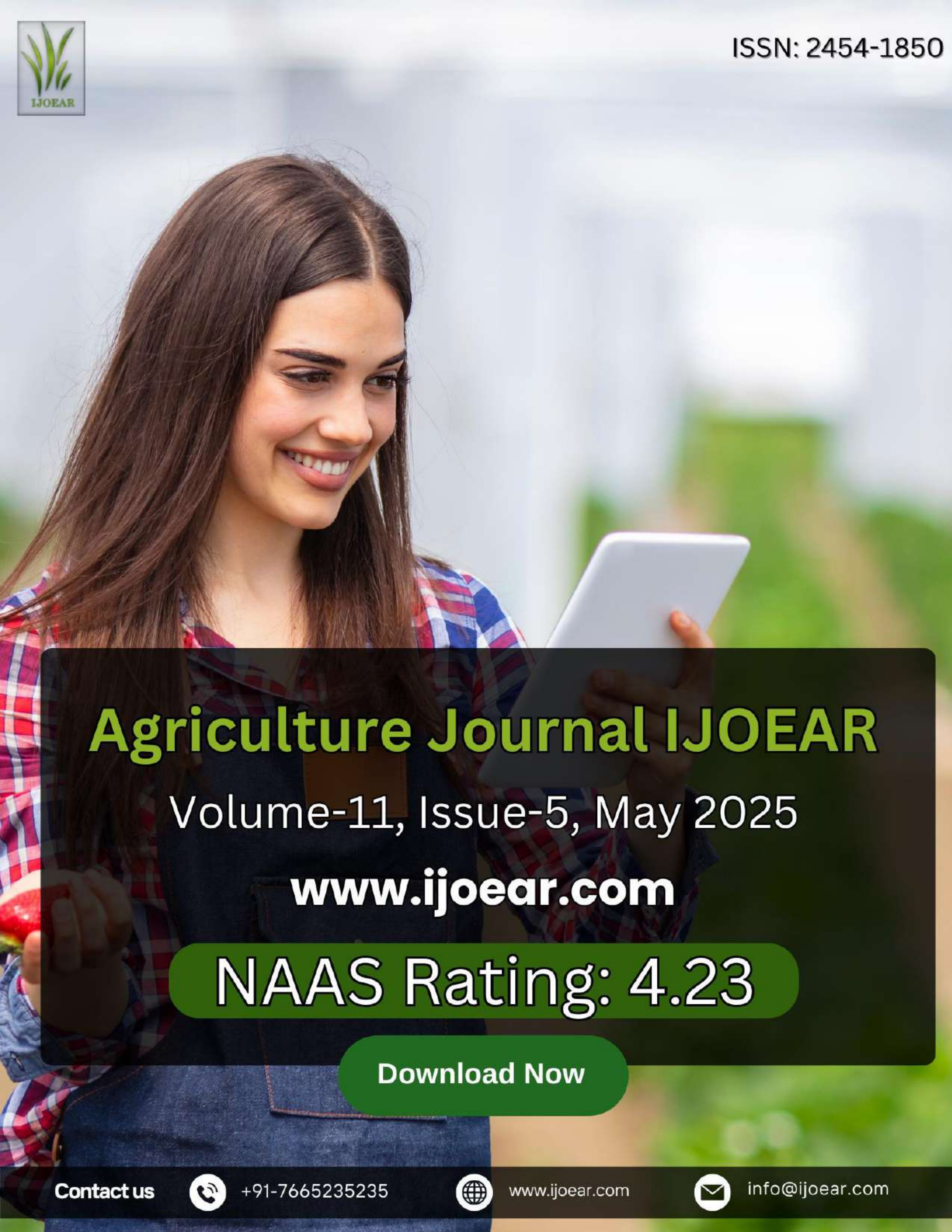
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Preface

We would like to present, with great pleasure, the inaugural volume-11, Issue-5, May 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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Environmental science and regulation, Ecotoxicology, Environmental health issues, Atmosphere and climate, Terrestric ecosystems, Aquatic ecosystems, Energy and environment, Marine research, Biodiversity, Pharmaceuticals in the environment, Genetically modified organisms, Biotechnology, Risk assessment, Environment society, Agricultural engineering, Animal science, Agronomy, including plant science, theoretical production ecology, horticulture, plant, breeding, plant fertilization, soil science and all field related to Environmental Research.

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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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Agricultural Sciences	
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Agricultural Management Practices	Agricultural Technology
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Mr. Aklilu Bajigo Madalcho

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Mr. Isaac Newton ATIVOR

MPhil. in Entomology, from University of Ghana.

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.

Mr. Bimal Bahadur Kunwar

He received his Master Degree in Botany from Central Department of Botany, T.U., Kirtipur, Nepal. Currently working as consultant to prepare CCA-DRR Plan for Hariyo Ban Program/CARE in Nepal/GONESA.

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Malnutrition among Young Girls: A Comprehensive Review

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Abstract— *Malnutrition is defined as excess or deficit in individual energy or nutrients intake. It is a persistent and multifaceted global public health challenge, with far-reaching implications for individual well-being, societal development, and future generations. Despite global efforts and progress towards reducing malnutrition rates, millions of people, particularly in low- and middle-income countries are suffering from malnutrition. It affects every segment of population irrespective of their age and sex but young and adolescents' girls are more affected by it. It prevails in the society in its various form like undernutrition includes (stunting, wasting and underweight) and overweight includes obesity or in certain cases both known as double burden of malnutrition. Another form is iron deficiency Anaemia. It is still a big public health issue in India. This demographic is at increased risk due to a combination of socio-economic factors like family income, parents' education, locality either urban or rural, cultural norms, inadequate dietary intake, gender-based discrimination, lack of access to nutritious food, and poor healthcare facilities. There are other social determinants of health classified into two structural and intermediary. Undernutrition causes mortality and morbidity, whereas overnutrition increases the risk of noncommunicable diseases. Their health issues have a direct impact on the newborn or can result in preterm or stillbirth, as well as maternal mortality. This review also discusses the intergenerational cycle of malnutrition. It is a global issue that necessitates a multi-sectoral strategy incorporating government measures, community participation, and nutrition education programs. Sustainable solutions are necessary to ensure the well-being and development of young girls, contributing to the general progress of the nation.*

Keywords— Global Burden, Malnutrition, Nutritional Status, Social Determinants of Health, Double Burden, Anaemia.

I. INTRODUCTION

Food insecurity, hunger, and malnutrition remain severe and widespread challenges for impoverished populations worldwide in the 21st century. The Food and Agricultural Organization (FAO) reported that between 2010 and 2012, more than 870 million people were persistently undernourished, with most of them residing in developing countries [1]. Nutrition is a critical factor in shaping health, overall well-being, and human development. Body size is often used to assess nutritional status: conditions like stunting and wasting indicate undernutrition, while overweight and obesity reflect overnutrition [2]. Malnutrition, which includes both nutrient deficiencies and excesses, affects energy and nutrient intake. Globally, about one-third of adults suffer from anaemia, and half are either underweight or overweight. In India, malnutrition poses a dual burden, with both underweight and overweight individuals coexisting. The problem escalates to a triple burden when micronutrient deficiencies—shortages of essential vitamins and minerals—are also present [3].

The youth demographic spans ages 10 to 24, with adolescence defined as ages 10–19 and youth as 20–24. Although malnutrition affects people of all ages and genders, this review focuses on adolescent girls, who represent a vital part of the population. A woman's nutritional health is shaped by her lifelong dietary habits, metabolism, and nutrient use. Many aspects influencing her nutrition originate even before birth and can have lasting effects. Nonetheless, there are opportunities to improve nutritional status during adolescence and beyond, including the pre- and post-pregnancy phases. Addressing the nutritional needs of adolescent girls and women—even those who don't become mothers can enhance their health, empower them, and strengthen their contributions to society [4].

Globally, women are more prone than men to being underweight or overweight/obese due to both biological (e.g., hormonal) and behavioural (e.g., early-life food scarcity and lack of physical activity) factors. Women at either extreme of the weight spectrum face higher risks of infertility and serious pregnancy complications, including miscarriage, premature birth, and infant mortality [5].

II. DIFFERENT FORMS OF MALNUTRITION

Malnutrition in young people can take several forms, including underweight, stunting, thinness, and overweight [6]. Wasting characterized by low weight relative to height—is a sign of acute undernutrition, often linked to food shortages or infections that result in rapid weight loss. In contrast, stunting—defined as low height for age is a marker of chronic undernutrition stemming from prolonged inadequate dietary intake that hampers optimal growth. The World Health Organization (WHO) recommends using Body Mass Index (BMI)-for-age and height-for-age as key indicators. Thinness is identified when the BMI-for-age (BAZ) falls below -2.0 standard deviations (SD), while stunting corresponds to a height-for-age (HAZ) also below -2.0 SD [7].

On the other hand, overnutrition includes both overweight and obesity, which involve unhealthy or excessive fat accumulation that may negatively impact health. Overweight is defined as a BMI-for-age that is more than one standard deviation above the WHO reference median, while obesity exceeds it by more than two standard deviations [7]. The Double Burden of Malnutrition (DBM) refers to the simultaneous presence of undernutrition and overnutrition—whether in the same individual, household, or population, across local or global contexts. An individual may experience DBM by being overweight while also suffering from vitamin or mineral deficiencies, or by having been stunted during childhood and becoming overweight as an adult. The pattern may also persist across generations, such as from a stunted mother to a stunted child, or within communities where undernourished children and overweight adolescents coexist. The concept of DBM was first introduced by the FAO and WHO at the International Conference on Nutrition (ICN) in 1992. [8].

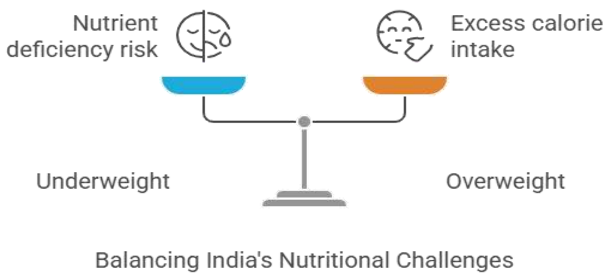

FIGURE 1: Balancing India Nutritional status

Iron deficiency anaemia is the most widespread micronutrient deficiency among adolescent girls and poses a major public health concern. Anaemia remains a complex, widely debated, and pressing issue within India's public health and nutrition landscape. It is medically defined by a drop in haemoglobin levels, red blood cell count, or packed cell volume below established thresholds. The 2017 National Health Policy, introduced by the Ministry of Health and Family Welfare, acknowledged anaemia as a critical health burden [9]. According to the World Health Organization, anaemia in women is diagnosed when haemoglobin levels fall below 120 g/l in non-pregnant women aged 15 and older, and below 110 g/l in pregnant women. Iron deficiency anaemia is especially common among women due to blood loss during menstruation. Globally, anaemia—predominantly caused by iron deficiency—contributes significantly to nutritional health problems. Although it can affect individuals across all age groups, it is particularly prevalent among women of reproductive age [10]. In low-resource settings, diets are often repetitive and lack nutritional diversity, which significantly raises the risk of micronutrient deficiencies across the globe, adolescent girls, and women of reproductive age are particularly vulnerable to these deficiencies. However, data on the micronutrient status of women outside of industrialized nations is limited. Despite the scarcity of detailed information, it is clear that micronutrient deficiencies among women are a widespread global concern, especially in developing countries [11].

TABLE 1
SYMPTOMS, NUTRITIONAL DEFICIENCY AND FOOD ITEM RECOMMENDED

Sites	Clinical symptoms	Nutritional Deficiency	Food to be eaten
Hairs	1. Alopecia,	Protein, Zn	Oysters, eggs nut dairy, yogurt and cheese
	2. Dry and brittle hair	Protein, Biotin	Kale, spinach, sweetcorn, shiitake mushroom etc.
Skin	1. Dry and flaky	Vitamin A, Zn	Avocado, carrots, sweet potatoes, almonds and cashew nuts
	2. Hyperpigmentation	Niacin	Fish poultry dairy peanuts, milk cheese.
Eyes	1. Night blindness	Vitamin A, Zn	Sweet potato and leafy greens
	2. Xerosis, conjunctivitis	Riboflavin, Vitamin A	Apricots, cantaloupe, Almonds, Milk cheese, tuna and salmon

According to the study, there is a high prevalence of nutritional insufficiency that can be treated with sufficient food intake [12]. The table is presented (*Table1).*

III. GLOBAL BURDEN AND PREVALENCE OF MALNUTRITION IN YOUNG

Nutrition is a cornerstone of human health and development across all stages of life. From fetal development to old age, it plays a crucial role in survival, physical and cognitive growth, productivity, and overall well-being. Currently, inadequate nutrition affects roughly one-third of the global population, making it one of the most pressing global health challenges [13]. Based on a large, representative sample, research has shown that while being underweight continues to be a significant public health issue—impacting about one in five women—overweight and obesity now affect an equal or even greater proportion, depending on the measurement standards used. Asian-specific BMI cutoffs tend to classify a larger number of women as overweight or obese, although the underlying factors are generally consistent [5]. Among individuals aged 15 to 49 years, women are more heavily affected by both undernutrition (23% of women vs. 20% of men) and overweight/obesity (21% of women vs. 19% of men) [14].

A study in northern India focusing on adolescent girls revealed that overweight and obesity were more common among those from nuclear families, with rates of 56.0% and 73.4%, respectively. Most overweight and obese individuals belonged to middle or upper socioeconomic groups (70.9% and 79.7%, respectively), while 88.8% of underweight girls came from lower socioeconomic backgrounds. Additionally, 59.6% of overweight and 67.2% of obese girls lived in urban areas, whereas 68.4% of underweight girls resided in rural settings [15].

Data from the National Family Health Survey (2015–2016) showed that 53% of Indian women suffer from anaemia, highlighting its persistently high prevalence (NFHS 2015–16; National Institute of Population Science). In Karnataka, one study found that mild anaemia was more common (21.5%) than moderate (14.2%) and severe (2%) cases. Similarly, a study in rural Tamil Nadu reported 2% severe, 6.3% moderate, and 36.5% mild anaemia. Most studies conducted in rural areas of India estimate severe anaemia to be between 2% and 6%. The relatively low detection of severe anaemia among adolescents suggests an "iceberg phenomenon," where the majority of mild and moderate cases remain undiagnosed or underreported [16]. (Figure 2) below shows the nutritional status of young women in five Indian states, based on data from NFHS-5).

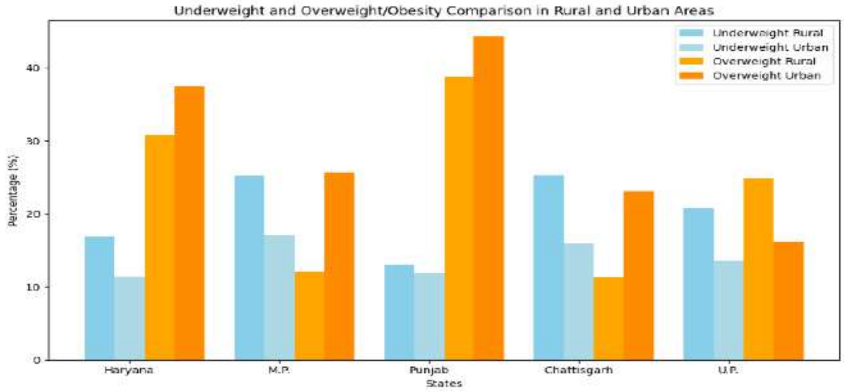

FIGURE 2: Nutritional status of young women among 5 states in India.

IV. FACTORS CONTRIBUTING TO MALNUTRITION IN YOUNG GIRLS:

Malnutrition among young females is driven by a wide range of interconnected factors. Across the globe, women and girls often face discrimination throughout their lives, with limited access to healthcare, education, and nutrition. They also face threats of violence and generally experience a lower quality of life [17]. Malnutrition is a multifaceted issue influenced by biological, environmental, social, and behavioural factors [2]. For example, a study in Ethiopia found that women of reproductive age who experienced chronic energy deficiency were more likely to live in rural areas, lack formal education, be unemployed, and report low well-being. Additionally, paternal attributes such as income, employment status, and household food security were strongly linked to gender disparities. Thinness continues to affect women and girls, and factors like age, dietary diversity, and access to community nutrition programs were directly associated with low BMI-for-age. According to a literature review, concerns around body image and negative self-perceptions also negatively impact young women's nutritional health [18]. Despite efforts over the past decade—including government initiatives, increased agricultural production, and economic growth—India has seen only limited success in reducing undernutrition. While some progress has been made, acute hunger and malnutrition persist, and the growing presence of an obesogenic environment is contributing to a rising Double Burden of Malnutrition (DBM) in the country [19]. To better understand and address malnutrition disparities, the concept of Social Determinants of Health (SDoH) is used. These determinants are categorized into structural and intermediary components. Structural determinants include ethnicity, education, occupation, and income, which influence food availability. Intermediary determinants are directly tied to nutritional outcomes and involve biological, psychological, and behavioral factors (Figure 3). Being underweight can result in poor pregnancy outcomes and other health risks, while being overweight increases the likelihood of early-onset noncommunicable diseases [20]. Adolescent girls are particularly vulnerable to iron deficiency anaemia (IDA) due to menstrual blood loss. Gender inequality further contributes to nutritional disparities—while this can occur even in middle-income households, it is more prevalent among poorer families. [21].

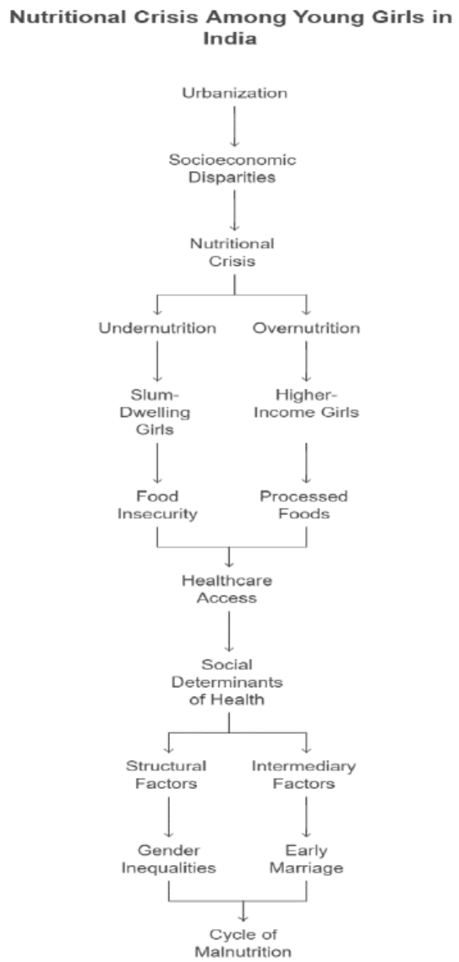

FIGURE 3: Nutritional status among young girls in India

V. CONSEQUENCES OF MALNUTRITION ON HEALTH AND DEVELOPMENT

All forms of malnutrition negatively impact health and development. Poor nutritional status during adolescence is a major predictor of adverse health outcomes, especially among girls [22]. In many developing countries, early marriage further heightens girls' risk of reproductive health complications and mortality. During adolescence, increased physical growth, the onset of menstruation, and gains in fat and muscle mass all demand higher nutritional intake. Girls who are undernourished during this critical period are more likely to become stunted women, which can lead to obstetric complications and the birth of low-weight infants. Without effective nutritional interventions, this cycle continues—low-birth-weight girls often grow into stunted mothers, perpetuating intergenerational malnutrition [23].

Women's lower social status also has widespread effects on child health. It limits their ability to make decisions regarding family planning, child healthcare, nutrition, and caregiving, all of which directly affect the well-being of future generations [24]. Being underweight before pregnancy can result in intrauterine growth restriction, premature delivery, low birth weight (LBW), and an increased risk of maternal complications such as haemorrhage and obstructed labor. Conversely, overweight and obese women are more likely to develop conditions like gestational diabetes and preeclampsia. Anaemia during pregnancy contributes to a range of severe maternal and fetal complications, including preeclampsia, premature labor, heart failure, low birth weight, intrauterine growth restriction, and stillbirth [3].

India faces a dual burden of malnutrition—undernutrition alongside rising rates of overweight and obesity—which further contributes to anaemia and other nutritional deficiencies. It is essential not only to understand the extent and evolving nature of these issues over time but also to address them effectively in order to meet the Sustainable Development Goals (SDGs). Focusing on women of reproductive age is particularly important for shaping data-driven policies and programs [19].

The SDGs emphasize equity, and Goal 5 aims to empower all women and girls. Therefore, assessing and advancing women's empowerment is vital for improving health and nutrition. The World Health Organization (WHO) underscores the importance of empowering adolescent girls, their families, and communities to enhance overall well-being [20].

Both undernutrition and overnutrition involve imbalances in calorie intake—either deficits or excesses—and each poses distinct risks. Undernutrition is linked to low bone density, increased illness, and higher mortality, while overweight and obesity are major contributors to chronic conditions like heart disease and type 2 diabetes [25].

VI. IMPORTANCE OF ADDRESSING MALNUTRITION IN YOUNG GIRLS

Women's nutritional and health status is critically important due to their roles in reproduction and caregiving. In many households, women are not only the primary caregivers but also the main providers of food and informal healthcare. They are also the key beneficiaries of most formal health and nutrition programs. Therefore, enhancing women's nutrition and overall health could be one of the most impactful strategies to improve public health outcome particularly in developing countries [26].

Despite this, there is limited research on the effectiveness of empowerment initiatives targeting adolescent girls. National surveys typically concentrate on reproductive outcomes, focusing mostly on married women aged 15 to 49. This leaves a gap in data on older adolescent girls and married young women, even though underweight and stunting remain prevalent, and rates of overweight and obesity are rising [21]. Late adolescent girls are especially vulnerable to malnutrition due to increased physiological demands during this phase of life, which can be negatively affected by imbalances in energy intake—whether from deficiency, excess, or poor-quality diets. Early marriage further exacerbates this issue by increasing the likelihood of adolescent pregnancy, which compounds the malnutrition burden and raises the risk of child mortality. Young mothers often lack sufficient knowledge about proper nutrition and dietary needs [27].

India has implemented a range of national programs aimed at addressing malnutrition, including the Integrated Child Development Services (ICDS), the National Health Mission (NHM), the Mid-Day Meal Scheme, and the National Food Security Mission. These initiatives offer evidence-based nutritional support. However, emerging challenges call for new strategies that tackle all types of malnutrition. "Double-duty actions"—interventions that simultaneously address undernutrition, overweight, and obesity—are particularly valuable. These approaches are essential in countries like India, which are experiencing rapid nutritional transitions, to ensure food security without inadvertently contributing to overnutrition [28].

VII. CONCLUSION

Malnutrition remains a widespread problem affecting nearly all segments of the global population. It arises from an imbalance in energy intake and presents in various forms, including both undernutrition and overnutrition. When these two conditions appear together within individuals or communities, it is referred to as the Double Burden of Malnutrition (DBM). One of the most prevalent nutritional issues, particularly among adolescent girls, is anaemia, which stems from a range of factors-physical, biological, environmental, and psychological. Additional contributing elements include parental education levels, gender inequality, poor dietary practices, household food insecurity, limited healthcare access, and inadequate utilization of available health services. Among young girls, misconceptions about ideal body weight and negative body image perceptions are major contributors to low BMI-for-age. In recent years, India has experienced notable shifts in dietary patterns. While undernutrition rates have declined, the prevalence of overweight and obesity has risen. Addressing this evolving nutritional landscape requires targeted efforts—such as enhancing nutrition-focused programs, increasing public awareness through education, and enacting supportive policies. These measures are essential for improving the health and nutritional status of adolescent and young girls, supporting their growth and long-term well-being.

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A New Functional Food Additive - Biomass Obtained by Bioconversion of Apple Juice Production Waste

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Abstract— *Functional food products rich in bioactive compounds (protein, lipids, vitamins, etc.) are especially demanded and popular in the modern food market. Due to its easy availability and low cost, apple juice production waste, which is produced in thousands of tons in Georgia, can be considered as a promising raw material for the production of functional food. Mycoprotein-enriched, easily digestible, non-toxic biomass was obtained as a result of successive experiments carried out in the biotechnology laboratory of the Durmishidze Institute of Biochemistry and Biotechnology of the Agrarian University of Georgia. The product obtained by microbial conversion contains Na, P, Ca and Mg, from as macro elements, and also is rich in Zn. The content of raw protein in the biomass is 18%, which fully corresponds to the standards of protein content in chicken feed for s. The raw fat content in a biomass is 7%. The amount of cellulose in the product corresponds to the standards as well. The product was found to have moderate antioxidant activity (60-70%). Based on all above mentioned, the biomass made on the basis of submerged fermentation of apple juice production waste can be recommended as a feed additive in poultry diet.*

Keywords— ***Apple juice industry waste, Mycelial fungi, Protein-rich biomass, Submerged fermentation, Functional food.***

I. INTRODUCTION

Global climate change and related environmental problems require the development of new approaches and concepts for sustainable development of the environment. One of the possible ways to solve the mentioned problem is a new economic model - circular bioeconomy, which allows the bioconversion of a colossal amount of waste generated in agro-industry.

Unpredictable population growth, massive urbanization and industrialization, as well as a high-protein diet, have all caused protein consumption to an exponential increase in recent years. Experts anticipate that pressure from demographic, societal, and economic reasons will lead to protein consumption to decline even further in the near future. Conflicts around the world, climate change, increased food prices, poor nutrition, a lack of protein, and micronutrients, among other factors, will make the problem of food shortage worse [1],[2],[3]. Based on this, the global scientific community is working intensively to find alternate solutions to the food scarcity. Nowadays, one of the most convenient, safe, and rapidly implemented technologies for replenishing food protein deficiency is the biotransformation of cellulose-containing raw materials (agricultural and food industry wastes) into protein-rich biomass and various valuable secondary metabolites of microbial origin [4].

Filamentous fungi attract special attention among the mass producers of valuable biologically active compounds of plant origin [5]. This group of microorganisms is regarded as an efficient producer of protein and physiologically active compounds due to their high ability to penetrate the substrate via their mycelium, the ability to synthesize some essential amino acids, semi-unsaturated linoleic and linolenic acids, various vitamins, and polysaccharides with pharmaceutical properties. In addition, they are highly valued in terms of nutrition and palatability [6],[7],[8].

Functional food products, rich in bioactive substances (proteins, lipids, vitamins, etc.) are especially demandable and popular at the market. Because of its easy reach and low cost the waste of apple juice industry, produced in thousand tons in Georgia, may be regarded as a perspective raw material for functional food industry. Together with the utilization of the waste, food supplements rich in protein and biologically active compounds may be prepared by the microbial conversion of the mentioned waste, which would make the industry even more profitable [9],[10].[11].

The microscopic fungus *Fusarium venenatum* is regarded as the most effective producer of commercial mycoprotein today. Due to its fast growth, filamentous structure, high protein content in biomass, absence of pigments, odors and toxins the fungus is recognized as the best producer of alternative protein.[12],[13][14]

The bioconversion of plant-origin waste with fungi is viewed as a real chance to alleviate the food scarcity and preserve the environment in accordance with all of the aforementioned points. Certainly, in this kind of technology, the industrial strain is crucial [15],[16]. Therefore, it is especially critical to find a potential perfect producer, optimize its cultivation conditions and develop the technological bases for the production feed additive [17],[18].

To reveal an active protein producer among the fungi of the genus *Fusarium* in the collection of microscopic fungi of the Durmishidze Institute of Biochemistry and Biotechnology of the Agrarian University of Georgia and bioconversion of apple juice industry waste into mycoprotein-rich biomass and other bioactive compounds with the selected strain, was the aim of the presented research.

II. RESEARCH OBJECT AND METHODS

2.1 Object of the study:

The non-pathogenic and non-toxic strains of the genus ***Fusarium*** from the mycelial fungi collection of the Durmishidze Institute of Biochemistry and Biotechnology of the Agrarian University of Georgia was the object of the study.

The residue of Georgian apple juice production (AJPW) was used as a biotransformation substrate.

2.2 fermentation of the AJPW:

The submerged fermentation of the AJPW (before the optimization of the composition of the nutrient medium) was carried out on a shaker, 180/rpm, at 30ºC, for 10 days. For this purpose 6 g of the absolute dry (ABW) waste(*Cole-Parmer® LB-200 Analytical Balances)*, ground in a laboratory mill (*RRH-A1000,High-speed Multi-function Comminutor*) into small particles (0.4-0.5 mm) was weighed and placed in 250 ml Erlenmeyer conical flasks. It was added 50 ml. of the following composition nutrient medium (g/l): $NaNO_3$- 3.0; KH_2PO_4 - 1.0; $MgSO_4$x7H_2O - 0.5; $FeSO_4$x7H_2O-0.02; the pH of the medium was adjusted to 6.5 with 0.5% solution of NAOH (*pH-meter MT30266628, FP20*. Flasks were sterilized by autoclaving at 1 atm. for 45 minutes. Cooled flasks were inoculated with 2 ml of fungal spore suspension. After the cultivation was completed, the content of the flasks was centrifuged for 5 min, 4000 rpm; the sediment was placed in a beaker brought to constant weight, and dried in a thermostat at 105ºC to a constant weight.

2.3 Determination of biomass composition:

The nutritional value of biomass was determined by modern standard methods: crude protein content according to ISO-5983-2: 2009/2016; crude fat - GOST 5668-68; humidity -GOST 13496 3-92; sodium - EN1551102017; magnesium - EN1551102017; zinc - EN 1551102017; phosphorus - EN 1551102017; ash by GOST 13979 6-69; potassium - EN 1551102017. The content of soluble sugars was determined by spectrophotometric method, using 3,5-*dinitrosalicylic acid* (Spectrophotometer SPECOL 11, Carl Zeiss, Germany. Total antioxidant activity was determined using diphenylpicrylhydrazyl (DPPH) [19].

2.4 Optimization of growing conditions and the composition of a nutrient medium:

Optimization of the nutrient medium composition and cultivation conditions of the selected strain was carried out according to the standard approach [20]. The cultivation conditions in which the producer accumulated the maximum amount of protein were considered optimal.

Reagents used. In the experiment, reagents from Sigma-Aldrich were used.

III. RESULTS AND DISCUSSION

High protein content in the biomass and fast growth of the strain are considered the main parameters in the selection of the industrial producer of mycoprotein [21]. Screening among the fungi of the genus Fusarium from the collection of microscopic fungi of Durmishidze Institute of Biochemistry and Biotechnology of the Georgian Agrarian University under the conditions of AJPW submerged fermentation was conducted, in order to select an effective agent for the bioconversion of AJPW. The strain Fusarium spp. S2 was found to accumulate the maximum amount of protein in the biomass on the eighth day of cultivation (Table 1). That is why it was considered appropriate to use this strain for further research.

TABLE 1
Screening of the protein active producers among the microscopic fungi of the genus Fusarium under the submerged fermentation conditions of AJPW (experimental conditions: temperature of cultivation 30°C, concentration of the substrate – 6g; pH of the nutrient medium – 6,5; duration of the fermentation – eight days)

Strain of the microscopic fungus	Content of a true protein, %
Fusarium spp. NP-7	4.7±0.8
Fusarium spp. A6	5.6±0.8
Fusarium spp. S2	6.4±1.0
Fusarium spp. S12	4,2±0.7
Fusarium spp. M11	3,8±0.4
Fusarium spp. M28	3,9±0.4
Fusarium spp. D18	3.8±0. 4

It is known that the growth and development of microorganisms significantly depends on the strain cultivation conditions; in particular, on pH, duration of cultivation and temperature. The optimization of cultivation conditions of *Fusarium* spp. S2 was started by determining the optimal duration of cultivation. For this purpose, the dynamics of protein accumulation by the selected strain was studied, depending on the duration of cultivation.

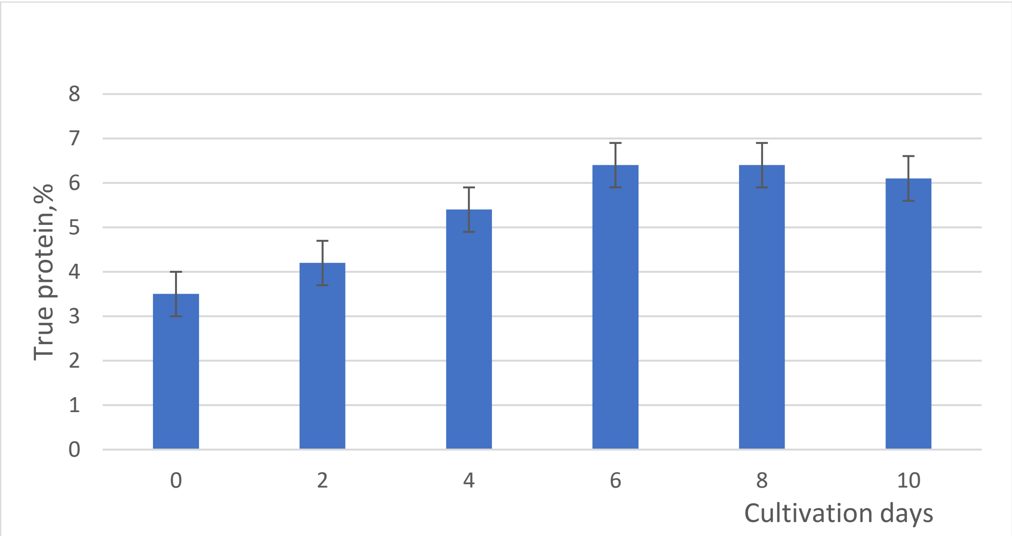

FIGURE 1: The dynamics of protein accumulation under the submerged cultivation conditions of *Fusarium* spp. S2 on AJPW (experimental conditions: cultivation T - 30°C; substrate concentration - 6g; nutrient medium pH - 6.5).

From the Fig.1 is clear that under the submerged fermentation of AJPW *Fusarium* spp. S2 accumulates maximum amount of protein on the sixth day; that is why it was considered appropriate to conduct cultivation of the strain during six days in further experiments.

To determine the optimal temperature of cultivation of *Fusarium* spp. S2 the submerged fermentation of the AJPW was conducted in wide range of temperature – 25ºC – 50ºC (with intervals of 5ºC).

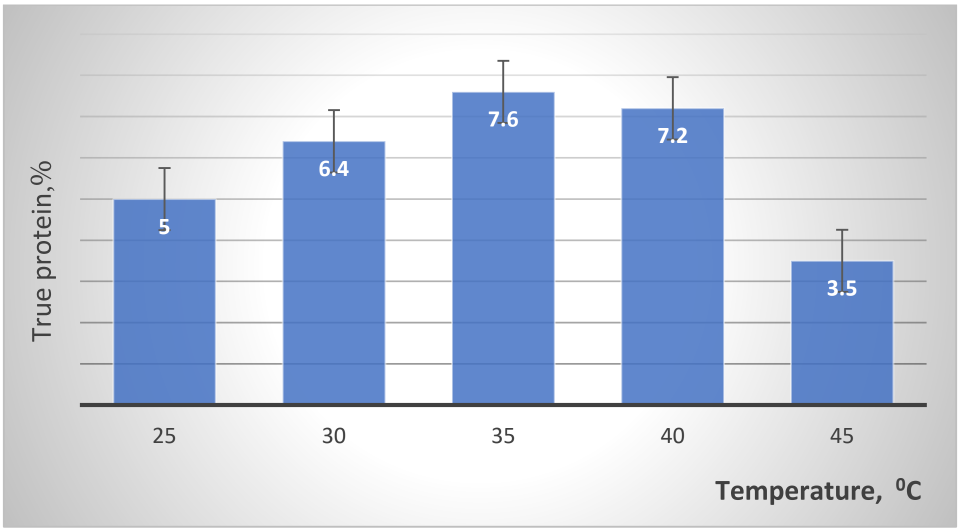

FIGURE 2: Influence of the cultivating temperature on the growth and development of *Fusarium* spp. S2 (experimental conditions: concentration of the substrate – 6g; pH of the nutrient medium – 6,5; duration of the fermentation – six days, temperature range 30ºC – 50ºC)

Fig. 2 demonstrates that *Fusarium* spp. S2 is a mesophylle and prefers cultivation at 35**ºC** that is why for further investigations this temperature was regarded as the optimal for substrate bioconvertion.

One of the significant parameters, which essentially affect the metabolic activity of microorganisms, is pH of the nutrient medium [18]. Accordingly at the next stage of the experiment the influence of pH of the nutrient medium on growth and development of *Fusarium* spp. S2, as well as on protein accumulation in the biomass was studied.

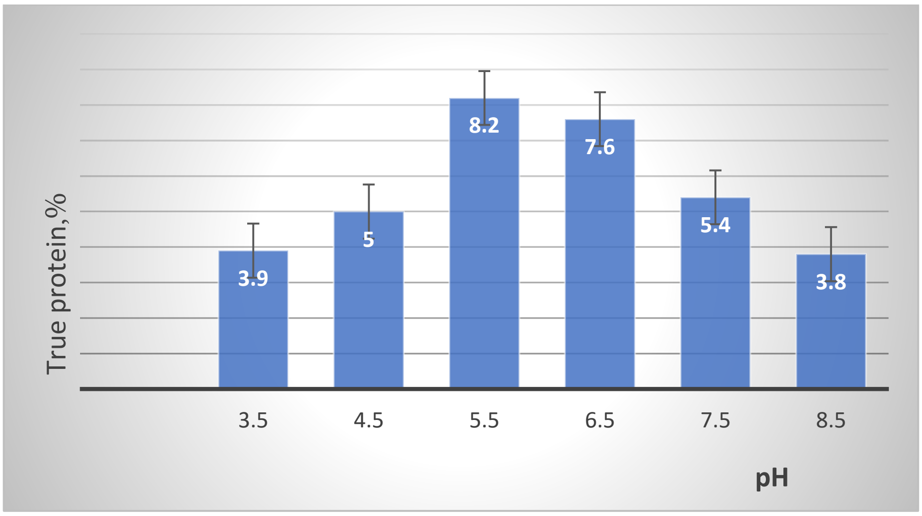

FIGURE 3: Influence of the initial pH of the nutrient medium on protein accumulation in the biomass of *Fusarium* spp. S2 (experimental conditions: concentration of the substrate – 6g; duration of the fermentation – six days, temperature 35ºC pH of the nutrient medium – from 3,5 till 8,5 (with 1,0 intervals))

From the Fig. 3 is evident that during the submerged fermentation on AJPW *Fusarium* spp. S2 accumulated maximum amount of protein (8,2%) when the initial pH of the fermentation area was 5,5. Accordingly it was decided to proceed submerged fermentation of AJPW with *Fusarium* spp. S2 under pH 5,5 conditions.

