

Preface

We would like to present, with great pleasure, the inaugural volume-11, Issue-2, February 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.

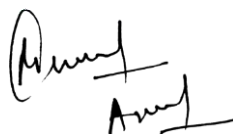
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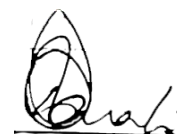
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Agriculture, Biological engineering, including genetic engineering, microbiology, Environmental impacts of agriculture, forestry, Food science, Husbandry, Irrigation and water management, Land use, Waste management and all fields related to Agriculture.

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



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Evaluation of the Chemical Composition of Biscuits from Soybean (*Glycine max*), and Corn (*Zea mays*) Seeds Flour Blends Supplemented with Date Palm (*Phoenix dactylifera*) Pulp

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Abstract—

Background/Objective: Development of biscuits from soybeans, and corn flour blends supplemented with date palm pulp and their chemical evaluation was necessitated by consumers demand for varied nutrient-dense foods, and informed food choices.

Methodology: The experimental study used standard methods to process flour from soybeans and corn; and pulp from date palm. The flour was formulated into three blends supplemented with the pulp in the ratios of 50:30:20, 40:40:20, 30:50:20. These were made into biscuits and evaluated for chemical compositions with standard procedures. IBM Statistical Product for Service Solution (SPSS) software version 21 was used to analyze data and presented as mean $\pm$ SD. The means were compared with Analysis of variance and separated with Duncan multiple test range.

Result: The proximate composition ranged from 7.37% to 8.66% moisture, 8.74% to 12.05% crude protein, 18.96% to 20.23% fat, 1.16% to 19.4% fiber, 1.24% to 1.93% ash, 57.81% to 60.27% carbohydrates, and 450.74Kcal to 458.09Kcal energy. The vitamin contents ranged from 12.58 μ g to 14.871 μ g beta-carotene, 0.56mg to 0.92mg thiamin, 25.52mg to 26.09mg riboflavin, 0.78mg to 1.38mg niacin, 0.27 μ g/g to 0.84 μ g/g folate, 15.67mg to 0.84mg Vitamin C. The mineral composition range was 34.74mg to 45.66mg calcium, 15.82mg to 23.47mg magnesium, 19.77mg to 37.62mg sodium, 54.75mg to 65.34mg potassium, 0.57mg to 0.91mg iron, 0.48mg to 0.65mg zinc. The phytochemical content range was 0.26mg to 0.68mg tannin, 0.35mg to 0.92mg flavonoids, 0.16mg to 0.39mg saponin, 0.23mg to 0.78mg phytate, 0.12mg to 0.45mg oxalate, and 0.11mg to 0.66mg phenols.

Conclusion: The biscuits have varied nutrients, fiber, and phytochemical content and will improve food intake.

Keywords— Chemical Composition; Biscuits; Soybeans; Corn; Date Palm Pulp.

I. INTRODUCTION

Recently, there has been a growing interest in exploring alternative food sources to eliminate dietary deficiencies, improve food security, and promote health. This is particularly pertinent in communities whose staples have inadequate nutritional components essential for life. The nutritional and health conditions of individuals depend on various factors amongst which, the kind of foods available for consumption is paramount. This affects all consumers both young and old. Malnutrition especially undernutrition can contribute to global mortality, especially in regions that rely greatly on starchy staples with insufficient nutrients, and high cost of quality proteins. Prolonged consumption of diets with inadequate nutritional components could have devastating effects on both younger generation with increased nutrients needs, and the older persons with nutrient maintenance requirements. Consequently, to ensure adequate intake of essential nutrients, as well as food security, available staples can be utilized in various ways to maximize nutrient intake. Recent studies have investigated the abilities of different food crops to improve nutrient consumption [1, 2, 3]. The food crops used are mainly cereals, legumes and starchy roots and

tubers. Common cereals include corn, millets, rice, sorghum, dawa, etc., legumes - soy beans, groundnuts, cowpea etc., and starchy roots and tubers - yam, cassava, potatoes etc. Traditionally, combined intake of these foods will ensure adequate intake of nutrients, but recently, nutrition transition and globalization have resulted in non-consumption of most indigenous foods, availability of foods from other regions, adoption of westernized diets with little economic power and a consequent consumption of cheap monotonous diets of poor nutrient profile. Many non-indigenous foods are available for consumption daily. They include highly processed confectionaries made mainly from refined wheat flours among which biscuits are topmost. Biscuit are small thin crisp bar made from unleavened dough. They are reported to be popular, convenient, and affordable snacks consumed by people of all ages [4, 5]. Common attractive features of biscuits include wider consumption, longer shelf-life, availability in varied taste and texture [6]. The ingredients have a great impact on the nutritional, textural, physical properties of biscuit; hence included in appropriate proportion to achieve the desired quality.

Traditionally wheat is most often used in biscuit making because of its unique baking properties; but it lacks essential nutrients and dietary fiber [7]. Thus, biscuits made from refined wheat flour whose amino acid composition is insufficient cannot support growth. It is reported that foods made from refined wheat and other cereals lack high-quality protein [8]. These biscuits are widely consumed by many daily. It is so much available and convenient that it has transited from snacks to the main and easy meals in many poor households. The implication of this transition is increased number of consumers with poor nutritional status. Fortunately, biscuits could serve as a vehicle for improving nutrition because of its favorite status. Updating the nutrient profile of biscuits by blending flours made from indigenous food crops could improve the food security status of these consumers. Soybeans for instance is a legume that has significant protein content, and corn has easy carbohydrates and fiber, both are food crops with varied nutrient composition that can complement to supply adequate nutrients to consumers. Cereals are low in lysine, and have sufficient methionine and cysteine, while legumes are rich in lysine but lack methionine and cysteine [9]. Cereal: legume ratio is particularly beneficial in plant-based diets to improve overall protein quality by ensuring both essential amino acids are present to prevent protein-energy malnutrition [10]. The addition of date palm pulp will improve vitamins, minerals, and fiber value of the biscuits. Consequently, this study produced biscuits made from blends of flours from soy beans, and corn seeds, pulp from date palm and evaluated the proximate (protein, fat, ash, crude fiber, moisture, carbohydrate), minerals (calcium, magnesium, potassium, phosphorus and iron), vitamins (Beta-Carotene, thiamin, riboflavin, niacin, vitamin C), and anti-nutrient (phytate, tannins, flavonoids, saponin, oxalate) compositions to ascertain their contribution to adequate and varied diets.

II. MATERIALS AND METHODS

2.1 Study design:

The study employed experimental design.

2.2 Study area:

The area of study is the University of Calabar established in 1975. The institution is situated in Calabar, the capital of Cross River State, in Southern Nigeria. The city is adjacent to Great Kwa-river and creeks of the Cross River (from its inland delta) which is the biggest tourist city in Western Africa and has an area of 406 square kilometers (157 sqm) and a population of 371,022, Density of 910 kilometers square, as at 2006 census [11]. The tertiary institution has many departments and faculties including the Faculty of Basic Medical Sciences of which the Department of Human Nutrition and Dietetics is domicile.

2.3 Source, Identification, and processing of the samples:

- **Source of the crops:** The soybeans, corn and date palm fruits, and biscuit ingredients (margarine, granulated sugar, salt and baking powder, vanilla, baking fruit, ground nutmeg) were purchased from different stalls in Watt market, Calabar, Cross Rivers State, Nigeria.
- **Identification of the samples:** The crops, Soybeans (*Glycine max*), Corn (*Zea mays*) and Date palm (*Phoenix dactylifera*) were identified by Prof. Osai, Ephraim Onukwu, a Professor of Crop Science from the Department of Crop Science, Faculty of Agriculture, University of Calabar.
- **Processing of Soybeans, Corn, and Date palm to flours and pulp:** Soybeans flour was prepared as described by [12]. The seeds were sorted to remove extraneous materials, washed, and soaked in tap water for 8 hours. It was dehulled, sun-dried for 12 hours, further dried to a constant weight for 36 hours at a temperature of 150°F in a digital food dehydrator (Excalibur® 3926TCDB). It was then milled into fine flour using a mechanical grinder (St Donkey

powder crusher- Leshan Dongchaun) with 5mm sieve. Corn flour was made as described by Onyeka [13]. The grains were sorted to remove extraneous materials, washed, and soaked in tap water for 24 hours, rinsed, and drained in 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 a temperature of 150°F. The dried corn was milled into fine flour using the mechanical grinder. The Date Palm fruit was sorted to remove extraneous particles, washed with tap water, and deseeded (Depitting) manually. The pulp with pericarp was cut into small pieces with knife, and oven dried at 75°C for 6-8 hours in a Uniscope Laboratory oven (Surgifriend Medigals England). The dried pulp was milled using hand milling machine (Corona) and sieved through a 0.35mm mesh sieve to obtain fine homogenized particles as described [14].

2.4 Formulation of the flours into blends:

The flours were blended based on the cereal: legume ratio used commonly in food formulations to enhances protein quality and nutritional balance of amino acids. The ratios of 50:30:20, 40:40:20, 30:50:20 was formulated and coded as SCDI, SCDII, and SCDIII where SCDI = soybean 50%: Corn 30%: date palm 20%, SCDII = soybean 40%: Corn 40%: date palm 20%, and SCDIII = soybean 30%: Corn 50%: date palm 20%. Wheat flour (100%) was coded as WFB.

2.5 Biscuit Production Procedure:

The biscuits were produced using traditional home method. The composite flour blends (1000g), salt (5g) and baking powder (2tsp) were manually mixed in a large bowl, the margarine (500g) was rubbed into the flour mixture using fingertips to form coarse crumbs. Sugar (200g), vanilla powder (2tsp), nutmeg (2tsp), and egg (40ml) were introduced into the bowl, the condensed milk (250ml) was added slowly with gentle hand kneading until the dough came together. The dough was then rolled out on a lightly floured surface to a uniform thickness using a wooden rolling pin, cut into different shapes with a biscuit cutter, and was placed on a baking tray. The cut dough was baked in an electric oven (Pyramid 22L Oven) at 150°C for 20 minutes.

2.6 Chemical analysis:

The proximate composition (moisture, ash, protein, fat, crude fiber, and carbohydrate) of the biscuits was determined by AOAC [15], total carbohydrates was obtained by difference, and the energy value was calculated using the Atwater factors methods. The minerals (calcium, potassium, iron, magnesium, and phosphorus), vitamins (beta carotene, thiamin, riboflavin, niacin, ascorbic acid), phytochemicals and antinutrients (phenol, flavonoids, saponin, tannin, oxalate, and phytate) were determined by standard methods [15].

2.7 Statistical analysis:

Data obtained from the study was analyzed using IBM Statistical Product for Service Solution (SPSS) version 21. The results were presented as means and standard deviation, means were compared with analysis of variance (ANOVA) and separated using Duncan's multiple range test at a 5% significance level ($p < 0.05$).

III. RESULT

The energy content of the biscuits ranged from 409.45Kcal in the control sample WFB (100% wheat) to 454.40Kcal in SCD III (soybean 30%: Corn 50%: date palm 20%) (table 1). The moisture content ranged from 12.20% in SCD III to 14.36% in the control sample WFB. Sample SCD II (soybean 40%: Corn 40%: date palm 20%) had the highest (12.36%) crude protein value while SCD III (soybean 30%: Corn 50%: date palm 20%) had the least 8.41% protein value, lower than 9.72% in the control WFB (100% wheat flour). Sample SCDII (soybean 30%: Corn 50%: date palm 20%) had the highest fat content (24.72%) while samples SCD I (soybean 50%: Corn 30%: date palm 20%), and SCD II (soybean 40%: Corn 40%: date palm 20%) had comparable values (23.20%, 23.21%) respectively. The ash content of the samples ranged from 2.12% in SCD I (soybean 50%: Corn 30%: date palm 20%) to 2.65% in SCDIII (soybean 30%: Corn 50%: date palm 20%). The control WFB (100% wheat flour) had appreciable ash value (2.29%) compared to 2.12% and 2.15% in samples SCDI (soybean 50%: Corn 30%: date palm 20%) and SCDII (soybean 40%: Corn 40%: date palm 20%) respectively. SCD III (soybean 30%: Corn 50%: date palm 20%) had the highest crude fiber value 2.45% while the control WFB (100% wheat flour) had the least (1.75%) fiber content. The carbohydrate range of the biscuits was 47.21% in SCDII (soybean 40%: Corn 40%: date palm 20%) to 55.27% in the control sample WFB.

TABLE 1
PROXIMATE COMPOSITION OF BISCUITS MADE FROM SOYBEANS AND CORN FLOUR BLENDS
SUPPLEMENTED WITH DATE PALM PULP

Sample	Moisture (%)	Protein (%)	Fat (%)	Ash (%)	Crude Fiber (%)	Carbohydrate (%)	*Energy (Kcal)
SCD I	13.40 ^b ± 0.03	10.86 ^b ± 0.02	23.20 ^b ± 0.02	2.12 ^c ± 0.03	1.88 ^b ± 0.01	48.56 ^c ± 0.01	446.48
SCD II	13.23 ^c ± 0.04	12.36 ^a ± 0.01	23.21 ^b ± 0.01	2.15 ^c ± 0.01	1.83 ^b ± 0.02	47.21 ^d ± 0.05	447.17
SCD III	12.20 ^d ± 0.03	8.41 ^d ± 0.01	24.72 ^a ± 0.03	2.65 ^a ± 0.03	2.45 ^a ± 0.02	49.57 ^b ± 0.05	454.4
WFB IV	14.36 ^a ± 0.03	9.72 ^c ± 0.02	16.61 ^c ± 0.02	2.29 ^b ± 0.02	1.75 ^c ± 0.03	55.27 ^a ± 0.02	409.45

*Means ± SD of triplicate determinations; means with different superscript on a column are significantly different. SCD I = soybean 50%: corn 30%: Date palm 20%, SCD II = soybean 40%: corn 40%: Date palm 20%, SCD III = soybean 30%: corn 50%: Date palm 20%, WFB IV = Wheat 100% flour; * = calculated.*

The calcium contents of the biscuits ranged from 40.64mg in SCD I (soybean 50%: Corn 30%: date palm 20%) to 47.81mg in SCD III (soybean 30%: Corn 50%: date palm 20%) in table 2. SCD I (soybean 50%: Corn 30%: date palm 20%) had the highest potassium content while the control WFB (100% wheat flour) had the least potassium value 96.81mg. The iron content of the samples ranged from 0.89mg in SCD II to 1.04mg the control WFB (100% wheat flour). SCD III (soybean 30%: Corn 50%: date palm 20%) had the highest magnesium content (47.75mg), while SCD II (soybean 40%: Corn 40%: date palm 20%) had the least magnesium value (33.62mg). The Phosphorus content of the samples ranged from 63.49 in SCD II (soybean 40%: Corn 40%: date palm 20%) to 71.59mg in WFB (100% wheat flour biscuits).

TABLE 2
MINERAL COMPOSITION OF BISCUITS MADE FROM SOYBEANS AND CORN FLOUR BLENDS SUPPLEMENTED
WITH DATE PALM SYRUP (mg/100g)

Sample	Ca (mg)	K (mg)	Fe (mg)	Mg (mg)	P (mg)
SCD I	40.64 ^d ± 0.02	104.62 ^a ± 0.02	0.93 ^b ± 0.01	34.77 ^c ± 0.04	64.77 ^c ± 0.04
SCD II	43.62 ^c ± 0.03	98.53 ^c ± 0.01	0.89 ^b ± 0.02	33.62 ^c ± 1.40	63.49 ^d ± 0.01
SCD III	47.81 ^a ± 0.01	101.73 ^b ± 0.02	0.92 ^b ± 0.03	47.75 ^c ± 0.02	69.55 ^b ± 0.07
WFB IV	45.32 ^b ± 0.02	96.81 ^c ± 0.01	1.04 ^a ± 0.02	36.79 ^b ± 0.01	71.59 ^a ± 0.01

Means ± SD of triplicate determinations; means with different superscript on a column are significantly different. SCD I = soybean 50%: corn 30%: Date palm 20%, SCD II = soybean 40%: corn 40%: Date palm 20%, SCD III = soybean 30%: corn 50%: Date palm 20%, WFB IV = Wheat 100% flour.

The vitamin composition of the biscuits (table 3) showed that carotenoid content ranged from 4.84mg in SCD I (soybean 50%: Corn 30%: date palm 20%) to 13.12mg in WFB (100% Wheat flour). Vitamin B1 ranged from 0.63mg in SCD I (soybean 50%: Corn 30%: date palm 20%) to 0.95mg in WFB (100% Wheat flour biscuits). SCD I (soybean 50%: Corn 30%: date palm 20%) had the highest riboflavin (B2) value (2.57mg) while 100% wheat flour biscuit (WFB) had the least value (2.08%). The vitamin B3 content of the samples ranged from 1.41mg to 1.76mg. Sample SCD I ((soybean 50%: Corn 30%: date palm 20%)) had the highest niacin (B3) value (1.76mg) while WFB (100% Wheat flour biscuit) had the least B3 value (1.41mg). Vitamin C was more (47.52mg) in sample SCD I (soybean 50%: Corn 30%: date palm 20%) than in SCD II (soybean 40%: Corn 40%: date palm 20%).

TABLE 3
VITAMIN COMPOSITION OF BISCUITS MADE FROM SOYBEANS AND CORN FLOUR BLENDS SUPPLEMENTED WITH DATE PALM SYRUP

Sample	VIT A (mg)	B1(mg)	B2 (mg)	B3 (mg)	Vit C (mg)
SCD I	4.84 ^d ± 0.03	0.63 ^d ± 0.03	2.57 ^a ± 0.02	1.76 ^a ± 0.01	47.52 ^a ± 0.02
SCD II	8.14 ^b ± 0.02	0.88 ^b ± 0.02	2.31 ^b ± 0.02	1.66 ^b ± 0.03	39.63 ^d ± 0.03
SCD III	7.15 ^c ± 0.03	0.77 ^c ± 0.02	2.19 ^c ± 0.02	1.52 ^c ± 0.03	41.74 ^c ± 0.03
WFB IV	13.12 ^a ± 0.01	0.95 ^a ± 0.02	2.08 ^d ± 0.03	1.41 ^d ± 0.02	44.21 ^b ± 0.01

Means ± SD of triplicate determinations; means with different superscript on a column are significantly different. SCD I = soybean 50%: corn 30%: Date palm 20%, SCD II = soybean 40%: corn 40%: Date palm 20%, SCD III = soybean 30%: corn 50%: Date palm 20%, WFB IV = Wheat 100% flour.

The anti-nutrient composition of the biscuit samples shown in table 4 revealed that sample SCD I had significantly higher tannin (1.02mg), saponin (0.39mg), phenol (0.49mg), phytate (0.31mg), and oxalate (0.53mg) than all the other samples. SCD III had the highest flavonoids value (0.21mg). The control had least value in tannin, saponin, phenol, phytate, and oxalate except flavonoids.

TABLE 4
ANTI-NUTRIENT COMPOSITION OF BISCUITS MADE FROM SOYBEANS AND CORN FLOUR BLENDS SUPPLEMENTED WITH DATE PALM (mg/100g)

Sample	Tan	FLAV	SAP	Phenol	Phytate	Oxalate
SCD I	1.02 ^a ± 0.01	0.18 ^{ab} ± 0.01	0.39 ^a ± 0.01	0.49 ^a ± 0.00	0.31 ^a ± 0.02	0.53 ^a ± 0.03
SCD II	0.67 ^b ± 0.02	0.14 ^b ± 0.01	0.32 ^b ± 0.02	0.44 ^{ab} ± 0.02	0.25 ^b ± 0.01	0.33 ^b ± 0.03
SCD III	0.55 ^c ± 0.01	0.21 ^a ± 0.01	0.27 ^c ± 0.01	0.38 ^c ± 0.02	0.19 ^c ± 0.02	0.20 ^c ± 0.01
WFB IV	0.48 ^d ± 0.03	0.15 ^b ± 0.01	0.23 ^c ± 0.01	0.34 ^c ± 0.02	0.12 ^d ± 0.02	0.19 ^c ± 0.01

Means ± SD of triplicate determinations; means with different superscript on a column are significantly different. SCD I = soybean 50%: corn 30%: Date palm 20%, SCD II = soybean 40%: corn 40%: Date palm 20%, SCD III = soybean 30%: corn 50%: Date palm 20%, WFB IV = Wheat 100% flour.

IV. DISCUSSION

The moisture content (12.20% to 13.40%) of the biscuits made from soybeans and corn flour blends supplemented with date palm pulp varied significantly ($p < 0.05$) among the blends and was appreciably higher than 5.90% to 7.90%; and 10.2% to 11.12% in biscuits made from unripe cooking banana, pigeon pea and sweet potatoes flour blends [16], and composite flour biscuits enriched with edible insect “kanni” [17] respectively. Moisture content of products has implication for shelf-life. Higher moisture (> 10 to 14%) will encourage spoilage [18, 19, 20] The moisture content was more in biscuits with higher soybean content. This could be attributed to the high-water holding capacity of soybean [21]. However, high moisture content of the biscuit (>10%) is an indication of low dry matter content and possibly lower the shelf-life. The study biscuits’ protein content (8.41% to 12.36%) was comparable to 8.14% to 10.38% reported by Ugo et al. [22], but lower than 11.85% to 15.95% in maize-soybean composite flour biscuits enriched with edible insect “kanni” [17]. The higher protein content of the latter maybe due to the addition of edible insects. Biscuits made from blends with equal content of soybean and corn had more proteins compared to blends with higher soybean value. This could be attributed to the constitution of the base crops (cereals and legumes) which when combined presents a protein value similar to animal proteins. Combination of legumes and cereals produce high quality protein [23]. The fat content of the biscuit (16.61% to 24.72%) was higher than 16.18% to 17.26% in maize-soybean composite flour biscuits enriched with edible insect “kanni” [17], and 15.38% to 18.37% in wheat-soybean-corn flours cookies [24]. Higher fat content is indicative of higher energy value needed for sustenance and vital for the metabolism of fat-soluble vitamins. The fiber content (1.75 to 2.15%) of the biscuit was lower than 3.84% - 4.22% reported by Okoye and Obi [25] but consistent with 1.70% and 1.74% – 2.12% reported on biscuits made from African breadfruit–sweet

potato-wheat flour composite blends and wheat flour-cocoyam-date palm fruit pulp as sugar substitute respectively [26, 27]. The variations could be attributed to the differences in composition, and the crops used. Fiber is an important component of food necessary for health since it enhances digestion, aids bowel movement, and increases fecal bulk. It also helps to prevent inflammatory bowel disease and colorectal cancer [28]. The study biscuits ash content (2.12 to 2.65%) was comparable with 2.41% and 2.40% reported on biscuit produced from wheat – breadnut flour composite blends [22, 29]. A range of 2.03 – 2.12% ash content for biscuit made from wheat – cocoyam flour composite which was slightly lower than that obtained from the present study was also reported [30]. However, higher ash values (4.29 – 5.45%) were reported for biscuit produced from wheat, cocoyam, and pigeon pea flour [27]. The ash content is a measure of the nutritionally important minerals present in a food material [31]. The carbohydrate contents of all the test biscuits samples were lower than the control and 62.60% and 64.54% reported [26, 29]. The addition of soyabean across the blends, increases the protein, fat, ash, and fiber contents and lowers the carbohydrate content of the biscuit samples. Although carbohydrate is the major energy source required in the body, the increase in proximate parameters (protein, fat, ash, and fiber) will be beneficial to daily consumers.

The study biscuits' calcium contents (40.64mg to 47.81mg) were lower than 42.63mg to 52.77mg reported Ugo et al [22], but higher than 10.28mg and 28.06mg, reported by Okoye and Obi and Nzeagwu and Onwudiwe [25, 32] respectively. Calcium is one of the most important minerals that the body requires and its deficiency is more prevalent than any other mineral [33]. Calcium, Phosphorus, and vitamin D work to eliminate rickets (in children) and osteomalacia (the adult rickets) as well as osteoporosis (bone thinning) among older people [34]. The potassium content (96.81mg to 104.62mg) of the biscuit samples were higher than 4.22mg to 5.08mg reported [25]. Potassium is essential in body water regulation, the maintenance of osmotic pressure of the body fluid, and the transport of CO₂ in the blood [35]. The known value of potassium will inform consumers concerned with maintenance of body fluids. The iron range (0.89mg to 1.04mg) of soybean-corn-date palm biscuits although small was comparable to 0.96mg to 1.27mg in biscuits reported [22, 25]. Iron is a component of hemoglobin which is responsible for the red color of the blood. When foods with iron are eaten, it is absorbed into proteins and helps these proteins take in, carry, and release oxygen throughout the body. The magnesium contents of the African yam bean-corn blended biscuits were within the range of 16.60 to 89.86mg of African walnut-Justicia carnea-wheat made biscuits [36]. The phosphorus contents were more than 9.40 to 11.40mg in African walnut-Justicia carnea-wheat made biscuits [36]. The mineral contribution of the study biscuits will contribute to daily intake. The importance of minerals in Human nutrition has been documented [37].

Carotenoid values ranged from 4.84mg to 13.12mg. The sample with equal content of cereal and legumes (40% soybean:40% corn: 20% date palm pulp) had higher carotenoids value than the other blends, though lower than the control (13.12mg) 100% wheat flour. The vitamin B1 and B2 content (0.63mg to 0.95mg; 2.08mg to 2.57mg) were higher than 0.40mg and 1.97mg obtained by Usman et al. [38]. The difference could be attributed to different composition and constitutions of the biscuits. Vitamin B acts as a coenzyme in the breakdown of fats, proteins, carbohydrate, and other nutrients in the liver, and assists eye and skin maintenance [39]. The study B3 content (1.41mg to 1.76mg) of the biscuits were higher than 0.13mg to 0.26mg reported by Usman [40]. B3 acts as a coenzyme for most essential chemical reactions. The vitamin C content (39.63 to 47.52mg) of the study biscuits were more than 0.17 to 0.27mg in Acha-tangerine peels flour made biscuits reported [41].

The study tannin and oxalate contents were similar to 0.67mg and 0.47mg respectively reported by Akujobi, Obicheozo and Nwokorie [42]. The study biscuits phenol and flavonoids contents were comparable to 0.41mg, and 0.14mg and 0.19mg reported by Okoye and Obi [25]; and Nanyen et al. [43] respectively. The saponin content result obtained from this present study were lower compare with those reported on biscuit produced from wheat–Acha and mung bean flour [43]. The phytate content of the study soybean-corn-date palm biscuits were much lower than 5.57 to 5.57mg reported for cookies made with wheat, African yam bean and Acha flours [44]. The antinutrients contents of the study soybean-corn-date palm made biscuits were within lower levels, which are reported to have phytochemical properties which includes antioxidant effect [45, 46].

V. CONCLUSION

Biscuit produced from composite flours of soybean, corn and date palm have varied nutritional potential and should be encouraged as a wheat flour substitute to baking industries. The protein, fat, ash, crude fiber, and energy values of the biscuit's blends were superior to 100% wheat flour biscuits. The vitamins and mineral values of the biscuit's blends were significantly comparable to wheat flour biscuits. The antinutrients compositions were in the level of phytochemicals and thus have beneficial effect to health. All the blends could serve various purpose for different life cycle stages and health conditions. The production of these biscuits will add variety and improve nutrient intakes; it will improve consumption of the base crops, motivate farmers to plant them, diversify the uses, provide nutrient rich and nutritious foods for all the consumers that have made biscuits part of their diet.

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Enhancing Food Safety with AI: Insights from Nigeria's Agricultural Household Dietary Diversity

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Abstract—

Objective: This study examined the determinants of Household Dietary Diversity Scores (HDDS) in agricultural households in Nigeria.

Methodology: Secondary data was sourced from General Household survey Dataset, wave 1-4. A two-stage sampling design was used to select 3,600 households from 360 enumeration areas based on the 2006 Population Census frame. A High-Dimensional Fixed Effects (HDFE) regression model and Multivariate Probit model was used to analyze and present the data collected.

Results: The model was highly significant (F -statistic: 223.73, $p < 0.000$), with an R -squared of 0.3057, indicating that 31% of the variance in HDDS was explained. The within R -squared (0.2353) showed that 23.5% of the variation in HDDS within geographical zones was explained by the independent variables. Key findings revealed that lagged HDDS (coefficient: 0.2304, $p < 0.000$), net income ($p < 0.05$), education ($p < 0.01$), and access to extension services ($p < 0.01$) had significant positive effects on dietary diversity. In contrast, household size had a weak negative effect, and household head age was not significant. Water source during the dry season significantly reduced HDDS ($p < 0.01$), highlighting the importance of water access. Sanitation facilities positively impacted HDDS ($p < 0.05$), while rural households showed marginally higher dietary diversity.

Conclusion: The study emphasizes the importance of income, education, water access, and community services in enhancing food security and dietary diversity among agricultural households in Nigeria.

Keywords— Dietary diversity, Agriculture, Income, Education, Food safety.

I. INTRODUCTION

Artificial Intelligence (AI) is defined as the tangible real-world capability of machines or non-human things to perform task, resolve issues, communicate, network, and behave logically as biological humans [1]. The integration of AI with agriculture is revolutionizing how we address food security challenges globally. This technological advancement is particularly relevant in Nigeria, where agriculture plays a vital role in both the economy and food security. Nigeria has unique agricultural, nutritional, and cultural landscape that necessitates the investigation of AI's ability to enhance farming practices and improve dietary outcomes. The Nigeria's agricultural sector supports many livelihoods. Agricultural sector is constantly challenged by post-harvest losses, limited access to modern farming technology, weak regulations, and policies; all of which affect food quality and nutrition [2]. Food safety, quality control and nutrition information are critical for agricultural sector. AI technological innovation offers potential solutions to improve these agricultural and supply chain issues [3]. Several studies have documented the use of AI in the agricultural space. AI can revolutionize agriculture through predictive analytics and supply chain improvements [4]. AI solutions can address specific challenges like pest detection and soil monitoring, and improve food distribution efficiency [5, 6]. AI can also empower farmers, through mobile-based applications and decision-support systems, to access vital information on best practices, market prices, and weather forecasts [7]. AI not only improve productivity but also enhance resilience to climate change. Climate change poses a growing threat to food security in most region [8]. It has

already been highlighted that AI can support policymakers and stakeholders in designing data-driven interventions that promote equitable access to nutritious food. For example, predictive models can identify regions most at risk of food safety and or food security, enabling targeted investments in infrastructure and agricultural extension services. AI-powered block-chain technology could enhance transparency and traceability in food supply chains, addressing concerns related to food fraud and contamination [9]. In addition to traceability, AI technologies can improve real-time safety monitoring, and storage efficiency, reducing spoilage and waste [10]. AI-powered advisory services provide farmers with timely, tailored advice to boost yields and manage pests, reducing crop failure and spoilage, thereby boosting food security [11]. AI tools, such as mobile applications, have been shown to educate consumers on the nutritional value and safety of foods [12]. These apps track dietary diversity and suggest improvements based on preferences and nutritional needs, enhancing household dietary diversity. In Nigeria, mobile technology solutions like the "Sell Harvest" app are being used to make farming more sustainable and secure food, thereby improving food safety/security and dietary diversity. Nigerian policymakers can leverage AI to analyze big data, predicting food safety risks and informing better regulations [13].

Nigeria faces significant challenges in food safety and quality control, with many at risk of food-borne illnesses due to inadequate food handling, poor storage, limited regulation enforcement, and low public awareness. These challenges have clear negative implication for household dietary diversity (HDD). HDD refers to the number of food groups consumed by *household* members over a 24-hour period. It is a critical indicator of nutritional adequacy and food security, that reflects the variety of food groups consumed by households within a specific period. A varied diet is associated with better nutrient intake and overall health. It is reported that female-headed households and those engaged in diverse on-farm production have more varied diets [14]. Factors like remoteness and limited market access significantly reduce dietary diversity [15]. The Household Dietary Diversity Score (HDDS) is a scale that assesses the diversity of household food consumption based on the number of food groups consumed in the previous 24 hours. HDDS serves as a proxy for food access and socioeconomic status [16].

Restricted access to safe and diverse food options leads to monotonous diets, increasing malnutrition risks. Other factors such as education, income, and access to clean water will also influence HDD. This study investigated the integration of AI in enhancing food safety and quality control within the framework of Nigeria's agricultural practices by specifically examining the determinants of Household Dietary Diversity (HDDS) and its variation across socio-economic and regional factors; and the relationship between HDDS and food safety indicators, focusing on access to clean water, sanitation, and socio-economic factors, and their implications for household health outcomes.

II. ANALYTICAL FRAMEWORK

2.1 High-dimensional fixed effects regression model (HDFE regression):

The HDFE linear regression is an advanced linear regression model designed to handle datasets with multiple levels of fixed effects efficiently. It builds on traditional linear regression by allowing for the absorption of high-dimensional fixed effects, such as those that represent multiple group-specific intercepts (e.g., fixed effects for regions, time periods, or industries). HDFE command makes it a robust choice for handling large, complex datasets with unobserved heterogeneity across multiple dimensions [17].

2.2 Multivariate Probit model:

The multivariate probit model estimates relationships between correlated binary dependent variables and independent variables, accounting for error correlations using a joint multivariate normal distribution. It typically uses simulated maximum likelihood methods to address high-dimensional integration challenges [18]. Common in econometrics and social sciences, the Mvprobit model analyzes complex decision-making, such as household resource allocation or participation in related programs [19]. By considering correlations among outcomes, it enhances parameter estimates and reflects real-world interdependencies. An in-depth discussion of multivariate models and their applications was documented [20].

III. METHODOLOGY

3.1 Study area:

The study area was Nigeria, located between latitude 9.082° n and longitude 8.6753° e [21]. Nigeria is a cardinal regional player in West Africa, accounting for about half of West Africa's population with approximately 205 million people (world population review 2020). It is a multi-ethnic, culturally diverse country consisting of 36 states and the federal capital territory. It is blessed with natural resources, Africa's biggest oil exporter, and largest natural gas reserves on the continent [22].

- **Data for the study:** The study collected secondary data from General Household survey Dataset, wave 1-4 [23].
- **Sampling Technique:** The study used the Nigerian General Household Survey (GHS) two-stage sampling design for national and zonal representativeness. Enumeration Areas (EAs) were selected based on the 2006 Population Census frame, with households systematically chosen in the second stage. The initial sample included 2,220 EAs, each contributing 10 households, resulting in 22,200 households. A panel of 5,000 households from 500 EAs supports longitudinal studies on agriculture and welfare. A partial refresh in subsequent waves added 3,600 households from 360 new EAs, maintaining national and zonal representativeness [23]. This design provided valuable data on agricultural practices, household welfare, and socio-economic indicators across Nigeria.
- **Data analysis:** The study used inferential statistics. High-dimensional fixed effects regression model (HDFE) was used to examine determinants of Household Dietary Diversity (HDDS) and its variation across socio-economic and regional factors; while the Multivariate Probit (mvprobit) model was used to investigate the relationship between HDDS and food safety indicators, focusing on access to clean water, sanitation, and socio-economic factors and their implications for household health outcome.

3.2 Model specification:

For **High-dimensional fixed-effects (HDFE)** Linear regression for **HDDS**:

$$HDDS_{it} = \beta_1 L.HDDS_{it} + \beta_2 crop_income_{it} + \beta_3 educat_{it} + \beta_4 hhsz_{it} + \beta_5 hhage_{it} + \beta_6 watersou_rainy_{it} + \beta_7 watersou_dry_{it} + \beta_8 sector_{it} + \beta_9 extserv_{it} + \beta_{10} toilet_type_{it} + \beta_{11} zone_{it} + \alpha_5 + \epsilon_{it}$$

Where

Dependent variable (HDDS_{it}): Household Dietary Diversity Score (HDDS), measured for individual i, at time t.

Lagged dependent variable (L.HDDS_{it}): The lagged value of HDDS to account for dynamic effects.

Explanatory variables ($\beta_1, \dots, \beta_{11}$):

Net income from (measured in Naira), Education level (number of years spent in school, Household size, Age of the household head (years), Water source in the rainy season, Water source in the dry season, **Sector** (Urban or rural residence), Access to extension services Type of toilet facility used, **zone**: Geographic zone or region.

Fix effects (α_5): Absorbed fixed effects for the variable **score** (as specified in the absorb(zone) option the your model).

Error term (ϵ_{it}): The residual variation not explained by the model.

3.3 The multivariate probit model was specified as follows,

First the 12 HDDS scores were further categorized into four binary categories to enable the investigation of the relationships between different levels of household dietary diversity and the explanatory variables

0 -3 **very poor (hdd1)**, 4 -6 **poor (hdd2)**, 7 - 9 **good (hdd3)**, 10 -12 **very good (hdd4)**.

$$hdd_{1i} = \beta_{1,0} + \beta_{1,1} \cdot watersou_dry_i + \beta_{1,2} \cdot watersou_rainy_i + \beta_{1,3} \cdot toilet_type_i + \beta_{1,4} \cdot extserv_i + \beta_{1,5} \cdot educat_i + \beta_{1,6} \cdot hh_age_i + \beta_{1,7} \cdot hhsz_i + \beta_{1,8} \cdot sector_i + \epsilon_{1i}$$

$$hdd_{2i} = \beta_{2,0} + \beta_{2,1} \cdot watersou_dry_i + \beta_{2,2} \cdot watersou_rainy_i + \beta_{2,3} \cdot toilet_type_i + \beta_{2,4} \cdot extserv_i + \beta_{2,5} \cdot educat_i + \beta_{2,6} \cdot hh_age_i + \beta_{2,7} \cdot hhsz_i + \beta_{2,8} \cdot sector_i + \epsilon_{2i}$$

$$hdd_{3i} = \beta_{3,0} + \beta_{3,1} \cdot watersou_dry_i + \beta_{3,2} \cdot watersou_rainy_i + \beta_{3,3} \cdot toilet_type_i + \beta_{3,4} \cdot extserv_i + \beta_{3,5} \cdot educat_i + \beta_{3,6} \cdot hh_age_i + \beta_{3,7} \cdot hhsz_i + \beta_{3,8} \cdot sector_i + \epsilon_{3i}$$

$$hdd_{4i} = \beta_{4,0} + \beta_{4,1} \cdot watersou_dry_i + \beta_{4,2} \cdot watersou_rainy_i + \beta_{4,3} \cdot toilet_type_i + \beta_{4,4} \cdot extserv_i + \beta_{4,5} \cdot educat_i + \beta_{4,6} \cdot hh_age_i + \beta_{4,7} \cdot hhsz_i + \beta_{4,8} \cdot sector_i + \epsilon_{4i}$$

$j=1,2,3,4$

$$= hdd_j = \begin{cases} 1 & \text{if } hdd_{ij} > 0 \\ 0 & \text{otherwise} \end{cases}$$

Dependent variables (hdd₁, hdd₂, hdd₃, hdd₄): These are binary indicators of different household dietary diversity outcomes or categories, where j=1,2,3,4 represents different outcomes, and iii represents the household.

hdd_{1i}^* , hdd_{2i}^* , hdd_{3i}^* , hdd_{4i}^* : Latent variables (unobserved) for each of the four categories (very poor, poor, good, very good).

Explanatory variables (β_{jk}): the same explanatory variables in objective (i)

Error terms (ϵ_{ij}): Jointly distributed error terms, typically assumed to follow a multivariate normal distribution with mean 0 and a variance-covariance matrix Σ .

IV. RESULT AND DISCUSSION

4.1 Determinants of household Dietary diversity in Agricultural households in Nigeria:

The analysis reveals several significant factors influencing household dietary diversity in Nigeria, with the model explaining approximately 31% of HDDS variance and 23.5% of variation within geographical zones. Past dietary patterns strongly influence current diversity, as indicated by a positive relationship between previous and current HDDS levels. Economic and educational factors play crucial roles. Higher net income significantly improves dietary diversity, suggesting that financial resources directly enable households to access more varied food options. Education levels show a strong positive correlation with HDDS, likely due to improved nutritional knowledge and better economic opportunities. Access to extension services, including food assistance and community support, also significantly enhances dietary diversity. Infrastructure and environmental factors demonstrate varying impacts. Water access emerged as a critical determinant, particularly during dry seasons where limited water availability significantly reduces dietary diversity. However, rainy season water sources show no significant impact. Improved sanitation facilities, measured through toilet type, correlate positively with higher dietary diversity, possibly due to better overall living conditions and health outcomes. Household characteristics show mixed effects. While household size shows a slight negative correlation with dietary diversity, the age of the household head appears to have no significant impact. The rural-urban divide shows only marginal significance, with rural households displaying slightly higher dietary diversity. The model's high statistical significance (F-statistic: 223.73, $p < 0.000$) confirms these relationships are not random. These findings suggest that improving household dietary diversity in Nigerian agricultural households requires a multi-faceted approach focusing on income generation, education, extension services, and infrastructure development, particularly water access during dry seasons.

TABLE 1
HIGH-DIMENSIONAL FIXED-EFFECTS (HDFE) LINEAR REGRESSION FOR HOUSEHOLD DIETARY DIVERSITY (HDDS)

HDDS	Coef.	Std. Err.	T	P> t	[95% Conf.	Interval]
HDDS L1.	0.2304113	0.0106403	21.65	0.000***	0.2095535	0.2512691
crop_income	1.12E-07	5.57E-08	2.01	0.044*	2.84E-09	2.21E-07
Educat	0.2448891	0.0146638	16.7	0.000***	0.2161443	0.2736339
extserv	0.3416214	0.0722932	4.73	0	0.1999078	0.483335
Score	0.0212818	0.0069906	3.04	0.002**	0.0075784	0.0349852
Hhsize	-0.0084023	0.0050759	-1.66	0.098	-0.0183523	0.0015477
hh_age	0.0009191	0.0011234	0.82	0.413	-0.0012831	0.0031212
watersou_rainy	-0.0486603	0.0404568	-1.2	0.229	-0.1279661	0.0306455
watersou_dry	-0.8427156	0.0357259	-23.59	0.000***	-0.9127477	-0.7726834
toilet_type	0.1087156	0.0499353	2.18	0.029*	0.0108295	0.2066018
Sector	0.0859522	0.0459509	1.87	0.061	-0.0041235	0.1760278
_cons	1.589532	0.1236236	12.86	0.000***	1.347198	1.831867
Model Statistics	Value					
Number of obs	8,014	F(11, 7997)	223.73		Prob > F	0
R-squared	0.3057	Adj R-squared	0.3043		Root MSE	1.4421
Absorbed degrees of freedom						
Absorbed FE Categories - Redundant = Num. Coefs						
zone 6 0 6 (MWFE estimator converged in 1 iterations)						

***, ** indicate significance of 1% and 5% respectively. Source: Nigeria GHS, Panel, Wave 4: National Bureau of Statistics, (2019)

4.2 Relationship between Household Dietary Diversity (HDDS) and food safety indicators, (access to clean water, sanitation, and socio-economic factors):

The multivariate probit model in table 2 showed a Wald $\chi^2(15) = 505.95$, with a p -value < 0.0000 , indicating statistical significance. The Log likelihood of 2938.9047 reflects the model's fit, with values closer to 0 suggesting better fit. The model estimates relationships between independent variables and the likelihood of being in one of four categories: very poor (hdd1), poor (hdd2), good (hdd3), and very good (hdd4). Coefficients represent the probability changes for each outcome relative to others. For hdd2 (poor), hdd3 (good), and hdd4 (very good), the coefficients for hdd2 (-7.095874), hdd3 (0.2562015), and hdd4 (-7.140424) were not significant, suggesting no difference from the very poor category (hdd1).

TABLE 2
MULTIVARIATE PROBIT MODEL

hdd1	Coef.	Std. Err.	z	P> z	[95% Conf. Interval]	
hdd1						
hdd2	-7.095874	131.423	-0.05	0.957	-264.6803	250.4886
hdd3	0.2562015	671.1693	0	1	-1315.211	1315.724
hdds4	-7.140424	565.5295	-0.01	0.99	-1115.558	1101.277
watersou_dry	0.6322208	0.0382349	16.54	0.000***	0.5572817	0.7071599
watersou_rainy	0.0680724	0.0451836	1.51	0.132	-0.0204859	0.1566306
toilet_type	-0.1048267	0.0547757	-1.91	0.056	-0.2121851	0.0025317
extserv	-0.1087722	0.0781235	-1.39	0.164	-0.2618914	0.044347
net_income	8.81E-10	6.18E-10	1.43	0.154	-3.29E-10	2.09E-09
Zone	-0.0922169	0.0129426	-7.13	0.000***	-0.1175838	-0.06685
dist_market	-0.0001432	0.0004256	-0.34	0.737	-0.0009774	0.000691
Educat	-0.1635386	0.0160198	-10.21	0.000***	-0.1949369	-0.1321403
hh_age	-0.0010324	0.0012479	-0.83	0.408	-0.0034781	0.0014134
hhsz	0.1494	6064	2.46	0.014**	0.0030549	0.0268251
GPSm_pltsize_ha	-0.0017598	0.0043563	-0.4	0.686	-0.010298	0.0067785
Sector	-0.1937633	0.0530086	-3.66	0.000***	-0.2976582	-0.0898684
Cons	1.725005	0.1471875	11.72	0.000***	1.436523	2.013487
Number of obs = 8,760 Wald $\chi^2(15) = 505.95$						
Log likelihood = -2938.9047 Prob > $\chi^2 = 0.0000$						

Households with dry season water sources were more likely to be in the poor category (hdd2). Education was negatively linked to being in the very poor category, indicating better dietary diversity among educated individuals. Larger households had a higher likelihood of being in the very poor category (hdd1), while rural households showed better food security. Better sanitation, indicated by toilet type, reduced the likelihood of being in the very poor category. Other variables were not significant.

V. CONCLUSION

Past dietary patterns strongly influence current diversity, emphasizing the need for sustained interventions. Economic factors, particularly household income and education were crucial for improving dietary diversity. Infrastructure, especially water access during dry seasons, was also critical. Household size had mixed effects, with larger households facing challenges in maintaining diversity, while rural households showed slightly better dietary diversity compared to urban areas.

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Technical Efficiency of Bell Pepper (*Capsicum frutescence* L.) Growing Farms- An Opportunity to Stabilize Agriculture Economy of Oman

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Abstract— Vegetables generally have significant importance with respect to their food value in terms of nutrition as they contain both essential vitamins and minerals. Bell pepper (*Capsicum frutescence* L.) is an economically important vegetable crop with the potential to increase farmers' incomes in open fields and greenhouses in Oman. Bell pepper is popular as *Capsicum* among customers and farmers in Arabian Peninsula countries, including Oman, with fairly moderate retail prices compared to other vegetables. Hence, a survey study investigated the technical efficiency of *Capsicum*-growing farms in the Sultanate of Oman. The research investigated the technical efficiency of *Capsicum*-growing farms of different governorates of Oman. A sample of 52 *Capsicum*-growing farms was selected using the multistage sampling method; the interview schedules with the farmers were conducted for data collection from January 2016 to December 2017. The data collected were subjected to the maximum likelihood method and the Cobb-Douglas stochastic frontier production model. The output of Frontier 4.1 was found to be a good fit after performing various software econometrics. Our study revealed that the mean technical efficiency for Bell pepper-growing farms in Oman was estimated to be 87%, ranging from 50% to 99%. This indicated a considerable scope of increasing capsicum yield by 13% with altering levels of inputs employed by the farmers. Further, it was observed that among the inefficiency factors, farmers' age and experience contributed significantly to the technical efficiency of the farms. Bell pepper-growing farms had enormous scope to improve their efficiency and increase productivity by following regular extension programs involving the farmers.

