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Preface

We would like to present, with great pleasure, the volume-12, Issue-3, March 2026 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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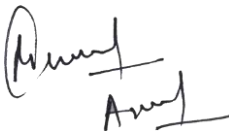
Each article in this issue provides an example of a concrete industrial application or a case study of the presented methodology to amplify the impact of the contribution. We are very thankful to everybody within that community who supported the idea of creating a new Research with *IJOEAR*. We are certain that this issue will be followed by many others, reporting new developments in the Environment and Agriculture Research Science field. This issue would not have been possible without the great support of the Reviewer, Editorial Board members and also with our Advisory Board Members, and we would like to express our sincere thanks to all of them. We would also like to express our gratitude to the editorial staff of AD Publications, who supported us at every stage of the project. It is our hope that this fine collection of articles will be a valuable resource for *IJOEAR* readers and will stimulate further research into the vibrant area of Environmental & Agriculture Research.



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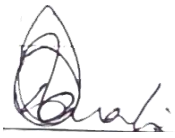

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Economical Method of Oyster Mushroom Cultivation in Urban Areas

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Abstract— *Mushrooms are a rich source of proteins and contain all essential amino acids. Eating mushrooms not only provides essential nutrients but also combats many diseases like cancer and diabetes. Oyster mushrooms are popular edible mushroom species which grow easily on paddy straw, wheat straw, paper waste, etc. The present paper discusses a very economical and eco-friendly method of growing mushrooms in urban areas like kitchen gardens and balconies. In the present protocol, no chemicals were used and steam sterilization was done to maintain aseptic conditions. The procedure to grow oyster mushrooms is affordable, and it is relatively easy to start a small-scale entrepreneurship business from home. Using this protocol, the first harvest was obtained within 25-30 days, and mushrooms were found fit for human consumption.*

Keywords— Oyster mushroom, Pleurotus ostreatus, urban farming, sustainable agriculture, food security.

I. INTRODUCTION

Mushrooms are saprophytic macro-fungi belonging to the family Basidiomycetes. Their mycelium penetrates deep into the substratum to absorb nutrition and forms the spore-bearing umbrella-shaped fruiting body above the substratum. The umbrella-like fruiting body is called the pileus and consists of gills and a stalk called the stipe. There are around 200 species of mushrooms that are edible. Oyster mushrooms (*Pleurotus* spp.), white button mushrooms (*Agaricus bisporus*), and milky mushroom (*Calocybe indica*) are widely grown mushrooms for edible purposes. The most cultivated mushroom species in the world is *Agaricus bisporus*, followed by *Lentinula edodes* and *Pleurotus* spp. (Aida et al., 2009). Mushrooms are highly nutritious, rich sources of antioxidants, potassium, zinc, and fibre. They are very good for weight loss as they are low in calories. Mushrooms have anti-cancer and anti-diabetic properties and also decrease cholesterol (Kurtzman, 1976; Priyadarshini and Kumar, 2020; Sahoo et al., 2021). Proteins of mushrooms have nutritional value comparable to meat, eggs, and milk, as they contain essential amino acids and have complete amino acid composition (Liu et al., 2025). They can serve as an alternative source to animal proteins in a vegetarian diet (Pashaei, 2024).

Foods that contain all essential amino acids are called complete proteins. Complete proteins are obtained from animal products like meat, poultry, and milk. Mushrooms contain all the essential amino acids, which include valine, isoleucine, phenylalanine, histidine, lysine, leucine, methionine, tryptophan, and threonine (Bach et al., 2017). These essential amino acids cannot be synthesized by humans and have to be supplied from the diet. Mushrooms are not only rich in all essential amino acids but also have dietary fibre and are complete proteins. They also contain β-glucans, which are polysaccharides present in the cell wall of mushrooms. They help in gastro-intestinal and bowel movement and are therefore good for digestion (Bach et al., 2017). β-glucans are also associated with prevention of cancer as they help in absorption of toxic substances in the body (Rosli et al., 2015; Ruthes et al., 2015; Bach et al., 2017).

Bach et al. (2017) evaluated nine edible mushrooms and concluded that they contain fibre content in the range of 24.4 to 46.62% and protein content varying from 16.47–36.96%, with very low fat content in the range of 1.40–2.08%. They also found that all nine mushrooms were rich in minerals like phosphorus, potassium, iron, copper, and zinc, and the sodium content was negligible.

In the present study, oyster mushroom *Pleurotus ostreatus* was used. It is known as "Dhingri" in India. It has a fan or oyster-shaped cap and can be grown easily on decaying wood or straw (Sharma, 2015).

The objective of the present study was to develop an economical and affordable protocol for the cultivation of oyster mushroom in small spaces like balconies using minimal methods without requiring much infrastructure. Our study shows that mushrooms can be grown easily in small spaces like kitchen gardens and urban balconies at low cost. It is a quite affordable way of growing them at home without using much infrastructure. It can be an alternative source to fulfil the requirement of animal proteins for vegetarians. Mushrooms can be grown in small areas, hence farmers with very small tracts of land can also make it a good source of livelihood.

II. MATERIALS AND METHODS

The kit to grow oyster mushroom was procured from a commercial seller on an online platform (Figure 1A). The kit consisted of very affordable and easily available materials. The kit had spawn, paddy straw substrate, two polypropylene (PP) growing bags, a spray bottle, a cutter, and a manual. The manual consisted of a step-by-step guide on how to make a bed for cultivating oyster mushroom. All the experiments were done in the month of October-November 2025. Clean conditions were maintained to prevent any contamination by other fungi and bacteria.

Protocol:

1. Paddy straw provided in the kit was taken according to one polypropylene (PP) plastic bag.
2. Paddy straw was soaked in clean tap water overnight, and excess water was removed by straining in a porous tray for 1-2 hours.
3. The straw was boiled in a big container for 1 hour, and it was kept covered for an hour for steam sterilization (Figure 1B).
4. After one hour, the straw was taken out and spread on a clean cloth. It was kept for air-drying for 3-4 days and covered with a very thin cloth to prevent contamination (Figure 1C).
5. To check whether the straw was ready for making the bed, one straw was taken and pressed between fingers. When no water came out but moisture was present, the straw was ready to make the bed.
6. The spawn provided in the kit was broken in a plate and divided into 8 parts. For one PP bag, four parts of spawn were taken (Figure 1D-F).
7. Each PP bag was filled with two inches of straw and spread with one part of spawn. Again, it was filled with two inches of straw and spread with one part of spawn. This was continued until the fourth part (Figure 1G).
8. Finally, the top spawn was covered by two inches of straw, and the top of the PP bag was tied tightly with the help of a rubber band.
9. For aeration, eight to ten small holes were made with the help of a safety pin in the PP bag (Figure 1G).
10. The PP bags were kept in a clean plastic hanging pot (cleaned with a hand sanitizer) and covered with a clean cloth. They were kept in the balcony in a shaded region behind palm leaves and covered with cloth in such a way that dark conditions were maintained (Figure 1H-I).
11. The bags were left undisturbed for 15-20 days and sprayed with a little water to keep the cloth moist until the straw turned whitish in colour.
12. After 15-20 days, some more holes were made with pins in the PP bag.
13. After 25-30 days, the fruiting bodies of oyster mushroom started emerging from the holes (Figure 1J-K).
14. The whole set-up was kept moist all the time to prevent dehydration.

15. The first harvest was ready for collection after 25-30 days. Mushrooms were harvested when fully mature (Figure 1L).
16. Mushrooms were harvested from the beds by twisting from the bottom, taking care not to damage the remaining mycelium (Figure 1L).
17. After harvest, oyster mushrooms were eaten and were found fit for human consumption.

III. RESULTS AND DISCUSSION

After the COVID-19 pandemic, people have become more health-conscious and seek healthy vegetarian food options. Herbal drugs and organic food have become very popular. Home-grown vegetables rich in fibre and protein have become healthy and popular food choices. People have started growing vegetables for daily consumption at home as well as for small start-up businesses. Mushrooms have emerged as an alternative source of animal proteins, as they are rich in all essential amino acids. They are also rich in vitamin D. Mushrooms contain large amounts of the plant sterol "ergosterol," which is a precursor of vitamin D (Sharma, 2015). When stimulated by light, it converts to vitamin D. Mushrooms have all the good qualities of a rich source of nutrients, proteins, and vitamin D.

Proteins for human consumption mainly come from animal sources like meat, milk, and eggs, which contain all essential amino acids. However, mushroom proteins can be an alternative source of animal proteins, as mushroom proteins show similarity to animal proteins (Liu et al., 2025). Their proteins have similarities to both animal and plant proteins (Kurtzman, 1976; Ayimbila and Keawsompong, 2023). Mushrooms contain an average of 23.80 g/100 g dry weight of proteins (Ayimbila and Keawsompong, 2023). Apart from proteins, mushrooms also contain niacin and biotin (Chand and Singh, 2022). Thus, mushroom proteins are complete sources of all essential amino acids and nutrients, but when compared to other vegetables, they are quite expensive to buy. However, they can be easily grown at home for daily consumption.

Mushrooms grow easily on paddy and wheat stalks. Agricultural waste products like straw, leaves, stems, and roots can be used to grow protein-rich mushrooms. The use of agricultural waste in the production of mushrooms helps in the conversion of waste to food. It also helps in combating air pollution as it reduces stubble burning.

While growing mushrooms, paddy straw can be sterilized by either steam sterilization or chemical fungicides. During steam sterilization, the paddy straw is boiled for one hour and then kept covered in steam for sterilization. Boiling makes the straw soft and sterilizes it by killing harmful fungi, bacteria, and other pathogens. Mushrooms grow well on soft surfaces. In the present study, the steam sterilization method was adopted to avoid any chemical fungicides. Polypropylene bags used to cultivate mushrooms were also sterilized with hand sanitizer before putting the straw and spawn bedding. Hands were sterilized properly during the whole procedure to prevent any contamination.

In the protocol used in our study, layering in the polybag was done with alternating layers of straw and spawn until four layers of spawn were added. The bag was closed tightly by tying a knot with the help of a rubber band, and tiny holes were made for aeration. Polybags were kept in a clean location in hanging pots covered with a cloth and away from direct sunlight. After around 20-25 days, the polybag turned white at the spawning stage. After two to three days, fruiting bodies started emerging from the holes. Fully grown oyster mushrooms were ready for the first harvest within 3-4 days of emergence of the fruiting body. Rapid growth of oyster mushroom was observed during evening and night compared to daytime. It was observed that the second harvest was less vigorous in yield compared to the first harvest. After harvest, oyster mushrooms were cooked and eaten and were found to be very delicious and fit for human consumption.

According to the United Nations, sustainable food diets are environmentally friendly and help in food and nutrition security for present and future generations. Sustainable diets help in the conservation of biodiversity. They are economical, affordable, nutritious, healthy, and optimize natural and human resources (Burlingame and Dernini, 2012).

By using the protocol described in the present study, mushroom cultivation can be done at home year-round. The protocol is completely chemical-free and uses only organic materials, making it very eco-friendly and pocket-friendly. The protocol is easy to follow and yields a good amount of oyster mushrooms for daily home consumption. If done on a slightly larger scale, it is affordable to start a mushroom-selling business from home, as it does not require much infrastructure. It can also be used

to generate income through small-scale entrepreneurship by the sale of oyster mushrooms. It is a sustainable way of living, mitigating pollution, and utilizing waste to produce wonder. Farmers with very little land and with meagre means can grow mushrooms throughout the year and generate income, as the procedure is affordable.

(A)

(B)

(C)

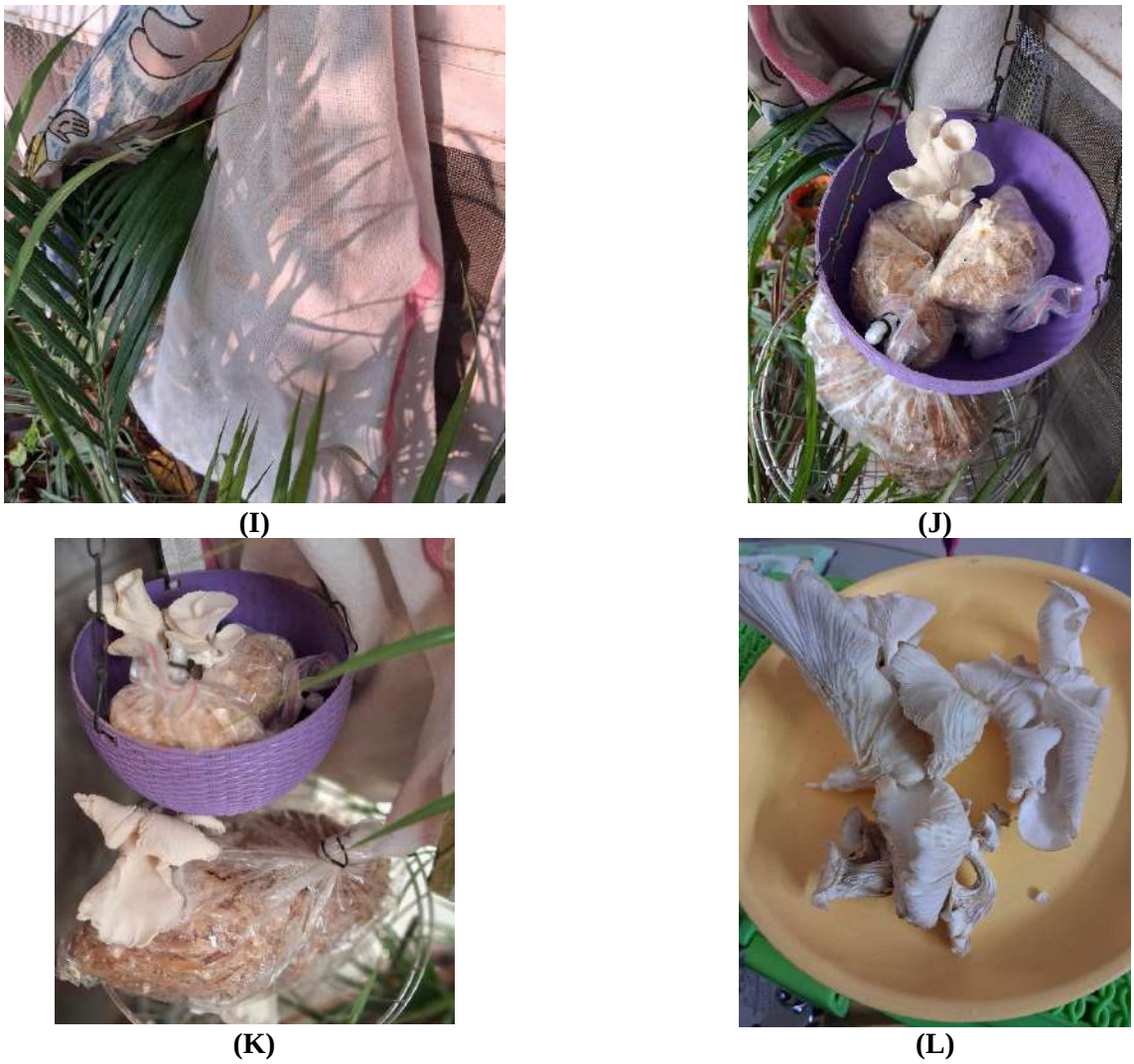
(D)

(E)

(F)

(G)

(H)



(I) **(J)**

(K) **(L)**

FIGURE 1: Steps in oyster mushroom cultivation:

(A) Commercial mushroom growing kit; (B) Steam sterilization of paddy straw; (C) Air-drying sterilized straw; (D-F) Spawn preparation and division; (G) Layering in polypropylene bag with aeration holes; (H-I) Bags placed in hanging pots covered with cloth; (J-K) Fruiting bodies emerging from holes; (L) Harvested mature oyster mushrooms.

IV. CONCLUSION

The present study demonstrates a simple, economical, and eco-friendly method for cultivating oyster mushrooms in urban spaces such as balconies and kitchen gardens. The protocol uses steam sterilization instead of chemical fungicides, ensuring chemical-free produce. The materials required are affordable and easily available, making this method accessible to urban dwellers and small-scale entrepreneurs. The first harvest was obtained within 25-30 days, and mushrooms were found to be of good quality and fit for consumption. This method not only provides a sustainable source of protein-rich food but also utilizes agricultural waste, thereby reducing environmental pollution. It offers an opportunity for income generation through small-scale entrepreneurship and can be particularly beneficial for farmers with limited land resources. The protocol aligns with sustainable development goals by promoting local food production, reducing waste, and supporting nutrition security.

CONFLICT OF INTEREST

The author declares no conflict of interest

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Genetic Variability and Qualitative Traits in Pole-Type French Bean (*Phaseolus vulgaris* L.) Genotypes

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***Abstract*—** *Thirty-two pole-type French bean genotypes were evaluated in a randomized block design with two replications during Rabi 2022–23 at the Regional Agricultural Research Station, Vijayapura, University of Agricultural Sciences, Dharwad, Karnataka, India. High genotypic and phenotypic coefficients of variation (GCV and PCV >20%) were recorded for protein content and reducing sugar content in pods, indicating substantial genetic variability with minimal environmental influence and good scope for improvement through direct selection. Moderate GCV and PCV (10–20%) for non-reducing sugar content in pods suggested the involvement of both additive and non-additive gene effects, reflecting adequate variability and the usefulness of phenotypic selection. High heritability (>60%) coupled with high genetic advance as per cent of mean (>20%) was observed for protein, reducing sugar, and non-reducing sugar contents in pods, indicating predominance of additive gene action. Therefore, direct selection would be effective for improving these traits. Evaluation of qualitative pod traits revealed significant variation for pod colour, shape, curvature, stringlessness, beak position, and beak orientation among genotypes. Genotypes IC-632961 and IIHR-01 were identified as superior based on round, stringless pods with attractive medium green colour, making them desirable for both consumer preference and commercial cultivation*.

***Keywords*— GAM, Heritability, GCV, PCV, Reducing sugar, Protein, Pole type French bean.**

I. INTRODUCTION

French bean (*Phaseolus vulgaris* L.) is one of the most common and widely grown vegetable crops in India with a chromosome number of 2n=22. According to Vavilov (1950), the origin of French bean is Southern Mexico and Central America, while the Peruvian-Ecuadorian-Bolivian area is considered to be a secondary centre of origin. It was originated from wild species *Phaseolus aborigineus* (L.) and domesticated in Mexico, Peru, and Colombia about 8000 years ago. This crop has extensive geographical distribution in the world.

French bean is known by several names related to its purpose of usage as vegetable viz., string bean, snap bean, salad bean, haricot bean, and green bean. However, the terms bean, dry bean, kidney bean, and navy bean are designated to pulses (George, 1985). Furthermore, string bean, dwarf bean, and pole bean pertain to distinct growth patterns. In different languages, it has diverse identities viz., rajmash in Hindi and tingala avare in Kannada. French bean varieties are categorized based on their growth habits i.e., bush type with compact segments, semi-pole type with more extended segment, and pole type with viny growth longer than semi-pole type (Prabhakar et al., 2016).

It is cultivated all over the world and has a wide geographical distribution. French bean is mainly used for immature green pods. Rajmash or dried pods are utilized as a pulse and provide a good source of proteins for humans (Abate, 2006). Immature pods are eaten fresh and can be easily preserved by freezing, canning, or dehydrating. Dried beans are eaten as

boiled, baked, fried, or ground into flour. It is highly nutritious as 100 g of green pods contain 1.7 g protein, 4.5 g carbohydrates, 221 IU vitamin A, 11 mg vitamin C, and 50 mg calcium (Gopalakrishnan, 2007). French bean can be used to some extent against diabetes and cardiac problems and is a wonderful natural cure for bladder burn. It has both carminative and reparative qualities in the treatment of constipation and diarrhea (Duke, 1981).

It is a cool season vegetable, extensively grown in temperate and subtropical areas, as well as in many parts of tropical regions with a temperature around 16 to 24 °C. Among prominent food legumes globally, French bean ranks third after soybean (*Glycine max* L.) and peanut (*Arachis hypogea* L.). Furthermore, it stands second position in terms of beans vegetables (Singh, 1999). The present study was undertaken to evaluate genetic variability and qualitative traits in pole-type French bean genotypes to identify superior lines for breeding programs.

II. MATERIALS AND METHODS

2.1 Experimental Site and Design:

The experiment was conducted at the Regional Agricultural Research Station (RARS), Vijayapura during *Rabi* 2022-23. Thirty-two genotypes of pole-type French bean were grown in a randomized block design with two replications. Ridges and furrows were opened at 120 cm and seeds of different genotypes were sown by dibbling on one side of the ridge at 30 cm. Plots were irrigated immediately after the completion of sowing. Where seeds did not germinate, gaps were filled by re-sowing within a week. All other activities were carried out as per the recommended package of practices (RPP) given by University of Horticultural Sciences (UHS), Bagalkot (Anon., 2022) to grow the crop. Observations were recorded visually on five randomly selected plants from each genotype.

2.2 Quality Parameters:

2.2.1 Pod Colour:

The colour of harvested pods for vegetable purpose was observed in each genotype under natural daylight and grouped into green/dark green/light green.

2.2.2 Pod Shape:

The shape of pod was observed at edible stage for all the genotypes and was grouped into round or elliptic shapes.

2.2.3 Pod Curvature:

The pod curvature was observed and recorded at edible stage for all the genotypes and was grouped into straight or curved or slightly curved.

2.2.4 Pod Stringlessness:

The pod stringlessness was observed by breakup of the pod at edible maturity stage for all the genotypes and grouped into stringed or stringless.

2.2.5 Pod Beak Position:

The pod beak position was observed visually at edible maturity stage for all the genotypes and grouped into marginal or non-marginal.

2.2.6 Pod Beak Orientation:

The pod beak orientation was observed visually at edible maturity stage for all the genotypes and grouped into straight or upward or downward.

2.2.7 Protein Content in Pods (g/100 g):

Protein content in pods from each genotype was estimated by following Lowry's method. A sample of harvested green pods at marketable maturity was shade dried and ground to fine powder. A sample of 0.5 g was taken and ground with five ml of phosphate buffer solution with the help of pestle and mortar. The powdered sample was centrifuged and supernatant was used. The standards of 0.2 ml, 0.4 ml, 0.6 ml and up to 3 ml were pipetted out from working solution of bovine serum albumin (BSA). The volume of each tube was made up to three ml. A blank with 3 ml of distilled water was also maintained. Exactly 0.1 ml of sample was taken into a test tube and made into 3 ml by adding distilled water. Five ml of alkaline copper reagent was added to each test tube and incubated for ten minutes at room temperature. 0.5 ml of folin-ciocalteau reagent

(FCR) was added to each test tube and kept for 30 minutes in dark condition and spectrophotometer readings were observed at 660 nm. The protein content in pods was calculated with the help of standard graph and expressed in g per 100 g of green pods (Lowry et al., 1951).

2.2.8 Reducing Sugar in Pods (%):

The reducing sugar in pods was estimated by using 3,5-dinitrosalicylic acid (DNS) method. A sample of harvested green pods at marketable maturity was shade dried and ground to fine powder. A sample of 100 mg was taken and ground in boiling 80% ethyl alcohol thoroughly in a mortar with pestle for 5-10 minutes to extract the sugars. The sample extract was centrifuged for 10 minutes and supernatant was collected and evaporated on boiling water bath. Ten ml of water was added to the supernatant to dissolve the sugars. The standards of 0.2 ml, 0.4 ml, 0.6 ml and up to 3 ml were pipetted out from working solution of glucose. One ml of alcohol-free extract was taken into test tubes and volume was made up to 3 ml with water in all test tubes. Three ml of DNS reagent was added to a blank and heated for 5 minutes in boiling water bath. After the colour development, one ml of sodium potassium tartarate solution was added and mixed thoroughly. The test tubes were cooled under running tap water and absorbance was measured at 510 nm using reagent and blank was adjusted to zero absorbance. The amount of reducing sugar in the sample was calculated using a standard graph prepared from working standard glucose solution in the same manner (Thimmaiah, 1999)..

$$\text{Reducing sugars in the sample (\%)} = \frac{\text{Sugar value from graph (µg)} \times \text{Total volume of alcohol free extract (10 ml)}}{\text{Aliquot of alcohol free extract used (ml)} \times \text{Weight of sample (100 mg)}} \times \frac{1}{1000} \quad (1)$$

2.2.9 Non-Reducing Sugar in Pods (%):

The non-reducing sugar in pods was estimated by using 3,5-dinitrosalicylic acid (DNS) method. A sample of harvested green pods at marketable maturity was shade dried and ground to fine powder. A sample of 100 mg was taken and ground in boiling 80% ethyl alcohol thoroughly in a mortar with pestle for 5-10 minutes to extract the sugars. The sample extract was centrifuged for 10 minutes and supernatant was collected and evaporated on boiling water bath. Ten ml of water was added to the supernatant to dissolve the sugars. One ml of extract was pipetted out and 1 ml of 1N H_2SO_4 was added to hydrolyze the mixture by heating at 49°C for 30 minutes (the acid hydrolysis is effective in splitting the sucrose-type linkages). After the test tubes were cooled, 1 or 2 drops of methyl red indicator was added and the contents were neutralized by adding 1N NaOH drop wise from a pipette. The standards of 0.2 ml, 0.4 ml, 0.6 ml and up to 3 ml were pipetted out from working solution of glucose. Three ml of DNS reagent was added and heated for 5 minutes in boiling water bath. After the colour development, one ml of sodium potassium tartarate solution was added and mixed thoroughly. The test tubes were cooled under running tap water and absorbance was measured at 510 nm using reagent and blank was adjusted to zero absorbance. The amount of non-reducing sugar in the sample was calculated using a standard graph prepared from working standard glucose solution in the same manner (Thimmaiah, 1999).

$$\text{Non-reducing sugars in the sample (\%)} = \frac{\text{Sugar value from graph (µg)} \times \text{Total volume of extract (10 ml)}}{\text{Aliquot sample (1 ml)} \times \text{Weight of sample (100 mg)}} \times \frac{1}{1000} \quad (2)$$

2.3 Statistical Analysis:

Genotypic and phenotypic coefficients of variation were calculated according to the method suggested by Burton and de Vane (1953). Heritability in broad sense and genetic advance as per cent of mean were estimated using the formula given by Johnson et al. (1955). The range, mean, and standard error were computed for each trait.

III. RESULTS AND DISCUSSION

3.1 Quality Parameters:

High PCV and GCV (Table 1) were observed for protein and reducing sugar content in pods, indicating the existence of a broad genetic base, which would be amenable for further selection. Similar results were also obtained by Singh et al. (2020).

Moderate PCV and GCV (Table 1) were observed for non-reducing sugar in pods, indicating the presence of moderate to good amount of variability for these traits in the genetic stock, which would be amenable for further selection. These results are in agreement with Tanuja et al. (2021) and Prakash and Ram (2014).

High heritability (>60%) along with high GAM (>20%) was recorded for protein, reducing sugar, and non-reducing sugar content in pods (Table 1). Therefore, the additive component is predominant and hence, direct selection would be more effective in improving these traits. Similar results were also noticed by Singh et al. (2020), Razvi et al. (2018), and Lad et al.

(2017). Jhanavi et al. (2018) and Prakash and Ram (2014) in their experiments reported similar findings for protein content in pods, and Singh et al. (2020) for reducing sugar and non-reducing sugar content in pods.

TABLE 1
ESTIMATES OF RANGE, MEAN, COMPONENTS OF VARIANCE, HERITABILITY AND GENETIC ADVANCE FOR QUALITY TRAITS IN POLE-TYPE FRENCH BEAN GENOTYPES

Sl. No.	Characters	Range	Mean ± S.Em	Components of variance					Genetic advance	
				PV (σ^2p)	GV (σ^2g)	PCV (%)	GCV (%)	h^2 (%)	GA	GAM (%)
	Quality parameters									
1	Protein content in pods (g/100 g)	0.96 - 2.27	1.66 ± 0.05	0.14	0.13	22.89	22.45	96.17	0.75	45.36
2	Reducing sugar in pods (%)	2.36 - 5.75	4.02 ± 0.18	0.88	0.81	23.31	22.38	92.14	1.78	44.25
3	Non-reducing sugar in pods (%)	0.95 - 1.93	1.24 ± 0.06	0.04	0.03	16.15	14.13	76.58	0.31	25.48

GV = Genotypic variance; PV = Phenotypic variance; GCV = Genotypic coefficient of variation; PCV = Phenotypic coefficient of variation; h^2 = Heritability (broad sense); GA = Genetic advance; GAM = Genetic advance as per cent over mean

3.2 Pod Colour:

The dark green pods (Table 2) were observed in genotypes IC-636225, IC-636226, IC-582514, IC-326977, and IC-026624. The genotypes IC-636224, IC-636241, IC-641919, IC-280818, IC-341797, IC-341807, IC-430379, EC-398555, IC-326978, IIHR-02, IC-313320, Super King, and US-2 were included in the light green pods group. Medium green pods were noticed in genotypes IC-636245, IC-313309, IC-632961, IC-582511, IC-538077, IC-328398, IIHR-01, Marlida, Arka Sukomol, and Lakshmi. The genotypes IC-636240 and IC-538039 were grouped under medium green with purple stripes. IC-341922 (medium green with red stripe) and IC-538073 (dark green with purple stripe) had different fruit colour patterns.

3.3 Pod Shape:

Elliptic shaped pods (Table 2) were noticed in genotypes IC-636224, IC-636225, IC-636226, IC-636241, IC-636245, IC-641919, IC-280818, IC-341797, IC-341807, IC-341922, IC-636240, IC-430379, IC-313309, EC-398555, IC-582514, IC-538073, IC-538039, IC-328398, IC-326978, IC-326977, IC-026624, IC-313320, IC-582511, IIHR-02, and IC-538077. The genotypes IC-632961, IIHR-01, Arka Sukomol, Lakshmi, Marlida, Super King, and US-2 were grouped under round shaped pods.

3.4 Pod Curvature:

Slightly curved pods (Table 2) were observed in genotypes IC-636226, IC-636241, IC-636245, IC-280818, IC-341797, IC-341807, IC-636240, IC-430379, IC-313309, EC-398555, IC-582514, IC-538073, IC-538039, IC-328398, IC-326978, IC-326977, IC-026624, IC-313320, IC-582511, IC-538077, IIHR-01, IIHR-02, Arka Sukomol, Marlida, and US-2. Straight pods were observed in genotypes IC-636224, IC-636225, IC-641919, IC-341922, and Lakshmi. The genotypes IC-636225, IC-632961, and Super King had curved pods.

3.5 Pod Stringlessness:

Stringed pods (Table 2) were observed in genotypes IC-636224, IC-636225, IC-636226, IC-636241, IC-636245, IC-641919, IC-280818, IC-341797, IC-341807, IC-341922, IC-636240, IC-430379, IC-313309, EC-398555, IC-582514, IC-538073, IC-538039, IC-328398, IC-326978, IC-326977, IC-026624, IC-313320, IC-538077, Arka Sukomol, and Lakshmi. Stringless pods were noticed in genotypes IC-632961, IC-582511, IIHR-01, IIHR-02, Marlida, Super King, and US-2.

3.6 Pod Beak Position:

Marginal pod beak position (Table 2) was observed in genotypes IC-636225, IC-636226, IC-636245, IC-641919, IC-280818, IC-341797, IC-341807, IC-636240, IC-430379, IC-313309, EC-398555, IC-582514, IC-538073, IC-538039, IC-328398, IC-326977, IC-026624, IC-313320, IC-538077, Arka Sukomol, Lakshmi, IC-632961, IC-582511, IIHR-01, IIHR-02, Marlida,

Super King, and US-2. Non-marginal pod beak position was observed in genotypes IC-636224, IC-341922, IC-636241, and IC-326978.

3.7 Pod Beak Orientation:

Straight pod beak orientation was observed in genotypes (Table 2) IC-636225, IC-636226, IC-636241, IC-641919, IC-280818, IC-636240, IC-582514, IC-538073, IC-538039, IC-328398, Super King, and US-2. Upward pod beak orientation was observed in genotypes IC-636224, IC-636245, IC-341797, IC-341807, IC-326977, IC-313320, IC-582511, IC-538077, Arka Sukomol, Lakshmi, and Marlida. Downward pod beak orientation was observed in genotypes IC-430379, IC-313309, EC-398555, IC-026624, IC-632961, IIHR-01, and IIHR-02.

TABLE 2
QUALITATIVE TRAITS OF POLE-TYPE FRENCH BEAN GENOTYPES

Sl. No.	Genotypes	Pod colour	Pod shape	Pod curvature	Pod stringness	Pod beak position	Pod beak orientation
1	IC-636224	Light green	Elliptic	Straight	Stringed	Non-marginal	Upward
2	IC-636225	Dark green	Elliptic	Curved	Stringed	Marginal	Straight
3	IC-636226	Dark green	Elliptic	Slightly curved	Stringed	Marginal	Straight
4	IC-636241	Light green	Elliptic	Slightly curved	Stringed	Non-marginal	Straight
5	IC-636245	Medium green	Elliptic	Slightly curved	Stringed	Marginal	Upward
6	IC-641919	Light green	Elliptic	Straight	Stringed	Marginal	Straight
7	IC-280818	Light green	Elliptic	Slightly curved	Stringed	Marginal	Straight
8	IC-341797	Light green	Elliptic	Slightly curved	Stringed	Marginal	Upward
9	IC-341807	Light green	Elliptic	Slightly curved	Stringed	Marginal	Upward
10	IC-341922	Medium green with red stripe	Elliptic	Straight	Stringed	Non-marginal	Straight
11	IC-636240	Medium green with purple stripe	Elliptic	Slightly curved	Stringed	Marginal	Straight
12	IC-430379	Light green	Elliptic	Slightly curved	Stringed	Marginal	Downward
13	IC-313309	Medium green	Elliptic	Slightly curved	Stringed	Marginal	Downward
14	EC-398555	Light green	Elliptic	Slightly curved	Stringed	Marginal	Downward
15	IC-582514	Dark green	Elliptic	Slightly curved	Stringed	Marginal	Straight
16	IC-538073	Dark green with purple stripe	Elliptic	Slightly curved	Stringed	Marginal	Straight
17	IC-538039	Medium green with purple stripe	Elliptic	Slightly curved	Stringed	Marginal	Straight
18	IC-328398	Light green	Elliptic	Slightly curved	Stringed	Marginal	Straight

Sl. No.	Genotypes	Pod colour	Pod shape	Pod curvature	Pod stringness	Pod beak position	Pod beak orientation
19	IC-326978	Light green	Elliptic	Slightly curved	Stringed	Non-marginal	Straight
20	IC-326977	Medium green	Elliptic	Slightly curved	Stringed	Marginal	Upward
21	IC-026624	Dark green	Elliptic	Slightly curved	Stringed	Marginal	Downward
22	IC-632961	Medium green	Round	Curved	Stringless	Marginal	Downward
23	IC-313320	Light green	Elliptic	Slightly curved	Stringed	Marginal	Upward
24	IC-582511	Medium green	Elliptic	Slightly curved	Stringless	Marginal	Upward
25	IC-538077	Medium green	Elliptic	Slightly curved	Stringed	Marginal	Upward
26	IIHR-01	Medium green	Round	Slightly curved	Stringless	Marginal	Downward
27	IIHR-02	Light green	Elliptic	Slightly curved	Stringless	Marginal	Downward
28	Arka Sukomol	Medium green	Round	Slightly curved	Stringed	Marginal	Upward
29	Lakshmi	Medium green	Round	Straight	Stringed	Marginal	Upward
30	Marlida	Medium green	Round	Slightly curved	Stringless	Marginal	Upward
31	Super King	Light green	Round	Curved	Stringless	Marginal	Straight
32	US-2	Light green	Round	Slightly curved	Stringless	Marginal	Straight

Note: Pod colour categories include dark green, light green, medium green, medium green with purple stripe, dark green with purple stripe, and medium green with red stripe. Pod shape: elliptic or round. Pod curvature: straight, slightly curved, or curved. Pod stringness: stringed or stringless. Pod beak position: marginal or non-marginal. Pod beak orientation: straight, upward, or downward.

IV. CONCLUSION

From the present study, the maximum protein content in pods was recorded in genotype EC-398555 (2.11 g per 100 g) followed by IC-538073 (2.07 g per 100 g) and IC-313320 (2.05 g per 100 g). These genotypes demonstrated superior nutritional potential compared to the others, indicating their suitability for improving dietary protein content through selection and breeding programs. Based on pod characteristics such as shape, stringlessness, and colour, the genotypes IC-632961 and IIHR-01 were identified as superior. These genotypes produced round, stringless pods with an attractive medium green colour, making them more desirable in comparison to the other genotypes studied. Their favourable pod traits suggest potential value for both consumer preference and commercial cultivation. The high heritability coupled with high genetic advance for protein and sugar contents indicates that additive gene action predominates, making direct selection effective for improving these nutritional traits.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Phylogenetic Relationships, Genetic Diversity, and Neutrality Tests of Nigerian Cattle Populations in Taraba State

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Abstract— *The research investigated the genetic diversity and genetic neutrality of cattle populations in Taraba State, Nigeria. The study analyzed 100 reference populations, and 28 blood samples were used for mitochondrial DNA sequencing using Flinders Technology Associates (FTA) paper, which covered five locations (Iware, Wukari, Donga, Gembu, and Jalingo) and five breeds (Bokoloji, Muturu, Red Bororo, White Fulani, Adamawa Gudali). The research utilized Tajima's Neutrality Test, Tajima's Relative Rate Clock Test, and phylogenetic analysis to determine patterns of molecular evolution and population structure. The analysis of location-based neutrality showed that all populations tested positive for Tajima's D, with Iware recording 2.73 D value, Wukari showing 3.33 D value, Donga presenting 1.99 D value, and Gembu achieving 4.04 D value. The nucleotide diversity (π) measured between 0.5759 in Donga and 0.6781 in Gembu, indicating moderate to high genetic variability, whereas Wukari and Gembu displayed the most segregating sites with S = 778 and 779. The findings demonstrate evolution that deviates from neutral patterns due to three factors: balancing selection, population subdivision, and historical demographic patterns. The breed-based analysis produced positive Tajima's D results, reaching peak values in White Fulani (D = 4.49) and Red Bororo (D = 4.05), with both breeds showing high nucleotide diversity (π = 0.6535 and 0.6989, respectively). The Bokoloji (D = 1.25) and Muturu (D = 1.00) results showed reduced polymorphism levels, with S = 562 and 512. The Tajima's Clock Test results showed that evolutionary rates differed significantly between study locations. Iware showed the highest number of identical sites (202) and very few divergent sites (6), but a pronounced imbalance in unique substitutions, specially in sequence A (536). Wukari, Donga, and Gembu showed more divergent sites, with their total counts reaching 244, 229, and 193 respectively, while their unique differences among sequences appeared to be distributed more evenly, which proved the molecular clock predictions to be less accurate. The analysis of phylogenetic relationships demonstrated that different breeds of cattle from different regions showed shared ancestry together with genetic mixing from different populations. The research results demonstrate that Taraba State cattle populations possess high genetic diversity together with non-neutral evolutionary patterns and different rates of evolutionary change, which affect both conservation efforts and breeding programmes.*

Keywords— Genetic diversity, Tajima's neutrality test, Cattle breeds, Phylogenetic analysis.

I. INTRODUCTION

Cattle (*Bos taurus* and *Bos indicus*) represent one of the most economically and culturally important livestock species globally, delivering essential resources that include meat, milk, hides, and draft power while functioning as vital components for both agricultural operations and rural community development (Mwai et al., 2021; Talenti et al., 2022). The cattle population in Nigeria serves as an essential resource for both food security and economic development while shaping the sociocultural traditions of northern communities who have practiced pastoralism for thousands of years (Sikiru et al., 2022). The country contains multiple native cattle breeds that developed through adverse environmental conditions, which include tropical weather patterns, transmission of trypanosomiasis and tick-borne illnesses, and the two different livestock management approaches of nomadic herding and stationary agriculture (Nwachukwu et al., 2022). The genetic structure, evolutionary links, and population history of these groups need to be examined because this knowledge helps develop

effective conservation approaches which create breeding systems that maintain production gains and safeguard essential adaptive genetic resources (Xu et al., 2025).

The evolutionary history of African cattle displays multiple complicated patterns that resulted from domestication, animal movement, and breeding between two different cattle types—humpless taurine cattle and humped zebu cattle—which began to separate from each other 150,000 to 500,000 years ago (Bonfiglio et al., 2024). Genetic and archaeological data show that taurine cattle originated in the Near East about 10,000 years ago and then spread to Africa, while zebu cattle developed from their wild ancestors in the Indus Valley about 8,000 years ago and spread throughout Africa (Pitt et al., 2022). The phenotypic diversity of contemporary African cattle results from their ability to adapt to different agro-ecological zones combined with their genetic heritage from multiple ancestral groups, which produce different taurine and indicine ancestry patterns throughout their diverse populations (Kim et al., 2023). Whole genome analysis shows that indigenous African cattle developed unique genetic characteristics through natural selection which enabled them to withstand environmental stresses, combat diseases, and survive high temperatures, thus making them key genetic resources for maintaining sustainable livestock production in tropical environments (Freitas et al., 2021; Kambal et al., 2023).

Recent genomic studies show that zebu cattle began entering African cattle herds during two major migration windows between 750 and 1,050 years ago. Research shows that African populations have strong genetic links to zebu through autosomal and Y-chromosomal zebu ancestry, while their mitochondrial DNA studies only show taurine maternal lineages, proving that male zebu ancestry entered Africa through the importation of zebu bulls from South Asia during the last three thousand years (Ward et al., 2022). This specific mitonuclear discordance pattern establishes evidence for mitonuclear coadaptation, because natural selection protects taurine mitochondrial genomes in African environments while populations maintain high levels of nuclear zebu ancestry (Kwon et al., 2022). African cattle populations today show the highest genetic diversity among all domestic livestock, with heterozygosity values between 0.30 and 0.37 and nucleotide diversity values exceeding those of European commercial breeds, because their populations maintain large effective sizes, experience minimal bottleneck events, and have continuous gene flow between groups (Freitas et al., 2021; Xu et al., 2025).

Nigeria holds the position of having the second largest cattle population in Africa, with more than 20 million cattle spread throughout its various agricultural regions (Sikiru et al., 2022). The country showcases its native cattle breeds through zebu-type breeds which include White Fulani, Red Bororo, Sokoto Gudali, Adamawa Gudali, Rahaji, and Wadara, and taurine breeds which consist of Muturu, N'Dama, and Keteku, and hybrid groups which display mixed traits (Mauki et al., 2022). Zebu cattle dominate the northern Nigerian Sahelo-Sudanian regions through extensive pastoral and transhumance practices which Fulani herders employ (Tijjani et al., 2022), while southern and Middle Belt regions house taurine breeds that benefit from their natural trypanotolerance to combat tsetse fly (*Glossina* spp.) transmitted trypanosomiasis (Nwachukwu et al., 2022). The northeastern Nigerian state of Taraba functions as an essential cattle genetic resource area because it contains multiple herds which include major genetic samples from the Mambilla Plateau, featuring its high-altitude grasslands and special weather patterns. Taraba State emerged as a key research destination for Nigerian cattle genetics studies because recent genomic research discovered 44 cattle samples from this region (Mauki et al., 2022). The combination of different environmental conditions throughout the state leads to multiple selection forces which result in different genetic patterns and adaptive abilities for regional cattle breeds that match the adaptations found in Ethiopian highland cattle (Terefe et al., 2022; Zegeye et al., 2022).

Researchers have studied the hypervariable displacement loop (D-loop) region of mitochondrial DNA through molecular analysis to study maternal ancestry, evolutionary relationships, and historical population movements among global cattle breeds (Dorji et al., 2022; Sällman Almén et al., 2022). The D-loop region exhibits high mutation rates and lacks recombination, thus researchers use it for tracing matrilineal descent and studying population distribution patterns (Demir et al., 2023). Researchers using mitochondrial DNA to conduct phylogenetic studies found distinct taurine cattle haplogroups (T, T1, T2, T3, T4, T5, P, Q, R) and zebu cattle haplogroups (I1, I2), which showed specific geographic patterns of distribution (Bonfiglio et al., 2024). African cattle studies showed that African taurine populations had a dominant T1 haplogroup distribution pattern, which indicates that strong maternal founder effects occurred during the colonization process of Africa (Pitt et al., 2022). Researchers have made recent advances in mitochondrial genomics through the development of whole mitochondrial genome sequencing, which enables better phylogenetic analysis and more precise determination of evolutionary timelines and population characteristics (Dorji et al., 2022; Sällman Almén et al., 2022). The mtDNA research of Nigerian cattle shows that the population has high haplotype diversity, because 80% of the population carries distinct haplotypes resulting from ongoing gene flow between groups (Adeola et al., 2021). Neutrality tests provide powerful

statistical frameworks for detecting departures from neutral evolution and identifying genomic regions subjected to natural or artificial selection.

The complete molecular analysis of Taraba State Nigerian cattle breeds remains unfinished because their economic value and unique genetic traits require further examination, which includes using genetic neutrality assessments to find specific genomic regions that show signs of selection (Sikiru et al., 2022; Mauki et al., 2022). This study will investigate three research areas by studying Taraba State Nigerian cattle populations through mitochondrial D-loop sequence analysis, which will provide haplotype data, establish phylogenetic links, reveal population genetic distribution, and show how evolution has deviated from neutral patterns to help create research-backed conservation methods for indigenous genetic resources which will enhance breeding efficiency and climate adaptability (Mwai et al., 2021; Xu et al., 2025).

II. MATERIALS AND METHODS

2.1 Study Area and Sample Collection:

Taraba State exists within northeastern Nigeria, extending between 6°25' N and 9°30' N and between 9°30' E and 11°45' E, covering 54,473 square kilometers. The state contains various landforms which include lowland Guinea savanna and the Mambilla Plateau, which reaches elevations above 1,600 meters to become one of West Africa's highest plateaus. The region experiences a tropical wet and dry climate which produces annual temperatures between 18°C and 35°C and yearly precipitation totals between 1,000 mm and 1,500 mm (Mauki et al., 2022).

2.2 Experimental Animals and Sample Collection:

The study used a reference population of 100 cattle, from which 28 blood samples were used for mitochondrial DNA sequencing. Blood samples were collected using Flinders Technology Associates (FTA) paper. The study used five breeds of cattle: Bokoloji, Muturu, Red Bororo, White Fulani, and Adamawa Gudali. Blood samples (5 mL) were collected from the jugular vein of apparently healthy adult cattle (>2 years old) using Flinders Technology Associates (FTA) paper. Phenotypic characteristics and herd owner information were used to identify the sampled animals. The Animal Ethics Committee of the Department of Animal Science, Federal University Wukari, Nigeria granted ethical approval for this research study, which used standard veterinary procedures after obtaining informed consent from cattle owners.

2.3 DNA Extraction:

The extraction of genomic DNA from whole blood samples was performed using a commercial DNA extraction kit which followed the manufacturer's instructions with slight adjustments that were developed specifically for cattle blood samples. The process required 200 µL of blood to be combined with 20 µL of proteinase K and 200 µL of lysis buffer (Buffer AL), which underwent incubation at 56°C for 10 minutes to achieve full cell destruction. The mixture was centrifuged at 8,000 rpm for 1 minute after the addition of 200 µL of absolute ethanol to the spin column. The column underwent two wash cycles with wash buffers (AW1 and AW2) before DNA was extracted using 100 µL of 70°C preheated elution buffer (Buffer AE). The researchers used a NanoDrop ND-1000 spectrophotometer (Thermo Scientific, Wilmington, USA) to evaluate DNA concentration and purity, which showed A260/A280 ratios between 1.8 and 2.0 during the assessment of suitable samples for downstream applications. The researchers confirmed DNA integrity through the use of 1% agarose gel electrophoresis which had been stained with ethidium bromide. The researchers kept extracted DNA samples in storage at -20°C until they conducted PCR amplification.

2.4 PCR Amplification of mtDNA D-loop Region:

The mitochondrial DNA D-loop hypervariable region was amplified using universal bovine-specific primers designed to amplify an approximately 600–900 bp fragment. The primer sequences used were: Forward primer (BovDL-F): 5'-CCACTATCAGCACCCAAAGC-3' and Reverse primer (BovDL-R): 5'-GCGGGTTGCTGGTTTCACG-3' (Demir et al., 2023). PCR amplification was performed in a 25 µL reaction volume containing 12.5 µL of 2× DreamTaq Green PCR Master Mix (Thermo Scientific), 1.0 µL (10 µM) of each primer, 2.0 µL of template DNA (approximately 50–100 ng), and 8.5 µL of nuclease-free water. Amplifications were carried out in a thermal cycler (Applied Biosystems Veriti, USA) using the following cycling conditions: initial denaturation at 95°C for 5 minutes; 35 cycles of denaturation at 95°C for 30 seconds, annealing at 58°C for 45 seconds, and extension at 72°C for 1 minute; followed by a final extension at 72°C for 10 minutes (Dorji et al., 2022; Demir et al., 2023). PCR products were visualized on 1.5% agarose gel stained with GelRed and documented using a gel documentation system. Amplicons showing clear single bands of expected size were purified using the QIAquick PCR Purification Kit (Qiagen) according to the manufacturer's instructions.

2.5 DNA Sequencing and Sequence Editing:

Purified PCR products were sequenced bidirectionally (forward and reverse) using the Sanger sequencing method on an ABI 3730xl automated sequencer (Applied Biosystems). Sequencing reactions were performed using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) with the same primers used for PCR amplification. Raw sequence chromatograms were visually inspected for quality using Chromas v2.6.6 software (Technelysium Pty Ltd, Australia). Forward and reverse sequences were aligned and edited manually to generate consensus sequences using BioEdit v7.2.5 (Hall, 1999). Ambiguous bases and poor-quality regions at sequence terminals were trimmed, and sequences were aligned to the bovine mitochondrial DNA reference sequence (GenBank accession number V00654) to verify correct amplification of the D-loop region. Final consensus sequences were trimmed to uniform length to facilitate comparative analysis.

2.6 Sequence Alignment and Haplotype Identification:

Multiple sequence alignment of all 28 D-loop sequences was performed using the MUSCLE (Multiple Sequence Comparison by Log-Expectation) algorithm implemented in MEGA11 software (Molecular Evolutionary Genetics Analysis version 11) (Tamura et al., 2021). The MUSCLE algorithm was chosen due to its superior accuracy and speed for aligning moderately sized datasets compared to ClustalW (Edgar, 2004). Alignment parameters were set to default values with gap opening penalty of -2.9 and gap extension penalty of 0. The alignment was manually inspected and refined where necessary to ensure correct positioning of indels and to remove ambiguous alignment regions. Unique haplotypes were identified using DnaSP v6.12.03 (DNA Sequence Polymorphism) software (Rozas et al., 2017), which collapses identical sequences into single haplotypes and assigns haplotype designations.

2.7 Phylogenetic Analysis:

The phylogeny was inferred using the Maximum Likelihood method and Tamura-Nei (1993) model of nucleotide substitutions, and the tree with the highest log likelihood (-20,110.51) is shown. The initial tree for the heuristic search was selected by choosing the tree with the superior log-likelihood between a Neighbor-Joining (NJ) tree and a Maximum Parsimony (MP) tree. The NJ tree was generated using a matrix of pairwise distances computed using the Tamura-Nei (1993) model. The MP tree had the shortest length among 10 MP tree searches; each performed with a randomly generated starting tree. The analytical procedure encompassed 24 coding nucleotide sequences using 1st, 2nd, 3rd, and non-coding positions, with 790 positions in the final dataset. Evolutionary analyses were conducted in MEGA12 utilizing up to 4 parallel computing threads.

2.8 Tajima's Test for Neutrality:

Tajima's D (Tajima, 1989) compares the average number of pairwise nucleotide differences (π) with the number of segregating sites (S). Under neutral evolution, Tajima's D $\approx$ 0. Significantly negative values ($D < 0$, $P < 0.05$) indicate an excess of rare alleles consistent with population expansion, purifying selection, or selective sweeps, while significantly positive values ($D > 0$, $P < 0.05$) suggest balancing selection or population contraction (Yurchenko et al., 2023). Fu and Li's D and F* (Fu and Li, 1993) compare the number of singleton mutations with the total number of mutations (D*) or with the average number of pairwise differences (F*). Significantly negative values indicate departures from neutrality consistent with population expansion or positive selection.

III. RESULTS AND DISCUSSION

3.1 Phylogenetic Analysis:

The results of phylogenetic analysis are displayed in Figure 1. The phylogenetic tree establishes genetic relationships among different indigenous Nigerian cattle breeds through its analysis of nucleotide sequence data. The branch lengths in the study show genetic distances through their measurement system, which uses shorter branches to indicate closer genetic relationships and longer branches to show greater evolutionary divergence (Kumar et al., 2022; Stecher et al., 2020). This phylogenetic approach enables the reconstruction of evolutionary histories and assessment of genetic differentiation among cattle populations, which is essential for conservation genetics and breeding program design (Weldenegker et al., 2024; Gobena et al., 2024).

The analysis included individuals from several indigenous Nigerian cattle breeds: White Fulani, a widely distributed zebu breed known for milk production and heat tolerance; Red Bororo, a zebu breed valued for meat production and pastoral adaptability; Muturu, an endangered West African shorthorn taurine breed with trypanotolerance; Bokoloji, a lesser-known

indigenous breed; and Adamawa Gudali, a large-framed zebu breed adapted to the Adamawa highlands. The breed name and location code are included in each individual label, which uses J to represent Jalingo, G to represent Gembu, W to represent Wukari, D to represent Donga, and I to represent Iware, along with an individual identifier and sometimes sex designation where M indicates male and F indicates female. The sampling strategy uses multiple locations to assess inter-breed genetic diversity and intra-breed genetic diversity, which is essential for understanding population structure in extensively managed pastoral systems (Kim et al., 2023; Upadhyay et al., 2021).

The phylogenetic tree displays multiple genetic patterning methods which enable researchers to study how Nigerian cattle breeds evolved their relations to one another. The tree shows that members from the same breed tend to cluster together, because their genetic links remain strong even when they travel to different locations. The study results demonstrate that genetic structure depends on breed identity, because Nigerian cattle breeds show distinct genetic patterns which continue to exist after interbreeding (Tijjani et al., 2021; Weldenegker et al., 2024). White Fulani individuals create the largest genetic group, which shows significant subgroups that display high genetic diversity within their breed. This breed shows genetic variation which stems from its distribution throughout Nigeria and the Sahel area because of its large breeding population and extensive movement of herders (Kim et al., 2023; Bahbahani et al., 2021). Research into population genomics has shown that Fulani zebu cattle possess mosaic genomes which result from historical breeding between indicine and taurine cattle breeds during different time periods (Kim et al., 2023; Gobena et al., 2024).

Red Bororo individuals form a distinct cluster separate from White Fulani, despite both being classified as zebu breeds. These two pastoral populations show genetic differences because they experienced separate reproductive isolation periods and faced different selection pressures (Smetko et al., 2021). Red Bororo cattle display breed-specific selection signatures which scientists discovered through genome-wide studies that link to their thermotolerance, disease resistance, and morphological traits (Zhou et al., 2023; Porto-Neto et al., 2022). The Muturu samples form a highly divergent branch, which proves their identity as West African shorthorn taurine cattle (*Bos taurus*) with only slight zebu ancestry. The genetic distance between these two groups represents the historical separation of taurine and indicine cattle lineages which happened about 250,000 years ago (Tijjani et al., 2021; Kim et al., 2023). The Muturu population maintains unique trypanotolerance and humid tropical adaptation alleles which serve as essential genetic assets, although the population faces endangered status according to recent genomic conservation research (Tijjani et al., 2021; Barbato et al., 2022). The Bokoloji samples create a small distinct cluster, which indicates that they have different genetic characteristics compared to more commonly studied breeds. The study must proceed with caution because it has a limited sample size of two individuals. The researchers need to conduct expanded sampling because it is necessary to establish the genetic position of this breed which exists within the broad range of Nigerian cattle diversity (Kardos et al., 2021).

The breed clusters display geographic patterns which become most evident through the analysis of White Fulani samples from different regions. This pattern arises from two factors which include localized adaptation to different agro-ecological zones and management practices that prevent gene flow and founder effects which exist in particular herding communities (Ginja et al., 2023; Weldenegker et al., 2024). Researchers found through population genomic research on African cattle that environmental factors and ethnic breeding methods created distinct genetic patterns in different populations (Kim et al., 2023; Upadhyay et al., 2021). The phylogenetic structure that this analysis reveals establishes two key effects which impact both scientific research on evolution and the development of effective conservation methods. Genetic testing showed clear distinctions between the five breeds which include White Fulani, Red Bororo, Muturu, Bokoloji, and Adamawa Gudali. The endangered breed Muturu displays special significance because it possesses exclusive taurine ancestry and special adaptive features which zebu breeds do not possess (Tijjani et al., 2021; Gobena et al., 2024). The subclustering observed within White Fulani and Red Bororo suggests maintenance of substantial genetic variation, which is favorable for adaptive potential and breeding programs. High within-breed diversity protects populations from inbreeding depression while supplying breeders with essential genetic material to select for their needs during times of environmental shifts (Kardos et al., 2021; Weldenegker et al., 2024). The tree displays breed-level differences, but some individuals show intermediate placements because of their historical or current blending with other groups. West African cattle already display through their genome-wide SNP studies which demonstrate their expected mutation patterns.

The current phylogenetic study which depends on restricted sequence information shows useful results about breed connections, although complete genome analysis with thousands of SNPs and whole-genome sequencing will provide better results for identifying minor population patterns, modern hybridization events, and selection signatures (Weldenegker et al., 2024; Gobena et al., 2024). The best method to fully assess Nigerian cattle genetic resources requires researchers to combine phylogenetic data with population genomic data and phenotypic data (Kim et al., 2023; Ginja et al., 2023).

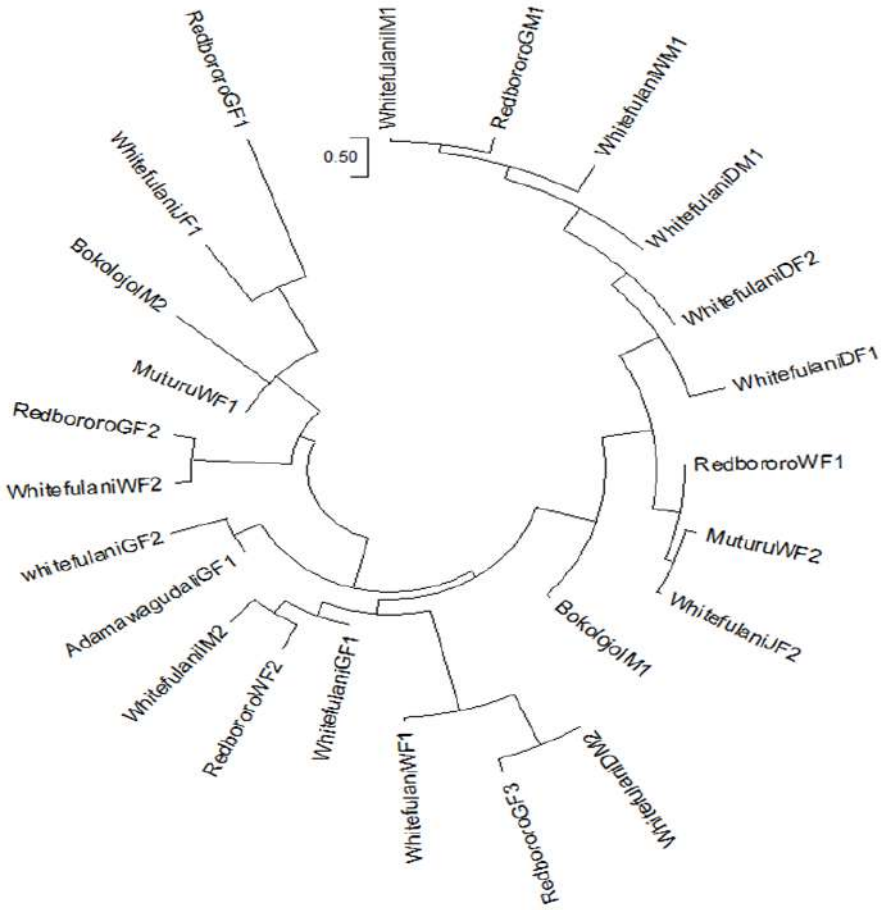

FIGURE 1: Evolutionary analysis of cattle in Taraba State

3.2 Tajima's Neutrality Test Results:

The results of Tajima's Neutrality Test for cattle populations from four locations in Taraba State are shown in Table 1, which uses nucleotide sequence data to study genetic variation patterns and neutral evolution deviations. The analyzed sequences across all locations showed similar results because they examined four to seven sequences and had 696 to 779 total sites. All studied populations showed high segregating sites (Ps) results which ranged from 0.887 to 0.991, which demonstrated substantial polymorphism between cattle populations in the entire research area. Watterson's theta (Θ), which estimates mutation rate through segregating sites, showed moderate values across locations, which indicates that all populations follow similar mutation patterns. Genetic variation estimation through this method remains strong for evaluating livestock genetic diversity across natural and human-driven breeding practices (Kardos et al., 2021). Nucleotide diversity (π) values in Donga reached 0.5759 while Gembu showed a higher value of 0.6781, which demonstrates moderate to high genetic diversity among both cattle populations. Populations with large effective population sizes show nucleotide diversity which results from multiple gene flow sources (Makina et al., 2020; Melka and Schenkel, 2021). Recent studies on African cattle breeds have documented comparable levels of nucleotide diversity which they attribute to admixture between indigenous and exotic breeds and to extensive pastoral mobility across agro-ecological zones (Smetko et al., 2021; Kim et al., 2020).

