error questions were asked in simple Bengali. After completion of each interview, it was checked to be sure that information had been properly recorded.

2.5 Variable of the Study:

In this study, the independent variables were: age, educational qualification, occupation, family size, farm size, homestead size, annual income. Total production of biomass and total utilization of biomass were dependent variables in the research. The procedure was followed to measure these variables are described below.

2.5.1 Measurement of independent variables:

2.5.1.1 Age:

The age of a respondent referred to the period of time from his birth to the time of interview. It was measured in complete year on the basis of his response to item no. 1 of the interview schedule.

2.5.1.2 Educational qualification:

Education level of a respondent was measured by the number of years of schooling he or she completed. The level of education score of a respondent was determined from his response to item no. 2 of the interview schedule (Appendix II). If a respondent did not know how to read and write his/her name literacy score was taken as zero. A score of one was given to that respondent who could sign his/her name only. Besides this, the respondent was given actual score of 1 for every year of schooling i.e. one for class one, 2 for class two, 10 for class SSC, 11 for HSC, 12 for graduation and 13 for masters.

2.5.1.3 Occupation:

The main income source of respondent in a year was assessed as his occupation. A score of zero was given when his occupation was agriculture and fishing, for service that was 1, for business that was 2 and for other occupation of a respondent's score was 3.

2.5.1.4 Family size

The family size of a respondent was measured by the number of his family member including himself, children, wife and parents.

2.5.1.5 Farm size:

Farm size of respondent was measured by the response to question item no. 5 of the interview schedule (Appendix II). Here farm size expressed as hectare and was computed by the following formula.

Farm size = Homestead area with pond+ own land under own cultivation+ land (1)

Taken from others on lease+1/2(own land given to others on *borga* + land taken from others on *borga*)- Land out to others on lease.

The farm was categorized into four sizes as shown in Table 2.

2.5.1.6 Homestead area:

It was measured by the area of the raised land in which the household has its entire dealing unit including living room, kitchen room, cattle shed, front yard, courtyard bushes, bamboo branches, etc. (BBS, 1987). It was expressed in hectare.

2.5.1.7 Annual income:

Annual income was defined as the total earning of the respondent from field crops, vegetables, timber, livestock, fish, service, business and other source. A score of (1) was assigned for each one thousand taka.

2.5.1.8 Problem faced on biomass production and utilization:

It refers to the problem faced by the respondents from different sources and also through their experiences of biomass production and utilization. The respondents were asked seven questions in item no. eight of interview schedule on different aspects of biomass production and utilization. The total assign score on all the questions was 35. A respondent answering a question correctly obtained the full score while for wrong answer he obtained zero score. A partial score was also given in case(s) of partial correct answer of questions. The total score obtained by the respondent was taken as his problem faced on biomass production and utilization.

2.5.2 Measurement of dependent variables:

2.5.2.1 Total production of biomass household^{-1}:

Total production of biomass household^{-1} was measured by the researcher. It was dependent on the total of different production components of a farm such as homestead trees, vegetables, field crops, livestock, households waste and weed etc. Some expert's opinions were taken by the researcher in this study (discussed later). It was expressed in tones.

2.5.2.2 Total utilization of biomass household^{-1}:

Total production of biomass household^{-1} was measured by the researcher himself with the help of respondent. Some expert's opinions were taken by the researcher in this study (discussed later). It was also dependent on the total of various utilization items such as fuel, organic fertilizers, animal feeds in each homestead. It was expressed in tones.

2.6 Biomass Estimation:

2.6.1 Estimation of biomass from field crops and vegetable:

The total amount of field crops and vegetable of selected farms during July 2012 to June 2013 were estimated with the help of SAAO experts. Biomass and productivity of crops were determined by the harvest method (Odum, 1960). Crops were harvested during their peak growth or ripening stage. First 10 sample plots of each crop/vegetable was selected. Then 5 to 10 number of quadrates (1m × 1m) was taken from each sample plots. The sample was dried in open sunlight then it was estimated in dry basis. The dry weight of sample was converted to hectare basis.

2.6.2 Estimation of biomass from trees in a homestead:

The total weight of leaves, twigs, branches wood etc. of trees homestead^{-1} during July 2012 to June 2013 was surveyed from 102 households in the village. Homestead^{-1} tree biomass was estimated by the Researcher himself with the help of expert's judgment where default values had to be used in each homestead.

2.6.3 Estimation of biomass from household wastes and weeds:

2.6.3.1 Household wastes:

The amount of household waste e.g. cooking waste, cleaning waste etc. (dry basis) were assessed from each household. Based on their opinions, the Researcher observed, weighed dry matter and was expressed in ton.

2.6.3.2 Weed biomass:

The data on weed biomass were taken from different crop lands and also from fallow land. The biomass of weeds was determined using a quadrate of (1m × 1m). The number of quadrate was 3 to 5 in each of five sampled plots for each

crop/vegetable/fallow land. Then weeds were cleaned, washed and air-dried and finally, dried in open sunlight and estimated in dry basis as done in crops and converted to hectare basis.

2.6.4 Estimation of biomass from livestock/poultry in a homestead:

2.6.4.1 Cowdung:

The total number of cattle present household^{-1} in July 2012 to June 2013 in the village was recorded. The quantity of cowdung (dry basis) was calculated by multiplication of the cowdung production head^{-1} year^{-1} and the number of cattle in the village. The cowdung (dry basis) quantity head^{-1} year^{-1} was estimated to 0.49t.

2.6.4.2 Goat faeces:

The dry biomass obtained from goat faces head^{-1} in the village was also estimated by same method as used in cattle. The quantity of biomass was estimated 18.25 kg head^{-1} year^{-1}.

2.6.4.3 Poultry excreta:

Similar method was also used for estimation of poultry excreta. The quantity of biomass was estimated 10.95 kg head^{-1} year^{-1} (Uddin, 1991).

2.6.5 Estimation of fuel biomass:

The respondents were the housewives of sampled farmers or the senior women member of the households. The respondent was asked about the fuel items and total amount of a day, she used usually. Based on her opinion, amount of those fuel items were weighed and was expressed in ton year^{-1}.

2.6.6 Estimation of organic fertilizer and other biomass which was used in selected farms:

The uses of total amount of organic fertilizer and other biomass in selected farms were surveyed and estimated by the researcher based on farmers' opinion and converted in t yr^{-1}.

2.7 Bio-energy Estimation:

After collection of biomass from all the available sources like field crops and vegetables, homestead trees, weeds and animals bio-energy were calculated from each source multiplied by their specific conversion factors. Each and every source had a definite factor that were given in Appendix III.

2.8 Biodiversity:

The farmers of the homestead in the village were asked about the names of trees, field crops, vegetable and weed species. Besides, the researcher himself identified the species diversity through frequent visit in the village.

2.9 Analysis of Data:

After completion of the field survey, the information obtained from all the respondents were coded, compiled, tabulated and analyzed according to the objectives of the study. Local units were converted into standard units. The responses to the questions in the interview schedule were transferred to a master to facilitate tabulation for statistical analysis. Statistical means such as number and percentage, distribution, range, mean and standard deviation were used to find out the relationship between selected variables of the farmers of homestead in Purbo Tethulia village and total production of biomass household^{-1} and total utilization of biomass household^{-1}. Pearson Product Moment correlation was used.

III. RESULTS AND DISCUSSION

In this chapter findings to address the objectives of the study have been presented in following parts- the Selected Characteristics of the Respondents, Biodiversity, Biomass Production, Bio-energy Production, Relationship between Variables and Policy Option.

3.1 Selected Characteristics of Respondents:

In this study, eight characteristics of the respondents such as (i) age, (ii) education, (iii) occupation, (iv) family size, (v) farm size, (vi) homestead area, (vii) annual income and (viii) problem faced on biomass production and utilization.

3.1.1 Age:

The age of the respondents ranged from 25-70 with an average 43.03 years with standard deviation 10.79 (Table 2). The distribution of farmers according to age 52.9% were middle aged, 24.5% old aged and 22.5% young aged. Thus, the majority of farmers were middle aged.

3.1.2 Educational qualification:

The education level of respondents ranged from 0-12 years of schooling with an average of 5.56 with standard deviation 4.43. In this study, 40.2% of respondents had primary level education categories, Agriculture and fish capturing were the major occupation of 62.75% respondents whereas 13.7% of them were illiterate, 28.4% had secondary level and 17.6% had upper secondary level education (Table 1).

3.1.3 Occupation:

The occupation of respondents were classified into four categories that ranged from 0-3 scaling with an average of 0.82 with 1.14 standard deviation.