Keywords— Technical efficiency, maximum likelihood method, stochastic frontier production, Frontier 4.1, *Capsicum*, Bell pepper.

I. INTRODUCTION

Capsicum Bell pepper (*Capsicum frutescence*) is an important agricultural food commodity because of its economic value and nutritional contents in terms of vitamins and minerals besides possessing natural colors from green, yellow, orange, red, etc., depending on differences in varieties and anti-oxidant compounds (Howard et al., 2012). Bell pepper is reported to be one of the world's most important fruit vegetables, along with tomatoes. Bell pepper provides essential minerals and vitamins like capsicums (Bosland & Votava, 2012). In 2020, the world's production of bell peppers was 36 million tons, led by China, which made up 46% of the total (Tridge, 2022). Globally, the capsicum market size was found to be USD 4575.3 million in 2023 and is projected to reach USD 7214.05 million by 2032. Asia-Pacific held a leading capsicum market share in 2023 (Industry Research Report, 2024).

In the Arabian Peninsula, vegetables are grown under open fields and greenhouse conditions. The investigations of ICARDA's APRP (Arabian Peninsula Research Program) have shown that growing vegetables adapting protected agriculture has proved very successful in raising the livelihoods and economic stability of the farmers in the Arabian Peninsula in general and in Oman in particular (Osman et al., 2017).

In Oman, the total cultivated area was found to increase by 3.9 percent to reach 276,000 acres by the end of 2022 compared to that in 2021 (266,000 acres) with a total agricultural production of 3.501 million tons. Vegetables with a total area of 69,074

acres produced 1.137655 million tons in 2022 (NCSI, 2022). Increasing vegetable production has been projected to contribute to government efforts to diversify the national economy (MAF, 2019). Interestingly, Capsicum production volume was also found to have surged gradually from 9.4 million kg in 2008 to 93.42 million kg in 2022, according to FAO report with code 0401 (Tridge, 2022). This increased nature of production has been considered a positive indicator of the potential opportunities for Capsicum Bell pepper cultivation in Oman and other countries (FAO, 2016; MAF, 2019).

The present study used Stochastic Production Frontier (SPF) to evaluate Bell pepper production efficiency in Oman. Earlier, many scientists used frontier applications in the field of production in non-agriculture production industries (Seiford et al., 1990; Ganley & Cubbins, 1992; Fare et al., 1994). During the same period, SPF was applied in forestry (Sanusi et al., 2017), agriculture (Fernandez-Cornejo, 1994), horticulture (Zeibet et al., 1999), olericulture (Shrestha et al., 2016; Bozoglu & Ceyhan, 2007; Julie et al., 2017) besides dairy sciences (Mbaga et al., 2003). Of late, SPF has been effectively applied to comprehend the technical efficiency of producing different vegetable crops (Wahid et al., 2017) and Okra (Ume et al., 2018; Alabi et al., 2023). Recently, Senthilkumar et al. (2018), Alabi et al. (2022), and Martinez-Reina et al. (2022) have attempted to study the technical efficiency of production in Capsicum/ Bell pepper. All these studies in agriculture have suggested effective measures for raising the production efficiency of crops. Given the above facts, the present study was undertaken to know the technical efficiency of the farms growing Bell pepper Capsicum in Oman.

II. MATERIALS AND METHODS

2.1 Data and Variables:

The primary data on Bell pepper growing farms were collected on all the aspects related to the study and recorded in the questionnaires through surveys during 2016 and 2017. The data were collected from 52 farmers growing Bell pepper Capsicum across four governorates of Oman, namely North Al Batinah, South Al Batinah, North Al Sharqiya, and Al Dakhiliya. The variables were selected based on previous efficiency studies (Belloumi & Matoussi, 2006; Mbaga, 2011; Wahid et al., 2017). The total output in kilograms was the dependent variable. In contrast, independent variables (inputs) were farm size (ha), fertilizer (kg/ha), labor (person-hours), seeds (kg/ha), irrigation water utilized (cubic meter/day), electricity charges (Omani Rials/month) and chemicals used (kg/ha). The study comprised three farm-specific variables used for the inefficiency model, viz. the farmer's age (years), the farmer's experience in farming (years), and the farmer's level of education as non-educated (zero (0)) and educated (1). The education level included illiteracy (no school), education till Class 12, and education beyond Class 12.

2.2 Analysis of Technical Efficiency:

The survey data were subjected to the analysis of the efficiency of Bell pepper production using both SHAZAM econometric software and the Coelli (1996) "FRONTIER 4.1" computer program. The software referred to as SHAZAM is a very comprehensive tool for measuring econometrics, statistics, and analytics. It is popular worldwide as it offers various computations such as building models, checking hypotheses, and explaining the variation among different variables.

2.3 Analysis of Technical Inefficiency:

The Stochastic Frontier Production (SFP) function model (approach) was used to assess the technical inefficiency of Bell pepper-growing farms in Oman, as suggested by Battese and Coelli (1995).

III. RESULTS AND DISCUSSION

The maximum likelihood estimates for Bell peppers are presented in Table 1. The model indicated that variables such as farm size, electricity, and water were positive for Bell pepper production output with a significance of about 5%. This meant that these parameters led to an increase in the output. The coefficient of electricity has the highest value, followed by water and farm size. The estimate of Bell pepper production as a result of a unit increase in electricity was found positive (34.74) and significant. This meant that 1 unit increase in input (electricity, OMR) would increase Bell pepper output by 34.7 kilograms. On the other hand, labor, seeds, chemicals, and fertilizer were found to be negative, with a significance of about 5%.

In the inefficiency model, we had the farmer's age, the farmer's experience, and education. Farmer's age and experience were found to be positive, which meant older and much more experienced farmers tended to be much less efficient, with a significance of 5%. The farmer's age coefficient was positive (0.2) by a priori expectations. This indicated that age increases inefficiency and reduces efficiency, meaning that older farmers tended to be much more technically inefficient than their relatively younger counterparts. The other two factors, like education level, had also negative signs but were insignificant ($p>0.05$), which was also observed by Haji and Andersson (2006).

TABLE 1
MAXIMUM LIKELIHOOD ESTIMATES OF THE COMMON STOCHASTIC PRODUCTION FRONTIER FOR BELL PEPPER

Variable Name	Parameter	Coef.	Standard Error	T-Ratio
Stochastic Frontier Model				
Constant	B_0	-1.08	0.88	-1.23
$\ln(X1)$ (Farm size)	β_1	2.22	0.87	2.57
$\ln(x2)$ (Fertilizer)	β_2	-2.65	0.78	-3.39
$\ln(X3)$ (Labor)	β_3	-5.79	0.88	-6.6
$\ln(X4)$ (Seeds)	β_4	-2.37	0.88	-2.69
$\ln(X5)$ (Water)	B_5	3.8	0.74	5.12
$\ln(X6)$ (Electricity)	B_6	34.74	7.99	4.35
$\ln(X7)$ (Chemicals)	B_7	-34.59	7.81	-4.43
$\ln(X1)*\ln(X1)$	B_8	0.45	0.74	0.61
$\ln(X2)*\ln(X2)$	B_9	-0.34	0.14	-2.5
$\ln(X3)*\ln(X3)$	B_{10}	-0.04	0.84	-0.04
$\ln(X4)*\ln(X4)$	B_{11}	-0.47	0.4	-1.17
$\ln(X5)*\ln(X5)$	B_{12}	-0.44	0.05	-9.56
$\ln(X6)*\ln(X6)$	B_{13}	-19.36	0.38	-50.42
$\ln(X7)*\ln(X7)$	B_{14}	-9.61	0.57	-16.98
$\ln(X1)*\ln(X2)$	B_{15}	-0.43	0.67	-0.64
$\ln(X1)*\ln(X3)$	B_{16}	-0.94	0.85	-1.11
$\ln(X1)*\ln(X4)$	B_{17}	-0.55	0.81	-0.68
$\ln(X1)*\ln(X5)$	B_{18}	-0.08	0.58	-0.14
$\ln(X1)*\ln(X6)$	B_{19}	-17.49	0.76	-23.09
$\ln(X1)*\ln(X7)$	B_{20}	16.27	0.76	21.55
$\ln(X2)*\ln(X3)$	B_{21}	-0.04	0.45	-0.08
$\ln(X2)*\ln(X4)$	B_{22}	-0.2	0.19	-1.03
$\ln(X2)*\ln(X5)$	B_{23}	-0.01	0.41	-0.02
$\ln(X2)*\ln(X6)$	B_{24}	-3.02	0.5	-6.09
$\ln(X2)*\ln(X7)$	B_{25}	3.15	0.63	5.03
$\ln(X3)*\ln(X4)$	B_{26}	0.35	0.75	0.47
$\ln(X3)*\ln(X5)$	B_{27}	0.29	0.63	0.46
$\ln(X3)*\ln(X6)$	B_{28}	85.55	8.77	9.75
$\ln(X3)*\ln(X7)$	B_{29}	-85.46	9.76	-8.76
$\ln(X4)*\ln(X5)$	B_{30}	-0.11	0.33	-0.34
$\ln(X4)*\ln(X6)$	B_{31}	6.37	0.69	9.26
$\ln(X4)*\ln(X7)$	B_{32}	-6.59	0.7	-9.36
$\ln(X5)*\ln(X6)$	B_{33}	-54.45	8.59	-6.34
$\ln(X5)*\ln(X7)$	B_{34}	53.36	9.56	5.58
$\ln(X6)*\ln(X7)$	B_{35}	28.33	9.71	2.92
Inefficiency Model				
Variable Name	Parameter	Coef.	Standard Error	T-Ratio
Constant (δ_0)	δ_0	-0.35	0.81	-0.43
Farmer's Age (Z1)	δ_1	-0.2	-0.1	2.03
Farmer's Experience (Z2)	δ_2	-0.22	-0.1	2.21
Education Dummy (Z3)	δ_3	-0.34	0.29	-1.2
Sigma Square	σ^2	1	0.24	4.18
Gamma	γ	0.01	0	6.77

The maximum likelihood estimates of the Stochastic Frontier for Bell peppers are presented in Table 2. The results revealed that the mean technical efficiency for Bell pepper farms in Oman was 87%, ranging from 50% to 99%. This indicated the possibility of increasing Bell pepper output to 13% with the current level of inputs used by farmers for cultivation. Further, more than half of the farms (55%), the Bell pepper growing farms in Oman, were observed to be efficient to 90 to 100%. This was followed by 19% of the efficient farms, between 80% and 90%. Similar observations have been noted from the histogram showing the distribution of technical efficiency scores of Bell pepper growing farms (Fig. 1). As many as 29 farms out of 52 had technical efficiency beyond 90%, which can be treated as the best farms. In contrast, the remaining 23 farms were found efficient from <60% (5) to 80 to 90% (10). These figures clearly showed at least a 10% scope for improving the efficiency of Bell pepper production with current levels of inputs used by farmers in Oman.

TABLE 2

A SUMMARY OF THE MAXIMUM LIKELIHOOD ESTIMATES OF THE COMMON STOCHASTIC FRONTIER FOR BELL PEPPER

Efficiency Index (%)	Study Sample	
	Number of Farms	Percentage (%)
Less than 60	5	10
Between 60–70	3	6
Between 70–80	5	10
Between 80–90	10	19
Between 90–100	29	55
Mean Efficiency	87%	
Median	95%	
Maximum	99%	
Minimum	50%	
Standard deviation	2	
Sample size	52	100

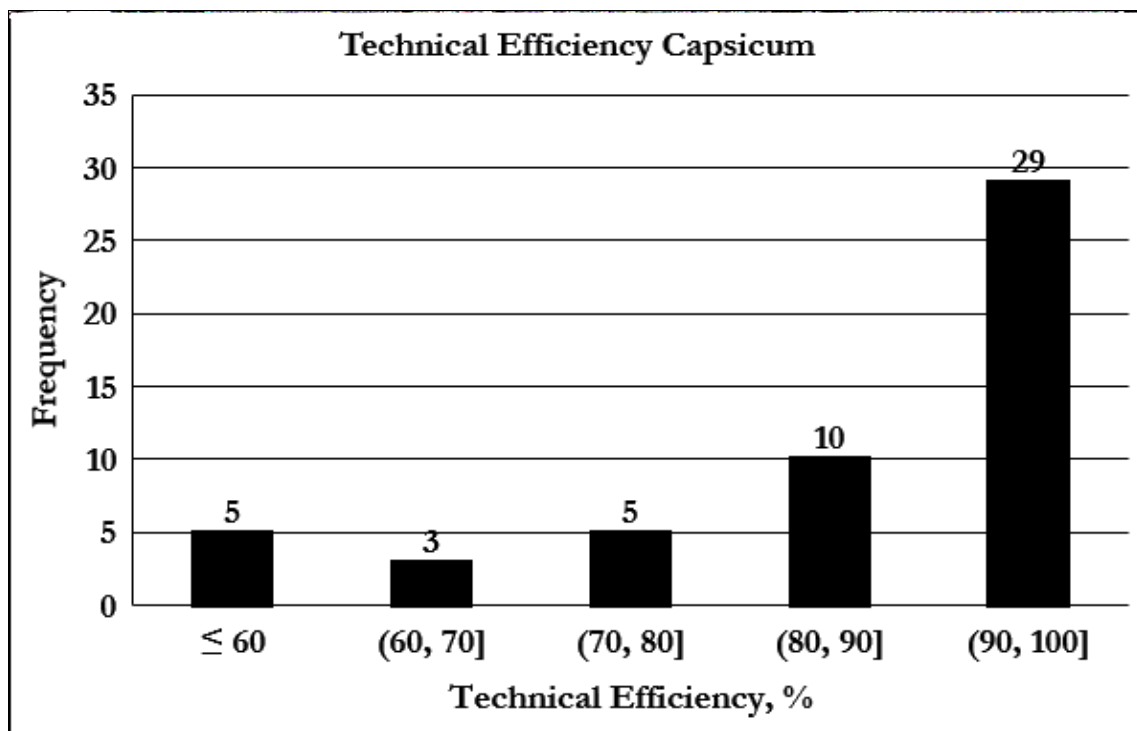


FIGURE 1: The distribution of technical efficiency scores of Bell pepper growing farms in Oman

The range of technical efficiency of Capsicum production observed in the present studies was similar to the findings of research conducted elsewhere in the world. Ogunbo et al. (2015) reported that the mean technical efficiency of pepper (*Capsicum annum* Mill) production was 0.64. The authors considered that the current production frontier was only relatively efficient and that improved high-yielding pepper cultivars and training in their use could improve pepper production efficiency in Nigeria. In the study of Hina et al. (2017), the mean technical efficiency was estimated to be 83.64 percent, indicating that output could be raised by 16.36 percent through efficient crop management practices without actually increasing the levels of inputs. Senthilkumar et al. (2018), however, reported the average technical efficiency of sampled capsicum farms under protected cultivation to be 83.64 percent with optimization of the production frontier of the capsicum cropping system by overcoming the existing level of technical inefficiencies related to farmers' experience in India. Martinez-Reina et al. (2022) reported the mean technical efficiency of the sweet chilli (*Capsicum chinense*) to 81% in the Colombian Caribbean, which was suggested for improvement with increasing labor and weed management inputs. Similarly, Alabi et al. (2022) observed that mean technical efficiency was 79%, leaving a gap of 21% for improvement for the smallholder pepper (*Capsicum* species) production in Abuja, Nigeria, through addressing the challenges of inadequate rainfall through proper irrigation policies.

The present study covers a sample of 52 farms growing Bell peppers across prominent vegetable-growing wilayats of four governorates of Oman, namely North Al Batinah, South Al Batinah, North Al Sharqiya, and Al Dakhiliya. The joint document of FAO with the Ministry of Agriculture & Fisheries on the Sustainable Agriculture and Rural Development Strategy (SARDS-2040) towards 2040 of the Sultanate of Oman listed Bell pepper as one of the lead vegetable crops in terms of land and water consumption. Our results revealed the potential of increasing Bell pepper production to 10-13% with levels of inputs/ variables studied. This indicated that an increase in vegetable production could be the key to attaining not only self-sufficiency in the agricultural sector but also to maintaining economic sustenance through diversification of crops for cultivation as highlighted in SARDS-2040 developed by FAO along with Ministry of Agriculture & Fisheries, Sultanate of Oman (FAO, 2016; AlSalmi et al., 2020). The above observations would also apply to all GCC countries (Gulf Cooperative Council), the Arabian Peninsula, and the world.

IV. CONCLUSION

The mean efficiency level of Bell pepper was about 87%, indicating that 13% of the scope existed to raise the efficiency of production to 100% with the levels of inputs considered in the study. Intensifying extension activities on Bell pepper cultivation among the farmers and organizing training programs to improve their skills would increase Bell pepper productivity.

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CONFLICT OF INTEREST

Authors declare that they do not have any conflict of interest.

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Detection of Herpesvirus Infection (RaHV-1) in amphibians from commercial, experimental, and recreational breeding in São Paulo State, Brazil

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Abstract— Frog farming represents an alternative animal production system with significant economic potential, especially in Brazil, recognized as one of the largest global producers of captive-bred frogs. However, inadequate management and environmental stressors may lead to outbreaks of infectious diseases, including those caused by herpesviruses (RaHV-1), posing severe health risks to animals and threatening production viability. This study aimed to detect the presence of herpesvirus in amphibians from commercial, experimental, and recreational facilities in São Paulo State, Brazil. Skin fragments and internal organs from 49 amphibians were analyzed using transmission electron microscopy and histopathological techniques. Findings revealed that 23 animals were positive for RaHV-1, showing pleomorphic viral particles, some enveloped, with diameters between 120 and 200 nm in both skin and organ fragments. Histopathological examinations also revealed intranuclear structures in various organs suggestive of RaHV-1. These findings indicate the circulation of RaHV-1 in Brazilian frog farms, highlighting the importance of further studies to better understand disease pathology and mitigate its impact on production.

Keywords— *Herpesvirus Infection (RaHV-1), Amphibians, Transmission Electron Microscopy, Histology.*

I. INTRODUCTION

Frog farming has become a promising activity in Brazil, which ranks as one of the largest producers of captive-bred frogs. Although Brazil is among the world's leading producers of frog meat, regulation of frog farming remains highly deficient, and the industry faces cultural, economic, governmental, and health-related obstacles, hindering its expansion in the country. Additionally, this sector encounters challenges related to inadequate management, environmental stress, and outbreaks of infectious diseases. Among the diseases that can affect amphibians, herpesvirus infections, belonging to the *Alloherpesviridae* family, have drawn attention due to their ability to cause skin lesions, tumors, and adenocarcinomas, negatively impacting animal development and producer profitability.

Herpesviruses, in general, are known for establishing long-term latent infections in their hosts. These viruses have large genomes with double-stranded DNA and are classified into three main subfamilies based on target cell types and replication cycles:

- 1) *Alphaherpesvirinae*: typically cause acute infections and establish latency in neurons.
- 2) *Betaherpesvirinae*: characterized by longer lytic cycles and latency in immune system cells.
- 3) *Gammapherpesvirinae*: tend to associate with lymphatic system cells and may be linked to cancers in their hosts.

However, herpesviruses that infect amphibians, such as RaHV-1, belong to the *Alloherpesviridae* family, distinct from the three subfamilies of herpesviruses that affect mammals, birds, and other warm-blooded vertebrates.

Amphibians are ecologically and economically significant (VALENCIA-AGUIAR et al., 2013). Due to their thin skin, which facilitates gas exchange between their bodies and the environment, these animals are highly susceptible to ecosystem changes (JORGENSEN, 1994). Most species undergo a life cycle with two phases, a predominantly aquatic larval phase and a terrestrial adult phase. This characteristic makes amphibians excellent bioindicators of environmental quality (SEWELL and GRIFFITHS, 2009). However, this sensitivity to environmental changes, such as deforestation, pollution, and global warming, has led to significant population declines (GRANT et al., 2020; DIETRICH et al., 2020).

This class of vertebrates is divided into three main orders: Anura (including frogs, toads, and tree frogs), Caudata (salamanders and newts), and Gymnophiona (caecilians or blind snakes) (FROST et al., 2021). Some species of the Anura order are traded globally for human consumption. Among them, the North American bullfrog (*Aquarana catesbeiana*) stands out due to its easy adaptation to various management systems worldwide (OLIVEIRA, 2015). Frog farming represents a significant economic potential for producing countries since, in addition to meat, various by-products such as flour, pâté, hamburgers, and preserves can be marketed (OLIVEIRA, 2015). However, international trade in frogs has contributed to the spread of emerging pathogens, as the species appears resistant to high infection levels (JENKINSON et al., 2016; SCHLOEGEL et al., 2009; BRUNNER et al., 2019).

Genetic studies attribute the co-evolution of this viral group with their hosts, where latent infections occur without causing severe diseases with high mortality, thereby favoring the spread of herpesviruses (FRANCO & ROECHE, 2007; CATROXO et al., 2003). Latency can be defined as viral persistence in which the virus remains within cells in a non-pathogenic form, with intermittent periods of reactivation and shedding (ADAMEK et al., 1996). Any latent carrier animal is a potential source of infection, as the virus can resume multiplication and be shed through secretions under low resistance conditions, promoting disease spread. During latency, the viral episomal genome becomes circular, and gene expression is limited. Reactivation is associated with various stressors, such as transport, adverse weather conditions, overcrowding, and concurrent infections (QUINN et al., 2005).

Herpesviruses spread primarily through three routes: cell-to-cell, bloodstream, and nervous system. Cell-to-cell transmission, common in these viruses, occurs through intercellular bridges that prevent antibody action. The virus can also reach the nervous system, being transported to specific ganglia neurons, such as the trigeminal or sacral, depending on the infection site. Blood circulation (in lymphocytes) also facilitates its dissemination, allowing the virus to reach reproductive organs, though viremia is brief. In animals with low immunity, infection can range from asymptomatic to mild but may worsen with high viral doses. As a result, some animals may be asymptomatic carriers of viruses and, when exposed to a drop in immunity, may trigger a viral process, even if the viral presence cannot be linked to the animal's cause of death, and it is unknown whether the viral particle quantity found contributes to worsening the condition (MEYER, 2001; LOPEZ & SILVA, 2019).

Studies on the presence of herpesvirus in bullfrogs (*Lithobates catesbeianus*) are limited in Brazil, motivating this research to detect the occurrence of this pathogen in commercial and experimental facilities in São Paulo State, Brazil.

II. OBJECTIVES

The primary objective of this study was to detect herpesvirus infection (Ra HV-1) in bullfrogs from commercial, experimental, and recreational fa

Specific objectives included:

- 1) Analyzing skin and internal organ samples using transmission electron microscopy (TEM) and light microscopy.
- 2) Identifying the presence of herpesvirus particles with characteristic morphology in the analyzed samples.
- 3) Assessing the infection frequency relative to the total number of animals and samples collected.

III. MATERIALS AND METHODS

3.1 Sample Collection:

Skin fragments and internal organs, such as liver, kidney, spleen, intestine, heart, lung, and brain lesions, were collected from 49 amphibians: 42 captive bullfrogs (*Lithobates catesbeianus*) raised at different locations in São Paulo State, as well as one horned frog (*Proceratophrys boiei*), two river frogs (*Hylodes asper*), two small frogs (*Leptodactylus podicipinus*), and two dart frogs (*Adelphobates galactonotus*) from the wild in various locations within São Paulo State. In total, 211 samples from the 49 individuals were processed and sent to the Biological Institute for microorganism analysis in organ and skin fragments.

3.2 Sample Processing:

Samples were processed at the Aquaculture Health Laboratory using routine H&E histological techniques and at the Electron Microscopy Laboratory with negative staining for rapid preparation.

3.3 Light Microscopy Analysis or Photomete:

H&E staining: Tissues from 49 amphibians were collected and fixed in 10% formalin for 36–48 hours, dehydrated in a graded series of alcohols (70°, 80°, 95°, and absolute), cleared in xylene, and embedded in paraffin at 58°C overnight. Sections 4 µm thick were placed on slides and stored at room temperature. For analysis, slides were deparaffinized with xylene, rehydrated in a descending alcohol series (absolute, 95°, 80°, 70°), and water, and stained with hematoxylin and eosin.

3.4 Transmission Electron Microscopy (TEM):

The fragments were suspended in 0.1M phosphate buffer at pH 7.0. Drops of this suspension were applied to metal grids covered with collodion and carbon, drained with filter paper, and contrasted with 2% ammonium molybdate at pH 5.0 (Brenner & Horne, 1959; Hayat & Miller, 1990; Madeley, 1997). The samples from 49 amphibians were examined using a Philips EM 208 transmission electron microscope.

IV. RESULTS

At necropsy, hemorrhagic and friable internal organs (Figure 1) were observed in a few amphibians, and some bullfrogs also exhibited skin lesions on the body and digits (Figure 2). Electron microscopy results revealed that out of the 49 amphibians, 23 (46.9%) tested positive for herpesvirus (Figures 12 and 13), including 16 captive bullfrogs (*Lithobates catesbeianus*), one horned frog (*Proceratophrys boiei*), two river frogs (*Hylodes asper*), two small frogs (*Leptodactylus podicipinus*), and two dart frogs (*Adelphobates galactonotus*) from the wild. Herpesvirus particles were pleomorphic, icosahedral, some of which were enveloped and measured between 120 and 200 nm in diameter. A total of 26 (53.1%) animals tested negative for herpesvirus. Histological examination showed various histopathological changes, such as hemorrhage, mononuclear and polymorphonuclear inflammatory reactions, edema, several melanomacrophage centers, necrosis, and the presence of inclusion bodies, primarily intranuclear, in various organs, including the skin, liver, kidney, and spleen, in 14 bullfrogs (Figures 3, 4, 5, and 6; Graphs 1, 2, 3, and 4).

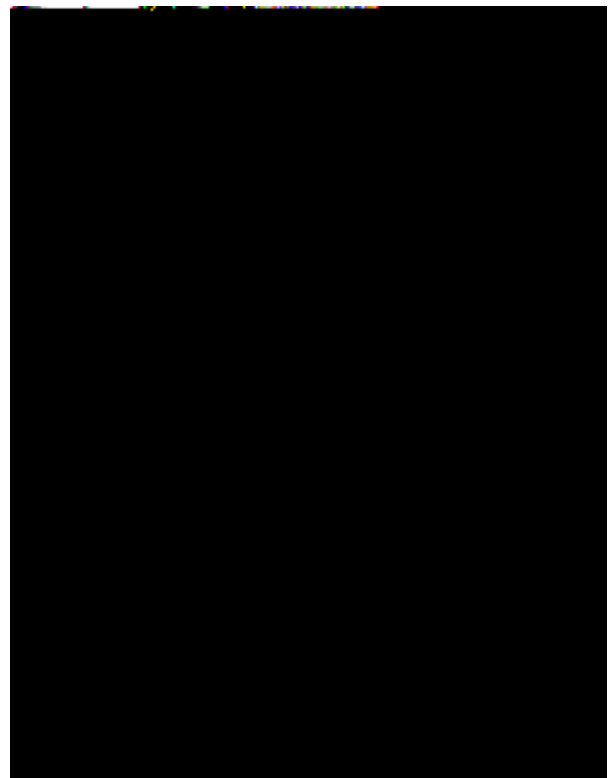
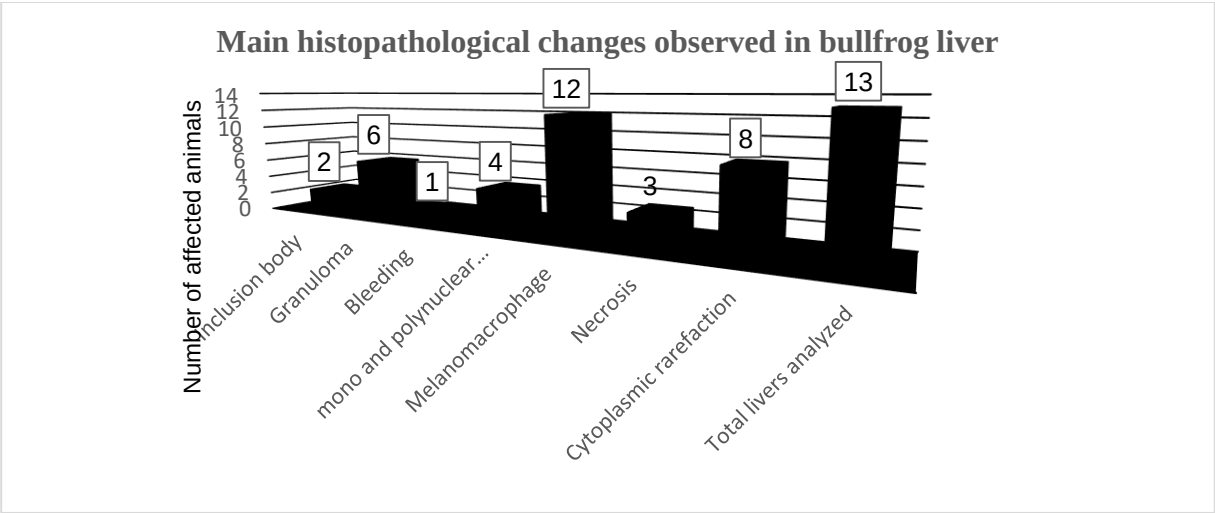


FIGURE 1: Bullfrog positive for herpesvirus showing altered organs (left). Macroscopic aspects of organs at necropsy (right)



GRAPH 1: Main histopathological changes observed in bullfrog liver and number of affected animals

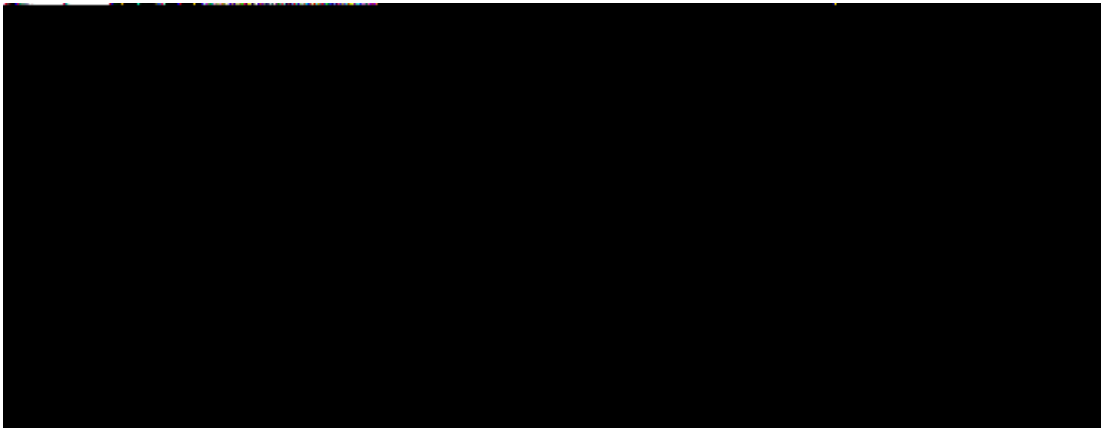
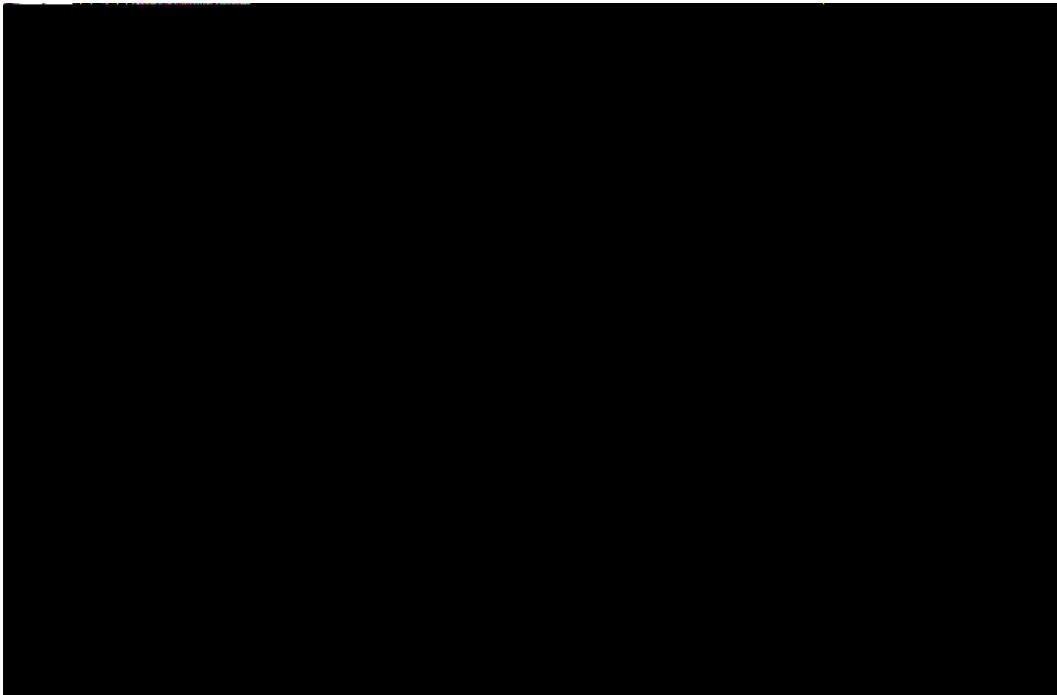


FIGURE 2: External macroscopic lesions observed in bullfrogs. Body skin lesions (A) and digit lesions (B) in bullfrogs



GRAPH 2: Main histopathological changes observed in bullfrog kidneys and number of affected animals



FIGURE 3: Panoramic photomicrograph of skin lesions in bullfrogs. H&E staining, 200x magnification



FIGURE 4: Photomicrograph of hyperplastic epidermal lesion and keratosis in bullfrog, showing neoplastic cells and inclusion bodies. H&E staining, 630x magnification



FIGURE 5: Photomicrograph of bullfrog liver histological section indicating basophilic nuclear inclusion bodies suggestive of herpesvirus (black arrow) and multiple melanomacrophage centers (blue arrow). H&E staining, 400x magnification

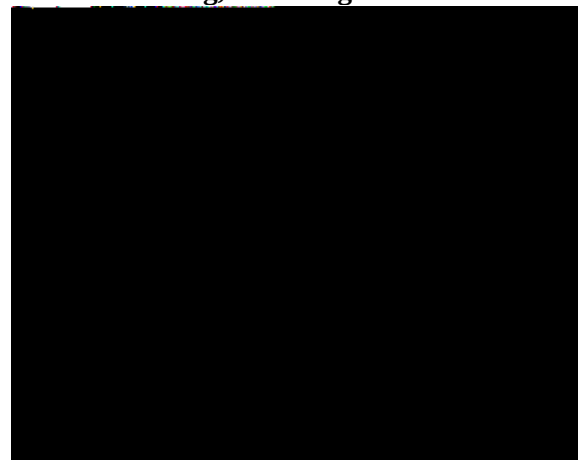
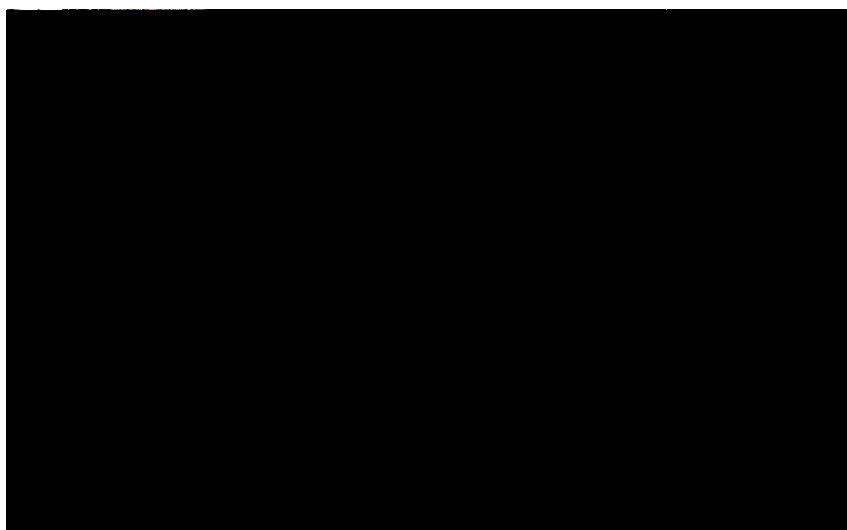
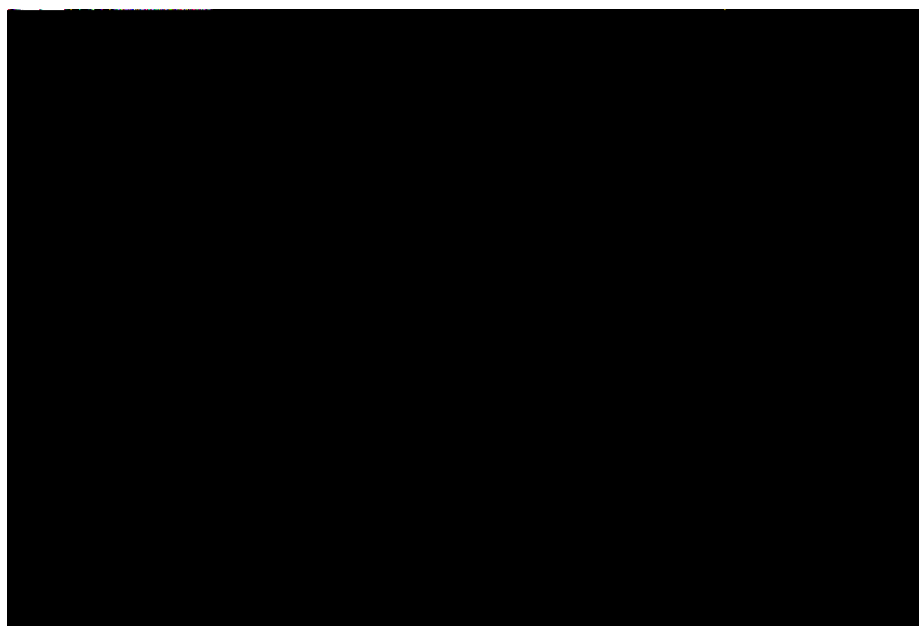


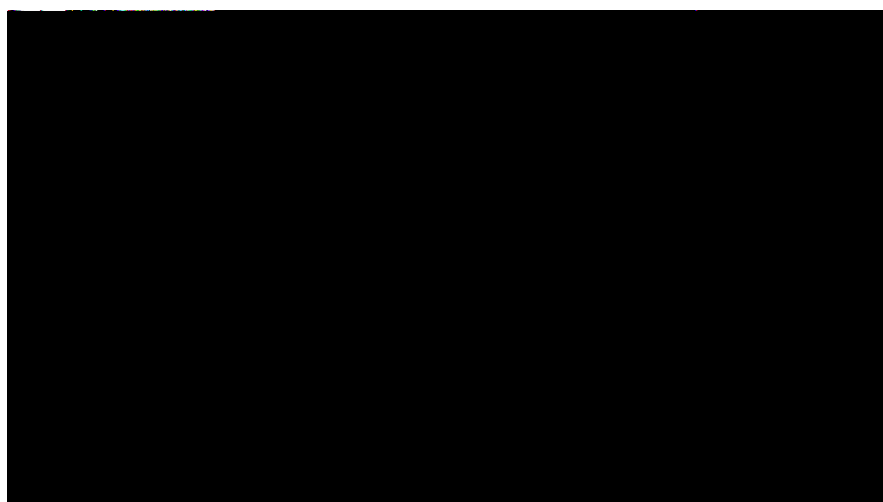
FIGURE 6: Photomicrograph of bullfrog liver histological section, arrow indicating basophilic nuclear inclusion body. H&E staining, 630x magnification



GRAPH 3: Main histopathological changes observed in bullfrog spleens and number of affected animals



GRAPH 4: Main histopathological changes observed in bullfrog skin and number of affected animals



GRAPH 5: Main histopathological changes observed in bullfrog lungs and number of affected animals

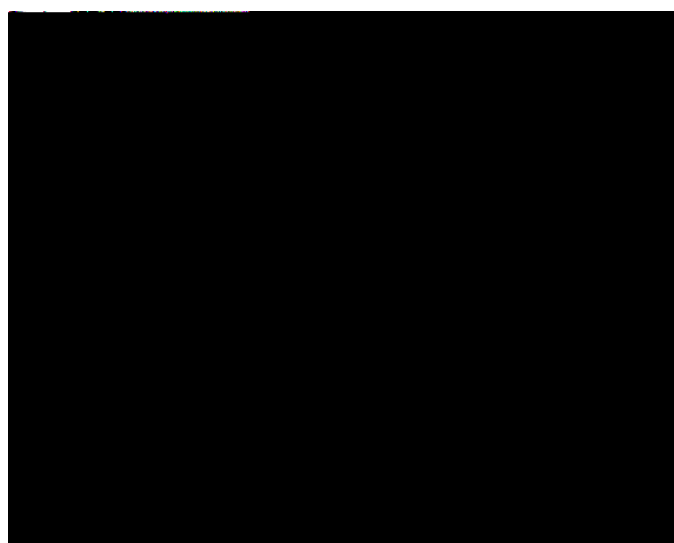


FIGURE 7: Enveloped herpesvirus particles in a bullfrog liver suspension, showing individual capsomeres (arrow). Bar: 140 nm

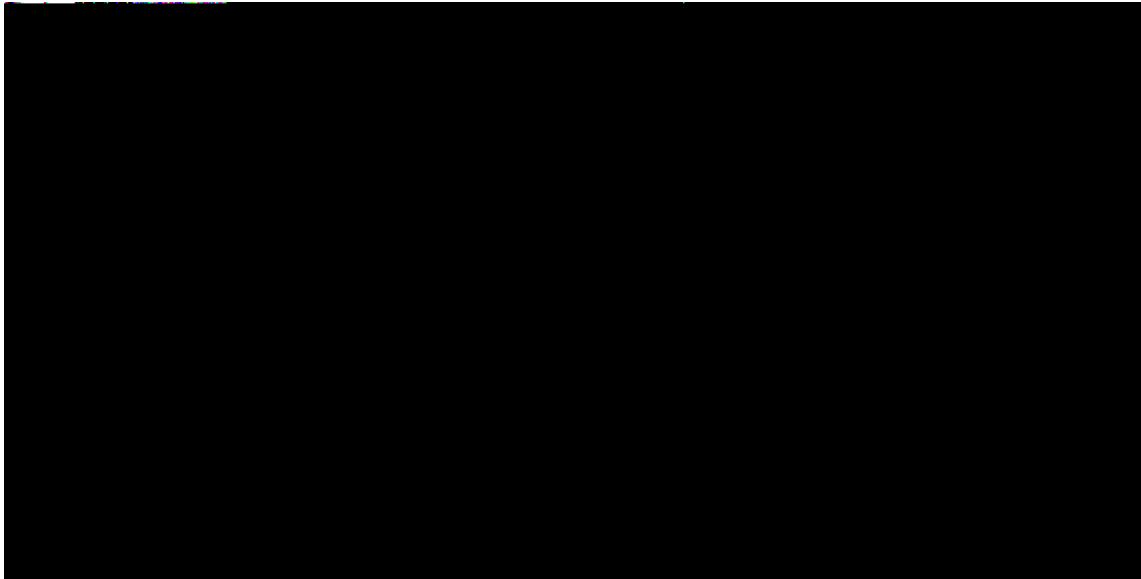


FIGURE 8: Herpesvirus particles, both enveloped (larger arrow) and non-enveloped (smaller arrow), in a bullfrog liver suspension. Bar: 150 nm

These transmission electron microscopy images provide detailed views of the herpesvirus particles found in infected bullfrog tissues, highlighting their pleomorphic nature and structural characteristics, such as the presence or absence of an envelope.

V. DISCUSSION

Frog farming holds significant potential for expansion within Brazilian agribusiness. However, several obstacles have hindered its progress nationally. In addition to previously mentioned economic challenges, the lack of governmental and private incentives, absence of specific regulations, and insufficient communication between research institutions and producers represent substantial barriers to advancing this activity within aquaculture. Improving access to knowledge for producers, especially regarding research on diseases affecting amphibians and appropriate prevention and treatment methods, would also be necessary.

Transmission electron microscopy revealed the presence of pleomorphic, icosahedral particles with herpesvirus-like morphology in the skin and organ fragments of 23 amphibians, some of which were enveloped and measured between 120 and 200 nm in diameter. Morphologically identical particles were visualized in other studies with bullfrogs from farms in São Paulo State, Brazil (HIPÓLITO et al., 2003; ANTONUCCI et al., 2014). The detection of the virus in multiple organs, including skin and liver, supports the hypothesis that this pathogen may affect multiple systems within the host. In total, 211 samples from 49 animals were processed using the negative staining technique (rapid preparation), with 23 (48.9%) animals testing positive for herpesvirus. A similar study by Antonucci et al. (2014) found herpesvirus in the small intestine of 7 (15.5%) out of 45 bullfrogs.

The most common clinical symptoms and signs observed in the amphibians in this study were sudden death and the presence of skin and digit lesions. HIPÓLITO et al. (2003) used the negative staining technique to detect herpesvirus particles in three animals with liver lesions, five animals with nodules in the leg muscles and back, and the liver and ascitic fluid of six animals. The occurrence of Lucké tumor, characterized by renal adenocarcinoma caused by herpesvirus, has been described in leopard frogs (*Rana pipiens*) (McKINNELL & CUNNINGHAM, 1982; CARLSON et al., 1994), with metastasis potentially mediated by low temperatures (TWEEDLE, 1989).

Regarding bullfrog histopathology, the most significant alteration observed in all bullfrogs (*L. castebianus*) was the presence of inclusion bodies in the liver, kidney, spleen, and ovary. These findings align with those of other authors studying the presence of Lucké tumor in leopard frogs (*Rana pipiens*) (GRANOFF, 1983; CARLSON et al., 1994).

We also observed hemorrhage in the liver, spleen, and skin, as well as lymphocytic inflammation in the kidney, spleen, and hepatic necrosis in some muscle areas and skin lesions. These findings were also reported by CARLSON et al. (1994) in the kidney of leopard frogs (*Rana pipiens*). However, other alterations we found, such as the presence of melanomacrophages in the liver, spleen, and lung, tubulonephritis in the kidneys, and monolymphocytic inflammatory reaction in the skin, were not reported in the literature. The variety of positive species affected suggests that multiple herpesvirus types may occur in Brazil.

The study by ORIGGI et al. (2021) investigated the pathophysiology and molecular mechanisms of herpesvirus in frogs and toads. Common and agile frogs (*Rana temporaria* and *R. dalmatina*) infected with RaHV3 and common toads (*Bufo bufo*) infected with BfHV1 were analyzed. At different developmental stages, six frogs and 14 toads exhibited transient skin lesions associated with and necessary for the viral replication cycle. Transcriptomic analysis in infected and non-infected animals (two frogs and two toads) revealed altered expression of signaling and cellular remodeling genes in infected individuals. Finally, viral transcriptomics indicated high expression of a viral immunomodulatory gene encoding a receptor for the tumor necrosis factor in the skin of infected hosts, suggesting a coordinated cycle between epidermis and virus involving host signaling, remodeling genes, and viral immunomodulatory genes.

Experimental transmission studies on ranid herpesvirus 1 (RaHV1, *Batravirus ranidallo*), the first characterized amphibian herpesvirus, indicate that viral-associated renal adenocarcinoma likely occurs when amphibians are infected during the early embryonic stage (pre-metamorphosis) but not in adult or juvenile stages (post-metamorphosis). We investigated the potential occurrence of RaHV3 infection in free-living, pre-metamorphic common frogs (ORIGGI et al., 2023).

