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Preface

We would like to present, with great pleasure, the inaugural volume-11, Issue-1, January 2025, of a scholarly journal, *International Journal of Environmental & Agriculture Research*. This journal is part of the AD Publications series *in the field of Environmental & Agriculture Research Development*, and is devoted to the gamut of Environmental & Agriculture issues, from theoretical aspects to application-dependent studies and the validation of emerging technologies.

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

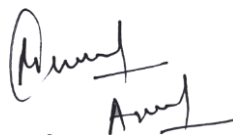
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Agriculture, Biological engineering, including genetic engineering, microbiology, Environmental impacts of agriculture, forestry, Food science, Husbandry, Irrigation and water management, Land use, Waste management and all fields related to Agriculture.

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



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He has extensive knowledge in tree fruit orchard pest management to evaluate insecticides and other control strategies such as use of pheromone traps and biological control to manage insect pests of horticultural crops. He has knowledge in agronomy, plant pathology and other areas in Agriculture which I can use to support any research from production to marketing.













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Mulberry: A Versatile Plant, its Potential Applications in different Industries

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Abstract— Mulberry is a deciduous, perennial plant that grows quickly in a variety of climatic, topographical, and soil conditions. The mulberry plant's rich constituents have led to its extensive application in a range of businesses. Mulberry has long been used as a functional food due to its high concentration of phytochemical components. Mulberry fruits are difficult to preserve due to their high water content; as a result, many value-added goods such as syrups, wines, teas, squashes, jams, breads, jellies and köme, pekmez (turkish by-products), yogurts, vinegar, parathas, biscuits, and many more are produced for optimal usage. Mulberries and their extracts contain antimicrobial, anti-hyperglycemic, anti-inflammatory, anti-hyperlipidemic, and anti-cancer activities and are used to treat a variety of acute and chronic conditions. *Morus* species' fruits, twigs, leaves, and bark exhibit significant anti-tyrosinase inhibitory activity, making it a viable cosmetic whitening agent. The current review focuses on the phytochemical composition, functioning, nutritional and nutraceutical potential of Mulberry, as well as its use as a common ingredient in many cosmetic products.

Keywords— Antimicrobial, Anti-Hyperglycemic, Anti-Inflammatory, Mulberry.

I. INTRODUCTION

Mulberry is a perennial plant that grows quickly and belongs to the genus *Morus* of the family *Moraceae*. It is an economically important plant and is extensively grown in the Asian countries to provide feed to the monophagous silkworm *Bombyx mori* L (Bhat *et al.*, 2024) which only feeds on the mulberry leaves, thus making mulberry an essential and crucial component of Sericulture Industry (Acharya *et al.*, 2022). Besides its importance in sericulture, the treasured constituents present in the mulberry plant has made an important application of this plant in various other industries (Jan *et al.*, 2021). According to the Chinese Ministry of Health in 1985, *M. alba* was recognized as the first fruit that was both therapeutic and edible (Yuan and Zhao, 2017), and its leaves and fruits were regarded as both pharmaceuticals and food (Wang *et al.*, 2014). The quantity and quality of protein, carbohydrates, dietary fiber, and the types of vitamins present inside any edible plant product are the primary factors that determine its nutritional content; in this regard, the Mulberry plants are highly valued. (Jan B *et al.*, 2021). Mulberry is well off with carbohydrates (Tantray *al* 2021), which can be found as simple sugars, starch, soluble and insoluble fibers, or both. They contain a lot of water and few calories. Fruits are mostly discovered to include iron, a good amount of potassium, vitamin C, vitamin E, and vitamin K. Additionally, they contain a lot of plant substances like anthocyanins, which give them the color and have beneficial health effects (Yu *et al.*, 2018). Mulberry fruits and leaves include significant components that make the plant eligible to be classified as a functional food that serves a purpose for health besides to its primary nutritional role. (Kadam *et al.*, 2019). Among the various species *Morus alba* (white mulberry), *Morus nigra* (black mulberry), and *Morus rubra* (red mulberry) are all well-known species in the genus *Morus* for their excellent medicinal capabilities. *M. alba* is one of the prominent species among all the others (Ercisli *et al.*, 2007). The food, pharmaceutical, and cosmetic industries may all gain from the valuable bioactive elements found in mulberry roots, bark, leaves, fruits stem and twigs (Ercisly *et al.*, 2007). In India this mulberry plant is called as “Kalpa Vruksha” for its flexible productiveness (Jan B *et al.*, 2021). The fruit is also known by the names toot and shahtoot (King's or "superior" mulberry). With numerous pharmacological qualities, almost all mulberry plant variants are traditionally acknowledged in Ayurveda, Unani, and Chinese systems of medicine. The *M. nigra*

fruits are one of the key ingredients of Tutiaswad, a Unani remedy thought to have anti-cancerous properties (Nursalam *et al.*, 2016). Anthocyanins belongs to the class of naturally occurring phenolic compounds that give fruits, flowers, and leaves their color. They are the best source of anti-inflammatory and antioxidant chemicals for health benefits. Chinese use mulberry fruit as a natural treatment. To help with urination, treat fever, strengthen the joints, control blood pressure, and protect against liver damage. Its fruits, leaves, and barks have been used in traditional Turkish Folk medicine as an antihelminthic agent, expectorant, urinary aid, blood pressure reducer, folk cure to treat dental disorders, diarrhea, and as a laxative, as well as diabetes, hypertension, arthritis, and anemia (Ozgen *et al.*, 2009). (Altaf *et al.*, 2024).

II. MULBERRY A MULTIFACETED PLANT:

The woody perennial plant known as the mulberry is included in both the genus *Morus*, which encompasses more than 15 species of deciduous plants, and the family Moraceae (Pihlanto *et al.*, 2008). Mulberry grows in a broad range of climatic, topographical, and soil conditions, from temperate to subtropical locations in the Northern Hemisphere to the tropics in the Southern Hemisphere. (Wen Peng *et al.*, 2019). Mulberry foliage has long supported the silk industry by serving as the main source of nourishment for silkworms (Hoy u *et al.*, 2002). The sole food the silkworm consumes is mulberry leaves, which it uses to create the cocoon that produces silk. Due to its numerous distinctive and peculiar traits, it has a significant commercial worth outside from sericulture (Jan B *et al.*, 2021). Mulberry being a source of of high concentration of phytochemical components, has long been used as a functional food. It is valued for both direct consumption and the manufacturing of high-value commodities. Mulberry fruits are regarded for their nutritional benefits in human health. (Sengül *et al.*, 2005). Mulberry fruits also include a variety of nutrients that are essential to human metabolism (Akbulut and Zcan, 2009). *M. alba* fruit is a great source of fiber, vitamins, minerals, lipids, protein, and carbohydrates. Fresh *M. alba* fruit has more protein than both strawberries (Rao *et al.*, 2010) and raspberries (Giampieri *et al.*, 2012) and is equivalent to blackberries in terms of protein content (Kaume *et al.*, 2012). Different types of mulberries species have bioactive substances that can lengthen life (vinkatesh *et al.*, 2008) Many scientists have studied the medicinal characteristics of mulberry plants, and they have discovered that a variety of biochemical substances, including hydroxymorcin, albafulan, albanol, Morusin, and kuwanol, which are generated from mulberry trees, are crucial in the pharmaceutical business. Discrete segments of the mulberry plant are utilized to guard the body and control oxidative stress, intestinal worms, sinusitis, throat soreness, hyperglycemia, inflammation, kidney disorders, cancer, cataracts and hepatoprotectivity, menstrual problems, depression, and migraines (Naowaboot *et al.*, 2009)

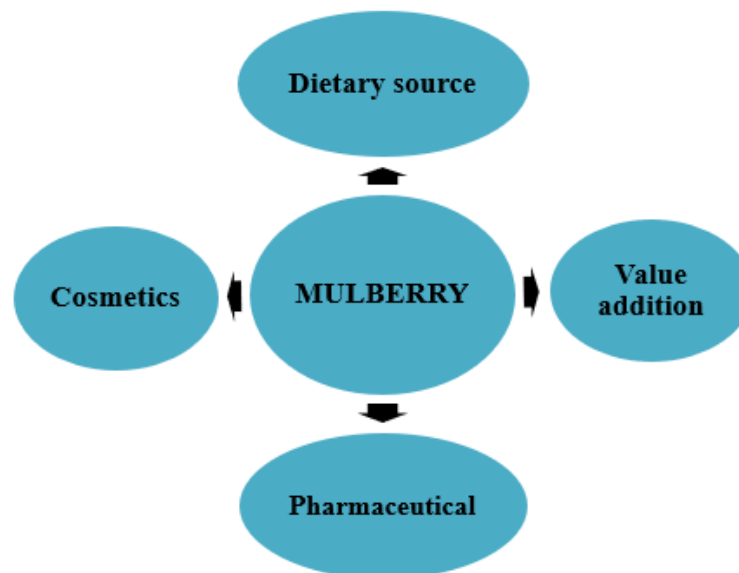


FIGURE 1: Potential utility of different parts of Mulberry

III. MULBERRY A BUDDING DIETARY SOURCE:

The flavor of fully matured mulberry fruit is incredibly tasty, enticing, and aromatic. It is prized for both direct use and the manufacture of high-value goods. Mulberry fruits are regarded for human health due to their high nutritional value (Sengül *et al.*, 2005). Mulberry fruits include a number of elements that are both beneficial and necessary for human metabolism (Akbulut *et al.*, 2009). The essential amino acid to total amino acid ratio is 42 percent, which is nearly as high as other high-protein foods

such as milk and seafood (Jiang et al., 2015). Consequently, it may be regarded as a great source of protein. Each variation of the *Morus* species includes a sizable amount of vitamin C, however *M. nigra* has the highest concentration of all the variants. The ascorbic acid concentration of *Morus alba* and *M. nigra* fresh fruit is 15.81 mg/100g and 12.81 mg/100g, respectively (Eyduan et al. (2015). Mulberries also include certain significant alkaloids that boost the immune system and activate macrophages, protecting the body from potential health risks (Kim et al., 2013). One-deoxynojirimycin (DNJ), 1, 4-dideoxy-1, 4-imino-D-ribitol, and 1, 4-dideoxy-1, 4-imino-D-arabinitol are the three most significant alkaloids discovered in mulberry leaves (Li et al., 2013, Sharma et al., 2010, Li et al.

TABLE 1
PHYSIOLOGICAL PARAMETERS OF MULBERRY

Physiochemical Properties	<i>Morus alba</i>
Protein (g ⁻¹ 100 g DW)	1.55
Fat (%)	1.1
Fiber g ⁻¹ 100 g	1.47
Ascorbic acid mg ⁻¹ 100 g	22.4
Malic acid (g 100 g ⁻¹ fw)	3.095
Citric acid (g 100 g ⁻¹ fw)	0.393
Calcium (mg ⁻¹ 100 g)	152
Mg (mg ⁻¹ 100 g)	106
K (mg ⁻¹ 100 g)	1668
Fe (mg ⁻¹ 100 g)	4.2
Nitrogen (%)	0.75
Fructose (g 100 g ⁻¹ fw)	6.269
Glucose (g 100 g ⁻¹ fw)	6.864

IV. VALUE-ADDITION

It is progressively gaining popularity with customers not only because of how good it tastes but also because of how highly nutritious and low in calories it is. The potential for using additional mulberry components as a source for processed foods is likewise quite strong. Due to this, mulberry has seen a rise in demand from the food processing sectors as well. *M.alba* squash fruits are used to make spiced squash and appetizers (Hamid et al., 2017). Pastry A pastry that has high fiber quantity and low calories is made using buckwheat flour, inulin, hulls chokeberry and *M. alba* extraction (Komolka et al., 2016). The concentrated *M. alba* paste used to create cupcakes (Jan et al., 2021), Syrup of the *M. alba* fruit is utilized directly and It can also be kept in the refrigerator for up to 6 months. (Thakur et al., 2017). Mulberry fruits can also be used to manufacture syrup since they contain high concentration of sugar. Mulberry syrup is a popular processed product in Vietnam. In the process of making syrup, the fresh fruit of mulberry and cane sugar are frequently mixed with a blender in a ceramic or a glass bottle, which can be stored indefinitely (Quang Trung et al., 2018). Anthocyanins obtained from dried fruits of *M. nigra* can be used to make chocolate (Gultekin-Ozguven et al., 2016). Producing pasta using *M. nigra* extraction, which has a hypoglycemic effect and lowers the glycemic index (Yazdankhah et al., 2019). Mulberry leaf methanolic extract increases the shelf life of minced beef (Yazdankhah et al., 2019). Anthocyanins from *M. rubra's* yogurt serve as a strawberry coloring ingredient. (Byamukama et al., 2014). Jam created with fruit jam made with a 70:30 ratio of rosella and mulberry fruit essence (Wongchalat et al., 2016). *M. alba* is used to manufacture vinegar (Karaagac et al., 2016). Alcoholic drink of *M. alba* fruit is used to make alcoholic beverages and make fruit wine. (Daris et al., 2003).

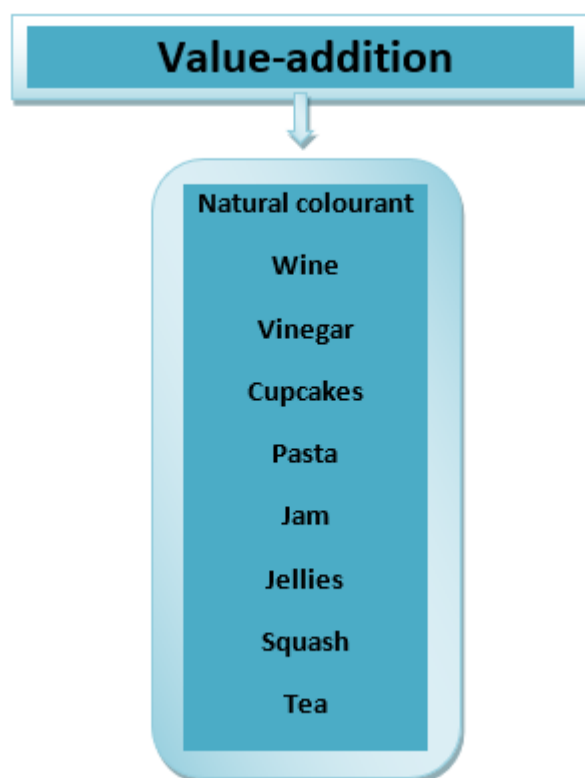


FIGURE 2: Value Addition

V. PHARMACEUTICAL USES OF MULBERRY:

Mulberry fruits, as previously indicated, are abundant in anthocyanins (Jiang *et al.*, 2013), which both academics and consumers due to their possible pharmacological effects on health (Carvalho *et al.*, 2013). Sugars, rutin, quercetin, have piqued the curiosity of volatile oil, amino acids, vitamins, and microelements. Mulberry leaves contain a variety of therapeutic compounds including lowering blood sugar, preventing hyperlipidemia, treating hypertension, and acting as an antibacterial and antiviral (Zou *et al.*, 2003). Low-density lipoprotein (LDL) oxidation can be prevented by anthocyanins found in mulberry fruits, and they can also remove free radicals (Du Q *et al.*, 2008).

5.1 Anticancer activity:

Cancer, in all of its manifestations, is the most fatal disease on the planet. There are several types of cancer therapies available on the market, but only a handful is successful and safe. Since the beginning of time, humans have utilized the mulberry plant as medicine. Mulberry has been shown in clinical studies that plants may impede cell development, most likely because to the presence of flavonoids, which are strong anticancer agents. Mulberries have been shown in animal studies to help prevent cancer. Purified anti-proliferative lectin from *M. alba* leaves caused apoptosis in human breast cancer (MCF-7) and colon cancer (HCT-15) cells by generating crucial morphological changes and DNA fragmentation associated with apoptosis. (Deepa *et al.*, 2012). Human lung metastasis when anthocyanins from the fruit of *M. alba* are isolated, A549 cancer cells invade. The leaves of this plant contain flavonoids, which have been isolated and found to have anticancer effects. (Khalid *et al.*, 2011). Albanol A (Mulberrofuran G) may be extracted from the root bark of mulberries; this chemical induces high cytotoxicity in HL60 (Human Leucemia Cell line) by inhibiting topoisomerase II activity (Kadam *et al.*, 2013)

5.2 Anti-inflammatory activity:

Multiple immune cells, including macrophages, use inflammation as an inherent immunological defense against potentially hazardous stimuli including bacteria and viruses (Nagarta *et al.*, 2005). Large levels of inflammatory mediators, such as nitric oxide (NO) and prostaglandin E2 (PGE2), are created as a result of an excessive inflammatory response (LeeYG *et al.*, 2008). If left unchecked for a while, it has the potential to harm the body and lead to the development of chronic diseases such as cancer, cardiovascular disease, and rheumatoid arthritis. Mulberry twigs and root bark include maclurin, morin, resveratrol, and isoquercitrin, while the fruits contain other vital fatty acids such as palmitic, linoleic, and oleic acids, which are also crucial for inflammatory responses (Kadam *et al.*, 2019). Methanolic extracts of *M. alba* root bark suppressed no future generations

by lowering iNOs over-expression in LPS- stimulated RAW264.7 9 (Eo *et al.*, 2014). *M.nigra* fruit contains anthocyanins, which have been shown to have anti-inflammatory effects. C3G and C3R have an anti-inflammatory impact in mice with carrageenan and xylene-induced paw edema via suppressing pro-inflammatory cytokines (chen *et al.*, 2014). *M. alba* root aqueous extract has strong anti-histamine and anti-allergic activity by reducing histamine release and systemic allergic responses produced by compound 48/80 in vitro and in vivo. *M. alba* root extract also suppresses mast cell-mediated allergy reactions. (Chai *et al.*, 2005).

5.3 Anti-hyperglycemic activity:

Diabetes mellitus (DM), the world's third most deadly metabolic illness, is characterized by hyperglycemia (high blood glucose levels). This chronic condition now affects more than 170 million people globally, and by 2030, the number is expected to double it is predicted to climb by 50%, with the greatest growth occurring in emerging nations including those in Asia, Africa, and South America (Wang *et al.*, 2013). In recent study *M. alba* leaves were found to help decrease blood sugar levels and minimize possible histo-pathological abnormalities in the pancreas and kidneys in brown rats (Kadam *et al.*, 2019). *M. alba* leaves help to reduce blood glucose levels and avoid any histo-pathological changes in the pancreas and kidneys that were discovered in brown rats in a recent study. (Kadam *et al.*, 2019).

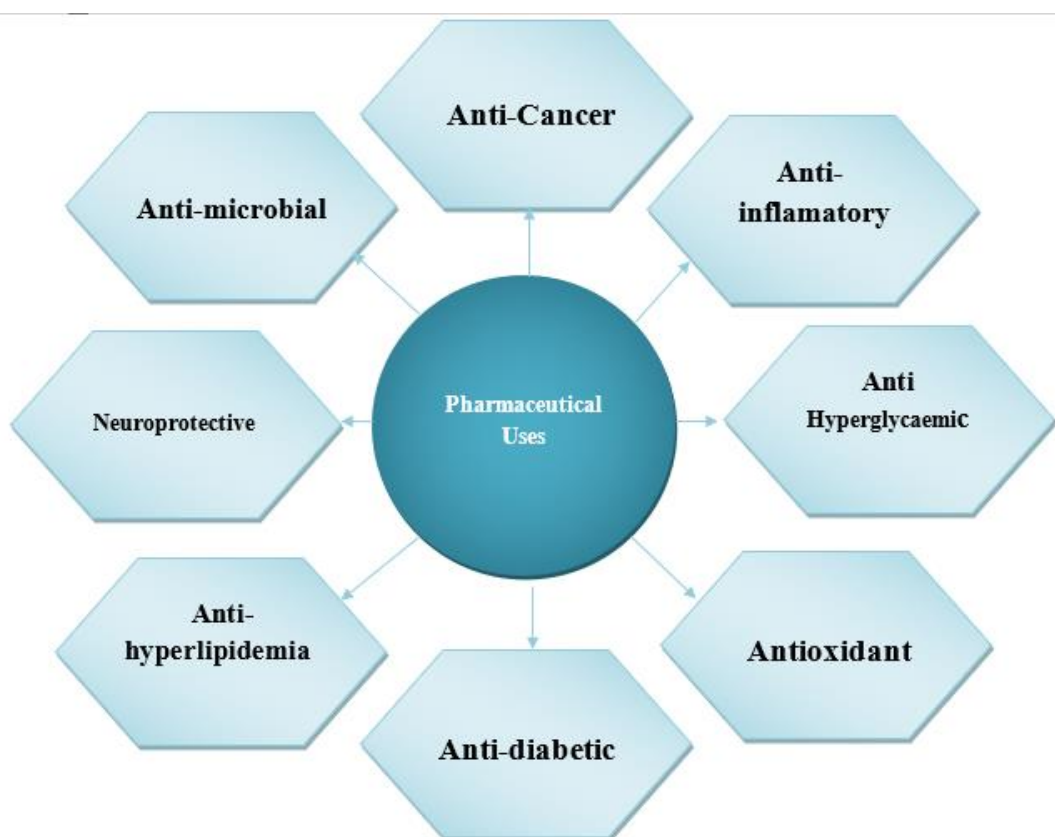


FIGURE 3: Anti-hyperglycemic activity

VI. COSMETIC INDUSTRY:

Skin lightening, sometimes referred to as skin whitening or depigmentation, is an aesthetic procedure that uses laser therapy or skin-lightening materials to reduce the appearance of dark skin spots and lighten the skin's overall tone (Rendon *et al.*, 2005). Exposure to UV rays or chemical irritants can result the darkening of skin or skin hyperpigmentation, which can also be a symptom of other skin conditions such melasma, solar lentigines, or post-inflammatory hyperpigmentation (Draelos *et al.*, 2013). In order to achieve a lighter skin tone, skin-lightening chemicals are used to decrease the release of the melanin production, the primary pigment in the skin (Kamakshi *et al.*, 2013). Paper mulberry extracts and its components shown potent tyrosinase activity inhibitory effects and have been used as skin-whitening agents in cosmetic products (Shivhare *et al.*, 2013). A face mask sheet made of paper mulberry and *Styela clava* extract is mixed for whitening purposes (Yun *et al.*, 2013). The skin responded favorably to a mask kit including paper mulberry (Go Un *et al.*, 2009). A cosmetic formulation for hydrating and smoothing the skin included paper mulberry and white ginseng (Slominski *et al.*, 2018).

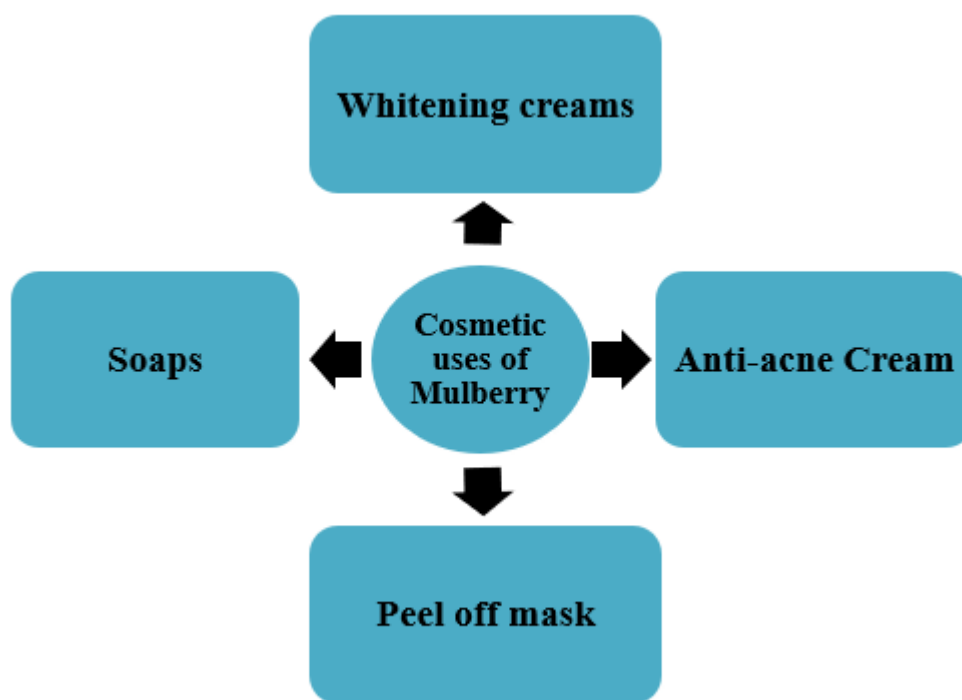


FIGURE 4: Cosmetic Industry

VII. CONCLUSION

The purpose of this mini review is to highlight the usefulness and applicability of *Morus* species in a variety of industries, and the debate demonstrated that Mulberry is a diversified and intriguing plant. The findings indicated that the *Morus* species might be suitable for a range of culinary products. This plant can be utilized in the creation of hypocaloric diets and as a new component to improve the functional properties of present meals because it has less calories. *Morus* is used to create value-added products such as jam, syrup wine, vinegar, jelly, tea, squash, and many others, allowing businesses to make better use of its fruits and leaves. The bioactive phytochemicals have been shown to have a wide range of biological activities including antioxidants, anti-inflammatory, anti-cancerous, anti-obesity, antihypertensive and hyperlipidemia effects. Varying species of mulberry plants have significantly varying nutritional contents, active component content and antioxidant activity. Cosmetics are widely used to cure hyperpigmentation. Because these conditions are frequently difficult to treat, cosmeceuticals and other skin-lightening solutions are required. As a result of current safety concerns, there is a growing need for alternative natural, safe and effective skin-lightening therapies. Similarly, academics and manufacturers should place greater attention on large-scale mulberry production for further betterment. By taking into account all of the facts about mulberry such as its potential for sericulture industry, human health and lifestyle improvement as supplementary diet, value addition in confectionary products, Cosmetic products. Thus, it can be considered a most ideal and beneficial plant for a sustainable development.

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Comparative Growth Performance of Arbor Acre and Ross Broiler Breeds under Different Lactobacillus Supplementation Levels

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Abstract— The experiment was designed to verify how supplementation chicken feed with *Lactobacillus* would affect the performance of Arbor Acre and Ross broilers. In total, 240-day-old chicks of both sexes were randomly allotted to four groups that had different *Lactobacillus* supplementation levels of 0%, 5%, 10%, and 15% for eight weeks, during which time all the performance parameters were measured, including initial and final body weight, total weight gain, feed intake, and feed conversion ratio. Results have shown that breeds are significantly different and so are the supplementation levels. The 10% *Lactobacillus* supplementation level proved the best, improving final body weight by 16.36%, average daily weight gain by 16.83%, and hence optimized feed conversion ratio by 17.65%. Arbor Acre always had higher initial body weight and feed intake compared to Ross breeds. Generally, the study has shown that targeted probiotic supplementation could serve as a viable alternative to antibiotic use in poultry besides determining an optimum inclusion level of *Lactobacillus* to enhance performance in broilers Light.

Keywords— *Lactobacillus*, broiler breeds, probiotics, growth performance, feed efficiency.

I. INTRODUCTION

Broiler chicken production continues to evolve with increasing demands for efficient meat production and improved animal health (Maharjan *et al.*, 2021). Genetic variations between breeds (like Arbor Acre and Ross), combined with nutritional interventions such as probiotic supplementation, offer potential strategies to optimize growth performance (Khomayezhi & Adewole, 2022). *Lactobacillus* probiotics have demonstrated promising effects on gut microbiota, nutrient absorption, and immune function in poultry (Kabir, 2009).

Probiotics have emerged as a promising alternative to antibiotics in poultry production, offering potential improvements in growth performance and overall bird health (Jha *et al.*, 2020). The use of *Lactobacillus* as a dietary supplement has garnered increasing attention due to its potential to enhance nutrient utilization, protect intestinal integrity, and stimulate animal growth (Kabir, 2009; Wu *et al.*, 2019).

Previous studies have demonstrated varied responses to probiotic supplementation, with some researchers reporting significant improvements in broiler performance (Ramlah and Tan, 1995; Uzabaci & Yibar, 2023), while others observed inconsistent results (Blajman *et al.*, 2014). The variability in outcomes has been attributed to factors such as probiotic strain, administration method, chicken genotype, and experimental conditions (Mountzouris *et al.*, 2007; Patterson & Burkholder, 2003).

Limited research exists on the comparative response of different broiler breeds to varying levels of *Lactobacillus* supplementation (Mountzouris *et al.*, 2007). This study aims to address this knowledge gap by systematically evaluating growth parameters across two prominent broiler breeds (Arbor Acre and Ross) under controlled probiotic interventions, thus addressing the existing knowledge gaps in probiotic application in poultry production.

II. MATERIALS AND METHODS

2.1 Experimental Site and Design:

The study was conducted at the Poultry Unit of the Teaching and Research Farm, Department of Animal Science and Technology, Nnamdi Azikiwe University, Awka (Latitude 6°12'25" N, Longitude 7°04'04" E, altitude 9 meters). A total of 240 day-old mixed-sex chicks from two broiler breeds (Arbor Acre and Ross) were sourced from Agrited Farms and CHI Farms in Ibadan, Nigeria.

2.2 Experimental Animals and Housing:

The chicks were delivered overnight and brooded for one week in deep litter. Houses were cleaned with detergent and Izal disinfectant, and space allowance was 0.05 m²/bird (0-3 weeks) and 0.11 m²/bird (4-8 weeks). Wood shavings were spread to a height of 5 cm, and heat was provided by 100-watt bulbs.

2.3 Lactobacillus Preparation:

Lactobacillus was isolated by fermentation using water yam wash water and raw cow milk. Isolation and identification were done using Bergey's Manual of Systematic Bacteriology, while confirmation was based on colony morphology, gram staining, biochemical tests (catalase, oxidase) and carbohydrate fermentation profiles.

2.4 Probiotic Culture Preparation:

The isolated Lactobacillus strains were:

- Inoculated in MRS broth
- Incubated at 37°C for 48 hours
- Centrifuged at 5000 rpm
- Washed with Phosphate Buffered Solution
- Preserved in 30% glycerol at -80°C

2.5 Experimental Design:

Chicks were randomly assigned to four treatment groups per breed, with three replicates of 10 birds each:

- Treatment 1 (T1): Control
- Treatment 2 (T2): 5 mL Lactobacillus
- Treatment 3 (T3): 10 mL Lactobacillus
- Treatment 4 (T4): 15 mL Lactobacillus

2.6 Feeding and Management:

- Commercial feed (New Hope) used for starter and finisher phases
- Ad libitum feeding
- Chick trays used initially, replaced with conical feeders
- Lactobacillus supplementation via drinking water daily for 8 weeks

2.7 Data Collection:

2.7.1 Performance Parameters:

- Individual bird weights recorded weekly using Camry EK5055 scale (± 0.01 g)
- Daily feed intake measured
- Feed conversion ratio calculated

2.7.2 Blood Parameters:

- Blood collected from wing web 24 hours after final dosage

Samples processed for: Hematological analysis (RBC, WBC, PCV, hemoglobin) and Serum biochemistry (protein, cholesterol, liver enzymes).

2.8 Data Analysis:

Data were analysed using SPSS version 20, two-way ANOVA within completely randomized design. Significant means were separated by Least Significant Difference (LSD) test at 5% level of significance.