TABLE 1
CATEGORY AND DISTRIBUTION OF THE SELECTED CHARACTERISTICS OF RESPONDENTS IN THE PURBO TETHULIA VILLAGE JULY 2012 TO JUNE 2013

Variable	Observed Range	Scoring method	Category	Farmer No	Farmer %	Mean	S.D
Age	20-70	Year	Young age(20-35)	23	22.5	43.03	10.79
			Middle age(36-50)	54	52.9		
			Old age(50)	25	24.5		
Education	0-12	Year of schooling (0-13)	Illiterate(no schooling)	14	13.7	5.56	4.33
			Primary level (0-5)	41	40.2		
			Secondary level (6-10)	29	28.4		
			Above Secondary (>10)	14	17.6		
Occupation	0-3	Scaling	Agriculture and fishing capture (0)	64	62.75	0.82	1.13
			Service (1)	13	12.75		
			Business (2)	14	13.72		
			other (3)	11	10.78		
Family size	13-Feb	Number of member	Small family (4)	37	36.2	5.73	2.43
			Medium family (5-8)	53	52		
			Large family (>9)	12	11.8		
Farm size	0-3.15	Farm size	Landless (<0.2 ha)	40	39.22	0.86	0.71
			Marginal and small (0.2-1.0) ha	29	28.43		
			Medium (1.0-2.0) ha	22	21.57		
			Large (>2.0 ha)	11	10.78		
Homestead area	0-.75	Farm size	Small (upto 0.02)	37	36.3	0.24	0.23
			Medium (0.021-0.05)	10	9.8		
			Large (>0.05)	55	53.9		
Annual income	25-210	Take in thousand	Very low income (upto 25)	29	28.4	94.1	55.03
			Low income (26-40)	35	34.3		
			Medium income (41-75)	12	11.8		
			High income (>75)	26	25.5		
Problem faced on biomass production and utilization	0-35	Marking (0-35)	No problem (upto 3)	14	13.7	14.94	10.12
			Minimum problem (4-15)	23	22.5		
			Medium problem (15-25)	29	28.4		
			More problem (>25)	36	35.3		

S.D= Standard deviation

3.1.4 Family size:

The family size scores of the farmers from 2-13 with mean 5.73 with standard deviation 2.43. Most of the farmers (52%) had medium family compared to 36.2% small family and 11.8% farmers had large family.

3.1.5 Farm size:

In the study the farm size ranged from 0.01-3.15 ha with mean of 0.86 and standard deviation 0.709 (Table 1). Most of the farmers (41.2%) had landless followed by 10.78% large, 21.57% medium, and 28.43% small and marginal.

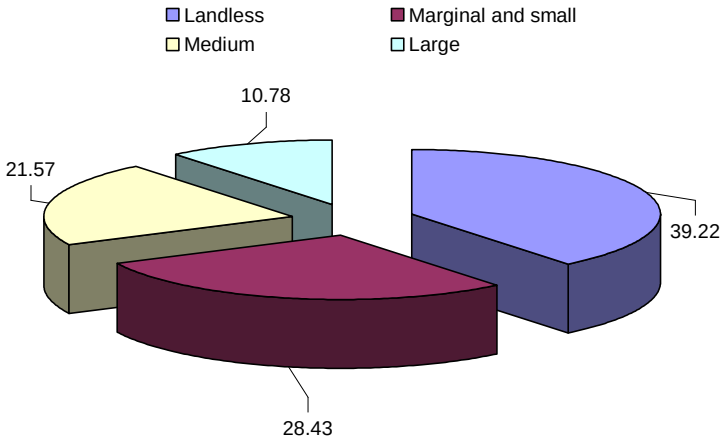

FIGURE 1: Categories of farmers according to farm size in Purbo Tethulia village, July 2012 to June 2013

3.1.6 Homestead area:

The homestead area of the farmer ranged from 0.01-0.75 ha with mean of 0.24 and standard deviation of 0.23. Among the farmers 36.3% was small, 9.8% was medium and 53.9% had large homestead area.

3.1.7 Annual income:

The annual income of the respondents varied from Tk. 25.9 thousand to Tk. 210 thousand with the mean of Tk. 94.10 thousand (Table 1) and standard deviation 55.03. Among the respondents 28.4% was in very low income, 34.3% in low income. 11.8% in medium income and 25.5% of respondents was in high income.

3.1.8 Problem faced on biomass production and utilization:

Knowledge on biomass production and utilization of respondents' sources ranged from 2-32 with mean 25.73 and standard deviation 5.77. The researcher observed that among respondents 13.7% of farmers no knowledge, 22.5% of farmers had minimum, 28.4% of farmer's had medium and 35.3% of farmer's had good knowledge on biomass production and utilization.

3.2 Biodiversity:

3.2.1 Species richness in study area:

In the study area, species richness and diversity were observed at different locations. Among the species, 16 fruit tree, 7 forest, 17 vegetable, 4 spice, 10 field crop species were found during July 2012 to June 2013.

3.2.1.1 Homestead plantation:

A wide variety of trees were found in selected homestead of the village during July 2012 to June 2013 (Table 4). A total number of 23 plant species of 22 families were observed in homestead area of which 16 were fruit species of 15 families and 7 forest species of 6 families. In the study area most available fruit tree species were mango. Jackfruit, litchi, coconut, guava, papaya were found. Among the forest species bamboo, Kadam, mahogani, hijal, karoch, etc. were also observed.

3.2.1.2 Field crop and vegetable:

The field crop and vegetable grown in selected farms in village Purbo Tethulia at different locations (homestead and field) are listed in Table 4. It was observed that 27 species of 13 families of crops and vegetable were grown by the respondents during July 2012 to June 2013. In this area cultivation was mainly crop based but rice was the main crop.

3.2.1.3 Weed vegetation:

In the study area 25 species of weeds belonging to 15 families were found which are given in Table 4. Farmers of the village opined that weed infestation was more in *Boro* season than in other seasons.

3.2.2 Livestock population:

The total number of cattle, goat, and duck and chicken in homestead were 527, 168 and 1102, respectively with 5.17, 1.65 and 10.80 farm^{-1}.

3.3 Biomass Production:

Biomass production is a flexible issue. It varies with land type, season, climate, soil, and other physical and biological environments. It also varies with farmers' choice, cultural, socio-political and socio- economic condition. In this study, the biomass production was estimated on field crops and vegetables, homestead plantations, weeds, household wastes and livestock excreta etc.

TABLE 2
TOTAL AMOUNT OF BIOMASS OBTAINED FROM DIFFERENT FIELD CROPS AND VEGETABLE ACCORDING TO FARM SIZE AND THE AMOUNT OF ENERGY OBTAINED FROM THE FIELD CROPS IN SELECTED HOMESTEAD OF PURBO TETHULIA VILLAGE, JULY 2012 TO JUNE 2013.

Field crops and vegetable biomass/Farm size	Estimated field crops and vegetable biomass production (t)**				Total biomass (t)	Estimated total bio-energy (GJ)*
	Large	Medium	Marginal and small	Landless		
Rice	58.99	56.85	43.03	5.52	164.39	2751.89
Dhaincha	1.32	0.81	0.36	-	2.49	48.01
Jute	2.36	1.32	-	-	3.88	76.13
Other (field crops and vegetable)	55.83	45.62	42.32	8.48	152.25	2987.15
Total	118.5	104.6	85.71	14	323.01	5863.18
Average farm^{-1}	10.77	4, 75	2.95	0.35		57.48

**** 1 t of rice biomass =16.74 GJ***
**** 1 t of dhaincha biomass =19.28 GJ***
**** 1 t of jute biomass =19.62 GJ***
**** 1 t of other crop and vegetable biomass biomass =16 GJ***

TABLE 3
TOTAL AMOUNT OF BIOMASS AND ENERGY FROM TREES ACCORDING TO FARM SIZE IN SELECTED HOMESTEADS OF PURBO TETHULIA, JULY 2012 TO JUNE 2013

Farm size	Estimated biomass		Estimated total energy (GJ)*
	Total	Farm^{-1}	
Large	20.23	1.84	303.45
Medium	21.85	0.99	327.75
Small and marginal	21.82	0.75	327.3
Landless	3.36	0.08	50.4
Total	67.26	-	1008.9

**** 1 t wood biomass = 15 GJ***

3.4 Bio-energy Production:

Bio-energy obtained from different sources in homesteads of Purbo Tethulia village are shown in Fig 10. In the study area, annual total production of bio-energy was estimated to 11324.11 GJ that was 111.02 GJ farm^{-1}. It came from different sources of biomass. The estimated energy obtained from different crops varied 15.79 GJ in banana to 19.28 GJ in *dhaincha.* The highest energy estimated 5863.18 GJ from rice biomass and the lowest 36.96 GJ from goat biomass. The total bio-energy obtained from field crop and vegetable in 102 selected homesteads was estimated 5863.18 GJ yr^{-1}.

Total tree bio-energy obtained in selected homesteads area 1008.9 GJ yr^{-1}. The total energy obtained in selected area from weeds and household waste was 3038.17 GJ yr^{-1} where 2458.46 GJ yr^{-1} from weeds and 487.14 GJ yr^{-1} from household waste

(Table 8) and from the livestock population in 102 selected homestead was 1413.86 GJ $yr^{-1.}$ Data in Table 11 indicated the total energy from different sources $farm^{-1}$. On the average 57.48, 9.89, 13.86 and 329.79 GJ $farm^{-1}$ yr^{-1} bio- energy were produced from field crop and vegetable, homestead plantation, animal excreta and homestead waste, weed, respectively.

TABLE 4
TOTAL AMOUNT OF BIOMASS AND ENERGY FROM WEEDS AND HOUSEHOLDS WASTE ACCORDING TO FIRM SIZE IN SELECTED HOMESTEADS OF PURBO TETHULIA, JULY 2012 TO JUNE 2013

Farm size	Estimated biomass (t yr^{-1})				Estimated energy (GJ)*yr^{-1}		
	Weed biomass	Household biomass	Total biomass	$Farm^{-1}$	Weeds	Household waste	Total
Large	27.2	18.56	45.76	4.16	353.6	107.65	461.25
Medium	66.47	30.1	96.57	4.38	864.11	174.58	1038.69
Marginal and small	85.25	32.09	117.34	4.09	1109.25	186.12	1295.37
Landless	10.2	3.24	13.44	0.33	132.6	18.79	157.5
Total	189.03	83.99	273.11	-	2458.56	487.14	3038.17

**** 1 t weed residue = 13 GJ energy***
**** 1 t dry household waste = 5.8 GJ energy***

TABLE 5
BIOMASS AND ENERGY FROM ANIMAL EXCRETA ACCORDING TO FIRM SIZE IN SELECTED HOMESTEADS OF PURBO TETHULIA VILLAGE, JULY 2012 TO JUNE 2013

Farm size\Biomass	Biomass estimation (t)			Total t yr^{-1}	$Farm^{-1}$	Estimated total energy (GJ)
	Cattle	Goat	Poultry			
Large	11	1.2	3.2	15.4	1.4	215.6
Medium	25	0.6	4.85	30.45	1.38	426.3
Marginal and small	39.63	0.84	9.25	50.93	1.75	676.2
Landless	1.7	0.16	2.34	4.2	0.11	58.8
Total	77.33	2.8	19.64	100.99	-	1413.86

**** 1 t of dry animal excreta = 14 GJ.***

3.5 Biomass Utilization:

In the study area, biomass was used in various purposes as bio-fuel, organic fertilizer and other purposes. Utilization of biomass for other purposes included animal feed, animal bedding, housing materials, mulching etc.