According to TWEEDLE (1989), electron microscopy and histopathology are the primary initial tools for diagnosing Lucké tumor. When histological analysis suggests a viral disease, transmission electron microscopy is a highly useful method for identifying the etiological agent. Numerous herpesviruses causing diseases in fish and frogs that could not be successfully isolated in cell cultures were detected via electron microscopy (HANSON et al., 2011).

In the animals testing positive via the negative staining technique, no organ preference was observed, as viral particles were present in all examined organs. In routine hematoxylin-eosin histology, the liver was the most affected organ. The liver can be chosen due to its easy access and relatively large size compared to the animal.

Although 26 animals tested negative for herpesvirus, the detected infection rate raises concerns about the potential impact of this infection on Brazilian frog farms, particularly in terms of economic losses. The correlation between inadequate management, environmental stress, and herpesvirus outbreaks in other animal species suggests that prophylactic measures, such as improved management practices and sanitary control, are essential for mitigating outbreak risks.

VI. CONCLUSION

The presence of pleomorphic particles, some enveloped, measuring between 120 and 200 nm, confirms herpesvirus-1 infection, consistent with previous studies in other amphibians. The detection of the virus in multiple organs, including the skin and liver, supports the hypothesis that this pathogen may affect multiple systems within the host. This study identified the presence of herpesvirus in captive bullfrogs in São Paulo State, Brazil, with a significant infection rate. These findings suggest that herpesvirus (RaHV-1) is circulating in Brazilian frog farms, potentially harming animal health and the economic viability of frog farming. Therefore, it is crucial to continue studies in this area to gain a better understanding of viral pathology and develop management and prevention practices that reduce losses caused by these infections.

In this regard, we emphasize the importance of a coordinated effort among various sectors within the country to ensure better prospects for the development of frog farming in Brazil.

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Effect of Methanolic Extract of (*Vernonia amygdalina*) on the Haematology and Lipid Profile of Rabbit Does

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Abstract— This study investigated the effects of methanolic extract of *Vernonia amygdalina* on the haematological and lipid profiles of rabbit does. A total of 24 rabbit does, aged 8–12 months, were randomly allocated to four treatment groups (T1, T2, T3, and T4) in a Completely Randomized Design (CRD), with six does per group. T1 served as the control and received no extract, while T2, T3, and T4 were administered 50 mg/kg, 100 mg/kg, and 200 mg/kg body weight of the extract, respectively, over a four-week period. Blood samples were collected at three time points: prior to extract administration, midway through the experiment, and at the end of the study. Haematological parameters were evaluated, and sera were analyzed for lipid profile components using standard laboratory procedures. The results revealed that *Vernonia amygdalina* extract exhibited dose-dependent immunostimulatory effects, maintained red blood cell indices, and significantly improved lipid profile parameters. Specifically, the extract reduced levels of total cholesterol, triglycerides, low-density lipoprotein cholesterol (LDL-C), and very-low-density lipoprotein cholesterol (VLDL-C) while increasing high-density lipoprotein cholesterol (HDL-C). These changes occurred without adverse physiological effects on the experimental rabbit does. In conclusion, the methanolic extract of *Vernonia amygdalina* demonstrated potential as a natural immunostimulant and lipid-modulating agent, suggesting its application in enhancing the health and productivity of livestock.

Keywords— Haematology, Methanolic extract, Rabbit does, Lipid profile, *Vernonia amygdalina*.

I. INTRODUCTION

Lipid profiling involves a panel of blood tests used to measure lipids, including cholesterol and triglycerides, which are synthesized in the liver and play critical roles in cell membrane integrity, hormone synthesis, immune function, energy storage, vitamin transport, and neural health (Cleveland Clinic, 2024; Eliza, 2023). It is a valuable diagnostic tool for detecting genetic disorders, cardiovascular diseases, pancreatitis, and liver diseases (Nigam, 2011; Cleveland Clinic, 2024).

Haematology, the study of blood's cellular components—erythrocytes, leucocytes, and thrombocytes—assists in diagnosing and managing diseases of the blood, bone marrow, and vascular systems (Merck Manual, 2012; Washington and Van Hoosier, 2012). Changes in haematological parameters are often indicative of environmental, nutritional, or pathological stress, providing insights into an animal's physiological status and responses to administered substances (Togun *et al.*, 2007; Khan and Zafar, 2005).

Vernonia amygdalina (bitter leaf) is a widely available perennial shrub in the African tropics, valued for its pharmacological properties, including antimicrobial, antioxidant, antimalarial, and antibacterial effects (Anibijuwon *et al.*, 2012; Ayoola *et al.*, 2008). These properties are attributed to its bioactive compounds such as flavonoids, saponins, alkaloids, and tannins (Jisaka *et al.*, 1993; Kiplimo *et al.*, 2011). Additionally, its leaves are rich in vitamins, minerals, and proteins, making it a potential feed supplement.

Given its phytochemical composition and availability in Nigeria, *V. amygdalina* holds promise as a functional ingredient in animal feed. This study aims to evaluate the physiological effects of methanolic extract of *V. amygdalina* on the haematological and lipid profiles of rabbit does, offering insights into its potential application in rabbit nutrition

II. MATERIALS AND METHODS

2.1 Experimental site:

The research was carried out at the Livestock Teaching and Research Farm of the College of Animal Science and Animal Production, Michael Okpara University of Agriculture, Umudike, Abia State. Geographically, Umudike is located in the Rain-forest zone of Southeastern Nigeria and lies on an altitude of 122m above sea level on latitude 5°29' North and longitude 7°21' East. The average highest daily temperature of the region ranges between 27°C and 36°C during the dry, hot season, while the average lowest temperature is from 20 to 26 during the rainy season. Average annual rainfall of the region is about 2100mm with double Maxima pattern (NRCRI, 2021).

2.2 Experimental Animals and Management:

A total of twenty-four (24) does aged between 8 months and one (1) year were purchased from a reputable rabbit farm. They were housed in standard cages under normal room temperature (25°C) with cross ventilation. The animals were acclimatized for a period of four (4) weeks before commencement of treatment during which they were dewormed and monitored. They were fed a compounded diet and good quality forage and provided with good quality portable water ad libitum. The extract was administered orally using a gavage, for a period of 4 weeks following the four-week acclimatization period.

TABLE 1
GROSS COMPOSITION AND CALCULATED NUTRIENTS OF EXPERIMENTAL DIETS FOR RABBIT DOES

Ingredients	T ₁	T ₂	T ₃	T ₄
Maize offal	15	15	15	15
Wheat offal	60	60	60	60
Palm kernel meal	9	9	9	9
Groundnut cake	12	12	12	12
Bone meal	3	3	3	3
Common salt	0.5	0.5	0.5	0.5
Vit./min. Premix*	0.5	0.5	0.5	0.5
Total	100	100	100	100
Calculated nutrients	T ₁	T ₂	T ₃	T ₄
Crude protein (%)	15.96	15.96	15.96	15.96
Metabolizable Energy (ME) (Kcal/kg diet)	2009.6	2009.6	2009.6	2009.6
Crude fibre (%)	8.58	8.58	8.58	8.58
Lysine (%)	0.50	0.50	0.50	0.50
Methionine (%)	0.1	0.1	0.1	0.1

T₁= 0mg/kg; T₂= 50 mg/kg; T₃= 100 mg/kg and T₄= 200 mg/kg of *Vernonia amygdalina* leaf extract.

*Premix composition (per kg of diet): vitamin A, 12,500 IU; vitamin D3, 2500 IU; vitamin E, 50.00mg; vitamin K3, 2.50mg; vitamin B1, 3.00mg; vitamin B2, 6.00mg; vitamin B6, 6.00mg; niacin, 40mg; calcium pantothenate, 10mg; biotin, 0.08mg; vitamin B12, 0.25mg; folic acid, 1.00mg; chlorine chloride, 300mg; manganese, 100mg; iron, 50mg; zinc, 45mg; copper, 2.00mg; iodine, 1.55mg; cobalt, 0.25mg; selenium, 0.10mg; antioxidant, 200mg.

2.3 Collection and Extraction of Plant Materials:

Fresh specimens of *V. amygdalina* were sourced from Umudike, Ikwuano Local Government Area, Abia state. The fresh leaves collected were air dried and then pulverized into a fine powder. The dried leaves were milled and grounded into coarse powder using Wiley machine. The powdered plant sample were soaked in methanol for 24 hours and filtered with Whatmann no. 1 filter paper to obtain the extract. The methanol extract was concentrated using rotary evaporator. The percentage yield (w/v) of the extract was be calculated using the formula:

$$\text{Percentage Yield (\%)} = \frac{\text{Weight of material extracted}}{\text{Weight of plant material}} \times 100 \quad (1)$$

(Source: Brian and Turner, 1975)

The extract was reconstituted to form a solution for oral administration by dissolving one gram (1g) of the extract in 100ml of distilled water. The dose (ml) of the liquid suspension of the extract was determined using the formula:

$$\text{Dosage} = \frac{\text{Administration rate} \times \text{concentration}}{\text{Body weight of the animal}} \quad (2)$$

(Source: Ukar *et al*, 2023)

2.4 Experimental Design:

The experimental design was a Completely Randomized Design (CRD) having four (4) treatments with three (3) replicates each. Each treatment group had six (6) does per treatment. Animals in treatment groups were fed the test ingredient as follows: treatment one (T_1) served as the Control; treatment two (T_2) received 50mg of the test ingredient per kilogram of body weight; treatment three (T_3) received 100mg of the test ingredient per kilogram body of weight; treatment four (T_4) received 200mg of the test ingredient per kilogram of body weight.

The following model was used:

$$Y_{ij} = \mu + T_i + e_{ij} \quad (3)$$

Where:

Y_{ij} = Single observation. = overall mean.

T_i = treatment effect,

e_{ij} = experimental error.

2.5 Data collection and Analysis:

Three does were randomly selected from each treatment group. Blood samples were collected via venipuncture from the major ear vein at three intervals: a day before extract administration (baseline), on day 12, and on day 26. The samples were analyzed for haematological parameters, including hematocrit, blood cell count, hemoglobin concentration, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and differential leukocyte count. Lipid profile parameters analyzed included total cholesterol, triglycerides, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and very low-density lipoprotein cholesterol (VLDL-C).

2.6 Statistical Analysis:

The data obtained were analyzed by ANOVA using Statistical Software Package System (SPSS version 20) software. All values were expressed as the mean value $\pm$ standard error of the mean and the level of significance $P < 0.05$ was considered statistically significant difference between tests and control groups for measured values. All statistical analysis were according to Steel and Torrie, (1980).

III. RESULTS AND DISCUSSION

TABLE 2
EFFECT OF METHANOLIC EXTRACT OF *VERNONIA AMYGDALINA* ON HAEMATOLOGICAL PROFILE OF RABBIT DOES

Treatment group						
Parameters	Collection	T ₁	T ₂	T ₃	T ₄	SEM
Haemoglobin (g/dl)	1 st	11.26 ^{ab}	10.93 ^b	11.80 ^a	11.40 ^{ab}	0.13
	2 nd	12.06	12.66	11.8	12.06	0.14
	3 rd	11.7	12.8	13.19	12.53	0.29
Packed Cell Volume (%)	1 st	29.66	28.33	34.33	31	1.12
	2 nd	31.33	34.33	30.33	31.66	0.67
	3 rd	30.00 ^b	33.36 ^{ab}	38.96 ^a	33.33 ^{ab}	1.23
Red Blood Cell ($\times 10^6/\mu\text{l}^3$)	1 st	4.82	4.57	5.56	5.02	0.18
	2 nd	5.16	5.56	4.99	5.19	0.1
	3 rd	5.16	5.56	4.99	5.19	0.2
TWBC ($\times 10^3$ cells/ μl)	1 st	7.98	6.88	7.45	7.31	0.32
	2 nd	8.11 ^b	8.80 ^{ab}	7.80 ^b	9.93 ^a	0.33
	3 rd	8.21	8.25	8.65	8.65	0.4
MCV (μm^3)	1 st	62.86	63.21	62.93	62.91	0.11
	2 nd	61.84	62.78	61.92	62.14	0.18
	3 rd	62.36	61.4	61.75	62.01	0.16
MCH (pg/cell)	1 st	23.56	23.99	21.45	22.75	0.53
	2 nd	23.37	22.75	23.66	23.25	0.19
	3 rd	23.53	22.64	20.48	22.65	0.41
MCHC (g/dL)	1 st	38.29	36.87	34.71	36.91	0.85
	2 nd	38.54	36.91	39	38.14	0.37
	3 rd	38.44	37.59	34.27	37.77	0.64

^{a-b}: means along the same row with different superscript letters across groups indicate significant ($p < 0.05$) difference.

SEM: Standard Error of Mean. TWBC: Total White Blood Cell; MCV: Mean corpuscular volume; MCH: Mean corpuscular haemoglobin; MCHC: Mean corpuscular haemoglobin concentration. T₁= 0mg/kg; T₂= 50 mg/kg; T₃= 100 mg/kg and T₄= 200 mg/kg of *Vernonia amygdalina* leaf extract.

The results presented in Table 2 highlight the effects of treatment and duration on the haematological parameters of rabbit does administered graded doses of *Vernonia amygdalina* leaf extract. Haemoglobin (Hb) values ranged from 10.93 g/dl to 13.19 g/dl, with significant differences observed only in the first collection. Packed cell volume (PCV) values showed no significant differences in the first two collections but were significantly higher in the third collection, with T₃ (100 mg/kg) recording the highest value (38.96%) and the control (T₁) the lowest (30.00%).

Red blood cell (RBC) counts ranged from 4.57 to $5.56 \times 10^6/\mu\text{l}$, showing no significant differences across treatments. Total white blood cell (TWBC) counts ranged from 6.88 to 9.93×10^3 , with significant increases observed in T₄ (200 mg/kg) during the second collection. Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) values remained consistent across treatments, showing no significant alterations.

In the first collection, PCV values for T₁ (29.66%) and T₂ (28.33%) were below the normal range (31–50%) (Washington and Van Hosier, 2012), indicating mild anemia. However, PCV values improved significantly in subsequent collections, demonstrating the anti-anaemic activity of *V. amygdalina*, consistent with findings by Tom-Otu *et al.* (2023). Similarly, RBC counts initially showed signs of hemolysis (Melillo, 2007) but normalized over time.

Overall, *V. amygdalina* did not cause significant alterations in Hb or RBC counts, while PCV and TWBC values improved significantly in T₂ and T₄ during the second and third collections, suggesting a dose-dependent immune-stimulatory effect.

These findings align with Pascal *et al.* (2021) and Johnson *et al.* (2014). The extract maintained erythropoiesis, exhibited anti-anaemic and immune-stimulatory properties, and showed no deleterious effects on haematological parameters, thus confirming its safety.

TABLE 3
EFFECT OF VERNONIA AMYGDALINA ON DIFFERENTIAL WHITE BLOOD CELL COUNT OF EXPERIMENTAL RABBITS DOES

Parameters	Collection	Treatment group				SEM
		T ₁	T ₂	T ₃	T ₄	
Lymphocyte (%)	1 st	60	59	59.33	59.66	0.35
	2 nd	60	61.33	57.66	58.33	0.71
	3 rd	59.00 ^b	63.38 ^a	61.01 ^{ab}	60.00 ^b	0.6
Neutrophils (%)	1 st	35.33	35.33	33.66	35.33	0.55
	2 nd	33.66	32.66	35	34.33	0.63
	3 rd	35.03 ^a	29.00 ^b	32.07 ^{ab}	34.03 ^a	0.82
Monocyte (%)	1 st	3	3.66	4.66	3.66	0.27
	2 nd	4.33	4.66	4.33	4.66	0.33
	3 rd	3.90 ^b	5.34 ^a	4.63 ^{ab}	4.53 ^{ab}	0.19
Eosinophils (%)	1 st	1.66	2	2.33	1.33	0.2
	2 nd	2.00 ^{ab}	1.33 ^b	3.00 ^a	2.66 ^a	0.26
	3 rd	1.99	1.33	2.03	1.63	0.21
Basophil (%)	1 st	0	0	0	0	0
	2 nd	0	0	0	0	0
	3 rd	0	0	0	0	0

^{a-b}: means along the same row with different superscript letters across groups indicate significant ($p < 0.05$) difference.

SEM: Standard Error of Mean.

The results presented in Table 3 reveal the effects of methanolic *Vernonia amygdalina* leaf extract on the differential leukocyte counts of rabbit does. Lymphocyte percentages ranged from 57.66% to 63.38%, with significant differences observed in the final collection. T₂ (50 mg/kg) recorded the highest lymphocyte value (63.38%), while the control group (T₁, 0 mg/kg) had the lowest (59.00%). Neutrophil percentages followed a similar trend but exhibited an inverse relationship, with T₂ significantly ($p < 0.05$) lower compared to T₁. Monocyte percentages aligned with the trend seen in lymphocytes, while basophilic cells were not detected in any samples. Eosinophil counts fluctuated across treatment groups but remained within normal values ($p > 0.05$) in the first and third collections.

Lymphocyte counts are known to increase in Vitamin B-12/folic acid anemia and decrease in iron/B-6 deficiency anemia or internal bleeding (Krishna Kumari *et al.*, 2007). Lymphocytes are the most abundant leukocytes in healthy rabbits, with fluctuations influenced by stress, disease, and time of day (Washington and Van Hoosier, 2012). Typically, lymphocyte values peak in the early morning and dip in the late afternoon and evening. Acute stress can cause lymphocytosis, while chronic stress may lead to lymphopenia (Melillo, 2007). The dose-dependent increase in lymphocyte counts observed in this study suggests that the methanolic extract of *Vernonia amygdalina* exhibits immune-stimulatory effects.

Neutrophil counts, which are lowest in the morning and peak in the afternoon (Melillo, 2007), were lower in this study due to the morning timing of blood sample collection rather than the extract's effect. Similarly, monocyte counts, the largest white blood cells in rabbits, also follow a diurnal rhythm, being lowest in the morning and highest in the afternoon (Washington and Van Hoosier, 2012). Elevated monocyte levels are linked to chronic inflammation and conditions like mastitis or empyema, though such increases were not observed in this study (Melillo, 2007).

Eosinophil counts, influenced by daily rhythm, stress, and disease, can indicate abscess presence or wound healing (Melillo, 2007). In this study, eosinophil counts remained within normal ranges, aligning with observations in healthy rabbits where low or absent eosinophil counts are typical (Washington and Van Hoosier, 2012).

Overall, the methanolic extract of *Vernonia amygdalina* demonstrated immune-stimulatory properties, as evidenced by the increased lymphocyte counts, while other leukocyte parameters remained within normal physiological ranges. These findings highlight the extract's potential as a safe immunomodulatory agent in rabbit does

TABLE 4
EFFECT OF METHANOLIC EXTRACT ON LIPID PROFILE OF RABBIT DOES

Parameters	Collection	Treatment group				SEM
		T ₁	T ₂	T ₃	T ₄	
Cholesterol (mg/dl)	1 st	122.69 ^a	100.67 ^b	94.96 ^c	90.31 ^d	3.74
	2 nd	121.92 ^a	99.90 ^b	94.25 ^c	89.94 ^d	3.71
	3 rd	122.18 ^a	100.16 ^b	94.49 ^c	90.06 ^d	3.72
Triglycerol (mg/dl)	1 st	78.00 ^a	64.48 ^b	62.23 ^c	61.29 ^c	2.04
	2 nd	78.53 ^a	64.20 ^b	62.26 ^c	60.87 ^c	2.13
	3 rd	78.35 ^a	64.29 ^b	62.25 ^c	61.01 ^c	2.09
HDL (mg/dl)	1 st	41.93 ^b	42.88 ^b	43.67 ^b	46.37 ^a	0.56
	2 nd	40.92 ^c	43.15 ^b	43.28 ^b	46.04 ^a	0.55
	3 rd	41.25 ^c	43.06 ^b	43.41 ^b	46.15 ^a	0.53
LDLC (mg/dl)	1 st	64.70 ^a	44.05 ^b	38.44 ^c	31.04 ^d	3.78
	2 nd	65.25 ^a	44.46 ^b	38.00 ^c	31.51 ^d	3.81
	3 rd	65.06 ^a	44.32 ^b	38.15 ^c	31.35 ^d	3.8
VLDL (mg/dl)	1 st	13.13 ^a	13.13 ^b	12.36 ^c	12.19 ^c	0.31
	2 nd	15.77 ^a	13.09 ^b	12.33 ^{bc}	12.04 ^c	0.45
	3 rd	15.44 ^a	13.11 ^b	12.40 ^c	12.09 ^c	0.39

^{a-b-c}: means along the same row with different superscript letters across groups indicate significant ($p < 0.05$) difference.

SEM: Standard Error of Mean. HDL: High-density lipoprotein; VLDL: Very-low-density lipoprotein; LDLC: Low-density lipoprotein cholesterol.

The result presented in table 4 showed the effect of the methanolic extract of *Vernonia amygdalina* on the lipid profile of rabbit does. The sera levels of Cholesterol, Triglyceride, LDLC and VLDL ranged from 89.94 to 122.69 mg/dl, 60.87 to 78.53 mg/dl, 31.04 to 65.25mg/dl and 12.04 to 15.77mg/dl respectively and they followed the same trend, with significant ($p < 0.05$) reduction (below the allowable amount in normal rabbit doe) across the extract administered groups in a dose dependent manner. However, serum HDL-cholesterol level, had values ranging from 40.94 to 46.37 mg/dl, was noticeably ($p < 0.05$) increased, above the normal serum level, as the extract doses were increased, suggesting that the extract exhibited better protection, with no compromise to the membrane integrity (its stability and fluidity) and their functions.

According to Basavanthappa *et al*, (2021), when serum lipids (cholesterol and triglyceride) values are elevated above reference range with low HDL value, it may suggest a possible risk of heart disease, circulatory problem as well as hazards on liver, kidney functions. From the lipid parameters profiled, *Vernonia amygdalina* extract improved the sera lipids, evidently possessed significant hypolipidemic effect, hepato- and cardio-protection by possibly preventing isoproterenol-induced myocardial damage in rabbits, as well as possess no hazards on liver functions. The lowering of cholesterol mean level in the sera of the experimental rabbit does in groups T₂, T₃ and T₄ compared with group T₁ might suggest that the ingredients (bioactive phyto-constituents) contained in the leaf extract at higher doses (dose dependently) was capable of inhibiting the activities of hepatic lipogenic and cholesterogenic enzymes, such as malic enzyme, fatty acid synthase, glucose 6-phosphate dehydrogenase and HMG-CoA reductase (Yeh and Yeh, 2003) which are all required for cholesterol synthesis. The low levels of serum triglyceride, LDLc and VLDLc in the extract treated groups may be as a result of some activity of the *Vernonia amygdalina* extract such as its ability to decrease the availability of fatty acids for esterification and encourage tissues lipases, activation of acetyl-CoA carboxylase (McCarty, 2021), or the production of triglycerides precursors such acetyl-CoA and glycerol phosphate (Campillo *et al.*, 2014). The result also demonstrated hypolipidemic potential, by influencing the liver metabolism towards decreasing the synthesis of lipids as the graded doses of extract were increased.

IV. CONCLUSION

The methanolic extract of *Vernonia amygdalina*, from this study showed immunostimulatory activity in a dose-dependent manner; had no significant effect on red blood cells and showed significant hypolipidemic activity by reducing the values of Cholesterol, Triglyceride, LDLC and VLDL while improving HDL values.

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Nitrogen Slow-Release Zeolite-Based Fertilizer on Rice Plants at Inceptisol Soil

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Abstract— Most of N fertilizer have low efficiency in paddy field due to it is easily lost through evaporation and runoff. Slow-release N fertilizer based on zeolite is potential N fertilizer in paddy field to improve the efficiency of N fertilizer. Slow release N fertilizer based on zeolite is also potential to improve the growth and production of rice. This study aimed to determine the effect of slow-release fertilizer on the growth and production of rice plants on inceptisols and knowing the best dosage for rice plants in the inceptisols. This study used a Completely Randomized Block Design (RAKL) with 2 treatment factors and 3 replications. The first factor is the application of N fertilizers, namely original N fertilizer, 1% coating slow-release N fertilizer, and 3% coating slow-release N fertilizer. The second factor is the dose of N fertilizer namely 0 kg/ha (N0), 100 kg/ha (N1), and 200 kg/ha (N2). The research variables are leaf area, leaf area index, number of tillers, number of productive tillers, the greenness of leaves, density and width of stomatal openings, plant dry weight, net assimilation rate, plant growth rate, relative growth rate, nitrogen uptake, panicle length, number of grains per malai, moisture content of the harvested grain, grain weight of 1000 seeds, the weight of grain in clumps, grain weight per hectare, and agronomic efficiency. Data were analyzed using ANOVA, and if it is significantly different, then further tested using DMRT at a 5% level. The results showed that slow-release N fertilizer improves plant growth and production components better than original N fertilizer. Provision of slow release zeolite-based fertilizers coating 3% with a dose of N 200 kg/ha gives the best results for plant growth and production.

Keywords— Rice, Nitrogen, zeolite, slow release.

I. INTRODUCTION

Nitrogen (N) is the main element needed by plants for growth. Nitrogen increases plant growth and production (Pan S et al., 2022). Nitrogen functions are a major component in forming proteins, nucleic acids, chlorophyll, and other organic compounds. Nitrogen also gives a green appearance to leaves as a component of chlorophyll, supports growth, increases plant height and the number of leaves, increases leaf size, increases seed size, and increases protein composition in seeds (Sumiyanah & Iman Sungkawa, 2018).

One of the problems of rice cultivation today is using fertilizer chemistry continuously with high doses, causing negative effects on the environment and reducing fertilization efficiency (Abdulrahman et al., 2008). The dosage of N fertilizer at the farm level is currently relatively high, reaching 400-600 kg urea/ha, especially in intensive rice fields in Java, which is above the national recommendation of 200-260 kg urea/ha (Triadiati et al., 2012).

Nikmah (2019) argues that the application of nitrogen (N) fertilizers in rice cultivation in Indonesia every year has increased. However, the ability of rice plants to absorb N is still low, around 30% -50%. The rest (50-70%) will be lost to the environment through evaporation and surface runoff (Coskun et al., 2017 Kharisun & Budiono, M.N, 2015). Even according to Oliveira et

al. (1997), loss of N can reach 94% when urea is applied to soil covered with straw. N loss is caused by dispersion (Sommer et al., 2004), volatility NH_3 (Li et al., 2017), and washed away by surface runoff (Cai et al., 2002).

The evaporation of gas from paddy fields is thought to be one of the contributors to the greenhouse effect (IPPC, 2013; Ye Yuan, 2019). Some gas emissions from fertilizing paddy soil are N_2O (Wu et al., 2017) and CH_4 (Shang et al., 2013). Nitrogen is a plant macronutrient that is very important for plant growth and production, and in most soils, monoculture cereals such as rice are deficient (Omara et al., 2019).

One of the things needed for nitrogen fertilizers is fertilization technology with low solubility but can provide sufficient nutrients for plants. Nitrogen technology Slow-release fertilizer is a fertilizer technology with low nutrient solubility and can provide nutrients gradually over a long period. The low nutrient release will enable nutrient absorption with high efficiency and nutrient loss due to the washing. Fertilizer NZEO-SR-Plus is a slow-release nitrogen fertilizer that uses natural zeolite as the main ingredient and is enriched with Si and humic acid, rice husk and clay minerals (montmorillonite) as binders and nano-zeolite coating and humic materials. The presence of nano-zeolite coating and humic materials can increase the soil's physical, chemical and biological fertility, especially on marginal soils. The coating is a layer applied to the surface (Ngguna et al., 2020).

Humic acid can stimulate and activate physiological and biological processes in living organisms in the soil, which makes humic acid act as a soil conditioner. The humic acid given to the urea fertilizer acts as a coating which helps protect the nitrogen elements from possible evaporation and dissolves with surface runoff. This is shown when N fertilizers such as urea are given humic acid, properties volatile (volatile) of urea fertilizer appear to be reduced. NH_2 from urea fertilizer changes into ammonium ion (NH_4^+).

Zeolite is an aluminosilicate mineral in three-dimensional crystals with a nano-sized pore channel structure resulting in a very large surface area. In NZEO SR Plus fertilizer, zeolite will bind NH_4^+ , released from the hydrolysis of N fertilizers such as urea. NH_4^+ ions are adsorbed on the negatively charged lattice in the nano-sized, making them inaccessible to microbes. The adsorbed ions can be released slowly and controlled through the cation exchange mechanism. According to Suwardi (2020), applying nitrogen fertilizer without adding zeolite facilitates high N leaching and volatilization, making it unavailable to plants (Suwardi, 2000). N fertilizer based on zeolite can work as a slow-release fertilizer (Kharisun et al. 2017).

Using nitrogen fertilizers released in a controlled manner using zeolite can increase nitrogen uptake efficiency and reduce the loss of nitrogen gas through ammonia evaporation and denitrification (Cabezas et al., 2005; Achmed O.H. et al., 2010; Palanivel P et al. 2015). Slowrelease N fertilizer based on zeolite and humic acid is expected to overcome the efficiency of nitrogen fertilizers and can increase sub-optimal soil fertility. This study aims to determine the effect of slow-release fertilizer (NZEO-SRPlus) on the growth and production of IR64 rice plants and determine the best dose for I.R. 64 rice plants in soil inceptisol, Karangsari, Cirebon.

II. RESEARCH METHODS

The research was conducted in the rice fields of Karangsari Village, Cirebon Regency, Indonesia from September 2020 to February 2021. The research was conducted in paddy fields with the Inceptisol soil type and IR 64 rice plants. The experimental plots were 4 meters x 5 meters with a spacing of 25 cm x 25 cm.

The experiment used a Completely Randomized Block Design (RAKL) which consisted of 2 treatment factors: the type of fertilizer and the dose of N fertilizers. The types of fertilizer consisted of original N fertilizer with 46 % N content (P1), slow release N zeolite-based fertilizer (NZEO SR Plus) with 20 % N content and coating of 1% of zeolite (P2), and NZEO SR Plus with 20 % N content and coating of 3% of zeolite (P3). The dose of N consists of 0 kg/ha (N_0), 100 kg N/ha (N_1), and 200 kg N/ha (N_2). There were 9 treatment combinations and 3 replications, so there were 27 experimental units.

Variables observed were leaf area, leaf area index, plant dry weight, number of tillers, green leaves, opening width and stomata density, number of productive tillers, panicle length, number of grains per malai, grain moisture content, the weight of 1000 seeds, grain weight per hill, net assimilation rate, plant growth rate, relative growth rate, nitrogen uptake, grain weight per hectare, and agronomic efficiency. The data obtained were analyzed statistically using the F-test analysis at an error level of 5%. If the results of the ANOVA show differences between the treatments, proceed with further testing using the DMRT (Duncan's Multiple Range Test) α 5%.

III. RESULTS AND DISCUSSION

3.1 The general condition of research location:

The research area is located at an altitude of 10 m above sea level with the soil type in Inceptisols with Soil fertility status is shown in Table 1. The average air temperature is 28°C-

32°C with humidity of 80%. The estimated air temperature in the morning is 24°C-27°C and during the day 29°C-32°C.

TABLE 1
STATUS OF INCEPTICOLS FERTILITY IN THE RESEARCH AREA

Analysis	Unit	Results	Dignity
H ₂ O pH	-	6.3	slightly acid
pH KCl	-	5.22	Acid
DHL	ds/cm	0.118	very low
Redox potential	Mv	130	Average
CEC	cmol(+)/kg	18.68	Average
C-Organic	%	0.11	very low
N-Available	ppm	21	High
P-Available	ppm	1675.95	very high
K-Available	cmol(+)/kg	1.43	very high
N-Total	%	0.22	Average
P-Total	ppm	1739.42	very high
K-Total	cmol _{K2O} _	5.21	very low
Si-Available	%	0.059	very low

Source: Result of analysis, soil laboratory, Agriculture, Unsoed

Based on Table 1, the inceptisol soil has pH_{KCl} and pH_{H₂O} of 5.22 and 6.3 respectively. The pH_{H₂O} is greater than the pH_{KCl}. This shows that the soil is negatively charged, meaning the soil is able to hold the cations that plants exchange. The status of C-organic and Si content is classified as very low. The availability of Si in soil is very low due to the desilication process so that the Si contained in the top layer of soil is leached into the bottom layer. The electrical conductivity (DHL) is also classified as very low. The redox potential is included in the medium category. Soil redox potential indicates the rate of oxidation and reduction reactions in the soil. The cation exchange capacity (CEC) on the research land is included in the medium category. The available N content in the land is high and the total N content in the land is moderate. The content of available P and total P in the land is very high. This is due to acidic condition of soil which is more easily to dissolve the P in soil. The available K content is very high while the total K is very low. This is due to the condition of flooding in rice field will increase the availability of Fe²⁺ and Mn²⁺ to replace K adsorbed by clay so that K is released into the soil solution. According to Prasetyo et al., (2004) flooding will reduce the redox potential (Eh) of the soil thereby increasing the solubility of Fe²⁺ and Mn²⁺.

3.2 Treatment of various type of fertilizers and doses of N on the growth and production of rice:

TABLE 2
EFFECT OF TYPE OF SLOW-RELEASE N AND DOSES OF N ON LEAF AREA, LEAF AREA INDEX, NUMBER OF TILLERS, AND PLANT DRY WEIGHT

Treatment	Number of Saplings	Number of Saplings	Number of Saplings	Dry Weight	Dry Weight	Dry Weight
	4 mst	6 mst	8 mst	4 mst	6 mst	8 mst
Fertilizer Type						
Original N Fertilizer	19.57 a	27.17 a	20.80 a	1.11 a	2.63 a	2.15 b
NZEO SR Plus Coating 1% .	20.15 a	27.08 a	20.68 a	1.17 a	2.28 b	2.40 a
NZEO SR Plus Coating 3% .	19.75 a	25.68 b	20.04 a	1.12 ab	2.36 b	2.36 ab
Dose N						
0 kg/ha	19.08 b	25.80 b	20.02 b	1.00 b	2.04 b	2.21 b
100 kg/ha	19. 80 ab	26.82 ab	19.53 b	1.21 a	2.23 b	2.14 b
200 kg/ha	20.60 a	27.33 a	21.97 a	1.19 a	3.01 a	2.71 a
Treatment	Leaf area	Leaf area	Leaf area	LAI	LAI	LAI
	4 ms	6 ms	8 ms	4 ms	6 mst	8 mst
Fertilizer Type						
Original N Fertilizer	1647.91 b	3982.17 a	2871.07 a	3.92 a	5,82 a	4,34 a
NZEO SR Plus Coating 1%	1865.04 a	4392.75 a	3025.84 a	3.98 a	6.08 a	4.68 a
NZEO SR Plus Coating 3%	1871.26 a	4189.02 a	3120.32 a	3.05 b	5.67 a	4.45 a
Dosis N						
0 kg/ha	1739.45 b	4053.35 a	2937.32 a	3.35 b	5.85 a	4.54 ab
100 kg/ha	1739.63b	4245.03 a	2879.14 a	3.79a	5.88 a	4.25 b
200 kg/ha	1905.12a	4265.56 a	3120.15 a	3.81 a	5.85 a	4.67 a

Note: Numbers followed by the same letters in the same column and treatment are not significantly different according to DMRT 5%.

Table 2 shows that application of type of fertilizer on agronomic characters varies, 5 of 12 variables provide a significant improvement. While the effect of fertilizer dosage on agronomic characters provides 8 of 12 variables in a significant improvement.

TABLE 3
EFFECT OF VARIOUS FERTILIZERS AND N DOSES ON LEAF GREENNESS, OPENING WIDTH AND STOMATA DENSITY, NAR, PGR, AND RGR

Treatment	Green Leaf (Unit)	Stomata Opening Width (μs)	Stomatal Density (mm ²)	NAR (g/dm ² / week)	PGR (g/cm ² / week)	RGR (g/g/week)
Kind of Fertilizer						
Original N Fertilizer	37.64 a	5,67 a	122.40a	0.23a	0.86a	1.05a
NZEO SR Plus Coating 1%	37.24 a	5.67 a	125,19 a	0.25 a	0,87 a	1.07 a
NZEO SR Plus Coating 3%	37.70 a	5.00b	129.27 a	0.27 a	0.88 a	1.08 a
Dose N						
0 kg/ha	37.76 a	5.11 a	134.49 a	0.25b	0.88 a	1.08 b
100 kg/ha	36.27 b	5.78 a	115.04 b	0.19 c	0.83 b	1.00 c
200 kg/ha	38.53 a	5.44 a	127.34 ab	0.32a	0.91a	1.13 a

Note: Numbers followed by the same letters in the same column and treatment are not significantly different according to DMRT 5%. NAR; Net Assimilation Rate, PGR = Plant Growth Rate, RGR = Relative Growth Rate

Table 3 shows that application of type of fertilizer on physiological characters only provides, 1 of 12 variables in a significant improvement (wide of stomata opening). While the effect of fertilizer dosage on physiological character provides, 5 of 12 variables in a significant improvement.

TABLE 4
INTERACTION OF FERTILIZER TYPES AND DOSES OF N ON GREENNESS OF LEAVES

Kind of fertilizer	N dose		
	0 kg/ha	100 kg/ha	200 kg/ha
Original N Fertilizer	38.14 aA	37.47aA	37.30bA
NZEO SR Plus coating 1%	37.94 aA	35.68bB	38.08bA
NZEO SR Plus coating 3%	37.22 aB	35.67 bC	40.20 aA

Note: Numbers followed by the same lowercase letters in the same column/same uppercase letter in the same row are not significantly different according to DMRT 5%

TABLE 5
INTERACTION OF FERTILIZER TYPES AND DOSES OF N ON DRY WEIGHT DOSES 8 WAP

Kind of fertilizer	N dose		
	0 kg/ha	100 kg/ha	200 kg/ha
Original Fertilizer	1.92 bB	2.16 aB	2.38b
NZEO SR Plus Coating 1%	2.71 aA	1.90 bB	2.60 bA
NZEO SR Plus Coating 3%	2.02 bC	2.35aB	3.17 aA

Note: Numbers followed by the same lowercase letters in the same column/same uppercase letters in the same row are not significantly different according to DMRT 5%

Tables 4 and 5. Show that there are interactions between application type of fertilizers and fertilizer dosage on the greenness of leaves and dry weight of plant 8 WAP. Both Tables show that the best combination of treatment is N slow-release fertilizer with the fertilizer dosage of 200 kg/ha.

3.3 Treatment of various types of fertilizers and doses of N on the yield components of rice:

TABLE 6
EFFECT TYPES OF SLOW-RELEASE FERTILIZERS AND DOSES OF N ON THE YIELD COMPONENTS OF RICE

Treatment	Productive chicks	Panicle length (cm)	Grains per panicle	Moisture content of harvested grain (%)	Nitrogen content (g)	Weight of 1000 seeds (g)	Grain weight per clump (g)	Weight of grain (ton/ha)	agronomic efficiency
Kind of fertilizer									
Original N Fertilizer	17.00 a.	25.80b	139.57b	16.51a	0.07b	26.62 ab	43.52 a	7.15 b	2.59a
NZEO SRPlus coating 1%	17.02a	26.60 a	157.71a	16.36 a	0.07 ab	26.15 b	41.36 b	7,09 b	2,50 a
NZEO SR Plus coating 3%	16.04 a	25.84 b	151.64 a	16.44 a	0.09 a	26.94 a	43.94 a	7.65 a	2.69 a
Dose N									
0 kg/ha	16.48 a	26.22 a	146,53a	16.66 b	0.05 c	26,00 a	42.82 a	6.93 b	-
100 kg/ha	16.35a	26.60 a	150.22a	16.25a	0.08b	26.79a	43.11 a	7.47 a	3.01a
200 kg/ha	17.22 a	26.00 a	152.17 a	16.40 ab	0.10a	26.91 a	43.34 a	7.46 a	2.17 b

Note: Numbers followed by the same letters in the same column and treatment are not significantly different according to DMRT 5%

Table 6. shows that application of type of fertilizer on component of yield of rice provide 6 of 9 variables in a significant improvement. While the effect of fertilizer dosage on component of yield of rice provide 4 of 9 variables in a significant improvement.

TABLE 7
INTERACTION OF FERTILIZER TYPES AND DOSES OF N ON PLANT NITROGEN UPTAKE (%)

Kind of fertilizer	N dose		
	0 kg/ha	100 kg/ha	200 kg/ha
Original N Fertilizer	2,48 aC	3.61 aB	3.21 cB
NZEO SR Plus coating 1%	2.08 bB	3.65 aA	3.54 bA
NZEO SR Plus coating 1%	2.09 bB	3.80 aA	3.88 aA

Note: Numbers followed by the same lowercase letters in the same column/same uppercase letter in the same row are not significantly different according to DMRT 5%

TABLE 8
INTERACTION OF FERTILIZER TYPES AND N DOSAGE ON GRAIN WEIGHT FOR HA

Kind of fertilizer	N dose		
	0 kg/ha	100 kg/ha	200 kg/ha
Original Fertilizer	6,74 bB	7.51 aA	7.20 bA
NZEO SR Plus coating 1%	6.86 aB	7,52aA	6,81bB
NZEO SR Plus coating 3%	7,20 aB	7,38aB	8.36 aA

Note: Numbers followed by the same lowercase letters in the same column/same uppercase letter in the same row are not significantly different according to DMRT 5%

Tables 7 and 8. Shows that there are interactions between application type of fertilizers and fertilizer dosage on plant nitrogen uptake and grain weight per ha (ton). Both Tables show that the best combination of treatment is N slow-release fertilizer coating 3 % and the fertilizer dosage of 200 kg/ha.

IV. DISCUSSION

Fertilizer slow release NZEOSR Plus increased 11 out of 27 total variables that observed the growth and yield of rice plants. Besides that, NZEOSR Plus fertilizer can also increase plant growth and production better than original N fertilizer fertilizer. This is because NZEO SR Plus fertilizer has slow-release properties that can control N nutrient release according to plant needs.

According to Suwardi & Wijaya (2013), slow-release effectively increases nutrient availability with the regular release. Zeolite minerals can increase the efficiency of N fertilizers such as urea. NH_4^+ and K^+ cations in the soil solution will be bound to the zeolite framework and slowly released around the root area to be absorbed by plants (Sastiono, 2004).

Humic acid has a negative charge that comes from the dissociation of H ions from functional groups, so it has a very high cation exchange capacity (more than 200 meq 100 g⁻¹) to bind, absorb and exchange cations to prevent nutrient loss (Suwardi & Darmawan, 2009).

Increasing the dose of N can increase the growth and yield of rice plants due to the adequacy of N nutrients in the soil for plant growth nutrition. The function of N nutrients is very important, especially in the formation of protein compounds in plants (Ibrahim & Kasno 2008), the formation of chlorophyll, protoplasm, proteins, and nucleic acids, photosynthetic activity, metabolic processes and respiration N elements to increase the growth and development of living tissue (Brady & Weil, 2002).

The interaction between NZEO SRPlus Coating fertilizer and nitrogen dosage indicates a strong influence between the type of fertilizer and dosage. This is presumably due to the nature of the element nitrogen, which is easily lost, requiring additional ingredients to minimize nitrogen loss after being added to the land. Zeolite and humic acid in NZEO-SR Plus fertilizer can control the release of nitrogen into the soil as long as the nitrogen availability is still high so that the nitrogen content in the solution remains stable and saves fertilizer that must be added.

In plants 4 WAP, the average leaf area was the widest in the treatment with NZEO SR Plus 3% fertilizer at a dose of N 200 kg/ha. The average leaf area was 2071.12 cm². The presence of zeolite, montmorillonite and humic acid in NZEO SR Plus fertilizer is thought to slow N solubility so that the availability of N around is guaranteed to root.

At 6 and 8 wp, nitrogen elements began to be lost by using metabolic processes, evaporation and washing, so the role of applying NZEO SR Plus fertilizer began to be seen according to its natural releases of nutrients slowly. At 8 weeks, the best leaf area was treated with 3% NZEO SR Plus Coating fertilizer with a dose of N 200 kg/ha.

At the beginning of plant growth, nitrogen application can increase leaf area because nitrogen availability in the soil is still sufficient. Then the availability of N will decrease due to leaching and evaporation. Nitrogen plays a role in vegetative growth as the main element of protein formation for plant cell division so that the number of leaves, leaf area and leaf thickness increases (Porter et al., 1990).

The NZEO SRplus Coating 3% fertilizer treatment affected the leaf area index of 4 mst with a leaf index value of 3.98 and at a dose of N 200 kg/ha producing the highest value is 3.81. The dose of N affected the leaf area index of 8 mst, and at a dose of 200 kg/ha, the leaf index was 4.67.

The leaf area index value increased until the age of 6 WAP, and then it decreased. This is because around the age of 6 WAP, there is a vegetative peak, and after that, the plants have started to enter a generative period so that nutrients will be more focused on panicle formation. The critical point LAI or LAI needed to absorb 95% of the solar radiation absorbed by the plant canopy ranges from 4-6. The critical point of LAI during generative growth is a prerequisite for obtaining maximum yields (Bachrain, 2005).

At 6 most plants, original N fertilizer fertilizer at a dose of N 200 kg/ha gave the best results, namely 27.86 tillers. Fertilizer NZEO SRplus Coating, 3% with a dose of N 200 kg/ha, increased the number of tillers by 8 mst, namely 22.4 tillers. The number of tillers indicates the growth of healthy or diseased rice plants. However, genetically the plant variety determines the number of tillers, and the role of N is very important in growing the number of tillers (Dara et al., 2017). Usman Made (2010) states that nitrogen nutrients greatly determine the development of plant meristem cells. With the availability of sufficient nitrogen, plants will form vegetative parts quickly.

NZEO SRplus Coating Fertilizer 3% with a dose of N 200 kg/ha gave the best green leaf yield of 40.20. This is because the greenness of the leaves is influenced by the availability of nitrogen with the addition of slow-release fertilizer at a fertilizer dose of 200 kg/ha, which will cause sufficient nitrogen availability so that the greenness of the leaves is high. This follows the opinion of Dwidjoseputro (1984). Plants need the nutrient N for shoot growth and green leaf coloring (chlorophyll). The N content in the soil can affect the amount of chlorophyll in the leaves so that the leaves turn green.

The NZEO SRplus Coating 1% fertilizer treatment increased the width of the stomata opening by 5.66 μm^2 . Stomata are important organs in the process of photosynthesis and also transpiration in plants. Stomata function as a place for exchanging CO₂ in the leaves for the process of photosynthesis and as a place for water evaporation in the process of transpiration (Arista et al., 2015).

NZEO SRplus Coating Fertilizer 1% at a dose of 200 kg/ha gave the best results at a 4-wm dry weight of 1.45 (g). NZEO SRplus Coating Fertilizer 3% with a dose of N 200 kg/ha gave the best results at 8 mst plant dry weight, namely 3.17 (g).

According to Putri et al. (2017), plant growth and development are shown by increasing the size and number of cells. Plants absorb nutrients for photosynthesis so that they produce photosynthesis. Photosynthate will be distributed and stored in the vegetative organs of plants, such as roots, stems and leaves, as food reserves. It is this storage of food reserves that will affect the wet weight and dry weight of plants.

Fertilizer NZEO SR Plus coating 1% with a dose of N 0 kg/ha (control) gave the best net assimilation rate of 0.39 g/dm²/week. The net assimilation rate shows the accumulation rate of the dry weight of material per unit of leaf area per unit of time. The value of the net assimilation rate indicates the efficiency of leaf photosynthesis in a cultivated plant community. The highest net assimilation rate value occurred in plants whose leaves were still exposed to sunlight (Aliinfallible et al., 2016).