The most important finding shows that all tested locations show positive Tajima's D results which range from 1.99 at Donga to 4.04 at Gembu. Positive Tajima's D results show that alleles exist at intermediate frequencies which exceed the standard neutral model prediction, thus indicating that evolutionary processes operate outside of standard neutral evolution (Tajima, 1989; Cadzow et al., 2022). The observed pattern occurs because of balancing selection, population structure or admixture, or recent population bottlenecks (Charlesworth, 2020; Simonsen et al., 1995). The particularly high Tajima's D values recorded in Gembu and Wukari show that these populations exhibit stronger neutrality deviations because their cattle populations experience higher genetic background admixture and face environmental adaptation selective pressures. Recent genomic studies on West African cattle have identified significant population stratification driven by historical introgression events and adaptation to distinct climatic conditions, including heat tolerance and disease resistance (Tijjani et al., 2021;

Smetko et al., 2021; Mbole-Kariuki et al., 2020). The elevated Tajima's D in Gembu may also reflect adaptation to the cooler highland environment of the Mambilla Plateau, where selective pressures differ markedly from lowland areas (Bahbahani et al., 2021).

Donga showed the lowest Tajima's D value among all competitors, but it maintained a positive value which demonstrated that Donga experienced less intense evolutionary forces than other competitors. The presence of these factors indicates that the region has undergone more recent genetic mixing or experiences weaker selection pressures when compared to other areas (Nielsen et al., 2020). The results of the Tajima's Neutrality Test demonstrate that Taraba State cattle populations do not follow the pattern of strict neutral evolution. The population shows non-neutral evolution which is driven by two factors that include balancing selection and population subdivision according to different agro-ecological zones and cattle movements throughout the area. The research results support existing genomic studies on African cattle because they demonstrate that African cattle show intricate evolutionary patterns which include hybridization with local populations and changes in population size (Upadhyay et al., 2021; Kim et al., 2023; Porto-Neto et al., 2022). The study expects to achieve better genomic selection through the use of genome-wide SNP data because it will help researchers find specific genomic areas affected by selection and allow them to measure how demographic history and adaptive mechanisms operated in the past.

TABLE 1
RESULTS FROM TAJIMA'S NEUTRALITY TEST OF CATTLE BASED ON LOCATION IN TARABA STATE

Location	m	n	S	Ps	Θ	π	D
Iware	4	786	758	0.964	0.526	0.662	2.725
Wukari	7	785	778	0.991	0.405	0.635	3.334
Donga	4	784	696	0.888	0.484	0.576	1.989
Gembu	7	790	779	0.986	0.402	0.678	4.036

Abbreviations: m = number of sequences, n = total number of sites, S = Number of segregating sites, ps = S/n, Θ = ps/a1, π = nucleotide diversity, and D is the Tajima test statistic*

Table 2 displays the results of Tajima's Neutrality Test for the four tested cattle breeds which include Bokoloji, Muturu, Red Bororo, and White Fulani. The number of sequences analyzed (m) varied across breeds, ranging from 3 in Bokoloji and Muturu to 16 in White Fulani, reflecting differences in sampling intensity among breeds. The results show that all breeds demonstrate strong evidence of non-neutral evolution. This finding holds true despite limited sample sizes in Bokoloji and Muturu which decrease statistical power for testing results. The positive Tajima's D values which showed consistent results across all breeds demonstrate that non-neutral evolutionary patterns have produced strong evolutionary evidence.

The segregating site proportion showed moderate results in Bokoloji at 0.715 and in Muturu at 0.655. Red Bororo showed high polymorphism levels which reached 0.984 and White Fulani reached 0.997. Watterson's theta (Θ) showed moderate values across different breeds because it estimates population mutation rate from segregating sites, which showed higher values in Bokoloji and Muturu than in Red Bororo and White Fulani. The difference in Θ results from how allele frequencies change across different polymorphic levels according to Tajima (1989) and Kardos et al. (2021). The high number of segregating sites found in Red Bororo and White Fulani aligns with genomic research which shows that West African cattle breeds experienced extensive zebu and taurine genetic mixing (Kim et al., 2023; Weldenegker et al., 2024).

The study found genetic diversity within all breeds to range from 0.536 in Muturu to 0.699 in Red Bororo. The high π values in Red Bororo and White Fulani suggest that their effective population sizes remain large while their gene flow patterns resemble those found in widespread indigenous cattle breeds which receive extensive management (Melka & Schenkel, 2021; Upadhyay et al., 2021). Recent whole-genome sequencing studies have confirmed that Fulani zebu breeds including White Fulani and Red Bororo exhibit elevated genomic diversity attributable to historical introgression events and continued admixture with multiple cattle populations across the Sahel region (Tijjani et al., 2021; Bahbahani et al., 2021).

The nucleotide diversity of Muturu shows lower values which match the breed's status as an endangered trypanotolerant shorthorn taurine breed that exists only in limited areas and has a small breeding population base according to research by Tijjani and his team (2021) and Barbato and his team (2022). The conservation genomic research findings have detected

historic population bottlenecks together with genetic drift patterns in Muturu populations which require specific breeding initiatives to safeguard their genetic diversity according to Smetko et al. (2021) and Gobena et al. (2024). The breeds all showed positive Tajima's D values which proved their evolutionary patterns had changed from neutral genetic evolution. The D values of Bokoloji and Muturu showed moderately positive D values which indicated the presence of weak balancing selection together with mild population structure. The moderate deviations show two possible explanations which include either localized environmental adaptation or recent demographic changes which include founder effects and bottlenecks according to Charlesworth (2020) and Nielsen (2021).

The study found that Red Bororo and White Fulani populations displayed highly positive Tajima's D values which demonstrated their intermediate allele frequencies. The observed patterns result from balancing selection and population subdivision and genetically distinct population mixing instead of recent population growth (Tajima, 1989; Cadzow et al., 2022; Simonsen et al., 1995). The genomic studies conducted recently showed that Red Bororo and White Fulani West African zebu cattle possess adaptive introgression from both African taurine and indicine sources, which scientists found through selection analysis of genes linked to thermotolerance, disease resistance, and milk production (Porto-Neto et al., 2022; Bahbahani et al., 2021; Ginja et al., 2023). The genetic changes in Red Bororo and White Fulani show strong deviation from neutrality because of their ability to breed across various geographical areas, their genetic mixing patterns, and their various environmental and management circumstances. The two factors create conditions which enable organisms to maintain their genetic variation together with their special adaptive traits that support their capacity to thrive in diverse environments (Kim et al., 2023; Weldenegker et al., 2024). The research on African zebu cattle through genome-wide association studies (GWAS) discovered loci under balancing selection which govern innate immunity, heat shock protein control, and parasite resistance—the vital abilities needed for survival in tropical climates (Smetko et al., 2021; Zhou et al., 2023).

The high Tajima's D values in these breeds test present genetic patterns which continue to evolve through multiple ancestral connections. The population genomic studies revealed that modern Fulani cattle populations maintain genetic contact with their neighboring breeds, resulting in ancestral patterns that produce intermediate-frequency alleles throughout their genomic structure (Kim et al., 2023; Upadhyay et al., 2021; Barbato et al., 2022). This should explain why intermediate allele frequencies decrease through genetic drift and inbreeding in Muturu dog breeds which face strong selection for adaptive traits (Gobena et al., 2024). The study results show that non-neutral evolutionary processes determine genetic differences between the investigated cattle breeds, with widespread breeds showing stronger effects than local and genetically distinct breeding groups. The research results demonstrate that African indigenous cattle conservation and breeding programs need to include demographic history and admixture patterns and selection pressure as essential elements for their success (Weldenegker et al., 2024; Zhou et al., 2023). Future research using high-density SNP arrays together with whole-genome sequencing will enable researchers to identify selection patterns while uncovering how organisms adapt through genetic changes that violate natural selection rules (Ginja et al., 2023; Nielsen, 2021).

TABLE 2
RESULTS FROM TAJIMA'S NEUTRALITY TEST OF CATTLE BASED ON BREEDS

Breeds	m	n	S	Ps	Θ	π	D
Bokoloji	3	786	562	0.715	0.477	0.577	1.246
Muturu	3	782	512	0.655	0.436	0.536	1
Red Bororo	6	790	777	0.984	0.431	0.699	4.055
White Fulani	16	786	784	0.997	0.321	0.654	4.486

Abbreviations: m = number of sequences, n = total number of sites, S = Number of segregating sites, ps = S/n, Θ = ps/a1, π = nucleotide diversity, and D is the Tajima test statistic*

3.3 Tajima's Clock Test Results:

Table 3 displays Tajima's Clock Test results for four Taraba State cattle populations which were tested in Wukari, Iware, Donga, and Gembu. The test examines sequence divergence among three representative sequences from each population, providing insight into molecular clock consistency and evolutionary rate variation among sites (Tajima, 1993). This method enables researchers to identify molecular clock violations which occur when different selection pressures, population structure, and demographic changes take place (Kumar & Hedges, 2021; Lartillot and Poujol, 2024).

The quantity of matching sites which appeared in all three genetic sequences showed significant differences between various testing sites. Iware preserved 202 sites which represent its most conserved elements because its sequence patterns show lower divergence from other genetic materials. Donga and Gembu displayed intermediate numbers of identical sites (91 and 116, respectively). Geographically nearby groups show different genetic patterns which result from distinct population histories and different environmental pressures according to Kardos et al. (2021) and Charlesworth (2020). The highest number of shared genetic differences between three sequences occurred at 244 sites in Wukari and 229 sites in Donga, which demonstrated extensive genetic variation. Gembu had 193 divergent sites while Iware had only 6, which supports its high number of identical sites. The pattern shows that Wukari and Donga cattle populations experience more genetic changes than Iware cattle because their populations exist in more complex structures. Recent studies of African cattle genomes show that higher genetic differences between populations develop when distinct genetic groups interbreed, especially between zebu and taurine ancestors according to Kim et al. (2023) and Weldenegker et al. (2024). The high genetic differences found in Wukari and Donga indicate ongoing genetic exchange between different cattle populations which have different evolutionary backgrounds, a phenomenon that researchers have documented throughout West African pastoral systems according to Gobena et al. (2024) and Upadhyay et al. (2021).

The study of unique sequence differences enables researchers to examine how different genetic variants exist within the same population. The three sequences from Wukari showed different levels of unique genetic differences because sequence A had 137 differences, sequence B had 106 differences, and sequence C had 143 differences, which showed that private genetic mutations appeared evenly among the studied people. The Iware sequences showed lower unique differences because they maintained higher sequence conservation, which results in their lower unique differences (4–536 range). The Iware sequence contains one sequence with an extremely high value of 536, which probably represents either a data error or a highly distinct genetic variant that entered the population through breeding with another genetically different group, which needs to be confirmed through research that involves more samples (Cadzow et al., 2022). Gembu showed significant genetic diversity through its sequence C results, which produced 286 unique differences. The unique environmental conditions of the Mambilla Plateau highland, where Gembu exists, create cooler temperatures and higher altitudes and different disease threats, which enable local populations to develop distinct genetic traits (Bahbahani et al., 2021; Porto-Neto et al., 2022). East African highland cattle genetic studies found genes that control hypoxia adaptation, thermoregulation, and immune function, which Gembu cattle populations use for their adaptive processes (Smetko et al., 2021; Zhou et al., 2023).

The discovered differences in sequence divergence patterns show significant effects on how scientists use molecular clocks in their research. The divergence time estimates and phylogenetic reconstructions get impacted by clock-like evolution violations which cause evolutionary rate changes across different lineages and throughout various genomic areas (Kumar & Hedges, 2021; Lartillot & Poujol, 2024). The dataset requires advanced molecular clock models because Taraba State populations show distinct patterns of divergent sites and unique genetic differences. Future phylogenomic analyses need to use relaxed clock models which handle rate heterogeneity according to Álvarez-Carretero et al. (2022) and Tamuri et al. (2021). The research results show that Wukari and Donga cattle populations show more genetic diversity and sequence divergence, which Iware population shows through its higher genetic similarity. The three geographic locations show different patterns of genetic variation which Tajima (1993) and Nielsen (2021) identify as results from historical population movements, gene exchanges, environmental adaptation, and different evolutionary forces that operate in each location. Recent research on population genomics has shown that West African cattle populations underwent multiple demographic changes which included several rounds of genetic mixing together with population growth and adaptation to various agro-ecological regions (Kim et al., 2023; Tijjani et al., 2021; Barbato et al., 2022).

The variation that Tajima's Clock Test shows proves scientists need to study population structures for their practical work on estimating evolution rates and using molecular clock methods to study cattle genetics research. Future investigations should incorporate genome-wide SNP data or whole-genome sequences to comprehensively characterize population structure, admixture patterns, and selection signatures across these populations (Ginja et al., 2023; Weldenegker et al., 2024). The combination of environmental data with phenotypic information would allow researchers to find adaptive alleles while they study how environmental factors cause genetic differences among Taraba State cattle populations (Gobena et al., 2024; Melka & Schenkel, 2021). Evidence-based conservation and breeding strategies need integrative approaches to maintain adaptive genetic variation in indigenous African cattle while they work to enhance productivity (Zhou et al., 2023; Smetko et al., 2021).

TABLE 3
RESULTS FROM TAJIMA'S CLOCK TEST BASED ON LOCATION

Parameters	Wukari	Iware	Donga	Gembu
Identical sites in all three sequences	59	202	91	116
Divergent sites in all three sequences	244	6	229	193
Unique differences in sequence A	137	536	138	104
Unique differences in sequence B	106	5	151	78
Unique differences in sequence C	143	4	170	286

IV. CONCLUSION

The research presents a comprehensive assessment of genetic diversity and evolutionary relationships among Taraba State Nigerian cattle populations. The presence of positive Tajima's D values at all testing sites and among all tested breeds demonstrates that the species exhibit genetic patterns which differ from neutral evolution because of balancing selection, population structure, and historical demographic changes. Gembu and Wukari populations showed the highest nucleotide diversity and strongest signals of non-neutrality, reflecting substantial within-population variation. The breed-level study found that White Fulani and Red Bororo cattle showed higher genetic diversity and more pronounced neutrality violations than Bokoloji and Muturu because different factors affected their population size, geographic distribution, and environmental conditions. The Tajima's Relative Rate (Clock) Test showed different evolutionary rates across the study, as Iware maintained its basic sequences while showing irregular substitutions among its specific lineages, which broke the basic molecular clock rules. The phylogenetic study showed that breeds and geographical regions formed distinct groups, which scientists used to trace shared ancestry and population mixing. The research demonstrates how Nigerian cattle developed through complex evolutionary changes, which researchers used to create fundamental data needed for conservation work, breeding programs, and genetic development methods.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Physiological and Biochemical Responses of Maize (*Zea mays* L.) Cultivars to Salinity Stress

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Abstract— *Salinity is a critical abiotic constraint to maize (Zea mays L.) production, affecting growth, photosynthesis and oxidative balance. This study examined the responses of five cultivars (DHM 144, NK 7720, SY 594, Sunny NMH 777 and Dragon NMH 1247) to 150 mM NaCl stress. Measurements at 0, 7 and 14 days after stress initiation included plant height, leaf area, chlorophyll content, stomatal conductance, antioxidant enzyme activities [superoxide dismutase (SOD), catalase (CAT), peroxidase (POD)], proline accumulation and malondialdehyde (MDA) levels. Salinity reduced morphological and physiological traits across genotypes, with Dragon NMH 1247 maintaining higher growth, chlorophyll content and stomatal conductance, coupled with enhanced SOD, CAT and POD activities and lower MDA accumulation. All cultivars had a higher proline content which was not always correlated with stress tolerance. Findings show that antioxidant capacity, chlorophyll retention and low oxidative damage are some of the main factors that determine salinity tolerance in maize. Dragon NMH 1247 emerged as the most tolerant genotype and is an attractive target for saline-prone environments and breeding programs.*

Keywords— Maize, salt stress, proline, antioxidant enzymes.

I. INTRODUCTION

Maize (*Zea mays* L.) is a universally significant cereal crop that is a staple food for millions of people, a critical component of livestock feed and a primary industrial raw material. Its capacity for adaptation to different agro-climatic factors has enabled its cultivation in different geographical areas, making it among the most extensively grown crops in the world (FAO, 2020). Nevertheless, its productivity is under threat from various abiotic stresses, especially salinity, which poses a considerable challenge to agricultural sustainability and food security.

Salinity stress negatively impacts plant growth and metabolism by interfering with water uptake, resulting in ionic toxicity and oxidative stress. These effects inhibit photosynthesis, nutrient assimilation and enzyme activity, ultimately leading to reduced yields (Munns and Tester, 2008). Even moderate levels of salt can cause significant losses in physiological performance and biomass gain in glycophytic crops such as maize, which is moderately sensitive to salinity (Cairns et al., 2013).

At the physiological level, salt stress affects plant height, leaf area, stomatal conductance and chlorophyll content—parameters used to measure plant vigor and photosynthetic performance under stress conditions. For example, reduction in stomatal conductance limits CO_2 uptake, thereby decreasing carbon assimilation and plant productivity (Zhang et al., 2006). Simultaneously, ionic and osmotic stress caused by salt induces symptoms including leaf chlorosis and stunted growth.

At the biochemical level, salinity causes excessive production of reactive oxygen species (ROS), leading to oxidative stress on cellular components. Plants respond by activating an antioxidant defense system containing enzymatic activities such as

superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) that mitigate ROS toxicity and maintain cellular integrity (Apel and Hirt, 2004). Additionally, osmolytes like proline accumulate in response to osmotic stress, contributing to osmotic adjustment, membrane stabilization and free radical scavenging (Ashraf and Harris, 2004). Lipid peroxidation byproducts such as malondialdehyde (MDA) serve as effective indicators of oxidative membrane damage and stress severity.

Maize possesses high genetic diversity, resulting in varied physiological and biochemical responses to salt stress among different cultivars. Characterizing this variability is essential for identifying stress-resilient genotypes suitable for salt-affected soils. Previous literature has demonstrated that certain cultivars exhibit enhanced antioxidant capacity, superior osmotic regulation and reduced MDA accumulation, contributing to superior salinity tolerance (Duvick, 2005; CIMMYT, 2019).

Therefore, evaluating a comprehensive range of physiological and biochemical parameters is important for understanding salt tolerance mechanisms in maize. The aim of this study was to examine how salinity influences key plant characteristics including height, leaf area, chlorophyll content, stomatal conductance and biochemical indices such as SOD, CAT and POD activities, proline content, and MDA levels across different maize cultivars. The findings will help identify maize genotypes capable of withstanding saline conditions and guide future breeding initiatives to develop resilient cultivars for saline-prone agroecosystems.

II. MATERIALS AND METHODS

2.1 Plant Material and Experimental Design:

The experiment was conducted with five maize (*Zea mays* L.) cultivars: DHM 144, NK 7720, SY 594, Sunny NMH 777 and Dragon NMH 1247. Seeds of each cultivar were surface sterilized followed by controlled germination to obtain uniform and healthy seedlings. After germination, seedlings were transplanted into pots filled with a homogenized and uniformly mixed soil medium. The experimental design was a completely randomized design (CRD) with two treatments (control and salinity stress), five cultivars and three biological replicates per treatment.

2.2 Salinity Stress Treatment

Salinity stress was imposed by applying 150 mM sodium chloride (NaCl) solution. Before stress initiation, all plants were irrigated with deionized water for seven days to ensure baseline uniformity. On Day 0, the treatment group received NaCl solution while the control group received deionized water. A soil salinity meter was used to monitor soil salinity levels and maintain consistent concentration across all treated pots. NaCl solution was applied every other day to sustain constant salinity stress.

2.3 Sampling Time Points

Physiological and biochemical measurements were performed at three time points: Day 0 (stress onset, baseline), Day 7 (mid-stress) and Day 14 (stress termination). Sample collection at each time point was conducted on both control and salt-treated plants.

2.4 Measurement of Physiological Parameters

- **Plant Height (PH)** was measured from the root-shoot junction to the tip of the youngest leaf using a ruler and recorded in centimeters (cm).
- **Leaf Area (LA)** was estimated using the formula: $LA = L \times W \times 0.75$, where L = leaf length and W = leaf width (both in cm). The coefficient 0.75 accounts for the elliptical shape of the leaf blade.
- **Chlorophyll Content (Chl)** was measured in fresh leaf samples using a SPAD-502 Plus chlorophyll meter (Konica Minolta, Japan).
- **Stomatal Conductance (SC)** was recorded using a portable porometer (SC-1 Leaf Porometer, Decagon Devices, USA) on the abaxial surface of fully expanded leaves.

2.5 Estimation of Biochemical Parameters

- **Superoxide Dismutase (SOD) Activity** was assayed by measuring the photochemical reduction of nitro blue tetrazolium (NBT) following the method of Beauchamp and Fridovich (1971). One unit of SOD activity was defined as the amount of enzyme required to inhibit 50% NBT photoreduction.

- **Catalase (CAT) Activity** was determined by monitoring the decomposition of H_2O_2 at 240 nm according to Aebi (1984).
- **Peroxidase (POD) Activity** was measured using guaiacol as substrate following the method of Chance and Maehly (1955).
- **Proline Content** was quantified using the ninhydrin method (Bates et al., 1973). Absorbance was read at 520 nm and results were expressed as µmol proline g^{-1} fresh weight.
- **Malondialdehyde (MDA) Content**, an index of lipid peroxidation, was determined using the thiobarbituric acid (TBA) reaction as described by Heath and Packer (1968). Absorbance was measured at 532 nm and corrected for non-specific turbidity at 600 nm.

2.6 Statistical Analysis:

Data were analyzed using two-way analysis of variance (ANOVA) with cultivar and treatment as fixed factors using SPSS software (Version XX). Duncan's Multiple Range Test (DMRT) was used to compare means at a significance level of $p \leq 0.05$. Correlation analysis of physiological and biochemical traits was performed using PAST software (Version 4.03).

III. RESULTS

3.1 Effect of Salinity on Morphological Traits:

3.1.1 Plant Height:

Salinity stress differentially affected plant height among maize cultivars over the experimental period (Figure 1). At Day 0, plant heights ranged from 188 cm to 229 cm across cultivars (SY 594 and Dragon NMH 1247, respectively). By Day 14, most cultivars showed either insignificant changes or slight decreases in height. Dragon NMH 1247 exhibited a gradual increase from 229 cm to 243 cm. DHM 144 and NK 7720 recorded slight incremental changes of 4-5 cm. SY 594 showed the highest reduction of 8 cm from its Day 0 height. The minimal reduction, or slight increase, in Dragon NMH 1247 height indicates greater tolerance to salt-induced growth retardation, possibly associated with efficient osmotic homeostasis and cell growth maintenance. Conversely, the height decrease in Sunny NMH 777 and SY 594 suggests higher sensitivity to salinity stress. These observations align with previous reports that stress-tolerant maize genotypes sustain shoot extension through hormonal and osmotic adaptation (Munns and Tester, 2008; Farooq et al., 2009; Hussain et al., 2018; Tang et al., 2023).

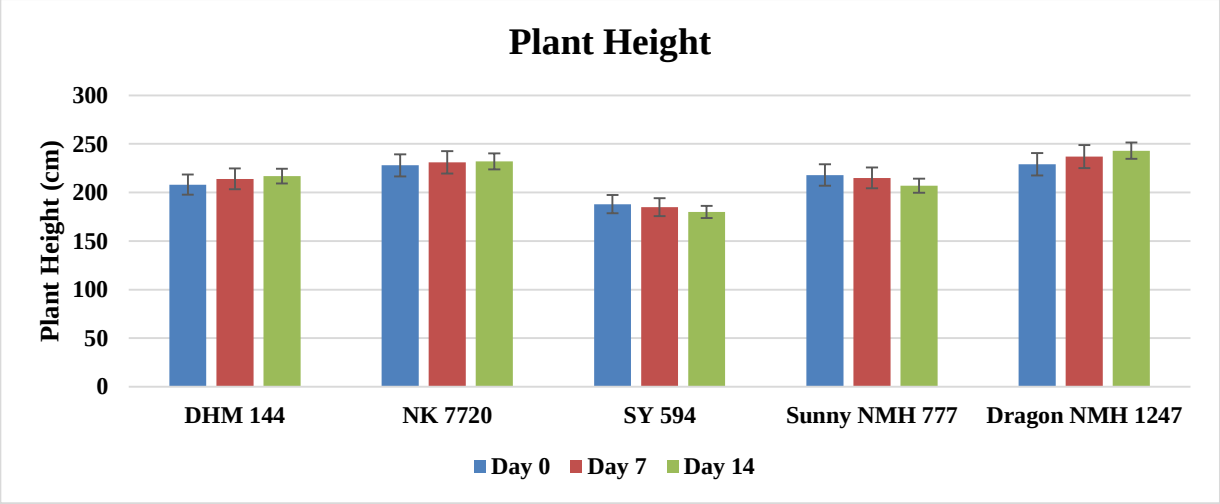

FIGURE 1: Effect of salt stress on the plant height of maize cultivars

3.1.2 Leaf Area:

Salinity caused progressive reduction in leaf area with considerable variation among cultivars (Figure 2). Dragon NMH 1247 maintained relatively greater leaf area under stress with minimal decreases, indicating robust cellular structure and sustained development (Romdhane et al., 2020). Yan et al. (2023) associated similar characteristics with enhanced biomass production in stress-tolerant lines. NK 7720 and DHM 144 exhibited moderate reductions, suggesting intermediate tolerance levels.

Conversely, SY 594 and Sunny NMH 777 showed sharp decreases, reflecting inhibited leaf expansion due to ionic toxicity and premature senescence as observed by Munns and Tester (2008).

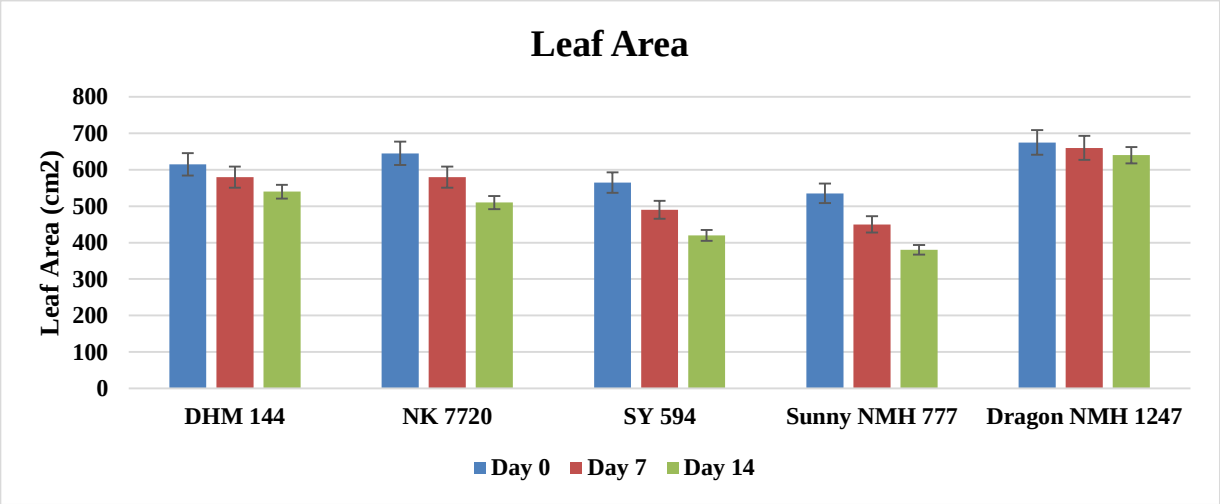

FIGURE 2: Effect of salt stress on the LA of maize cultivars

3.2 Physiological Responses:

3.2.1 Chlorophyll Content:

Salinity significantly reduced chlorophyll content across all genotypes, particularly after Day 14 (Figure 3). Sunny NMH 777 displayed the lowest chlorophyll levels while Dragon NMH 1247 and DHM 144 maintained higher SPAD values. These results are consistent with Ali et al. (2024) and Moharramnejad et al. (2019) who reported enhanced chlorophyll retention as a characteristic of tolerant genotypes. Salt-induced chlorophyll degradation is associated with oxidative damage and impaired pigment biosynthesis (Ashraf and Harris, 2004). Higher chlorophyll levels in resistant genotypes indicate effective antioxidant protection and reduced membrane damage, in agreement with findings by Akhtar et al. (2024).

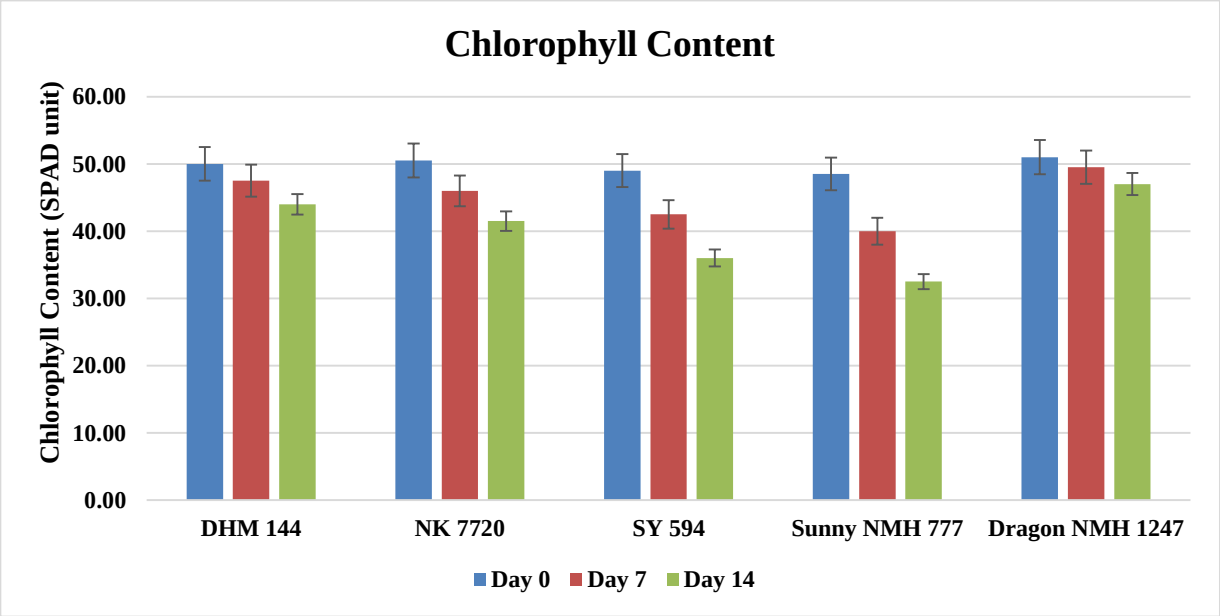

FIGURE 3: Effect of salt stress on the Chlorophyll Content of maize cultivars

3.2.2 Stomatal Conductance:

All cultivars exhibited reduced stomatal conductance under salt stress, indicating decreased gas exchange and photosynthetic activity (Figure 4). However, the reduction was smaller in DHM 144 and Dragon NMH 1247, suggesting better stomatal regulation under stress (Liao et al., 2022). Reduced stomatal conductance is a well-documented protective response to minimize water loss, though it limits carbon assimilation and consequently yield (Beauclaire et al., 2024).

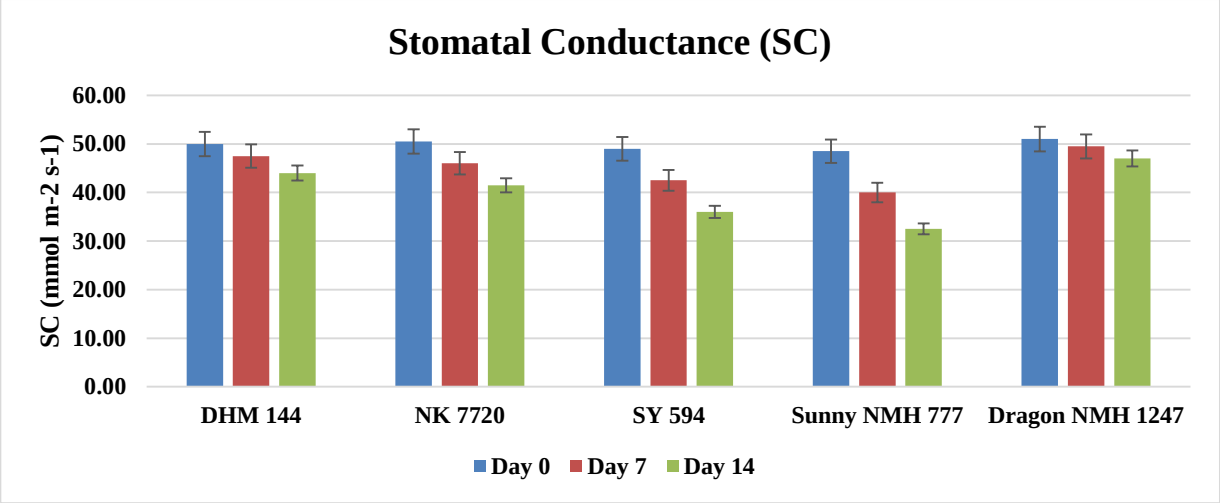

FIGURE 4: Effect of salt stress on the Stomatal Conductance (SC) of maize cultivars

3.3 Biochemical Responses:

3.3.1 Antioxidant Enzyme Activities:

Salt stress induced pronounced increases in antioxidant enzyme activities (SOD, CAT, POD) across all maize cultivars, with the highest activities observed in Dragon NMH 1247 followed by Sunny NMH 777 (Table 1). This elevation reflects effective ROS-scavenging capacity, essential for stress tolerance. SOD provides the initial defense by converting superoxide radicals to hydrogen peroxide, which is subsequently neutralized by CAT and POD (Mittler, 2002). These results align with Shehu et al. (2023) and Ali et al. (2024), who reported that enhanced SOD activity correlates with improved salt and drought tolerance in maize and other cereals.

CAT decomposes toxic H_2O_2 produced by SOD activity into water and oxygen. All maize varieties showed increased CAT activity in response to salt stress, indicating a universal stress response. Similar patterns were reported by Hussain et al. (2018) and Ali et al. (2024), where CAT activity positively correlated with yield stability under abiotic stress.

POD contributes to oxidative stress mitigation by catalyzing H_2O_2 reduction using phenolic substrates, often complementing CAT activity. Up-regulation of POD was observed across all varieties, with NK 7720 showing the highest induction, suggesting enhanced oxidative detoxification potential and synergy between CAT and POD pathways. These findings are supported by Ali et al. (2024) and Munavvar et al. (2025), emphasizing the importance of POD in stress adaptation.

TABLE 1

EFFECT OF SALT STRESS ON ANTIOXIDANT ENZYME ACTIVITIES IN MAIZE CULTIVARS

Cultivar	SOD Activity (U mg^{-1} protein)			CAT Activity (µmol min^{-1} mg^{-1} protein)			POD Activity (µmol min^{-1} mg^{-1} protein)		
	Day 0	Day 7	Day 14	Day 0	Day 7	Day 14	Day 0	Day 7	Day 14
DHM 144	21.13 ± 0.22	18.84 ± 0.14	16.80 ± 0.24	1.41 ± 0.01	2.32 ± 0.02	3.04 ± 0.02	0.70 ± 0.01	1.12 ± 0.01	1.44 ± 0.02
NK 7720	21.38 ± 0.34	18.18 ± 0.23	16.36 ± 0.36	1.45 ± 0.01	2.27 ± 0.01	3.18 ± 0.03	0.70 ± 0.01	1.06 ± 0.01	1.50 ± 0.08
SY 594	21.01 ± 0.46	17.39 ± 0.34	13.33 ± 0.22	1.38 ± 0.01	2.26 ± 0.02	3.11 ± 0.02	0.69 ± 0.01	1.04 ± 0.01	1.44 ± 0.06
Sunny NMH 777	20.74 ± 0.22	17.00 ± 0.12	11.54 ± 0.32	1.33 ± 0.02	2.20 ± 0.02	2.56 ± 0.02	0.67 ± 0.02	1.00 ± 0.02	1.15 ± 0.07
Dragon NMH 1247	21.28 ± 0.43	20.00 ± 0.23	18.71 ± 0.28	1.49 ± 0.02	2.48 ± 0.01	2.95 ± 0.01	0.74 ± 0.01	1.21 ± 0.02	1.44 ± 0.01
S.Em±	1.62			0.032			0.01		
CD (5%)	4.77			0.092			0.03		
CD (1%)	6.51			0.125			0.05		

Values represent mean ± standard error (n=3)

3.3.2 Proline Accumulation:

Salt stress significantly increased proline levels across all cultivars, with the highest concentrations observed in Dragon NMH 1247 and Sunny NMH 777 at Day 14 (Figure 5), indicating activation of osmotic adjustment mechanisms. However, despite high proline content, Sunny NMH 777 showed poor performance in physiological and antioxidant parameters, suggesting that proline accumulation alone is insufficient for effective stress tolerance. Similar findings were reported by Ibrahim et al. (2022) and Shahimoghadam et al. (2024), indicating that proline concentration in sensitive genotypes may reflect stress severity rather than stress resilience. Proline functions as an osmoprotectant, stabilizing proteins and membranes while scavenging free radicals under osmotic stress (Szabados and Savouré, 2010).

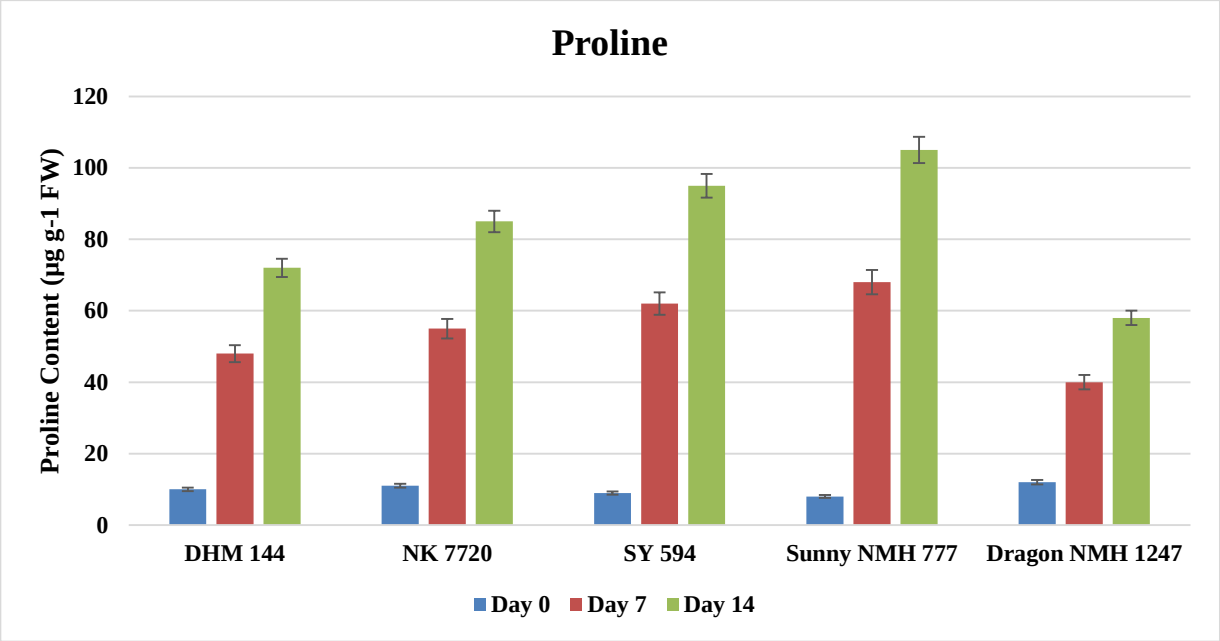

FIGURE 5: Effect of salt stress on the Proline Content of maize cultivars

3.3.3 Malondialdehyde Content:

MDA levels, indicating lipid peroxidation and membrane damage, increased significantly under salt stress (Table 2). The highest MDA content was recorded in Sunny NMH 777 while the lowest was observed in Dragon NMH 1247 at Day 14. This pattern demonstrates superior oxidative damage mitigation in Dragon NMH 1247 compared to other cultivars, consistent with Ali et al. (2024) who emphasized that coordinated enzymatic activity is essential for membrane stability under stress.

TABLE 2
EFFECT OF SALT STRESS ON MDA CONTENT (µmol g^{-1} FW) IN MAIZE CULTIVARS

Cultivar	Day 0	Day 7	Day 14
DHM 144	2.45 ± 0.12	4.89 ± 0.18	6.78 ± 0.22
NK 7720	2.38 ± 0.10	4.76 ± 0.15	6.54 ± 0.19
SY 594	2.52 ± 0.14	5.23 ± 0.21	7.45 ± 0.26
Sunny NMH 777	2.61 ± 0.13	5.67 ± 0.24	8.12 ± 0.31
Dragon NMH 1247	2.31 ± 0.09	4.12 ± 0.14	5.23 ± 0.17

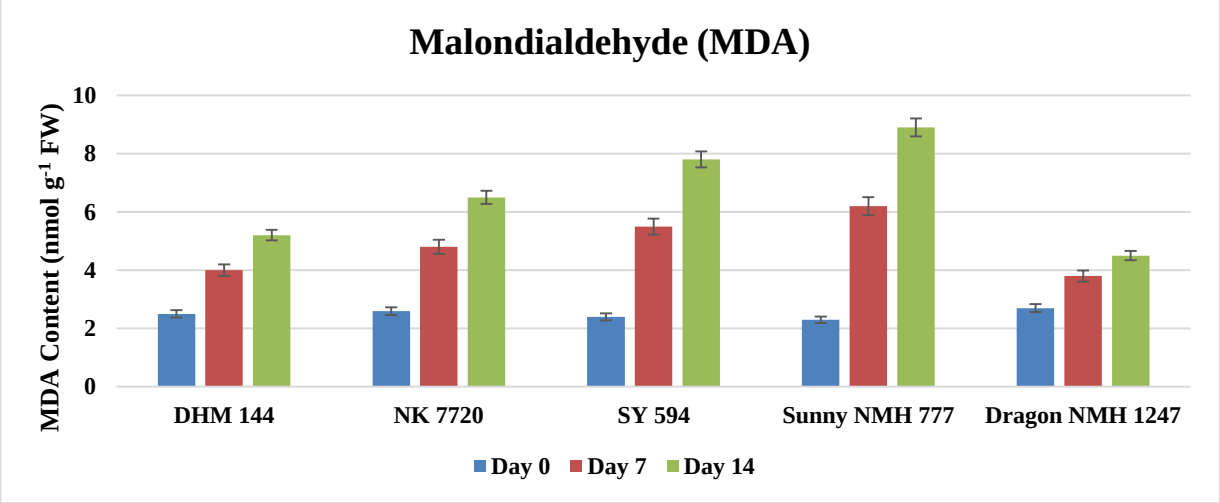

FIGURE 6: Effect of salt stress on the Malondialdehyde of maize cultivars

IV. DISCUSSION

Salinity stress adversely affected morphological, physiological and biochemical parameters in all maize cultivars, though the magnitude of impact varied considerably among genotypes. Dragon NMH 1247 consistently demonstrated superior performance across most measured traits, maintaining greater plant height, leaf area, chlorophyll content and stomatal conductance under stress conditions. This was accompanied by enhanced antioxidant enzyme activities and reduced MDA accumulation, indicating effective ROS scavenging and membrane protection.

The maintenance of plant height and leaf area in Dragon NMH 1247 under salinity suggests efficient osmotic adjustment and sustained cellular division and expansion. Similar observations were reported by Romdhane et al. (2020) in salt-tolerant maize lines, where preserved growth correlated with better ion homeostasis and water relations. The minimal reduction in chlorophyll content in this cultivar indicates protection of photosynthetic pigments from salt-induced degradation, consistent with findings by Moharramnejad et al. (2019) who identified chlorophyll retention as a key tolerance trait.

Stomatal conductance responses revealed that Dragon NMH 1247 and DHM 144 maintained relatively higher stomatal opening under stress, suggesting better control of gas exchange while managing water loss. This partial stomatal opening may enable continued carbon assimilation, contributing to sustained growth. Beauclaire et al. (2024) similarly reported that tolerant genotypes optimize the trade-off between water conservation and CO_2 uptake through modulated stomatal behavior.

The coordinated up-regulation of SOD, CAT and POD activities in Dragon NMH 1247 represents an efficient antioxidant defense system. SOD catalyzes the dismutation of superoxide radicals to H_2O_2, while CAT and POD subsequently detoxify H_2O_2, preventing accumulation of harmful ROS (Mittler, 2002). The simultaneous enhancement of all three enzymes suggests integrated regulation of the antioxidant network, which likely contributed to reduced oxidative damage as evidenced by lower MDA levels. These findings align with Ali et al. (2024) and Shehu et al. (2023), who reported similar antioxidant coordination in stress-tolerant maize genotypes.

Proline accumulation occurred in all cultivars under salinity, confirming its role as an osmotic stress response. However, the lack of correlation between proline content and overall stress tolerance—particularly in Sunny NMH 777 which accumulated high proline but performed poorly—indicates that proline alone is insufficient for stress adaptation. This observation supports the view of Ibrahim et al. (2022) and Shahimoghadam et al. (2024) that proline accumulation in sensitive genotypes may reflect stress severity rather than adaptive capacity. Effective tolerance apparently requires integrated responses including antioxidant defense, osmotic adjustment and membrane stability rather than any single protective mechanism.

MDA content, reflecting lipid peroxidation and membrane damage, was lowest in Dragon NMH 1247 and highest in Sunny NMH 777. This inverse relationship between antioxidant activity and MDA accumulation confirms that efficient ROS scavenging protects membrane integrity under stress. Similar patterns were reported by Ali et al. (2024), where tolerant lines exhibited lower MDA alongside elevated antioxidant enzyme activities.

The observed genotypic variation in salinity responses underscores the importance of genetic diversity in maize for stress adaptation. Dragon NMH 1247 emerged as the most salt-tolerant cultivar, combining sustained growth, chlorophyll retention,

stomatal regulation, coordinated antioxidant defense and membrane stability. Sunny NMH 777 and SY 594 showed greater sensitivity, characterized by growth reduction, chlorophyll degradation, weaker antioxidant responses and higher oxidative damage. DHM 144 and NK 7720 exhibited intermediate responses, suggesting moderate tolerance levels.

V. CONCLUSION

Salinity stress significantly impacted physiological and biochemical characteristics in maize, with considerable genotypic variation among the five cultivars evaluated. Dragon NMH 1247 demonstrated the highest salt tolerance, maintaining superior growth, chlorophyll content, stomatal conductance and antioxidant enzyme activities while accumulating less MDA. Sunny NMH 777 and SY 594 were more sensitive, exhibiting greater growth reduction, chlorophyll degradation, weaker antioxidant responses and higher oxidative damage. DHM 144 and NK 7720 showed intermediate tolerance levels.

The findings indicate that effective salinity tolerance in maize requires integrated mechanisms including sustained photosynthetic pigment retention, modulated stomatal behavior, coordinated antioxidant enzyme activity and membrane stability. Proline accumulation, while universally induced by salinity, does not independently confer tolerance and may reflect stress intensity rather than adaptive capacity in sensitive genotypes.

Dragon NMH 1247 represents a promising genetic resource for cultivation in saline-prone areas and for inclusion in breeding programs aimed at developing salt-tolerant maize varieties. Further research should investigate the molecular mechanisms underlying the superior performance of this cultivar, including expression patterns of stress-responsive genes and ion transport regulation. Field validation under diverse saline conditions would also be valuable to confirm the consistency of these findings across environments.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Effects of Amino Acid Supplementation in Low-Crude Protein Diets on Growth Performance, Footpad Health, and Economics of Hubbard Broilers Raised in a Deep-Litter System

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Abstract— *This study evaluated the effects of reduced crude protein (CP) diets supplemented with amino acids on the growth performance and profitability of Hubbard colored broilers. Three isocaloric finisher rations were formulated with 19%, 17%, and 15% CP, with the CP-reduced diets supplemented with methionine, lysine, threonine, and tryptophan. A total of 120 broilers were randomly assigned to these three treatments with four replicates each. Results showed significant differences ($p<0.05$) in final weight, total weight gain, and average daily gain. Broilers fed reduced CP diets (17% and 15%) exhibited superior growth performance compared to those on the 19% CP control. Furthermore, reduced CP treatments significantly decreased the incidence of footpad dermatitis and increased net profit and return on investment. These findings suggest that lowering CP levels with amino acid supplementation offers a cost-effective and environmentally sustainable strategy for broiler production.*

Keywords— Crude Protein, Amino Acids, Hubbard Broilers, Growth Performance, Footpad Dermatitis, Economics.

I. INTRODUCTION

In the Philippines, poultry production, particularly chicken meat production, continues to grow due to its demand and lower price in the market. The demand for poultry products brought its success in the animal enterprise due to lack of supply and higher cost of pork brought on by the African Swine Fever (ASF) outbreak (Acosta, 2022). In the Cordillera region, backyard raising for livestock and poultry is practiced for consumption and for additional income. Rearing poultry is more likely to increase through the years due to its lower cost in financing compared to livestock such as pigs and ruminants.

Recent industry trends emphasize improving animal welfare, mitigating environmental pollution, and reducing production costs, primarily those associated with feed. The product of metabolism in poultry such as undigested dietary protein and uric acid is excreted into the environment that could become a source of ammonia (Nahm, 2003). According to Poultry World (2019), unused amino acids are changed into nitrogen which results in higher ammonia levels in the poultry house that affects the animal as well as the workers. Generally, production of 1 kg chicken meat generates 1.1 kg CO_2 equivalents and it has also been predicted that it will contribute 7.1% of greenhouse gases (Fiola, 2008).

Current research focuses on mitigating the environmental impact of livestock production, specifically the emission of greenhouse gases. Studies demonstrate that developing reduced-crude protein (CP) diets supplemented with crystalline (non-bound) amino acids significantly lowers gas emissions (Chrystal et al., 2020; Meda et al., 2019). Furthermore, Applegate (2008) identified CP reduction through amino acid supplementation as the most effective strategy for decreasing nitrogen excretion in poultry waste. While formulating reduced-CP diets is an established concept, the modern availability of synthetic amino acids now allows for a substantial reduction in both dietary CP and reliance on soybean meal.

The determination of ideal dietary CP levels has been a subject of debate since the recognition that protein requirements are essentially a requirement for a specific profile of amino acids (Pesti, 2009). Beyond nutritional balance, manipulating these

levels serves as an environmental tool; Ferguson et al. (1998) asserted that reducing CP during the grower and finisher phases, supplemented with synthetic amino acids, is the most cost-effective method for controlling ammonia (NH_3) emissions.

High costs of corn and soybean meal often drive up feed prices, prompting producers to seek cost-reduction strategies that maintain flock performance. While Son et al. (2024) observed that reducing dietary CP lowered total feed costs without altering net profit, performance results vary. Consequently, this study aims to validate the performance of Hubbard broilers reared on deep litter when fed CP-reduced diets.

II. MATERIALS AND METHODS

2.1 Pre-experimental Phase:

2.1.1 Preparation of Brooding Pens:

One week prior to chick arrival, the experimental area was cleaned and disinfected. Broilers were housed in a corrugated GI-roofed pen with 4-inch deep rice hull litter and brooding paper. The area was pre-heated for 12 hours using six 100-watt incandescent bulbs, which served as the primary heat and light source throughout the brooding period.

2.1.2 Brooding Management:

During the entire brooding period, ventilation, lighting, and temperature were closely monitored to ensure the overall health of the brood. The chicks were fed a commercial booster diet from day 1 to 17 and provided potable water ad libitum.

2.2 2.2 Experimental Phase:

2.2.1 2.2.1 Experimental Design:

At 22 days of age, 120 Hubbard colored broilers (60 males and 60 females) were selected from an initial flock of 200 and assigned to three dietary treatments using a Randomized Complete Block Design (RCBD). Each treatment consisted of four replicates, with 10 birds per replicate (5 males and 5 females). The experimental diets were:

- **T0** - (Control) at 19% CP
- **T1** - 17% CP with amino acid (AA) supplementation
- **T2** - 15% CP with amino acid (AA) supplementation

Data were analyzed using the Univariate Analysis of Variance (UNIANOVA) for Randomized Complete Block Design (RCBD) using Statistical Analysis System. Differences in treatment means were compared using the Duncan Multiple Range Test (DMRT) at 5% level of significance.

2.2.2 Experimental Area:

A 4-inch deep litter using rice hull was used in rearing the birds until the termination of the study. Each pen had a dimension of 1.02 m × 2.57 m, using plastic slats as pen dividers.

2.2.3 Preparation of Feeds:

The experimental finisher rations (Table 1) were formulated according to the Hubbard Broiler Management Manual (WinMix Soft, 2014) specifications for birds with a 2.0 kg target slaughter weight. While the manual recommends a crude protein (CP) range of 19–21% and a metabolizable energy (ME) of 3,150–3,200 kcal/kg, this study utilized the minimum requirements (19% CP) as the baseline for the control diet. Across all treatments, 3,150 kcal/kg ME was used to formulate isocaloric diets.

Major ingredients (soybean meal, corn, and rice bran) were analyzed at the Charoen Pokphand Foods Philippines Corporation Feed Laboratory to ensure precise formulation of target CP levels. Diets were prepared in 20-kg batches, with ingredients weighed via digital scale. Micro-ingredients were premixed separately, then incorporated into macro-ingredients that had been mechanically mixed for 5 minutes, followed by an additional 5 minutes of mixing. Coconut oil was subsequently added, and the complete diet was mixed for 10 minutes to ensure homogeneity.

TABLE 1
FEED INGREDIENTS AND NUTRIENT COMPOSITION OF FINISHER DIET

Ingredients	19% CP	17% CP + AA	15% CP + AA
Yellow Corn	58	58	58
Soybean Meal	28.802	24.165	19.483
Rice Bran	5	5	5
Coconut Oil	4.605	5.8	7
Limestone	0.913	0.948	0.983
MDCP	1.74	1.791	1.843
Salt	0.3	0.3	0.3
Vitamin Premix	0.03	0.03	0.03
Mineral Premix	0.1	0.1	0.1
Refined Sand	0.51	3.517	6.557
Amino Acids			
Methionine	-	0.123	0.165
Lysine	-	0.161	0.345
Tryptophan	-	0.039	0.07
Threonine	-	0.026	0.124
Nutrient Composition (Calculated)			
Metabolizable Energy (kcal) *	3,150.08	3,150.42	3,150.13
Crude Protein **	19%	17%	15%

ME is computed values based on book values by PHILSAN.
CP is computed values based on the analysis of individual raw ingredients.

2.2.4 General Care and Management:

Experimental birds were managed uniformly, differing only in their respective dietary treatments. The pens were equipped with sanitized feeders and drinkers; water was refreshed twice daily, and litter was turned three times weekly to maintain quality. Feed was provided twice daily (8:00–9:00 AM and 3:00–4:00 PM) at an amount that is 20 g/bird above the previous day's intake to ensure ad libitum access, with refusals weighed every morning. Individual body weights were recorded weekly (±1 g), and Newcastle disease vaccinations were administered intraocularly at weeks 2 and 4.

III. RESULTS AND DISCUSSION

3.1 Body Weight:

The initial (22 d) and final (50 d) body weights are presented in Table 2. Initial body weights did not differ significantly among treatments (709.10–716.03 g; $p > 0.05$), indicating uniformity of the experimental birds at the start of the trial. Thus, subsequent differences in final body weight can be attributed to dietary treatments rather than baseline variation.

At 50 days, significant differences ($p < 0.05$) were observed in growth performance. Broilers on the 15% CP + AA and 17% CP + AA diets achieved significantly higher body weights (2,220.58 g and 2,199.18 g, respectively) compared to those on the unsupplemented 19% CP control (1,966.65 g). These results indicate that amino acid supplementation effectively compensated for reduced dietary protein, thereby sustaining or enhancing growth. However, it is important to note that final body weights across all treatments remained below the Hubbard JA787 (2023) breed standard of 2,526 g for birds at 50 days of age.

TABLE 2
INITIAL AND FINAL BODY WEIGHTS (G) OF HUBBARD BROILERS FED CRUDE PROTEIN-REDUCED DIETS SUPPLEMENTED WITH ESSENTIAL AMINO ACIDS

Treatment	Body Weight (G)	Final
	Initial	
19% CP	709.1	$1,966.65^{b}$
17% CP + AA	716.03	$2,199.18^{a}$
15% CP + AA	715.43	$2,220.58^{a}$

Means with different superscripts differ significantly ($p < 0.05$, LSD).

These results corroborate existing literature suggesting that high crude protein (CP) levels are unnecessary provided that the essential amino acid profile is precisely balanced. Sterling et al. (2005) demonstrated that broilers achieve optimal performance on reduced-CP diets when supplemented with limiting amino acids such as lysine, methionine, and threonine.

The reduced final weight observed in the 19% CP group may be attributed to the metabolic inefficiency of utilizing excess dietary protein. Excessive protein intake accelerates deamination, resulting in elevated nitrogen excretion and a significant metabolic cost to the bird (Kidd et al., 2004). This physiological process diverts dietary energy away from tissue accretion toward the detoxification and elimination of nitrogenous waste, potentially explaining the stunted growth in broilers fed the highest CP ration.

These results corroborate the findings of van Harn et al. (2019) and Belloir et al. (2019), who observed that moderate CP reductions do not compromise final body weight when diets are formulated based on digestible amino acid requirements. Similar performance stability in reduced-CP diets has been attributed to the supplementation of essential amino acids, specifically methionine and lysine (Khan et al., 2011). This aligns with earlier evidence from Corzo et al. (2006), which demonstrated that lysine supplementation enhances both live weight and feed conversion. Collectively, as emphasized by Baxter et al. (2020) and Lisnahan et al. (2017), these studies highlight that optimal growth is a function of a balanced amino acid profile rather than a high crude protein concentration.