TABLE 6
BIOMASS PRODUCTION $FARM^{-1}$ IN PURBO TETHULIA VILLAGE, JULY 2012 TO JUNE 2013

Source/Farm size	Total biomass production (t $farm^{-1}yr^{-1}$)				
	Large	Medium	Small and marginal	Landless	All
Field crops and vegetable	10.77	4.75	2.95	**0.35**	4.71
Homestead plantation	1.84	0.99	0.75	0.08	0.92
Household waste and weeds	4.16	4.38	4.09	0.33	3.25
Livestock excreta	1.4	1.38	1.75	0.11	1.16
Total	17.11	11.5	9.54	0.87	10.04

TABLE 7
TOTAL ENERGY VALUE PRODUCED FROM DIFFERENT BIO SOURCES IN SELECTED HOMESTEADS AT PURBO TETHULIA VILLAGE, JULY 2012 TO JUNE 2013

Source\Energy	Total energy produced (GJ yr^{-1})	Estimated annual energy (GJ $farm^{-1}$)
Field crops and Vegetable	5863.18	57.48
Homestead Plantation	1008.9	9.89
Household waste and weeds	3038.17	29.79
Livestock excreta	1413.86	13.86
Total	11324.11	111.02

TABLE 8
SOURCE AND UTILIZATION OF BIOMASS IN THE STUDY AREA OF VILLAGE PURBO TETHULIA, JULY 2012 TO JUNE 2013

Source of biomass	Product	Utilization
A. Field crops and Vegetable	a. Rice straw	1. Animal feed
		2. Animal bedding
		3. Mulching
		4. Housing material
		5. Fuel
	b. Rice polish	1. Poultry
		2. Cattle feed
	c. Rice husk	1. Fuel
		2. Poultry bedding
	d. Jute stick	1. Fuel
	e. Mustard plants	1. Fuel
	f. Vegetable plants	1. Fuel
	g. Weeds	1. Fuel
		2. Fodder
		3. Compost
B. Homestead plantation	a. Leaves, twigs and branches	1. Fuel,
		2. Fodder
	b. Wood	1. Furniture
		2. Fuel
C. Animal excreta / waste	a. Cowdung	1. Manure
		2. Fuel
	b. Poultry excreta	1. Manure
	c. Cattle bedding material	1. Compost
	d. Goats faces	1. Manure
D. Household waste	a. Kitchen waste	1. Manure
		2. Animal feed
	b. Cleaning waste	1. Compost

3.6 Total utilization of biomass:

The total amount of biomass utilization in selected homestead with bio-fuel, organic fertilizer and other purposes are shown in Table 15. It shows annual biomass used 14.89 t farm^{-1}. According to farm size, large farm used more (20.75 t farm^{-1} yr^{-1}) than others. The landless, marginal and small, and medium used 1.53, 16.24, 21.78 t farm^{-1} yr^{-1}, respectively.

TABLE 9
BIOMASS UTILIZATION IN SELECTED HOMESTEADS AT PURBO TETHULIA VILLAGE, JULY 2012 TO JUNE 2013

Purpose	Biomass utilization t farm^{-1}yr^{-1}				
	Large	Medium	Small and marginal	Landless	All
Bio-fuel	6.31	6.98	5.77	0.95	5
Organic fertilizer	8.16	8.21	4.92	0.25	5.39
Other (animal feed, animal bedding, housing materials etc)	6.25	6.59	5.55	0.33	4.16
Total	20.75	21.78	16.24	1.53	14.55

3.7 Problem Faced by the Farmers on Biomass Production and Utilization:

The major problem by the farmers on biomass production and utilization was sometime decomposition of biomass by rain opined by 65% respondents, who felt the problem of "wet fuel materials caused severe problem for fire". Some of the respondents (38%) said that insects and diseases were increased by the manure or organic fertilizer and storage problem (due to lack of labour) also found in 65% respondents. Some respondents (45%) measured other problems like flood caused unfavorable effect on trees, crops and animals which were the source of biomass. somewhere stolen problem, sometime epidemic exposes on livestock, crop fields etc. 10% of respondents mentioned that they did not feel any problem on production and utilization of biomass.

TABLE 10
PROBLEM FACED BY THE RESPONDENTS ON BIOMASS PRODUCTION AND UTILIZATION IN SELECTED HOMESTEADS OF PURBO TETHULIA VILLAGE, JULY 2012 TO JUNE 2013

Problem	% of respondents
1. Biomass decomposed by rain some time	65
2. Increased intensity of insects and diseases by the manure/ organic fertilizer	38
3. Storage problem due to lack of labor	65
4. Wet fuel materials caused severe problem for fire	70
5. Other	45
6. No problem	10

TABLE 11
FARMERS' CHOICE IN SITE SELECTION FOR TREE PLANTATION IN SELECTED HOMESTEADS OF PURBO TETHULIA VILLAGE, JULY 2012 TO JUNE 2013

Place of tree plantation	% of respondents
Homestead area	98
Crop land	5
Other (Road side, river bank)	15
No response	3

3.8 Relationship Between the Selected Characteristics of the Farmers vs. effect of their Total Production of Biomass and Total Utilization of Biomass:

This section deals with the relationships between selected characteristics (independent variables) of the respondents and effect of their total production of biomass and total utilization of biomass (dependent variables). Pearson's Product Moment Correlations Coefficient "r" was used to test the hypothesis concerning the relationship between two variables and 0.05 level of significant was used. A null hypothesis rejected when the observed "r" value was greater than tabulated value of "r" at 0.05 percent level of probability. The possible correlation between 8 independent and 2 dependent variables are presented in Table 18.

TABLE 12
RELATIONSHIP BETWEEN THE SELECTED CHARACTERISTICS OF THE FARMERS VS. EFFECT OF THEIR TOTAL PRODUCTION OF BIOMASS AND TOTAL UTILIZATION OF BIOMASS

	Computed ' r value (df = 102)	
Characteristics	**Total production of biomass**	**Total utilization of biomass**
Age	0.476**	0.381**
Education qualification	0.488**	0.403**
Occupation	-0.074^{NS}	0.195*
Family size	0.105^{NS}	-0.225*
Farm size	0.871**	0.567**
Homestead area	0.311**	0.238*
Annual income	0.555**	0.281**
Problem faced on biomass production and utilization	-0.026^{NS}	-0.057^{NS}

***** = Significant level at 1%***
**** = Significant level at 5%***
NS = Not Significant

IV. CONCLUSION AND RECOMMENDATION

In Bangladesh, population growth is high but per capita land area is decreasing day by day. The growing population need food, fuel-energy, shelter, etc. which need biomass resources. The biomass production increases with the increasing farm size. It also competes with the biomass utilization. There exists conflict between fuel and organic fertilizer. Bio-energy puts great stress on biomass resources. A severe imbalance between production and utilization of biomass as fuel prevails in the village and ultimate pressure face by the poor farmers specially the landless. Biomass production was not equally proportionate among large, medium, small and landless farmers. It might be due to the difference in ability, technology know-how and variation in land resource of the farmers.

From the study it is evident that farmers from all categories had to maintain the useful energy status for their livelihood. But scarcity of fuel wood and lower utilization efficiency caused much hardship for the farmers specially poor farmers. The most alarming situation was that cowdung and households waste were mostly used as fuel instead of maturing in crop field. The possibility intervention by homestead agro-forestry practices should be considered as the urgent awaiting thought.

The study suggested to:

a) Increase biomass production through creating awareness about energy crisis among general people.

b) Judicious and efficient use of biomass energy with the improved Chula to overcome this situation.

c) Government initiative is needed.

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Determinants of Farmers' Willingness to Pay for Improved Varieties of Pigeon Pea in Oyo State, Nigeria

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Abstract— *Despite the pigeon pea's significance as a nutritious and affordable protein source, its production remains low due to the lack of improved, high-yielding seed varieties. This study, therefore, examines the factors influencing farmers' willingness to pay (WTP) for improved pigeon pea varieties in Oyo State, Nigeria. Using a multi-stage sampling technique, 150 pigeon pea farmers were selected, and data were collected through a structured questionnaire. The contingent valuation method and logistic regression model were employed to analyze WTP and its determinants. Results indicate that while farmers lacked awareness of improved varieties, all respondents expressed a willingness to pay for seeds with desirable traits such as drought tolerance, early maturity, and high yield. Key determinants of WTP included age, farming experience, farm size, marital status, and farm income. The study concludes that improving access to enhanced seed varieties and raising farmer awareness are crucial steps toward increasing pigeon pea production and strengthening food security in Nigeria.*

Keywords— Pigeon Pea, Willingness to Pay, Improved Varieties, Farmers, Nigeria.