NZEO SRplus Coating Fertilizer 3% with a dose of N 200 kg/ha gave the best results on growth rate (LPT), which was 0.96 cm²/week. Nitrogen plays an important role in forming photosynthetic tools, namely chlorophyll and the enzyme RuBP carboxylase, which function in the fixation of CO to be reduced to sugar. The high and efficient photosynthesis rate allows hoarding to occur in biomass plant dryness and is measured by increasing the value of LPT (Lakitan, 2007).

NZEO SR plus Fertilizer SR plus Coating 3% with a dose of N 200 kg/ha gave the best results relative to the growth rate with a yield of 1.21 g/week. Relative growth rate (LPR) is an increase in plant dry weight in a certain time interval (Gardner, 1991). LPR is determined by the shape of the leaf canopy (Horie, 2001; Yoshida, 1981).

Fertilizer NZEO SRPlus Coating, 1% with a dose of N 200 kg/ha, gave the best results for the number of productive tillers, namely 17.73. The number of productive tillers describes the tillers that produce panicles. Nitrogen plays a role in the formation of the number of productive tillers. The higher the nitrogen content, the more productive tillers increase (Winarso, 2005). Fertilizer NZEO SRPlus Coating, 1% with a dose of N 200 kg/ha, gives the best results on the number of grains per malai, namely 167.6 points. The high and low number of grains per panicle can be determined by the productivity level of rice plants (Azalika et al., 2018). Using fertilizers with zeolite and humate coatings effectively increases the availability and absorption of nutrients in plants to increase the number of grains per malai. Aina & Jumadi (2018) said that nitrogen affects the quality and quantity of corn kernels.

The NZEO SRPlus Coating 3% fertilizer treatment with a dose of N 200 kg/ha gave the best results for nitrogen uptake, namely 3.88%. The soil's adequacy of the N nutrient will allow it to absorb the nutrient according to its needs. According to Mengel et al. (2001), when the macronutrients in the soil increase, the amount of uptake by plants will increase. This follows the opinion of Suminarti (2010) that there is a close relationship between the level of soil nutrient availability and the level of N uptake by plants.

Fertilizer NZEO SRPlus Coating, 1% with a dose of N 200 kg/ha, gave the lowest yield of grain moisture content, namely 16.10%. According to Wibowo (2010), grain moisture content can be used to predict grain storage after harvest. Rice generally has a grain moisture content at harvest of about 25%. The lower the grain moisture content indicates the cooking process grain is maximum.

NZEO SRPlus Coating 3% fertilizer gave the highest 1000 seed weight yield of 26.46 g, and the N dose of 200 kg/ha gave the highest 1000 seed weight yield of 26.42 g. The weight of 1000 seeds indicates the quality of the grain. The higher the weight of 1000 seeds, the higher the quality of the grain. NZEO SRPlus Fertilizer can increase the weight of 1000 seeds because the fertilizer can increase the availability of Nutrients in a controlled manner. According to Haryati & Anna (2016), nutrients absorbed from the soil by plants will be accumulated into protein to form seeds of maximum size and weight.

Fertilizer NZEO SR Plus Coating, 3% with a dose of N 200 kg/ha, gives yields per hectare the highest was 8.58 tons/ha. These results indicate that the fertilizer can increase production better than original N fertilizer fertilizer. According to Sarief (1990) and Aisyah (1990), using N fertilizer with zeolite can increase the efficiency of N fertilizer use and grain yield.

Fertilizer treatment with NZEO SRPlus Coating 1% at a dose of 100 kg/ha. Provides agronomic efficiency of 2.87. According to Schulze & Caldwell (1995) and Zheng (2007), applying N fertilizer with the appropriate dose will increase the efficient use of nitrogen. However, the application of excessive doses will reduce plant growth.

V. CONCLUSION

Application of slow-release zeolite-based N fertilizer (NZEO-SRPlus) is better than original N fertilizer in increasing plant growth and production components (agronomic and physiologic kharacteristic. Provision of fertilizers NZEO SR Plus coating 3% with a dose of 200 kg N/ha gives the best results for plant growth and production.

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Effect of Commercial Feed Formula and Feed Constituted with Industry Ready-Made Concentrates on the Growth Performance of Broilers

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Abstract— Optimal nutrition directly impacts the efficiency and growth performance of broilers, which are critical factors determining the profitability and sustainability of broiler production. Despite the widespread use of both commercial and reconstituted concentrate-based feeds, there has been limited empirical data directly comparing the impact of these feeds under controlled conditions. This lack of comprehensive, comparative studies creates uncertainty for farmers in making informed decisions about the more cost-effective and productive feed to use. This study compared the growth performance of 120 broiler chicks fed a commercial feed formula and a feed constituted with industry-ready-made concentrates. The chicks were randomly assigned to two dietary experimental groups (T1 – Commercial feed; T2 – feed formulated with ready-made concentrate from the feed industry) and fed for eight weeks. The results of the experiment were analyzed using a two-tailed t-test. The findings of the study revealed that although the average feed intake, feed conversion rate, and weight gain of the two groups were not significantly different ($P > 0.05$), broilers fed with reconstituted concentrate-based feed showed significant weight gain in the 6th ($p = 0.002$), 7th ($p = 0.019$), and 8th ($p = 0.002$) weeks of the experiment. The results of the findings showed that the use of reconstituted concentrate-based feed allows for better growth performance in broilers. Thus, the use of reconstituted concentrate-based feed by farmers is recommended.

Keywords— Commercial feed, Reconstituted concentrate-based feed, Broiler, Growth performance.

I. INTRODUCTION

The broiler production business offers one of the quickest returns on investment for poultry farmers, with high feed conversion rates and the production of one of the cheapest and best sources of animal protein (Akpaeti & Agom, 2018). However, animal protein consumption in Nigeria is grossly below the average required for good health, in line with the Sustainable Development Goals (SDGs) and the World Health Organization (WHO) (Akpaeti & Agom, 2018; Adekunmi *et al.*, 2017). This low availability of animal protein might be attributed to the challenges of broiler production faced by farmers, which have slowed down the rate of production in the industry (Anosike *et al.*, 2020).

With the growth in the production and rearing of broilers in recent years, industries have launched different commercial feed factories, some of which have benefits and disadvantages for broiler farmers (Wognaa *et al.*, 2023). Yadollah and Ali (2016) observed that there is a significant difference between different production concentrates with respect to weight gain and aviculture duration. Using factory concentrates compared to feeds formulated on a poultry farm had positive effects on the diet behaviors of broilers, such that the rate of leftover protein, calcium, and phosphorus in dishes with concentrate feeds was less than that in feeds formulated on a poultry farm.

Mixing ingredients is an essential process in feed manufacturing, as these ingredients must be combined effectively to be supplied as a complete feed to animals (Rocha *et al.*, 2022). The general objective of poultry nutrition is to maximize the economic production performance of birds. Diets are formulated to provide specific levels of nutrients needed for optimum performance (Uchegbu *et al.*, 2009). Variations in nutrient composition, digestibility, and bioavailability between commercial feeds and those formulated with ready-made concentrates may lead to differences in broiler performance, which need to be

understood to optimize feeding practices. Therefore, this study aims to fill this knowledge gap by rigorously comparing the effects of commercial feeds and feeds reconstituted with ready-made concentrates on the performance of broilers.

II. MATERIALS AND METHODS

2.1 Procurement of Experimental Animals:

A total of one hundred and twenty (120) day-old chicks of mixed sexes, comprising one breed (Arbor Acre), was purchased from Amo Farm Sieberer Hatchery, located at Awe. The chicks were transported by night using Divine Favor Company's supply van to a collection point at Awka, and a tricycle was used to transport them to the school farm where the experiment was conducted. The birds were acclimatized for one week, each breed per compartment. After the acclimatization process, they were randomly assigned to the two treatment groups (T1 = commercial feed; T2 = feed formulated with ready-made concentrate from the feed industry). These two treatments were replicated three times each.

2.2 Housing:

The birds were housed in three pens measuring 2.60 square meters (1.22 x 2.13 meters) at the Poultry Unit of the Teaching and Research Farm, UNIZIK, Awka, with similar conditions for both groups. The pens were further demarcated to 0.05 m² per bird for 0-3 weeks and 0.10 m² per bird from 4-8 weeks, in accordance with Briese and Hartung [8]. Each group was kept in separate but identical pens to prevent cross-contamination.

2.3 Feeding/Watering:

The chicks were fed 6 kg of feed ad libitum (free access) during the first week and ad libitum throughout the experiment. This was to ensure that the birds ate as much as they wanted at any time to support optimal growth and performance. The feed was provided in clean feeders of 25 cm, preventing spillage. Clean, fresh water was made available ad libitum to all the broilers at all times in clean, easily accessible Dinoplast and Loveleen plastic drinkers.

2.4 Sanitation/Bio-Security:

The pens, feeders, and drinkers were thoroughly cleaned and disinfected with hypo and Izal before the arrival of the chicks. A strict cleaning schedule was followed throughout the experiment (i.e., every morning, the Dinoplast drinkers were washed thoroughly with clean water, and fresh water was poured into the clean drinkers for the broilers). Also, the feeders were inspected every morning, scrubbed with detergent and a sponge, and left to dry under the sun before feed was introduced into the feeders for the broilers. Clean, dry bedding (i.e., wood shavings) was provided in each of the three pens used. The bedding was replaced as needed to maintain a dry and comfortable environment, with complete bedding changes done every week.

Entry to the housing area was restricted as much as possible. Footbaths and clean protective clothing were used by personnel before entering the broiler area. Equipment such as pens, feeders, and drinkers were designated for each group to avoid cross-contamination.

2.5 Data Collection:

The weight and daily feed consumption of the birds in each pen were weighed using a Camry weighing balance and calculated weekly. The total feed consumed in a week was divided by the number of birds to get the average weekly feed intake, while the average body weight gained was calculated by subtracting the weight at the start of the week from the weight at the end of the week and dividing by the number of weeks. The FCR was computed by dividing the total feed intake of the broilers by the total weight gained for each group.

2.6 Statistical Analysis:

Comparisons between the two groups were made using a two-tailed t-test. Significant differences were determined at the level of $P < 0.05$ (i.e., $p < 0.05$ shows a significant difference; while $p > 0.05$ shows no significant difference).

III. RESULTS AND DISCUSSION

3.1 Feed Intake of the Broiler Chicks:

The results of the feed intake (Figure 1) revealed that there was no statistically significant difference between broilers fed with commercial feed or reconstituted concentrate-based feed, both in the starter ($p = 0.233$) and finisher ($p = 0.681$) phases. The overall feed intake also showed no statistically significant difference ($p = 0.360$). This might be because both feed formulations

are designed to meet the essential dietary requirements of broilers, including protein, carbohydrates, fats, vitamins, and minerals, ensuring growth and development. Also, broilers tend to adjust their intake based on energy needs and palatability, which might likely be similar in both feed types. Therefore, despite slight variations in composition, the overall energy and nutrient density remain sufficiently balanced, thus leading to similar feed consumption levels. This finding aligns with the results of Khalid *et al.* (2015), who found that the feed intake of broilers when commercial feed is replaced with reconstituted concentrate-based feed was the same, and had a similar effect on the performance of the broiler chicks with no significant difference. Additionally, the works of Lee *et al.* (2021) and Ammani *et al.* (2012) were in agreement with the findings of this study.

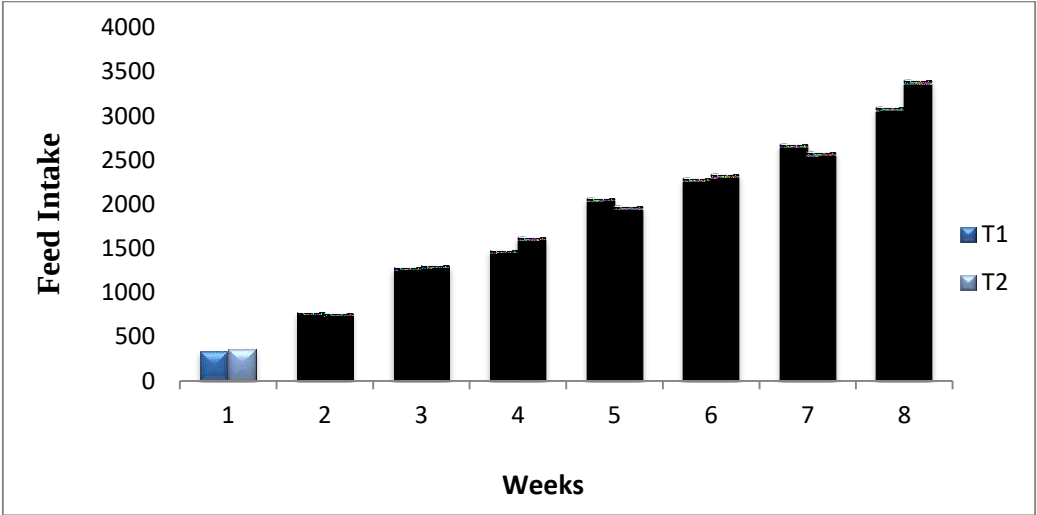


FIGURE 1: Feed Intake of Broiler Chicks fed with commercial feed and reconstituted concentrate-based feed

3.2 Weight Gain of the Broiler Chicks:

The average weight gain of broilers (Figure 2) showed no statistically significant difference in the first 5 weeks ($p>0.05$), but was statistically significant in the 6th ($p = 0.002$), 7th ($p = 0.019$), and 8th ($p = 0.002$) weeks. This suggests that the reconstituted concentrate-based feed may offer a higher concentration of key nutrients essential for optimal growth, such as proteins, fats, and energy-rich ingredients, crucial for supporting the rapid growth phase of broilers, especially in later stages (Leeson & Summers, 2001). This result contrasts with the findings of Ammani *et al.* (2012) and Hassan *et al.* (2021), who reported higher broiler weights with commercial feed compared to locally formulated feed.

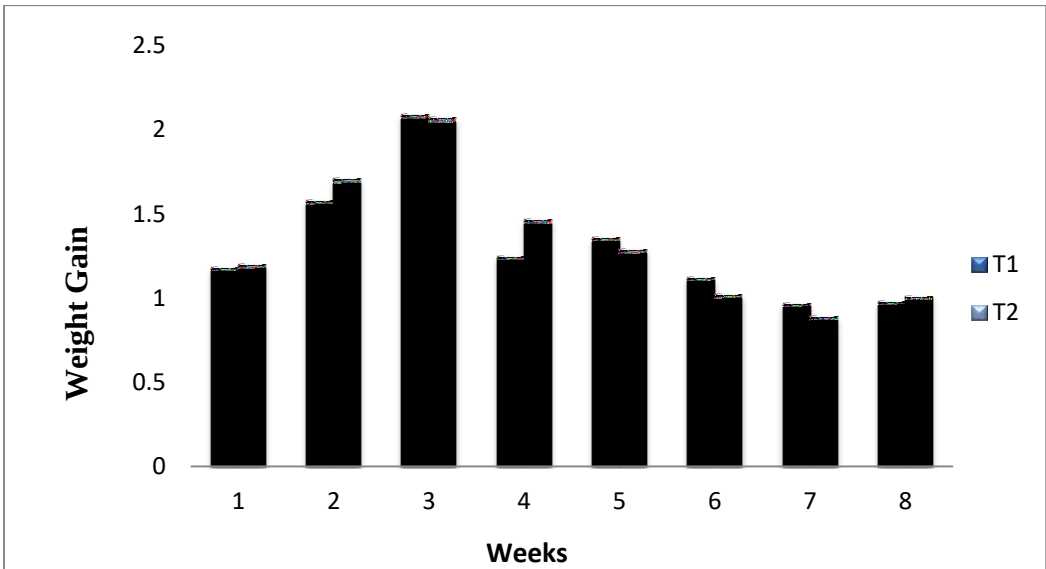


FIGURE 2: Weight gain of Broiler Chicks fed with commercial feed and reconstituted concentrate-based feed

3.3 Feed Conversion Rate of the Broiler Chicks:

The feed conversion ratio (FCR) showed no statistically significant difference ($p = 0.691$) between broilers fed commercial feed and those fed reconstituted concentrate-based feed. This similarity might be due to a similar balance of essential nutrients (carbohydrates, fats, vitamins, and minerals) in both feeds. Additionally, consistent experimental conditions (environment, management practices, and health status) may have reduced FCR variability. These findings are consistent with studies that found similar FCRs in broilers fed commercial and diluted concentrate-based feeds (Khalid *et al.*, 2015; Kuleile, 2020; Yadollah & Ali, 2016).

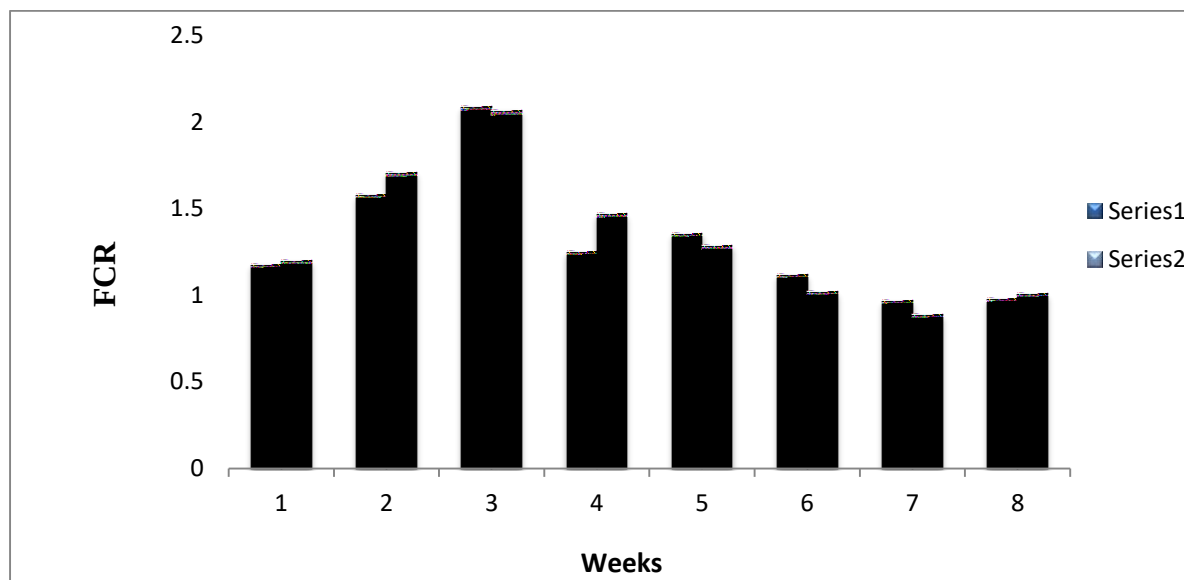


FIGURE 3: Feed conversion ratio (FCR) of Broiler Chicks fed with commercial feed and reconstituted concentrate-based feed

IV. CONCLUSION

This study showed that broilers fed reconstituted concentrate-based feed exhibited greater weight gain in the later stages of the experiment. This suggests that reconstituted concentrate-based feed may be a viable alternative to commercial feed for maximizing broiler growth performance. Further research is needed to determine the optimal formulation of reconstituted concentrate-based feeds for different broiler growth stages.

CONFLICT OF INTEREST

There is no conflict of interest

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Extraction of Leaf gel from *Cyclea peltata* and Its Utilization in Ice cream Manufacture

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Abstract— Ice cream is a frozen dessert that consists of ice crystals, air cells, fat globules, and liquid syrup. Stabilizers are added to reduce meltdown, increase viscosity, and add smoothness to the ice cream. Gelatin is an important stabilizer used in ice cream manufacture, which is produced from animal origin. Thus, consumers of lacto-vegetarian are highly affected. This study was conducted to find out the most suitable grams of leaf gel from the *Cyclea peltata* plant to produce a gelatin substitute and investigate the physico-chemical properties and storage of ice cream incorporated with *Cyclea peltata* leaf gel with 8.0 g (T1), 9.6 g (T2), 11.2 g (T3), 12.8 g (T4), and 14.4 g (T5) and a control sample with 8 g of gelatin. The results of this study revealed that the pH and melting rate were significantly decreased with the increasing of leaf gel content in ice cream. Titratable acidity, overrun, and fat were increased significantly. According to the study, it could be concluded that the 11.2 gL⁻¹ of *Cyclea peltata* leaf gel can be used as a substitute for gelatin used in ice cream manufacture.

Keywords— Ice cream, Stabilizer, Gelatin, leaf gel.

I. INTRODUCTION

Ice cream is a complex colloidal system, usually formed by four structural compounds: air bubbles, fat and ice crystals dispersed in a matrix consisting of a solution of sugars, proteins, stabilizers, emulsifiers, dyes and scents (Silva *et al.*, 2014). Stabilizers are substances that, despite their low usage level in ice cream mix, have very important functions, such as increase in viscosity of ice cream mix, aeration improvement, cry protection, and control of meltdown.

Presently ice cream industries are using stabilizers/emulsifiers blends, which are imported and very costly (Murtaza *et al.*, 2004). Commercial gelatin was made from bone and cow or pork skin, because of that, imported gelatin is still questionable of halal status (Ayudiarti *et al.*, 2022). Further, the gelatine price itself is high and thus, the producers are looking for alternatives to replace gelatine (Mariod and Adam, 2013).

Cyclea peltata belongs to the family Menispermaceae commonly known as “paatha” (Chellappan *et al.*, 2011). *Cyclea peltata* leaf gel incorporated yoghurt could be prepared by incorporating 6 gL⁻¹ of leaf gel as a total substitute for gelatine in yoghurt preparation (Siriwardhana *et al.*, 2018).

The research aimed to replace gelatin in ice cream production using was *Cyclea peltata* leaf gel. The objectives were to prepare gelatin substitute, determine suitable grams of leaf gel, analyses physico-chemical properties of Ice cream during preparation and storage and assess consumer acceptability of the leaf gel based ice cream.

II. MATERIALS AND METHODS

2.1 Experimental Site and Design:

This study was undertaken at the Food Science Laboratory of the Department of Agricultural Chemistry, Faculty of Agriculture, Eastern University, Sri Lanka for a period of six months from June to December 2022.

2.2 Experimental Design:

The experiment was laid out in a Completely Randomize Design (CRD). Experiment has six treatments with three replicates.

- Treatment 1 (T1): 08.00g of Gelatine (Control)
- Treatment 2 (T2): 08.00g of leaf gel
- Treatment 3 (T3): 09.60 g of leaf gel
- Treatment 4 (T4): 11.20 g of leaf gel
- Treatment 5 (T5): 12.80 g of leaf gel
- Treatment 6 (T6): 14.40 g of leaf gel

2.3 Preparation of *Cyclea peltata* leaf gel:

Cyclea peltata leaves were cleaned by using distilled water to remove the adulterations. Afterwards the leaves were cut into smaller pieces and blended by adding 100ml of water to extract the leaf gel. When blending the leaf gel was formed. This leaf gel was squeezed out by using a clean cotton cloth. The collected gel was stored at 4°C

2.4 Sterilization of plastic cups and equipment:

Plastic cups were washed with tap water and detergent was used to remove the dust and dirty in the cups. Then plastic cups were washed with boiled water and they were allowed to dry in shade. Stainless steel pans and steel spoons were cleaned with tap water and detergent was used to remove dust and dirt.

2.5 Preparation of ice cream:

Milk was boiled for 15-20 minutes until the temperature of the milk reached 90°C - 95°C and it was cooled for 40°C. Small amount of milk was taken and 200g of milk powder/1L was dissolved in it. 100g of corn flour were dissolved in cooled milk separately. Then dissolved milk powder, 200g/1L sugar and corn flour were added subsequently to boiling milk and stirred well. Boiling was stopped and allow to cool 5 minutes.

Then leaf gel with different grams and gelatine (as a control) were added to relevant concentration and beat about 5-8 minutes. Mixture was kept in refrigerator for 1 hour and it was beat 2 times with 1 hour interval. It was filled in to sterilized plastic cups and sealed with lids. Then sealed plastic cups were labelled according to the date of produced. Finally, ice cream cups were put in deep freezer.

III. RESULTS

Physico-chemical properties such as pH, titratable acidity, Melting rate, overrun and fat of ice cream samples for different treatments are shown in below Table 1.

TABLE 1
PHYSICO-CHEMICAL PROPERTIES OF ICE CREAM SAMPLES FOR DIFFERENT TREATMENTS

Treatments/ Analysis	T ₁	T ₂	T ₃	T ₄	T ₅	T ₆
pH	6.5566± 0.00333 ^a	6.55000± 0.00000 ^a	6.51333± 0.00333 ^b	6.4766± 0.00333 ^c	6.4466± 0.00333 ^d	6.3033± 0.00333 ^e
Titratable acidity	0.1633± 0.00333 ^d	0.17000± 0.00000 ^{cd}	0.18667± 0.00333 ^{bc}	0.1966± 0.00333 ^b	0.2033± 0.00667 ^b	0.2233± 0.00333 ^a
Melting rate	29.363± 0.00333 ^e	31.7133± 0.00333 ^a	30.5133± 0.00333 ^b	30.006± 0.00667 ^c	29.383± 0.00333 ^d	29.386± 0.00333 ^d
Overrun	54.186± 0.00333 ^c	53.3567± 0.00333 ^f	53.4467± 0.00333 ^e	53.466± 0.00333 ^d	54.213± 0.00333 ^b	54.236± 0.00333 ^a
Fat	10.130± 0.0000 ^d	10.1400± 0.0000 ^{cd}	10.1467± 0.00333 ^c	10.160± 0.0000 ^b	10.167± 0.00333 ^b	10.183± 0.00333 ^a

The values are means of 3 replicates ± standard error

The means with the same letters are not significantly different from each other on Tukey's test

The pH is a direct measurement of acid content (H^+). According to Tukey's test, the value of pH decreased significantly with the increasing of leaf gel content respectively in all ice cream formulations. T1 which formulated with 8g of gelatine had the highest pH value while T6 (14.4 g of leaf gel) had the lowest value of pH.

The titratable acidity of the ice cream sample varied significantly and increased from 0.1633 % to 0.22333 % with the content of leaf gel from 8g to 14.4 g. And ice cream formulated with 8g of gelatine had 0.16333 % of titratable acidity which present the lowest acidity content among all the treatments.

The lower melting rate was observed in T1 (8g of gealtin), T5 (12.8 g of leaf gel) and T6 (14.4 g of leaf gel) the he highest melting rate was observed in T2 (8g of leaf gel). There was a decreasing of melting rate along with the increasing of leaf gel content in ice cream. According to the turkey also, there was a significant deference between treatments.

The lowest overrun was in T2 which is the ice cream formulated with low level of leaf gel content (8g). The highest overrun was observed in T6 (sample with 14.4g of leaf gel). Fat content had increased gradually with the content of leaf gel.

IV. DISCUSSION

The pH is a direct measurement of acid content (H^+). According to Tukey's test, the value of pH decreased significantly with the increasing of leaf gel content respectively in all ice cream formulations. This decrease of pH of ice cream samples which formulated with leaf gel may be attributed by the presence of acidic compound present in the leaf gel.

The titratable acidity of the ice cream sample varied significantly and increased from 0.17000 % to 0.22333 % with the content of leaf gel from 8g to 14.4 g. These results are agreement with the results reported by Choo et al. (2010) and in ice cream formulated with virgin coconut oil and with ginger juice by Gabbi et al. (2018). The acidity of leaf gel added ice-cream may have been influenced by the presence of acidic compounds, which are dominated by resin and saponin (Sukanya & Bhat, 2019).

Cyclea peltata leaf gel which was added to ice cream was different level (8.0 – 14.4 g) and that may be disturbed to the microstructure of ice cream samples and this may be the reason for significant difference of melting rate of ice cream. Ice cream samples incorporated with gelatine and T4, T3, T5 treatments showed the low level of melding down thus may be the indication that *Cyclea peltata* leaf gel inhibit the formation growth of ice crystals responsible for the meltdown of leaf gel added ice cream. The reason for the highest melting rate in T2 may be the lowest content of leaf gel.

There was an increasing of over run along with increasing of leaf gel grams added to ice cream. Because Stabilizers bind the water by means of hydrogen bonds, resulting in increased viscosity and overrun. According to turkey also, there was a significant different between all treatments. The higher the overrun the softer the ice cream (Thaiudon, et al., 2008).

Fat content had increased gradually with the content of leaf gel. These results are conformity with the results obtained by Erkaya et al. (2012) who studied the influence of cape goose berry in to ice cream. Fat present in leaf gel may interfere the fat content of ice cream.

V. CONCLUSION

Increasing leaf gel content led to decreased pH and melting rate values, increased titratable acidity, overrun and fat in formulated ice cream. Sensory evaluations showed that the most preferred ice cream formulations were T1, T3, T4 and T5. During at -12 °C, the most preferred ice cream samples showed significant differences in chemical and organoleptic qualities. *Cyclea peltata* leaf gel is a new substitute for gelatin and the combination of 1l of ice cream with 11.2g of *Cyclea peltata* leaf gel is the best combination for maintaining physic-chemical and organoleptic qualities without significant changes, making it safe for consumption.

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Efficacy of Newer Fungicides against Leaf and Fruit Spot of Custard Apple Caused by *Colletotrichum Gloeosporioides*

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Abstract— Custard apple (*Annona squamosa* L.) belong to family *Annonaceae* is one of the finest fruits gifted to India by tropical America. The crop is badly infected with leaf and fruit spot disease caused by *C. gloeosporioides*. Five systemic, five contact and five ready mixed fungicides were evaluated against *C. gloeosporioides* under in vitro by poison food technique. Among the systemic fungicides, carbendazim 50WP was recorded highest mean mycelial inhibition (88.50%) followed by tebuconazole 25EC (81.10%). Among the non-systemic fungicides, copper oxychloride 50WP found superior for mean mycelial inhibition (94.43%) of *C. gloeosporioides* followed by mancozeb 75WP (89.10 %). Carbendazim 12% + mancozeb 63% WP recorded maximum mean mycelial growth inhibition (95.89%) which was at par with tebuconazole 50% + trifloxystrobin 25% WG (94.43%) in ready mixed fungicides.

Keywords— Custard apple, leaf and fruit spot, *Colletotrichum gloeosporioides*, fungicides.

I. INTRODUCTION

Custard apple (*Annona squamosa* L.), is the most important fruit crop of the tropical and sub-tropical regions of the world it belonging to the family *Annonaceae*. It has hardy in nature and commercially grown on marginal land and hillocks with minimum inputs. *Annona squamosa* Linn. is a small, semi-deciduous tree, 3 - 7 m in height, with a broad, open crown or irregularly spreading branches, widely available in different agro-climatic zones of India. This fruit is sometimes also considered as “poor man’s rich food” in the arid zones of India and required dry climate with mild winter.

In India, custard apple was cultivated over 47 million hectares with an annual production of 402 MT (Anon, 2022). The area under cultivation of custard apple is increased only due to high economic returns obtained from this crop and that too at very low input cost. Since last 13-15 years, the crop is found to be badly infected with blackening of fruits i.e., dry fruit spot caused by *Colletotrichum gloeosporioides*. It is prevalent in almost all custard apple growing areas of country. Now a day it has become a severe melody to this crop. About 60 - 70 per cent losses have been reported due to the disease (Somwanshi *et al.*, 2021). Considering the severity of this disease and frequent occurrence in the fields, it has been felt necessity to develop effective management strategies. Thus, attempts have been made to evaluate the efficacy of newer fungicides against leaf and fruit spot disease.

II. MATERIALS AND METHODS

The *in vitro* efficacy of five systemic, five contact and five ready mixed fungicides were tested against *C. gloeosporioides* by poison food technique (Nene and Thapliyal, 1993) with Completely Randomized Design. This experiment was conducted in Department of Plant Pathology, C. P. College of Agriculture, S. D. Agricultural University, Sardarkrushinagar, Gujarat during

2022-23. The measured quantities of fungicides were incorporated in melted sterilized PDA medium and poured it into sterilized Petri dishes aseptically. The Petri dishes were inoculated with pathogen in the centre by placing seven days old mycelial discs of 5 mm diameter and then incubated at $25 \pm 1^{\circ}\text{C}$ temperature. Simultaneously, a suitable control was maintained by growing the fungus on fungicide free PDA medium. Radial mycelial growth of fungus was recorded on daily basis in control plates starting from the initiation of the fungal growth in correspondence to treatment plates till full mycelial growth of fungus was observed in control. The per cent inhibition of growth of the fungus in each treatment in comparison with control was calculated by the following equation (Vincent, 1927):

$$\text{PGI} = \frac{C-T}{C} \times 100 \quad (1)$$

Where,

PGI = Per cent growth inhibition

C = Growth of the test fungus in untreated control plates

T = Growth of test fungus in treated plates.

III. RESULTS AND DISCUSSION

3.1 Efficacy of systemic fungicides against *C. gloeosporioides*:

Among systemic fungicides, the highest mean mycelial growth inhibition was obtained with carbendazim (88.50%) followed by tebuconazole (81.10%) and difenoconazole (77.11%). Azoxystrobin recorded least inhibition of only 67.55 per cent mean mycelial growth inhibition of the fungus. Carbendazim and tebuconazole at 1000 ppm showed significant maximum growth inhibition of the pathogen *i.e.* 98.51 and 94.81 per cent. The next best treatment in order of merit were carbendazim at 750 ppm, pyraclostrobin at 1000 ppm and azoxystrobin at 1000 ppm which inhibited 93.33, 93.33 and 91.85 per cent mycelial growth inhibition, respectively. Azoxystrobin at 100 ppm found the least inhibition of only 52.22 per cent mycelial growth of test pathogen (Table 1). Watve *et al.* (2009) also found carbendazim and tebuconazole inhibited the growth and sporulation to the extent of 100 per cent of *C. gloeosporioides*. Patil *et al.* (2009) reported carbendazim and Ravi *et al.* (2021) reported tebuconazole as most effective fungicides for inhibiting the growth of *C. gloeosporioides*.

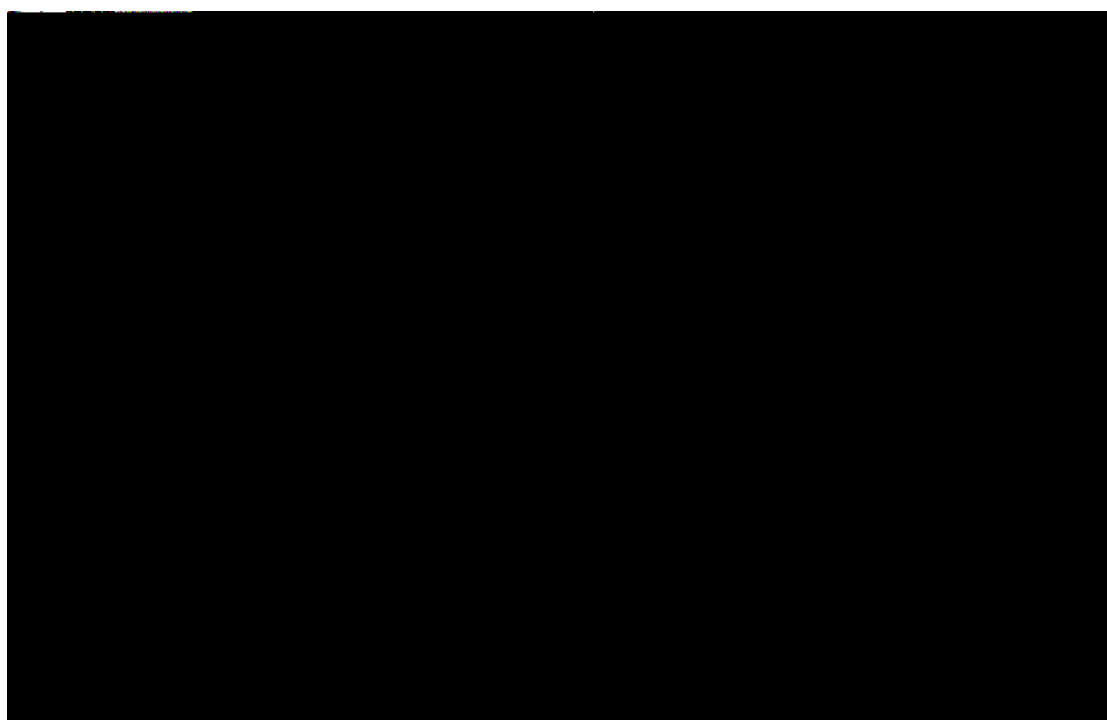


PLATE 1: Growth inhibition of *C. gloeosporioides* by systemic fungicides at different concentration *in vitro*

TABLE 1
EFFICACY OF DIFFERENT SYSTEMIC FUNGICIDES AGAINST *C. GLOEOSPORIOIDES* IN VITRO

Tr. No.	Treatments	Per cent growth inhibition over control Concentration (ppm)					
		100	250	500	750	1000	Mean
T1	Carbendazim 50WP	62.95	66.27	70.59	75.62	84.43	71.97
		(78.8)*	83.3	88.48	93.3	98.51	88.5
T2	Tebuconazole 25EC	59.21	61.43	63.23	64.85	77.48	65.24
		73.33	76.7	79.25	81.5	94.81	81.1
T3	Difenoconazole 25EC	53.23	59.21	62.45	65.77	69.95	62.12
		63.7	73.3	78.14	82.6	87.77	77.1
T4	Pyraclostrobin 20WG	47.6	56.61	58.73	68.05	75.92	61.32
		54.07	60	72.59	85.6	93.33	75
T5	Azoxystrobin 23 SC	46.54	51.03	53.91	56.38	73.92	56.36
		52.22	60	64.81	68.8	91.85	67.6
T6	Control	4.5	4.5	4.5	4.5	4.5	4.5
		0	0	0	0	0	0
Mean		45.88	50.05	52.44	56.07	64.52	-
		53.69	60.4	63.88	68.6	77.71	
		Treatment		Concentration		Treatment x Concentration	
S. Em.±		0.271		0.248		0.607	
C.D. at 5%		0.768		0.701		1.716	
C.V.%		1.95					

**Figures in parentheses are original values and outside are arc-sine transformed values*

3.2 Efficacy of non-systemic fungicides against *C. gloeosporioides*:

Among the seven different non systemic fungicides, copper oxychloride was found most effective with highest mean mycelial growth inhibition with 94.43 per cent found significantly superior over rest of fungicides. Next best fungicide was mancozeb with 89.10 per cent mean mycelial growth inhibition. Captan was found next effective to inhibit 82.29 per cent mycelial growth inhibition of the fungus and significantly superior over propineb (69.10 %).

Copper oxychloride at 2500, 2000 and 1500 ppm showed significantly maximum growth inhibition of pathogen i.e. 98.51, 97.03 and 94.44 per cent, respectively. The next best fungicide was mancozeb at 2500 ppm which inhibited the fungal growth as 93.70 per cent. Fungal growth of the pathogen generally decreased with increased concentration of the tested fungicides (Table 2). Rewale *et al.* (2016) found copper oxychloride and mancozeb as most effective with highest average mycelial growth inhibition of *C. graminicola*. Devamma *et al.* (2012) also found mancozeb as highly effective in inhibiting the mycelial growth of pathogen.

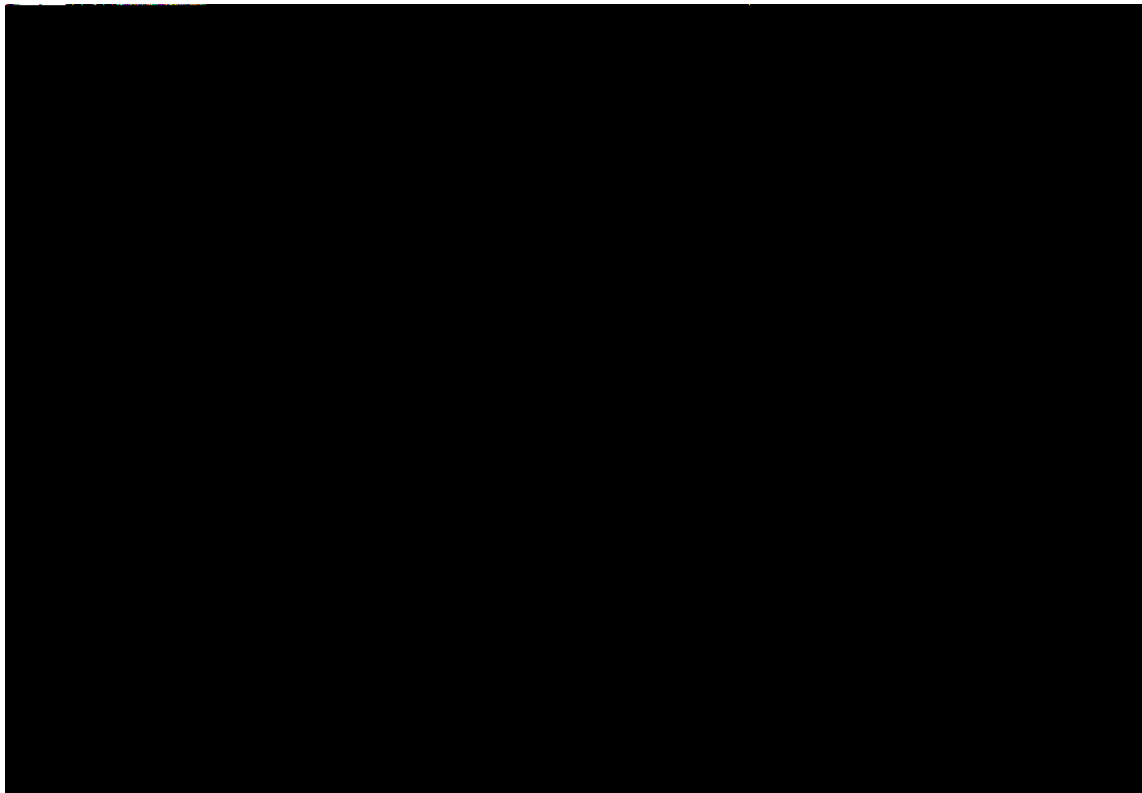


PLATE 2: Growth inhibition of *C. gloeosporioides* by non-systemic fungicides at different concentration *in vitro*

TABLE 2
EFFICACY OF NON-SYSTEMIC FUNGICIDES AGAINST *C. GLOEOSPORIOIEDS* IN VITRO

Tr. No.	Treatments	Per cent growth inhibition over control Concentration (ppm)					
		500	1000	1500	2000	2500	Mean
T1	Copper oxychloride 50WP	72.03	74.34	77.02	80.98	84.43	77.76
		(89.99)*	92.2	94.4	97	98.5	94.43
T2	Mancozeb 75WP	67.14	68.98	71.32	73.52	76.04	71.4
		84.44	86.7	89.3	91.5	93.7	89.1
T3	Chlorothalonil 75WP	47.17	50.17	51.47	52.78	57.07	51.73
		53.33	58.5	60.7	63	70	61.1
T4	Propineb 70WP	48.88	51.69	53.44	60.44	70.97	57.08
		56.29	61.1	64.1	75.2	88.9	69.1
T5	Captan 75WP	57.31	62.72	65.13	69.95	75.22	66.07
		70.36	78.5	81.9	87.8	93	82.29
T6	Control	4.5	4.5	4.5	4.5	4.5	4.5
		0	0	0	0	0	0
Mean		49.71	52.27	54.02	57.23	61.58	-
		59.07	62.8	65.1	69.1	74	
		Treatment		Concentration		T x C	
S. Em.±		0.245		0.224		0.549	
C.D. at 5%		0.694		0.633		1.551	
C.V.%		1.73					

**Figures in parentheses are original values and outside are arc-sine transformed values*

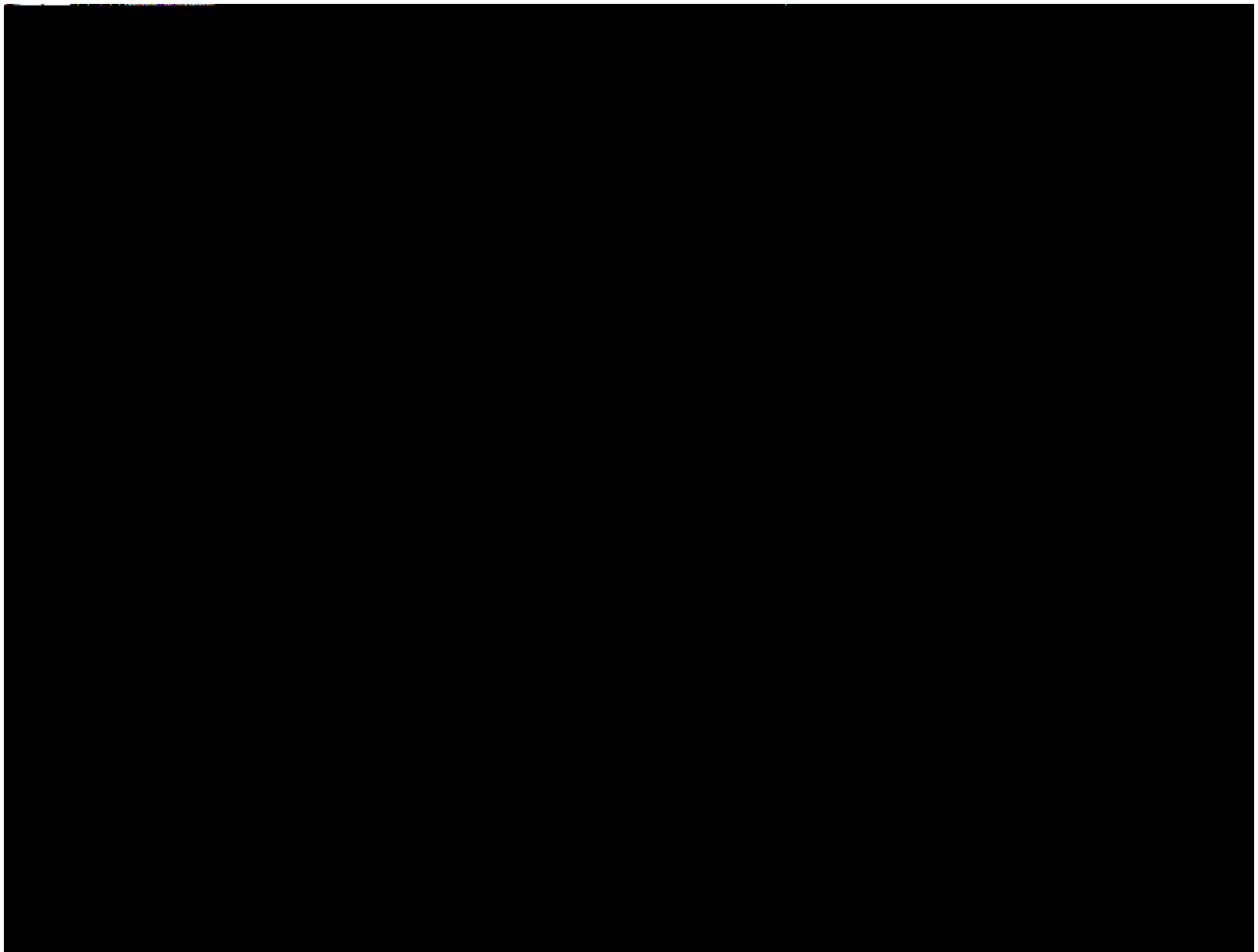


PLATE 3: Growth inhibition of *C. gloeosporioides* by ready mixed fungicides at different concentration *in vitro*

3.3 Efficacy of ready mixed fungicides against *C. gloeosporioides* under *in vitro*:

All the ready mixed fungicides were found effective at 2500 ppm concentration. Among them, carbendazim + mancozeb was found significantly higher than the rest of the ready mixed fungicides with 95.89 per cent mean mycelial growth inhibition followed by tebuconazole + trifloxystrobin, azoxystrobin + difenoconazole and metiram + pyraclostrobin with percentages of 94.43, 86.38 and 73.69, respectively. Azoxystrobin + mancozeb recorded the least mean mycelial growth inhibition of only 66.27 per cent of test fungus.