It is well known that the efficiency of the bioconversion of plant origin substrate significantly depends on the nature and concentration of carbon source. That is why on the next stage of experiment the optimal concentration of AJPW – the bioconversion substrate - was determined. For this purpose different concentrations of the substrate (2, 4, 6, and 8grams) were taken for the submerged fermentation.

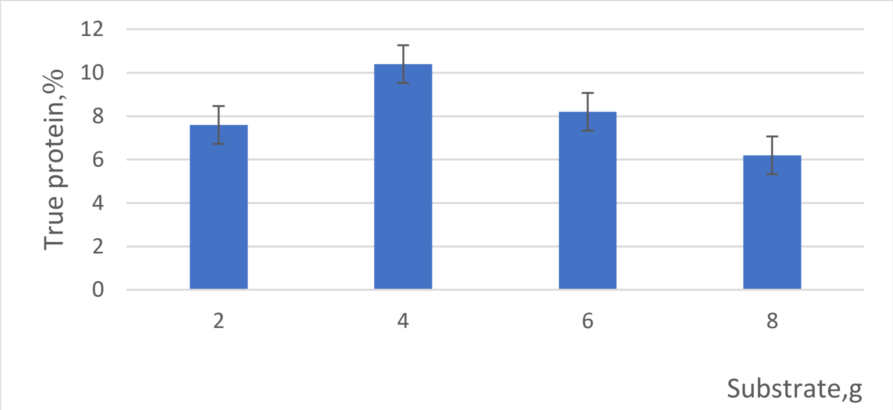

FIGURE 4: Influence of the different concentrations of carbon source on the protein accumulation in *Fusarium* spp. S2 biomass (experimental conditions: concentration of the substrate – 2, 4, 6 and 8g; duration of the fermentation – six days, temperature 35ºC pH of the nutrient medium – 5,5)

From the Fig. 4 is clear that *Fusarium* spp. S2 accumulates maximum amount of protein while cultivated on 4g of the substrate. That is why in further experiments the submerged cultivation of *Fusarium* spp. S2 was conducted on 4g of the substrate.

For the purpose to increase the content of protein in the biomass selection of the optimal source of nitrogen for *Fusarium* spp. S2 was aimed on the next step of study.The selected strain was cultivated on peptone, yeast and malt extract containing nutrient mediums with different sources of nitrogen – $NaNO_3$, KNO_3, NH_4NO_3 and $(NH_4)_2SO_4$.

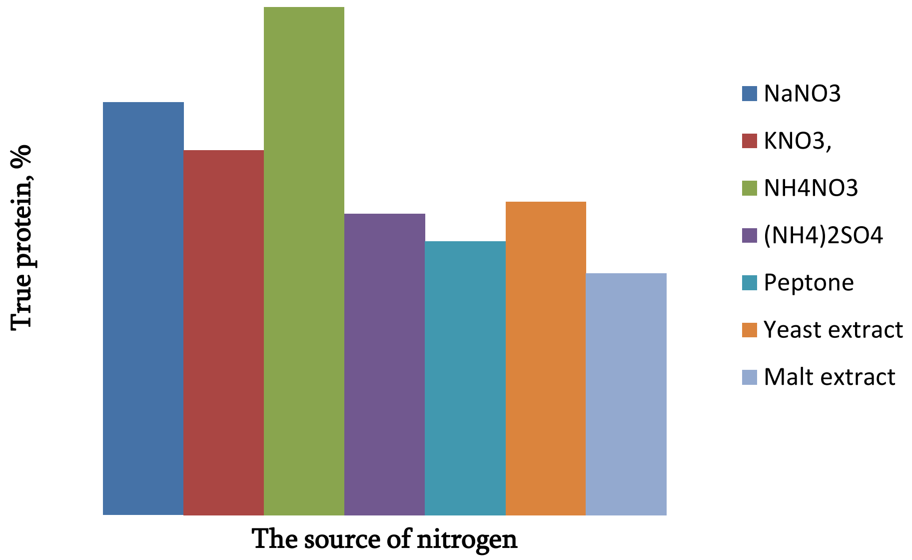

FIGURE 5: Content of true protein in *Fusarium* spp. S2 biomass following additional sources of nitrogen (experimental conditions: concentration of the substrate 4g; duration of the fermentation – six days, temperature 35ºC pH of the nutrient medium – 5,5; organic and inorganic sources of nitrogen with concentration 108g/4g of substrate)

As it is clear from the Fig. 5 *Fusarium* spp. S2 accumulates maximum amount of protein when NH_4NO_3 is used as an additional source of nitrogen in the nutrient medium. That is why this salt was used in further experiments.

Determining the optimal concentration of NH_4NO_3 was the last step in the optimization of *Fusarium* spp. S2 cultivating conditions.

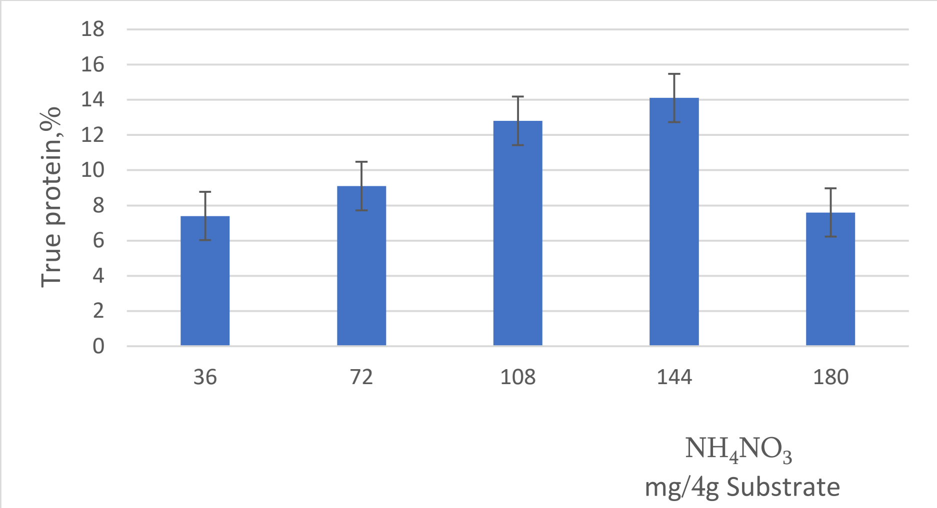

FIGURE 6: Influence of different concentrations of nitrogen source of protein content in *Fusarium* spp. S2 biomass (experimental conditions: concentration of the substrate 4g; duration of the fermentation – six days, temperature 35ºC pH of the nutrient medium – 5,5; NH_4NO_3 concentrations: 36mg, 72mg, 108mg, 144mg and 180mg per 4g of the substrate)

Fig. 6 clearly demonstrates that the fungal strain accumulated maximum amount of protein when concentration of NH_4NO_3 in the nutrient medium was 144mg per 4g of substrate.

Thus, according to series of subsequent experiments on the base of bioconversion of AJPW by means of *Fusarium* spp. S2 was obtained enriched with mycoproteins, easily digestible biomass, where the content of protein was 4times higher, compared to the initial substrate. The nutrient value of the prepared potential food additive was determined at the last step of experiment.

TABLE 2
Content of micro and macro elements, true protein and crude fat in AJPW bioconversion (with *Fusarium* spp. S2) products (calculated on absolutely dry weight (ADW)

	Components	Content in biomass, ADW
1	Humidity	4,2±0.8
2	Sodium	1,0%±0.2
3	Zinc	11 mg /kg±1.2
4	Phosphorus	0,29%±0.2
5	Ash	3,3%±0.4
6	Magnesium	0,28%±0.02
7	Potassium	0,70%±0.03
8	True protein	14,1%±1.2
9	Crude fat	6,5 %±1.0
10	Total antioxidant activity	65%±1.5

From the Table 2 is clear that the product of microbial conversion contains Na, P, Ca and Mg, as microelements and is rich in Zn as well. The crude fat in the biomass is 6,5%, which is higher than the norm determined for combined feed of egg laying hens. The product has moderate antioxidant activity (60-65%).

The fact that FAO annually implements a number of political activities to improve the market access and investment for these products to enhance market access and investment for these products as well as support the competitiveness of small and medium-sized businesses illustrates the urgency of the production of such alternative sources of protein[22].

According to all above mentioned the biomass obtained as a result of bioconversion of AJPW by means of *Fusarium* spp. S2, may be recommended as a food additive in poultry feed.

IV. CONCLUSIONS

1) Possibility of biotransformation of AJPW into protein- and other biotic compounds rich biomass by means of microscopic fungus *Fusarium spp.* S2 has been established.
2) As a result of the optimization of submerged cultivation conditions and nutrient medium composition of *Fusarium* spp. S2 the true protein content of the biomass obtained from the biotransformation of AJPW increased from 3.5% to 12.8%.
3) Biomass is rich in crude fat, micro- and macro elements, and has antioxidant properties; accordingly it can be recommended for use as a feed additive in poultry diet.

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This article has been retracted

Nutritional and Functional Properties of Oyster Mushroom (*Pleurotus ostreatus*) Based Protein Bar: A Review

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***Abstract*—** *Oyster mushrooms (Pleurotus ostreatus) have emerged as a versatile and nutritionally valuable fungi with significant potential in the food industry. Native to temperate and subtropical regions, these mushrooms have been cultivated globally due to their ease of growth, high yield, and adaptability to diverse agricultural by-products as substrates. Their production involves environmentally sustainable practices, utilizing lignocellulosic waste materials such as straw, sawdust, and husks, thereby contributing to waste reduction and promoting circular agricultural systems. Pleurotus ostreatus is known for its remarkable nutritional profile, including high- quality proteins rich in essential amino acids, dietary fibers, vitamins, and bioactive compounds like β-glucans and polyphenols, which are associated with numerous health benefits such as immune system support, cholesterol regulation, and antioxidant effects. This review highlights the innovative application of oyster mushrooms in the formulation of protein bars, leveraging their functional properties such as binding, texture enhancement, and prolonged shelf stability. The incorporation of these mushrooms into protein bars not only enriches their nutritional value but also aligns with consumer demands for plant-based, nutrient-dense food products. Additionally, the potential of Pleurotus ostreatus in addressing sustainability challenges and catering to modern dietary preferences underscores its relevance in functional food development. By tapping into the nutritional and functional properties of Pleurotus ostreatus, this paper aims to contribute to the advancement of innovative and sustainable food products that align with contemporary health and environmental goals.*

Keywords*— *Oyster Mushroom, Nutritional Properties, Functional Food, Protein Bar.

I. INTRODUCTION

Mushrooms are regarded as functional foods, with health advantages that extend beyond the usual nutrients they contain[1]. However, until the last decade, knowledge of the composition and nutritional worth of culinary mushrooms was limited in comparison to vegetables and medicinal mushroom species. Because culinary mushrooms have always been regarded as a delicacy, their usage in many developed countries has been marginal, making them of little interest to researchers. However, the situation has begun to alter noticeably: the number of original papers published each year is now many times higher than it was 10-15 years ago[2]. Among the abundance of edible mushrooms, the Pleurotus genus is a prolific producer of unique "Mycochemicals"

Pleurotus was initially cultivated in Germany during World War I as a subsistence measure for food preservation, and was the first to document the crop. Today, various species of Pleurotus are cultivated commercially because of their rich mineral content and therapeutic characteristics, short life cycle, reproducibility in the recycling of certain agricultural and industrial wastes, and minimal demand for resources and technology[4]. There is a greater need than ever for food items that meet both sustainable standards and nutritional needs. The oyster mushroom (Pleurotus ostreatus), a diverse fungus with excellent nutritional properties, is one item gaining traction in this space. These mushrooms are simple to grow and thrive on agricultural byproducts such as straw and sawdust, making them an effective tool for encouraging waste recycling and environmentally friendly farming practices.[5],[6] Pleurotus ostreatus is known for its high protein content, essential amino acids, dietary fibers, and bioactive substances such β-glucans and polyphenols.[7] These components not only add nutritional value but also provide a variety of health benefits, such as immunological boosting, cholesterol management, and antioxidant capabilities.[8] Furthermore, oyster mushrooms have functional features such as binding and texture enhancement, making them ideal for use in novel food formulations such as protein bars.[9] Protein bars have been a popular dietary choice among health- conscious consumers due to their convenience, nutrient density, and flexibility to accommodate certain dietary preferences, such as plant-based and environmentally friendly options.[10] However, the use of sustainable ingredients such as oyster mushrooms in protein bars is an underexplored field with enormous potential for innovation. Many studies from around the world have confirmed that the Pleurotus mushroom is highly nutritious and contains a variety of bioactive compounds such as terpenoids, steroids, phenols, alkaloids, lectins, and nucleotides, which have been isolated and identified from the fruit body, mycelium, and culture broth of mushrooms and have shown to have promising biological effects.[11]

II. TAXONOMIC CLASSIFICATION OYSTER MUSHROOM:

- Kingdom : Fungi
- Phylum : Basidiomycota
- Class : Agaricomycetes
- Order : Agaricales
- Family : Pleurotaceae
- Genus : Pleurotus
- Species : P. ostreatus

2.1 Cultivation of oyster mushroom:

Oyster mushrooms (Pleurotus spp.) are grown with simple techniques and sustainable practices that include agricultural byproducts like wheat straw, sawdust, and paddy husks. The process starts with base preparation, in which lignocellulosic materials are chopped, soaked, and pasteurized with hot water or steam to remove impurities. This is followed by spawning, in which fungal mycelium is implanted into sterilized grains under sterile conditions to produce spawn, which is then placed on the substrate in ventilated bags or trays that provide air circulation and moisture for mycelial growth.[12]

During incubation, the base material is kept in a controlled dark and humid environment at temperatures ranging from 20°C to 30°C to ensure effective mycelial colonization. After colonization is complete, the substrate is exposed to light and fresh air to start fruiting, which typically occurs within 15-20 days. Mushrooms are collected by carefully twisting them from the base material. Proper handling preserves the mushrooms' nutritional and functional characteristics, making them acceptable for ingestion or further processing. Oyster mushroom production promotes sustainable agriculture by converting agricultural waste into useful food resources.[13],[14]

2.2 Nutritional Profile of Oyster Mushroom:

Oyster mushrooms are well-known for their outstanding nutritional profile, making them an important functional food. Their balanced composition comprises macronutrients and micronutrients that are beneficial to general health.

2.2.1 Macronutrients:

- **Protein:** Oyster mushrooms are a good source of plant-based protein, with 17-42 g per 100 g of dried mushrooms. They are high in essential amino acids, making them an excellent vegetarian protein source.[15]
- **Carbohydrate:** These mushrooms contain 50-60% carbs, mostly in the form of polysaccharides, which provide dietary fiber and promote intestinal health. Pleurotus eryngii has the greatest carbohydrate content of any species, with around 41 g per 100 g.[16]
- **Fat:** Oyster mushrooms are low in fat and mostly contain linoleic acid. Comparative studies have revealed that lipid content varies by species, with Pleurotus sajor-caju having up to 0.61 g per 100 g.[16]

2.2.2 Micronutrients:

- **Vitamins:** These mushrooms are high in B-complex vitamins, including thiamine, niacin, and folic acid. However, they contain very little vitamin B12.
- **Minerals:** Oyster mushrooms contain vital minerals such as potassium, selenium, zinc, and iron. These minerals are essential for maintaining health, including immunological function and lowering oxidative stress.[17]

2.3 Bioactive Compounds:

Oyster mushrooms contain bioactive compounds such as β-glucans, polyphenols, and antioxidants. These chemicals have been related to a variety of health advantages, including cholesterol management, immune system strengthening, and anti-inflammatory activity.[18]

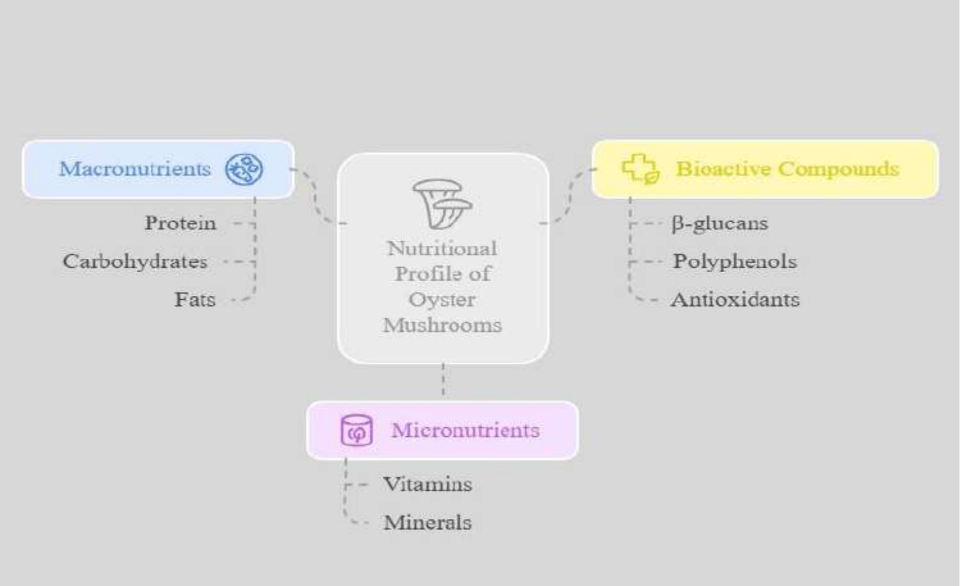

FIGURE 1: Nutritional Profile of Oyster Mushroom

2.4 Functional Profile of Oyster Mushroom:

Oyster mushrooms offer a diverse array of functional properties that contribute to their applications in health, nutrition, and food industries.

2.4.1 Antioxidant Properties:

Oyster mushrooms are rich in bioactive compounds such as ergothioneine, phenolics, and flavonoids, which demonstrate remarkable antioxidant properties. These compounds protect cells by neutralizing harmful free radicals, thereby reducing oxidative stress and preventing cellular damage.[15],[23],[21]

2.4.2 Immunomodulatory Effects:

Polysaccharides like β-glucans found in oyster mushrooms are well-known for their immunomodulatory activity. They enhance immune function by stimulating immune cell activity, including the proliferation and differentiation of lymphocytes, which are key to maintaining a healthy immune system17],[22]

2.4.3 Antimicrobial Properties:

Studies have demonstrated that oyster mushroom extracts exhibit antimicrobial activity against various pathogens. Phenolic compounds and tannins found in these mushrooms contribute to their antibacterial and antifungal effects by disrupting microbial membranes and inhibiting protein synthesis[20]

2.4.4 Prebiotic Potential:

The high dietary fiber content in oyster mushrooms contributes to their prebiotic potential by supporting the growth of beneficial gut microbiota. This not only improves gut health but also enhances overall digestion[16],[18].

2.4.5 Anti-Cancer Properties:

Oyster mushrooms contain bioactive compounds that have demonstrated anti-cancer properties in various studies. Extracts from these mushrooms have shown the ability to suppress tumor growth and inhibit the multiplication of cancer cells. For instance, water extracts of *Pleurotus* species have been effective in reducing the proliferation of MCF-7 human breast cancer cells.[17]

2.4.6 Anti-Inflammatory Effects:

Oyster mushrooms exhibit anti-inflammatory properties that help reduce inflammation under both acute and chronic conditions. Extracts from various *Pleurotus* species have been shown to alleviate inflammatory responses, making them beneficial for managing inflammation- related conditions.[23],[9]

2.4.7 Functional Food Applications:

The functional properties of oyster mushrooms, such as their binding ability and texture- enhancing properties, make them ideal for use in innovative food formulations. They are increasingly incorporated into functional foods like protein bars, meat substitutes, and dietary supplements to meet modern health and sustainability standards[9],[10]

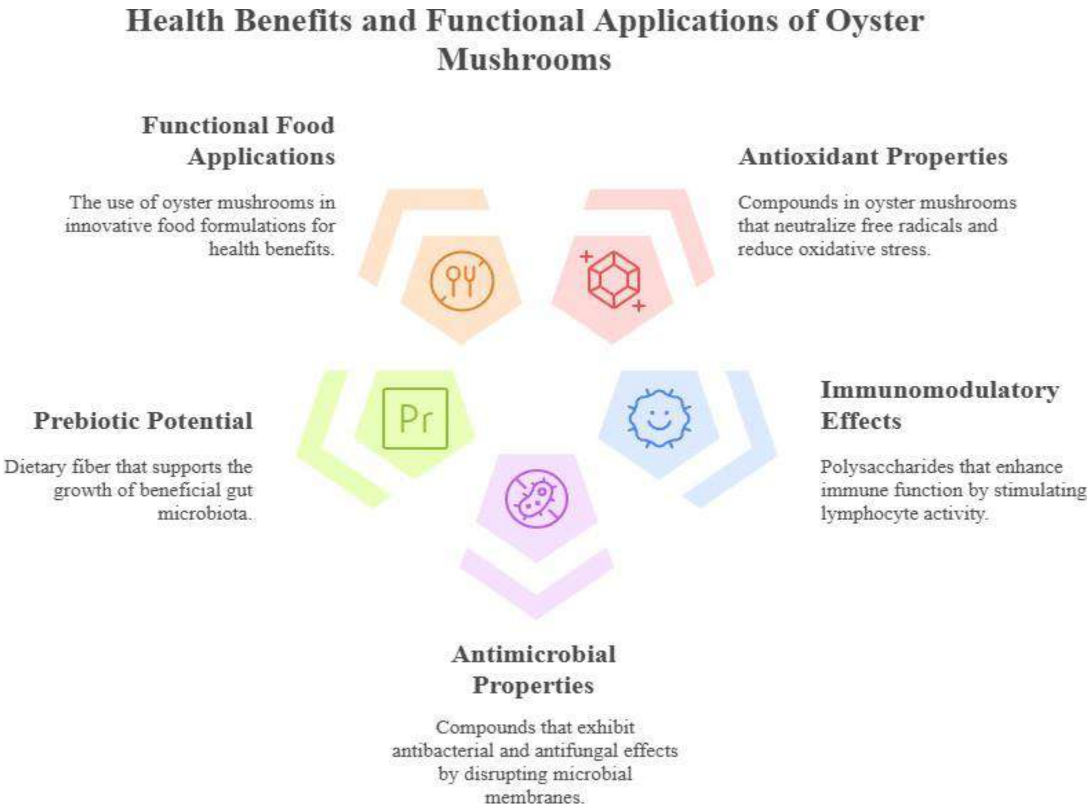

FIGURE 2: Health Benefits of Functional Applications of Oyster Mushroom

2.5 Uses of oyster mushroom:

Oyster mushrooms (Pleurotus spp.) have many uses in the food sector due to their exceptional nutritional and functional qualities. These mushrooms are commonly used as natural flavor enhancers, particularly in vegetarian and vegan meals, because their umami flavor mimics the savory taste of meat. This makes them a necessary component of plant-based substitutes such vegan burgers and sausages[24]. Furthermore, oyster mushrooms are used to boost the nutritional value of a variety of food products, including energy bars, cereals, and supplements, by offering a high concentration of protein, vitamins, and minerals[19]. Their functional properties, such as binding and texture- enhancing abilities, enable their incorporation into innovative food formulations like protein bars and fortified snacks, catering to modern consumer demands for healthy and sustainable food options[9].

Furthermore, oyster mushrooms have antibacterial qualities derived from bioactive chemicals, making them an important element in food preservation. These chemicals prevent the growth of spoilage microorganisms, increasing the nutritional value of both fresh and processed foods [20]. The food sector also uses oyster mushroom fermentation to produce enzymes, organic acids, and bioactive peptides that improve the sensory and nutritional quality of fermented foods and beverages [24]. Overall, oyster mushrooms' diversity in terms of flavor, nutrition, preservation, and functionality demonstrates their critical role in developing food science and addressing the growing demand for health- conscious and environmentally friendly foods.

2.6 Effective uses of oyster mushroom:

Flavor Enhancement	Oyster mushrooms provide a natural umami flavor, making them ideal for vegetarian and vegan meals. They are commonly used in plant-based substitutes like vegan burgers and sausages.[24]
Nutritional Value	Rich in protein, vitamins, and minerals, oyster mushrooms enhance the nutritional value of food products such as energy bars, cereals, and supplements.[19]
Functional Properties	Their binding and texture-enhancing abilities allow their incorporation into protein bars and fortified snacks, aligning with modern health and sustainability trends[9].
Antibacterial Properties	Bioactive compounds in oyster mushrooms inhibit spoilage microorganisms, aiding in food preservation and maintaining nutritional value[20].
Fermentation Applications	Used in fermentation to produce enzymes, organic acids, and bioactive peptides, which improve the sensory and nutritional quality of fermented foods and beverages.[24]
Overall Role in Food Science	Oyster mushrooms contribute to food innovation by enhancing flavor, nutrition, preservation, and functionality, supporting the demand for health-conscious and eco-friendly foods.

III. CONCLUSION

Oyster mushrooms (Pleurotus spp.) are well-known for their nutritional value, functional characteristics, and multiple uses in the food sector. They include high-quality protein, necessary vitamins, minerals, and bioactive substances, making them perfect for health- conscious individuals and vegans. Their minimal fat and calorie content make them ideal for vegans. Oyster mushrooms have significant antioxidant and anti-inflammatory properties, making them useful for food preservation. They include prebiotic fibers, which enhance gut health and have potential medicinal applications such as immunomodulation, anticancer, and cholesterol-lowering properties. Oyster mushrooms are utilized in plant-based foods, meat substitutes, energy bars, morning cereals, dietary supplements, protein bars, and fortified snacks. They also contribute to fermentation by creating enzymes, organic acids, and bioactive peptides. Oyster mushrooms are a sustainable and environmentally favorable food innovation alternative, surviving on agricultural byproducts and helping to recycle trash. Their variety, health benefits, and environmental sustainability make them essential ingredients in today's diets.

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The Effects of Different Seed Priming Chemicals on Germination and Seedling Growth Rate of Maize (*Zea mays L.*) in Lesotho

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Abstract**—** *Maize is grown widely in Lesotho as a staple crop for the nation and is accepted in cultural dishes. Its productivity is low necessitating importation from neighboring South Africa. Low productivity is attributed to low germination rate and poor stand in the field. The objective of the study is to identify the most potent chemical compounds that enhance high germination and seedling growth rate of maize. The study was conducted at the National University of Lesotho domiciled in Maseru, Lesotho. The experiment was performed in the laboratory of Department of Crop Science. Complete Randomized Design with PANNAR Seed Company maize cultivar of PAN 12 and four priming chemical compounds varied in amounts and duration immersed in the solutions. Chemical compounds were Indole Butyric Acid, Calcium chloride, Sodium chloride and Copper sulphate, while distilled water was control. Seed germination and seedlings parameters measured were germination rate, germination percentage, germination index, radical length, plumule length, plumule fresh weight and dry weight, radical fresh weight and dry weight, and coleoptile length. Data from parameters were subjected to perform Analysis of Variance and least significant differences. Results showed no significant difference among concentrations of Indole Butyric Acid on all parameters. Three other chemical compounds revealed significant to highly significant differences. Priming maize seed with Calcium chloride for a duration of 18hrs to 24hrs increased seed germination and seedling growth rate significantly. Similarly, varying concentrations of Calcium chloride compound used showed significantly different responses on measured parameters of maize seed. Moreover, results on copper sulphate indicated that the higher the concentration of copper sulphate, the lower the maize seed germination and seedling growth rate. In conclusion, maize seed primed with water generated better results compared Sodium chloride, Calcium chloride and Copper sulphate based on sequence of potency.*

Keywords**— Maize, PAN 12, seed germination, seedling growth rate, seed priming, Lesotho.**

I. INTRODUCTION

Lesotho is among the nations with inhabitants consuming maize as a staple food prepared in various dishes. Almost all farming households grow maize for both home consumption and animal feeding. Morojele and Sekoli (2016) [1] state that maize is a major staple crop in Lesotho as evidenced by number of farmers (195,958) involved in its production, proportion of area under which it is grown (146,313 ha) and the rate at which it is consumed (266,755 metric tons) [2]. Maize ranks first among cereal crops grown in Lesotho, followed by sorghum, wheat and beans [3]. It is cultivated across all agro-ecological zones of Lesotho, namely; low-lands, foot-hills, mountains and Orange River valley. Among the four ecological zones of Lesotho, lowlands account for the highest maize production reaching 15, 800 tons on 118 586 hectares. The lowest production is in the Orange River Valley where 1,475 tons is achieved on 2,757 hectares (2021). Foothills and mountain zones are at par. In the lowlands, arable area put under maize is larger than in the other zones because of both suitable climatic and edaphic conditions prevailing and a long growing season, from October to December. In the foothills and mountain zones, planting can only commence in September and terminate in November due to early chilling and freezing injuries that the crop suffers [3]. The Orange River Valley is prone to drought and is very hot and dry in summer resulting in maize crops being adversely affected in terms of growth, development and grain yield [1], [4].

The Basotho nation perceives maize as one of the crops contributing significantly to the household economy, particularly when all farmers consume it and sell the surplus to community members in the neighborhood generating income for purchasing other household needs [5], [6]. The importance of maize to the Basotho nation cannot be over-emphasized as a main source of nutrients particularly energy and other elements. Nutritionally, carbohydrates (70-87 %), proteins (6-13 %), fiber (7%), oil (2-6 %), vitamin B and minerals constitute maize grain [7], [8]. For maize to be a balanced diet, it is complemented by the common bean, which has essential amino acids devoid in maize such as lysine. Lysine plays a critical role in post-translational modifications facilitated by enzymes Lysol hydroxylase and Lysol oxidase, which are directly involved in the synthesis and maturation of collagens [9]. Farmers cook and prepare maize with various forms of relish such as immature pods of beans and peas, pumpkin, indigenous and exotic vegetables. Besides maize being consumed by Basotho, it is also used to feed domestic animals as grains or plant residues during the winter, when feed resources are very scarce [1]. The economic importance of maize in Lesotho cannot be over-emphasized as afore-mentioned necessitating a boast in productivity, which will translate into increased national production. It is therefore imperative to identify factors that determine the optimum growth rate, good standability in the field, high grain yield and quality of maize crops in Lesotho.

These factors when applied accordingly will enable Lesotho to meet its requirement for maize and export surplus elsewhere. According to Anazco *et al.*, (2023) [9], the primary causes of low productivity and poor standability are low seed germination rate and emergence of weak seedling above the soil surface. Reed *et al.*, (2022) highlighted that seed germination and quality tests are performed in the laboratory before the seed is released to the farmers for sale with a germination percentage of 85%, which must be declared officially on the label. In Lesotho, such seeds with declared germination percentages result in low germination rates and weak seedling emergence above the soil surface in the field. The low germination emergence rate is attributed to the time elapsed from planting to emergence above the soil surface. Longer elapsed period results in low germination, which in turn culminate into poor crop stand. It is only through seed priming that germination rate and period can be enhanced, which triggers germination mechanism and processes [10], [11]. Seed priming is crucial as the main source of water for germinating seed and producing stronger seedlings where soil moisture is inadequate to initiate a germination process [12]. Insufficient soil moisture can contribute to delay or uneven germination, resulting in poor seedling establishment and crop yield [13]. Several studies validate the notion that when maize seeds are soaked in water before sowing, germination is enhanced, thus such practice increases the chances of growing faster than when such a method is not applied at all [14], [15]. Besides, many researchers discovered that certain chemical compounds are more potent in enhancing germination than pure distilled water when proper procedure is followed and modified accordingly [16], [17]. The seed priming methods such as hydro-priming, halo-priming, osmo-priming and solid matrix priming were introduced and adopted to enhance seed germination elsewhere [18], [19]. Hence, the study explored the effects of different seed priming chemicals on the germination and seedling growth rate of maize (*Zea Mays L.*). Specific objective of the study was to (i) identify the most potent chemical compounds that enhance high germination and seedling growth rate of maize.

II. MATERIALS AND METHODS

2.1 Study Area:

The study was conducted in the National University of Lesotho, Faculty of Agriculture, in the Department of Crop Science at the Roma Valley. The National University of Lesotho is domiciled approximately 34km south east of Maseru, the capital town of Lesotho. The coordinates of the University are 29^0 26’ 48 South latitude and 27^0 42’ 29 East longitudes with an altitude of 1,610m above sea level. The experiment was undertaken in the laboratory thereof.

2.2 Experimental Design:

Maize seed cultivar of PAN 12 bred by PANNAR Seed Company was obtained in the Department of Crop Science at the National University of Lesotho. This cultivar was the only one used for an experiment. Completely Randomized Design with three replications of four treatments was applied. Four seed-priming chemical compounds (treatments) were used to determine their potential to enhance seed germination, each tested at different concentration levels. The compounds were Indole butyric acid (IBA), 1% Calcium chloride ($CaCl_2$), Sodium chloride (NaCl) and copper sulphate ($CuSO_4$) having different volumes of distilled water such as 100ml and 250ml respectively, and distilled water was used as a control. Healthy maize seeds were collected and sterilized for 5 minutes using 2% sodium hypochlorite and washed thoroughly with distilled water. A total number of 570 seeds of maize were placed in 57 Petri dishes, each bowl containing 10 maize seeds. PAN 12 cultivar of maize seeds commonly used by farmers was used for this study. Three replications were used for priming chemical compounds. Several methods mentioned were applied below following procedures by respective authorities.

2.3 Priming with Distilled Water (H2O) (Hydro-priming):

2.3.1 Procedure:

The procedure described by Forti *et al*., (2020) and Heydecker (1973) [20], [21] for seed priming with distilled water was adopted in this study. The total number of 570 seeds were soaked with distilled water for 24 hours, after which were dried with a paper towel and placed in petri dishes. Within each petri dish, 10 maize seeds were placed and transferred to the growth chamber set at 24^0C. The light intensity of 61.2 lux was provided both day and night to provide consistency; particularly where seeds require light to trigger germination through phytochromes, with 70-80% relative humidity. Distilled water was applied regularly to the seeds until the seeds displayed sprouting. After 14 days, all parameters such as germination percentage, germination index, coleoptile, plumule length, radicle length, plumule fresh weight, radicle fresh weight, plumule dry weight, and radicle dry weight measurements were recorded.

2.4 Priming with Indole butyric acid:

2.4.1 Procedure:

The procedure outlined by Banerjee and Roychoudhury (2018) [22] for seed priming with indole butyric acid was adopted in this study. Indole Butyric Acid of the following magnitudes were weighed; 0, 37.5mg, 75mg and 112,5mg, and after which they were transferred to individual 1000ml volumetric flasks. An amount of 200ml distilled water was added to each flask and mixed thoroughly. A magnetic sterilizer was used to mix butyric acid until dissolved, and then distilled water was added to the top mark of a volumetric flask [23]. Seeds were then placed in Petri dishes according to the sample size (10 seeds per petri dish), four concentrations, and three replications of each concentration. A set of 12 petri dishes containing 10 seeds were soaked with different concentrations of Indole Butyric Acid (0, 37.5mg, 75mg, and 112,5mg) for 24 hours. After which they were removed, dried and put back to their respective petri dishes. The petri dishes were transferred to the growth chamber set at 25ºC with a relative humidity of 70-80% and the light intensity was 61.2 lux. The seeds were watered with the distilled water every day until day 7. The seed germination was recorded daily at a certain time for 7 days. After 7 days, the coleoptile length of all germinated seeds was measured followed by other parameters [23].

2.5 Priming with Calcium Chloride (CaCl2) (Osmo-priming):

2.5.1 Procedure:

The method adopted followed that of Di Girolamo and Barbanti (2019) [24] for seed priming with Calcium chloride whereby 1% calcium solution and 10g calcium salt were placed in a measuring flask with a volume made up to 1000ml with distilled water. Distilled water was utilized to wash selected seeds, while subsequently dipping them into sodium hypochlorite 0.05% solution for 5 minutes for surface sterilization. Seeds were then dried at room temperature up to moisture content <10% on a dry weight basis of the seeds following standard conditions for seed storage [25]. Seeds were immersed in distilled water to regulate hydro priming and halo priming (1% $CaCl_2$) each for 12, 18, 24 and 30hrs respectively, as per treatments put separately at room temperature. Ten seeds were placed in petri dishes of double layers of Whatman no.5 filter paper, moistened with 5ml distilled water for up to 7 days. Each priming treatment was replicated three times in a completely randomized design (CRD). Then petri dishes were transferred into the growth chamber under controlled conditions set at 25^0 C with 60% relative humidity and 61.2 lux as light intensity. Upon germination, seedlings were counted for two weeks. The number of normal seedlings was recorded according to the international seed testing agency [26]. Other parameters such as germination percentage, germination index, radical and plumule length, fresh and dry weight of radical and plumule finally the Coleoptile were recorded.

2.6 Priming with Sodium Chloride (NaCl) (Osmo-Priming):

2.6.1 Procedure:

The procedure developed by Rajpar *et al.* (2006) and Toklu *et al*. (2015) [27], [28] for seed priming using sodium chloride was also adopted in this study. The following amounts of Sodium chloride were weighed (2, 5, 10, and 15g) to prepare different concentrations. Each concentration was placed into a measuring flask and the volume was made up to 1000ml with distilled water. Seeds were then sterilized by dipping maize seeds into sodium hypochlorite 0.05% solution for 5min, removed, and rinsed thoroughly in distilled water. Seeds were soaked in 200ml of each of the concentrations for 24 hours, removed and rinsed with distilled water. The sample size of 10 seeds was planted on a moistened filter paper in each of the already labeled petri dishes. Each treatment was replicated three times and then transferred into a growth chamber at 25^0C temperature with 80% relative humidity and a light intensity of 61.2 lux. The germination count was taken daily for five days, while other parameters were germination percentage, germination index, radical and plumule length, fresh and dry weight of radical and plumule finally the Coleoptile were also recorded.

2.7 Priming with Copper sulphate (CuSo4) (Osmo-priming):

2.7.1 Procedure:

The method followed was explained by Rajpar *et al*. (2006) and Toklu *et al*. (2015) [27], [28] for priming maize seeds using Copper sulphate. The following amounts of copper sulphate were prepared to develop different concentrations (20, 40, 60, and 80g). Each concentration was placed into a measuring flask and volume was made up to 100ml with distilled water. Seeds were then sterilized by dipping maize seeds into sodium hypochlorite 0.05% solution for (5min), removed and rinsed thoroughly in distilled water. Seeds were soaked in each concentration for 24 hours, removed and rinsed with distilled water. The sample size of 10 seeds was planted on a moistened filter paper in each of the already labeled petri dishes. Each concentration was replicated three times and then transferred into a growth chamber set at 25^0C temperature with 70-80% relative humidity and a light intensity of 61.2 lux. The germination count was taken daily for five days, while other parameters as afore-mentioned were measured for five days after the germination.