I. INTRODUCTION

Pigeon pea *(Cajanus cajan)* is a crucial member of the pulse family, recognized for its diversity in colors, sizes, and shapes. This leguminous crop is celebrated globally for its high nutritional value, comparable to other significant legumes such as cowpea, groundnut, and soybean. In 2022, global pigeon pea production reached approximately 5.33 million tonnes, cultivated over 6.03 million hectares (FAO, 2022). The crop is cultivated in over 82 countries, with India being the dominant producer, accounting for about 79.10% (4.22 million tonnes) of total production, followed closely by Myanmar (Ahamad and Kiresur, 2016). Pigeon pea plays a crucial role in India's food security and economy, contributing substantially to both food processing and the pharmaceutical industry.

In Africa, pigeon pea production was approximately 0.72 million tonnes in 2022, serving as a vital export crop in nations like Malawi, Tanzania, and Uganda (FAO, 2022). These countries have invested in research to enhance the productivity and acceptability of pigeon pea, recognizing its potential to foster economic growth through exports. Despite the crop's importance, pigeon pea production in Nigeria remains limited, with an estimated cultivation area of only 190,000 hectares—less than 4% of global production (Egbe and Vange, 2008). The scarcity of research and data on pigeon pea in Nigeria underscores its underutilization, with most efforts historically concentrated on major food crops (Popoola, 2020; Kuraz, 2022; Munialo, 2024).

Pigeon pea is a valuable source of affordable protein, providing a necessary dietary supplement for rural communities predominantly reliant on starch-based diets (Tanimonure et al., 2023). In light of the Sustainable Development Goals (SDGs) aimed at eradicating poverty and hunger, there is a growing emphasis on underutilized crops such as pigeon pea as avenues for

economic empowerment and nutritional security. As an alternative to increasingly unaffordable animal protein sources, leguminous crops like pigeon pea present an essential solution for rural households (Popoola et al., 2022).

Despite its economic and nutritional benefits, pigeon pea productivity in Nigeria has stagnated, primarily due to the lack of high-yielding improved varieties and the unfavorable cooking characteristics of local varieties (Fatokimi and Tanimonure, 2021; Esan & Ojemola, 2018). Recent studies indicate that the area allocated to pigeon pea cultivation in Nigeria is insufficient, with farmers likely to maintain their current farm sizes without significant interventions, such as the introduction of improved varieties and increased awareness of the crop's nutritional advantages (Fatokimi & Tanimonure, 2021; Tanimonure et al., 2023). To enhance productivity, there is a pressing need for improved pigeon pea varieties that align with both farmer and consumer preferences (Tanimonure et al., 2023).

Research initiatives led by the International Crops Research Institute for Semi-Arid Tropics (ICRISAT) have focused on developing early-maturing pigeon pea varieties resistant to diseases such as Fusarium wilt and pod borer. These advancements aim to facilitate large-scale production and commercialization (Vales et al., 2012; Zhao et al., 2020). However, assessing farmers' willingness to pay (WTP) for these improved varieties is crucial. Understanding this willingness will inform researchers, policymakers, and breeders about the preferences of both farmers and consumers, ultimately enhancing the successful adoption of improved pigeon pea varieties.

Government and non-governmental organizations (NGOs) play a pivotal role in making improved seed varieties accessible to smallholder farmers through subsidized initiatives or free distributions. Increased productivity through technology adoption is imperative for the income generation and nutritional security among rural populations, forming a foundation for achieving a world free from poverty and hunger (Kumar and Sultana, 2010; IFAD, 2024).

While existing literature has documented farmers' willingness to pay for improved varieties across various crops, including cowpeas, soybeans, millet, and teff (Ongudi et al., 2017; Lugamara *et al*., 2021; Adjei-Nsiah *et al*., 2022; Tilahun and Tadesse, 2022; Agossou et al., 2023), there is a notable lack of research on the willingness to pay for improved pigeon pea varieties that offer desirable traits such as early maturity, drought tolerance, and high yield. This knowledge gap may hinder efforts aimed at promoting pigeon pea production in Nigeria. Therefore, analyzing farmers' willingness to pay is essential for informing breeders and enhancing the widespread cultivation of pigeon pea in Nigeria.

Consequently, this study seeks to investigate farmers' willingness to pay for improved varieties of pigeon pea in Oyo State, Nigeria, addressing the critical challenges that currently constrain its production and identifying pathways for enhancing its contribution to food security and economic development. The objectives of this study therefore are to: 1. describe the socioeconomic characteristics of the pigeon pea farmers; 2. describe their level of awareness of improved varieties of pigeon pea and 3. analyze their willingness to pay for improved varieties of pigeon pea.

II. METHODOLOGY

The research was conducted in Oyo State, Nigeria. The state was purposively selected because of high level of the farmers involvement in pigeon pea production in the derived savanna area of the State. Oyo State was created on February 3, 1976, in southwestern Nigeria. The state shares boundaries with Kwara State in the north, Osun State in the east, and Ogun State and the Republic of Benin in the west. Oyo State has a total land area of 28,454 square kilometers. It is situated within the tropics and lies between latitudes 7^0 3′ and 9^0 12′ north of the equator and longitudes 2^o 47′ and 4^0 23′ east of the Meridian, with a temperature that ranges between 21^{o}C and 39^{o}C. The population of Oyo State is estimated to be 5.58 million, according to the National Population Census (NPopC, 2006), with 33 Local Government Areas (LGAs). Rainforest and derived savanna are the two ecological zones in the state. The most commonly cultivated crop are yam, maize, millet, rice, cocoa, cowpea, cashew, plantains and pigeon pea among others. According to OYSADEP, Oyo State is stratified into four agricultural zones A to D (Ibadan, Saki, Oyo, and Ogbomoso zones).

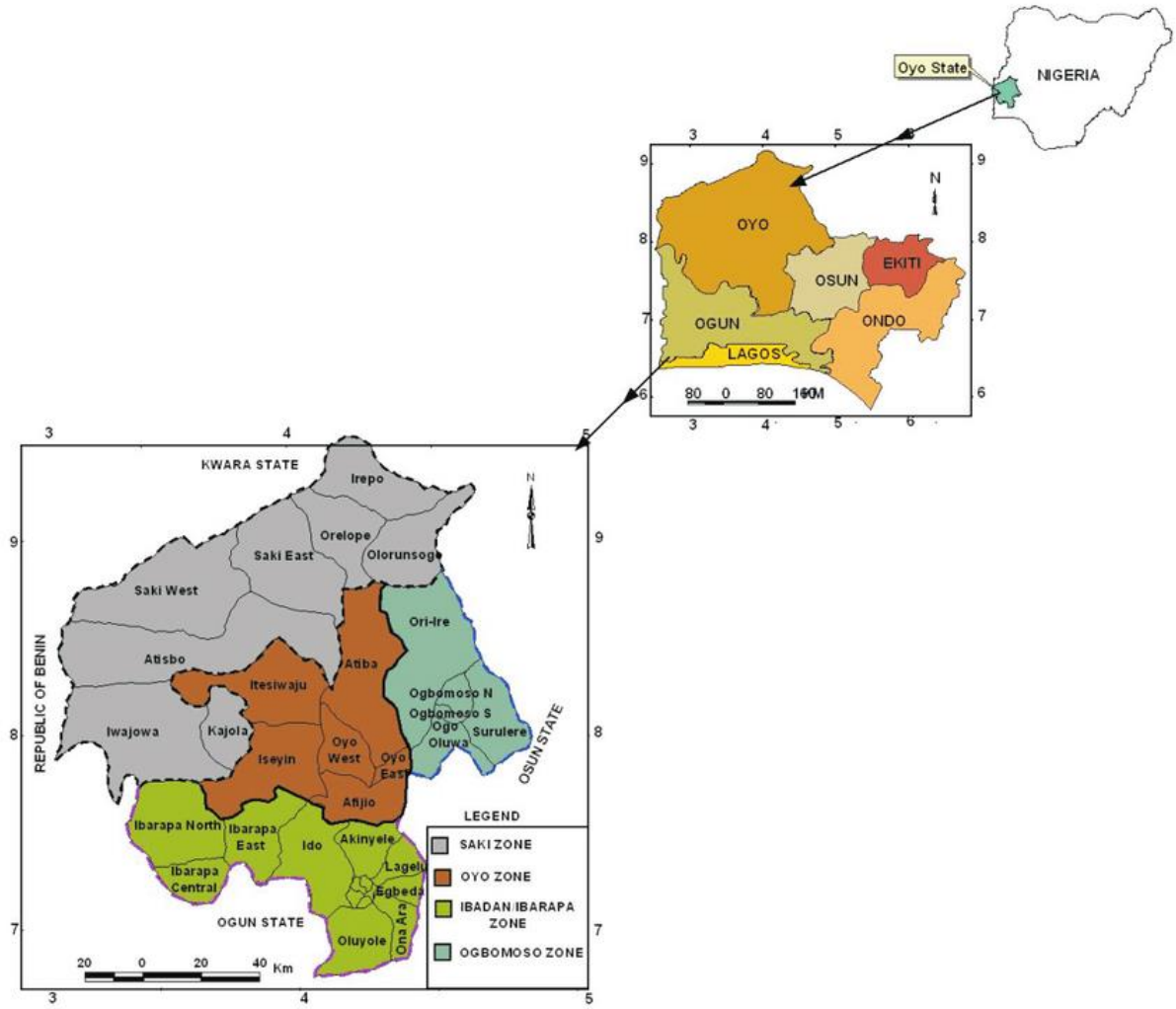

FIGURE 1: Map of Oyo State, Nigeria, Showing the Study Area (Saki Zone) for Pigeon Pea Farmer Survey

A Multi-stage sampling procedure was adopted to select the farmers for the study. The first stage involved the purposive selection of Zone B (Saki Zone), which is located in the derived savanna of Oyo State where pigeon pea is commonly cultivated. The zone comprises 8 LGAs. At the second stage, five LGAs (Saki East, Saki West, Oorelope, Atisbo, and Kajola), areas with high numbers of pigeon pea farmers, were purposively selected.