2.9 Ethical Considerations

The experiment was conducted with due considerations to institutional animal care, and welfare, and, as much as possible, distress during sample collection was minimized.

III. RESULTS

The mean growth performance of Arbor Acre and Ross broiler breeds and the mean growth performance of broilers injected different levels of lactobacillus bacterial are presented in Tables 1 and 2.

TABLE 1
MEAN GROWTH PERFORMANCE OF ARBOR ACRE AND ROSS BROILER BREEDS

Parameter	Breed		SEM	P-value
	Arbor Acre	Ross		
IBWT (g)	182.88a	144.12b	0.93	0
FBWT (g)	3943.2	3891.76	42.12	0.396
TWG (g)	3760.31	3747.63	41.51	0.831
ADWG (g)	67.15	66.92	0.74	0.829
ADFI (g)	124.01a	112.40b	0.47	0
TFI (g)	6944.44a	6282.25b	22.05	0

IBWT= initial body weight, FBWT= Final body weight, TWG= total weight gain, ADWG = average daily weight gain, ADFI= average daily feed intake, TFI= total feed intake, FCR= feed conversion ratio.

TABLE 2
MEAN GROWTH PERFORMANCE OF BROILERS INJECTED DIFFERENT LEVELS OF LACTOBACILLUS BACTERIAL

Parameter	Levels of Lactobacillus				SEM	P-value
	0%	5%	10%	15%		
IBWT (g)	157.36b	163.95a	167.20a	165.49a	1.317	0
FBWT (g)	3510.79b	4019.88a	4084.99a	4054.24a	59.56	0.396
TWG (g)	3353.43b	3855.93a	3917.76a	3888.75a	58.7	0.831
ADWG (g)	59.88b	68.86a	69.96a	69.44a	1.05	0.829
ADFI (g)	121.17a	116.17b	117.77b	117.72b	0.66	0
TFI (g)	6760.50a	6505.50b	6594.88b	6592.50b	32.04	0
FCR	2.04b	1.69a	1.68a	1.69a	0.04	0.003

a-b =Means on the same row with different superscripts are significantly different(P<0.05)

IBWT= initial body weight, FBWT= Final body weight, TWG= total weight gain, ADWG = average daily weight gain, ADFI= average daily feed intake, TFI= total feed intake, FCR= feed conversion ratio.

The initial body weight (IBWT) showed significant variations across different experimental conditions. In the breed comparison, Arbor Acre chickens demonstrated a significantly higher initial body weight (182.88g) compared to Ross breed chickens (144.12g), with a statistically significant difference (P=0.000) (Table 4.1). When examining the impact of

Lactobacillus supplementation, a notable trend in IBWT was observed (Table 2). The control group (0% Lactobacillus) had the lowest initial body weight (157.36g), while groups supplemented with 5%, 10%, and 15% Lactobacillus showed significantly higher initial weights (163.95g, 167.20g, and 165.49g, respectively). The 10% Lactobacillus group recorded the highest initial body weight among all treatment groups. These findings suggest that both breed type and Lactobacillus supplementation can significantly influence the initial body weight of broiler chickens, with statistically significant differences detected across groups ($P < 0.05$).

The final body weight (FBWT) and total weight gain (TWG) exhibited interesting patterns across different experimental conditions (Table 4.1 and 4.2). In the breed comparison, Arbor Acre and Ross chickens showed minimal differences in final body weight (3943.20g vs. 3891.76g, respectively), with no statistically significant variation ($P = 0.396$). Similarly, total weight gain was comparable between the two breeds (3760.31g for Arbor Acre vs. 3747.63g for Ross, $P = 0.831$). Lactobacillus supplementation demonstrated more pronounced effects. The control group (0% Lactobacillus) showed the lowest final body weight (3510.79g), while groups supplemented with 5%, 10%, and 15% Lactobacillus exhibited significantly higher final weights (4019.88g, 4084.99g, and 4054.24g, respectively). Correspondingly, total weight gain was significantly improved in the supplemented groups, with the 10% Lactobacillus group achieving the highest total weight gain (3917.76g). Again, these results suggest that Lactobacillus supplementation can effectively enhance broiler growth performance.

Total feed intake (TFI) and feed conversion ratio (FCR) revealed significant differences across experimental groups. In the breed comparison, Arbor Acre chickens demonstrated a substantially higher total feed intake (6944.44g) compared to Ross breed chickens (6282.25g), with a statistically significant difference ($P = 0.000$). The Lactobacillus supplementation groups showed marked variations in feed intake and efficiency. The control group (0% Lactobacillus) exhibited the highest total feed intake (6760.50g), while supplemented groups showed reduced intake (6505.50g to 6594.88g). Correspondingly, feed conversion ratio was significantly improved in the Lactobacillus-supplemented groups.

The control group had the least efficient feed conversion ratio of 2.04, whereas groups supplemented with 5%, 10%, and 15% Lactobacillus demonstrated improved FCR values (1.69-1.68), with statistically significant differences ($P = 0.003$). These findings still suggest that Lactobacillus supplementation can enhance feed efficiency in broiler chickens, potentially reducing overall feed consumption while maintaining growth performance.

Lactobacillus supplementation demonstrated significant improvements across various growth performance parameters. At progressive supplementation levels, notable enhancements were observed. The initial body weight increased from 157.36g in the control group (0% Lactobacillus) to a peak of 167.20g at 10% supplementation, representing a 6.26% improvement. Final body weight showed a substantial increase from 3510.79g in the control group to 4084.99g at 10% supplementation, indicating a 16.36% enhancement. Correspondingly, average daily weight gain improved from 59.88g to 69.96g, representing a 16.83% increase in growth rate. Most remarkably, feed conversion ratio demonstrated significant optimization, improving from 2.04 in the control group to 1.68 at 10% Lactobacillus supplementation. This 17.65% improvement in feed efficiency suggests that Lactobacillus supplementation can effectively enhance broiler chicken metabolism, nutrient absorption, and overall growth performance. These findings highlight the potential of targeted probiotic supplementation in poultry nutrition.

IV. DISCUSSION

The present study investigated the application of Lactobacillus on growth performance between two broiler breeds, Arbor Acre and Ross, and provided useful information regarding probiotic interventions in poultry production. The results agree and extend previous studies conducted to investigate potential benefits of probiotic supplementation in broiler nutrition.

Initial body weight and feed intake are significantly different between Arbor Acre and Ross breeds. Initial body weight was 182.88g and the total feed intake was 6944.44g for Arbor Acre chickens. These are significantly higher when compared to Ross chickens. This evidences that breed consideration is important in nutritional intervention programs. Genetic differences in broiler performance have been echoed elsewhere. Havenstein *et al.* (2003) suggested that nutritional strategies may be needed, which are tailored to a particular genetic line.

The most interesting results were obtained in the supplementation groups treated with Lactobacillus. Such probiotic supplementation with different concentrations exhibited regular enhancements regarding growth performance parameters. Thus, with a supplementation level of 10% Lactobacillus, more encouraging results may be obvious, improving initial body weight, final body weight, and feed conversion ratio. The improvement in feed conversion ratio (FCR) from 2.04 in the control group to 1.68 in supplemented groups is particularly noteworthy. This 17.65% enhancement in feed efficiency aligns with

research by Mountzouris *et al.* (2007), who highlighted the potential of probiotics to optimize nutrient utilization and metabolic efficiency in broiler chickens. The mechanism behind this improvement likely involves enhanced gut microbiota composition and improved nutrient absorption (Kabir, 2009).

The significant increase in average daily gain from 59.88g in the control group to 69.96g in the 10% Lactobacillus group represents a 16.83% improvement. This result supports previous studies by Begdildayeva *et al.* (2024) showing that probiotics have the potential to enhance growth performance in broiler chickens. This increase in weight gain could partly be explained by a number of factors including gut health, increased nutrient digestion, and changes in the intestinal microbiota (Fesseha *et al.* 2021).

Interestingly, this study demonstrated a non-linear relationship in the performances due to supplementation with Lactobacillus. While 5%, 10%, and 15% supplementation groups outperformed the control group, 10% was the most effective among those, suggesting an optimal dosage range beyond which further supplementation additions do not translate into greater improvements, in a similar vein to the dose-dependent response of many probiotic intervention studies (Patterson & Burkholder, 2003).

V. CONCLUSION

The study clearly shows the most promising role of Lactobacillus supplementation in broiler chickens and an optimum inclusion level at 10% is clearly indicated. The results have showed that there is great improvement in feed conversion ratio, weight gain, and overall growth performance, which recommend probiotics as a promising substitute for conventional antibiotics in poultry nutrition. This study forms a valuable addition to already existing knowledge regarding economic and performance benefits of targeted probiotic interventions.

While encouraging, the present findings call for additional studies concerning the exact modes of action of probiotics, longer-term effects, and possible strain-specific differences. More studies will be needed altogether to explain the overall Lactobacillus supplementation effects given under variable conditions of diet and genetics.

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Haematological and Serum Biochemical Responses of Broiler Chickens to Lactobacillus Probiotic Supplementation

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Abstract— This study evaluated the haematological and serum biochemical responses of two broiler chicken breeds, Arbor Acre and Ross, to different levels of Lactobacillus probiotic supplementation. This experiment was a completely randomized design with 2 × 4 factorial arrangements where 240 day-old mixed-sex broiler chicks of both Arbor Acre and Ross chicken breeds were allotted to four groups and fed 0, 5, 10, and 15 mL of Lactobacillus, respectively. The results of the haematological analysis indicated that the packed cell volume, RBC count, and haemoglobin were significantly enhanced, and the best enhancements were with 10 mL supplementation level. These changes were more pronounced in Ross chickens, breed specifically. The promising results in serum biochemistry took in renal function markers, lowered total cholesterol levels, and possible immunomodulatory consequences. Dietary supplementation with Lactobacillus positively altered the blood characteristic within the normal physiological range. Significantly different mineral profiles, enzymatic activities, and protein metabolisms between breeds and levels of dietary supplementation were recorded. The results suggest that probiotic supplementation can optimize broilers' health, and the daily dosage of 10 ml seems to impart the most pronounced benefits.

Keywords— Lactobacillus, broiler chickens, probiotic supplementation, haematology, serum biochemistry.

I. INTRODUCTION

Poultry production is one of the mainstays in ensuring global food security, with broiler chicken meat being one of the key protein sources for millions of people around the world. With growing demand for improved efficiency and sustainable meat production, scientists and agricultural professionals are turning more toward nutritional interventions that could optimize health, performance, and immune function in birds. Probiotics have emerged as one of the promising strategies in improving broiler chicken production because they have certain advantages over antibiotic interventions (Gadde *et al.*, 2017).

In general, probiotics have been a good area for animal nutrition scientists as they modulate gut microbiota, digestion, and immunity. These populations can attach to the gastrointestinal tract as a competitor against pathogenic microorganisms to promote better health in birds as evidenced by Mohd Shaufi *et al.*, (2015). The modes of action for these are rather complex because of interactions between microbial population populations with intestinal epithelium cells and the immune system as a whole, making this an interesting area of science to venture into.

Haematological and serum biochemical parameters are vital in assessing the physiological response of broilers to dietary manipulations. These parameters are sensitive indicators of metabolic status, immunity and health, thus providing insight to the researcher on nutritional supplements, including probiotics, which influence the physiology of birds (Bah *et al.*, 2022). Probiotics have also been seen in various studies to alter characteristics of blood profiles across different animals, which may indicate better metabolic efficiency and immunity.

Despite the increased interest in probiotic applications, results have been highly variable due to a variety of probiotic strains, dosages, and conditions of the experiment (Al-Shawi *et al.*, 2020). That variability showed the need for broad-based studies that assess the effects of certain Lactobacillus strains on several health markers of broiler chickens. According to Shokryazdan *et al.* (2017), understanding such disparate responses is crucial in developing target nutritional strategies that will enhance the production potential and welfare of broiler chickens.

This present study is aimed at comprehensively investigating the haematological and serum biochemical responses of broiler chickens fed with *Lactobacillus* probiotic supplementation. A battery of physiological parameters was employed to see the possible mechanism through which these useful microorganisms impact the health and performance of birds. This work also contributes to the ever-growing knowledge in the application of probiotics in poultry nutrition and helps to foster more efficient, sustainable agricultural practices.

II. MATERIALS AND METHODS

2.1 Experimental Site and Design:

The study was conducted at the Poultry Unit of the Teaching and Research Farm, Department of Animal Science and Technology, Nnamdi Azikiwe University, Awka campus, which is located at Latitude 6°12'25"N and Longitude 7°04'04"E, with an average elevation of 9 metres above sea level. Two hundred and forty (240) day-old mixed-sex broiler chicks of two breeds (Arbor Acre and ROSS) were procured from Agrited Farms and CHI Farms, Ibadan. Birds were then taken to the experimental site and allowed to acclimate for one week.

The experimental design was a 2×4 factorial arrangement in a completely randomized design. Birds were divided into four treatments: T1 (control), T2 (5 ml *Lactobacillus*), T3 (10 ml *Lactobacillus*), and T4 (15 ml *Lactobacillus*). Each treatment group per breed was replicated thrice with ten chicks per replicate.

2.2 Animal Housing and Management:

The birds were brooded on deep litter within detergent and Izal-disinfected pens. 0.05m²/bird was provided for 0-3 weeks and 0.11m²/bird from 4-8 weeks. The pens were spread with five centimetres of wood shavings while 100-watt bulbs were provided as the source of heat. New Hope commercial feeds were provided ad libitum during both starter and finisher phases; water was supplied through three-litre drinkers.

2.3 Probiotic Preparation and Administration:

The *Lactobacillus* cultures were isolated from the fermented yam wash water and raw cow milk. As identified by Bergey's Manual of Systematic Bacteriology (Kandler and Weiss, 1986), the technique adopted for identification included a check on the colony morphology, Gram staining and biochemical tests. The cultures were prepared in MRS broth, centrifuged, washed with buffered salt solution and stored in 30% glycerol at -80°C. The *Lactobacillus* was administered daily through drinking water with different volumes including 5mL, 10mL and 15mL for eight weeks.

2.4 Blood Sample Collection and Analysis:

Blood was collected from three birds per treatment randomly selected at the termination of the experiment (day 55) with the aid of wing web venipuncture. The blood was collected into heparinized and nonheparinized bottles with utmost care taken to prevent haematoma and stress on the animals.

Haematological parameters were made following standard procedures. The red blood cell count and white blood cell count were made by haemocytometer using Natt-Herrick solution. Haematocrit (packed cell volume) and haemoglobin were estimated using micro haematocrit and Sahli's method respectively (Brundha and Priyadharshini, 2019). Peripheral blood leukocyte percentage was done using May Grunwald-Giemsa stained blood smears (Chari and Prasad, 2018).

2.5 Serum Biochemistry Analysis:

Serum was harvested following centrifugation of blood at 930.7g for 10 minutes. Biochemical assay was carried out for different biochemical parameters, viz., total protein by biuret reaction, creatinine by Jaffe reaction, and cholesterol by the method of Kaneko *et al.* (2008). Alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphatase were assayed calorimetrically using Randox reagent kit.

2.6 Statistical Analysis:

Data were analysed using Analysis of Variance (ANOVA) through Statistical Package for Social Sciences (SPSS version 20). Significant means were separated using Least Significant Difference (LSD) at a 5% significance level.

III. RESULTS AND DISCUSSION

3.1 HAEMATOLOGICAL Biochemical Responses:

TABLE 1
MEAN HAEMATOLOGY PERFORMANCE OF ARBOR ACRE AND ROSS BROILER BREEDS

Parameter	Breed		SEM	P-value
	Arbor Acre	Ross		
PCV (100%)	27.67b	29.83a	0.31	0
RBC($10^{12}/\text{mm}^3$)	1.74b	2.25a	0.03	0
HB(g/dl)	9.19b	10.00a	0.12	0
MCV(fl)	158.92a	134.33b	1.54	0
MCH(pg)	52.79a	45.02b	0.55	0
MCHC(%)	33.22a	33.77b	0.15	0.21
WBC($10^3/\text{mm}^3$)	57.92b	59.06a	1.18	0.504

a-c Means on the same row with different superscripts are significantly different(P<0.05)

PCV= parked cell volume, RBC= Red blood cell, HB= Haemoglobin, MCV= mean corpuscular volume, MCH= mean corpuscular haemoglobin, MCHC= mean corpuscular haemoglobin concentration, WBC= white blood cell

TABLE 2
MEAN HAEMATOLOGY PERFORMANCE OF BROILERS INJECTED DIFFERENT LEVELS OF LACTOBACILLUS BACTERIA

Parameter	Levels of Lactobacillus				SEM	P-value
	0%	5%	10%	15%		
PCV (100%)	26.00 ^C	28.17 ^b	30.83 ^a	30.00 ^a	0.44	0
RBC($10^{12}/\text{mm}^3$)	2.39 ^b	2.59 ^a	2.77 ^a	2.62 ^a	0.44	0
HB(g/dl)	8.65 ^C	9.43 ^b	10.28 ^a	10.02 ^a	0.17	0
MCV(fl)	153.67 ^a	143.83 ^b	145.00 ^b	144.00 ^b	2.18	0.026
MCH(pg)	51.17 ^a	48.03 ^b	48.37 ^b	48.05 ^b	0.78	0.032
MCHC(%)	33.27	33.32	33.32	33.9	0.21	0.189
WBC($10^3/\text{mm}^3$)	54.28b	59.72a	59.78a	60.17a	1.67	0.072

a-c Means on the same row with different superscripts are significantly different(P<0.05)

PCV= parked cell volume, RBC= Red blood cell, HB= Haemoglobin, MCV= mean corpuscular volume, MCH= mean corpuscular haemoglobin, MCHC= mean corpuscular haemoglobin concentration, WBC= white blood cell

TABLE 3
MEAN HAEMATOLOGY PERFORMANCE OF ARBOR ACRE AND ROSS BROILER BREEDS INJECTED WITH DIFFERENT LEVELS OF LACTOBACILLUS SUPPLEMENTATION

Parameter	Breed x Level of Lactobacillus								SE M	Pvalue
	AAx0%	AAx5%	AAx10%	AAx15%	Rx0%	Rx5%	Rx10%	Rx15%		
PCV (100%)	25.67	27	29.33	28.67	26.33	29.33	32.33	31.33	0.62	0.285
RBC($10^{12}/\text{L}$)	1.64c	2.73a	2.80a	2.20b	1.75c	2.24b	2.54ab	2.45ab	0.57	0
HB(g/dl)	8.5	8.93	9.73	9.6	8.8	9.93	10.83	10.43	0.24	0.363
MCV(fl)	157.00a b	142.00b	162.67a	160.00ab	150.33 b	131.67 c	127.33 c	128.00 c	3.08	0.001
MCH(pg)	52.00ab	51.53ab	54.07a	53.57ab	50.33b	44.53c	42.67c	42.53c	1.1	0.001
MCHC(%)	33.13	33.1	33.13	33.5	33.4	33.87	33.5	34.3	0.3	0.752
WBC($10^3/\text{mm}^3$)	55.6	57.03	58.7	60.33	52.97	62.4	60.87	60	2.36	0.396

a-c Means on the same row with different superscripts are significantly different(P<0.05)

PCV= parked cell volume, RBC= Red blood cell, HB= Haemoglobin, MCV= mean corpuscular volume, MCH= mean corpuscular haemoglobin, MCHC= mean corpuscular haemoglobin concentration, WBC= white blood cell

Haematological examination between chickens revealed marked significant differences due to the different levels of Lactobacillus supplementation and chicken breed, as presented in tables 1, 2 and 3. The Pack Cell Volume was significantly different ($p < 0.05$). The birds treated with 10 ml Lactobacillus had the highest value of PCV as 30.83%. Though very important, all are within the normal physiological value that is expected in chickens, ranging from 22 to 35%. For breeds, Ross chickens were significantly higher with a PCV of 29.83% compared to Arbor Acre chickens, accounting for 27.67%. The bird's blood composition and oxygen carrying will be improved, though its normal physiological range is between 22 and 35%. Breed variations between Ross and Arbor Acre chickens also hint at genetic variations affecting the blood cell concentration.

Haemoglobin levels showed a significant difference ($p < 0.05$) due to Lactobacillus inclusion levels. Thus, the T3 treatment group revealed significantly higher levels of haemoglobin when compared to the control group. Range for the observed haemoglobin was between 8.65 g/dl to 10.28 g/dl. No significant differences were witnessed due to breed or their interactions with Lactobacillus. In RBCs, a significant difference was noted on Lactobacillus inclusion levels with the highest of 2.77×10^6 observed in T3. Significant differences were also found between Arbor Acre and Ross; however, no significant interaction was noted between breed and Lactobacillus inclusion. The increase in such aspects indeed reflects an increase in the production of blood cells, probably an improved immune system and overall optimization of blood health. These differences among breeds further point out that the interaction between supplementation and genetic makeup is complicated.

MCV has presented significant differences ($p < 0.05$), and the highest value was expressed by the control group. Values ranged between 143.83 fl - 153.67 fl. Significant differences were observed among breeds and in breed-Lactobacillus interactions. In MCH, there was a significant difference ($p < 0.05$), and the control group (T1) is the highest with a value of 51.17 pg, while T2 had the lowest with 48.03 pg. The Arbor Acre chickens had presented a higher value of 52.79 than that of Ross chickens, which was 45.02. Significant differences had been obtained in the interaction between breed and Lactobacillus.

While MCHC did not show significant variation across the different levels of Lactobacillus inclusions, significant variations were recorded among breeds with no significant breed-Lactobacillus interaction detected. This stability only creates a consistent concentration of haemoglobin inside the cells, hence a sign that supplementation does not alter the basic cellular haemoglobin density. White Blood Cell values also varied significantly across the different inclusions of Lactobacillus ($p < 0.05$). Significant variations were also obtained among breeds, though no significant breed \times Lactobacillus inclusion interaction was detected. These variations may result from possible alterations to inflammatory responses or because breeds show different immune cell production.

The comprehensive haematological analysis conducted in the study provides an overview of the broiler chicken physiological responses due to the supplementation of Lactobacillus probiotic. From the result, it can be observed that the supplementation of Lactobacillus showed positive effects on the various haematological parameters across studies. There was a significant elevation at 10 mL (T3) of PCV, RBC, and haemoglobin. Significant increase of Red Blood Cell and Packed Cell Volume upon the administration of Lactobacillus probiotics may indicate better erythropoiesis and absorption of iron. This is in agreement with our finding, where Pertiwi and Mahendra (2021) stated that probiotics may enhance nutrient absorption and increase erythrocytes production.

The differences noted between Arbor Acre and Ross breeds on all parameters underline genetic factors that are very important in probiotic response. Ross chickens gave consistently higher values, indicating breed-specific differences in metabolic efficiencies and immune responses. The noted rise in White Blood Cell count in Lactobacillus-supplemented diet indicates a possible immunomodulation effect. This finding further supports the postulation of Ashraf and Shah (2014) that 'probiotics have the capability of enhancing both specific and nonspecific immune mechanisms.' All the haematological parameters measured were within their respective normal physiological ranges, hence Lactobacillus supplementation did not disrupt fundamental blood characteristics. Consistently, the improvements in most of the parameters suggest that positive impacts may have occurred at the level of general health in broilers.

Improvement in haematological profile by Lactobacillus probiotic supplementation has been demonstrated to be possible in broiler chickens. The best results appear to be yielded by the 10 ml/day dose; hence, finding an optimal dose may yield new strategies in improving poultry production and health.

The findings of the present study showed remarkable consonance and divergence with existing literature on avian haematology with probiotic supplementation. In this connection, Pourgholam *et al.* (2017) reported marginal improvement in haematological parameters with supplementation of Lactobacillus spp., whereas the present study showed more pronounced changes in RBC count and haemoglobin levels.

This study is contrasted with Zhao *et al.* (2022), who reported no consistent haematological response to different probiotic strains. Here, there is more consistency in improvement in a majority of parameters. The breed variations in the present study agree with the suggestion of van Baarlen *et al.* (2013) that genetic predisposition significantly influences the probiotic response. But this is the first report showing these variations in multiple haematological indices, providing a better insight into breed variations than previously reported.

The immunomodulatory effects evidenced in the WBC analyses agree with suggestions by Isolauri *et al.* (2019) that probiotics have the potential as immune system modifiers. However, this study has provided more detailed information on the breed-specific immune response-a dimension missing in most of the earlier works. The trend of increase in WBC in different levels of Lactobacillus suggests a strong immunological mechanism which may be further elucidated. The methodology adopted in this research has overcome some limitations inherent in several of the earlier works. While studies such as those conducted by Pertiwi and Mahendra (2021) were restricted to single breed experiments, the Arbor Acre and Ross breeds used in this comparative analysis have given a better understanding of the interaction effect of probiotics. The approach has thus brought out certain genetic influences which were not addressed by earlier literature and provides a more sophisticated understanding of probiotic supplementation.

3.2 Serum Biochemical Responses:

The serum mineral profile studied showed a minimum significant difference among the experimental treatments (Table 4, 5 and 6).

TABLE 4
MEAN BIOCHEMICAL PARAMETERS OF ARBOR ACRE AND ROSS BROILERS INJECTED WITH DIFFERENT LEVELS OF LACTOBACILLUS BACTERIA

Parameters	Breed x Level of Lactobacillus								SEM	P-value
	AAx0%	AAx5%	AAx10%	AAx15%	Rx0%	Rx5%	Rx10%	Rx15%		
Na+	147	146	168.9	147.53	144.3	147.87	149.5	147.5	10.08	0.71
K+	4.83	4.83	5.63	5.33	4.27	4.63	4.27	4.33	0.38	0.467
HCO ₅	16.43ab	14.87ab	14.50ab	14.47ab	13.90b	16.37ab	17.27a	17.33a	0.96	0.04
CL	103	103	118.4	104.77	103.33	104.33	111.33	105	4.98	0.822
Urea	1.03	1.12	1.2	1.1	1.35	0.78	0.97	0.72	0.22	0.38
Creatinine (mg/dl)	30.37	21.23d	22.6	31.13ab	29.63d	22.2	23.7	24.47	1.99	0.012
TC(g/dl)	3.46	3.17	3.38	3.94	4.26	4.69	3.76	3.98	0.33	0.166
TP(g/dl)	2.67a	1.72ab	2.40ab	1.78ab	1.14b	2.58a	2.02ab	2.18ab	0.4	0.043
HDL(g/dl)	1.61b	1.74b	1.66b	1.79b	2.36a	1.66b	1.85b	1.85b	0.12	0.022
LDL(g/dl)	1.11c	0.94c	1.24bc	1.42bc	1.45bc	2.19a	1.65b	1.34bc	0.15	0.004
ALT(IU/l)	6.97	7.13	7.43	8.23	9.57	8.3	18.37	22.7	0.27	0.074
AST(IU/l)	272.20cd	284.60cd	457.50a	326.43bcd	249.67d	429.13ab	386.23ab	428.50abc	36.2	0.028
ALP(IU/l)	3044.53	2702.6	2289.4	2785.57	3238.6	1863.53	2148	2169.33	313.2	0.377
Uric acid(mg/dl)	170.63	166.17	238.93	255.3	282.03	295.1	338.77	410.17	49.93	0.95

Notably, chlorine was the only mineral that showed a statistically significant difference among the treatments. The increase was thus in agreement with an earlier study by Dousa *et al.* (2015) in which certain serum minerals likewise showed significant increases with probiotic supplementation. This thus places inclusion of lactobacillus as having an effect that is subtle but not nuanced on the mineral composition of broiler serum.

Mineral parameters also showed some breed variations within the same treatment (Table 5). The sodium and potassium values were significantly higher for the Arbor Acre breed when compared with the Ross breed. These variations between breeds bring into focus the need to study genetic variations as well when considering supplementation strategies for poultry.

TABLE 5
MEAN BIOCHEMICAL PARAMETERS OF ARBOR ACRE AND ROSS BROILER BREEDS

Parameter	Breed		SEM	P-value
	Arbor Acre	Ross		
Na+	152.36 ^a	147.29 ^b	5.04	0.488
K+	5.16 ^a	4.38 ^b	0.19	0.01
HCO ₅	15.07 ^b	16.22 ^a	0.48	0.108
CL	107.29 ^a	106.00 ^b	2.49	0.718
Urea	1.11 ^a	0.96 ^b	0.11	0.325
Creatinine (mg/dl)	28.83 ^a	23.75 ^b	0.99	0.002
TC(g/dl)	3.49 ^b	4.17 ^a	0.16	0.009
TP(g/dl)	2.14 ^a	1.98 ^b	0.2	0.577
HDL(g/dl)	1.70 ^b	1.93 ^a	0.06	0.017
LDL(g/dl)	1.18 ^b	1.65 ^a	0.08	0
ALT(IU/l)	7.44 ^b	14.73 ^a	1.36	0.002
AST(IU/l)	373.38 ^b	2705.53 ^a	18.1	0.155
ALP(IU/l)	2354.87 ^a	207.76 ^b	156.61	0.133
Uric acid (mg/dl)	331.52 ^b	373.38 ^a	156.61	0.003

^{a-b} means on the same row with different superscript are significantly different (P<0.05)

TC=total cholesterol, TP=total protein, HDL= High density lipoprotein, LDL =low density lipoprotein, AST=Aspartate amino transferase, ALT= Alanine amino transferase, ALP=Alkaline phosphate transferase

A significant and promising finding was the decrease in kidney function markers across lactobacillus-supplemented groups. Urea, uric acid, and creatinine values were significantly lower in the supplemented groups compared to the control. These results corroborate recent study by Chauhan *et al.* (2021), which reported similar reductions in these kidney function indicators when probiotics were introduced to broiler diets.

TABLE 6
MEAN GROWTH PERFORMANCE OF BROILERS INJECTED WITH DIFFERENT LEVELS OF LACTOBACILLUS BACTERIA

Parameter	Levels of Lactobacillus				SEM	P-value
	0%	5%	10%	15%		
Na+	145.650 ^a	146.933 ^a	159.200 ^a	147.517 ^a	7.13	0.522
K+	4.550 ^a	4.733 ^a	4.950 ^a	4.833 ^a	0.27	0.757
HCO ₅	15.167 ^a	15.617 ^a	15.883 ^a	15.900 ^a	0.68	0.856
CL	103.167 ^b	103.667 ^b	114.867 ^a	104.883 ^{ab}	3.52	0.099
Urea	1.387 ^b	.950 ^a	1.018 ^a	0.910 ^a	0.15	0.579
Creatinine (mg/dl)	25.000 ^b	23.217 ^a	22.150 ^a	22.800 ^a	1.41	0.036
TC(g/dl)	3.862 ^a	3.430 ^b	3.467 ^b	3.562 ^b	0.23	0.615
TP(g/dl)	1.905 ^a	2.150 ^a	2.212 ^a	1.978 ^a	0.28	0.855
HDL(g/dl)	1.987 ^a	1.700 ^b	1.753 ^b	1.820 ^{ab}	0.09	0.15
LDL(g/dl)	1.178 ^b	1.562 ^a	1.443 ^a	1.378 ^a	0.11	0.347
ALT(IU/l)	8.267 ^b	7.717 ^b	12.900 ^{ab}	15.467 ^a	1.93	0.032
AST(IU/l)	260.933 ^b	356.867 ^a	421.867 ^a	377.467 ^a	25.6	0.003
ALP(IU/l)	3141.567 ^a	2283.067 ^b	2218.700 ^b	2477.450 ^b	221.14	0.036
Uric acid(mg/dl)	226.333 ^a	170.633 ^a	188.850 ^a	182.733 ^a	35.31	0.144

^{a-b} means on the same row with different superscript are significantly different (P<0.05)

TC=total cholesterol, TP=total protein, HDL= High density lipoprotein, LDL =low density lipoprotein, AST=Aspartate amino transferase, ALT= Alanine amino transferase, ALP=Alkaline phosphate transferase.