In combine fungicides, tebuconazole + trifloxystrobin and carbendazim + mancozeb at 2500 ppm both inhibited 98.51 per cent mycelial growth inhibition and it was at par with both same fungicides at 2000 ppm. The next best treatment was carbendazim + mancozeb at 1500 ppm and metiram + pyraclostrobin at 2500 ppm recorded with 96.29 and 95.18 per cent mycelial growth inhibition. Azoxystrobin + mancozeb and Metiram + pyraclostrobin both at 500 ppm recorded the least mycelial growth inhibition of only 51.85 and 52.59 per cent, respectively. Similar results were obtained in the investigations made by Kadam *et al.* (2014) who found carbendazim + mancozeb completely inhibited growth of *C. gloeosporioides*. Ann and Mercer (2017) also observed that tebuconazole + trifloxystrobin showed complete mycelial inhibition of *C. gloeosporioides*.

IV. CONCLUSION

Among systemic fungicides, carbendazim, followed by tebuconazole and difenoconazole, exhibited the highest mean mycelial growth inhibition. Among non-systemic fungicides, copper oxychloride was the most effective, exhibiting the highest mean mycelial growth inhibition against *C. gloeosporioides*. Carbendazim + mancozeb showed higher mean mycelial growth inhibition against *C. gloeosporioides*.

TABLE 3
EFFICACY OF READY MIXED FUNGICIDES AGAINST *C. GLOEOSPORIOIDES* IN VITRO

Tr. No.	Treatments	Per cent growth inhibition over control Concentration (ppm)					
		500	1000	1500	2000	2500	Mean
T1	Tebuconazole50+ Trifloxystrobin 25 WG	72.03	74.34	77.02	80.98	84.43	77.76
		(89.99)*	92.22	94.44	97.03	98.51	94.43
T2	Azoxystrobin11.5+ Mancozeb 30 WP	46.32	51.69	55.18	58.49	63.26	54.99
		51.85	61.1	66.92	72.22	79.25	66.27
T3	Carbendazim 12 WP + Mancozeb 63 WP	75.62	77.02	79.67	80.89	84.43	79.53
		93.33	94.44	96.29	96.88	98.51	95.89
T4	Metiram 55 + Pyraclostrobin 5 WG	46.75	55.47	57.07	66.27	78.06	60.72
		52.59	67.4	69.99	83.33	95.18	73.69
T5	Azoxystrobin18.2+ Difenconazole11.4 SC	66.27	68.35	69.62	68.8	70.97	68.8
		83.33	85.92	87.4	86.4	88.88	86.38
T6	Control	4.5	4.5	4.5	4.5	4.5	4.5
		0	0	0	0	0	0
Mean		52.12	55.43	57.38	60.19	64.48	-
		61.84	66.84	69.17	72.64	76.72	
		Treatment		Concentration		Treatment x concentration	
S. Em.±		0.325		0.297		0.728	
C.D. at 5%		0.921		0.84		2.05	
C.V.%		2.18					

***Figures in parentheses are original values and outside are arc-sine transformed values**

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The Prevalence of Gastrointestinal Protozoa Infection in Pigs in Umuahia South, Abia State Nigeria

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Abstract— Pig production plays a major role in boosting the economy in most regions of the world by serving as a source of income and satisfying the growing demand for livestock and livestock products. However, the challenges affecting pig production are enormous, such as gastrointestinal parasitism in the form of protozoan infection, which often is not diagnosed early until damage is done, reducing economic gains and resulting in losses. A cross-sectional study of six small-scale pig farms in Umuahia South LGA, with the objective of determining the prevalence of gastrointestinal protozoans of the pigs was undertaken. A total of 116 pigs were randomly selected comprising of 29 pigs categorized as young less than 6 months and 87 pigs as adult aged above 6 months, 50 male and 66 female, 96 pigs raised intensively and 20 pigs reared under extensive management was sampled from July to September for protozoan infection. Faecal samples were obtained from pigs of various ages, sexes, and those raised under intensive and extensive farm systems and analyzed in the laboratory using standard techniques. The results of the frequency of protozoan infection were calculated and presented as percentages. Four different types of protozoans were found to infect pigs in Umuahia South LGA of Abia State, namely: *Cryptosporidium* spp. (33.6%), *Balantidium* spp. (34.5%), *Entamoeba* spp. (14.7%) and *Giardia* spp. (42.2%). The occurrence of the various protozoan parasites was higher in females, adult and intensively reared pigs when compared with males, young and extensively reared pigs. These protozoan parasites are capable of causing considerable loss in pig production and can cause infection in humans. Therefore, farmers in the study area should be sensitized on the herd health programmes and practice good hygienic measures to reduce the incidence of gastrointestinal protozoa in pig herds.

Keywords— Abia State, Gastrointestinal, Pig, Protozoa, Umuahia South, Zoonotic.

I. INTRODUCTION

Pigs (*Sus Scrofa Domesticus*) generally referred to as swine is one of the abundant livestock species raised in Nigeria, most especially in the middle-belt and southern part of the country accounting for about nine million pigs out of the fourteen million pigs reared in the West African sub-region (Bernard *et al.*, 2021). Pigs are reared primarily as a source of animal protein such as ham, pork, bacon and gammon and other meat products, raw materials for biomedical products and manure (Ejinaka and Onyali, 2020; Bernard *et al.*, 2021). Pig production is among the fastest growing sectors in the livestock industry, and it is projected to continue rapidly growing in the years to come with the thought of bridging the deficiency in animal-derived protein and as a useful tool to fight poverty in low-and middle-income countries (Nwachukwu and Udegbumam, 2020). Pigs are inexpensive to raise, making them a popular and more acceptable choice among farmers with limited resources especially in the rural communities (Adesehinwa *et al.*, 2024). The choice of pig rearing over other food-producing animals such as ruminants is due to their polytocous nature, high feed conversion efficiency, early maturation rate, short gestation duration, and are very hardy in tolerating less-than-ideal tropical environmental conditions (Bernard *et al.*, 2021).

Various constraints affect the sustainable development of the swine industry, with diseases caused by gastrointestinal parasites being the most common (Unanam and Lekko, 2023). Pigs are known to harbor parasites, some of which are zoonotic, including

helminths and protozoans which can negatively impact feed conversion, growth rate, and weight gain and can result in condemnation of organs following slaughter and huge financial losses (Ajibo *et al.*, 2020). Certain parasites of pigs with the potential to affect humans become major public health issues as there have been outbreaks of waterborne diseases reportedly caused by *Giardialamblia* and *Cryptosporidium* spp. (Abiola *et al.*, 2018).

Internal parasites of pigs are widespread globally and its occurrence is of great importance most especially in Nigeria with favorable climatic conditions that allows for easy transmission of these parasites in pigs (Ejinaka and Onyali, 2020). There are several studies that have been carried out in different parts of Nigeria on the gastrointestinal parasites of pigs focusing on nematodes (helminths) and cestodes (tapeworms) with less emphasis on protozoans of pigs; Fabiyi (1979) in Jos reported 15 helminth species in pigs, and Sowemimo *et al.* (2012) identified 5 species of parasites from pigs in Ibadan. Hence, there is paucity of information on the gastrointestinal protozoans affecting pigs, especially in the study area. This study aims to provide epidemiological data on the prevalence and intensity of protozoans affecting pigs in Umuahia South, Abia State, Nigeria.

II. MATERIALS AND METHODS

2.1 Study area:

The study was conducted in Umuahia South local government area of Abia State, Nigeria, and lies within longitude 7°29'40.60" E latitude 5°31'29.68" N. Umuahia South has an area of 140km² and a population of 138,570 according to the 2006 National population census estimation (NPC, 2007). This study was carried out between July and September 2023 during which sampling took place from the various farms: GGAG farm (old Umuahia), JEN venture farm (Olokororo), Unique farm (Umuopara), Chizoba ENT (Ubakala), and Ofovic farm (Old Umuahia) used for the survey.

2.2 Sample collection:

Faecal samples were randomly collected from the rectum of 116 pigs in the various farms in the study area. The faeces were collected using sterile plastic gloves into a clean sterile sample bottle labelled appropriately according to the age, sex, and location taking note of the management practices. The sample bottles containing the faecal samples were placed in a cooler and transported to the parasitology laboratory of the Department of Veterinary Parasitology and Entomology, College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike. The samples were immediately analyzed for gastrointestinal protozoans.

2.3 Laboratory analysis:

The faecal samples were grossly analyzed for adult parasites. Microscopic examination for various protozoan oocysts/cysts were carried out using direct smear method and modified Ziehl-Neelsen method to detect acid fast organisms as described by Rekha *et al.* (2016) and viewed under the microscope using x 10 and x 40 objective lens. A small amount of the feces was emulsified in a drop of 1% iodine placed in the center of a spotless, grease-free glass slide using applicator sticks. The smear was viewed under a microscope using a x 40 objective lens after being covered with a cover slip as described by Karaye *et al.* (2016). Using the formalin-ether concentration method as described by Brhanie *et al.* (2014), approximately 1 g of the fecal sample was added to a clean 15 ml conical centrifuge tube filled with 7 ml of 10% formalin using an applicator stick. The applicator stick was used to properly mix the sample. The resultant suspension was put back into the original tube after the filtrate was transferred from the sieve into a beaker. Following the addition of 3 ml of diethyl ether, the mixture was manually agitated and centrifuged for 3 minutes at 2000 rpm. Following centrifugation, the residue was spread out on microscope slides, covered with cover glass, and examined under the microscope with the x 40 objective lens after the supernatant was carefully discarded after centrifugation. The oocysts and cysts were identified according to structural and morphometric standards (Soulsby, 1982).

2.4 Statistical analysis:

The collected data were analyzed using descriptive statistics as described by Swai *et al.* (2010). The prevalence (P) of the various gastrointestinal protozoans in different sex, age and management system were calculated and presented in tables using the formula $P = d/n$, where d is the number of positive samples analyzed at that point in time and n = total number of pigs sample analyzed at that point in time (Thrusfield, 2005). The prevalence was calculated in percentage.

III. RESULTS

3.1 Microscopy:

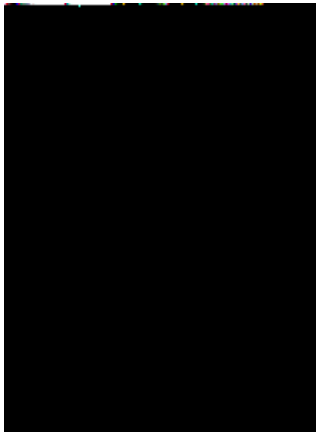


FIGURE 1:
Cryptosporidium Oocyst

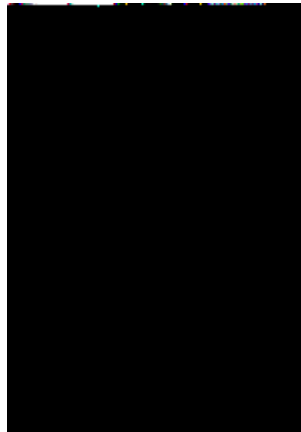


FIGURE 2: Balantidium
Oocyst



FIGURE 3: Giardia
Oocyst



FIGURE 4: Entamoeba
Oocyst

In the survey, 116 faecal samples of pigs were analyzed and all were found to be infected with one or more different gastrointestinal protozoa (Table 1). Thus, the prevalence of gastrointestinal protozoans of pigs observed in this study were *Giardia* spp. (42.2%) which had the highest prevalence, followed by *Balantidium* spp. (34.5%); *Cryptosporidium* spp. (33.6%), and *Entamoeba* spp. (14.7%) with the lowest prevalence recorded in this study.

The prevalence of *Giardia* spp. in Table 2 was higher in adult pigs (69.0%) than in piglets (31.0%). The prevalence in females (63.6%) was higher when compared to males (36.0%). *Giardia* spp. was more prevalent in intensively reared pigs (33.3%) and the protozoan was not observed in extensively managed pigs.

The prevalence of *Balantidium* spp. is presented in Table 3. The prevalence was higher in adult pigs (75.7%) than in piglets (24.1%). The prevalence in females (56.0%) was higher than in males (44.0%). Intensively reared pigs (24.0%) had a higher prevalence than in extensively reared pigs (5.0%).

The prevalence of *Cryptosporidium* spp. in Table 4 was higher in adult pigs (79.3%) than in piglets (20.7%). The prevalence in females (54.5%) was higher when compared to males (46.0%). The prevalence was higher in intensively reared pigs (21.9%) than in extensively managed pigs (5.0%).

The prevalence of *Entamoeba* spp. in Table 5 was higher in adult pigs (93.1%) than in piglets (6.9%). The prevalence in females (63.6%) was higher when compared to males (36.0%). The prevalence was higher in intensively reared pigs (7.3%) than in extensively managed pigs (5.0%).

TABLE 1
PREVALENCE OF DIFFERENT SPECIES OF GASTROINTESTINAL PROTOZOAN FROM FAECAL SAMPLES OF 116 PIGS IN UMUAHIA SOUTH

Protozoan type	Number examined	Frequency of infection	Prevalence (%)
<i>Giardia</i> spp.	116	49	42.2
<i>Balantidium</i> spp.	116	40	34.5
<i>Cryptosporidium</i> spp.	116	39	33.6
<i>Entamoeba</i> spp.	116	17	14.7

TABLE 2
PREVALENCE OF *GIARDIA* SPP. IN EACH CATEGORY OF PIGS SAMPLED FROM UMUAHIA SOUTH

Population type	Number examined	Number infected	Prevalence (%)
Young	29	9	31
Adult	87	60	69
Male	50	18	36
Female	66	42	63.6
Intensive management	96	32	33.3
Extensive management	20	0	0

TABLE 3
PREVALENCE OF *BALANTIDIUM* SPP. IN EACH CATEGORY OF PIGS SAMPLED FROM UMUAHIA SOUTH

Population type	Number examined	Number infected	Prevalence (%)
Young	29	7	24.1
Adult	87	66	75.7
Male	50	22	44
Female	66	37	56
Intensive management	96	23	24
Extensive management	20	1	5

TABLE 4
PREVALENCE OF *CRYPTOSPORIDIUM* SPP. IN EACH CATEGORY OF PIGS SAMPLED FROM UMUAHIA SOUTH

Population type	Number examined	Number infected	Prevalence (%)
Young	29	6	20.7
Adult	87	69	79.3
Male	50	23	46
Female	66	36	54.5
Intensive management	96	21	21.9
Extensive management	20	1	5

TABLE 5
PREVALENCE OF *ENTAMOEBAS* SPP. IN EACH CATEGORY OF PIGS SAMPLED FROM UMUAHIA SOUTH

Population type	Number examined	Number infected	Prevalence (%)
Young	29	2	6.9
Adult	87	81	93.1
Male	50	18	36
Female	66	42	63.6
Intensive management	96	7	7.3
Extensive management	20	1	5

IV. DISCUSSION

The protozoan parasites observed in this study belonged to four genera namely; *Balantidium*, *Cryptosporidium*, *Entamoeba*, and *Giardia*. This is comparable to the study of Gagman *et al.* (2015) in Jos, Plateau State who identified five different protozoa including *G. lamblia* (6.77%), *Isospora* spp. (7.90%), *Eimeria* spp. (7.14%), *B. coli* (9.40%), and *Entamoeba* spp. (4.70%). However, the prevalence of *Giardia* spp. (42.2%), *Balantidium* spp. (34.5%) and *Entamoeba* spp. (14.7%) in this study were higher than what was obtained in Gagman *et al.* (2015). *Eimeria* and *Isospora* spp., were not identified in this study, and this could be due to differences in diagnostic techniques used in both studies. The findings in this study where all the fecal samples analyzed were positive for one or more gastrointestinal protozoan parasites show that there is a high prevalence of gastrointestinal protozoan infection of pigs in Umuahia South. This is similar to the report of Nwoha and Ekwurike (2011) who recorded one or more parasite in all the fecal samples of intensively managed pig farms in Umuahia City of Abia state examined for gastrointestinal parasites. There was varying prevalence of the four species of protozoan parasites observed in this study with *Giardia* spp. being the most prevalent while *Entamoeba* spp. had the lowest prevalence. This contrasts the study of Ejnaka and Onyali (2020) who reported *Balantidium coli* and *Entamoeba histolytica* having the highest frequencies in their study and are the only two of the four protozoa parasites observed in this study. The varying prevalence could be attributed to differences in the breed, diet, herd management techniques, and even genetic resistance of the pigs (Ejinaka and Onyali, 2020).

The various protozoan parasites of pigs observed in this study were higher in females than in males. This however agrees with Tamboura *et al.* (2006) in Burkina Faso which reported that the frequency of pig parasites was higher among female scavengers but contrasts the studies of Nsoso *et al.* (2000), Kumar *et al.* (2002), and Sowemimo *et al.* (2012) where higher prevalence was recorded in males than in females. This could be due to the reason that females are usually kept for breeding for a longer time unlike males that are typically and sold off thus, exposing the females to a higher parasitic burden for longer periods (Akanni *et al.* 2017).

The protozoan infection was lower in young pigs (piglets) in relation to age as compared with adult mature pigs including boars and sows of reproductive ages. This is in agreement with the studies of Atawalna *et al.* (2016) and Akanni *et al.* (2017). It is suggested that low prevalence of parasites in piglets could be due to maternal immunity acquired from the sow through milk while higher frequency of parasites in adult pigs could be a result of pre-exposure, weaker immune systems, and possibly inadequate nutrition over time (Ejinaka and Onyali, 2020).

The higher prevalence of protozoa infection in intensive system of management as compared to the extensive system observed in this study could be due to the higher population size of intensively reared pigs in this study, poor environment and managerial practices like high stocking density, inadequate nutrition and low immunity status observed in the farms visited. This contrasts the study of Ogbe *et al.* (2023) who reported a high prevalence of gastrointestinal parasites in extensively managed pigs in Aba, Abia State.

V. CONCLUSION

In conclusion, the protozoan type observed in this study are zoonotic and they are also capable of causing adverse health conditions in pig herd. Therefore, it is advocated that regular monitoring of pig farms by veterinarians to screen faeces for protozoan parasites and administration of appropriate anthelmintic be done to reduce the burden of parasites in pig farms. Farmers should also be encouraged to attend trainings on the importance of herd health management, adopt modern day practices for better productivity in order to decrease the burden of gastrointestinal protozoa infection of pigs. This study also shows that protozoan parasites capable of being implicated in zoonotic infection of humans either via water or food can be found in faecal samples of pigs thus, creates a burden on public health. In Abia State where pork is widely consumed, further studies on investigation and public health assessment of the impact of these zoonotic parasites should be carried out and more public awareness raised towards the prevention of protozoan infection in humans.

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First Record of Leucism in Albino *Gazella bennettii* from the Western Thar Desert, Barmer, Rajasthan, India

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Abstract— The leucism research study carried out in the Western part of Thar Desert in the Barmer district and this research is completely based on the observation and interview of the local people for Leucism in Albino *Gazella bennettii* (Indian Gazelle) locally called Chinkara. The *Gazella bennettii* (Indian Gazelle) is considered an endangered species by the International Union for Conservation of Nature (IUCN). In this Paper we describe the occurrence of leucism in Albino Indian Gazelle in Barmer first time. at Siyago ka tala, VP Kheda, Dhanau village of the Barmer district. General eco-behavior of this animal is noted which are similar to the normal Indian Gazelle.

Keywords— Albino, Chinkara, *Gazella bennettii*, leucism.

I. INTRODUCTION

The Indian Gazelle, scientifically known as *Gazella bennettii* and commonly referred to as the Chinkara, belongs to the class Mammalia, order Cetartiodactyla, and family Bovidae. This species is easily identifiable by its reddish-brown fur on the underside and pale yellowish coat with faint white markings. The gazelle's fur undergoes seasonal changes, adapting to its environment. During winter, its coat turns a dark grayish-sandy hue, providing effective camouflage against predators, while in summer, it transitions to a deeper shade of brown. Both male and female Indian Gazelles possess horns, though there are notable differences. Males have longer, thicker horns with prominent rings and slightly curved tips, while females have shorter, slimmer horns with less pronounced rings. On average, the Indian Gazelle measures between 0.9 to 1.2 meters in length and stands 0.6 to 0.8 meters tall at the shoulder. Adult males typically weigh between 20 to 25 kilograms, whereas females are slightly smaller, weighing less and often standing up to 10 centimeters shorter than males (Jerdon, 1874; Groves, 1993).

A rare and noteworthy observation in the region was the recording of an albino gazelle in 2015, exhibiting abnormal body coloration (Khagendra et. All, 2016). Occurrences of fur color anomalies, such as albinism, leucism, and melanism, are quite rare in mammals and are not well-documented in scientific literature, especially concerning wild species. Most studies on mammalian coloration have concentrated on domesticated animals and laboratory mice, making sightings in the wild particularly important for understanding biological adaptations. In February 2024, a leucistic Indian Gazelle fawn was observed in the Western Thar Desert of Barmer District, Rajasthan, marking the first confirmed case of a leucistic gazelle in this area. This finding emphasizes the unique biodiversity of the Thar Desert and highlights the necessity for more research into the adaptive significance of these color variations in wild populations. Indian Gazelles have a polygynandrous mating system, where both males and females engage with multiple partners. Their diet primarily consists of desert vegetation, including grasses, cereals, legumes, fruits, and shrubs such as *Crotalaria burhia* (Bui), *Ziziphus nummularia* (Ber), *Prosopis cineraria* (Khejri), *Tecomella undulata* (Rohida), and *Lasiurus scindicus* (Sevan). These plants are well-suited to the dry environment and are vital food sources for gazelles. Indian Gazelles play an important role in the ecosystem by helping to maintain ecological balance and acting as indicators of environmental health. Unfortunately, their survival is increasingly at risk due to human activities. The growth of agricultural land, habitat fragmentation from fencing, and the use of chemical fertilizers and pesticides have greatly affected their populations. They also face dangers from natural predators like feral dogs and wolves, as well as conflicts with humans. Farmers often see gazelles as pests that damage their crops, which can lead to hunting or accidental harm. Research shows that the extensive use of agrochemicals not only impacts gazelles but also threatens

other wildlife, insects, and birds, disrupting the fragile balance of the ecosystem (Rajpurohit and Chena Ram, 2011). Conservation efforts are crucial to address these threats and help ensure the survival of this iconic desert species.

II. MATERIAL AND METHODS

Barmer, located in the western part of Rajasthan, India, is part of the vast Great Indian Desert, commonly referred to as the Thar Desert. This area is known for its dry landscape, featuring extensive sand dunes and a challenging climate. Within the Barmer District, you'll find the village of Siagon Ka Tala, which is part of the revenue village of Kheda, under the administrative oversight of Tehsil Dhanau, Panchayat Samiti, and Gram Panchayat Deengarh. Geographically, this region is situated at around 25.75°N latitude and 71.5°E longitude. The land is mainly covered with sand dunes, and the climate is extreme, with temperatures reaching between 45–52°C during the hottest months of May and June, while in winter, particularly in December and January, temperatures can plummet to as low as 0°C. Rainfall is limited and unpredictable, with about 90% of the annual precipitation occurring during the monsoon season, which generally takes place from July to September. The average annual rainfall is around 277 mm, making water scarcity a significant challenge for both the local population and the ecosystem.

The vegetation in Barmer mainly consists of xerophytic plants that are well-suited to the dry climate. Common species found in the area include *Acacia senegal*, *Prosopis cineraria* (known as the "khejri" tree, which is culturally and ecologically important in Rajasthan), *Capparis decidua*, *Prosopis juliflora*, *Calotropis procera*, *Crotolaria buhia*, *Salvadora persica*, *Salvadora oleoides*, *Ziziphus nummularia*, *Tecomella undulata*, and *Calligonum polygonoides*. These plants are essential for stabilizing the sandy soil, providing fodder for livestock, and supporting local biodiversity. The varying environmental conditions, characterized by extreme temperatures and scarce water resources, have created unique habitats that host a range of wildlife, including the Indian Gazelle (*Gazella bennettii*), commonly referred to as the "chinkara." This species is well-adapted to the desert and is frequently spotted in the Barmer region.

A study conducted in Siagon Ka Tala, a village within the Kheda revenue village, aimed to explore the ecological dynamics and biodiversity of the area. The village is managed by Gram Panchayat Deengarh, Panchayat Samiti, and Tehsil Dhanau, and is located at approximately 25.32°N latitude and 71.07°E longitude. During the fieldwork, researchers used visual observation techniques and photography to capture the flora and fauna of the region. They also gained valuable insights through discussions with local residents, who shared traditional knowledge about natural resources, wildlife, and the environmental challenges faced by the community. This collaborative approach not only enhanced the study but also emphasized the strong connection between the local people and their environment. The findings highlight the resilience of both the ecosystem and the inhabitants who have adapted to the harsh conditions of the Thar Desert.

III. OBSERVATION AND DISCUSSION

After September 2015, extensive field studies spanning over nine years were conducted in Western Rajasthan to document the presence of Albino *Gazella bennettii*. However, the sighting of a leucistic individual in the Thar region marks a significant first. In February 2024, villagers from Siagon Ka Tala discovered a small fawn of the Indian Gazelle with unusual coloration. This discovery prompted my visit to the area, located approximately 81 kilometers west of Barmer, as part of my fieldwork focused on studying fawns of the Indian Gazelle and exploring the region's faunal and floral diversity.

Upon observation, the primary distinction between the leucistic fawn and normal gazelles was its unique coloration. Unlike typical albino animals, which exhibit a complete lack of pigment, this fawn retained pigmentation in its eyes, a characteristic feature of leucism. This situation is quite unique, as leucism represents a partial form of albinism where pigment loss occurs in specific areas rather than entirely. To gain further understanding, I spoke with local residents, who provided valuable insights about their experiences with albino gazelles in the area. The fawn is currently being cared for at the Wildlife Conservation Centre in Devda, Dhorimanna, Barmer, Rajasthan.

This facility offers a secure environment for the animal while enabling researchers and conservationists to observe its health and behavior. The finding of this leucistic fawn not only showcases the genetic diversity within the *Gazella bennettii* population but also emphasizes the need for ongoing conservation efforts in the Thar region. Such rare events present unique chances to explore the genetic and ecological factors that affect pigmentation anomalies in wildlife, enhancing our overall understanding of biodiversity and species preservation.

3.1 Role of Bishnoi Community in Wildlife Conservation in the Thar Region:

The Bishnoi community is well-known for its strong dedication to wildlife conservation, particularly in the Thar Desert. Their cultural and religious beliefs prioritize the protection of animals, especially the Indian Gazelle (Chinkara), blackbucks, and other

native species. This commitment contributes to the higher biodiversity in these areas, as the Bishnoi community actively participates in conserving wildlife in the region. They treat these gazelles as if they were their own children, working diligently to protect them from poachers and habitat loss. Their deep-rooted traditions forbid the hunting of animals and the felling of green trees which is significantly contributing to maintaining ecological balance in the region. Bishnoi community efforts in wildlife conservation have led to flourishing populations of endangered species. By protecting natural habitats, opposing deforestation and preventing poaching they have played a critical role in sustaining biodiversity in the arid Thar landscape. Community participation to wildlife conservation always plays an effective role and the Bishnoi example serves as inspiration for sustainable coexistence between humans and nature.

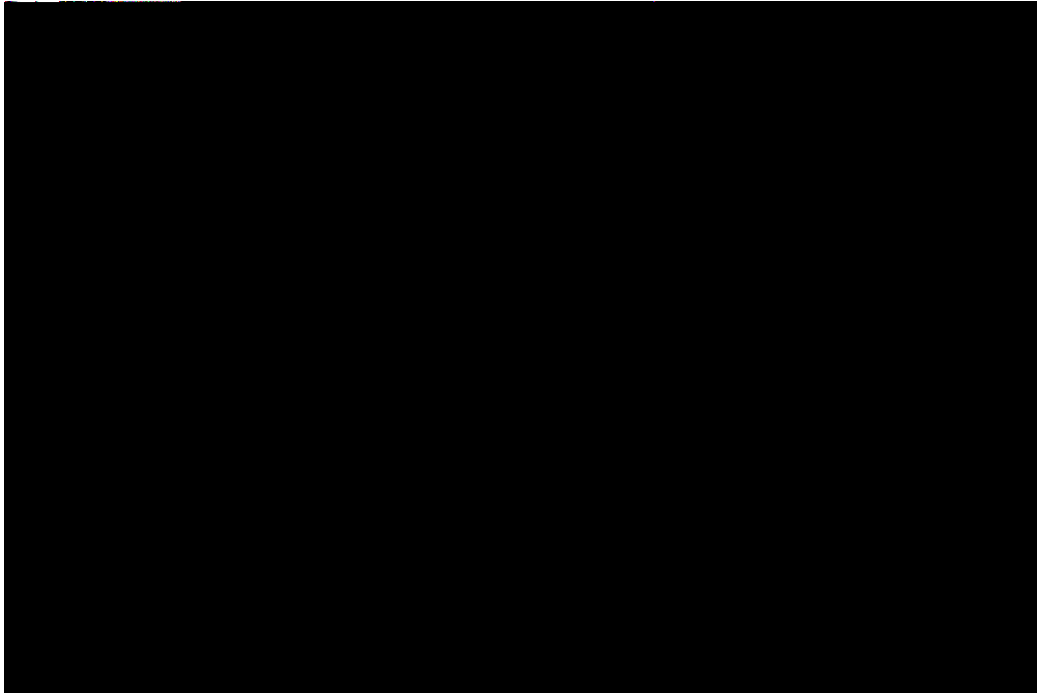


FIGURE 1. While feeding the fawn with goat's milk

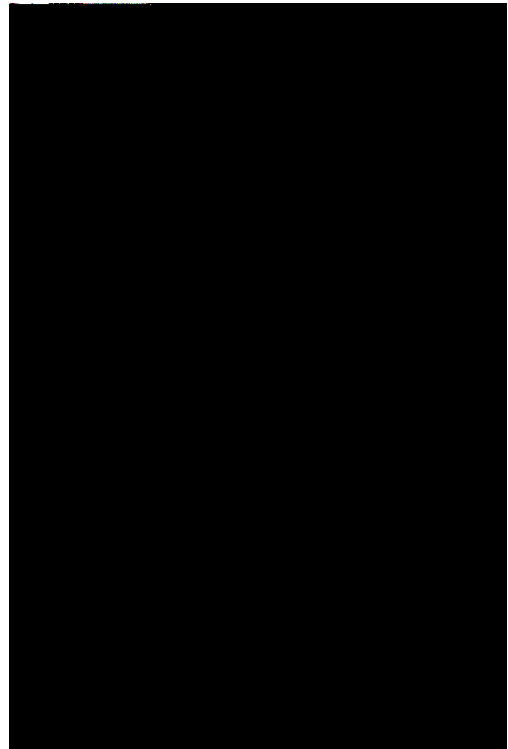


FIGURE 2: Fawn with boy and on field.

3.2 GPS location:

Siagon Ka Tala is a village located within the revenue village of Kheda, under the jurisdiction of Gram Panchayat Deengarh, Panchayat Samiti and Tehsil Dhanau, in Barmer District, Rajasthan, India. The approximate geographical coordinates are 25.32°N latitude and 71.07°E longitude.

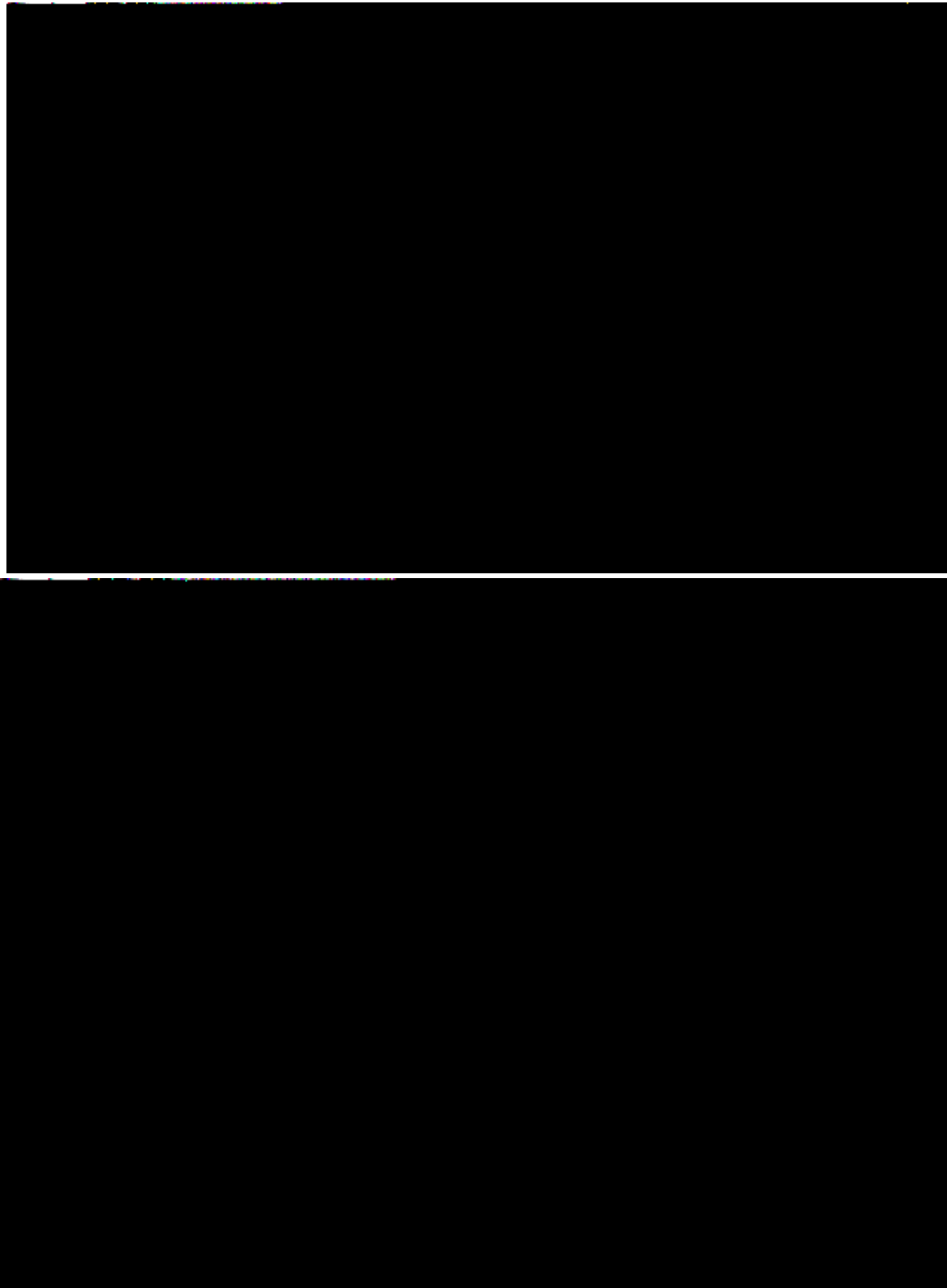


FIGURE 3: Location of Deengarh, Panchayat Samiti and Tehsil Dhanau, in Barmer District, Rajasthan, India

3.3 What is Leucism?

Melanin, a natural pigment, is responsible for the coloration of hair, skin, and eyes in humans and animals. It is produced by specialized cells called melanocytes. While the number of melanocytes remains consistent, variations in melanin production lead to differences in coloration, resulting in light or dark skin, hair, and eyes. The amount of melanin produced is influenced by genetic and environmental factors, which is why offspring often share similar skin tones with their parents. Melanin is categorized into five types: eumelanin, pheomelanin, neuromelanin, allomelanin, and pyomelanin. In humans, melanin primarily exists in three forms: eumelanin, pheomelanin, and neuromelanin.

Eumelanin, which includes brown and black subtypes, is responsible for darker shades in hair, skin, and eyes. The combination of black and brown eumelanin determines hair color, while red hair results from equal amounts of pheomelanin and eumelanin. Strawberry blonde hair, on the other hand, arises from a mix of brown eumelanin and pheomelanin. Pheomelanin gives pinkish hues to body parts such as the lips, nipples, and genitalia. Neuromelanin, found in neurons, influences the coloration of certain brain regions and plays a role in preventing cell death in specific areas. A deficiency in neuromelanin has been linked to Parkinson's disease, a neurological disorder. Beyond coloration, melanin also protects the skin by absorbing and dispersing harmful UV rays, preventing deep penetration and reducing the risk of damage such as oxidative stress, premature aging, diabetes, and even cancer.

Albinism is a genetic disorder characterized by abnormal coloration of the skin, hair, feathers, scales, or eyes (Hiler 1983). True or complete albinism involves the total absence of pigmentation in the skin and retina, while partial albinism occurs when pigmentation is reduced or absent in specific areas (Sandoval-Castillo et al. 2006). Leucism, a form of partial albinism, is marked by the retention of color in the eyes, (Berdeen and Otis 2011) bill, and legs, but a lack of pigment in the skin or plumage (Forrest and Naveen 2000). Albinism is caused by multiple genes (Summers 2009), whereas leucism is typically controlled by a single recessive allele (Owen and Shimmings 1992). Albino animals are rare due to the recessive nature of the alleles involved. Partial albinism is more common in small, isolated populations, where inbreeding increases the likelihood of such genetic traits being expressed (Holyoak 1978).

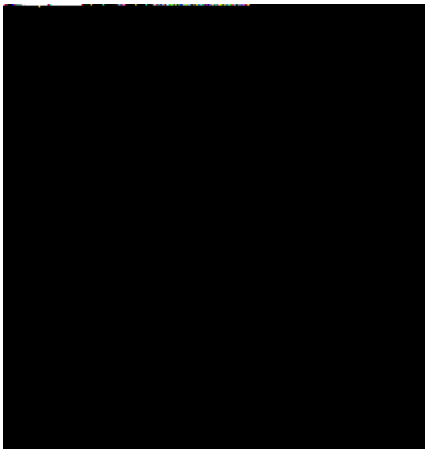


FIGURE 3: Eumelanin

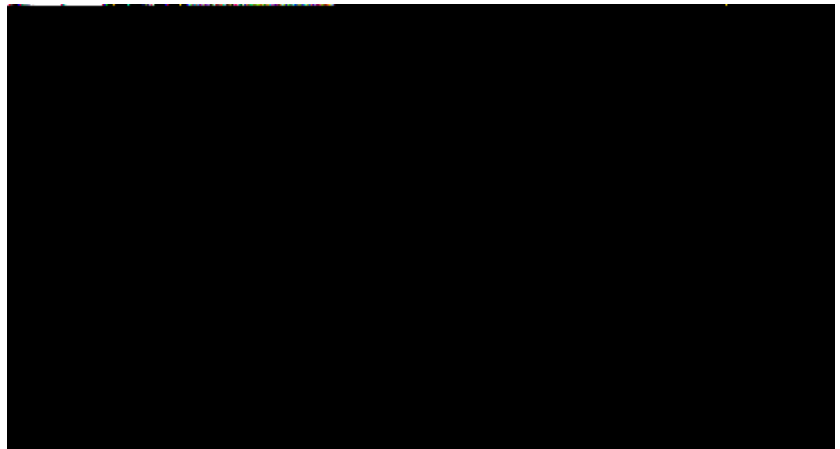


FIGURE 4: Pheomelanin

IV. CONCLUSION

Biodiversity faces significant threats due to various developmental pressures, including population growth, urbanization, industrialization, and the expansion of human civilization. Protecting biodiversity is crucial for sustaining life on Earth. The recent sighting of an Albino *Gazella bennettii* exhibiting leucism in the Thar Desert presents exciting new possibilities for wildlife conservation across varied landscapes. This finding underscores the urgent need for environmental awareness, greater public involvement, and a collective responsibility to safeguard and preserve biodiversity.

Ecologists view albinism as a significant disadvantage for wild animals for several reasons. The most apparent issue is the loss of camouflage, as white animals are more visible to predators compared to those that blend into their surroundings. Additionally, albino animals often suffer from poor eyesight due to a lack of pigment in their eyes, which hinders their ability to locate food or evade threats. Another challenge is intraspecific interactions; even if an albino animal survives to adulthood,

its ability to reproduce may be compromised. The lack of coloration can make these individuals more conspicuous to predators and potentially less attractive to mates, further threatening their survival.

AUTHOR'S CONTRIBUTIONS

This Research study has been carried out at Western Thar Desert of Barmer Rajasthan. The establishment of Methodology, literature collection, preparation of questionnaires, interviews of local people, photography of field and conductance of survey carried out by the Dr. Khagendra Kumar and Dr. CP Gharu.

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A Study of Common Pathogens in Poultry Industries in Ifite-Ogwari, Anyamelum Local Government Area of Anambra State, Nigeria

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Abstract— Poultry production is an essential sector in global agriculture, contributing significantly to food security by providing affordable animal protein. However, the prevalence of infectious diseases caused by various pathogens presents substantial economic losses and public health risks. This study dealt with isolation and characterization of common pathogens in poultry flocks within Ifite-Ogwari, Ayamelum local government area of Anambra State, Nigeria. Primary objectives include isolating pathogens from fecal samples of birds raised in the area, characterizing the pathogens and determination of antibiotics resistance profiles of the pathogens using standard biochemical techniques, and assessing their prevalence and distribution across the area. Key bacterial pathogens identified include *Salmonella enterica*, *Campylobacter* spp., *Escherichia coli*, *Enterococcus* spp., and Methicillin-Resistant *Staphylococcus aureus* (MRSA). Most of these pathogens from different farms were resistant to various antibiotics indicating subnormal cum improper applications by farmers. The study therefore underscores the urgent need for the government to detail Extension Agents to Ifite-Ogwari 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 Ifite-Ogwari. The use of alternative bio security measures such as herbs are recommended in order to mitigate the imminent danger of using antibiotics.

Keywords— Pathogens, Ifite-Ogwari, Antibiotic Resistant, Zoonotics, Bacterial Profile.

I. INTRODUCTION

The poultry industry is susceptible to a wide range of bacterial, viral, fungal, and parasitic pathogens. Bacterial pathogens, including *Salmonella* spp., *Escherichia coli*, *Campylobacter* spp., and *Staphylococcus aureus* are among the most common culprits responsible for disease outbreaks in poultry. They cause high mortality and productivity losses in poultry (Tamer *et al.*, 2020).

Salmonella enterica causes foodborne enteric disease worldwide, representing the second most commonly reported zoonotic pathogen in the world (Lorenzo-Rebenaqu *et al.*, 2022). It is responsible for disease outbreaks associated with significant morbidity and mortality (Ricke *et al.*, 2021). And up to 25% of human *Salmonella* outbreaks, illnesses and hospitalizations are related to poultry sources (Chai *et al.*, 2017). *Salmonella* are gram-negative, facultative anaerobic bacteria belonging to the Enterobacteriaceae family, and are considered commensals of the gut microbiota of mammals, birds, reptiles, amphibians, fish and shellfish (Cosby *et al.*, 2015). *S. enterica* includes more than 2650 serovars (Issenhuth-Jeanjean *et al.*, 2014), and many are implicated as contaminants of poultry meat and eggs, representing a serious concern for public health (Antunes *et al.*, 2016). In poultry, diseases due to *S. enterica* may be grouped into three namely fowl typhoid, pullorum disease and avian paratyphoid. Young poultry are particularly susceptible to gastrointestinal tract (GIT) colonization by *S. enterica*. Its excretion in feces may

result in the contamination of the environment and the infection of nearby birds (Cosby *et al.*, 2015). Moreover, poultry meat contaminated with digesta during slaughter is a major risk to public health (Alali *et al.*, 2018).

Campylobacter spp. are ubiquitous bacteria that can be found in various environments, including soil and water, and as commensals of the GIT of poultry. Despite this, they can cause disease in animals and humans and constitute an important cause of foodborne diseases worldwide (Silva *et al.*, 2011). This bacterial genus can be responsible for acute bacterial diarrhea, which is mainly caused by *C. jejuni* and *C. coli*. Although other sources can be responsible for human infection, poultry products are considered the predominant source of human campylobacteriosis (Rickie *et al.*, 2021). *Campylobacter spp.* can be introduced in the production farms by wild animals, pests or humans. When infecting poultry, it colonizes the GIT of animals, invades the intestinal epithelium and multiplies rapidly in the intestinal mucus, avoiding clearance and persisting in the animal's GIT (Van Deun *et al.*, 2008). In this way, avian hosts constitute a natural reservoir for *Campylobacter spp.*, namely *C. jejuni* and *C. coli* (Sahin *et al.*, 2015). According to the European Food Safety Authority (EFSA) and European Centre for Disease Prevention and Control (ECDC), the highest prevalence of *Campylobacter* is observed in fresh meat from broilers (37.5%). Although carriers of *Campylobacter spp.* chickens generally do not exhibit clinical signs. Antibiotics have a limited role in the elimination of *Campylobacter spp.* by these animals due to its high occurrence and commensal character in avian species, and can promote the emergence of resistant strains; therefore, biosecurity practices are the most important method for reducing *Campylobacter* infection at the production level (Hermans *et al.*, 2012).

Human infections are usually associated with the handling, preparation and consumption of contaminated poultry products, and occupational transmission has also been observed. In humans, these pathogens cause gastroenteritis associated with diarrhea, abdominal pain, fever, nausea and vomiting, which usually occur between two and five days after infection. Symptoms are often mild and self-limiting. Antibiotic treatment is not usually required, but severe cases may be treated with macrolides, such as clarithromycin, azithromycin and erythromycin. Ciprofloxacin is not currently used, as resistance to quinolones is now considered to be too high for these antibiotics to be used as an empirical treatment (Sahin *et al.*, 2015). Studies on the antimicrobial drug resistance profile of *Campylobacter spp.* Isolated from broilers, laying hens, chicken carcasses and Chicken meat revealed high frequencies of resistance to nalidixic acid, ampicillin, cephalixin, ciprofloxacin, erythromycin, Gentamicin and tetracycline (de Saraiva *et al.*, 2022).

E.coli is a gram-negative bacillus belonging to the Enterobacteriaceae family (Daneshmand *et al.*, 2019). It is an important bacterial species in the Human–animal–environment triad, since it is a commensal inhabitant of the digestive tract of animals, including birds, being widely spread via fecal material (Islam *et al.*, 2021). This species is often studied as a marker of antimicrobial drug resistance, mainly Due to its widespread distribution and capacity to harbor several genes in mobile genetic elements, serving as a source of Antimicrobial drug resistance determinants to other bacteria (Bass *et al.*, 1999). Most *E. coli* are nonpathogenic; however, certain pathogenic serotypes may induce disease. There are several *E. coli* Pathotypes, which can be divided into extra intestinal *E. coli* (ExPEC) and diarrhoeagenic *E. coli* (DEC). Avian pathogenic *E. Coli* (APEC), an ExPEC, may induce colibacillosis in domestic birds, a disease characterized as a local or systemic syndrome That can be transmitted by oral or vertical routes or through inhalation. *E. coli*-associated infections are widely distributed among poultry of all ages. Birds can be asymptomatic until sudden death or present various forms of disease, such as Septicemia, coligranuloma (Hjarre's disease), air sac disease (chronic respiratory disease), swollen-headsyndrome, venerea Colibacillosiscellulitis, peritonitis, salpingitisorchitis, osteomyelitis/synovitis, panophthalmitis, omphalitis/yolk sac infection and enteritis (Nolan *et al.*, 2013). Colibacillosis constitutes the most frequent infectious bacterial disease found in poultry, being responsible for significant economic losses due to the loss of productivity, increased mortality and condemnations of carcasses (Żbikowska *et al.*, 2020).