Weekly body weight monitoring from placement through week 3 (Figure 1) indicated a consistent growth trajectory across all treatments. Notably, broilers on reduced-CP diets exhibited superior body weight compared to the 19% CP control. These results reinforce the principle that absolute crude protein levels are not the primary drivers of growth performance. Instead, precise formulation based on a balanced profile of digestible amino acids appears more critical for optimizing nutrient utilization and supporting early development.

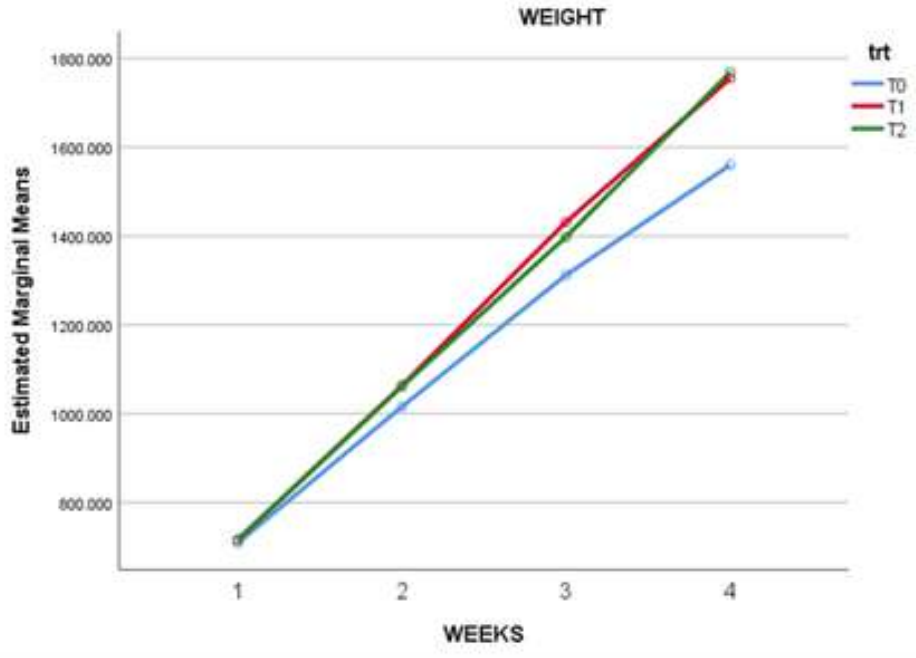

FIGURE 1: Weekly weight of broilers fed (g)

3.2 Gain in Weight:

Total weight gain (TWG) and average daily gain (ADG) for the experimental period are presented in Table 3. Broilers fed the 15% CP + AA and 17% CP + AA diets achieved significantly higher ($p < 0.05$) TWG (1,505.15 g and 1,483.16 g, respectively) than those on the 19% CP control (1,257.55 g). Similarly, the ADG for the 15% and 17% CP treatments (53.76 g and 52.97 g) surpassed the 44.91 g recorded for the control group. These findings demonstrate that dietary CP reduction does not compromise growth performance, provided it is accompanied by precise amino acid supplementation.

The present findings are consistent with those reported by van Harn et al. (2019) and Belloir et al. (2019), who demonstrated that broilers fed reduced crude protein diets supplemented with essential amino acids maintained or improved total weight gain and average daily gain compared with birds fed higher-protein diets.

TABLE 3
TOTAL WEIGHT GAIN (TWG) AND AVERAGE DAILY GAIN (ADG) OF HUBBARD BROILERS FED CRUDE PROTEIN-REDUCED DIETS SUPPLEMENTED WITH ESSENTIAL AMINO ACIDS

Treatment	TWG (g)	ADG (g)
19% CP	1,257.55[b]	44.91[b]
17% CP + AA	1,483.16[a]	52.97[a]
15% CP + AA	1,505.15[a]	53.76[a]

Means with different superscripts differ significantly (p < 0.05, LSD).

The weekly body weight gain (BWG) and average daily gain (ADG) are illustrated in Figures 2a and 2b. Broilers fed the 15% CP + AA diet exhibited a consistent upward trend, demonstrating the efficacy of amino acid supplementation. Conversely, a sharp decline in BWG was observed in the 19% CP and 17% CP + AA groups during week 3, primarily due to a temporary reduction in feed intake over a two-day period. However, these groups demonstrated a robust recovery by week 4, likely reflecting compensatory growth triggered by increased feed consumption. The nearly identical trends in total weight gain and ADG indicate a direct correlation between these two performance metrics.

The temporary decline in weight gain was likely an isolated occurrence unrelated to the dietary treatments, as only a single replicate per treatment was affected. No clinical signs of disease or adverse environmental factors were observed that could account for this localized decrease, as adjacent pens maintained normal performance. Despite the weight gain fluctuations in Treatment 1, broilers on reduced-CP diets consistently outperformed those in the 19% CP group. While partial molting was observed, there is insufficient evidence to definitively link this physiological process to the transient reduction in feed intake.

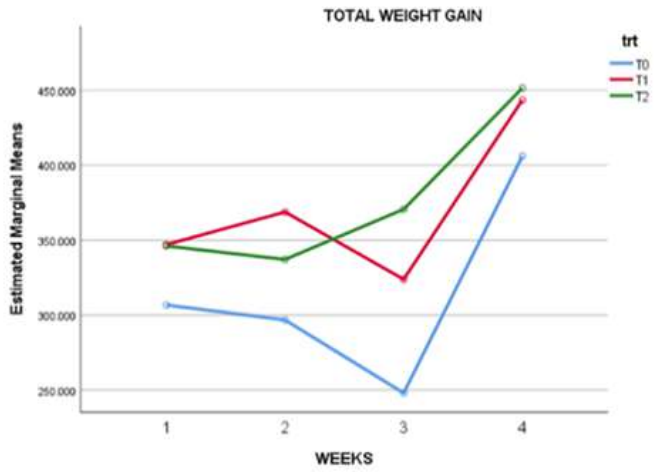

FIGURE 2a. Weekly total weight gain of Hubbard broilers

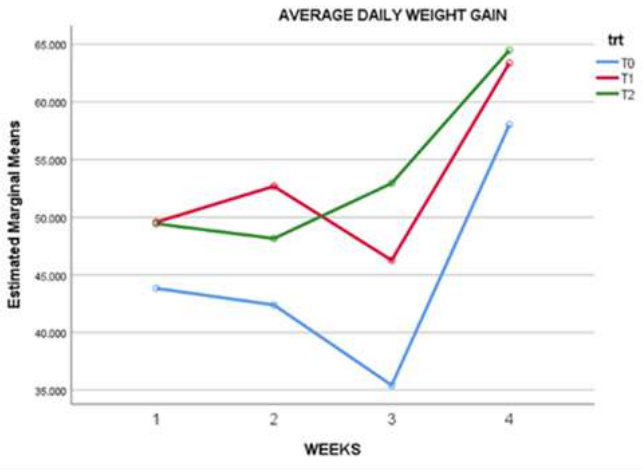

FIGURE 2b. Average daily gain in weight of Hubbard broilers

3.3 Feed Intake:

Total feed intake (TFI) and average daily feed intake (ADFI) are summarized in Table 4. No significant differences ($p > 0.05$) were observed among treatments for either TFI or ADFI (as-fed and dry matter basis). However, broilers on the 15% CP + AA and 17% CP + AA diets exhibited numerically higher TFI and ADFI compared to the 19% CP control. These findings align with Songuine et al. (2025), who reported improved intake following methionine supplementation. Conversely, the poorer performance of the 19% CP group mirrors observations by Hossain et al. (2017), who attributed reduced growth in high-protein diets to impaired nutrient digestibility.

TABLE 4
AVERAGE DAILY FEED INTAKE (ADFI) AND TOTAL FEED INTAKE (TFI) OF HUBBARD BROILERS FED CRUDE PROTEIN-REDUCED DIETS SUPPLEMENTED WITH ESSENTIAL AMINO ACIDS (g)

Treatment	ADFI As Fed	ADFI DM	TFI As Fed
19% CP	121.17[a]	105.10[b]	3,392.63[a]
17% CP + AA	126.33[a]	110.42[ab]	3,537.12[a]
15% CP + AA	129.72[a]	114.53[a]	3,632.03[a]

Means with different superscripts differ significantly (p < 0.05, LSD).

Figures 3a and 3b illustrate the weekly total feed intake and weekly average daily feed intake (ADFI) of broilers across dietary treatments. From the onset of the study, birds fed the 19% CP diet consistently exhibited lower weekly total feed intake and ADFI compared with those receiving reduced crude protein diets supplemented with amino acids. This pattern contributed to the lower cumulative feed intake observed in the 19% CP group over the entire experimental period. The results suggest that reducing dietary crude protein does not adversely affect feed intake when essential amino acids are supplemented to maintain nutritional adequacy.

A temporary decline in feed intake was observed in one replicate each of Treatment 0 and Treatment 1. This reduction may have been associated with environmental fluctuations, particularly elevated daytime temperatures followed by afternoon rainfall, which could have influenced feeding behavior. No clinical signs of disease were observed during this period, and feed intake returned to normal levels in subsequent days. The recovery in intake supports the assumption that the transient reduction was likely due to short-term environmental stress rather than treatment effects or health-related factors.

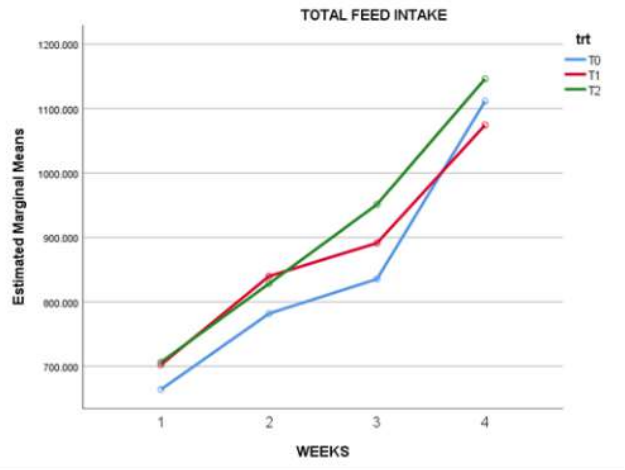

FIGURE 3a. Weekly total feed intake (as-fed basis) of Hubbard broilers

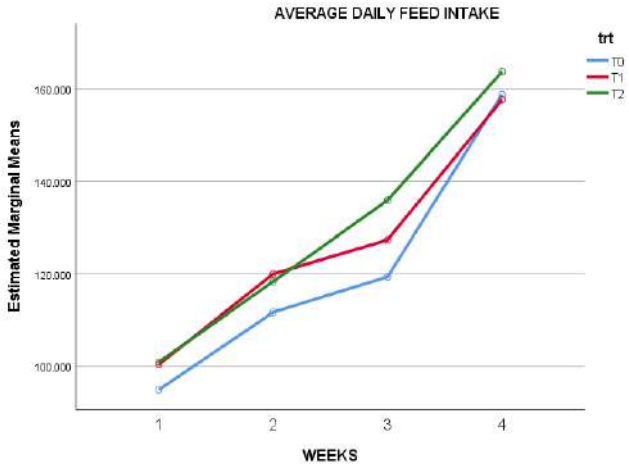

FIGURE 3b. Weekly average daily feed intake (as-fed basis) of Hubbard broilers

3.4 Feed Efficiency:

Table 5 presents the feed conversion ratio (FCR) and gain-to-feed (G:F) ratio of broilers across treatments. A significant difference ($p < 0.05$) was observed among dietary treatments for FCR, expressed on both as-fed and dry matter bases. Despite the reduction in dietary crude protein, broilers fed the amino acid–supplemented reduced-CP diets exhibited improved FCR and G:F values compared with those fed the 19% CP diet. These results indicate that when limiting amino acids are adequately supplied, reductions in crude protein do not compromise feed efficiency. Notably, the FCR values obtained in the present study were lower (i.e., better) than the average FCR (1.63) reported for standard Hubbard JA787 broilers from 23 to 50 days of age.

The findings of this study are consistent with those of van Harn et al. (2019) and Belloir et al. (2017), who reported that reducing dietary crude protein by 2% to 4% did not adversely affect feed conversion when diets were balanced with essential amino acids. These results support the concept that feed efficiency is primarily determined by amino acid balance rather than crude protein concentration per se. The higher FCR observed in broilers fed the 19% CP diet may be associated with less efficient nutrient utilization, potentially linked to impaired intestinal function. van Harn et al. (2019) suggested that high-protein diets may negatively influence gut health and performance. Similarly, Liu et al. (2017), Apajalahti and Vienola (2016), and Qaisrani et al. (2015) reported that excessive dietary protein can increase undigested protein flow to the hindgut, promoting microbial fermentation and the production of harmful metabolites, which may compromise intestinal health and growth performance. Reducing crude protein levels may therefore decrease the production of toxic protein fermentation products in the ceca, contributing to improved nutrient utilization.

Although intestinal morphological and histological parameters were not evaluated in the present study, previous research provides supporting evidence. Buwjoom et al. (2010) reported improvements in intestinal characteristics following dietary protein reduction. Furthermore, Macelline et al. (2020) demonstrated that broilers fed low-protein diets supplemented with amino acids exhibited upregulation of tight junction-related genes (e.g., ZO-1 and claudin-1), which are essential for

maintaining intestinal barrier integrity. Wang and Peng (2008) emphasized that the efficiency of dietary protein utilization is closely associated with gastrointestinal tract structure and function, underscoring the critical role of intestinal health in nutrient absorption and feed efficiency.

TABLE 5
FEED CONVERSION RATIO (FCR) AND GAIN-TO-FEED (G:F) RATIO OF HUBBARD BROILERS FED CRUDE PROTEIN-REDUCED DIETS SUPPLEMENTED WITH ESSENTIAL AMINO ACIDS

Treatment	FCR As Fed	FCR DM	Gain to Feed Ratio
19% CP	2.80[a]	2.43[a]	0.37[b]
17% CP + AA	2.41[b]	2.11[b]	0.43[a]
15% CP + AA	2.41[b]	2.13[b]	0.42[a]

Means with different superscripts differ significantly (p < 0.05, LSD).

Figures 4a and 4b illustrate the weekly feed conversion ratio (FCR) and gain-to-feed (G:F) ratio. Throughout the four-week experimental period, birds receiving the control diet (Treatment 0; 19% CP) consistently exhibited a higher FCR compared to those on reduced crude protein (CP) diets ($p < 0.05$). A notable performance fluctuation was observed during Week 3, where Treatments 0 and 1 showed a sharp increase in FCR and a corresponding drastic decline in G:F. This shift was attributed to a transient reduction in voluntary feed intake, which limited the nutrient availability for weight gain during this phase.

In contrast, Treatment 2 maintained a linear, gradual increase in FCR and a steady decline in G:F efficiency. This progression aligns with the physiological maturation of the birds, as metabolic partitioning shifts from lean tissue deposition toward maintenance and fat accretion as age increases. Overall, the data suggest that reducing CP levels improved feed utilization efficiency, with Treatment 2 demonstrating the most stable growth trajectory and superior nutrient conversion compared to the 19% CP control.

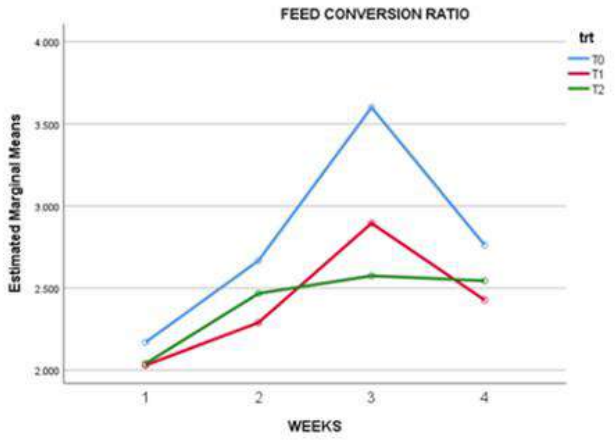

FIGURE 4a. Effects of dietary crude protein reduction and amino acid supplementation on feed conversion ratio (FCR) of Hubbard broilers over a 4-week period

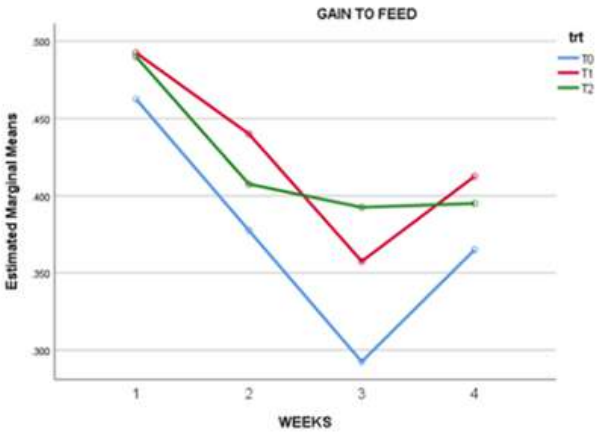

FIGURE 4b. Effects of dietary crude protein reduction and amino acid supplementation on gain-to-feed (G:F) ratio of Hubbard broilers over a 4-week period

3.5 Mortality:

Mortality data are summarized in Table 6. Overall survival was high across all groups, with a single mortality event (2.50%) recorded in the 17% CP + AA treatment group. Based on necropsy findings and environmental conditions, this mortality was not attributed to the dietary treatments. The bird exhibited clinical signs consistent with acute heat exhaustion, characterized by pallor of the pectoralis major (Plate 1), a common indicator of heat-induced myopathy in broilers (Srivastava, 2022). These findings align with the high ambient temperatures recorded during the summer rearing period. Consequently, the isolated loss was classified as an environmental rather than a nutritional casualty.

TABLE 6
CUMULATIVE MORTALITY RATE (%) OF HUBBARD BROILERS ACROSS DIETARY TREATMENTS

Treatment	Mortality	Percentage (%)
	Number	
19% CP	0	0
17% CP + AA	1	2.5
15% CP + AA	0	0

This data was not subjected to statistical analysis.

PLATE 1: Gross pathological changes in a 17% CP + AA bird; the pale coloration of the breast tissue suggests heat-related mortality rather than nutritional deficiency (cf. Srivastava, 2022)

3.6 Footpad Dermatitis:

The incidence and severity of footpad dermatitis (FPD) during Weeks 3 and 4 are summarized in Table 7, based on the scoring system established by Nagaraj et al. (2007). At Week 3, birds receiving the 15% and 17% CP + AA diets exhibited Score 0 (no lesions), whereas the 19% CP control group showed a 10% incidence of Score 1 lesions. By Week 4, although FPD was observed across all treatments, the severity was inversely proportional to dietary CP levels: Score 1 incidence was highest in the 19% CP group (12.50%), followed by the 17% CP + AA (10.00%) and 15% CP + AA (2.50%) groups. Notably, no lesions reaching Score 2 were recorded during the experimental period.

These findings align with previous research indicating that reduced dietary protein intake significantly mitigates the risk of FPD (Son et al., 2024; van Harn et al., 2019). The reduction in FPD incidence in low-CP groups is likely mediated by a decrease in excess nitrogen excretion, which consequently lowers voluntary water intake (Alfonso-Avila et al., 2022). Lower water intake reduces excreta moisture and maintains litter friability, preventing the prolonged contact with wet substrate that triggers pododermatitis and hock burns (Swiatkiewicz et al., 2017). Additionally, consistent litter management, including maintaining adequate depth and periodic agitation, likely contributed to the low overall severity of lesions observed in this flock.

TABLE 7
INCIDENCE AND SEVERITY OF FOOTPAD DERMATITIS (%) IN HUBBARD BROILERS AT WEEK 3 AND WEEK 4

Treatment	Week 3			Week 4		
	0	1	2	0	1	2
19% CP	90	10	0	87.5	12.5	0
17% CP + AA	100	0	0	90	10	0
15% CP + AA	100	0	0	97.5	2.5	0

This data was not statistically analyzed.

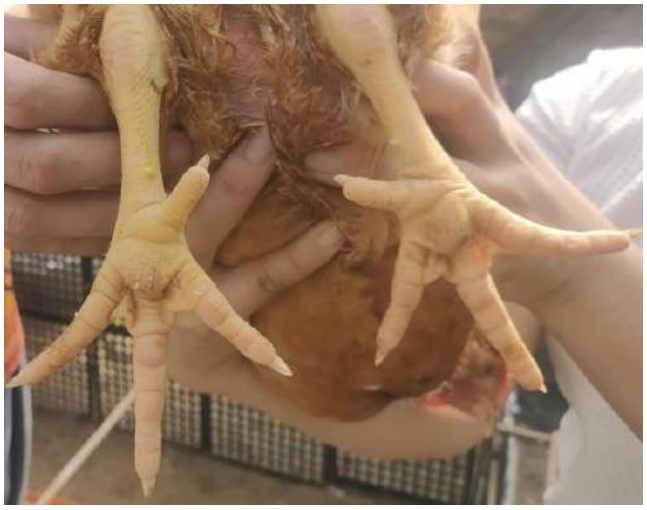

PLATE 3a: Visual assessment of footpad health: normal intact skin (Score 0)

PLATE 3b: Visual assessment of footpad health: mild plantar pododermatitis (Score 1). Scoring based on the scale by Nagaraj et al. (2007)

3.7 Profitability:

3.7.1 Production Costs and Sales:

The economic performance of Hubbard broilers under different dietary CP levels is summarized in Table 8. The total cost of production increased as dietary CP was reduced, a trend primarily driven by the higher inclusion levels of crystalline amino acid supplements and associated labor costs. Consequently, these nutritional inputs directly elevated the cost of production per bird in the reduced CP treatment groups.

Net sales were calculated based on a prevailing farmgate price of ₱165.00/kg live weight. Despite the lower production costs in the control group (19% CP), this treatment yielded a negative net profit and profit per bird (-₱418.50 and -₱10.46, respectively), largely due to significantly lower final body weights. Conversely, birds receiving the 17% + AA and 15% CP + AA diets achieved positive economic margins. This indicates that the enhanced growth performance and higher market weights of birds on crude protein-reduced diets more than compensated for the increased feed costs, resulting in superior net profit, ROI, and profit per bird.

Break-even point (BEP) analysis further validated these results. To reach the BEP in the 19% CP group, a minimum of 48 birds was required. In contrast, the 17% + AA and 15% CP + AA groups exceeded their respective BEP thresholds by 9 and 7 birds, respectively, confirming the economic feasibility and profitability of utilizing amino acid-supplemented, reduced-CP diets in broiler production.

TABLE 8
INFLUENCE OF DIETARY CRUDE PROTEIN (CP) AND AMINO ACID (AA) LEVELS ON THE ECONOMIC EFFICIENCY OF BROILER PRODUCTION

Parameters	Treatment		
	19% CP	17% CP + AA	15% CP + AA
Total Expenses (₱)	13,399.05	13,794.00	14,128.86
Total Sales (₱)	12,980.55	14,515.05	14,655.30
Net Profit (₱)	-418.5	721.05	526.44
Profit per Bird (₱)	-10.46	18.03	13.16
Cost of Production per Bird (₱)	334.98	344.85	353.22
Return on Investment (%)	-3.12	5.23	3.73
Breakeven Point	48	31	33

This data was not statistically analyzed.

IV. CONCLUSION

Reducing dietary crude protein (CP) by up to 4% (15% CP) with amino acid supplementation significantly enhances feed intake, final body weight, and average daily gain in Hubbard colored broilers. These improvements in growth parameters resulted in superior feed conversion ratios (FCR) and feed efficiency compared to the 19% CP control. Beyond performance, the reduced-CP diets notably decreased the incidence of footpad dermatitis, suggesting improved welfare and litter quality. While the inclusion of synthetic amino acids increased initial production costs, the 15% and 17% CP treatments yielded a higher net profit, return on investment (ROI), and profit per bird. Consequently, amino acid-supplemented, low-CP diets represent a physiologically superior and more profitable strategy for finisher broiler production.

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CONFLICT OF INTEREST

The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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An Exploratory Case Study of Google Meet and Zoom as Real-Time Digital Extension Tools for Aquaculture Advisory at Brazil Farm, Abuja, Nigeria

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Abstract— *Limited reach and delayed responsiveness of conventional agricultural extension services may constrain fish farming productivity in Nigeria. Inadequate farm visits and delayed access to technical expertise can hinder timely problem resolution and informed decision-making. This exploratory case study examined the use of Google Meet and Zoom as real-time digital extension tools for supporting aquaculture advisory interactions at Brazil Farm, Abuja, Nigeria. A field-based case-study design was adopted, and live advisory sessions were conducted on both platforms to connect a farm manager with an aquaculture specialist located remotely. Structured observation was used to assess technical performance, interaction quality, advisory clarity, and farmer engagement during the live sessions. Both platforms enabled real-time visual assessment, interactive diagnosis, and immediate technical feedback, suggesting that synchronous video platforms may support real-time aquaculture advisory interactions in similar contexts. However, performance differences were observed. Zoom demonstrated more stable connectivity and clearer audio transmission, supporting smoother interaction during the technical discussion. Google Meet provided easier access and simpler navigation, facilitating quicker session initiation for users with limited prior experience. The observations from this exploratory case study suggest that platform functionality may be influenced by technological reliability, user familiarity, and local infrastructural conditions. Although limited to a single-case field study, the research provides empirical insight into the potential of real-time digital advisory systems to complement conventional extension approaches. Further multi-site and quantitative investigations are recommended to enhance generalizability and inform policy adoption.*

Keywords— Digital extension; Aquaculture advisory; Real-time ICT; Google Meet; Zoom; Agricultural extension; Video conferencing; Fish farming; Nigeria.

I. INTRODUCTION

Agriculture remains fundamental to global food security, rural livelihoods, and economic development. However, agricultural systems face mounting pressures from rapid population growth, environmental change, and the depletion of natural resources. The global population is projected to approach 10 billion by 2050, intensifying the demand for food production while requiring greater resilience and sustainability in agricultural practices (World Bank, 2019; FAO, 2022). These pressures are particularly acute in developing countries, where smallholder farmers contribute substantially to agricultural production yet remain vulnerable to climatic variability, market fluctuations, and limited access to technical support services.

Within the agricultural sector, aquaculture, particularly fish farming, has emerged as one of the fastest-growing food-producing subsectors worldwide. Fish farming contributes significantly to food and nutrition security, income generation, and employment creation, especially in Africa and Asia (FAO, 2016; Beveridge et al., 2013). In sub-Saharan Africa, aquaculture enhances protein intake and provides livelihood opportunities along value chains encompassing production,

processing, and marketing (Brummett et al., 2008; Kassam & Dorward, 2017). In Nigeria, which grapples with persistent fish supply deficits, aquaculture has the potential to strengthen rural economies, reduce poverty, and improve household nutrition due to the growing demand for fish as an affordable source of animal protein (FAO, 2010; World Bank, 2014).

Despite its potential, fish farming in Nigeria continues to face persistent operational challenges that constrain productivity and profitability. These challenges include inadequate extension services, limited access to quality inputs such as fish seed and feed, disease outbreaks, poor water-quality management, weak market linkages, and constrained access to finance (Opara, 2008; Ogello et al., 2013; Chenyambuga et al., 2014). The limited reach of conventional agricultural extension, characterized by infrequent farm visits, high operational costs, and insufficient staffing, widens the information gap between farmers and subject-matter experts, often resulting in delayed or sub-optimal decision-making at the farm level. Access to timely, accurate, and relevant agricultural information is therefore critical for improving farm management decisions and productivity (Demiryurek et al., 2008; Das et al., 2016).

To achieve the primary goal of agricultural extension effectively, extension practitioners must be knowledgeable and skillful in communicating and disseminating information (Okunade & Oladosu, 2006). The rapid emergence and evolution of information and communication technologies (ICTs) have revolutionized information delivery across sectors, including agriculture (Alwahaishi & Snášel, 2013). In agricultural extension, ICTs have significantly transformed communication processes by facilitating faster, broader, and more interactive dissemination of information (Meera et al., 2004; Taiwo & Amoo, 2021; Nyarko & Kozari, 2021). Digital communication tools have the potential to enable extension systems to provide farmers with more timely information that may support improved production decisions and farm management practices.

In Nigeria, ICT adoption has expanded the reach of extension services by enabling communication through mobile phones, internet applications, and digital platforms, thereby mitigating some limitations of conventional extension approaches (Okeke et al., 2014). Social media and other internet-based platforms have further enhanced rapid information exchange, peer learning, and stakeholder engagement, particularly in resource-constrained rural settings (Alhassan et al., 2022). However, while digital tools have been widely used for meetings, training sessions, and coordination, especially during the COVID-19 pandemic, their structured application as real-time advisory tools in the fisheries sector remains limited.

Among emerging digital tools, synchronous video conferencing platforms such as Google Meet and Zoom present a promising but under-explored medium for real-time agricultural advisory services. Unlike asynchronous communication methods, video conferencing enables live, interactive engagement between farmers and subject-matter experts. Through real-time audio-visual interaction, experts can observe farm conditions remotely, diagnose problems instantly, and provide context-specific recommendations without physical presence. This capability is particularly valuable in aquaculture, where delays in addressing issues such as disease outbreaks or water-quality deterioration can result in rapid stock losses.

Live video-conferencing tools offer unique advantages for aquaculture advisory services because they enable visual demonstration and immediate feedback. Through real-time video interactions, extension agents can demonstrate practical techniques such as water-quality testing procedures, feeding management, disease symptom identification, pond sanitation practices, and aeration system adjustments, while farmers are able to ask questions and receive instant clarification. Davis and Sulaiman (2016) note that interactive ICT tools enhance learning outcomes in agricultural extension because they combine visual, auditory, and participatory elements that are particularly important in production systems requiring practical skill acquisition. In aquaculture, where management errors can lead to rapid stock mortality, the ability to visually assess pond conditions and provide immediate corrective guidance may significantly improve farm outcomes. Furthermore, the increasing penetration of smartphones in Nigeria has enhanced the feasibility of using live digital platforms for extension delivery. Empirical evidence indicates that farmers who utilize mobile-based advisory tools report improved access to technical information and greater confidence in adopting recommended practices (Salau & Saingbe, 2019).

Although both Google Meet and Zoom offer similar functionalities, including live video interaction, chat features, screen sharing, and session recording, they differ in interface design, connectivity stability, participant capacity, cost structure, and data requirements. These differences may influence their usability, reliability, and effectiveness in rural and peri-urban farming contexts. Despite the growing integration of ICTs in extension delivery, empirical evidence comparing the effectiveness of specific video conferencing platforms for real-time aquaculture advisory in Nigeria remains scarce. Existing studies largely focus on general ICT adoption rather than comparative evaluations of platform performance in practical farm problem-solving scenarios.

Given these realities, it is essential to assess which real-time digital platforms perform better under local infrastructural and socio-economic conditions. Comparative evaluation of platforms such as Google Meet and Zoom may provide useful insights for designing effective aquaculture advisory interventions. Nigeria's National Electronic Extension Platform (NEEP) underscores this need by promoting "interactive, cost-effective, and technology-driven" extension delivery that minimizes security risks to field agents and expands coverage to underserved farming communities (Federal Ministry of Agriculture and Rural Development [FMARD], 2023). Understanding the relative strengths and limitations of Google Meet and Zoom as real-time ICT advisory tools will provide evidence-based guidance for extension practitioners and policymakers seeking to improve aquaculture advisory services in Brazil Farm, Gosa Village, and similar communities across Nigeria.

Against this backdrop, this study conducts a comparative assessment of Google Meet and Zoom as real-time digital extension tools for addressing fish farming challenges at Brazil Farm, Gosa Village, Abuja Municipal Area, Nigeria. The study evaluates how each platform facilitates live expert–farmer interaction, enhances problem diagnosis, supports timely decision-making, and improves advisory outcomes. By systematically comparing platform performance, usability, and advisory effectiveness, this research contributes to emerging scholarship on digital extension models and provides empirical evidence to guide technology selection and policy interventions aimed at strengthening aquaculture advisory services in Nigeria and similar developing-country contexts.

1.1 Objectives of the Study:

The broad objective was to examine how Google Meet and Zoom supported real-time observation and discussion of fish farming challenges during remote advisory sessions. The specific objectives are to:

1. Evaluate the effectiveness of Google Meet and Zoom in facilitating real-time diagnosis and resolution of fish farming challenges.
2. Explore how real-time digital advisory interactions may support farmers' understanding of recommended aquaculture management practices.
3. Compare the technical performance of Google Meet and Zoom in terms of connectivity stability, audio-visual clarity, and ease of use during live advisory sessions.
4. Document farmer reflections and experiences regarding the usability of Google Meet and Zoom during the advisory sessions.

II. RESEARCH METHODOLOGY

2.1 Research Design:

This study adopted a comparative field-based exploratory case study design to examine the use of Google Meet and Zoom as real-time digital extension tools during aquaculture advisory interactions at Brazil Farm, Abuja, Nigeria.

The research was conducted through live advisory sessions at Brazil Farm, Gosa Village, Abuja Municipal Area Council, Nigeria. The study simulated a realistic digital extension scenario in which a farm manager received remote advisory support from an aquaculture subject-matter specialist who was not physically present on the farm but was located at the University of Abuja.

Each advisory session involved:

- The farm manager and farm staff (on-site participants)
- Two researchers present at the farm (facilitators and structured observers)
- One aquaculture specialist located at the University of Abuja, Department of Fisheries
- Two supporting researchers with the remote specialist (technical support and independent observation)

These settings enabled replication of practical digital extension conditions while maintaining structured comparative observation for methodological rigor.

To enhance internal validity and minimize participant-related bias, the roles of facilitators and observers were rotated between the Google Meet and Zoom sessions. This role rotation ensured that observed differences in interaction quality,

technical performance, or user experience were attributable primarily to platform characteristics rather than individual participant influence.

Both sessions were conducted under comparable farm and network conditions to maintain procedural consistency. Each session lasted approximately 45-60 minutes, allowing sufficient time for farm assessment, problem diagnosis, and advisory discussion.

2.2 Data Collection Method:

Data were collected through structured field observation during live advisory sessions conducted via Google Meet and Zoom.

Observers (both on-site and remote) systematically recorded predefined indicators, including:

- Audio clarity
- Video quality
- Network stability and connectivity
- Ease of joining and navigating the platform
- Ease of interaction between farmer and specialist
- Responsiveness and clarity of advisory communication
- Level of farmer engagement and participation
- Occurrence and duration of technical disruptions

The use of predefined observational indicators enhanced consistency across sessions.

No structured questionnaires or formal surveys were administered, as the study focused on real-time functional performance under field conditions. However, post-session reflections and informal feedback from participants were documented to supplement observational findings.

2.3 Data Collection Procedure:

Data collection was conducted through live on-farm advisory interventions at Brazil Farm.

Step 1: Physical Farm Assessment:

The research team conducted an initial on-site assessment with the farm manager. Observations focused on pond conditions, fish health status, water quality management practices, infrastructure adequacy, and operational challenges.

Step 2: Google Meet Session:

A live advisory session was initiated using Google Meet with the remotely located aquaculture specialist. Real-time video of ponds, equipment, and farm conditions was transmitted to the specialist, who provided immediate technical feedback following visual assessment and clarifying questions. Google Meet was selected for the initial session due to the farm manager's limited familiarity with Zoom and the ease of accessing Google Meet via a shared link.

Step 3: Zoom Session:

A comparable advisory session was subsequently conducted using Zoom under similar farm and network conditions. The same categories of production challenges were discussed. Observers documented differences in technical performance, interaction dynamics, connectivity stability, and overall advisory effectiveness.

2.4 Identified Farm Challenges:

During the advisory sessions, the following key challenges were identified:

- Shortage of trained personnel and limited technical expertise
- Climate-related fluctuations affecting water quality
- Fish mortalities linked to inadequate monitoring practices

- Infrastructural limitations, including insufficient equipment
- Limited access to aquaculture health services

2.5 Expert Advisory Intervention:

During both live sessions, the aquaculture specialist provided real-time recommendations, including:

- Establishing linkages with aquaculture health professionals
- Scheduling periodic technical visits
- Implementing structured training programs for farm personnel
- Strengthening equipment maintenance and sanitation practices
- Conducting routine water-quality monitoring
- Adopting proactive fish health management strategies

The comparative evaluation focused on how effectively each platform facilitated clear communication, visual assessment, timely diagnosis, and immediate expert response.

2.6 Data Documentation and Analysis:

Observational indicators were systematically reviewed and comparatively analyzed across both platforms. The analysis focused on identifying differences in:

- Technical performance
- Quality of interaction
- Clarity and responsiveness of advisory communication
- Farmer engagement and participation
- Overall effectiveness in addressing real-time production challenges

A descriptive comparative analytical approach was employed to determine the relative suitability of Google Meet and Zoom as real-time digital extension tools under the prevailing field conditions.

III. RESULTS AND DISCUSSION

3.1 Effectiveness in Real-Time Diagnosis and Problem Resolution:

Both Google Meet and Zoom enabled real-time visual interaction between the farm manager and the aquaculture specialist, allowing remote observation of pond conditions, feeding practices, and farm infrastructure. Through live video streaming, the specialist was able to identify observable issues such as poor water coloration, inadequate aeration practices, and irregular monitoring routines.

However, differences in technical performance influenced the efficiency of real-time diagnosis. During the observed session, Zoom appeared to provide relatively more stable connectivity and clearer audio transmission under the prevailing network conditions at the farm site. This facilitated an uninterrupted explanation of recommended corrective measures, particularly during detailed discussions of water-quality monitoring and disease-prevention protocols.

Google Meet, while accessible and easy to initiate, experienced minor audio distortions and brief connectivity lags during the session. Although these interruptions did not prevent advisory delivery, they occasionally required repetition of instructions, slightly reducing communication efficiency.

These findings align with existing research emphasizing that ICT effectiveness in extension is influenced not only by availability but also by functional reliability under field conditions (Meera et al., 2004; Taiwo & Amoo, 2021). In aquaculture, where rapid decision-making is essential to prevent stock losses, platform stability becomes a critical factor. This observation highlights the importance of technological reliability in digital extension delivery.

3.2 Influence on Understanding of Recommended Practices:

The live advisory format facilitated immediate clarification of recommended management practices, including structured water-quality monitoring and routine sanitation procedures. The ability to visually demonstrate pond conditions enhanced mutual understanding between the expert and farm personnel.

Informal reflections from the farm manager suggested increased clarity and understanding of the recommended management practices following the Zoom session, primarily due to clearer audio communication and smoother interaction flow. The reduced need for repetition allowed for more focused technical explanation.

These findings support the assertion by Davis and Sulaiman (2016) that interactive ICT tools enhance learning outcomes in agricultural extension by combining visual, auditory, and participatory elements. Real-time interaction may enhance farmers' understanding of recommended aquaculture practices and facilitate clearer communication between experts and farm personnel compared to delayed or text-based advisory methods.

3.3 Technical Performance and Usability Comparison:

Comparative assessment across predefined indicators revealed observable differences between the two platforms, as summarized in Table 1.

TABLE 1
COMPARATIVE PERFORMANCE OF GOOGLE MEET AND ZOOM DURING LIVE ADVISORY SESSIONS

Indicator	Google Meet	Zoom
Ease of joining	Easier (direct link, minimal steps)	Slightly more steps required
Connectivity stability	Minor audio distortions and brief lags	More stable, fewer disruptions
Audio-visual clarity	Adequate with occasional interruptions	Clearer audio transmission
Interaction flow	Brief interruptions requiring occasional repetition	Smoother conversational exchange
User familiarity	Higher (Google account integration)	Lower initially for this user

These findings reflect broader observations that platform performance may vary depending on bandwidth optimization and data management design (Alwahaishi & Snášel, 2013). In peri-urban Nigerian contexts where network stability is inconsistent, platform adaptability to low-bandwidth conditions becomes a decisive usability factor.

3.4 Farmer Perception and Satisfaction:

Informal post-session reflections indicated that the farm manager found both platforms useful for remote advisory support. Google Meet was perceived as easier to initiate, particularly for first-time users. However, Zoom was considered more reliable for sustained technical discussion.

The farmer noted that uninterrupted communication improved comprehension of water-quality management strategies and disease-prevention protocols. This suggests that perceived usefulness is closely linked to communication clarity and system stability.

These observations align with technology adoption research, which highlights perceived ease of use and perceived usefulness as central determinants of technology acceptance (Demiryurek et al., 2008; Nyarko & Kozari, 2021).

3.5 Comparative Effectiveness and Advisory Impact:

Overall, both platforms demonstrated potential as real-time digital extension tools in aquaculture advisory. However, comparative analysis suggests that Zoom offered relatively stronger performance in technical stability and interaction continuity under the observed field conditions.

Google Meet's simplicity and accessibility make it suitable for rapid initiation and low-barrier engagement, especially where users are less technologically experienced. Conversely, Zoom's superior interaction stability may provide advantages in scenarios requiring prolonged technical consultation.

These findings contribute to ongoing discussions on digital extension models in Nigeria, particularly within the framework of the National Electronic Extension Platform (FMARD, 2023), which advocates interactive, technology-driven advisory delivery. The observations from this exploratory case study suggest that synchronous video platforms may help reduce

geographical barriers and support real-time problem discussion between farmers and specialists. However, platform selection should consider local network infrastructure, user familiarity, cost implications, and advisory complexity.

IV. LIMITATIONS OF THE STUDY

While this study provides insights into the comparative use of Google Meet and Zoom as real-time digital extension tools in aquaculture, several limitations should be acknowledged.

First, the study was conducted as a single-case field experiment at one fish farm in Abuja. As a result, the findings reflect the specific infrastructural, network, and managerial conditions of that farm. Variations in farm size, technological readiness, staff capacity, and regional connectivity conditions may influence platform performance differently. Therefore, the generalizability of the findings to other geographic locations or production systems remains limited.

Second, the study represents a short-term intervention focusing on immediate advisory effectiveness rather than long-term outcomes. Although both platforms facilitated real-time problem diagnosis and technical guidance, the sustained adoption of recommended practices, long-term productivity improvements, and measurable economic impacts were not systematically assessed. The absence of longitudinal monitoring limits conclusions regarding enduring behavioral or performance changes.

Third, the evaluation relied primarily on structured observation rather than quantitative performance metrics. While observational indicators such as connectivity stability, interaction flow, and user engagement provided practical insights, the study did not incorporate statistical measurement, cost–benefit analysis, or standardized usability scales. Consequently, conclusions regarding comparative efficiency are interpretive rather than statistically validated.

Fourth, the presence of researchers during the advisory sessions may have influenced participant behavior, potentially introducing observer-related bias. Although efforts were made to rotate roles and maintain consistency, the complete elimination of such effects in field experiments is difficult.

Despite these limitations, the study provides practical, field-based evidence on the feasibility of integrating synchronous video conferencing platforms into aquaculture extension systems in Nigeria. The findings offer an important exploratory foundation for larger-scale, multi-site, and longitudinal studies that can incorporate quantitative performance metrics and economic impact assessment to strengthen generalizability and policy relevance.

V. CONCLUSION

This study comparatively assessed Google Meet and Zoom as real-time digital extension tools for addressing fish farming challenges at Brazil Farm, Abuja, Nigeria. The findings suggest that synchronous video conferencing platforms can effectively facilitate remote advisory interaction between aquaculture specialists and farm managers under real field conditions.

Both platforms enabled live visual assessment of pond conditions, interactive problem diagnosis, and immediate technical feedback, confirming the operational feasibility of integrating real-time ICT-based approaches into aquaculture extension systems. The study, therefore, contributes field-based evidence to the growing discourse on digital agricultural extension in developing-country contexts.

Comparative analysis revealed functional differences between the two platforms. Zoom demonstrated relatively stronger performance in connectivity stability and audio clarity, which enhanced continuity of interaction during extended technical discussions. In contrast, Google Meet provided easier access and simpler navigation, making it more suitable for rapid deployment and users with limited familiarity with video conferencing tools.

The findings indicate that the effectiveness of digital extension platforms depends not only on technological features but also on contextual factors such as network stability, infrastructural constraints, user familiarity, and advisory complexity. While Zoom may be preferable where stable connectivity can be reasonably maintained, Google Meet may offer advantages in resource-constrained settings where ease of access is prioritized.

As a single-case field study, these findings are context-specific and should not be generalized without further multi-site investigation. Nonetheless, the study highlights the potential of structured real-time digital advisory systems to complement conventional face-to-face extension models and strengthen responsiveness within aquaculture production systems.

VI. RECOMMENDATIONS FOR FUTURE RESEARCH

Based on the exploratory findings of this single-case study, the following recommendations are proposed primarily to guide future research and methodological refinement in the application of digital platforms for aquaculture extension.

6.1 Need for Multi-Site Comparative Studies:

Future studies should examine the use of synchronous video conferencing platforms across multiple fish farms and geographic locations. Conducting research with larger samples will enable more robust comparison of platform performance under diverse infrastructural and production conditions and improve the generalizability of findings.

6.2 Incorporation of Quantitative Performance Metrics:

Subsequent investigations should incorporate measurable indicators such as session duration, number of technical disruptions, connectivity stability scores, and communication latency. The use of standardized quantitative metrics would strengthen the empirical assessment of digital extension platforms.

6.3 Assessment of Farmer Adoption and Behavioural Outcomes:

Longitudinal studies are needed to evaluate whether real-time digital advisory interactions influence the long-term adoption of recommended aquaculture management practices. Tracking behavioral changes, productivity improvements, and farm performance over time would provide stronger evidence of advisory impact.

6.4 Evaluation of User Experience and Technology Acceptance:

Future research should incorporate structured surveys or standardized usability scales to systematically measure farmer satisfaction, perceived usefulness, and ease of use of video conferencing platforms. Such data would complement observational findings and improve understanding of technology acceptance in rural farming contexts.

6.5 Cost-Effectiveness and Operational Feasibility Analysis:

Further studies should investigate the economic feasibility of integrating video conferencing tools into agricultural extension systems. Comparative analysis of operational costs, data consumption, and time efficiency relative to conventional farm visits would provide valuable insights for extension program design.

6.6 Exploration of Training and Digital Literacy Needs:

Additional research should assess the digital literacy levels of farmers and extension agents to determine how familiarity with communication technologies influences the effectiveness of video-based advisory interactions.

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CONFLICT OF INTEREST

The authors declare no conflict of interest. This research was conducted independently without funding from Google, Zoom, or any commercial entity involved in video conferencing services. All assessments and conclusions are based solely on the observed performance under the specific field conditions described

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Effects of Bay Leaf (*Laurus nobilis* L.), Potato (*Solanum tuberosum* L.) Peel and Banana (*Musa* Species) Peel Extracts on Bio-Chemical Indicators of Some Upland Rice (*Oryza sativa* L.) Crop under Higher Iron in Acid Soil Condition

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Abstract—

Background: *The occurrence of high rainfall in Assam and leaching of base cations cause the soils to become acidic in reaction. The high mobility of iron in acid soil and its absorption raises the iron concentration, and it damages the physiology of crop plants. That is why excessive iron is regarded as one of the limiting factors responsible for lowering growth, development and yield of upland rice crop under irrigation through shallow tube wells. An innovative approach using extracts of potassium- and antioxidant-rich bio-inputs might be a novel approach for deterring and halting physio-biochemical aberrations due to higher iron in acid soil condition.*

Method: *A pot experiment (CRBD with three replications) was carried out to investigate the effects of bay leaf, potato peel, and banana peel extracts on biochemical indicators of some upland (Ahu) rice crop (varieties: Inglongkiri, Dehangi (Fe tolerant), Lachit (Fe susceptible), and Luit) under higher iron in acid soil conditions during the Autumn season (March-September, 2024). The five treatments were: (1) 100 ppm $FeSO_4$ as basal at vegetative stage (control), (2) 100 ppm $FeSO_4$ as basal at vegetative stage plus root dip treatment before transplanting and foliar spray with bay leaf extract at 20 days after transplanting, (3) 100 ppm $FeSO_4$ as basal at vegetative stage plus root dip treatment before transplanting and foliar spray with banana peel extract at 20 days after transplanting, (4) 100 ppm $FeSO_4$ as basal at vegetative stage plus root dip treatment before transplanting and foliar spray with potato peel extract at 20 days after transplanting; (5) Natural soil without any treatment (absolute control).*

Result: *In general, compared to the control, the treatments significantly increased biochemical indices viz., total chlorophyll (10.80-21.90%), chlorophyll 'a' (6.39-20.93%), chlorophyll 'b' contents (7.27-22.35%) at maximum tillering stage; total chlorophyll (10.96-21.38%), chlorophyll 'a' (11.88-21.89%), chlorophyll 'b' contents at heading stage (7.22-15.79%), NR activity at maximum tillering (12.43-35.52%) and heading (15-36.23%) stages, carbohydrate content (7.51-13.81%) and reduced iron content (10.57-21.34%) in grain at harvest. The bio-input extracts lessened cation leakage at maximum tillering (2.25-1.63%) and heading (3.52-2.83%) stages, and lipid peroxidation at maximum tillering (30.03-10.55%) and heading (29.64-10.96%) stages, despite the presence of high iron in the soil (initial: 200 ppm, harvest: 106 ppm). In the experiment, the intensity of blue colour following Perl's Prussian Blue staining was directly related to iron content in grain, with darker staining indicating higher iron accumulation, and this was altered by the bio-input treatments.*

Keywords— Banana peel, Bay leaf, Chlorophyll, CMS, Cation leakage, Carbohydrate, Iron, Potato peel, Rice.

I. INTRODUCTION

Rice is one of the staple food crops in Assam cultivated as Winter (70%), Autumn rice (23%) and Summer rice (7%) covering 2.54 million hectares. The higher iron content in the acid soil (80% of geographical area i.e. 25 m ha) of the region is one of the considerable factors for lower productivity (< 3 t ha^{-1}) of traditional genotypes. The high rainfall (>2000 mm) makes the soil acidic in nature (pH<5.0) due to leaching of basic cations (Mandal, 1995; Mandal et al., 2019). Consequently, the ground water contains higher iron (0.25-67.0 ppm), and its absorption by plant roots enriches the concentration of iron in plants. The excess iron (150-450 ppm) in plants affects the growth and development of rice crop, which is manifested by appearance of different physiological symptoms viz., potassium deficiency, yellowing of green leaves, dark brown or bronze spots, and death of plants (Baruah et al., 1983; Bora and Borkakati, 1997). Although many efforts have been made to ameliorate the aberrations due to iron toxicity in plants (Singh and Singh, 1987; Borah and Nath, 1979; Bey, 2022; Bey et al., 2026), there is a paucity of information on how bay leaf, banana peel, and potato peel extracts halt and rectify the degradation of biochemical indices in rice crop. As the extracts of these bio-inputs contain various nutrients and bio-molecules (Burton, 1989; Nguyen et al., 2003; Buckenhüskes, 2005; Emaga et al., 2007; Cakmak et al., 2013; Sidhu and Zafar, 2018; Batool et al., 2020; Karan, 2023), it is worthwhile to investigate their effects on biochemical indices in upland (Ahu) rice crop in the presence of high iron conditions.

II. MATERIALS AND METHODS

The laboratory pot experiment (March-September 2024) was conducted at Assam Agricultural University (Geographical position: 26°45' N Latitude, 94°12' E Longitude, 87 meters above mean sea level). The study comprised four upland (Ahu) cultivars: Inglongkiri, Dehangi (iron tolerant), Lachit (iron susceptible), and Luit. The 25 to 30 days old seedlings of the cultivars were transplanted in pots filled with a mixture of sand, loam soil, and farm yard manure. All management practices including application of recommended NPK fertilizers @ 60:40:40 kg ha^{-1} based on the volume of soil per hectare, irrigation (2-3 cm) and prophylactic measures against insect pests were taken up when necessary.

Iron solution of 100 ppm strength was prepared using $FeSO_4$ (MW: 151.908 g). Each pot (made leak proof by sealing bottom with mud) received a basal application of the $FeSO_4$ solutions (10 days prior to transplanting). Locally collected matured bay leaves, ripe banana peel, and potato peel were dried at 55°C in a hot air oven. Ten grams of each dried and ground material was combined with 100 ml of distilled water separately, then the mixtures were heated to 50°C for 60 minutes in a thermostatic bath. The extracts were cooled, filtered (with a pore size of 0.45 mm), and kept in dark at 4°C (Gebre Christos et al., 2020). Before transplanting, root dip treatments (RDT) of 30-day-old seedlings were administered overnight (100 seedlings per litre solution) with the extracts of banana, potato, and bay leaf.

Soil pH was measured as suggested by Jackson (1973). Biochemical parameters viz., leaf chlorophyll contents (Hiscox and Israelstam, 1979), nitrate reductase (NR) activity (Thimmaiah, 1999), carbohydrate content in grain (Hedge et al., 1962), cation leakage (Leopold et al., 1981), and lipid peroxidation (Heath and Packer, 1968) were estimated at different growth stages. Iron content in grain was determined by colourimetric method (Sandell, 1950; Yoshida et al., 1971). The presence of iron in grains was also visualized using Perl's Prussian Blue staining technique (Velu et al., 2006) with 5% potassium ferrocyanide and 5% hydrochloric acid. Data were analyzed using the ANOVA technique suggested by Panse and Sukhatme (1967). The "F" value was calculated and critical difference between a pair of means was compared to the tabulated value at 5% significance level using appropriate statistical software.

III. RESULTS AND DISCUSSION

3.1 Experimental Conditions:

The experimental environmental conditions were congenial: Temperature ranged from 17.4 to 35.1°C, rainfall from 14.8 to 83.6 mm, and bright sunshine hours from 2.4 to 7.2 hours. The soil was acidic with pH ranging from 4.93 to 5.60, and initially deficient in N (200.35 kg ha^{-1}), P (33.08 kg ha^{-1}), and K (133.03 kg ha^{-1}), which was corrected later as suggested by Baruah and Borthakur (1997). The amelioration of physiological aberrations of indigenous upland Ahu rice genotypes caused by the presence of higher available iron (at transplanting: 200 ppm; at harvest: 106 ppm) by application of bay leaf, potato peel, and banana extracts was also reported by Bey et al. (2026).

3.2 Chlorophyll Content:

3.2.1 Total Chlorophyll Content:

The results revealed significant variations in total chlorophyll content at maximum tillering stage (Table 1a) among the treatments. Compared to the 100 ppm $FeSO_4$ (control), total chlorophyll increased by 21.90% with 100 ppm $FeSO_4$ plus aqueous bay leaf > 100 ppm $FeSO_4$ plus aqueous banana peel (18.61%) > 100 ppm $FeSO_4$ plus aqueous potato peel (11.13%) > natural soil (10.80%). Overall, total chlorophyll content decreased in the variety Lachit (27.05%) > Inglongkiri (14.95%) > Luit (13.91%) as compared to Dehangi (iron tolerant).

At heading stage (Table 1b), total chlorophyll content varied significantly among treatments. Compared to control, total chlorophyll increased by 21.38% with 100 ppm $FeSO_4$ plus aqueous bay leaf > 100 ppm $FeSO_4$ plus aqueous banana peel (17.72%) > 100 ppm $FeSO_4$ plus aqueous potato peel (15.23%) > natural soil (10.96%). Overall, total chlorophyll content decreased in the variety Lachit (23.22%) > Inglongkiri (15.14%) > Luit (12.07%) as compared to Dehangi.

TABLE 1
EFFECT OF BAY LEAF, POTATO PEEL, AND BANANA PEEL EXTRACTS ALONG WITH Fe TREATMENTS ON TOTAL CHLOROPHYLL CONTENT (mg g^{-1} f.w.)

Variety	Treatments					
	100 ppm $FeSO_4$ (control)	100 ppm $FeSO_4$ + aqueous Bay leaf	100 ppm $FeSO_4$ + aqueous Banana peel	100 ppm $FeSO_4$ + aqueous Potato peel	Natural soil	Mean
(a) at Maximum tillering stage						
Inglongkiri	1.833	2.433	2.4	2.267	2.067	2.2
Dehangi	2.367	2.767	2.667	2.533	2.6	2.587
Lachit	1.633	2.167	2.033	1.7	1.9	1.887
Luit	1.9	2.533	2.4	2.2	2.1	2.227
Mean	**1.933**	**2.475**	**2.375**	**2.175**	**2.167**	
	S.Ed(±)	CD(0.05)				
Variety (V)	0.02	0.06				
Treatment (T)	0.02	0.05				
V × T	0.04	0.11				
(b) at Heading stage						
Inglongkiri	1.5	2	1.933	1.867	1.833	1.827
Dehangi	1.833	2.433	2.3	2.2	2	2.153
Lachit	1.5	1.8	1.7	1.667	1.6	1.653
Luit	1.667	2.033	1.967	1.933	1.867	1.893
Mean	**1.625**	**2.067**	**1.975**	**1.917**	**1.825**	
	S.Ed(±)	CD(0.05)				
Variety (V)	0.02	0.06				
Treatment (T)	0.02	0.06				
V × T	0.04	0.11				

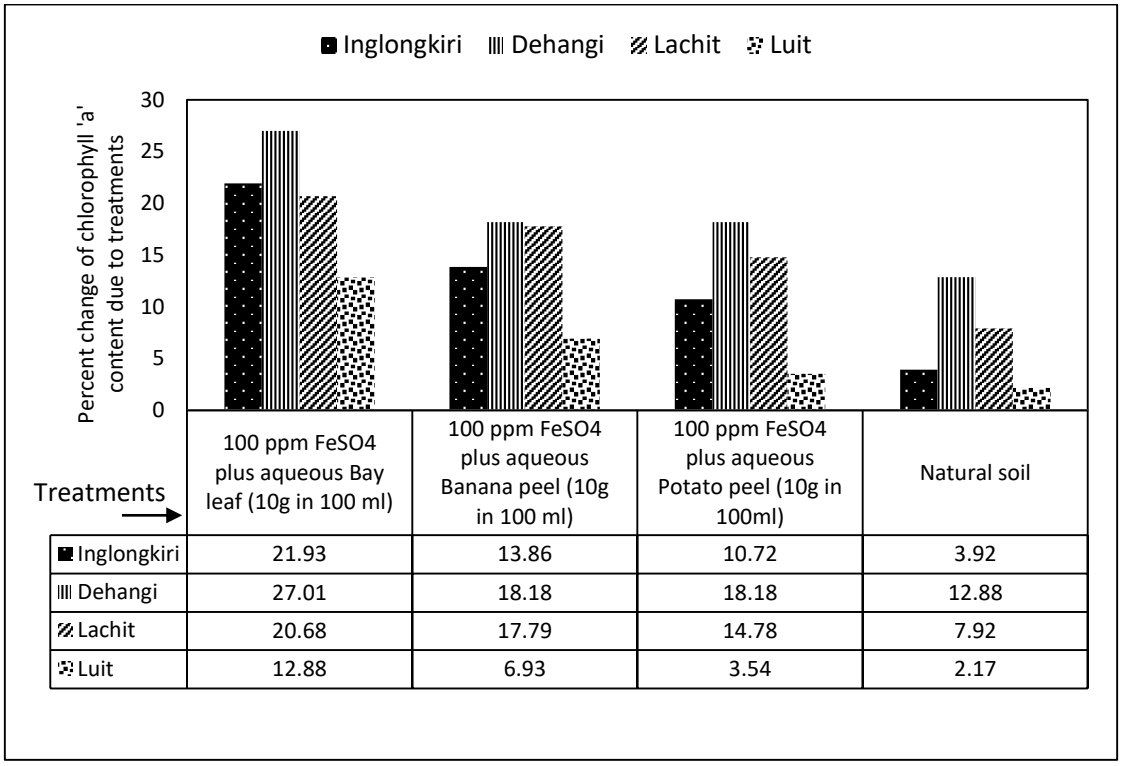
Note: Values are means of three replications. Higher values indicate better chlorophyll retention. S.Ed = Standard Error of Difference; CD = Critical Difference at 5% level.