Also, the hypothesis advanced by the researcher was that probiotic microorganisms use creatinine, urea, and uric acid as substrates for their own development; hence, they could explain such reductions in their levels. In other words, this mechanism can involve a renal-protective effect of probiotic supplementation, which might imply some important reasons for broiler health and management.

The levels of total protein were significantly affected with supplementation of lactobacillus. The mean plasma total protein values differed significantly among the different levels of lactobacillus and breeds. This is in agreement with Pourakbari *et al.* (2016), where there was an increase in plasma protein and an improvement in growth performance following supplementation with probiotics. The probable mechanism has been ascribed to the ability of the lactic acid bacteria to competitively inhibit the growth of pathogenic bacteria, reducing protein catabolism and enhancing utilization of amino acids. This therefore suggests that probiotic application may result in improved protein metabolism in broilers.

Probably the most exciting results were recorded in the cholesterol profile. The supplementation of Lactobacillus significantly lowered plasma total cholesterol compared to the control group (Table 6). The finding agreed with previous study by Pourakbari *et al.* (2016). Apparently, the cholesterol-lowering mechanism is multifaceted. Lactic acid bacteria were able to assimilate cholesterol in the intestinal tract, decrease the expression of cholesterol absorption proteins, and produce bile salt hydrolase, hence facilitating the excretion of bile acids. In contrast with total and LDL cholesterol, variations in HDL cholesterol levels were more complex across treatments and breeds.

The study revealed significant breed-specific difference across many parameters. Two important breeds of broiler chickens, Ross and Arbor Acre breeds, exhibited differential responses to lactobacillus supplementation. Significant differences in total protein, cholesterol levels, and enzymatic activities are shown in Table 5. It then seems that breed-specific characteristics should be considered within a nutritional approach.

IV. CONCLUSION AND RECOMMENDATION

The holistic investigation into Lactobacillus probiotic supplementation in broilers justifies the utilization of targeted nutritional interventions within poultry, as represented by a number of physiological gains. The clear trends of optimum improvement in haematological and biochemical indices, as established in this study, were evident at the 10-ml supplementation level. This work has clearly demonstrated that it is possible to positively modulate blood cell production, immune response, and metabolic efficiency through supplementation with probiotics.

The apparent variability between Arbor Acre and Ross chickens strengthens the argument for consideration of genetics in formulating nutritional programs. Such differences in response suggest that future research is warranted directly toward developing breed-specific probiotic protocols which better realize the utmost in the expression of individual genetic potential. In this aspect, further normalization in reduced kidney function markers and cholesterol levels may be considerate of the health benefits emanating from strategic probiotic supplementation.

Based on this, supplementing 10 mL of Lactobacillus probiotics daily, especially to the Ross breed chicken, is recommended for poultry farms. This provides strong evidence, especially in enhancing the haematological profile, probably by modulating the immune system and optimizing metabolism. Nevertheless, the full elucidation of these mechanisms of improvement and more refined supplementation strategies require further long-term studies. Further studies may want to focus on the exact strains of Lactobacillus, the effect of those on breed genetics, and long-term use implications on health and productivity.

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Efficacy of Commonly used Insecticides against Sucking Pests on PGPR treated Okra Plants

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Abstract— A field experiment was conducted during Kharif 2021 at the Agricultural College and Research Institute, Killikulam, Vallanadu, Tamil Nadu, India to assess the efficacy of commonly used insecticides against sucking pests on okra plants treated with *Bacillus subtilis* Bbv57, a plant-growth-promoting rhizobacterium (PGPR). The experiment was designed in a completely randomized block design with three replications. Okra seeds were treated with a talc-based formulation of *B. subtilis* Bbv57, and the PGPR was also applied to the soil before sowing. Insecticides, including imidacloprid, thiamethoxam, acetamiprid, and thiacloprid, were sprayed on both PGPR-treated and untreated plots. Pest populations, including aphids and leafhoppers, were monitored at 3, 7, 10, and 14 days after treatment. The results indicated that insecticide treatments on PGPR-treated plants significantly reduced pest populations compared to untreated plants. Acetamiprid 20 SP at 100 g ha⁻¹ was the most effective, reducing aphid and leafhopper populations to the lowest levels on PGPR-treated plants. Furthermore, PGPR treatment enhanced pest resistance, likely through induced biochemical changes. The highest yield (18.55 tonnes ha⁻¹) and benefit-cost ratio (1:2.53) were observed in PGPR-treated plants treated with acetamiprid. This study demonstrates that combining PGPR with insecticide treatments provides an effective, sustainable solution for managing sucking pests in okra, offering both improved pest control and higher economic returns.

Keywords— *Bacillus subtilis*, Okra, PGPR, Sucking pests, Yield.

I. INTRODUCTION

Okra (*Abelmoschus esculentus* [L.] Moench), commonly known as bhindi or lady's finger, is a widely cultivated vegetable in tropical and subtropical regions globally (Elkhalifa et al., 2021). However, its cultivation faces significant challenges due to pest infestations. In India, okra is commercially grown over approximately 0.53 million hectares, yielding an annual production of 6.46 million tonnes contributing 62% of the global output and playing a vital role in meeting the nation's vegetable demand (Mohapatra et al., 2024). Despite its importance, okra is highly susceptible to various insect pests which include shoot and fruit borers (*Earias insulana* [Boisd.] and *Earias vittella* [Fab.]), leafhopper (*Amrasca biguttula biguttula* [Ishida]), leaf roller (*Sylepta derogata* Fab.), whitefly (*Bemisia tabaci* Genn.), aphid (*Aphis gossypii* Glover), and mite (*Tetranychus cinnabarinus* Boisduval) (Kodandaram et al., 2017) and yield loss range between 50.00% and 63.41% (Asi et al., 2008 and Mohapatra et al., 2024). Sap-sucking pests like leafhoppers extract chlorophyll, disrupting photosynthesis and causing leaf cupping, yellowing, and bronzing, which slow crop growth. Leafhoppers can cause production losses of 50% to 63.41% (Mohapatra et al., 2024). Aphids, especially *A. gossypii*, harm young plants, causing stunted growth, wilting, and plant death in severe cases. Their honeydew encourages sooty mold, further blocking photosynthesis and damaging buds, flowers, and fruits (Murovhi et al., 2020; Kedar et al., 2014).

Okra growers opt synthetic insecticides based pest management as a primary strategy (Jan et al., 2022). However, prolonged use of insecticides exerts selection pressure, leading to insecticide resistance in pest populations globally (Cerna et al., 2013; Szczepaniec et al., 2019) and escalates production costs and reduces profitability. In India, okra farmers often apply 10–12 pesticidal sprays in a single growing season⁻¹ to manage sucking pests and fruit borers, resulting in fruits with high pesticide

residues, posing serious risks to consumer health (Ounis et al., 2024). Therefore, adopting sustainable practices, particularly Integrated Pest Management (IPM), is imperative for safeguarding both the environment and human health. Plant growth promoting rhizobacteria (PGPR), present a sustainable alternative in agriculture (Santoyo et al., 2021; Harris, 2009) by enhancing plant growth by facilitating nitrogen uptake, phytohormone synthesis, mineral solubilization, and iron chelation (Bowen and Rovira, 1999). PGPR also enhance resistance against pests and pathogens by inducing physical and chemical defenses in plants, a phenomenon termed induced systemic resistance (Kloepper et al., 2004; Nelson, 2004 and Bostock, 2005). This resistance mechanism has been extensively documented in plant–pathogen and plant–insect interactions (Zehnder et al., 2001; Conrath et al., 2006).

PGPR are characterized by their ability to colonize root surfaces, survive and multiply in competitive microbial environments, and express growth-promotion and protection activities (Mohanty et al., 2021; Kloepper and Okon, 1994). About 2–5% of rhizobacteria exert beneficial effects on plant growth when inoculated into soils with competitive microflora (Kloepper, 1978). These bacteria, thriving in the rhizosphere, enhance plant growth via diverse mechanisms (Vocciante et al., 2022; Vessey, 2003). The below-ground colonization of PGPR triggers various biological processes, altering interactions with above-ground herbivores through changes in plant abundance, nutritional quality, and defenses (Hartley and Gange, 2009; Grunseich et al., 2019). Thus, incorporating PGPR into pest management frameworks offers a promising approach to reduce reliance on synthetic pesticides, mitigate environmental hazards, and enhance agricultural sustainability (Basu et al., 2021). This study was conducted to evaluate the efficacy of commonly used insecticides in controlling sucking pests on plants treated with plant-growth-promoting rhizobacteria (PGPR). By integrating PGPR treatment with insecticide use, the study aimed to explore potential improvements in pest management, plant resistance, and overall crop health, providing insights into sustainable pest management strategies.

II. MATERIALS AND METHODS

Field experiment was conducted in completely randomized block design with three replications at Agricultural College and Research Institute, Killikulam, Vallanadu, Tamil Nadu, India farm during Kharif 2021 (8°46 N latitude and 77°42 E longitude) to study the efficacy of commonly used insecticides against sucking pests of okra on PGPR treated plants. Okra F₁ hybrid CoBh4 seeds were treated with talc-based formulation (containing 1×10^8 cfu g⁻¹) of *B. subtilis* Bbv57 @ 10 g kg⁻¹ of seed which has been identified as the effective PGPR strain from the pot culture and field experiments. Okra seeds were sown in plots of size 6x5 m². Soil application of *B. subtilis* Bbv57 @ 2.5 kg hectare⁻¹ was done before sowing. Similarly, untreated plots without any seed treatment were maintained to study the efficacy of insecticides. The insecticides were applied on *B. subtilis* Bbv57 treated plots and untreated plots with high volume knapsack sprayer using solid cone nozzle. Observations on sucking pests before the application of insecticide and 3,7,10 and 14 days after treatment were recorded. The field experiment was conducted with following treatments.

TABLE
TREATMENTS USED IN FIELD EXPERIMENT

T ₁	<i>Bacillus subtilis</i> Bbv57 (ST-SA)	T ₆	Imidacloprid 17.8 SL @ 100 ml ha ⁻¹
T ₂	<i>Bacillus subtilis</i> Bbv57 (ST-SA) - Imidacloprid 17.8 SL @ 100 ml ha ⁻¹	T ₇	Thiamethoxam 25 WG @ 100 g ha ⁻¹
T ₃	<i>Bacillus subtilis</i> Bbv57 (ST-SA) - Thiamethoxam 25 WG @ 100 g ha ⁻¹	T ₈	Acetamiprid 20 SP @ 100 g ha ⁻¹
T ₄	<i>Bacillus subtilis</i> Bbv57 (ST-SA) - Acetamiprid 20 SP @ 100 g ha ⁻¹	T ₉	Thiacloprid 21.7 SC @ 500 ml ha ⁻¹
T ₅	<i>Bacillus subtilis</i> Bbv57 (ST-SA) - Thiacloprid 21.7 SC @ 500 ml ha ⁻¹	T ₁₀	Untreated control

The fruit yield was recorded at each harvest and pooled. Gross income, net income and benefit cost ratio (BCR) were worked out for each treatment.

III. RESULTS AND DISCUSSION

Microplot experiments and biochemical analysis showed that *Bacillus subtilis* strain Bbv57 was the best PGPR strain in reducing the incidence of major sucking insect pests of okra. Hence the efficacy of commonly used insecticides on *B. subtilis* Bbv57 treated plants for the management of sucking pests was evaluated under field conditions. The field experiment revealed that all the chemical insecticides significantly reduced the population of sucking pests up to 14 days after treatment (DAT) on plants treated with *B. subtilis* Bbv57. Among them, acetamiprid 20 SP @100 g ha⁻¹ was better in reducing the aphid and leafhopper population on *B. subtilis* Bbv57 treated and untreated plants (Table 1 and 2).

TABLE 1
EFFICACY OF COMMONLY USED INSECTICIDES AGAINST A. GOSSYPHII ON PGPR TREATED OKRA DURING KHARIF 2021

S.No.	Treatments	Number of aphids plant ⁻¹ *				
		PTC	3 DAT	7 DAT	10 DAT	14 DAT
T ₁	<i>Bacillus subtilis</i> Bbv57 (ST-SA)	38.42	27.32	30.55	45.66	69.88
		-6.24	(5.27) ^b	(5.57) ^d	(6.79) ^d	(8.39) ^f
T ₂	<i>Bacillus subtilis</i> Bbv57 (ST-SA) - Imidacloprid 17.8 SL @100 ml ha ⁻¹	37.98	0	5.41	12.36	22.64
		-6.2	(0.71) ^a	(2.43) ^{bc}	(3.59) ^{bc}	(4.81) ^{bcd}
T ₃	<i>Bacillus subtilis</i> Bbv57 (ST-SA) - Thiamethoxam 25 WG @100 g ha ⁻¹	40.11	0	0	4.88	17.21
		-6.37	(0.71) ^a	(0.71) ^a	(2.32) ^a	(4.21) ^{abc}
T ₄	<i>Bacillus subtilis</i> Bbv57 (ST-SA) - Acetamiprid 20 SP @100 g ha ⁻¹	42.29	0	0	3.82	10.54
		-6.54	(0.71) ^a	(0.71) ^a	(2.08) ^a	(3.32) ^a
T ₅	<i>Bacillus subtilis</i> Bbv57 (ST-SA) - Thiacloprid 21.7 SC @500 ml ha ⁻¹	44.58	0	6.21	14.59	29.11
		-6.71	(0.71) ^a	(2.59) ^{bc}	(3.88) ^{bc}	(5.44) ^{cde}
T ₆	Imidacloprid 17.8 SL @100 ml ha ⁻¹	41.14	0	9.44	21.66	38.74
		-6.45	(0.71) ^a	(3.15) ^c	(4.71) ^c	(6.26) ^e
T ₇	Thiamethoxam 25 WG @100 g ha ⁻¹	38.85	0	3.18	8.31	18.73
		-6.27	(0.71) ^a	(1.92) ^b	(2.97) ^{ab}	(4.39) ^{abc}
T ₈	Acetamiprid 20 SP @100 g ha ⁻¹	41.02	0	0	7.21	15.33
		-6.44	(0.71) ^a	(0.71) ^a	(2.78) ^{ab}	(3.98) ^{ab}
T ₉	Thiacloprid 21.7 SC @500 ml ha ⁻¹	43.66	0.22	8.54	19.11	35.98
		-6.65	(0.85) ^a	(3.01) ^{bc}	(4.43) ^c	(6.04) ^{de}
T ₁₀	Untreated control	45.22	51.72	66.54	80.22	86.94
		-6.61	(7.07) ^c	(8.01) ^e	(8.78) ^e	(9.14) ^f
CD (p=0.05)		ns	1.01 ^{**}	1.15 ^{**}	1.26 ^{**}	1.32 ^{**}

DAT – Days after treatment

PTC – Pretreatment count

*Mean of three replications

Figures in parentheses are $\sqrt{x + 0.5}$ transformed values.

In a column, means followed by common letters are not significantly different by LSD (P=0.05)

Among the insecticides, acetamiprid 20 SP @100 g ha⁻¹ effectively reduced the aphid population (3.82 and 10.54 aphids plant⁻¹ on 10 DAT and 14 DAT respectively) on *B. subtilis* Bbv57 treated plants and untreated plants (7.21 and 15.33 aphids plant⁻¹ on 10 DAT and 14 DAT respectively). The efficacy of all insecticides on *B. subtilis* Bbv57 treated plants was high when compared with untreated plants which received the same insecticide (Table 1). Similarly, no incidence of leafhopper was recorded up to 7 DAT on *B. subtilis* Bbv57 treated plants with acetamiprid 20 SP @100 g ha⁻¹. Furthermore, it recorded 0.24 and 0.61 hoppers plant⁻¹ on 10 DAT and 14 DAT respectively on *B. subtilis* Bbv57 treated plants. Thiamethoxam 25 WG @100 g ha⁻¹ on *B. subtilis* Bbv57 treated plants also recorded less number of leafhopper population (0.43 and 1.08 hoppers plant⁻¹ respectively) on 10 DAT and 14 DAT. The hopper population was high in the untreated plants (5.76, 6.17, 6.98 and 7.91 hoppers plant⁻¹) on 3,7,10 and 14 DAT respectively when compared with *B. subtilis* Bbv57 treated plants (4.21, 4.16, 5.27, 6.91 and 7.64) (Table 2). The present findings are in line with Sharma (2020) and Reddy and Gowdar (2006) who showed that

acetamiprid 20 SP @20 g a.i ha⁻¹ significantly reduced the population of sucking pests in okra. However, the efficacy of insecticides persisted more in *B. subtilis* Bbv57 treated plants when compared with untreated plants.

TABLE 2
EFFICACY OF COMMONLY USED INSECTICIDES AGAINST A. DEVASTANS ON PGPR TREATED OKRA DURING KHARIF 2021

S.No.	Treatments	Number of aphids plant ⁻¹ *				
		PTC	3 DAT	7 DAT	10 DAT	14 DAT
T ₁	<i>Bacillus subtilis</i> Bbv57 (ST-SA)	4.21	4.16	5.27	6.91	7.64
		-2.17	(2.12) ^b	(2.36) ^b	(2.67) ^b	(2.85) ^d
T ₂	<i>Bacillus subtilis</i> Bbv57 (ST-SA) - Imidacloprid 17.8 SL @100 ml ha ⁻¹	3.65	0	0.24	0.76	1.15
		-2.04	(0.71) ^a	(0.86) ^a	(1.12) ^a	(1.28) ^{ab}
T ₃	<i>Bacillus subtilis</i> Bbv57 (ST-SA) - Thiamethoxam 25 WG @100 g ha ⁻¹	4.14	0	0	0.43	1.08
		-2.15	(0.71) ^a	(0.71) ^a	(0.96) ^a	(1.26) ^{ab}
T ₄	<i>Bacillus subtilis</i> Bbv57 (ST-SA) - Acetamiprid 20 SP @100 g ha ⁻¹	4.11	0	0	0.24	0.61
		-2.15	(0.71) ^a	(0.71) ^a	(0.86) ^a	(1.05) ^a
T ₅	<i>Bacillus subtilis</i> Bbv57 (ST-SA) - Thiacloprid 21.7 SC @500 ml ha ⁻¹	3.55	0	0.61	0.85	2.06
		-2.01	(0.71) ^a	(1.05) ^a	(1.16) ^a	(1.6) ^{bc}
T ₆	Imidacloprid 17.8 SL @100 ml ha ⁻¹	3.14	0.12	0.45	0.91	2.57
		-1.91	(0.79) ^a	(0.97) ^a	(1.19) ^a	(1.75) ^c
T ₇	Thiamethoxam 25 WG @100 g ha ⁻¹	4.01	0	0.18	0.51	1.22
		-2.12	(0.71) ^a	(0.82) ^a	(1.00) ^a	(1.31) ^{ab}
T ₈	Acetamiprid 20 SP @100 g ha ⁻¹	3.66	0	0	0.33	1.05
		-2.04	(0.71) ^a	(0.71) ^a	(0.91) ^a	(1.24) ^{ab}
T ₉	Thiacloprid 21.7 SC @500 ml ha ⁻¹	3.89	0	0.66	1.06	2.71
		-2.09	(0.71) ^a	(1.07) ^a	(1.25) ^a	(1.79) ^c
T ₁₀	Untreated control	4.18	5.76	6.17	6.98	7.91
		-2.12	(2.46) ^b	(2.53) ^b	(2.68) ^b	(2.84) ^d
CD (<i>p</i> =0.05)		ns	0.40**	0.45**	0.50**	0.38**

DAT – Days after treatment

PTC – Pretreatment count

*Mean of three replications

Figures in parentheses are $\sqrt{x + 0.5}$ transformed values.

In a column, means followed by common letters are not significantly different by LSD (*p*=0.05)

The yield, net income and benefit cost ratio (BCR) were high in PGPR treated plants sprayed with insecticides compared to the untreated plants. On PGPR treated plants, acetamiprid 20 SP @100 g ha⁻¹ recorded higher yield of 18.55 tonnes ha⁻¹ followed by thiamethoxam 25 WG @100 g ha⁻¹ (18.21 tonnes ha⁻¹) compared to untreated plants (14.39 tonnes ha⁻¹). The benefit cost ratio (BCR) was also high for acetamiprid 20 SP @100 g ha⁻¹ on *B. subtilis* Bbv57 treated plants (1:2.53) than the untreated plants (1:1.80) (Table 3). The prolonged efficacy of chemical insecticides against the insect pests on *B. subtilis* Bbv57 treated plants may be due to the increased levels of biochemicals which might have reduced the feeding preference and affected the physiology of insect pests on okra as reported by Barman et al (2024) and Singh et al. (2022). The present findings are in line with the reports of Kahia et al. (2021) and Myresiotis et al. (2015) where *B. subtilis* strain FZB24 significantly enhanced the root uptake of thiamethoxam in corn, *Zea mays* L. thereby reduced the usage of chemical insecticides.

TABLE 3
EFFICACY OF INSECTICIDES AGAINST SUCKING PESTS ON PGPR TREATED OKRA PLANTS - YIELD AND ECONOMICS

S. No.	Treatments	Yield	Cost of cultivation (₹)	Gross income (₹)	Net income (₹)	BCR
		(t/ha)				
T ₁	<i>Bacillus subtilis</i> Bbv57 (ST-SA)	16.02 ^c	94554	288360	193806	01:02.1
T ₂	<i>Bacillus subtilis</i> Bbv57 (ST-SA) - Imidacloprid 17.8 SL @ 100 ml ha ⁻¹	17.63 ^{abcd}	94829	317340	222511	01:02.4
T ₃	<i>Bacillus subtilis</i> Bbv57 (ST-SA) - Thiamethoxam 25 WG @ 100 g ha ⁻¹	18.21 ^{ab}	94954	327780	232826	01:02.5
T ₄	<i>Bacillus subtilis</i> Bbv57 (ST-SA) - Acetamiprid 20 SP @ 100 g ha ⁻¹	18.55 ^a	94714	333900	239186	01:02.5
T ₅	<i>Bacillus subtilis</i> Bbv57 (ST-SA) - Thiacloprid 21.7 SC @ 500 ml ha ⁻¹	17.99 ^{abc}	95954	323820	227866	01:02.4
T ₆	Imidacloprid 17.8 SL @ 100 ml ha ⁻¹	16.65 ^{de}	92894	299700	206806	01:02.2
T ₇	Thiamethoxam 25 WG @ 100 g ha ⁻¹	17.20 ^{bcd}	92950	309600	216650	01:02.3
T ₈	Acetamiprid 20 SP @ 100 g ha ⁻¹	17.41 ^{abcd}	92630	313380	220750	01:02.4
T ₉	Thiacloprid 21.7 SC @ 500 ml ha ⁻¹	16.96 ^{cde}	92900	305280	212380	01:02.3
T ₁₀	Untreated control	14.39 ^f	92550	259020	166470	01:01.8
CD ($p=0.05$)		1.15 ^{**}				

*Mean of three replications Okra fruits sold @18 kg⁻¹

In a column, means followed by common letters are not significantly different by LSD ($p=0.05$)

IV. CONCLUSION

The combination of PGPR-treated plants and acetamiprid 20 SP @ 100 g ha⁻¹ significantly reduced aphid and leafhopper populations, increasing okra yields compared to untreated plants. This synergistic effect of *B. subtilis* Bbv57 and insecticide is likely due to the biochemical changes induced by the PGPR, which enhances plant resistance and boosting the effectiveness of insecticide treatments. This integrated approach offers a sustainable strategy for managing sucking pests in okra with reduced environmental impact, balancing pest control with ecological considerations.

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Exploring the Role of Moringa Oleifera Leaf Extract (MLE) in Enhancing Seed Germination, Seedling Growth, and Plant Development of Chili

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Abstract— This study investigates the effect of Moringa oleifera leaf extract (MLE) on chili seed germination, seedling development, and growth parameters. Utilizing a randomized complete block design, six treatments of MLE concentrations (1%-5%) and a control (distilled water) were applied to chili seeds. Results revealed that MLE significantly enhances germination, with the 2% concentration yielding the highest germination rate (99%) and germination index (12.353). Moreover, the 2% MLE treatment demonstrated superior seedling vigor index (1513.3), leaf count (7.78 leaves/plant), branch count (9.6 branches/plant), and overall plant height (15.134 cm), outperforming all other treatments. Root length (6.5 cm) and biomass results further confirmed the effectiveness of 2% MLE in promoting root growth and development. Additionally, chlorophyll content peaked at a SPAD value of 64.868 in the 2% MLE treatment, signifying enhanced photosynthetic capacity. This study concludes that the application of 2% MLE is the most effective for improving germination, seedling growth, and overall chili plant development.

Keywords— Priming, Moringa leaf extract (MLE), Chilies.

I. INTRODUCTION

Chilli (*Capsicum annum* L.) belongs to the family Solanaceae and it is one of the most important spices cultivated for its fruits. They are widely used as both green and ripe dried form for its pungency (Jalgaonkar et al., 2024) It is mainly used as a vegetable and also a condiment but the topmost use of chilli throughout the world is as a spice due to its pungency and pleasant flavor. Chilli is one of the most essential cash crops grown in Sri Lanka and it has become a vital ingredient in Sri Lankan meals (Mihiranie et al., 2020). The history of chilies dates back thousands of years, with their origins believed to be in the Americas (Andrews, J. (1995). Indigenous peoples in Central and South America were the first to cultivate and use chilies, incorporating them into their diets as well as using them for medicinal purposes (Foster, N., & Cordell, L. S. (Eds.). (1992). When Christopher Columbus arrived in the New World, he introduced chilies to Europe, sparking a worldwide exchange of crops during the Columbian Exchange, which led to chilies being integrated into the cuisines of Asia, Africa, and beyond. Today, chilies are an essential ingredient in many iconic dishes. From the fiery curries of India to the spicy salsas of Mexico and the heat-filled stir-fries of Thailand, chilies play a pivotal role in defining the flavor profile of these cuisines (Cai et al., 2021). Whether they are used fresh, dried, ground into powder, or made into sauces, chilies offer a range of flavors—from smoky and fruity to intensely hot—allowing for a vast spectrum of culinary applications (Peterson, J. (2017). Chilies also hold cultural significance, often being seen as a symbol of hospitality, vitality, and resilience in many societies. For example, in countries like Mexico and

India, chilies are used not only in cooking but also in traditional rituals, such as offerings to gods or as protective charms against evil spirits (Etkin, N. L. (2008). Beyond their culinary uses, chilies have been the subject of scientific research due to their potential health benefits. Capsaicin, the compound that gives chilies their heat, has been studied for its analgesic, anti-inflammatory, and metabolism-boosting properties. Moreover, the rich vitamin content of chilies, particularly vitamin C, adds to their nutritional value, making them both a flavorful and health-conscious addition to any diet (Gupta, E., & Mishra, P. (2021). *Moringa oleifera*, commonly known as the drumstick tree, is a fast-growing, drought-resistant plant native to parts of Africa and Asia (Kumar et al., 2020). Its leaves are a rich source of essential nutrients, including vitamins, minerals, and antioxidants, which have garnered significant attention for their potential applications in agriculture (El-Ramady et al., 2022). Recent studies have explored the use of *Moringa oleifera* leaf extract (MLE) as a natural biostimulant to enhance plant growth and development (Mashamaite et al., 2022). The bioactive compounds present in MLE, such as cytokinins, phenolic acids, and flavonoids, are believed to promote seed germination, stimulate root and shoot growth, and improve overall plant health (Hafeez et al., 2022).

II. MATERIAL METHOD

The experiment was carried out according to a randomized complete block design with three replicates. It was comprised of six treatments, viz. (T₁= Control-no priming), T₂ = Priming with 1% of MLE, T₃ = Priming with 2% of MLE, T₄ = Priming with 3% of MLE, T₅ = Priming with 4% of MLE, T₆ = Priming with 5% of MLE. The control treatment was represented by priming with distilled water.

III. RESULTS AND DISCUSSION

3.1 Germination Percentage:

The (Figure 1 a) showing the results Regarding germination percentage was statistically significant. The highest germination rate was observed in the treatment with 2% MLE (99%), followed by 5% MLE (76%) and 3% MLE (80%). The control group (T₁) exhibited the lowest germination percentage at 50%.

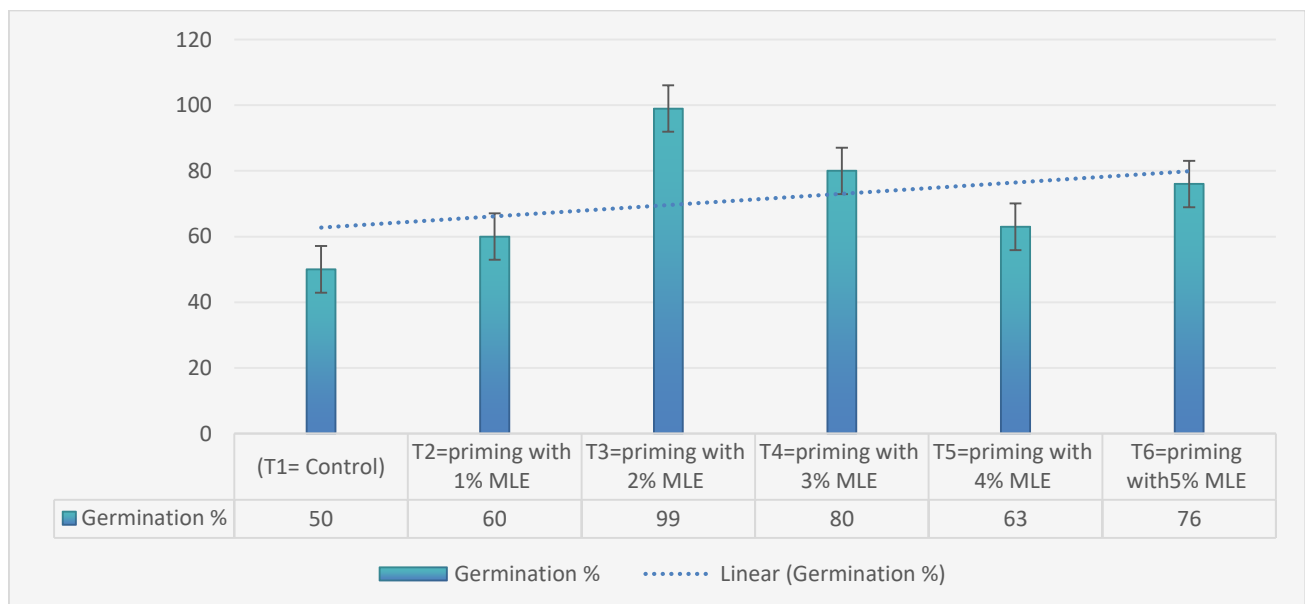


FIGURE 1 (a): Germination Percentage (%) of Chilli under the Influence of Varying Priming Treatments with MLE

3.2 Germination Index (GI):

The (Figure 1 b) showing the results Regarding germination Index (GI) was statistically significant. The 2% MLE treatment also recorded the highest GI (12.353), indicating faster and more uniform germination compared to other treatments. The control group had the lowest GI (10.313), suggesting slower germination.