2.8 Data collection:

The following seed germination and seedlings parameters were measured; germination rate was measured from day 1 after planting until day 14. Other parameters such as germination percentage, germination index, radical length, plumule length, plumule fresh weight, dry weight, radical fresh weight, dry weight, and Coleoptile length depended on different methods adopted.

2.9 Data Analysis:

Data collected from the four procedures were separately analyzed using SPSS software (version 21) and subjected to ANOVA to establish the differences among the seed priming chemical compounds, after which mean separation using the least significant difference was performed at P<0.01 and P<0.05.

III. RESULTS AND DISCUSSIONS

3.1 Calcium Chloride as a Seed Primer:

Analysis of variance depicted in Table 1 showed a highly significant difference (P<0.01) among Calcium chloride priming durations on maize cultivar and the reaction of maize (PAN 12) evaluated for germination percentage, plumule fresh weight and radical fresh weight. A significant difference (P<0.05) was observed for germination index and radical dry weight. No significant difference was obtained for coleoptile length, plumule length and plumule dry weight.

TABLE 1
SUMMARIZED ANALYSIS OF VARIANCE FOR SEED GERMINATION PARAMETERS PRIMED WITH $CaCl_2$

Source of Variation	Df	Mean Square								
		Germination %	Germination index	Coleoptile length	Plumule length	Radicle length	Plumule fresh weight	Radicle fresh weight	Plumule dry weight	Radicle dry weight
Treatment	7	703.14**	7.54*	909.99	14149.91**	1604.95	2.35**	1.34**	0.51	0.01*
Error	16	126.83	1.50	555.42	3512.29	1011.29	0.58	0.10	0.01	0.03
Total	23	6951.33	76.81	15256.63	155246.00	2741.33	25.67	10.96	0.58	0.12

*****High Significant (P<0.01), *Significant (P<0.05)***

Table 2 showed that the highest germination percentages were 96% and 93% obtained from control (distilled water) at 24hrs and 18hrs durations, respectively. The germination percentage of 93% at 18hrs was the same as calcium chloride primed for 12hrs. The lowest germination percentage was 50% found on a control where seeds were soaked for 30 hours. The highest germination index of 7.9333 was expressed by control where seeds were primed for 24hrs, while the lowest germination seed index was 2.7333 found at 30hrs on a control. The germination index showed a grand mean of 4.9167. Coleoptile measured the longest length of 102.333mm, followed by 85mm when immersed in $Cacl_2$ for 18 hours. The shortest coleoptile length was

49.333mm on control soaked for 30hrs. The grand mean of coleoptile length stood at 72.1250mm. The longest plumule length was 334.6667mm where seeds were placed in $Cacl_2$ for 24 hours, while the shortest length was 139.3333mm obtained from control soaked for 12 hours. The grand mean for plumule length was 207mm captured from a control. Radicle length revealed a grand mean of 73.6667mm. The longest radical length was 110.6667mm obtained on duration of 24hrs, while the shortest radicle length was 53.6667mm found on control in 30hrs. Plumule fresh weight for calcium chloride had a grand mean of 2.8958, with the highest weight of 4.1667g and the lowest weight of 1.8000g obtained on a control for 24hrs and at $Cacl_2$ primed for 30hrs same as 30hrs on a control, respectively. The highest radicle fresh weight was 2.2333g on a control soaked for 24 hours. The grand mean of radicle fresh weight was 0.8500g. The lower radicle fresh weight found on $Cacl_2$ soaked for 30 hours was 0.2333g. The grand mean of plumule dry weight grand mean was detected to be 0.4417g with the highest weight of 0.6667g, and the lowest weight being 0.2667g, which were all obtained from the control within 24hrs and 30hrs, respectively. Finally, the radicle dry weight grand mean was 0.1083g. At 24hrs, on a control, 0.2333g showed as the highest weight, whereas at 30hrs of 1% $Cacl_2$, the lowest weight was 0.0333g.

TABLE 2
MEANS FOR $CaCl_2$ DURATIONS

Duration	Germination %	Germination Index	Coleoptile	Plumule Length	Radicle Length	Plumule Fresh Weight	Radicle Fresh Weight	Plumule Dry Weight	Radicle Dry Weight
12hrs	93.33	5.37	67.00	205.00	66.33	3.70	.63	.57	.10
18hrs	86.67	5.37	85.00	277.67	103.00	3.40	.70	.50	.10
24hrs	76.67	5.77	83.00	214.00	66.00	3.23	.77	.43	.10
30hrs	76.00	3.27	73.00	148.00	46.67	1.80	.23	.30	.03
0.12hrs	90.00	4.73	61.67	139.33	60.00	2.23	.43	.40	.10
0.18hrs	93.33	4.83	55.67	187.00	83.00	2.83	1.43	.40	.13
0.24hrs	96.66	7.93	102.33	334.67	110.67	4.17	2.23	.67	.23
0.30hrs	50.00	2.73	49.33	150.33	53.67	1.80	.37	.27	.07
Grand mean	82.83	4.92	72.13	207.00	73.67	2.8958	.85	.44	.11
CV	13.60	24.91	32.68	28.63	43.17	26.25	37.28	25.73	49.85
LSD	19.49	2.12	40.79	102.58	55.04	1.32	0.55	0.19	0.09

Priming seeds for approximately 24 hours with distilled water improved the germination parameters. This implied that the absence of inhibiting factors can be observed because the seeds absorbed more water which assisted in speeding up the enzyme activities. When the enzyme activities were activated, they easily hydrolyzed starch, protein and lipids into simplex compounds to provide energy for the growing embryo. The above experimental results correlated with Shrestha *et al*. (2019) [29] who revealed that water was necessary for enzymatic reactions and the mobilization of seed storage reserves, including lipids, carbohydrates, and proteins. Similarly, Chen *et al*., (2022) [30] induced germination in sorghum using $Cacl_2$. As shown in Table 2 above, the following germination parameters: 1) plumule length, 2) radicle length, 3) plumule fresh weight, 4) radicle fresh weight, 5) plumule dry weight, and 6) radicle dry weight showed different germination increases, for instance, $CaCl_2$ for 12hrs, 18hrs, and 24hrs. This result conformed to Yousof (2013) [31] who reported the significance of halo priming between 12hrs and 24hrs, while water uptake was increased when the priming time was prolonged for 24hrs. When priming time for 36 hours, the osmotic potential to (-1.25mpa) water uptake decreased. Generally, $CaCl_2$ induced the germination processes it helped in killing or neutralizing toxic substances found in the soil [32].

3.2 Sodium Chloride as a Seed Primer:

Table 3 below indicated analysis of variances where there was a highly significant difference (P<0.01) among sodium chloride concentrations on maize cultivar and the reaction of maize (PAN 12) evaluated for germination percentage, germination index, coleoptile, plumule length, radicle length, plumule fresh weight and radicle dry weight. No significant difference (P<0.05) was observed for plumule dry weight.

TABLE 3
SUMMARIZED ANALYSIS OF VARIANCE FOR SEED GERMINATION PARAMETERS PRIMED WITH NaCl.

Source of Variation	Df	Mean Square								
		Germination %	Germination Index	Coleoptile	Plumule Length	Radicle Length	Plumule Fresh Weight	Radicle Fresh Weight	Plumule Dry Weight	Radicle Dry Weight
Treatment	4	1140.00**	1.37**	255.93**	474.17	1688.90**	.42**	.18**	.001	.003**
Error	10	80.00	.119	47.097	513.93	94.33	.099	.23	.003	.001
Total	14	5360.00	6.696	1494.40	7036.00	7698.93	2.66	.93	.036	.017

High Significant (P<0.01), *Significant (P<0.05)

Table 4 below revealed that the highest germination percentages (83% and 60%) were obtained from 15g and 10g concentrations of sodium chloride, respectively. The lowest germination percentage was 33% found on 2g concentration of sodium chloride. The highest germination index of 3.03 was observed where the seed was soaked with 15g sodium chloride solution. Moreover, the lowest germination seed index was 1.36 in a 2g concentration of sodium chloride. The germination index showed a grand mean of 2.1600. Coleoptile measured the longest length of 43.67mm where seeds were soaked in 5g sodium chloride solution, while the shortest length was 23.33mm obtained from a control. The grand mean of coleoptile length stood at 33.80mm. The longest plumule length was 85.00mm found on the concentration of 10g sodium chloride, while the shortest length was 54.00mm obtained from a 2g concentration of sodium chloride. The grand mean for plumule length was 69.00mm. Radicle length revealed a grand mean of 72.26mm. The longest radicle length was 97.33mm followed by 87.67mm obtained from 15g sodium chloride concentration and on a control respectively. The shortest radicle length was 38.33mm found on where seeds were soaked with 2g sodium chloride solution. The plumule fresh weight had a grand mean of 0.820g, with the highest weight of 1.20g found on 15g of sodium chloride concentration solution, and the lowest weight of 0.23g was obtained from 2g sodium chloride concentration. The highest radicle fresh weight was 0.80g obtained from where the seed was soaked with 10g sodium chloride solution with the lowest weight of 0.20g found on 2g concentration of sodium chloride solution. The grand mean of radicle fresh weight was 0.566g. The grand mean for plumule dry weight was 0.160g, with the highest weight of 0.167g obtained on a control, 2g, 5g, and 15g of NaCl concentration solution. The lowest plumule fresh weight was 0.133g found on 10g NaCl solution. Finally, the radicle dry weight grand mean was 0.113g. The highest radicle dry weight was 0.167g obtained on control, with the lowest weight of 0.100g found on all four concentrations of NaCl solution.

TABLE 4
MEANS FOR NaCl CONCENTRATION

Concentration of NaCl	Germination %	Germination Index	Coleoptile	Plumule Length	Radicle Length	Plumule Fresh Weight	Radicle Fresh Weight	Plumule Dry Weight	Radicle Dry Weight
0	53.3333	2.2000	23.3333	60.3333	87.6667	1.0667	.5667	.1667	.1667
2g	33.3333	1.3667	24.6667	54.0000	38.3333	.2333	.2000	.1667	.1000
5g	40.0000	1.6333	43.6667	77.6667	58.6667	.7333	.5000	.1667	.1000
10g	60.0000	2.5667	40.0000	85.0000	79.3333	.8667	.8000	.1333	.1000
15g	83.3333	3.0333	37.3333	68.0000	97.3333	1.2000	.7667	.1667	.1000
Grand mean	54.0000	2.1600	33.8000	50.47	69.00	.8200	.5667	.1600	.1133
CV	16.56	15.95	20.30	30.32	32.86	38.31	26.96	36.08	22.78
LSD	16.27	0.63	12.48	27.84	31.24	0.57	0.28	0.11	0.05

The above table highlights that there was a significant difference in the germination process, which showed an impact on germination parameters such as 1) germination percentage, 2) germination index, 3) radicle length, 4) plumule fresh weight, 5) plumule dry weight and 6) radicle dry weights where seeds were primed with 15g concentration of sodium chloride solution. Also, in a 10g concentration solution, there was a significant difference in coleoptile, plumule length and radicle fresh weight. However, the results of this study revealed that no was significant reaction of sodium chloride as it did not show any negative impact on metabolic processes within the seed, implying no observation of osmotic stress. Instead, more water was absorbed which initiated the enzyme activity to resume. These results are in line with Marthandan *et al*. (2020) [33]and Gebreegziabher (2017) [34] who highlighted that pre-sowing treatment of maize seeds with sodium salt can initiate various reactions, which included an early metabolic process, activated various enzymes, and enhanced physiological activities inside the seed. Moreover, Naim *et al.* (2012) [35] reported that seed germination percentage was likely to be increased by a low level of salinity. In general, this study showed that salt stress at 2g, enhanced germination percentage, and all the parameters were salt sensitive at 5g, 10g, and 15g. Pannar cultivar had germinated well under salt stress. This meant that the Pannar 12 cultivar, which was used in this study was salt tolerant during the germination process as per se the result. This result was consistent with Naim *et al*. (2012) [35] who revealed that crop cultivars germinated effectively under salt stress.

3.3 Copper Sulphate as a Seed Primer:

Table 5 revealed a highly significant difference (P<0.01) among copper sulphate concentration on maize cultivar and the reaction of maize (PAN 12) evaluated for germination percentage, germination index, coleoptile, plumule length, plumule fresh weight, and radicle fresh weight. A significant difference (P<0.05) was observed only for radicle length. No significant difference was found between plumule dry weight and radicle dry weight.

TABLE 5
SUMMARIZED ANALYSIS OF VARIANCE FOR SEED GERMINATION PARAMETERS PRIMED WITH $CuSO_4$

Source of Variation	Df	Mean Square								
		Germination %	Germination Index	Coleoptile	Plumule Length	Radicle Length	Plumule Fresh Weight	Radicle Fresh Weight	Plumule Dry Weight	Radicle Dry Weight
Treatment	4	1623.33**	3.82**	570.83**	441.10**	951.23*	.097**	.199**	.004	.004
Error	10	126.67	.09	24.67	33.73	264.00	.009	.014	.002	.001
Total	14	7760.00	16.14	2530.00	2101.73	6444.93	.476	.936	.037	.029

Table 6 indicated the highest germination percentage (70%) found in a control (distilled water). The lowest germination percentage (13.3%) was recorded on 80mg of copper sulphate solution. The highest germination index was 3.167 obtained from control, while the lowest germination index was 0.400 found on 80mg with a grand mean of 1.260. The coleoptile had the longest length of 45.33mm obtained from 40mg copper sulphate and the shortest length was 10.00mm obtained from 80mg copper sulphate solution. The grand mean was 23.00. The longest plumule length was 41.00mm found on control with the shortest length of 12.66mm found on 80mg of copper sulphate. The grand mean was 27.46. The longest radicle length was 60.66mm found where distilled water was used as a control with the shortest length of 16.33mm obtained from 80mg copper sulphate solution. The grand mean was 33.93. Plumule fresh weight for copper sulphate has a grand mean of 0.360 with the highest weight of 0.566g obtained from control and the lowest weight of 0.166g found on 80mg. The highest radicle fresh weight was 0.86g on control with the lowest weight of 0.20g obtained from 80mg. The radicle fresh weight grand mean was 0.44. The plumule dry weight grand mean was 0.467g with the highest weight of 0.03g obtained on a control. The lowest weight, that is, 0.00 was attained from 20mg copper sulphate solution. Lastly, the radicle dry weight grand mean is 0.07g with the highest weight of 0.10g obtained from control and 40mg while the lowest radicle dry weight was 0.03g obtained from 80mg and 20mg copper sulphate solution.

TABLE 6
MEANS FOR $CuSO_4$ CONCENTRATIONS

Concentration of $CuSO_4$	Germination %	Germination Index	Coleoptile	Plumule Length	Radicle Length	Plumule Fresh Weight	Radicle Fresh Weight	Plumule Dry Weight	Radicle Dry Weight
0	70.00	3.17	26.33	41.00	60.67	.57	.87	.03	.10
20g	26.67	.83	17.67	22.67	23.00	.23	.37	.00	.03
40g	43.33	1.37	45.33	39.00	42.67	.53	.47	.10	.10
60g	16.67	.53	15.67	22.00	27.00	.30	.30	.07	.10
80g	13.33	.40	10.00	12.67	16.33	.17	.20	.03	.03
Grand mean	34.00	1.27	22.87	24.87	32.80	.29	.36	.05	.07
CV	27.38	25.49	21.98	18.72	51.18	23.29	42.43	95.83	49.79
LSD	16.94	0.59	9.14	8.47	30.54	0.12	0.28	0.08	0.07

Table 6 above expressed a significant difference in the germination process where maize seed was treated with distilled water that improved most of the germination parameters as follows: 1) germination percentage, 2) germination index, 3) plumule length, 4) radicle length, 5) plumule fresh weight, 6) radicle fresh weight and 7) radicle dry weight. This improvement was because of the free osmotic potential of water. Water plays a significant role by enhancing the activities of enzymes which helped to break the stored sugars into simplest sugars to provide energy for embryo development. El-Sanatawy *et al*. (2021) [36] showed that seed priming with water enhanced seed yield in maize. There was also a significant difference in coleoptile, plumule dry weight and radicle dry weight where maize seed was primed with a 40mg of copper sulphate solution. This implied that an increase of copper sulphate slightly increased the germination process and later decreased the germination process when the concentration up-surged above 40mg. These results conformed with Akram *et al*. (2020) [37] who reported that seed priming with 40 µM of $CuSO_4$ produced exceptional results but priming with $CuSO_4$ above 40 µM started a decline in plant growth and reached the lowest point when seeds were primed with 80 µM $CuSO_4$ because at higher concentration, seedling weight was reduced due to toxic effects of copper.

3.4 Indole Butyric Acid as a Seed Primer:

Analysis of variance depicted in Table 7 showed no significant difference ($P<0.01$) among indole butyric acid on maize cultivar and the reaction of maize (PAN 12) evaluated on all parameters. A significant difference ($P<0.05$) was also not observed on all parameters.

TABLE 7
SUMMARIZED ANALYSIS OF VARIANCE FOR SEED GERMINATION PARAMETERS WITH IBA

Source of Variation	Df	Germination %	Germination Index	Coleoptile	Plumule Length	Radicle Length	Plumule Fresh Weight	Radicle Fresh Weight	Plumule Dry Weight	Radicle Dry Weight
Treatment	3	319.444	.512	874.972	1312.667	1237.889	.139	.163	.002	.003
Error	8	291.667	.418	496.750	931.833	1148.250	.108	.127	.008	.003
Total	11	3291.667	4.872	6598.917	11392.667	12899.667	1.277	1.503	.067	.029

Table 8 exhibited the highest germination percentage (50%) obtained from 75g of indole butyric acid solution, with the lowest of 26.7% found at 37.5g of butyric acid concentration. The grand mean value was 40.83. The highest germination index of 1.86 was recorded on the concentration of 112.5g and 75g of indole butyric acid while the lowest was 1.00 obtained from 37,5g of butyric acid. The grand mean was 1.62. The longest coleoptile obtained from 112.5g indole was 70.33mm with the shortest length of 35mm obtained from 75g of indole butyric acid solution. The grand mean was 49.92mm. The longest plumule length was 86mm where seeds were soaked with 112.5g indole butyric acid. The current study showed the shortest plumule length (37mm) was obtained from a 75g concentration of butyric acid with a grand mean of 61.33mm. The indole butyric acid grand mean for radicle length was 62.83mm, with the longest length of 88.33 found on a control with 45.33 as the shortest length obtained from 37.5g butyric acid solution. The plumule fresh weight of indole butyric acid has a grand mean of 0.62g. The highest weight was 0.80g obtained from 112.5g butyric acid concentration with the lowest length of 0.33 found from 37.5g indole solution. The highest radicle fresh weight was 0.70g found on the 112.5g solution of indole butyric acid and the lowest was 0.20 obtained from 37.5g of indole acid. The grand mean weight for indole acid was 0.48g. Plumule dry weight had the highest weight of 0.16g obtained from control with the lowest weight of 0.10g found from 37.5g of indole butyric acid solution and the grand mean value was 0.13g. Finally, the highest radicle dry weight where seeds were soaked with 112.5g of indole was 0.10g with the lowest value of 0.03g found on a control and 37.5g concentration while the grand mean was 0.05. Mean values are presented in Table 8 below. Contrarily, Kumari *et al*, (2017) [38] found a significant difference in seed germination and seedling vigor in maize when comparing effects of halo and hormonal priming in India. Again, Mahmood *et al*. (2016) [39] conducted a research priming seeds of maize using plant growth promoting rhizobacteria and obtained a high seed germination rate and seedling growth. In line with previous researchers, Mustafa and Khan (2015) [40] investigated effects of indole butyric acid on sugar-cane root development and found to increase root proliferation, diameter and length.

TABLE 8
MEANS FOR IBA CONCENTRATIONS

Concentration of Indole Butyric Acid	Germination %	Germination Index	Coleoptile	Plumule Length	Radicle Length	Plumule Fresh Weight	Radicle Fresh Weight	Plumule Dry Weight	Radicle Dry Weight
0	40.0000	1.7667	57.6667	69.0000	88.3333	.7667	.6333	.1667	.0333
37.5g	26.6667	1.0000	36.6667	53.0000	45.3333	.3333	.2000	.1000	.0333
75g	50.0000	1.8333	35.0000	37.3333	47.6667	.5667	.3667	.1333	.0667
112.5g	46.6667	1.8667	70.3333	86.000	70.0000	.8000	.7000	.1333	.1000
Grand Mean	40.83	1.62	49.92	61.33	62.83	.62	.48	.13	.058
CV	39.15	36.01	51.44	57.28	59.91	56.69	81.84	70.71	80.81
LSD	31.94	1.16	51.30	70.19	75.20	0.69	0.78	0.19	0.09

IV. CONCLUSION AND RECOMMENDATION

Among the four chemical compounds evaluated for potency to enhance both germination and seedling parameters, distilled water was found to be the best and is recommended. It is followed by Sodium chloride, Calcium chloride and lastly Copper sulphate. While Indole-acetic acid seemed to have no perceptible influence on all germination and seedling parameters implying that it should not be used at all to enhance germination and seedling growth in maize to be specific. The procedure developed by Forti *et al*. (2020) [20] using distilled water superseded the other three, followed by Raiparet *et al* (2006) and Toklu *et al*., (2015) [28]using sodium chloride, then Di Girolamo and Barbanti (2012) [24] with calcium chloride and lastly, copper sulphate by Baneijee and Roychoudhury (2018) [22]. Four differing concentrations of each compound were applied.

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Agricultural Pest Identification Enhanced with Deep Learning Features and Machine Learning Models

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Abstract— *The research proposes a novel approach combining deep feature extraction using machine learning and traditional machine learning techniques to classify 12 agricultural pests. Individual features were extracted through AlexNet, GoogLeNet, and feature fusion; afterwards, they were classified using K-Nearest Neighbors, Support Vector Machine, and Random Forest. GoogLeNet achieved 86.21% accuracy with SVM, while the fused features achieved 82.03% with Random Forest. The proposed method makes good use of deep learning with feature representation and classical models for accurate and computationally efficient pest identification in agricultural applications.*

Keywords— Pest Classification, Googlenet, Alexnet, Deep features, SVM.

I. INTRODUCTION

Agrology is recognized as essential in ensuring global food security and economic development, especially when a significant portion of the population relies on agriculture for employment opportunities in a specific region [1]. However, agricultural productivity faces a continuous threat from pest infestations, which are a major contributing factor to crop damage and yield losses. Critical pest damage and losses need timely identification along with accurate pest recognition to determine the appropriate control measure to be put in place to curtail such losses[2]. With pest recognition relying on manual examination and the expertise of specialists, such methods, while effective, tend to be labor-intensive and ineffective for large-scale employment in agricultural settings.

Recent years have seen remarkable advances with the integration of Artificial Intelligence (AI) and Computer Vision (CV) into agriculture. Crop field monitoring and pest identification using deep learning techniques have received attention due to the high level of accuracy and autonomy they offer [3][4] Image classification has seen the adoption of Convolutional Neural Networks (CNNs), which can hierarchically learn complex representations from raw data to perform advanced classification tasks. Today, numerous CV applications such as object detection, image recognition, and classification rely heavily on previously developed models such as AlexNet and GoogLeNet[9][18]

The use of deep learning methods accomplishes remarkable outcomes, though their training requires enormous data alongside computational power, resources that are difficult to obtain in pre-established agricultural settings. To counter this challenge, the use of pre-trained CNNs for feature extraction is a practical substitution. With this method, models trained on benchmark datasets like ImageNet are employed to extract information from images about a specific field, with no training required. Such features are sufficient to train simple, low-cost classifiers that, without the need for significant resources, achieve accurate performance.

In this work, we propose a new approach to classifying 12 categories of agricultural pests that combines different methods. Features from two popular deep learning networks, AlexNet and GoogLeNet, have been incorporated. To this end, both individual feature extraction and feature fusion approaches have been adopted. In the first case, features were extracted independently from each model. In the second case, the features that were extracted from the individual networks were merged to create a single comprehensive feature set.

The innovation of this study is the combination of deep feature extraction with traditional machine learning techniques, thus providing better efficiency regarding computational resources while maintaining high performance. By framing the problem as pest classification without training an end-to-end deep learning model and employing pre-trained networks for feature extraction, this study proposes an effective and scalable approach. Such an approach is important for practical use in agricultural settings with limited resources.

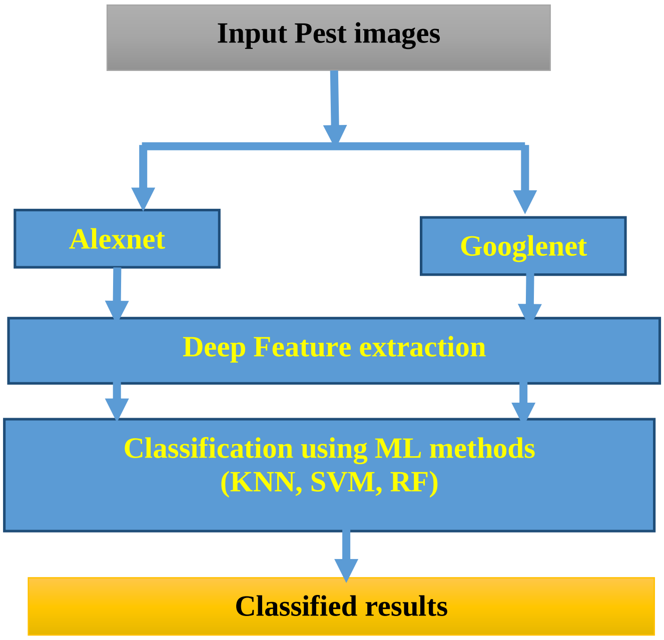


FIGURE 1: Block diagram of proposed method

II. LITERATURE SURVEY

This literature survey reviews recent state-of-the-art research in pest classification, focusing on lightweight models, transformer-based architectures, data augmentation techniques, and explainable machine learning. Each study offers unique contributions toward enhancing classification accuracy, computational efficiency, and practical deployment, particularly in real-world agricultural settings.

IN [15] Proposed PestNet, an optimized MobileNet-V2 architecture that incorporates an attention mechanism and dual-branch feature fusion to enhance pest classification performance while reducing model complexity and computational cost. It achieved superior accuracy and efficiency compared to ResNet-50 and EfficientNet-[14] introduced GNViT, a Vision Transformer-based model trained on the IP102 dataset for classifying pests in groundnut crops, achieving an accuracy of 99.52% and outperforming existing state-of-the-art models. In the work [17] proposed a novel Hybrid Pooled Multihead Attention (HPMA) model that enhances the feature-capturing ability of vision transformers, attaining high accuracy across multiple pest datasets by integrating both local and global feature extraction. [2] tackled the long-tailed data distribution issue using diffusion model-based data augmentation, which generates realistic synthetic images to balance pest datasets, significantly boosting classification performance on the IP102 dataset. [21] explored various transfer learning strategies for cotton boll weevil classification, showing that parameter- and instance-based methods can greatly improve accuracy even with limited data or features. [11] Proposed DEMNet, a ResNet50-based lightweight model for classifying Tomicus pests, offering a 90% reduction in parameter count and a 9.5% increase in accuracy, making it ideal for embedded pest management systems. [6] introduced DWViT-ES, a Dilated-Windows-based Vision Transformer that utilizes efficient and suppressive self-attention to boost the receptive field and accuracy while significantly reducing model parameters, validated on the IP102 and CPB datasets. [20] Enhanced pest detection using a YOLOv7-based spatio-temporal framework, addressing environmental noise and overlapping images in sticky trap data to achieve an F1-score improvement from 0.93 to 0.98. [5] addressed the open-world pest classification problem by developing a lightweight ResNet8-based matching network trained with NT-Xent loss, enabling high performance without retraining when encountering new pest classes. [23] Proposed InsectMamba, an innovative framework that combines CNNs, MSA, and SSMs for effective pest classification. The model outperformed competitors across five datasets due to its adaptive feature aggregation strategy. [16] Used explainable machine learning to forecast pest outbreaks in olive and grape crops. By applying SHAP and ICE plots, the models identified key environmental predictors, offering actionable insights for pest control. [17] presented MobileENet, a compact model for pest identification using deep feature extraction and optimization techniques, achieving 98.83% accuracy on the IP102 dataset while minimizing computation and overfitting.

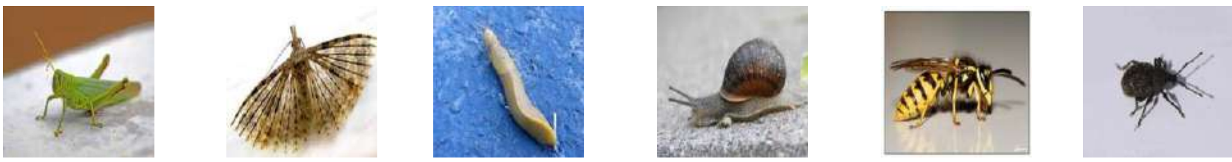
This study investigated twelve recent works centered on the use of deep learning and machine learning for insect pest identification in agriculture, highlighting two specific works that utilized both approaches. Specifically, the examined works proposed PestNet and MobileENet CNNs, GNViT and DWViT-ES based on transformers, and other newer techniques, including state space models, transfer learning, and data augmentation using diffusion models. Several works employed standard datasets IP102 and dealt with long-tailed data distribution, open-set identification, and real-time performance on mobile platforms. All models enhanced accuracy, efficiency, and generalizability, reflecting AI's advancement in pest classification, aiding the efforts towards sustainability in agriculture, and precision pest management.

III. PROPOSED METHOD

3.1 Dataset details:

This experiment was carried out by considering the standard dataset [3]. The Agricultural Pest Image Dataset comprises images representing 12 distinct types of agricultural pests, including ants, bees, beetles, caterpillars, earthworms, earwigs, grasshoppers, moths, slugs, snails, wasps, and weevils.

a. Ants b. Bees c. Beetle d. Caterpillar e. Earthworms f. earwigs



g. Grasshopper h. Moth i. Slug j. Snail k. wasp l. weevil

FIGURE 2: Sample images

The process begins by extracting deep features from pest images using two pre-trained convolutional neural networks (CNNs): AlexNet and GoogLeNet. In the initial step, the deep features were extracted from pest images using two pre-trained convolutional neural networks: AlexNet and GoogLeNet. These architectures were selected for their proven effectiveness in classification tasks, owing to their capacity to learn complex feature representations from input data (Krizhevsky et al., 2012; Szegedy et al., 2015).

3.2 AlexNet:

Image classification on the ImageNet dataset was greatly enhanced by AlexNet, a complex deep neural network conceived of by [9] in the year 2012. The network structure contains eight layers in total, five of which are convolutional layers and the other three are fully connected layers. The first convolutional layer employs a kernel size of 11×11 with a stride of four, allowing it to capture rough features from the images. In the second layer, a 5 ×5 kernel is used, and in the third, fourth, and fifth layers, 3×3 sized kernels are employed. The convolutional layers are also mixed with ReLU activation function, local response normalization, max pooling, and other methods to enhance training speed and generalization. The output is passed through two fully connected layers, each containing four thousand and ninety-six neurons, flattened before that, and then passed into one shared fully connected layer with 1000 neurons, which serve the purpose of classifying them. Dropout is also used in the fully connected layers to prevent overfitting. Class probabilities are yielded with the softmax function at the end.

3.3 GoogLeNet (Inception v1):

GoogLeNet, introduced by [19] in 2015, is a 22-layer deep CNN that introduced the Inception module as its core innovation. Rather than stacking standard convolutional layers, GoogLeNet uses Inception modules that apply parallel convolutions of varying kernel sizes (1×1, 3×3, 5×5) and a 3×3 max-pooling operation within the same layer, concatenating their outputs. This design allows the network to capture features at multiple scales efficiently. To maintain computational efficiency, 1×1 convolutions are used as bottleneck layers before the 3×3 and 5×5 convolutions to reduce dimensionality. GoogLeNet includes nine Inception modules, followed by an average pooling layer and a fully connected layer with 1000 units for classification. Unlike AlexNet, it reduces reliance on large fully connected layers, decreasing the model's parameters and memory usage significantly. GoogLeNet also uses auxiliary classifiers during training to combat the vanishing gradient problem and improve convergence.

The feature outputs from both networks were then fused using a joint representational learning approach to form a unified composite feature set. This fusion strategy was employed to preserve and leverage the individual strengths of each network [7]. Pest classification is achieved with the help of classical machine learning classifiers after extracting and fusing the features. The selected classifiers are K-Nearest Neighbors (KNN), Support Vector Machine (SVM), and Random Forest (RF). Among these, the GoogLeNet features plus SVM gave the best classification results with an accuracy of 86.21%. For the fused features, the classification accuracy was 82.03% with Random Forest. This demonstrates the effectiveness of combining deep feature extraction with lightweight classifiers, allowing for reduced computational complexity during the classification phase

This hybrid approach leverages the robust feature representation capabilities of deep CNNs and the interpretability and efficiency of traditional classifiers, offering a scalable and accurate solution for pest recognition in precision agriculture.

IV. RESULTS AND DISCUSSIONS

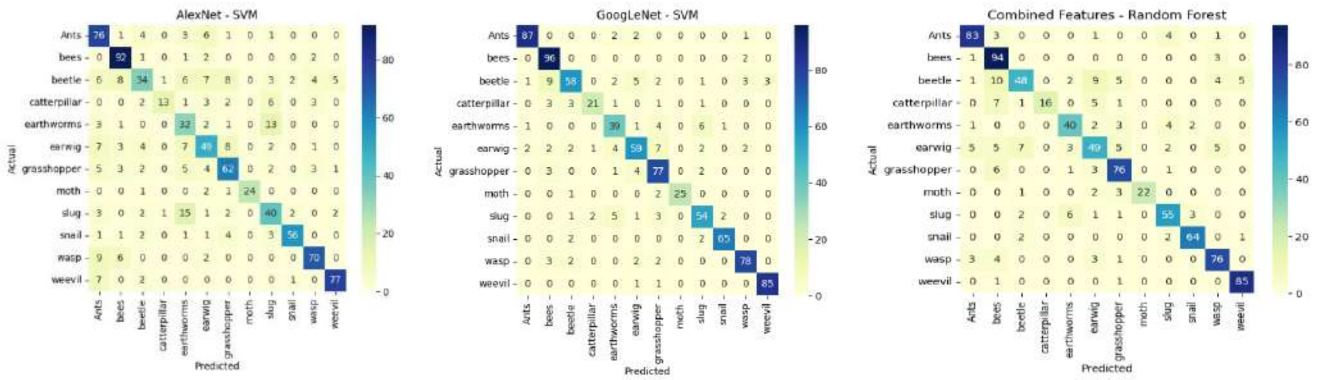
To evaluate the effectiveness of the proposed pest classification framework, multiple experiments were conducted using deep features extracted from AlexNet, GoogLeNet, and their fused representations. These features were then classified using three classical machine learning algorithms: K-Nearest Neighbors (KNN), Support Vector Machine (SVM), and Random Forest (RF). The results were assessed based on classification accuracy as the primary performance metric.

4.1 Performance Analysis:

The classification outcomes across various combinations of feature extraction methods and classifiers are summarized in Table 1.

TABLE 1
CLASSIFICATION ACCURACY FOR DIFFERENT FEATURE-CLASSIFIERS COMBINATIONS

Feature Source	Classifier	Accuracy (%)
AlexNet	KNN	75.68
AlexNet	SVM	80.21
AlexNet	Random Forest	78.14
GoogLeNet	KNN	83.12
GoogLeNet	SVM	86.21
GoogLeNet	Random Forest	84.05
Feature Fusion	KNN	79.87
Feature Fusion	SVM	81.44
Feature Fusion	Random Forest	82.03



(a) Alexnet with SVM classifier **(b) Googlenet with SVM classifier** **(c) Feature fusion with Random Forest Classifier**

FIGURE 3: Classification Accuracy for Different Feature-Classifiers Combinations

The following shows the confusion matrix of the highest recognition rate from each section.

Following Table 2. Shows the classification results based on the Precision, Recall, and F1-score

TABLE 2
PROPOSED EXPERIMENT RESULTS IN PRECISION, RECALL AND F1-SCORE

Model	Precision	Recall	F1-Score
AlexNet with KNN	0.5215	0.4461	0.4444
AlexNet with SVM	0.7343	0.7242	0.7213
AlexNet with Random Forest	0.6703	0.6674	0.6500
GoogLeNet with KNN	0.7983	0.7914	0.7826
GoogLeNet with SVM	0.8641	0.8621	0.8607
GoogLeNet with Random Forest	0.8202	0.8157	0.8120
Features Fusion with KNN	0.5572	0.4971	0.4879
Features Fusion with SVM	0.7839	0.7786	0.7751
Features Fusion with Random Forest	0.8256	0.8203	0.8162

The results obtained from this study demonstrate the viability and strength of combining deep learning-based feature extraction with traditional machine learning classifiers for pest identification. The experimental outcomes reveal several key insights regarding model performance, feature representation, and classification efficiency. Following table 3 shows the comparative analysis paper, proposed work with other research work. This study shows the robustness of the proposed method.

TABLE 3. COMPARATIVE ANALYSIS

Author(s) & Year	Proposed Method	Recognition Accuracy (%)
Ayan **et al. [1]**	GAEnsemble (Inception-V3, Xception, MobileNet)	67.13
Ung et al. **[22]**	CNN Ensemble with Attention & Feature Pyramid	74.13
Zhou & Su **[26]**	ExquisiteNet (Lightweight CNN)	52.32
Nguyen et al. [13]	DeWi (Deep-Wide Learning Assistance)	76.44
Liu et al. [12]	ResNet with Feature Fusion	55.43
Zhang et al. **[25]**	EfficientNetV2 + Coordinate Attention	73.70
Wu et al. **[24]**	ResNet-based Feature Fusion	68.34
Proposed (Current Study)	**GoogLeNet + SVM (Deep feature extraction + traditional ML)**	**86.21**

The comparative analysis presented in Table 1 demonstrates the superior performance of the proposed method, which integrates GoogLeNet-based deep feature extraction with a traditional Support Vector Machine (SVM) classifier. Achieving a recognition accuracy of 86.21% on the standard dataset [3], this approach significantly outperforms several recent state-of-the-art techniques.