Bay leaf extracts are known to enhance the availability and uptake of essential nutrients like nitrogen and magnesium, which are critical for chlorophyll synthesis. Phenolics and flavonoids in bay leaf extracts mitigate oxidative stress, which often leads to chlorophyll degradation. The bioactive compounds in bay leaf extracts inhibit chlorophyll-degrading enzymes like chlorophyllase. By stabilizing chloroplast structures and reducing damage caused by reactive oxygen species (ROS), bay leaf extract enhanced chlorophyll accumulation. Furthermore, phenolic compounds caused an increase in the activity of photosynthetic pigments (chlorophyll 'a', chlorophyll 'b', total chlorophyll, and carotenoid) in plants (Aina et al., 2022). An improvement of 28% in chlorophyll content was observed by the application of Moringa leaf (Rashid et al., 2021).

3.2.2 Chlorophyll 'a' Content:

The results revealed significant variations in chlorophyll 'a' content at maximum tillering stage (Fig. 1a) among treatments. Compared to control, chlorophyll 'a' increased by 20.93% with 100 ppm $FeSO_4$ plus aqueous bay leaf > 100 ppm $FeSO_4$ plus

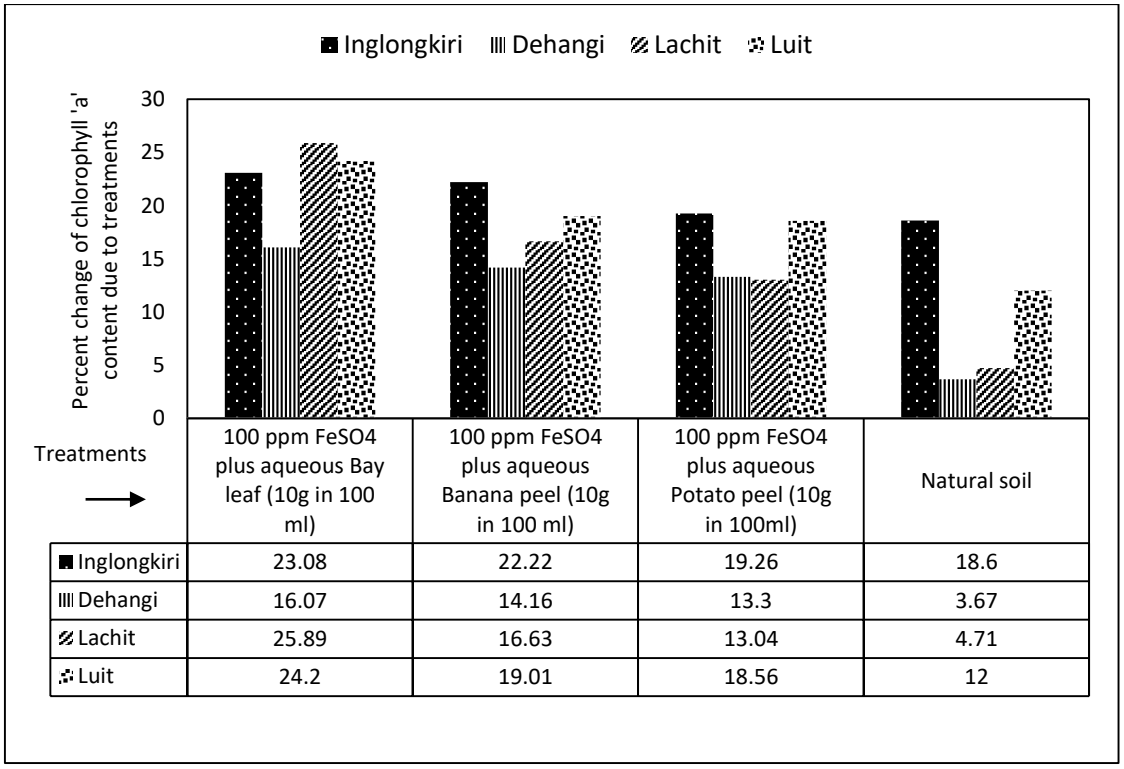
aqueous banana peel (14.31%) > 100 ppm $FeSO_4$ plus aqueous potato peel (12.10%) > natural soil (6.39%). Overall, chlorophyll 'a' decreased in the variety Lachit (17.98%) > Inglongkiri (13.04%) > Luit (11.74%) as compared to Dehangi.



	100 ppm FeSO4 plus aqueous Bay leaf (10g in 100 ml)	100 ppm FeSO4 plus aqueous Banana peel (10g in 100 ml)	100 ppm FeSO4 plus aqueous Potato peel (10g in 100ml)	Natural soil
Inglongkiri	21.93	13.86	10.72	3.92
Dehangi	27.01	18.18	18.18	12.88
Lachit	20.68	17.79	14.78	7.92
Luit	12.88	6.93	3.54	2.17

FIGURE 1(a): Chlorophyll 'a' in comparison with control at Maximum tillering stage

At heading stage (Fig. 1b), significant variations in chlorophyll 'a' content were observed among treatments. Compared to control, chlorophyll 'a' increased by 21.89% with 100 ppm $FeSO_4$ plus aqueous bay leaf > 100 ppm $FeSO_4$ plus aqueous banana peel (17.56%) > 100 ppm $FeSO_4$ plus aqueous potato peel (15.20%) > natural soil (11.88%). Overall, chlorophyll 'a' decreased in the variety Lachit (20.68%) > Inglongkiri (12.40%) > Luit (10.34%) as compared to Dehangi.



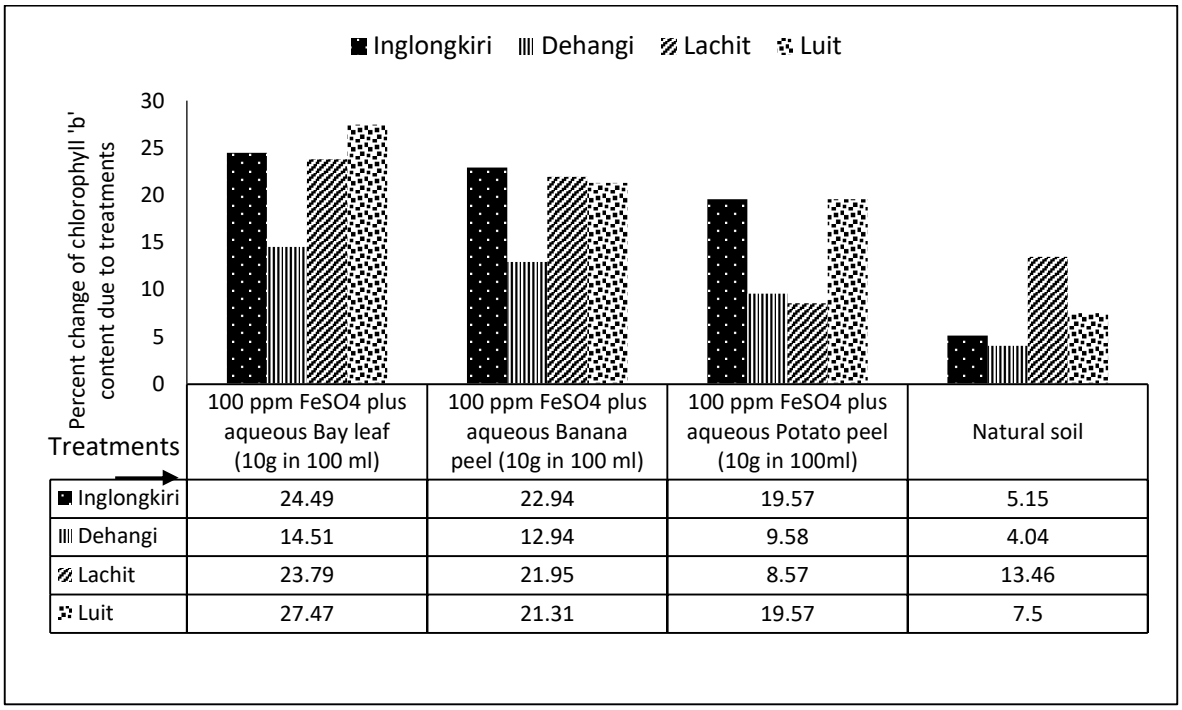
	100 ppm FeSO4 plus aqueous Bay leaf (10g in 100 ml)	100 ppm FeSO4 plus aqueous Banana peel (10g in 100 ml)	100 ppm FeSO4 plus aqueous Potato peel (10g in 100ml)	Natural soil
Inglongkiri	23.08	22.22	19.26	18.6
Dehangi	16.07	14.16	13.3	3.67
Lachit	25.89	16.63	13.04	4.71
Luit	24.2	19.01	18.56	12

FIGURE 1(b): Chlorophyll 'a' in comparison with control at Heading stage

3.2.3 Chlorophyll 'b' Content:

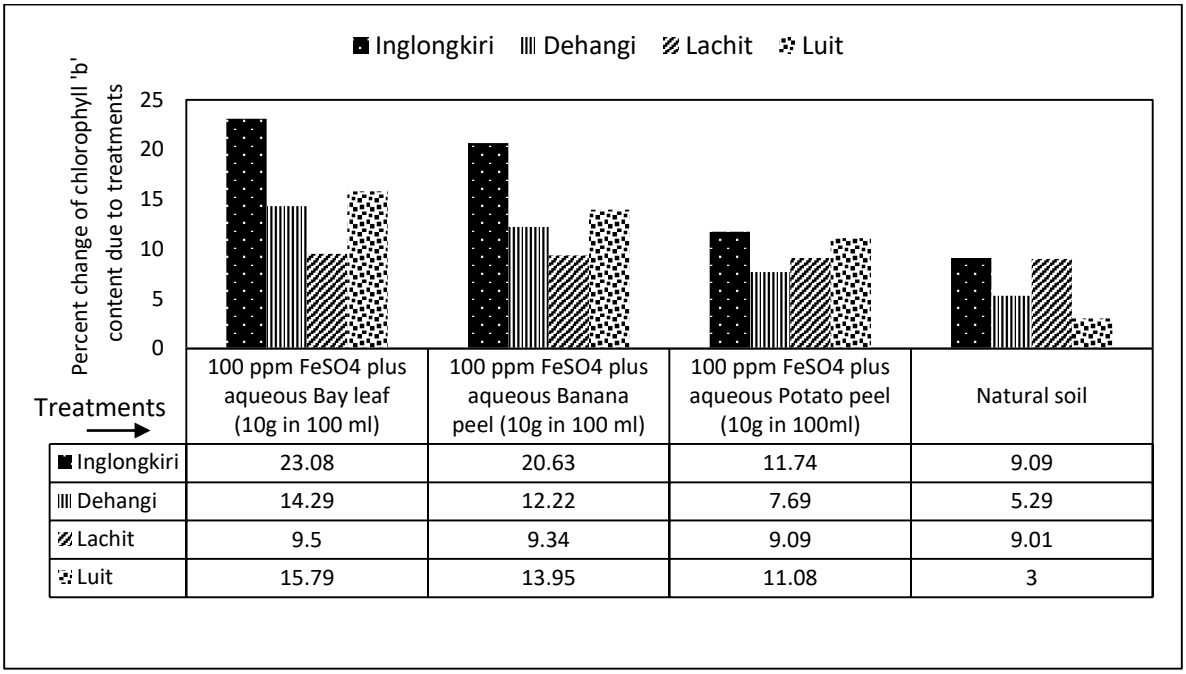
The results revealed significant variations in chlorophyll 'b' content at maximum tillering stage (Fig. 2a) among treatments. Chlorophyll 'b' content increased by 22.35% with 100 ppm $FeSO_4$ plus aqueous bay leaf > 100 ppm $FeSO_4$ plus aqueous banana peel (19.46%) > 100 ppm $FeSO_4$ plus aqueous potato peel (14.54%) > natural soil (7.27%). Overall, chlorophyll 'b' decreased in the variety Lachit (27.20%) > Inglongkiri (14.76%) > Luit (14.01%) as compared to Dehangi.

At heading stage (Fig. 2b), chlorophyll 'b' content increased significantly among treatments. Total chlorophyll content increased by 15.79% with 100 ppm $FeSO_4$ plus aqueous bay leaf > 100 ppm $FeSO_4$ plus aqueous banana peel (13.46%) > 100 ppm $FeSO_4$ plus aqueous potato peel (10.49%) > natural soil (7.22%). Overall, chlorophyll 'b' decreased in the variety Lachit (17.36%) > Inglongkiri (11.24%) > Luit (9.71%) as compared to Dehangi.



Treatments	100 ppm FeSO4 plus aqueous Bay leaf (10g in 100 ml)	100 ppm FeSO4 plus aqueous Banana peel (10g in 100 ml)	100 ppm FeSO4 plus aqueous Potato peel (10g in 100ml)	Natural soil
Inglongkiri	24.49	22.94	19.57	5.15
Dehangi	14.51	12.94	9.58	4.04
Lachit	23.79	21.95	8.57	13.46
Luit	27.47	21.31	19.57	7.5

FIGURE 2 (a): Chlorophyll 'b' in comparison with control at Maximum tillering stage



Treatments	100 ppm FeSO4 plus aqueous Bay leaf (10g in 100 ml)	100 ppm FeSO4 plus aqueous Banana peel (10g in 100 ml)	100 ppm FeSO4 plus aqueous Potato peel (10g in 100ml)	Natural soil
Inglongkiri	23.08	20.63	11.74	9.09
Dehangi	14.29	12.22	7.69	5.29
Lachit	9.5	9.34	9.09	9.01
Luit	15.79	13.95	11.08	3

FIGURE 2 (b): Chlorophyll 'b' in comparison with control at Heading stage

3.3 Nitrate Reductase (NR) Activity:

The results revealed significant variations in NR activity at maximum tillering stage (Table 2a) among treatments. Compared to control, NR activity increased by 35.52% with 100 ppm $FeSO_4$ plus aqueous bay leaf > 100 ppm $FeSO_4$ plus aqueous banana peel (30.94%) > 100 ppm $FeSO_4$ plus aqueous potato peel (24.56%) > natural soil (12.43%). Overall, NR activity decreased in the variety Lachit (32.84%) > Inglongkiri (24.61%) > Luit (17.46%) as compared to Dehangi.

At heading stage (Table 2b), NR activity varied significantly among treatments. NR activity increased by 36.23% with 100 ppm $FeSO_4$ plus aqueous bay leaf > 100 ppm $FeSO_4$ plus aqueous banana peel (31.06%) > 100 ppm $FeSO_4$ plus aqueous potato peel (24.44%) > natural soil (15.00%). Overall, NR activity decreased in the variety Lachit (33.50%) > Inglongkiri (25.20%) > Luit (18.42%) as compared to Dehangi.

TABLE 2

EFFECT OF BAY LEAF, POTATO PEEL, AND BANANA PEEL EXTRACTS ALONG WITH Fe TREATMENTS ON NITRATE REDUCTASE (NR) ACTIVITY (µmole NO_3^- g^{-1} f.w. hr^{-1})

Variety	Treatments					
	100 ppm $FeSO_4$ (control)	**100 ppm $FeSO_4$ + aqueous Bay leaf**	**100 ppm $FeSO_4$ + aqueous Banana peel**	**100 ppm $FeSO_4$ + aqueous Potato peel**	**Natural soil**	**Mean**
(a) at Maximum tillering stage						
Inglongkiri	0.767	1.2	1.1	1	0.833	0.98
Dehangi	1	1.5	1.467	1.4	1.2	1.3
Lachit	0.7	1	0.967	0.9	0.8	0.873
Luit	0.8	1.367	1.267	1.033	0.9	1.073
Mean	**0.817**	**1.267**	**1.183**	**1.083**	**0.933**	
	S.Ed(±)	CD(0.05)				
Variety (V)	0.01	0.02				
Treatment (T)	0.01	0.02				
V × T	0.02	0.04				
(b) at Heading stage						
Inglongkiri	0.8	1.267	1.167	1	0.9	1.027
Dehangi	1.067	1.6	1.433	1.467	1.3	1.373
Lachit	0.7	1.067	1	0.933	0.867	0.913
Luit	0.833	1.4	1.333	1.1	0.933	1.12
Mean	**0.85**	**1.333**	**1.233**	**1.125**	**1**	
	S.Ed(±)	CD(0.05)				
Variety (V)	0.01	0.03				
Treatment (T)	0.01	0.02				
V × T	0.02	0.05				

Note: Values are means of three replications. Higher values indicate better nitrogen assimilation. S.Ed = Standard Error of Difference; CD = Critical Difference at 5% level.

Bay leaf enhanced NR activity and nitrogen metabolism in crops. Phenolic compounds in bay leaf extracts may serve as natural activators of NR by stabilizing its structure and increasing enzymatic activity. These compounds can also reduce oxidative stress, preventing NR degradation under unfavourable conditions. Bay leaf extracts enhance root function and nutrient uptake, ensuring a higher nitrate supply for conversion by NR. Improved nitrogen metabolism leads to better protein synthesis, promoting growth and biomass accumulation. NR is sensitive to oxidative damage; antioxidants in bay leaf extracts protect NR from ROS, sustaining its activity under environmental stress (Sarkar et al., 2010; Rice-Evans et al., 1997). Bay leaf is rich in potassium, which plays a crucial role in NR activity, an essential enzyme in nitrogen metabolism. Application of foliar

mineral KNO_3 significantly increased nitrate content as well as NR activity in sunflower and safflower plants irrespective of their growth under non-saline or saline conditions (Jabeen and Ahmad, 2011).

3.4 Cation Leakage:

The results revealed significant variations in cation leakage at maximum tillering stage (Table 3a) among treatments. Compared to control, cation leakage decreased by 1.93% with 100 ppm $FeSO_4$ plus aqueous bay leaf > 100 ppm $FeSO_4$ plus aqueous banana peel (1.78%) > 100 ppm $FeSO_4$ plus aqueous potato peel (1.63%) > natural soil (2.25%). Overall, cation leakage increased in the variety Lachit (0.93%) > Luit (0.53%) > Inglongkiri (0.50%) as compared to Dehangi.

At heading stage (Table 3b), significant variations in cation leakage were observed among treatments. Cation leakage decreased by 3.27% with 100 ppm $FeSO_4$ plus aqueous bay leaf > 100 ppm $FeSO_4$ plus aqueous banana peel (3.13%) > 100 ppm $FeSO_4$ plus aqueous potato peel (2.83%) > natural soil (3.52%). Overall, cation leakage increased in the variety Lachit (1.11%) > Luit (0.48%) > Inglongkiri (0.41%) as compared to Dehangi.

TABLE 3

EFFECT OF BAY LEAF, POTATO PEEL, AND BANANA PEEL EXTRACTS ALONG WITH Fe TREATMENTS ON CATION LEAKAGE (%)

Variety	Treatments					
	100 ppm $FeSO_4$ (control)	**100 ppm $FeSO_4$ + aqueous Bay leaf**	**100 ppm $FeSO_4$ + aqueous Banana peel**	**100 ppm $FeSO_4$ + aqueous Potato peel**	**Natural soil**	**Mean**
(a) at Maximum tillering stage						
Inglongkiri	95.167	93.633	94.433	95	93.367	94.32
Dehangi	99.733	89.167	91	93.367	88.267	92.307
Lachit	98.1	94.3	95.8	96.567	93.7	95.693
Luit	96.833	94.1	94.867	94.9	91.467	94.433
Mean	**97.458**	**92.8**	**94.025**	**94.958**	**91.7**	
	S.Ed(±)	CD(0.05)				
Variety (V)	0.61	1.79				
Treatment (T)	0.54	1.55				
V × T	1.22	3.1				
(b) at Heading stage						
Inglongkiri	92.067	89.467	90.367	90.4	89.033	90.267
Dehangi	89.467	88.233	89.033	89.1	88.167	88.8
Lachit	98.567	90.5	90.567	93.467	89.467	92.513
Luit	91.077	89.767	89.8	91.067	89.233	90.187
Mean	**92.792**	**89.492**	**89.942**	**91.008**	**88.975**	
	S.Ed(±)	CD(0.05)				
Variety (V)	0.5	1.48				
Treatment (T)	0.45	1.28				
V × T	1	2.56				

Note: Values are means of three replications. Lower values indicate better membrane integrity. S.Ed = Standard Error of Difference; CD = Critical Difference at 5% level.

The phenolic and flavonoid compounds in bay leaf extracts strengthen cell membranes by reducing lipid peroxidation and maintaining membrane integrity, thereby preventing excessive cation loss. Reactive oxygen species cause oxidative damage to membrane lipids, leading to increased cation leakage. The antioxidant properties of bay leaf extracts neutralize ROS, protecting cell membranes from oxidative stress. Bay leaf bioactive compounds may enhance the regulation of ion transporters such as H^+-ATPases and Na^+/K^+ transporters, maintaining ionic homeostasis and preventing excessive cation efflux. Lipid peroxidation, a major cause of membrane damage, is mitigated by the bioactive compounds in bay leaf extracts, leading to

lower levels of malondialdehyde (MDA), a key indicator of membrane deterioration. Bay leaves are a good natural source of potassium. Under drought conditions, excess ROS production in plants may exaggerate cellular lipid peroxidation, leading to an increase in cellular membrane permeability, evidenced by increases in electrolyte leakage and MDA content (Fazeli et al., 2007; Degenkolbe et al., 2009). Soleimanzadeh et al. (2010) reported that an adequate supply of K significantly decreased MDA content under water shortage condition, clearly indicating the role of K in mitigating oxidative stress. The use of K against cadmium (Cd) toxicity confirmed the positive effect of this element by ameliorating Cd-induced oxidative damage in broad bean (Siddiqui et al., 2012).

3.5 Cell Membrane Stability (CMS):

At maximum tillering stage (Table 4a), there were significant variations in CMS among treatments. Compared to control, CMS increased by 52.02% with 100 ppm $FeSO_4$ plus aqueous bay leaf > 100 ppm $FeSO_4$ plus aqueous banana peel (44.76%) > 100 ppm $FeSO_4$ plus aqueous potato peel (31.40%) > natural soil (26.14%). Overall, CMS decreased in the variety Lachit (47.87%) > Inglongkiri (30.52%) > Luit (27.45%) as compared to Dehangi.

At heading stage (Table 4b), CMS varied significantly among treatments. Compared to control, CMS increased by 37.22% with 100 ppm $FeSO_4$ plus aqueous bay leaf > 100 ppm $FeSO_4$ plus aqueous banana peel (30.68%) > 100 ppm $FeSO_4$ plus aqueous potato peel (20.02%) > natural soil (16.22%). Overall, CMS decreased in the variety Lachit (34.30%) > Inglongkiri (21.86%) > Luit (19.66%) as compared to Dehangi.

TABLE 4
EFFECT OF BAY LEAF, POTATO PEEL, AND BANANA PEEL EXTRACTS ALONG WITH Fe TREATMENTS ON CELL MEMBRANE STABILITY (CMS)

Variety	Treatments					
	100 ppm $FeSO_4$ (control)	**100 ppm $FeSO_4$ + aqueous Bay leaf**	**100 ppm $FeSO_4$ + aqueous Banana peel**	**100 ppm $FeSO_4$ + aqueous Potato peel**	**Natural soil**	**Mean**
(a) at Maximum tillering stage						
Inglongkiri	3.06	4.12	4.02	3.22	3.127	3.509
Dehangi	3.297	6.63	5.81	4.853	4.667	5.051
Lachit	1.123	3.95	3.22	2.75	2.12	2.633
Luit	2.16	5.39	4.4	3.23	3.14	3.664
Mean	**2.41**	**5.023**	**4.363**	**3.513**	**3.263**	
	S.Ed(±)	CD(0.05)				
Variety (V)	0.038	0.077				
Treatment (T)	0.034	0.068				
V × T	0.075	0.153				
(b) at Heading stage						
Inglongkiri	5.057	6.123	6.02	5.223	5.127	5.51
Dehangi	5.3	8.63	7.807	6.853	6.67	7.052
Lachit	3.123	5.953	5.22	4.75	4.12	4.633
Luit	4.16	7.393	6.4	5.23	5.14	5.665
Mean	**4.41**	**7.025**	**6.362**	**5.514**	**5.264**	
	S.Ed(±)	CD(0.05)				
Variety (V)	0.071	0.144				
Treatment (T)	0.063	0.129				
V × T	0.142	0.288				

Note: Values are means of three replications. Higher values indicate better membrane stability. S.Ed = Standard Error of Difference; CD = Critical Difference at 5% level.

In the study, CMS was decreased by iron and ameliorated by the bio-input extracts, especially bay leaf having higher potassium. The fall in CMS is linked to increase in lipid peroxidation regulated by ROS generation (Bharali and Bates, 2004; Bharali et al., 2015; Bharali et al., 2016) under higher iron in acid soil condition. Free iron directly or indirectly as ferritin of ferredoxin catalyzes the formation of the highly damaging hydroxyl radical (OH^-) from superoxide or its dismuted product H_2O_2. The OH^- becomes the most likely form of activated oxygen initiating peroxidative breakdown of lipids, which in turn results in disruption of the plasma membrane, reduces CMS, and increases membrane leakage (Price and Hendry, 1991).

3.6 Lipid Peroxidation:

The results revealed significant variations in lipid peroxidation at maximum tillering stage (Table 5a) among treatments. Compared to control, lipid peroxidation decreased by 17.49% with 100 ppm $FeSO_4$ plus aqueous bay leaf > 100 ppm $FeSO_4$ plus aqueous banana peel (14.29%) > 100 ppm $FeSO_4$ plus aqueous potato peel (10.55%) > natural soil (30.03%). Overall, lipid peroxidation increased in the variety Lachit (13.76%) > Inglongkiri (5.77%) > Luit (3.72%) as compared to Dehangi.

At heading stage (Table 5b), significant variations in lipid peroxidation were observed among treatments. Lipid peroxidation decreased by 17.78% with 100 ppm $FeSO_4$ plus aqueous bay leaf > 100 ppm $FeSO_4$ plus aqueous banana peel (13.28%) > 100 ppm $FeSO_4$ plus aqueous potato peel (10.96%) > natural soil (29.64%). Overall, lipid peroxidation increased in the variety Lachit (14%) > Inglongkiri (5.67%) > Luit (3.70%) as compared to Dehangi.

TABLE 5

EFFECT OF BAY LEAF, POTATO PEEL, AND BANANA PEEL EXTRACTS ALONG WITH Fe TREATMENTS ON LIPID PEROXIDATION (µmol MDA g^{-1} fresh weight)

Variety	Treatments					
	100 ppm $FeSO_4$ (control)	**100 ppm $FeSO_4$ + aqueous Bay leaf**	**100 ppm $FeSO_4$ + aqueous Banana peel**	**100 ppm $FeSO_4$ + aqueous Potato peel**	**Natural soil**	**Mean**
(a) at Maximum tillering stage						
Inglongkiri	6.233	5.067	5.267	5.6	4.433	5.32
Dehangi	5.8	4.9	5.067	5.167	4.133	5.013
Lachit	6.8	5.6	5.9	6.133	4.633	5.813
Luit	6.133	5.033	5.167	5.433	4.267	5.207
Mean	**6.242**	**5.15**	**5.35**	**5.583**	**4.367**	
	S.Ed(±)	CD(0.05)				
Variety (V)	0.04	0.1				
Treatment (T)	0.03	0.09				
V × T	0.07	0.18				
(b) at Heading stage						
Inglongkiri	6.467	5.233	5.5	5.733	4.633	5.513
Dehangi	6.033	5.067	5.267	5.333	4.3	5.2
Lachit	7	5.867	6.267	6.3	4.8	6.047
Luit	6.367	5.1	5.4	5.667	4.467	5.4
Mean	**6.467**	**5.317**	**5.608**	**5.758**	**4.55**	
	S.Ed(±)	CD(0.05)				
Variety (V)	0.04	0.11				
Treatment (T)	0.03	0.09				
V × T	0.07	0.19				

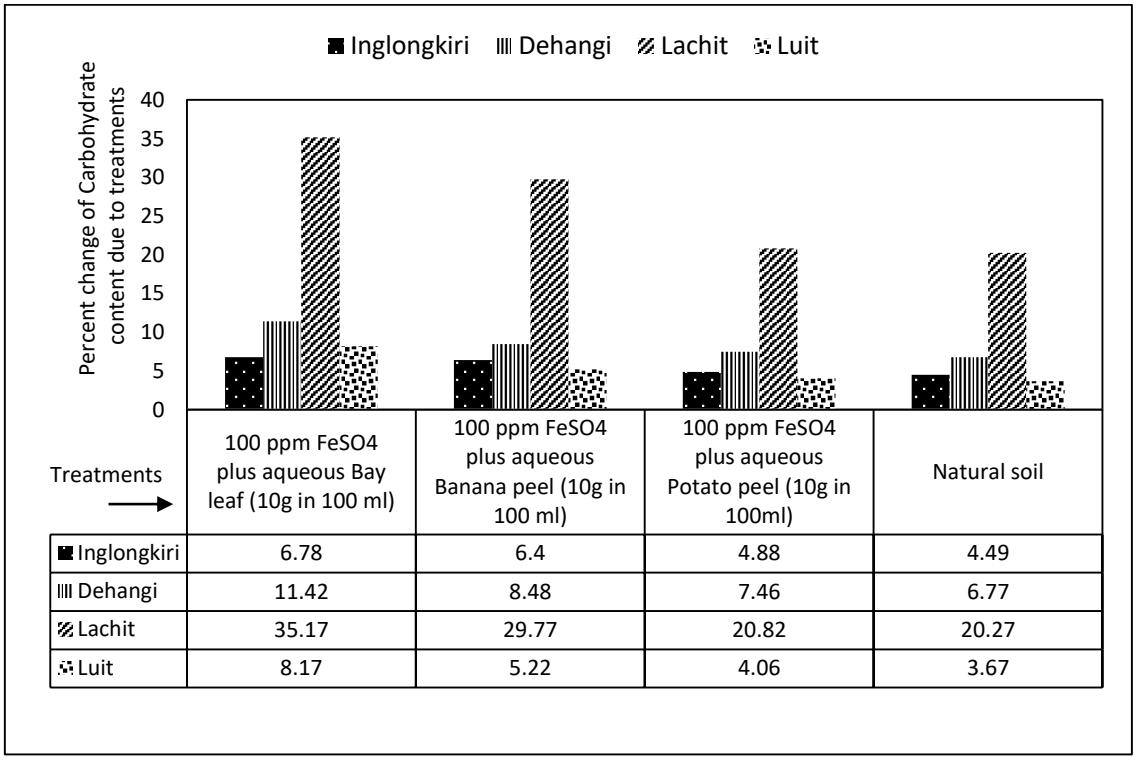
Note: Values are means of three replications. Lower values indicate less oxidative damage. MDA = malondialdehyde. S.Ed = Standard Error of Difference; CD = Critical Difference at 5% level.

Bay leaf extracts contain high potassium levels and potent antioxidants such as quercetin, kaempferol, and eugenol, which scavenge ROS, preventing oxidative damage to membrane lipids. Malondialdehyde (MDA) is a byproduct of lipid peroxidation

and a key indicator of membrane damage. Studies suggest that plant-derived phenolics, like those in bay leaf extracts, significantly reduce MDA accumulation, leading to improved cell stability. Song et al. (2015) experimented with peach plants using elevated exogenous K (10 mM) against Zn toxicity (2 mM). They observed that Zn damages the plant by altering physiological processes and nutritional balance. However, K mitigated Zn toxicity by improving photosynthesis, antioxidant defence systems, and plant K nutritional status.

3.7 Carbohydrate Content in Grain:

The results revealed significant variations in carbohydrate content in grain at harvesting stage (Fig. 3) among treatments. Compared to control, carbohydrate content increased by 13.81% with 100 ppm $FeSO_4$ plus aqueous bay leaf > 100 ppm $FeSO_4$ plus aqueous banana peel (10.87%) > 100 ppm $FeSO_4$ plus aqueous potato peel (8.03%) > natural soil (7.51%). Overall, carbohydrate content decreased in the variety Lachit (42.61%) > Inglongkiri (8.03%) > Luit (7.50%) as compared to Dehangi.



	100 ppm FeSO4 plus aqueous Bay leaf (10g in 100 ml)	100 ppm FeSO4 plus aqueous Banana peel (10g in 100 ml)	100 ppm FeSO4 plus aqueous Potato peel (10g in 100ml)	Natural soil
Inglongkiri	6.78	6.4	4.88	4.49
Dehangi	11.42	8.48	7.46	6.77
Lachit	35.17	29.77	20.82	20.27
Luit	8.17	5.22	4.06	3.67

FIGURE 3: Carbohydrate content in grain compared with control at harvest

The current studies on phenolic-rich plant extracts (bay leaf) might help in enhancing carbohydrate metabolism and grain quality. Bay leaf extracts improve chlorophyll stability, leading to increased photosynthetic activity. Higher photosynthesis rates result in greater production of photo-assimilates like sucrose, which are translocated to developing grains. Bioactive compounds in bay leaf extracts may upregulate the activity of starch-synthesizing enzymes, such as ADP-glucose pyrophosphorylase and starch synthase, leading to higher starch accumulation in grains. Bay leaf extracts may enhance phloem loading and sucrose transport from leaves to grains, ensuring a steady supply of carbohydrates for grain filling. The antioxidant properties of bay leaf extracts help mitigate stress effects, maintaining efficient carbohydrate metabolism. Improved nitrogen and potassium uptake facilitated by bay leaf extracts supports energy metabolism, promoting carbohydrate biosynthesis and storage in grains. Flavonoids constitute a wide range of substances that play an important role in protecting biological systems against the harmful effects of oxidative processes on macromolecules, such as carbohydrates, proteins, and lipids (Halliwell and Gutteridge, 1989). Potassium takes part in protein synthesis, carbohydrate metabolism, and enzyme activation (Wang et al., 2013).

3.8 Iron Content in Grain:

The results revealed significant variations in iron content in grain among treatments (Table 6). Compared to control, iron content in grain decreased by 7.33% with 100 ppm $FeSO_4$ plus aqueous bay leaf > 100 ppm $FeSO_4$ plus aqueous banana peel (5.77%) > 100 ppm $FeSO_4$ plus aqueous potato peel (3.49%) > natural soil (2.23%). Overall, iron content in grains increased in the variety Lachit (21.34%) > Luit (15.57%) > Inglongkiri (10.57%) as compared to Dehangi (Fig. 4).

TABLE 6
EFFECT OF BAY LEAF, POTATO PEEL, AND BANANA PEEL EXTRACTS ALONG WITH FE TREATMENTS ON IRON CONTENT IN GRAINS (ppm)

Variety	100 ppm $FeSO_4$ (control)	100 ppm $FeSO_4$ + aqueous Bay leaf	100 ppm $FeSO_4$ + aqueous Banana peel	100 ppm $FeSO_4$ + aqueous Potato peel	Natural soil	Mean
Inglongkiri	120	113.44	114.43	117.85	118.74	116.89
Dehangi	115.24	95.06	100.39	104.65	107.34	104.53
Lachit	135.01	130.65	131.3	133	134.45	132.88
Luit	126.57	121.21	122.01	124	125.21	123.8
Mean	**124.2**	**115.09**	**117.03**	**119.87**	**121.43**	
	S.Ed(±)	CD(0.05)				
Variety (V)	2.16	4.42				
Treatment (T)	2.49	5.1				
V × T	4.31	8.83				

Note: Values are means of three replications. Lower values indicate reduced iron accumulation in grains. S.Ed = Standard Error of Difference; CD = Critical Difference at 5% level. CD values have been corrected using the formula CD = S.Ed × t-value (at error df).

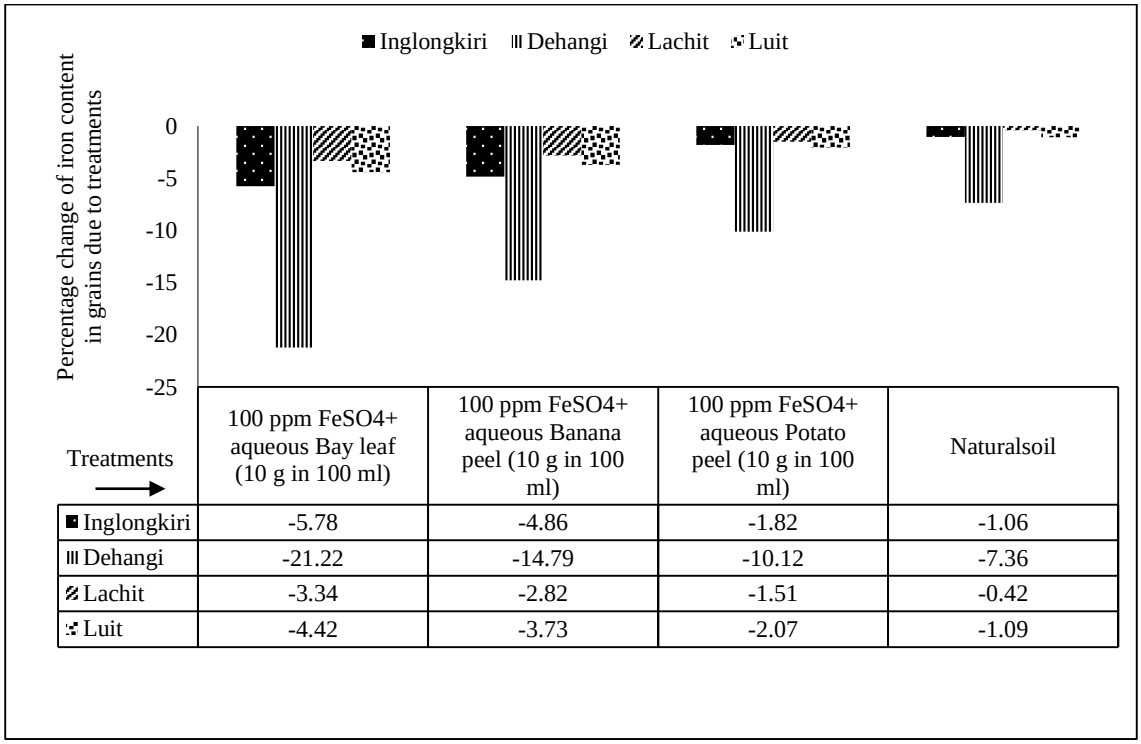


FIGURE 4: Iron content in grain as compared to the control at harvest

3.9 Histochemical Localization of Iron in Grain:

There were variations in intensity of colour among the genotypes under the treatments depending upon the iron contents in grain. Higher iron content in grain resulted in greater intensity of blue colour (Table 7a). Genotypes with relatively high iron developed medium to dark blue colour intensity, and those with low iron level developed light blue or no colour. Thus, the genotypes could be sorted for iron content in grain with varying scores (Table 7b). Genotypes with more than 8 ppm iron received a score of '4', those with 4-6 ppm received a score of '3', and those with less than 4 ppm iron received a score of '2' or '1'. Using this technique, genotypes were categorized as having very low (score 1), low (score 2), medium (score 3), and high (score 4) iron contents (Purusothaman, 2010).

TABLE 7(a)

CATEGORY OF GENOTYPES UNDER THE TREATMENTS BASED ON THE IRON CONTENT IN RICE GRAIN AT HARVEST AS DETERMINED BY PERL'S PRUSSIAN BLUE STAINING METHOD (Velu et al., 2006)

Treatment	Variety	Fe Score	Colour Intensity	Fe Category
100 ppm $FeSO_4$ (Control)	Inglongkiri	4	Dark blue	High
	Dehangi	4	Dark blue	High
	Lachit	4	Dark blue	High
	Luit	4	Dark blue	High
100 ppm $FeSO_4$ + Aqueous Bay Leaf (10 g/100 ml)	Inglongkiri	3	Medium blue	Medium
	Dehangi	2	Light blue	Low
	Lachit	3	Medium blue	Medium
	Luit	2	Light blue	Low
100 ppm $FeSO_4$ + Aqueous Banana Peel (10 g/100 ml)	Inglongkiri	3	Medium blue	Medium
	Dehangi	2	Light blue	Low
	Lachit	3	Medium blue	Medium
	Luit	2	Light blue	Low
100 ppm $FeSO_4$ + Aqueous Potato Peel (10 g/100 ml)	Inglongkiri	3	Medium blue	Medium
	Dehangi	3	Medium blue	Medium
	Lachit	3	Medium blue	Medium
	Luit	3	Medium blue	Medium
Natural Soil Condition	Inglongkiri	1	No colour	Very Low
	Dehangi	1	No colour	Very Low
	Lachit	1	No colour	Very Low
	Luit	1	No colour	Very Low

TABLE 7 (b)

INTERPRETATION USED FOR SORTING OUT THE GENOTYPES FOR IRON CONTENT IN GRAIN (RELATIVE SCALE FOR EXPERIMENTAL CONDITIONS)

Score	Fe Content (ppm)	Category	Colour Intensity
4	> 120	High	Dark blue
3	100–120	Medium	Medium blue
2	80–100	Low	Light blue
1	< 80	Very Low	No colour

Note: Scoring thresholds adjusted to match actual experimental values from Table 6 (range: 95-135 ppm). This relative scale allows for meaningful comparison within the experimental context. Staining intensity based on Perl's Prussian Blue reaction with 5% potassium ferrocyanide and 5% HCl (Velu et al., 2006).*

In the treatment with 100 ppm $FeSO_4$ (control), all varieties (Inglongkiri, Dehangi, Lachit, and Luit) scored 4 with dark blue colour. In the treatment with 100 ppm $FeSO_4$ plus aqueous bay leaf, varieties Inglongkiri and Lachit scored 3 with medium blue colour, while Dehangi and Luit scored 2 with light colour. Under the treatment with 100 ppm $FeSO_4$ plus aqueous banana peel, Inglongkiri and Lachit scored 3 with medium blue colour, while Dehangi and Luit scored 2 with light colour. In the treatment with 100 ppm $FeSO_4$ plus aqueous potato peel, all varieties (Inglongkiri, Dehangi, Lachit, and Luit) scored 3 with medium blue colour. Under natural soil condition, all varieties scored 1 and exhibited no colour change. Shobhana et al. (2013) stated that the different blue colour intensities developed during the treatment provide a steadfast selection criterion for grain micronutrient (iron) contents in cereal crops. This technique could, therefore, be cost-effectively used to categorize genotypes for higher grain iron content quickly.

IV. CONCLUSION

Among bay leaf, banana peel, and potato extracts, bay leaf was found to be most suitable for enhancing key biochemical parameters viz., chlorophyll contents, NR activity, CMS at different growth stages, and carbohydrate content in grain. Moreover, lipid peroxidation, cation leakage, and CMS were lowered by bay leaf, particularly in the variety Dehangi, due to

iron-induced ROS activity in the plant. This was evidenced by the iron content in grain and its correspondence with the varying intensity of grain colour determined in the experiment.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest in publishing the manuscript in the journal regarding the publication of this article.

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Eco-toxicological and Nutritional Hazards of Tembotrione: A Multi-Species Evaluation After Long-Term Use

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Abstract— *Environmental contamination from herbicide applications for weed control remains a significant concern. This study presents an ecological and dietary risk assessment of tembotrione following its long-term use in maize cultivation, focusing on potential impacts on humans, animals, and aquatic organisms. Residue levels and degradation behavior of tembotrione were monitored in soil, maize plants, and grains at various intervals post-application to estimate exposure risks. The half-life showed a positive correlation with tembotrione persistence in soil, as well as with its water solubility and volatility. Risk Quotient (RQ) values indicated a risk ranging from high to negligible for soil macro-organisms over a 60-day period, with a moderate risk at 90 days. Health Quotient analysis revealed that the risk to animal health from consuming contaminated maize straw varied from high to low. Human dietary exposure posed a relatively low risk. However, aquatic organisms exhibited moderate to high ecological risk. These findings underscore the importance of tembotrione residues and its long-term ecological footprint in agricultural systems.*

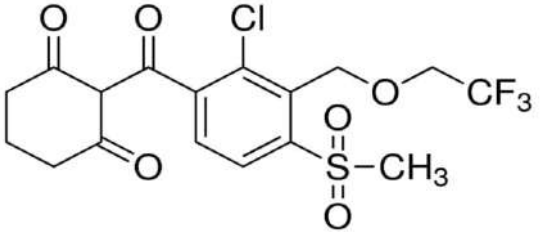
Keywords— Risk assessment; Fish; Health; Macro-organisms; Pesticides; Maize cultivation; Herbicide persistence.

I. INTRODUCTION

Herbicides are among the most widely used pesticides for effective weed control, with global consumption estimated at approximately 1.4 million metric tons annually (Statista, 2020). However, their frequent application has led to growing concerns, including the development of herbicide-resistant weed species, phytotoxic effects on non-target crops, and the accumulation of chemical residues in soil and aquatic ecosystems (Zhang et al., 2019; Wang et al., 2022; Heap, 2023; Sahand et al., 2021; Sondhia & Waseem, 2020; Syafrudin et al., 2021). As a result, many herbicides have been banned or subjected to regulatory restrictions in various countries (Sondhia, 2014; 2018; Zhou et al., 2019; Sondhia & Waseem, 2020; Syafrudin et al., 2021). Maize, the second most extensively cultivated crop worldwide, occupies around 193.7 million hectares with an average yield of 5.75 tons per hectare (FAOStat, 2020; ICAR-IIMR, 2022). Looking ahead, maize is projected to dominate global cereal consumption for animal feed, growing at an annual rate of 1.3%. By 2031, global maize production is expected to increase by 161 million tons, reaching approximately 1.33 billion tons (OECD-FAO, 2022).

Tembotrione is a triketone-class herbicide widely used for post-emergence weed control in maize cultivation across the globe (Christos et al., 2018; Khanna et al., 2022). Its mode of action involves inhibition of the enzyme 4-hydroxyphenylpyruvate dioxygenase (HPPD), which disrupts carotenoid biosynthesis in target weed species. The global market value of tembotrione was estimated at USD 33.4 million in 2020 and is projected to grow at a compound annual growth rate (CAGR) of 13.6%, reaching USD 81.54 million in the coming years (Global Tembotrione Market, 2023). Chemically, tembotrione is hydrolytically stable and non-volatile (EPA, 2007; Zemełka, 2015), with a water solubility of 28.3 g/L at pH 7 (PubChem NCBI, 2023; Table 1). It undergoes transformation into xanthenedione derivatives, which exhibit greater toxicity than the parent compound (Wang et al., 2022).

TABLE 1
IMPORTANT PHYSICO-CHEMICAL PROPERTIES OF TEMBOTRIONE

Tembotrione chemical structure	
Chemical name	Tembotrione, 2-[2-chloro-4-(methylsulfonyl)-3-[(2,2,2-(trifluoroethoxy)methyl]benzoyl]-1,3-cyclohexanedione
molecular formula	C17H16ClF3O6S
Mol wt g/mol	440,82
Melting point	117 ºC
pH @ 24 °C	3.63
Water solubility (g/L @ 20 °C)	28.3 at pH 7
Vapor pressure (Torr, 20 °C)	8.25×10^{-11}
Dissociation constant (pKa)	3.2 (means weak acid)
Octanol/water partition coefficient (Pow @ 24 °C)	0.0807 at pH 7.0
Acute Oral – rat LD50 (mg/kg)	> 2000
Tolerance Levels:	
Corn, field, grain	0.03 ppm
Corn, field, forage	0.60 ppm

Concerns have emerged regarding tembotrione's residual persistence in soil, with some studies reporting its presence for up to 200–300 days post-application (PMRA, 2012). Such persistence has been linked to phytotoxic effects on sensitive rotational crops including potato (Dias et al., 2019), beet (Silva et al., 2019), and carrot (Bontempo et al., 2016). Additionally, tembotrione has been shown to negatively impact soil biological health by reducing dehydrogenase and alkaline phosphatase enzyme activities, suppressing arbuscular mycorrhizal fungi populations, and impairing root colonization (Goalla et al., 2022).

Although chemical weed control methods offer efficient alternatives to manual weeding and enhance overall weed management, they also raise concerns regarding environmental safety. In particular, the long-term persistence and residue behavior of herbicides like tembotrione in soil, plant tissues, and water bodies warrant comprehensive investigation. Existing studies have primarily focused on short-term degradation patterns and residue mobility, often overlooking the extended dietary risks associated with consuming maize cultivated in tembotrione-treated fields. These earlier assessments have emphasized residue concentrations and leaching potential, without evaluating chronic exposure effects on humans and non-target organisms.

To address this gap, the present study aims to evaluate the ecological and long-term dietary risks of tembotrione through a multi-year analysis of its residues in maize soil, plant tissues, water, and aquatic organisms. Conducted over three consecutive growing seasons, this research provides a more holistic understanding of tembotrione's environmental footprint and its implications for food safety and ecosystem health.

II. MATERIALS AND METHODS

2.1 Field experiment:

Field experiments were conducted during the rainy seasons of 2017, 2018, and 2019 on black cotton soils (Vertisols) to evaluate the effects of tembotrione application in maize cultivation. The soil at the experimental site was classified as sandy clay loam, comprising 66.5% sand, 23.3% clay, and 10.2% silt, with an electrical conductivity (EC) of 200 µS/m, pH of 7.01, and organic carbon content of 0.89%. A hybrid maize variety (4212) was cultivated following standard agronomic practices recommended

for the central province. Tembotrione was applied as a post-emergence herbicide at the recommended dose of 120 g active ingredient per hectare (g a.i./ha) during the maize growing seasons. Control plots were maintained without herbicide application. Each experimental plot measured 16 meters in length and 10 meters in width, with raised soil boundaries approximately 30 cm in height and width on all sides to prevent cross-contamination. Meteorological data for the study years (2017–2019) is presented in Figure 1.

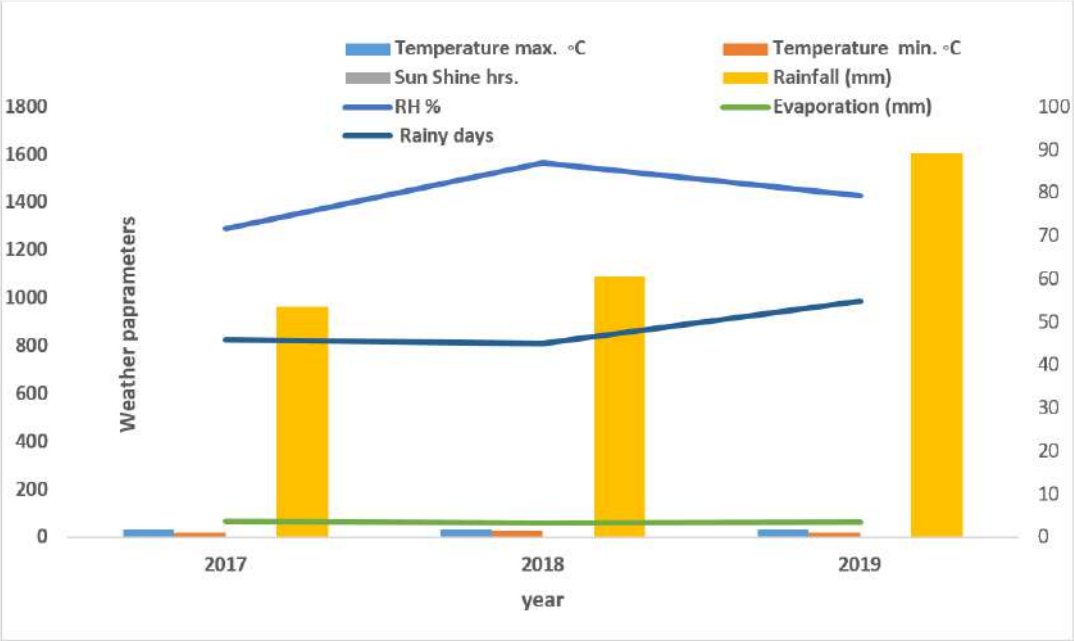


FIGURE 1: Minimum and maximum temperature, relative humidity, sunshine, and total rain during the maize growing stage period in each year

Periodic sampling of soil (0–15 cm depth), maize plants, and water was conducted from 2 hours after the application of tembotrione up to harvest. Mature maize plant samples were collected at harvest from both treated and untreated plots to compare residue levels. To assess bioaccumulation and estimate tembotrione residues in aquatic organisms, fish samples were collected from neighboring ponds between 20 and 100 days after herbicide application. Fish mortality was observed in adjacent ponds receiving tembotrione through drift and surface runoff from treated maize fields. All collected samples were appropriately stored and processed for tembotrione residue analysis using Ultra-Fast Liquid Chromatography (UFLC).

2.2 Herbicide extraction and quantification:

Tembotrione was procured in two forms—analytical grade standard and commercial formulation (Tembotrione 34.4% SC)—from Bayer Crop Sciences India Pvt. Ltd. All analytical-grade chemicals and solvents used in the study were obtained from Merck (Darmstadt, Germany). Quantification of tembotrione residues in soil, water, fish, and maize plant samples was performed using a validated Ultra-Fast Liquid Chromatography (UFLC) method, as described by Sondhia (2016). The analytical procedure was validated in accordance with WHO (2020) and SANTE (2021) guidelines, assessing key parameters including recovery rates at 0.1 mg/L and 0.5 mg/L, selectivity, linearity, limits of detection (LOD) and quantification (LOQ), precision, and accuracy.

Tembotrione residues in soil, water, fish, and maize samples were analyzed using a Prominence UFLCXR system (Shimadzu, Japan), equipped with an SPD-MP00A VP diode array detector, LC-c0 AD pump, DHU degasser, 20A3 unit, and an autosampler. Chromatographic separation was achieved on an XR ODS II column (75 mm length × 3 mm internal diameter) using a mobile phase composed of acetonitrile and water containing 0.01% H_3PO_4 in a 70:30 (v/v) ratio. The flow rate was maintained at 0.35 mL/min. A 20 µL aliquot of tembotrione standard solution (ranging from 0.001 to 10 mg/L in acetonitrile) was injected via the autosampler. Detection was performed at 231 nm, and peak areas (µabs) were recorded.

Under these conditions, tembotrione exhibited a retention time of 3.13 minutes, with a total run time of 6.5 minutes per sample. Calibration was performed by plotting peak area (mabs/sec) against tembotrione concentration (µg/mL), and the data were fitted to a linear regression model to generate a standard curve. Tembotrione concentrations in test samples were calculated using the regression equation: $y = 30788x + 43543$ with a correlation coefficient $R^2 = 0.998$, indicating excellent linearity.

2.3 Risk assessment:

Tembotrione residues detected over time in soil, water, maize plants, and fish were analyzed to assess the ecological risk and potential toxicity to various taxa. This evaluation followed the frameworks established by EFSA (2019), FAO/WHO, and the EPA Risk Assessment Guide (2022), utilizing toxicological reference data from EPA (2007), EFSA (2013), and USDA (2022). The ecological risk was quantified using the Risk Quotient (RQ) method, wherein calculated RQ values were compared against the EPA's Level of Concern (LOC). According to EPA guidelines, the risk is considered acceptable when RQ < LOC. The RQ approach, widely adopted for preliminary environmental screening, serves as a standardized tool to estimate the potential hazard of pesticide residues across environmental compartments.

A risk quotient (RQ) as prescribed by the US Environmental Protection Agency is used to evaluate the ecological risk of pesticides (Equation 1).

$$\text{Risk Quotient} = \text{Exposure} / \text{Toxicity} \quad (1)$$

In the referenced equation, exposure refers to the Estimated Environmental Concentration (EEC), while toxicity corresponds to an effect level or endpoint derived from ecotoxicological studies, such as the median lethal concentration (LC_{50}) or the No Observed Effect Concentration (NOEC). Level of Concern (LOC) values were calculated using EEC in conjunction with LC_{50} or NOEC data, following the methodology prescribed by the U.S. Environmental Protection Agency (EPA) and adopted by Shobha et al. for ecological risk assessment. Additionally, dietary exposure to tembotrione was evaluated in accordance with World Health Organization (WHO) guidelines and Dietary Risk Index (DRI) was calculated as described by Benbrook and Davis (2020) and given as equation 2:

Dietary exposure = Σ (Concentration of pesticide in food × Food consumption) / Body weight (kg)

$$\text{DRI} = (\text{Pesticide concentration} \times \text{Serv}) / (\text{cRfD} \times \text{BW}) \quad (2)$$

Where, Pesticide concentration (mg/kg); Serving (kg), cRfd (mg/kg bw); BW Body weight (kg).

And the risk characterization for tembotrione was performed by estimation of the Hazard Quotient (HQ), i.e., the estimated total chronic dietary exposure divided by the Acceptable Daily Intake (ADI) (mg/kg bw). Ecological and health assessment was described based on correlation among the calculated dietary risk exposure assessment and residues of tembotrione detected in various samples taken in this study where tembotrione was applied continuously for three years at recommended dose of 120 g/ha.

2.4 Data analysis:

Regression analyses were performed using Microsoft Excel's graphing tools and data analysis functions on the Windows platform. The dissipation behavior of tembotrione in maize field soil was characterized through a linear regression model. Residue concentrations were expressed as mean ± standard deviation (S.D.) and statistically evaluated using analysis of variance (ANOVA). Significant correlations between mean tembotrione residues, soil physico-chemical properties, and meteorological parameters were assessed using a Pearson correlation matrix. The dissipation kinetics of tembotrione were modeled using a first-order reaction equation: Ct = C0 e-kt, where Ct (mg/kg) denotes the concentration at time t (min), C0 (mg/kg) the starting concentration in the soil, and k (per minute) is the rate constant.

III. RESULTS AND DISCUSSION

3.1 Dissipation and degradation behavior of tembotrione in maize field soil:

Ultra-Fast Liquid Chromatography coupled with a diode array detector (UFLC-DAD) was employed for the quantification of tembotrione residues across various environmental and biological matrices, owing to its high sensitivity and suitability for multi-residue pesticide analysis. The method demonstrated satisfactory recovery rates ranging from 81% to 92% across maize plants, grain, straw, soil, water, and fish samples. The relative standard deviation (RSD) for all matrices was below 5%, indicating good analytical precision. The limit of detection (LOD) for tembotrione was determined to be 0.001 mg/kg.

In soil, tembotrione dissipation was monitored over three consecutive years following its application in maize fields. In 2017, an initial concentration of 0.7176 mg/kg was detected two hours post-application, which declined to 0.4425 mg/kg at five days, 0.2239 mg/kg at 20 days, and 0.077 mg/kg by 90 days (Figure 2). In 2018, residues measured 0.641 mg/kg at two hours, decreasing to 0.443 mg/kg at 10 days, 0.1925 mg/kg at 20 days, and 0.010 mg/kg by 90 days. Similarly, in 2019, the initial

residue concentration was 0.541 mg/kg, which dissipated to 0.0223 mg/kg at 60 days and 0.010 mg/kg at 90 days. By harvest in all three years, residue levels in soil were consistently below the LOD (<0.001 mg/kg).