Enterococcus species are ubiquitous and are commensals of the gastrointestinal microbiota of both humans and animals. Some enterococcal strains have been used as probiotics (Kabir *et al.*, 2009), while others are known to be pathogenic, including in birds (Souillard *et al.*, 2022). The transmission of enterococci can occur via vertical and horizontal routes. *E. cecorum* and *E. faecalis* are the most Important species associated with avian disease. Pathogenic strains of *E. cecorum* have been associated with free thoracic Vertebra (FTV) osteomyelitis in broilers (Devriese *et al.*, 2002), resulting in the paralysis of the posterior limbs, and with septicemia related to Pericarditis or hepatitis, which can lead to death. In turn, *E. faecalis* can cause omphalitis

and yolk sacculitis, which can lead To sepsis and the death of chicks in the first week of life. Surviving animals may develop chronic diseases, such as valve endocarditis, which can also lead to death (de Saraiva *et al.*, 2022).

Enterococcus spp. can easily acquire resistance determinants and, therefore, play a central role in AMR dissemination. Vancomycin-resistant *Enterococcus* (VRE) has been associated with economic losses in animal production and healthcare And associated with infections in humans (Khan *et al.*, 2015). Humans are exposed to enterococci from a variety of sources, including other Humans, the environment and foods contaminated with animal's intestinal microbiota. Certain species, such as *E. faecalis* and *E. faecium*, are a prominent cause of opportunistic infections in hospitalized humans, causing mild to fatal diseases, such as Endocarditis, urinary tract infections or septicemia (Dea *et al.*, 2019). Studies previously performed have identified high levels of Resistance against aminoglycosides (streptomycin), tetracyclines (doxycycline and tetracycline) and quinolones (ciprofloxacin and enrofloxacin) in enterococci isolated from poultry (de Saraiva *et al.*, 2022). Vancomycin resistance, which is reported as infrequent, can be higher in isolates from chickens affected with FTV.

In view of these havocs emanating from these bacterial pathogens, this study was designed to isolate and characterize the major pathogens associated with poultry production in Ifite-ogwari of Ayamelum local government area of Anambra State, Nigeria.

II. MATERIALS AND METHOD

2.1 Study Area:

The research was carried out in ifite-ogwari, which is a town in Ayamelum LGA of Anambra state. The town has five villages namely Amah, Amadie, Aniocha, Isudala and Umuawah. Ifite-ogwari coordinates are latitude: 6.60 412, longitude: 6.950 69.

2.2 Sampling Techniques:

Simple random sampling techniques were used to select three villages from the total villages in ifite-ogwari. Then three biggest farms in each of three villages were selected for this study. The villages selected were Amah, Umuawah and Amadie.

Then, a total of 30 freshly defecated fecal samples were collected from the selected broiler farms in Amah, Umuawah and Amadie. The samples were collected in sterile containers. After collection, the samples were labeled and placed in a sterile plastic containers and was taken to Prof. J. C. Okonkwo and Prof. I. F. Okonkwo Research Laboratory, Nnamdi Azikiwe University, Awka, Anambra State for Analysis.

2.3 Analysis of the Samples:

Each sample was prepared by making dilution in distilled water. They were inoculated by streaking using a wire loop into both MacConkey and Salmonella-shigella ager respectively, and incubated at 37°C for 24 hours in an incubator. The colonies obtained were sub-cultured to obtain pure colonies.

The pure colonies were subjected to various biochemical tests for proper identification using the methods as described by Chessburgh (2010). Standard biochemical techniques were used to carried out Gram staining, Catalase test, Coagulase test, Oxidase test, Citrate utilization, Indole test, Urease test, Methyl red test, Vloges prostain test, Hydrogen sephide test and Motility test.

2.4 Sensitivity test:

The various pure isolates were subjected to sensitivity test with commercial antibiotic discs using disc diffusion method. They were incubated at 37°C for 24 hrs. After which the zone of inhibition were measured using a metre rule.

III. RESULT

3.1 Bacteria Isolates:

Tables 1 to 3 present the colony, morphology, gram reaction, and biochemical test results of the isolates from Amah, Umuawa and Amadia, respectively.

3.1.1 Amah Village:

A total of six (6) dormant isolates were obtained from 30 faecal samples collected from Amah town, Table 1.

TABLE 1
BIOCHEMICAL CHARACTERISTICS OF CHICKEN DROPPINGS IN AMAH

Colony morphology	Organisms	Gram rxn	Catalase	Coagulase	Oxidase	Indole	Methyl red	Citrate	Vp	Urease	H ₂ S	Motility test
Colourless small colony	<i>Enterococcus spp</i>	+	-	-		-	-	-	+	-	-	-
Pink smooth colony	<i>E. coli</i>	-	+	-		+	+	-	-	-	-	+
Dark colonies	<i>Salmonella</i>	-	+	-	+	-	+	+	-	-	+	+
Cocci in clusters	<i>Staphylococci</i>	+	+	+	-	-	+	-	+	+	+	-
Small cocci in chains	<i>Streptococci</i>	+	-	-	-	-	-	-	-	-	-	-

Biochemical tests revealed the organisms to be *Enterococcus spp*, *E. coli spp*, *Salmonella spp*, *Streptococcal spp*, *Staphylococcal spp* and *Shigella spp*. These isolates are subjected to antibiotic sensitivity tests with commercial antibiotic discs using disc diffusion methods and the results showed Enterobacter app was sensitive to only referyricin with zone diameter of 20mm Also Streptococcal sp was sensitive to Ciproflaxin with 20mm zone diameter, lerofloxacin 20mm and Erythnycin 10mm. Salmonella spp was sensitive to Ciproflaxin 12mm, E.coli Spp was resistant to all the antibiotics. Shigella spp was sensitive to centanycin 10mm and ciproflaxin 7mm. For Staphylococcal spp heroflaxinzone gene zone diameter of 20mm, Rofanpricin 20mm, Ciproflaxin 12mm, Amoxicillin, 12mm and Ampiclox 5mm.

3.1.2 Umuawa Village:

A total of five (5) dormant isolates were obtained from 30 faecal samples collected from Umuawa village in Ifite-ogwari, and biochemical tests revealed the organisms to be *Salmonella Spps*, *Shigella spps*, *E.coli spps*, *streptococcal apps* and *Enterococcus spps* (Table 2).

TABLE 2
BIOCHEMICAL CHARACTERISTICS OF BACTERIA ISOLATES FROM DROPPING IN UMUAWA

Colony morphology	Organism	Gram rxn	Catalase	Coagulase	Oxidase	Indole	Methyl red	Citrate	Vp	Urease	H ₂ S	Motility
Dark brown	<i>Salmonella</i>	-	+	-	+	-	+	-	-	-	+	+
Small pink colonies	<i>Shigella spp</i>	-	+	-	-	+	+	-	-	-	-	-
Round white coloring in chains	<i>Streptococci</i>	+	-	-	-	-	-	-	-	-	-	-
Pink smooth colonies	<i>E.coli</i>	-	+	-	-	+	+	-	-	-	-	+
Colorless small colony	<i>Enterococi</i>	+	-	-	-	-	-	-	+	-	-	-

These isolates were subjected to sensitivity tests with commercially prepared antibiotics discs using disc diffusion method. The results showed that all the isolates were resistant to all the antibiotics except *Streptocci spp* which was sensitive to Gataryin (10mm diameter) and herofloxacin (8mm diameter).

3.1.3 Amadia village:

As presented in Table 3, four (4) dormant isolates were recovered from 30 faecal samples obtained from Amadia village in Ifite-ogwari, and biochemical tests evinced the organisms to be *Salmonella spp*, *E. coli spp*, *Streptocci spp* and *Bacillus spp*.

TABLE 3
BIOCHEMICAL CHARACTERISTICS OF BACTERIA ISOLATES FROM DROPPING IN AMADIA

Colony morphology	Organism	Gram rxn	Catalase	Coagulase	Oxidase test	Indole test	Methyl red	Citrate	Vp test	Urease	H2S test	Motility test
Dark colour	<i>Salmonella</i>	-	+	-	+	-	+	-	-	-	+	+
Pink smooth colonies	<i>E.coli</i>	-	+	-	-	+	+	-	-	-	-	+
White small colonies in chain	<i>Streptococci</i>	+	-	-	-	-	-	-	-	-	-	-
Long rod	<i>Bacillus</i>	-	+	-	-	-	-	+	+	-	+	+

When these isolates were subjected to sensitivity tests with commercially prepared antibiotic discs using disc diffusion methods, *Salmonella* was resistant to all the antibiotics, *E. coli* was resistant to all the antibiotics, *Streptococcal spp* was sensitive to Streptorycin (15mm diameter) and levofloxacin (10mm) and *Bacillus spp* was sensitive to streptomycin (20mm), Ciproflaxin (10mm) and Gentamicin (15mm).

3.2 Summary of Isolates in Ifite-Ogwari:

The summary of the bacterial isolates identified in Amah (T1), Umuawa (T2) and Amadia (T3) are presented in Table 4.

TABLE 4
THE BACTERIAL ISOLATES IDENTIFIED IN AMAH (T1), UMUAWA (T2) AND AMADIA (T3)

Isolates	T1(AMAH)	T2(UMUAWA)	T3(AMADIA)
<i>Salmonella spp</i>	+	+	+
<i>Shigella spp</i>	+	+	-
<i>E coli spp</i>	+	+	+
<i>Streptococci spp</i>	+	+	+
<i>Enterococci spp</i>	+	+	-
<i>Staphylococci spp</i>	+	-	-
<i>Bacillus spp</i>	-	-	+
Key = + indicates presence of a given isolate while – indicates its absence.			

In Amah, virtually all the bacteria isolate studied were present while, *shigella spp* and *Enterococcus spp* was absent in Amadia. *Staphylococcal spp* was absent in Umuawa and Amadia.

IV. DISCUSSION

Generally, four to seven different bacterial isolates were obtained from the study area, and these include *Salmonella spp*, *Shigella spp*, *E-coli spp.*, *Enterococcus spp.*, *Streptococcal spp.*, *Staphylococcal spp.* and *Bacillus spp*. Most of these species have been reported by different researchers in different areas (Adegunloye, 2006; Omojowo and Omojasola, 2013)

Salmonella spp is prevalent in all the three villages studied, and agrees with the report of Omojowo and Omojasola (2013) and Adegunloye(2006) who maintained that *Salmonella spp* is rampant in poultry industries. *Salmonella spp.* causes significant challenge in poultry farming due to their implications on public health, economic stability, and animal welfare. These bacteria are zoonotic pathogen, implying that they can be transmitted from animals to humans. Poultry products, particularly undercooked meat and eggs are common vectors for *Salmonella* transmission, leading to infections that range from mild gastrointestinal illness to severe systemic conditions in humans. Vulnerable populations, such as immune compromised individuals, the elderly, and young children, are at an increased risk of severe disease, making *Salmonella* a critical public health concern (Lund *et al.*, 2011). Economically, *Salmonella* infections in poultry flocks lead to increased mortality rates, reduced growth performance, and lowered egg production. Farmers face additional expenses for treatment, control, and management practices required to mitigate infections. In cases of outbreaks, product recalls and export restrictions can also contribute to significant financial losses. Furthermore, the welfare of infected poultry is compromised, as infections can cause enteritis and septicemia, particularly in young birds, leading to adverse conditions in poultry operations (Marshall & Levy,

2011). *Salmonella* infections can cause ailment in poultry, manifesting as enteritis and septicemia in young birds, leading to poor welfare conditions. High infection rates in flocks also necessitate the use of antibiotics, which may lead to adverse health effects due to drug side effects. *Salmonella spp.* in poultry has demonstrated resistance to various antimicrobials. Several factors contribute to *Salmonella* resistance to antibiotics. First, the overuse and misuse of antibiotics in agriculture and animal husbandry have accelerated resistance in bacteria. In poultry farming, for example, antibiotics are often used as growth promoters and to prevent infections, even in healthy animals, which can promote resistant *Salmonella* strains (M samtiya *et al.*, 2022). Additionally, improper dosing or incomplete antibiotic courses can leave some bacteria alive, allowing them to adapt and develop resistance. Another factor is the genetic adaptability of *Salmonella*, which can acquire resistance genes through horizontal gene transfer from other resistant bacteria in the environment. This process allows *Salmonella* to rapidly acquire and share resistance traits, particularly in intensive farming environments where bacteria are in close contact (Shu-Kee *et al.*, 2015). Furthermore, the spread of multidrug-resistant strains is exacerbated by global trade and movement, making containment and treatment of resistant strains even more challenging. In recent years, *Salmonella* strains are resistant to multiple antibiotics, including fluoroquinolones, cephalosporins, and aminoglycosides, have been reported. Multidrug-resistant (MDR) *Salmonella* is especially concerning because it limits treatment options and can prolong illness in affected individuals. For example, strains like *Salmonella* Typhi and *Salmonella* Typhimurium have been observed with significant resistance profiles, making them harder to control. Its infections can lead to prolonged and more severe symptoms, as the typical antibiotics are ineffective. These infections may require extended hospitalization and carry a higher risk of complications, such as bloodstream infections. In severe cases, if alternative treatments fail or the infection is left untreated, antibiotic-resistant *Salmonella* can result in death. Additionally, drug-resistant genes can spread to other bacteria, compounding the problem of antimicrobial resistance. The growing resistance of *Salmonella* to common treatments emphasizes the need for better antibiotic stewardship, improved surveillance, and research into alternative therapies.

4.1 *Shigella spp.*:

Shigella spp. was present in Amah and Umuawa village of Ifite-ogwari Ayamelum LGA Anambra State Nigeria. *Shigella spp.*, in Amah was sensitive to Gentamicin -10mm and Ciproflaxin 7mm and resistant to all antibiotics in Umuawa and Amadia. *Shigella* is generally known as a human pathogen, but its presence in poultry farming can have significant implications, particularly for zoonotic transmission and food safety concerns. *Shigella* species are less commonly found in poultry compared to other pathogens like *Salmonella* or *Escherichiacoli*, but when they do infect poultry, it can lead to contamination of poultry products, posing a risk to humans who consume or handle these products. *Shigella*-contaminated poultry products can cause shigellosis, a bacterial infection in humans, resulting in symptoms like diarrhea, fever, and abdominal cramps. This can pose a major food safety issue. Poultry can serve as asymptomatic carriers of *Shigella*, transmitting the bacteria to humans indirectly through contaminated food or directly through handling. The contamination risks associated with *Shigella* can lead to product recalls, affecting the poultry industry economically. *Shigella* species have shown high resistance to commonly used antibiotics like ampicillin, tetracycline, and trimethoprim-sulfamethoxazole. This resistance in poultry isolates could limit treatment options if an outbreak occurs. Drug resistance in *Shigella* is often attributed to its ability to acquire resistance genes from other bacteria through horizontal gene transfer mechanisms, including conjugation and transposable elements, within the gut microbiome of animals. Possible Reasons for Drug Resistance in *Shigella* are Antibiotic Misuse and Horizontal Gene Transfer.

4.2 *E-coli spp.*:

Ecoli spp. was present in Amah, Umuawa and Amadia village of Ifite-ogwari Ayamelum LGA Anambra State Nigeria. In all the villages *E.coli spp.* Was resistant to all the antibiotics, which agrees with the report of This observation was consistent with previous reports (Atnafu *et al.*, 2021) that multidrug-resistant bacteria were isolated in poultry litters. *Escherichia coli* (*E. coli*), particularly pathogenic strains like avian pathogenic *E. coli* (APEC), can lead to a range of diseases in poultry, including colibacillosis. This condition can cause septicemia, peritonitis, air sacculitis, and other infections that significantly impact poultry health and production. It can lead to increased mortality rates, poor weight gain, reduced egg production, and economic losses due to the costs of treatment and prevention. Additionally, *E. coli* infections can compromise the immune system, making birds more susceptible to other infections, which can further reduce farm productivity.

Over the past decades, there has been a notable increase in antimicrobial resistance (AMR) among *E. coli* strains isolated from poultry. Studies have shown that pathogenic *E. coli* in poultry often exhibits resistance to common antibiotics such as tetracycline, sulfonamides, and quinolones, which are frequently used in poultry farming (Nhung *et al.*, 2017). Overuse and Misuse of Antibiotics In poultry farming, Inadequate sanitation and overcrowding in poultry farms can facilitate the spread of resistant *E. coli* strains, making it difficult to control infections without the use of antibiotics and also Administration of

antibiotics via feed or water can expose large populations of bacteria to sub-lethal antibiotic concentrations, encouraging the selection of resistant strains (Ajit K *et al.*, 2006) are possible reasons of drug resistant to *E. Coli spp.*

4.3 *Enterococcus spp.*:

Enterococcus spp. was present in Amah and Umuawa village of Ifite-ogwari Ayamelum LGA Anambra State Nigeria. From the sensitivity test, In Amah village, *Enterococcus spp.* Was sensitive to only Referyricin with Zone diameter of 20mm and was resistant to all antibiotics in Umuawa. *Enterococcus* species, particularly *Enterococcus faecalis* and *Enterococcus faecium*, are naturally found in the intestinal tracts of animals and can serve as indicators of fecal contamination. In poultry farming, they are significant because they can act as both commensals and opportunistic pathogens, often contributing to infections in poultry as well as posing zoonotic risks to humans. According to Hammerum (2012), *Enterococcus spp.* are opportunistic pathogens that can lead to infections like septicemia and endocarditis in poultry under stress or weakened immune conditions. They can exacerbate other infections and reduce the overall health and productivity of flocks. (Devriese *et al.*, 1992) also highlight that some strains of *Enterococcus* are highly virulent and can cause outbreaks in poultry farms, especially when hygiene standards are low or birds are overcrowded. *Enterococcus spp.* has shown high levels of resistance to various antibiotics, including vancomycin, a last-resort antibiotic for treating certain infections in humans. Resistance in poultry-associated *Enterococcus* strains is a major public health concern as it can spread to humans through direct contact, the food chain, or environmental exposure (Valeria *et al.*, 2016). (Jessica *et al.*, 2023) found multidrug-resistant *Enterococcus* strains in poultry farms, particularly resistant to tetracyclines, macrolides, and aminoglycosides. They attributed this to the use of these antibiotics in poultry feed, which selects for resistant strains over time. (Joshua *et al.*, 2004) Also found out that *Enterococcus* strains isolated from poultry farms are frequently identical to those causing infections in humans. This highlights the zoonotic potential of these bacteria and the risks posed by handling poultry or consuming undercooked poultry products.

4.4 *Streptococcal spp.*:

Streptococcal spp. Was present in Amah, Umuawa and Amadia of Ifite-ogwari Ayamelum LGA Anambra State Nigeria. The antimicrobial sensitivity profile observed in the *Streptococcus spp.* isolated from Amah, Umuawa, and Amadia reveals varying levels of susceptibility to antibiotics, specifically Ciprofloxacin, Levofloxacin, Erythromycin, and Streptomycin. This variability in sensitivity patterns can be linked to geographical differences, local antimicrobial usage patterns, and potential genetic variation within the bacterial populations.

Streptococcus spp. from Amah showed sensitivity to Ciprofloxacin and Levofloxacin, with zone diameters of 20 mm, indicating a strong inhibition. Additionally, a moderate sensitivity to Erythromycin was observed with a 10 mm zone. The high sensitivity to Ciprofloxacin and Levofloxacin suggests that these fluoroquinolones are effective in this area, possibly due to limited previous exposure or use of these antibiotics in Amah.

In Umuawa, *Streptococcus spp.* sensitivity to Levofloxacin was significantly lower, with a zone diameter of 8 mm, indicating reduced efficacy. The lower sensitivity might be attributed to increased exposure to Levofloxacin in Umuawa, potentially leading to increased resistance. This trend aligns with studies indicating regional differences in resistance, where frequent antibiotic use tends to decrease effectiveness.

Higher sensitivity of *Streptococcus spp.* was observed for Streptomycin (15 mm) and Levofloxacin (10 mm), indicating a moderate level of inhibition in Amadia. The sensitivity to Streptomycin in Amadia may suggest limited prior exposure to this antibiotic, aligning with reports from other rural areas where less commonly used antibiotics show retained efficacy. The moderate sensitivity to Levofloxacin is consistent with findings in similar regions where *Streptococcus spp.* display variable susceptibility based on antibiotic exposure history.

Studies conducted in various regions have reported similar observations. For instance, Canton *et al.* (2003) found that *Streptococcus spp.* isolates in urban areas showed higher resistance to fluoroquinolones, particularly Levofloxacin, than those in rural settings, suggesting that usage patterns influence susceptibility (Carbon *et al.*, 2003). Hayes *et al.* (2019) reported varying Erythromycin sensitivity in *Streptococcus spp.*, with resistance patterns aligning closely to local prescribing practices and historical exposure, similar to the moderate sensitivity noted in Amah. These findings indicate that antibiotic susceptibility among *Streptococcus spp.* is not only species-specific but also influenced by regional antibiotic use. The data emphasize the need for region-specific guidelines on antibiotic usage to prevent resistance development. Certain strains of *Streptococcus* found in poultry have developed resistance to various antibiotics. Common antibiotics to which *Streptococcus* has shown resistance include: Tetracyclines, Penicillin, Lincomycin, Clindamycin etc.

4.5 *Staphylococcal spp.*:

Staphylococcal spp. Was present in Amah village of Ifite-ogwari Ayamelum LGA Anambra State Nigeria. The antimicrobial sensitivity profile observed in the *Staphylococcal spp.* Isolated in Amah village displayed varied sensitivity to the antibiotics tested, with levofloxacin showing the highest zone of inhibition at 20 mm, followed by ciprofloxacin and amoxicillin, each at 12 mm, and Ampiclox at only 5 mm. Levofloxacin's 20 mm zone of inhibition against *Staphylococcal spp.* reflects strong effectiveness. Studies have shown that fluoroquinolones like levofloxacin remain highly effective against various strains of *Staphylococcus spp.*, particularly methicillin-sensitive strains. For instance, Andrejs *et al.* (2024) reported that levofloxacin maintained a high level of efficacy against *Staphylococcal* isolates in clinical samples, with zones of inhibition ranging from 18–25 mm, highlighting its potency as an effective treatment option. Ciprofloxacin, showing a 12 mm inhibition zone in my findings, has also demonstrated moderate effectiveness against *Staphylococcal spp.* in similar studies. In research conducted by Aref *et al.* (2022), ciprofloxacin showed zones of inhibition between 10–14 mm against *Staphylococcal spp.* isolated from hospital settings, suggesting that while effective, ciprofloxacin's efficacy may be limited due to rising resistance trends in some *Staphylococcal* strains. With a 12 mm inhibition zone, amoxicillin showed intermediate sensitivity to *Staphylococcal spp.* in your results. Amoxicillin has generally shown lower efficacy against *Staphylococcus spp.*, especially in cases where beta-lactamase production or methicillin resistance is present. Research by Supatcharee (2016) supports these findings, where *Staphylococcal* isolates had zones of inhibition for amoxicillin averaging around 10–13 mm, which is consistent with my results. Ampiclox had a minimal inhibition zone of 5 mm, indicating poor effectiveness against *Staphylococcal spp.* This outcome is also supported by recent studies, such as one conducted by Sabina *et al.* (2023) which showed similarly low sensitivity rates for Ampiclox against *Staphylococcus spp.*, with zones of inhibition below 7 mm. This low effectiveness is often due to the resistance mechanisms that *Staphylococcus spp.* develop against beta-lactam/beta-lactamase inhibitor combinations. certain species of *Staphylococcus*, particularly *Staphylococcus aureus*, are known to be resistant to multiple drugs. The most notable drug-resistant strain is Methicillin-resistant *Staphylococcus aureus* (MRSA), which shows resistance to methicillin and other commonly used antibiotics, including penicillin and amoxicillin.

4.6 *Bacillus spp.*:

Bacillus spp. was found in Amadia village of Ifite-ogwari Ayamelum LGA Anambra State Nigeria. This current study shows that *Bacillus spp.* isolated from Amadia showed sensitivity to streptomycin (20 mm), ciprofloxacin (10 mm), and gentamicin (15 mm). (Christian *et al.*, 20) found that *Bacillus* isolates from poultry in Pakistan displayed high resistance to tetracycline but showed sensitivity to aminoglycosides such as streptomycin and gentamicin, which agrees with this current study. However, ciprofloxacin resistance was more common in their study, suggesting that geographical and environmental factors can significantly influence antibiotic susceptibility patterns. (Anthony *et al.*, 2016) discussed the emergence of antibiotic resistance among *Bacillus spp.* in various clinical settings. Their study noted increased resistance to fluoroquinolones, which contrasts with your findings of sensitivity to ciprofloxacin. This discrepancy highlights the need for localized studies to assess resistance patterns accurately. *Bacillus cereus*, a common pathogenic species, is often resistant to multiple antibiotics, including beta-lactams, aminoglycosides, and some macrolides. Resistance can be due to the production of beta-lactamases or other mechanisms. Other *Bacillus* species, like *Bacillus subtilis*, are generally less pathogenic and may show some sensitivity to antibiotics, but resistance can still occur, particularly in strains that have been exposed to antibiotics over time. *Bacillus cereus*, can cause foodborne illnesses, which can lead to gastrointestinal issues, such as diarrhea and vomiting. In more severe cases, it can cause infections in individuals with weakened immune systems, leading to more serious conditions such as bacteremia or meningitis.

V. CONCLUSION

The common pathogens threatening poultry industry in Ifite-Ogwari include *Salmonella spp.*, *Shigella spp.*, *E. coli spp.*, *Enterococcus spp.*, *Streptococcal spp.*, *Staphylococcal spp.*, and *Bacillus spp.* The economic implications to farmers include increased mortality rates, reduced production, and the costs associated with managing these infections.

Furthermore, most of these pathogens are zoonotic and resistance to commonly used antibiotics. The presence of multidrug-resistant strains poses a serious threat not only to poultry health but also to the safety of poultry products for human consumption. Therefore, effective management strategies, including improved antibiotic stewardship and enhanced biosecurity measures, are essential to mitigate the impact of these pathogens and promote sustainable poultry farming practices in the region.

RECOMMENDATIONS

- 1) **Implement Antibiotic Stewardship Programs:** Farmers should adopt responsible antibiotic use practices to minimize the development of resistance. This includes using antibiotics only when necessary and adhering to prescribed dosages.
- 2) **Enhance Biosecurity Measures:** Poultry farms should implement stringent biosecurity protocols to prevent the introduction and spread of pathogens. This includes measures such as proper sanitation, controlling access to farms, and regular health monitoring of flocks.
- 3) **Regular Surveillance and Monitoring:** Establish ongoing surveillance programs to monitor the presence of bacterial pathogens and their resistance patterns in poultry populations. This data will be crucial for informing treatment decisions and management practices.
- 4) **Education and Training:** Provide training and resources for poultry farmers on the risks associated with antibiotic resistance and the importance of maintaining good hygiene practices. Workshops and extension services can help disseminate this knowledge.
- 5) **Research into Alternative Treatments:** Invest in research to explore alternative therapies and management strategies for controlling bacterial infections in poultry. This includes evaluating the use of probiotics, vaccines, and other non-antibiotic interventions.
- 6) **Collaboration with Public Health Authorities:** Foster partnerships between poultry farmers, veterinary services, and public health officials to address the broader implications of zoonotic diseases and develop integrated approaches to disease management.
- 7) **Consumer Awareness Programs:** Educate consumers about the importance of proper cooking and handling of poultry products to reduce the risk of foodborne illnesses associated with these pathogens.

By adopting these recommendations, stakeholders in the poultry industry can enhance flock health, safeguard public health, and promote economic viability within the sector. Implementing these strategies will be crucial in combating the growing challenge of antimicrobial resistance and ensuring a sustainable future for poultry farming in Ifite-Ogwari.

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Performance of Phosphorus Levels and Varieties on Growth Yield and Quality of Pea (*Pisum sativum* L.)

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Abstract— A field experiment was conducted during the Rabi season in 2023-2024 at the Research farm, Department of Agronomy, AKS University, Satna (M.P.), to evaluate the effect of phosphorus levels and varieties on growth, yield, and quality of pea (*Pisum sativum* L.). The experiment was laid out in Factorial Randomized Block Design comprising four phosphorus levels viz. 0kg P₂O₅ ha⁻¹, 20kg P₂O₅ ha⁻¹, 40kg P₂O₅ ha⁻¹, 60kg P₂O₅ ha⁻¹ and three pea varieties viz. KN-5, Aman, KS-10, with treatments replicated thrice. Results revealed that different levels of phosphorus significantly affected the growth parameters as well as yield attributes and yield of pea. Application of 60kg P₂O₅ ha⁻¹ (P₃) recorded maximum plant height at 90 DAS (62.10cm), number of pods per plant (14.32), pod length (8.47cm), grain yield (47.65q ha⁻¹), stover yield (85.23q ha⁻¹), test weight (18.32g), and protein content (23.24%). However, phosphorus levels of 40kg P₂O₅ ha⁻¹ (P₂) showed slightly higher harvest index (32.10%). Among the varieties, Aman exhibited the best performance, recording maximum grain yield, protein content, and other yield attributes.

Keywords— Phosphorus levels, pea varieties, growth, yield, quality, *Pisum sativum*.

I. INTRODUCTION

Pea (*Pisum sativum* L.) is one of the most important pulse crops grown during the winter season and is highly valued for its rich nutritional content, including high levels of digestible protein, carbohydrates, vitamins, and minerals. In India, pea is cultivated over an area of 5.97 lakh hectares, contributing to a total production of 8.60 lakh tonnes (Anonymous, 2020) [1]. Madhya Pradesh is a key pea-growing state, with approximately 0.58 lakh hectares under cultivation, producing around 0.48 lakh tonnes (Anonymous, 2020) [2]. Phosphorus is an essential macronutrient, playing a crucial role in plant development, particularly in processes like root formation, energy transformation, and nitrogen fixation. Phosphorus is a component of important molecules like nucleic acids, phospholipids, and ATP. In legumes such as pea, adequate phosphorus availability enhances root nodulation and symbiotic nitrogen fixation, which are vital for improving plant growth and yield (Tsvetkova and Georgiev, 2007) [3]. A deficiency of phosphorus can lead to poor nodulation and lower yields, particularly in low-nutrient soils.

Research has demonstrated that phosphorus application can significantly improve pea growth parameters such as plant height, number of branches, and root development. For instance, Gupta et al. (2000) [4] found that phosphorus application leads to deeper root systems and better nutrient absorption. In pea cultivation, phosphorus enhances pod formation, grain weight, and protein content, making it a key nutrient for maximizing crop productivity (Bhat et al., 2013) [5]. Varietal selection also plays a crucial role in determining crop yield and quality. Different pea varieties exhibit varying responses to phosphorus fertilization, with some showing greater tolerance to low phosphorus conditions or higher yields under optimal phosphorus levels (Singh and Singh, 2017) [6]. Hence, the interaction between phosphorus levels and varieties must be optimized to achieve higher yields and better quality pea production. This study aims to evaluate the performance of different phosphorus levels and varieties of pea on growth, yield, and quality, thereby providing insights into optimal agronomic practices for pea cultivation in phosphorus-deficient regions.

II. MATERIALS AND METHODS

The present investigation was carried out during 2023-2024 in the Rabi season at the Research farm, Department of Agronomy, AKS University, Satna (M.P.). The mean temperature during the experimental period ranged from 5.68°C (min) to 34.47°C (max), with relative humidity ranging from 72% (morning) to 30% (evening). The total rainfall received was 50.90 mm. The soil of the experimental field was clay loam in texture, medium in organic carbon (0.58%), available nitrogen (224.33 kg ha⁻¹), available phosphorus (11.87 kg ha⁻¹), and medium in available potassium (204.12 kg ha⁻¹), with a pH of 7.24 and EC of 0.16 ds/m. Twelve treatment combinations (P₀V₁, P₀V₂, P₀V₃, P₁V₁, P₁V₂, P₁V₃, P₂V₁, P₂V₂, P₂V₃, P₃V₁, P₃V₂, P₃V₃) were laid out in Factorial Randomized Block Design and replicated thrice. The treatments consisted of four phosphorus levels: P₀ = Control (0kg P₂O₅ ha⁻¹), P₁ = 20kg P₂O₅ ha⁻¹, P₂ = 40kg P₂O₅ ha⁻¹, P₃ = 60kg P₂O₅ ha⁻¹, and three varieties of pea (*Pisum sativum* L.): V₁ = KN-5, V₂ = Aman, V₃ = KS-10

The experimental plots were fertilized as per the treatments. Single super phosphate (16% P₂O₅) was used as the phosphorus source, while muriate of potash (60% K₂O) was applied as the potassium source. A full recommended dose of potassium (25 kg K₂O ha⁻¹) and nitrogen (25 kg N ha⁻¹) was uniformly applied to each plot as a basal dose before sowing. Phosphorus was applied as per the treatment at the time of sowing. The seeds were sown on November 4, 2023, at a rate of 80kg ha⁻¹ in rows spaced 45 cm apart, with 10 cm plant spacing within rows. Proper field preparation, including plowing and leveling, was done prior to sowing. Irrigation was provided whenever necessary, with three irrigations applied during the crop growth period. Standard agronomic practices such as weeding, thinning, and pest control were followed throughout the season.

III. RESULTS AND DISCUSSION

3.1 Experimental results on the effect of treatments are explained as under:

The beneficial effect of different levels of phosphorus on mean plant height at 60 DAS, number of leaves per plant at 60 DAS, number of branches per plant, root nodulation, grain yield (q ha⁻¹), stover yield (q ha⁻¹), test weight, harvest index, and protein content were evident during the active growth and maturity period of the pea crop (*Pisum sativum* L.). Incorporation of 60kg P₂O₅ ha⁻¹ (P₃) produced significantly higher mean plant height at 60 DAS (62.10cm), number of leaves per plant at 60 DAS (28.32), and number of branches per plant at 60 DAS (12.45), followed by incorporation of 40kg P₂O₅ ha⁻¹ (P₂), while the control treatment (P₀) recorded the lowest values (Table 1). Data on yield and yield-contributing traits such as the number of pods per plant, pod length (cm), grain yield (q ha⁻¹), stover yield (q ha⁻¹), test weight, and harvest index, as influenced by different phosphorus levels, was found to be significant and is presented in Table 2. Incorporation of 60kg P₂O₅ ha⁻¹ (P₃) produced the maximum number of pods per plant (14.32), pod length (8.47cm), grain yield (47.65 q ha⁻¹), stover yield (85.23 q ha⁻¹), and test weight (18.32g), followed by incorporation of 40kg and 20kg P₂O₅ ha⁻¹, respectively. However, the highest harvest index (32.10%) was observed when 40kg P₂O₅ ha⁻¹ was applied (P₂). Protein content was also influenced by different levels of phosphorus. The highest protein content (23.24%) was observed with the application of 60kg P₂O₅ ha⁻¹ (P₃), followed by 40kg and 20kg P₂O₅ ha⁻¹, respectively. The beneficial effect of different varieties on mean plant height at 60 DAS, number of leaves per plant, number of branches per plant, and root nodulation was also evident during the active growth and maturity period of the pea crop. The variety Aman (V₂) recorded maximum plant height at 60 DAS (63.32cm), followed by KS-10 (V₃) (60.89cm). The highest number of pods per plant (14.32), pod length (8.47cm), and grain yield (47.65 q ha⁻¹) were also recorded in Aman (V₂), which was statistically at par with KS-10 (V₃). The yield and yield-contributing traits like number of pods per plant, pod length, grain yield (q ha⁻¹), stover yield (q ha⁻¹), test weight, and harvest index were significantly influenced by different varieties. The highest grain yield (47.65 q ha⁻¹) was obtained with Aman (V₂), which was statistically at par with KS-10 (V₃). In addition, Aman also gave the highest test weight (18.32g) and harvest index (32.10%). Protein content was also influenced by different varieties. Aman recorded the highest protein content (23.24%) compared to other varieties, which might be due to its better nutrient absorption efficiency. These results are in conformity with those of Singh et al. (2021) and Bhat et al. (2013) [5, 9]. The interaction between phosphorus levels and varieties was significant in terms of plant growth, yield, and protein content. The combination of 60kg P₂O₅ ha⁻¹ and Aman (P₃V₂) resulted in the best overall performance, including the highest grain yield (47.65 q ha⁻¹), stover yield (85.23 q ha⁻¹), and protein content (23.24%) [5, 9].

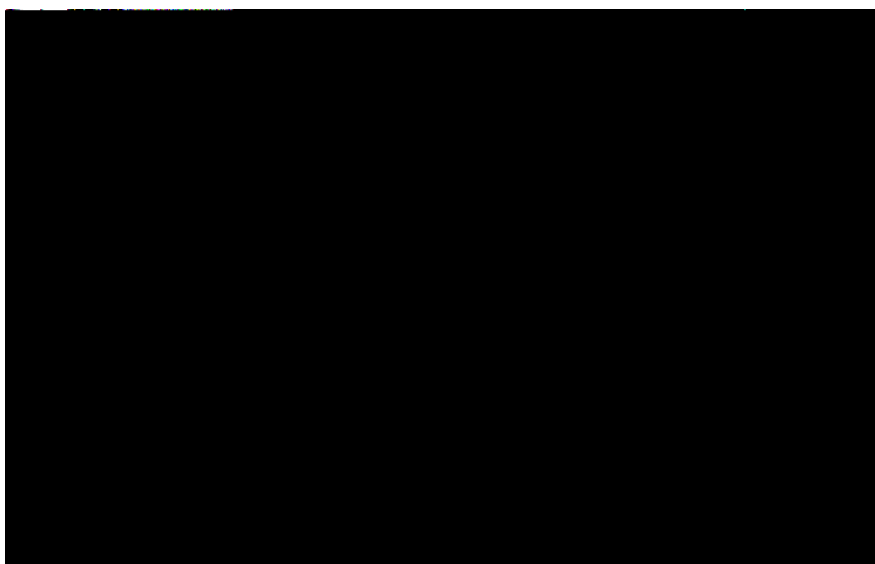


FIGURE 1: Effect of Phosphorus Levels on Plant Height of Pea

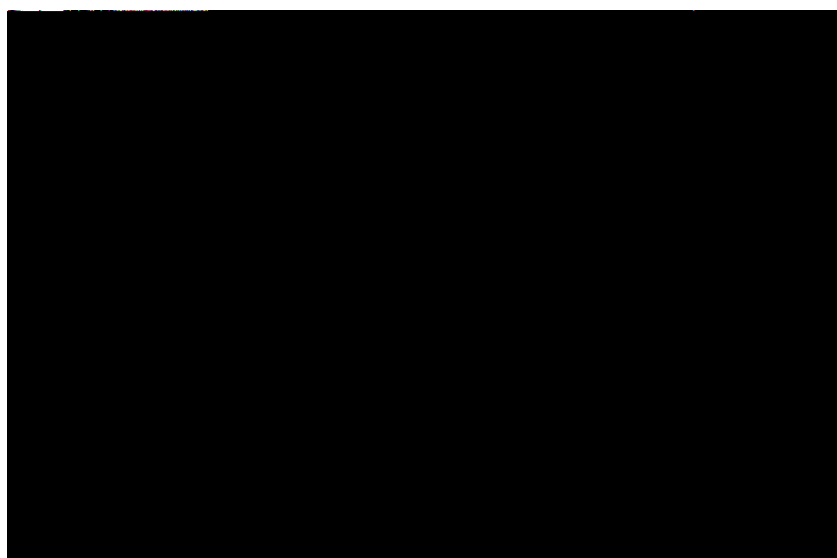


FIGURE 2: Effect of Phosphorus Levels on Number of Pods per Plant of Pea

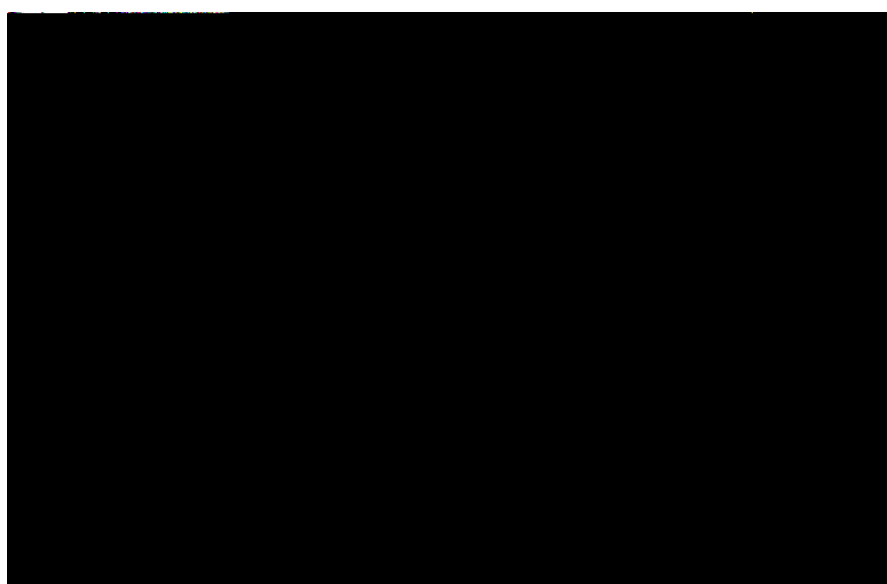


FIGURE 3: Effect of Phosphorus Levels on Pod Length (cm) of Pea

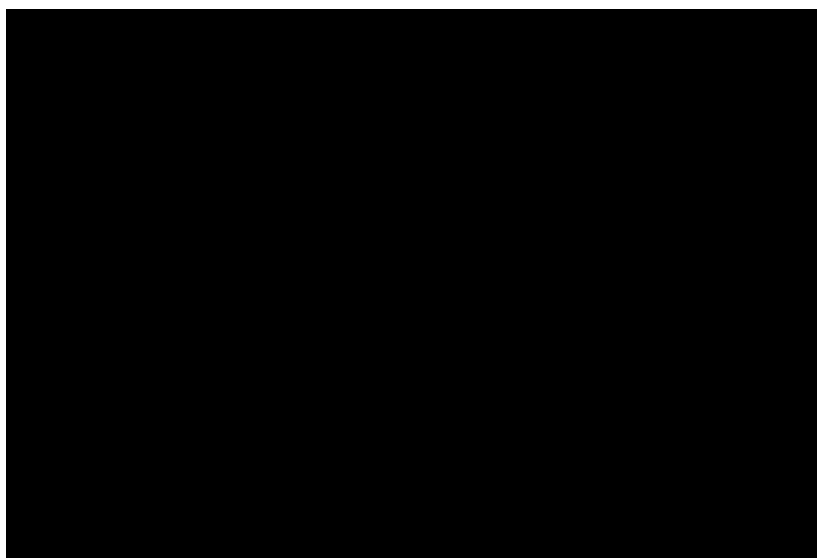


FIGURE 4: Effect of Phosphorus Levels on Grain Yield (q/ha) of Pea

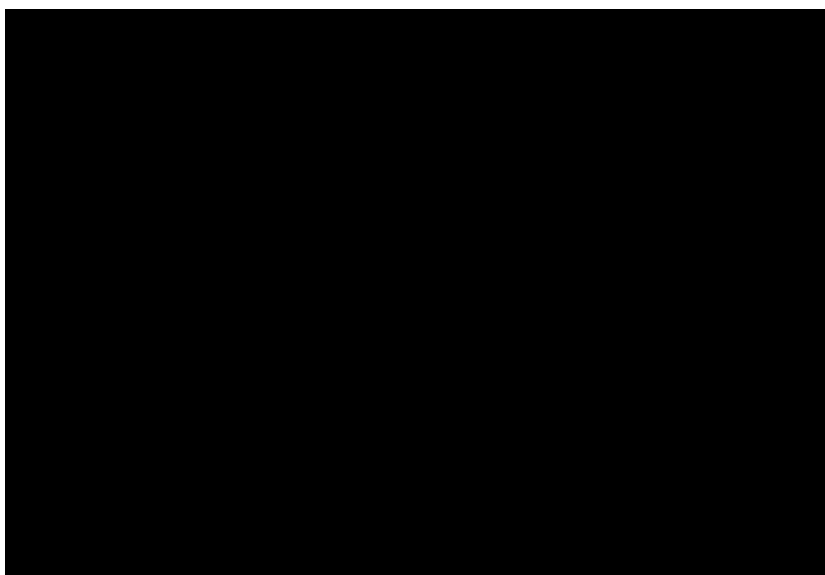


FIGURE 5: Effect of Phosphorus Levels on Stover Yield (q/ha) of Pea

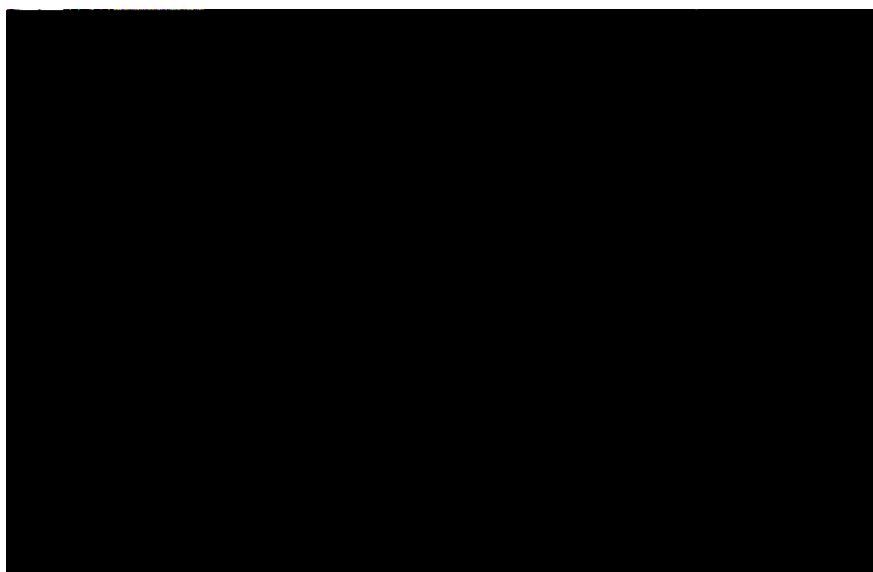


FIGURE 6: Effect of Phosphorus Levels on Test Weight (g) of Pea

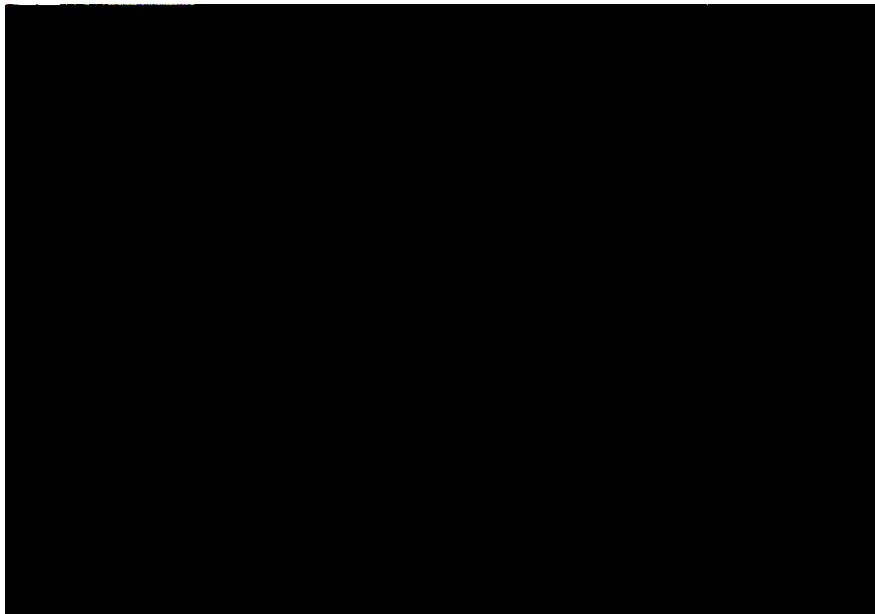


FIGURE 7: Effect of Phosphorus Levels on Harvest Index (%) of Pea

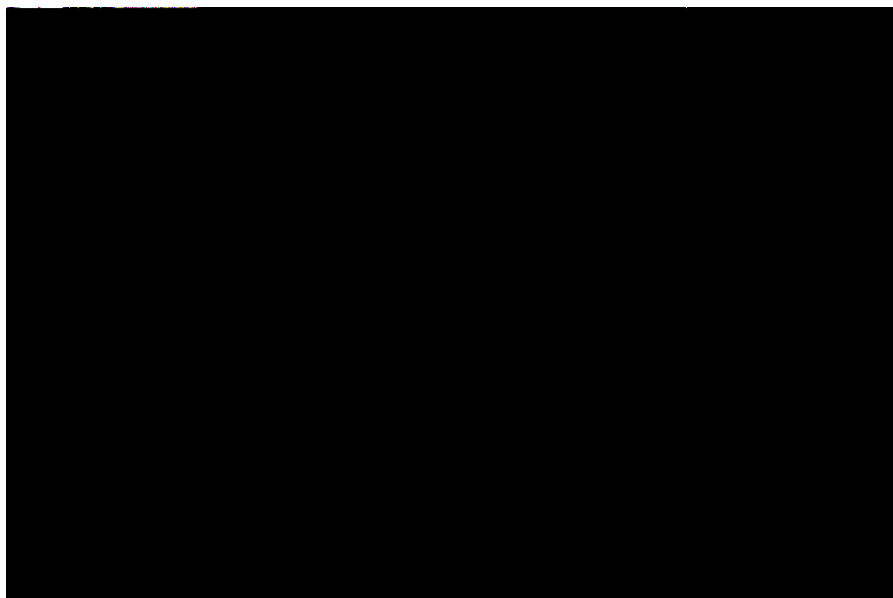


FIGURE 8: Effect of Phosphorus Levels on Protein Content (%) of Pea

IV. SUMMARY AND CONCLUSION

The present study investigated the effect of different phosphorus levels and pea varieties on growth, yield, and quality parameters. The application of phosphorus significantly influenced all growth parameters, including plant height, number of leaves, and branches per plant, with the highest values observed at 60kg P₂O₅ ha⁻¹. Among the varieties, Aman demonstrated superior performance in terms of plant height, number of pods per plant, and overall yield. The maximum grain yield, test weight, and protein content were achieved with the application of 60kg P₂O₅ ha⁻¹, particularly when combined with the Aman variety. This treatment combination produced the highest grain yield per hectare, along with improved stover yield and protein content. In conclusion, the application of 60kg P₂O₅ ha⁻¹ is recommended for optimal pea growth and yield, especially when using the Aman variety. These findings suggest that the interaction between phosphorus levels and variety selection plays a critical role in enhancing pea crop productivity, and phosphorus fertilization is essential for maximizing both yield and quality.