V. CONCLUSION AND FUTURE WORK

The study proposed a hybrid pest classification framework that integrates GoogLeNet-based deep feature extraction with a Support Vector Machine classifier, evaluated using the standard dataset [3]. The method achieved a recognition accuracy of 86.21%, outperforming several existing deep learning-based approaches. These results highlight the effectiveness of combining deep feature representations with classical machine learning techniques for accurate and resource-efficient pest identification.

Future research may focus on incorporating feature selection methods to reduce feature dimensionality and improve model interpretability. Additionally, the framework could be adapted for deployment on edge devices to facilitate real-time pest monitoring in agricultural settings and expanded to include a wider range of crop-pest datasets for enhanced generalizability.

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Role of Dietary Fiber Supplementation in Prevention of Diabetes Mellitus

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Abstract— *Type 2 diabetes mellitus (T2DM) is a common metabolic disease that is frequently associated with obesity and insulin resistance. Dietary fiber greatly improves glycemic control, cholesterol management, and weight regulation, even while drugs are necessary. Because of their solubility, viscosity, and gel-forming qualities, soluble fibers—like psyllium, black cumin, flaxseeds, basil seeds, and guar gum—have a beneficial effect on metabolic health. Based on clinical studies, fiber supplementation improves waist circumference, BMI, and glucose levels in a variety of populations, including individuals with type 2 diabetes, prediabetes, and pregnant women. Reduced incidence of diabetes, fasting glucose, HbA1c, body weight, and cholesterol are highlighted by randomized controlled trials. Viscose and soluble fibers improve metabolic health by delaying the absorption of glucose. One easy and efficient way to avoid diabetes is to incorporate fiber into your diet. The purpose of this study is to assess the therapeutic effectiveness of dietary fiber supplements in the management and prevention of diabetes.*

Keywords— Diabetes, Supplements, Dietary Fiber, Types of Fiber Supplements, Management.

I. INTRODUCTION

Diabetes mellitus (DM), a metabolic disorder, is characterized by persistently high blood sugar levels and variable levels of impairment in the metabolism of proteins, lipids, and carbohydrates. One of the oldest diseases that people have ever encountered is probably diabetes mellitus. It was initially recorded in an Egyptian papyrus approximately 3,000 years ago [1]. In 1936, the distinction between type 1 and type 2 diabetes was made. Type 2 diabetes was first recognized as a component of the metabolic syndrome in 1988 [2]. Diabetes mellitus can have many different causes and origins, but abnormalities in either insulin secretion, response, or both are always present at some point over the course of the disease. The majority of people with diabetes mellitus have either type 1 diabetes (idiopathic or immune-mediated). The most prevalent kind of diabetes mellitus, known as type 2 DM (non-insulin dependent DM), is typified by hyperglycemia, insulin resistance, and relative insulin insufficiency [3]. Genetic, environmental, and behavioral risk factors interact to cause type 2 diabetes [4] [5]. Additionally, genetic problems, various diseases, the hormonal milieu during pregnancy, and some medications can all be linked to diabetes [6]. The inflammatory process that kills beta cells is the characteristic of type 1 diabetes mellitus, sometimes referred to as juvenile diabetes, and usually leads to total insulin insufficiency [7]. Type 1 diabetes is usually indicated by the presence of insulin, islet cell, or anti-glutamic acid decarboxylase antibodies, which identify the autoimmune processes responsible for beta cell loss. To maintain normoglycemia, insulin therapy will eventually be necessary for all patients with type 1 diabetes. There has been and will continue to be debate regarding the relative significance of abnormalities in insulin secretion or the hormone's peripheral activity in the development of type 2 diabetes mellitus. Eighty to ninety percent of all reported cases are Type 2 Diabetes Mellitus. Insulin resistance is intimately linked to intra-abdominal (visceral) obesity, which is seen in most people with Type 2 diabetes. These people also frequently have dyslipidemia (high triglyceride and low HDL cholesterol; postprandial hyperlipidemia) and hypertension. Furthermore, women who develop diabetes mellitus during pregnancy are identified by an operational classification known as gestational diabetes mellitus, rather than a pathophysiologic disease. Gestational Diabetes Mellitus (GDM) is the term used to describe women who acquire Type 1 diabetes mellitus during pregnancy as well as women who have undetected asymptomatic Type 2 diabetes mellitus that is identified during pregnancy. The third trimester of pregnancy is when GDM typically first appears in women."Other Specific Types" is a classification that includes diabetes mellitus types with a variety of known etiologies. This group, known as Maturity-Onset Diabetes in Youth (MODY), comprises less than 10% of cases of diabetes mellitus and includes people with genetic defects of beta-cell function or insulin action, exocrine pancreatic diseases like pancreatitis or cystic fibrosis, people with dysfunction linked to other endocrinopathies (like acromegaly), and people with pancreatic dysfunction brought on by drugs, chemicals, or infections.

A nutritional supplement designed to supply nutrients that may be lacking or insufficiently taken in a person's diet, such as vitamins, minerals, fiber, fatty acids, amino acids, and probiotics. There are several kinds of dietary supplements, but the most popular kind is for vitamins and minerals. They can be obtained as single nutrients or as a blend of many micronutrients [8]. The Institute of Medicine (IOM) defines fiber supplements in the United States as a subcategory of effective dietary fiber. Supplements containing fiber come in powder, pill, and capsule form. Dietary supplements used by bodybuilders and athletes are known as bodybuilding supplements. These can be taken in place of meals, to help you gain weight, lose weight, or perform better in sports. Overall, the most utilized substances are glutamine, essential fatty acids, meal replacements, creatine, weight reduction products, and testosterone boosters.

The complex chronic metabolic disease known as type 2 diabetes mellitus (T2DM) is typified by dysregulation of the metabolism of macronutrients, including fat, protein, and carbohydrates, which is brought on by insulin resistance or decreased insulin production [9]. Even though the standard clinical treatment for type 2 diabetes involves taking anti-diabetic medications orally, insulin therapy is eventually required because of the gradual decline in β-cell capacity [10]. One of the most reliable and secure methods of regulating blood sugar levels is still dietary treatments, sometimes referred to as "Medical Nutrition Therapy" [11]. The therapeutic benefits of dietary fiber, which is neither absorbed nor digested in the human small intestine and is strongly linked to gut hormone and gut microbiota, are one of the most researched dietary patterns [12]. Particularly with regard to the connection to type 2 diabetes. Viscous β-glucan is an example of soluble fiber that might positively impact short-term gut hormone responses, insulin, glucose, and satiety [13]. However, insoluble fiber is typically poorly fermented and may have an impact on human health through the gut microbiota and its byproducts [14]. Indeed, consumption of dietary fiber has been confirmed to be inversely related to the progression of type 2 diabetes [15] and can flatten glycemic and insulinemic responses [16]. However, there have been mixed results about the effects of dietary fiber in reducing glycated hemoglobin (HbA 1c), varying by the type and dose of fiber, with only soluble fiber being fully explored [17]. In addition, due to minor side effects like diarrhea, bloating in the abdomen, and flatulence, no meta-analysis evidence was found to clarify the negative effects of fiber with respect to a specific dose [18]. Therefore, the present study aims to provide clinical efficacy of dietary fiber supplementation in prevention of Diabetes Mellitus.

II. MANAGEMENT OF DIABETES

One of the biggest challenges facing healthcare professionals today is managing the continuing needs and expectations of individuals with chronic diseases like diabetes [19]. For those with diabetes, routine follow-up visits with the doctor are essential to avoiding long-term effects. Studies have demonstrated that strict metabolic control can prevent or delay the onset of diabetes-related issues [20] [21]. Large, randomized trials including patients with type 1 diabetes or newly diagnosed or established type 2 diabetes have shown that controlling glycemia delays the onset and slows the progression of microvascular issues, including retinopathy, neuropathy, and nephropathy [22] [23]. In addition to maintaining proper glucose control, diabetic patients also require rehabilitation, disability limitation, and the prevention of complications. Due to the public's low health literacy and negative attitude toward the illness, some Indian studies found extremely low adherence to treatment plans [24] [25]. Psychosocial constraint is one factor that should be considered when establishing glycemic objectives [26]. Patients with "hypoglycemia unawareness" should have their glucose goals lowered for extended periods of time as they wait for the condition to possibly reverse [27] [28]. The objective is to prevent non-ketonic hyperosmolar coma, infections, water and electrolyte loss, and clinically significant glycosuria in patients with serious coexisting illnesses that might make the treatment strategy difficult. Insulin is recommended for people with type 1 or type 2 diabetes who have insulinopenia whose hyperglycemia does not improve with diet treatment alone or in conjunction with oral hypoglycemic medications [29]. The most successful blood glucose-lowering treatment may be insulin-assisted blood glucose control [30]. Insulin treatment will eventually be necessary for many type 2 diabetic patients. Insulin needs can surpass 1 unit/kg/day because type 2 diabetes is linked to insulin resistance [31]

III. BENEFITS OF DIETARY FIBER SUPPLEMENTS ON HEALTH

Four main aspects influence clinical efficacy of fiber supplements: solubility, degree/rate of fermentation, viscosity, and gel formation. A fiber supplement's solubility determines whether it will dissolve in water (soluble) or stay as distinct insoluble particles [32] [33]. The four therapeutically relevant parameters listed below can be used to classify fiber supplements: Insoluble fiber (e.g., wheat bran): Does not dissolve or gel; low fermentation; coarse particles provide a laxative effect via mechanical gut stimulation. Soluble, non-viscous, readily fermented fiber (e.g., inulin): Dissolves but does not form gel; rapidly fermented, producing gas and calories; lacks laxative or gel-related benefits; may affect gut microbiota but with no proven clinical benefits. Soluble, viscous, gel-forming fiber (e.g., oats): Dissolves, forms gel, slows nutrient absorption, improves glycemic control, lowers cholesterol; loses gel properties during fermentation, offering no laxative or diarrhea attenuation effects. Non-fermented, viscous gel-forming fiber (e.g., psyllium): Dissolves, forms gel, slows nutrient absorption, improves glycemic control, lowers cholesterol; remains gelled in the bowel, normalizing stool consistency by softening constipation and firming diarrhea. There are various dietary fiber supplements available on the market, including Psyllium husk, Black cumin seeds, Flax seeds, Basil seeds, Guar Gum, Chia seeds and etc.

3.1 Psyllium husk:

It belongs to the kingdom Plantae, genus Plantago Family Plantaginaceae, Species Plantago ovata. The species is renowned for its seeds, which are widely used for their medicinal and dietary fiber benefits. Seeds are used commercially to make mucilage, are commonly referred to as psyllium. Psyllium supplementation for three weeks or more may reduce blood glucose levels in individuals with type 2 diabetes and lower blood cholesterol levels in those with increased cholesterol [34] [35]. Psyllium has been used to thicken frozen delicacies like ice cream. The binding characteristics of psyllium mucilage at a weight/volume ratio of 1.5% are better than those of starch mucilage at a weight/volume ratio of 10% [36]. Psyllium husk is a very viscous soluble fiber that lowers low-density lipoprotein cholesterol and stabilizes blood sugar levels. "Psyllium husk may reduce the risk of type 2 diabetes, although the FDA has concluded that there is very little scientific evidence for this claim [37]

3.2 Black cumin seeds:

It belongs to the species Nigella sativa and Elwendia persica, two quite distinct plants that are both used as spices, can be referred to as black cumin belongs to the Genus Nigella, Family Ranunculaceae, Kingdom Plantae. Numerous medicinal qualities, such as antioxidant, anti-inflammatory, immunomodulatory, anticancer, neuroprotective, antimicrobial, antihypertensive, cardioprotective, antidiabetic, gastroprotective, nephroprotective, and hepatoprotective qualities, are largely responsible for the traditional uses of N. sativa seeds [38]. Thymoquinone, one of the key bioactive substances that was shown to have a protective effect against diabetes, is primarily responsible for the therapeutic actions of NS [39]

3.3 Flax seeds:

It is commonly known as linseed or common flax, is a flowering plant that belongs to the Linaceae family. A half ounce of flaxseed provides 20 to 25 percent of daily fiber needs, making it the richest plant lignan source [40]. It has been demonstrated to be helpful in decreasing blood glucose, cholesterol, weight, and acting as a laxative. It includes both soluble and insoluble fibers. It has been demonstrated that the insoluble fiber in flax seeds slows down the blood's sugar release, which significantly lowers blood glucose levels [41]

3.4 Basil seeds:

It is referred to by the generic term *Ocimum basilicum* or *Ocimum tenuiflorum*, also known as *Ocimum sanctum*, and belongs to the Lamiaceae family. These seeds are typically tiny, black, and after 15 minutes of soaking in water, they take on the consistency of gel and have a mild, nutty flavor [42]. Phytochemicals found in basil seeds have a variety of uses. Rich in fiber, basil seeds lower cholesterol and sugar levels, relieve constipation, and aid in weight loss [43]. Basil seeds prevent blood sugar spikes by postponing the conversion of carbohydrates to glucose. Another advantage of their high fiber content is improved glycemic management.

FIGURE 1: Types of dietary fiber supplements

3.5 Guar Gum:

Guar gum, often referred to as guaran, is a galactomannan polysaccharide that is extracted from guar beans and has thickening and stabilizing properties that make it suitable for usage in feed, food, and industrial settings [44]. Guar gum is produced from the seeds of the drought-tolerant plant Cyamopsis tetragonoloba, a member of the Leguminosae family [45]. It has been demonstrated that the gel-forming properties of guar gum lower cholesterol and blood sugar. In addition to lowering blood glucose levels, its gelling properties help people lose weight and avoid obesity. Furthermore, the gel-forming properties of guar gum soluble fiber cause the stomach to empty more slowly, which promotes fullness [46].

3.6 Chia seeds:

Chia seeds are edible seeds from the flowering plant *Salvia hispanica*, which belongs to the Lamiaceae family of mints. They aid in weight control by reducing obesity and helping to manage Type 2 diabetes. Chia seeds are high in soluble dietary fats, omega-3 and omega-6 polyunsaturated fatty acids, and other nutrients.

IV. CLINICAL STUDIES ON PREVENTION OF DIABETES THROUGH DIETARY FIBER

Studies have demonstrated the therapeutic benefits of high-fiber diets in the treatment of diabetes. This is because the soluble fiber content slows down the small intestine's absorption of glucose, which may help avoid the blood glucose increase that occurs after a meal or snack [47]. In diabetic patients, fiber consumption has been linked to improved insulin sensitivity and decreased postprandial glucose levels. Soluble fiber's gelling and/or viscous qualities were blamed for these effects [48]

Ruixue Li et al. (2024) conducted study on long-term clinical efficacy of dietary fiber supplementation in middle-aged and elderly prediabetic patients. This study evaluated the long term benefits of DF supplementation on body composition, glucose-lipid metabolism, and clinical regression in middle-aged and elderly patients with prediabetes. In this study Participants were randomized into a control group receiving health education and an intervention group consuming DF supplement daily before meals (15 g of mixed fiber per serving) for 6 consecutive months based on health education. Follow-up was 1 year with a 6-month cycle. Blood and anthropometric parameters were assessed at baseline and 6 months and 12months of follow-up. Fifty-four participants were included in the study, 27 in each group. After 6 months, waist circumference, waist-to-hip ratio, fasting plasma glucose (FPG), 2-hour plasma glucose (2h PG), and postprandial insulin levels were significantly lower in the intervention group compared to baseline. FPG, 2h PG, glycosylated hemoglobin, triglyceride/high-density lipoprotein cholesterol values and diabetes incidence were lower than in the control group. After 12 months, blood glucose and diabetes incidence remained lower in the intervention group. DF supplementation can reduce the degree of central obesity, the levels of FPG and 2h PG, and the incidence of diabetes in middle-aged and older patients with prediabetes.

Caroline Honsek et al. (2018) study conducted on fibre supplementation for the prevention of type 2 diabetes and improvement of glucose metabolism. A modified version of the one-year Prevention of Diabetes Self-management (PREDIAS) lifestyle training program was administered to 180 people with impaired glucose tolerance. Individuals with impaired glucose tolerance were given 7.5 grams of insoluble fiber twice daily for a duration of two years. The study, a randomized controlled trial, found a significant reduction in 2-hour OGTT (Oral Glucose Tolerance Test) levels in both the fiber and placebo groups. However, there was no significant difference observed between the two groups.

Dong-Yao Zhang et al. (2022) conducted study on preventing gestational diabetes mellitus in women who were overweight or obese before to pregnancy by dietary fiber supplements. Randomized controlled trial was conducted in Shanghai General Hospital from June 2021 to March 2022. Women with pre-pregnancy overweight or obesity were given 12 grams of dietary fiber twice daily from 20 to 24+6 gestational weeks. The study observed a reduction in the incidence of gestational diabetes mellitus (GDM), fasting plasma glucose, body weight gain, and preterm birth. However, there was an increase in triglycerides (TG) and the TG/HDL-C ratio.

Ayman S et al. (2018) conducted study on the Effect of Soluble Fiber Supplementation on Metabolic Syndrome Profile among Newly Diagnosed Type 2 Diabetes Patients. The study aims to determine role of soluble dietary fiber supplementation improve Metabolic syndrome profile for 8 weeks of intervention in newly diagnosed type 2 diabetes (T2D) adult patients. This study utilized an experimental design, called clinical randomized controlled trial. The study reported significant reductions in fasting blood sugar (FBS), triglycerides, total cholesterol, systolic and diastolic blood pressure, as well as waist circumference.

Abutair et al. (2016) conducted study on soluble fibers from psyllium improve glycemic response and body weight among diabetes type 2 patients (randomized control trial) : Forty type 2 diabetes patients, non-smoker, aged >35 years were stratified to different strata according to sex, age, body mass index (BMI) and fasting blood sugar level (FBS) and randomly assigned into two groups. Type 2 diabetes patients consumed 10.5 grams of soluble fiber daily for 8 weeks. The study demonstrated significant reductions in BMI, fasting blood sugar (FBS), HbA1c, insulin levels, C-peptide, and HOMA-IR, along with improvements in HOMA-β%.

V. CONCLUSION

Chronic type 2 diabetes mellitus (T2DM) is characterized by insulin resistance and hyperglycemia and is associated with lifestyle variables such as obesity, poor food, and inactivity. Although drugs are still essential, dietary fiber supplements have demonstrated potential for enhancing metabolic regulation. Frequent consumption of fiber improves lipid profiles, lowers insulin, and lowers fasting and postprandial blood glucose, HbA1c. Although longer-term effects require further research, clinical trials indicate fiber lowers the risk of diabetes and helps regulate blood sugar. An all-encompassing strategy that incorporates medication, dietary changes, and lifestyle adjustments enhances long-term health outcomes and efficiently supports diabetes control.

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Influence of Integration of Inorganic Nutrient Sources with Organic Manures on Growth and Yield Attributes of Wheat (*Triticum aestivum* L.)

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Abstract— *A field study was conducted during rabi season 2022-23 and 2023-24 at the Department of Agriculture, Sri Guru Granth Sahib World University, Fatehgarh Sahib on Influence of Integration of inorganic nutrient sources with organic manures on growth and yield of Wheat (Triticum aestivum L.). The experiment was laid out in Randomized Block Design in three replications with 14 treatments. The values for growth attributes such as plant height (81.66 cm), leaf area (6.14 m^2), dry matter per meter row length (302.48 g) and no. of tillers per meter row length (124.33) were recorded maximum under T_9 (100 % RDN through chemical fertilizer + 25% RDN through poultry manure) for both years. This was statistically at par with treatment T_2 (100 % RDF (125 kg ha^{-1})), T_5 (100 % RDN through chemical fertilizer + 25% RDN through FYM) and T_7 (75 % RDN through chemical fertilizer + 25% RDN through poultry manure). On the contrast, the lowest values were observed under treatment T_1 (control) for growth attributes. Similarly, the yield attributing characters viz., no. of spikes, spike length, total no. of grains and 1000 grain weight were maximum under T_9 and also at par with treatment T_2 and T_7. The lowest values were observed under treatment T_1 (no nutrient source) for given parameters. This study revealed that integration of organic nutrient sources with inorganic sources showed better results for mentioned growth and yield attributes of wheat crop.*

Keywords— Wheat, FYM, Poultry manure, Biogas Slurry, Nitrogen.

I. INTRODUCTION

Wheat (*Triticum aestivum* L.) a member of family *Poaceae,* is chief staple food which supplies approximately 35 percent of total food consumed by the global population (Mohammadi-Joo *et al* 2015). It is one of the most important cereal crops of the world, which globally stand in second position both in terms of area and production next to rice. In Punjab, it is cultivated over an area of 35.17 lakh hectares with annul production of 165.67 lakh tonnes and average productivity of 47.10 quintals ha^{-1} (Anonymous, 2022-23). The demand for wheat is expected to increase due to increase in population and affordability due to improved income status of the people (Gangwar *et al* 2018).

Integrated nutrient management refers to the combination of all possible sources of nutrients like organic, inorganic and biological sources in a judicious way for obtaining an ecologically sound environment and economically optimal farming system (Jat *et al* 2015). Integrated use of organic and inorganic nutrient sources helps in gaining sustainable yield and improved soil quality for enhanced production (Brar *et al* 2015). Continuous application of organic manures year after year improves physical and chemical conditions by providing a favourable soil structure, enhanced soil cation exchange capacity, increased quantity and availability of plant nutrients, increase humus content and substrate for microbial activities (Bohme and Bohme, 2006).

II. MATERIAL AND METHODS

The present studies were carried out at Agriculture Farm, Sri Guru Granth Sahib World University, Fatehgarh Sahib during *rabi* season in the year 2022-2023 and 2023-2024. It was 14 treatments and three replications with RBD design. The variety was sown in PBW-826. The crop was sown row to row spacing with 20 cm.

III. RESULT AND DISCUSSION

3.1 Growth Attributes:

The study evaluated the effect of different nutrient sources on plant growth attributes, including plant height, leaf area index, dry matter accumulation and the number of tillers per meter row length, for two consecutive years (2023 and 2024) data were show in tabular (table 1). The highest plant height, leaf area, dry matter and number of tillers per meter row length were recorded in T_9 (100% RDN through poultry manure) with 81.15 cm, 6.16 m², 133.45 q ha^{-1} and 124.33 and in 2024 and 82.17 cm, 6.16 m^2, 133.22 q ha^{-1} and 124.67 in 2023. This was statistically at par with T_2 – (100 % RDF (125 kg ha^{-1}) from chemical fertilizers), T_5 – (100 % RDN through chemical fertilizer + 25 % RDN through FYM), T_7 - (75% RDN through chemical fertilizer + 25% RDN through poultry manure) and T_{13} - (100% RDN through chemical fertilizer + 25% RDN through biogas slurry) in both the years. The control treatment (T_1) showed the lowest result in all the growth parameters. Due to this, the study concludes that nutrient management significantly influences plant growth parameters. Among the treatments, T_9 exhibited the best results across all growth attributes, including plant height, leaf area index, dry matter production, and tiller count, making it a promising organic nutrient source for sustainable crop production. However, the control treatment (T_1) resulted in significantly lower growth attributes, emphasizing the necessity of nutrient application for optimal plant development.

3.2 Yield Attributes:

The analysis of yield attributes across different treatments reveals significant variations under the integrated nutrient management strategies show in tabular (table. 2). The highest number of spikes $plant^{-1}$,spike length and number of grains $spike^{-1}$ were observed in treatment T_9 - (100% RDN through chemical fertilizer + 25% RDN through poultry manure) with 245.33, 8.17 cm and 53.33 in 2023 and 247.33, 8.33 cm and 54.67 in 2024, which was statistically at par with T_2 – (100 % RDF (125 kg ha^{-1}) from chemical fertilizers), T_5 – (100 % RDN through chemical fertilizer + 25 % RDN through FYM), T_7 - (75% RDN through chemical fertilizer + 25% RDN through poultry manure) and T_{13} - (100% RDN through chemical fertilizer + 25% RDN through biogas slurry). The lowest number of spikes was recorded in the treatment T_1 - control (no nutrient source) in all the yield attributes parameters. Because of this, the findings suggest that T_9 resulted in the highest yield attributes, surpassing even chemical fertilizers (T_2). This could be due to the slow release of nutrients and better soil health under organic amendments. The results were non-significant for test weight during both the years.

FIGURE 1: The analysis of different attributes across different treatments reveals significant variations

TABLE 1
EFFECT OF INORGANIC NUTRIENT SOURCES WITH ORGANIC MANURES ON GROWTH OF WHEAT

	Growth attributes	Plant Height (cm)		Pooled	Leaf area index (m^2)		Pooled	Dry matter accumulation q h^{-1}		Pooled	Number of tillers per meter row length		Pooled
		2023 year	2024 year		2023 year	2024 year		2023 year	2024 year		2023 year	2024 year	
T_1	Control (No nutrient sources)	73.46	74.11	73.78	4.38	4.61	4.50	119.51	120.24	119.87	102.00	104.33	103.17
T_2	100 % RDF (125 kg a^{-1}) from chemical fertilizers	80.83	81.40	81.12	6.13	6.14	6.14	132.05	132.93	132.49	123.67	124.33	124.00
T_3	75 % RDN (93.75 kg ha^{-1}) through chemical fertilizer + 25% RDN through farm yard manure (FYM)	79.20	79.57	79.38	5.68	5.81	5.75	129.94	130.83	130.39	120.33	122.33	121.67
T_4	50 % RDN (62.5 kg ha^{-1}) through chemical fertilizer + 50 % RDN through FYM	76.67	77.50	77.08	5.56	5.62	5.59	127.82	128.38	128.10	120.00	121.00	120.50
T_5	100 % RDN through chemical fertilizer + 25 % RDN through FYM	80.03	80.93	80.48	6.10	6.13	6.12	131.61	132.23	131.92	122.67	123.67	123.17
T_6	100 % RDN through FYM	76.47	77.20	76.83	4.53	4.74	4.64	122.24	122.95	122.59	117.33	119.33	118.33
T_7	75 % RDN through chemical fertilizer + 25% RDN through poultry manure (PM)	80.20	81.17	80.68	6.12	6.14	6.13	131.81	132.60	132.21	123.00	124.33	123.67
T_8	50 % RDN through chemical fertilizer + 50 % RDN through PM	77.42	78.22	77.82	5.53	5.66	5.60	127.13	127.80	127.46	119.33	120.00	119.67
T_9	100 % RDN through chemical fertilizer through PM + 25% RDN through poultry manure (PM)	81.15	82.17	81.66	6.16	6.16	6.16	133.22	133.45	133.33	124.33	124.67	124.33
T_{10}	100 % RDN through PM	76.23	77.13	76.68	5.45	5.54	5.49	124.76	125.48	125.12	118.00	119.00	118.50
T_{11}	75 % RDN through chemical fertilizer + 25% RDN through Biogas Slurry	78.73	79.40	79.07	5.63	5.70	5.66	129.86	129.80	129.83	120.33	122.00	121.50
T_{12}	50 % RDN through chemical fertilizer + 50 % RDN through Biogas Slurry	76.17	76.80	76.48	5.45	5.52	5.48	123.46	124.36	123.91	117.00	120.00	118.50
T_{13}	100 % RDN through chemical fertilizer + 25 % RDN through Biogas Slurry	79.93	80.40	80.17	6.09	6.13	6.11	131.36	132.04	131.70	122.00	123.00	122.50
T_{14}	100 % RDN through Biogas Slurry	75.50	76.23	75.87	4.92	5.08	5.00	121.01	122.25	121.63	114.67	116.00	115.33
	SEM	0.62	0.63	0.54	0.16	0.11	0.13	0.68	0.52	0.52	1.19	0.59	0.72
	C.D. (0.05)	1.80	1.84	1.56	0.47	0.33	0.37	1.98	1.51	1.50	3.45	1.73	2.08

TABLE 2
EFFECT OF INORGANIC NUTRIENT SOURCES WITH ORGANIC MANURES ON YIELD ATTRIBUTES OF WHEAT

	Yield attributes	Number of spikes plant^{-1}		Pooled	Spike length (cm)		Pooled	Number of grains spike^{-1}		Pooled	1000 grain weight (g)		Pooled
		2023 year	2024 year		2023 year	2024 year		2023 year	2024 year		2024 year	2024 year	
T_1	Control (No nutrient sources)	170.67	171.67	171.17	8.17	8.33	8.25	53.33	54.67	54.00	38.00	37.67	37.83
T_2	100 % RDF (125 kg a^{-1}) from chemical fertilizers	244.67	246.67	245.67	11.50	12.33	11.92	64.00	65.67	64.83	42.67	43.00	42.83
T_3	75 % RDN (93.75 kg ha^{-1}) through chemical fertilizer + 25% RDN through farm yard manure (FYM)	239.67	241.33	240.50	10.50	11.00	10.75	62.00	63.00	62.50	41.33	42.00	41.67
T_4	50 % RDN (62.5 kg ha^{-1}) through chemical fertilizer + 50 % RDN through FYM	235.00	236.67	235.83	10.17	10.83	10.50	60.67	62.00	61.33	40.33	41.33	40.83
T_5	100 % RDN through chemical fertilizer + 25 % RDN through FYM	243.33	244.67	244.00	11.17	12.00	11.58	63.67	65.00	64.33	42.00	42.33	42.17
T_6	100 % RDN through FYM	198.33	199.33	198.83	9.83	10.17	10.00	59.00	60.00	59.50	39.33	40.33	39.83
T_7	75 % RDN through chemical fertilizer + 25% RDN through poultry manure (PM)	244.33	245.33	244.83	11.50	12.17	11.83	63.67	65.00	64.33	42.67	42.67	42.67
T_8	50 % RDN through chemical fertilizer + 50 % RDN through PM	230.00	232.00	231.00	10.50	10.83	10.67	60.33	61.33	60.83	39.67	41.33	40.50
T_9	100 % RDN through chemical fertilizer through PM+ 25% RDN through poultry manure (PM)	245.33	247.33	246.33	12.00	12.50	12.25	64.67	66.00	65.33	43.00	43.00	43.00
T_{10}	100 % RDN through PM	229.00	231.33	230.17	10.00	10.33	10.17	59.33	60.33	59.83	39.33	41.00	40.17
T_{11}	75 % RDN through chemical fertilizer + 25% RDN through Biogas Slurry	239.00	241.33	240.17	10.33	10.67	10.50	62.00	62.67	62.33	41.33	41.67	41.50
T_{12}	50 % RDN through chemical fertilizer + 50 % RDN through Biogas Slurry	233.67	236.00	234.83	9.83	10.33	10.08	58.67	60.33	59.50	40.00	40.00	40.00
T_{13}	100 % RDN through chemical fertilizer + 25 % RDN through Biogas Slurry	243.00	243.67	243.33	11.17	11.83	11.50	63.33	65.33	64.33	42.00	42.33	42.17
T_{14}	100 % RDN through Biogas Slurry	196.00	198.33	197.17	9.67	10.00	9.83	58.33	60.00	59.17	39.33	40.00	39.67
	SEM	1.66	1.29	1.21	0.39	0.31	0.29	0.60	0.50	0.37	NS	NS	NS
	C.D. (0.05)	4.83	3.74	3.51	1.13	0.90	0.84	1.75	1.45	1.07	NS	NS	NS

IV. CONCLUSION

Based on present study it was concluded that the combination of RDF and organic amendments helps to improve the growth and yield attributes of wheat which plays important role in the sustainable agriculture. Moreover, the significant improvement in growth parameters viz. plant height, leaf area index, dry matter accumulation and number of tillers per meter row length and yield parameters such as number of spikes plant^{-1}, spike length and number of grains spike were also recorded under T_9 (100% RDN through chemical fertilizer + 25% RDN through poultry manure) as compared to other treatments. But, 1000 grain weight show no significant variations among all the treatments. The treatment T_1 Control (no nutrient source) recorded minimum results under all the growth and yield attributes of wheat in both the years.

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A Review on the Nutritional and Hepatoprotective Properties of *Madhuca indica* Flower Extracts

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Abstract— *Mahua (Madhuca indica), a member of the Sapotaceae family, is an important economic tree from India. Mahua flowers were rich in bioactive compounds such as carbohydrates, minerals- calcium,iron, antioxidants, flavonoids, and phenolic acids. Mahua flowers are nutritional and have medicinal properties. The paper reviews the Mahua flower's nutritional qualities and health-protective functions in the paracetamol-induced liver damage in the Wistar rat models. Traditional application records indicated that Mahua flowers have anti-inflammatory, anticancer, and anthelmintic properties, and help against the causative agents like bronchitis, diabetes, eczema, and digestive disorders. Mahua seeds arenutritionally significantas a good source of proteins, fats, fiber, different sugars, carbohydrates, and minerals. Mahua methanol extracts have been proven hepatoprotective and non-toxic and can be used to treat liver diseases.*

Keywords— Mahua flower, Nutritional properties, Health Benefits, and Hepatoprotective properties.

I. INTRODUCTION

The genus Madhuca belongs to the Sapotaceae family and is botanically known as *Madhuca indica J.F. Gmel*. The Mahua or Indian butter tree is a self-growing tree with the name meaning "Madhu" for sweet and "indicus" for India from the Sanskrit language. The Mahua tree grows well in mixed deciduous forests in South Asia, including India, Nepal, and Sri Lanka. The tree has several names depending on the area. This evergreen tree is called Mahwa in Hindi, and other familiar names are Mahua, mahwa, mohwa, mohwra, llupai, honey tree, butter tree [English]; illipe, arbre à beurre, bassie, madhuca [French]. The tree has white, juicy blossoms that eventually turn into rectangular, yellow fruits, which are ripe from March to June annually. Mahua is an essential economic resource because of its use in food, medicine, and various industrial processes, including oil extraction, soap and detergent manufacturing, and cosmetics. These substances support several therapeutic applications, including the management of ulcers, tonsillitis, diarrhea, rheumatism, eczema, bleeding gums, skin conditions, and respiratory ailments[1,2]. Because of its galactogenic, analgesic, and vomiting-inducing properties, mahua seed oil treats piles, skin conditions, and pneumonia. With its use, especially in the nutritional field, mahua proves to be a versatile plant. The Mahua seeds have a high percentage of lipids (between 50 and 61%), with the main fatty acids being stearic, oleic, linoleic, and palmitic acids. The seeds also contain considerable protein (16.3–29.4 %).However, it is underutilized due to a lack of processing and consumption data[3]. Mahua flowers also supply essential minerals, including iron, calcium, magnesium, and potassium, and they are a source of carbohydrates that significantly increase calorie intake. Mahua is used in local cuisines and is known for its high-energy seeds. Its blossoms and seeds are used in various ways, enhancing the nutritional landscape[4]. Barfi, kheer, [5,6,], butter [7], candy [8], fermented drinks, and milkshake are just a few of the many products that have been developed from Mahua. [9, 10,], Jam jimjam Jam [11], pickle [12], wine/vermouth [13]. Bioethanol [14,15,16]aging and fining agent [17]. Mahua cake is a byproduct that promotes general health and production by providing wholesome feed for animals. Beyond its apparent advantages, Mahua's bioactive components and antioxidant qualities show promise for improving health. The soil and ambient conditions are among the elements that affect the variances in nutritional and bioactive components in Mahua. The nutritional analysis of Mahua guides its successful incorporation into customs and more comprehensive nutritional plans, opening up new study directions in the food and human health domains. Therefore, this review study aims to comprehend the nutritional and functional characteristics, Hepatoprotective properties, and mahua health advantages. The scientific classification is also discussed below in Table 1.

FIGURE 1: Photographs of the *madhuca indica* tree with yellowish flowers

1.1 Taxonomy:

TABLE 1
SCIENTIFIC CLASSIFICATION OF MAHUA PLANT (*Madhuca indica*)

Kingdom	Plantae
Division	Angiosperms
Clade	Asterids
Order	Ericles
Family	Sapotaceae
Genus	Madhuca
Species	*M.longifolia*

1.2 Mahua Flowers in Nutrition:

The Corolla parts of Mahua flowers are edible and form an article of the diet of tribals. Tribes used to consume raw or cooked flowers, make medical decoctions, and make country liquor. Flowers are rich in sugars, varying with the flower habitat source. Flowers are safe for human consumption and non-toxic in the mouse models.

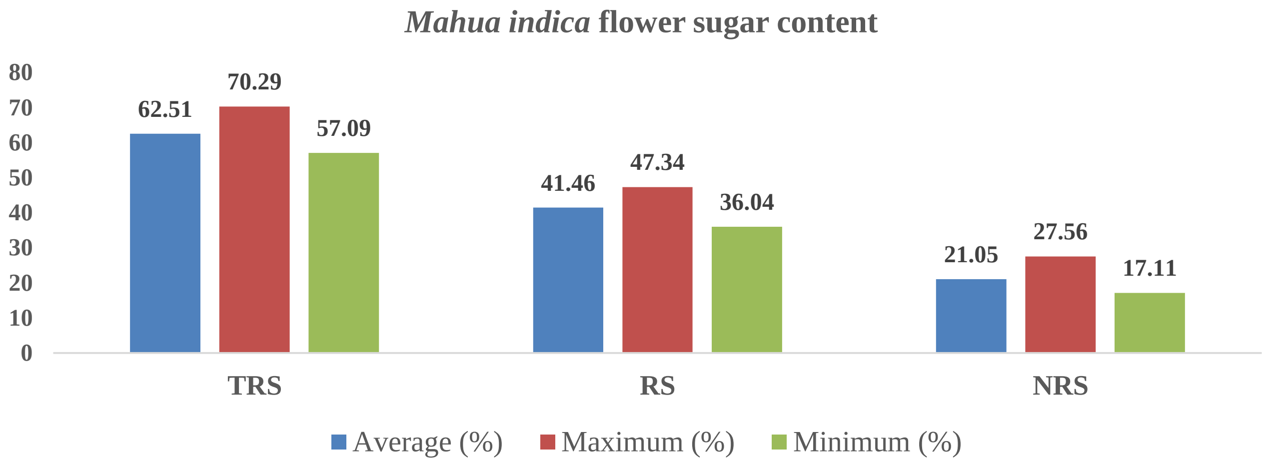

FIGURE 2: Total sugar content in the mahua flowers data collected from suryawanshi & mokat, 2020; trs= total reducing sugar, rs= reducing sugar, nrs =non-reducing sugar.

Flowers have total reducing sugars (TRS), reducing sugars (RS), and non-reducing sugars (NRS). The TRS content ranged from 57.09 to 70.29 %, the RS ranged from 36.04 to 47.34 %, and the NRS ranged from 17.11 to 27.56 % in Mahua flowers.Sugar syrup is prepared by various researchers using dried Mahua flowers that contain sucrose, glucose, fructose, arabinose, maltose, and rhamnose [18].Flowers contain 2-acetyl-1-pyrroline, D-glucose, L-rhamnose, D-xylose, L-arabinose, D-glucuronic acid, stearic acid, lactic acid, myristic acid, arachidic acid, oleic acid, linoleic acid, palmitic acid, as active constituents[19].