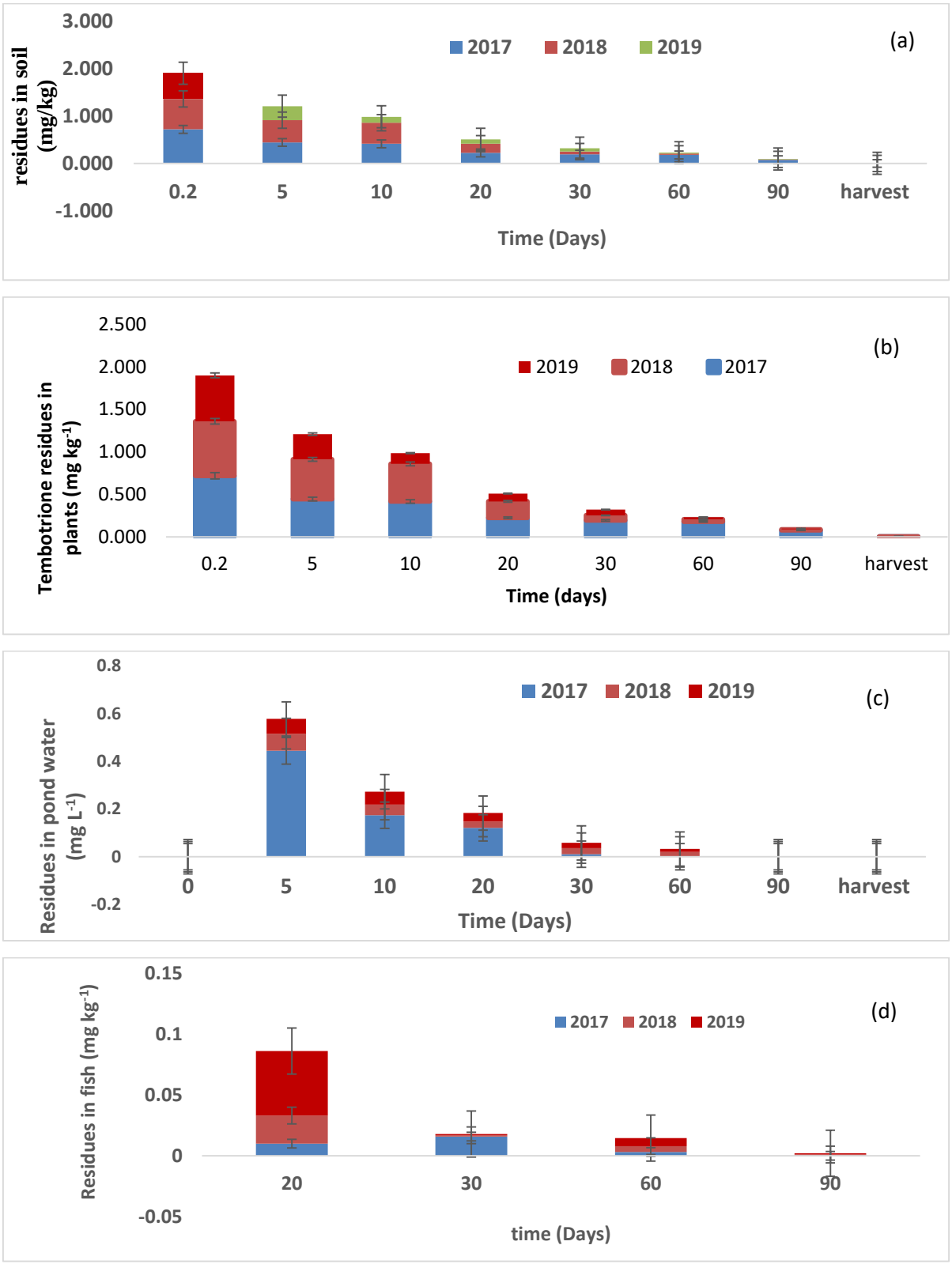


FIGURE 2: Detection of tembotrione residues in the soil of maize field (a), maize plants (b) adjacent pond water (c) and fishes (d) during 2017, 2018 and 2019

The dissipation of tembotrione in soil followed first-order kinetics, with regression equations for each year as follows:

- 2017: $Y = -0.0442x + 4.3009$ ($R^2 = 0.710$)
- 2018: $Y = -0.0560x + 4.1095$ ($R^2 = 0.941$)
- 2019: $Y = -0.0496x + 3.5251$ ($R^2 = 0.919$)

Corresponding half-lives of tembotrione in soil were calculated as 15.67, 12.38, and 13.97 days for 2017, 2018, and 2019, respectively (Table 2; Figure 3). The average half-life across the three years was estimated at 14.0 days. By 90 days post-application, more than 90% of the applied tembotrione had degraded in all study years (Figure 4), indicating its relatively rapid dissipation under field conditions.

TABLE 2
RATE KINETICS EQUATIONS AND HALF-LIFE OF TEMBOTRIONE AND IN THE SOIL OF MAIZE FIELD AND MAIZE PLANTS DURING 2017 TO 2019

Herbicides	Rate kinetics equation	R^2	Calculated time for 50% Dissipation	Average Half-life (days)
Soil				
2017	Y= -0.0442x + 4.3009	0.71	15.67	14
2018	Y= -0.056x + 4.1095	0.941	12.37	
2019	Y= -0.0496x + 3.5251	0.919	13.97	
Maize plants				
2017	Y= = -0.0749x + 5.2194	0.982	9.252	11.4
2018	Y= = -0.0553x + 4.9684	0.935	12.53	
2019	Y= -0.0551x + 4.2900	0.801	12.57	

3.2 Tembotrione degradation in maize plants, grain and straw:

Tembotrione residue levels in maize plants were monitored over three consecutive years following application for weed management. Initial residues detected at one-day post-application were 0.718, 0.641, and 0.541 mg/kg in 2017, 2018, and 2019, respectively. These concentrations progressively declined to 0.180, 0.027, and 0.022 mg/kg by 60 days, indicating substantial dissipation. By 90 days, residue levels were further reduced to 0.077 mg/kg (2017) and 0.010 mg/kg (2018 and 2019).

The dissipation of tembotrione in maize plants followed first-order kinetic models, with calculated half-lives of 9.25, 12.53, and 12.57 days in 2017, 2018, and 2019, respectively (Table 2). Rapid metabolic degradation of tembotrione was observed in the maize plants, particularly during the early growth stages. More than 90% of the initial residues were degraded within 60 days in 2018 and 2019, while similar levels of degradation were reached by 90 days in 2017.

At harvest, tembotrione residues in both maize grain and straw were below the analytical method's limit of detection (0.001 mg/kg), indicating the absence of residual contamination in edible parts of the plant (Figures 3 and 4).

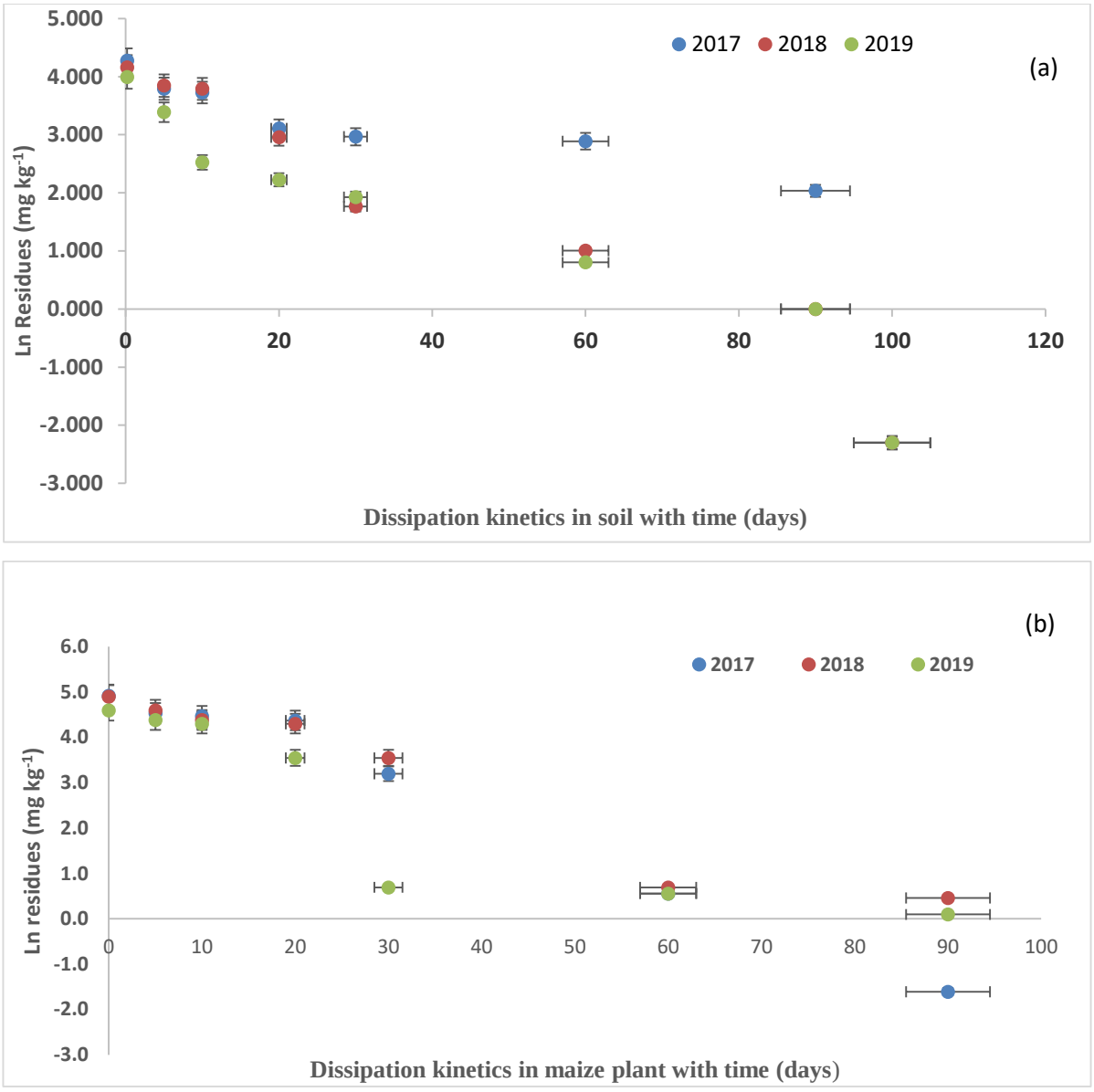
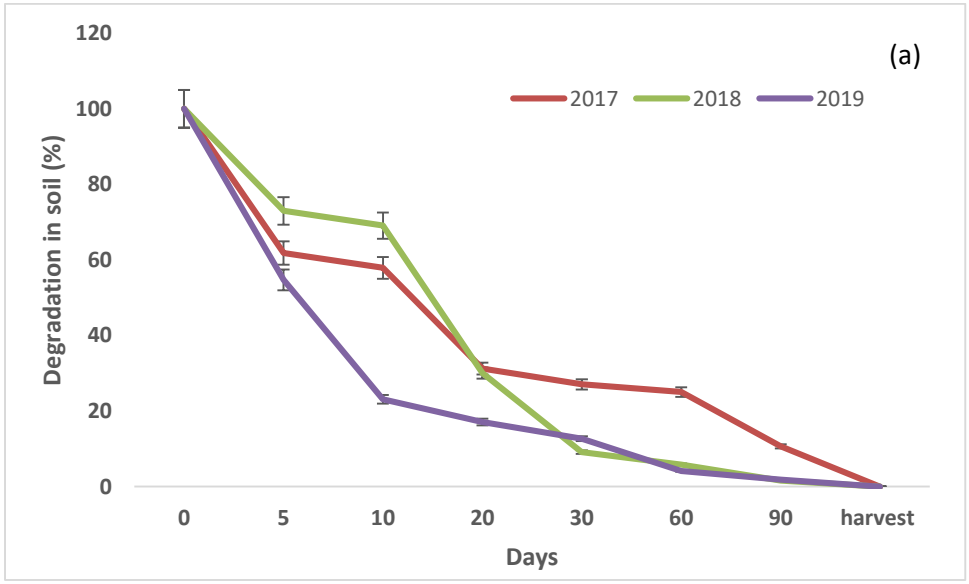


FIGURE 3: First order dissipation pattern of tembotrione residues in the soil of maize grown field (a) and maize plant (b) during 2017, 2018 and 2019



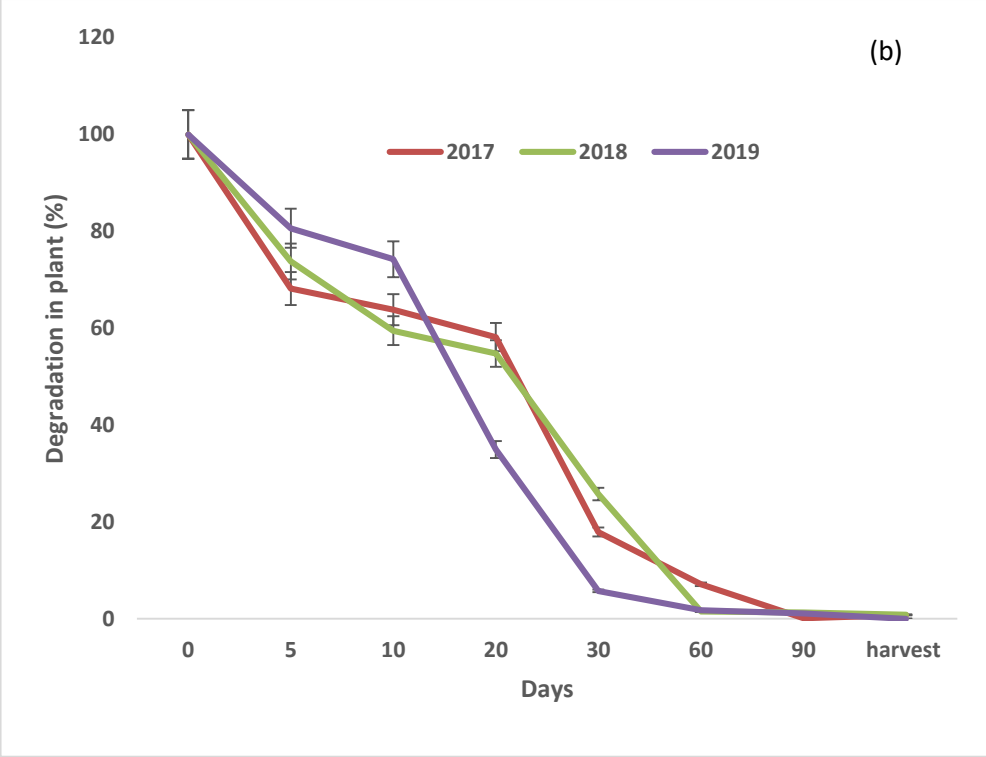


FIGURE 4: Tembotrione degradation residues in maize field soil (a) and maize plants (b) during the years 2017, 2018, and 2019

3.3 Tembotrione residues and mortality in the fish:

Tembotrione residues were detected in water samples from a nearby pond following application in maize fields. In the first year, residue levels reached 0.444 mg/L at 5 days post-application and declined to 0.0121 mg/L by day 20. In the second year, concentrations ranged from 0.0720 mg/L at 5 days to 0.0261 mg/L at 30 days. During the third year, residues varied between 0.0619 mg/L and 0.0361 mg/L at 5 and 20 days, respectively (Figure 2). Across all three years (2017–2019), residue levels in pond water dropped to below 0.001 mg/L by 90 days post-application.

Residue accumulation and potential toxicological effects on fish were also evaluated. Tembotrione was transported into the adjacent pond via rainwater runoff during the experimental period. Consequently, residue levels in fish muscle tissue at 60 days were recorded as 0.067, 0.0048, and 0.0031 mg/kg in 2017, 2018, and 2019, respectively. These levels suggest that fish may not be suitable for consumption at this stage. However, residue concentrations declined to below the detection limit (0.001 mg/kg) by 90 days in all years.

Importantly, no instances of fish mortality or observable toxic symptoms were recorded in the pond on any sampling day throughout the study period.

3.4 Risk assessment of tembotrione in aquatics and other taxon:

Risk quotient (RQ) values were calculated based on field-derived residue data, and the results are presented in Table 3. The acceptable daily intake (ADI) for tembotrione is 0.0004 mg/kg body weight (bw) (EPA, 2007; EFSA, 2013). For dietary risk assessment, an average body weight of 65 kg for humans and 450 kg for cattle was assumed, in accordance with EFSA (2019) and as adopted by Benbrook et al. (2020). The RQ values for all assessed organisms were found to be well below 1 and remained below the established levels of concern (LOC), indicating minimal ecological and human health risks.

TABLE 3

Risk assessment based on Risk quotient and level of concern of tembotrione on aquatic life and other taxon based on residues detected in the adjacent aquatic ponds near to maize field where tembotrione was applied at recommended dose continuously for three years (risk assessment was calculated based on residues as well as NOEC data available at Environmental Protection Agency (EPA) or European Food and Safety Assessment, (EFSA) website

Organism	Selected species	NOEC (mg/L)	LOC Presumtions	RQ Presumtions	LOC	RQ_f Risk assessment based on mean residues (mg/L) in water at various days						
						0.2	5	10	20	30	60	90
Fathead minnow	Pimephales promelas	0.604	ARU	Moderate	>0.05 to <0.5	NA						
Rainbow trout	Oncorhynchus mykiss	100	NR	Negligible	<0.05	NA						
water flea	Daphnia magna	18	NR	Negligible	<0.05	NA						
Bluegill sunfish	*Lepomis macrochirus*	100	NR	Negligible	<0.05	NA						
algal growth inhibition-	*Pseudokirchneriella subcapitata*	0.2	AHR	Moderate to negligible	>0.05 to >0.5	NA						
freshwater blue-green alga	*Anabaena flosaqua*	39	NR	Negligible	<0.05	NA						
Duckweed	Lemna gibba	0.0032	CR to ARU	High to moderate	> 1 to > 0.5	NA						
Earthworm	*Eisenia fetida*	1.25	ARU to NR	Moderate to negligible	<0.5 to <0.05	NA						
Soil macro-organisms: Collembola	*Folsomia candida*	2.68	AES to NR	Low risk to negligible	>0.05 to <0.05	NA						
Mammalian acute (Rat)	*Rattus norvegicus*	2500#	NR	Negligible	<0.05	NA						
Dogs	-	26.7	NR	Negligible	<0.05	NA						

 # Acute LD_{50} value were used in absence of NOEC (EFSA 2013)

Level of concern (LOC), 0.5 or above, Acute High Risk (AHR); 0.1 Acute Restricted Use (ARU); 0.05 Acute Endangered Species (AES); 1 Chronic Risk (CR); <0.05, No risk;

RQ	Risk	RQ	Risk	RQ	Risk	RQ	Risk
≥1	High	≥0.1 and 1	Moderate	≥ 0.1 - 0.01	Endurate (Low risk)	<0.01	Negligible

The results confirm that chronic dietary exposure to tembotrione, when applied at recommended agricultural doses, poses negligible health risks to humans, remaining well within the ADI threshold. Table 3 also presents RQ values for various taxa, along with corresponding LOC benchmarks defined by the U.S. Environmental Protection Agency (EPA). According to EPA criteria, LOC values exceeding 0.5, 1.0, 0.05, and 1.0 indicate acute high risk, acute restricted use, acute endangered species risk, and chronic risk, respectively. These LOC thresholds are used by regulatory agencies to interpret ecological risk and to determine the need for further regulatory actions.

For aquatic and soil organisms—including fish, algae, and macroflora—risk assessment was conducted using NOEC (No Observed Effect Concentration) or LC_{50}/EC_{50} values, as detailed in Table 3. This approach is consistent with methodologies reported by Vasickova et al. (2019) and Zhou et al. (2019), who also utilized NOEC and LC_{50} values for ecological risk assessments. Additionally, hazard quotient (HQ) values at harvest were found to be below 1, further supporting the conclusion of low risk from dietary exposure. Risk characterization based on the detection of tembotrione residues in soil and water was conducted using risk quotient (RQ) values for representative non-target organisms. For soil macro-organisms, the RQ values for *Folsomia candida* (Collembola) indicated a low to negligible risk up to 90 days post-application. In aquatic environments, *Lemna gibba* (duckweed) exhibited RQ values ranging from high to negligible risk up to 60 days, with a moderate risk persisting at 90 days. The fathead minnow (*Pimephales promelas*), used to evaluate early life stage risk in fish, showed moderate risk up to 20 days, with RQ values declining to non-significant levels thereafter.

For *Pseudokirchneriella subcapitata*, representing algal growth inhibition, RQ values indicated a moderate risk up to 60 days. In the case of *Eisenia fetida* (earthworms), a moderate risk was observed at 5 days post-application, which diminished to negligible levels from day 10 onward. RQ values were consistently negligible for *Oncorhynchus mykiss* (rainbow trout), *Daphnia magna* (water flea), *Lepomis macrochirus* (bluegill sunfish), and *Anabaena flos-aquae* (freshwater blue-green alga).

Regarding mammalian toxicity, *Rattus norvegicus* (rat) and domestic dogs were selected as representative species. For both, RQ values remained negligible across all sampling intervals, indicating minimal chronic risk from environmental exposure to tembotrione residues (Table 3).

3.5 Dietary risk assessment of tembotrione:

Chronic dietary exposure to tembotrione was evaluated using the deterministic approach outlined in WHO guidelines (WHO, 2019; 2020). In accordance with the International Programme on Chemical Safety (IPCS, 2009), such assessments are mandated for all registered crops under evaluation. In India, tembotrione is registered for use on maize, with a maximum residue limit (MRL) established at 0.02 mg/kg (EPA, 2007). Based on mean residue data collected over a three-year period, chronic dietary exposure for humans did not exceed the acceptable daily intake (ADI) on any of the sampling days. Correspondingly, hazard quotient (HQ) values for humans remained below 1 across all sampling intervals, indicating no appreciable risk of adverse health effects from the consumption of maize grains or fish containing tembotrione residues.

Conversely, dietary risk to livestock, particularly cattle, was found to be higher. HQ values exceeded 1 when maize fodder aged 30 to 60 days was considered as animal feed, suggesting a potential health risk during this period. At harvest, however, HQ values were below 1, indicating a low risk of adverse effects if fodder is provided at this stage. Specifically, chronic dietary exposure based on residue levels in 30-day-old maize fodder exceeded the ADI for cattle, while exposures at 60, 90 days, and at harvest remained within acceptable safety margins (Table 4).

TABLE 4

Calculated dietary risk assessment of tembotrione based on ADI and RFD and level of concern on human and animal life based on residues detected in the fish, plant, maize grain and straw samples collected from maize field where tembotrione was applied continuously for three years

	Parameter	Fish	Maize Grain	Plant	Maize Straw
Mean Residues (mg/kg)	30 days	0.02	NA	0.2	NA
	60 days	0.06	NA	0.02	NA
	90 days	0	NA	0.01	NA
	Harvest	0.001	0.001	0.007	0.008
Rfd (mg/kg)		0.00008	0.00008	0.00008	0.00008
Tolerance Limit (mg/L)		0.02*	0.02	1	0.5
Consumption of Food (kg/day)		0.1	0.08	15	15
Chronic Dietary Risk Exposure (mg/kg/day)	30 days	$2.44E^{-05}$	NA	0.0023	NA
	60 days	$8.85E^{-05}$	NA	0.0002	NA
	90 days	$1.54E^{-06}$	NA	0.0001	NA
	Harvest	$2.37E^{-10}$	$1.23E^{-06}$	$8.18E^{-05}$	0.0002
ADI (mg/kg bw/day)		0.0004	0.0004	0.0004	0.0004
% ADI Risk		<ADI	<ADI	>ADI	<ADI
Dietary Exposure Index	30 days	0.31	NA	85	NA
	60 days	1.11	NA	7.63	NA
	90 days	$1.54E^{-05}$	NA	4.23	NA
	Harvest	$1.54E^{-05}$	0.00008	0.00022	3.233
Hazard Quotient	30 days	0.25	NA	68	NA
	60 days	0.89	NA	6.1	NA
	90 days	0.02	NA	3.38	NA
	Harvest	$2.37E^{-06}$	0.012	0.818	0.862

3.6 Correlation among tembotrione physicochemical properties, residues in soil, and weather parameters:

Correlation analysis revealed that tembotrione residues in maize field soil were significantly and positively correlated with initial residue concentrations ($R^2 = 0.84$, $p = 0.05$), residue levels at 60 days ($R^2 = 0.84$, $p = 0.05$), and overall persistence ($R^2 = 0.83$, $p = 0.05$). In contrast, significant negative correlations were observed with degradation percentage at 60 days ($R^2 = –0.83$, $p = 0.05$), octanol–water partition coefficient (Pow, 24 °C; $R^2 = –0.81$, $p = 0.05$), total rainfall ($R^2 = –0.97$, $p = 0.05$), and number of rainy days ($R^2 = –0.805$, $p = 0.05$). Other factors, including vapor pressure (Torr), temperature, water solubility (g/L), humidity, evaporation rate, and dissociation constant (pKa), showed no significant correlation with initial residue levels.

Tembotrione degradation in soil was positively correlated with Pow ($R^2 = 1.00$, $p = 0.05$) and humidity ($R^2 = –0.86$, $p = 0.05$), and negatively correlated with herbicide water solubility ($R^2 = –0.879$, $p = 0.05$) and evaporation ($R^2 = –0.879$, $p = 0.05$). No significant correlations were observed for vapor pressure, temperature, rainy days, or pKa in relation to degradation rates. The half-life of tembotrione in soil exhibited positive correlations with persistence at 60 days ($R^2 = 0.87$, $p = 0.05$), water solubility ($R^2 = 1.00$, $p = 0.05$), and evaporation ($R^2 = 0.99$, $p = 0.05$), indicating moderate environmental stability. Conversely, half-life was negatively correlated with degradation at 60 days ($R^2 = –0.88$, $p = 0.05$), average maximum temperature ($R^2 = –0.87$, $p = 0.05$), humidity ($R^2 = –1.00$, $p = 0.05$), and Pow ($R^2 = –0.89$, $p = 0.05$). Other physicochemical properties, including vapor pressure, temperature, evaporation rate, and pKa, showed no significant influence on half-life (Table 5).

TABLE 5

Pearson correlation matrix for various soil and herbicides parameters describing degradation of tembotrione in soil of maize field

	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.	14.	15.	16.
year	1															
Initial Residues	-1	1														
Residues at 60 days (µg g^{-1})	-0.88	0.84	1													
Av. Half-life (days)	-0.52	0.45	0.86	1												
Persistence at 60 day	-0.87	0.83	1	0.87	1											
Degradation at 60 day (%)	0.87	-0.83	-1	-0.88	-1	1										
Total rainfall	0.94	-0.97	-0.67	-0.21	-0.66	0.66	1									
Av max Temperature	-0.3	0.37	-0.19	-0.66	-0.21	0.21	-0.6	1								
Av max Temperature	0.04	0.04	-0.51	-0.87	-0.53	0.53	-0.29	0.94	1							
Herbicide water solubility (mg/L)	-0.5	0.43	0.85	1	0.86	-0.86	-0.18	-0.67	-0.89	1						
vapor pressure (Torr, 20 °C)	0	0	0	0	0	0	0	0	0	0	1					
Humidity (%)	0.5	-0.43	-0.85	-1	-0.86	0.86	0.18	0.67	0.89	-1	0	1				
Av number of rainy days	0.8	-0.85	-0.42	0.09	-0.4	0.4	0.96	-0.81	-0.56	0.11	0	-0.12	1			
Dissociation constant (pKa)	0	0	0	0	0	0	0	0	0	0	1	0	0	1		
Octanol water partition coefficient (Pow 24 °C)	0.85	-0.81	-1	-0.89	-1	1	0.63	0.24	0.56	-0.88	0	0.88	0.37	0	1	
Evaporation (mm)	-0.38	0.31	0.78	0.99	0.79	-0.79	-0.05	-0.77	-0.94	0.99	0	-0.99	0.24	0	-0.81	1

Tembotrione dissipation in both soil and maize plants followed first-order kinetic models. The calculated DT_{50} (half-life) values ranged from 12.57 to 15.67 days in soil and from 9.25 to 12.57 days in maize plants, with mean half-lives of 14.0 and 11.4 days, respectively. Faster degradation rates were observed in the second and third years of the study, likely influenced by climatic and edaphic factors (Figure 4).

Similar dissipation behavior was reported by Zhang et al. (2015), who observed first-order kinetics for tembotrione in low pH Red-Yellow Latosol soils, although with substantially longer half-lives ranging from 61 to 90 days. In contrast, Silva et al. (2019) documented significantly higher persistence of tembotrione in medium- and clay-textured soils, with detectable residues persisting for up to 200 days in humid conditions and 300 days under water-deficit conditions. These findings highlight the influence of soil properties and moisture regimes on tembotrione persistence.

Elevated half-life values, such as those observed in our study, may indicate a potential carryover risk, as previously reported by Zhang et al. (2015). The interaction between sorption and degradation is well-established for other triketone herbicides, including mesotrione. Mendes et al. (2017) noted that rapid degradation in soil can be constrained by the adsorption capacity of soil particles, which limits bioavailability.

Austria (2011) reported 105 days for 90% dissipation of tembotrione under field conditions, while laboratory studies by PMRA (2014) indicated persistence beyond 342 days, underscoring the variability in degradation depending on environmental conditions. Similarly, slow degradation in Vertisol soils was documented by Zemelka (2015). Prolonged persistence has been linked to phytotoxic effects in subsequent crops, including sugar beet (Silva et al., 2019), potato (Dias et al., 2019), and carrot (Bontempo et al., 2016).

Conversely, rapid degradation in maize fields was reported by Sue et al. (2020), who found tembotrione residues in maize grain and corncob matrices to be below 0.02 mg/kg, consistent with the findings of our study regarding low residue levels at harvest.

The highest tembotrione half-life in the soil of maize field was found in 2017 when compared to that observed in the same soil with other years; this can be correlated with the physicochemical properties of the soil as well as weather conditions prevailed during the experimental period, especially high rainfall and humidity that might have contributed to more microorganism degradation in 2018 and 2019 (Zhang et al., 2015; Sondhia et al., 2016). A direct correlation between soil clay content, soil organic matter and half-life was reported by Silva et al. (2019). Lower half-life of tembotrione is reported at high pH soil due

to a decrease in the number of cationic molecules present in the soil (Trigo et al., 2014). Since tembotrione is a weak acid (pKa, 3.2) and is negatively charged in solution, hence, it is repulsed in those circumstances where soil pH is increased and inhibits microbial degradation.

In this study, less quantity of tembotrione residues were detected in the pond water samples than in maize field soil. However, detection of tembotrione residues at 30 and 60 days in fish limits its consumption at these days. However, in this study, tembotrione residues in fish were not detected (<0.001 mg/kg) after 90 and 100 days. The hazard quotient at all sampling days was also found to be <1 for humans and indicates that there is no appreciable risk of adverse health effects following dietary exposure to tembotrione through consumption of fish or maize grains. Negligible dietary risk after tembotrione application in maize field was also reported by Su et al. (2020) and Cao et al. (2022). However, due to tembotrione residues in the pond and fish up to 60 days, an appropriate remediation procedure is required to limit its release into neighboring water systems. Similarly, due to relatively high to moderate RQ of tembotrione toxicity to aquatics, such as fathead minnow and duckweed, the release of maize herbicides through runoff into receiving water canals should be minimized through appropriate measures.

Rainfall and temperature variations played a significant role in the degradation dynamics of tembotrione in maize field soil over the three-year study period. Lower cumulative rainfall totals in 2017 and 2018 (967.1 mm and 1092.1 mm, respectively) contrasted with the substantially higher precipitation recorded in 2019 (1604.8 mm), likely facilitating the more rapid degradation of tembotrione observed during the third year. Correspondingly, tembotrione residue concentrations at 20 days post-application were higher in 2017 (0.224 mg/kg) and 2018 (0.193 mg/kg) compared to 2019 (0.093 mg/kg), suggesting that increased rainfall contributed to enhanced dissipation in the latter year.

While elevated rainfall may accelerate tembotrione degradation in soil, it simultaneously increases the potential for herbicide runoff into adjacent aquatic ecosystems, raising concerns regarding off-site contamination during the maize cropping season (Sondhia et al., 2013; 2016). Although tembotrione dissipation consistently followed first-order kinetics each year, degradation rates in both soil and maize plants exhibited inter-annual variability, likely driven by fluctuating environmental conditions.

IV. CONCLUSION

This study provides a comprehensive evaluation of the long-term environmental and health behavior of tembotrione, a triketone herbicide widely used in maize cultivation. Under field conditions, tembotrione demonstrated rapid soil dissipation, indicating low persistence in subtropical agroecosystems. Human dietary exposure through maize grains and fish consumption consistently yielded health risk quotients below one across all sampling intervals, suggesting minimal risk. In contrast, a potential dietary risk to livestock, particularly cattle, was identified when maize plants were harvested 30 to 60 days post-application, with risk quotients exceeding one. Additionally, aquatic organisms may be temporarily affected by runoff shortly after application. While soil macro-organisms exhibited a wide range of risk levels over time, from high to negligible, this risk is mitigated by the herbicide's low volatility and limited environmental stability. Health risk assessments revealed varying degrees of concern for animals, but negligible risk for humans. Overall, the findings support the safe use of tembotrione in maize production when integrated with appropriate risk management strategies, particularly concerning livestock feeding schedules and mitigation of aquatic exposure.

CONFLICTS OF INTEREST

The authors declare no known competing financial or personal interest in the work of this paper.

DATA AVAILABILITY STATEMENT

All data are found within the main manuscript and original sources as stated

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Molecular Characterization and Stability Analysis of Maize (*Zea mays* L.) Genotypes under Environmental Stress Conditions

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Abstract— *Maize (Zea mays L.) is one of the most important cereal crops cultivated worldwide and plays a significant role in food security, livestock feed, and industrial applications. However, environmental stresses such as drought, heat stress, and irregular rainfall patterns have severely affected maize productivity in many regions. The identification of stable and stress-tolerant maize genotypes is therefore essential for improving crop productivity under changing climatic conditions. The present study aimed to analyze molecular diversity and yield stability among selected maize genotypes under different environmental conditions. Field experiments were conducted to evaluate important agronomic traits including plant height, days to tasseling, cob length, and grain yield. Molecular characterization was performed using simple sequence repeat (SSR) markers to identify genetic variation among maize genotypes. Statistical analyses including analysis of variance (ANOVA), principal component analysis (PCA), and cluster analysis were conducted to evaluate variability and stability among genotypes. The results revealed significant genetic variability among the evaluated maize genotypes. Certain genotypes exhibited superior yield stability and adaptability under stress conditions. The findings suggest that integrating molecular tools with conventional breeding approaches can accelerate the development of climate-resilient maize varieties for sustainable agricultural production.*

Keywords— Maize, Molecular diversity, Genetic variability, Stress tolerance, Crop improvement, Climate resilience.

I. INTRODUCTION

Maize (*Zea mays* L.) is one of the most widely cultivated cereal crops globally and ranks third after wheat and rice in terms of production. The crop is widely used for human consumption, livestock feed, and industrial applications such as starch production and biofuel manufacturing. Due to its high yield potential and adaptability, maize plays a crucial role in agricultural economies around the world.

Despite its importance, maize production is increasingly threatened by environmental stresses such as drought, high temperature, and erratic rainfall patterns. These stresses negatively affect plant growth and physiological processes, leading to reduced crop productivity. Climate change has further intensified these challenges, making it necessary to develop improved maize varieties with enhanced stress tolerance.

Genetic diversity is a key component in crop improvement programs because it provides the basis for selection and breeding of superior genotypes. Plant breeders utilize diverse germplasm resources to identify desirable traits such as high yield, disease resistance, and environmental stress tolerance.

Advances in molecular biology have provided powerful tools for studying genetic diversity in crop plants. Molecular markers such as simple sequence repeats (SSR) and single nucleotide polymorphisms (SNP) allow researchers to analyze genetic variation at the DNA level. These technologies have significantly improved the efficiency of crop breeding programs by enabling the identification of superior genotypes.

Therefore, the evaluation of molecular diversity and yield stability among maize genotypes is essential for developing improved varieties capable of sustaining productivity under environmental stress conditions.

II. OBJECTIVES OF THE STUDY

1. To evaluate molecular diversity among maize genotypes using SSR markers.
2. To analyze yield performance and stability of maize genotypes under field conditions.
3. To identify stress-tolerant maize genotypes suitable for crop improvement programs.

III. REVIEW OF LITERATURE

- Prasanna, B.M., et al. (2015) conducted studies on modern maize breeding strategies and reported that hybrid breeding combined with molecular tools significantly improves maize productivity.
- Zaidi, P.H., et al. (2016) conducted research on drought tolerance in maize hybrids and observed that drought-tolerant genotypes maintained higher grain yield under water stress conditions.
- Bänziger, M., et al. (2017) conducted studies on stress-tolerant maize varieties and emphasized the importance of selecting genotypes capable of performing well in both optimal and stress environments.
- Cairns, J.E., et al. (2017) conducted research on climate-resilient maize breeding and highlighted the role of genetic diversity in improving drought tolerance.
- Chen, B., et al. (2018) conducted molecular diversity analysis in maize populations using SSR markers and reported significant genetic variation among genotypes.
- Xu, Y., et al. (2018) reported that genomic selection techniques accelerate crop breeding programs by identifying superior genotypes.
- Li, X., et al. (2019) conducted genome-wide association studies and identified genes associated with yield improvement and stress tolerance in maize.
- Khan, A., et al. (2020) reported significant genetic variability among maize genotypes for agronomic traits and grain yield.
- Barbosa, P.A.M., et al. (2021) conducted research on maize germplasm diversity and highlighted the importance of landrace populations as valuable genetic resources.
- Prasanna, B.M., et al. (2021) reported that integrating molecular breeding techniques with conventional breeding significantly enhances maize productivity.
- Shiferaw, B., et al. (2022) studied global maize production challenges and emphasized the need for developing climate-resilient maize varieties.
- Zhang, Y., et al. (2023) reported that molecular markers are useful tools for analyzing genetic diversity in maize breeding programs.
- Liang, X., et al. (2024) conducted studies on genomic technologies in maize improvement and reported that next-generation sequencing accelerates breeding programs.
- Peer, L.A., et al. (2025) conducted research on molecular mechanisms of drought tolerance and identified several stress-responsive genes in maize plants.
- Singh, R., et al. (2026) reported that integrating molecular tools with traditional breeding methods improves crop productivity and stress tolerance.

IV. MATERIALS AND METHODS

4.1 Experimental Site and Design:

The present study was conducted at the research farm under suitable agro-climatic conditions. Several maize genotypes were selected to evaluate molecular diversity and yield stability. The experiment was laid out in a Randomized Block Design (RBD) with three replications. Standard agronomic practices including irrigation, fertilization, and pest management were followed throughout the crop growth period.

4.2 Morphological Data Collection:

Data were recorded on important agronomic traits including plant height (cm), days to tasseling, cob length (cm), and grain yield (t/ha). Observations were recorded from five randomly selected plants in each replication.

4.3 Molecular Analysis:

Leaf samples were collected from maize plants at the vegetative stage for molecular analysis. DNA extraction was carried out using the CTAB method described by Doyle and Doyle (1990). PCR amplification was performed using SSR molecular markers to detect genetic variation among maize genotypes. The amplified DNA fragments were separated through gel electrophoresis and visualized under UV light.

4.4 Statistical Analysis:

Statistical analyses including analysis of variance (ANOVA), principal component analysis (PCA), and cluster analysis were performed using statistical software to evaluate genetic diversity among maize genotypes.

V. RESULTS

5.1 Morphological Performance:

The results of the study revealed significant variation among maize genotypes for several agronomic traits including plant height, days to tasseling, cob length, and grain yield.

TABLE 1
MEAN PERFORMANCE OF MAIZE GENOTYPES

Genotype	Plant Height (cm)	Days to Tasseling	Cob Length (cm)	Grain Yield (t/ha)
G1	178	60	17	5.1
G2	172	58	16	4.7
G3	185	62	19	5.6
G4	180	61	18	5.3
G5	168	57	15	4.5
G6	182	60	18	5.4
G7	188	63	20	5.8
G8	175	59	17	5
Mean	178.5	60	17.5	5.18
CV (%)	4.2	3.1	5.8	6.3
CD (5%)	8.5	2.4	1.6	0.42

The analysis of variance indicated statistically significant differences among genotypes for most of the measured traits, suggesting the presence of substantial genetic variability. Genotype G7 recorded the highest plant height (188 cm), latest days to tasseling (63 days), longest cob length (20 cm), and highest grain yield (5.8 t/ha). Genotype G5 exhibited the lowest values for most traits, indicating poor adaptation to the growing conditions.

5.2 Molecular Diversity Analysis:

Molecular marker analysis revealed considerable genetic diversity among maize genotypes. The SSR markers produced polymorphic bands, indicating genetic variation at the DNA level.

TABLE 2
PCA DATA TABLE

Genotype	PC1	PC2
G1	1.45	0.82
G2	1.12	0.65
G3	2.01	1.21
G4	1.76	0.98
G5	0.98	0.54
G6	1.67	1.05
G7	2.1	1.34
G8	1.32	0.77

The PCA plot illustrated the distribution of maize genotypes based on genetic diversity. Genotypes G3, G4, G6, and G7 clustered together, indicating closer genetic relationships. Genotype G5 was positioned separately, suggesting distinct genetic background. The first two principal components accounted for approximately 68% of the total variation, effectively capturing the major patterns of genetic diversity among the genotypes.

TABLE 3
GENETIC DISTANCE MATRIX

Genotype	G1	G2	G3	G4	G5
G1	0	0.25	0.4	0.35	0.3
G2	0.25	0	0.42	0.38	0.27
G3	0.4	0.42	0	0.33	0.36
G4	0.35	0.38	0.33	0	0.29
G5	0.3	0.27	0.36	0.29	0

The dendrogram grouped the eight maize genotypes into distinct clusters based on genetic similarity. Cluster I comprised genotypes G3, G4, G6, and G7, which exhibited higher yield potential and better agronomic performance. Cluster II included genotypes G1, G2, and G8, which showed moderate performance. Genotype G5 formed a separate cluster, indicating its genetic distinctness from the other genotypes.

5.3 ANOVA for Grain Yield:

TABLE 4
GRAIN YIELD DATA (t/ha)

Genotype	Rep 1	Rep 2	Rep 3	Mean
G1	4.8	5.1	5.2	5.03
G2	4.5	4.9	4.7	4.7
G3	5.5	5.8	5.6	5.63
G4	5.2	5.4	5.3	5.3
G5	4.3	4.6	4.4	4.43
G6	5.3	5.5	5.4	5.4
G7	5.7	6	5.8	5.83
G8	4.9	5.2	5	5.03

TABLE 5
ANOVA TABLE FOR GRAIN YIELD

Source	DF	SS	MS	F	Significance
Genotypes	7	3.85	0.55	6.42	**
Error	16	1.37	0.09		
Total	23	5.22			

***** Significant at p < 0.01***

The ANOVA results revealed highly significant differences ($p < 0.01$) among genotypes for grain yield, confirming the presence of substantial genetic variability. Genotype G7 recorded the highest mean grain yield (5.83 t/ha), followed by G3 (5.63 t/ha) and G6 (5.40 t/ha). Genotype G5 recorded the lowest grain yield (4.43 t/ha), indicating poor adaptation to the growing conditions.

5.4 Identification of Stress-Tolerant Genotypes:

Based on the combined analysis of morphological performance and molecular diversity, genotypes G3, G6, and G7 were identified as superior performers with high yield potential and good adaptability. These genotypes exhibited consistent performance across replications and showed desirable agronomic traits including optimal plant height, appropriate days to tasseling, and longer cob length. Genotype G5 was identified as poorly adapted to the growing conditions and may require further evaluation under different environments.

VI. DISCUSSION

The results of the present study indicate that genetic diversity plays an important role in maize improvement programs. The observed variability among maize genotypes for agronomic traits such as plant height, days to tasseling, cob length, and grain yield provides opportunities for plant breeders to select superior genotypes for crop improvement. These findings are consistent with previous studies conducted by Prasanna et al. (2015) and Barbosa et al. (2021), who emphasized the importance of genetic diversity in maize breeding programs.

The significant differences observed among genotypes for grain yield ($p < 0.01$) suggest that the evaluated germplasm possesses substantial genetic variability, which can be exploited for developing improved varieties. The identification of genotypes G3, G6, and G7 as superior performers aligns with the findings of Khan et al. (2020), who reported significant genetic variability among maize genotypes for agronomic traits and grain yield.

Molecular markers provide valuable tools for identifying genetic variation and selecting desirable traits in crop plants. The SSR markers used in this study successfully detected polymorphism among the maize genotypes, confirming their utility for genetic diversity analysis. The clustering patterns observed in the PCA plot and dendrogram reflect the genetic relationships among genotypes and can guide parental selection in breeding programs. These results corroborate the findings of Chen et al. (2018) and Zhang et al. (2023), who reported that molecular markers are useful tools for analyzing genetic diversity in maize breeding programs.

The integration of molecular markers with conventional breeding techniques can significantly enhance the efficiency of crop improvement programs. Marker-assisted selection allows breeders to identify desirable traits at the seedling stage, reducing the time and cost associated with field evaluation. This approach is particularly valuable for selecting stress-tolerant genotypes, as demonstrated by Peer et al. (2025) and Singh et al. (2026), who identified stress-responsive genes and reported improved crop productivity through integrated breeding approaches.

The identification of genotypes with superior yield stability and stress tolerance is essential for developing climate-resilient maize varieties. As reported by Bänziger et al. (2017) and Cairns et al. (2017), selecting genotypes capable of performing well under both optimal and stress environments is crucial for sustainable maize production in the face of climate change. The genotypes identified in this study (G3, G6, and G7) warrant further evaluation under multiple environments to confirm their stability and adaptability.

VII. RECOMMENDATIONS

Based on the results of the study, the following recommendations are suggested:

1. **Utilize diverse maize germplasm resources in breeding programs:** The significant genetic variability observed among genotypes highlights the importance of maintaining and utilizing diverse germplasm collections for crop improvement.
2. **Integrate molecular breeding techniques with conventional breeding methods:** The combined use of SSR markers and phenotypic evaluation enables more efficient selection of superior genotypes, reducing the time required for variety development.
3. **Conduct further research on drought-tolerant maize genotypes:** The genotypes identified as superior performers (G3, G6, and G7) should be evaluated under managed stress conditions to confirm their drought tolerance potential.
4. **Evaluate promising genotypes across multiple locations:** Multi-environment trials are essential to assess the stability and adaptability of the selected genotypes under diverse agro-climatic conditions.
5. **Utilize genomic selection approaches for accelerating breeding programs:** Advanced molecular techniques such as genomic selection and genome-wide association studies (GWAS) can further enhance the efficiency of maize improvement programs.
6. **Strengthen collaboration between research institutions:** Collaborative efforts can facilitate the exchange of germplasm, data, and expertise, accelerating the development of climate-resilient maize varieties.

VIII. CONCLUSION

The present study revealed significant genetic diversity among maize genotypes and identified promising varieties with improved yield stability and stress tolerance. Genotypes G3, G6, and G7 exhibited superior agronomic performance and desirable traits, making them suitable candidates for further evaluation and potential release as improved varieties. The integration of molecular tools with conventional breeding approaches will play a crucial role in developing climate-resilient maize varieties for sustainable agricultural production. Continued research efforts focusing on stress tolerance mechanisms and molecular breeding techniques will contribute to ensuring food security in the face of changing climatic conditions.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Bamboo Seeds as a Resource for the Future: A Review of Germination, Storage, Phytochemistry, and Biotechnological Applications

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Abstract— *Flowering and seed set is enigmatic in bamboos. Massive seeding is followed by death of the entire clump, and seeds also remain viable for a short span of time. This limitation restricts the utility of seeds for various purposes. The solution for this loss of viability can be obtained by improving seed storage methods, such as cryopreservation, to maintain viability over extended periods. Alternative approaches using in vitro techniques including somatic embryogenesis (micropropagation) followed by artificial seed production, as well as in vitro flowering with subsequent seed set, offer promising solutions. Seeds can be utilized for multiple applications through innovative, practical, and commercial approaches that address the unpredictable seeding behavior of bamboos. This review examines the current state of knowledge on bamboo seed germination, storage requirements, phytochemical composition, and biotechnological applications including micropropagation, somatic embryogenesis, artificial seeds, and in vitro flowering.*

Keywords— Bamboo seeds, Monocarpy, Somatic embryogenesis, Cryopreservation, Bamboo rice, Artificial seeds, In vitro flowering.

I. INTRODUCTION

Bamboos are monocarpic plants of the grass family (Poaceae). Flowering is cyclic in bamboos and manifested as transformation of the entire vegetative plant into inflorescence (Janzen, 1976; John and Nadgauda, 1999; Varmah and Bahadur, 2011). Seeds are produced in huge amounts (massive seeding) but lose viability within a short span of 2 to 3 months (Banik, 1994). Despite this limitation, seeds have potential for large-scale and inexpensive propagation naturally. The seeds can also be utilized for other purposes such as germplasm conservation and genetic improvement through biotechnological approaches (Nadgauda, 1999; Sharma et al., 2017), in addition to raising large-scale plantations both in vitro and in vivo. All these applications of seeds can be enhanced by increasing shelf life and preventing loss of viability within the short period after production.

There is limited information on uses of bamboo seeds (Kiruba et al., 2007), probably due to their scarcity resulting from limited viability. Seeds of bamboo species such as *Bambusa arundinacea* are collected and used as food grains (bamboo rice) by tribal communities of Kanyakumari district in Tamil Nadu, India (Kiruba et al., 2007). Bamboo seeds are nutritionally superior to rice and wheat (Rao et al., 1955). Bamboos are grouped under non-timber forest products, but they have transcended from 'poor man's timber' to 'the timber of the 21st century' (Singh et al., 2017).

Although bamboo is part of the grass family, which has been well studied in genomics, genome data for the subfamily Bambusoideae remains far from complete. Construction of bamboo genetic populations and genetic maps is difficult because bamboo flowering is unpredictable, with cycles ranging from 40 to 120 years (Sood et al., 2018). Research on methods to enhance viability of bamboo seeds can help address this paucity of genetic studies, particularly comparative genomic studies in bamboos. Full-length cDNA cloning and sequencing in bamboos has revealed close relationships with other Poaceae members including rice, wheat, and barley (Peng et al., 2010). This review aims to synthesize current knowledge on bamboo seed biology and explore potential applications for sustainable utilization of this valuable resource.

II. GERMINATION OF BAMBOO SEEDS

Bamboos are characterized by prolonged periods of flowering accompanied by massive seeding and subsequent death of the entire clump. Germination of seeds to produce saplings occurs with high percentage under shade within a few days of production (Sharma et al., 2017). Under in vitro conditions, plant growth regulators are known to affect the germination of seeds of various bamboo species (Singh and Nayyar, 2000; Gopichand and Sood, 2008; Sharma et al., 2016).

Seeds may be classified as orthodox or recalcitrant based on their germination characteristics. Orthodox seeds typically germinate within 2 to 24 days, while recalcitrant species such as *Melocanna* and *Ochlandra* exhibit shorter germination periods (Sharma et al., 2017). A third category of seeds exhibiting intermediate characteristics between orthodox and recalcitrant types has also been established (Ellis et al., 1990). Understanding these germination patterns is essential for developing effective propagation protocols for different bamboo species.

III. STORAGE OF BAMBOO SEEDS

Storage conditions significantly influence the viability of bamboo seeds, which is naturally limited to a short period (Banik, 1994). During storage, environmental factors including relative humidity and temperature play crucial roles in maintaining viability by affecting seed moisture content and consequently metabolic rate (Banik, 1994). Dried seeds stored at low temperatures in the range of 8 to 12°C have been found to remain viable for up to one year (Somen and Seethalakshmi, 1989; Midya, 1994).

More recently, Scherwinsk-Pereira et al. (2021) suggested the use of cryopreservation technology for long-term storage of bamboo seeds to maintain viability on an extended basis. Cryopreservation offers potential for preserving genetic resources from unpredictable flowering events and ensuring availability of germplasm for future research and propagation programs.

IV. PHYTOCHEMISTRY OF BAMBOO SEEDS

Bioactive compounds of plant origin are termed phytochemicals. Bamboo seeds have not been reported to contain toxic secondary compounds, unlike seeds of many tropical trees (Watt, 1889). Preliminary phytochemical analysis of bamboo rice from *Bambusa arundinacea* revealed the presence of tannins, phlobatannins, flavonoids, cardiac glycosides, reducing sugars, and phenols in aqueous extracts (Saravanamoorthy et al., 2016).

Bamboo rice is traditionally consumed by tribal populations of the Kanyakumari region in Tamil Nadu, India, for enhancing fertility (Kiruba et al., 2007). Alcoholic seed extracts of bamboo rice have shown the presence of flavonoids, tannins, phenols, quinones, sterols, carbohydrates, and amino acids (Thamizharasan et al., 2015). The extraction and analysis of phytochemicals in bamboo seeds is still in its infancy and requires further experimentation to fully characterize the bioactive compounds and their potential health benefits.

V. BIOTECHNOLOGICAL APPLICATIONS WITH BAMBOO SEEDS

Seeds are available only during the limited flowering periods in bamboos. Various biotechnological approaches have been developed to utilize seeds and seed-derived tissues for propagation, conservation, and genetic improvement of bamboo species.

5.1 Micropropagation Using Seed Explants:

Seed tissues have been widely used for micropropagation of bamboos. Examples include the use of embryonic axes of *Bambusa arundinacea* (Mehta et al., 1982) and *Bambusa bambos* var. *gigantea* (Kapoor and Rao, 2006), seed embryos of *Dendrocalamus farinosus* (Hu et al., 2011), zygotic embryos of *Bambusa vulgaris* (Rout and Das, 1994), inflorescence

explants of *Bambusa oldhamii* (Yeh and Chang, 1986a) and *Bambusa beecheyana* (Yeh and Chang, 1986b), and pseudospikelets of *Bambusa balcooa* (Gillis et al., 2007). These approaches enable rapid multiplication of selected genotypes during limited seed availability windows.

5.2 Somatic Embryogenesis:

Natural seed formation in bamboos is a single-time occurrence in the life cycle and is followed by death of the entire clump. The use of biotechnological tools for production of somatic embryos from any available part of bamboo theoretically provides a solution to this limitation. Somatic embryogenesis allows for micropropagation and provides a source for developing genetically modified variants. Conventional breeding methods to induce hybrid vigor and obtain varieties with desired traits are almost impossible in bamboos due to long flowering cycles. Somatic embryogenesis opens a gateway for obtaining varieties with desired traits in this valuable resource.

An attempt at hybrid seed production using conventional breeding methods in bamboos was reported by Alexander and Rao (1968), marking the beginning of tissue culture research in bamboos. Seeds of hybrid bamboo (*Bambusa* × *Saccharum*) were germinated on sucrose-enriched medium (Alexander and Rao, 1968). Since naturally formed embryos are rare in bamboos, the importance of artificially generated seeds (somatic embryos) is immense, with advantages accruing as a substitute for natural embryogenesis or seed set.

Mehta et al. (1982) reported regeneration of plantlets of *Bambusa arundinacea* from somatic embryos. Subsequently, numerous scientific reports have documented production of somatic embryos in various bamboo species (reviewed by Singh et al., 2013). Godbole et al. (2002) achieved germination of somatic embryos and generation of plantlets in *Dendrocalamus hamiltonii*. While somatic embryogenesis has been achieved in various bamboos with sustainable success (reviewed by Singh et al., 2013), lab-to-land transfer rates still require improvement to enhance efficiency.

5.3 Artificial Seed Production:

Production of somatic embryos followed by their encapsulation with alginates generates artificial seeds. This technology holds promise for raising bamboo plantations (Singh et al., 2013) and mitigating shortage of this resource with multiple uses. Artificial seeds offer advantages including ease of handling, transport, and storage, as well as potential for large-scale propagation of elite genotypes.

5.4 In Vitro Flowering and Seed Set:

Flowering in bamboos is enigmatic and seeds are not produced regularly (John and Nadgauda, 1999). In vitro flowering followed by seed set can potentially help in understanding issues related to bamboo seeds and provide for ready availability of seeds at will. As early as 1990, Nadgauda et al. and Rao and Rao reported in vitro flowering in bamboos. According to Singh et al. (2013), despite considerable research in this area, it remains in its juvenile phase with no reports on practical and commercial exploitation. Further research is needed to achieve consistent and reproducible in vitro flowering and seed set that can be utilized for breeding and propagation programs.

VI. CONCLUSION

Bamboo seeds are available only for a short span of time during flowering events that occur at long, unpredictable intervals. Unlike seeds of other plants, bamboo seeds cannot be readily utilized and exploited, particularly for genetic studies and variety improvement programs. These limitations also restrict research on various aspects of bamboo biology and utilization.

In vitro techniques including somatic embryogenesis via micropropagation, artificial seed production, and in vitro flowering with subsequent seed set are practically possible and have been attempted on various scales to address these limitations, though with limited success to date. Further experiments are needed to obtain seeds through alternative methods and to improve research and development of bamboos for long-term sustainability. Cryopreservation offers potential for long-term storage of viable seeds and germplasm conservation. Phytochemical analysis of bamboo seeds reveals the presence of various bioactive compounds that warrant further investigation for potential health and nutritional applications. Continued research efforts combining conventional and biotechnological approaches will be essential for unlocking the full potential of bamboo seeds as a resource for the future.

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CONFLICT OF INTEREST

The author declares no conflict of interest.

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Canine Parvoviruses. Rapid Diagnostic by Transmission Electron Microscopy and Histopathology Techniques

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Abstract— *Canine parvovirus infection is a highly contagious disease of significant clinical and epidemiological relevance in veterinary medicine. It carries a high risk of severity and lethality, posing a particular threat to young, unvaccinated, or immunocompromised animals, and is one of the leading causes of severe gastroenteritis and mortality in dogs. Canine parvovirus (CPV-2) belongs to the family Parvoviridae and the genus Protoparvovirus. Two distinct parvoviruses are known to infect dogs: CPV-1, also called the minute virus of canines (MCV), and the pathogenic CPV-2. MCV may cause pneumonia, myocarditis, and enteritis in young pups, or transplacental infections in pregnant dams, leading to embryo resorption and fetal death. CPV-2, the causative agent of acute hemorrhagic enteritis and myocarditis in dogs, is one of the most important pathogenic viruses, with high morbidity (100%) and frequent mortality—up to 10% in adult dogs and 91% in puppies. This study aimed to diagnose canine parvovirus in fecal samples, rectal swabs, and organ fragments from dogs using transmission electron microscopy and histopathological techniques. Between 1995 and 2016, approximately 665 fecal specimens or small intestine fragments from dogs with diarrhea were sent to the Electron Microscopy Laboratory of the Biological Institute of São Paulo, SP, Brazil, for viral diagnosis. The samples were processed using transmission electron microscopy (negative staining, immunoelectron microscopy, immunocytochemistry with colloidal gold labeling, and resin embedding) and routine histopathological techniques. Using a Philips EM 208 transmission electron microscope, all samples were analyzed by the negative staining technique. In 62 samples (9.32%), a large number of parvovirus particles were observed—non-enveloped, isometric, and characterized as "complete" and "empty," measuring approximately 20 nm in diameter. Positive results in immunoelectron microscopy were confirmed by the presence of aggregates formed through antigen-antibody interactions. In immunocytochemistry, the antigen-antibody reaction was strongly enhanced by dense colloidal gold particles in all 62 positive samples. Histopathological analysis revealed hemorrhagic small intestine with villous necrosis, multiple hepatic lobules with moderate vacuolar degeneration of hepatocytes, kidneys with extensive areas of cortical coagulative necrosis, severe pulmonary edema, and moderate splenic white pulp reaction.*

Keywords— Clinical cases, Anatomopathology, Dog disease.

I. INTRODUCTION

Canine parvovirus infection is a highly contagious disease with significant severity and lethality, posing a major threat particularly to young animals. It is one of the leading causes of severe gastroenteritis and mortality in unvaccinated and immunocompromised dogs. The disease occurs worldwide in domestic dogs and other members of the Canidae family, and outbreaks have been reported in several countries, including Italy (Mira et al., 2024), Thailand (Charoenkul et al., 2024), Turkey (Ulas et al., 2024), Egypt (Magouz et al., 2023), China (Fu et al., 2022), the United States (Hong et al., 2007), Chile (Castillo et al., 2020), and India (Abhiram et al., 2023).

In Brazil, thousands of animals are infected every year, with numerous cases reported in different states, such as Rio de Janeiro (Castro et al., 2007), Rio Grande do Sul (Oliveira et al., 2018), Paraíba (Souto et al., 2018), Minas Gerais (Silva et al., 2022), and São Paulo (Cappellaro et al., 1995; Catroxo et al., 2015).