In the third stage, a list of the registered farmers cultivating pigeon pea was obtained from the Oyo State Agricultural and Development program (OYSADEP) and proportionate sampling technique was used to select 50% of the farmers from each LGA to arrive at 150 pigeon pea farmers. A Structured questionnaire was used for data collection. Information like age, gender, farming experience, household size, level of education, farm size of pigeon pea, cost of local seed, yield, economic value of the crop which was measured using its market value at sowing, among other information were gathered using interview schedule approach. For willingness to pay, the method of willingness to pay was the stated preference question type with dichotomous choice contingent valuation methods (Agossou et al., 2023). Also, information on the premium amount farmers were willing to pay for the improved pigeon peas was also gathered.

2.1 Method of analysis:

All analyses were performed using Stata 15. Descriptive statistics (mean, standard deviation, frequences and percentages) were used for the socioeconomic characteristics data. The Contingent valuation method was used to capture farmers' WTP. Logistic regression model was used to analyze the determinants of the farmers' willingness to pay for improved pigeon pea variety.

Contingent Valuation approach involves several stages of calculation. The first stage is to create a hypothetical market to provide explanations of the potential attributes of the varieties such as drought tolerance, disease resistance, shorter cooking time, early maturity among others. By this explanation, it is expected that the farmers will become aware of the importance of

the provision of the improved seed technology. At the second stage, the bidding game technique, which is a technique used by giving questions repeatedly to respondents until they get a certain amount of willingness to pay was used by using the price of the current varieties cultivated by the farmers and increasing it by 20%, 50% and 100%.

2.2 Logistic regression model:

A Logistic regression model was used to analyze the determinants of pigeon pea farmer's willingness to pay for improved varieties of pigeon pea. The logistic regression analysis is the task of estimating the log odds of an event. The log odds ratio is the ratio of two odds and it is a summary measure of the relationship between two variables (Yila etal, 2023). Mathematically, Logistic regression estimates a multiple linear regression function defined as;

$$P) = \text{Log } log\left[\frac{(y=1)}{1-(p=1)}\right] = \beta + \beta x + \beta x + \cdots \ldots \ldots \ldots \ldots + \beta x$$

For i= 1,2,3,…….n

Logistic regression can be binomial, ordinal or multinomial. Binomial or binary regression deals with situation in which the observed outcome for a dependent variable can only have two possible types. '0' and '1'. Multinomial logistic regression deals with situations where the outcome can have three or more possible types that are not ordered. Ordered logistic regression deals with dependent variables that are ordered.

Binary logistic regression was used tin this analysis to predict the odds of being a case based on the values of the independent variables (predictors) the odds are defined as the probability that a particular outcome is a case divided by the probability that it is not a case.

In the case of this study, The Logit model is presented as;

$$P = \frac{\exp(z)}{1+\exp(z)} \quad (1)$$

Where P is the proportion of occurrence

$$Z = \beta_0 + \beta_1 X_1 + \ldots\ldots\ldots + \beta_n X_n \quad (2)$$

Where X_1..... X_n are the explanatory variables. The inverse relation of Equation 1 is:

$$P = In\frac{P}{(1-P)} \quad (3)$$

That is, the natural logarithm of the odds ratio, known as the logit. It transforms P which is restricted to the range [0, 1] to a range $[-\infty, \infty]$.

The dummy variable willingness to pay will be used as a dependent variable for the analysis to estimate the coefficient of factors that determine willingness to pay for improved pigeon pea varieties.

The explicit form of the functional relationship of the variables expressed above is:

$$y^* = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_4 + \beta_5 X_5 + \beta_6 X_6 + \beta_7 X_7 + ei \quad (4)$$

The independent variables include:

Y* = Individual willingness to pay for improved variety of pigeon pea 1 for farmers willing to pay a premium for the improved varieties and 0 otherwise.

X_1= age of the respondent (in years)

X_2= Years of Experience (duration in years)

X_3= Farm size

X_4= years of formal education

X_5= Marital status (Married = 1, not married =0)

X_6=off farm income, (specified in Naira)

X_7=farm income, (specified in Naira)

X_8= full time farming

X_9= Access to credit (Yes = 1, No = 0)

X_{10}= Amount willing to pay (specified in Naira)

ei = independently distribution error term

III. RESULTS AND DISCUSSION

3.1 Socioeconomic factors:

The most significant socio-economic factors relevant to determine the respondent's willingness to pay for improved quality of pigeon pea at the threshold price of ₦600 which is equivalent to $1.5 at the time of data collection in 2019.

TABLE 1
SOCIOECONOMIC CHARACTERISTICS OF THE RESPONDENTS

Variables	Observation	Percentage
Total observation	150	-
Mean age	49.07(11.6)	-
Marital status		
Yes	141	94
No	9	6
Access to credit		
Yes	131	87.33
No	19	12.67
Membership of association		
Yes	123	82
No	27	18
Mean household size	5.97(3.98)	-
Number of years of education	8.48(5.09)	-
Mean Off farm income	685,902.02(926,926.40)	-
Mean farm income	670652.34(783500.00)	-
Mean years of experience	18.21(7.93)	-
Mean farm size (in acres)	1.72(1.50)	-

Source: Field survey, 2019.
Values in bracket are standard deviation.

3.2 Awareness on improved pigeon pea varieties among farmers:

The awareness of farmers on pigeon pea improved varieties was very low as shown in Table 2, none of the farmers was aware or has any information about the availability of improved varieties. Despite lack of awareness, majority of the farmers are WTP for the improved seed with preferred attribute such as high yielding, early maturing, shorter cooking time, better taste, and drought tolerance if made available.

TABLE 2
AWARENESS OF IMPROVED PIGEON PEA VARIETY

Improved varieties awareness	Frequency	Percentage
Are you aware of improved pigeon pea varieties		
Yes	0	0
No	150	100
If you have improved varieties with shorter cooking time, are you willing to pay?		
Yes	150	100
No	0	0
If you have early maturing varieties, are you willing to pay?		
Yes	150	100
No	0	0
If you have varieties with high drought tolerant, are you willing to pay?		
Yes	150	100
No	0	0
If you have varieties with better taste, are you willing to pay?		
Yes	150	100
No	0	0
If you have high yielding varieties, are you willing to pay?		
Yes	150	100
No	0	0
Are you willing to pay for improved seed (if made available)		
Yes	0	100
No	150	0

Source: Field survey, 2019.

TABLE 3
WILLINGNESS TO PAY FOR IMPROVED PIGEON PEA VARIETIES AT DIFFERENT PRICE BIDDING SCENARIOS

Willingness to pay	Respondent	Frequency	Mean WTP (₦)
WTP If the price of new variety is same as the old			
Yes	150	100	320.47
No	0	0	
WTP If the price of old variety increased by 20%			
Yes	149	99.3	384.56
No	1	1.7	
WTP If the price of old variety increased by 50%			
Yes	138	92	480.7
No	12	8	
WTP at double the local variety price ₦600 (1.5 USD).			
Yes	127	84.7	640.93
No	23	15.3	
WTP at less than the local price ₦600 (1.5 USD)			
Yes	148	98.7	
no	2	1.3	
Total			1826.66
Average			456.67

Source: field survey, 2019.

3.3 Amount the farmers are willing to pay:

Based on table 3 above, it can be seen that the mean value of WTP obtained in this study in each 1826 naira. Majority of respondents who are willing to pay for improved pigeon pea varieties and they are more concentrated at zero price increase and at 20% increase of the old variety. Additionally, good number of the farmers are still willing to pay even at 100% price increment of the old varieties.

3.4 Determinants of WTP for improved pigeon pea seed varieties:

The farmers' decision regarding their WTP was regressed against the set of independent variables. A logit form was estimated using the dummy variable of WTP, where farmers that are willing to pay at least the threshold price of #600 ($1.5) were scored 1 and those who were not were scored 0. The logit model revealed that the log-likelihood value (-11.179), the pseudo R^2 (0.55), and the chi-square value (27.40) were significant ($P \leq, 0.0001$), which indicates that the overall models were well fitted and the independent variables used in the model were collectively able to explain the pigeon peas farmers' decisions regarding their WTP for highly improved pigeon pea varieties in the study area. Many of the included variables were significant for determining the farmers' WTP for the product.

The results revealed that socioeconomic characteristics of the pigeon pea farmers had significant effects on their WTP for improved pigeon pea varieties that is early maturing, drought tolerance and diseases resistance as shown in table 4. Age of the farmers was negatively and significantly associated with WTP for improved pigeon pea seed (P> 0.01). Years of farming experience was positively and significantly correlated with WTP for Pigeon pea (P >0. 01). The farm size allocated to pigeon pea production was significant at (P> 0.01) and positively correlated with WTP. Marital status of the respondents was significant at (P> 0.01), this indicated that married people are always willing to expand their crop business due to their level of responsibility. Farm income was positively correlated to WTP and it was significant at (P< 0.1).