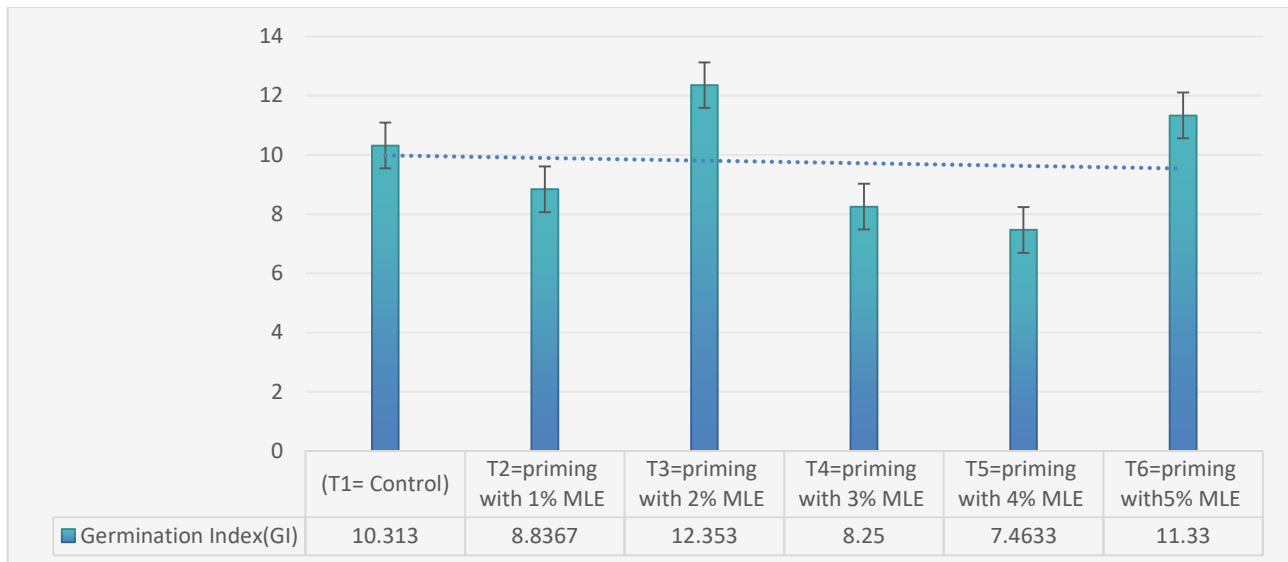


FIGURE 1 (b): Germination Index (GI) of Chilli under the Influence of Varying Priming Treatments with MLE.

3.3 Seedling Vigour Index (SVI):

The (Figure 1 c) showing the results Regarding **Seedling Vigour Index (SVI)** was statistically significant. The 2% MLE priming treatment also had the highest SVI (1513.3), suggesting better seedling growth and vigor. The control group had a significantly lower SVI (303.33), indicating weaker seedlings.

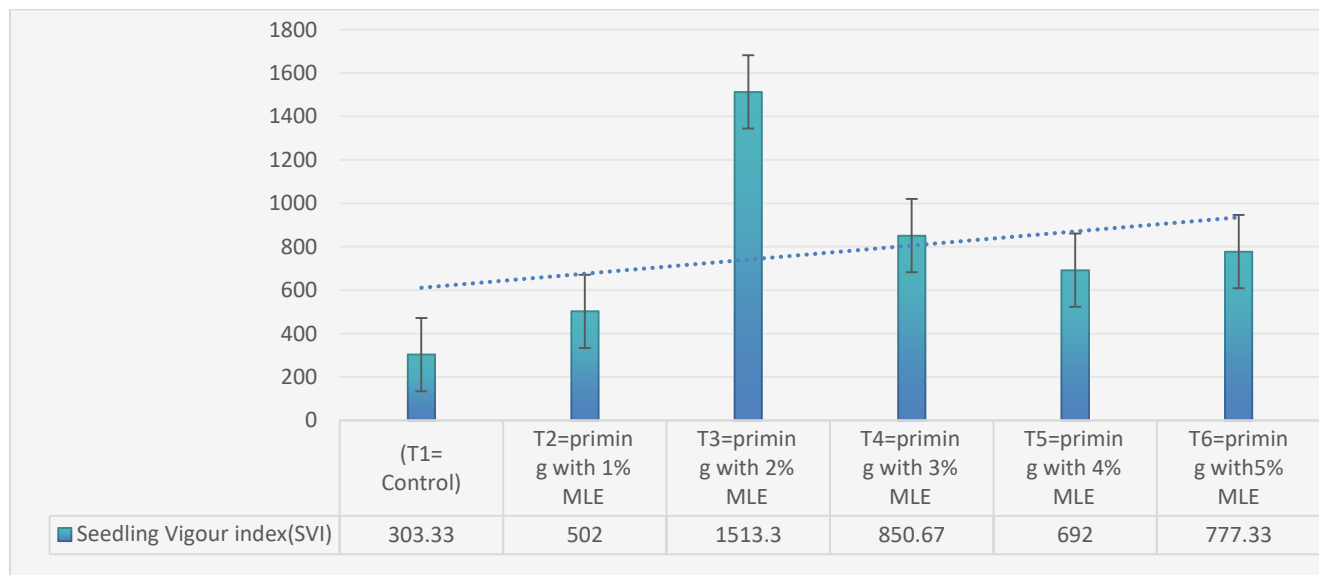


FIGURE 1 (c): Seedling Vigour index (SVI) of Chilli under the Influence of Varying Priming Treatments with MLE.

The (Figure 2 a) showing the results Regarding number of leaves per plant was statistically significant. The highest number of leaves per plant was observed with the 2% MLE priming treatment, which resulted in an average of 7.78 leaves. This suggests that 2% MLE is the most effective concentration for promoting leaf growth in chili plants. In comparison, the control group (T1), which did not receive any priming, had the lowest leaf count with only 4.89 leaves per plant. Other priming treatments showed varying levels of improvement in leaf number. For example, priming with 1% MLE (T2) resulted in 5.2 leaves per plant, while the 3% MLE treatment (T4) yielded 6.23 leaves per plant. Similarly, the 4% MLE treatment (T5) produced 5.89 leaves, and the 5% MLE priming (T6) resulted in 5.23 leaves per plant. Although these treatments led to an increase in leaf number compared to the control, they did not surpass the results of the 2% MLE treatment.

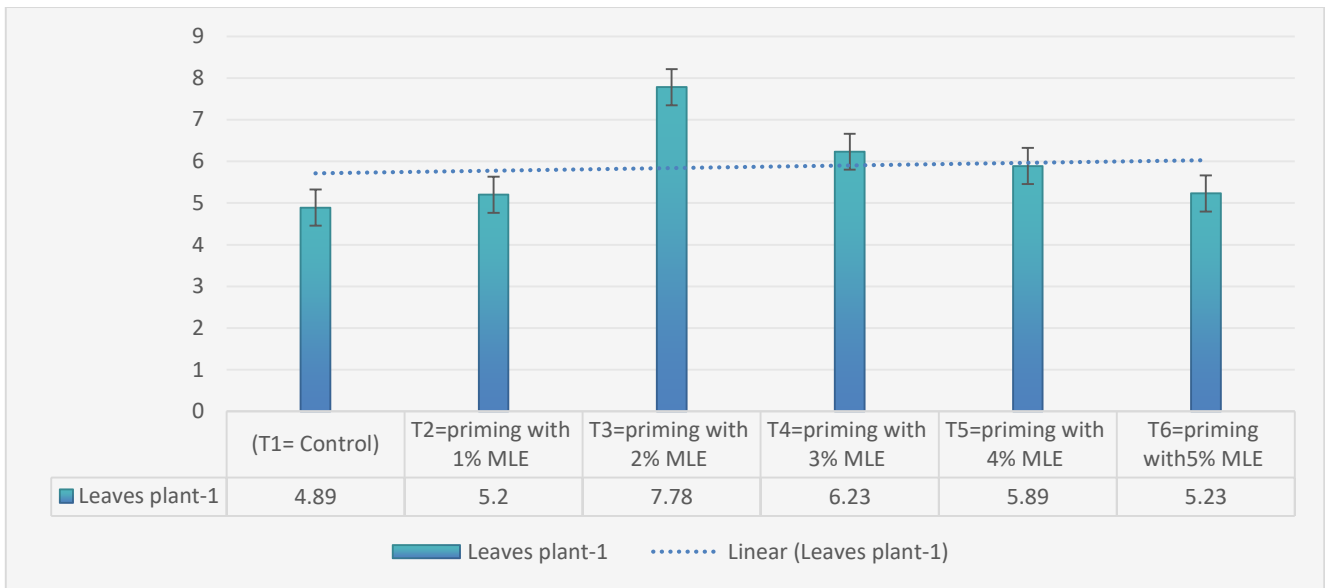


FIGURE 2 (a): Leaves plant-1 of Chili under the Influence of Varying Priming Treatments with MLE.

The (Figure 2 b) showing the results Regarding number of branches per plant was statistically significant. Among the different treatments, the 2% MLE priming (T3) proved to be the most effective, resulting in the highest number of branches per plant, with an average of 9.6 branches. This indicates that the 2% MLE concentration promotes optimal branching in chili plants. In contrast, the control group (T1), which did not receive any priming, had the lowest number of branches, with only 3.56 branches per plant. Priming with 1% MLE (T2) also showed a significant improvement, leading to 5.34 branches per plant. The 3% MLE treatment (T4) resulted in 6.34 branches per plant, demonstrating a moderate increase in branching. However, treatments with 4% MLE (T5) and 5% MLE (T6) produced fewer branches, averaging 4.23 and 4.67 branches per plant, respectively. While these treatments showed some improvement over the control, their effect was not as pronounced as that of the 2% MLE treatment. The enhanced differentiation of shoots and leaves in pepper plants in response to moringa leaf extracts according to our results might be due their content of hormones, namely cytokinins, that cause higher cell extension which promotes enhanced metabolite production and thus higher food translocation to young expending shoots (Nouman et al., 2012, Rady et al., 2015).

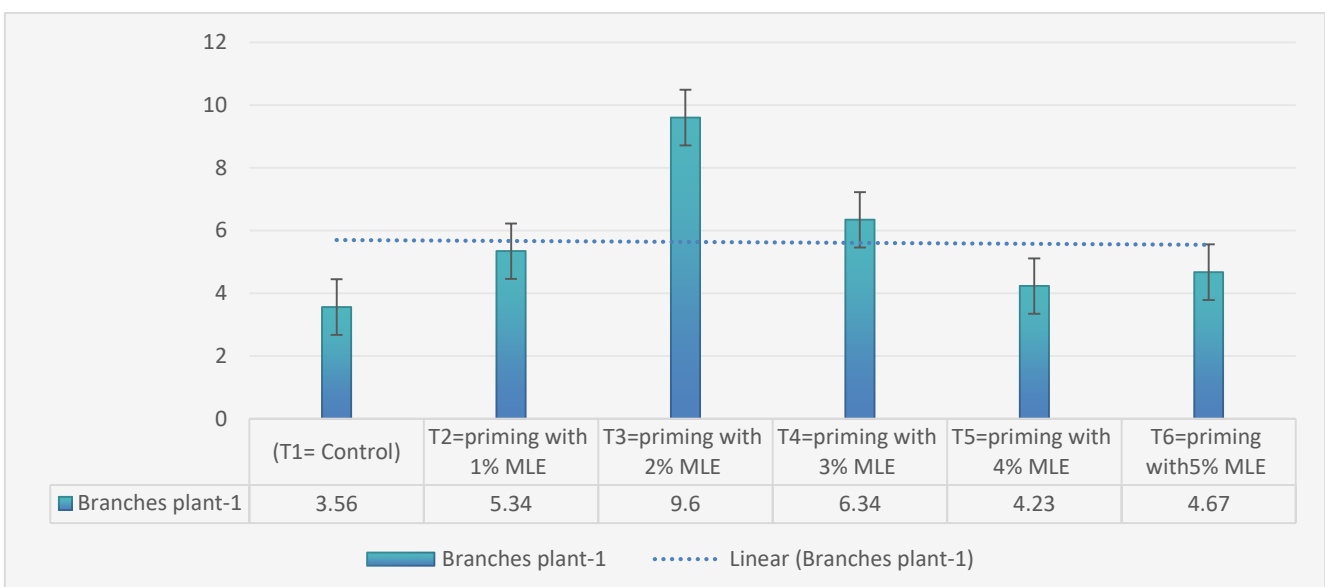


FIGURE 2 (b): Branches plant-1 of Chili under the Influence of Varying Priming Treatments with MLE.

The (Figure 2 c) showing the results Regarding Seedling height (cm) was statistically significant. The 2% MLE treatment (T3) resulted in the tallest seedlings, with an average height of 15.134 cm, making it the most effective treatment for promoting seedling growth. In contrast, the control group (T1), which did not undergo any priming, showed the shortest seedlings, with a height of only 7.06688 cm. Seedlings treated with 1% MLE (T2) exhibited a moderate increase in height, averaging 8.3334

cm. Similarly, priming with 3% MLE (T4) led to a noticeable improvement, with seedlings reaching an average height of 10.634 cm. The 4% MLE treatment (T5) also resulted in taller seedlings, averaging 11 cm, while the 5% MLE treatment (T6) produced seedlings with an average height of 10.134 cm.

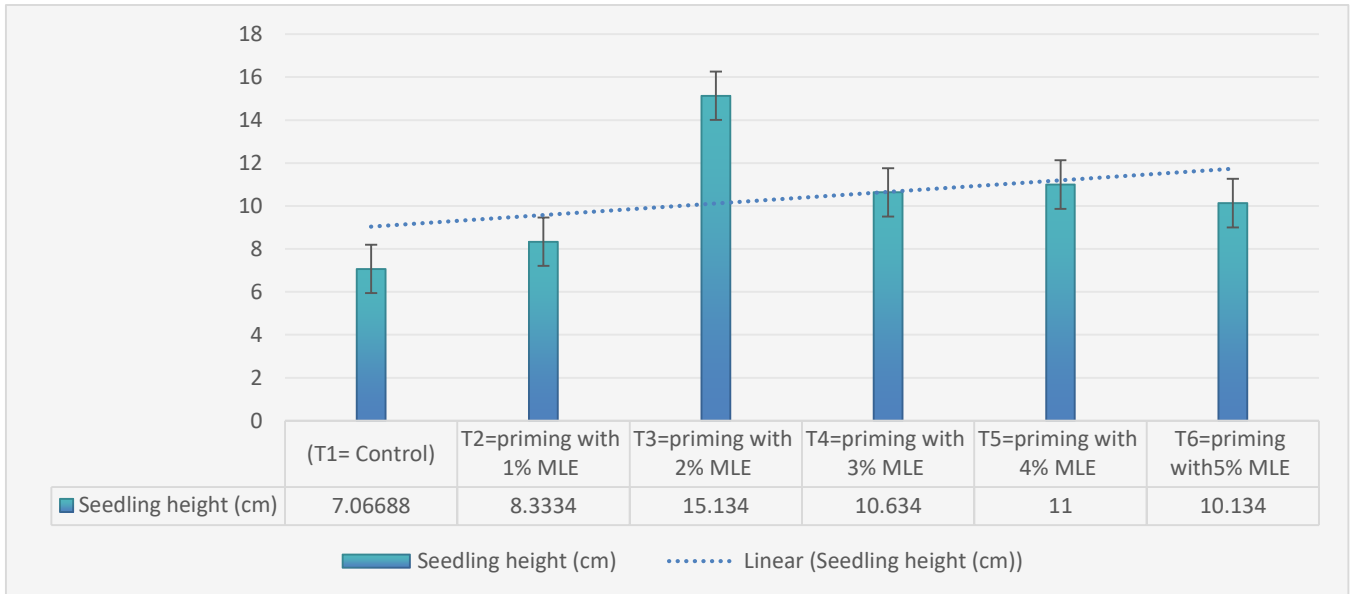


FIGURE 2 (c): Seedling height (cm) of Chilli under the Influence of Varying Priming Treatments with MLE.

The (Figure 3 a) showing the results Regarding Root length (cm) was statistically significant. The 2% MLE priming treatment (T3) resulted in the longest roots, with an average length of 6.5 cm, indicating that this concentration was the most effective for promoting root growth. In contrast, the control group (T1), which did not receive any priming, showed the shortest root length, measuring only 1.3668 cm. Priming with 1% MLE (T2) led to a moderate increase in root length, with an average of 2.3668 cm. Similarly, priming with 3% MLE (T4) resulted in roots averaging 3.5 cm in length, which is a noticeable improvement compared to the control. The 4% MLE treatment (T5) also promoted root growth, with an average root length of 5.4667 cm, while the 5% MLE treatment (T6) produced roots with an average length of 4.7 cm. In agreement with the results of the present study several reports indicated the favorable effects of Moringa on various chillies species which included significant effects on plant height, no. of leaves and branches, seedlings fresh and dry weight, leaf chlorophyll content, yield components and mineral content (Abou El-Nour and Eweis,2017, Dunsin and Odeghe, 2015, Aluko, 2016, Weerasingha and Harris, 2020).

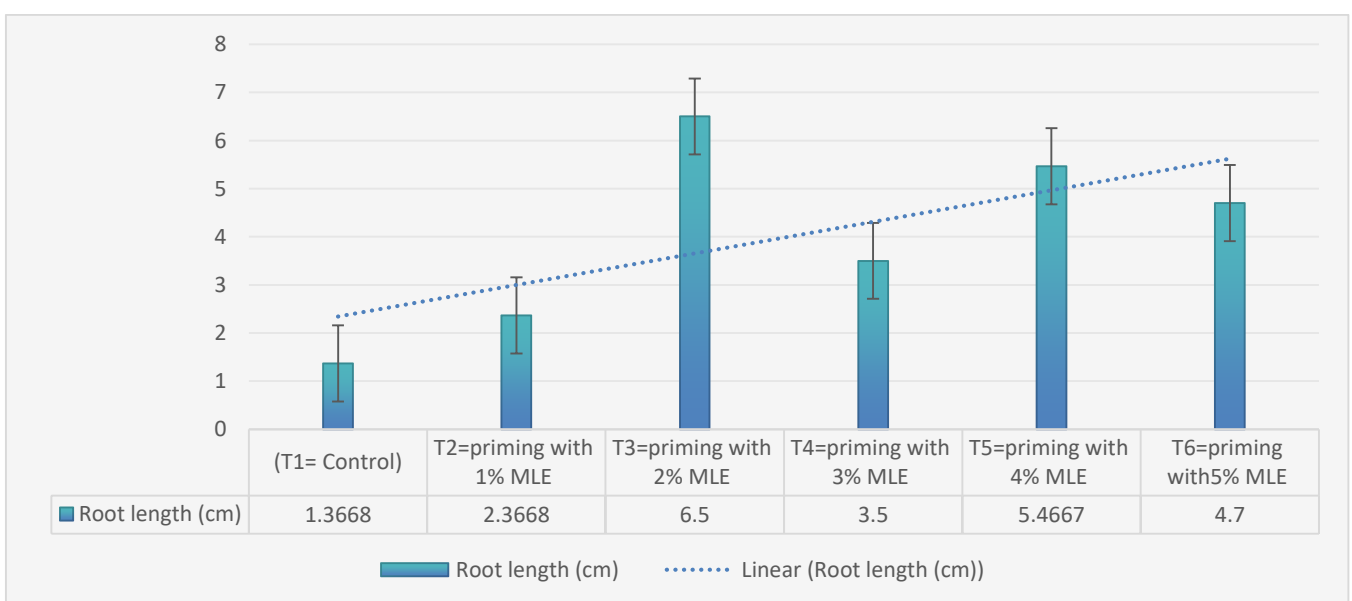


FIGURE 3 (a): Root length (cm) of Chilli under the Influence of Varying Priming Treatments with MLE.

The (Figure 3 b) showing the results Regarding Root Biomass (g) was statistically significant. The 2% MLE priming treatment (T3) resulted in the highest root biomass, with an average of 0.8668 g. This indicates that 2% MLE was the most effective treatment for promoting root biomass development in chili plants. In comparison, the control group (T1), which did not receive any priming, exhibited the lowest root biomass, with only 0.4634 g. Priming with 1% MLE (T2) led to an increase in root biomass, with an average of 0.58 g, while the 3% MLE treatment (T4) resulted in a root biomass of 0.63 g. Similarly, the 4% MLE treatment (T5) showed a moderate increase in root biomass, with an average of 0.6533 g. The 5% MLE treatment (T6), however, had a slight decrease in root biomass compared to other treatments, with an average of 0.52 g.

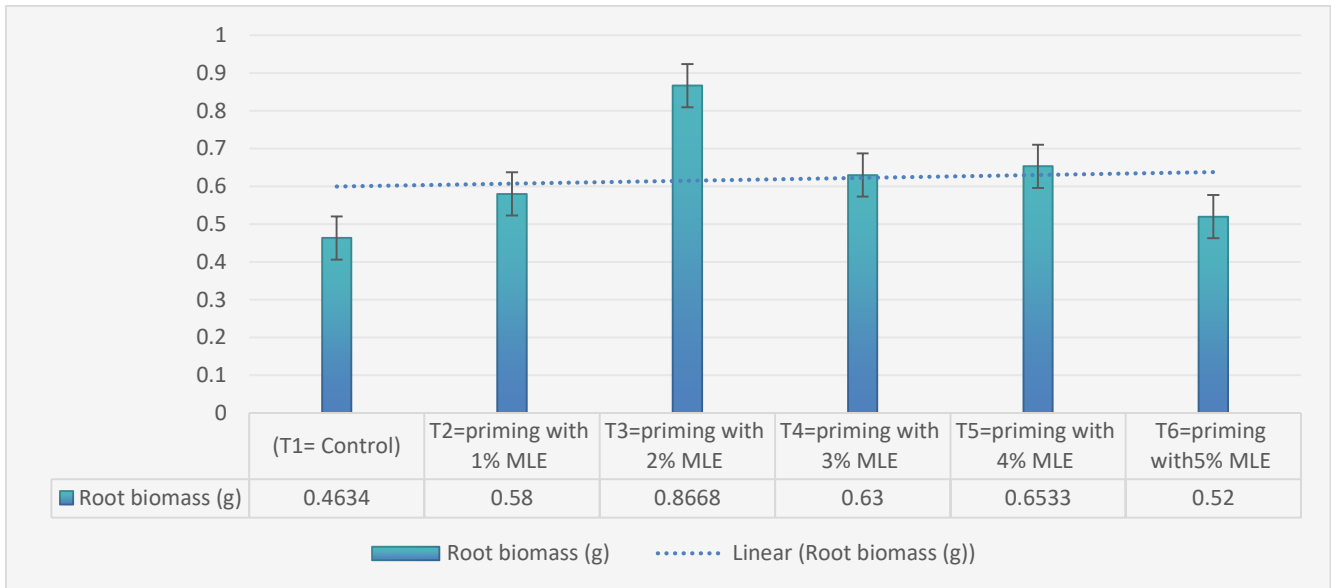


FIGURE 3 (b): Root biomass (g) of Chilli under the Influence of Varying Priming Treatments with MLE.

The (Figure 3 c) showing the results Regarding Shoot Biomass (g) was statistically significant. The highest shoot biomass was observed in the 2% MLE priming treatment (T3), with an average value of 1.3234 g. This suggests that priming with 2% MLE was the most effective for promoting shoot biomass accumulation in chili plants. In contrast, the control group (T1), which did not undergo any priming, showed a lower shoot biomass of 0.5868 g. Priming with 1% MLE (T2) resulted in a substantial increase in shoot biomass, with an average of 0.9368 g. The 3% MLE treatment (T4) also led to an increase in shoot biomass, with a value of 0.6668 g. However, priming with 4% MLE (T5) resulted in the lowest shoot biomass among all treatments, with only 0.5533 g. The 5% MLE treatment (T6) led to a moderate increase, yielding a shoot biomass of 0.63 g.

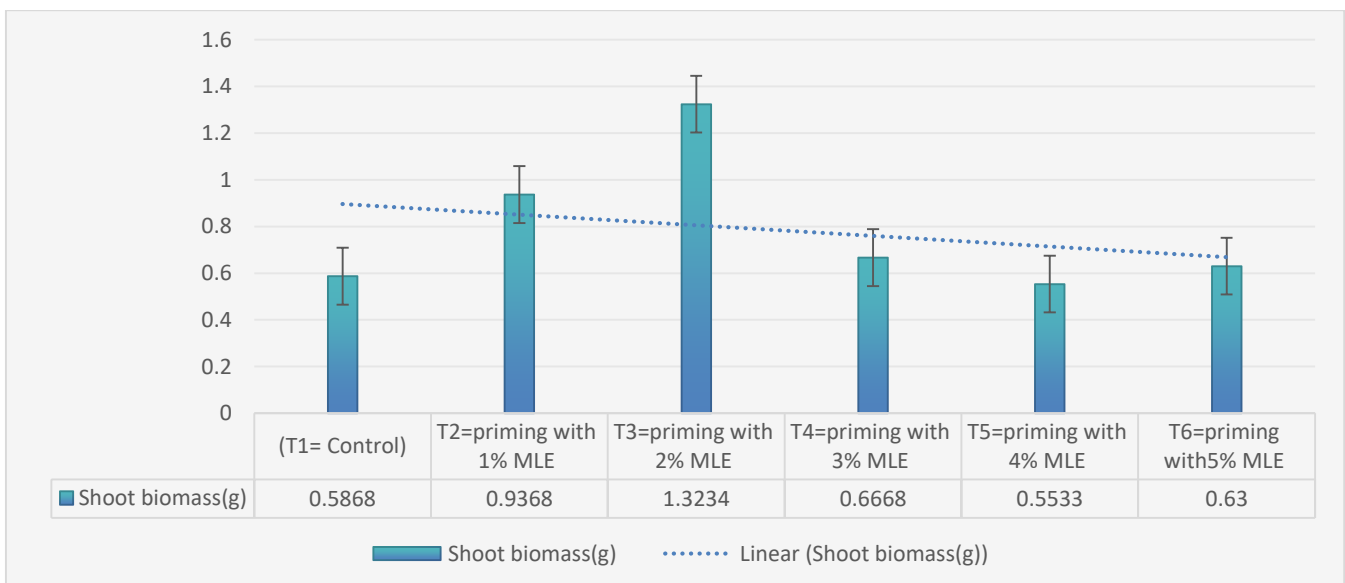


FIGURE 3 (c): Shoot biomass (g) of Chilli under the Influence of Varying Priming Treatments with MLE.

The (Figure 3 d) showing the results Regarding chlorophyll content was statistically significant. The 2% MLE priming treatment (T3) resulted in the highest chlorophyll content, with a SPAD value of 64.868. This indicates that 2% MLE priming was the most effective in enhancing chlorophyll production, which is essential for photosynthesis and overall plant health. In comparison, the control group (T1), which did not receive any priming, had the lowest chlorophyll content, with a SPAD value of 28.2. Priming with 1% MLE (T2) also led to an increase in chlorophyll content, with a SPAD value of 34.068. The 3% MLE treatment (T4) showed a moderate improvement, with a SPAD value of 35.9. Additionally, priming with 4% MLE (T5) resulted in a chlorophyll content of 42.634, and the 5% MLE treatment (T6) showed a SPAD value of 57.434, indicating that higher concentrations of MLE also enhanced chlorophyll content, though not as significantly as the 2% MLE treatment. Fuglie (2008) had reported that application of moringa leaf extract can increase yield of any crop by 25 to 30%. Nagar *et al.* (2006) pointed out that moringa leaves are rich in zeatin (naturally occurring cytokinin) hormone that enhances plant growth. Mvumi *et al.* (2012 and 2013) confirmed this on maize, beans and tomato buttressing the work of Fuglie (2000) that there was increased crop yields when crops were sprayed with and priming with extract from moringa leaves.

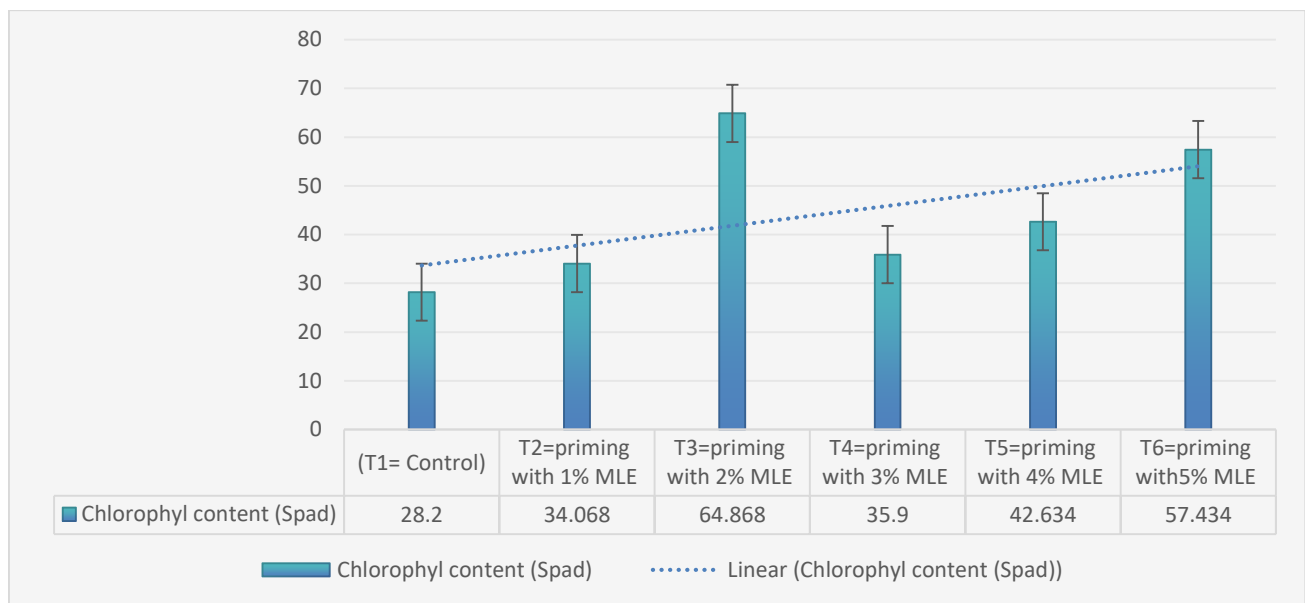


FIGURE 3 (d): Chlorophyll content of Chilli under the Influence of Varying Priming Treatments with MLE

IV. CONCLUSION

The priming with 2% MLE increased the germination of chili seeds, seedling growth, and plant development. This concentration showed the highest germination percentage, germination index, seedling vigor index, number of leaves and branches per plant, plant height, root length, root biomass, shoot biomass, and chlorophyll content. These results can be used to support the use of 2% MLE as a priming enhancing chili plant productivity and performance, thus providing valuable information for agricultural practices. Further research and field trials are therefore recommended to assess the practicality and benefits of MLE in chili production.