Canine parvovirus (CPV-2) belongs to the family *Parvoviridae* and the genus *Protoparvovirus* (Khatri et al., 2017). These viruses are isometric, non-enveloped, measuring 18–26 nm in diameter, with icosahedral symmetry and 32 capsomers surrounding a core containing single-stranded DNA approximately 5.2 kb in length (Bennett et al., 2008). Parvoviruses encode two nonstructural proteins, NS1 and NS2. NS1 is associated with nuclear processes required for viral replication (Moon et al., 2013). A recent study demonstrated that NS2 interacts with chromatin, regulating cellular proteins (Mattola et al., 2022).

Two distinct parvoviruses are known to infect dogs: CPV-1, also known as minute virus of canine (MCV), and the pathogenic CPV-2 (Nandy & Kumar, 2010). MCV may cause pneumonia, myocarditis, and enteritis in young puppies, as well as transplacental infections in pregnant dams, leading to embryonic resorption and fetal death (Carmichael et al., 1994). CPV-2, the causative agent of acute hemorrhagic enteritis and myocarditis in dogs, is one of the most important pathogenic viruses, with high morbidity (up to 100%) and frequent mortality rates reaching up to 10% in adult dogs and 91% in puppies (Nandy & Kumar, 2010).

Canine parvovirus is transmitted through oral contact with infected feces or contaminated surfaces, such as soil, shoes, dog toys, and other fomites (Decaro & Buonavoglia, 2012). Following an incubation period averaging 4 to 14 days, the virus replicates in the crypts of the small intestine, causing destruction of enterocytes, rupture of the mucosal barrier, and atrophy of intestinal villi. Affected animals may shed the virus in feces for more than 10 days.

The acute disease begins with depression, anorexia, high fever, profuse vomiting, and severe diarrhea. The diarrhea is abundant, often containing mucus and/or blood, and dehydration develops rapidly. The clinical scenario has become more complex due to the emergence of several variants over the years, including CPV-2a, CPV-2b, new CPV-2a, new CPV-2b, and CPV-2c, as well as the involvement of domestic and wild canids, causing serious damage to kennels (Nandy & Kumar, 2010).

The main histopathological alterations include atrophy of intestinal villi and dilation of crypts due to selective viral replication in enterocytes, bronchiolar epithelial necrosis in the lungs, vacuolar degeneration of hepatocytes, kidneys with areas of necrosis, and lymphoplasmacytic myocarditis in the heart (Kumari et al., 2020; Al-Bayati et al., 2016; Decaro & Buonavoglia, 2012; Fagbohun et al., 2020).

In breeding kennels and animal shelters, parvovirosis represents a frequent veterinary concern due to the rapid spread of the virus, resulting in significant animal losses and economic damage. Rapid diagnosis is essential, as the disease favors the development of secondary infections, accelerating clinical progression. Death in unvaccinated animals or those with ineffective vaccination protocols may occur within 2 to 3 days after the onset of clinical signs (Santana et al., 2019).

The objective of this study was to diagnose canine parvovirus in fecal samples, rectal swabs, and organ fragments from dogs, using transmission electron microscopy and histopathological techniques.

II. MATERIAL AND METHODS

2.1 Clinical Cases:

Between 1995 and 2016, approximately 665 stool specimens or small intestine fragments from dogs with clinical disease were submitted for viral diagnosis to the Electron Microscopy Laboratory of the Biological Institute of São Paulo (São Paulo State, Brazil). Of these 665 total samples, 75 were selected for histopathological examination and resin embedding analysis based on sample adequacy and clinical history. The animals ranged in age from 20 days to 3 years, included both sexes, and originated from the municipalities of São Paulo, Campo Limpo, Jundiaí, and Guarulhos, São Paulo State, Brazil.

The clinical signs and symptoms observed included acute hemorrhagic gastroenteritis, anorexia, vomiting, prostration, watery feces containing mucus and blood, fever, abdominal pain, nausea, seizures, salivation, dehydration, loss of consciousness, hypovolemic shock, and death.

2.1.1 Outbreak 1 Description:

In 1995, an outbreak of diarrhea occurred in a kennel in the State of São Paulo, Brazil, affecting approximately 10 young weaned dogs aged 50 to 60 days. The animals presented clinical signs of hemorrhagic gastroenteritis, characterized by profuse hemorrhagic diarrhea, anorexia, dehydration, and death. No information regarding the vaccination status of the dogs was available.

2.1.2 Outbreak 2 Description:

In April 2015, an outbreak of diarrhea occurred in a kennel of Pug dogs located in São José do Rio Preto, São Paulo State, Brazil, affecting a litter of 20-day-old puppies, which presented bloody diarrhea and dehydration followed by death. Two dogs

were submitted to the Pathology and Electron Microscopy Laboratories of the Biological Institute for necropsy, histopathological examination, and negative staining. In this case, information regarding the immunization status of the dogs was also unavailable.

2.2 Transmission Electron Microscopy:

The samples were processed for transmission electron microscopy utilizing negative staining (rapid preparation), immunoelectron microscopy, immunocytochemistry (immunolabeling with colloidal gold particles), and resin embedding techniques.

2.2.1 Negative Staining Technique (Rapid Preparation):

In the negative staining process, the clinical samples were suspended in phosphate buffer 0.1 M and pH 7.0 and placed in contact with metallic grids. Next, the grids were drained with filter paper and negatively stained with 2% ammonium molybdate, pH 5.0 (Brenner & Horne, 1959).

2.2.2 Immunoelectron Microscopy Technique:

In this technique, copper grids previously prepared with collodion film and stabilized with carbon were first incubated with protein A (1 mL/mL) placed in contact with the virus-specific antibody (antiserum from sick dogs infected with parvovirus). Afterward, grids were washed in PBS drops, incubated with the viral suspension of the 75 samples of small intestine, washed with drops of water, and negatively stained with 2% ammonium molybdate, pH 5.0 (Berthiaume et al., 1981; Katz & Kohn, 1984; Doane & Anderson, 1987; Hayat & Miller, 1990; Padrón, 1998).

2.2.3 Immunocytochemistry Technique:

In the immunolabeling technique with colloidal gold particles for negative staining, the copper grids were placed in contact with viral suspension of the samples of feces, rectal swab, and small intestine fragments and, after removing excess with filter paper, were placed on specific primary antibody drops. After successive washings in PBS drops, the grids were incubated in protein A drops, in association with 10 nm colloidal gold particles (secondary antibody). Grids were then contrasted with 2% ammonium molybdate, pH 5.0 (Knutton, 1995).

2.2.4 Resin Embedding Technique:

All 75 fragments of small intestine samples were fixed in 2.5% glutaraldehyde in 0.1 M, pH 7.0 phosphate buffer and post-fixed in 1% osmium tetroxide in the same buffer. After dehydration in acetone series, the fragments were embedded in Spurr resin (González-Santander, 1969; Luft, 1961). Ultrathin sections were cut on the LKB ultratome and mounted on copper grids. The sections were contrasted with uranyl acetate-lead citrate (Watson, 1958; Reynolds, 1963).

2.3 Histopathology:

2.3.1 Routine Histological Technique:

All 75 fragments of the small intestine were fixed in 10% buffered formalin, dehydrated, diaphanized, and embedded in paraffin. Five-µm-thick sections were performed and stained with hematoxylin and eosin technique (H&E).

III. RESULTS

3.1 Clinical Cases:

Of the 665 fecal samples and small intestine fragments from dogs with diarrhea processed using the negative staining technique and examined by transmission electron microscopy (Philips EM208), 62 (9.32%) were positive for parvovirus particles.

Considering the age of the positive animals, most were up to 11 months old (39/62; 62.90%). Only three adult dogs (4.83%) were infected with parvovirus, while in 20 cases (32.5%) the age was not identified. Among the 62 parvovirus-positive dogs, 22 (35.40%) were females and 24 (38.73%) were males; in 16 samples (25.80%), sex could not be determined.

Regarding coinfections, 21 cases (33.87%) were coinfected with coronavirus, 11 (17.74%) with paramyxovirus, three (4.83%) with coronavirus and *Mycoplasma* spp., and one (1.61%) with coronavirus and paramyxovirus. All 10 fecal samples from outbreak 1 (16.12%) were coinfected with *Mycoplasma* spp., and all affected dogs died.

3.2 Transmission Electron Microscopy:

3.2.1 Negative Staining (Rapid Preparation) Technique:

In all 62 positive fecal samples and small intestine fragments examined by transmission electron microscopy using the negative staining technique, a large number of parvovirus particles were observed. The particles were non-enveloped and isometric, and were characterized as "complete" particles and "empty" particles, with an average diameter of approximately 20 nm (Fig. 1). In 10 stool samples, pleomorphic formations similar to *Mycoplasma* spp. (Fig. 4), measuring between 100 and 800 nm, were also observed.

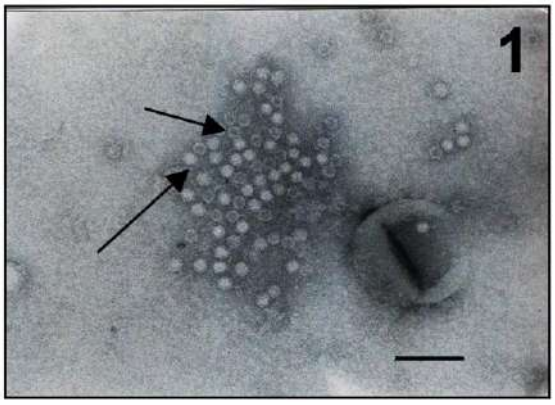

FIGURE 1: Negatively stained parvovirus particles, non-enveloped and isometric, "complete" (big arrow) and "empty" (small arrow), measuring 20 nm in diameter. Scale bar: 100 nm.

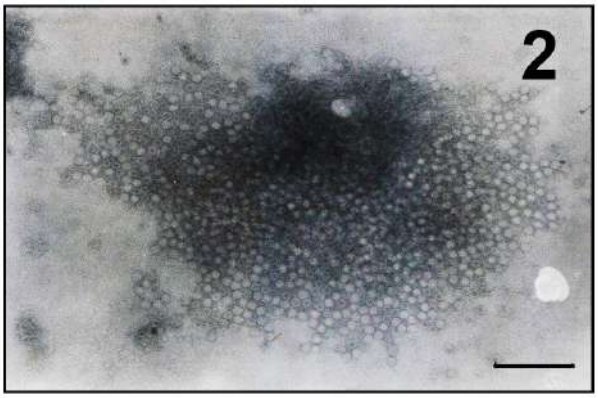

FIGURE 2: In the immunoelectron microscopy technique, the parvovirus particles were aggregated by antigen-antibody interaction. Scale bar: 180 nm.

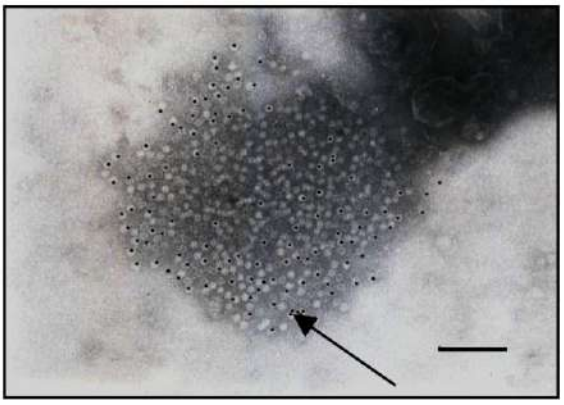

FIGURE 3: Antigen-antibody interaction strongly enhanced by the dense gold particles over the parvoviruses (arrow). Scale bar: 160 nm.

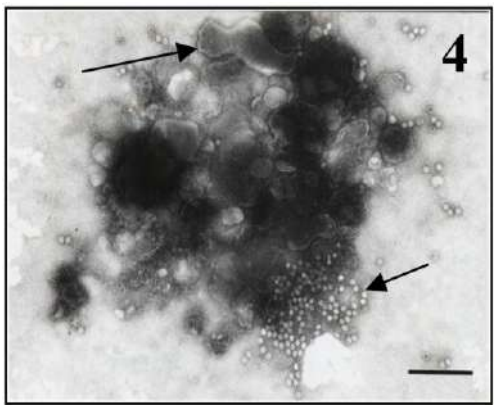

FIGURE 4: Negative staining of dog feces showing the simultaneous presence of parvovirus (small arrow) and *Mycoplasma* (large arrow). Scale bar: 190 nm.

3.2.2 Immunoelectron Microscopy Technique:

Positive immunoelectron microscopy results for parvovirus were characterized by the presence of aggregates formed through antigen-antibody interactions (Fig. 2) in all 62 positive samples.

3.2.3 Immunocytochemistry Technique:

In the immunocytochemistry technique, the antigen-antibody reaction was strongly enhanced by the dense colloidal gold particles on parvovirus in all 62 positive samples (Fig. 3).

3.2.4 Resin Embedding Technique:

In ultrathin sections of the small intestine, shortening and reduction of the microvilli were observed in the intestinal cells (Fig. 5), as well as misshapen nuclei with rounded inclusions (Fig. 6) containing parvovirus particles.

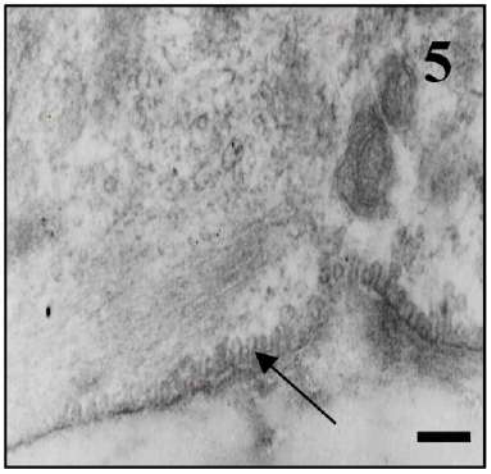

FIGURE 5: Ultrathin section of the intestine, showing shortening and reduction of the microvilli (arrow). Scale bar: 220 nm.

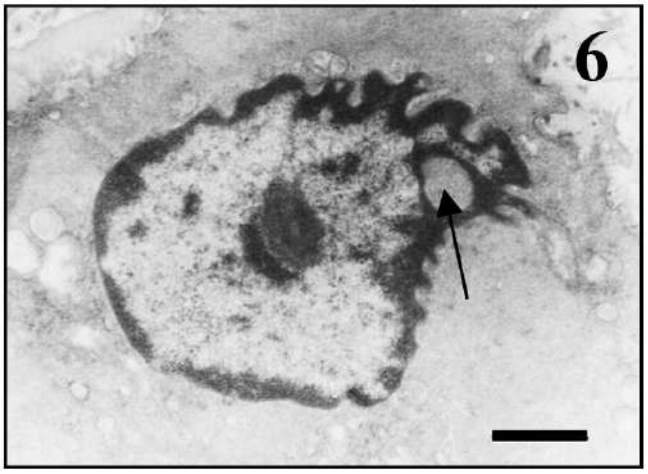

FIGURE 6: Ultrathin section of a dog's intestine. Misshapen nucleus containing a rounded inclusion (arrow). Scale bar: 800 nm.

3.3 Histopathology:

The histopathological findings demonstrated necrotizing hemorrhagic enteritis of the small intestine, characterized by necrosis of the intestinal villi (Fig. 7). The liver showed moderate vacuolar degeneration of hepatocytes (Fig. 8), distributed across multiple hepatic lobules. The kidneys exhibited extensive coagulative necrosis in the cortical region (Fig. 9), consistent with severe renal injury. Severe pulmonary edema was also observed (Fig. 10), along with moderate hyperplasia/reaction of the splenic white pulp, suggestive of a systemic immune response (Fig. 11).

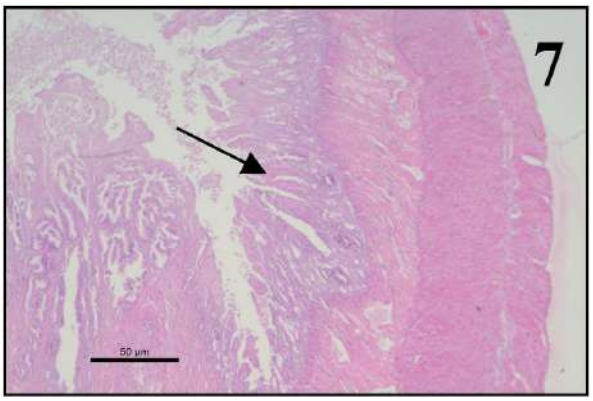

FIGURE 7: Histopathology of the small intestine, H&E staining (40x). Villous necrosis.

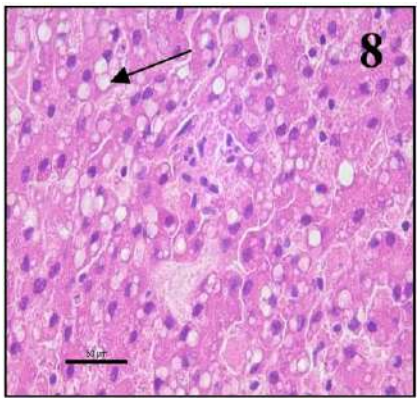

FIGURE 8: Histopathology of the liver, H&E staining (630x). Hepatocyte vacuolization.

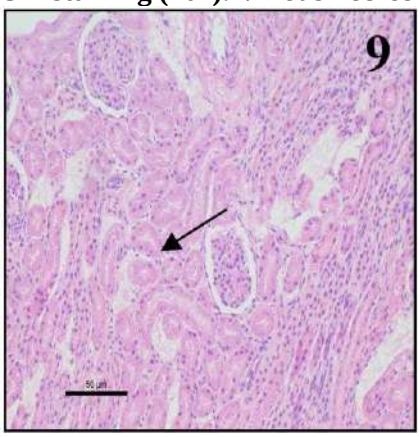

FIGURE 9: Histopathology of the kidney, H&E staining (200x). Focal necrosis.

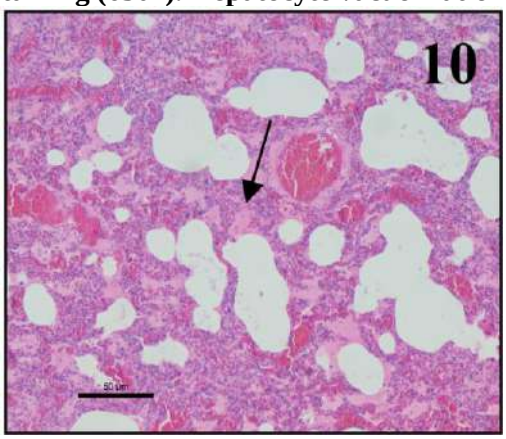

FIGURE 10: Histopathology of the lung, H&E staining (100x). Severe pulmonary edema and congestion.

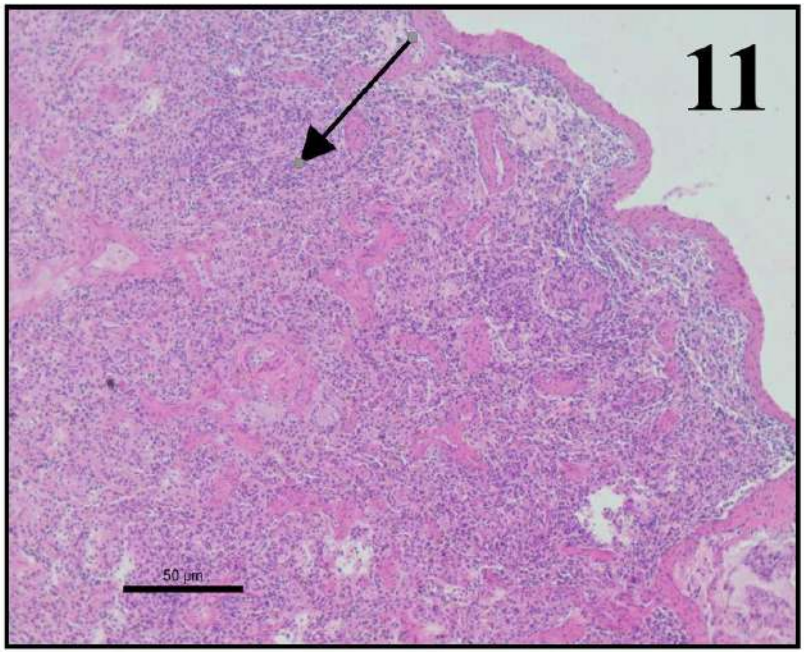

FIGURE 11: Histopathology of the spleen, H&E staining (100x). Moderate splenic pulp reaction.

IV. DISCUSSION

Canine parvovirosis is a contagious viral disease that primarily affects newborns, and infection may result in the total loss of entire litters. Diagnosis using conventional methods has shown low sensitivity, especially in the late stages of infection (Decaro & Buonavoglia, 2012).

In this study, 665 samples of feces or small intestine fragments from dogs with diarrhea were examined by transmission electron microscopy. The detection rate of 9.32% for parvovirus in this study is lower than rates reported in most other studies. Most studies on canine parvovirosis conducted in various countries have reported higher detection rates, ranging from 100% in Turkey (Vural & Alcigir, 2011); 100% and 82.5%, respectively, in China (Zhao et al., 2013; Magouz et al., 2023); 88.9% in the USA (Hong et al., 2007); 87.2% and 66.5%, respectively, in Italy (Zobba et al., 2021; Mira et al., 2024); and 46% in Chile (Castillo et al., 2020). Lower rates (17%) were reported by Del Amo (1999) in Turkey and by Biezus et al. (2020) in the State of Santa Catarina, Brazil. In other Brazilian states, positivity rates ranged from 100% to 68.7% in Rio Grande do Sul (Oliveira et al., 2018); 46% in Rio de Janeiro (Castro et al., 2007); 54% in Mato Grosso (Fontana et al., 2013); and 65% in Goiás (Martins et al., 2017).

The main clinical signs and symptoms induced by CPV in the animals of our study were characterized by acute hemorrhagic gastroenteritis, nausea, anorexia, persistent vomiting, prostration, fever, abdominal pain, watery feces with mucus and blood, dehydration, loss of consciousness, hypovolemic shock, and death. Most of these clinical signs have also been reported by other authors, although diarrhea and vomiting are the most frequently described manifestations (Robinson et al., 1980; Del Amo, 1999; Catroxo et al., 2007; Hong et al., 2007; Fontana et al., 2013; Zhao et al., 2013; Oliveira et al., 2018; Jaune et al., 2019; Biezus et al., 2020; Castillo et al., 2020; Zobba et al., 2021; Fu et al., 2022; Magouz et al., 2023; Mira et al., 2024; Ulas et al., 2024). One of the animals in our study presented neurological signs, such as seizures and vocalization, corroborating the findings of Oliveira et al. (2018), who described these clinical signs in three animals in their study. Souto et al. (2018) reported the occurrence of the cardiac form of parvovirosis, characterized by hyperacute cardiorespiratory alterations and death of the affected puppies.

Considering the age of the infected animals, the majority (39 dogs) were up to 11 months old, corresponding to 62.90%. Other authors have also reported higher positivity rates in animals under one year of age: 90.79% (Biezus et al., 2020) and 70.83% (Oliveira et al., 2018); 82.3% in animals younger than 6 months (Magouz et al., 2023); 2 to 12 months (Vural & Alcigir, 2011); 1 to 6 months (Ulas et al., 2024); up to 6 months (Castro et al., 2007); between 6 weeks and 6 months (Del Amo, 1999); 3 to 8 months (Hong et al., 2007); and 55% with a mean age of 4 months (Martins et al., 2017). Only three adult animals (1 and 3 years old) (4.83%) became ill. In contrast, Dezengrini et al. (2007) reported a 72% positivity rate in animals aged 1 to 2 years. The high infection rate in puppies may be explained by the fact that their immune system is still developing, making them more

susceptible to infection. Additionally, the virus has a high affinity for rapidly dividing cells, such as those of the intestinal tract, which are in constant proliferation in young animals. This favors viral replication and results in a more severe form of the disease (Appel & King, 1992; Carpenter & Meyer, 2003).

Regarding sex, positivity rates did not show significant differences, as 35.40% were females and 38.70% were males. This finding is consistent with Castro et al. (2007), who reported 49.40% in females and 43.30% in males, and with Martins et al. (2017), who detected 31.7% in females and 33.3% in males. A rate of 56.53% in females and 47.37% in males was reported by Biezus et al. (2020).

Regarding co-infections, among the examined samples, 21 (33.87%) were co-infected with coronavirus, 11 (17.74%) with paramyxovirus, 3 (4.83%) with coronavirus and *Mycoplasma*, and 1 (1.61%) with coronavirus and paramyxovirus. Other studies have also reported dual and triple infections when investigating canine parvovirosis (Roseto et al., 1980; Decaro & Buonavoglia, 2012; Licitra et al., 2014; Zhao et al., 2016; Headley et al., 2018; Zobba et al., 2021; Catroxo et al., 2023, 2024). All 10 fecal samples from outbreak 1 (16.12%) were co-infected with *Mycoplasma* sp., and all affected dogs died. *Mycoplasma* sp. is considered an opportunistic agent that may remain asymptomatic in the host and cause clinical manifestations following episodes of immunosuppression (Nascimento et al., 2012). Co-infection with parvovirus and other agents is favored by the virus-induced reduction of the immune response, since it affects cells of the immune system and the intestinal tract, which are essential for host defense against other infectious agents. Immunocompromised dogs are more susceptible to co-infections. Due to its high infectivity and ability to suppress the immune response, parvovirus creates a favorable environment for the proliferation of other pathogens (Buonavoglia et al., 2001).

The distinctive ultrastructural features of parvovirus particles observed in this study have also been described in other studies on canine parvovirosis using this technique (McAdaragh et al., 1979; Burtonboy et al., 1979; Roseto et al., 1980; Williams, 1980; Meunier et al., 1981; Muneer et al., 1988; Harrison et al., 1992; Drane et al., 1994; Finlaison, 1995; Hurtado et al., 1996; Del Amo et al., 1999; Nelson et al., 2008; Schulz et al., 2008; Catroxo et al., 2013; Areshkumar et al., 2018; Jaune et al., 2019; Zhao et al., 2023). Martinello et al. (1997) detected parvovirus particles in fecal samples from wolves (*Canis lupus*) in Italy.

The immunoelectron microscopy technique applied to all 62 positive samples confirmed the presence of parvovirus through the formation of antigen-antibody aggregates (Fig. 2). This technique has also been used in other studies on canine parvovirosis (Karasaki, 1966; Durigon et al., 1987; Sherding, 1992; Casal, 1999; Schmitz et al., 2009; Catroxo et al., 2013, 2015; Feng et al., 2014; Singh et al., 2022). Similarly, the strong labeling of parvovirus particles with colloidal gold in the immunocytochemistry technique (Fig. 3) has been reported by Suikkanen et al. (2002, 2003) and Catroxo et al. (2013).

The histopathological findings observed in this study—including hemorrhagic small intestine with villous necrosis, multiple hepatic lobules with vacuolar degeneration of hepatocytes, kidneys with extensive areas of cortical coagulative necrosis, as well as severe pulmonary edema and moderate splenic white pulp reaction—have also been reported by other authors in cases of canine parvovirosis (Robinson et al., 1980; Vural & Alcigir, 2011; Zhao et al., 2013; Al-Bayati et al., 2016; Oliveira et al., 2018; Fagbohun et al., 2020).

Transmission electron microscopy and histopathology contributed decisively to the diagnostic confirmation of canine parvovirosis during the outbreaks, allowing the etiological identification of the agent involved and revealing morphological alterations consistent with the parvovirus's tropism for highly mitotically active cells. The combination of these techniques provided complementary and reliable diagnostic support, reinforcing the interpretation of clinical findings and contributing to the understanding of the disease's pathogenesis.

Considering that canine parvovirosis is a disease with high morbidity and mortality, especially in young and unvaccinated dogs, rapid and accurate diagnosis and immediate intervention are essential for a favorable prognosis. In the absence of a specific antiviral therapy, treatment is based on intensive clinical support, emphasizing fluid therapy, control of gastrointestinal signs, antibiotic therapy to address immunosuppression caused by panleukopenia, protection of the intestinal mucosa, early nutritional support, and analgesia. The integrated application of these measures significantly contributes to clinical stabilization, reduction of secondary complications, and increased survival rates.

Adjuvant therapies, such as the use of immunoglobulins, may be considered in specific situations, although their use depends on clinical criteria and availability. Finally, preventive vaccination remains the main strategy for controlling canine parvovirosis, highlighting the essential role of veterinarians in guiding pet owners and reducing disease incidence (Santana et al., 2019; Melo et al., 2021; Larson et al., 2024; Ulas et al., 2024).

The combination of the techniques used is highly effective for the rapid diagnosis of canine parvovirus and can be applied in routine procedures to identify the viral agent responsible for this important disease.

V. CONCLUSION

This study successfully demonstrated the utility of transmission electron microscopy techniques—including negative staining, immunoelectron microscopy, immunocytochemistry with colloidal gold labeling, and resin embedding—combined with histopathological analysis for the rapid and accurate diagnosis of canine parvovirus infection. Of the 665 samples analyzed, 62 (9.32%) were positive for CPV-2, with the highest prevalence observed in animals up to 11 months of age. Co-infections with coronavirus, paramyxovirus, and *Mycoplasma* spp. were identified in a significant proportion of cases. The ultrastructural and histopathological findings were consistent with the characteristic tropism of parvovirus for rapidly dividing cells, particularly in the intestinal crypts, and revealed systemic involvement including hepatic, renal, pulmonary, and splenic alterations. The combination of these diagnostic techniques provides reliable support for clinical diagnosis and contributes to the understanding of disease pathogenesis. Rapid diagnosis remains essential for effective clinical management and implementation of control measures, while preventive vaccination continues to be the primary strategy for reducing the impact of this significant veterinary disease.

VI. CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. This research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest

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Effect of Compost and Urea Fertilizers on Sugarcane Quality at the Kenana Sugar Scheme, Sudan

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Abstract— *Sugarcane (Saccharum officinarum L.) is recognized as one of the world's most important cash crops. The climatic conditions and soil types in Sudan, particularly within the central clay plains, are highly suitable for its cultivation. Organic fertilizer (compost) is a vital resource for improving soil fertility by increasing organic matter content, enhancing soil structure, and stimulating microbial activity, which collectively improve nutrient uptake and crop productivity. In contrast, urea is a synthetic nitrogen fertilizer that provides a highly concentrated and readily available source of nitrogen, a macronutrient essential for vigorous vegetative growth, tillering, and the synthesis of proteins and chlorophyll in plants. This study aimed to evaluate the effects of compost and urea fertilizer on quality of sugarcane (var. Co6806). A field experiment was conducted over two consecutive seasons (2023/24 and 2024/25) at the Research and Development Farm of the Kenana Sugar Scheme, Sudan. The treatments were arranged in a 4×4 factorial in split-plot design with four replications. Urea was assigned to the main plots at four levels (0, 119, 238, and 357 kg/ha), while compost was applied to the sub-plots at four rates (0, 12, 24, and 36 ton/ha). Data were collected on juice quality parameters (Pol%, Brix%, Fiber%, Purity%, Moisture%, and Estimated Recoverable Sucrose Content (ERSC%). The results showed that the main effects of compost and urea, both individually and in combination, on sugarcane quality parameters were non-significant across both seasons. Seasonal variation was the dominant factor influencing juice quality, indicating that environmental conditions played a greater role than fertilization in determining sucrose accumulation. Based on the results of this study, it could be recommended that to obtain high cane yield with maintained quality under similar soil and climatic conditions, the crop should be fertilized with compost at the rate of 36 tons/ha in combination with urea at the rate of 357 kg/ha.*

Keywords— Sugarcane, Compost, Urea, Juice quality, Pol%, Brix%, Purity%, ERSC%, Kenana Sugar Scheme, Sudan.

I. INTRODUCTION

Sugarcane (*Saccharum officinarum* L.) is a globally vital crop, primarily cultivated for sugar production (accounting for ~80% of world sugar) and increasingly as a renewable source for bioethanol (40% of global bioethanol) (Nair & Sachan, 2022; Voora et al., 2023). As a C4 plant, it possesses high photosynthetic efficiency, enabling rapid growth and substantial biomass accumulation (Tew & Cobill, 2008). Originating in New Guinea, it is now grown extensively in tropical and subtropical regions, including Sudan, and adapts to various soil types from sandy loams to heavy clays (Moore et al., 2013; Mwasinga, 2018). The crop's economic value lies in its ability to store high concentrations of sucrose in its stalk internodes (James, 2008).

Intensive monoculture and continuous ratooning deplete soil nutrients, necessitating regular inorganic fertilizer application (Kusumawati & Noviyanto, 2025). However, soil degradation under sugarcane monoculture is severe, with documented losses of 30-40% of original soil organic carbon after 20 years of continuous cultivation (Bottinelli et al., 2020). This degradation directly reduces yield potential by up to 35% in degraded soils (Obour et al., 2017) and negatively impacts sucrose accumulation, with reductions of 2-4% absolute sucrose content (Verma et al., 2024). Additionally, heavy machinery use causes soil compaction, restricting root penetration, water movement, and ultimately reducing yields (Shaheb et al., 2021).

Sugarcane has a high nitrogen demand (200–300 kg N/ha), but nitrogen use efficiency in current systems is only 30–50% (Otto et al., 2016; Thorburn et al., 2017). This inefficiency results in significant nitrogen losses through leaching, volatilization, and denitrification, causing environmental and economic consequences, including groundwater nitrate contamination often exceeding WHO limits (Canton, 2021; Chen et al., 2022), significant N_2O emissions estimated at 1.5–5.0 kg N_2O-N/ha/year—a potent greenhouse gas (Van Beneden et al., 2010)—and annual economic losses of approximately $1.2 billion in wasted fertilizer globally (De Luca & Müller, 2023).

Sugarcane cultivation and processing generate substantial organic wastes, including bagasse, filter mud, molasses, trash, and vinasse (Singh et al., 2021; Salman et al., 2023). For each ton of sugarcane processed, approximately 250 kg of bagasse, 36 kg of filter mud, and 100 kg of tops are generated (Mena et al., 1985). Recycling these by-products into compost offers a sustainable strategy to enhance soil fertility, improve soil physical properties, and reduce reliance on chemical fertilizers (Iqbal, 2018). Compost application has been shown to improve soil organic carbon, total nitrogen, available phosphorus, and potassium (Teshome et al., 2014), increase soil water holding capacity by 15–25% (Diacono & Montemurro, 2011), enhance microbial biomass carbon by 30–50% (Sá et al., 2001), and improve cane growth parameters such as height and stalk weight, as well as sugar quality traits including Brix and sucrose content, particularly when combined with nitrogen fertilizer (Bekheet et al., 2018; Zeng et al., 2020).

In Sudan, sugarcane yields are considerably lower (about 60 t/ha) compared to other irrigated cane production areas worldwide (Ibrahim, 2020). The Kenana Sugar Scheme, located on the eastern bank of the White Nile, is characterized by Vertisol soils with high clay content, low nitrogen, and low organic matter (Emam & Musa, 2011; Ganawa & Kheiralla, 2011). Currently, the scheme relies exclusively on chemical fertilizers such as urea, ammonium sulphate, and di-ammonium phosphate (Hamid & Dagash, 2014). However, in response to sustainability challenges, the Kenana Scheme has begun recycling sugarcane by-products and animal waste into compost for field application.

Despite advances, most research has evaluated compost or urea fertilizer in isolation, with limited studies on their combined, synergistic effects (Aouass & Kenny, 2024). There is currently no consensus on the extent to which compost can replace urea fertilizer without compromising yield and quality, nor on the optimal compost-to-nitrogen fertilizer ratios (Reimer et al., 2023). With the increasing need to conserve natural resources and reduce pollution, recycling sugar industry wastes as compost offers a promising strategy to lower fertilizer costs and mitigate environmental and health risks. Accordingly, a research project was initiated over two consecutive seasons (2023/24 and 2024/25) at the Kenana Sugarcane Estate with the following objectives:

Main Objective:

- To evaluate the effects of compost and urea fertilizer applications on the quality of sugarcane at the Kenana Sugar Scheme.

Specific Objectives:

- To study the effects of compost and urea fertilizers on sugarcane quality.
- To determine the optimum application rates of compost and urea fertilizer to achieve the highest yield of sugarcane without compromising quality.

II. LITERATURE REVIEW

2.1 Sugarcane: Origins, Biology, and Agronomic Requirements:

Sugarcane is a tropical C4 plant domesticated in New Guinea approximately 10,000 years ago (Grivet et al., 2004; Sánchez-Ken, 2019; Zhang et al., 2023). It is characterized by high photosynthetic efficiency under intense solar radiation (Mehdi et al., 2024; Sage et al., 2013). Optimal growth requires warm temperatures (18–33°C), adequate water (300–2,500 mm annual rainfall or irrigation), and well-structured soil (Bonnett et al., 2006; Devi et al., 2022). In Sudan, sugarcane is predominantly cultivated on heavy clay soils, which, despite their challenges, support high yields with proper management (Bakker, 2012). The crop is vegetatively propagated via setts, and its productivity is determined by critical growth stages including tillering, stalk elongation, and sucrose accumulation (Terefe et al., 2017; Impollonia et al., 2024).

2.2 Economic and Industrial Importance:

Globally, sugarcane is a major commercial crop for sugar and bioethanol production, with expanding applications in bioplastics and organic fertilizers (Huang et al., 2020; Lee et al., 2023). The industry contributes significantly to agricultural GDP and rural livelihoods, particularly in developing economies (Solomon, 2016; Mohan, 2017).

2.3 Compost: Production, Composition, and Soil Benefits

Composting is a biological process that stabilizes organic wastes (e.g., bagasse, filter mud, press mud) into a humus-rich amendment (Haug, 2018). The process requires controlled moisture and aeration to achieve thermophilic temperatures that eliminate pathogens (Raviv, 2013). Compost derived from sugarcane residues is rich in organic carbon (~28.6%), macronutrients (N: 0.35–0.65%, P: 0.04–0.15%, K: 0.40–0.50%), and micronutrients (Fe, Zn, Mn) (Fortes et al., 2013; Tajmirriahi et al., 2021). Its application improves soil physical properties (structure, water-holding capacity, aeration), chemical properties (organic matter, CEC, nutrient availability), and biological activity (microbial biomass and diversity) (Dotaniya et al., 2016; Khan et al., 2023; Miháliková et al., 2025).

2.4 Effect of Compost on Sugarcane Quality:

Compost application can positively influence sugarcane juice quality parameters, particularly Brix (total soluble solids), Pol (sucrose percentage), and juice purity. However, these effects are highly variable and depend on several interacting factors, including the type of organic manure used, prevailing soil conditions, and the specific sugarcane variety being cultivated (Teama et al., 2017; Ghallab et al., 2024). A critical factor in maintaining or improving quality is the implementation of balanced nutrition. Specifically, avoiding excessive nitrogen application is essential, as an over-supply of nitrogen, even from organic sources, can delay crop maturity and reduce sucrose accumulation in the stalks (Zeng et al., 2020).

2.5 Effect of Urea (Nitrogen) on Sugarcane Yield:

Nitrogen is essential for sugarcane, promoting tillering, biomass accumulation, and photosynthesis (Gopalasundaram et al., 2012; Aslam et al., 2024). Urea (46% N) is the most widely used nitrogen fertilizer (Poultney et al., 2024). Moderate nitrogen rates (e.g., 46–220 kg N/ha) increase cane yield, but excessive application can delay maturity, reduce sucrose concentration (Pol% and Brix), lower juice purity, and increase impurities (Muchow et al., 1996; Skocaj et al., 2013; Yang et al., 2019). The optimal nitrogen rate for balancing yield and quality is typically around 220 kg N/ha, beyond which sugar recovery declines (Ahmed et al., 2008; Yahaya et al., 2010).

2.6 Integrated Nutrient Management: Compost and Urea:

Integrating compost with urea produces synergistic effects, often outperforming either amendment alone. This combination enhances nutrient use efficiency, sustains soil health, and reduces environmental impacts such as nitrate leaching and N_2O emissions (Bokhtiar et al., 2015; Tang et al., 2022). Studies in Ethiopia and elsewhere confirm that applying compost (15 t/ha) with reduced nitrogen rates (46 kg N/ha) maximizes cane and sugar yields while improving economic returns (Teshome et al., 2014; Xu et al., 2021). The slow-release nature of compost complements the readily available nitrogen from urea, ensuring balanced nutrition throughout the crop cycle (Priya et al., 2024).

2.7 Long-Term and Environmental Benefits:

Long-term compost use builds soil organic matter, enhances carbon sequestration, and improves soil resilience to drought and climate variability (Diacono & Montemurro, 2011; Wright et al., 2022). Recycling sugarcane industry by-products (bagasse, filter mud, vinasse) as compost aligns with circular economy principles, reducing waste and chemical fertilizer dependency (Raza et al., 2021; Sathiyapriya et al., 2024). Economically, compost reduces input costs and increases profitability over multiple seasons (Noor et al., 2023; Stephen et al., 2024).

III. MATERIALS AND METHODS

3.1 Experimental Site Description:

The experiment was conducted at the Research and Development Farm of Kenana Sugar Scheme, Sudan, over two consecutive seasons (2023/24 and 2024/25). Kenana is geographically situated between the White Nile and Blue Nile rivers, at approximately 33° E longitude and 13° N latitude, with an elevation of 410 meters above sea level (Ibrahim & Workneh, 2023). The site is located about 330 km south of Khartoum, the capital city of Sudan, and 30 km southeast of Rabak Town (Ahmed & others, 2016). The climate of the area is characterized as tropical aridic, with a distinct summer rainy season lasting approximately five months, from June to October, peaking in August. The average annual rainfall for the two seasons under study was 379 mm, although rainfall varies considerably from year to year. Temperature extremes range from a mean maximum of 42 °C in May to a minimum of 13.7 °C in January. Relative humidity fluctuates between 20.5% and 79.8%. The soil at the experimental site is classified as a brown, heavy clay Vertisol. The top 60 cm soil profile consists of cracking clay with a clay content ranging from 40% to 60% (Mohamed, 2018). Soil pH values range from 7.50 to 8.50 (Antille et al., 2016). More than

90% of the upper soil horizon exhibits electrical conductivity values below 3 mS/cm³. Extractable sodium percentage (ESP) ranges between 510 and 770 ppm (Mohammed, 2006).

3.2 Experimental Layout Design and Treatments:

This study examined the individual and interactive effects of compost and urea fertilization on quality parameters of sugarcane. The experimental material consisted of four urea levels (0, 119, 238, and 357 kg/ha) and four compost levels (0, 12, 24, and 36 tons/ha). The experiment design was a 4×4 factorial in split-plot design with four replications. Urea levels were assigned to the main plots, and compost levels to the subplots. The total plot area was 60 m² (plot size: 4 furrows, each 10 meters long and 1.5 meters wide). The test variety used was Co6806. Data were collected on key agronomic and quality parameters and subjected to analysis of variance (ANOVA), with means separated using Duncan's Multiple Range Test (DMRT).

3.3 Cultural Practices:

3.3.1 Fertilizer and Compost Application:

Compost was applied as a single dose and uniformly spread along the ridges at the time of planting. Urea fertilizer was also applied as a single dose at planting. All agronomic practices including irrigation, weeding, and other management operations were carried out uniformly across all experimental plots, following the standard protocols of the Sugar Estate.

The compost used in this study was produced by the Kenana Sugar Company using the windrow composting method. Windrow composting involves piling organic materials, such as agricultural and industrial by-products, into long rows (windrows) that are regularly turned to ensure adequate aeration, moisture distribution, and temperature control. This aerobic process accelerates the decomposition of organic matter, reduces odor, and minimizes the risk of soil and water pollution. The temperature of the windrows is monitored to ensure the process passes through the necessary mesophilic and thermophilic phases, which are critical for pathogen reduction and compost stabilization.

Before field application, the maturity of the compost was assessed by evaluating its odor and colour, which are reliable indicators of stability and readiness for use. Additional parameters, such as the C/N ratio and cation exchange capacity, may also be used to confirm compost maturity and biological stability.

The compost formula consisted of organic raw materials with balanced nutrient content, specifically tailored for agricultural use by the Kenana Sugar Company. The composition was as follows: filter mud (45–50%), green cane trash (25–30%), cow manure (8–10%), poultry manure (4–5%), and vinasse sludge (3–5%). This blend provides a rich source of macro- and micronutrients, improves soil structure, and enhances water-holding capacity, contributing to long-term soil fertility and sustainability.

3.3.2 Land Preparation:

Land under continuous sugarcane cultivation in two locations was used. The stubble of the previous crop was uprooted using a disc plow, and the land was then left fallow during the summer months and rainy period. When it was dry, it was deeply plowed using the same disc plow, disk harrowed by a wide level disc, levelled using a planer, and ridged at 1.5 meters spacing using a ridger.

3.3.3 Planting Date and Method:

Planting was carried out using the continuous double-set furrow method. Seed cane was obtained from ten-month-old stalks of the plant crop, which were cut into short setts, each containing three buds. Following fertilizer application, these setts were uniformly placed in the furrows at a rate of 264 setts per plot. To protect the setts from termite damage, the insecticide Regent was applied directly by spraying at a rate of 2.38 L/ha. After treatment, the setts were manually covered with soil and irrigated immediately to ensure proper establishment. Planting was done in the first season on 2nd December 2023, and the second season on 1st December 2024.

3.3.4 Weed Control:

A combination of the herbicides Stomp (pendimethalin) and Gezaprim (Atrazine) was applied as a pre-emergence treatment, following commercial recommendations, just prior to the second irrigation. The application rates were 1.43 L/ha for Stomp and 1.79 kg/ha for Gezaprim. To ensure effective weed control, plots were maintained weed-free by supplementary hand weeding whenever necessary throughout the growing seasons.

3.3.5 Hilling-Up Practice:

Hilling up of the plant rows was performed three months after planting. This involved raising the soil around the cane plants by employing the split ridging technique to cover the furrows in which the cane was planted. This practice helps improve soil aeration, moisture retention, and supports healthy crop growth.

3.3.6 Irrigation Management:

During the germination phase, setts were irrigated at 12-day intervals to ensure optimal moisture for sprouting. After the completion of germination, subsequent irrigations were applied as needed based on crop requirements and prevailing environmental conditions.

3.3.7 Pre-Harvest Drying Off:

Prior to each harvest, irrigation was withheld from the plots scheduled for harvesting for a period of one month to allow the fields to dry adequately. This pre-harvest drying off facilitates easier harvesting and improves cane quality.

3.3.8 Harvesting Procedure:

The harvested area for each plot was 30 m^2, consisting of two rows, each 10 meters in length and 1.5 meters in width. Harvesting was conducted manually. Stalks were cut precisely at the soil surface to maximize yield and ensure uniformity. After cutting, all stalks were thoroughly cleaned by removing leaves and tops, ensuring that only the cane stalks were retained for subsequent analysis determination. This standardized harvesting method ensures accurate assessment of quality parameters across all experimental plots.

3.3.9 Collection of Data for Cane Quality Parameters:

Cane quality data were collected one day prior to harvest. Cane analysis was conducted in the Sucrose Laboratory of the Kenana Sugarcane Research Department, following the procedures outlined by the International Commission for Uniform Methods of Sugar Analysis (ICUMSA, 1994). For each plot, a random sample of 10 stalks (5 stalks per row) was collected from the effective harvested area at harvest time. The sampled stalks were immediately stripped of leaves, topped, and prepared for laboratory analysis. The stalks were chopped and ground using a Jeffco Cutter Grinder to obtain crushed cane. From the crushed material, a 50 g subsample was randomly selected to determine cane moisture content by oven drying at 105°C for 5 hours. The remaining quality parameters were assessed using the Jeffco Wet Disintegrator method. A 100 g sample of crushed cane was disintegrated in two liters of distilled water for 20 minutes using the Jeffco Wet Disintegrator Machine. From the disintegrated material, 100 ml was filtered through Whatman No. 42 filter paper and used to determine Brix% extract with an RFM 340 refractometer (B+S Ltd). Another 150 ml aliquot, mixed with three teaspoons of Octapol, was filtered and the filtrate was analyzed for Pol% extract using an AA-10 Polarimeter (Optical Activity Ltd). The following cane quality components were determined according to standard ICUMSA calculation procedures:

- Pol% cane
- Brix% cane
- Fiber% cane
- Moisture% cane
- Purity% cane
- ERSC% (Estimated Recoverable Sucrose Content)

3.4 Data Statistical Analysis:

The data were analyzed using standard analysis of variance (ANOVA) appropriate for the split-plot design, utilizing the MSTATC statistical software package. Means found to be significant were separated using Duncan's Multiple Range Test (DMRT) as described by Gomez and Gomez (1976). This approach ensured robust evaluation of treatment effects and reliable comparison among means.

IV. RESULTS AND DISCUSSION

4.1 Effect of Compost and Urea Fertilizers on Pol% Cane:

Table 1 presents the effect of compost and urea fertilizer levels, as well as their interaction, on Pol% (sucrose content) in sugarcane. The results showed that neither compost nor urea fertilizer, nor their interaction, had a significant effect on Pol% in both seasons. In the first year (2023/24), all mean values for compost (ranging from 14.6 to 15.1) and urea (from 14.6 to 14.9) were statistically similar. Similarly, in the second year, all treatment combinations were statistically equivalent, confirming the absence of a significant treatment response. These findings are consistent with those of Teshome et al. (2014), who reported that compost application did not significantly affect sucrose content (Pol%), although it significantly enhanced sugar yield by increasing cane biomass. This suggests that the primary contribution of compost to sugar production is through increased biomass rather than direct improvements in juice quality. Similarly, Nawaz et al. (2017) found that moderate rates of compost and urea fertilizer do not significantly influence sucrose content, which is more strongly affected by varietal characteristics, crop maturity, and climatic conditions. It is noteworthy that Yousif et al. (2021) observed that high urea rates can reduce Pol% by promoting vegetative growth at the expense of sucrose accumulation. However, the stability of Pol% observed in the present study suggests that the fertilizer rates applied were within safe and optimal limits, avoiding any detrimental effects on sucrose content.

Although compost and urea significantly improved physical growth parameters and overall yield, they had little effect on the sugar concentration (Pol%) of the cane. The only major factor influencing Pol% was season, which was highly significant ($P < 0.01$). This suggests that climatic conditions between the two years played a much larger role in sucrose accumulation than the nutrient treatments. The lack of a significant change in Pol% despite the increased yields is positive, as it shows that the higher biomass produced by the compost and urea did not 'dilute' the sugar content of the stalks.

TABLE 1
EFFECT OF COMPOST AND UREA FERTILIZERS ON POL% CANE

Seasons	**First year (2023/2024)**					**Second year (2024/2025)**				
Treatments	**Urea (kg/ha)**									
Compost (t/ha)	**0**	**119**	**238**	**357**	**Means**	**0**	**119**	**238**	**357**	**Means**
0	15.4	15.6	14.5	15	15.1 A	14.4	14.1	14.4	14.7	14.4 A
12	14.8	14.5	14.7	14.5	14.6 A	14.4	13.7	14.5	14.6	14.3 A
24	15.2	14.6	14.6	14.2	14.7 A	14.7	13.6	13.7	13.5	13.9 A
36	14	14.9	14.9	15	14.7 A	14.6	14.6	13.6	14.7	14.4 A
Means	14.9 A	14.9 A	14.6 A	14.7 A		14.5 A	14.0 A	14.0 A	14.4 A	
	Statistical Parameters									
	Compost	**Urea**	**Interaction**			**Compost**	**Urea**	**Interaction**		
SE±	0.24	0.26	0.53			0.28	0.2	0.4		
CV%	7.2					5.69				

Note: Values are means of four replications. Means followed by the same letter (A) within each main effect (urea or compost) are not significantly different at $P \leq 0.05$ according to Duncan's Multiple Range Test (DMRT).

4.2 Effect of Compost and Urea Fertilizers on Brix% Cane:

Table 2 presents the effects of compost and urea fertilizer rates, and their interaction, on sugarcane juice quality measured as Brix% (total soluble solids). The results indicate that the main effects of the treatments were largely non-significant, with their interaction also showing no consistent influence across the two seasons. In the first season, neither compost nor urea application had a statistically significant effect on Brix%. Mean values for compost treatments ranged from 17.0% to 17.4%, while those for urea rates ranged from 16.9% to 17.4%. This pattern was consistent in the second season for the urea treatments, with all mean values falling within a narrow range of 15.5% to 15.9%. In contrast to the first year, compost application exhibited a statistically significant, though non-dose-dependent, effect on Brix% during the second season. The highest Brix% values were recorded in the 0 t/ha (16.1%) and 36 t/ha (15.8%) compost treatments, which were statistically similar. Conversely, the intermediate rate of 24 t/ha produced a significantly lower mean Brix% of 15.3%.

These findings are consistent with Balaganesh et al. (2020), who reported that Brix% remains stable under moderate fertilization and is primarily influenced by water status and crop maturity rather than nutrient supply, except in cases of severe

deficiency or excess. Similarly, Kumara and Bandara (2002) concluded that urea application did not significantly affect Brix%, purity, or commercial cane sugar (CCS%) content in sugarcane.

TABLE 2
EFFECT OF COMPOST AND UREA FERTILIZERS ON BRIX% CANE

Seasons	**First year (2023/2024)**					**Second year (2024/2025)**				
Treatments	**Urea (kg/ha)**									
Compost (t/ha)	**0**	**119**	**238**	**357**	**Means**	**0**	**119**	**238**	**357**	**Means**
0	17.7	18	16.7	17.2	17.4 A	15.8	16.3	15.8	16.4	16.1 A
12	17	17	17.1	16.9	17.0 A	15.9	15.2	16	16.1	15.8 A
24	17.5	17.2	16.6	16.8	17.0 A	16.1	15.1	15.1	14.9	15.3 A
36	16.2	17.4	17.2	17.5	17.1 A	16.1	16	15.1	16.1	15.8 A
Means	17.1 A	17.4 A	16.9 A	17.1 A		15.9 A	15.7 A	15.5 A	15.9 A	
	Statistical Parameters									
	Compost		**Urea**	**Interaction**		**Compost**		**Urea**		**Interaction**
SE±	0.25		0.27	0.53		0.3		0.17		0.35
CV%	6.2					4.41				

Note: Values are means of four replications. Means followed by the same letter (A) within each main effect (urea or compost) are not significantly different at P ≤ 0.05 according to DMRT.

4.3 Effect of Compost and Urea Fertilizers on Fiber% Cane:

Table 3 presents the effect of compost and urea fertilizer levels, as well as their interaction, on fiber percentage in sugarcane. The results showed that the response of fiber to compost and urea application varied between the two seasons. In the first season, compost application had a statistically significant effect on fiber percentage. The treatments receiving 12 t/ha and 24 t/ha of compost produced the highest fiber percentages, with means of 18.1% and 18.0%, respectively, significantly higher than the control (17.4%). In contrast, the main effect of urea application was not significant. In the second season, no significant differences were observed in the main effects of either compost or urea on fiber content. Although the main effects of urea were consistently non-significant, the interaction between compost and urea produced notable results in the first season. These findings are consistent with Teshome et al. (2014), who reported that while compost and urea significantly influenced stalk girth, stalk weight, cane yield, and sugar yield, fiber percentage remained unaffected by compost, nitrogen, or their interaction.

TABLE 3
EFFECT OF COMPOST AND UREA FERTILIZERS ON FIBER% CANE

Seasons	**First year (2023/2024)**					**Second year (2024/2025)**				
Treatments	**Urea (kg/ha)**									
Compost (t/ha)	**0**	**119**	**238**	**357**	**Means**	**0**	**119**	**238**	**357**	**Means**
0	17.5	17.2	17.3	17.6	17.4 A	20	19.5	18.4	17.9	19.0 A
12	18.8	18.6	16.9	18.1	18.1 A	19.4	19.1	17.7	18.8	18.7 A
24	17.8	17.2	19	18	18.0 A	19.1	19.4	17.7	18	18.5 A
36	18.4	16.4	16	16	16.7 A	19.5	18.1	18.5	19.2	18.8 A
Means	18.1 A	17.3 A	17.3 A	17.4 A		19.5 A	19.0 A	18.1 A	18.5 A	
	Statistical Parameters									
	Compost		**Urea**	**Interaction**		**Compost**		**Urea**		**Interaction**
SE±	0.33		0.46	0.91		0.37		0.34		0.67
CV%	10.4					7.17				

Note: Values are means of four replications. Means followed by the same letter (A) within each main effect (urea or compost) are not significantly different at P ≤ 0.05 according to DMRT.

4.4 Effect of Compost and Urea Fertilizers on Purity% Cane:

Table 4 presents the effect of compost and urea fertilizer levels, as well as their interaction, on juice purity percentage (Purity%) in sugarcane. The results indicated that the main effects of the treatments were consistently non-significant across both seasons.

In the first season, neither compost nor urea application had a statistically significant impact on Purity% as a main effect. All mean values for the compost levels, ranging from 86.0% to 86.9%, and for the urea levels, ranging from 85.8% to 86.8%, were statistically equivalent. This pattern was repeated in the second season, with no significant differences observed. The highest individual Purity% recorded across the entire study was 91.72%, achieved in the second year with the absolute control treatment (0 t/ha compost and 0 kg/ha urea). These findings are consistent with Kwong and Pasricha (2002) and Kumara and Bandara (2002), who reported that juice purity remains stable under balanced fertilization regimes and is more strongly influenced by harvest timing and ripening conditions.

TABLE 4
EFFECT OF COMPOST AND UREA FERTILIZERS ON PURITY% CANE

Seasons	**First year (2023/2024)**					**Second year (2024/2025)**				
Treatments	**Urea (kg/ha)**									
Compost (t/ha)	**0**	**119**	**238**	**357**	**Means**	**0**	**119**	**238**	**357**	**Means**
0	86.8	86.8	86.7	87.3	86.9 A	91.7	86.6	91.2	89.5	89.8 A
12	86.7	85.5	86.1	85.6	86.0 A	90.6	89.8	90.8	90.8	90.5 A
24	87.4	85.4	87.8	84.8	86.3 A	91.6	90.4	90.7	90.7	90.8 A
36	86.3	85.6	86.5	85.6	86.0 A	90.7	91.6	90.2	91.2	90.9 A
Means	86.8 A	85.8 A	86.8 A	85.8 A		91.1 A	89.6 A	90.7 A	90.5 A	
	Statistical Parameters									
	Compost		**Urea**		**Interaction**	**Compost**		**Urea**		**Interaction**
SE±	0.8		0.5		1.01	0.79		0.65		1.31
CV%	2.3					2.89				

Note: Values are means of four replications. Means followed by the same letter (A) within each main effect (urea or compost) are not significantly different at P ≤ 0.05 according to DMRT.

4.5 Effect of Compost and Urea Fertilizers on Moisture% Cane:

Table 5 shows the effect of compost and urea fertilizer levels, as well as their interaction, on moisture percentage in sugarcane. The analysis of cane moisture content revealed contrasting results between the two seasons. In the first year, no significant differences were observed in the main effects of either compost or urea on moisture percentage. However, in the second season, compost application exhibited a statistically significant effect. The treatment receiving 24 t/ha of compost produced a significantly higher mean moisture content (66.2%) compared to both the control (0 t/ha) and the highest compost rate (36 t/ha). These findings are consistent with Balaganesh et al. (2020) and Caetano et al. (2023), who reported that cane moisture content is predominantly influenced by climatic factors rather than fertilization, except in cases where excessive vegetative growth is induced by very high urea rates.