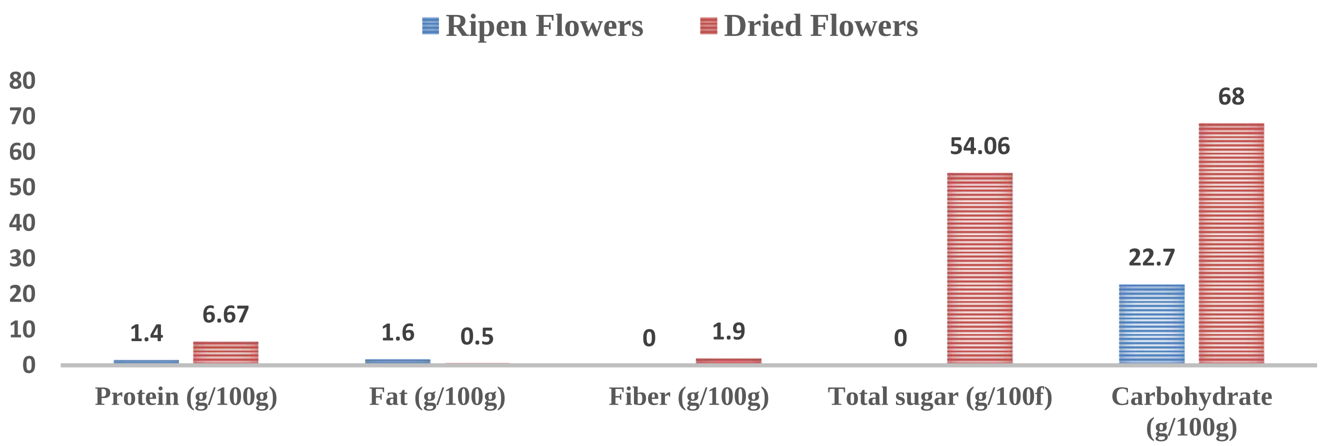

FIGURE 3: Nutritional components in fresh mahua and dried flowers, data collected from [20].

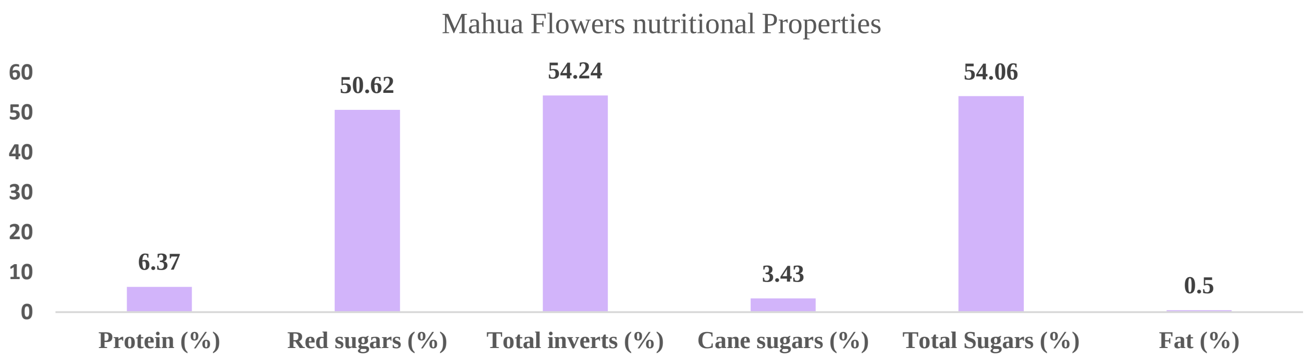

FIGURE 4: Nutritional properties of madhuca longifolia flowers data collected from [21].

II. FUNCTIONAL CHARACTERISTICS:

The mahua tree is regarded as one of the non-timber forest products (NTFP) nutritional powerhouses. All parts of the mahua tree are used to make food goods, make alcohol, make biodiesel, or treat illnesses. The most abundant natural hard fat is mahua fat, which makes chocolate instead of ghee or cocoa butter. Because of its emulsifying qualities, Mahua seed fat is used to make lubricants and laundry soaps. Purification and characteristics of human erythrocyte glutathione peroxidase, Awasthi demonstrate Mahua flowers are rich in nutrients such as reducing and non-reducing sugar, polysaccharides, dietary fibers, protein, fat, minerals, vitamins, enzymes, and other organic acids. It was discovered that dried Mahua flowers have a 40–70% sugar content. The geographical location, flower harvest, and Mahua variety affect the amount of sugar in the blooms [22]. According to Sutaria and Magar, the concentration of reducing and non-reducing sugars varies from 3–18 and 48–57%, respectively. The mahua flower was extracted using both the hydrolyzed and unhydrolyzed extraction methods to extract various polysaccharides [23].

The hydrolyzed extract contained galacturonic acid, maltose, glucose, arabinose, fructose, and rhamnose. There was no sugar in this extract. Polysaccharide structures were also examined after their extraction and separation from the mahua flower. Mahua flowers are used to make fresh juice, and it was found that these flowers are high in sugar and a natural sweetener, namely 1:2.1:2.3 sucrose, glucose, and fructose [24]. The amount of protein and nitrogen fluctuates according to the flower's development stage. Compared to mature flowers, tender flowers are rich in nitrogen content. Protein and nitrogen percentages were found to be 4.4–7% and 0.65–1.1%, respectively. Mahua blooms are high in protein content, and their flowers are high in vital amino acid content [25].

III. FLOWER JUICE UNDER SACCHAROMYCES CEREVISIAE FERMENTATION

Dried mahua flowers are used for alcohol production. The boiled and crushed, de-pulped, and pasteurized flowers were allowed to ferment using *Saccharomyces cerevisiae.* The highest ethanol was produced at 108 hours with 150 rpm purification. Fermentation increased the % alcohol by volume (Fig. 5C) and decreased the total soluble solids (5A), specific gravity (5B), and pH (5D) in the final yield. The sugars fructose, glucose, and sucrose were utilized at different rates during fermentation. The fermented beverage contained flavor compounds of alcohols, terpenes, esters, acids, and ethers [26].

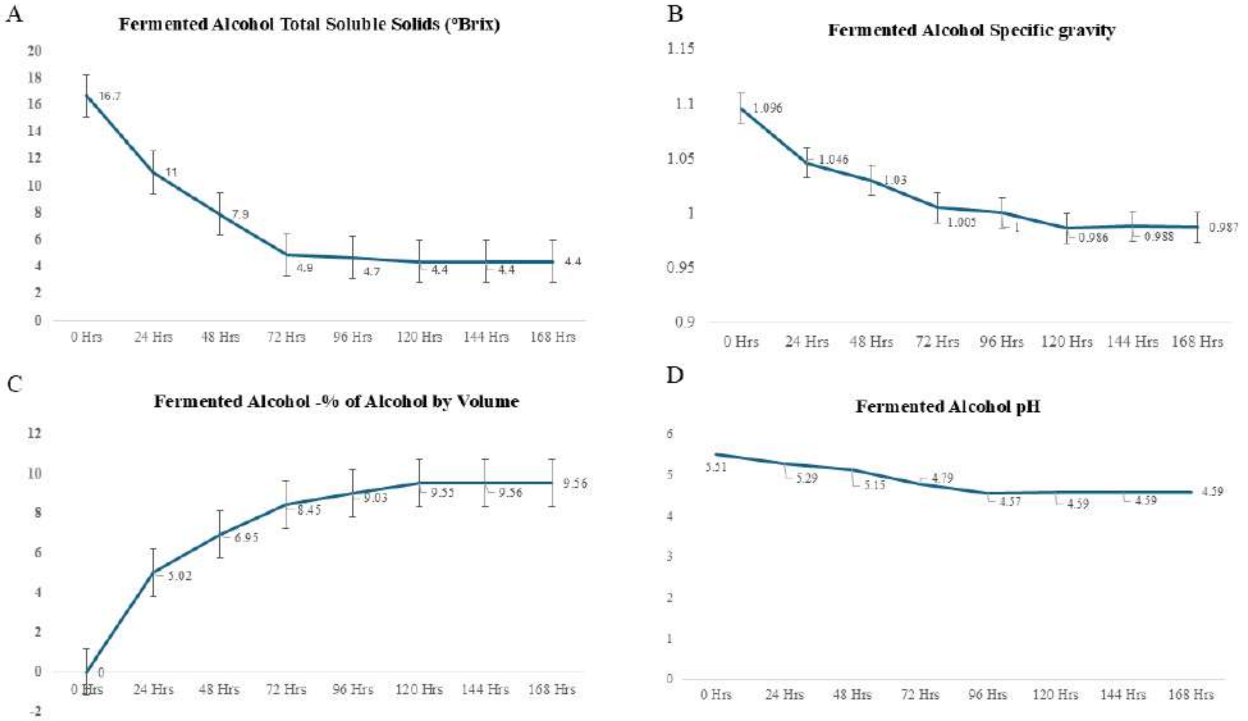

FIGURE 5: Physico-chemical characteristics of fermented alcoholic beverages.

IV. HEALTH BENEFITS OF THE MAHUA PLANT

The Mahua flower (Madhucalongifolia) offers many health benefits (Figure 1), many of which have been traditionally recognized and utilized across generations. Here is a detailed explanation of some of its remarkable therapeutic uses:

- **Bronchitis:** Chronic bronchitis, a disorder characterized by ongoing airway inflammation, can be effectively treated with mahua flowers. Because of its calming qualities, eating the blossoms can help reduce cough and other respiratory problems Orchitis
- **Testis Inflammation:** Madhuca leaves are boiled to treat orchitis by lowering inflammation and accelerating healing. This conventional use demonstrates the plant's possible anti-inflammatory qualities.
- **Rheumatism:** A decoction prepared by boiling the bark in water can be used internally to relieve rheumatic symptoms. Additionally, to relieve pain and inflammation brought on by injured joints or tissues, Madhuca seed oil is administered to the afflicted regions.
- **Diabetes Mellitus:** Madhuca bark decoction has demonstrated promise in controlling blood sugar levels, making it advantageous for diabetics. Frequent ingestion could aid in the natural stabilization of blood glucose levels.
- **Pile:** Madhuca seed oil helps those with persistent constipation and piles by acting as a natural laxative. Its calming properties can aid in bowel regularity and pain reduction.
- **Eczema:** Madhuca leaves can help with eczema when they are cooked and sprayed with sesame oil. Applying the prepared leaves to the afflicted region of the skin aids in wound healing, inflammation reduction, and irritation relief.
- **Gums:** The liquid extract made from Madhuca bark can be gargled after being diluted with water (4 milliliters extract to 300 milliliters). This medication promotes gum health by successfully reducing bleeding, spongy gums, and other dental discomforts.
- **Tonsillitis:** The remedy for gum disease also aids in treating pharyngitis and both acute and chronic tonsillitis. It is a multipurpose treatment that reduces inflammation and soothes sore throats.
- **Burns:** Ghee and leaf ash are administered to the afflicted region to treat burns and scalds. Bark paste is often used to improve healing and lessen irritation.
- **Lactation:** Madhuca seeds and blossoms are thought to help nursing moms produce more milk. Because of this characteristic, it is beneficial for promoting breastfeeding and guaranteeing that babies receive better nutrition.
- A veritable gold mine of health benefits, the mahua plant can help with digestive, skin, and respiratory disorders, among other ailments. Its uses demonstrate the diversity of traditional medicine and the need for more research to support and broaden its application [27].

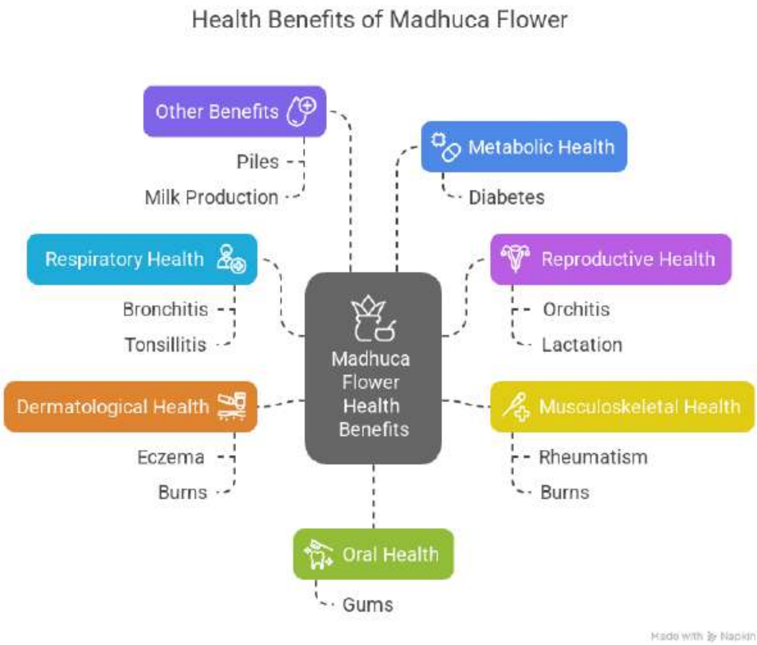

FIGURE 6: Demonstrate the wide health benefits of the mahua flower (*Madhucalongifolia)*

V. MEDICINAL POTENTIAL OF MAHUA FLOWERS

Traditional medicine has long acknowledged the pharmacological qualities of Mahua flowers (Madhucalongifolia), and current research endeavors seek to confirm their therapeutic advantages in various applications.

Anthelmintic properties: Mahua has been investigated extensively, especially regarding how well they work against parasitic worms. The ethanol and methanol extracts of Madhucalongifolia flowers have been shown to have strong worm-killing effects in studies on the Indian earthworm, *Pheretimaposthuma*. The study by Katiyar *et al.* (2011) supported the traditional therapeutic usage of these extracts by confirming their effectiveness against helminthic illnesses. The anthelmintic effects of saponins, flavonoids, alkaloids, and tannins are among the bioactive substances found in mahua flowers. These substances cause paralysis and death in parasites by interfering with their metabolic processes. With encouraging outcomes, the effectiveness of Mahua flower extracts has been contrasted with that of common anthelmintic medications such as piperazine citrate and albendazole [28].

Antioxidant activity: Ethanolic extracts of Madhucasylvestris bark demonstrate the strong antioxidant qualities of mahua flowers. Both in vitro and in vivo methods were used to evaluate the antioxidant activities. The extracts demonstrated strong reducing power and free radical scavenging capabilities against superoxide and hydroxyl radicals in vitro. Reduced lipid peroxidation and elevated tissue glutathione (GSH) levels in vivo validated the extract's antioxidant efficacy. In order to compare the results with ascorbic acid, a well-known natural antioxidant, experiments used techniques such as DPPH free radical scavenging activity, reducing power tests, and superoxide scavenging assays. Additionally, at 500 mg/kg and 750 mg/kg body weight, ethanolic extracts of Madhucasylvestris leaves showed protective properties against acetaminophen-induced toxicity in rats [6].

Anticancer activity: Research also shows that Mahua leaves have cytotoxic properties. Several *in-vitro* cytotoxic tests assessed acetone and ethanol extracts against Ehrlich Ascites Carcinoma (EAC) cell lines. Compared to the acetone extract, the ethanol extract showed more cytotoxic action, suggesting it might be used in cancer treatment. The findings corroborate the anticancer potential of Madhucalongifolia, opening the path for future study into its active components [29].

Analgesic properties: Madhucasylvestris flowers' have been assessed using tail-flick, hot plate, and chemical writing techniques. Studies on rats and mice showed that both alcoholic and aqueous extracts had dose-dependent analgesic effects. Similarly, when examined utilizing an acetic acid-induced nociception response, the crude methanolic extract from *Madhucaindica* aerial portions demonstrated analgesic effectiveness [30].

Cytotoxic properties: A brine shrimp lethality experiment evaluated Madhucalongifolia. When compared to vincristine sulfate as a reference (LC_{50} = 8.84 µg/ml), the crude extracts, especially those from the leaves and bark, showed considerable cytotoxicity with LC_{50} values of 17.09 µg/ml and 45.96 µg/ml, respectively [31].

Antiulcer properties: Methanolic extracts of Madhucasylvestris were investigated for their antiulcer properties at different oral dosages (100, 200, and 400 mg/kg) in rat models of pylorus ligation, ethanol-induced, and naproxen-induced stomach ulcers. The results demonstrated that by strengthening the protective mucin layer and lessening the negative effects of pepsin and stomach acid, the methanolic extract from Madhucaindica leaves had potent antiulcer benefits. In rats with pylorus-ligated ulcers, ethanolic extracts of Madhucalongifolia flowers also showed promise. In summary, Mahua flowers have a variety of pharmacological activities, such as anthelmintic, antioxidant, and anticancer qualities that support their traditional ethnomedical uses. These findings highlight the necessity of more research to completely understand this amazing plant's medicinal potential [32].

Hepatoprotective properties: The shade-dried flowers' methanol extract was hepatoprotective in the Wistar Albino rat models. Umadevi et al. (2011) reported that methanol extracts are non-toxic and decrease the paracetamol-induced liver markers, such as serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), serum alkaline phosphatase (ALKP), and total bilirubin. Methanol extracts decreased the elevated SGOT (Fig. 6A), SGPT (6B), ALKP (6C), and bilirubin levels (6D) at 200 mg/kg doses in the Wistar rats [33].

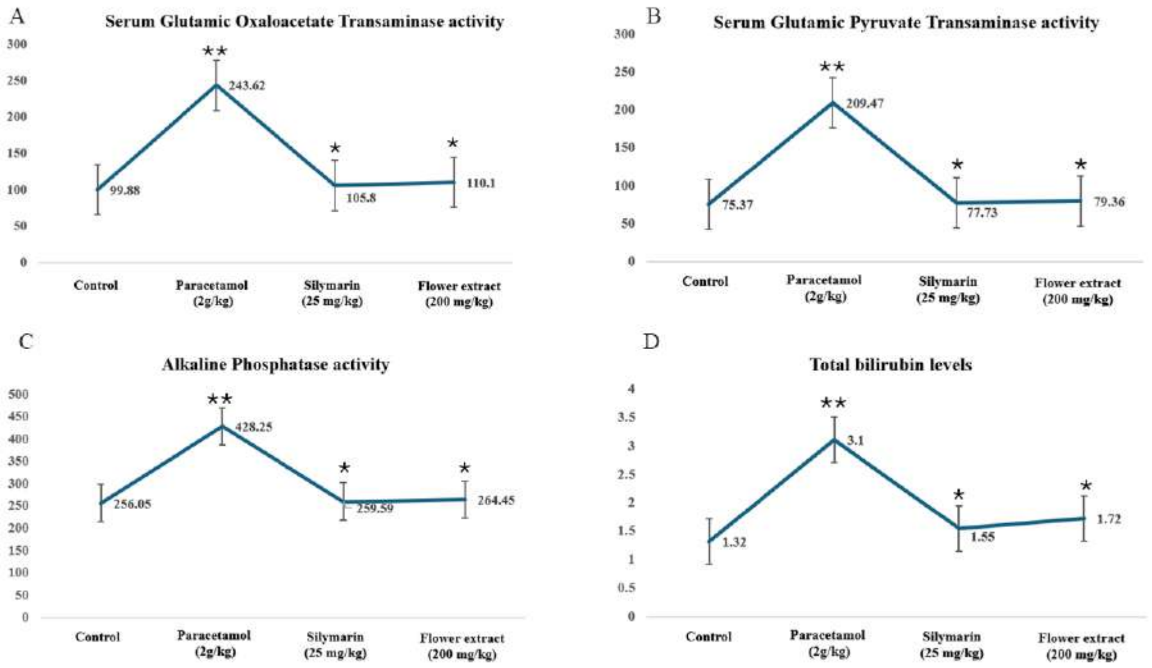

FIGURE 7: Effect of flower extracts on liver markers such as sgot (iu/l), sgpt (iu/l), alkaline phosphatase activity (iu/l), and total bilirubin content (mg/dl); data from (27).

VI. CONCLUSION

The Mahua tree (Madhucaindica) is a unique resource that is neglected while having enormous potential for industrial, medicinal, and nutritional uses. Every component has a special use, from its nutrient-dense seeds and fragrant blooms to its bark and fruits. Rich in bioactive substances, including flavonoids, phenolic acids, and saponins, mahua has pharmacological, anti-inflammatory, and antioxidant qualities, making it useful for treating conditions like eczema, diabetes, and bronchitis. Value-added products from Mahua, such as jams, alcoholic drinks, and baked goods, demonstrate the region's economic potential. Mahua is still underutilized despite its many advantages, and a lack of infrastructure and knowledge hampers its widespread adoption. Mahua offers an incredible chance for sustainable practices and broad growth. Educating people about its nutritional and health advantages through seminars and advertising is essential to maximizing its potential. Product development should concentrate on producing Mahua-based products, including snacks, drinks, and dietary supplements to take advantage of its adaptability. Working with local communities can ensure the preservation of indigenous knowledge while combining conventional wisdom with modern techniques. A significant way to fight hunger and improve food security is to incorporate Mahua into nutrition programs, such as school meals and community projects.Mahua flowers were protective against paracetamol-induced hepatotoxicity. As suggested by the research authors, further experiments are needed to use them on the human population.

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Awareness and Knowledge about Vitamin D among College Students: A Review

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***Abstract*—** *Vitamin D deficiency constitutes a widespread public health issue, especially among college students whose lifestyles frequently restrict sun exposure and dietary consumption of this essential nutrient. This review investigates the awareness of Vitamin D among college students in Lucknow, a swiftly urbanizing metropolis in North India, by a systematic analysis of global and regional studies. This research identifies substantial knowledge gaps by synthesizing data from many contexts and applying them to Lucknow's distinct socio-cultural and environmental backdrop, despite a foundational awareness of Vitamin D's significance. Elements including academic discipline, gender, dietary practices, and availability of health education influence awareness levels, revealing significant differences among demographics. The paper examines the ramifications for health promotion, identifies obstacles to knowledge diffusion, and suggests customized interventions to bridge these gaps. This report provides a detailed analysis, highlighting the immediate necessity for specific educational interventions to reduce Vitamin D insufficiency risks among college students in Lucknow.*

***Keywords*— *Vitamin D, awareness, knowledge, college students, public health, health education, deficiency*.**

I. INTRODUCTION

1.1 The Multifaceted Role of Vitamin D in Human Health:

Vitamin D, sometimes referred to as the "sunshine vitamin," is a fat-soluble nutrient distinguished by its dual role as both a dietary element and a hormone produced by the body. The principal source is endogenous synthesis in the skin, where ultraviolet B (UVB) radiation from sunshine transforms 7-dehydrocholesterol into cholecalciferol (Vitamin D3). The molecule undergoes hydroxylation in the liver to make 25-hydroxyvitamin D (25(OH)D), the circulating form utilized to evaluate Vitamin D status, and is subsequently activated in the kidneys to produce 1,25-dihydroxyvitamin D (calcitriol), the physiologically active form. This complex metabolic route highlights Vitamin D's function as a prohormone, engaging with Vitamin D receptors present in almost all tissues, including bones and brain cells. Historically acknowledged for its function in calcium regulation and skeletal health, preventing rickets in children and Osteomalacia or osteoporosis in adults, the importance of Vitamin D has significantly increased in recent decades. As Fig.1 shows the important role and deficiency factors of Vitamin D. Also, research has demonstrated its role in immunological regulation, decreasing the likelihood of diseases such as tuberculosis and influenza by augmenting innate immunity via cathelicidin synthesis. In addition to its role in immunity, Vitamin D affects cell proliferation and differentiation, providing preventive benefits against malignancies, including colorectal and breast cancer. Cardiovascular health is enhanced, since research associates sufficient Vitamin D levels with reduced risks of hypertension and atherosclerosis, facilitated by its anti-inflammatory effects and modulation of the renin-angiotensin system. Mental health constitutes another domain where the influence of Vitamin D is becoming increasingly apparent. Reduced levels have been linked to depression, anxiety, and seasonal affective disorder, disorders common among college students experiencing scholastic stress. Anglin et al. [2] conducted a meta-analysis that revealed a statistically significant association between Vitamin D insufficiency and depressive symptoms, indicating a potential neurobiological function perhaps related to vitamin D receptors in the hippocampus and prefrontal cortex. These findings are especially relevant for young adults, whose cerebral development persists into their early twenties, rendering Vitamin D an essential nutrient during this period. In India, despite enough sunlight, Vitamin D insufficiency impacts 70-100% of the population, a contradiction influenced by urbanization, pollution, and cultural habits. College students in urban centre's such as Lucknow exemplify this concern, as their scholastic obligations and indoor lifestyles restrict sun exposure, while food sources are limited in conventional vegetarian diets. This section examines these dynamics comprehensively, establishing a foundation for comprehending awareness levels among college students in Lucknow.

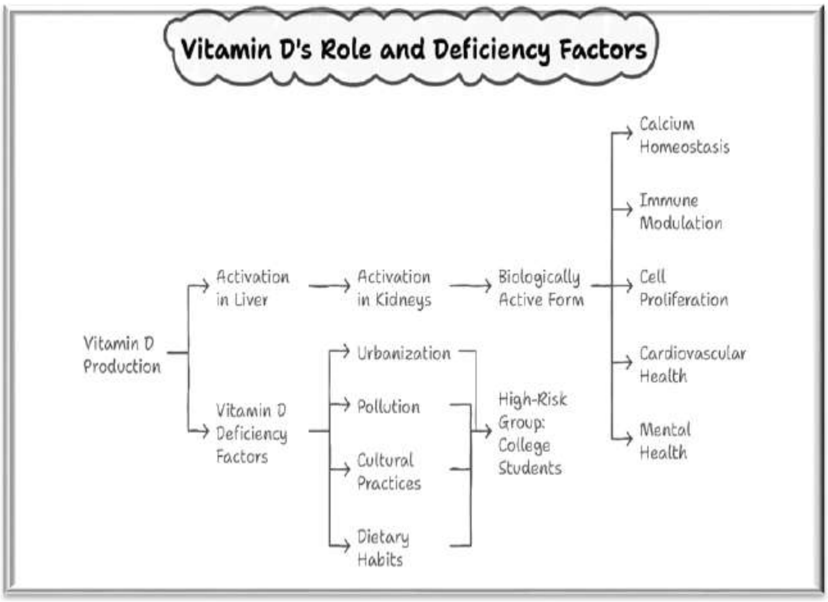

FIGURE 1: Vitamin D's Role and Deficiency Factor

1.2 Global and Indian Context of Vitamin D Deficiency:

Worldwide, Vitamin D insufficiency constitutes a silent epidemic, impacting nearly one billion individuals, according to Holick [9]. In affluent countries, the fortification of foods such as milk and cereals has alleviated severe deficiencies; yet, inadequate amounts remain due to diminished sun exposure resulting from sedentary lifestyles and the application of sunscreen. Conversely, developing nations such as India encounter a distinct challenge: plentiful sunshine is compromised by environmental and behavioural influences. Research demonstrates that 25(OH)D levels below 20 ng/ml, classified as inadequate, are prevalent throughout all age demographics in India, with urban populations being especially susceptible. The Indian context is characterized by a convergence of variables. Urbanization has concentrated populations inside, diminishing UVB exposure vital for Vitamin D production. Air pollution, a critical concern in urban areas such as Lucknow, disperses and absorbs UVB rays, hence reducing cutaneous synthesis. Cultural conventions intensify this phenomenon: the inclination towards pale skin promotes sunscreen application and sun evasion, while conventional attire, particularly for women, conceals the majority of the body. Harinarayan et al. [8] discovered that, despite the abundant sunlight in southern India, insufficiency rates surpass 80% owing to these obstacles. Dietary consumption provides minimal relief. Natural sources of Vitamin D, such as fatty fish, egg yolks, and liver, are scarce in predominantly vegetarian Indian diets, particularly in regions like Uttar Pradesh. Fortified foods, although accessible in metropolitan markets, are rarely extensively consumed, especially by budget-conscious students dependent on hostel meals or street food. Ritu and Gupta [18] emphasize that fortified milk is underutilized due to cost and awareness deficiencies, resulting in supplementation being infrequently pursued. The dietary deficiency, along with environmental limitations, categorizes college students as a high-risk demographic, warranting a more thorough investigation of their knowledge.

1.3 Challenges Specific to College Students in Lucknow:

Lucknow, the capital of Uttar Pradesh, amalgamates heritage and modernity, including esteemed institutions such as Babasaheb Bhimrao Ambedkar University, Lucknow. College students, numbering in the tens of thousands, encounter distinct problems that exacerbate the hazards of Vitamin D insufficiency. Academic timetables are demanding, involving extensive hours in classrooms, libraries, or dormitories, which limit sun exposure. A proposed day schedule may encompass morning lectures, afternoon study periods, and evening tasks, resulting in minimal opportunity for outdoor pursuits. Even physical education sessions, when available, are frequently conducted indoors or during early morning hours when UVB rays are inadequate. Environmental factors exacerbate this condition. Lucknow is classified as one of India's most polluted cities, with particulate matter (PM2.5) concentrations often beyond permissible thresholds. This pollution disperses UVB photons, diminishing their availability for Vitamin D production. Research conducted by Harinarayan et al. [8] in Hyderabad, a similarly polluted urban centre, revealed that air aerosols reduced UVB penetration by as much as 50%, a phenomenon presumably reflected in Lucknow. Seasonal fluctuations, including the city's fog-laden winters, significantly restrict sunshine, especially from November to February, coinciding with students' peak academic engagement. The conservative mindset of Lucknow culturally impacts student behaviour. Female students, comprising a substantial segment of the college demographic, frequently don full-length garments such as salwar kameez or sarees, thereby minimizing skin exposure. The cultural preference for pale skin deters tanning, especially among male students who may otherwise participate in outdoor sports. Dietary practices mirror regional conventions: vegetarianism is predominant, with hostel menus primarily featuring grains, lentils, and vegetables, lacking Vitamin D-rich items. Fast food, a mainstay for students, provides calories without any nutritious compensation for this deficiency. Socioeconomic diversity introduces an additional dimension. Students from wealthy homes may utilize supplements or fortified items, whilst those from lower-income backgrounds depend exclusively on sunlight and limited nutritional sources. This discrepancy undoubtedly affects awareness, as affluent students may get health information via private healthcare or media, in contrast to their counterparts reliant on public resources. These problems underscore the necessity to evaluate and improve Vitamin D awareness among college students in Lucknow.

1.4 The Importance of Awareness in Preventive Health:

Awareness is the cornerstone of preventative health, connecting information and action. The Health Belief Model (HBM) asserts that behavioural modification depends on perceived susceptibility, severity, advantages, and obstacles. Students must identify their risk factors for Vitamin D deficiency (e.g., sedentary indoor lifestyles), comprehend the potential repercussions (e.g., osteoporosis, depression), acknowledge the advantages of proactive measures (e.g., enhanced immunity), and surmount obstacles (e.g., time limitations). However, awareness is inadequate without precise, actionable knowledge. Research indicates that college pupils, notwithstanding their degree, possess misunderstandings regarding Vitamin D. Srinivasan et al. [22] discovered that several individuals overestimate the necessary sun exposure, supposing that several hours are essential, whereas 15-30 minutes of midday exposure on the face and arms is adequate for lighter skin tones (darker complexion, common in India, necessitates more). Many individuals are oblivious to food sources or supplements, mistakenly believing that sunlight alone suffices for their requirements—an erroneous assumption in polluted, metropolitan environments. These deficiencies highlight the necessity for focused education that dispels misconceptions and offers pragmatic solutions.

In Lucknow, awareness is further hindered by restricted health literacy beyond medical fields. Non-medical students, including engineers, artists, or commerce majors, may lack exposure to nutritional science and depend on informal sources such as peers or social media, potentially perpetuating misconceptions. Manandhar et al. [14] discovered that even medical students possess only moderate knowledge, indicating that awareness efforts should be comprehensive yet customized. This review seeks to analyse these dynamics, providing a basis for actions that align with Lucknow's varied student population.

1.5 Objectives of the Review:

This study aims to achieve four principal objectives:

- **Evaluate Awareness Levels:** Determine the existing knowledge of Vitamin D among college students in Lucknow, integrating global and Indian data to infer local trends.
- **Ascertain Determinants:** Identify determinants affecting awareness, including education, gender, and socioeconomic status, to inform targeted interventions.
- **Examine Demographic Disparities:** Investigate variations in awareness among subgroups, pinpointing inequalities that require intervention. And Suggest Interventions: Identify research deficiencies and propose practical measures to improve awareness, specifically suited to the context of Lucknow. This comprehensive analysis aims to highlight a significant public health concern, promoting educated and proactive health behaviours among college students. To enhance health
- maintenance and address vitamin D deficiency, one should incorporate comprehensive dietary sources of vitamin D, including natural food sources, fortified foods, and supplements administered in appropriate dosages and formulations

as recommended by a healthcare professional tailored to the individual patient. Some sources are illustrated in Table 1. Also factors which influences the vitamin D levels shown in fig.2

TABLE 1
SOURCES OF VITAMIN D, THEIR TYPES, AND ESTIMATED INTAKE LEVELS

Source Type	Examples	Vitamin D Content	Type of Vitamin D	Approximate Dose (IU per serving)
Natural Food Sources	Fatty fish (salmon, mackerel, tuna, sardines)	High	Vitamin D3 (Cholecalciferol)	400–600 IU per 3.5 oz (100g)
	Egg yolks	Moderate	Vitamin D3 (Cholecalciferol)	40–50 IU per yolk
	Mushrooms (especially sun-exposed)	Moderate	Vitamin D2 (Ergocalciferol)	200–500 IU per 3.5 oz (100g)
	Cod liver oil	Very High	Vitamin D3 (Cholecalciferol)	1,360 IU per 1 tablespoon
Fortified Foods	Fortified dairy products (milk, yogurt, cheese)	Varies (depends on fortification level)	Vitamin D3 (Cholecalciferol)	100–150 IU per cup (milk)
	Fortified plant-based milk (soy, almond, oat)	Varies	Vitamin D2 or D3 (Varies by brand)	100–150 IU per cup
	Fortified cereals and orange juice	Moderate	Vitamin D2 or D3 (Varies by brand)	40–100 IU per serving
Supplements	Vitamin D2 and D3 supplements	Controlled dose	Vitamin D2 or D3 (Depends on the supplement)	400–5,000 IU per dose (varies)
Sunlight Exposure	UVB rays from sunlight	Essential for skin synthesis	Helps skin synthesize Vitamin D3	10,000–25,000 IU (15–30 min midday sun exposure)

Note: The figures of International Units (IU) are estimations and may fluctuate according on the brand, fortification levels, and portion size.

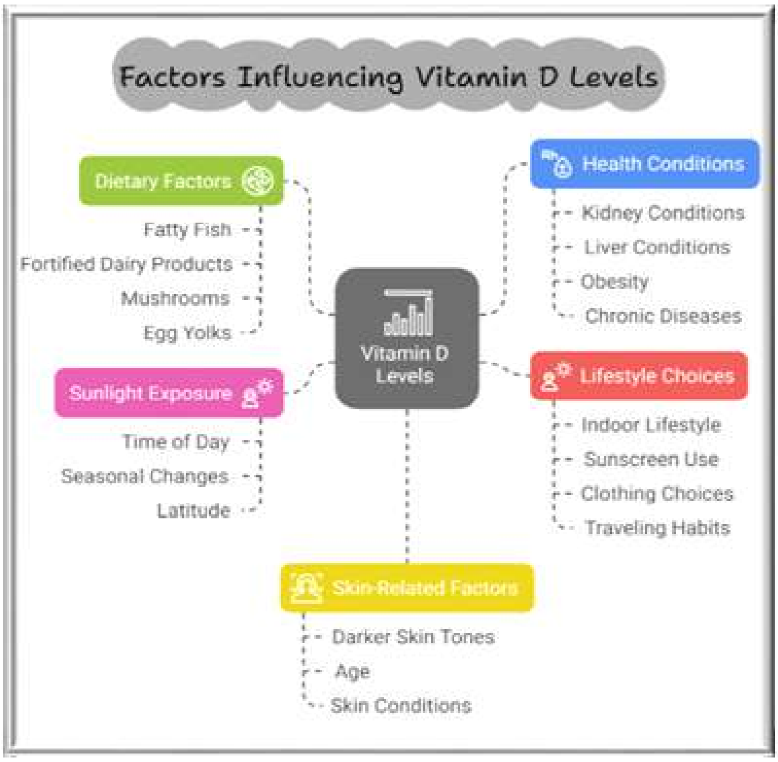

FIGURE 2: Factors Influencing Vitamin D Levels

II. METHODOLOGY

2.1 Thorough Literature Retrieval Approach:

A systematic literature search was performed across three principal databases: PubMed, Google Scholar, and Scopus, to establish a solid foundation for this review. These platforms were selected for their comprehensive coverage of biological, public health, and educational research, guaranteeing a wide but pertinent array of studies. The search encompassed literature from January 2010 to October 2023, capturing current insights about Vitamin D knowledge while omitting obsolete perspectives. Only English-language publications were chosen to ensure analytical consistency; however, this decision recognizes the possible omission of regional research in Hindi or other languages. The search approach utilized a combination of keywords and Boolean operators to enhance results. Key phrases encompassed “Vitamin D,” “awareness,” “knowledge,” “college students,” “university students,” “young adults,” “health education,” “India,” “South Asia,” and “urban populations.” To refine the findings, terms such as “Lucknow,” “Uttar Pradesh,” and “North India” were incorporated; nonetheless, they produced limited direct results, requiring further extrapolation. The filters narrowed the search to peer-reviewed publications, cross-sectional studies, and surveys, emphasizing empirical data rather than reviews or opinion pieces. The preliminary search yielded more than 500 papers, indicating the global scientific significance of Vitamin D. A two-stage screening process occurred. Initially, titles and abstracts were assessed according to inclusion criteria, excluding research centred on clinical outcomes (e.g., prevalence of deficiencies without awareness data) or unrelated demographics (e.g., children, old individuals). This narrowed the selection to 120 studies. Subsequently, full texts were examined for their pertinence to college students and Vitamin D understanding, resulting in the selection of 10 principal papers for examination. Prominent among these was the study by Manandhar et al. [14], a Nepalese investigation involving medical students, chosen for its scientific rigor and geographical relevance to North India. Supplementary searches in grey literature, including conference proceedings and university theses, conducted through Google Scholar, produced minimal peer-reviewed material, hence underscoring dependence on official publications. The procedure was iterative, with search criteria modified (e.g., include “nutrition education”) to encompass related research, so establishing a broad evidence base despite the lack of Lucknow-specific data.