TABLE 4
DETERMINANTS OF WILLINGNESS TO PAY (WTP) FOR IMPROVED PIGEON PEA IN THE STUDY AREA

Explanatory variable	Odd ratio	Standard error	Z	p>z
Age	0.762872	0.093	-2.22**	0.03
Farming experience	1.324797	0.177	2.11**	0.04
Farm size (acres)	38.46803	62.76	2.24**	0.03
Years of education	1.100352	0.136	0.77	0.44
Marital status	9114.521	36562.35	2.27**	0.02
Off farm income	0.999995	0	-1.32	0.19
Farm income	1.000007	0	1.73*	0.08
Full time farming	0.098085	0.209	-1.05	0.3
_cons	0.000916	0.006	-1.07	0.28

Source: Field survey, 2019.

****, **,*** represent significant level at 1%, 5% and 10% respectively***

This study was conducted to estimate the determinants of WTP for improved pigeon pea seed among pigeon pea farmers in the northern part Oyo State, Nigeria. This was with a view to provide results that could be useful for guiding agricultural research institutions and policy makers on the need for improved pigeon pea seed to the smallholder farmers in Nigeria. The contingent valuation method was employed to analyse farmers' willingness to pay by direct farmer response based on previous variety presently cultivated, a scenario of 100% price increase was presented and the percentage of farmers WTP the threshold was recorded using descriptive statistics. Descriptive statistics were also used to describe and compute statistics such as mean, standard deviation, and frequency distributions. Econometric modelling was employed to determine the factors influencing farmers' WTP for improved pigeon pea seed if it were available. The majority of the farmers (84.7%) are willing to pay 100% price increase of the local variety. The mean WTP estimates for pigeon pea improved seed was ₦600 ($1.5). The average age of the respondents negatively and significantly influenced WTP for the product. The negative value of the coefficient was in line with the a priori expectation that aged farmers tends to have low productivity and therefore have a lower WTP for improved technology). This result is in line with findings of previous studies in which the respondent's age influenced his or her WTP because he or she would lack the strength and agility needed for agricultural activities ((Aye and Edoja 2021,). In this study, education was not significant in influencing WTP decisions this may be due to the fact that willingness is about perceptions of the benefit. Farming experience positively and significantly influenced the farmers' WTP for improved pigeon pea. Farm size cultivated by pigeon pea positively influence farmers willing to pay. The more the land area cultivated by farmers the higher

to tendency for WTP. The regression results also indicated that farmers with higher income were more likely to pay for the improved technology because they had lower liquidity constraints. This finding is supported by other studies on the effect of wealth status on use of agricultural technologies (Amirnejad and Tonarkbar, 2015).

IV. CONCLUSION

This study explored the willingness of farmers to pay for improved pigeon pea varieties in Oyo State, Nigeria. The findings revealed that while farmers lacked awareness of improved varieties, they showed strong interest in paying for seeds with improved traits, such as high yield, early maturity, and drought resistance. Age, marital status, farming experience, farm size, and income significantly influenced farmers' WTP. Older farmers showed less willingness, while those with larger farms and higher incomes were more likely to pay for improved seeds. The results indicate that there is potential to increase pigeon pea production in Nigeria if improved varieties are made available, affordable, and accessible to farmers.

RECOMMENDATION

To increase the adoption of improved pigeon pea varieties in Oyo State, the following actions are recommended:

1. **Raise Awareness:** Extension workers should educate farmers about the benefits of improved varieties through community meetings, farm demonstrations, and training sessions.
2. **Improve Seed Accessibility:** The government and agricultural agencies should provide improved seeds at subsidized prices to make them affordable for smallholder farmers.
3. **Enhance Credit Access:** Financial institutions should offer credit facilities to help farmers invest in improved seeds and farming technologies.
4. **Align Seed Traits with Farmer Preferences:** Breeding programs should focus on developing varieties with traits such as early maturity, drought resistance, and high yield to meet farmers' needs.

By implementing these recommendations, policymakers, researchers, and development partners can help boost pigeon pea production, enhance farmer incomes, and improve food security in Nigeria.

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Resilient Orchids: Understanding the Heat Tolerance of Vanda tessellata in Changing Climate in the Western Ghats, Wayanad

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Abstract— *This study examines the heat resistance and adaptive strategies of Vanda tessellata, a resilient wild orchid species thriving in the semi-arid conditions of Wayanad, Western Ghats. Over five years (2019–2024), comprehensive field observations, physiological assessments, and environmental monitoring were conducted to evaluate its ability to withstand extreme temperatures and minimal water availability.*

Our findings highlight that Vanda tessellata exhibits multiple survival mechanisms, including Crassulacean Acid Metabolism (CAM) photosynthesis, which allows it to minimize water loss by absorbing CO_2 at night. The orchid also develops thickened cuticles and an extensive aerial root system that enhances moisture retention and nutrient uptake from humid air. Additionally, anthocyanin pigmentation was observed to provide photoprotective benefits by reducing oxidative stress under intense solar radiation. Microhabitat selection played a crucial role in heat tolerance, with orchids growing in shaded areas demonstrating higher survival rates than those in direct sunlight. Soil and substrate analysis revealed that the species primarily thrives on host trees with high water-holding capacity, suggesting a strong dependency on specific ecological conditions.

The study underscores the importance of habitat conservation, particularly in the context of climate change, which threatens the stability of these microhabitats. Conservation strategies should focus on protecting key forest patches, promoting assisted propagation techniques, and implementing long-term monitoring programs to track climate-induced changes in orchid populations. By understanding the adaptive strategies of Vanda tessellata, this research contributes to broader conservation efforts aimed at preserving heat-resistant orchid species in tropical ecosystems.

Keywords— Vanda tessellata, heat resistance, Western Ghats, Wayanad, CAM photosynthesis, orchid adaptation, climate resilience, aerial roots, habitat conservation, anthocyanin pigmentation.

I. INTRODUCTION

1.1 Background:

Orchids are one of the most diverse and ecologically significant plant groups in the Western Ghats, playing a crucial role in maintaining ecosystem balance. They serve as bio-indicators of environmental health due to their sensitivity to climatic changes. Among them, *Vanda tessellata* is a robust, epiphytic orchid well-adapted to extreme heat conditions, making it a key species for studying plant resilience under stress. As global temperatures rise, understanding the physiological and ecological mechanisms enabling this species to endure high temperatures and moisture fluctuations becomes increasingly important.

This orchid species is known for its ability to thrive in exposed environments with limited water availability, thanks to specialized adaptations like CAM (Crassulacean Acid Metabolism) photosynthesis, aerial roots, and thickened cuticles. These features help it survive in semi-arid and dry deciduous forests where other orchids struggle. Given the increasing threat posed

by climate change and deforestation, a comprehensive study of *Vanda tessellata*'s resilience is crucial for developing effective conservation strategies.

1.2 Objectives:

- To investigate the morphological adaptations of *Vanda tessellata*, including leaf structure, cuticle thickness, and root system modifications that enhance heat tolerance.
- To analyze the physiological mechanisms, such as CAM photosynthesis and stomatal regulation, that contribute to its survival under extreme environmental conditions.
- To assess the ecological factors influencing its distribution, including temperature fluctuations, moisture availability, and symbiotic relationships with host trees and mycorrhizal fungi.
- To propose effective conservation measures, including habitat preservation, assisted propagation, and long-term monitoring, to ensure the sustainability of wild orchid populations in the face of climate change.

By addressing these objectives, this study aims to provide valuable insights into the resilience of *Vanda tessellata* and inform broader conservation efforts for heat-resistant orchid species in tropical ecosystems.

II. METHODOLOGY

2.1 Study Area:

The study was conducted in the Wayanad district of the Western Ghats, covering elevations between 700–1200 meters. Selected study sites included dry deciduous forests and exposed rocky terrains where *Vanda tessellata* is naturally found.

TABLE 1
EXPERIMENTAL DESIGN AND DATA COLLECTION FOR *VANDA TESSELLATA* STUDY

Parameter	Methodology	Purpose
Climate Monitoring	Automated weather stations recorded temperature, humidity, and precipitation levels	Assess environmental conditions affecting *Vanda tessellata*
Morphological Assessments	Microscopy used to measure leaf thickness, root structure, and cuticle characteristics	Evaluate physical adaptations to heat stress
Physiological Studies	Diurnal gas exchange measurements and chlorophyll fluorescence analysis conducted	Confirm CAM activity and assess photosynthetic efficiency
Soil and Substrate Analysis	Water retention and nutrient levels in host trees and rock surfaces analyzed	Understand substrate influence on orchid survival
Survival Rate Monitoring	Annual surveys recorded population trends and plant health over five years	Track long-term viability and response to climate change

2.2 Data Collection:

A combination of field observations, laboratory analysis, and remote monitoring techniques were employed to ensure comprehensive data collection. The following parameters were examined:

TABLE 2
KEY MORPHOLOGICAL ADAPTATIONS CONTRIBUTING TO HEAT TOLERANCE IN *VANDA TESSELLATA*

Morphological Feature	Adaptation	Function/Benefit
Thick, succulent leaves	Leaves are fleshy and store water	Reduces water loss, helps retain moisture during dry periods
Waxy cuticle on leaves	A thick, waxy outer layer on leaf surfaces	Minimizes water loss through transpiration
Extensive aerial root system	Roots extend outwards into the air	Absorbs atmospheric moisture, provides hydration in humid environments
Minimal leaf surface area	Smaller or fewer leaves in relation to plant size	Reduces transpiration and water loss under heat stress

This structured approach allowed for a holistic understanding of *Vanda tessellata*'s adaptation strategies and its resilience to environmental stressors.