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The Organic Agri Export Conundrum: Growth and Opportunities in India

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Abstract— India holds the sixth position globally in terms of organic agricultural area and ranks first in terms of the total number of producers in 2021 (FIBL & IFOAM Year Book 2023). The country has seen significant growth in organic cultivated area and production from 0.5 to 5.39 million hectare (Mha), and 1.3 million tones (MT) to 2.95 MT respectively, during 2012-13 to 2022-23. Madhya Pradesh, Maharashtra, and Rajasthan have the highest area and production share under organic farming in India. These three states have a combined share of over 60% of the total organic area and 70% of the total organic production in the country. Madhya Pradesh, in particular, has emerged as the leader in organic farming in India with the highest area (0.618 Mha) and production (1.26 MT) in 2021-22.

In the wake of the current scenario of organic export from the country to major export destinations, an effort has been made through this paper to provide an in-depth analysis of the export of organic agricultural produce from India, examining trends, growth, and instability in the area and production. By leveraging secondary data from various governmental sources like the Agricultural and Processed Food Products Export Development Authority (APEDA) and the Ministry of Agriculture and Farmers Welfare (MoA&FW), the paper explores the potential of the organic sector to contribute to India's economic growth and agriculture sustainability, strengthening the nation's position as a key player in the global organic market. Time series data spanning the period 2012-2022 on area, production, and export of organic produce was utilized and the export of organic produce from India and major states was studied using CAGR, coefficients of variation (CV), and the Cuddy-Della Valle Index (CDVI) for instability.

Keywords— Organic, Export, Growth, Agri, Certification and CDVI.

I. INTRODUCTION

In the realm of agriculture, organic practices have emerged as a prominent force, championed for their holistic approach to maintaining sustainable food systems. As defined by the Food and Agriculture Organization/ World Health Organization (FAO/WHO) Codex Alimentarius Commission (1999), "organic agriculture is a holistic production management system which promotes and enhances agro-ecosystem health, including biodiversity, biological cycles, and soil biological activity. It emphasizes the use of management practices in preference to the use of off-farm inputs, considering that regional conditions require locally adapted systems. This is accomplished by using, where possible, agronomic, biological, and mechanical methods, as opposed to using synthetic materials, to fulfill any specific function within the system. This global shift towards organic production has been further fueled by the COVID-19 pandemic, as consumers increasingly prioritize healthy and immunity-boosting foods, propelling the organic market to new heights.

According to the World Organic Agriculture (2023) report, the global organic agricultural land area reached 76.4 Mha in 2021, representing 1.6% of the world's total agricultural land. The major players i.e., Australia (35.69 Mha), Argentina (4.07 Mha), and France (2.78 Mha) possess almost 70% of the global organic area. China, Uruguay, India, Spain, United States, Italy, and Germany also possess remarkable areas of organic farming (Fig.1).

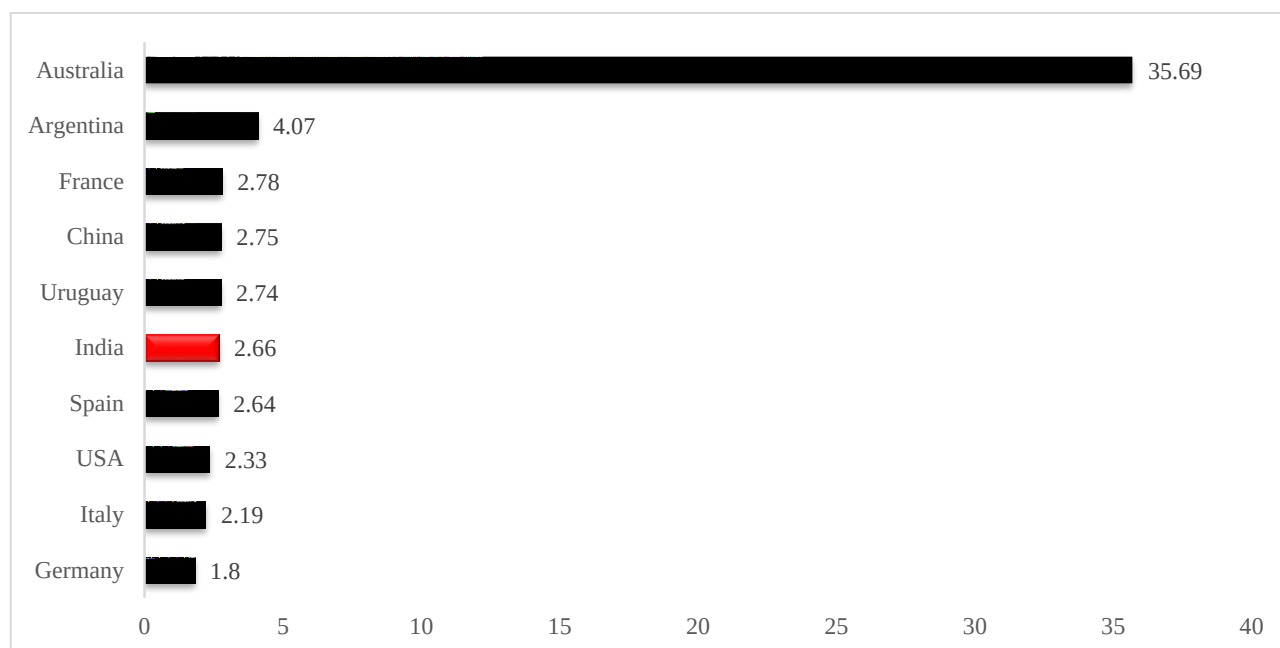


FIGURE 1: Country wise area under Organic Farming (Mha)
(Sources: WOA, 2023)

Globally, there were 3.7 million organic agri producers in 2021, with more than 91% in Asia, Africa, and Europe. India stands top among these countries, followed by Uganda and Ethiopia. With the highest number of certified organic farmers in the world, India has a vast potential to cater to the increasing global demand for organic food basket.

1.1 Unlocking India's Potential as a Major Organic Produce Exporter:

India has carved a significant niche for itself in the global organic agriculture landscape. India holds the sixth position globally in terms of organic agricultural area and ranks first in terms of the total number of producers in 2021 (FIBL & IFOAM Year Book 2023). The country has seen significant growth in organic cultivated area and production from 0.5 to 5.39 Mha, and 1.3 MT to 2.95 MT respectively, from 2012-13 to 2022-23 (Fig. 3). As per the latest statistics of APEDA, India produced around 2.9 MT (2022-23) of certified organic products which include all varieties of food products namely oil seeds, fibre, sugar cane, cereals & millets, cotton, pulses, aromatic & medicinal plants, tea, coffee, fruits, spices, dry fruits, vegetables and processed foods, etc. The production is not limited to the edible sector but also produces organic cotton fiber, functional food products, etc. Madhya Pradesh, Maharashtra, and Rajasthan have the highest area and production share under organic farming in India. These three states have a combined share of over 60% of the total organic area and 70% of the total organic production in the country. Madhya Pradesh, in particular, has emerged as the leader in organic farming in India with the highest organic area (0.618 Mha) and production (1.263 MT) in 2021-22. This accounts for 38.19% of the total organic area and 42.8% of the total organic production in India (Fig. 2&3). The Madhya Pradesh government has taken several initiatives to promote organic farming and provide support to farmers to adopt organic practices. These include the establishment of organic farming clusters, subsidies on organic inputs, training programs for farmers, etc. Maharashtra is the second leading state with a share of 13.89% in the total area under organic, followed by Rajasthan (12.66%), Gujarat (5.05%), Sikkim (4.64%), and Odisha (4.50%) (Fig. 4).

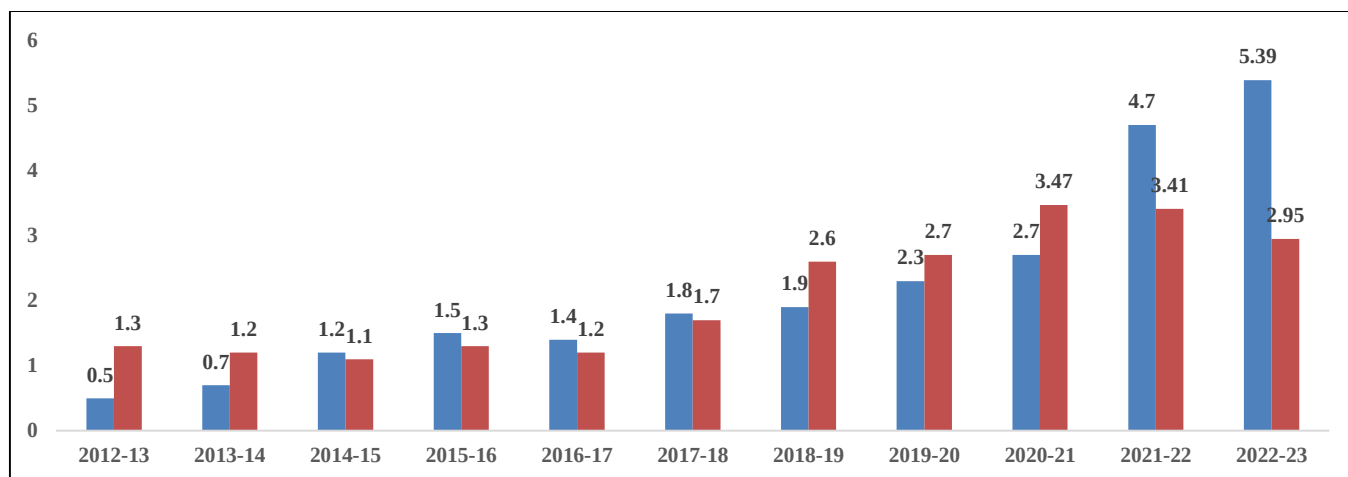


FIGURE 2: India's total certified organic cultivated area (Mha) and production (MT)
(Source: APEDA)

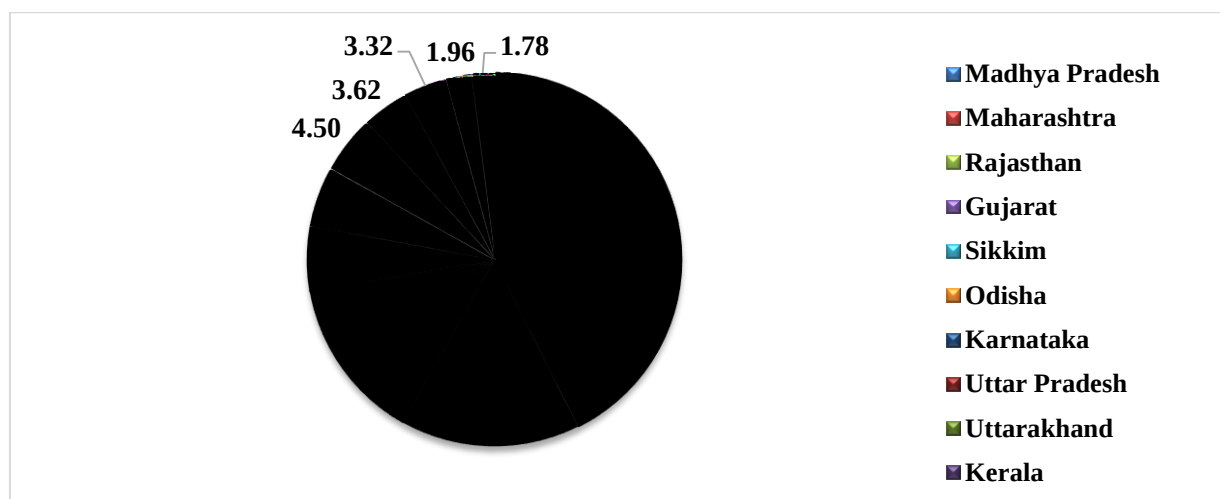


FIGURE 3: Percentage share of organic area in different states of India (2021-22)
(Source: APEDA)

As far as organic production is concerned, Maharashtra and Rajasthan stand in second and third position after Madhya Pradesh with a production share of 19.7% and 11.2% respectively (Fig. 4). Even though there is a significant increase in the area under organic farming, the absolute quantity of organic produce remained same in 2021-22 from the previous year.

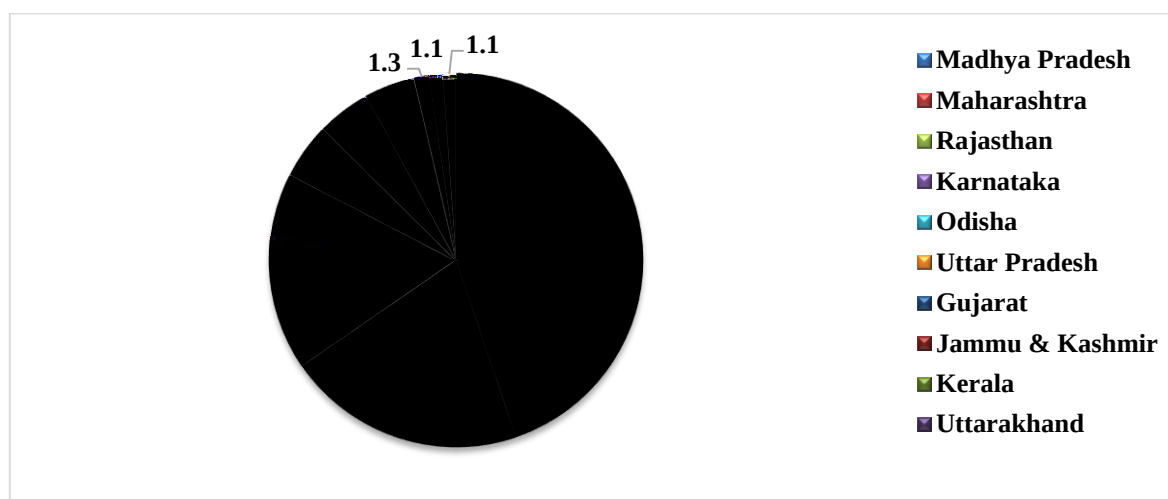


FIGURE 4: Percentage share of different states in organic agri production (2021-22)
(Source: APEDA)

Processed food, oilseeds, and cereal & millet are the leaders among the commodities exported from the country. These three commodities have a combined share of about 87% of the total organic export. Processed food possessed the top position in total organic export with 61.09% share, followed by oilseeds (12.85%), cereal & millets (12.71%), and sugar (4.76%). Rest commodities contributed only 8.58% of total organic export (Table 1).

TABLE 1
COMMODITY WISE ORGANIC AGRI EXPORT-2021-22

Commodity name	Exported quantity (in tons)	% to total
Processed food	281190.452	61.09
Oil seeds	59168.419	12.85
Cereals & millets	58513.835	12.71
sugar	21932.591	4.76
Spices & condiments	7957.918	1.73
Tea	6060.466	1.32
Pulses	5433.505	1.18
Fodder	4972.904	1.08
Medicinal plant products	4072.861	0.88
Coffee	3916.192	0.85
Dry fruits	3433.509	0.75
Fresh fruits & vegetables	1580.125	0.34
Tuber products	922.138	0.2
Miscellaneous	730.847	0.16
Flowers	243.293	0.05
Essential oil	188.346	0.04
Oils & Oleoresins	2.999	0
Total	460320.4	100

Source: APEDA

1.2 Government Initiatives to Bolster India's Organic Produce Exports:

The Government of India has actively fostered organic agriculture through dedicated schemes like the Paramparagat Krishi Vikas Yojana (PKVY) and Mission Organic Value Chain Development in North East Region (MOVCDNER) since 2015-16 to cater to the needs of domestic and export markets respectively. Both the schemes stress end-to-end support to organic farmers i.e., from production to certification and marketing. Post-harvest management support including processing, packaging, and marketing is made integral part of these schemes to encourage organic farmers. Under PKVY, a unique quick certification program “Large Area Certification” (LAC) was launched to harness the potential areas such as areas in hills, tribal districts, deserts, and rainfed areas in India that continue to remain free from chemical input usage. The government is also implementing its Large Area Certification program to transform “Traditional Organic Areas” into certified organic production hubs. In addition, Organic cultivation on either side of River Ganga and Large Area Certification have also been introduced under PKVY to increase acreage under organic cultivation using organic manure / bio-fertilizers. Organic Farming has also been supported under other schemes viz. Rashtriya Krishi Vikas Yojana (RKVY) and Mission for Integrated Development of Horticulture (MIDH), Network Project on Organic Farming under the Indian Council of Agricultural Research (ICAR). Natural Farming is promoted in India as Bharatiya Prakritik Krishi Paddhati (BPKP) under the centrally sponsored scheme PKVY. India is all set to launch a National Mission on Natural Farming to promote the adoption and upscaling of these practices across

the country. As per the MoA&FW, more than 1 Mha area (i.e., 10,05,623 ha) is under natural farming and more than 1.6 million (i.e. 16,78,693) farmers are practicing this (GoI, 2023).

1.3 Organic Certification:

In India, organic certification can be obtained either from the National Program for Organic Production (NPOP) or through the Participatory Guarantee System-India (PGS). However, certification for export is mandated through NPOP only. The NPOP is the central regulatory system for organic agriculture in India, established by the APEDA under the Ministry of Commerce and Industry in 2001. This program was originally meant for exporters and requires adherence to stringent standards. The NPOP is based on the International Federation of Organic Agriculture Movements (IFOAM) standards and includes rules for the accreditation of certification authorities, guidelines for certifying growers, and rules for the use of India's organic label. Mainly the Organic certification is carried out by a third-party inspection agency under the NPOP's purview, and the government maintains the "India Organic" national seal, which is used by exporters, processors, and manufacturers.

In NPOP, the exporter can get a certificate, valid for one year, from one of the 28 third-party certification agencies accredited by the APEDA. The other option is the Participatory Guarantee System-India (PGS), under which a group of farmers come together and vouch for each other's produce.

The NPOP and PGS both are process-based certification systems. They look at the processes and practices of farming and food processing. Testing of food for pesticide residues is a limited part of the scheme. NPOP is far more expensive than PGS and therefore, preferred by big farmers, companies, and exporters.

The organic agriculture sector has experienced significant growth, supported by government initiatives and increasing consumer demand. However, there are areas where further improvement can enhance the organic food processing industry and boost exports. Efforts are undertaken to refine certification systems to better accommodate small and marginal farmers, ensuring they receive adequate support. While multiple certificates are currently necessary for exports to various countries following the cessation of the equivalence agreement with the US certification system, this presents an opportunity to streamline processes and enhance compliance standards. Additionally, initiatives are being developed to improve traceability within the certification and export system, addressing a key challenge and further strengthening the organic food industry's global competitiveness. In 2013, India embarked on a journey to enhance its processed food exports by reevaluating its unilateral equivalence arrangement with the European Union (EU), a pivotal market for Indian organic exports. Concurrently, consumer surveys in India revealed a robust preference for organic food among consumers, underscoring a growing awareness and appreciation for healthier dietary choices. Moreover, consumer survey highlighted an evolving consumer sentiment, where concerns about the quality of organic products are being addressed through increased transparency, stringent quality control measures, and heightened vigilance against adulteration and fraudulent practices. (Ramesh and Divya, 2015; Sharma et al. 2016).

Recognizing the current landscape of organic exports from India this paper delves into a comprehensive analysis of the nation's organic agricultural produce exports; examining trends, growth patterns, and any instabilities observed in area, production, and export volumes of organic agri produce.

II. METHODOLOGY

By leveraging secondary data from various governmental sources like APEDA and the MoA&FW, the paper explores the potential of the organic sector to contribute to India's economic development and agriculture sustainability. Time series data spanning the period 2012-2022 on area, production, and export of organic produce was utilized and the export of organic produce from India and major states was studied using CAGR, coefficients of variation (CV), and the Cuddy-Della Valle Index (CDVI) for instability.

2.1 Compound Annual Growth Rate (CAGR) Analysis:

CAGR is used to assess trends in organic produce area, production, and exports from India. This analysis leverages an exponential growth function to calculate the CAGR. Time-series data encompassing the chosen period will be fitted to this exponential trend equation. Subsequently, by solving the resulting equation, the CAGR for each metric (area, production, and

exports) is precisely determined. This approach provides a clear and concise understanding of the annual growth rate experienced within the organic produce sector over the specified timeframe.

The exponential trend equation used in this case is of the following form:

$$Y_t = ab_t \quad (1)$$

Where, Y_t = Parameters of land-utilization and cropping pattern

a = constant

t = time variable with an annual frequency (Year = 1, 2, ..., n)

b = coefficient that shows the rate of change

Converting eq. 1 into a semi log exponential form:

$$\log(Y_t) = a + tb \quad (2)$$

Eq. 1 can also be written as:

$$Y_t = Y_o (1+r)^t \quad (3)$$

Taking log on both sides:

$$\log(Y_t) = \log(Y_o) + t \log(1+r) \quad (4)$$

Comparing eq. 2 and eq. 4, we get:

$$a = \log(Y_o)$$

$$b = \log(1+r)$$

The Compound Annual Growth Rate can be calculated by using the following method:

$$b = \log(1+r)$$

$$\text{Antilog}(b) = 1+r$$

$$\text{Antilog}(b) - 1 = r$$

$$\text{i.e. } r = \text{Antilog}(b) - 1$$

When this value of r is multiplied by 100, it gives the percentage compound annual growth rate as per the formula.

$$\text{CAGR} = (\text{Antilog of } (b) - 1) \times 100$$

2.2 Cuddy Della Valle Instability Index (CDVI):

Cuddy and Valle (1978) created the CDVI to measure the instability in time series data that is characterized by trends. The coefficient of variation (CV) was calculated using the expression to investigate the stability of processed vegetable exports. The Cuddy Della Valle Index is used to measure the instability in this study. The CDVI de-trends the given series first, indicating the direction of instability. The use of the CV as a measure of instability in time series data has significant drawbacks. If the time series data show any trend, the variation assessed by CV can be overstated, i.e., if CV is used to quantify instability, the region with expanding output at a constant rate will score high in instability. In contrast, the CDVI uses the coefficient of determination to de-trend the coefficient of variation.

The CV is a statistical measure of data points' dispersion around the mean in a data series, and a useful statistic for assessing the degree of variation between two data series, even if the means are radically different. It indicates the ratio of the standard deviation to the mean.

$$\text{Coefficient of Variation (CV)} = \frac{\text{Standard Deviation (SD)}}{\text{Mean}} \times 100 \quad (5)$$

The CV (%) was calculated to determine the extent of fluctuation in processed vegetable exports in terms of quantity and value over time. The instability index was also calculated to look at the volatility of processed vegetable exports from the country in terms of quantity and value over time using the following formula:

$$\text{Instability Index (I)} = \text{CV} \times \sqrt{1 - \text{Adjusted } R^2} \quad (6)$$

Where,

CV = Coefficient of Variation

Adj R^2 = Coefficient of determination

The ranges of CDVI (Sihmar, 2014) are given as follows:

Low instability = between 0 and 15

Medium instability = greater than 15 and lower than 30

High instability = greater than 30

III. RESULTS AND DISCUSSION

India has a long history of organic farming practices, with a large number of farmers engaged in traditional organic farming methods. However, the country's formal organic agriculture sector has been growing in recent years, with increasing domestic and international demand.

India's organic agriproduct exports increased from 0.286 MT worth 327 Million USD in 2014-15 to 0.888 MT worth 1041 Million USD in 2020-21. India's organic exports have continuously shown positive growth with constant increase in exports till 2020-21. In 2021-22 it remained at 0.46 MT with a value of 772 million USD (i.e., Rs. 5249 Crores), registering a decline of 51% from the previous year (Fig. 5).

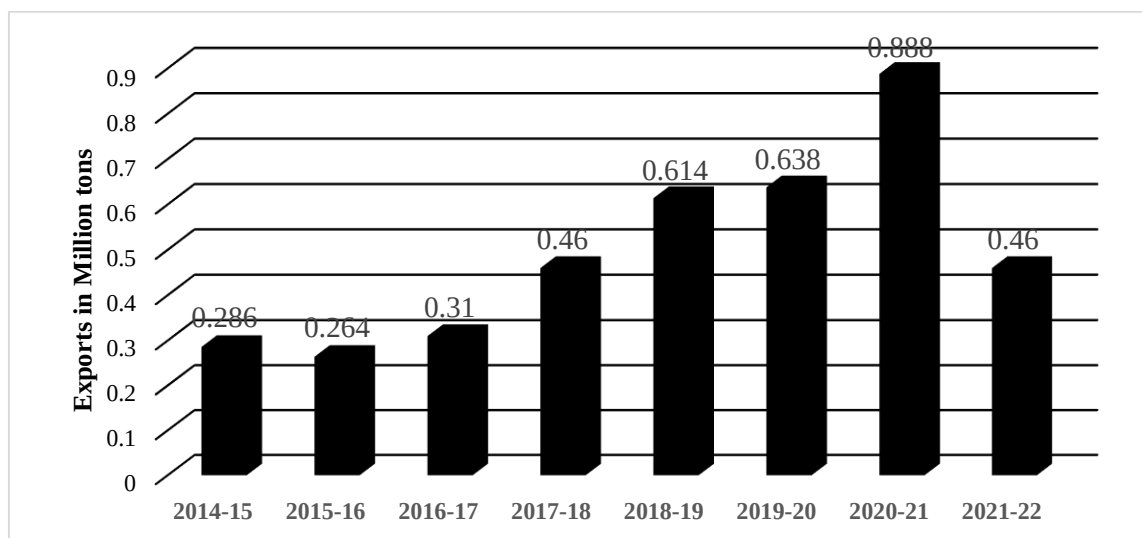


FIGURE 5: Export of India's Organic Agri Produce (MT)
(Source: APEDA)

Overall, the country has seen a significant growth in organic export, with a magnitude of 0.065 MT/ha/year with a CAGR of 15.14% from 2014 to 2022.

The largest export destination for Indian Organic Agri produce is United States and EU, which together account for over three-quarters of the total export volume and value. Other significant export destinations include Canada, Great Britain, Turkey, Switzerland, Vietnam, Ecuador, Korea Republic, and Australia, etc. India export 86.42% share of its organic export to United States, EU, and Canada of which the highest 40.48% exported to United States followed by EU (37.10%), Canada (8.84%), Switzerland (1.10%), and Australia (0.34%) (Table 2).

TABLE 2
MAJOR ORGANIC EXPORT DESTINATIONS FOR INDIA (TONS)

Country	2017-18	2018-19	2019-20	2020-21	2021-22
United States	223854	334112	376070	500936	186339
	-48.50%	-54.40%	-58.85%	-56.40%	-40.48%
European Union (EU)	129546	155253	175674	267076	170762
	-28%	-25.30%	-27.50%	-30.10%	-37.10%
Canada	82133	101942	64225	69142	40677
	-17.80%	-16.60%	-10.05%	-7.80%	-8.84%
Switzerland	8925	6199	5192	3924	5142
	-1.93%	-1.01%	-0.81%	-0.44%	-1.10%
Australia	2690	2130	2357	2923	1574
	-0.57%	-0.35%	-0.37%	-0.33%	-0.34%
Total Export	458339	614089	638998	888179	460320

(Figure in parenthesis is percentage to total export) Source: APEDA

In 2017-18, Madhya Pradesh possessed the highest, 51.5% share of exports of organic agri produce, followed by Gujarat (14.3%), New Delhi (13.9%), and Maharashtra (7%). Rajasthan, Daman & Diu, Karnataka, Haryana, Uttar Pradesh, and Andhra Pradesh also contributed with a small share in India's organic export basket, ranging from 0.5% to 2.6%. The percentage share of organic exports increased for all the states during 2017 to 2021 except, New Delhi, Andhra Pradesh, and Gujarat where the same has been decreased by more or less similar quantity during the period. However, in 2021-22, there was a drastic decline in the percentage share of exports for Madhya Pradesh, which dropped to 38.3%. This was accompanied by an increase in the percentage share of exports of Maharashtra, Gujarat, and New Delhi, although their share remained lower than that of Madhya Pradesh (Table 3).

TABLE 3
STATE-WISE EXPORT OF ORGANIC PRODUCE IN INDIA DURING 2017 TO 2022 (x000 TONS)

State	2017-18	2018-19	2019-20	2020-21	2021-22
Madhya Pradesh	237 (51.5%)	314(51%)	351 (54.9%)	500 (56.3%)	176 (38.3%)
Maharashtra	32 (7%)	75 (12.26%)	73 (11.4%)	126 (14.2%)	85.5 (18.6%)
Gujarat	66 (14.3%)	64.9 (10.50%)	58 (9.1%)	65.5 (7.4%)	60 (13%)
Haryana	4.7 (1%)	26.67 (4.83%)	31 (4.9%)	38.9 (4.4%)	29.1 (6.3%)
New Delhi	64 (13.9%)	40.15 (6.50%)	20.7 (3.2%)	31.7 (3.6%)	24.8 (5.4%)
Karnataka	6.7 (1.5%)	11.89 (1.94%)	21.8 (3.4%)	17.4 (2%)	22.1 (4.8%)
Daman & Diu	10 (2.2%)	25.50 (4.16%)	36.2 (5.7%)	42.4 (4.8%)	16.5 (3.6%)
Rajasthan	12 (2.6%)	23.27 (3.78%)	14.5 (2.3%)	24.7 (2.8%)	9.1 (2%)
Andhra Pradesh	2.48 (0.5%)	3.73 (0.60%)	2.3 (0.4%)	1.83 (0.2%)	2.7 (0.6%)
Uttar Pradesh	2.43 (0.5%)	4.66 (0.76%)	5.2 (0.8%)	12.14 (1.4%)	6.9 (1.5%)
Total Export	460	614	639	888	460

Figure in parenthesis is percentage to total (Source: APEDA)

The reason behind this deceleration might be a sizeable reduction in the production of organic agri produce in some states like Maharashtra and Karnataka due to heavy rainfall, cyclones, and COVID-19 pandemic.

3.1 Trend, Growth, and Instability in Organic Area and Production in Major States (2017 -2022):

Area: The growth and instability of area under organic farming in major states have been analyzed from 2017 to 2022 and found that, over the years, the absolute area under organic farming increased in all the major States and the country as well (Table 4).

TABLE 4
TREND, GROWTH, CHANGES, AND INSTABILITY IN ORGANIC AREA OF MAJOR STATES

State	Base year (2017)	Current year	Absolute Change	Relative Change (%)	CAGR (%)	Instability
		2022			(P-Value)	
Madhya Pradesh	328420	618,080	289660	88.2	17.56** -0.0003	2.15
Maharashtra	108029	224,787	116758	108.08	19.65** -0.0028	10.54
Rajasthan	76002	204,871	128869	169.56	27.89** -0.0077	6.79
Gujarat	49298	81,701	32403	65.73	12.68** -0.012	7.05
Sikkim	74094	75,168	1074	1.45	0.42 -0.2	0.81
Odisha	61981	72,758	10777	17.39	3.95 -0.212	7.46
Karnataka	24669	58,613	33944	137.6	19.72 -0.165	28.32
Uttar Pradesh	39406	53,701	14295	36.28	8.23** -0.007	3.32
Uttarakhand	19746	31,739	11993	60.74	15.06** -0.013	8.92
Kerala	17523	28,743	11220	64.03	13.63** -0.0005	3.75
Total	884875	1,618,464	733589	82.9	16.36** -0.0003	3.24

** Significant at 1 percent; Source: Author's calculation

The maximum increase in area under organic farming was observed in Rajasthan (169.56%), followed by Karnataka (137.60%), Maharashtra (108.08%), Madhya Pradesh (88.20%), Gujarat (65.73%), Kerala (64.03%), Uttarakhand (60.74%), Uttar Pradesh (36.28%), Odisha (17.39%), and Sikkim (1.45%) respectively. As far as the growth of area under organic farming is concerned, the Compound Annual Growth Rate (CAGR) of the same was found highly significant and positive in all the major states during the period from 2017 to 2022. The maximum growth in CAGR was observed in Rajasthan (27.89%) followed by Karnataka (19.72%), Maharashtra (19.65%), Madhya Pradesh (17.56%), Uttarakhand (15.06%), Kerala (13.63%), Gujarat (12.68%), Uttar Pradesh (8.23%), Odisha (3.95%), and Sikkim (0.42%) during the reference period. The growth of area under organic farming in the country was found highly significant and positive during the period.

The fluctuation was found through CDVI and observed that the fluctuation in areas under organic farming was recorded as very low in all the major states except in Karnataka, where the instability was observed medium as the CDVI value was found 28.32. This indicates that the persistence of volatility may be higher in Karnataka than in other states. In the rest of the states, the CDVI value ranged from 0.81 (Sikkim) to 10.54 (Maharashtra) indicating the lowest instability over the period of five years. Low instability in area under organic farming in major states shows that the farmers are moving towards organic farming in these states.

Production: The absolute quantity of organic agri-produce increased in the country and all the major states during the reference period. At the country level, organic production was increased by 77.28 % during the reference period. Among the States, Karnataka had the highest increase (510.65%) in organic production, followed by Maharashtra (437.97%), Rajasthan (336.70%), Madhya Pradesh (284.56%), Uttar Pradesh (234.50%), Jammu & Kashmir (185.63%), Gujarat (147.79%), Odisha (119.42%), Kerala (82.42%) and Uttarakhand (60.64%) in the current year over the base year (Table 5).

TABLE 5

TREND, GROWTH, CHANGES, AND INSTABILITY IN ORGANIC AGRICULTURE PRODUCTION IN MAJOR STATES

State	Base year	Current year	Absolute Change	Relative Change (%)	CAGR (%)	Instability
					(P-Value)	
Madhya Pradesh	328420	1262967	934547	284.56	37.59*	12.14
					-0.028	
Maharashtra	108029	581164	473135	437.97	38.16	53.02
					-0.312	
Rajasthan	76002	331901	255899	336.7	42.13**	10.2
					-0.0012	
Karnataka	24669	150641	125972	510.65	43.2	83.24
					-0.373	
Odisha	61981	135999	74018	119.42	21.38*	12.44
					-0.03	
Uttar Pradesh	39406	131813	92407	234.5	30.54	35.59
					-0.19	
Gujarat	49298	122155	72857	147.79	26.48**	11.92
					-0.0058	
Jammu & Kashmir	13528		25113	185.63	25.75	22.98
		38641			-0.1	
Kerala	17523	31965	14442	82.42	13.8	11.23
					-0.058	
Uttarakhand	19746	31720	11974	60.64	15.06	25.85
					-0.181	
Total	1664549	2950920	1286371	77.28	14.63	13.51
					-0.074	

*Note: *Significant at 5percent; ** Significant at 1 percent; source: Author's estimation*

All the major states and the country showed an increasing trend of growth rates in production during the period. Among the states, the highest CAGR was seen in Rajasthan (42.13%) and Gujarat (26.48%) which was significant at 1% level. Whereas, the growth of organic production in Madhya Pradesh (37.59%) and Odisha (21.38%) was positive and significant at 5% level. The rest of the states showed not significant growth of organic production and the same was highest in Karnataka (43.20%), followed by Maharashtra (38.16%), Uttar Pradesh (30.54%), Jammu & Kashmir (25.75%), Uttarakhand (15.06%) and Kerala (13.80%). The production in Uttar Pradesh, Karnataka, and Maharashtra showed quite high fluctuation over the reference period with CDVI values ranging from 35.59 to 83.24. Uttarakhand (25.85) and Jammu & Kashmir (22.98) showed medium fluctuation in organic production. In Odisha, Madhya Pradesh, Gujarat, Rajasthan, and Kerala, the fluctuation in organic production was minimal during the reference period with CDVI values ranging from 10.20 to 12.44.

3.2 Growth and Instability in Export of Organic Produce to Major Export Destinations (2017 -2022):

The results reveal that over the period, the absolute quantity of export of organic produce to all the major export destinations has decreased except for the EU, experiencing an acceleration of 31.82% per annum during the period. The export to Canada showed the highest decline (50.47%), followed by Switzerland (42.39%), Australia (41.49%), and the United States (16.76%) respectively. As far as the growth of export of organic produce is concerned, CAGR of export to the United States (0.38%) and EU (11.57%) was found positive but not significant whereas, the same was found negative and highly significant to Canada (16.42%) and Switzerland (14.44%). The CAGR of the export of organic produce to Australia was found negative and not significant (7.28%). The CAGR of total organic agri export from India was found positive but not significant during the reference period (Table 7). The CDVI was analyzed to know the fluctuation in exports and the result indicated that the instability of the quantity exported from the country was at a medium level for all the export destinations except the United States. The export to the United States showed high instability over the reference period as the CDVI value is found to be 44.42 (Table6).

TABLE 6
TREND, GROWTH, CHANGES, AND INSTABILITY IN EXPORT OF ORGANIC PRODUCE TO MAJOR EXPORT DESTINATIONS (2017-2022)

Country	Base Year	Current year	Absolute change (MT)	Relative change (%)	CAGR (%)	Instability
					(P-Value)	
USA	223854	186339	-37515	-16.76	0.38	44.42
					-0.98	
EU	129546	170762	41216	31.82	11.57	27.95
					-0.236	
Canada	82133	40677	-41456	-50.47	-16.42	21.47
					-0.082	
Switzerland	8925	5142	-3783	-42.39	-14.44	20.92
					-0.093	
Australia	2690	1574	-1116	-41.49	-7.28	23.28
					-0.396	
Total Export	458339	460320	1981	0.43	3.85	32.12
					-0.725	

Source: Author's estimation

The results further reveal that the absolute quantity of export of organic produce increased in all the major exporting states during the reference period. The highest increase in the export of organic produce was found in New Delhi (108.08%), followed by Karnataka (96.38%), Haryana (91.47%), Uttar Pradesh (71.34%), Maharashtra (68.33%), Daman & Diu (24.89%), Madhya Pradesh (19.33%), Rajasthan (17.35%), Gujarat (9.54%), and Andhra Pradesh (8.44%) during the period. The absolute quantity of total export was more or less stagnant during the period under study (Table 7).

As far as the growth of export of organic produce is concerned, the CAGR of export was found positive and not significant in five major states while in the rest of the five States, it was negative and insignificant. Among the major exporting states, Haryana showed the highest positive but not significant growth rate of 47.95% per annum followed by Uttar Pradesh (35.59%), Karnataka (31.89%), Maharashtra (28.20%), and Daman & Diu (16.30%). Whereas, New Delhi, Andhra Pradesh, Rajasthan,

Gujarat, and Madhya Pradesh showed negative and not significant growth rates with 19.20, 5.28, 4.82, 1.79, and 1.29% per annum during the period. The CAGR of total export was found positive but not significant during the period (Table 8).

TABLE 7

TREND, GROWTH, CHANGES, AND INSTABILITY IN EXPORT OF ORGANIC PRODUCE FROM MAJOR EXPORT STATES (2017 – 2022)

State	Base Year	Current year	Absolute Change (MT)	Relative Change (%)	CAGR (%)	Instability
					(P-Value)	
Madhya Pradesh	237	176	61	19.33	-1.29	45
					-0.93	
Maharashtra	32	85.5	54	68.33	28.2	33.08
					-0.116	
Gujarat	66	60	6	9.54	-1.79	5.79
					-0.402	
Haryana	4.7	29.1	24	91.47	47.95	39.27
					-0.174	
New Delhi	64	24.8	39	108.08	-19.2	32.79
					-0.131	
Karnataka	6.7	22.1	15	96.38	31.89	24.06
					-0.055	
Daman & Diu	10	16.5	7	24.89	16.3	55.48
					-0.499	
Rajasthan	12	9.1	3	17.35	-4.82	47.61
					-0.769	
Andhra Pradesh	2.48	2.7	0	8.44	-5.28	29.45
					-0.587	
Uttar Pradesh	2.43	6.9	4	71.34	35.59	47.32
					-0.088	
Total	460	460	0	0	3.76	32.07
					-0.73	

Source: Based on Authors' estimation

The CDVI was analyzed to know the fluctuation in export and the result showed that the highest fluctuation was found in Daman & Diu, Rajasthan, Uttar Pradesh, Madhya Pradesh, Haryana, Maharashtra, and New Delhi with a CDVI value of 55.48, 47.61, 47.32, 45.00, 39.27, 33.08, and 32.79 respectively during the period. The fluctuation of the quantity exported was found low and medium in Gujarat and Karnataka with an instability value of 5.79 and 24.06 respectively. In all the states, the instability of Daman & Diu was quite higher than the others, which indicates that the persistence of volatility may be higher as compared to other states (Table 7).

IV. CONCLUSION AND WAY FORWARD

Despite holding the highest number of organic farmers globally and experiencing rising global demand for organic produce, India's organic export share remains below 1%, which shows the global market is enough space for untapped potential and opportunities of Indian organic produces. To solidify the position in global market, we can leverage technologies to enhance traceability throughout the organic value chain. Establish organic agri export facilitation centers in the state having more area under organic but less export. To further enhance and capitalize on the immense potential of the organic export sector, India must prioritize strengthening its certification processes while exploring additional strategies. Furthermore, fostering collaboration with Krishi Vigyan Kendras (KVKs) and agricultural universities can significantly leverage their existing infrastructure, expertise, and research capabilities within the organic certification process. Use of satellite imagery, AI based analytics and digital tracking might be helpful for addressing the issues of traceability of production, stocks and wastage.

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A Comparative Economic Analysis of Improved and Local Maize Seed System in Udayapur, Nepal

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Abstract— The primary aim of this study is to identify significant predictors affecting the deployment of upgraded seed in Udayapur, Nepal. With minimum prior study and insufficient information in the survey area, this research tries to fill the knowledge gap by investing socioeconomic, institutional, financial and technical factors affecting the adoption of improved seed. A sample size of 75 respondents were interviewed at farm level. Benefit Cost ratio, Binary Logistic regression and Scaling index techniques are employed for the research. The benefit-cost ratio (BC ratio) for improved seed adoption was found to be 1.37, which is higher than of local seed (1.10). The total area under cultivation ($p = 0.010$) and training status of farmers ($p = 0.050$) were significant factors affecting the adoption of improved seed. For every increase in one-unit, area (hectare) and trained farmer, the odds of planting improved seed increase by 15.1 % and 4.168% respectively. The untimely availability of improved seed (0.71), fragmented land holdings (0.65) and lack of technical knowledge (0.53) were top three ranked factors hindering the adoption of premium seeds. To further encourage the use of high graded seed, technical and financial assistance, timely supply of input, land consolidation, and cooperative's roles become crucial. To streamline the future plans and programs for maize cultivation in the research area, an endeavor is made to study possible pathways for interventions.

Keywords— Adoption, Binary Logistic Regression, Improved seed, Scaling index.

I. INTRODUCTION

Increasing agricultural productivity is crucial for economic growth and food security (SQCC, 2013). There are numerous factors diminishing food security, one of which is inefficient inputs application (FAO, 2024). The impacts of climate change across the globe will exacerbate issues of alimentary security, famine and global hunger especially, notably for the resources poor developing countries, and more especially in South Asia, including Nepal (Masso, 2012). Studies show that the use of improved seed can increase crop yields by up to 30 %, emphasizing the importance of seed in agriculture (SQCC, 2013). An access to affordable high quality seeds is crucial for improving productivity (Sector & Strategy, 2013).

Maize, known as the “queen of cereals” for its high yield potential, cultivated across diverse regions, with notable variation in adaptation (Revilla et al., 2022; Prakash et al., 2019). It covers total production area of 985,565 hectares (ha), production 3,106,397 Metric ton (Mt), and 3.15 Mt/ha Yield. In Udayapur district, maize is grown on 17,945 hectares (ha), production 43,714 Metric ton (Mt), yielding 2.4 Mt/ha (MoALD, 2020). Maize, once a subsistence staple, is now increasingly cultivated on commercial level (Dhakal et al., 2015) due to increase in demand by 5 % annually, (Sapkota & Pokhrel, 2013) especially from poultry industry.

Nevertheless, its productivity in Nepal lags behind neighboring countries, despite increasing in its demand (Subedi, 2015). The introduction of superior maize type is one potential solution to enhance productivity and meet market needs (S. P. Adhikari et al., 2019). The preference of maize varieties is affected by various factors (Kalle Hirvonen, Elia Machado, 2024b) among which socioeconomic factor is studied in this paper.