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Effects of *Musa Paradisiaca* Stem Juice on the Haematology Indices of Rabbits Induced with Organophosphate based Pesticide

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Abstract— To achieve crop productivity, pesticides are extensively used all over the world to minimize the damage caused by pests, and in turn pose serious challenges to farm animals, including rabbits which depend on crop residues and grasses. In this study, physiological effects of *Musa paradisiaca* juice (PSJ) on the haematological indices. To achieve crop productivity, pesticides are extensively used all over the world to minimize the damage caused by pests, and in turn pose serious challenges to farm animals, including rabbits which depend on crop residues and grasses. In this study, physiological effects of *Musa paradisiaca* juice (PSJ) on the haematological indices of organophosphate based pesticide (OPBP) exposed rabbits were assessed using thirty-six male rabbits. The study was conducted in a 3 x 4 factorial design assessing three concentration levels of organophosphates-based pesticide (0ml, 1.5ml and 3ml) and four levels of *Musa paradisiaca* juice (0ml, 5ml, 10ml and 15ml). At the end of twelve weeks trial, standard methods were used to determine the haematological indices which include white blood cells (WBC), red blood cells (RBC), haemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), platelets and WBC differential count. This study revealed that OPBP exerted well-defined effect on all the parameters studied; PSJ had moderate effect in some of the indices examined though all the values are within normal range, and PSJ was able to mitigate the effect of OPBP. It is therefore recommended that at least 5ml of PSJ should be given to rabbits in order to ward-off reproductive dysfunction in male rabbits which might arise as a result of OPBP residue in their feed.

Keywords— Haematology, *Musa paradisiaca* stem juice, organophosphates-based pesticide.

I. INTRODUCTION

Over time, the increasing human population significantly relies on agriculture for food production and nutrition (Mulla *et al.*, 2020). Pesticide use has grown increasingly in order to decrease crop damage and boost land production. The environmental quality of both human and animal health are impacted by the load of pesticides used for crop protection (Siriwong, 2006).

Due to the ease of purchase and high effectiveness in pest control, farmers are applying pesticides in large quantities. One of the most widely used groups of pesticides is the organophosphate pesticides as they are highly effective in eradication of pests. However, the extensive use of these pesticides leads to the contamination of environmental surroundings (Barceló *et al.*, 1991). It has also been observed that various pesticides affect human beings and animals' health due to their capability to interact with living systems, especially the endocrine system (Encarnaçao *et al.*, 2019)

Organophosphates are neurotoxins that act by preventing acetylcholine esterase in the central and peripheral nervous system, causing choline and acetate formation, and resulting in the blocking of the nerves (Mulla *et al.*, 2020). These inhibitions due to exposure by animals lead to convulsion, paralysis, and lastly death for insects, and to lesser extent mammals (Singh and Walker, 2006). Additionally, organophosphates can cause genotoxic and carcinogenic effects on animals (Kaushik *et al.*, 2009).

As stated by Alabi *et al.* (2013), *Musa paradisiaca* (plantain) is a crop in the genus *Musa* and all members of the genus are indigenous to the tropical and sub-tropical. Various parts of *Musa paradisiaca* promote healthy digestion, enhance stability, help retain, and serve as good sources of potassium, calcium, phosphorus, and nitrogen for animals. Plantain also helps in the

development and regeneration of body tissues and is a rich source of iron and vitamins, especially Vitamins C and E (Alabi *et al.* 2013).

Literature reports abound that in some traditional settings, the leaves, roots, and fruits of plantain are used effectively in the purification management of male sexual dysfunction (Alabi *et al.* 2013). Park *et al.* (2017) and Fakher *et al.* (2019) claim that the inclusion of plant extracts in diets improves blood circulation, biochemical indices, semen parameters, androgen status, and fertility index, and has a positive influence on sperm quality in males.

Therefore, research works conducted using natural diets like plantain showed that its consumption by men could enhance some reproductive functions and alleviate certain reproductive dysfunctions.

The aim of this study, therefore, is to determine the efficacy of using plantain stem juice to detoxify the effect of organophosphate based pesticide on the haematological indices of normal adult rabbits induced with organophosphate based pesticide. Specifically, the research was designed to ascertain:

- 1) The effect of plantain stem juice on the hematological profile (PCV, WBC, RBC, HB, Differential counts) of rabbits
- 2) The effect of organophosphate based pesticide on the hematological profile (PCV, WBC, RBC, HB, Differential counts) of rabbits
- 3) The plantain juice x organophosphate based pesticide interaction effect on hematological profile (PCV, WBC, RBC, HB, Differential counts) of rabbits

II. MATERIALS AND METHOD

2.1 Experimental site and Location:

The experiment was conducted at the Rabbit unit of the Teaching and Research Farm of Department of Animal science and Technology, Faculty of Agriculture, Nnamdi Azikiwe University, Awka campus, Anambra State, Southeast of Nigeria. The town, Awka is located at latitude 6 12'25"N and longitude 7 04'04'E (6.212090 N and 7.071990 E respectively) with the altitude of 9m above sea level.

2.2 Experimental Subjects:

Organophosphate based pesticide was purchased from a chemical store at Eke Awka market in Anambra state. The fresh stem of a matured plantain was harvested from the school, in Nnamdi Azikiwe University premises close to the farm area and dirt was removed and washed. The plantain stem was chopped and crushed using a blender, the juice was then extracted using a sieve and it was administered to the rabbit by drench. This test experiment lasted for a period of three months (sixty days for application of test factors and one month for collection of data).

2.3 Experimental Animals:

A total of thirty-six male rabbits were procured from a reputable farm at Nnobi in Anambra state and transported to the Departmental Teaching and Research Farm of Animal Science and Technology, Nnamdi Azikiwe University Awka. They were transported early in the morning to avoid harsh weather. The animal was kept in a hutch of 3m x 2mx1m high and they were acclimatized for one week and fed with fresh leaves for this period.

2.4 Experimental Design:

The experiment was conducted using 3x4 factorial design and the model proposed is:

$$Y_{ijk} = \mu + P_i + O_j + PO_{ij} + \epsilon_{ijk} \quad (1)$$

Where:

Y_{ijk} is the kth observation on the jth rabbit within the ith group. Otherwise called response variable.

μ = population mean.

P_i - is the effect of Plantain stem juice

O_j - organophosphate effect

PA_{ij} - Interaction effect

Eijk= Experimental error.

2.5 Experimental Layout:

A total of thirty-six (36) rabbits were randomly assigned for this study. The Experimental layout is as shown in Table 1.

TABLE 1
THE EXPERIMENTAL LAYOUT

Treatment					
Organophosphates	Replicate	T ₁ PJ 0 ml	T ₂ PJ 5 ml	T ₁ PJ 10 ml	T ₁ PJ 15 ml
0 ml	1	T ₁ R ₁	T ₂ R ₁	T ₃ R ₁	T ₄ R ₁
	2	T ₁ R ₂	T ₂ R ₂	T ₃ R ₂	T ₄ R ₂
	3	T ₁ R ₃	T ₂ R ₃	T ₃ R ₃	T ₄ R ₃
1.5ml in 250ml of H ₂ O	1	T ₁ R ₁	T ₂ R ₁	T ₃ R ₁	T ₄ R ₁
	2	T ₁ R ₂	T ₂ R ₂	T ₃ R ₂	T ₄ R ₂
	3	T ₁ R ₃	T ₂ R ₃	T ₃ R ₃	T ₄ R ₃
3ml in 250mL of H ₂ O	1	T ₁ R ₁	T ₂ R ₁	T ₃ R ₁	T ₄ R ₁
	2	T ₁ R ₂	T ₂ R ₂	T ₃ R ₂	T ₄ R ₂
	3	T ₁ R ₃	T ₂ R ₃	T ₃ R ₃	T ₄ R ₃

2.6 Management of experimental Animals:

The rabbit hutch, drinkers and feeding troughs were thoroughly cleaned and disinfected using detergent and clean water and later rinsed. Feeds were also provided for the rabbits, the rabbits were fed on forages, which was harvested within the university premises and commercial grower ration was supplemented for the rabbit's nutrition twice daily. Water was also provided daily for them; medication was administered to them on arrival, and subsequently given anti stress, while antibiotics were administered if necessary. Daily standard management practices were carried out throughout the experiment.

III. DATA COLLECTION

3.1 Blood Sample:

The blood sample was collected from the ear vein of the bucks. Using the sterile gauges and syringes, about 5ml of blood was expelled gradually into a bottle, containing ethylenediamine tetra acetic acid (EDTA). The bottle was capped, and the content mixed gently to avoid coagulation, the samples were sent to the laboratory haematological studies.

3.2 Haematological studies:

All the haematological parameters were determined following the procedure outlined by Lamb (1981). The parameters determined include Red Blood Cell volume (RBC) count, White Blood Cell (WBC) count, Packed Cell Volume (PVC), Haemoglobin Concentration (HB), and Differential Count.

3.3 Statistical Analysis:

Data obtained were subjected to Analyses of Variance (ANOVA) using SPSS to determine the differences between treatments. Differences between treatments means were separated using Duncan's Multiple Range Test (DMRT).

IV. RESULTS

4.1 Effect of Plantain Stem Juice on Haematological Profile of Rabbits:

Table 2 shows the effect of PSJ on the haematological profile and differential counts of rabbits.

TABLE 2
EFFECT OF PLANTAIN STEM JUICE ON HAEMATOLOGICAL PROFILE OF RABBITS

Parameters	T1 (0ml)	T2 (5ml)	T3 (10ml)	T4 (15ml)	SEM
WBC ($\times 10^3/\text{mm}^3$)	4.94	4.58	4.98	3.92	0.23
RBC ($\times 10^6/\text{mm}^3$)	4.86	4.99	4.42	4.07	0.17
Hb (g/dl)	9.689	9.79	9.04	8.5	0.26
MCV (FL)	50.07	48.42	46.99	45.77	1.41
MCH (pg.)	20.24	19.7	20.02	20.73	0.38
MCHC (g/dl)	40.96	43.48	42.6	42.76	0.88
PCV (%)	24.99	24.49	22.17	24.69	0.83
Platelet	104.11	99.08	132.33	81.11	10.91
Differential count					
Lymphocyte	50.91 ^b	57.92 ^{ab}	58.01 ^{ab}	58.56 ^a	1.28
Neutrophil	42.92	37.59	36.54	35.88	1.3
Monocyte	3.44	2.89	3	3.11	0.1
Eosinophil	2.22 ^a	1.33 ^b	1.89 ^{ab}	1.67 ^{ab}	0.12
Basophil	0.87 ^a	0.39 ^b	0.68 ^{ab}	0.80 ^{ab}	0.08

^{ab} means bearing different superscripts along the same row are significantly different ($P < 0.05$), SEM- Standard error of the mean. Hb – Haemoglobin, PCV – Packed Cell Volume, WBC – White Blood Cell, RBC – Red Blood Cell, MCV – Mean Corpuscular Volume, MCH – Mean Corpuscular Haemoglobin, MCHC – Mean Corpuscular Haemoglobin Concentration.

Plantain stem juice did not exert any significant effect ($P > 0.05$) on the haematological profile of the rabbits, except for differential counts.

Rabbits given 15 ml of PSJ (T4) were significantly higher ($P < 0.05$) in the number of lymphocyte count than those in 0ml (T1) of PSJ, but were not significantly different from those in 5 ml (T2) and those in 10 ml (T3). Eosinophil (2.2) and basophil (0.87) were significantly higher ($P < 0.05$) in rabbits with 0 ml of PSJ (T1) than those in 5 ml of PSJ (T2). However, the values were not significantly different ($P > 0.05$) from those receiving 10 ml (T3) and those receiving 15 ml (T4).

4.2 Effect of Organophosphate Based Pesticide on Hematological Profile and Differential count of Rabbits:

The effects of Organophosphate Based Pesticide on Hematological Profile and Differential count of Rabbits are presented in Table 3.

TABLE 3
EFFECT OF ORGANOPHOSPHATE BASED PESTICIDE ON HEMATOLOGICAL PROFILE OF RABBITS

Parameters	T1 (0ml)	T2 (1.5ml)	T3 (3ml)	SEM
WBC ($\times 10^3/\text{mm}^3$)	4.64 ^{ab}	3.68 ^b	5.49 ^a	0.23
RBC ($\times 10^6/\text{mm}^3$)	5.07 ^a	4.32 ^b	4.00 ^c	0.17
Hb (g/dl)	9.40 ^a	9.00 ^b	8.05 ^c	0.26
MCV (FL)	59.88^a	55.94^b	48.99^c	1.41
MCH (pg.)	20.83^a	20.13^b	18.59^c	0.38
MCHC (g/dl)	47.35^a	45.00^b	39.05^c	0.88
PCV (%)	24.13 ^a	20.00 ^b	17.05 ^c	0.5
Platelet	125.08 ^a	99.39 ^b	88.00 ^c	9.22
Differential count				
Lymphocyte	52.73 ^b	56.48 ^{ab}	59.84 ^a	1.28
Neutrophil	43.09 ^a	37.29 ^{ab}	34.32 ^b	1.3
Monocyte	2.88 ^b	3.33 ^a	3.17 ^{ab}	0.1
Eosinophil	1.25 ^b	2.17 ^a	1.92 ^a	0.12
Basophil	0.49	0.81	0.76	0.08

^{ab} means bearing different superscripts along the same row are significantly different ($P < 0.05$), Hb – Haemoglobin, PCV – Packed Cell Volume, WBC – White Blood Cell, RBC – Red Blood Cell, MCV – Mean Corpuscular Volume, MCH – Mean Corpuscular Haemoglobin, MCHC – Mean Corpuscular Haemoglobin Concentration, SEM- Standard error of the mean

Significant differences ($P < 0.05$) were observed in all the major blood parameters studied. WBC counts did not follow any definite pattern, but RBC counts of rabbits that received 0.00ml (T1) of OBP were significantly higher ($P < 0.05$) than the RBC counts of those that received 1.5ml of OBP, and at the same, the RBC counts of those that received 1.5ml of OBP were higher ($P < 0.05$) than those that were given 3ml OBP. Similar trend was observed in Hb, MCV, MCH, MCHC, PCV and platelet counts, where rabbits in control group (T1) recorded higher values ($P < 0.05$) than those in T2 with 1.5ml OBP, and the least ($P < 0.05$) being those in T3 with 3ml OBP.

Again, significant differences ($P < 0.05$) were also observed in all the differential counts except for basophil. Lymphocyte was significantly higher ($P < 0.05$) in rabbits administered 3ml of OBP (T3) but not significantly different ($P > 0.05$) from those given 1.5ml OBP (T2). Neutrophil was higher ($P < 0.05$) in those without OBP (T1), but similar with those given 1.5ml OBP (T2). Rabbits with 1.5ml OBP (T2) was significantly higher ($P < 0.05$) in monocyte, with the mean values of 3.33 but not significantly different ($P > 0.05$) from those given 3ml OBP (T3). T2 and T3 were significantly higher ($P < 0.05$) than T1 in mean eosinophil with the mean values of 2.17 and 1.92, respectively

Plantain Stem Juice (PSJ) x Organophosphate Based Pesticide (OBP) on Hematological Profile of Rabbits.

TABLE 4
SHOWED THE INTERACTION EFFECT OF OBP AND PSJ ON HAEMATOLOGICAL PROFILE OF RABBITS

OBP	PSJ	WBC	RBC	Hb	MCV	MCH	MCHC	PCV	Platelet
0ml	0ml	4.3	4.1867	8.9667	46.6	19.5	39.3667 ^c	24.2	95.6667 ^b
	5ml	5.2667	4.2	9.3	51.6333	19.7	42.7000 ^b	24.0667	93.3333 ^b
	10ml	5.3667	4.5	9.5333	49.3567	21.2667	44.6000 ^b	24.6667	102.2333 ^a
	15ml	5.6333	4.5867	9.6667	52.4	23.1	49.4333 ^a	23.6	106.3333 ^a
1.5ml	0ml	3.3333 ^d	3.2667 ^c	6.7000 ^d	34.2000 ^d	19.4000 ^c	39.9333 ^c	25.5667 ^b	75.3333 ^d
	5ml	3.6333 ^c	3.7333 ^b	8.2667 ^c	46.5667 ^c	20.6667 ^{bc}	40.7333 ^c	26.3667 ^b	82.0000 ^c
	10ml	3.8633 ^b	5.1000 ^{ab}	10.6000 ^b	49.2000 ^b	20.9000 ^b	45.8333 ^{ab}	26.8000 ^{ab}	92.3333 ^b
	15ml	5.1333 ^a	5.1833 ^a	10.4333 ^a	51.6333 ^a	21.5667 ^a	47.7667 ^a	26.9333 ^a	102.3333 ^a
3ml	0ml	5.1333 ^d	4.3700 ^c	9.1	42.0000 ^d	16.9333 ^c	38.3333 ^d	23.2000 ^c	68.0000 ^d
	5ml	5.4000 ^c	5.0233 ^b	9.3333	47.8000 ^c	18.2333 ^b	39.2667 ^c	23.5000 ^{bc}	102.6667 ^c
	10ml	5.5000 ^b	5.2133 ^b	9.5	48.6000 ^b	18.8000 ^b	40.2667 ^b	23.9000 ^b	114.3333 ^b
	15ml	5.9333 ^a	5.6700 ^a	9.6667	53.7667 ^a	22.0333 ^a	41.1333 ^a	25.2000 ^a	215.3333 ^a
SEM		0.672	0.517	0.823	4.646	1.054	2.734	1.463	29.081

^{ab} means bearing different superscripts along the same column are significantly different ($P < 0.05$), SEM- Standard error of the mean

At 0ml of OBP, no significant ($P > 0.05$) interaction effect was observed at all levels of PSJ except for MCHC and platelet counts. MCHC was highest ($P < 0.05$) at 15ml addition of PSJ and least at 0ml inclusion of PSJ. The platelet counts followed the same trend with MCHC.

But at 1.5ml inclusion level of OBP, significant differences ($P < 0.05$) were recorded at all inclusion levels of PSJ. As PSJ inclusion level increase from 0ml to 15ml, virtually all the blood indices increase progressively. However, increase in PCV was not significant ($P > 0.05$) between 0ml and 5ml inclusion levels, and between 10ml, and 15ml levels of PSJ addition. Equally, for MCHC, 0ml and 5ml PSJ inclusion levels exerted similar effect, and 10ml and 15ml PSJ addition level exhibited the same effect.

Furthermore, at 3ml of OBP level, sharp increments ($P < 0.05$) were observed from 0ml to 5ml, 5ml to 10ml and 10ml to 15ml inclusion levels of PSJ.

4.3 Plantain Stem Juice (PSJ) x Organophosphate Based Pesticide (OBP) on Differential Counts of Rabbits:

The interaction effect between Plantain Stem Juice (PSJ) x Organophosphate Based Pesticide (OBP) on Differential Counts of Rabbits is given in Table 5.

TABLE 5
EFFECT OF PLANTAIN STEM JUICE (PSJ) X ORGANOPHOSPHATE BASED PESTICIDE (OBP) ON THE DIFFERENTIAL COUNT OF RABBITS

OBP	PSJ	Lymphocytes	Neutrophil	Monocyte	Eosinophil	Basophil
0ml	0ml	55.4	51.5333	2.6667	1	0.5167
	5ml	55.1667	47.8667	3	1	0.1
	10ml	56.7333	48.6667	2.6667	1.6667	0.6
	15ml	55.6333	49.3	3	1.3333	0.7333
1.5ml	0ml	57.3	34.8	4.0000 ^a	3.0000 ^a	0.9
	5ml	52.8	42.9667	2.6667 ^b	1.3333 ^b	0.4667
	10ml	61.6	32.5	3.0000 ^b	2.0000 ^b	0.9333
	15ml	54.2000 ^c	38.9	3.6667 ^{ab}	2.3333 ^b	0.9333
3ml	0ml	50.0333 ^c	42.4333 ^a	3.6667 ^a	2.6667	1.2
	5ml	67.8000 ^a	26.9333 ^b	3.0000 ^b	1.6667	0.6
	10ml	55.7000 ^b	38.4667 ^a	3.3333 ^b	2	0.5
	15ml	65.8333 ^a	29.4333 ^b	2.6667 ^b	1.3333	0.7333
SEM		3.222	3.039	0.255	0.255	0.249

^{ab} means bearing different superscripts along the same column are significantly different ($P < 0.05$), SEM- Standard error of the mean.

No significant interactions effect ($P < 0.05$) was detected at 0ml of OBP irrespective of the level of PSJ. However, at 1.5ml of OBP, significant differences were observed in monocytes and eosinophil. But these differences have no definite inclination. Again at 3ml level of OBP, significant differences were experiential in lymphocytes, neutrophil and monocyte counts. However, the differences did not follow any definite pattern.

V. DISCUSSION

Mitruka and Rawnsley (1977) reported the haematological indices of rabbits as follow: PCV 30 – 35%, Hb 9.3 – 19.3g/dl and RBC 4.00 – 8.60 ($\times 10^6/\text{mm}^3$). The values obtained in this study were in line with the report of Mitruka and Rawnsley (1977), except for PCV, which was lower than the 30-35% earlier reported. Furthermore, Poole (1987) reported a PCV range of 30 – 50%. Lower PCV values observed in this study could be due to the harmful effects of the treatments. However, Olabanji *et al.* (2007) reported the range of 21.0– 30.0 $\times 10^6/\text{mm}^3$, which concur with the PCV values obtained in this study.

The Hb values obtained in this study agreed with the findings of RAR (2009), Saleh *et al.* (2014) and Ragab *et al.* (2014) who reported the range of 10-15, 11.70 and 8.90g/dl, respectively as the normal values for Hb in rabbits. Olabanji *et al.* (2007) reported the range of 7.02– 9.92g/dl which is also within the same range.

Most of the white blood cell counts (WBC) obtained in this study is within normal range going by the work of Burke (1994), RAR (2009), Saleh *et al.* (2014) and Ragab *et al.* (2014) who reported the WBC count in rabbits to be 4.5-11, 12.90 and 7.38 $\times 10^3/\text{mm}^3$, respectively. However, when 15ml of PSJ (T4) and 1.5ml of OBP (T2) were administered, lower ranges were obtained. Nwosu (1979) reported that higher WBC count may explain the reason for disease resistance which has been reported or the prevalence of disease condition. It may also explain longevity as reported by Mbanasor *et al.* (2003). Lower than normal White Blood Cells (WBC) count suggests a greater challenge to the immune system of rabbits. Eheba *et al.* (2008) noted that a decrease in WBC count, however, reflected a fall in the production of defensive mechanism to combat infection. The fact that all the treatments in this study except for T4 (15ml of PSJ) and T2 (1.5ml OBP) fell within normal physiological range indicates that the rabbits' immune systems were well developed. This also demonstrated that administering 15ml of PSJ and 1.5ml OBP in rabbits had a negative impact on their immune systems.

Saleh *et al.* (2014) and Ragab *et al.* (2014) reported the RBC counts in rabbits to be 5.5 and 3.43 $\times 10^6/\text{mm}^3$, respectively. Olabanji *et al.* (2007) also reported the range of 3.53– 5.05 $\times 10^6/\text{mm}^3$ for healthy rabbits, which is within the range obtained in this study. The RBC count helps in the depiction of anaemia. The within normal physiological range values recorded for all the groups is an indication of the absence of vulnerability of the rabbits to anaemia-related disease conditions (Jiwuba *et al.*, 2021).

The MCV range of 45.77 – 50.07 fl obtained in table 2 and 45.40 – 50.00fl in table 3, were lower than the range of 59.4– 59.8fl (Olabanji *et al.*, 2007), 78-95 (RAR, 2009) and 68.80 (Saleh *et al.*, 2014). The MCH values obtained in Tables 1 and 2 were within the range 21.00pg (Saleh *et al.* (2014) and 19.7– 19.8pg (Olabanji *et al.*, 2007). However, the MCH of T3 were lower the values earlier reported. The MCHC range of 40.96-43.48g/dl obtained in Table 2 and 39.75-43.54 in Table 3 were within the range reported by some authors. RAR (2009) reported the range of 27-37 g/dl, 30.60g/dl (Saleh *et al.*, 2014), 33.1– 33.3g/dl (Olabanji *et al.*, 2007).

Various lymphocyte values may represent different levels of immune status in farm animals, according to Aikhuomobhogbe and Orheruata (2006), while low lymphocyte levels may suggest either a deficient immune system or a high neutrophil level in an active infection, according to Lazzaro (2001). Neutrophil, Lymphocytes, Monocytes and Eosinophils range of 32.17 – 34.5%, 52.17– 59.3%, 0.67– 1.50% and 0%, respectively has been reported by Olabanji *et al.*, (2007). The Lymphocyte counts obtained in T2 (57.92%), T3 (58.01%) and T4 (58.56%) in Table 2 and T1 (52.73%), T2 (56.48%) and T3 (59.84%) in Table 3 fall within the range recorded by Olabanji *et al.*, (2007). Togun *et al.* (2007) reported that a significantly lower lymphocyte count was an indication of a reduction in the ability of the experimental rabbits to produce and release antibodies when infections occur (Campbell and Lasley, 1975). The neutrophil count was higher than the range of 32.17 – 34.5% reported by Olabanji *et al.* (2007). Neutrophil count in T1 and T2 were higher than that reported by same authors, while T3 (34.32%) falls within the range. Monocyte counts ranged from 2.89 – 3.44% in Table 2 and 2.88 – 3.33% in Table 3, these values were much higher than the range of 0.67– 1.50% that reported by Olabanji *et al.* (2007). Similarly, the eosinophil counts were higher than the 0% reported by same authors.

Amir *et al.* (2022) observed that Hb, and PCV were significantly decreased in baby and juvenile rabbits exposed to Malathion which is an organophosphate pesticide. They attributed to it be because of failing of hematopoietic system in rabbit exposed to malathion. As toxicants exposure exerts an adverse effect on the hematopoietic organs which in turn could alter blood parameters (Sharmin *et al.*, 2016). A significant decrease in Hb concentration to malathion may be due to less oxygen supply to different tissues, resulting in a sluggish metabolic rate and low energy output (Mrong *et al.*, 2021). This decline in Hb and RBC values could happen due to the breakdown of iron synthesizing machinery (Sethuraj *et al.*, 1992). The observations by the above authors were in contrast with the findings in this work; this may be because the rabbits used were adults. PCV values obtained in this study, although at the lower range readings are important in determining the effect of pesticides and in determining the oxygen-carrying capacity of the blood.

In our study, although not significant ($P>0.05$), the neutrophil and eosinophil concentrations were decreased in Table 1, while in Table 2, neutrophil counts decreases while eosinophil increased These cells play a key role in combating a great variety of weapons and diverse chemicals as chemotactic triggers that are directed against different kinds of pathogens. The granules secreted from eosinophils and neutrophils into the extracellular space upon activation consist of a variety of important mediators that are crucial for the function of granulocytes within innate immunity (Gigon *et al.*, 2021).

VI. CONCLUSION

The administration of 5ml to 15ml of plantain stem juice to male rabbits induced with Organophosphate based Pesticide was capable of detoxifying the rabbits going by the haematological characteristics studied. Thus, PSJ is one of the efficient natural detoxifiers, which is environmentally friendly, cheap and readily available in our local environment. Rabbit farmers, especially those using forage, and or direct crop residue are encouraged to occasionally give their rabbits PSJ for sustainable rabbit production. This will invariably reduce toxicity due to organophosphate based pesticides often reported in rabbit industries.

The study recommends that 5 to 15 ml of plantain stem juice should be given to rabbit bucks being raised in our local environment where farmers often use organophosphate based pesticides for eradication of pests and pathogens in their fields.

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Effect of Nano Urea on Increasing Nitrogen use Efficiency and Productivity of Wheat under Restricted Irrigation Condition

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Abstract— Nanotechnology has made a real revolution in the agriculture. The use of nano fertilizers has led to the increased productivity, reduced production costs in the last decade and also led to the increased production stability due to reduction of biotic and abiotic stresses. Nano fertilizer, the most important field of agriculture has drawn the attention of the soil scientists as well as the environmentalists due to its capability to increase yield, improve soil fertility, induce drought tolerance, reduce pollution and make a favourable environment for microorganisms. So considering these aspects a field experiment was conducted during rabi, 2021-22 to evaluate the “Effect of nano urea on increasing nitrogen use efficiency and productivity of wheat under restricted irrigation condition”. The trial comprised of different nine treatments with three replications in Randomized Block Design. The result revealed that effect of different nano urea treatment affected significantly on yield and yield attributes of wheat. Significantly higher grain yield (44.40 q/ha), biomass yield (107.29 q/ha) effective tillers per metre square (387) and grains per spike (38) were recorded by application of Recommended N + two spray of nano urea at tillering (40-45 DAS) and jointing (60-65 DAS) (T₃), however other yield attributing characters like plant height (78.80 cm) and length of spike (7.25 cm) were recorded significantly by application of Rec. N + one spray of urea (5%) + nano urea at tillering (40-45 DAS) (T₈) while, there was no significant effect of any treatments on 1000 grain weight.

Keywords— Wheat, Nano urea, 1000 grain weight, tillers, nitrogen use efficiency, food security.

I. INTRODUCTION

Wheat (*Triticum* spp.) belongs to poaceae family and is the second important food grain crop of India being next to rice. Among the different wheat species, common bread wheat [*Triticum aestivum* L. emend Fiori and Paol. (6n=42)], occupying more than 85 per cent of the total area under wheat cultivation. Wheat has its own outstanding importance as a human food, rich in carbohydrates and protein. About 35 per cent of the World’s population directly or indirectly depends upon wheat for food and providing 20 per cent of human dietary and energy supply and serving as the main source of protein in developing nations (Braun *et al.*, 2010). In the world, wheat is grown in China, India, Thailand, Indonesia and U.S.A. with an area of 221.17 million hectares and produced 774.66 million tonnes with an average productivity of 3.5 tonnes per hectare (WAP, 2022). In India wheat is an important cereal crop covering an area of 30.46 million hectares and produced annually 10.77 million tonnes with the productivity of 3537 kg/ha during 2021-22 (WAP, 2022). Wheat is cultivated almost in all the states of India but extensive cultivation is confined to Uttar Pradesh, Punjab, Haryana, Madhya Pradesh, Rajasthan and Gujarat.

In Gujarat, wheat is an important *rabi* crop and is grown in Ahmedabad, Junagadh, Sabarkantha, Rajkot, Banaskantha, Kheda and Mehsana districts of the state and produced 4.01 million tonnes from 12.53 lakh hectares area with productivity of 3205 kg/ha (DOA, 2021-22). But actual yield potential of wheat in Gujarat is 6617 kg/ha. As compared to 2012- 13, the area,

production and productivity of wheat in Gujarat increased to the tune of 2.22, 7.12 and 4.64 per cent, respectively. Major constraints of lower productivity of wheat are the deteriorating effect of unjudicious use of fertilizer on soil health poor quality and facilities of irrigation, lack of adoption of agronomic techniques and light textured soil etc. Nanotechnology has made a real revolution in the agriculture. The use of nano-fertilizers has led to the increased productivity, reduced production costs in the last decade and also led to the increased production stability due to reduction of biotic and abiotic stresses. More solubility than the other similar non-nano-fertilizers is the important property of these fertilizers. Numerous reports have suggested that the application of nano particles increases the plant resistance against the drought stress by activating the antioxidant enzymes, facilitating the uptake process in the plants and enhancing their growth. (Ahmadian *et al.*, 2021).