III. RESULTS AND DISCUSSION

3.1 Morphological Adaptations:

The morphological features of *Vanda tessellata* were observed to be crucial for its survival in its native environment. Key adaptations include:

TABLE 3
PHYSIOLOGICAL RESPONSES TO ENVIRONMENTAL STRESS IN *VANDA TESSELLATA*

Physiological Response	Observation/Characteristic	Function/Benefit
CAM photosynthesis	Nocturnal CO_2 uptake, storing CO_2 in vacuoles during cooler night temperatures	Minimizes water loss by performing photosynthesis at night, conserving moisture
High levels of anthocyanin pigments	Pigment synthesis observed, especially in exposed individuals	UV protection and reduction of oxidative stress caused by intense sunlight
Low stomatal conductance	Stomata remained closed during peak heat stress, reducing water loss	Helps the plant conserve water during extreme temperatures

3.2 Physiological Responses:

Physiological responses to environmental stressors were significant, contributing to the orchid's ability to conserve water and thrive in dry, hot climates.

TABLE 4
ENVIRONMENTAL INFLUENCES ON *VANDA TESSELLATA* GROWTH AND DISTRIBUTION

Environmental Factor	Influence on *Vanda tessellata*	Adaptation/Observation
Shaded microhabitats	Populations in shaded areas exhibited better water retention and overall health	Shaded areas reduce temperature extremes, leading to more consistent water availability
Exposure to sunlight and heat	Orchids in exposed sites developed thicker cuticles and smaller stomata	Thicker cuticles and smaller stomata help reduce water loss under intense sun
Population density and humidity	Positive correlation between population density and humidity fluctuations	Higher density populations may influence local microclimates, providing better humidity for growth

3.3 Environmental Influence:

The environmental factors observed greatly influenced the growth and distribution of *Vanda tessellata*. Notably, the following patterns were recorded:

TABLE 5
CONSERVATION STRATEGIES FOR *VANDA TESSELLATA*

Conservation Strategy	Action Required	Reason/Benefit
Habitat protection	Protect host trees in dry zones from deforestation and climate change	These trees are crucial for orchid survival, providing necessary support and microhabitats
Assisted propagation techniques	Explore seed banking and tissue culture techniques to propagate the orchid	Helps preserve genetic diversity and increase populations for conservation purposes
Monitoring programs	Establish programs to track the distribution and shifts of orchid populations	Tracks effects of climate change, helping to adapt conservation efforts and assess long-term viability

3.4 Conservation Implications:

The findings suggest several important conservation strategies for *Vanda tessellata* to ensure its continued survival and sustainability.

IV. CONCLUSION

This five-year study underscores **Vanda tessellata** as one of the most heat-resistant orchid species in Wayanad, demonstrating its remarkable adaptability to extreme environmental conditions. Despite prolonged exposure to high temperatures and minimal water availability, this species has exhibited exceptional resilience, making it a key candidate for conservation in the face of climate change.

The ability of **Vanda tessellata** to withstand such harsh conditions highlights its ecological significance. As rising temperatures and unpredictable rainfall patterns threaten the survival of many plant species, this orchid's natural resilience offers valuable insights into climate-adaptive conservation strategies. Its ability to thrive in high-heat environments suggests that it could serve as a model species for studying stress tolerance mechanisms in orchids and other epiphytic plants.

Given the increasing threats of habitat destruction and climate change, future conservation efforts should prioritize **ex-situ propagation** and **habitat restoration**. Establishing controlled propagation programs in botanical gardens and research institutions will help ensure the survival of the species outside its natural habitat. Additionally, restoring degraded habitats through afforestation and microclimate management could support the natural regeneration of wild populations.

Further studies should also explore the genetic basis of its heat resistance, which could provide valuable information for breeding programs aimed at developing more resilient orchid varieties. Collaborative efforts between conservationists, researchers, and local communities will be crucial in safeguarding **Vanda tessellata** for future generations, ensuring that this resilient species continues to thrive in its natural ecosystem despite ongoing environmental challenges.

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This study would not have been possible without their generous assistance and shared passion for orchid preservation. We sincerely appreciate their contributions and look forward to future collaborations aimed at furthering the conservation and sustainable management of orchid species.

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Orchid Disorders Demystified: Early Detection, Control, and Recovery

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Abstract— *Orchid cultivation is a widely admired horticultural practice, yet it presents numerous challenges due to the susceptibility of orchids to various diseases. This comprehensive three-year study aims to document the most common diseases affecting orchid plants, their symptoms, and effective prevention and treatment methods. By examining fungal and bacterial infections, the research highlights their detrimental impact on plant health and productivity, while offering actionable solutions for mitigation. Fungal diseases such as black root, root rot, petal blight, and leaf spots were found to be prevalent, often exacerbated by environmental factors like excess moisture and poor air circulation. The study identifies effective strategies for managing these conditions, including the use of fungicides, improved drainage, and enhanced air circulation. Similarly, bacterial diseases, particularly bacterial soft and brown rot, were observed to spread rapidly under hot and humid conditions. Immediate intervention, such as the removal of infected material and the application of copper fungicides or hydrogen peroxide, proved crucial in controlling these outbreaks.*

Preventive measures such as sanitation, environmental monitoring, and routine inspections emerged as critical components of disease management. The study also emphasizes the importance of integrating cultural practices, such as proper potting mediums and strategic plant spacing, to minimize disease risk. By combining traditional horticultural knowledge with modern scientific approaches, this research provides a holistic framework for maintaining orchid health.

The findings of this study serve as a valuable resource for orchid growers, researchers, and horticulturists, addressing the growing need for sustainable and efficient orchid disease management. This work underscores the significance of early detection, rapid response, and long-term preventive measures in safeguarding the biodiversity and aesthetic value of these exquisite plants.

Keywords— Orchid diseases, Plant pathology, Orchid pests and pathogens, Fungal infections in orchids, Bacterial diseases in orchids, viral orchid diseases, Disease identification, Plant disease diagnosis, Orchid disease symptoms, Disease prevention strategies, integrated pest management (IPM), Biological control of plant diseases.

I. INTRODUCTION

Orchids, belonging to one of the largest families of flowering plants, hold a unique place in horticulture and botany due to their exceptional diversity, beauty, and ecological significance. With over 25,000 species and countless hybrids, orchids thrive in a wide range of habitats, from tropical rainforests to temperate regions. Their intricate blooms and adaptability have made them a favorite among cultivators and enthusiasts worldwide. However, despite their allure, orchids are highly sensitive to environmental changes, cultural practices, and diseases, making their cultivation both rewarding and challenging.

The majority of cultivated orchids originate from rainforest regions where conditions are humid, warm, and shaded. These specific environmental requirements often create vulnerabilities when orchids are grown outside their natural habitats. Factors such as excess moisture, inadequate air circulation, and poor drainage significantly increase the likelihood of disease occurrence. Moreover, the global popularity of orchids has led to large-scale cultivation, where the risk of disease transmission is heightened due to close plant proximity and shared resources.

Orchid diseases can broadly be categorized into fungal and bacterial infections, each posing distinct threats to plant health. Fungal diseases, including black rot, petal blight, and root rot, are commonly associated with poor water management and sanitation practices. Bacterial diseases, such as bacterial soft and brown rot, often thrive in hot and humid conditions, spreading rapidly through splashing water and contact with infected material. Left untreated, these diseases can compromise plant vitality, diminish aesthetic value, and even lead to the complete loss of specimens.

The need for effective disease management in orchid cultivation cannot be overstated. This study was initiated to address the gaps in understanding the etiology, symptoms, and control measures of common orchid diseases. By systematically documenting disease progression and evaluating various treatment strategies, this research aims to provide a comprehensive resource for orchid growers, researchers, and horticulturists. The focus extends beyond reactive measures, emphasizing the importance of preventive practices, such as sanitation, cultural adjustments, and environmental monitoring, to mitigate disease risks proactively.

Furthermore, the study highlights the economic and ecological implications of orchid diseases. As orchids play a significant role in the ornamental plant industry, ensuring their health is vital for sustaining market demand and preserving biodiversity. The susceptibility of orchids to diseases also underscores the need for integrating traditional horticultural knowledge with modern scientific approaches to develop sustainable cultivation practices.

The introduction of this study sets the stage for an in-depth exploration of the challenges and solutions in managing orchid diseases. By shedding light on the intricacies of orchid care, the research aims to equip cultivators with the tools and knowledge needed to maintain healthy plants, prevent outbreaks, and foster a deeper appreciation for these extraordinary plants. This endeavor not only contributes to the scientific community but also supports the global effort to preserve and promote one of nature's most captivating plant families.

II. MATERIALS AND METHODS

This study was conducted over three years in both controlled greenhouse environments and natural orchid cultivation sites. The research focused on identifying, monitoring, and addressing common diseases in a variety of orchid species, including Phalaenopsis, Vanda, and Dendrobium. The methodology encompassed environmental monitoring, symptom documentation, and the application of treatment strategies. Below is a summary of the materials and methods used:

TABLE 1
EXPERIMENTAL SETUP AND DATA COLLECTION PROTOCOL

Aspect	Description
Study Location	Controlled greenhouses and natural orchid sites in temperate and tropical climates.
Orchid Species Studied	Phalaenopsis, Vanda, Dendrobium, and other widely cultivated species.
Environmental Factors Monitored	Humidity, temperature, air circulation, and drainage conditions.
Disease Identification	Visual inspection for symptoms such as black spots, leaf discoloration, and petal damage.
Sample Collection	Infected plant parts collected using sterile tools for microscopic examination and laboratory analysis.
Treatment Methods	Application of fungicides (e.g., copper-based), hydrogen peroxide, and sanitation techniques.
Preventive Measures	Routine cleaning of tools, disinfection of potting mediums, and ensuring proper airflow.
Data Documentation	Progress of infection, recovery rates, and recurrence documented through photographs and field notes.
Evaluation Criteria	Plant recovery rates, reduction in disease spread, and improvement in plant health.