Nepal is highly vulnerable to natural disasters (Masso, 2012), which pose significant risks to maize production. The areas such as Nepal, dependent on rain-fed agriculture, have been slower to benefit from Green Revolution technologies (Pingali, 2012).

Further marginal farms often devoid of the resources to completely adopts these advancements (George, 2015). The utilization of progressive farm practices, such employing progressive over traditional seeds can aid in enhanced productivity (Pandya-lorch, 2009).

The affordability, timeliness along with availability of quality seeds, and varietal options, is crucial for embracing superior maize strains and bridging the yield gap (Mishra, R., P., Joshi, G., R. dan Dilli K., 2017). The adoption rate of improved maize varieties in Nepal is about 64% with approximately 40% of the total maize plantation is performed with refined type seeds and the remaining proportion with local or traditional varieties (Paudel & Matsuoka, 2008). The rate of adoption is higher in Terai regions as compared with hilly areas of the country, as access to inputs and infrastructure is poor in these areas (MOAD, 2014). To remedy this, production of Maize in Udayapur district has been supported by HMRP, CBSP (MoALD, 2021) and more.

There is no prior research on the determinants influencing the incorporation of improved seeds in Udayapur, Nepal. This research aims to assess the cost of production, income, benefit cost ratio for farmers with improved and local seed usage, the socioeconomic variables affecting the integration and constrains in adoption of superior strains.

II. RESEARCH METHODOLOGY

2.1 Data Source and methodology:

The study district for the comparative economics of improved and local seed system was decided purposively. The random sampling technique was applied to a sample size of 75 respondents. Data were collected by using a structured interviewer-administered questionnaire. The questionnaire covered socioeconomic, demographic and related characteristics of two seed system. Focused group discussions and key informant's interview were done to validate the survey statistic. The data was conducted using IBM SPSS (Version-27.0) and MS-Excel.

2.2 Model Specification

Benefit-Cost ratio was calculated for comparative economics of improved and local seed system. The Binary logistic regression model was applied for identify variables affecting the integration of superior maize seeds strain. The model has a binary outcome for seed adoption, assigning '1' to hybrid and '0' to non-hybrid or local seed. The equation for the model is

$$\text{Log} \left(\frac{P}{1-P} \right) = \beta_0 + \beta_1 \text{ education} + \beta_2 \text{ years of farming} + \beta_3 \text{ cultivation area} + \beta_4 \text{ training} + \beta_5 \text{ market proximity} \quad (1)$$

Note:

$$\text{Log} \left(\frac{P}{1-P} \right) = \log \text{ odds}$$

P = Probability of adopting improved seeds ($0 \leq P \leq 1$)

$1 - P$ = Probability of not adopting improved seeds

B_0 = Constant term

β_i = Regression coefficient for each independent variable ($i = 1, 2, \dots, 5$)

Scaling technique is employed to study factors hindering the adoption of improved seeds,

$$I = \frac{\sum S_i F_i}{N} \quad (2)$$

Where,

I = Index value which ranges between 0 and 1.

S_i = Value obtained by i^{th} category

F_i = frequency of the respondent

N = total survey respondents

III. RESULT AND DISCUSSION

TABLE 1
ECONOMIC COMPARISON OF HYBRID AND LOCAL SEEDS IN UDAYAPUR, NEPAL

A. Production cost	Improved	Local Seed
1. Average variable cost	(ha)	(ha)
Human Labor	18,847	14,429
Bullock Labor	6,429	3,102
Machinery cost	19,662	20,233
Seed cost	2,099	2,200
Manure Cost	1,391	1,330
DAP Cost	1,503	1,478
Urea Cost	1,398	1,049
Pesticide Cost	690	663
Other Cost	1,007	921
2. Fixed cost		
Land Cost	41	42
Farm Equipment, Depreciation and Maintenance	95	87
Total cost of production	53,162	45,544
B. Marketing at nearest market		
Yield	3,100	24,07
Market Price	23.5	21
Income	72,850	50,547
B.C ratio	1.37	1.1

The table presents the comparative economic analysis of maize production with two seed types. The outcomes show that production cost and the productivity (ton/hectare) for improved seed is higher than that of local seed by 0.27 units. In the research site, the B:C ratio for superior seeds and local seeds were 1.37 and 1.10 respectively.

TABLE 2
BINARY LOGISTIC REGRESSION FOR THE ADOPTION OF SUPERIOR SEEDS IN MAIZE CULTIVATION, UDAYAPUR, NEPAL

Predictors	β	S.E.	Wald	Sig.	Exp(β)
Educational status	-0.065	0.53	0.015	0.902	0.937
Years of farming	0.017	0.022	0.615	0.433	1.018
Cultivation area	0.141	0.055	6.549	0.01	1.151
Training	1.428	0.731	3.813	0.05	4.169
Market proximity	-0.09	0.547	0.027	0.869	0.914
Constant	-2.613	1.187	4.847	0.028	0.073
Omnibus test of model coefficient					
Model significance = 0.023					
- 2 log likelihood = 89.504					
Nagelkerke R square = .512					

The table demonstrates the findings from the Binary Logistic Regression for incorporation of superior seeds strains (0-local seed, 1-improved seed). The predictors for the model are educated (0-no, 1-yes), years of farming, cultivation area (katha), training (0-unskilled, 1-skilled) and market proximity (0- far, 1-near) were employed as predictors. The table examines the

relation between the predictor variables and likelihood of adoption of improved seed. Education has -0.065 beta coefficient, indicating that higher education decreases the odds of the implementing superior hybrid strains. However, this effect is not statistically significant ($p=0.902$). The years of farming has a coefficient of 0.017, indicating that higher the farming experience, the odds of employing improved seed increases, but this association is not statistically significant ($p=0.433$). The cultivation area has an affirmative and statistically significant effect ($p = 0.010$), indicating the larger cultivation areas increase the odds of improved seed use. This implies, for every increase in one unit (hectare) area, the odds of adopting improved seeds increase by 15.1%. Trained farmers and employ of superior seed were found to have affirmative and statistically significant relationship ($p = 0.050$), suggesting that training increases the odds of the outcome by a factor of 4.169 %. Market proximity has a coefficient of -0.090, indicating that closer market proximity decreases the likelihood of the employing superior seeds, but the effect is insignificant ($p=0.0869$).

The final equation after logistic regression results,

$$\text{Log} \left(\frac{P}{1-P} \right) = -2.613 - 0.065 \text{ education} + 0.017 \text{ years of farming} + 0.141 \text{ cultivation area} + 1.428 \text{ training} - 0.090 \text{ Market proximity} \quad (3)$$

The Omnibus test of model coefficient has p value of 0.023, imply that the predictors in the model is statistically significant and explains substantial effects on the adoption of improved seed. The -2-log likelihood and Nagelkerke R square values are 89.504 and 0.512 respectively, suggesting that model is fit and explains approximately 51.2% of the variance in the adoption is explained by the model.

TABLE 3
CONSTRAINTS IN THE INTEGRATION OF IMPROVED SEEDS IN MAIZE CULTIVATION, UDAYAPUR, NEPAL

S. No.	Constraints	Index value	Rank
1	Untimely availability	0.71	I
2	Fragmented and small land holdings	0.65	II
3	Lack of technical awareness	0.53	III
4	Crop management	0.46	IV
5	High price of seeds	0.29	V

This table highlights the primary challenges faced by farmers in adoption of improved seeds. The highest ranked constraint was found to be untimely availability of improved seed with the index score of 0.71. Fragmented small land holdings and lack of technical awareness were key hinderance factors with 0.65 and 0.53 index values. Crop management and high seed price were other constraints in the adoption of improved seed.

IV. CONCLUSION

The research offers clear evidence that the employing superior seeds over the traditional, is associated with greater Benefit-Cost ratio by 0.27%. Additionally, two predictor variables, cultivation area and training status of farmers were found to have an affirmative and significant linkage on the adoption of enhanced seeds. This implies, for every increase in one unit (hectare) area and trained farmer, the odds of employing superior seed strains increase by 15.1%. and 4.169 % respectively. The untimely availability of improved seed, small land holdings and lack of technical knowledge were top ranked hindering factors in the adoption of improved seed.

To remedy the major barriers in adopting improved seeds, several recommendations are propose based on the study. There is an urge to supply adequate high qualities seeds on time through a robust distribution network. Establishing efficient supply and distribution network by interlinking all the channels of value chain should be promoted. Supporting marginalized farmers with target specific programs such as land consolidation, farmers' cooperative, subsidies and incentives, is pivotal. The financial and technical assistance, and disseminating technical knowledge at grass root level will encourage the adoption of

improved seeds. Implementing these programs and policies will incentivize better adoption of improved seeds by pooling available resources, increasing, encourage bulk supply, strengthening community seed banks and increasing bargaining powers of farmers.

DECLARATIONS

I, Saroj Dhakal, declare that the research entitled “A Comparative Economic Analysis of Improved and Local Seed Systems in Udayapur, Nepal” is our original work and has not been submitted previously in any form to any other university or institution. To the best of my knowledge and belief, this work is free from plagiarism. I understand that my research may be used as a reference in future research or publications, and I have no objection to its use in that context.

AUTHOR CONTRIBUTION STATEMENT

- S. Dhakal*: Conceptualization, Methodology, Data Collection, Data Analysis, Writing - Original Draft, Writing - Review & Editing.
- P. Gyawali: Methodology, Data Analysis, Supervision, Writing - Review & Editing.
- S. Pandey: Conceptualization, Resources, Writing - Review & Editing.
- B. Sharma: Data Collection, Visualization, Writing - Review & Editing.
- S. Dhakal: Project Administration, Funding Acquisition, Writing - Review & Editing.

CONFLICTS OF INTEREST

The authors certify no conflicts of interest concerning the research, authorship, and publication of this report. We have conducted the study without any biasness.

ETHICS CONSENT

All contributors provided their informed consent before their inclusion in the study, ensuring their voluntary participation. The confidentiality and anonymity of participants were maintained throughout the research process, and all data were securely stored.

CONSENT FOR PUBLICATION

We provide consent to the publication of materials related to our participation in the research study titled ‘A Comparative Economic Analysis of Improved and Local Seed Systems in Udayapur, Nepal’. We understand that our information will be used for research purposes and that our identity will remain confidential.

DATA AVAILABILITY

Additional data, questionnaire, data entry or any other requirements will be provided upon request.

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Decision-Making and Participation in Labour Markets: The Case of Rural Households of Office du Niger area at Mali

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Abstract— *Developing countries' agriculture sectors were characterised by low productivity and low capital-intensive, dominated by smallholder agriculture exposed to imperfect markets. However, the study aims to examine rural labour market participation decisions in the Office du Niger region in the republic of Mali. This article analyses the behaviour of rural households using the agricultural household model by applying the multinomial logit model. The data used in this study were obtained through stratified random sampling. According to the sampling method, 400 rice farmers were studied, and the data was obtained through a household survey conducted from March to June 2020.*

The results showed that the household head age, livestock value, dummy savings, distance from the financial institution to the village, and distance from the farm to the town lowered the probability of participating in the supply labour regime. However, the number of active women and the off-farm income increased the likelihood of household participation in the supply regime. The results showed that credit constraints negatively influenced the probability of the farm using hired labour in household production. However, off-farm income is essential in selecting the hiring labour regime. Furthermore, the other characteristics of households and farms were a critical factor in selecting labour regime participation.

Reducing credit constraints will increase hired labour use in agricultural production and increase production levels in the study area. To reduce credit constraints, policymakers should revise cadastral laws allowing farmers to use their land as collateral when applying for loans.

Keywords— *Agricultural household, Imperfect market, Labour demand, Mali, Office du Niger, Smallholder.*

I. INTRODUCTION

Mali's economy is based on the agricultural sector, dominated by small-scale farms, characterised by low productivity and the persistence of low-capital-intensity agriculture, and it is subject to imperfections. Market imperfection often leads to the non-separability between production and consumption decisions. However, the rapid increase in the agricultural population, coupled with the failure of rural markets and the effects of climate change, are today the determining factors in the decline in agricultural productivity of rural households. It poses the challenge of food insecurity in most developing countries. Today more than a billion people live in food insecurity (IED, 2014).

However, the significant problems that rural households face in developing countries, which need to be solved to understand household behaviour in the labour market, remain, as do how these households make their production and consumption decisions. In the rural market of developing countries, household production and consumption decisions are non-separable (Benjamin, 1992). Otherwise, production and consumption decisions are made independently in rural markets.

Households in developing countries frequently confront imperfect or even non-existent markets. Even if the markets exist, transaction costs or quantity constraints (labour markets, credit markets, input markets) prevent them from participating in these markets. When all markets function perfectly, farms with sufficient family labour will transfer their surplus to those with insufficient family labour to maintain production levels. However, when the market does not function perfectly (the main characteristic of developing countries), the household withdraws into itself (autarky). Production and consumption decisions are made instantly. In this case, we find ourselves in subsistence farming (autarky), where the household produces for its own consumption, not for the market. Under this assumption, there will be excess labour on farms, reducing labour productivity per worker. The downward trend in agricultural production (farm profit) per active worker would, in the long term, create a situation of food insecurity and poverty within households. The surplus labour must be transferred to off the farm to compensate for the loss of farm productivity. Due to the necessity for the market to operate effectively, income derived from agriculture needs to be improved to fulfil the requirements of family members. Consequently, family labourers migrate to non-agricultural activities or to the regions where labour remuneration is better.

According to the perfect market hypothesis, households base their decisions on comparative advantage and wages rather than household endowments. Market imperfections generally affect the organisation and use of resources within small farms (Bell, 1988; Conning & Udry, 2007; Ali et al., 2014). However, market imperfections in developing countries affect small-scale farms' production levels and technical efficiency (Stiglitz et Weiss, 1981; Eswaran et Kotwal, 1986; Ma & Abdulai, 2019).

However, studies on rural households' composition give divergent results but are of great interest in agricultural policy formulations. Lopez (1984) rejects the hypothesis of separability between household production and consumption decisions in the case of Canada. Benjamin (1992), in his famous paper on household composition and labour demand in the labour market in India, accepts the separability hypothesis, while Grimard (2000) studies the rural household behaviour in Côte d'Ivoire and rejects the separability notion between production and consumption decisions. De Janvry and Sadoulet (2002) assert that the household participates in the labour market according to its endowment of working time and disposable income. However, it allocates time between leisure, family, and off-farm work. The household hires labour in his farm when it needs it or sells it at the market wage rate w (Benjamin, 1992; Porgo et al., 2017; Ma & Abdulai, 2019) on the labour market. Examination of household involvement in the labour market was approached from various perspectives. (Huffman, 1980; Lopez, 1984; Benjamin et al., 1993; Porgo et al., 2017). Benjamin (1993) divided them into eight different regimes and then analysed the men's and women's behaviour outside the farm. However, in the case of Burkina Faso, Porgo et al. (2017) divided the work into four work regimes and then added a credit constraint to determine the impact of this on the different work regimes.

The decision to participate in the labour market was associated with the gender of the household's head, the farm's size, and the household's size. We note that farms with too few farm workers employ more salaries labour in their farms than farmers with more workers. In general, the demand for labour depends on the type of crop cultivated by the household. The need for labour evolves according to the type of activity, the crop grown on the plot and the growing season. In Mali and the Office du Niger area, for example, activities such as ploughing, sowing, fertiliser spreading, herbicide use, and plant protection products are generally carried out by family labour. However, transplanting, weeding, and harvesting activities often require external support, as they depend on experience and technical expertise. We also note that the demand for labour is higher during the rainy season than during the low-water period when water levels in the dams are lower. It raises the issue of using family and hired labour in farm activities.

II. MATERIALS AND METHODS

2.1 Material:

Empirical research on the analysis of rural households' behaviour uses either aggregated or primary data. The research relied on primary data gathered from households within the Office du Niger region. Using primary data in research studies improves

the accuracy of information and makes better recommendations for agricultural development policies. A well-structured questionnaire was developed and administered to selected households to obtain information on socio-economic and demographic factors. However, all the rice producers' households in the Office du Niger area constitute the primary basis of this study. To carry out the study list of all households engaged in rice production was acquired from the statistics department of the Office du Niger. Subsequently, this list was utilised for sampling.

2.2 Sampling technique:

Rice-producing households operating in the Office du Niger area constitute the base population of this study. The Office du Niger is subdivided into seven agricultural zones, including, among others, Niono, Macina, M'Bèwani, N'Débougou, Kouroumani, Molodo, and Kolongo. The Niono zone comprises four irrigated areas and 45 villages, the Maciana zone 3 irrigated areas and 46 villages, the Kolongo zone 4 irrigated areas and 60 villages, the Kouroumani zone 5 irrigated areas with 39 villages, the M'bewani zone 5 irrigated area with 62 villages, the Molodo zone 4 irrigated areas and 33 villages and the N'Debougou zone 4 irrigated areas and 49 villages. To undertake this study, two agricultural areas were selected, and within each agricultural area, three irrigated areas were chosen. The primary data collected through this method constitutes the main material of the study. This framework determined the sample volume using the stratified random sampling method. The results thus obtained through this method have made it possible to increase the precision and quality of the data and adequately represent the different sections of the population studied. Thus, the sample volume was determined using the stratified random sampling method (Yamane, 1967).

$$n = \frac{N \sum (N_h S_h)^2}{N^2 D^2 + \sum N_h S_h^2} \quad (1)$$

$$D = d^2 / Z^2$$

n= number of samples

N= number of farmers in the population

N_h = number of farmers in stratum h

S_h^2 = the variance of stratum h

Z= the value of Z in the standard normal distribution table as a function of the error term.

After briefly describing the survey methodology, the sample size was established at 400 households with a 95% confidence interval and a 5% margin of error.

2.3 Analytical model:

The household maximises its utility, a function of its consumption, the endowment of its total available time and characteristics. The household program is formalised as follows:

$$\text{Max } U_h(C, l, \theta) \text{ s/c } c, l, L^o, L^h, L^f \longrightarrow \text{household utility function} \quad (2)$$

$$q = f(L; Q; G; A) \longrightarrow \text{production function} \quad (3)$$

$$c = F(L; A) - wL^h + wL^o + y \longrightarrow \text{budget constraint} \quad (4)$$

$$l + L^f + L^o - L^h = T(\theta) \longrightarrow \text{Time constraint} \quad (5)$$

$$L^o \leq H \longrightarrow \quad (6)$$

The household utility $U_h = u(C, l, \theta)$ is defined twice as derivable and quasi-concave on consumption (c) and leisure (l). θ is a vector containing the demographic characteristics of the household (size and sex of the household members). c , designates the household's total consumption, which includes goods produced by the household and goods purchased on the goods and services market. The household produces with the technology represented by the convex and twice differentiable production function such that $Q = f(L, A)$, where L is the sum of family labour and hired labour (L^f, L^h), A designates the land, which is an exogenous and fixed variable. De Janvry et al. (2002) assert that the household participates in the labour market according to its available resources (working time endowment and disposable income). He allocates his total time between leisure, family work and off-farm work. He can also hire labour when needed or sell his labour force at the market wage rate w (Benjamin, 1992; Abdulai, 2000; Porgo et al., 2017).

$U(.)$ is the household utility function, which is quasi-concave and strictly increasing. θ is the vector of the demographic characteristics of the household, such as the number of people in the household and the level of education of the head of the household. The variable c designates the total consumption of the household. L^f and L^h denote family and hired labour in the household; L^o denotes the family labour supply by household; l leisure time; w the market wage rate; A is a fixed variable. Constraint (6) above means that the market allows a maximum of H hours of labour to be sold. If the family labour supply is less than the labour market need ($L^o < H$), the household could sell all its surplus labour at the market wage rate. In such a condition, we will say that the market is functioning perfectly. However, if the constraint is saturated, i.e., $L^o = H$, the household would like to offer more labour, but the market can only offer H work hours. Under these conditions, we find ourselves in a surplus of labour within the family, which cannot be transferred to the market because of the constraints linked to the imperfect. There would be disguised unemployment in the labour market (Sen, 1966), a direct consequence market of market imperfection.

2.4 Empirical model:

Empirically, several studies have focused on analysing the behaviour of agricultural households worldwide. Some studies have analysed rural households' labour market participation decisions (Lass & Gempesaw, 1992; Gunter, 1992; Lovo, 2012; Bedemo et al., 2013; Porgo et al., 2017). Other studies have focused on the participation decisions of men and women on and off the farm (Huffman & Lange, 1989; Lass & Gempesaw, 1992; Benjamin et al., 1993). The decision to allocate total household time between family work, off-farm work and the demand for hired labour depends on the marginal productivity of labour (shadow price) and the market wage rate.

However, as specified in the literature, the household faces four types of regimes: (1) a regime in which it offers only its family labour off-farm, (2) a regime which its only hired on-farm labour, (3) a regime which simultaneously offers and hired labour, and (4) a regime which neither supply nor hired labour (Porgo et al., 2017). However, his participation depends on the market wage rate. As a result, he chooses the regime with the highest internal wage rate. Let r_j (j with $j=1,2,3,4$) be the wage rate of the regime. The household will choose the regime with the highest wage rate among the four alternatives described as preconditions for participating in the labour market.

Generally, the rural markets of developing countries are incomplete or imperfect. The decisions taken by the household in such a scenario are taken simultaneously (hypothesis of non-separation). The internal wage rate in such a situation is endogenous and depends on the characteristics of the household. The characteristics of the household being heterogeneous, we can then write the utility function of the household as follows:

$$r_{kj} = \beta_j' X_k + \varepsilon_{kj} \text{ avec } k = 1 \dots n \text{ et } j = 1 \dots 4 \quad (7)$$

Thus r_{kj} denotes the indirect utility function of household k in regime j and β_j' is the vector of parameters to be estimated. X_k , the vector containing the explanatory variables of the model for each household, ε_{kj} , the error term of the indirect utility

function of household k in regime j . Let us introduce a binary variable to consider the internal wage rate relative to each regime, which is not observable. Let us call this variable μ_{kj} and define it as follows:

$$\mu_{kj} = \begin{cases} 1 & \text{if } r_k \max(r_1, r_2, r_3, r_4) \\ 0 & \text{otherwise} \end{cases} \quad (8)$$

The probability that household k is found in the j^{th} alternative is given by the following formula:

$$\begin{aligned} Pr_{kj} &= \Pr(\mu_{kj} = 1) = \Pr(r_{kj} > r_{ki}) \forall j \neq i \\ &= \Pr(\beta_j' X_k + \varepsilon_{kj} > \beta_i' X_k + \varepsilon_{ki}) \forall j \neq i \\ &= \Pr\{(\beta_j' X_k - \beta_i' X_k > \varepsilon_{ki} - \varepsilon_{kj})\} = \Pr\{(\beta_j' - \beta_i') X_k > \varepsilon_{ki} - \varepsilon_{kj}\} \forall j \neq i \end{aligned} \quad (9)$$

In such a situation, the residuals are assumed to be independent and identically distributed according to Weibull law (McFadden, 1974; Porgo et al., 2017). Thus, (Maddala, 1983) suggests defining a multinomial model with definite probability as follows:

$$\Pr(\mu_{kj} = 1) = e^{(\beta_j' X_k) / \sum_{j=1}^4 \beta_j' X_k} \quad (10)$$

For simplification, one regime is chosen as the reference regime to standardise the system. Here, regime (4) was chosen as the reference regime by constraining its coefficient to zero. The reduced model is then written as follows:

$$\log \frac{Pr_{kj}}{Pr_{k4}} = \beta_j' X_k, \quad j = 1, 2, 3 \quad (11)$$

III. RESULTS AND DISCUSSION

Before proceeding with multinomial logit model estimation, it is recommended to consider whether it is appropriate to perform multinomial logit model analysis. First, the test for Independence of Irrelevant Alternative (IIA) is performed. A significant value of χ^2 would indicate a violation of the IIA hypothesis. In this study, the χ^2 values obtained are all insignificant at the 10% level. This shows that the independence assumption of irrelevant alternative cannot be rejected (Hausman & McFadden, 1984; Long & Freese, 2006). This allows us to conclude that the multinomial logit model analysis should be continue. Hausman's hypothesis test is given in Table 1.

TABLE 1
HAUSMAN TESTS OF THE IIA HYPOTHESIS

Omitted	Chi ²	P>Chi ²	Evidence
Autarky	15,83	0,15	For Ho
Hire labour	0,00	10,000	For Ho
Supply labour	2,31	0,9995	For Ho
Supply and Hire Labour	4,37	10,000	For Ho

The estimation results of the multinomial logit model are presented in Table 2 below. However, it is not easy to interpret the coefficients of the multinomial logit model directly because the sign of the estimated coefficient associated with a variable does not indicate in which direction the probability of the j^{th} regime varies with a variation of this variable. Therefore, it is recommended to calculate the marginal effects first. The results of the marginal effects are calculated and given in Table 2.

TABLE 2
REGRESSION COEFFICIENT OF MULTINOMIAL LOGIT MODEL

Variables	Supply labour			Hire labour			Supply and hire labour		
	Regime 1			Regime 2			Regime 3		
	Coefficients	Z	Marginal effects	Coefficients	Z	Marginal effects	Coefficients	Z	Marginal effects
Household characteristics									
Farm size	-0,20	-	-0,002	-0,15	-	-0,03	0,63	-	-0,00002
	(0,34)	0,59		(0,19)	0,78		(0,60)	1,04	
Gender of household head	-0,80	-	-0,14	-0,88	-	-0,003	4,66	0,01	0,00004
	(2,28)	1,23		(1,64)	0,53		(699,18)		
The age of the head of household	-0,55***	-	-0,008	-0,24	-	-0,04	0,36	0,84	0,00002
	(0,22)	2,53		(0,148)	1,59		(0,43)		
Squared age of head of household	0,004**	2,17	0,00006	0,0022	1,51	0,0003	-0,0030	-	-0,001
	(0,0020)			(0,0014)			(0,0039)	0,76	
Household head's level of education	-0,53	-	-0,004	-0,47	-	-0,08	-0,086	-	-0,02
	(0,72)	0,74		(0,48)	0,96		(0,74)	0,12	
% primary education labour	-2,47	-	-0,07	0,54	0,32	0,14	-1,91	-	-0,00007
	(2,37)	1,04		(1,66)			(3,50)	0,55	
Active men	0,001	0,00	0,0014	-0,08	-	-0,01	1,43	1,25	0,00005
	(0,67)			(0,42)	0,19		(1,14)		
Active women	2,60***	3,22	0,05	0,12	0,29	-0,02	2,38**	2,08	0,00007
	(0,81)			(0,42)			(1,14)		
Dependency ratio (%)	1,18	1,06	0,011	0,90	1,47	0,15	0,28	1,06	0,00002
	(1,12)			(0,61)			(1,21)		
Credit constraint	170	1,49	0,10	-2,50***	-	-0,48	-2,66*	-	-0,00003
	(1,14)			(0,66)	3,73		(1,47)	1,80	
Farm characteristics									
Farm size (da)	-0,43	-	-0,009	-0,04	-	-0,0008	-5,73**	-	-0,0002
	(0,76)	1,15		(0,22)	0,21		(2,90)	1,98	
Log input (FCFA)	0,85	1,23	0,05	0,82*	1,76	0,14	0,64	0,64	0,0046
	(0,38)			(0,46)			-1,003		
Log Farm Income (FCFA)	-0,09	-	-0,004	-0,39	-	-0,07	0,21	0,19	0,00002
	(0,70)	0,12		(0,44)	0,89		(1,07)		
Off-farm income (FCFA)	0,004***	4,01	0,00004	0,0028***	3,06	0,0005	0,0046***	3,77	0,003
	(0,0009)			(0,0009)			(0,0012)		
Livestock value	-	-	-0,04	-0,000016	-	-0,004	-0,00010**	1,93	-0,0004
	0,00008***	2,35		(0,16)	2,75		(0,00006)		
Village characteristics									
Dummy savings	-1,18	-	-0,02	-0,27	-	-0,04	0,71	0,39	0,0002
	(1,36)	0,87		(0,96)	0,28		(1,83)		
Distance from the financial institution to the village	-0,60***	-	-0,006	-0,44***	-	-0,07	1,72**	0,95	0,00007
	(0,25)	3,05		(0,16)	4,84		(0,89)		
Distance from farm to village	-0,16***	-	-0,001	-0,16***	-	-0,03	0,12	-	0,020
	(0,05)	2,73		(0,032)	1,07		(0,13)	1,82	
Constant	15,45*	1,68	-	12,04***	2,10	-	37,96	-	-
	(9,17)			(5,74)			(699,44)	0,05	

Note: Pseudo R² =0,5888; Log likelihood=-94,88 Restricted Likelihood=-230,75. * Significant at 1%, ** at 5%, * at 10%.**

Table 2 shows that the age of the household head has a non-linear and significant effect on the decision to participate to the labour market. However, the coefficient associated with the variable age of the head of household is statistically significant and negative in the labour supply regime. Indeed, the effect of the age of the household head on the supply of household labour outside the farm decreases until the age of 47 and then increases. In other words, compared to the reference regime (autarky regime), the probability that household headed by young household heads supply their labour outside the farm decreases by 0.80%. In contrast, the supply of labour employment of elderly households' heads increases significantly. According to this result, it is concluded that households headed by the young are less endowed with family labour and are less able to supply family labour outside the farm than households headed by the old ($p < 0.01$). Household headed by older are more likely to supply their labour outside their farm than younger ones.

The coefficient associated with the active women on the farm is positive and significant in regimes 1 and 3. This coefficient means that the increase in the active women in each of these regimes increases the probability of labour by 5% and 0.007% respectively compared to the reference regime (autarky). This result is consistent with empirical results found in previous studies, which state that as the number of active women in a household increases family labour use also increases (Porgo et al., 2017). It would be due to the possibility of substituting family labour on the farm.

The results confirm that credit constraints strongly influence the hiring regime for hiring labour. The credit constraint also impacts the regime in which the household is simultaneously a supplier and hiring labour. The results show that the credit constraint reduces the probability that the household participates in the hire labour regime by 48% compared to autarky households ($p < 0.01$). However, the credit constraint variable was negative and significant in regime-3. This variable means that credit constraint reduces the probability of the household being into the regime in which the household is simultaneously hiring and supplying labour at 0.003% ($p < 0.01$). In the presence of capital-intensive agriculture, access to agricultural credit remains one of the alternatives for coping with the cost of hiring labour and the short-term consumption needs of poor households.

Regarding the characteristics of the farm, we find that the area variable is negative and significant in regime-3. That means that the increase in the agricultural area reduces by 0.20% the probability that the household will be in the regime where the household is simultaneously hiring and supplying labour ($p < 0.05$). However, the variable represented the input expenditure is positive and significant at 10%. That means that the increase in input expenditure increases by 14% the probability that the household being into hiring labour regime compared to the autarky regime ($p < 0.10$).

The variable represented the non-agricultural income was statistically significant and positive at the 1% threshold. However, any increase in non-agricultural income leads to an increase of 0.004% and 0.05% in the probability of the household being in the supplying and hiring labour regime, respectively ($p < 0.01$). This result could be explained by households using only hired labour generally working in non-agricultural sectors. That means an increase in off-farm income will increase the demand for hiring labour on the farm. The increase in non-agricultural income increases by 0.3% the probability of the household being in the regime where the household is a hiring and supplying labour compared to the autarky situation ($p < 0.01$). The agricultural income variable positively affects rural households' decision to participate in the market. Under credit constraints, the local labour market is a natural alternative to generating non-agricultural income, making it possible to meet short-term liquidity needs. However, this strategy remains optimal if labour productivity on the farm is at least equal to the market wage. When the market wage is lower than the productivity of farm labour, the decision to participate in off-farm labour becomes a consumption-slowness strategy rather than an income-maximizing strategy. It can lead to loss of welfare for the household.

Village characteristics showed that the distance from the village to the microfinance institution has a negative effect on the probability of supplying family labour outside the farm and the likelihood of using hired labour on the farm. However, we also find that the distance variable from the village to the microfinance institution positively affects the probability of the household being in the regime where the household is simultaneously hiring and supplying labour. On the other hand, the distance between village to the microfinance institution reduces the probability of employing family labour outside the farm by 0.6% and the probability of using hiring labour in the farm by 0.007% compared to the reference situation ($p < 0.01$ and $p < 0.05$).

The variable represented village-to-farm variable also affects the household's decision to participate in the labour market. The distance from the village to the farm is negative in regimes 1 and 2. It also negatively affects the household's decision to participate in the labour market. The further the farm is from the village; the more labour costs increase. The further the farm is from the village, the more the probability that the household being in the supply labour and hiring labour regime decreases by 0.1% and 3% compared to the reference situation ($p < 0.01$ and $p < 0.01$).

IV. CONCLUSION

The agricultural sector in developing countries is characterised by low productivity and low capital-intensity agriculture and is dominated by small farms exposed to incomplete markets. Based on this, this study examines the rural labour market participation decisions in the Office du Niger zone in Mali. The data used in this study were obtained through stratified random sampling. According to the sampling method, 400 rice farmers were studied, and the data was obtained through a household survey conducted from March to June 2020.

This article analyses the behaviour of rural households using the agricultural household model. However, the multinomial logit model examined rural labour market participation decisions. To carry out this study, the households were classified into four work regimes, namely: the regime in which the household is only a supplier of labour, a regime in which the household is only a labour demander, the regime in which the household is simultaneously a supplier and a demander of labour and the regime where the household is neither a supplier nor a demander of labour.

The results show that young heads of households are less endowed with family labour and are less likely to offer their labour off the farm, while the labour supply off-farm by elderly household heads is increasing significantly. In other words, younger household heads are less likely to participate in the labour market as labour supplier than older household heads. However, households headed by women participate more in the labour market as labour supplier than households headed by men.

The results shows that the credit constraint strongly influences the household's decision to hire labour on the farm. Indeed, the increase in input expenditure encourages households to employ only hire labour on their farm. In contrast, increased off-farm income encourages households to participate in all three labour regimes. The presence of livestock, the household's proximity to a microfinance institution and the distance from the village to the farm reduce the household's participation in the labour supply market. However, the household's proximity to a microfinance institution and the distance from the village to the farm have been determined as factors to discourage the household's participation in the wage labour market.

According to the results obtained, improving human capital level within a household would give agricultural households more flexibility in their decision to participate in the labour market. In addition, measures taken to improve the human capital of a farm will promote the use of hired labour and allow households to work off the farm at the same time.

Furthermore, it is essential to promote subsidy policies for phytosanitary products to reduce family labour supply outside the farm. Policies to increase fertiliser use on farms would allow households to use wage labour on the farm and work off their farm simultaneously. In addition, to improve the labour market in the Office du Niger zone, the government must promote access to the credit market, likely to promote the use of hired labour on farms.

The farmers in the area are small-scale farms and need to be equipped with agricultural machinery and equipment to ensure the farm's activities. However, promoting agricultural machinery and equipment would be an excellent way to improve the use of agricultural machinery and equipment, promoting household participation in the labour market and increasing agricultural production.

Farms distant from villages are less likely to hire labour on their farms due to high transaction costs. Therefore, interventions to improve feeder roads would likely increase household participation in labour markets

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The Role of Pyrenomycetous Fungi in Environmental Ecology – A Review

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Abstract— Pyrenomycetous fungi, the diverse group within the Ascomycota phylum, play a critical role in environmental ecology through their involvement in the decomposition of plastic, organic matter and nutrient cycling. These fungi inhabit a variety of ecological niches, including soil, leaf litter, and decaying wood, where they contribute significantly to the breakdown of complex organic compounds. This decomposition process releases essential nutrients back into the ecosystem, promoting soil fertility and supporting plant growth. Pyrenomycetous fungi exhibit a wide range of enzymatic capabilities, including cellulases, laccases, esterases and peroxidases, which enable them to degrade cellulose, lignin, plastic and other complex organic molecules. Their activity accelerates the conversion of organic matter into humus, enhancing soil structure and water retention. Additionally, these fungi form intricate relationships with other soil microorganisms, facilitating the formation of stable soil aggregates and promoting microbial diversity. The ecological importance of saprophytic pyrenomycetous fungi extends beyond nutrient cycling. They play a pivotal role in carbon sequestration by decomposing organic material and regulating carbon dioxide release from soils. Their presence influences the dynamics of soil organic carbon pools, contributing to the mitigation of climate change. Furthermore, these fungi are involved in the detoxification of soil pollutants, including heavy metals and xenobiotics, through various biochemical pathways. Research into the biodiversity and functional roles of saprophytic fungi are essential for understanding ecosystem processes and developing sustainable land management practices.

Keywords— Ecology, Environment, Organic Matter, Pyrenomycetous Fungi, Saprophyte.

I. INTRODUCTION

Pyrenomycetes are fungi that mainly grow as tiny, flask-shaped fruiting bodies called perithecia that contain asci and ascospores. We can find them on a variety of surfaces like soil, animal waste, decaying leaves, wood, and even other fungi. These "little black dots" make up the largest group of fungi in the Ascomycota phylum. Pyrenomycetes are both ecologically and economically important because they include fungi which are often used in research, as well as harmful pathogens that cause diseases like chestnut blight, Dutch elm disease, and beech bark disease. These fungi are found all over the world in different ecosystems and geographical areas primarily as saprobes, playing a key role in breaking down organic matter and recycling nutrients. Most of them are hard to spot without a keen eye since their fruiting bodies are usually small (less than 1 mm in diameter) and dark-coloured, often blending into the surface of plant material, both living and dead. Many Pyrenomycetes also have a stroma, a much larger structure embedding the perithecia in fungal tissue. In narrow sense Pyrenomycetes encompass the class Sordariomycetes, but some species of Dothideomycetes, Eurotiomycetes and Lecanoromycetes with superficially similar fruiting bodies. The terms "Pyrenomycetes" is no longer used in a taxonomic sense and have been replaced by the terms Sordariomycetes, to refer to one major Class of fungi in the Phylum Ascomycota. In this paper the term "pyrenomycetes" is used in a general sense to refer to the fungi that occur in the class Sordariomycetes.

Fungi are the principal decomposers in terrestrial ecosystems and one of the most important ecological roles of fungi, is there function as decomposer also known as saprophyte. Fungi breakdown Complex organic matters such as dead plants, animals and other organic materials, into simpler compounds. This decomposition process is vital for nutrient cycling in ecosystems. Without this, organic materials would accumulate and essential nutrients like carbon, nitrogen and phosphorus would become trapped, preventing their return to the soil and the food chain. fungi secrete enzymes that breakdown tough substances such as

cellulose, Lignin and chitin which most other organisms cannot degrade efficiently. Pyrenomycetous fungi not only decompose organic matters but also play an important role in plastic biodegradation. The main classes of enzymes involved in plastic biodegradation are hydrolases and oxidoreductases. These enzymes have been extensively studied due to their involvement both in natural and industrial processes. For example, in nature and industry they are essential in lignocellulose biodegradation, fungal pathogenesis and the hydrolysis of triacylglycerol to fatty acids. These enzymes also have applications in the food and textile industries and in bioremediation processes.

1.1 Systematic Position of Pyrenomycetes:

Pyrenomycetes (Sordariomycetes of Eriksson and Winka 1998) is a class name formerly used to refer to a group of species with a particular shared morphology. It now is used to designate a clade that includes the orders Diaporthales, Halosphaeriales, Hypocreales, Lulworthiales, Microascales, Ophiostomatales, Phyllachorales, Sordariales and Xylariales [25]. Although the taxa in the clade encompass a wide range of macromorphologies and micromorphologies, the vast majority have a flask-shaped ascomata or perithecia. The Pyrenomycetes clade also includes numerous lineages of cleistothecial fungi, which represent multiple and repeated losses of the perithecial neck and ostiole [25]. Although almost all perithecial ascomycetes are included in the pyrenomycetes clade, there are a few exceptions. Pyxidiophora species and the Laboulbeniales are arthropod-associated ascomycetes comprising a separate clade based on SSU rDNA analyses.

In traditional taxonomy ascomycetes were classified according to their ascoma-types, with the class Pyrenomycetes including all taxa having perithecia. Later, the development of ascomata and the type of ascus were employed for higher-level classification, and consequently, Loculoascomycetes was separated from Pyrenomycetes [7,9,13]. However, molecular studies show that even these revised classifications were too coarse. The Loculoascomycetes fall into two distinct and not closely related groups, which are placed in two clades: Chaetothyriomycetidae and Dothideomycetes [14,16]. Ascospore morphology has been widely used in taxonomy of ascomycetes, and Sordariales is a prominent example of this. Molecular data suggest that ascomatal wall morphology is a better predictor of phylogenetic relationships in these fungi. Further, the molecular data helped to redefine the circumscription of Sordariales. The majority of non-lichenized pyrenomycetes form a monophyletic group: Sordariomycetes. However, the lichenized pyrenomycetes are highly polyphyletic.

Pyrenocarpous lichen-forming fungi occur in several lineages each in Dothideomycetes, Chaetothyriomycetidae and Lecanoromycetes, whereas no lichenized forms are currently known in the classical Sordariomycetes [27]. Sordariomycetes are also known as Pyrenomycetes, from the Greek *πύρην* - 'the stone of a fruit' - because of the usually somewhat tough texture of their tissue [5].

II. MATERIALS AND METHODS

Literature Review was the primary method of present study. The data gathered herein was from a variety of sources, including published research, online databases, and direct communications. Information on plastic-degrading fungi mostly came from platforms like Google Scholar, ResearchGate, PubMed, and Web of Science.

III. DISCUSSION

The members of Pyrenomycetes are found in very diverse condition in nature. They exist in a wide range of Habitats as biotrops, necrotrophs and as well as saprotrophs. The biotrobes are ecologically obligate parasite and 'in vivo' obtain nutrients only from living host cells. Many of the Pyrenomycetes are saprotrophs and cause damage to various objects like paper, leather, food stuffs and wood. They utilize non-living materials, other than that killed by the fungus itself. Saprotrophic Pyrenomycetes are usually found on decaying plant materials such as logs and stumps, dead twigs and branches of tree, dead leaves and stems of herbaceous plants. Others are coprophilous, inhabiting the dung of animals. Some of these perithecial Ascomycetes also are well known for their production of mycotoxins in stored grains, and these metabolites cause adverse effect in mammals. Pyrenomycetes are a group of fungi that are ecologically important and play a role in many ecosystems:

3.1 Lignin Degradation:

Lignin is a complex aromatic polymer found in the cell walls of vascular plants, providing rigidity and resistance to microbial attack. Saprophytic pyrenomycetous fungi possess specialized enzymes, such as lignin peroxidases and manganese peroxidases, which allow them to break down lignin. This process is essential for converting wood and other lignin-rich materials into simpler compounds, making nutrients available to other organisms. Effective lignin degradation contributes to soil formation and carbon cycling, as it releases carbon dioxide and other nutrients back into the ecosystem. The ability of

several Pyrenomycetes (*Biscogniauxia nummularia*, *Daldinia concentrica*, *Graphostroma platystoma*, *Hypoxylon howeanum*, *H. multifforme*, *H. truncatum*, *Rosellinia aquila* and *Ustulina deusta*) fungi to degrade Japanese beech (*Fagus crenata*) wood, microcrystalline cellulose and lignin-related dimeric compounds was studied. Changes in the lignin component of the wood during decay were also measured [24].

3.2 Cellulose Decomposition

Cellulose is a primary structural component of plant cell walls, consisting of long chains of glucose molecules. Pyrenomycetous fungi produce cellulases, enzymes that hydrolyze cellulose into simpler sugars. This decomposition process is crucial for recycling carbon and other nutrients, enabling the growth of plants and microorganisms in the soil. By breaking down cellulose, these fungi play a vital role in enhancing soil fertility and maintaining healthy ecosystems, especially in forested areas where plant debris accumulates.

3.3 Organic Matter Recycling:

Organic matter recycling refers to the breakdown of dead plant and animal material into simpler organic compounds by saprophytic fungi. Pyrenomycetous fungi are key players in this process, as they decompose various organic substrates, including leaf litter, dead wood, and animal remains. Through enzymatic activity, they convert complex organic materials into simpler forms that can be assimilated by soil microorganisms and plants. This process not only enriches the soil with essential nutrients but also helps in maintaining soil structure and health, promoting biodiversity and ecosystem stability.

3.4 Faunal Interaction:

Some pyrenomycetous fungi, such as *Trichoderma*, can trap nematodes, using them as a nutrient source while influencing soil food webs.

3.5 Plastic Biodegradation:

Plastic waste accumulates in the environment to hazardous levels, because it is inherently recalcitrant to biological degradation. Biodegradation is a complex process of physico-chemical transformation of polymers into smaller units mediated by microorganisms. Microorganisms, including pyrenomycetes fungi, are able to biochemically degrade, assimilate and metabolise complex organic compounds, xenobiotics and recalcitrant substances for their energy needs. The main classes of enzymes involved in plastic biodegradation are hydrolases and oxidoreductases. These enzymes have been extensively studied due to their involvement both in natural and industrial processes. For example, in nature and industry they are essential in lignocellulose biodegradation [6], fungal pathogenesis [3] and the hydrolysis of triacylglycerol to fatty acids [13]. *Chaetomium globosum*, *Cladosporium* [2] *Curvularia senegalensis* and *Trichoderma harzianum* [23] etc. improve and promote the biodegradation of complex and polluting polymers through enzyme like esterases, Laccases and Peroxidases.

3.6 Bioremediation:

Bioremediation is a process that occurs by using microorganisms that pollutants could transit or degrade into low-degree pollution. Bacteria, fungus, algae, and plants use in this method. In other words, enzymes of these microorganisms attack the pollutants and convert them to low risk pollutants [1,8]. In bioremediation after degradation of pollutions, the society of microorganisms reduced, and the presence of microorganisms don't create more pollutions [13]. Bioremediation is a new technology which can be used for treatment of a different group of environmental pollutants with other physical and chemical methods of treatment, simultaneously [17,26].

3.6.1 Microbial bioremediation:

Microbial bioremediation are a process that use spent biomass, enzymes, or microorganisms for removing environmental pollutions. Pollutants in the various environmental sections, always have contact with microorganisms due to the microorganisms' existence everywhere. Microbes allow the pollutant to be channeled into the normal microbial metabolic pathway for biotransformation and degradation. Microbes transform or break down the pollutants via their intrinsic metabolic processes with or without slight pathway modifications. In the bioremediation process, by using naturally occurring microbial catabolic capabilities, most of the synthetic compounds such as metals, radionuclides, polyaromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and hydrocarbons (e.g., oil) can be accumulated, transform or degrade [8, 22]. Microbial bioremediation includes processes such as aerobic process, anaerobic process, and combination of these two methods. Cellules that need the oxygen molecules presence during the cellular processes named aerobic, and cellulose that do not require for the

presence of oxygen molecules named anaerobic [15,17]. Microorganisms include fungi adsorb pollutions into their active sites and degrade the pollutions and convert them in other forms and finally remove the pollutions.

Some species of pyrenomycetous fungi (Sordariomycetes) are economically important as bio-control agents, [11] and other genera can produce a wide range of chemically diverse metabolites, that are important in agricultural, medicinal and other biotechnological industries. [12]

IV. CONCLUSION

Pyrenomycetes are a remarkably diverse and functionally significant group of fungi. Their roles in **decomposition, biodegradation, bioremediation, biocontrol, and industrial applications** highlight their ecological and economic importance. While some species pose challenges as plant pathogens, their potential in sustainable environmental solutions and industrial applications cannot be overlooked. Further research into their enzymatic pathways and genetic mechanisms will enhance our ability to harness their capabilities for environmental conservation, waste management, and biotechnological advancements.

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