Nano fertilizer, the most important field of agriculture has drawn the attention of the soil scientists as well as the environmentalists due to its capability to increase yield, improve soil fertility, reduce pollution and make a favourable environment for microorganisms. Nano fertilizers play role in boosting nutrients uptake and nutrients use efficiency, reducing losses through leaching and gaseous emissions along with reducing the risk of nutrient toxicity for ensuring food security achieved through higher productivity and economic turn outs by practicing the sustainable farming practices (Ahmadian *et al.*, 2021). Nano Urea (Liquid) is a source of nitrogen. It emerged as a novel fertilizer for targeted and precise application of nitrogen. It can easily transported. It is environment friendly and its production also doesn't pollute or harm environment in any way.

Hence, keeping the above facts in view, a field investigation entitled, Effect of nano urea on increasing nitrogen use efficiency and productivity of wheat under restricted irrigation condition

II. MATERIALS AND METHODS

A field experiment was conducted during the *Rabi*, 2021-22. The experimental soil was sandy loam with pH-7.47, electrical conductivity (EC) 0.19 ds/m, organic carbon 0.23 % with available N (192.85 kg/ha), P₂O₅ (78.68 Kg/ha) and K₂O (316.22 kg/ha). The experiment was laid out in Randomized Block Design with 9 treatments in 3 replications. The treatment consists different levels of urea and nano urea, *i.e.* T₁: One spray nano urea at tillering (40-45 DAS) T₂: Two spray nano urea at tillering (40-45) and jointing (60-65 DAS) T₃: Recommended N (1/3rd basal, 2/3rd CRI - Rec. N) T₄: Rec. N + one spray of nano urea at tillering T₅: Rec. N + two spray of nano urea at tillering and jointing T₆: Rec. N + one spray of urea (5%) at tillering T₇: Rec. N + two spray of urea (5%) at tillering and jointing T₈: Rec. N +one spray of urea (5%) + nano urea at tillering T₉: Absolute control (No nitrogen). Quantity of nano urea will be 4 ml/litre of water. Quantity of spray solution will be 400 litre of water/ha and urea spray according to treatment and percentage of spray. All other cultivation practices were adopted as per recommendations. There was no rainfall during the crop season. Wheat variety GW 451 sown at 20.0 cm spacing between two row @ 100 kg/ha seed rate and crop fertilized according to treatment, however recommended dose of fertilizer is 90-60-40 kg/ha N-P₂O₅-K₂O, respectively. Sowing was done on 18th November, 2021 and crop harvested on 16th March, 2022. Only three irrigation applied at CRI, Tillering and flowering stage under restricted irrigation condition. The values off "F" was worked out and compared with the values of table F at 5 per cent level of significance. The value of S.Em.±, C.D. and C.V. per cent were also calculated (Cochran and cox, 1967).

III. RESULTS AND DISCUSSION

3.1 Initial plant stand/m²:

Initial pant stand per metre square was not affected significantly by different nano urea treatments (Table 1).

TABLE 1
EFFECT OF DIFFERENT TREATMENTS ON GROWTH AND YIELD ATTRIBUTES OF WHEAT UNDER RESTRICTED IRRIGATION CONDITION

Treatments	Initial plant stand/m ²	Plant height at harvest (cm)	Effective tillers / m ²	Length of spike (cm)	No. grains / spike	1000 grain wt. (gm)
T ₁ : One spray nano urea at tillering (40-45 DAS)	285	65.33	313	6.20	34	47.37
T ₂ : Two spray nano urea at tillering (40-45) and jointing (60-65 DAS)	258	66.07	294	6.75	30	48.91
T ₃ : Recommended Nitrogen (1/3 rd basal, 2/3 rd CRI - Rec. N) (90 kg/ha)	274	77.00	353	6.63	36	48.26
T ₄ : Rec. N + one spray of nano urea at tillering (40-45 DAS)	256	74.67	341	7.19	33	49.46
T ₅ : Rec. N + two spray of nano urea at tillering(40-45 DAS) and jointing (60-65 DAS)	288	76.53	387	6.81	38	51.24
T ₆ : Rec. N + one spray of urea (5%) at tillering(40-45 DAS)	285	78.40	379	7.05	35	50.02
T ₇ : Rec. N + two spray of urea (5%) at tillering(40-45 DAS) and jointing (60-65 DAS)	320	76.13	381	7.03	35	48.16
T ₈ : Rec. N +one spray of urea (5%) + nano urea at tillering (40-45 DAS)	261	78.80	366	7.25	36	51.92
T ₉ : Absolute control (No nitrogen)	271	66.33	308	4.73	29	45.54
S.Em±	20.27	1.60	20.33	0.29	1.40	1.84
CD @ 5%	NS	4.80	60.90	0.90	4.20	NS
CV%	12.65	3.79	10.15	7.63	7.14	6.50

RDF: 90:60:40 N P₂O₅ K₂O kg/ha (under restricted irrigation condition)

3.2 Plant height (cm) at harvest:

Plant height at harvest affected significantly by different treatment. Among the treatments, Rec. N + one spray of 5% urea + nano urea at tillering (T₈) observed significantly higher plant height at harvest (78.80 cm) as compare to other treatments (Table 1). However, it was statistically at par with treatment T₃, T₄, T₅, T₆, and T₇. The lowest plant height (65.33 cm) was observed in treatment T₁ (one spray of nano urea).

3.3 Number of effective tillers per metre square (No.):

The number of effective tillers per metre square affected significantly by different nano urea treatments. Significantly higher number of effective tillers per metre square was recorded at harvest (387) in treatment T₅ (Rec. N + two spray of nano urea at tillering and jointing) as compare to other treatments (Table 1). and it was at par with treatment T₃, T₄, T₆, T₇, and T₈. The lowest number of effective tillers per metre square (294) was noted in treatment T₂ (Two spray of nano urea at tillering and jointing).

3.4 Length of spike (cm):

Different treatment has affected significantly on length of spike at harvest. Application of Rec. N +one spray of urea (5%) + nano urea at tillering (T₈) had maximum spike length (7.25 cm) which was at par with T₂, T₃, T₄, T₅, T₆ and T₇ and lowest spike length (4.73 cm) was recorded under Absolute control T₉ (Table 1). Due to the spray of nano urea and 5% chemical urea, the plant received more nutrition for development and growth, resulting in healthier spike length growth as compared to absolute control (Table 1).

3.5 Grain per spike (No.):

At harvest, different treatment has affected significantly on grains per spike, among the different treatment application of Rec. N+ two spray of urea (5%) at tillering and jointing (T₅) had maximum number of grains per spike (38), and the lowest number of grains was recorded under the treatment control T₉ (29) (Table 1).

3.6 1000 grain weight (g):

Effect of different treatments does not exerted any significant effect on 1000 grain weight of wheat, however numerically higher value of 1000 grain weight (51.92 g) was observed in treatment T₈ (Rec. N +one spray of urea (5%) + nano urea at tillering (40-45 DAS). The lowest 1000 grain weight (45.54 g) was recorded under treatment T₉ (Table 1).

3.7 Grain yield (q ha⁻¹):

The result revealed that effect of different treatments was significantly affected on grain yield of wheat. Application of Rec. N + two spray of nano urea at tillering (40-45 DAS) and jointing (60-65 DAS) (T₅) produced significantly higher grain yield (44.40 q ha⁻¹) however it was at par with T₃, T₄, T₆, T₇ and T₈ further the lowest grain yield was recorded (14.56 q ha⁻¹) under treatment T₉ (Absolute control) (Table 2). This may be due to the provision of N through nano urea spray at later growth stages which might have enhanced accumulation of assimilates in the grains and thus resulting in heavier grains of wheat. Such type of result were also found by Ojha *et al.* (2023), Patidar *et al.* (2022), Rawate *et al.* (2022), Khaled *et al.* (2021) and Baloch *et al.* (2019) Rahman *et al.* (2014).

TABLE 2

EFFECT OF DIFFERENT TREATMENTS ON YIELD OF WHEAT UNDER RESTRICTED IRRIGATION CONDITION

Treatments	Grain yield (q/ha)	Straw Yield (q/ha)	Biomass yield (q/ha)
T ₁ : One spray nano urea at tillering (40-45 DAS)	15.92	30.67	46.58
T ₂ : Two spray nano urea at tillering (40-45) and jointing (60-65 DAS)	14.6	30.31	47.71
T ₃ : Recommended Nitrogen (1/3 rd basal, 2/3 rd CRI - Rec. N) (90 kg/ha)	39.52	56.48	96
T ₄ : Rec. N + one spray of nano urea at tillering (40-45 DAS)	38.59	53.5	92.08
T ₅ : Rec. N + two spray of nano urea at tillering (40-45 DAS) and jointing (60-65 DAS)	44.4	62.9	107.29
T ₆ : Rec. N + one spray of urea (5%) at tillering (40-45 DAS)	42.46	63.13	105.58
T ₇ : Rec. N + two spray of urea (5%) at tillering (40-45 DAS) and jointing (60-65 DAS)	43.13	63.21	106.34
T ₈ : Rec. N +one spray of urea (5%) + nano urea at tillering (40-45 DAS)	42.04	63.04	105.08
T ₉ : Absolute control (No nitrogen)	14.56	33.15	44.92
S.Em+	2.15	3.61	5.19
CD @ 5%	6.5	10.8	15.6
CV%	11.37	12.32	10.77

RDF: 90:60:40 N P₂O₅ K₂O kg/ha (under restricted irrigation condition)

3.8 Straw yield (q ha⁻¹):

Effect of different treatment affected significantly on straw yield of wheat. Application of 100% RDN with two spray of nano urea (T₇) produced significantly higher straw yield (63.21 q ha⁻¹) and it was at par with T₃, T₄, T₅, T₆ and T₈ further the lowest straw yield was recorded (30.31 q ha⁻¹) under absolute control T₉ (Table 2). This may be due to the provision of N through nano urea spray at later growth stages which might have enhanced accumulation of assimilates in the grains and thus resulting in heavier grains of wheat. Such type of result were also found by Ojha *et al.* (2023), Patidar *et al.* (2022), Rawate *et al.* (2022), Khaled *et al.* (2021) and Baloch *et al.* (2019) Rahman *et al.* (2014).

3.9 Biomass yield (q ha⁻¹):

Effect of different treatment was found significant on biomass yield of wheat. Treatment T₅ (Rec. N + two spray of nano urea at tillering (40-45 DAS) and jointing (60-65 DAS) recorded significantly higher biomass yield (107.29 q ha⁻¹) and it was at par

with treatment T₃, T₄, T₆, T₇ and T₈ further the lowest biomass yield was recorded (44.92 q ha⁻¹) under treatment of absolute control (T₉) (Table 2). Its might be due to by increasing nitrogen level with foliar application of nano urea and urea on wheat crop increased photosynthetic rate and higher leaf area that increased total biomass production. The finding of Ojha *et al.* (2023), Patidar *et al.* (2022), Rawate *et al.* (2022), Khaled *et al.* (2021) and Baloch *et al.* (2019) Rahman *et al.* (2014) is in similar pattern of the present study.

IV. CONCLUSION

Based on the above findings it is concluded that the application of Recommended dose of Nitrogen (90 kg/ha) along with two spray of nano urea at tillering (40-45 DAS) and jointing (60-65 DAS) with common application of phosphorous (60 kg/ha) and potash (30 kg/ha) performs positively and improves the growth parameters yield and yield attributes of Wheat. Since the results are based on a single season, further trails could be needed for additional confirmation.

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Farmers' Response to the use of Organic Fertilizers on Butternut Squash in Pamekasan Regency

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Abstract— Farmers still rely on chemical fertilizers for cultivating butternut squash in Pamekasan Regency. Agricultural extension programs have been conducted to educate farmers about the benefits of organic fertilizers for butternut squash plants. The objective of this study is to determine farmers' responses to the use of organic fertilizers in butternut squash cultivation. The research method employed is qualitative descriptive analysis. The results indicate that the adoption rate of organic fertilizers in butternut squash cultivation in Pamekasan Regency is low. Only 13% of butternut squash farmers use organic fertilizers as a supplement to chemical fertilizers, while 87% still rely entirely on chemical fertilizers. This low adoption rate is influenced by farmers' perceptions that organic fertilizers may reduce income, a lack of knowledge about organic fertilizer management, and dependence on chemical fertilizers, which are considered more practical. The factor influencing farmers' response to the use of organic fertilizers on butternut squash is farming experience.

Keywords— *Butternut Squash; Organic Fertilizers; Farmers' Responses.*

I. INTRODUCTION

A healthy diet has been proven to have a positive impact on maintaining both physical and mental health in society (Özenoğlu et al., 2021). A healthy diet can be defined as a pattern of food intake that has beneficial effects on health or at least does not cause any harmful effects (Stevenson, 2017). The first step in adopting a healthy diet begins with balancing calorie intake and expenditure, limiting fat intake, switching from saturated fats to unsaturated fats, eliminating industrially produced trans fats, and restricting sugar and salt consumption (World Health Organization, 2019).

Butternut squash is a healthy food alternative that can be chosen by the community. In addition to being filling due to its high carbohydrate content, butternut squash is also beneficial for health as it contains a rich amount of fiber, vitamins A, C, and E, as well as essential minerals. These nutrients can boost the immune system and help fight free radicals. Additionally, the orange color of butternut squash indicates the presence of beta-carotene, which can help reduce the risk of cancer. Moreover, butternut squash contains B-complex vitamins such as niacin, folate, vitamin B6, pantothenic acid, and thiamine, as well as minerals like calcium, phosphorus, and copper (Logistik BPPI, 2016 in Kurniati et al., 2018).

One of the health benefits of butternut squash is its ability to help control blood sugar levels, which is attributed to the presence of ethanolic extract in the fruit (Junita et al., 2017; Marbun, 2022). Another benefit of butternut squash is its role as an antidiabetic (Adams et al., 2011; Chang et al., 2014), it helps in insulin production and improves pancreatic cells (Jin et al., 2013; Makni et al., 2010), additionally, the oil from butternut squash seeds, which contains chromium, plays a role in carbohydrate metabolism by enhancing chromium absorption (Glew et al., 2006).

The development of butternut squash cultivation has been growing in line with the healthy lifestyle adopted by society. This development primarily occurs in areas that meet the growth requirements of butternut squash, which include a temperature range of 18–30°C, humidity levels of 65%, and an elevation of 0–1200 meters above sea level (Lolliani, 2017).

Pamekasan Regency is one of the areas with characteristics that are suitable for the growth requirements of butternut squash. Pamekasan Regency is located at an elevation of 6–312 meters above sea level, with an average temperature ranging from

27.3°C to 28.9°C, and humidity levels between 44% and 98% (Badan Pusat Statistik, 2024). As a result, many farmers in Pamekasan Regency have started cultivating butternut squash.

The cultivation of butternut squash by farmers in Pamekasan Regency still relies heavily on chemical fertilizers. The use of chemical fertilizers on plants can increase the residue of chemicals on butternut squash fruit. Additionally, the use of chemical fertilizers on butternut squash crops also has an adverse impact on environmental degradation (Savci, 2012).

Based on this, the researcher is interested in providing education to farmers about the benefits of organic fertilizers for butternut squash. The researcher conducts extension activities based on trials using various types of organic fertilizers on butternut squash plants. The aim of this research is to determine farmers' response to the use of organic fertilizers on butternut squash.

II. METHODOLOGY

2.1 Location and Time of Research:

This research was conducted in Pamekasan District, Pamekasan Regency. The study took place in December 2024.

2.2 Method of Identifying Respondents:

The respondents in this study are butternut squash farmers in Pamekasan District, Pamekasan Regency. There are 15 butternut squash farmers in Pamekasan District, Pamekasan Regency. The census method was used to identify the respondents. This method was chosen because the research population is small, so all members of the population were selected as respondents.

2.3 Collecting Data Method:

Data for the study was collected through interviews and the completion of questionnaires by farmers. The researcher conducted a Focus Group Discussion (FGD) and distributed questionnaires during the FGD. The aim was to gain insights into farmers' responses to organic fertilizers and the challenges they face in its implementation. The questionnaire was used as an instrument to gather more detailed information about farmers' attitudes, knowledge, and readiness to implement the use of organic fertilizers on a larger scale.

2.4 Method of Data Analysis:

This study uses a qualitative descriptive analysis method to determine farmers' responses to the use of organic fertilizers on butternut squash. This method involves providing a detailed description of the farmers' responses based on the results of interviews and the completion of questionnaires by the farmers.

III. RESULT AND DISCUSSION

3.1 The Use of Organic Fertilizers on Butternut Squash by Farmers:

The use of organic fertilizers in butternut squash cultivation in Pamekasan Regency, prior to the socialization of the research findings, showed a low level of implementation among farmers. Out of the 15 farmers butternut squash, 87% (13 people) still rely on chemical fertilizers such as urea, NPK, and ZA, while only 13% (2 people) use a combination of organic fertilizers from cow manure and chemical fertilizers. This indicates that the use of organic fertilizers is still relatively rare, despite many farmers not fully realizing their benefits. Another reason is the farmers' mindset, which remains heavily reliant on chemical fertilizers, as they are considered more practical to use.

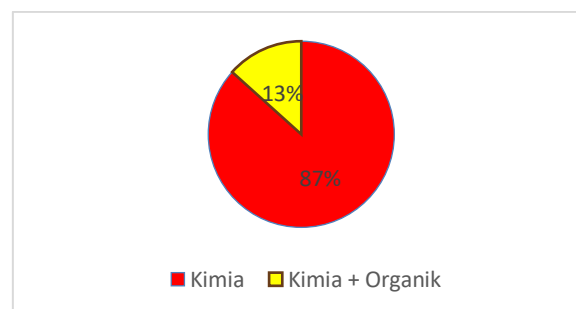


FIGURE 1: Percentage of Farmers Based on the Use of Organic Fertilizers (Primary Data 2024, processed)

The limited use of organic fertilizers by farmers is due to the belief that organic fertilizers may reduce their income. This is consistent with the findings of Wang et al. (2018), which showed that most farmers in developing countries prefer using

chemical fertilizers over organic fertilizers, primarily because they are concerned about losing income if they switch to organic fertilizers. The lack of interest among farmers in using organic fertilizers needs serious attention, especially considering the abundant availability of animal manure waste in the areas where these farmers are located.

Based on interviews with farmers who use a combination of fertilizers, they expressed that this method helps reduce fertilizer costs. For example, for a 500 square meter plot planted with 1,000 plants, around 50 kg of chemical fertilizer was previously required to achieve optimal yields. However, with the addition of organic fertilizer, farmers only need the same 50 kg of chemical fertilizer. The organic fertilizer itself is produced by the farmers using cow manure, so there are no additional costs for the organic fertilizer. Therefore, the use of organic fertilizers can reduce production costs, provide economic benefits to farmers, and decrease dependence on chemical fertilizers..

This is in line with research findings that the use of a combination of organic bioslurry fertilizer (200 mL/L) and chemical NPK fertilizer results in heavier butternut squash fruits compared to using chemical NPK fertilizer alone. In addition to fruit weight, organic fertilizers also play a role in determining fruit quality. Butternut squash plants treated with a combination of bioslurry organic fertilizer and NPK fertilizer produced sweeter fruits, with a sweetness level of 10.37 °Brix, while those treated with only chemical fertilizers reached a sweetness level of 8.5 °Brix. (Dermawan et al., 2020).

The results of the research on the testing of organic and chemical fertilizers indicate that the organic fertilizer made from goat manure provides the best results. Meanwhile, the use of NPK fertilizer did not show a significant difference compared to other types of manure fertilizers.

The results of this study, after the socialization efforts with butternut squash farmers, show that it has changed their perspective on the use of organic fertilizers. This is evidenced by the farmers' readiness to implement organic fertilizers on butternut squash crops. The reason for this is the abundant availability of organic fertilizers in Pamekasan Regency. Among the 15 farmers cultivating butternut squashes, 3 farmers have goats, 5 farmers have cattle, and all of them have chickens.

The main challenge in the implementation of organic fertilizers is that farmers are still accustomed to using chemical fertilizers. Many believe that using organic fertilizers may reduce their production yields. This is consistent with the findings of (Wang et al., 2018), which show that farmers prefer chemical fertilizers because they are concerned about losing income. The limited understanding of the long-term benefits of organic fertilizers, both from an economic and environmental sustainability perspective, poses a significant challenge. However, the use of organic fertilizers can reduce dependence on increasingly expensive chemical fertilizers, which have negative environmental impacts, such as groundwater contamination caused by excess chemical fertilizers that cannot be absorbed by plants.

The use of organic fertilizers in agriculture offers various benefits, as stated by butternut squash farmers who use a combination of organic and chemical fertilizers. Organic fertilizers are particularly essential during the vegetative phase of plants. Animal manure-based organic fertilizers, which contain nitrogen (N), are crucial for horticultural crops, including butternut squash. The nitrogen content in organic fertilizers is particularly needed during the vegetative phase of horticultural plants (Bergstrand, 2022). Plants treated with organic fertilizers during the vegetative phase tend to grow taller and have broader leaves.

In the socialization efforts, farmers were also provided with information on the benefits of using organic fertilizers as decomposing bacteria that enhance nutrient absorption by plants. Organic fertilizers contain microorganisms that can improve soil structure, increase its biological activity, and accelerate the decomposition of organic matter into nutrients that are more easily absorbed by plants. Regular use of organic fertilizers helps make the soil more fertile and prevents resistance to fertilizers, which is often observed with excessive use of chemical fertilizers. This allows the soil to maintain long-term fertility, increases the plant's ability to absorb nutrients, and supports environmentally sustainable farming practices.

Another challenge in the implementation of organic fertilizers on butternut squash in Pamekasan Regency is the low level of farmers' understanding regarding effective organic fertilizer management techniques. Although raw materials for organic fertilizers, such as livestock manure, are easily available, the process of producing high-quality organic fertilizers requires specific knowledge and skills that most farmers have not mastered. This becomes a barrier for them to produce organic fertilizers independently. On the other hand, the initial costs required for organic fertilizer production, although cheaper in the long run, can be a burden for farmers who are accustomed to the more practical and quicker use of chemical fertilizers.

In addition, the socialization regarding the production methods and benefits of organic fertilizers is still limited. Farmers, who are more frequently exposed to information about chemical fertilizers, are hesitant to switch to organic fertilizers without guarantees that the results will be comparable to those obtained from chemical fertilizers. However, in the long run, the use of

organic fertilizers can improve soil fertility and soil structure, allowing plants to absorb more nutrients, reduce dependence on chemical fertilizers, and enhance ecological balance. Therefore, in addition to socialization, it is crucial for relevant parties to provide technical training to farmers on the production, application, and benefits of organic fertilizers, so that farmers feel more confident and ready to adopt this technology in their cultivation practices.

3.2 Farmers' Response to the Use of Organic Fertilizers on Butternut Squash:

Farmers' response to the use of organic fertilizers was measured after they received extension services from the researcher regarding the benefits of using organic fertilizers on butternut squash. The farmers' response is a combination of knowledge, attitude, and behavior aspects towards the use of organic fertilizers on butternut squash.

The scoring method using a Likert scale was employed to determine the scores for each question based on the indicators of knowledge, attitude, and skills. Subsequently, a proportion test was conducted to assess the level of farmers' responses.

Farmers' responses were divided into three categories: low, medium, and high. A low response was categorized if the farmer's score fell within the interval of 8 to 20, a medium response was categorized within the interval of 21 to 33, and a high response was categorized within the interval of 34 to 46. Based on this categorization, the distribution of farmers' responses to the use of organic fertilizers on butternut squash is as follows:

TABLE 1
DISTRIBUTION OF FARMERS BASED ON FARMERS' RESPONSE

Categories	Number (people)	%
Low	0	0
Medium	13	87
High	2	13
Total	15	100

Source: Primary Data Analysis, 2024

Based on Table 1, it is known that 2 farmers, or 13%, had a high response to the use of organic fertilizers on butternut squash. Meanwhile, 13 farmers, or 87%, had a medium response to the use of organic fertilizers on butternut squash. No farmers had a low response to the use of organic fertilizers on butternut squash after the extension activities. This indicates that the extension activities were able to change the farmers' perspective on the use of organic fertilizers on butternut squash.

3.3 Factors Affecting Farmers' Response to the Use of Organic Fertilizers on Butternut Squash:

Farmers' response to the use of organic fertilizers on butternut squash is influenced by several factors, including the characteristics of the farmers and cultivation activities. The factors that influence farmers' response to the use of organic fertilizers on butternut squash in Pamekasan Regency are as follows:

TABLE 2
FACTORS AFFECTING FARMERS' RESPONSE TO THE USE OF ORGANIC FERTILIZERS

Variable	Regression Coefficient	t-value	Sig	Note
Gender	0,086	0,259	0,801	ns
Age	-0,003	-0,259	0,801	ns
Farming Experience	0,502	4,321	0,002	*
Farm Area	21,640	1,664	0,127	ns
Constant	0,778			
R square	0,786			
Adj. R square	0,700			
F-value	9,179			

*Notes: * = significant (p-value < 0.05) / ns = not significant*

The regression analysis results show an Adjusted R square value of 0.700. This means that 70 percent of the farmers' response to the use of organic fertilizers is explained by the independent variables, namely gender, age, duration of farming, and farm area. The remaining 30 percent is explained by other variables outside the model. The F-value is 9.179 with a significance of 0.002. This indicates that all independent variables together are able to explain the dependent variable.

Based on the results of the multiple linear regression analysis, it can be concluded that the variable of farming experience influences the farmers' response to the use of organic fertilizers on butternut squash crops. Meanwhile, the variables of gender, age, and farm area do not have an impact on the farmers' response to the use of organic fertilizers.

The regression coefficient for farming experience is 0.502 with a significance of 0.002. This means that for each additional year of farming experience, the farmers' response to the use of organic fertilizers on butternut squash increases by 0.502 units. In other words, as farming experience increases, the farmers' response to the use of organic fertilizers also improves.

Experienced farmers tend to have better cultivation techniques. This is in line with research that states farming experience significantly influences farmers' decision-making regarding organic farming (Puspasari et al., 2018).

Based on the data on farmer characteristics, the farmers who use organic fertilizers in butternut squash cultivation are those with five years of farming experience. This suggests that farmers with more experience have already applied the use of organic fertilizers to their butternut squash crops. They are familiar with the benefits of organic fertilizers on butternut squash.

IV. CONCLUSION

1. The use of organic fertilizers in butternut squash cultivation in Pamekasan Regency is still very low, with only 13% of farmers using it as a supplement to chemical fertilizers, while 87% of farmers rely entirely on chemical fertilizers. This low adoption rate is influenced by farmers' belief that organic fertilizers may reduce their income.
2. The extension activities conducted have successfully changed the views of some farmers in Pamekasan Regency, who are now more open to the use of organic fertilizers due to their abundant availability. However, the main challenges in its implementation are the limited understanding of organic fertilizer production techniques, the initial production costs, and the farmers' habit of relying on chemical fertilizers. Therefore, more intensive education and technical training are needed so that farmers can optimally adopt organic fertilizers for more sustainable agriculture.
3. The factor of farming experience significantly influences farmers' responses to the use of organic fertilizers on butternut squash.

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Sustainable Technologies for Rain fed Agriculture in India

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Abstract— India's agrarian economy contributes 21% to the country's GDP and provides employment to 60% of the population. Rainfed agriculture constitutes 67% of the net sown area, contributing 44% of food grains and supporting 40% of the population. To meet the increasing demand for food grains, it is crucial to enhance the productivity of rainfed areas from the current 1 t/ha to 2 t/ha over the next two decades. However, the quality of natural resources in these regions is deteriorating due to overexploitation, and rainfed areas face biophysical and socio-economic constraints affecting crop and livestock productivity.

This paper critically examines economically viable technologies for rainfed agriculture, including soil and rainwater conservation, efficient crops and cropping systems tailored to the growing season, and suitable implements for timely sowing and labor-saving. Sustainable practices such as integrated nutrient and pest management (INM and IPM) are emphasized. Additionally, the paper highlights interventions in dryland technologies to utilize marginal lands through alternative land-use systems like silvopasture, rainfed horticulture, and tree farming, demonstrated on a watershed basis. The integration of livestock with arable farming systems and the incorporation of indigenous knowledge are also discussed. Finally, the paper advocates for the formation of self-help groups, innovative extension tools such as portable rainfall simulators, and focus group discussions to facilitate the adoption of rainfed technologies by farmers. A farming systems approach in rainfed agriculture not only addresses income and employment challenges but also ensures food security.

Keywords— Rainfed agriculture technologies, sustainable, indigenous knowledge.

I. INTRODUCTION

India's economy heavily relies on agriculture, which contributes 21% of GDP and provides employment for 60% of the workforce. Over the past three decades, the country has achieved self-sufficiency and surplus food production. However, feeding the growing population remains a formidable challenge, with projections indicating the need to support 1.3 billion people by 2020, requiring an annual increase of 5-6 million tons of food grains. Despite advancements, issues like poverty and malnutrition continue to hinder national food security, particularly in rural areas where over 70% of the population resides. Rainfed agriculture accounts for 67% of the net sown area, producing 44% of food grains and supporting 40% of the population. Even after realizing the full irrigation potential, 50% of the net sown area will remain rainfed.

Currently, rainfed agriculture constitutes 95% of the area under coarse cereals, 91% under pulses, 80% under oilseeds, 65% under cotton, and 53% under rice. Livestock forms an integral component, with two-thirds of animals thriving in rainfed regions. These areas encompass diverse environments, including semi-arid to sub-humid climates, shallow to deep soils, and varying crop-growing periods of 90-180 days.

II. METHODS AND MATERIALS

This study employs a descriptive and explorative methodology, utilizing secondary data from government reports, research institutions, and published sources such as websites, periodicals, and reports.

2.1 Scenario of Food Demand and Resources:

India's food grain demand is projected to reach 243 million tons by 2007-08 and 308 million tons by 2025 under a low population growth scenario. For a higher growth scenario, the requirement is 320 million tons. Ensuring protein security is becoming increasingly critical due to the predominantly vegetarian diet and limited availability of pulses, with current consumption at 25 g/person/day against the dietary requirement of 70 g.

Agricultural production increased significantly from 50 million tons in 1950 to over 200 million tons by 2000, driven by the Green Revolution. However, this progress came at the cost of land and water degradation, biodiversity loss, and environmental pollution. Moving forward, ecological sustainability and economic access to food will be major concerns.

2.2 Shrinking Natural Resources:

Per capita agricultural land in India decreased from 0.46 hectares in 1951 to 0.15 hectares in 2000, compared to the global average of 0.6 hectares. The population density on net-cropped land rose from 3 persons/hectare in 1951 to 6.5 in 2000 and is projected to reach 8 by 2025. Competing demands for food, fiber, fuel, and urbanization exacerbate the pressure on shrinking natural resources.