The synthesis employed a thematic methodology, categorizing data into "awareness levels," "determinants," "demographic variations," and "gaps." A narrative synthesis amalgamated findings, juxtaposing knowledge across research (e.g., medical versus non-medical students) and contrasting regional influences (e.g., South Asia versus the Middle East). Quantitative synthesis, including meta-analysis, was hindered by different measurements; nonetheless, qualitative patterns were thoroughly analysed. Due to the data deficiency in Lucknow, the conclusions were extrapolated.

Employing proxy cities such as New Delhi and Mumbai while accounting for pollution, vegetarianism, and educational facilities. Hypothetical situations, such as a survey including 300 students from Lucknow, were developed based on averages from analyzed research to estimate local awareness. This adaptive synthesis harmonized empirical rigor with contextual significance.

III. RESULTS OR KEY FINDINGS

3.1 Comprehensive Examination of Chosen Research Ten studies constituted the evidence foundation, each analysed for methodology, findings, and applicability to Lucknow:

Manandhar et al. [14] conducted a survey including 157 first-year medical students in Nepal, encompassing MBBS, BDS, and Nursing programs. A 51-point questionnaire was utilized, revealing that 13.3% exhibited strong knowledge, 73.9% demonstrated average knowledge, and 12.8% displayed low knowledge. Strengths: comprehensive scoring; limitations: limited sample size, novice pupils.

Lhamo et al. [13] evaluated 200 Indian medical students in New Delhi, observing inadequate awareness of dietary sources, with just 40% correctly identifying fish. Strengths: urban emphasis; limitations: only medical sample.

Junaid et al. [11] conducted a survey of 340 Pakistani medical students, revealing that 54% exhibited proficient knowledge. Strengths: substantial sample size; limitations: curricular bias.

Alshamsan et al. [1] conducted a study involving 250 Saudi students, revealing that 72.3% were aware of the roles of bone health, although only 30% identified its sources. Strengths: comprehensive inquiries; limitations: regional specificity.

- Nowreen and Hameed Examined Indian female students (n=180), correlating awareness with health education. Strengths: emphasis on gender; limitations: restricted scope.

- Safdar et al. [19]: Australian healthcare students (n=200) exhibited enhanced knowledge following educational intervention. Strengths: insights from interventions; limitations: context outside of India.

Srinivasan et al. [22]: A study involving 220 Indian students revealed the prevalence of misconceptions regarding sun exposure. Strengths: erroneous data; limitations: extensive demographic.

- Ajay Gupta [18]: Analysed the factors contributing to deficiencies in India. Strengths: contextual profundity; limitations: lack of specificity regarding consciousness.
- Christie and Mason [6]: UK citizens (n=160) exhibited enduring disparities. Strengths: comparative perspective; limitations: lack of student emphasis.

These research, however varied, offer a framework for hypothesizing Lucknow's awareness landscape, tailored to urban similarities and cultural intersections.

3.2 Degrees of Vitamin D Awareness:

Awareness exhibited considerable variability. Manandhar et al. [14] found that 13.3% of medical students have high knowledge, while 73.9% shown average adequacy for basic recognition (e.g., bone health), but exhibited deficiencies in specifics (e.g., UVB duration). Junaid et al. [11] observed that 54% exhibited proficient knowledge, presumably attributable to exposure to an advanced curriculum, whereas Lhamo et al. [13] reported a superficial comprehension with just 40% dietary awareness. Alshamsan et al. [1] corroborated this finding: 72.3% were aware of skeletal functions, whereas knowledge of sources was only 30%.

3.3 Factors and Indicators:

Prominent influencers emerged:

Medical students exhibited superior performance compared to their peers, with BDS students demonstrating 17.9% proficiency in knowledge, while Nursing students achieved 8.5%.

- **Gender:** Females had greater awareness [15], potentially attributable to health-seeking behaviour.
- **Educational Access:** Workshops enhanced understanding [19].
- **Diet:** Non-vegetarians possessed greater knowledge of sources [1].
- **Socioeconomics:** Affluent pupils are likely to utilize superior resources (implied). D. Demographic Variations
- **Discipline:** Medical (77.1% average) compared to Nursing (74.3%) and BDS (66.7%) as reported by Manandhar et al. [14].
- **Gender:** Females 65% favourable versus Males 45% [15].
- **Diet:** Non-vegetarians surpassed vegetarians in performance [1].
- **Residence:** Hostel students may experience delays owing to dietary and sunlight limitations (hypothesized).

3.4 Research Deficiencies:

- **Absence of Local Data:** Studies particular to Lucknow are lacking.
- **Cross-Sectional Bias:** Absence of longitudinal insights.
- **Intervention Scarcity:** Limited assessments of educational effectiveness.
- **Cultural Depth:** The influence of norms remains inadequately examined.

These limitations necessitate primary research in Lucknow to corroborate projections.

IV. DISCUSSION

4.1 Analysing Findings through Public Health Perspectives:

The moderate awareness levels (e.g., 73.9% average in Manandhar et al. [14]) correspond with the Diffusion of Innovations theory: early adopters (medical students) comprehend the significance of Vitamin D, while laggards (non-medical individuals) do not. Social Cognitive Theory posits that self-efficacy discrepancies exist—students recognize the importance of Vitamin D yet lack the competencies to implement effective actions (e.g., maximizing sun exposure). In Lucknow, this inadequate understanding jeopardizes health due to the widespread presence of deficiencies.

4.2 Implications for Health Promotion:

- **Curriculum:** Incorporate Vitamin D into all curricula.

- **Peer Education:** Employ female medical students as representatives.

Utilize social media platforms for initiatives aimed at dispelling myths.

- **Health Camps:** Conduct screenings and provide education on campus.

4.3 Obstacles:

- **Misinformation:** Myths around sun exposure endure.
- **Lifestyle:** Indoor activities restrict solar exposure.
- **Environment:** Pollution obstructs UVB radiation.
- **Cultural norms around attire and preferences for fair skin limit exposure:** Proposed solutions encompass concise, secure sun exposure recommendations and the advocacy of supplements.

4.4 Suggestions:

- **Local Surveys:** Evaluate students in Lucknow directly.
- **Interventions:** Evaluate workshops and applications.
- **Cultural Studies:** Examine the influence of norms.
- **Longitudinal Research:** Monitor changes in awareness.

V. CONCLUSION

Vitamin D is essential for preserving bone health, immune function, and mental wellness; nevertheless, its shortage is a growing issue among college students in Lucknow. This research highlights a significant public health issue: despite the broad acknowledgment of Vitamin D's significance, students' awareness remains insufficient and lacking. Research such as that conducted by Manandhar et al. [14] indicates that medical students, anticipated to possess comprehensive health knowledge, have just a moderate level of understanding, with merely 13.3% displaying "good" knowledge. The disparity is much more pronounced among non-medical students, who frequently lack access to fundamental nutritional knowledge. Various factors influence this understanding, including academic discipline and gender, whereas obstacles such as misconceptions regarding sun exposure and lifestyle limitations impede advancement. In Lucknow, a city obscured by pollution that obstructs UVB rays, together with cultural norms that restrict sun exposure and diets deficient in Vitamin D, pupils encounter increased dangers. The repercussions are significant—osteoporosis, compromised immunity, and mental health disorders pose substantial risks to both individual prospects and social welfare. Absent prompt intervention, this covert epidemic may inundate healthcare systems and impair the prospects of an entire generation. Nevertheless, remedies are attainable. Colleges must incorporate Vitamin D teaching throughout all curricula, establishing it as a fundamental element rather than a secondary consideration. Social media channels, extensively utilized by students, provide a significant opportunity to dispel misconceptions and advocate for evidence-based practices such as safe sun exposure and dietary modifications. Additionally, localized research is necessary to identify Lucknow's distinct difficulties, including regional eating habits and environmental influences, and to develop customized solutions. Educators, legislators, and health researchers must unite to close the awareness gap. By converting disjointed knowledge into effective action, we can protect the well-being of Lucknow's college students and ensure a more robust, healthier future for the city.

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Use of Artificial Intelligence and IoT for Seed Quality Testing

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Abstract— *The quality of seeds is a fundamental factor in ensuring agricultural productivity and food security. Traditional seed quality assessment methods, such as manual inspection and laboratory-based testing, are often time-consuming, labor-intensive and prone to human error. To overcome these limitations, modern agricultural technologies have increasingly integrated Artificial Intelligence (AI) and the Internet of Things (IoT) to enhance seed quality evaluation. AI-driven models, including deep learning and computer vision techniques, have demonstrated high accuracy in detecting seed defects, predicting germination potential and classifying seeds based on various parameters (Kundu et al., 2021). Additionally, IoT-based smart sensors enable real-time monitoring of critical environmental factors such as humidity, temperature and storage conditions, ensuring optimal seed preservation (Kler et al., 2023). The fusion of AI and IoT facilitates automated, high-throughput and non-destructive seed quality testing using hyperspectral imaging, near-infrared spectroscopy and cloud-based analytics. While these advancements offer numerous benefits, challenges such as high implementation costs, data security concerns and the need for technical expertise hinder widespread adoption. This review demonstrate that integrating IoT-driven sensing with advanced AI methods offers a scalable, objective solution for seed certification, with potential extensions to disease detection, phenotypic trait analysis, and adaptive sorting in commercial processing lines.*

Keywords— ***Seed quality, Artificial Intelligence (AI), Internet of Things (IoT) sensing, Deep learning, Smart sensors and Hyperspectral imaging.***

I. INTRODUCTION

Seed quality is a fundamental determinant of agricultural productivity, influencing germination rates, crop uniformity, and overall yield. The growing global population, projected to reach 9.7 billion by 2050 (FAO, 2021), has intensified the demand for high-quality agricultural produce. Ensuring optimal crop yields starts with high-quality seeds, which directly influence germination rates, plant vigor and resistance to environmental stresses (ISTA, 2020). Traditional seed quality assessment methods, including manual inspection, germination tests and biochemical analyses, are widely used but often suffer from inefficiencies due to their labor-intensive nature and susceptibility to human error (McDonald, 1998). These limitations necessitate the adoption of advanced technologies that can enhance precision, efficiency and scalability in seed quality testing.

Recent advancements in Artificial Intelligence (AI) and the Internet of Things (IoT) have revolutionized the agricultural sector, offering innovative solutions for seed quality assessment. AI, particularly machine learning (ML) and deep learning (DL), enables the automated analysis of seed traits from digital images and sensor data, facilitating rapid and accurate classification, damage detection, and viability prediction (Zhou *et al.*, 2021). Convolutional Neural Networks (CNNs) have shown high accuracy in checking seed quality by analyzing features like size, shape, and color. They can also find small defects such as cracks or fungus that are hard to see with the human eye, making seed testing faster and more reliable (Zhou *et al.*, 2021; Sharma *et al.*, 2020). Additionally, IoT-enabled smart sensors facilitate real-time monitoring of seed storage conditions, including temperature, humidity, and moisture levels, ensuring optimal preservation and reducing post-harvest losses (Kler *et al.,* 2023). The integration of AI and IoT allows for automated, non-destructive and highly accurate seed quality assessment, addressing the limitations of conventional methods.

This review explores the latest advancements in the integration of Artificial Intelligence (AI) and the Internet of Things (IoT) for seed quality testing, emphasizing their applications, benefits, challenges, and future prospects within the context of agricultural innovation. By utilizing AI-driven techniques and IoT-enabled systems, the agricultural sector stands to significantly improve seed quality management, optimize crop yield, and ultimately contribute to global food security. These technologies offer promising solutions to enhance precision, sustainability, and efficiency in seed testing, paving the way for smarter and more resilient agricultural practices.

II. OBJECTIVE

- Enhance Accuracy of Seed Quality Assessment
- Automate and Speed Up the Testing Process
- Enable Real-Time Monitoring and Data Collection
- Improve Detection of Damaged or Non-Viable Seeds
- Ensure Consistency and Standardization in Testing
- Improve Seed Traceability and Quality Control

2.1 Traditional Methods of Seed Quality Testing:

Conventional seed quality assessment encompasses physical, physiological and biochemical evaluations:

- **Physical Inspection**: Manual examination of seed attributes like size, shape and color.
- **Germination Tests**: Assessing seed viability through controlled germination experiments.
- **Biochemical and Genetic Analyses**
- Advanced seed quality testing includes biochemical and molecular techniques to assess seed vigor, purity and resistance to diseases. These methods include:
- **Tetrazolium Test (TZ Test)**
- The tetrazolium test is a rapid biochemical method used to assess seed viability. Seeds are soaked in a tetrazolium chloride solution, which stains living tissues red. This test provides quick results but requires expert interpretation.
- **Enzyme Activity Tests**
- Seed vigor is often linked to enzyme activity. Tests measuring the activity of catalase, peroxidase and ehydrogenase enzymes help determine seed viability. Higher enzymatic activity generally indicates better metabolic function and vigor.
- **Molecular Marker Techniques**
- DNA-based techniques such as RAPD (Random Amplified Polymorphic DNA), SSR (Simple Sequence Repeat) and AFLP (Amplified Fragment Length Polymorphism) help assess genetic purity and seed authenticity. These methods are crucial for ensuring that seeds meet quality standards, particularly in hybrid seed production.

TABLE 1
COMPARISON OF TRADITIONAL VS. AI AND IOT-BASED SEED QUALITY TESTING

Method	Accuracy	Time Required	Cost	Automation
Manual Inspection	Moderate	High	Low	No
Germination Tests	High	High	Medium	No
AI-Based Image Processing	Very High	Low	High	Yes
IoT-Enabled Monitoring	High	Real-time	Medium	Yes

Traditional seed testing methods have limitations, including being time-consuming, labor-intensive, and prone to subjectivity. Some tests, like the tetrazolium test are destructive, making seeds unfit for planting. These challenges highlight the need for automated and real-time seed quality assessment technologies.

2.2 Artificial Intelligence in Seed Quality Testing:

Artificial Intelligence (AI) has significantly enhanced seed quality assessment by providing automated, accurate and high-throughput testing solutions. AI-powered models utilize machine learning (ML), deep learning and computer vision to analyze seed characteristics efficiently, reducing reliance on manual inspection. Here's a list of different techniques used in AI-driven seed quality assessment:

- **Image Processing and Machine Vision:** AI-based image analysis enables the identification of seed defects, classification based on size, shape and color and detection of impurities. Advanced deep learning techniques, such as Convolutional Neural Networks (CNNs) and You Only Look Once (YOLO) models, have shown high accuracy in seed classification (Kundu *et al.,* 2021).

  Example: Sachin Sonawane and Basant Kumar Mohanty (2021) n Improved Image Processing Scheme for Automatic Detection of Harvested Soybean Seeds. This research proposes an enhanced image processing method for the automatic detection of harvested soybean seeds. The scheme focuses on improving the accuracy of seed detection by analyzing topological features and object count, contributing to more efficient seed quality assessment.

- **Spectral Analysis:** AI-driven models process hyperspectral imaging, near-infrared (NIR) spectroscopy and X-ray imaging to assess seed composition, internal structure, moisture levels and viability (Shan *et al.,* 2022). These techniques offer a non-destructive approach, preserving seed integrity for further use.

  Example: Chu Zhang and colleagues applied hyperspectral imaging combined with chemometrics for variety classification of maize seeds, achieving high classification accuracy and safety inspection, highlighting its applications across various crops.

NIRS is commonly employed to assess moisture, oil, and protein content in seeds such as wheat, soybean, and maize, this method is a standard practice in seed quality assessment.

- **Predictive Modeling:** Machine learning algorithms analyze historical seed quality data, genetic information and environmental factors to predict germination rates, potential yield, and resistance to diseases. Support Vector Machines (SVMs), Random Forest and Artificial Neural Networks (ANNs) have been widely applied in seed viability and vigor prediction (Zhao *et al.,* 2021).

  Example: Singh *et al.* (2010) used Artificial Neural Networks (ANN) to predict wheat seed germination based on electrical conductivity and moisture content. The model provided a fast, non-destructive method for assessing seed viability, demonstrating ANN's effectiveness in seed quality testing.

- **Automated Sorting and Grading**: AI-integrated systems sort seeds based on quality parameters such as purity, weight and damage level, improving efficiency in seed processing plants (Liu *et al.,* 2022).
- **AI-Powered Seed Health Assessment**: Machine learning algorithms can detect fungal infections, insect damage and genetic mutations, ensuring that only disease-free, high-quality seeds are selected for cultivation (Singh *et al.,* 2023).

By integrating AI with **real-time data analytics and cloud computing**, seed testing becomes faster, more accurate and scalable, benefiting both small-scale farmers and large agribusinesses.

III. INTERNET OF THINGS IN SEED MONITORING AND QUALITY CONTROL

The Internet of Things (IoT) has emerged as a transformative technology in agriculture, offering real-time monitoring and data collection capabilities essential for maintaining seed quality. By integrating IoT devices with advanced analytics, farmers and agronomists can ensure optimal conditions for seed storage, germination and growth.

- **Environmental Monitoring**: IoT sensors are deployed to continuously monitor environmental parameters critical to seed quality, such as temperature, humidity and soil moisture. Maintaining these parameters within optimal ranges is vital for seed preservation and successful germination. For instance, Kler *et al.* (2023) developed an Arduino-based IoT framework that monitors air and soil moisture, enhancing sunflower seed yield by ensuring ideal growing conditions.
- **Automated Sorting Systems**: IoT-enabled devices, when integrated with Artificial Intelligence (AI) models, facilitate the automation of seed sorting and grading processes. These systems utilize sensors and imaging technologies to

assess seed attributes such as size, shape and color in real-time, classifying seeds based on predefined quality metrics. This automation not only increases efficiency but also reduces human error in seed quality assessment. Elmasry *et al.* (2019) highlighted the application of multispectral imaging combined with IoT for seed phenotyping and quality monitoring, demonstrating improved accuracy in seed sorting operations.

- **Data Analytics and Cloud Computing**: IoT devices generate vast amounts of data that, when analyzed through cloud-based platforms, provide actionable insights for improving seed quality and storage practices. Real-time data analytics enable the detection of environmental fluctuations that may adversely affect seed viability, allowing for prompt corrective actions. Moreover, historical data analysis aids in optimizing storage conditions and predicting germination success rates. Prakash *et al.* (2023) discussed the role of IoT and wireless communication in smart farming, emphasizing how data analytics and cloud computing enhance decision-making processes in seed quality management.

The integration of IoT in seed monitoring and quality control not only streamlines agricultural practices but also contributes to sustainable farming by minimizing resource wastage and ensuring high-quality seed production.

3.1 Integration of AI and IoT: A Synergistic Approach

The convergence of AI and IoT technologies offers a holistic approach to seed quality testing:

- **Enhanced Data Accuracy**: IoT devices provide continuous data streams, which AI models analyze to detect patterns and anomalies in seed quality.
- **Automation and Efficiency**: The integration reduces the need for manual intervention, streamlines operations, and accelerates decision-making processes.
- **Scalability**: AI and IoT systems can be scaled to accommodate large volumes of seeds, making them suitable for both small-scale and industrial applications.

IV. APPLICATIONS

Several studies have demonstrated the efficacy of AI and IoT in seed quality assessment:

- **Automated Seed Quality Testing**: Nagar *et al.* (2021) developed a computer vision-based system that employs Generative Adversarial Networks (GANs) and active learning to automate seed quality testing. Their approach includes a novel seed image acquisition setup capturing images from multiple angles, and an annotation tool leveraging Batch Active Learning to minimize manual labeling efforts. This system achieved an accuracy of 91.6% in assessing the physical purity of corn seeds.
- **Seed Classification Using UAV Imagery**: Margapuri *et al.* (2021) made a significant contribution to seed classification using synthetic data and advanced deep learning methods. Their study, which utilized low-altitude UAV imagery, seems to be a promising approach for large-scale seed monitoring in breeding environments. By incorporating domain randomization and combining several powerful CNN architectures (ResNet-100, VGG-16, and VGG-19), they achieved an impressive accuracy of 94.6%. The scalability of this method could indeed be crucial for improving seed certification and quality assessment processes. These case studies underscore the potential of integrating AI and IoT technologies to enhance the efficiency and accuracy of seed quality assessment processes.
- **Seed Yield Estimation via Ground Robot and Deep Learning**: Feng, J., Blair, S. W., *et al.* (2024). Robust soybean seed yield estimation using high-throughout ground robot video. A ground robot carrying fisheye cameras collects video across soybean plots. Frames are processed by P2PNet-Yield (a deep feature-extraction + regression architecture) to count seeds and predict plot yields. The system cut data-collection time by 32% and achieved an 83% genotype ranking accuracy.

V. CHALLENGES

Despite the promising advancements, several challenges persist in integrating AI and IoT into seed quality assessment:

- **High Initial Investment**: Implementing AI and IoT infrastructure requires substantial financial resources, which can be prohibitive, especially for smallholder farmers. The costs associated with acquiring advanced technologies, such as precision farming equipment and AI-powered systems, range from $20,000 to $100,000, posing significant barriers to adoption.

- **Data Management and Security**: The deployment of IoT devices in agriculture leads to the generation of vast amounts of data. Ensuring the privacy and security of this data is paramount, as breaches can compromise sensitive information about farming operations. Additionally, the lack of standardized data collection methods results in fragmented datasets, complicating the integration and analysis processes necessary for effective AI application.
- **Technical Expertise**: The effective utilization of AI and IoT technologies necessitates a workforce proficient in these advanced systems. Many farmers face challenges in hiring or training personnel with the requisite technical skills, leading to slower adoption rates and underutilization of the technologies potential benefits.

Addressing these challenges requires collaborative efforts among technology developers, policymakers and the agricultural community to create cost-effective solutions, establish robust data management frameworks and provide comprehensive training programs.

VI. FUTURE DIRECTIONS

The integration of AI and IoT for seed quality testing is expected to continue evolving with several promising advancements on the horizon:

6.1 Integration with Blockchain:

Block chain technology holds great potential for improving seed traceability. By incorporating block chain, seed quality data can be securely and transparently recorded at each stage of the supply chain. This ensures that the origin and quality of seeds are traceable, preventing fraud and increasing confidence in the testing process. Additionally, it allows stakeholders to access verified data, supporting decisions related to seed quality management (Al-Bassam *et al.*, 2022).

6.2 Edge Computing:

Edge computing enables real-time data processing closer to the source, such as in the field or storage areas. This reduces the need for data to travel to central servers, minimizing latency and ensuring faster, more responsive seed quality assessments. By deploying edge computing in AI and IoT systems, seed testing processes will become quicker and more efficient, making it possible to perform on-the-spot evaluations of seed quality (Singh & Mehta *et al.*, 2021).

6.3 Advanced Sensor Technologies:

IoT sensors are expected to become more sophisticated, with capabilities to detect a wider range of environmental and biological factors that influence seed quality. Future sensors may be able to detect chemical imbalances, microbial contamination, or even assess the genetic traits of seeds, providing a comprehensive understanding of their quality. Such advancements will lead to more precise and reliable testing methods, enhancing the overall seed evaluation process (Chakraborty *et al.*, 2021).

6.4 Global Collaboration:

Increased global collaboration between researchers, technology developers, and agricultural stakeholders will drive further innovation in AI and IoT applications. By sharing knowledge and resources, these collaborations will accelerate the development of more effective, adaptable, and accessible seed quality testing systems. The integration of local and global expertise will help address challenges like data quality, standardization, and affordability, ensuring that advancements in seed quality testing are accessible to farmers and industries worldwide (Choudhury *et al.*, 2022).

VII. CONCLUSION

The convergence of Artificial Intelligence (AI) and the Internet of Things (IoT) marks a transformative leap in digital technology, creating systems that are not only interconnected but also context-aware, autonomous, and adaptive. IoT provides the sensory infrastructure—an expansive network of sensors and devices generating vast amounts of real-time data. AI, in turn, acts as the cognitive engine, processing this data to extract insights, recognize patterns, and make intelligent decisions without human intervention.

This synergy empowers applications ranging from predictive maintenance in industrial IoT to personalized healthcare, precision agriculture, and autonomous systems in smart cities. Advanced AI models, particularly in machine learning and deep learning, elevate the potential of IoT by enabling real-time anomaly detection, demand forecasting, resource optimization, and autonomous control.

However, successful integration demands addressing critical challenges: data privacy and security, standardization, latency, computational limitations on edge devices, and the need for robust infrastructure. The evolution of edge AI, 5G connectivity, and federated learning is helping bridge these gaps, paving the way for scalable and secure intelligent systems.

In conclusion, the fusion of AI and IoT is not merely a technological enhancement—it's a foundational pillar for next-generation digital ecosystems. As this integration matures, it promises to redefine operational models, foster innovation, and accelerate the shift toward truly intelligent environments.

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Unravelling of Soil pH Dynamics under Flooded Environment: A Review

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***Abstract*—** *Global climate change has increase the frequency and severity of extreme weather conditions including heavy rainfall followed by subsequent flooding. Soil pH is one of the most influential chemical parameters that have an impact on directly affecting the nutrient availability status, microbial activity and overall plant health. During the flooding period of paddy crop the soil pH for initially < 6.5 it was increased to approximately 7.0. Rice cultivated under continuous flooded conditions when soil pH became varied from 6.1 to 6.5 throughout the crop growth period. Agricultural crops such as rice benefit from mild increases of soil pH in submerged conditions to enabling improved nutrient solubility and reduced aluminum toxicity. In this contrast, crops like maize, wheat and legumes are inhibited the growth and nutrient uptake under flooded soil due to shifting of soil pH environments. Future research and development must focus on deeper understanding and management of these soil chemical shifts to ensure resilient cropping systems.*

Keywords*— *Agriculture, Climate change, Flooding, Nutrient, Soil pH and Rainfall.

I. INTRODUCTION

Global climate change has increase the frequency and severity of extreme weather circumstances with heavy rainfall followed by subsequent flooding. It is a direct effect of prolonged waterlogging and inundation stress altering soil physio-chemical and microbial changes in agricultural soils. Consequently, the understanding of how soil pH responds to these conditions has become critical. Flooding-related disruptions due to soil pH changes can lead to substantial agricultural losses. When soil pH shifts from the optimal range typically 6.0–7.0 for most crops, nutrient solubility becomes impaired, toxic elements may become bioavailable (e.g., aluminum, manganese) and microbial function is inhibited. Global estimated acid sulfate soils alone affect over 49 million hectares, predominantly in Asia. Where water management is key challenges cause significant crop failure due to post-flood pH depression (Dent, 1986). In India, economic losses due to reduced soil productivity under waterlogged and pH-altered conditions in lowland rice fields are estimated in the billions of rupees annually (Singh & Sharma, 2016). Horticultural crops like tomatoes, chillies, and bananas, which are particularly sensitive to pH changes, often exhibit stunted growth and fruit loss in these settings (Yadav *et al.,* 2017). Moreover, these losses are increased by input cost especially for lime application in acidic soils or sulfur amendments in alkaline soils and long-term degradation of soil fertility.

The most immediate effect of anaerobic soil conditions on plant is a reduction in aerobic respiration in roots. The particular end product of anaerobic respiration is partially dependent on soil pH. At a pH above neutrality, lactate fermentation is dominant, and as pH decreases (due to partially lactate fermentation), ethanol fermentation is induced. Rapid drop in cytosolic pH called acidosis is thought to be one of the main reasons why cells die in response to flood. In flood tolerant plants the pH drop may be counteracted by an alkaline process (Crawford *et al.* 1994).

Soil pH is one of the most influential chemical parameters that have an impact on directly affecting the nutrient availability status, microbial activity and overall plant health. Alteration of soil pH has a significant role on the sustainable cultivation of agricultural and horticultural crop production under flooded conditions. In this present review is mainly focused on critical examination of the mechanisms behind the modification of the soil pH under the flooding conditions. To assess the impact of soil pH changes on different crop species and explore soil management practices that mitigate adverse environmental effects

II. DIFFERENT TYPES OF SOIL AND PH CHANGES OCCURRED DURING FLOODED CONDITIONS

1) **In acidic soils (pH < 5.5) commen in the area of tropical regions having red or yellow and laterites soils are initially low pH or acidic nature. But, when flooded condition pH is increased. Because, when the flooded conditions** oxygen

is depleted through microbes begin reducing **Fe^{3+} to Fe^{2+} and Mn^{4+} to Mn^{2+}.** Hence, the reduction processes may **consume the hydrogen ions (H^+)** → leads to a **rise in pH. Ponnamperuma (1984) reported i**n tropical India and Southeast Asia, paddy cultivated in the acidic red soils which showed an increase from pH 4.5 to pH 6.2 during the growing season.

2) Arid/semi-arid zones, calcareous soils, vertisols **are i**nitial pH is high alkaline **(pH > 7.5 and** pH trends during the flooding m**ay be decreased. In the f**looded soil accumulates **CO_2**, which dissolves in water to form **carbonic acid (H_2CO_3) its having** slightly **low** acid pH. If sulfur compounds are present (e.g., gypsum), **sulfate reduction** can increase alkalinity again later. *Abou El-Naga et al., 2019 noticed that the p*arts of Egypt's Nile Delta, pH in calcareous soils dropped from 8.1 to 7.4 during extended flooding durations.

3) **Neutral soils having** alluvial plains and many productive crop lands initially near neutral pH **(pH ~6.5–7.5)** at the time of flooding mild fluctuation which was happened. Those soils have **moderate buffering capacity** due to clay minerals and organic matter. The pH may be initially dropped slightly due to CO_2, then increase slightly as Fe and Mn reduction occurred. Often stabilizes around **pH 6.5–7.0**, ideal for rice crop. In Indo-Gangetic plains of India, neutral loamy soils showed pH variation only between 6.7 and 7.3 under flooding condition *(Sahrawat, 2003).*

4) **Peaty and organic soils commonly found in m**arshes, peatlands, lowland of tropics. In the initial pH was often slightly acidic (pH 5–6). The pH is during flooding period c**an raise**, but post-drainage time **sharply dropped. U**nder flooding condition reduction of Fe/Mn cases **pH id increased.** After drainage, o**xidation of sulfides** (especially pyrite FeS_2) produces **sulfuric acid** → severe acidification (pH < 4). These soils become **toxic for crops after drying** unless properly managed. *Minh et al., 2008 reported in* Vietnam's Mekong Delta, acid sulfate soils increased to pH 6.0 under flooding then dropped to pH 3.8 upon drainage.

5) **In the saline and sodic soils of c**oastal zones, arid inland regions, initial pH is often > 8.0. Flooding duration pH became c**omplex in nature.** Flooding can **leach soluble salts**, reducing pH slightly. But in sodic soils, poor structure leads to clay dispersion → **low permeability**, **stagnant water**, limited leaching. Without leaching, **pH remains high** unless gypsum or acid-forming fertilizers are added In western India, sodic soils flooded for rice showed only minor pH decline (8.6 to 8.2) unless amendments were applied *(Sharma & Gupta, 2005).*

TABLE 1
SOIL TYPE VS. PH CHANGE UNDER FLOODING CONDITIONS

S. No.	Soil type	Initial soil pH	Flooding effect on soil pH	Cause
1	Acidic lateritic soils (e.g. Ultisols, Oxisols)	4.5–5.5	Increases	Iron (Fe^{3+}) and manganese (Mn^{4+}) are reduced to Fe^{2+} and Mn^{2+}, consuming H^+ ions and increasing pH.
2	Alkaline soils (e.g. Aridisols, Calcareous)	>7.5	Soil pH decreases (slight)	CO_2 accumulation followed by carbonic acid and also ammonia accumulation
3	Neutral loamy soils (e.g. Mollisols, Alfisols)	6.5–7.5	Minor soil pH changes occurred during the flooding	Balanced buffering from clay/OM and Buffering capacity keeps stabilized soil pH; redox changes may mildly alter nutrient solubility.
4	Peaty/acid sulfate (Histosols)	~5.5	Increase/ more acidic under flooding, decrease after drainage	Sulfide oxidation reduction take place post flooding; pyrite oxidation after drainage (↓)
5	Sodic saline soils	8.5–10	Initially pH may be decreased then rebound after flooding	Low permeability; clay dispersion and exchangeable sodium reactions; limited leaching
6	Clayey soils (Vertisols)	6.0–8.0	pH changes slowly	High buffering capacity and shrink-swell clays delay redox equilibrium.
7	Sandy soils (Entisols)	On the flooding occasions soil pH varied from often acidic in nature	Rapid but limited pH shifts	Low buffering capacity, fast water movement and fewer redox-active minerals.

III. MECHANISMS OF SOIL PH CHANGE UNDER FLOODED CONDITIONS

When flooded conditions, soil become transition from aerobic (oxygen-rich) to anaerobic (oxygen-depleted) environment. This shift triggers a series of redox reactions activities that significantly influence in soil pH. One of the primary mechanisms is the reduction of iron and manganese oxides. Under anaerobic conditions, iron (Fe^{3+}) and manganese (Mn^{4+}) oxides are biologically reduced to their soluble forms, Fe^{2+} and Mn^{2+}, respectively. The reduction of ferric iron (Fe^{3+}) can be represented as,

$$Fe^{3+} + e^- \rightarrow Fe^{2+} \tag{1}$$

Similarly, manganese reduction occurs as,

$$MnO_2 + 4H^+ + 2e^- \rightarrow Mn^{2+} + 2H_2O \tag{2}$$

These reactions consume hydrogen ions (H^+), thereby reducing acidity and causing a rise in soil pH, especially in acidic soils (Ponnamperuma, 1972).

A second major mechanism is nitrate reduction via microbial de-nitrification. In the absence of oxygen, facultative anaerobic bacteria use nitrate (NO_3^-) as an electron acceptor, reducing it to gaseous forms like nitrogen (N_2) or nitrous oxide (N_2O). The representative de-nitrification reaction is,

$$2NO_3^- + 10e^- + 12H^+ \rightarrow N_2 + 6H_2O \tag{3}$$

This process also consumes protons (H^+), further it was increasing soil pH under flooded condition (Tiedje, 1988). De-nitrification process is common in waterlogged agricultural soils and significantly affects nitrogen availability and pH dynamics.

Another important factor is the accumulation of ammonium (NH_4^+). Under normal aerobic conditions, ammonium is oxidized to nitrate via nitrification, a process that releases H^+ and acidifies the soil. However, in flooded soils, oxygen is limited, and nitrification is suppressed. Consequently, ammonium accumulates:

$$\text{Organic N} \rightarrow NH_4^+ \tag{4}$$

While NH_4^+ is slightly acidic, its accumulation under flooded conditions may lead to a slight decrease in pH, although this effect is typically overshadowed by the pH-increasing reactions described above (Patrick & Reddy, 1976).

In calcareous soil, flooding leads to form carbonate dissolution, which acts as pH buffer. Microbial respiration under anaerobic conditions produces CO_2, which dissolves in water to form carbonic acid:

$$CO_2 + H_2O \rightarrow H_2CO_3 \rightarrow H^+ + HCO_3^- \tag{5}$$

This weak acid reacts with calcium carbonate ($CaCO_3$),

$$CaCO_3 + H^+ \rightarrow Ca^{2+} + HCO_3^- \tag{6}$$

This reaction neutralizes acidity and maintains or slightly raises pH in alkaline soils (Sah & Mikkelsen, 1986).

Anaerobic decomposition of organic matter also plays a crucial role in soil pH. Initially, this process generates short-chain organic acids (e.g., acetic, butyric acids), which can temporarily reduce pH. However, as decomposition continues, microbial by-products such as bicarbonates (HCO_3^-), ammonia (NH_3), and hydroxides (OH^-) accumulate,

$$\text{Organic matter + microbes} \rightarrow CH_4 + CO_2 + HCO_3^- + NH_3 \tag{7}$$

The net effect over time is typically an increase in soil pH, particularly in soils with high organic matter (Ponnamperuma, 1984).

In sulfate-rich soils, sulfate-reducing bacteria (SRB) contribute to pH regulation by converting sulfate (SO_4^{2-}) into hydrogen sulfide (H_2S),

$$SO_4^{2-} + 8e^- + 8H^+ \rightarrow H_2S + 4H_2O \tag{8}$$

Hydrogen sulfide reacts with iron to form insoluble iron sulphide,

$$H_2S + Fe^{2+} \rightarrow FeS \text{ (precipitate)} \tag{9}$$

This reaction removes acidic components and contributes to a rise in pH in reduced soils (Patrick & De Laune, 1977).

In flooded conditions initiate a cascade of redox transformations in soil that predominantly result in a rise in pH, especially in acidic soils. While processes like ammonium accumulation and organic acid production may initially lower pH, dominant mechanisms such as iron/manganese reduction, nitrate reduction, carbonate dissolution, and sulfate reduction typically outweigh them in the long term. The final impact on soil pH is governed by the soil's original pH, redox status, microbial populations, and organic matter content. Mechanism of flooding is essential for sustainable soil and crop management in flood prone regions.

TABLE 2
SOIL PH CHANGE AND NUTRIENT STATUS UNDER FLOODING CONDITIONS

S. No.	Electron acceptor	Reaction	Effect on soil pH	Approximate time after flooding
1	Nitrate (NO_3^-)	Denitrification to N_2 gas	Releases H^+ → lowers pH	1–3 days
2	Manganese (Mn^{4+})	Reduced to Mn^{2+}	Consumes H^+ → raises pH	3–7 days
3	Iron (Fe^{3+})	Reduced to Fe^{2+}	Consumes H^+ → raises pH	7–14 days
4	Sulfate (SO_4^{2-})	Reduced to H_2S gas	Slight H^+ use, but H_2S toxic	2–3 weeks
5	CO_2 (Carbon dioxide)	Methanogenesis → CH_4 gas	Neutral/slightly alkaline	3–4 weeks and beyond

IV. FACTORS INFLUENCING SOIL PH CHANGES DURING FLOODED CONDITIONS

Flooding drastically alters the soil's chemical environment and the pH which shift during and after flooding depends on interacting between soil **physical, chemical, biological and environmental** factors. The following factors that influence the soil pH under flooded conditions,

1) **Soil Redox Potential (Eh):** The soil redox potential is a measure of the soil's oxidation-reduction status. It affects soil pH as redox potential drops (becomes more reducing), protons are consumed in redox reactions, often increasing pH in acidic soils (Ponnamperuma, 1972).

   Chemical process: Oxygen reduction → NO_3^- reduction → Mn^{4+} reduction → Fe^{3+} reduction → SO_4^{2-} reduction → Methanogenesis.

2) **Soil organic matter content:** Soils having more organic matter equally contain more microbial activity (Reddy and De Laune, 2008)**.** When reduction processes taken place which consume H^+ ionic potential to increasing soil pH. But, excessive decomposition of post-drainage time can acidify the soil due to sulfur or organic acid accumulation.