TABLE 5
EFFECT OF COMPOST AND UREA FERTILIZERS ON MOISTURE% CANE

Seasons	**First year (2023/2024)**					**Second year (2024/2025)**				
Treatments	**Urea (kg/ha)**									
Compost (t/ha)	**0**	**119**	**238**	**357**	**Means**	**0**	**119**	**238**	**357**	**Means**
0	64.7	64.3	66.1	65.2	65.1 A	64.2	64.2	65.8	65.7	65.0 A
12	64.2	64.5	66	65	64.9 A	64.8	65.8	66.4	65.1	65.5 A
24	64.8	65.7	64.4	65.3	65.1 A	64.9	65.5	67.3	67.2	66.2 A
36	65.4	66.2	66.8	66.6	66.2 A	64.5	65.9	66.4	64.7	65.4 A
Means	64.8 A	65.2 A	65.8 A	65.5 A		64.6 A	65.3 A	66.5 A	65.7 A	
	Statistical Parameters									
	Compost		**Urea**		**Interaction**	**Compost**		**Urea**		**Interaction**
SE±	0.38		0.35		0.77	0.46		0.35		0.71
CV%	2.4					2.15				

Note: Values are means of four replications. Means followed by the same letter (A) within each main effect (urea or compost) are not significantly different at P ≤ 0.05 according to DMRT.

4.6 Effect of Compost and Urea Fertilizers on ERSC% Cane:

Table 6 shows the effect of compost and urea fertilizer levels, as well as their interaction, on Estimated Recoverable Sucrose Content (ERSC%) in sugarcane. ERSC% showed no significant differences for any main effects or interactions across the two years. These findings are consistent with Balaganesh et al. (2020), who reported that moderate rates of compost and urea do not significantly affect recoverable sucrose, a parameter known to be more sensitive to extreme nutritional imbalances or water stress conditions. Teshome et al. (2014) reported that while compost and urea applications significantly influenced stalk girth, stalk weight, cane yield, and sugar yield, the ERSC% and related quality parameters including purity and sucrose content did not respond significantly to either compost, nitrogen, or their interaction.

TABLE 6
EFFECT OF COMPOST AND UREA FERTILIZERS ON ERSC% CANE

Seasons	**First year (2023/2024)**					**Second year (2024/2025)**				
Treatments	**Urea (kg/ha)**									
Compost (t/ha)	**0**	**119**	**238**	**357**	**Means**	**0**	**119**	**238**	**357**	**Means**
0	13.1	13.3	12.2	12.8	12.8 A	12.5	11.8	12.5	12.6	12.3 A
12	12.4	12.1	12.4	12.1	12.3 A	12.3	11.7	12.6	12.6	12.3 A
24	13	12.3	12.3	11.8	12.3 A	12.7	11.6	11.8	11.7	11.9 A
36	11.8	12.6	12.7	12.7	12.4 A	12.5	12.8	11.7	12.7	12.4 A
Means	12.5 A	12.5 A	12.4 A	12.3 A		12.5 A	12.0 A	12.1 A	12.4 A	
	Statistical Parameters									
	Compost	**Urea**	**Interaction**			**Compost**	**Urea**	**Interaction**		
SE±	0.25	0.28	0.55			0.29	0.23	0.46		
CV%	8.9					7.57				

Note: Values are means of four replications. Means followed by the same letter (A) within each main effect (urea or compost) are not significantly different at P ≤ 0.05 according to DMRT.

V. CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions:

Based on the results of the two-year trial, the study arrived at the following conclusions:

1. Despite significant yield improvements (reported elsewhere), all juice quality parameters (Pol%, Brix%, Purity%, and ERSC%) remained statistically unaffected by compost or urea application. Higher productivity was achieved without any dilution of sucrose content or reduction in juice purity.
2. Season was the most dominant factor affecting quality parameters, with highly significant effects on Pol%, Brix%, and Purity%. Climatic conditions play a greater role in sucrose accumulation than moderate variations in fertilizer inputs.
3. The progressive improvement in sugarcane response from the first to the second season suggests that compost application enhances soil health over time through increased organic matter, improved nutrient cycling, and enhanced soil physical properties.
4. Composting sugar industry by-products, specifically filter mud, bagasse, and vinasse, offers a strategic approach to recycling agricultural wastes, which enhances soil health and supports sustainable intensification while mitigating the negative impacts of soil degradation.
5. The findings suggest that a fundamental combination of "nitrogen-centric" and "organic matter-centric" sugarcane nutrition strategies may be warranted. Therefore, a transformation to compound fertilizer recommendations is needed and opens new possibilities for sustainable production intensification.

5.2 Recommendations:

Based on the results, to obtain high sugarcane yield with maintained quality under similar soil and climatic conditions, fertilization with compost at the rate of 36 tons/ha in combination with urea at the rate of 357 kg/ha is recommended. These

rates represent the highest levels tested and produced optimal growth responses without adversely affecting juice quality parameters.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this article

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Comparative Analysis of Agricultural Productivity in India and China: Structural Constraints and Policy Implications

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Abstract— *Agriculture continues to play a fundamental role in sustaining the economies of many developing countries by ensuring food availability, generating rural employment, and supporting overall economic stability. Among the major agricultural economies of the world, India and China occupy a prominent position as they together account for a substantial share of the global population and agricultural production. Despite similarities in demographic pressures and dependence on agriculture, the two countries display significant differences in agricultural productivity, technological advancement, and institutional support mechanisms. This study provides a comparative analysis of agricultural production in India and China by examining production statistics, crop yields, policy reforms, and structural characteristics of the agricultural sector. The analysis reveals that while India possesses a large agricultural workforce and extensive cultivated land, China has achieved significantly higher productivity with cereal yields averaging 5,800–6,000 kg/ha compared to India's 3,000 kg/ha. China's total food grain production of approximately 695 million tonnes substantially exceeds India's 354 million tonnes. These differences are primarily attributed to China's greater investments in large-scale mechanization, stronger irrigation infrastructure, advanced agricultural research systems, and coordinated institutional reforms. The study also evaluates the impact of economic liberalization and market-oriented reforms on Indian agriculture, particularly in the context of rising input costs, market volatility, and structural constraints faced by small and marginal farmers. The findings suggest that improving agricultural productivity in India requires greater investment in agricultural research, expansion of irrigation infrastructure, promotion of farm mechanization, land consolidation, and strengthening of post-harvest supply chains.*

Keywords— India-China comparison, agricultural productivity, farm mechanization, structural constraints, agricultural policy, food security.

I. INTRODUCTION

Agriculture continues to play a fundamental role in sustaining the economies of many developing countries by ensuring food availability, generating rural employment, and supporting overall economic stability. Among the major agricultural economies of the world, India and China occupy a prominent position as they together account for a substantial share of the global population and agricultural production. Despite similarities in demographic pressures and dependence on agriculture, the two countries display significant differences in agricultural productivity, technological advancement, and institutional support mechanisms. Understanding these differences is important for identifying policy strategies that can strengthen agricultural performance and improve farmer livelihoods.

India and China are among the largest agricultural producers in the world and together support more than one-third of the global population. Despite similarities in demographic pressures and historical dependence on agriculture, the two countries exhibit significant differences in agricultural productivity, technological adoption, and institutional support systems. This study provides a comparative analysis of agricultural production in India and China by examining production statistics, crop yields, policy reforms, and structural characteristics of the agricultural sector. The analysis highlights that although India possesses a large agricultural workforce and extensive cultivated land, China has achieved significantly higher productivity through large-

scale mechanization, stronger irrigation infrastructure, advanced research and development systems, and coordinated institutional reforms.

Another important factor influencing the trajectory of Indian agriculture since the early 1990s has been the gradual integration of the sector with global markets. Economic reforms introduced under the framework of Liberalization, Privatization, and Globalization (LPG) reduced several trade restrictions and encouraged greater participation of private enterprises in agricultural input markets and supply chains. These policy changes aimed to improve efficiency and competitiveness by exposing domestic agriculture to market forces. However, the transition towards market-oriented agriculture has also created new challenges for farmers, particularly in relation to price volatility, fluctuating input costs, and increased exposure to international competition.

In the contemporary policy context, particularly during the 2020–21 to 2024–25 period, these market-oriented reforms became more pronounced through initiatives aimed at restructuring agricultural markets, encouraging private investment, and integrating domestic agricultural production with global value chains. The introduction of the Farm Laws in 2020 represented a significant attempt to liberalize agricultural marketing by allowing farmers to sell produce outside regulated mandis, promoting contract farming arrangements, and relaxing stockholding restrictions on agricultural commodities. Although these reforms were eventually repealed following large-scale farmer protests, they reflected a broader policy orientation towards market liberalization in agriculture consistent with LPG reforms.

One of the underlying assumptions of such reforms has been that exposure to global markets and competition would enhance agricultural efficiency and productivity. Under this framework, import restrictions were reduced and agricultural trade was gradually liberalized to allow Indian agriculture to compete internationally. However, this transition has also exposed farmers to greater price volatility and competition from heavily subsidized agricultural systems in developed countries, where farmers benefit from advanced mechanization, technological innovation, and substantial government support.

Furthermore, the liberalization process has facilitated the entry of multinational corporations in the agricultural input sector, particularly in seeds, fertilizers, and agrochemicals. While the presence of these companies has expanded the availability of improved technologies and hybrid seeds, it has simultaneously increased farmers' dependence on commercial inputs and raised the overall cost of cultivation. In many regions, especially those dominated by small and marginal farmers, these rising input costs combined with unstable output prices have intensified agrarian distress.

In this context, several international and national institutions have provided policy advisories aimed at improving the resilience and competitiveness of Indian agriculture while addressing farmer distress. The World Bank has recommended that India strengthen agricultural productivity by investing in irrigation infrastructure, improving supply chains, promoting climate-resilient farming practices, and enhancing farmers' access to modern technologies and markets. Similarly, the Food and Agriculture Organization (FAO) has emphasized the need for sustainable agricultural intensification, diversification of cropping patterns, and strengthening farmer organizations to improve bargaining power in markets.

At the national level, NITI Aayog has suggested structural reforms such as modernization of agricultural marketing systems, expansion of digital agricultural platforms, improved agricultural logistics, and development of integrated value chains linking farmers directly with processors and exporters. The institution has also highlighted the importance of strengthening the Minimum Support Price (MSP) procurement system and income support schemes to protect farmers against market fluctuations.

Despite these policy recommendations, the implementation of market-oriented reforms without adequate institutional safeguards continues to raise concerns regarding the vulnerability of farmers in an increasingly liberalized agricultural economy. Rising input costs, uncertain market prices, climate-related risks, and limited access to remunerative markets have continued to place significant financial pressure on agricultural households.

Therefore, while the current LPG-oriented policy framework aims to modernize and globalize Indian agriculture, the success of these reforms depends on balancing market liberalization with strong policy support mechanisms, including price assurance, institutional credit, risk-mitigation systems, and investments in rural infrastructure. Without such complementary measures, the continued liberalization of agricultural markets may inadvertently exacerbate farmer distress rather than resolve the structural challenges faced by the agricultural sector.

This study addresses the following research questions: (1) What are the key structural determinants of agricultural productivity differences between India and China? (2) How have policy reforms in both countries shaped agricultural development trajectories? (3) What policy lessons can India draw from China's agricultural modernization experience?

II. REVIEW OF LITERATURE

Several scholars have examined the role of institutional reforms, technological adoption, and public investment in improving agricultural productivity in developing countries. Comparative studies between India and China particularly emphasize how policy reforms and modernization strategies have influenced agricultural growth trajectories in the two countries.

One of the earliest and most influential studies is by Justin Yifu Lin (1992), who analyzed the impact of rural reforms introduced in China during the late 1970s. His study highlights that the introduction of the Household Responsibility System significantly increased agricultural productivity by providing stronger incentives to farm households. The reforms shifted production decisions from collective farming institutions to individual households, which improved efficiency and output. The findings demonstrate that institutional reforms and secure land-use rights played a crucial role in stimulating agricultural growth in China.

Further research by Shenggen Fan and Philip G. Pardey (1997) examined the relationship between agricultural research investment and productivity growth in China. Their analysis shows that increased public expenditure on agricultural research and extension services contributed significantly to improvements in crop productivity and overall agricultural output. The study emphasizes that sustained investments in agricultural research and technology development are critical drivers of long-term productivity growth.

In the context of agricultural market integration and value chains, Pratap S. Birthal, P. K. Joshi, and Ashok Gulati (2007) examined vertical coordination in high-value agricultural commodities. Their study highlights how contract farming and improved supply chain coordination can enhance market access for smallholder farmers and improve farm incomes. The authors argue that institutional arrangements linking farmers with processors and markets play an important role in modernizing agricultural systems.

Research focusing specifically on China's agricultural modernization has been conducted by Jikun Huang and Scott Rozelle (2018), who demonstrate that China's rapid agricultural transformation has been driven by technological innovation, strong rural institutions, and investments in irrigation and infrastructure. Their findings suggest that policy reforms combined with technology adoption have enabled China to significantly improve crop productivity and rural incomes.

Studies on India's agricultural development highlight the importance of public investment and policy support. For example, Shenggen Fan, Ashok Gulati, and Sukhadeo Thorat (2008) examined the impact of government investment and subsidies on rural poverty reduction in India. Their findings indicate that investments in rural infrastructure, agricultural research, and irrigation have a stronger long-term impact on productivity and poverty reduction compared to subsidies alone.

Recent studies have also emphasized the importance of technological innovation and climate resilience in modern agriculture. Mahajan, Gupta, and Sharma (2023) compared rice production in India and China and found that China achieves higher yields primarily due to better irrigation infrastructure, improved seed technology, and higher levels of mechanization. Their research underscores the significant productivity gap between the two countries.

Similarly, international organizations such as the Food and Agriculture Organization and the World Bank highlight the importance of sustainable intensification, climate-resilient farming practices, and improved supply chains to enhance agricultural productivity. Reports from the Organisation for Economic Co-operation and Development also emphasize that policy reforms, technological innovation, and efficient resource management are essential for improving agricultural productivity and ensuring long-term food security.

Overall, the existing literature indicates that agricultural productivity improvements are strongly influenced by institutional reforms, investments in research and infrastructure, and the adoption of modern technologies. However, comparative studies suggest that while China has successfully implemented coordinated policy reforms and large-scale agricultural modernization strategies, India continues to face structural constraints such as fragmented landholdings, limited mechanization, and uneven irrigation coverage. These findings provide an important foundation for the present study, which examines the structural determinants of agricultural productivity differences between India and China.

III. METHODOLOGY

This study employs a comparative analytical approach to examine agricultural productivity differences between India and China. The analysis draws upon secondary data from multiple sources including government publications, international organizations, and academic research. The primary data sources include the Ministry of Agriculture and Farmers Welfare

(Government of India), NABARD annual reports, Reserve Bank of India reports, Economic Survey of India, Fertiliser Association of India, Ministry of Chemicals and Fertilizers, and comparable Chinese agricultural statistics from the National Bureau of Statistics of China and FAO databases.

The temporal scope of the study covers approximately four decades from 1978 to 2024, allowing examination of long-term structural changes and policy reforms in both countries. The analysis focuses on key indicators including food grain production, crop yields, landholding patterns, mechanization levels, irrigation coverage, agricultural research investment, and institutional credit flows. Comparative analysis is structured around six structural determinants: farm size and land consolidation, mechanization and technological adoption, irrigation infrastructure, agricultural research and extension systems, post-harvest infrastructure, and institutional support frameworks. Policy reforms are analyzed chronologically to trace the evolution of agricultural development strategies in both countries.

IV. AGRICULTURAL STRUCTURE AND POLICY CONTEXT IN INDIA

4.1 Declining Share of Agriculture in GDP:

One of the most visible outcomes of economic reforms has been the steady decline in agriculture's share in GDP. While this is partly due to rapid growth in the services sector, the agricultural sector has not experienced proportional improvements in productivity and farmer incomes.

TABLE 1
YEAR-WISE SHARE OF AGRICULTURE IN GDP

Years	Share of Agriculture in GDP
1990-91	30%
2000-01	23%
2010-11	17%
2023-24	15%-16%

Source: Ministry of Agriculture, Government of India; Economic Survey of India

The declining share of agriculture in India's Gross Domestic Product (GDP) is a significant structural feature of the country's economic transformation. While agriculture once constituted a dominant component of the national economy, its relative contribution has steadily declined over the past three decades. This decline can largely be attributed to the rapid expansion of the industrial and services sectors, which have experienced higher growth rates due to technological advancements, urbanization, increased investment, and policy support under economic liberalization.

In contrast, the agricultural sector has continued to face several structural constraints, including fragmented landholdings, limited irrigation coverage, inadequate infrastructure, low levels of mechanization, and vulnerability to climate variability. Furthermore, agricultural productivity growth has remained relatively slow compared to other sectors of the economy. The shift of labour from agriculture to non-agricultural sectors, along with increasing urbanization, has also contributed to the declining share of agriculture in GDP. Although agriculture still employs nearly 45 percent of India's workforce, its contribution to GDP has declined to around 15–16 percent in recent years, highlighting a significant structural imbalance between employment and output in the sector.

4.2 Institutional Credit in Indian Agriculture:

Another important dimension influencing the performance of the agricultural sector is the availability of institutional credit. Over the years, the Government of India has attempted to improve farmers' access to formal financial institutions through commercial banks, cooperative credit institutions, and regional rural banks. Institutions such as the National Bank for Agriculture and Rural Development (NABARD) play a crucial role in refinancing agricultural loans and strengthening rural financial systems.

According to recent estimates, institutional credit to agriculture has increased significantly in the past decade. The agricultural credit target set by the Government of India reached Rs. 20 lakh crore for the year 2023–24, reflecting the expanding role of formal financial institutions in supporting the agricultural sector. NABARD has played an important role in channelizing credit

to rural areas through cooperative banks and regional rural banks, thereby facilitating investment in agriculture, irrigation, farm mechanization, and allied activities.

TABLE 2
INSTITUTIONAL CREDIT GROWTH IN INDIA (2020-21 TO 2023-24)

Year	Amount (in crore)
2020-21	15,00,000
2021-22	16,50,000
2022-23	18,50,000
2023-24	20,00,000

Source: NABARD, Annual Report (various issues); Government of India

Cooperative credit institutions continue to remain an important source of agricultural finance, particularly for small and marginal farmers. Data from NABARD indicates that cooperative banks and cooperative societies account for nearly 15–17 percent of institutional agricultural credit, while commercial banks contribute the largest share. Short-term crop loans are largely disbursed through Primary Agricultural Credit Societies (PACS) operating under the cooperative credit structure.

TABLE 3
INSTITUTIONAL SHARE IN AGRICULTURAL CREDIT IN INDIA (RECENT YEARS)

Institutions	Share in Agriculture Credit (%)	Amount Disbursed (lakh crore)
Commercial Banks	79-81%	Rs. 19-20
Regional Rural Banks	10-11%	Rs. 3.0-3.1
Cooperative Banks	8-10%	Rs. 2.3-2.4

Source: NABARD, Annual Report (2023–24); RBI, Report on Trend and Progress of Banking in India; Ministry of Agriculture and Farmers Welfare

The institutional agricultural credit system in India operates under a multi-agency framework, with commercial banks playing the dominant role due to their extensive branch networks and larger capital base. Recent data indicates that commercial banks account for around 79–81 percent of total agricultural credit, while Regional Rural Banks contribute approximately 10–11 percent, and cooperative banks and societies account for about 8–10 percent of total agricultural lending.

Within the cooperative credit structure, Primary Agricultural Credit Societies (PACS) serve as the primary grassroots institutions responsible for disbursing short-term crop loans to farmers. These societies operate at the village level and are linked to District Central Cooperative Banks (DCCBs) and State Cooperative Banks (SCBs), forming a three-tier cooperative credit system designed to provide affordable credit to small and marginal farmers.

Despite their crucial role in rural finance, the relative share of cooperative institutions in agricultural credit has gradually declined over the years due to the expansion of commercial banking networks and technological advancements in banking services. However, cooperative institutions continue to remain important for last-mile credit delivery in rural areas, particularly for farmers who face difficulties accessing formal banking services.

Despite the expansion of institutional credit, several challenges persist. A significant proportion of small farmers still rely on non-institutional sources of credit, including moneylenders and informal lenders, often at high interest rates. Moreover, access to institutional finance remains uneven across regions and categories of farmers. In many cases, rising input costs, market volatility, and climate-related risks have limited the effectiveness of credit in improving farm incomes.

Thus, while institutional credit flows to agriculture have increased over time, the persistent structural challenges faced by the sector continue to constrain its overall contribution to economic growth. Addressing these challenges requires comprehensive policy reforms focusing on productivity enhancement, improved market access, diversification of crops, and strengthening of rural financial institutions to ensure sustainable agricultural development.

V. RISING INPUT COSTS IN INDIAN AGRICULTURE

Economic liberalization reduced government controls over markets and gradually shifted agricultural inputs toward market-determined prices. As a result, farmers have faced rising costs of various inputs.

5.1 Fertilizers:

Fertilizers constitute one of the most significant inputs in modern agricultural production. With the spread of intensive cropping systems, particularly after the Green Revolution, the consumption of chemical fertilizers has increased considerably across states. However, fertilizer use remains highly concentrated in a few agriculturally advanced states.

Data indicate that states such as Uttar Pradesh, Madhya Pradesh, Maharashtra, Punjab, Karnataka, Rajasthan, Gujarat, Bihar, Telangana, and Andhra Pradesh collectively account for more than 88 percent of total fertilizer consumption in India. Punjab is among the highest consumers of fertilizers in the country, with an average consumption of about 223 kg per hectare, which is significantly higher than the national average of around 90 kg per hectare.

TABLE 4
FERTILIZER CONSUMPTION BY MAJOR STATES (APPROXIMATE)

State	Share of National Fertilizer Consumption (%)
Uttar Pradesh	17%
Madhya Pradesh	10%
Maharashtra	9.50%
Punjab	6.40%
Karnataka	6.30%
Rajasthan	6.20%
Gujarat	6.10%
Bihar	5.80%
Telangana	5.80%

Source: Fertiliser Association of India; Agricultural Development Report (2024)

High fertilizer consumption is generally associated with intensive cultivation of crops such as wheat, rice, and sugarcane. However, the excessive use of fertilizers has also raised concerns regarding soil degradation, environmental sustainability, and rising production costs.

5.2 Pesticides:

Pesticide use has increased in India as farmers attempt to control crop losses caused by pests and diseases. The adoption of chemical pest control measures varies widely across states depending on cropping patterns and climatic conditions.

Research indicates that Uttar Pradesh records the highest pesticide consumption, followed by Maharashtra, Andhra Pradesh, and Punjab. Over the last decade, pesticide consumption has increased significantly in several states, including Chhattisgarh, Andhra Pradesh, and Maharashtra, while some states such as Punjab and Karnataka have experienced moderate declines. For instance, Punjab alone consumes approximately 5,270 metric tonnes of pesticides annually, making it one of the largest pesticide-consuming states in India. High pesticide use is particularly associated with commercial crops such as cotton, vegetables, and fruits, which are more vulnerable to pest attacks.

TABLE 5
STATE-WISE CONSUMPTION OF CHEMICAL PESTICIDES IN INDIA (METRIC TONNES)

State	Pesticide Consumption (MT)
Uttar Pradesh	11,824 – 13,275
Maharashtra	6,814 – 8,718
Punjab	5,130 – 5,257
Telangana	~4,920
Haryana	~4,064
West Bengal	~3,700 – 4,081
Andhra Pradesh	~1,828 – 1,940
Rajasthan	~1,865 – 1,898
Gujarat	~1,835
Karnataka	~1,830
Chhattisgarh	~1,781
Odisha	~1,144
Bihar	~995
Madhya Pradesh	~599
Kerala	~529
Jharkhand	~455
Himachal Pradesh	~277
Goa	~35

Source: Ministry of Chemicals & Fertilizers and PPQS statistical database (2023–24)

5.3 Hybrid Seeds:

Hybrid seeds represent another important component of modern agricultural technology. The use of hybrid and high-yielding varieties (HYVs) has expanded rapidly since the Green Revolution, particularly in crops such as maize, cotton, sunflower, and vegetables. The adoption of hybrid seeds is highest in states with commercialized agriculture, including Punjab, Haryana, Maharashtra, Gujarat, Andhra Pradesh, Telangana, and Karnataka.

These states have higher levels of agricultural productivity due to improved seed varieties. However, hybrid seeds are often produced and marketed by private companies, which increases farmers' dependence on commercial seed markets. The cost of hybrid seeds is generally much higher than traditional varieties, and farmers are required to purchase new seeds every season, thereby increasing the cost of cultivation.

5.4 Diesel and Electricity:

Energy inputs such as diesel and electricity play a crucial role in agricultural production, particularly for irrigation and mechanized farming operations. Diesel is widely used for operating tractors, pump sets, and harvesting machines, while electricity is used primarily for groundwater irrigation. States with extensive irrigation infrastructure such as Punjab, Haryana, Uttar Pradesh, and Gujarat have relatively high electricity consumption for agriculture. In contrast, states with lower irrigation coverage rely more on diesel-powered pump sets.

Rising global crude oil prices and periodic increases in electricity tariffs have significantly increased the cost of agricultural operations. Since irrigation is essential for crops such as rice, wheat, and sugarcane, higher energy costs directly affect farm profitability.

5.5 Farm Machinery:

Agricultural mechanization has expanded rapidly in recent decades as farmers adopt tractors, harvesters, threshers, and other modern equipment to improve productivity and reduce labour dependency. Mechanization levels are highest in states like Punjab, Haryana, Uttar Pradesh, Gujarat, and Maharashtra. These states have a higher density of tractors and agricultural machinery due to larger farm sizes and better access to credit. However, the rising cost of machinery, fuel, and maintenance has increased the financial burden on farmers. Although mechanization improves efficiency and reduces labour requirements, the high capital investment required for machinery often forces farmers to rely on institutional or non-institutional credit, thereby increasing indebtedness.

In recent years, fertilizer prices and diesel costs have increased substantially, raising the cost of cultivation. Small and marginal farmers, who constitute more than 85 percent of total farmers in India, are particularly vulnerable to these rising input costs.

VI. COMPARATIVE ANALYSIS OF AGRICULTURAL PRODUCTION: INDIA AND CHINA

Agriculture remains one of the most important sectors in developing countries. Both India and China have historically relied on agriculture to support rural livelihoods and ensure food security. These countries together account for nearly 36 percent of the world's population, making agricultural productivity crucial for global food supply.

Over the past four decades, China has undergone rapid agricultural transformation through policy reforms, technological adoption, and infrastructure development. In contrast, India's agricultural growth has been slower and more uneven due to structural challenges such as fragmented landholdings, limited mechanization, and dependence on monsoon rainfall.

6.1 Food Grain Production Comparison:

Agriculture continues to play a crucial role in ensuring food security in both India and China, which together account for a significant share of global food grain production. However, the scale and productivity of agricultural production differ considerably between the two countries.

TABLE 6
FOOD GRAIN PRODUCTION COMPARISON

Country	Food Grain Production
India	~354 million tonnes
China	~695 million tonnes

Sources: Ministry of Agriculture, Government of India; National Bureau of Statistics of China

In 2024–25, India produced approximately 354 million tonnes of food grains, which include major crops such as rice, wheat, maize, and pulses. This production reflects consistent growth over the past decades due to the expansion of irrigation, improved seed varieties, and government support programs. Despite this progress, productivity levels remain relatively moderate compared to other major agricultural economies.

China, on the other hand, produces around 695 million tonnes of cereals, almost double the food grain production of India. The higher output in China can largely be attributed to greater crop productivity, intensive cultivation practices, higher mechanization levels, and extensive adoption of modern agricultural technologies. China has also invested heavily in agricultural research, irrigation infrastructure, and rural mechanization, which has significantly improved yield levels.

Consequently, although India possesses comparable agricultural land and a large farming population, China's higher yield per hectare and technologically advanced farming systems enable it to achieve substantially greater food grain production. These differences highlight the importance of improving productivity, mechanization, and technological adoption in Indian agriculture to narrow the production gap.

TABLE 7
MAJOR CROP-WISE COMPARISON: INDIA VS CHINA

Crop	India Production (MMT)	China Production (MMT)	India Yield (t/ha)	China Yield (t/ha)	Key Gap
Rice	~147–150	~145	~4.3	~7.1	China produces similar output with much less land due to higher yield
Wheat	~113–117	~140	~3.5	~5.5	China has much higher productivity per hectare
Maize (Corn)	~35	~280–290	~3.2	~6.1	Very large production and yield gap
Soybean	~12–13	~20	~1.2	~1.9	China has higher yield and mechanized cultivation
Potato	~60	~95	~24	~33	China leads in productivity and processing industry

MMT: Million Metric Tonnes; t/ha: tonnes per hectare
Sources: Ministry of Agriculture, Government of India; National Bureau of Statistics of China; FAO Statistical Database

VII. STRUCTURAL DETERMINANTS OF AGRICULTURAL PRODUCTIVITY GAP

The comparative analysis of major crops between India and China reveals that the difference in agricultural output is primarily driven by productivity-related structural factors rather than land availability. While India possesses comparable or even larger areas under cultivation for several crops, China consistently achieves higher production due to superior technological adoption, irrigation infrastructure, mechanization, and institutional support mechanisms. These factors collectively contribute to a substantial productivity gap between the two countries.

TABLE 8
COMPARATIVE AGRICULTURAL PERFORMANCE INDICATORS

Indicator	India	China
Average cereal yield	~3000 kg/ha	~5800–6000 kg/ha
Rice yield	~3.5 t/ha	~4–6.5 t/ha
Wheat yield	~3.5 t/ha	~5–6 t/ha

7.1 Farm Size and Land Consolidation:

One of the most significant structural differences between the two countries lies in the size and organization of agricultural landholdings. In India, agriculture is characterized by highly fragmented and small landholdings, with the average farm size being less than two hectares. Such fragmentation limits the efficient use of modern agricultural machinery, irrigation systems, and large-scale farm management practices. Small farmers often lack the financial capacity to invest in improved seeds, fertilizers, and mechanized equipment.

In contrast, China has undertaken extensive land consolidation and cooperative farming initiatives, which have enabled larger operational farm units. Larger farm sizes facilitate the use of modern machinery, efficient irrigation systems, and advanced agricultural practices. As a result, Chinese farmers are able to achieve higher productivity per hectare, thereby increasing total agricultural output.

7.2 Mechanization and Technological Adoption:

Agricultural mechanization is another critical determinant of productivity. China has made substantial investments in farm mechanization, including tractors, combine harvesters, automated transplanting machines, and precision farming technologies. These technological advancements significantly reduce labor constraints, improve the efficiency of farm operations, and enhance crop yields.

In India, although mechanization has improved in certain regions such as Punjab and Haryana, many parts of the country still rely on traditional farming practices and manual labor. Limited access to mechanized equipment, particularly among small and marginal farmers, results in lower efficiency in land preparation, sowing, irrigation, and harvesting. Consequently, crop productivity remains relatively lower compared to China.

7.3 Irrigation Infrastructure and Water Management:

The availability and management of irrigation infrastructure play a crucial role in determining agricultural productivity. China has invested heavily in large-scale irrigation networks, reservoirs, and water management systems, ensuring that a significant proportion of agricultural land remains irrigated throughout the year. Reliable irrigation reduces dependence on rainfall and enables farmers to maintain consistent crop yields.

India, on the other hand, still relies heavily on monsoon rainfall, especially in rain-fed agricultural regions. Although irrigation facilities have expanded in recent decades, uneven distribution of irrigation infrastructure across states leads to variability in crop productivity. Regions with inadequate irrigation often experience lower yields and higher vulnerability to climatic fluctuations, thereby affecting overall agricultural output.

7.4 Agricultural Research, Development, and Extension Services:

Investment in agricultural research and development (R&D) is another factor that contributes to China's productivity advantage. China has established strong agricultural research institutions that focus on developing high-yield crop varieties, hybrid seeds, and advanced cultivation techniques. These innovations are rapidly disseminated to farmers through effective extension services.

While India also has a robust agricultural research network through institutions such as the Indian Council of Agricultural Research (ICAR), the transfer of technology to farmers remains uneven and slower. Limited awareness, insufficient extension services, and financial constraints often prevent farmers from adopting improved agricultural technologies at a large scale.

7.5 Post-Harvest Infrastructure and Market Integration:

China's agricultural sector is supported by well-developed post-harvest infrastructure, including cold storage facilities, transportation networks, food processing industries, and efficient supply chains. These systems reduce post-harvest losses and enable farmers to obtain better market prices, thereby incentivizing higher production.

In contrast, India faces significant challenges related to inadequate storage facilities, limited cold-chain infrastructure, and inefficient marketing systems. A substantial portion of agricultural produce is lost during storage and transportation, reducing the effective output and income of farmers. These constraints discourage investments in productivity-enhancing technologies.

7.6 Institutional Support and Government Policies:

China's agricultural development has been supported by strong institutional frameworks and coordinated government policies aimed at improving productivity. Government programs provide subsidies for farm machinery, irrigation equipment, and technological innovations. In addition, policies promoting rural infrastructure development and farmer training have strengthened the agricultural sector.

India also provides substantial policy support through subsidies, minimum support prices, and agricultural schemes. However, the fragmented implementation of policies and regional disparities often limit their overall effectiveness. As a result, the benefits of agricultural modernization are not uniformly distributed across the country.

VIII. COMPARATIVE AGRICULTURAL POLICY REFORM TIMELINE (1978–2024)

The following timeline presents key agricultural policy reforms in India and China over the past four decades, providing context for understanding divergent development trajectories.

TABLE 9
COMPARATIVE AGRICULTURAL POLICY REFORM TIMELINE (1978–2024)

Period	China – Major Agricultural Reforms	India – Major Agricultural Reforms
1978–1984	Introduction of the Household Responsibility System, which replaced collective farming with household-based production incentives and significantly increased productivity.	Post-Green Revolution consolidation. Expansion of irrigation, fertilizers, and HYV seeds to increase food grain production.
1985–1993	Liberalization of agricultural markets; reduction of state procurement monopoly and introduction of market pricing for agricultural commodities.	Expansion of Minimum Support Price (MSP) and procurement system to ensure price support for major crops like wheat and rice.
1994–2003	Land tenure reforms allowing transfer of land-use rights and promotion of township and village enterprises to diversify rural income.	Economic liberalization influences agriculture; emphasis on diversification into horticulture and dairy sectors.
2004–2012	Introduction of annual "No.1 Central Document" focusing on agriculture, rural development, and farmer income growth; increased subsidies for seeds, machinery, and irrigation.	Launch of major national schemes including National Food Security Mission (2007) and Rashtriya Krishi Vikas Yojana (2007) to increase crop productivity and investment in agriculture.
2013–2017	Promotion of large-scale farming, land consolidation, agricultural mechanization, and modernization of supply chains.	Launch of schemes such as Pradhan Mantri Krishi Sinchai Yojana (2015) and Pradhan Mantri Fasal Bima Yojana (2016) to improve irrigation and risk management.
2018–2020	Policies promoting digital agriculture, rural revitalization strategy, and modernization of agricultural value chains.	Income support scheme Pradhan Mantri Kisan Samman Nidhi launched in 2019.
2020–2021	Continued agricultural modernization and rural revitalization policies focusing on mechanization and high-tech farming.	Introduction of 2020 Indian agriculture acts to liberalize agricultural markets (later repealed in 2021).
2022–2024	Expansion of smart agriculture, digital platforms, and high-value agriculture to enhance productivity and rural incomes.	Focus on climate-resilient agriculture, digital agriculture mission, and strengthening farmer producer organizations (FPOs).

Sources: Lin (1992); Huang & Rozelle (2018); Ministry of Agriculture, Government of India; various policy documents

IX. REFORMS AND POLICIES REQUIRED TO BOOST AGRICULTURAL PRODUCTION

Agricultural production in many developing countries, particularly India, can be significantly enhanced through a comprehensive set of institutional, technological, and market-oriented reforms. Lessons from countries such as China, which has achieved substantial gains in agricultural productivity through coordinated policies and modernization strategies, indicate that a combination of structural reforms and technological investments is essential for improving farm output and farmer incomes.

One of the most important reforms is the strengthening of agricultural research and innovation systems. Increased public investment in agricultural research institutions, universities, and extension services can accelerate the development and dissemination of improved seed varieties, biotechnology applications, and climate-resilient crops. Enhanced collaboration between research institutions and farmers would ensure that scientific innovations are effectively translated into field-level productivity gains. Expanding agricultural extension networks and promoting digital knowledge platforms can further improve farmers' access to modern farming techniques and best practices.

Another crucial reform involves expanding agricultural mechanization and the adoption of modern technologies. Mechanization reduces labour costs, increases efficiency in farm operations, and minimizes post-harvest losses. Governments

can promote mechanization by providing subsidies for farm machinery, establishing custom hiring centers for small and marginal farmers, and encouraging the adoption of precision agriculture tools such as drones, sensors, and satellite-based crop monitoring systems. The integration of digital technologies and artificial intelligence in agriculture can help optimize resource use and improve productivity.

Improving irrigation infrastructure and water management systems is also essential for enhancing agricultural output. In many regions, agricultural production remains heavily dependent on rainfall, which makes farmers vulnerable to climate variability. Expanding irrigation coverage, promoting micro-irrigation technologies such as drip and sprinkler systems, and improving water-use efficiency can significantly stabilize crop production and increase yields.

Land reforms and farm consolidation represent another important policy area. Fragmented landholdings reduce economies of scale and limit the adoption of mechanized farming practices. Policies that encourage land leasing, cooperative farming, and the formation of farmer producer organizations can help small farmers pool resources, access modern technologies, and improve their bargaining power in agricultural markets.

Finally, strengthening agricultural markets and supply chains is essential for ensuring that higher production translates into higher farmer incomes. Investments in rural infrastructure, storage facilities, cold chains, and food processing industries can reduce post-harvest losses and enhance value addition. Market reforms that improve price transparency and expand access to national and international markets can also encourage farmers to diversify into high-value crops.

X. DISCUSSION

The comparative analysis presented in this study reveals significant structural differences between Indian and Chinese agriculture that explain the persistent productivity gap between the two countries. While both nations began their post-independence development trajectories with similar challenges of food insecurity and rural poverty, their policy choices and investment priorities have led to divergent outcomes.

China's agricultural transformation can be understood as the result of coordinated institutional reforms initiated in the late 1970s. The Household Responsibility System represented a fundamental shift in incentive structures, allowing farmers to retain surplus production above state procurement quotas. This institutional change, combined with sustained investments in irrigation, mechanization, and agricultural research, created conditions for rapid productivity growth. Importantly, China's reforms were implemented in a sequenced manner, with institutional changes preceding technological investments, allowing farmers to respond to market signals with improved productive capacity.

India's agricultural trajectory, by contrast, has been characterized by a different policy mix. The Green Revolution of the 1960s and 1970s successfully increased food grain production through the adoption of high-yielding varieties, expansion of irrigation, and government procurement at guaranteed prices. However, the institutional framework that supported this growth—centered on state procurement, input subsidies, and regulated markets—has proven more difficult to reform than in China. The fragmented nature of agricultural policymaking in India's federal system, combined with the political sensitivity of agricultural issues, has constrained the implementation of comprehensive reforms.

The structural constraints identified in this analysis—fragmented landholdings, limited mechanization, uneven irrigation coverage, and weak post-harvest infrastructure—are interconnected. Fragmented landholdings limit the economic viability of mechanization; without mechanization, productivity remains low; low productivity reduces farmers' capacity to invest in irrigation and other improvements; and weak infrastructure reduces the returns to any productivity gains achieved. Addressing these constraints requires coordinated policy interventions rather than piecemeal reforms.

The policy reform timeline presented in this study illustrates the different reform trajectories. China's reforms began earlier (1978) with fundamental institutional changes that transformed the incentive structure for farm households. Subsequent reforms built upon this foundation, gradually liberalizing markets while maintaining strong public investment in agricultural research and infrastructure. India's reforms, particularly since 1991, have focused more on market liberalization and reduced state intervention, but have been less successful in addressing the underlying structural constraints of the sector. The recent experience with the 2020 farm laws, which were ultimately repealed, illustrates the political challenges of agricultural reform in India.

The implications for policy are clear. Improving agricultural productivity in India requires a comprehensive approach that addresses structural constraints while building institutional capacity. Increased investment in agricultural research and extension is essential for developing and disseminating improved technologies. Expansion of irrigation infrastructure,

particularly in rain-fed regions, can reduce vulnerability to climate variability and enable productivity improvements. Policies that facilitate land consolidation and promote cooperative farming can help overcome the diseconomies of small farm size. And strengthening post-harvest infrastructure and supply chains can ensure that productivity gains translate into improved farmer incomes.

However, these reforms must be implemented with attention to their distributional consequences. Small and marginal farmers, who constitute the majority of India's agricultural workforce, are particularly vulnerable to market volatility and rising input costs. Any reform strategy must include adequate safety nets, including well-functioning price support mechanisms, affordable institutional credit, and risk management tools such as crop insurance.

XI. CONCLUSION

This study examined the comparative performance of the agricultural sectors in India and China, with particular emphasis on production patterns, structural characteristics, and policy frameworks influencing agricultural productivity. The analysis indicates that although India possesses extensive cultivated land and a large agricultural workforce, its productivity levels remain significantly lower than those of China. China has achieved higher crop yields and overall agricultural output primarily through sustained investments in irrigation infrastructure, widespread mechanization, stronger agricultural research systems, and coordinated institutional reforms. In contrast, Indian agriculture continues to face structural constraints such as fragmented landholdings, uneven irrigation coverage, limited technological adoption, and inadequate post-harvest infrastructure.

The findings suggest that improving agricultural productivity in India requires a comprehensive policy approach focusing on strengthening agricultural research and extension services, expanding irrigation infrastructure, promoting farm mechanization through accessible technologies, and improving agricultural supply chains and storage facilities. Policies encouraging land consolidation, farmer producer organizations, and digital agricultural platforms can further enhance efficiency and market access for farmers. At the same time, policy frameworks should balance market-oriented reforms with institutional safeguards that protect farmers from price volatility and climate-related risks.

The comparative policy timeline demonstrates that China's agricultural transformation resulted from coordinated reforms implemented over several decades, combining institutional change with sustained public investment. India can draw lessons from this experience while adapting strategies to its own institutional context and political economy constraints. The challenge for Indian agricultural policy is to address structural constraints without undermining the livelihoods of the small and marginal farmers who form the backbone of the sector.

Future research may focus on quantitative analysis of productivity determinants using econometric models, region-specific assessments of technological adoption, and the role of digital agriculture and climate-resilient farming practices in enhancing long-term agricultural sustainability. Such studies would provide deeper insights into policy interventions that can help bridge the productivity gap and promote sustainable agricultural development.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Incidence of Major Pest and Diseases under Natural Field Condition in Pole Type French Bean (*Phaseolus vulgaris* L.) Genotypes

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Abstract— *The present investigation was undertaken to identify promising genotypes resistant to major pests and diseases in pole-type French bean. Thirty-two genotypes were evaluated during the Rabi season of 2022–23 at the Regional Agricultural Research Station, Vijayapura, under the University of Agricultural Sciences, Dharwad, Karnataka, India. The experiment was laid out in a Randomized Complete Block Design (RCBD) with two replications under natural field conditions. Significant variation among the genotypes was observed in their response to biotic stresses. Genotypes IC-636224, IC-636225, IC-341797, IC-341922, IC-636240, IC-313309, EC-398555, IC-582514, IIHR-01, and IIHR-02 showed resistance to Fusarium wilt with 0–10% mortality. These same genotypes exhibited no symptoms of Bean common mosaic virus and were classified as immune. Similarly, genotypes IC-636224, IC-341797, IC-341807, IC-341922, IC-636240, IC-430379, IC-313309, IC-582514, IC-538073, IC-632961, IC-313320, IC-538077, IC-326977, IIHR-01, and IIHR-02 showed resistance to pod borer with 1–12% pod damage. Furthermore, genotypes IC-538077, IC-582511, IC-632961, IC-326978, IC-328398, IC-313309, IC-430379, and IC-641919 showed no aphid infestation. These lines may be effectively utilized in breeding programmes to develop new varieties with enhanced resistance to insect pests and diseases.*

Keywords— Fusarium wilt, Bean common mosaic virus, Pod borer, Aphids, Pole-type French bean.

I. INTRODUCTION

French bean (*Phaseolus vulgaris* L.) is one of the most common and widely grown vegetable crops in India with a chromosome number of 2n=22. According to Vavilov (1950), the origin of French bean is Southern Mexico and Central America, while the Peruvian-Ecuadorian-Bolivian area is considered to be a secondary centre of origin. It originated from the wild species *Phaseolus aborigineus* (L.) and was domesticated in Mexico, Peru, and Colombia about 8000 years ago. This crop has extensive geographical distribution in the world.

It is an important legume vegetable belonging to the family Leguminosae or Fabaceae and has worldwide significance as a source of food and feed. The genus *Phaseolus* contains about 70-80 species, among which *Phaseolus vulgaris* (L.) accounts for 90 per cent of cultivated species around the world (Evans, 1979). There are four cultivated species of *Phaseolus* viz., *Phaseolus vulgaris*, *P. coccineus*, *P. lunatus*, and *P. acutifolius* var. *latifolius*, wherein all the species are self-pollinated except *P. coccineus*, which is cross-pollinated.

French bean is known by several names related to its purpose of usage as a vegetable viz., string bean, snap bean, salad bean, haricot bean, and green bean. However, the terms bean, dry bean, kidney bean, and navy bean are designated to pulses (George, 1985). Furthermore, string bean, dwarf bean, and pole bean pertain to distinct growth patterns. In different languages, it has

diverse identities viz., rajmash in Hindi and tingala avare in Kannada. French bean varieties are categorized based on their growth habits i.e., bush type with compact segments, semi-pole type with more extended segment, and pole type with viny growth longer than semi-pole type (Prabhakar et al., 2016).

French bean has a wide range of genetic variations in terms of growth habit (determinate vs. indeterminate), days to maturity, seed size, colour and quality (cooking ability and palatability), vegetative and reproductive growth, pigmentation, leaf size, shape and orientation, and resistance to pests (Leakey, 1970). The choice of promising genotypes from a diverse genetic base and their subsequent utilization for hybridization is one of the strategies for improving the productivity of any crop including beans.

It is cultivated all over the world and has a wide geographical distribution. French bean is mainly used for immature green pods. Rajmash or dried pods are utilized as a pulse and provide a good source of protein for humans (Abate, 2006). Immature pods are eaten fresh and can be easily preserved by freezing, canning, or dehydrating. Dried beans are eaten boiled, baked, fried, or ground into flour. It is highly nutritious as 100 g of green pods contain 1.7 g protein, 4.5 g carbohydrates, 221 IU vitamin A, 11 mg vitamin C, and 50 mg calcium (Gopalakrishnan, 2007). French bean can be used to some extent against diabetes and cardiac problems and is a wonderful natural cure for bladder burn. It has both carminative and reparative qualities in the treatment of constipation and diarrhea (Duke, 1981).

French bean is a short-duration crop; hence, it can be grown in various cropping systems across the hills and plains of India. This vegetable is predominantly cultivated in the states of Himachal Pradesh, Punjab, Haryana, Uttar Pradesh, Bihar, Gujarat, Madhya Pradesh, Maharashtra, Karnataka, Andhra Pradesh, and Tamil Nadu. The total cultivation area for French bean in India is around 2.9 lakh hectares with an annual production of 27.25 lakh tonnes and an average productivity of 9.09 tonnes per hectare. In Karnataka, this crop is grown in an area of 0.17 lakh hectares with 1.94 lakh tonnes of production and productivity of 11.07 tonnes per hectare.

The use of resistant varieties offers a practical and cost-effective approach to managing pests and diseases in green beans. The development and assessment of high-yielding legume varieties with resistance to pests and diseases are crucial for maximizing the crop's production potential (Neritu, 2008). An integrated pest management (IPM) strategy is considered the most effective method for controlling pest problems in beans, as it combines multiple proven techniques to keep pest populations at manageable levels. These methods include cultivating resistant varieties, encouraging natural predators, applying organic and bio-pesticides, practicing crop rotation, and adopting cultural measures such as removing field debris.

This study was undertaken to identify high-yielding bean varieties with strong tolerance to pests and diseases. Accordingly, the present investigation was designed to evaluate insect and disease incidence in pole-type French bean genotypes grown under open conditions.

II. MATERIALS AND METHODS

2.1 Experimental Design and Layout:

The experiment was laid out in a Randomized Complete Block Design (RCBD) with two replications. The genotypes were sown in a plot size of one row each of 3.6 m length. The spacing between rows was 120 cm and the distance between plants was 30 cm.

2.2 Preparation of Experimental Plot and Sowing

The experimental field was ploughed repeatedly and brought to a fine tilth. The recommended dose of farm yard manure (25 tonnes per hectare) and fertilizer dosage of 62.5:100:75 kg NPK per hectare was applied. According to the fertilizer schedule, the full quantity of phosphorous and potassium and half dose of nitrogen were applied as a basal dose, and the remaining half of the recommended nitrogen was top-dressed at 30 and 60 days after sowing. After the layout, the treatments were assigned to different plots in each replication by utilizing a randomization method. The main and sub-water irrigation channels were laid out by considering the slope of the site. The ridges and furrows were opened at 120 cm, and seeds of different genotypes were sown by dibbling on one side of the ridge at 30 cm, and plots were irrigated immediately after the completion of sowing. Wherever seeds did not germinate, gaps were filled by re-sowing seeds within a week. All other activities were carried out as per the recommended package of practices (RPP) given by the University of Horticultural Sciences (UHS), Bagalkot, to grow the crop. The following disease and pest incidences were noticed. The disease and pest incidences under natural epiphytic conditions were calculated by following methodologies.

2.2.1 Fusarium Wilt Incidence (%):

Per cent wilting was calculated by the following formula

$$\text{Per cent wilting} = \frac{\text{Number of plants infected} \times 100}{\text{Total number of plants observed}} \quad (1)$$

ICRISAT established a screening technique for Fusarium wilt incidence (Nene et al., 1981), which was used in this study.

Per cent wilting (mortality)	Disease reaction
0-10% mortality	Resistance
10.1-20% mortality	Moderately resistant
20.1-30% mortality	Moderately susceptible
30.1-50% mortality	Susceptible
Above 50% mortality	Highly susceptible

2.2.2 Bean Common Mosaic Virus (BCMV) Incidence (%):

Per cent damage was calculated by the following formula:

$$\text{Per cent damage} = \frac{\text{Number of plants infected} \times 100}{\text{Total number of plants observed}} \quad (2)$$

Diwakar and Mali (1976) developed the Bean common mosaic virus scale (BCMV), which was used in the present experiment.

Scale	Category	Description
0	Immune	No plants showing BCMV symptoms
1	Resistant	1-5 per cent of plants showing BCMV symptoms
2	Moderately resistant	6-15 per cent of plants showing BCMV symptoms
3	Moderately susceptible	16-25 per cent of plants showing BCMV symptoms
4	Susceptible	26-50 per cent of plants showing BCMV symptoms
5	Highly susceptible	>50 per cent of plants showing BCMV symptoms

2.2.3 Pod Borer Damage (%):

Per cent pod damage was calculated by the following formula:

$$\text{Per cent pod damage} = \frac{\text{Total number of infested pods} \times 100}{\text{Total number of pods observed}} \quad (3)$$

A pod borer incidence scale given by Krishna et al. (2006) was adopted in this study.

Pod damage (%)	Category
01-Dec	Resistant
13-24	Moderately resistant
25-30	Moderately susceptible
>30	Susceptible

2.2.4 Aphid Population:

Five tagged plants were observed in the plot to record the aphid population. The top three leaves were counted, and the average population was calculated.

III. RESULTS AND DISCUSSION

An excellent, cost-effective alternative for pest and disease control is host plant resistance. Pest and disease control in agricultural systems is facilitated by the use of pest and disease-resistant cultivars, which both increase productivity and lower costs. These cultivars also conserve natural resources, cut costs, save time, and are more environmentally friendly than alternative pest and disease management techniques. In general, the growth and yield of pole-type French beans are highly vulnerable to the occurrence of pests and diseases. Among several pests and diseases, Fusarium wilt, Bean common mosaic virus, pod borer, and aphids are particularly important. Hence, considerable importance has been given to finding resistant cultivars since these pests and diseases drastically reduce crop growth, yield, and quality of the pods by adversely affecting the growth of the crop.

3.1 Fusarium Wilt Incidence (%):

Table 1 presents the reaction of pole-type French bean genotypes to Fusarium wilt disease incidence under natural field conditions.

TABLE 1
REACTION OF POLE-TYPE FRENCH BEAN GENOTYPES TO FUSARIUM WILT DISEASE INCIDENCE UNDER NATURAL FIELD CONDITION

Percent wilting (mortality)	Disease reaction	Genotypes
0-10	Resistance	IC-636224, IC-636225, IC-636245, IC-280818, IC-341797, IC-341922, IC-636240, IC-313309, EC-398555, IC-582514, IC-026624, IC-313320, IC-582511, IIHR-01, IIHR-02, Arka Sukomol, Marlida, Super King, US-2
10.1-20	Moderately resistant	IC-636226, IC-636241, IC-641919, IC-341807, IC-430379, IC-538073, IC-538039, IC-326978, IC-326977, IC-538077, Lakshmi, IC-632961
20.1-30	Moderately susceptible	IC-328398
30.1-50	Susceptible	-
>50	Highly susceptible	-

The genotypes viz., IC-636224, IC-636225, IC-636245, IC-280818, IC-341797, IC-341922, IC-636240, IC-313309, EC-398555, IC-582514, IC-026624, IC-313320, IC-582511, IIHR-01, IIHR-02, Arka Sukomol, Marlida, Super King, and US-2 were found to be resistant with per cent mortality of 0-10 per cent. Conversely, it was noted that the genotypes IC-636226, IC-636241, IC-641919, IC-341807, IC-430379, IC-538073, IC-538039, IC-326978, IC-326977, IC-538077, Lakshmi, and IC-632961 were moderately resistant with 10.1-20 per cent incidence. This indicates that the resistant genotypes can be used as donors of resistance to Fusarium wilt disease in further hybridization programmes. These results are in agreement with those reported by Tabasia et al. (2021) and Buruchara and Camacho (2000).

3.2 Bean Common Mosaic Virus Incidence (%):

Table 2 presents the reaction of pole-type French bean genotypes to Bean common mosaic virus (BCMV) disease incidence under natural field conditions.

TABLE 2
REACTION OF POLE-TYPE FRENCH BEAN GENOTYPES TO BEAN COMMON MOSAIC VIRUS (BCMV) DISEASE INCIDENCE UNDER NATURAL FIELD CONDITION

Scale	Disease reaction	Genotypes
0	Immune	IC-636224, IC-636225, IC-641919, IC-341797, IC-341807, IC-341922, IC-636240, IC-430379, IC-313309, EC-398555, IC-582514, IC-328398, IC-326978, IC-632961, IC-538077, IIHR-01, IIHR-02, Marlida, US-2
1	Resistant	IC-538073, IC-313320, Super King
2	Moderately resistant	IC-636241, IC-636245, IC-280818, IC-538039, IC-026624, Lakshmi, IC-636226, Arka Sukomol, IC-326977
3	Moderately susceptible	IC-582511
4	Susceptible	-
5	Highly susceptible	-

The genotypes viz., IC-636224, IC-636225, IC-641919, IC-341797, IC-341807, IC-341922, IC-636240, IC-430379, IC-313309, EC-398555, IC-582514, IC-328398, IC-326978, IC-632961, IC-538077, IIHR-01, IIHR-02, Marlida, and US-2 did not show any symptoms and were classified as immune. Conversely, genotypes with 1-5 per cent incidence (IC-538073, IC-313320, and Super King) belonged to the resistant group, while the moderately resistant group with 6-15 per cent symptoms included IC-636226, IC-636241, IC-636245, IC-280818, IC-538039, IC-326977, IC-026624, Arka Sukomol, and Lakshmi. It is possible to take advantage of resistance breeding for Bean common mosaic virus in French beans with these resistant genotypes. These results are consistent with the findings of Salgar et al. (2021) and Meena et al. (2019).

3.3 Pod Borer Damage (%):

Table 3 presents the reaction of pole-type French bean genotypes to pod damage incidence under natural field conditions.

TABLE 3
REACTION OF POLE-TYPE FRENCH BEAN GENOTYPES TO POD DAMAGE (%) INCIDENCE UNDER NATURAL FIELD CONDITION

Pod damage (%)	Reaction	Genotypes
01-Dec	Resistant	IC-636224, IC-636226, IC-636241, IC-636245, IC-280818, IC-341797, IC-341807, IC-341922, IC-636240, IC-430379, IC-313309, IC-582514, IC-538073, IC-538039, IC-328398, IC-326978, IC-632961, IC-313320, IC-538077, IC-326977, IIHR-01, IIHR-02, Arka Sukomol, Marlida, US-2, Super King, Lakshmi
13-24	Moderately resistant	IC-636225, IC-582511, IC-641919, EC-398555, IC-026624
25-30	Moderately susceptible	-
>30	Susceptible	-

The genotypes like IC-636224, IC-636226, IC-636241, IC-636245, IC-280818, IC-341797, IC-341807, IC-341922, IC-636240, IC-430379, IC-313309, IC-582514, IC-538073, IC-538039, IC-328398, IC-326978, IC-632961, IC-313320, IC-538077, IC-326977, IIHR-01, IIHR-02, Arka Sukomol, Marlida, Super King, Lakshmi, and US-2 were classified into the resistant group with 1-12 per cent pod damage. Meanwhile, the moderately resistant group with 13-24 per cent pod damage included genotypes IC-636225, IC-582511, IC-641919, EC-398555, and IC-026624. This suggests that in a future hybridization programme, the resistant genotypes may be utilized as donors of resistance to the pod borer. Similar findings were also reported by Sahu et al. (2021) and Bharathi et al. (2020).

3.4 Aphid Population:

Table 4 presents the aphid population on the top three leaves of pole-type French bean genotypes under natural field conditions.

TABLE 4
APHID POPULATION ON THE TOP THREE LEAVES UNDER NATURAL FIELD CONDITION

Sl. No.	Genotype	Number of aphids
1	IC-636224	1.68
2	IC-636225	5.35
3	IC-636226	6.3
4	IC-636241	5.7
5	IC-636245	2.05
6	IC-641919	0
7	IC-280818	2.37
8	IC-341797	1.95
9	IC-341807	3.1
10	IC-341922	2.77
11	IC-636240	1.55
12	IC-430379	0
13	IC-313309	0
14	EC-398555	6.1
15	IC-582514	2.9
16	IC-538073	5.02
17	IC-538039	4.15
18	IC-328398	0
19	IC-326978	0
20	IC-326977	4.5
21	IC-026624	3.3
22	IC-632961	0
23	IC-313320	2.07
24	IC-582511	0
25	IC-538077	0
26	IIHR-01	2.15
27	IIHR-02	1.5
28	Arka Sukomol	2.85
29	Lakshmi	3.5
30	Marlida	1.65
31	Super King	2.75
32	US-2	0

The highest number of aphids on the top three leaves was found in genotypes IC-636226 (6.30) followed by EC-398555 (6.10), IC-636241 (5.70), IC-636225 (5.35), IC-538073 (5.02), IC-326977 (4.50), IC-538039 (4.15), Lakshmi (3.50), IC-026624 (3.30), IC-341807 (3.10), IC-582514 (2.90), Arka Sukomol (2.85), IC-341922 (2.77), Super King (2.75), IC-280818 (2.37), IIHR-01 (2.15), IC-313320 (2.07), IC-636245 (2.05), IC-341797 (1.95), IC-636224 (1.68), Marlida (1.65), IC-636240 (1.55), and IIHR-02 (1.50). However, genotypes IC-538077, IC-582511, IC-632961, IC-326978, IC-328398, IC-313309, IC-430379, and IC-641919 did not show any aphid infestation. These genotypes may be utilized in the development of new varieties of pole-type French bean. Similar findings of aphid-less genotypes were also reported by Sahu et al. (2021).