2.3 Constraints of Production in Rainfed Areas:

Rainfed regions face numerous challenges, including:

- Low and erratic rainfall
- Land degradation
- Poor productivity
- Limited input use and technology adoption
- Inadequate draft power
- Insufficient fodder and low-productivity livestock
- Resource-poor farmers and limited credit availability

2.4 Strategies for Sustained Food Production in Rainfed Regions:

A number of economically viable rainfed technologies have been developed over the years in the country to address the challenges of food production in rainfed agriculture. Through CRIDA (Central Research Institute for Dryland Agriculture) and its network centers over the last three decades, these technologies have been refined and validated in farmers' fields using approaches like Operational Research Projects (ORPs), Institute Village Linkage Program (IVLP), and farm science centers.

Key innovations include simple yet effective practices such as off-season tillage in rainfed Alfisols and related soils, aimed at improving moisture conservation and weed control. Farmers in Operational Research Project areas of Hyderabad, for example, adopted this practice for crops like sorghum and castor, achieving yield increases of up to 40% compared to traditional methods.

However, a significant barrier to the widespread adoption of these practices is the lack of adequate draft power among small-scale farmers. Custom hiring of tractors has emerged as an effective solution, enabling farm mechanization on these rainfed lands and helping farmers adopt these sustainable practices.

2.5 Soil and Rainwater Conservation Techniques:

Efficient conservation of rainwater is the central issue in successful dryland farming. Extensive trials conducted by the soil conservation and dryland research centers have led to the identification of a number of inter-terrace land treatments besides contour and graded bunds (Sharma et al., 1982). These techniques are location specific, and the benefits from their adoption are highly variable depending on the rainfall intensity, slope, and texture of the soil, besides the prevailing crop/cropping system (Katyal and Das, 1993).

Farmers have not widely adopted mechanical measures like contour bunds, graded bunds, grassing of waterways, and construction of farm ponds without government support due to financial constraints. However, studies at Hyderabad, Bangalore, and Anantapur revealed that more than 80 percent of farmers follow simple conservation measures like sowing across the slope, opening of dead furrows, and keyline cultivation. The yield improvement by adopting soil and water conservation measures varies between 12 and 20 percent, which are at times not convincing enough to farmers. However, cumulative effects are significantly visible at some locations. Since such measures help in the long-term conservation of resources, these are implemented through the Government of India or the respective State Government-sponsored watershed management programs.

2.5.1 Timely Planting of Crops:

Timely sowing ensures better plant stand, higher yields, and optimal rainfall use. Demonstrations in farmers' fields through programs like ORPs and KVKs have shown significant yield reductions when sowing is delayed by 15 days. Availability of farm implements is critical to overcoming these constraints.

2.5.2 Adoption of Improved Crop Varieties:

Improved varieties tailored to rainfed conditions have increased farmers' benefits by Rs. 2,000-4,000/ha. Examples include improved sorghum, castor, and sunflower varieties.

2.5.3 Efficient Crops and Cropping Systems:

Efficient intercropping and sequential cropping systems have been recommended based on soil type, rainfall, and growing season. For example, the sorghum-pigeonpea (2:1) system and finger millet-pigeonpea (8:1) system have been successful in different regions. However, adoption challenges include preferences for fodder genotypes, lack of suitable farm implements, and delayed planting.

2.5.4 Farm implements:

Proper tillage and precise placement of seed and fertilizers in the moist zone are most critical to for successful crop establishment in drylands. Since the sowing of crops must be completed in a short span of time, use of appropriate implements is necessary to cover large area before the seed zone dries out. Suitable implements have been recommended for various locations to meet this requirement. These are designed to suit the soil type, crop and the draught power availability. In many cases, the existing local implement used by the farmers have been improved to increase their working efficiency (Gupta and Sriram, 1987).

Studies at CRIDA in farmers' fields of Telangana indicated that use of the drill plough for sowing of castor and sorghum crops showed no variation in yields of the crops and plant as compared to farmers practice resulted 1 ½ times more coverage compared to farmers' method of seeding. Two labourers who are required for placement of seed and fertilizer in farmers method can be saved with the drill plough. Thus a saving of Rs. 187/ha is possible with a drill plough compared to the traditional plough and plant system.

2.5.5 Nutrient management:

Fertilizer recommendations in rainfed crop production have been made primarily for NPK along with the conjunctive use of chemical, organic and bio-fertilizer (Rao and Das, 1982). Inclusion of legumes in cropping systems can supplement fertilizer N to the extent of about 20 kg N per ha. Conjunctive use of fertilizer N with FYM, croppings of luecaena and gliricidia help in reducing the requirement of fertilizer by 50 percent (Reddy et al., 1996).

2.5.6 Integrated pest management (IPM):

Pests and deceases constitutes a major constraint to increased food production. Crop losses due to pest attack range from 10-30 percent depending on the crop and environment. Complete crop failure may occur in case of serious attack. Indiscriminate use of the pesticides in rainfed crops will lead to harmful side effects such as direct toxically to the applicator or consumer, development of strains or pests resistant to pesticides, resurgence of pest species, outbreak of secondary pesticides, destruction of non-target organisms such as parasites and predators and accumulation of harmful residues of food products. Integrated pest management is one of the alternatives for the chemicals used for pest management. IPM encourages the most comfortable and ecologically sound combination of available pest suppression techniques and to keep the pest population below economic threshold. Easily adaptable and economically viable integrated pest management strategies have been developed for the control of major pest in rainfed crops like cotton and pulses.

2.5.7 Alternate Land use Systems:

Despite evolving a number of production technologies, arable cropping in dry lands continues to suffer from instability due to aberrant weather. To provide stability to farm income and also utilize the marginal lands for production of fodder, fuel wood and fibre, a number of alternative land use systems were evolved based on location specific experimentation and cafeteria studies (Singh, 1988). In addition to the above general guidelines, specific experiments have been carried out to develop land use practices for different categories of soils across the centres integrating annual crops with the perennial component in order

to utilize the off-season rainfall (Katyal et al., 1994). Different alternate land use systems include agri-silviculture, silvi-pasture, agri-horticulture, alley cropping etc.

2.5.8 Integration of live stock with rain fed farming systems:

Live stock is treated as a part of farming system in rainfed agriculture in India. The soil, plant, animal cycle is the basis for all feed used by the animals. The livestock in the rain fed regions are weak. Farmers in this area often sell their cattle due to the scarcity of fodder. In India the land holdings are being reduced with increased population pressure. Hence, land not suitable for agriculture has to be diverted for raising fodder need of animals through the appropriate alternate land use system such as improved pasture, silvipasture, hortipasture and tree techniques.

2.5.9 Integration of the technologies through watershed approaches:

The concept of watershed is important in efficient management of water resources. As the entire process of agricultural development depends upon the status of water resources, the watershed with distinct hydrological boundary is considered ideal for taking up a development programme. In brief, planning and designing of all soil conservation structures are carried out considering the peak runoff. In this context, the watershed concept is of practical significance. Also, the entire development needs are to be taken up on topographic considerations from ridge to valley.

2.6 Resource conservation measures:

Details about conservation measures adopted in cultivated lands have been delineated by Katyal et al., (1995) and Sharma and Mishra (1995). Based on the nature and type of barriers and their cost, the conservation measures in arable lands can be divided into three categories: (i) Hardware treatments (ii) Medium software treatments and (iii) Software treatments.

2.7 Farming system approach:

Of late, it has been increasingly recognized that unlike irrigated areas, it is difficult to develop profitable technologies for heterogeneous agro-ecological and socio-economic conditions of small holders in arid and semi-arid regions (Osten et al., 1989). Since, the problems are complex, addressing only a component of the farming system, e.g crop variety, fertilizer use or even crop husbandry per se is not expected to bring about a significant increase in the productivity as witnessed in irrigated areas. The extension strategy should be such as to match this challenge. The farming systems perspective, dovetailed on watershed approach therefore can be the appropriate management strategy for such regions (Chambers, 1991).

III. CONCLUSION

The following steps constitute the farming systems mode for research, both on-station and on-farm (Watershed):

- PRA and assessment of socio-economic conditions of people.
- Identification of ITK (indigenous technical knowledge)
- Collection of available technological knowledge on various components of the farming system – arable farming, animal husbandry, water harvesting, management of wastelands and alternate land use systems etc.
- Focus group (farmers) interaction to identify appropriate technology for different categories of farmers.
- Identification of lead farmers to function as facilitator in technology application and adoption.
- Identification of points of synergy among systems components.
- Structuring of technological components with maximum synergy.
- Phasing of program over the project period.

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Study of the Avian Diversity in Jasder Dham, Barmer, Rajasthan

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Abstract— *Jasder Dham is a unique site in Barmer, Rajasthan. Despite being located in an arid region, the presence of a pond supports a significant diversity of aquatic birds. This research study aims to investigate the avian diversity within Jasder Dham, driven by the need to understand the ecological significance of this unique habitat and its role in supporting avian populations.*

Avian diversity studies in the arid zones of Rajasthan have been relatively limited. This research seeks to fill this gap by providing valuable insights into the bird species of various families inhabiting Jasder Dham.

The findings of this study will contribute to our understanding of the avian community structure in arid ecosystems, particularly in the context of Barmer, Rajasthan. Furthermore, the results may have implications for conservation efforts, as the identification of key bird species and their ecological requirements can guide conservation strategies aimed at preserving biodiversity in this unique habitat. Overall, this research serves as a valuable addition to the knowledge base on avian diversity in arid regions, emphasizing the importance of holistic conservation approaches for sustaining ecological balance.

Keywords— *Jasder Dham, Avian, Diversity, Barmer, Birds, Desert.*

I. INTRODUCTION

Rajasthan, situated in northwestern India, is a state renowned for its rich birdlife despite facing water scarcity. Remarkably, this state, comprising only 10% of India's geographical area, is home to approximately 40% of the country's avifauna (1224 species). This high avian diversity can be attributed to the region's diverse habitats and its location within a major migratory bird flyway.

An estimated 510 bird species are believed to occur in Rajasthan (Grimmett and Inskipp 2003), with 496 species recorded to date (Devarshi, D. 2004), representing around 40% of the Indian avifauna.

Our understanding of Barmer's avian fauna has been enriched by several studies. Rahmani (1997) conducted a comprehensive survey, documenting 213 species, including 35 in Barmer, highlighting the presence of diverse avian populations, even in this arid region. Subsequent studies, such as those by Sivaperuman et al. (2005) and Kumar et al. (2006), further expanded our knowledge. Kumar et al. (2006), focusing on wetland birds, listed 53 species from Barmer in their 'Geo-spatial Atlas for the wetland birds of GITD.'

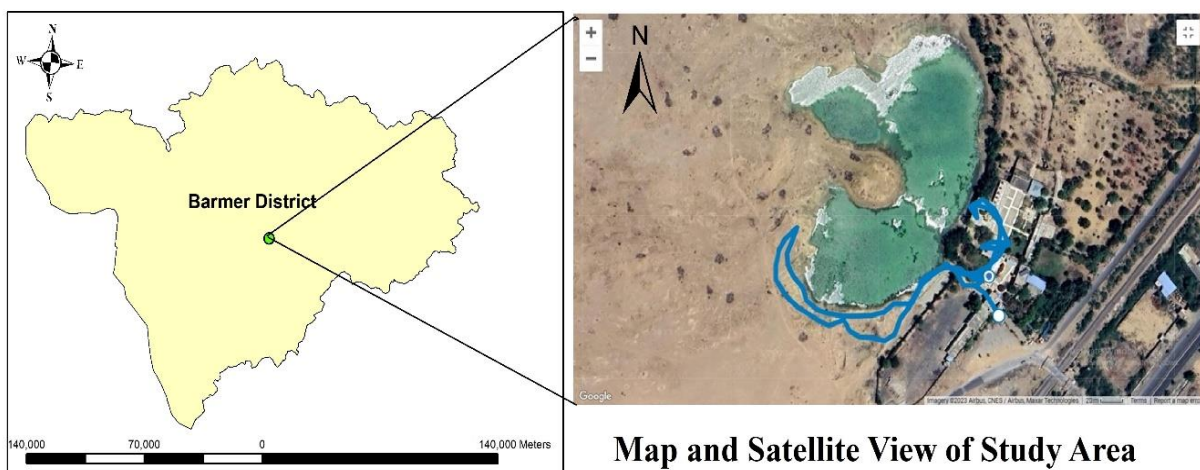
More recently, as part of the Zoological Survey of India's assessment of the impact of proposed lignite mining in the Kapurdi and Jalipa Blocks and the surrounding 10-kilometer radius of Barmer district, "General Faunistic Surveys" were conducted. These surveys, covering approximately 850 square kilometers, meticulously recorded avian species, resulting in a comprehensive bird checklist for the Barmer district. This checklist encompasses a total of 153 species, representing 103 genera and 46 families (Tak et al., 2009).

II. MATERIALS AND METHODS

This study was conducted from July 2022 to January 2024 at and around Jasder Dham (25.773069, 71.425928), located in Barmer district, Rajasthan, India. Barmer, situated in the westernmost part of Rajasthan, forms a significant portion of the Great Indian Thar Desert (GITD). The study area exhibits an arid climate with an average annual rainfall of 150-280 mm and extreme pre-monsoon temperatures ranging between 44 and 52 degrees Celsius. High wind speeds, averaging 10-40 km/hour during summers, contribute to high rates of evaporation and transpiration (1000-1200 mm annually).



FIGURE 1: The study area



Map and Satellite View of Study Area

FIGURE 2: Map and Satellite View of Study Area (Jasder Dham, Barmer) (Map Source- ArcGIS and Google Map)

Field surveys were conducted regularly during all seasons, with morning sessions (05:00 AM to 10:00 AM) and evening sessions (04:00 PM to 07:00 PM). Each visit involved a 1-2 km transect within and around Jasder Dham. Data was collected seasonally to compare avian diversity across different periods.

2.1 Data Collection Techniques:

- **Bird Observation:** Bird sightings were recorded using 10x50 DPS I binoculars (Olympus).
- **Photography:** Canon B600 cameras were used to photograph observed bird species.

- **Location Tracking:** A Garmin eTrex GPS device and the eBird app were used to record the location of bird sightings.
- **Bird Identification:** Bird species were identified using the field guide "The Book of Indian Birds" by Dr. Salim Ali.

2.2 Data Analysis:

- **Point-Count Surveys (PCS):** The Point-Count Survey method was employed to assess avian biodiversity at fixed locations within the study area for a predetermined time period. This method is suitable for year-round surveys and allows for the recording of both seen and heard birds (Kumar, 2018).
- **Seasonal Analysis:** Seasonal grand totals were calculated, and a Seasonal Index (SI) was determined for each species by dividing the seasonal total of individual species by the seasonal grand total of all species and multiplying by 100.
- **Relative Diversity Index (RDi):** The RDi of each bird order was calculated using the following formula:

$$\text{RDi} = (\text{Number of bird species in an order} / \text{Total number of bird species}) \times 100 \quad (1)$$

III. OBSERVATIONS

Extensive field surveys were conducted within a 2.11 km² area encompassing Jasder Dham and its surroundings. These surveys involved systematic observations along transects, covering approximately 6 km around the pond circumference. Observations were conducted regularly during all seasons, with morning sessions (05:00 AM to 10:00 AM) and evening sessions (04:00 PM to 07:00 PM).

A total of 65 bird species belonging to 35 families were documented within the study area. The relative diversity of avian fauna is presented in Table 1 and Figure 4. A complete checklist of the documented avian fauna is provided in Appendix 1.

Notably, 19 species (7 families) were identified as waterbirds, highlighting the importance of the pond as a crucial habitat for aquatic avifauna. White-throated Kingfishers and Pied Kingfishers were commonly observed. The presence of a diverse small bird community attracted a variety of raptors, including hawks, kites, eagles, and vultures. Figure 3 showcases photographs of some of the bird species observed within the study area.



FIGURE 3: Photographs of some birds taken during field work (Upper Row- from Left→Right: White wagtail, Indian Rollar, Black naped ibis, Lesser Cormorant; Middle Row→ Black drongo, White throated kingfisher, Common sandpiper, Shikra; Lower Row→ Black-Winged Stilt, Eurasian coot, House crow, Indian Pond Heron)

TABLE 1
RELATIVE DIVERSITY (RDi) OF VARIOUS AVIAN FAMILIES AT JASDER DHAM, BARMER

S.N.	Avian Family	No. of Species	RDi
1	Accipitridae	6	9.2
2	Alaudidae	1	1.5
3	Alcedinidae	2	3.1
4	Anatidae	5	7.7
5	Anhingidae	1	1.5
6	Ardeidae	5	7.7
7	Burhinidae	1	1.5
8	Caprimulgidae	1	1.5
9	Charadriidae	1	1.5
10	Ciconiidae	1	1.5
11	Columbidae	4	6.2
12	Coraciidae	1	1.5
13	Corvidae	2	3.1
14	Cuculidae	3	4.6
15	Dicruridae	1	1.5
16	Falconidae	1	1.5
17	Laniidae	1	1.5
18	Laridae	1	1.5
19	Leiothrichidae	1	1.5
20	Meropidae	1	1.5
21	Motacillidae	2	3.1
22	Muscicapidae	4	6.2
23	Passeridae	1	1.5
24	Phalacrocoracidae	2	3.1
25	Phasianidae	2	3.1
26	Psittacidae	1	1.5
27	Pteroclididae	1	1.5
28	Pycnonotidae	2	3.1
29	Rallidae	2	3.1
30	Recurvirostridae	1	1.5
31	Scolopacidae	1	1.5
32	Strigidae	1	1.5
33	Sturnidae	2	3.1
34	Threskiornithidae	2	3.1
35	Upupidae	1	1.5

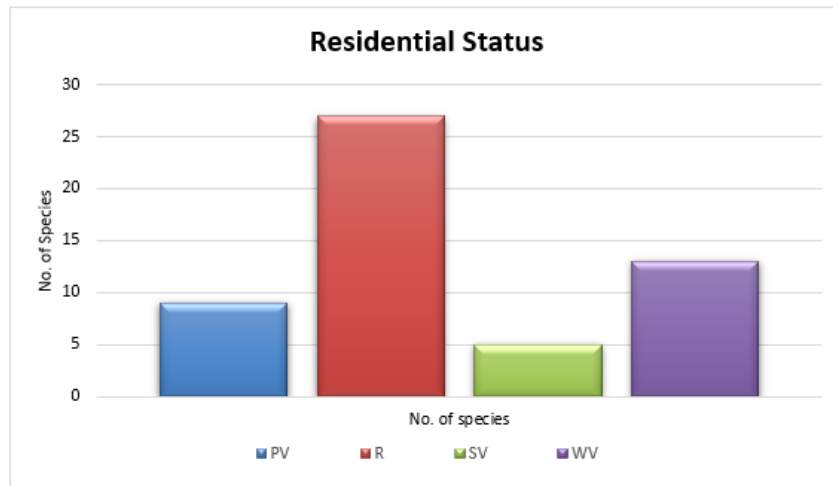


FIGURE 4: Chart showing comparative residential status of avian diversity at study area.
 (Note: Residential Status- (R=Resident: PV= Passage visitor: SV= Summer visitor WV= Winter visitor)

3.1 Threats and Challenges:

The study also investigated the threats and challenges faced by avian fauna in and around the study area. Ongoing development activities pose a significant risk to avian populations by leading to habitat loss and fragmentation.

3.2 Seasonal Variation in Bird Abundance:

The frequency of bird species sightings was recorded for each season: summer (April-June), monsoon (July-October), and winter (November-March). The seasonal grand totals were 343, 418, and 603, respectively.

A Seasonal Index (SI) was calculated for each species to assess its relative abundance in each season. SI values greater than 1 indicate higher abundance in that season compared to the average across all seasons. Conversely, SI values less than 1 indicate lower abundance. The SI values for each species were plotted to understand seasonal patterns of occurrence.

Statistical analysis revealed that the mean and median number of reported birds were highest in summer. The standard deviation was also highest in summer, indicating greater variability in bird abundance during this season. Boxplots (Figure 5) were used to visualize the distribution of bird species frequencies across different seasons. Figure 6 presents the seasonal variation in the total number of bird species reported, demonstrating that winter had the highest species diversity (65 species) while summer had the lowest (52 species).

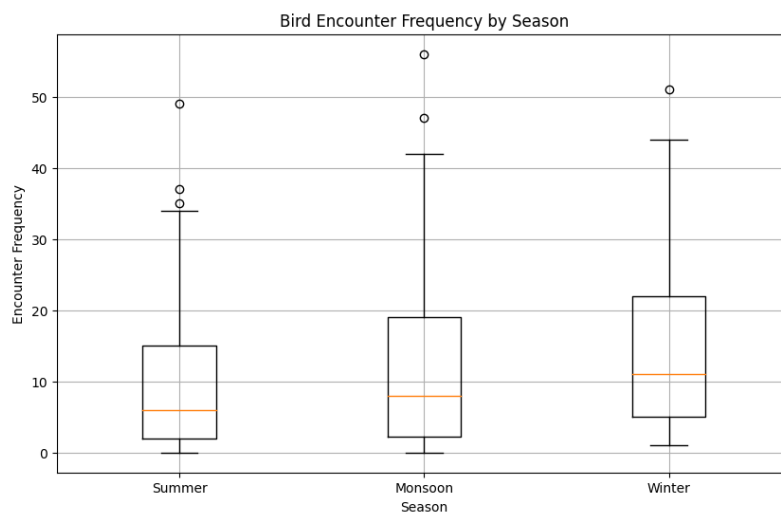


FIGURE 5: Boxplot showing bird encounter frequency in summer, monsoon and rainy season at Jasder Dham

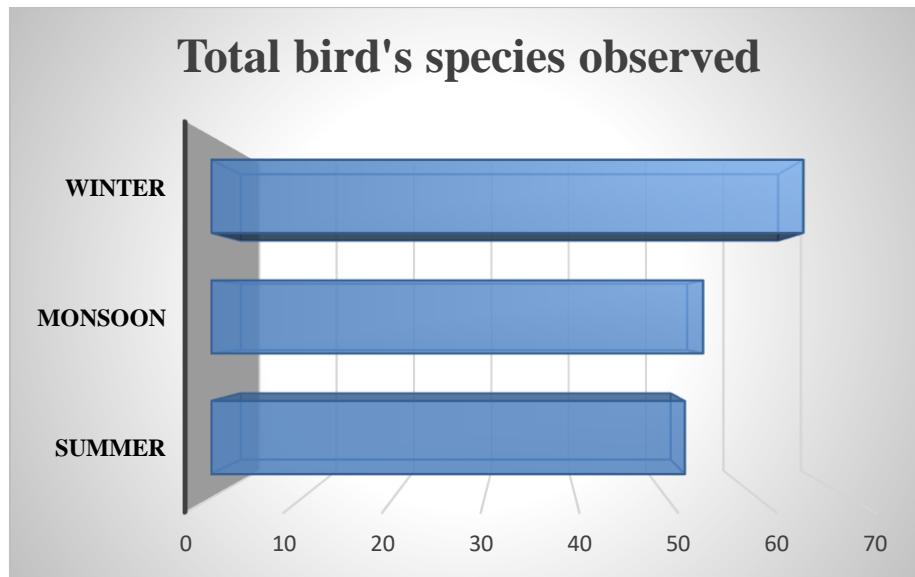


FIGURE 6: 3-D Clustered bar graph showing total bird species recorded in each seasons

3.3 Observations on Endangered Species:

The study area provides suitable habitat for endangered vulture species, particularly the Egyptian Vulture (*Neophron percnopterus*). The presence of carcass dumping sites near the wetland provides a reliable food source for these scavengers. Egyptian Vultures were frequently observed resting on Khejri trees (*Prosopis cineraria*) and actively feeding on carcasses. This suggests that the study area plays a crucial role in supporting the conservation of endangered vulture species, including the Egyptian Vulture, White-rumped Vulture (*Gyps bengalensis*), and Indian Vulture (*Gyps indicus*).

3.4 Threats to Avian Diversity:

Several threats are impacting avian diversity in and around Jasder Dham:

- **Water Pollution:** The immersion of Lord Ganesha idols, often made of harmful chemicals, during the festival of Ganesh Chaturthi contaminates the pond water. This pollution can lead to the depletion of dissolved oxygen and the accumulation of toxic sediments, posing a serious threat to aquatic life, including birds.
- **Kite Flying Hazards:** The use of thin but sharp threads during Makar Sankranti (kite flying festival) poses a significant threat to birds. These threads can entangle and injure birds, often resulting in fatalities.
- **Improper Waste Disposal:** The dumping of domestic and commercial waste, along with the improper disposal of dead animals, can contaminate the environment and pose health risks to birds.
- **Human Disturbance:** Increased human activity in the area, such as recreational activities and urbanization, can disrupt bird breeding, feeding, and roosting behaviors.
- **Electrocution:** Power lines and transmission towers pose a significant threat to birds, particularly large raptors, through electrocution.
- **Predation by Feral Dogs:** Feral dog populations can prey on ground-nesting birds and their eggs, impacting avian populations.

IV. RESULTS AND DISCUSSION

This study documented 65 bird species belonging to 35 families within and around Jasder Dham, demonstrating a rich avian diversity that includes resident, resident-migratory, and migratory species. Despite the presence of human settlements, the study area supports a significant avian population.

4.1 Seasonal Variation:

Bird species encounters varied across seasons. Summer exhibited the highest activity, followed by winter and monsoon. The highest variability in sightings was observed during summer, suggesting a wider range of species observed during this period.

- **Summer:** Average Bird Count (ABC) was approximately 10.54. Red-wattled Lapwing was the most frequently sighted species, while Eurasian Griffon Vulture, Black-shouldered Kite, Long-legged Buzzard, Comb Duck, Northern Pintail, Common Pochard, Great Cormorant, and Common Kestrel were observed less frequently.
- **Monsoon:** ABC was approximately 12.37. India Pigeons and Doves were the most frequently sighted, while Common Pochard, Common Kestrel, Great Cormorant, Common Sandpiper, and Red-naped Ibis were less frequent.
- **Winter:** ABC was approximately 10.11. Pigeons, doves, vultures, and kites were frequently observed, while Oriental White Ibis, Northern Pintail, Eurasian Griffon, Common Pochard, Great Cormorant, Common Kestrel, and Red-naped Ibis were less frequent.

This quantitative seasonal analysis indicates significant variations in bird species encounter frequencies across different seasons. Notably, winter exhibited the highest diversity of migratory bird species.

4.2 Community Composition:

Analysis of the species checklist (Appendix 1) revealed that resident birds dominate the avifauna. Carnivorous birds comprise a significant portion of the resident population, suggesting a diverse prey base within the habitat. Passage visitors, primarily carnivorous birds, likely utilize the area as a foraging ground during their migration.

4.3 Conservation Status:

The majority of bird species observed were classified as "Least Concern" by the IUCN Red List, indicating relatively stable populations. However, the presence of endangered (E) species like the Egyptian Vulture and near-threatened (NT) species like the Indian Thick-knee highlights the need for focused conservation efforts.

4.4 Ecological Significance:

The presence of diverse bird families, such as Accipitridae, Ardeidae, and Columbidae, indicates a diverse habitat supporting various ecological niches.

4.5 Threats and Conservation:

The study identified several threats to avian populations, including:

- Water pollution from idol immersions during festivals.
- Bird fatalities due to kite flying with sharp threads during Makar Sankranti.
- Improper waste disposal and habitat degradation.
- Electrocution from power lines.
- Predation by feral dogs.

To mitigate these threats, it is crucial to implement conservation measures and raise awareness among local communities about the importance of environmental protection and habitat conservation.

V. CONCLUSION

This study provides valuable insights into the avian community composition, ecological interactions, and conservation needs within the Jasder Dham region. Conservation strategies should prioritize the protection of critical habitats, maintaining ecological connectivity, and mitigating threats such as habitat loss, pollution, and climate change.

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APPENDIX I

List of avian diversity observed in and around Jasder Dham, Barmer, Rajasthan, India.

S.N.	Name of Bird	Scientific Name	Family	Residential Status	IUCN STATUS	Feeding Status
1.	Egyptian Vulture	<i>Neophron percnopterus</i>	Accipitridae	PV	E	Carnivores
2.	Eurasian Griffon	<i>Gyps fulvus</i>		PV	LC	Carnivores
3.	Black Kite	<i>Milvus migrans</i>		R	LC	Carnivores
4.	Black Shouldered Kite	<i>Elanus caeruleus</i>		PV	LC	Carnivores
5.	Shikra	<i>Accipiter badius</i>		R	LC	Carnivores
6.	Long Legged buzzard	<i>Buteo rufinus</i>		PV	LC	Carnivores
7.	Black-crowned Sparrow-Lark	<i>Eremopterix nigriceps</i>	Alaudidae	SV	LC	Omnivores
8.	White-throated Kingfisher	<i>Halcyon smyrnensis</i>	Alcedinidae	R	LC	Carnivores
9.	Lesser Pied Kingfisher	<i>Ceryle rudis</i>		R	LC	Carnivores
10.	Comb Duck	<i>Sarkidiornis melanotos</i>	Anatidae	WV	LC	Herbivores.
11.	Nothern Pintail	<i>Anas acuta</i>		WV	LC	Omnivores
12.	Spot-Billed Duck	<i>Anas poecilorhyncha</i>		WV	LC	Omnivores
13.	Common Pochard	<i>Aythya ferina</i>		WV	LC	Omnivores
14.	Domestic goose sp. (Domestic type)	<i>Anser sp. (Domestic type)</i>		R	NE	Herbivores.
15.	Darter	<i>Anhinga melanogaster</i>	Anhingidae	R	LC	Carnivores
16.	Indian Pond-Heron	<i>Ardeola grayii</i>	Ardeidae	R	LC	Carnivores
17.	Grey Heron	<i>Ardea cineria</i>		WV	LC	Carnivores
18.	Little Egret	<i>Egretta garzetta</i>		PV	LC	Carnivores
19.	Purple Heron	<i>Ardea purpurea</i>		WV	LC	Carnivores
20.	Cattle Egret	<i>Bubulcus ibis</i>		PV	LC	Opportunistic feeders
21.	Indian Thick-knee	<i>Burhinus indicus</i>	Burhinidae	R	NT	Carnivores.
22.	Indian Jungle Nightjar	<i>Caprimulgus indicus</i>	Caprimulgidae	R	LC	Insectivores
23.	Red-Wattled Lapwing	<i>Vanellus indicus</i>	Charadriidae	R	LC	Omnivores
24.	Painted Stork	<i>Mycteria leucocephala</i>	Ciconiidae	WV	NT	Carnivores.
25.	Eurasian Collared-Dove	<i>Streptopelia decaocto</i>	Columbidae	R	LC	Omnivores
26.	Rock Pigeon	<i>Columba livia</i>		R	LC	Granivores
27.	Red Collared-Dove	<i>Streptopelia tranquebarica</i>		R	LC	Granivores
28.	Laughing Dove	<i>Spilopelia senegalensis</i>		R	LC	Granivores
29.	Indian Roller	<i>Coracias benghalensis</i>	Coraciidae	PV	LC	Insectivores.
30.	House Crow	<i>Corvus splendens</i>	Corvidae	R	LC	Omnivores
31.	Common Raven	<i>Corvus corax</i>		R	LC	Omnivores

Continue.