3) **Duration and depth of flooding:** there are three different categories in the flooding was reported by Sahrawat (2004).
   a. **Short-term flooding**: Causes minor, often reversible pH changes.
   b. **Prolonged flooding**: Promotes stronger reduction reactions, more dramatic pH shifts and
   c. **Deep vs. shallow waterlogging** is affects oxygen diffusion and microbial stratification.

4) **Soil texture and type:**
   a. **Clay soils**: High buffering capacity; slower pH changes.
   b. **Sandy soils**: Quick but limited pH changes; poor buffering.
   c. **Peaty soils**: Can become very acidic after drainage due to sulfide oxidation Brady & Weil (2008).

5) **Temperature:** When soil temperature during the flooding occasion can influences microbial metabolism. The high temperature due to accelerates redox activity reactions and cold soils are delay pH changes due to slower microbial activity (Fageria *et al.,* 2010).

6) **Type of crops grown:** Under the flooded condition some crops like rice can modify rhizosphere pH through oxygen transport and root exudates. Legumes are sensitive to low pH, affecting nodulation and N-fixation. Deep-rooted vs shallow-rooted crops experience different pH zones (Kirk, 2004).
7) **Microbial community composition:** Mitsch & Gosselink (2015) reported that the anaerobic microbes dominate under flooded conditions and enzyme activities also shifts, altering organic acid production. Their respiration products (e.g., NH_4^+, CH_4, H_2S) affect soil pH either by consuming or producing protons.
8) **Presence of acid sulfate minerals:** Dent (1986) studied the acid sulphate soils after flooding the oxidation of FeS_2 leads to release of sulfuric acid → severe drop in pH (<4) and often found that coastal and deltaic soils (e.g., Pyrite, Mekong, Sundarbans**)**
9) **Fertilizer and amendment history:** Pre-flooded conditions the fertilizer types either nitrate vs. ammonium can affect soil pH trajectory. Lime/amendment residues buffer pH differently in flooded vs non-flooded soils (Fageria and Baligar, 2003).
10) **Repeated flooding and drainage cycles:** Repeated flooding and drying can cause cyclical pH swings (e.g., Paddy-upland rotation systems: rice-wheat, rice-legume) (Singh and Ladha, 1977).

V. INFLUENCE OF SOIL PH AND FLOODING DYNAMICS ON THE PERFORMANCE OF AGRICULTURE AND HORTICULTURAL CROPS

According to the IPCC, 1966 year report, flooding causes the increase soil pH in acidic soil and decrease in alkaline soil. The primary cause of the pH rise in acidic soils is the conversion of acidic Fe^{3+} to Fe^{2+}, which also lowers the Eh. Soil pH of both acidic and alkaline soils tends to converge around neutral after flooded conditions (Ponnamperuma, 1972). Whereas, Crowley soil with an initial pH of 5.7 to it was gradually increased after being flooded/submerged condition around soil pH of 6.9.

According to Abdullahi *et al.* (2011), flooded soil had a pH range varied from 5.97 to 6.28 and was fairly acidic. Akter *et al.* (2011) also revealed the finding of result showed pH (water) value of soils ranged between 5.4 un-flooded conditions and it was increased flooded condition around 7.0. Because, release of silicon and sesquioxides from disintegration of clay lattice from seasonal flooding and doughtiness. Due to their silica-rich parent materials (acidic to intermediate crystalline rock) which was found in majority of flooded soils of Nigeria are to be acidic pH 5.5 or less (Isah, 2017; Aliyu, 2011). Nancy (2011) stated that pH were greater in flooded soils compared to non-flooded soil. On the other hand, Vincent *et al.* (2014) established that the control soil (well drained) is weakly alkaline than those of the flood affected farmland areas. Jusop *et al.* (2014) recorded that the post effect of flooding on affected farmlands was evident in the reduction in pH values, tending the soils towards acidity.

Sani Idris (2019) studied the three different soil depth viz., 0–30cm, 30-60 cm and 60-90cm under seasonally flooded field (SFF) and non-flooded field (NFF).

Two techniques were employed to measure the pH of the soil to water (1:2.5); soil to CaCl2 solution (1:2.5). Among the method taken into consideration, soil pH values of 5.93, 5.29(0-30cm), 6.41, 5.67(30-60cm) and 7.03, 6.26 (60-90cm) were found in SFF it was significantly higher than NFF soil pH of 5.663, 4.85(0-30cm), 6.12, 5.52(30-60cm) and 6.65, 5.96 (60-90cm). The presence of more exchangeable basic cations, which may have been deposited by flood water from the upland areas due to the pH increase, and the higher amount of organic matter complexes in SFF, which can buffer soil acidity. Similarly kind of result were obtained under acidic conditions of flooded soils (Idoga, 2006; Egharevba and Mayah, 2001; Brady and Weil, 1999; Miller and Donahue, 1992).

Soil pH of the paddy crop during flooding phase it was increased from less than 6.5 to about 7.0. Whereas, rice was grown in continuously flooded conditions with soil pH ranging from 6.1 to 6.5 (Ding *et al*., 2019). Kashem and Singh (2001) reported that the soil pH during flooding time increased. The soil pH increased by roughly 2.0, 1.0, and 0.6 units in the tannery, city sewage and alum shale soils for rice (*Oryza sativa* L.) throughout the 65-day submersion condition.

While rice (*Oryza sativa*) is naturally adapted to low-pH flooded environments and many legumes crops, vegetables and fruit trees are exhibit reduced growth, nutrient uptake and yield when subjected to acidic or alkaline deviations from their optimal pH ranges (Marschner, 2012; Fageria *et al.,* 2011). A major challenge lies in understanding how different soil types and soil pH fluctuations under flooding conditions to tailoring crop choices and management strategies accordingly.

In the flooded soil, rice soil at a sample depth of 15 cm the pH varied from 4.5 to 5.2 (Schulz *et al*., 2024). The pH conditions' geographical heterogeneity decreased over the next few weeks. The pH rose throughout the course of 16 weeks, ranging from 6.0 to 6.2.

For problematic soils, flooding is an excellent way to neutralize pH it was reported by Rangasami *et al*., 2022. The release and availability of plant nutrients are after floods, often positively induced by the neutralization activity of acidity in acid soils and alkalinity in alkaline soils. Wetland rice can better absorb nutrients from soils with a neutral pH, which can be achieved by adding or retaining a moderate amount of organic matter. Because of their decreased solubility due to elevated pH, toxic quantities of Al and Mn in soil solution are lowered.

Zheng and Zhang (2011) investigated three distinct moisture regimes in paddy cultivated soils. The Soil pH was 5.06, 5.08 and 5.81 under 75% field capacity, wetting drying cycle and flooding regimes during the initial stage. It was changed that the soil pH is increased from 5.81 (day 5) to 6.69 (day 150) under flooding regime with an increment of 0.88 units. When biological and microbiological processes in wet paddy soil are coupled with restricted oxygen diffusion and/or depleted, reducing conditions. This leads to a notable shift of soil pH increase towards neutrally and decrease in Eh (Narteh and Sahrawat, 1999).

Borin *et al*. (2016) reported that the initial soil pH value of the experimental region was originally very low 4.2 and when the flooded condition increasing in soil pH values of rice field which observed since 21 days after flooding existence around 6.2. In contrast of 61 days after flooding, the pH of the soil in the Cl-treated field samples reached about 6.8. In acidic soils, where Fe oxides are the main oxidants, even that exist the proton release due to CO_2 dissociation and rhizosphere oxidation, the H^+ consumption is higher than the release of soil pH increase up to7.0 (Sousa *et al.,* 2009).

According to Gambrell and Patrick (1978) reported that the soil pH of the mango cultivar "Tommy Atkins" was initially 7.4, which was indicative of anaerobic soil conditions within three days of flooding. The pH reduced around 7.0 after 21 days of flooding (Larson and Schaffer, 1991).

Cao *et al.,* 2020 reported that the soil pH under NF soil was 6.71, while in the rhizosphere of *Salix integra*, Cu stressed flooded soils significantly increased in soil pH of 6.83–6.89.

The effects of organic materials in the soils of Geriyo irrigation project were studied by Solomon *et al.,* 2014. They found that applying organic materials like poultry droppings generally increase the soil pH about 8.2 under submerged conditions. The soil pH was increased by these organic anion is consumption of protons. For this elevated soil pH is increased could be exchange of protons (H+) between the soil and when adding organic materials (Tang *et al*., 1999).

TABLE 3
HORTICULTURAL CROP RESPONSES TO FLOODING-INDUCED PH CHANGES

S. No.	Crop	Flooding response	pH sensitivity range	Impact
1	Tomato	Root rot, Fe toxicity in low pH soils	6.0–6.8	Chlorosis, poor yield
2	Potato	Soil pH drop and oxygen stress due to tuber cracking	5.5–6.5	Internal browning
3	Onion	Poor growth in acidic, waterlogged soils	6.0–6.8	Poor bulb formation, rot
4	Cabbage	Tolerates slight flooding, but not low pH	6.0–7.0	Leaf malformation, slow heading
5	Mango	Sensitive to root pathogens in flooded soils	6.0–7.5	Collar rot, low flowering
6	Banana	Root suffocation in poorly drained soils	5.5–6.5	Stunted growth, pseudostem rot
7	Citrus	Decline in Fe, Zn at high pH; root asphyxiation	5.5–7.0	Yellowing, dieback

TABLE 4
SOIL TYPE, EFFECT OF SOIL PH AND CROP RESPONSES UNDER FLOODED CONDITIONS

S. No.	Soil type	Crop(s)	Flood effect on soil pH	Notable observations under flooded conditions	Reference
1	Alluvial, Lateritic	Rice	Increase soil pH from ~5.2 to ~6.5	Fe/Mn reduction increased pH	Poonia & Ponnamperuma (1976)
2	Alfisols	Rice	Increase soil pH from acidic to near neutral	Denitrification reduces acidity	Singh & Tripathi (2008)
3	Calcareous, clayey	Rice and Wheat	Stable soil pH near to neutral pH	Buffering capacity of soil due to carbonates	Yaduvanshi *et al.* (2005)
4	Acid sulfate	Rice	Increase soil pH during flooded condition, decrease soil pH below 4 after drainage	Pyrite oxidation post-flooding	Minh *et al*. (2012)
5	Silt loam	Rice and Jute	Increase soil pH up to 6.8 under flood	Better Phosphorus availability, reduced Al toxicity	Islam *et al*. (2014)
6	Vertisols	Soybean, Maize	Increase soil pH under temporary flooding	Reduced O_2 triggers acidification	Reddy & Delaune (2008)
7	Peaty soils	Vegetables and Pasture	Decrease soil pH due to organic acid accumulation	Anaerobic decay increases acidity	Koerselman *et al.* (1993)
8	Sodic clay	Cotton	Decrease soil pH due to Fe/Mn reduction	Improved micronutrient solubility	McKenzie *et al.* (2004)
9	Red sandy loam	Groundnut	Stable soil pH during short floods	High soil buffering capacity	Sreenivas *et al.* (2013)
10	Peaty wetland	Taro, Banana, Coconut	Decrease under stagnant water	Soil organic matter fermentation enhances acidification process	Nair *et al.* (2008)
11	Saline-sodic clay	Rice and Pulses	Increase soil pH upto ~7.2 during flooding, decrease when post-flooded conditions	Stabilized soil pH under rice; pulses affected by acid shock	Natesan *et al.* (2010)
12	Black clay loam	Banana, Turmeric, Tapioca	Decrease soil pH from 6.5 to 5.5	Increased root rot risk, micronutrient imbalance	Jayakumar et al. (2016)
13	Sandy/red lateritic soils	Tomato and Brinjal	Decrease soil pH from 6.8 to 5.3	Poor drainage slowed pH recovery	Loganathan et al. (2018)
14	Clay loam	Coconut and Arecanut	Decrease soil pH from 6.3 to 5.0	Root hypoxia, increased sulfur stress	Nair & George (2007)
15	Acid sulfate coastal soil	Rice, Chilli and Sunflower	Decrease soil pH up to < 4 post-drainage	Acid sulfate soils highly reactive upon drying	Subramanian & Thiyagarajan (2005)

VI. SOIL PH MANAGEMENT STRATEGIES UNDER FLOODED CONDITIONS

Soil pH management under flooded conditions is crucial for optimizing crop yield and health, as pH influences nutrient availability, microbial activity, and soil structure. The fluctuating pH levels during flooding—due to anaerobic conditions—can be detrimental or beneficial depending on the crop type and soil properties. Effective management strategies help mitigate adverse pH changes, improving crop productivity in flood-prone agricultural areas.

6.1 Water management practices:

1) **Alternate wetting and drying (AWD)**: AWD is a widely practiced water management strategy for rice cultivation, especially in areas with limited water resources. It involves alternate wetting and drying of rice fields rather than continuous flooding. This method helps regulate pH by preventing continuous anaerobic conditions that could lead to excessive acidification of the soil. Studies show that AWD can reduce methane emissions, limit the reduction of iron and manganese, and stabilize soil pH (Gao et al., 2018).

2) **Controlled drainage**: Controlled drainage systems, which allow for the controlled release of water after flooding, can help restore soil oxygen levels, thus regulating microbial activity and preventing drastic pH shifts. This practice is particularly beneficial in regions prone to waterlogging (Kowalska et al., 2021).

3) Soil amendments:

a. Lime application: The addition of lime (calcium carbonate) is a common strategy for neutralizing acidic soils. In regions where flooding leads to soil acidification (e.g., due to the reduction of iron and sulfur compounds), liming can raise the pH to levels that are more favorable for crop growth. This is particularly important in rice paddies, where flooding promotes the formation of sulfuric acid in the soil (Sharma & Saha, 2017). The use of lime helps in maintaining an optimal pH range (5.5-7) for most crops.

b. Organic matter addition: Incorporating organic matter (compost, manure) into soils before and after flooding can help buffer pH changes by increasing soil buffering capacity. Organic amendments also encourage microbial diversity and promote the activity of beneficial microorganisms that can mitigate the negative effects of acidic conditions (Schipper et al., 2019).

4) Selection of crop varieties:

a. **Flood-tolerant crops**: Selecting crop varieties that are more tolerant to pH fluctuations and waterlogged conditions is another important strategy. Rice, for instance, has developed physiological mechanisms, such as aerenchyma (air spaces in roots), which allow it to thrive in low oxygen environments typical of flooded conditions. However, other crops like maize or legumes may suffer if soil pH falls below optimal levels (Becker et al., 2020).

b. **Leguminous crops and pH sensitivity**: Leguminous crops are particularly sensitive to pH fluctuations, especially if pH drops below 5.0. Choosing varieties that are more resistant to pH-induced stress can help optimize yield in flood-prone regions (Patil et al., 2021).

5) Microbial activity and redox management:

a. **Microbial management**: Microbial communities play a significant role in regulating soil pH under flooded conditions. Denitrification and methane production in anaerobic environments often lead to acidification, while the reduction of iron and manganese can increase soil pH. Managing microbial activity through proper soil management practices, such as the use of bio-inoculants, can help stabilize pH fluctuations (Smith et al., 2017).

b. **Inoculation with nitrifying bacteria**: Inoculating soils with nitrifying bacteria, such as *Nitrosomonas* and *Nitrobacter*, can help balance the nitrogen cycle, thus reducing the accumulation of ammonium and preventing acidification (Sullivan *et al.*, 2018).

6) Monitoring and adjusting soil pH:

a. **Regular pH monitoring**: Regularly monitoring soil pH during flooding events is essential to detect early signs of pH fluctuations. Soil testing at different stages of crop growth allows for timely intervention using appropriate amendments. Automated pH monitoring systems can help farmers track pH levels in real time and adjust water management practices accordingly (Rasmussen *et al.*, 2020).

b. **Fertilizer management**: Fertilization practices should be adapted based on the soil pH and the nutrient requirements of the crop. For instance, applying ammonium-based fertilizers in flooded fields with acidic soils may exacerbate pH reduction. Instead, ammonium nitrate or urea may be preferred in such scenarios, as they are less likely to contribute to soil acidification (Nandal et al., 2019).

VII. CONCLUSION

Flooding alters the soil environment drastically and one of the most significant consequences is shifting of soil pH due to oxygen depletion and redox reaction activities. Under flooded conditions, soils transition from aerobic to anaerobic condition to prompting a cascade of microbial and chemical changes that impact nutrient availability based on soil pH status (Ponnamperuma, 1984; Reddy & Delaune, 2008). The direction of pH change occurring, whether increasing or decreasing the soil pH depends on soil type, initial pH, soil buffering capacity and the duration including frequency of flooding (Patrick & Delaune, 1977). Whereas, the acidic soils are typically experience a temporary increase of soil pH due to proton consumption during reduction reaction process, while alkaline soils may acidify due to organic acid production and CO_2 accumulation (Brady & Weil, 2017).

Agricultural crops such as rice benefit from mild increases of soil pH in submerged conditions to enabling improved nutrient solubility and reduced aluminum toxicity. In this contrast, crops like maize, wheat and legumes are inhibited the growth and nutrient uptake under flooded soil due to shifting of soil pH environments (Setter & Waters, 2003). The effects on nutrient solubility such as iron, manganese, phosphorus and sulphur are sensitive to soil pH, leading to either deficiencies or toxicities depending on the redox-pH dynamic potential (Fageria et al., 2011). This emphasises are the importance of managing soil pH to sustain crop productivity during and after flooding events.

Horticultural crops, which generally require soil pH for optimal growth. The crops like tomatoes, citrus, grapes and peppers show chlorosis, stunted growth or even plant death when pH moves outside optimal threshold level (Ehret *et al*., 2010; Zhang *et al.*, 2019). In addition, microbial shifts in the rhizosphere also affect nutrient cycling and disease susceptibility in these high-value crops. Flooding not only changes the crop root-zone soil pH but also alters beneficial symbiotic microorganism such as mycorrhizae and rhizobia, which are crucial role in nutrient uptake in many horticultural crops (Kozlowski, 1997).

To mitigate these effects, strategic management practices such as lime application, raised beds, controlled drainage and the use of pH-tolerant crop varieties are essential for maintain the soil pH. Also monitoring real-time soil redox activities and soil pH changes can guide fertilizer application and drainage strategies to minimize long-term degradation (Pezeshki, 2001; Sharma *et al*., 2005). This is more helpful for understanding the dynamic relationship between soil pH, flooding and crop responses for ensuring resilient agricultural and horticultural systems in flood-prone conditions.

VIII. FUTURE PROSPECTS

Future prospects of soil pH under flooded conditions in agricultural and horticultural crop cultivation.

In the global climate change has marked by increasing frequency and intensity of rainfall pattern is making flooding or waterlogging is more common in many agricultural crop cultivated lands. One of the key challenges under these conditions is the rapid and often unpredictable fluctuation of soil pH. Future research and development must focus on deeper understanding and management of these soil chemical shifts to ensure resilient cropping systems (Ponnamperuma, 1984; Reddy & Delaune, 2008).

One of the most promising areas lies in advanced soil monitoring technologies. Precision agriculture tools, especially Internet of Things (IoT)-enabled soil sensors, now offer the possibility of continuously monitoring soil pH, redox potential (Eh) and moisture in real time. These tools could allow farmers to respond to unfavorable soil pH shifts with lime or sulfur applications, drainage modifications or chancing of fertilization schedules. Additionally, artificial intelligence (AI) models could predict soil pH trends based on real-time flooding events, enabling proactive soil pH correction strategies (Gebbers & Adamchuk, 2010; Sudduth *et al.*, 2013).

Breeding of agriculture and horticulture crops varieties which more scope for flood-resilient and pH-tolerant are another critical path. In the case of rice, extensive studies have shown its unique adaptations like aerenchyma formation and root oxygenation. These traits, along with rhizosphere acid–base balancing mechanisms, need to be bred into other cereals and horticultural crops like tomato, citrus and eggplant. Modern genomic tools, especially CRISPR/Cas9, offer the potential to accelerate development of such crops by targeting genes related to pH tolerance, nutrient uptake under flooding and organic acid metabolism (Setter & Waters, 2003; Zhang *et al*., 2019; Chen et al., 2019).

Finally, there is a need for policy support and farmer-level capacity building. Flood-induced soil pH stress is often underappreciated at the policy level and many farmers are unaware of how waterlogging changes soil chemistry. Governments and institutions must invest in farmer training, soil testing infrastructure and incentives for adopting sustainable water and soil pH management practices. Focused research on local soil types and crop responses to flooding across different agro-ecological zones will be crucial in forming future flooding issues eradication strategies (FAO, 2020).

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Performance and Egg Quality Responses of Laying Hens to High Copper Supplementation during the Early Production Phase

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Abstract— *As the poultry industry moves toward antibiotic-free production systems, identifying effective alternatives to antibiotic growth promoters has become a priority. Copper, an essential trace mineral, has gained attention for its antimicrobial properties and potential to enhance poultry performance. This study aimed to evaluate the effects of high copper hydroxychloride supplementation on the performance and egg quality of laying hens during the early production phase.*

A total of 400 Hy-line laying hens at 16 weeks of age were randomly assigned to two dietary treatments: a control group receiving a basal diet with 180g/T Virginiamycin (AGP) and a test group supplemented with IntelliBond copper at 216g/T (IBC). The trial lasted for 24 weeks including a 4-week adaptation period, with performance parameters including hen-day egg production, feed intake, feed conversion ratio, egg weight, egg mass, and egg quality traits monitored throughout.

Results showed no significant differences in egg production rate or feed intake between treatments. However, hens fed the IBC-supplemented diet exhibited a significantly improved FCR, heavier eggs, and greater egg mass. Additionally, IBC supplementation enhanced yolk weight and yolk color without affecting eggshell quality, albumen height, or the incidence of defective eggs.

In conclusion, IBC offers a reliable alternative to AGPs in laying hen diets. It improves feed efficiency and egg quality, particularly yolk color, while maintaining egg integrity and production performance, making it a valuable strategy for sustainable, antibiotic-free poultry production.

Keywords— Antibiotics, Copper hydroxychloride, Egg production, Egg quality.

I. INTRODUCTION

Optimal nutrition is the foundation of productivity and well-being in laying hens, especially during the early laying phase when the birds experience rapid physiological changes to support the demands of egg production. For decades, the poultry industry has relied on antibiotic growth promoters (AGPs) to enhance feed efficiency, control disease, and maintain flock performance. However, mounting concerns over antimicrobial resistance and the potential presence of antibiotic residues in animal-derived food products have led to tighter regulations and growing consumer awareness (Donoghue, 2003; Castanon, 2007). This shift has placed increasing emphasis on identifying safe, effective, and economically sustainable alternatives to AGPs in poultry production systems.

Among the strategies being explored, high copper supplementation has gained considerable attention. Copper is an essential trace mineral that plays a vital role in immune function, antioxidant defense, bone health, iron metabolism, and the activity of several critical enzymes involved in physiological and metabolic processes (Arredondo and Núñez, 2005). While copper is typically supplied in poultry diets at nutritional levels ranging from 5 to 30 ppm to meet basic physiological needs, research has shown that higher dietary copper levels can deliver additional benefits beyond simple nutrient supplementation. When provided at level above standard nutritional requirements, copper exhibits bacteriostatic and bactericidal properties, which can help improve gut health, enhance feed conversion, and support growth and egg production by modulating intestinal microbiota and reducing harmful microbial metabolites (Pesti and Bakalli, 1996; Wang et al., 2008).

IntelliBond Copper hydroxychloride (Trouw Nutrition, The Netherlands) has emerged as a preferred copper source due to its superior stability, bioavailability, and lower risk of antagonistic interactions compared to conventional inorganic copper sources (Klasing, 1998; Lim and Paik, 2006). Although widely used in broiler diets, limited research has explored the effects of high dietary copper levels from hydroxychloride sources in laying hens, especially during the early production phase.

This study was therefore designed to evaluate the impact of high-level copper supplementation on the performance and egg quality of laying hens during the early production phase. The aim is to provide practical, evidence-based insights for poultry producers seeking effective, antibiotic-free nutritional strategies to support flock health and productivity.

II. MATERIALS AND METHODS

2.1 Birds and Management

The hens were housed in battery cages in an open-sided housing system equipped with a trough feeder shared by adjacent cages and a waterer. A total of 400 Hy-line layers, 16-week-old and weighing 1.10± 0.01 kg, were used. Before the trial, the hens received the treatment diets for a four-week (pre-lay) adaptation period. Hens were fed *ad libitum* according to their assigned treatment and had free access to water from the start until the termination of the trial. Throughout the study, they were provided with 16 hours of light and 8 hours of darkness per day.

Vaccinations were administered following breed recommendations. During periods of stress, vitamin and electrolyte supplementation via drinking water was considered.

2.2 Ethical statement

All the methods used in this experiment were conducted with strict adherence to the approved experimental guidelines and procedures by the Central Luzon State University Institutional Animal Care and Use Committee- CLSU-IACUC Reference No. 20230412-04.

2.3 Experimental Design and Treatments

The trial was set up in a Randomized Complete Block Design, with cage location (2-tier) as the blocking factor. Each cage, containing 8 hens, was considered an experimental unit. Hy-line layers (n=400) were randomly assigned to one of two dietary treatments, with 25 replicates per treatment. The treatments tested included: (1) a basal diet with 180g/T Virginiamycin plus 60 g/T copper sulfate (AGP) and (2) a basal diet with 216 g/T IntelliBond copper (IBC).

2.4 Diet Formulation and Feed Processing

A practical corn-soybean meal diet was formulated to meet or exceed the nutrient recommendations for layers set by the Philippine Society of Animal Nutritionists Feeding Standard (PHILSAN, 2010). A master batch of the basal diet, which was free from AGP, was initially prepared in mash form. The test products were then added to the basal diet, thoroughly mixed, and placed into pre-marked feed bags (Table 1).

TABLE 1
INGREDIENTS AND CALCULATED COMPOSITION OF THE BASAL DIET

Ingredient	Percent
Corn, yellow	47.45
Rice bran (D1)	15.00
Coconut oil	1.18
Soybean meal (HP)	22.25
Meat and Bone meal	3.00
Limestone (F)	4.45
Limestone (C)	4.50
Mono-dicalcium phosphate	1.00
Salt	0.35
DL-methionine	0.16
Lysine	0.01
Vit Premix[1]	0.15
Min Premix[2]	0.15
Choline Chloride	0.113
Toxin binder	0.25
Calculated nutrient content[3]	
Metabolizable energy, kcal/kg	2728
%Crude protein	18.09
% Crude fat	4.19
% Crude fiber	3.08
% Lysine	1.01
% Meth+cysteine	0.82
% Threonine	0.69
% Tryptophan	0.21
% Calcium	3.62
% Available phosphorus	0. 40

[1]Supplied per kilogram of diet: vitamin A (as retinyl acetate), 5,500 IU; vitamin D (as cholecalciferol), 550 IU; vitamin E (as α-tocopheryl acetate), 30 IU; vitamin K (as menadione dimethylpyrimidinol bisulfate), 4.4 mg; riboflavin, 11.0 mg; d-pantothenic acid, 22.05 mg; niacin, 33.0 mg; and vitamin B_{12} (as cyanocobalamin), 33.0 mg.

[2]Supplied per kilogram of diet: iodine (as Ca $(IO_3)\cdot H_2O$), 0.25 mg; iron ($FeSO_4\cdot 2H_2O$), 125 mg; manganese (MnO),15 mg; selenium (Na_2SeO_3), 0.3 mg; and zinc ($ZnSO_4\cdot H_2O$), 125 mg.

[3]Book values were obtained from the PHILSAN Feed Reference Standards (PHILSAN, 2010).

2.5 Feed sampling at the feed mill and feed analysis

After the individual diets were prepared, representative pooled samples of 5 kg per treatment were analyzed for dry matter, crude protein, crude fat, crude fiber, and ash content, following the proximate analysis procedures of the Association of Official Analytical Chemists (AOAC, 1990)

2.6 Measurements and records

Feed consumption was recorded weekly, and mortalities were noted as they occurred, with the total number of deaths calculated over the entire study period. Eggs were collected and weighed daily, and egg mass was calculated as the product of egg production and egg weight. The feed conversion ratio (FCR) was determined by calculating grams of egg weight produced per gram of feed consumed. Performance parameters were analyzed for the entire duration of the experiment (21-40 weeks).

Eggshell defects, including cracked, dirty, soft-shelled, and shell-less eggs, were recorded daily, and the percentage of saleable eggs was calculated. External and internal egg quality were assessed at 24, 28, 32, 36, and 40 weeks of age. At each of these time points, 10% of eggs from each replicate were collected on the last day of the corresponding 4-week period. Shell thickness and albumen height were measured (at three different points) using a digital vernier caliper. The average of these three measurements was used to calculate shell thickness and albumen height for each cage. Yolk color was assessed using a DSM yolk color fan. Yolk weight and albumen weight were measured using a digital scale after separating the yolk from the albumen. Eggshell weight was determined by drying the shells overnight at room temperature and weighing them using a digital scale.

2.7 Statistical Analysis

Data were tested for normality and homogeneity using Levene's test. The GLIMMIX procedure in SAS was used to analyze the data, with treatment included as a fixed effect. Cage was considered the experimental unit and included as a random effect for both production performance and egg quality parameters. Performance and egg quality were summarized across the entire study period, and results were expressed as least squares mean (LS-means). Variability in the data is presented as the standard error of the mean (SEM), with statistical significance set at p-values < 0.05. A trend toward significance was considered for p-values between 0.05 and 0.10. Comparisons among significantly different means were performed using Tukey's honest significant difference (HSD) test.

III. RESULT

3.1 Nutrient Analysis of the Experimental Diets

The dry matter, crude protein content, crude fat, and crude fiber contents were similar across diets (Table 2). However, the ash content was higher in the diets containing IBC, which may be attributed to the carriers used in the product, possibly containing clay minerals.

TABLE 2
PROXIMATE ANALYSIS OF THE DIETARY TREATMENTS

Parameter	Treatments	
	AGP	IBC
Dry matter, %	88.1±0.7	89.0±0.9
Crude protein, %	18.3±0.9	18.5±0.8
Ash, %	16.4±0.5	19.7±0.7
Crude fat, %	2.1±0.2	2.1±0.2
Crude fiber, %	2.7±0.8	2.9±0.6

3.2 Egg production

Table 3 shows the production performance (21 to 40 weeks) of laying hens fed different dietary treatments during the 20-week feeding trial, following a 4-week adaptation period. No significant differences (p > 0.05) were observed in hen-day egg production and average daily feed intake (ADFI) between the AGP and IBC groups. However, FCR was significantly improved (p = 0.03) in the IBC group (2.13) compared to the AGP group (2.20). Egg weight was significantly higher (p < 0.0001) in the IBC-supplemented hens, averaging 57.06 g compared to 55.62 g in the AGP group. Correspondingly, egg mass was also significantly greater (p = 0.02) in the IBC group (49.33 g) compared to the AGP group (47.57 g).

TABLE 3
PERFORMANCE OF HENS FED DIETS CONTAINING AGP AND IBC

Treatment	Hen-day (%)	ADFI, (g/b/d)	FCR (g/g)	Egg Weight (g)	Egg mass (g)
AGP	85.29	103.05	2.20 ^{a}	55.62 ^{b}	47.57 ^{b}
IBC	86.33	103.98	2.13 ^{b}	57.06 ^{a}	49.33 ^{a}
SEM	0.99	0.79	0.02	0.22	0.54
***p*-value**	0.47	0.94	0.03	<0.0001	0.02

^{a,b}***Means with different superscript are significantly different (p<0.05)***

3.3 Egg quality

The effects of dietary treatments on egg quality parameters and the occurrence of egg defects are summarized in Table 4.

A significant difference (p = 0.04) was observed in yolk weight, with hens in the IBC group producing heavier yolks (14.84 g) compared to those in the AGP group (14.53 g). Similarly, the yolk color score was significantly higher in the IBC-supplemented group (7.21) than in the AGP group (6.58)

No significant differences were recorded for eggshell weight, eggshell thickness, or albumen height between the two dietary groups, indicating that shell quality and internal egg freshness were unaffected by the dietary treatments.

Regarding egg defects, no significant differences were found in the percentage of cracked, dirty, shell-less, or soft-shell eggs between AGP and IBC groups (p > 0.05 for all parameters).

TABLE 4
EGG QUALITY OF HENS FED BASAL DIET WITH AGP AND IBC

Treatment	Yolk Weight (g)	Yolk color score	Eggshell weight (g)	Eggshell thickness (mm)	Albumen Height (mm)	Cracked (%)	Dirty (%)	Shell-less (%)	Softshell (%)
AGP	14.53^{b}	6.58^{b}	5.74	0.42	9.29	0.30	3.52	0.13	0.27
IBC	14.84^{a}	7.21^{a}	5.62	0.42	8.99	0.33	3.23	0.22	0.21
SEM	0.10	0.11	0.05	0.004	0.37	0.04	0.16	0.03	0.09
***p*-value**	0.04	0.0003	0.11	1	0.56	0.64	0.20	0.09	0.65

^{a,b}Means with different superscript are significantly different (p<0.05)

IV. DISCUSSION

4.1 Egg Production

The present study demonstrated that replacing traditional antibiotic growth promoters (AGPs) with high copper (IBC) in the diets of laying hens significantly enhanced key production performance parameters during the early laying phase. Although hen-day egg production and feed intake did not differ significantly between dietary treatments, hens receiving IBC exhibited a significantly improved FCR, along with increased egg weight and egg mass.

These findings are consistent with previous research highlighting copper's vital role in egg production, due to its involvement in numerous metabolic, immune, and nutrient utilization pathways (Richards et al., 2010). Beyond its nutritional importance, copper when supplemented above standard dietary requirements has demonstrated notable antibacterial effects within the gastrointestinal tract (Pesti and Bakalli, 1996; Wang et al., 2008). This antimicrobial action likely contributes to improved gut health and nutrient absorption, which together enhance overall production efficiency, as evidenced by the improved FCR in the IBC group in this study. Similar improvements in FCR (Attia et al., 2012; Olgun et al., 2013), egg mass (Olgun et al., 2013), and egg weight (Lim and Paik, 2006) have been documented with various copper sources, including copper hydroxychloride. These effects are believed to result from copper's stimulatory influence on digestive enzyme activity. Supporting this, Xia et al. (2004) reported increased activities of lipase, amylase, and protease in broilers supplemented with elevated copper levels, suggesting that enhanced nutrient digestion and assimilation may contribute to the improved feed efficiency and performance outcomes observed in the present study. Additionally, the superior performance observed with IBC supplementation may be partly attributed to its enhanced bioavailability compared to inorganic trace mineral sources. Inorganic trace minerals tend to dissociate in the upper gastrointestinal tract, where they can interact with other dietary components, reducing their absorption efficiency and increasing excretion (Miles et al., 1998). In contrast, hydroxychloride trace minerals possess a unique crystalline structure (Hawthorne & Sokolova, 2002) and low water solubility (Cao et al., 2002), which allow them to dissolve more gradually in the digestive tract. This slower disintegration reduces undesirable interactions with feed constituents, thereby improving mineral stability and uptake (Miles et al., 1998; Pang & Applegate, 2007). Several studies have reported the higher bioavailability of copper hydroxychloride compared to inorganic sources (Batal et al., 2001; Cao et al., 2002). This enhanced stability and absorption likely contribute to the improved production parameters observed in the present study. Corroborating these findings, Toghyani et al. (2019) reported that supplementation with hydroxychloride trace minerals significantly improved egg mass, FCR, and hen-day egg production during the post-peak laying period.

4.2 Egg Quality

In addition to performance benefits, IBC supplementation had a positive influence on selected egg quality traits. Notably, hens in the IBC group produced eggs with significantly higher yolk weight (p = 0.04) and yolk color score (p = 0.0003) compared to those in the AGP group. The improved yolk weight and color with IBC supplementation may be linked to copper's role in promoting gut health and nutrient absorption, thereby enhancing the uptake and deposition of dietary pigments like carotenoids in the yolk as earlier mentioned by several authors. Gül et al. (2014) noted that a more favorable intestinal environment, often achieved through pH reduction and microbial modulation, can increase carotenoid absorption in maize-based diets, a likely contributing factor in the present study's IBC group. While dietary carotenoid content is a primary determinant of yolk pigmentation (Kljak et al., 2021), improvements in nutrient absorption mechanisms facilitated by copper hydroxychloride could amplify these effects.

Other egg quality parameter including albumen height, eggshell weight, and eggshell thickness did not differ significantly between treatments, and the rates of cracked, dirty, shell-less, and soft-shelled eggs remained comparable. These results agree with findings from Lim and Paik (2006) and Olukosi et al. (2018), who observed that while copper supplementation may reduce egg defects, such improvements may not always be reflected in measurable changes in shell thickness or weight. As noted by Xiao et al. (2015), improvements in eggshell quality are often attributed to better mineral bioavailability and shell formation efficiency rather than increases in shell dimensions alone.

Overall, the current study's egg quality results reinforce the potential of IBC to selectively improve internal egg traits, particularly yolk-related characteristics, without compromising external egg quality or increasing the incidence of defective eggs.

V. CONCLUSION

The findings of this study demonstrate that high IBC supplementation is an effective alternative to antibiotics in laying hen diets during the early laying phase. While overall egg production and feed intake remained comparable between the AGP and copper-supplemented groups, hens receiving IBC exhibited significantly better feed conversion efficiency, along with improvements in egg weight and egg mass.

In addition to performance benefits, IBC supplementation positively influenced specific egg quality traits, notably yolk weight and yolk color, without compromising eggshell strength, internal egg freshness, or increasing the occurrence of defective eggs. The enhanced yolk pigmentation is particularly valuable in markets where yolk appearance plays a key role in consumer preference and marketability.

These results highlight the potential of IBC as an antibiotic-free feed additive capable of supporting both production efficiency and egg quality in modern poultry systems. Its ability to maintain egg integrity while improving feed efficiency and yolk characteristics makes it a reliable option for producers seeking alternatives in response to growing restrictions on AGP use.

Future research could further explore the long-term effects of high copper supplementation across different laying phases and under varying production conditions, as well as its interactions with other nutritional strategies in antibiotic-free systems.