The study employed a combination of observational and experimental approaches. Regular inspections were carried out to detect early signs of diseases, and symptomatic plants were isolated to prevent cross-contamination. A significant emphasis was placed on sanitation practices, including the use of a 10% bleach solution to disinfect tools and growing areas.

The findings from this methodical approach were analyzed to determine the efficacy of various treatments and cultural modifications. This systematic documentation provides a foundation for developing best practices in orchid disease management, ensuring sustainability and resilience in cultivation practices.

III. RESULTS AND DISCUSSION

3.1 Overview of Key Findings:

The study systematically identified prevalent diseases affecting orchids, analyzed their symptoms, and evaluated the efficacy of various treatment and preventive measures. The results are summarized below, categorized by fungal and bacterial diseases.

TABLE 2
SUMMARY OF ORCHID DISEASES, SYMPTOMS, CAUSES, AND TREATMENTS

Disease	Symptoms	Causes	Treatment and Prevention
Black Rot	Dark black spots on foliage, rapid spread	Standing water, fungal spores	Remove affected areas; apply fungicides
Root, Rhizome, and Pseudobulb Rots	Root decay, above-ground symptoms	Non-sterile potting soil, excess water	Excise infected roots; fungicide drench
Petal Blight	Black or brown spots on petals	High humidity, Botrytis fungus	Remove infected flowers; improve airflow
Southern Blight	Rapid plant collapse, rotting of roots and leaves	Warm, humid weather, poor sanitation	Fungicides; improve circulation
Leaf Spots	Water-soaked or discolored areas on leaves	Multiple fungal or bacterial organisms	Ensure dry foliage; apply fungicides
Bacterial Soft and Brown Rot	Water-soaked lesions with yellow halos, foul odor	Hot, moist conditions	Sterile tools; copper fungicides

3.2 Key Observations:

3.2.1 Fungal Diseases:

- Black rot emerged as a primary concern, particularly in Phalaenopsis species, where its rapid progression could devastate plants within days.
- Root and pseudobulb rots were prevalent in plants grown in poorly draining media. Use of sterilized soil and regular root inspections significantly reduced cases.
- Petal blight caused by Botrytis fungus frequently appeared during high-humidity periods. Removal of affected petals combined with fungicide applications proved effective.

3.2.2 Bacterial Diseases:

- Soft and brown rot were found to spread rapidly through splashing water. Vanda species exhibited translucent lesions, while Dendrobium showed sunken black patches.
- Hydrogen peroxide emerged as a versatile treatment, particularly in species sensitive to copper fungicides.

3.3 Case Study Insights:

3.3.1 Black Rot Management:

- Affected plants showed significant recovery when treated with systemic fungicides and maintained in well-ventilated environments. Preventive measures, including avoiding overhead watering, reduced new infections by 70%.

3.3.2 Southern Blight Control:

- High humidity in greenhouses led to a 30% increase in southern blight cases during peak summer months. Improved air circulation and regular cleaning of growing areas brought this number down to negligible levels within the study period.

IV. DISCUSSION

The study highlights the critical role of early detection and integrated disease management in maintaining orchid health. Fungal diseases like black rot and root rot can be effectively controlled with cultural adjustments and targeted fungicides, while bacterial infections demand strict sanitation and rapid intervention.

Environmental factors, including humidity and airflow, emerged as key contributors to disease prevalence. Simple changes, such as spacing plants appropriately and using fans, significantly mitigated risks. The effectiveness of hydrogen peroxide as a broad-spectrum disinfectant underscores its utility in disease management without chemical residues.

Preventive practices, particularly regular monitoring and sanitation, proved invaluable. For example, routine inspections helped identify early signs of bacterial rot, enabling timely treatment and limiting disease spread. Combining these measures with modern fungicides and organic approaches ensures a sustainable pathway for orchid cultivation.

By addressing both the biological and environmental aspects of orchid diseases, this study provides a comprehensive framework for growers. These findings not only enhance orchid care but also contribute to broader horticultural practices, emphasizing the importance of integrated solutions for plant health.

4.1 Bacterial Diseases:

4.1.1 Bacterial Soft and Brown Rot:

This bacterial infection, common in hot and moist conditions, caused water-soaked lesions with yellow halos. The disease spread quickly, especially in Phalaenopsis and Vanda species, and produced a foul odor.

- **Treatment:** Removal of infected areas with sterile tools and application of copper fungicides or hydrogen peroxide. Hydrogen peroxide was effective in controlling spread when sprayed on both infected and neighboring plants.

4.1.2 Preventive Measures and Cultural Practices:

Preventive measures and cultural practices are critical in minimizing the occurrence and impact of orchid diseases. These practices not only reduce the risk of infection but also promote overall plant health and resilience. The table below summarizes key preventive strategies and their benefits:

TABLE 3
PREVENTIVE MEASURES AND CULTURAL PRACTICES FOR ORCHID DISEASE MANAGEMENT

Preventive Measure	Description	Benefits
Sanitation	Regular cleaning of tools, pots, and growing areas using disinfectants like bleach or hydrogen peroxide.	Prevents the spread of pathogens and reduces the risk of contamination.
Proper Drainage	Use of well-draining potting mediums such as bark, perlite, or coconut husk to avoid waterlogging.	Prevents root rot and other water-related fungal infections.
Environmental Monitoring	Regularly checking and maintaining optimal levels of temperature, humidity, and light.	Creates a stable environment that discourages disease development.
Air Circulation	Installing fans or spacing plants adequately to enhance airflow.	Reduces humidity around plants and prevents fungal and bacterial growth.
Watering Practices	Avoiding water accumulation on leaves and flowers; watering early in the day.	Reduces standing water that facilitates fungal and bacterial infections.
Early Detection and Removal	Regular inspection of plants for signs of disease and immediate removal of infected parts.	Prevents the spread of infection to neighboring plants.
Use of Sterile Tools	Always using sterilized knives, scissors, and other tools during pruning or repotting.	Minimizes the introduction of pathogens during plant handling.
Cultural Adjustments	Adapting practices to suit the specific requirements of orchid species (e.g., light and humidity levels).	Supports healthy growth and reduces stress-related vulnerabilities.
Isolation of New Plants	Quarantining newly acquired plants for observation before integrating them with the main collection.	Prevents introduction of external diseases to existing plants.
Fungicide and Bactericide Use	Applying preventative treatments such as copper fungicides or hydrogen peroxide sprays as needed.	Provides an additional layer of protection against infections.

By incorporating these measures into routine orchid care, growers can significantly lower the risk of diseases and promote robust plant development. For instance, regular sanitation and air circulation reduce humidity levels, which is a primary factor for fungal infections. Similarly, choosing the right potting medium ensures that excess water is efficiently drained, protecting roots from decay.

Implementing these cultural practices requires consistency and attention to detail, as even minor lapses can create opportunities for pathogens to thrive. This integrated approach, combining traditional horticultural knowledge with proactive management strategies, ensures that orchids remain healthy and flourish in both natural and controlled environments.

V. CONCLUSION

This three-year study provides an in-depth analysis of common orchid diseases, their causes, and the strategies needed to manage them effectively. Orchids, as delicate and highly valued ornamental plants, are particularly susceptible to diseases driven by environmental factors and improper cultivation practices. The findings underscore the critical importance of integrating preventive measures, early detection, and timely intervention to mitigate the impact of these diseases.

Fungal infections, such as black rot, root rot, and petal blight, were identified as major threats, often exacerbated by excessive moisture, poor drainage, and inadequate air circulation. Similarly, bacterial infections, including bacterial soft and brown rot, spread rapidly in humid conditions, posing a significant challenge to orchid growers. This research highlights the effectiveness of treatment methods such as fungicides, hydrogen peroxide applications, and sanitation protocols in controlling these diseases. However, the study also emphasizes that prevention remains the most effective strategy. Practices like maintaining optimal environmental conditions, sterilizing tools and potting mediums, and conducting routine plant inspections are pivotal.

Beyond disease management, this study contributes to a broader understanding of sustainable orchid cultivation. By combining traditional horticultural knowledge with scientific advancements, growers can preserve the health and beauty of orchids while ensuring ecological balance. The research also highlights the economic implications, as maintaining healthy orchids is essential for meeting the demands of the ornamental plant market and safeguarding biodiversity.

In conclusion, the findings from this study provide a valuable resource for orchid growers, researchers, and horticulturists. By adopting the outlined strategies and fostering a culture of proactive care, it is possible to overcome the challenges posed by

orchid diseases. This work reinforces the importance of a holistic approach to plant health, ensuring that these extraordinary plants continue to thrive for future generations.

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The team at EUNOIA Orchid Garden offered access to a wide variety of orchid species, allowing for a comprehensive examination of fungal and bacterial diseases across different genera. Their commitment to maintaining a pristine and controlled environment for orchid cultivation played a crucial role in ensuring accurate and reliable data collection. The garden's staff, with their extensive knowledge and hands-on experience, provided critical insights into disease symptoms, propagation challenges, and effective management practices.

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Finally, we are deeply thankful to the plant pathology laboratories and academic institutions involved in this project, whose analytical support and guidance were instrumental in validating our findings. This collaborative effort underscores the importance of partnerships in advancing our understanding of orchid diseases and promoting sustainable cultivation practices. Together, these contributions have laid the foundation for impactful research in the field of ornamental horticulture.

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