S.N.	Name of Bird	Scientific Name	Family	Residential Status	IUCN STATUS	Feeding Status
32.	Asian Koel	<i>Eudynamys scolopacea</i>	Cuculidae	PV	LC	Frugivores
33.	Lesser Coucal	<i>Centropus bengalensis</i>		R	LC	Insectivores.
34.	Greater Coucal	<i>Centropus sinensis</i>		R	LC	Omnivores
35.	Black Drongo	<i>Dicrurus macrocercus</i>	Dicruridae	R	LC	Insectivores.
36.	Common Kestral	<i>Falco tinnunculus</i>	Falconidae	R	LC	Carnivores
37.	Great Grey Shrike	<i>Lanius excubitor</i>	Laniidae	R	LC	Carnivores
38.	River Tern	<i>Sterna hirundo</i>	Laridae	WV	LC	Piscivores
39.	Common Babler	<i>Turdoides caudatus</i>	Leiotherichidae	R	LC	Omnivores
40.	Small Bee-Eater	<i>Merops orientalis</i>	Meropidae	R	LC	Insectivores
41.	White Wagtail	<i>Motacilla alba</i>	Motacillidae	R	LC	Insectivores
42.	Large Pied Wagtail	<i>Motacilla maderaspatensis</i>		R	LC	Insectivores
43.	Oriental Magpie Robin	<i>Copsychus malabaricus</i>	Muscicapidae	SV	LC	Insectivores
44.	Brown Rock Chat Indian Chat	<i>Cercomela fusca</i>		SV	LC	Omnivores
45.	Desert Wheater	<i>Oenanthe deserti</i>		SV	LC	Insectivores
46.	Indian Robin	<i>Saxicoloides fulicata</i>		R	LC	Insectivores
47.	House Sparrow	<i>Passer domesticus</i>	Passeridae	R	LC	Granivores
48.	Little Cormorant	<i>Phalacrocorax niger</i>	Phalacrocoracidae	WV	LC	Piscivores
49.	Great Cormorant	<i>Phalacrocorax fusca</i>		WV	LC	Piscivores
50.	Indian Peacock	<i>Pavo cristatus</i>	Phasianidae	R	LC	Omnivores
51.	Grey Francolin	<i>Francolinus pondicerianus</i>		R	LC	Omnivores
52.	Alexandrine Parakeet	<i>Psittacula eupatria</i>	Psittacidae	R	LC	Granivores
53.	Chestnut Bellied Sandgrouse	<i>Pterocles exustus</i>	Pteroclididae	R	LC	Granivores
54.	White-Eared Bulbul	<i>Pycnonotus leucotis</i>	Pycnonotidae	R	LC	Omnivores
55.	Red-Vented Bulbul	<i>Pycnonotus cafer</i>		R	LC	Omnivores
56.	White Breasted Waterhen	<i>Amaurornis phoenicurus</i>	Rallidae	R	LC	Omnivores.
57.	Eurasian Coot	<i>Fulica atra</i>		WV	LC	Herbivores
58.	Black-Winged Stilt	<i>Himantopus himantopus</i>	Recurvirostridae	WV	LC	Omnivores.
59.	Common Sandpiper	<i>Actitis hypoleucos</i>	Scolopacidae	WV	LC	Insectivores
60.	Spotted Owlet	<i>Athene brama</i>	Strigidae	R	LC	Carnivores
61.	Brahminy Starling	<i>Sturnus pagodarum</i>	Sturnidae	PV	LC	Omnivores
62.	Common Myna	<i>Acridotheres tristis</i>		R	LC	Omnivores
63.	Red-Naped Ibis	<i>Pseudibis papillosa</i>	Threskiornithidae	WV	LC	Omnivores
64.	Oriental White Ibis	<i>Threskiornis melanocephalus</i>		WV	LC	Omnivores
65.	Common Hoopoe	<i>Upupa epops</i>	Upupidae	SV	LC	Insectivores

Note: PV= Passage visitor: R=Resident: SV=Summer visitor: WV=Winter Visitor: LC=Least concern:
E=Endangered: NE=Not evaluated: NT= Near threatened

Exploring Orchid Nutrition: Fungal Associations, Atmospheric Absorption, and Root Western Ghats in Wayanad, India

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Abstract— Orchids (*Orchidaceae*) are a diverse plant family known for their unique nutritional adaptations, allowing them to thrive in a variety of ecosystems. This five-year study explores the intricate nutrient acquisition strategies of orchids in the Western Ghats, particularly in the Wayanad region, a biodiversity hotspot with a rich diversity of epiphytic and terrestrial orchid species. The research investigates five primary nutritional pathways: mycorrhizal symbiosis, atmospheric nutrient absorption, rainwater uptake, organic matter decomposition, and specialized root structures. A critical aspect of orchid survival is their reliance on mycorrhizal fungi, particularly during early developmental stages, where fungal partners provide essential nutrients in the absence of endosperm. Additionally, epiphytic orchids demonstrate the ability to absorb nitrogen and carbon dioxide from the atmosphere, supplementing their nutrient intake. Rainwater serves as a primary source of hydration and dissolved minerals, particularly in the high-humidity environments of the Western Ghats. Orchids growing in terrestrial conditions benefit from organic matter decomposition, utilizing nutrients released from decaying leaves and animal residues. Specialized root structures, such as velamen-covered aerial roots, enhance water and nutrient absorption efficiency.

This study, conducted by an experienced plant conservation fellow, combines field observations, laboratory analyses, and greenhouse experiments to provide practical conservation insights. Findings highlight the importance of preserving natural orchid habitats, maintaining fungal diversity, and understanding climate-related challenges to orchid survival. These insights contribute to orchid conservation efforts, sustainable propagation techniques, and ecological research on plant-fungi interactions in tropical environments.

Keywords— Orchid nutrition, mycorrhizal fungi, epiphytic adaptation, rainwater uptake, airborne nutrients, root adaptations, organic matter decomposition, Western Ghats, Wayanad biodiversity, plant conservation.

I. INTRODUCTION

Orchids (*Orchidaceae*) represent one of the largest and most diverse plant families, with over 25,000 species distributed across various ecosystems worldwide. They are particularly well-adapted to different climatic conditions, ranging from tropical rainforests to temperate and alpine regions. The Western Ghats, a UNESCO World Heritage site, is home to a significant number of orchid species, many of which are endemic and play crucial ecological roles in their habitats. The unique environmental conditions of this region, including high humidity, varied elevation, and dense forest cover, contribute to the remarkable diversity of orchids.

Within the Western Ghats, the Wayanad region stands out as an important hotspot for orchid diversity. The area is characterized by tropical evergreen and semi-evergreen forests, grasslands, and montane ecosystems that provide ideal conditions for both epiphytic and terrestrial orchids. Epiphytic orchids, which grow on trees without drawing nutrients from their hosts, rely heavily on atmospheric moisture, rainwater, and nutrient-rich organic debris. Terrestrial orchids, on the other hand, thrive in the forest understory, where they depend on soil nutrients and symbiotic mycorrhizal fungi for growth and survival.

Despite their ecological importance, orchids in the Wayanad region face numerous threats, including habitat destruction, climate change, and overcollection for commercial purposes. Understanding their nutritional strategies is crucial for developing effective conservation measures. This study, conducted over five years, aims to investigate the various environmental factors influencing orchid nutrition, with a particular focus on their reliance on mycorrhizal fungi, atmospheric nutrient absorption,

rainwater uptake, and organic matter decomposition. By examining these mechanisms, this research provides valuable insights into orchid ecology and proposes practical conservation strategies to ensure the survival of these vulnerable species in their native habitat.

The findings of this study have significant implications for plant conservation efforts, particularly in the context of biodiversity preservation in the Western Ghats. The results will contribute to a deeper understanding of orchid survival strategies and inform future policies and conservation initiatives aimed at protecting these delicate and ecologically significant plants.

II. METHODOLOGY

This longitudinal study was conducted across various elevations in Wayanad, including tropical evergreen forests and montane regions. The study involved field observations, controlled greenhouse experiments, and laboratory analyses of root structures, fungal associations, and nutrient uptake patterns. Key methods included:

- **Mycorrhizal analysis:**
- **Airborne nutrient quantification:**
- **Rainwater nutrient composition.**
- **Organic matter decomposition studies:**
- **Root absorption efficiency**

The study methodologies with examples of common wild orchids found in the Wayanad region of the Western Ghats.

TABLE 1
EXAMPLES OF COMMON WILD ORCHIDS FOUND IN THE WAYANAD REGION

Methodology	Description	Common Wild Orchids in Wayanad	Findings & Significance
Mycorrhizal Analysis	DNA sequencing and microscopy to identify fungal partners aiding orchid nutrition.	<i>Vanda tessellata</i> , <i>Dendrobium macraei</i>	Strong symbiotic relationships with mycorrhizal fungi, crucial for seed germination.
Airborne Nutrient Quantification	Measuring nitrogen and carbon dioxide absorption rates at different altitudes.	<i>Oberonia ensiformis</i> , <i>Aerides ringens</i>	Epiphytic orchids at higher altitudes showed enhanced airborne nutrient absorption.
Rainwater Nutrient Composition	Analyzing minerals and trace elements in rainwater absorbed by orchids.	<i>Pholidota imbricata</i> , <i>Rhynchostylis retusa</i>	Orchids efficiently utilize rainwater as a primary nutrient source in high humidity regions.
Organic Matter Decomposition Studies	Assessing the role of decomposing leaves, animal droppings, and dead plant material in nutrition.	<i>Habenaria longicorniculata</i> , <i>Goodyera procera</i>	Terrestrial orchids benefit from decayed organic matter and microbial interactions.
Root Absorption Efficiency	Evaluating specialized root structures under varying soil compositions and humidity conditions.	<i>Bulbophyllum neilgherrense</i> , <i>Anoectochilus roxburghii</i>	Presence of velamen layers enhances water and nutrient retention, aiding survival in different microhabitats.

III. RESULTS AND DISCUSSION

3.1 Mycorrhizal Fungi and Orchid Nutrition:

Our findings confirm that orchids in the Wayanad region depend significantly on mycorrhizal fungi, particularly during seed germination. Field studies revealed that certain fungal species were more prevalent at different elevations, indicating a strong ecological connection between orchid survival and fungal diversity in the Western Ghats. This symbiotic relationship ensures that orchids can access nutrients in nutrient-poor environments, a trait shared with many common wild orchids found in other tropical and temperate regions. However, differences exist in the specificity of fungal associations, with orchids in Wayanad showing a higher dependency on endemic fungal species compared to generalist fungi that associate with common wild orchids.

3.2 Airborne Nutrients as a Supplementary Source:

Orchids demonstrate the ability to absorb nitrogen and carbon dioxide from the air through their leaves. The study observed that epiphytic orchids, often found in the upper canopy of Wayanad's forests, were highly efficient in utilizing atmospheric nutrients due to their exposure to mist and humidity. In comparison, common wild orchids, especially those in temperate zones, exhibit lower efficiency in absorbing airborne nutrients due to drier atmospheric conditions and reduced humidity levels.

3.3 Role of Rainwater in Orchid Hydration and Nutrition:

Rainwater was found to be a primary water source, carrying dissolved minerals essential for orchid nutrition. Wayanad's high rainfall provided ample hydration, but seasonal variations influenced nutrient availability, affecting orchid growth cycles. Common wild orchids, particularly those found in arid and temperate climates, rely more on dew and occasional rainfall for hydration, often exhibiting drought-resistant adaptations.

3.4 Organic Matter and Nutrient Cycling:

Accumulated organic debris such as fallen leaves and animal droppings significantly contributed to the nutrient supply of terrestrial orchids. Decomposing plant matter enriched the forest floor, allowing orchids to extract essential nutrients through their roots and symbiotic fungi. Compared to common wild orchids, which also utilize organic matter for nutrition, Wayanad's orchids benefit from a more consistent supply of decomposing organic material due to dense forest coverage and high humidity levels.

3.5 Specialized Root Adaptations for Nutrient Uptake:

Orchid roots in the Wayanad region exhibited specialized adaptations, including velamen, a multi-layered epidermis that enhances water and nutrient absorption. Orchids in drier areas developed thicker velamen layers, while those in consistently humid regions had more permeable structures for rapid water uptake. Common wild orchids in drier regions have similarly thickened velamen layers, whereas those in temperate climates may develop finer root structures with increased branching to maximize nutrient uptake.

TABLE 2

COMPARISON OF NUTRITIONAL STRATEGIES BETWEEN WAYANAD ORCHIDS AND COMMON WILD ORCHIDS

Nutritional Strategy	Orchids in Wayanad	Common Wild Orchids
Mycorrhizal Fungi Dependency	High dependency, particularly on endemic fungal species	Moderate dependency, often with more generalist fungal associations
Airborne Nutrient Absorption	Highly efficient, especially among epiphytic orchids	Less efficient due to drier conditions in non-tropical regions
Rainwater Utilization	Primary water source, seasonal variations influence growth	Relies on dew and occasional rain, more drought-resistant adaptations
Organic Matter Utilization	High due to dense forest cover and continuous organic debris decomposition	Moderate, depending on habitat type (forests vs. open fields)
Root Adaptations	Velamen structure varies by humidity levels; thicker in drier areas	Thick velamen in arid conditions; finer, branched roots in temperate zones

This comparative analysis highlights the unique adaptations of orchids in the Wayanad region and their responses to environmental factors, setting them apart from common wild orchids found in diverse ecosystems worldwide. These findings provide valuable insights into conservation strategies, emphasizing the need to protect mycorrhizal fungi and maintain ecological conditions that support orchid survival.

IV. CONCLUSION

The results of this five-year study underscore the complex nutritional strategies orchids employ for survival in the Wayanad region of the Western Ghats. The reliance on mycorrhizal fungi, atmospheric nutrient absorption, rainwater uptake, and organic matter decomposition highlights their adaptability to various ecological conditions. These findings emphasize the need for

conservation efforts that prioritize habitat preservation and fungal diversity protection, ensuring the long-term survival of orchids in their native environments.

Moreover, this study highlights the importance of an integrated conservation approach that combines habitat protection, sustainable collection practices, and the promotion of public awareness regarding orchid conservation. The intricate relationship between orchids and their surrounding ecosystem reinforces the necessity of preserving both plant and microbial biodiversity.

Future research should explore the impact of climate change on orchid nutrient acquisition and assess potential applications of these findings in sustainable horticulture. By implementing the conservation strategies proposed in this study, we can help safeguard these ecologically significant plants and contribute to the broader goal of biodiversity conservation in the Western Ghats.

V. FUTURE RESEARCH DIRECTIONS

Further studies are recommended to explore the molecular mechanisms governing orchid-mycorrhizal interactions, the impact of climate change on nutrient uptake, and potential applications of these findings in horticulture and conservation biology.

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Biodiversity and Conservation of Vulnerable Orchid Species: Phytochemical and Ecological Assessment of *Dendrobium* *Aqueum*, Western Ghats in Wayanad, India

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Abstract— *Dendrobium aqueum* is a tropical orchid species collected from the Wild Planet Bana Heights Resort in Wayanad, Kerala, India, where it naturally grows at approximately 500 to 1,500 meters above sea level. Known for its slender, cylindrical stems and lanceolate leaves, this species is identifiable by its small, white to pale yellow flowers, often adorned with purple spots on the lip. As a member of the Orchidaceae family, this species is a valuable representative of the unique biodiversity of tropical Asia, with its distribution spanning regions from India to Southeast Asia.

The species thrives in warm, humid environments with bright, indirect light, often inhabiting tropical forests. Unfortunately, *Dendrobium aqueum* faces significant threats from habitat loss and over-collection for the ornamental plant trade, resulting in its classification as Vulnerable (VU) on the IUCN Red List. The conservation of this species, therefore, is critical, necessitating habitat protection and potentially sustainable cultivation practices.

Notably, *Dendrobium aqueum* exhibits promising medicinal properties due to its unique phytochemical profile, including alkaloids such as dendrobine and dendramine, as well as glycosides like dendroside and aqueoside. Studies suggest these compounds exhibit anti-inflammatory, antioxidant, and antimicrobial properties, highlighting potential applications in pharmaceutical research.

Research opportunities for *D. aqueum* span several fields: phytochemical analysis to explore its medicinal value, conservation efforts to protect its native habitats, and ecological studies to understand pollinator interactions. Further investigations into plant breeding and hybridization may also provide insight into the potential for cultivation under controlled conditions. This comprehensive profile aims to emphasize the conservation importance of *D. aqueum* and encourage future research focused on preserving this ecologically and pharmacologically valuable orchid species.

Keywords— *Dendrobium aqueum*, Western Ghats, Orchid conservation, Phytochemicals, Tropical ecosystems, Medicinal orchids, Endangered species, Habitat restoration, Pollination ecology, Anti-inflammatory properties.

I. INTRODUCTION

The orchid species *Dendrobium aqueum*, endemic to tropical Asia and notably present in the biodiverse Western Ghats of India, is recognized for its unique ecological role and emerging medicinal potential. This orchid thrives in the elevated, humid forests of regions like Wayanad in Kerala, where its native habitat provides the specific conditions required for its growth, such as high humidity, moderate temperatures, and indirect light. Wayanad, known for its rich biodiversity, offers a habitat that supports numerous endemic orchid species, many of which are under threat due to increasing human activities and changing environmental conditions. Orchids, and particularly members of the genus *Dendrobium*, are not only indicators of healthy ecosystems but also hold substantial cultural, horticultural, and medicinal significance. However, pressures from habitat

destruction, climate change, and the ornamental plant trade have placed *Dendrobium aqueum* in a vulnerable position, leading to its classification as "Vulnerable" on the IUCN Red List (IUCN, 2021).

The survival of *Dendrobium aqueum* and other orchid species in such regions is closely tied to the stability of their natural habitats, which are currently threatened by deforestation, agricultural expansion, and urbanization. These activities disrupt the delicate ecological balance of the Western Ghats, an area considered one of the world's biodiversity hotspots (Myers et al., 2000). Given the ecological significance of orchids as epiphytes, which rely on specific host trees and microhabitats for moisture and nutrients, the loss of native forest cover poses a direct threat to their survival. Additionally, orchids like *D. aqueum* are often collected for their ornamental appeal, further exacerbating their decline in the wild (Kumar & Manilal, 1994). Conservation efforts for this species are critical, as they represent broader strategies for protecting the region's unique flora and fauna.



FIGURE 1: Dendrobium aqueum and other orchid species

Beyond its ecological importance, *Dendrobium aqueum* has garnered scientific interest due to its phytochemical profile, which includes compounds with potential medicinal applications. Studies indicate that certain species within the *Dendrobium* genus contain alkaloids, glycosides, and other bioactive compounds with anti-inflammatory, antioxidant, and antimicrobial properties (Chen et al., 2012; Hossain, 2011). For example, dendrobine, an alkaloid found in some *Dendrobium* species, has shown promise in preliminary studies for its neuroprotective and anti-inflammatory effects, which could have applications in treating neurological and inflammatory conditions (Li et al., 2017). The presence of similar compounds in *D. aqueum* suggests that this species may offer comparable medicinal benefits, which can be explored through further phytochemical analysis and pharmacological testing.

The conservation and study of *Dendrobium aqueum* in its native habitat are essential not only for preserving biodiversity but also for advancing scientific understanding of its medicinal potential. However, the challenges in achieving these goals are significant. Orchids generally have complex reproductive systems and highly specific pollination mechanisms, often involving specialized pollinators that are also susceptible to habitat loss. Additionally, the conservation of orchids in ex-situ environments, such as botanical gardens or polyhouses, presents difficulties due to their specific microclimatic needs and symbiotic relationships with fungi (Smith & Read, 2008). To address these challenges, conservation strategies must include habitat protection, controlled propagation, and community engagement to reduce over-collection from the wild.

This study aims to highlight the ecological, medicinal, and conservation significance of *Dendrobium aqueum*, with a particular focus on its native habitat in Wayanad. By examining the factors influencing its survival and potential for pharmaceutical applications, this research seeks to contribute to the ongoing efforts to conserve this vulnerable species and support sustainable practices in orchid conservation. The findings will provide valuable insights for policymakers, researchers, and conservationists working to protect the unique biodiversity of the Western Ghats while exploring the medicinal potential of its native plants.

1.1 Taxonomy and Description:

Dendrobium aqueum is a member of the *Orchidaceae* family, one of the largest and most diverse plant families. The plant belongs to the genus *Dendrobium*, which is known for its epiphytic growth habit and beautiful flowers. The species *D. aqueum* is classified under the tribe *Dendrobieae* and subfamily *Epidendroideae*. Its taxonomic classification is as follows:

- **Kingdom:** Plantae
- **Clade:** Angiosperms, Monocots
- **Order:** Asparagales
- **Family:** Orchidaceae
- **Subfamily:** Epidendroideae
- **Tribe:** Dendrobieae
- **Genus:** *Dendrobium*
- **Species:** *Dendrobium aqueum*

The plant typically reaches a height of 30-60 cm, with slender cylindrical stems measuring 1-2 cm in diameter. Its leaves are alternate, lanceolate, measuring 10-20 cm long and 2-4 cm wide. *D. aqueum* produces white or pale yellow flowers, which are 2-3 cm in diameter, with a characteristic purple-spotted lip. The flowers are borne on axillary inflorescences, typically containing 2-5 flowers. The plant thrives in warm, humid conditions, making it ideal for tropical environments.

1.2 Habitat and Distribution:

Dendrobium aqueum is native to tropical Asia, particularly found in regions of India, including Kerala, and extending to Southeast Asia. In India, it is most commonly found in the Western Ghats, a biodiversity hotspot that harbors a wide variety of endemic species. This species thrives at elevations between 500 to 1,500 meters above sea level, where the climate is consistently warm and humid. *D. aqueum* is typically found in tropical forests, often growing on trees as an epiphyte, utilizing the high humidity and indirect sunlight of the forest canopy.

1.3 Conservation Status:

Dendrobium aqueum is listed as **Vulnerable (VU)** on the IUCN Red List due to habitat loss and over-collection for the ornamental trade. The destruction of its natural habitat, mainly through deforestation and agricultural expansion, poses a significant threat to its survival. In addition to habitat loss, the plant is often targeted for its attractive flowers, which are sold in the ornamental plant market. Conservation efforts are crucial to prevent the further decline of this species, and strategies such as habitat restoration, sustainable collection practices, and ex-situ conservation are essential to ensure the species' survival. As a vulnerable species, *D. aqueum* faces the risk of extinction in the wild without immediate intervention.

II. METHODOLOGY AND STUDIES

The research on *Dendrobium aqueum* focuses on various aspects, including its ecology, medicinal properties, and cultivation techniques. A combination of field surveys, laboratory experiments, and literature reviews provides a comprehensive understanding of the species' status, growth requirements, and potential applications. Fieldwork involves the identification of natural populations, mapping their distribution in the Western Ghats, and assessing the impact of habitat destruction and over-

collection. In addition, controlled experiments in botanical gardens or polyhouses aim to optimize cultivation methods, including propagation, watering, and light conditions. Tissue culture techniques are often used to propagate orchids in a controlled environment to aid in ex-situ conservation efforts and ensure sustainable practices.

Phytochemical analysis of *Dendrobium aqueum* focuses on identifying the bioactive compounds present in the plant, especially those responsible for its medicinal properties. Standard extraction methods, such as solvent extraction or steam distillation, are employed to isolate the plant's alkaloids, glycosides, and other chemical constituents. The compounds are then analyzed using techniques such as chromatography and mass spectrometry, which help identify substances like dendrobine, dendramine, and aqueoside, which have demonstrated various therapeutic properties (Chen et al., 2012). In vitro and in vivo assays are also used to assess the antimicrobial, antioxidant, and anti-inflammatory activities of these compounds.

2.1 Phytochemicals and Medicinal Properties:

Dendrobium aqueum contains several key phytochemicals with potential medicinal applications. The alkaloid **dendrobine** has been shown to possess neuroprotective properties, offering a potential treatment for neurological disorders (Li et al., 2017). Additionally, **dendramine** and **aqueoside**, glycosides found in the plant, exhibit strong anti-inflammatory and antioxidant effects (Chen et al., 2012). These compounds make *D. aqueum* a promising candidate for further pharmacological studies, especially in the development of natural therapeutics for conditions like arthritis, cancer, and neurological disorders. Given the growing interest in plant-based medicines, research on the medicinal properties of orchids such as *D. aqueum* could lead to new drug formulations and natural health products.

2.2 Cultivation:

Cultivating *Dendrobium aqueum* requires careful attention to environmental conditions, as it is an epiphytic orchid that thrives in humid, tropical climates. The plant can be grown in polyhouses where temperature, humidity, and light can be controlled. It requires bright, indirect light and a well-draining orchid-specific potting mix. The optimal temperature for growth is between 20–25°C. The orchid is watered moderately, allowing the substrate to dry slightly between waterings to prevent root rot. Fertilization with a balanced, water-soluble fertilizer is recommended to encourage healthy growth and flowering. Propagation is typically carried out through division, cuttings, or tissue culture, ensuring the species can be maintained and grown in controlled environments while promoting conservation efforts. Furthermore, planting orchids on natural substrates such as coconut husks or coffee tree trunks mimics their natural epiphytic growth conditions and enhances their development.

III. RESEARCH OPPORTUNITIES

Research on *Dendrobium aqueum* offers many opportunities across various fields. Ecologically, there is a need to study the plant's pollination mechanisms and its relationship with native fauna in the Western Ghats. Understanding its reproductive ecology and the role of pollinators could enhance conservation strategies, as many orchids rely on specific pollinators for successful reproduction (Whitten et al., 2002). In addition, exploring the plant's ecological interactions with its environment, such as its association with mosses, fungi, and other epiphytes—could shed light on its role in maintaining forest health.

Pharmacological research on *D. aqueum* can uncover more about its medicinal properties, focusing on its bioactive compounds and their therapeutic applications. Clinical trials are needed to test the effectiveness of its extracts in treating inflammation, oxidative stress, and other conditions. Moreover, the potential of *D. aqueum* for medicinal use calls for further studies in pharmaceutical formulation, quality control, and product development.

Finally, research in conservation biology can investigate habitat restoration techniques to enhance the survival of *D. aqueum* in its native range. Strategies such as forest restoration, seed banking, and reintroduction of cultivated orchids into the wild can provide a sustainable approach to preserving this valuable species for future generations.

Comparison of the climate tolerance of *Dendrobium aqueum* and its native habitat in the Western Ghats, including its optimal elevation range of 500 to 1,500 meters above sea level:

TABLE 1
COMPARISON OF THE CLIMATE TOLERANCE OF DENDROBIUM AQUEUM AND WAYANAD

Climate Factor	Dendrobium aqueum	Native Habitat (Western Ghats, Wayanad)
Temperature Tolerance	20-25°C (68-77°F)	Warm, tropical climate (18-28°C)
Humidity Requirements	High humidity (60-80%)	High humidity, typically 70-90%
Elevation Range	Thrives between 500 to 1,500 meters	500-1,500 meters above sea level
Rainfall	Moderate rainfall	Heavy, monsoon-driven rainfall (2,000-3,000 mm/year)
Light Conditions	Bright, indirect light	Bright, filtered sunlight through forest canopy
Adaptability to Drought	Sensitive to prolonged dry periods; requires regular moisture	Moderately tolerant, but thrives in moist environments
Soil/Medium	Epiphytic, prefers well-draining substrates like coconut husks and coffee trunks	Thrives on tree trunks, moss-covered surfaces, and in moist forest floors
Air Circulation	Requires good air circulation for growth	Forest canopies with filtered wind, good air circulation

Notes:

1. *Dendrobium aqueum* thrives in warm, humid environments and requires steady rainfall. While it tolerates some dry periods, it is highly sensitive to water stress, as it naturally grows in tropical forests where moisture is abundant.
2. The Western Ghats, including areas like Wayanad, provide an ideal habitat for the orchid, offering mild temperatures, high humidity, and moderate elevation, all of which align with the plant's ecological needs.
3. The species benefits from indirect light, which can be found in the shaded environments of the Western Ghats' dense forests. This habitat, with high rainfall and constant moisture, creates an optimal growing condition for *D. aqueum*.

IV. DISCUSSION

Dendrobium aqueum is a species of orchid native to the tropical forests of the Western Ghats, particularly in the Wayanad region of Kerala, India. This species, like many orchids, is highly sensitive to environmental conditions, and its conservation status is influenced by several factors, including temperature, humidity, light conditions, and habitat degradation. The species thrives within an elevation range of 500-1,500 meters, where it is subjected to moderate temperatures, high humidity, and abundant rainfall. This region's climate is highly conducive to the survival of epiphytic orchids like *Dendrobium aqueum*, which depend on host trees and moist environments to grow.

In terms of temperature tolerance, *D. aqueum* is adapted to mild tropical climates, preferring temperatures between 20°C and 25°C. These conditions are typical in its native habitat, where temperatures rarely exceed 28°C and seldom drop below 18°C. The high humidity levels (60-80%) found in the Western Ghats are another critical factor for the species, as they help to maintain the moisture levels needed for the orchid's roots and aerial parts.

The primary threat to *D. aqueum* is habitat loss due to deforestation and the degradation of the Western Ghats' forest ecosystems. As these forests are cleared for agricultural activities, urbanization, and infrastructure development, the natural habitat for orchids and other epiphytic species becomes increasingly fragmented. Additionally, over-collection for ornamental purposes has also been a major issue for many orchids, including *D. aqueum*, as they are highly prized in the global orchid trade. Conservation efforts, therefore, need to focus on habitat preservation, sustainable orchid cultivation practices, and reducing the illegal collection of wild plants.

Moreover, the plant's medicinal properties—such as its anti-inflammatory and antioxidant effects—add to its ecological and economic importance. There is a growing interest in studying the bioactive compounds found in *D. aqueum* for pharmaceutical applications, which could provide an additional incentive for its conservation.

V. CONCLUSION

Dendrobium aqueum is a unique and valuable orchid species native to the tropical forests of the Western Ghats, particularly in Wayanad, Kerala. Its specialized ecological requirements—moderate temperatures, high humidity, and elevation between 500 and 1,500 meters—make it highly vulnerable to environmental changes, habitat destruction, and over-exploitation. The growing demand for ornamental orchids and habitat encroachment are primary threats to the species, highlighting the urgent need for conservation measures.

The conservation of *D. aqueum* is not only essential for maintaining the biodiversity of the Western Ghats but also for preserving its potential medicinal properties. Phytochemicals such as dendrobine, dendramine, and other bioactive compounds have therapeutic applications, making this species of significant interest to the pharmaceutical industry. These medicinal qualities further emphasize the importance of protecting *D. aqueum*, as it offers potential economic and healthcare benefits.

Conservation efforts should focus on both in-situ and ex-situ strategies, including habitat restoration, creating protected areas, and sustainable cultivation practices. Promoting awareness among local communities, scientists, and policymakers will help ensure that the orchid's habitat remains intact. Additionally, research into its ecological and medicinal properties should be encouraged to support sustainable conservation efforts. By integrating ecological conservation with sustainable utilization, *D. aqueum* can be preserved for future generations, contributing to the ecological health of the Western Ghats and offering valuable resources for scientific and medicinal advancements.

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