IV. CONCLUSION

The results of the present investigation revealed significant variability among the thirty-two pole-type French bean genotypes with respect to resistance against major pests and diseases. Genotypes such as IC-636224, IC-341797, IC-341922, IC-636240, IC-313309, EC-398555, IC-582514, IIHR-01, IIHR-02, Marlida, and US-2 exhibited promising resistance to Fusarium wilt and Bean common mosaic virus, indicating their potential use as donor parents in future hybridization programmes. Likewise, genotypes IC-538077, IC-632961, IC-328398, IC-326978, IC-430379, and IC-313309 demonstrated resistance to pod borer and aphids and may serve as valuable genetic resources for developing pest-resistant varieties. Overall, these identified genotypes can be effectively utilized in breeding programmes aimed at developing high-yielding pole-type French bean varieties with enhanced resistance to major pests and diseases.

DISCLAIMERS

The authors present only their findings and take full responsibility for the accuracy and quality of the content. The views expressed are solely those of the authors.

INFORMED CONSENT

No animals were used during the research. Plant material used in this study was obtained from the germplasm collection.

CONFLICT OF INTEREST

Neither author has a conflict of interest. No funding or sponsorship was involved in the design or article preparation

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Metagenomics: Concepts, Methodologies and Transformative Applications

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***Abstract*—** *Metagenomics has emerged as a paradigm-shifting approach in microbiology, enabling direct genomic analysis of entire microbial communities from their natural environments without the constraints of laboratory cultivation. This comprehensive review synthesizes current methodologies, computational challenges, and breakthrough applications of metagenomic approaches. We examine the evolution from single-organism genomics to community-level genomic analysis, highlighting how technological advances in sequencing platforms have overcome traditional cultivation limitations. The review covers critical aspects including environmental sampling strategies, next-generation sequencing technologies, assembly algorithms, taxonomic binning approaches, and functional annotation pipelines. The profound implications for understanding microbial ecology, symbiotic relationships, and the discovery of novel gene families are discussed. Current computational challenges and emerging solutions are evaluated, along with the transformative potential of third-generation sequencing technologies. This review positions metagenomics as a foundational technology driving discoveries across environmental microbiology, clinical diagnostics, biotechnology, and our fundamental understanding of microbial contributions to planetary processes.*

***Keywords*— metagenomics, microbial communities, environmental genomics, next-generation sequencing, taxonomic binning, functional annotation.**

I. INTRODUCTION

The intimate relationship between higher organisms and their associated microbial communities has become increasingly recognized as fundamental to understanding biological systems. Humans and animals harbor more bacterial cells than their own somatic cells, emphasizing the critical importance of microbial genomics in comprehending host-microbe interactions and ecosystem dynamics. Because of the intimate relationship of humans and animals with microbes, sequencing the genomes of microbes is necessary as this would facilitate better understanding of the role of microbes in the biosphere. Traditional microbiology, constrained by the requirement for axenic cultures, has provided insights into only a minute fraction of the microbial world. Only a small percentage of the microbes in nature can be cultured, which means that extant genomic data are highly biased and do not represent a true picture of the genomes of microbial species.

The field of genomics experienced its first revolution with the sequencing of complete microbial genomes, beginning with bacteriophages MS2 and φ-X174 in the late 1970s, followed by the landmark sequencing of *Haemophilus influenzae* in 1995. This single-organism approach, while revolutionary, faced inherent limitations: the cultivation bias that excluded the vast majority of environmental microorganisms, and the failure to capture the complex interactions within natural microbial communities. Metagenomics, literally meaning "beyond the genome," represents a paradigmatic shift that circumvents these

limitations by enabling direct genomic analysis of entire microbial communities. Sequence data taken directly from the environment are called metagenomes, and the study of sequence data taken directly from the environment is metagenomics. This approach harnesses environmental DNA (eDNA) extracted directly from natural habitats, providing unprecedented access to the genetic potential of uncultivated microorganisms and their ecological interactions. New sequencing technologies and the drastic reduction in the cost of sequencing have helped tremendously in overcoming these limitations. We now have the ability to obtain genomic information directly from microbial communities in their natural habitats. Suddenly, instead of looking at a few species individually, we are able to study tens of thousands all together.

II. METHODOLOGICAL FRAMEWORK

2.1 Environmental Sampling Strategies:

The foundation of any metagenomic investigation lies in representative sampling of the target microbial community. Unlike traditional microbiological approaches where target organisms are visible, metagenomic sampling must account for the invisible microbial world and its inherent heterogeneity.

Sample Size and Replication: To estimate the fraction of species sequenced, rarefaction curves are typically used. A rarefaction curve plots the number of species as a function of the number of individuals sampled. The curve usually begins with a steep slope, which at some point begins to flatten as fewer species are being discovered per sample. For microbial samples, different operational taxonomic units (OTUs) are typically characterized by 16S (prokaryotic) or 18S (eukaryotic) rDNA, also referred to as ribotypes.

Filtration and Size Fractionation: When filtering an environmental sample, the goals are: (1) obtain as much as possible of what is desired and (2) exclude as much as possible of what is not desired. Computational filtering can be used after sequencing. Genomic material that is obviously within the clades of interest can be filtered in using similarity searches against annotated sequence databases. Care must be taken with false negatives: relevant genomic material may be filtered out simply because homologues have never been deposited in existing databases.

Metadata Collection and Standardization: Keeping strict and comprehensive records of metadata is as important as the sequence data. Metadata are the "data about the data": where the samples were taken from, when, and under which conditions. Many metagenomic studies are driven by discovery and data mining, rather than by hypothesis. These studies seek statistically significant correlations between the metagenomic data and the habitat-associated metadata, which may lead to biologically significant discoveries.

2.2 Sequencing Technologies and Their Evolution:

First-Generation Sanger Sequencing: Until recently, prokaryotic genomes have been typically sequenced using Sanger shotgun sequencing. The first step is shearing the DNA content of a genomic clone into random fragments, hence the "shotgun." The fragments are then cloned into plasmid vectors that are grown in monoclonal libraries to produce enough genomic material for sequencing. Another disadvantage of shotgun sequencing is the "cloning bias." Some genes cannot be incorporated into the library vector, usually because of toxicity to the vector expressing them.

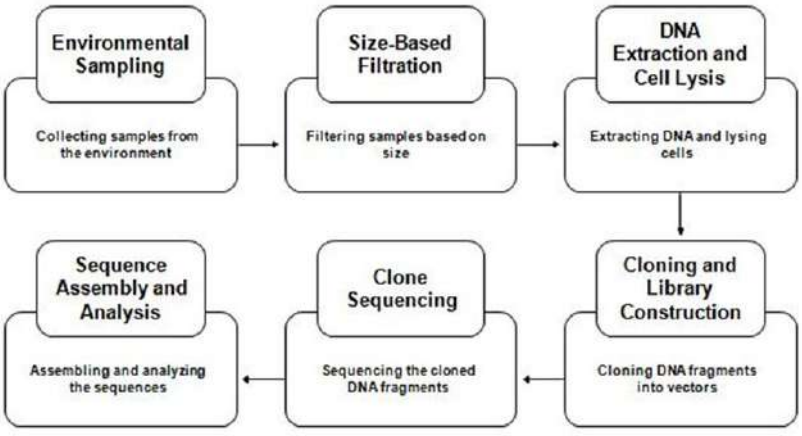

FIGURE 1: Environmental shotgun sequencing workflow: (A) Environmental sampling; (B) Size-based filtration; (C) DNA extraction and cell lysis; (D) Cloning and library construction; (E) Clone sequencing; (F) Sequence assembly and analysis.

Second-Generation High-Throughput Sequencing: Second-generation sequencing methods have been rapidly gaining ground and are replacing Sanger sequencing for small-sized genomes and environmental genomics. A common denominator among second-generation methods is the generation of "polymerase colonies" or polonies. Polonies are PCR amplicons derived from a single molecule of nucleic acid.

In pyrosequencing methods such as Roche 454 sequencing, sequencing is performed by polymerase extension of a primed template. Single nucleotide species are added at each cycle. If the particular nucleotide species added to the polymerase reaction pairs with the one on the template, the incorporation causes a luciferase-based light reaction.

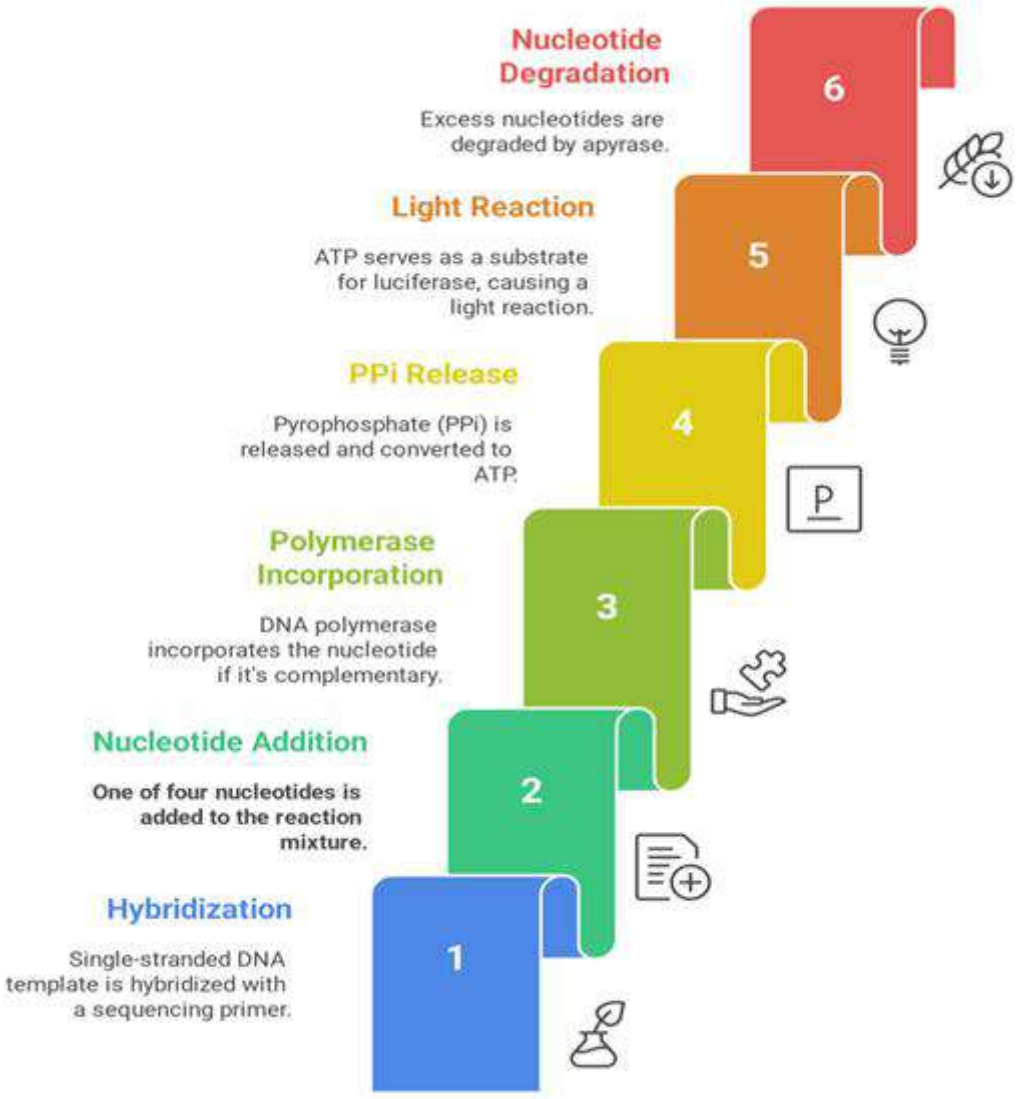

FIGURE 2: Pyrosequencing mechanism: Single stranded DNA template is first hybridized with the sequencing primer and mixed with the two substrates adenosine 5'-phosphosulfate (APS) and luciferin. In each cycle, (1) one of four nucleotides is added to the reaction. (2) If the nucleotide is complementary to the base in the template strand, then the DNA polymerase incorporates it into the growing strand. (3) Pyrophosphate (PPi) is released and converted to ATP by sulfurylase. (4) ATP serves as a substrate to luciferase, causing a light reaction. (5) Excess nucleotides are degraded by apyrase.

Sequencers such as Illumina produce shorter reads (50-300 bp) but generate very large volumes of DNA per sequencing run. Despite the individual short read lengths, these technologies provide a viable alternative for sequencing whole genomes, due to the large volume of DNA sequenced.

Emerging Third-Generation Technologies: Third-generation sequencing, loosely defined as technology that is capable of sequencing long sequences without amplification, represents an advanced development. Long-read sequencing technologies promise to address current assembly limitations by generating contiguous sequences spanning repetitive regions and complete

operons. These technologies, including platforms from PacBio and Oxford Nanopore, enable the sequencing of DNA molecules up to tens of kilobases in length, facilitating the assembly of complex microbial communities and the resolution of repetitive genomic regions.

2.3 Assembly Algorithms and Computational Approaches:

Challenges in Metagenomic Assembly: For sequencing a whole genome, the reads are assembled into progressively longer contiguous sequences or contigs and finally to the whole genome. The incomplete and fragmentary nature of metagenomic data presents challenges. In contrast, in all but the most species-poor metagenome, a full assembly is not possible—first, because the sampling is incomplete and many if not all species' genomes are partially sampled, if at all; second, because the species information itself is incomplete, and it is difficult to map individual reads to their species of origin.

Graph-Based Assembly Strategies: Phrap, Forge, Arachne, JAZZ, and the Celera Assembler are the assembly programmes developed for single genome assembly from Sanger sequencing. They seem to provide good results even when assembling metagenomic sequence data from Sanger sequencing. Most of these assembly algorithms use mate-pair information to check the scaffolds or the assembled intermediaries between raw reads and whole chromosomes.

For short reads, these techniques are not suitable. The solution to a Hamiltonian path is an NP-complete problem, meaning that the time necessary for a solution grows exponentially with the number of nodes. The EULER assembler was the first to present an alternative technique using de Bruijn graphs, which represent sequences as overlaps of k-mers rather than individual reads, making assembly of short reads computationally feasible.

Specialized Assembly Approaches: Recently, assembly methods have been developed that find putative open reading frame (ORF) regions first, and then assemble those regions. This method, dubbed ORFome assembly, increases assembly accuracy for ORF regions at the expense of losing noncoding regions.

III. ANALYTICAL METHODOLOGIES

3.1 Coverage Assessment and Genome Size Estimation:

Coverage Calculations: Coverage of a genome is defined as the mean number of times a nucleotide is sequenced. If DNA shearing and sequencing are treated as random events, then the Poisson distribution model can be used to estimate the number of reads required to sequence an entire genome. This model is given by the Lander-Waterman equation:

$$C = \frac{L \times N}{G} \tag{1}$$

Where L is the read length, N is the number of reads, G is the genome length, and C is coverage. The fraction of sequence covered would be given as:

$$P_0 = 1 - e^{-C} = 1 - e^{-(LN/G)} \tag{2}$$

Effective Genome Size (EGS) Estimation: An effective genome size (EGS) measure has been suggested that includes multiple plasmid copies, inserted sequences, and associated phages and viruses. EGS uses the density of single copy marker genes to extrapolate the EGS:

$$EGS = \frac{a + b \times L^{-c}}{x} \tag{3}$$

Where L is the read length, x is the marker gene density, and a, b, and c are empirical parameters.

3.2 Gene Identification and Functional Prediction:

Gene Calling Challenges: Genes are the basic functional unit in the genome, which may constitute larger functional units such as operons, transcriptional units, and functional networks. In the Global Ocean Sampling (GOS) data, which were Sanger-sequenced, the mean number of whole reading frames per assembly is 4.7.

For a high complexity metagenomic dataset, gene prediction on assemblies can be as accurate as 85% of the originally predicted genes in the constituting genomes, and for a low complexity set this goes up to 90%. For genes with known homologs, BLASTing against known databases is a common approach. For new families and new genes that have no homologs in known databases, *ab initio* gene prediction tools are used. GeneMark.hmm is a programme that uses inhomogeneous Markov models based on monocodon frequency analysis for gene calling.

Innovative Gene Discovery Approaches: A different approach to gene finding involves beginning with simple ORF identification of consecutive translatable regions that translate to at least 60 amino acids and then clustering those sequences using an all-against-all BLAST search.

3.3 Taxonomic Classification and Binning:

Composition-Based Binning: There are two binning strategies: composition-based binning and similarity-based binning. GC content of bacterial genomes is used routinely for higher-level systematics. Tetranucleotides are used by the TETRA program. PhyloPythia is a supervised method that trains a set of support vector machines (SVMs) to bin sequences of a length greater than 1 kb. Growing Self Organizing Maps and Seeded GSOM (S-GSOM) use a variant of the machine learning algorithm self-organizing maps.

A composite supervised method that uses both TETRA and codon usage statistics has been developed to classify fragments in the 100-300-bp range.

Similarity-Based Binning: MEGAN implements similarity-based binning by reading a BLAST file output. MEGAN assigns each read to the lowest common ancestor on the phylogenetic tree. Phymm uses interpolated Markov models to characterize variable length DNA sequences by their phylogenetic groupings.

3.4 Diversity Assessment and Community Structure Analysis:

Alpha Diversity Quantification: Shannon's index is used to calculate α-diversity:

$$H'_{\alpha} = -\sum_{i=1}^{S} p_i \ln p_i$$

Where $p_i = n_i/N$, S is the total number of OTUs, n_i is the number of clones in each OTU, and N is the total number of individuals.

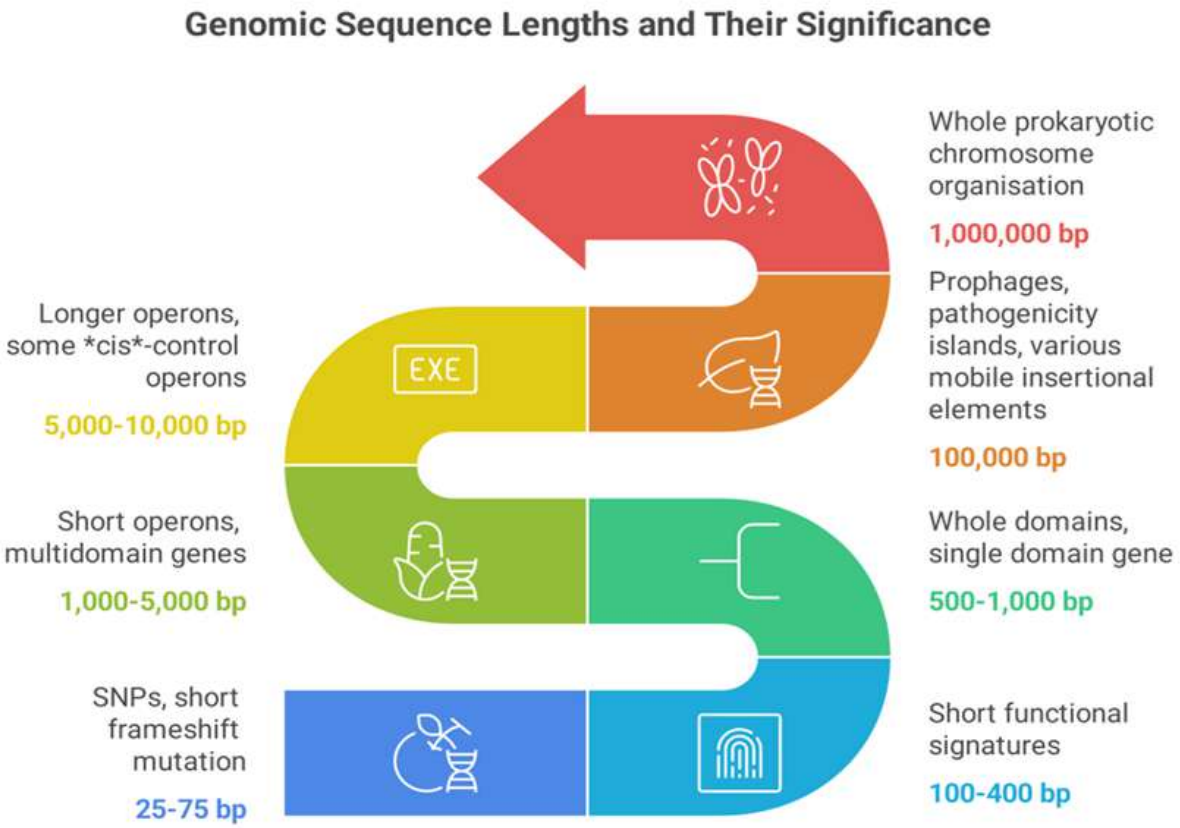

FIGURE 3: Rarefaction curves interpretation: Green curve indicates comprehensive species sampling; blue curve shows incomplete habitat sampling; red curve represents species-rich environments with minimal sampling coverage.

Phylogenetic Marker Considerations: Using 16S/18S rDNA as a proxy for OTU identification is not without limitations. Evidence of horizontal gene transfer involving rDNA may confound its reliability. 16S rDNA may exist in multiple different sequence copies in a single bacterium: the mean number of bacterial ribosomal operons per genome is 4.1, but 16S rDNA gene copy numbers may vary between 1 and 15. Alternative markers, such as single copy housekeeping genes including *rpoB*, *amoA*, *pmoA*, *nirS*, *nirK*, *nosZ*, and *pufM* have been suggested.

Several software packages are useful for biodiversity analysis. EstimateS contains a rich set of biodiversity analysis modules. MOTHUR is tailored towards microbial diversity analysis. QIIME is a very powerful and versatile package for analysis of genomic and metagenomic microbial ecology data. PHACCS is specialized software geared to the analysis of viral metagenomic data.

IV. TRANSFORMATIVE APPLICATIONS

4.1 Environmental Microbiology and Ecosystem Function:

Metagenomic approaches have revolutionized understanding of microbial contributions to global elemental cycles. Functional gene surveys reveal metabolic potential for nitrogen fixation, sulfur oxidation, and carbon cycling across diverse environments. Comparative metagenomics across environmental gradients elucidates how community composition and metabolic capacity respond to geochemical variations.

Many comparative analyses make use of ordination statistics when several metagenomic datasets are involved. Principal component analysis (PCA) and nonmetric multidimensional scaling (NM-MDS) are typically used to visualize the data. Recent studies have suggested how to locate multivariate correlations between metagenomic data and environmental attributes, identifying covariation in amino acid transport and cofactor synthesis in nutrient-poor ocean areas.

4.2 Host-Associated Microbiomes and Human Health:

One notable study examined the connection between the gut microbiome and obesity. Researchers discovered that the metagenome in obese mice was enriched in carbohydrate-active enzymes over that of lean mice. A separate biochemical experiment confirmed that the microbiome in obese mice has a larger energy harvesting capacity than in lean mice and concluded that the gut microbiome contributes to obesity through this feed-forward cycle.

Metagenomic approaches are transforming clinical microbiology by enabling culture-independent pathogen detection and antimicrobial resistance gene surveillance. Recent molecular-based discoveries of highly prevalent viral infections highlight the need for a better understanding of the human viral flora.

4.3 Symbiotic Systems and Evolutionary Biology:

In many cases, symbiotic bacteria living in an animal host consist of a small number of species, which are often phylogenetically distant. Researchers sequenced the environmental shotgun sequencing (ESS) data from bacterial symbionts living in the glassy-winged sharpshooter, which is an insect that lives solely on tree sap, a nutrient-poor diet. By binning the ESS data, it has been inferred that one symbiont synthesizes amino acids for the host insect, while another synthesizes cofactors and vitamins.

Another study of the marine gutless worm *Olavius algarvensis* has revealed the different roles of its four symbionts in generating nutrients and processing the worm's waste. None of the symbionts in the insect or in the worm study could be cultured under the reported conditions. Metagenomics thus became the chosen avenue for these studies.

4.4 Biotechnological Innovation and Gene Discovery:

Another type of study enabled by metagenomics is the search for new members of a gene family. The previously small bacterial Eukaryotic Protein Kinase Like (ELK) family has been enriched several folds by the Global Ocean Sampling (GOS) project. Many new members of known families were identified, as well as new families.

4.5 Viral Metagenomics and Genetic Diversity:

Metagenomic studies have enriched our knowledge of viral diversity and the role viruses play as facilitators of microbial genetic diversity. Sequence similarity analyses of viral metagenomic data have shown that approximately 90% of the sequences have no similarity to GenBank sequences, a phenomenon often referred to as "viral dark matter."

The existence of photosynthetic genes in cyanophages—viruses infecting cyanobacteria—has been known for some time. However, metagenomic studies have revealed the extent of this phenomenon: it is estimated that 60% of the *psbA* genes, a component of Photosystem II, in surface water are of phage origin.

V. COMPUTATIONAL TOOLS AND PIPELINES

5.1 Annotation Pipelines:

The versatile and useful annotation pipelines for metagenomics include MG-RAST. MG-RAST accepts a 454 dataset as input, normalizes it, and then performs gene calling and annotation by a variety of sequence similarity searches against various sequence databases, including 16S rDNA.

RAMMCAP uses the fast clustering algorithm CD-HIT to cluster translated ORFs by high sequence similarity. The sequences are then compared to the profile HMM databases TIGRfam using HMMer for functional annotation. Motif Extraction (MEX) is an unsupervised motif creation method that is successful in identifying enzymes in genomic and metagenomic data.

5.2 Comparative Analysis Tools:

Besides using MEGAN as a binning software, it can also be used to compare the OTU composition of two or more frequency-normalized samples. MG-RAST provides a comparative functional and sequence-based analysis for uploaded samples. Other software used for the comparison of microbial populations include UniFrac and MetaStats. Galaxy provides online workbench capabilities for comparative metagenomic analysis. ShotgunFunctionalizeR is a stand-alone analysis tool written in R.

VI. FUTURE DIRECTIONS AND EMERGING TECHNOLOGIES

6.1 Third-Generation Sequencing Integration:

Long-read sequencing technologies, including platforms from PacBio (Single Molecule Real-Time sequencing) and Oxford Nanopore Technologies, promise to address current assembly limitations by generating contiguous sequences spanning repetitive regions and complete operons. These platforms enable the sequencing of native DNA molecules without amplification bias, and some technologies can differentiate between cytosine and methyl-cytosine during sequencing, providing additional epigenetic information directly from environmental samples.

6.2 Data Management Challenges:

A growing problem is that of data management. Sequencing centers are working to equip themselves with computational infrastructure to meet the flow of sequence data. However, many research institutes who request sequencing do not have the computational infrastructure needed to deal with analysis and long-term storage of these data. The development of cloud-based platforms and standardized data repositories represents an ongoing effort to address these challenges.

VII. CONCLUSIONS AND IMPLICATIONS

We are in the midst of the fastest growing revolution in molecular biology, perhaps in all of life science, and it only seems to be accelerating. Assembly, quality control, binning, and annotation all require ingenious algorithms combined with the latest computational power. Metagenomics represents a transformative technology that has fundamentally altered our understanding of microbial life and its planetary significance. By circumventing cultivation limitations, metagenomic approaches have revealed the vast extent of microbial diversity, complex ecological interactions, and the genomic basis of ecosystem function.

The applications discussed demonstrate metagenomics' broad impact across environmental science, medicine, biotechnology, and evolutionary biology. From understanding symbiotic relationships and discovering novel gene families to tracking viral diversity and uncovering the role of the microbiome in human health, metagenomics has become an indispensable tool in modern biological research.

As computational capabilities continue expanding and sequencing costs decline, metagenomics will undoubtedly drive further revolutionary discoveries in our understanding of life's microbial foundations. The integration of metagenomics with other omics approaches—metatranscriptomics, metaproteomics, and metabolomics—promises to provide even deeper insights into the functional dynamics of microbial communities. Continued development of computational infrastructure, standardized data sharing practices, and accessible analysis tools will be essential to ensure that the potential of metagenomics is fully realized across the global scientific community.

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CONFLICT OF INTEREST

The author declares no competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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A Study of Enhancing Cataloguing and Collection Management Operations Using RFID Technology in Libraries: A Modern Approach

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Abstract— *Radio Frequency Identification (RFID) technology is instrumental in transforming traditional university libraries into smart, automated, and user-centric environments. The traditional security system of libraries is usually based on manual inspection and access control, which leads to low security and low efficiency. Based on the intelligent perception system, the location and state of books can be monitored and controlled in real-time, so as to prevent books from being lost or stolen. By implementing self-service checkouts, real-time inventory management, and improved security measures, RFID enhances security by deterring unauthorized removal of materials and optimizes inventory management through swift, bulk item scanning. The advent of Radio Frequency Identification (RFID) technology has initiated a significant transformation in library operations. RFID, a wireless technology that utilizes electromagnetic fields, presents an innovative method for identifying and tracking library materials. RFID simplifies library operations and enhances the user experience. Its integration promotes effective resource tracking, diminishes manual workload, and aids in the digital advancement of academic libraries. Applications in various universities have illustrated RFID's contribution to improving operational efficiency and service quality, establishing it as a fundamental element in the evolution of smart university libraries. This study aims to assess user awareness of RFID technology, examine its implementation status in university libraries, identify areas of application, and evaluate its impact on library operations. The findings indicate that while 80% of users are aware of RFID technology, its implementation remains limited. Among libraries that have adopted RFID, the technology is primarily used for self-check-in/check-out (59%) and security/anti-theft purposes (41%). The majority of respondents view RFID as a crucial tool for transforming traditional libraries into smart, automated, and user-friendly environments.*

Keywords— RFID, Smart Libraries, Security System, Automation, Self-Service, Inventory Management.

I. INTRODUCTION

RFID (Radio Frequency Identification) is a technology that uses radio waves to identify and track objects. In libraries, RFID is used to manage books and other materials more efficiently. In the 21st century, university libraries are undergoing rapid digital transformation to meet the dynamic needs of students, researchers, and faculty. Among the various emerging technologies, Radio Frequency Identification (RFID) has become a key tool in making academic libraries more efficient, user-friendly, and smart. RFID uses electromagnetic fields to automatically identify and track tags attached to books and other library materials. Its integration into library systems supports automation in circulation, inventory management, security, and user services.

The need for smart university libraries arises from increasing student populations, demand for real-time access, and expectations for self-service. RFID bridges the gap between traditional services and modern expectations by enabling faster check-in/check-out processes, reducing human errors, and allowing real-time tracking of materials. It also enhances security by preventing theft through gate sensors and improves collection management through automated stock verification. Many universities globally and in India have adopted RFID-based systems to increase operational efficiency and reduce manual workload on library staff. This has led to better service delivery, enhanced user satisfaction, and optimal utilization of library resources.

1.1 Objectives of the Study:

1. To assess the awareness level of users about RFID technology
2. To examine whether the university/institute library has implemented RFID technology
3. To identify the specific areas of library operations where RFID technology is being utilized
4. To assess the impact of RFID technology on the efficiency and effectiveness of library operations
5. To evaluate the role of RFID technology in transforming libraries into smart, digital, automated, and user-friendly spaces

1.2 What is RFID (Radio Frequency Identification)?

The abbreviation for radio frequency identification is RFID. It is a technology that transmits and remembers information using high frequency radio waves in order to give items a distinct identity. The British needed to be able to distinguish between Allied aircraft and enemy aircraft on their radar in real time, which is when RFID was first used in the 1930s. After that, the technology evolved to become the RFID that we know today. Among its many uses are contactless payments and object tracking. In many applications, radio frequency identification is very helpful, especially in Big Data and the Internet of Things. An RFID tag is often a flat square containing an antenna and an electrical chip on a substrate, like a patch.

1.3 Origin of RFID:

The origin of radio frequency identification (RFID) technology dates back to the Second World War. During this time radar systems were developed for detecting approaching aircraft. But distinguishing between friendly and enemy aircraft presented a challenge. To tackle this problem, the UK developed the Friend or Foe Identification (IFF) system, which is considered a forerunner of modern RFID technology. The IFF system allowed ground-based radar to identify friendly aircraft by sending out a signal that would trigger a response from the aircraft transponder, effectively classifying the aircraft as friendly.

In 1948, Harry Stockman published the paper "Communication by Means of Reflected Power," which laid the theoretical foundation for RFID. Stockman's work explored the concept of using reflected radio waves to transmit information, a principle central to RFID technology. During the 1950s and 1960s, RFID technology was mostly theoretical. In the 1970s, however, companies and research institutes began to develop practical applications. For example, academic institutions like Northwestern University and companies like RCA and Fairchild invested in RFID research, focusing on applications such as car tracking for tolls, animal tracking, and automated assembly.

The 1980s and 1990s saw significant advancements in RFID technology. In 1986, Norway implemented the first toll collection system using RFID. By 1989, the Dallas North Turnpike in the U.S. adopted RFID for toll collection. In 1990, seven toll agencies in the northeastern U.S. formed the E-Z Pass Interagency Group to develop a regionally compatible toll collection system. In the 1990s, Texas Instruments developed the TIRIS system, which found applications in gas stations, vehicle access management, ski pass scanning, and even in casinos. Further advancements by companies such as IBM allowed RFID tags to be built using a single integrated circuit, reducing their size and expanding their potential applications. Today, RFID technology is widely used in various industries, including retail, logistics, healthcare, and libraries, offering efficient and automated solutions for tracking and identification.

1.4 The Development of RFID Technology in Library Perspective:

1.4.1 Early Exploration and Adoption (Late 1990s–Early 2000s):

RFID technology, initially developed during World War II for military applications, began finding commercial uses in the late 20th century. By the early 2000s, libraries started exploring RFID to streamline circulation and inventory processes. For

instance, a 2002 article highlighted libraries considering RFID to replace barcodes for efficient book tracking and theft prevention.

1.4.2 Standardization and Global Implementation (2000s–2010s):

As RFID technology matured, libraries worldwide began adopting it to improve efficiency and service quality. Organizations like the American Library Association (ALA) provided guidelines to ensure consistent and secure RFID implementation in libraries.

1.4.3 Integration with Advanced Technologies (2010s–Present):

Modern libraries have integrated RFID with self-service kiosks, automated sorting systems, and real-time inventory management. Case studies include:

- **National Library of Singapore:** Implemented RFID in 2015, reducing checkout times by 30% and increasing inventory accuracy by 20%.
- **Hong Kong University Library:** Adopted RFID in 2018, leading to a 40% reduction in book processing time and a 35% decrease in theft.
- **New York Public Library:** Integrated RFID in 2020, resulting in a 50% reduction in theft and a 25% increase in user satisfaction.

1.4.4 Future Trends:

The future of RFID in libraries includes integration with Artificial Intelligence (AI) and the Internet of Things (IoT) to enable predictive analytics and intelligent resource management. For example, AI can analyze borrowing patterns to optimize book placement and staffing.

1.5 How RFID Works in Libraries:

An RFID system consists of different components:

1. **RFID Transponder Tags:** RFID tags are tracking devices that identify objects using radio frequency technology. Data is transferred from the tag to a reader via radio waves, and the reader forwards the data to an RFID computer program. Incorporated into library materials, these tags hold distinct identifying information. Another name for an RFID tag is an RFID chip.
2. **RFID Readers:** An RFID reader is a radio frequency device that sends out a signal via an antenna. RFID tags receive this signal and respond when the reader interrogates them. The reader reads the responses and can communicate with all RFID tags in its field using various protocols. There are three types of readers in general:
    - **Portal readers:** Stationary readers that RFID tags travel through
    - **Handheld readers:** Portable devices that can scan RFID tags
    - **Reader/writer devices:** Gadgets that read or write data by interacting with tags
3. **Antennas:** Antennas are an essential component of RFID. They help RFID readers effectively transmit and receive radio waves from RFID tags. As the key link between the tag and the reader, the antenna has an important influence on the range, speed, and accuracy of RFID tag scanning.
4. **RFID Software:** RFID systems often incorporate software to collect and manage RFID data from readers. This software processes data from readers and integrates with the library management system.
5. **Server:** The server acts as a communication gateway between different components. It receives information from one or more readers and verifies it against its own database or exchanges information with the circulation database of the Integrated Library Management System. Typically, the server includes a transaction database to produce reports.
6. **Handheld Reader:** A handheld reader can be moved over items on the shelf without touching the documents. The data is sent to a storage unit that can later download it to a server, or it is transferred to a server using wireless

technology. The handheld reader is used to scan a complete collection of books on the shelf for inventory control, to search for books that are incorrectly shelved, and to search for individual required books.

7. **Anti-Theft Gate Reader:** The anti-theft gate reader is mounted at the exit gate. It consists of pedestal gates fixed on the left and right of the exit, with visible and audible alarms for detecting theft. RFID exit gate sensors read book tags and ID tags. Information about items passing through is transmitted to the server. The server, after checking the circulation database, triggers an alarm if the material has not been checked out properly.
8. **Book Shelf Check-In/Check-Out Machine (Charging and Discharging Drop Desk):** RFID reduces the time needed to carry out circulation operations. For users, RFID speeds up lending and borrowing. It helps librarians to be more productive and to focus on more interesting library tasks. Book shelf check-in and checkout machines enable self-service for book submission, self-return, and RFID card reading.
9. **Inventory Management:** Real-time tracking of books and materials enables quick stock audits and reduces misplaced items.

These components work together to automate circulation, enhance inventory management, and improve security.

1.6 Applications of RFID in Academic Libraries:

- RFID technology is widely used in universities for asset tracking, library management, student identification, access control, and attendance monitoring.
- Asset tracking using RFID enables efficient inventory management and reduces the time spent on locating and managing university resources.
- RFID-based library systems improve the accuracy and speed of book check-in/out, inventory control, and shelf management.
- RFID-based student identification and authentication systems enhance security and streamline various administrative processes.
- Access control systems based on RFID technology provide secure and convenient access to buildings, labs, and facilities.
- RFID-enabled attendance monitoring systems automate attendance tracking, reducing manual effort and improving accuracy.

1.7 Advantages of RFID for Libraries:

A new era of innovation has begun with the introduction of Radio Frequency Identification (RFID) technology in libraries, providing numerous advantages that greatly enhance user experiences and library operations.

- **Efficiency:** RFID's contribution to library management is based on efficiency. RFID simplifies the library's fundamental operations by automating processes including material check-in and check-out, inventory management, and lost item recovery. Transactions can now be completed quickly by customers, cutting down on wait times and administrative effort.
- **Accuracy:** In library operations, accuracy is crucial, and RFID excels at it. The accuracy of the system ensures that circulation and inventory management errors are kept to a minimum. Accurate tracking of materials helps to eliminate inconsistencies and guarantee that the library's holdings are methodically arranged.
- **Enhanced Security:** One important benefit is increased security. RFID technology can be used to recover misplaced or lost items as well as to deter theft. Alarms may be generated by the system if items are incorrectly checked out, strengthening the protection of library materials.
- **Improved User Experience:** The goal of the library is to improve the user experience, and RFID is essential to this goal. Self-service kiosks have made it possible for customers to handle their transactions on their own. Fast check-in and check-out procedures reduce wait periods, making library visits more efficient and enjoyable.

1.8 Updated Technologies Used in Libraries:

Libraries today are rapidly adopting advanced technologies to enhance their services and provide better access to information. One of the most impactful technologies is Radio Frequency Identification (RFID). In addition to RFID, many libraries are implementing self-service kiosks, barcode systems, and integrated library management software (LMS) to streamline operations. Cloud-based systems are becoming popular, allowing libraries to store data securely and provide remote access to e-resources. Digital libraries and e-resources give students and researchers the ability to access books, journals, and databases from anywhere, anytime.

Libraries are also using smart shelves integrated with RFID readers to automatically detect misplaced books, making it easier to maintain order. Biometric authentication and smart ID cards enhance security and access control. Furthermore, artificial intelligence (AI) and data analytics are being explored to understand user behavior, recommend resources, and optimize services.

1.9 Future Potential of RFID in Academic Libraries:

- **Comprehensive Library Automation:** RFID technology facilitates the automation of routine library operations, including book check-ins and check-outs, inventory management, and user authentication. For instance, Rajendra Prasad Central Agricultural University (RPCAU) has fully automated its central library by implementing RFID technology and launching the 'MyLib@FT' (My Library on Finger Tips) app. This advancement allows users to access the library's resources remotely and makes the entire book issuance and return process wireless and automated.
- **Integration with Smart Campus Services:** RFID-enabled smart ID cards are being utilized to streamline various campus services beyond the library. Banaras Hindu University (BHU) has launched RFID-enabled smart identity cards in a pilot phase at the Sayaji Rao Gaekwad Central Library to enhance student convenience and modernize campus services. These smart cards aim to streamline library operations by allowing quicker book issuance and returns, easier tracking, and digital management of entry and attendance. The RFID system will eventually expand to departmental and faculty libraries across the campus.
- **Enhanced Self-Service and User Experience:** RFID technology enables self-service kiosks for book borrowing and returns, reducing the need for staff intervention and minimizing wait times. This automation not only improves user satisfaction but also allows library staff to focus on more specialized tasks.
- **Integration with AI and IoT for Predictive Analytics:** The future of RFID in libraries includes integration with Artificial Intelligence (AI) and the Internet of Things (IoT) to enable predictive analytics and intelligent resource management. For example, AI can analyze borrowing patterns to optimize book placement and staffing.
- **Development of Smart and Unmanned Services:** RFID technology is driving libraries toward more intelligent, unmanned service models. For example, RFID-powered self-service checkouts, automatic inventory management, and smart shelving are expected to become more common. Users will be able to borrow and return books or documents through self-service stations without human intervention, and they will be able to locate needed items or access records through self-service kiosks. The widespread adoption of RFID technology will reduce the reliance on manual labor, optimizing resource allocation and allowing for more efficient management.

II. MATERIALS AND METHODS

To meet the objectives of the research, a structured questionnaire was prepared and responses were collected from participants. The questionnaire was designed to gather information on the frequency of library usage, awareness of RFID technology, implementation status of RFID in libraries, areas of RFID application, and user perceptions about the role of RFID in transforming library services.

The study population consisted of library users from select university libraries. Data was collected through convenience sampling, and responses were obtained from a diverse group of users including undergraduate students, postgraduate students, research scholars, and faculty members. The sample size for this study was 100 respondents. The data collected was organized and presented in bar chart form for clarity. The following sections present the findings, highlighting the frequency of library usage, the level of awareness about RFID technology, its implementation status, and the main areas where it is applied.

The following figures present the findings from the survey conducted among library users.

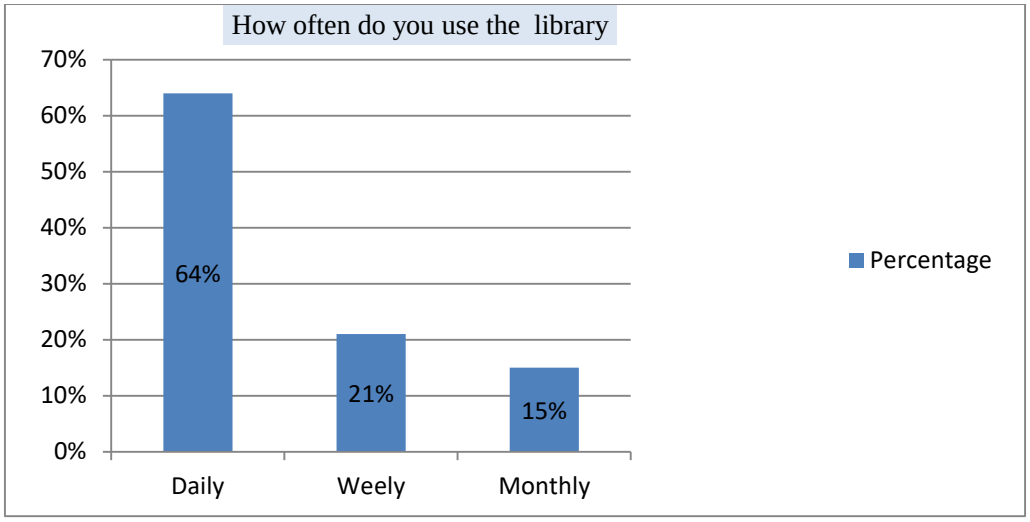

FIGURE 1: Frequency of Library Usage

[Bar chart showing: 64% daily, 21% weekly, 15% monthly]

The data shows that most users (64%) visit the library daily, demonstrating strong regular engagement with the library. Meanwhile, 21% use it weekly and 15% monthly, indicating that the library is an essential part of users' academic routines.

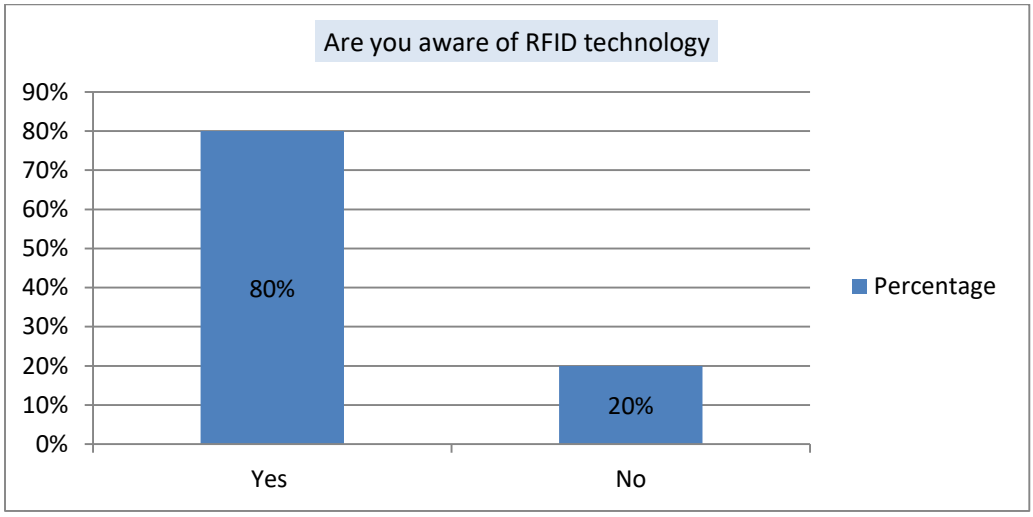

FIGURE 2: Awareness of RFID Technology

[Bar chart showing: 80% aware, 20% not aware]

The data shows that 80% of respondents are aware of RFID technology. This indicates a high level of awareness about RFID technology among library users.

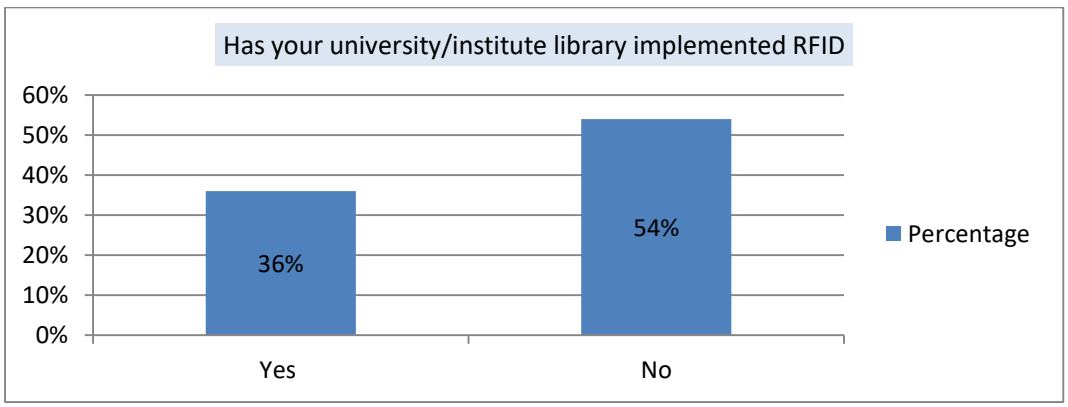

FIGURE 3: Implementation Status of RFID in Libraries

[Bar chart showing: Majority indicating RFID not yet implemented]

The data indicates that RFID technology is still not widely adopted in most libraries. The implementation of RFID remains in the early stage and is not yet widespread across institutions.

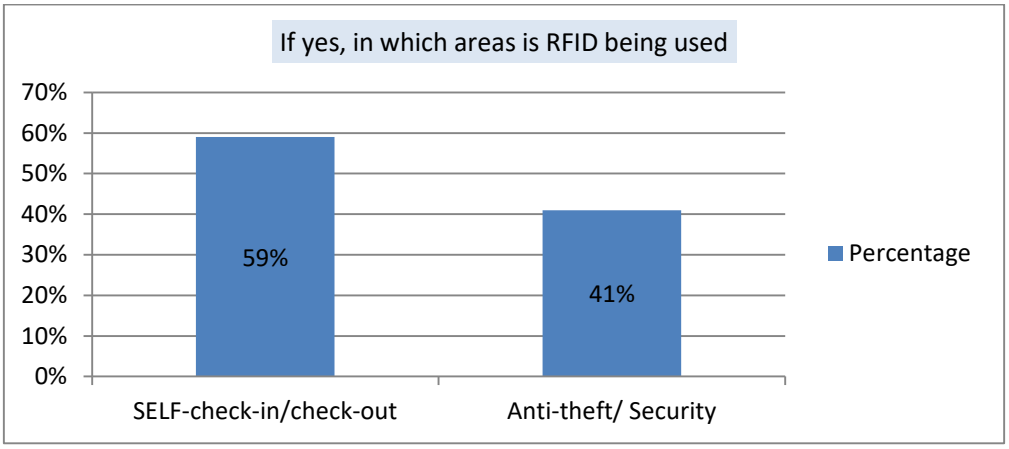

FIGURE 4: Areas of RFID Application in Libraries

[Bar chart showing: 59% for self-check-in/check-out, 41% for security/anti-theft]

The library uses RFID technology primarily for self-check-in and self-check-out (59%), which improves circulation efficiency. Additionally, 41% of users reported its utilization for security and anti-theft purposes, which helps safeguard library resources. This suggests that RFID is used in library administration for both convenience and security reasons.

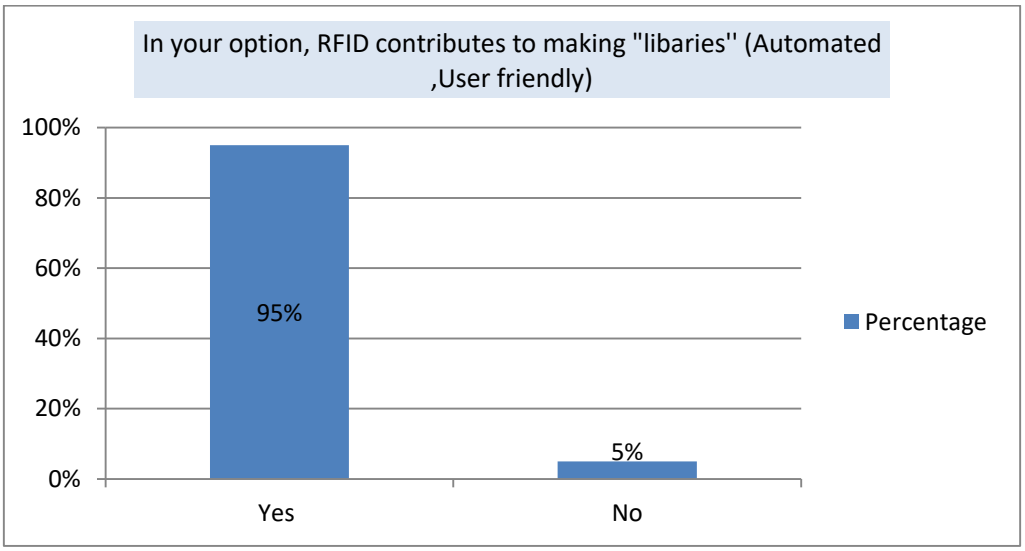

FIGURE 5: Perception of RFID's Role in Transforming Libraries

[Bar chart showing: Majority agree that RFID makes libraries smart, automated, and user-friendly]

The majority of respondents think RFID is a crucial tool for upgrading libraries since it makes them "smart," automated, and user-friendly. This indicates strong positive perception about the transformative potential of RFID technology.

The findings of this study provide valuable insights into the current state of RFID technology adoption in university libraries and user perceptions regarding its potential.

- **Library Usage Patterns:** The finding that 64% of respondents use the library daily reflects the continued importance of physical library spaces and services in academic life. This high frequency of use suggests that libraries remain central to academic activities, making them appropriate sites for technological enhancements such as RFID implementation. The regular engagement of users also means that any improvements in efficiency or service quality through RFID would have significant impact on a large number of users.
- **Awareness and Implementation Gap:** A notable finding of this study is the significant gap between user awareness of RFID technology (80%) and its actual implementation in libraries. While users are generally aware of RFID technology and its potential benefits, its adoption in libraries remains limited. This gap may be attributed to several

factors including the cost of implementation, lack of technical infrastructure, staff training requirements, and institutional budget constraints. Understanding this gap is important for library administrators considering future technology investments.

- **Primary Applications of RFID:** Among libraries that have implemented RFID, the technology is predominantly used for self-check-in/check-out operations (59%) and security/anti-theft measures (41%). This pattern reflects the immediate operational benefits that RFID offers in terms of reducing staff workload at circulation desks and enhancing collection security. The emphasis on self-service and security aligns with the core objectives of RFID adoption—improving efficiency and protecting library assets. However, the limited application of RFID in other areas such as inventory management, shelf management, and real-time tracking suggests that many libraries may not yet be fully utilizing the technology's capabilities.
- **User Perception of RFID's Transformative Potential:** The positive perception of RFID as a tool for transforming libraries into smart, automated, and user-friendly environments indicates strong user support for technological modernization. This finding is encouraging for library administrators considering RFID adoption, as it suggests that users are receptive to such changes and may actively embrace enhanced services enabled by RFID technology.
- **Comparison with Existing Literature:** The findings of this study are consistent with case studies from other institutions. For example, the National Library of Singapore's experience of reduced checkout times and improved inventory accuracy [3] reflects the potential benefits that users in this study anticipate. Similarly, the Hong Kong University Library's success in reducing book processing time [3] aligns with user expectations that RFID can improve operational efficiency.
- **Implications for Library Practice:** The findings suggest that while awareness and positive perceptions exist, there remains significant opportunity for expanding RFID implementation in university libraries. Library administrators should consider conducting cost-benefit analyses, exploring phased implementation approaches, and developing staff training programs to facilitate successful RFID adoption. Additionally, as RFID technology continues to evolve with integration into AI and IoT systems, libraries that adopt RFID now will be better positioned to leverage future technological advancements.

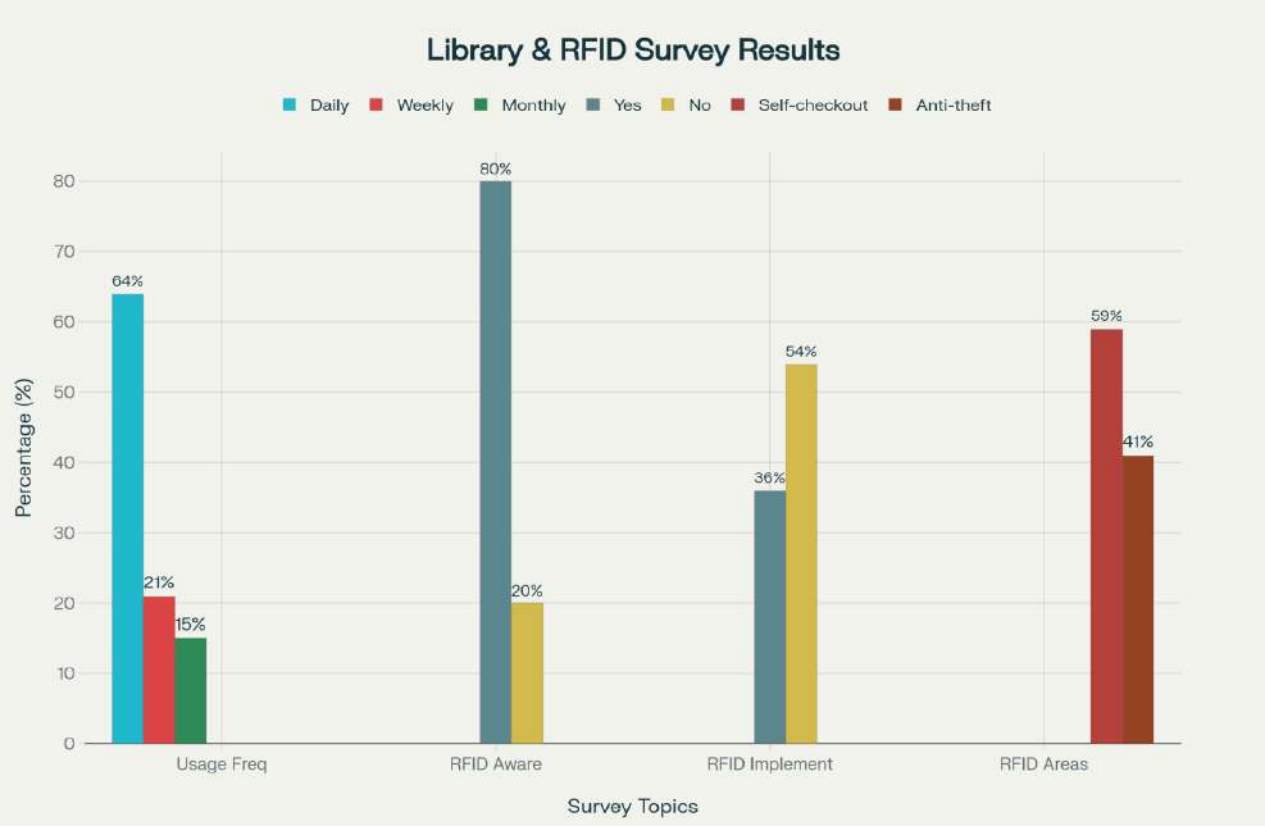

FIGURE 6: Library and RFID's Survey Results

III. CONCLUSION

The purpose of this study was to assess the awareness level of users about RFID technology, examine the implementation status of RFID in university/institute libraries, identify specific areas of application, and evaluate its impact on library operations. The findings reveal a user group that is highly engaged with the library and open to technological modernization. The large number of daily library visits highlights how important the library is to users' daily routines. The vast majority of respondents demonstrated substantial awareness of RFID technology, which aligns with this strong foundation of frequent use.

However, there is a significant disconnect between this awareness and actual implementation, suggesting that RFID adoption is still in its infancy in many institutions. Where RFID technology has been deployed, it is primarily used to enhance operational security and efficiency, with a particular focus on self-service checkout and anti-theft measures. This indicates that libraries are leveraging RFID for immediate operational benefits while potentially underutilizing its capabilities for comprehensive inventory management and resource tracking.

Ultimately, the results validate that users view RFID as an essential instrument for converting conventional libraries into contemporary, automated, and user-focused "smart" environments. The positive perception of RFID's transformative potential provides strong support for continued investment in this technology. As libraries face increasing pressure to deliver efficient, accessible, and secure services, RFID technology offers a proven pathway for meeting these demands while enhancing user satisfaction.

IV. RECOMMENDATIONS

Based on the findings of this study, the following recommendations are proposed:

1. **Expand RFID Implementation:** Library administrators should consider implementing RFID technology to improve operational efficiency and meet user expectations for modern services.
2. **Comprehensive Application:** Beyond self-checkout and security, libraries should explore RFID applications for inventory management, shelf verification, and real-time resource tracking to maximize return on investment.
3. **Staff Training:** Successful RFID implementation requires adequate training for library staff to ensure effective use and maintenance of the system.
4. **User Awareness:** While awareness is already high, libraries should continue to educate users about RFID-enabled services to encourage adoption and utilization.
5. **Future Planning:** Libraries should consider the integration potential of RFID with emerging technologies such as AI and IoT when planning for technology upgrades.

FUTURE RESEARCH DIRECTIONS

Future research may focus on comparative studies examining the cost-effectiveness of RFID versus traditional barcode systems, longitudinal studies measuring the impact of RFID on user satisfaction and operational efficiency, and qualitative research exploring the challenges faced during RFID implementation in developing country contexts.

CONFLICT OF INTEREST

The authors, Akshay Kumar, Aamir Muzafar Shah, and Sangeeta Tandia, declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. This research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest

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