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Biochar based Seed Biopriming for Enhancing Yield Quality Attributes of Spinach

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Abstract— *Spinach (Spinacia oleracea), a winter season leafy vegetable, is widely consumed due to its high nutritional value, involving Vitamin C, Vitamin K, and iron. Enhancing the productivity and nutritional quality of spinach through eco-friendly practices has become an essential component in the context of sustainable agriculture. One such promising approach involves the use of biochar, an organic carbon-rich material which produced through the pyrolysis of organic biomass. Apart from soil application, biochar can also be used as a seed biopriming agent to enhance germination rate, seedling vigor, and stress tolerance in crops. This study is designed to evaluate the combined and individual effects of biochar used as a seed biopriming agent and soil amendment on the growth and yield parameters of spinach. The field experiment was conducted at the Amity Institute of Organic Agriculture in Randomized Block Design (RBD) with six treatments as biochar at 2%, 5%, and 10% (v/v) in both seed priming and soil application, seed priming alone, soil application alone, and a control without biochar.*

Keywords— Biochar, spinach, sustainable agriculture, biopriming.

I. INTRODUCTION

Spinach, scientifically known as *Spinacia oleracea*, a fast-growing crop with culinary and nutritional interests all over the world is an important vegetable crop belonging to the family Amranthaceae. It is an annual vegetable crop grown for its nutrient-rich, dark green leaves originated in Southwest Asia and was introduced to Europe in 12th century. In India, major spinach producing states are Gujarat, Maharashtra, West Bengal and Uttar Pradesh (Rani et al., 2020). It is rich in iron, calcium, vitamin A, C and K that plays a crucial role in supporting bone health, immune functioning and thereby improving health. Spinach is a cool season crop that grown well under cool weather with well-drained soil and consistent moisture conditions. The crop faces challenges due to decline in soil fertility, water retention capacity and thereby need is there for some organic amendments for sustainable agricultural practices that improve soil health with crop growth and yield.

Biochar is an organic carbon-rich material which is produced through the action of pyrolysis of the biomass under limited oxygen conditions that has gained considerable attention as a promising tool for sustainable agriculture (Lehmann et al., 2006). The characteristics of biochar involving its porous structure, high cation exchange capacity with its ability to enhance soil fertility and water retention capacity thereby makes it a suitable candidate for its utilization as soil amendment (Liang et al., 2006; Chan et al., 2007; Ghori et al., 2019; Enaime et al. 2021; Ramamoorthy et al., 2024). Biochar being a organic compound has the potential to influence soil microbiome with increase enzymatic activity (Lehmann et al., 2011). Seed health is as important as soil heath for promoting the crop productivity. Seed biopriming, a component of seed priming is a pre sowing treatment of seeds with biological agents that activates the enzymatic reactions inside the seed and thereby conditioning it by activating defense reactions for better adaptability to growing conditions. In recent years, biochar has also been utilized as a seed biopriming agent with a potential to enhance seed germination, seedling vigor and stress tolerance (Joseph et al., 2013; Farooq et al., 2020). Seed biopriming is a type of physiological treatment strategy that enhances the plant growth even under stress conditions by utilizing hydration to promote water absorption before the radicle emergence (Takoliya et al., 2018). This method not only increases the rate and uniformity of seed germination but also strengthens the defense response of plants against the biotic stress by improving the activity of beneficial microbial activity on spermosphere (Parsad et al., 2015). Utilization of biochar as soil amendment has a potential in maintain soil pH, preventing nutrient leaching and enhancing the activity of nutrients and organic matter in degraded soil, aids in carbon sequestration and thereby aligning with the goals of sustainable agriculture (Jeffery et al., 2011; Hussain et al., 2017; Ahmad et al., 2024).

Biochar application enhances the seed germination, vigor index with increase in plant height and shoot length which are indicative of their improved vegetative growth and biomass accumulation thereby contributing to improved quality and yield of crop (Naggar et al., 2019; Abborova et al., 2023). The present study was carried out with aim to study the potential of biochar as seed biopriming and soil amendment treatment both in combination or as alone on growth parameters of spinach for further utilization in crop growth and productivity.

II. MATERIALS AND METHODS

2.1 Experimental location and climatic conditions:

The experimental trial was carried out during the Rabi season of cropping year 2025 at Research farm of Amity Institute of Organic Agriculture, Amity University, Noida, Uttar Pradesh. The geographic coordinates of the location were 77°19′51.64″E (77.331007) longitude and 28°32′37.64″N (28.543793) latitude, and an altitude of 200 meters above sea level. The cropping area falls under Trans-Gangetic plains agro-climatic zone, which has a semi-arid, subtropical climate with hot, dry summers and freezing winters. The cultivation of the spinach crop was carried out during January as this month has the lowest temperatures of 14ºC to 17ºC suitable for cultivation of spinach crop. The region receives an average annual rainfall of about 700 mm, primarily from July to September, followed by dry weather from December to February, thereby making it ideal for growing cool season crops like spinach.

2.2 Seed treatments and sowing:

Firstly, the field was prepared by ploughing to a fine tilth followed by layout of experiment as per design (Table 1). For seed biopriming, spinach seeds were soaked in distilled water for 12 hours to initiate hydration before radicle emergence. After draining the water, the seeds were dried partially and then combined with biochar slurry (prepared by combining biochar with adequate water) and incubated in a shady, aerated conditions for 24 hours. For soil amendment, biochar was added to the topsoil layer as per the treatment details to enhance soil structure and nutrient retention. Sowing was carried out by implementing the broadcasting method of application for the already bio primed seeds. The seeds were then lightly covered with a thin layer of soil and thereby immediate irrigation was provided to ensure appropriate seed-soil contact and moisture availability to the sown seeds.

2.3 Treatment details:

A Randomized Block Design (RBD) with six treatments and four replication was laid out. Each plot measured 2m x 2m with 0.5m distance between plots and about 1m between the blocks. The data pertaining to different growth parameters like seed germination per centage, shoot length (cm), root length (cm) and yield (kg/plot) was recorded and the data recorded was later analyzed for the presence of variance using ANOVA tests from OPSTAT.

TABLE 1
DETAILS OF THE TREATMENTS

Sr. No.	Treatments	
1	T1	Biochar (Seed priming + Soil application) 2%
2	T2	Biochar (Seed priming + Soil application) 5%
3	T3	Biochar (Seed priming + Soil application) 10%
4	T4	Biochar (Seed priming)
5	T5	Biochar (Soil application)
6	T6	Control

III. RESULTS AND DISCUSSION

The different data recorded was analysed statistically and presented in Table 1. It was evident from observations that, among different treatments, increase in number of foliage was recorded from T4 involving alone application of seed biopriming with biochar followed by treatment combination of seed biopriming and soil application with biochar 5 % (T2) which are statistically at par with each other. However, the treatment combinations T3 and T1 also resulted in significantly increase in number of foliage as compared to control. Another growth attribute in spinach plants pertaining to plant height was recorded as highest in T4 involving seed biopriming with biochar (23 cm) followed by treatment T2 (21 cm) which are significantly different from each other.

It is evident from Table 1, the shoot length of spinach plants was significantly improved by biochar application in comparison to control as T4 observed the highest shoot length (19.25 cm) followed by T2 with shoot length of 18 cm which are significantly different from each other. The different treatment combinations of T3 and T1 also presented significant increase in plant height. Similar results were also observed in terms of root length as the highest value was recorded for T4 treatment combination with an average value of 9 cm followed by T2 and T3 treatment combinations with value of 8.25 and 7.75 cm, respectively.

The highest yield was recorded in treatment combination involving seed biopriming with biochar as 2.5 kg per plot followed by treatment combination T2 and T3 with average yield of 2.4 and 2.2 kg per plot, respectively (Figure 1).Thus, it was evident from the different parameters that seed biopriming with biochar alone had the most positive impact on growth in spinach followed by combined application of seed biopriming with soil application.

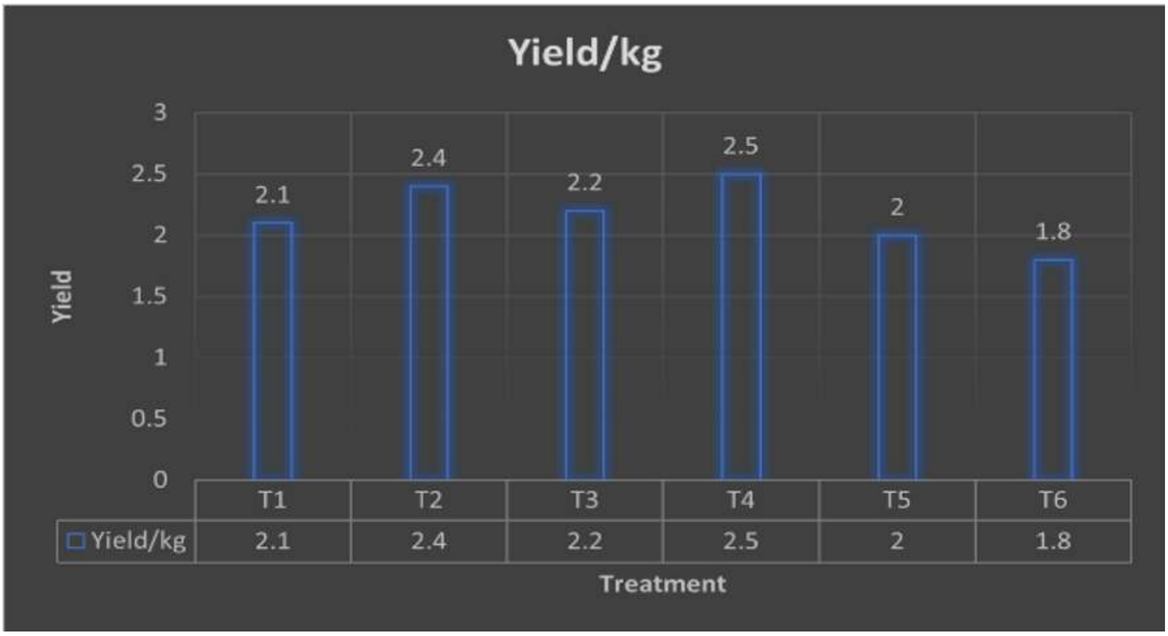

FIGURE 1: Effect of biochar combinations on yield in spinach

The results are in conformation with studies conducted by Ahmad et al. (2024) as priming of metallic nanoparticles resulted in increased growth in spinach crop under abiotic stress conditions. Similar results have been reported by some other research workers that also depicts the potential of biochar treatment in enhancing the plant growth and its different attributes (Saxena et al., 2013; Trupiano et al., 2017; Jabborova et al. 2021).

TABLE 2
EFFECT OF BIOCHAR AS SEED BIOPRIMING AND SOIL APPLICATION ON DIFFERENT GROWTH PARAMETERS OF SPINACH

Treatment	No. of leaves	Plant height (cm)	Shoot length (cm)	Root length (cm)
T1: Biochar (Seed priming + Soil application) 2%	7.50±0.28	18.50±0.24	15.50±0.28	7.25±0.18
T2: Biochar (Seed priming + Soil application) 5%	9.00±0.40	21.00±0.44	18.00±0.40	8.25±0.14
T3: Biochar (Seed priming + Soil application) 10%	8.50±0.28	19.50±0.25	17.50±0.28	7.75±0.14
T4: Biochar (Seed priming)	9.70±0.25	23.00±0.40	19.20±0.47	9.00±0.20
T5: Biochar (Soil application)	6.70±0.25	17.50±0.28	14.50±0.28	6.75±0.14
T6: Control	6.20±0.25	16.00±0.40	13.00±0.40	6.00±0.20
CD $_{(0.05)}$	**0.76**	**0.83**	**0.87**	0.4

IV. CONCLUSION

The aim of this study was to assess the effect of biochar as soil amendment and seed biopriming on key growth parameters in spinach. It can be concluded that the treatment T4 (biochar applied as both seed biopriming and soil amendment) significantly enhanced plant height and number of leaves compared to the control. The rise in leaf number reflects enhanced photosynthetic capacity, contributing to better biomass accumulation. Biochar-based biopriming likely facilitated early seed metabolism, moisture retention, and nutrient availability, leading to improved seedling establishment and overall plant performance. These findings suggest that biochar-based seed biopriming is an effective strategy to enhance early growth traits closely linked to yield and quality in spinach.

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Geospatial Assessment of Chilling Requirements for Temperate Fruit Cultivation in Meghalaya, India

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Abstract— *Chilling hour (CH) accumulation is a critical climatic factor influencing dormancy release and flowering synchronization in temperate fruit crops. In this study, the spatial variability of chilling hours across Meghalaya, a northeastern hill state of India, has been assessed to evaluate the agro-climatic suitability for seven temperate fruit crops: apple, apricot, kiwi, persimmon, blueberry, peach, and walnut. Using ERA5-Land hourly gridded temperature data (9 km resolution), average annual CH accumulation was calculated over a 20-year period (2003–2023). The state was stratified into seven chilling hour zones based on cumulative CH thresholds relevant to crop-specific chilling requirements, determined in consultation with the Department of Horticulture, Government of Meghalaya.*

Results revealed that 55.33% of the state receives <100 CH annually, rendering these areas unsuitable for most temperate crops. However, higher-elevation districts such as West Khasi Hills and East Khasi Hills exhibit favorable chilling regimes, supporting the potential cultivation of low- and moderate-chill crops. Suitability analysis indicates that peach (CH >100) has the highest spatial suitability (45.25%), followed by blueberry (150–600 CH; 33.04%), kiwi and persimmon (CH >200; 28.98% each), apple and apricot (CH >300; 21.52% each), and walnut (CH >600; 4.00%).

The study provides the first comprehensive chilling hour-based agro-climatic zoning for Meghalaya and presents a scalable framework for site-specific temperate horticultural planning in subtropical highland regions. The findings support the horticulture department of the state in decision-making for varietal selection, land-use allocation, and horticultural diversification under changing climate scenarios.

***Keywords—* Chilling hours, temperate fruits, agro-climatic zoning, GIS, Meghalaya, suitability assessment.**

I. INTRODUCTION

Chilling requirements are a critical factor in the successful cultivation of temperate fruit crops. These requirements refer to the cumulative number of hours during which temperatures remain between 0°C and 7.2°C (32°F to 45°F) during the dormant period of deciduous fruit trees. This cold exposure is essential for breaking dormancy and ensuring proper flowering and fruiting in the subsequent growing season. In India, varying climatic conditions across regions influence the accumulation of chilling hours, thereby affecting the viability of temperate fruit cultivation.

The concept of chilling requirements has been essential for temperate fruit cultivation. Weinberger (1950) first emphasized the need for adequate winter chilling to ensure successful bud break in deciduous fruit trees. Since then, several models have been developed to estimate chilling accumulation, including the Chilling Hours Model (CU Model), the Utah Model, and the Dynamic Model (Fishman et al., 1987), each varying in their sensitivity to temperature fluctuations and regional adaptability.

Globally, chilling hour mapping has gained importance in light of climate change, which has led to significant reductions in winter chill in traditional temperate fruit-growing regions (Luedeling et al., 2009). For instance, studies in California, Spain, and Chile have documented how declining winter chill affects both the geographical range and productivity of temperate fruit crops. To address this, researchers have increasingly turned to gridded climate datasets and geospatial tools for assessing chilling patterns at regional and sub-regional levels (Campoy et al., 2011).

In the Indian context, the Himalayan states—particularly Himachal Pradesh, Jammu & Kashmir, and Uttarakhand—have been focal points of research on chilling hour distribution and its impact on apple and pear cultivation (Singh & Pal, 2014).

Traditional high-chill varieties of apples, pears, and plums thrive well in the Himalayan regions, due to the ample accumulation of chilling hours. For instance, studies in Himachal Pradesh have reported chilling hours ranging from 779 to 1,155 hours, depending on the specific location and microclimate (Conversely, in the subtropical plains of Punjab, Haryana, and Uttar Pradesh, the accumulation of chilling hours is significantly lower, ranging between 150 to 350 hours (Tyagi et.al, 2022). This variation necessitates the selection of appropriate cultivars suited to the local chilling conditions. However, in the Northeastern Region (NER), particularly Meghalaya, very few studies have explored the application of chilling-based agro-climatic zonation for temperate fruit crops.

Meghalaya, a state located in the northeastern hill region of India, is characterized by varied elevations, topographic complexity, and a subtropical highland climate, which together create microclimatic conditions suitable for diversified horticulture. While traditionally dominated by crops suited to humid subtropical environments, interest in cultivating temperate fruits has grown due to rising market demand and favorable high-altitude pockets. However, systematic agro-climatic zoning based on chilling hour accumulation is lacking in the region. Most decisions related to temperate crop cultivation are based on elevation alone, overlooking the temporal and spatial variation in actual chilling accumulation.

Earlier assessments in Meghalaya focused largely on altitude-based suitability, rainfall, slope and soil characteristics for horticultural development (Borthakur et al., 2015, NESAC, 2021). While useful, such approaches lack temporal climate resolution. Recent advancements in remote sensing and the availability of reanalysis datasets like ERA5 have opened opportunities for high-resolution agro-climatic modeling. Studies utilizing ERA5-Land data in Mediterranean and South American regions have demonstrated its utility in accurately estimating chilling accumulation over complex terrains (de Melo-Abreu et al., 2004; Luedeling & Brown, 2011).

Despite these advancements, a gap remains in applying these tools in the Northeast Indian context. Moreover, crop-specific chilling thresholds must be localized, as cultivars and growing conditions vary widely. By integrating ERA5-Land temperature data with chilling hour models and expert inputs from the Meghalaya Horticulture Department, this study presents a novel, region-specific approach to temperate fruit crop planning.

II. MATERIALS AND METHODS

2.1 Study Area:

Meghalaya is a mountainous state in Northeast India, located between latitudes 25°1′N to 26°5′N and longitudes 89°45′E to 92°47′E. The state's varied elevation, ranging from 50 m to over 1,900 m above sea level, results in diverse microclimates. The climate is predominantly subtropical with high annual rainfall, but high-altitude regions experience cooler winters, making them potentially suitable for temperate fruit cultivation. Location of study area is depicted in Figure 1.

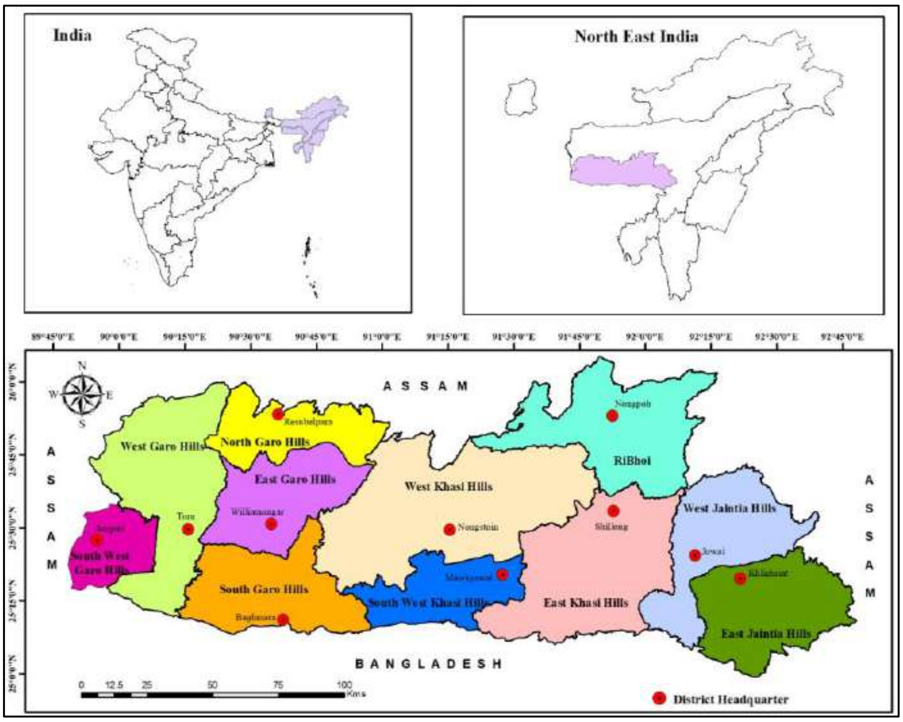

FIGURE 1: Location of study area

2.2 Chilling Hour Estimation:

Chilling accumulation was assessed using ERA5-Land hourly temperature data at a spatial resolution of 9 km, obtained from the Copernicus Climate Data Store. The data spanned 20 years (2003–2023), covering the winter dormancy period from November to February. The standard Chilling Hours Model, which counts the number of hours between 0°C and 7.2°C, was applied. Hourly temperatures were processed using Python to compute daily chilling hours and aggregated to produce annual CH totals.

2.3 Crop-Specific Thresholds and Suitability Criteria:

Crop-specific chilling thresholds were determined through consultation with the Department of Horticulture, Government of Meghalaya (Table 1). The selected temperate fruit crops—apple, apricot, peach, blueberry, kiwi, persimmon, and walnut—were assigned minimum CH requirements based on established horticultural standards and field experience. Suitability was assessed by comparing the observed CH against these thresholds.

2.4 Spatial Analysis and Agro-Climatic Zoning:

Using ArcGIS 10.8.2, annual CH maps were generated and averaged to produce a long-term mean CH map. This composite map was classified into seven CH zones: <100, 100–200, 200–300, 300–400, 400–500, 500–600, and >600 hours. Each zone was reclassified as suitable or not suitable for each crop, based on whether observed CH met or exceeded the required threshold.

2.5 District- and Block-Level Suitability Mapping:

To assess spatial suitability at administrative levels, district and block boundaries were overlaid on the CH suitability maps and area statistics for each crop were extracted using spatial overlay tools in ArcGIS.

The flowchart of the methodology followed for the study is depicted in figure 2.

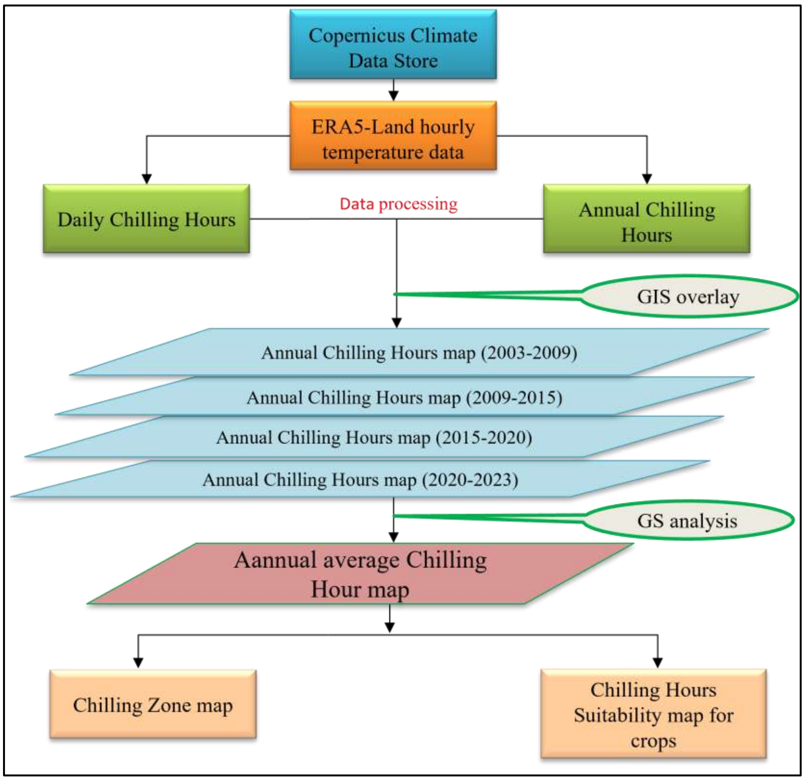

FIGURE 2: Flowchart of the Methodology

III. RESULTS

3.1 Chilling Hour Distribution in Meghalaya:

The spatial analysis of average annual chilling hours (CH) over a 20-year period (2003–2023) revealed considerable variability across Meghalaya (Figure 3 and Table 1). CH accumulation ranged from as low as 0.09 hours to a maximum of 707 hours during the winter dormancy period (November–February). The composite CH map categorized the state into seven zones, with over 55.33% of the area receiving <100 CH, thereby falling under the 'Very Low Chill' zone. These areas are largely unsuitable for most temperate fruit crops due to insufficient winter chilling. The remaining area was distributed across progressively higher chill zones: 100–200 CH (15.89%), 200–300 CH (7.44%), 300–400 CH (7.45%), 400–500 CH (5.46%), 500–600 CH (4.47%), and >600 CH (3.97%). These findings highlight the potential of mid- to high-altitude regions—particularly in the West and East Khasi Hills-for temperate horticulture.

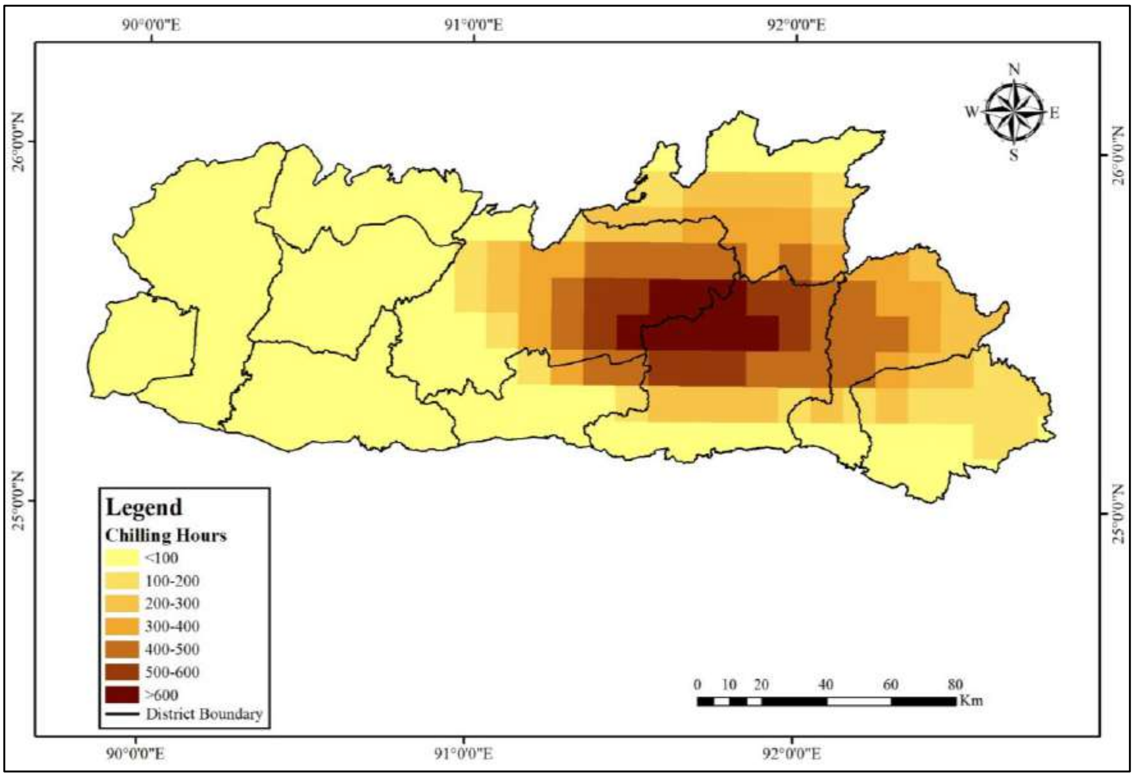

FIGURE 3: Chilling hour map of Meghalaya

TABLE 1
SPATIAL DISTRIBUTION OF CHILLING HOUR ZONES OF MEGHALAYA

Sl. No.	Chilling Hour Class (CH)	Class Name	Area (%)
1	<100	Very Low Chill Zone	55.33%
2	100–200	Low Chill Zone	15.89%
3	200–300	Moderate Chill Zone-I	7.44%
4	300–400	Moderate Chill Zone-II	7.45%
5	400–500	High Chill Zone-I	5.46%
6	500–600	High Chill Zone-II	4.47%
7	600–707	Very High Chill Zone	3.97%
Total	—	—	100.00%

3.2 Suitability Analysis for Temperate Fruit Crops:

Crop-specific suitability analysis based on chilling thresholds showed a clear inverse relationship between chilling requirement and suitable land area (Table 2). Peach (>100 CH) was found to be the most adaptable, suitable across 45.25% of the state's area. Blueberry (150–600 CH) followed with 33.04%, and both kiwi and persimmon (>200 CH) were each suitable in 28.98% of the area. Apple and apricot (>300 CH) each had 21.52% suitable area, while walnut (>600 CH) was restricted to only 4.00% of the total area. This gradient demonstrates the advantage of low-chill cultivars in expanding horticulture into regions previously deemed marginal. The suitability map (Figure 4) highlights the importance of crop-climate matching in temperate fruit planning.

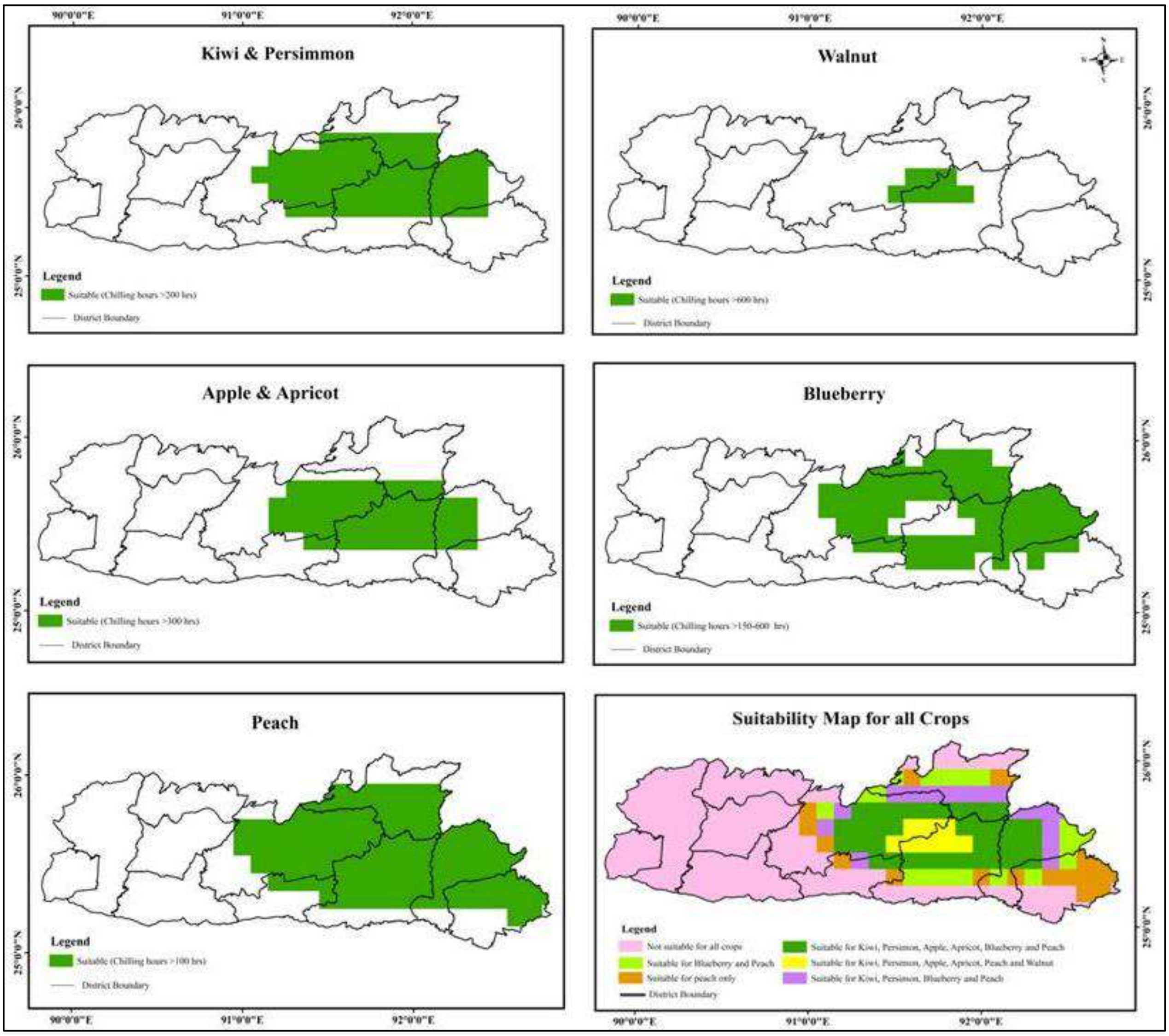

FIGURE 4: Suitable areas for temperate fruits in Meghalaya

TABLE 2
CROP WISE SUITABLE AREAS AVAILABLE IN MEGHALAYA

Crop	Suitability Criteria (CH)	Suitable area (%)
Peach	> 100	45.25
Blueberry	150–600	33.04
Kiwi	> 200	28.98
Persimmon	> 200	28.98
Apple	> 300	21.52
Apricot	> 300	21.52
Walnut	>600	4

3.3 District-Level Suitability Assessment:

District-wise analysis revealed West Khasi Hills and East Khasi Hills as the most promising regions. West Khasi Hills had the highest suitability for apple, apricot (37.89% each), kiwi, persimmon (35.36%), and walnut (40.41%). East Khasi Hills showed peak suitability for walnut (59.59%) and substantial potential for other crops. Moderate suitability was observed in West Jaintia Hills and Ri Bhoi, especially for low- to mid-chill crops like blueberry and peach. East Jaintia Hills and South West Khasi Hills exhibited limited suitability due to insufficient CH accumulation (Table 3).

TABLE 3
DISTRICT WISE SUITABLE AREAS FOR DIFFERENT TEMPERATE FRUITS IN MEGHALAYA

SL No.	Districts	Area %						
		Peach	Blueberry	Kiwi	Apple	Apricot	Persimmon	Walnut
1	RiBhoi	16.9	19.88	14.5	7.62	7.62	14.49	
2	East Jaintia Hills	12.2	4.82	2.25	1.56	1.56	2.25	
3	East Khasi Hills	22.2	21.72	25.7	34.57	34.57	25.67	59.59
4	South West Khasi Hills	4	3.05	3.47	3.3	3.3	3.47	
5	West Jaintia Hills	16.6	21.54	18.8	15.07	15.07	18.76	
6	West Khasi Hills	28.1	29	35.4	37.89	37.89	35.36	40.41

3.4 Block-Level Suitability Trends:

At the block level, spatial variability was more nuanced. Blocks such as Mawphlang and Mawsynram (East Khasi Hills), and Mairang and Nongstoin (West Khasi Hills), emerged as highly suitable for multiple temperate crops including high-chill varieties (Table 4). These micro-zones offer focused opportunities for introducing niche temperate horticulture through customized cultivar selection.

The overall results highlight the value of CH-based agro-climatic zoning in optimizing crop distribution and aligning cultivar selection with local climatic regimes. This approach provides a scientific basis for expanding temperate horticulture in Meghalaya and can be adapted for other subtropical highland regions.

TABLE 4
BLOCK WISE SUITABLE AREAS FOR SELECTED TEMPERATE FRUITS IN MEGHALAYA

District	Blocks	Area %						
		Peach	Blueberry	Kiwi	Apple	Apricot	Persimmon	Walnut
East Jaintia Hills	Khliehriat	4.15	3.41	2.04	1.56	1.56	2.04	-
	Saipung	8.01	1.41	0.21	-	-	0.21	-
East Khasi Hills	Mawkdok	1.82	1.38	2.85	3.84	3.84	2.85	9.29
	Mawkynrew	4.04	4.67	5.37	7.24	7.24	5.37	3.3
	Mawphlang	2.06	0.13	3.22	4.34	4.34	3.22	22.29
	Mawryngkneng	2.4	3.07	3.74	5.04	5.04	3.74	1.75
	Mawsynram	5.32	4.65	5.7	7.68	7.68	5.7	17.98
	Mylliem	1.71	1.86	2.67	3.6	3.6	2.67	4.02
	Pynursla	2.67	3.02	1.65	2.23	2.23	1.65	0.97
	Shella_bholaganj	2.16	2.95	0.45	0.61	0.61	0.45	-
RiBhoi	Jirang	5.01	5.49	2.42	-	-	2.42	-
	Umling	3.24	4.09	0.58	-	-	0.58	-
	Umsning	8.7	10.3	11.49	7.62	7.62	11.49	-
South West Khasi Hills	Mawkyrwat	3.98	3.05	3.47	3.3	3.3	3.47	-
	Ranikor	0.02	-	-	-	-	-	-
West Jaintia Hills	Amlarem	2.25	1.94	0.89	1.19	1.19	0.89	-
	Laskien	6.14	8.41	5.18	2.13	2.13	5.18	-
	Thadlaskien	8.17	11.19	12.69	11.75	11.75	12.69	-
West Khasi Hills	Mairang	8.32	7.71	12.99	13.45	13.45	12.99	30.39
	Mawshynrut	5.35	4.28	3.16	0.5	0.5	3.16	-
	Mawthadraishan	5.15	5.84	7.96	10.15	10.15	7.96	10.01
	Nongstoin	9.25	11.16	11.25	13.78	13.78	11.25	

IV. CONCLUSION

This study provides a comprehensive geospatial assessment of chilling hour distribution and its implications for temperate fruit cultivation in Meghalaya. The analysis reveals that over half of the state (55.33%) falls under the very low chill zone (<100 CH), rendering it unsuitable for most traditional temperate fruits. Only about 13.9% of the total area receives more than 400 chilling hours, confining the cultivation of high-chill crops such as apple, apricot, and walnut to a few high-altitude pockets.

Conversely, low- to moderate-chill crops such as peach, blueberry, kiwi, and persimmon show broader adaptability, particularly in mid-altitude zones. The inverse relationship between chilling requirement and suitable area underscores the importance of selecting crop varieties based on localized climatic conditions. District-wise analysis identifies West Khasi Hills and East Khasi Hills as the most promising regions for temperate horticulture, with both low- and high-chill zones present. Ri Bhoi and West Jaintia Hills offer moderate potential, while East Jaintia Hills and South West Khasi Hills are less suitable due to inadequate chilling accumulation.

Strategic crop selection, focusing on low-chill cultivars and site-specific planning, will be essential to realize the temperate horticulture potential in Meghalaya under current and future climatic conditions. The successful introduction and cultivation of low-chill temperate fruit varieties in Meghalaya highlight the potential for expanding horticultural activities in the region. Continued research and development, along with farmer training and support, are essential to optimize cultivation practices and improve yields. By leveraging the state's unique agro-climatic conditions and adopting suitable cultivars, Meghalaya can enhance its temperate fruit production, contributing to agricultural diversification and economic growth.

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Effect of Seaweed Extracts as Bio Stimulant on Growth Parameters in Spinach

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Abstract— *Spinach (Spinacia oleracea), is an important cool season vegetable crop which is widely consumed due to its high nutritional content and eco-friendly techniques has a great role in enhancing the productivity and nutritional quality of spinach in context of human health and sustainable agriculture. Among different organic amendments, seaweed is an important organic extract which can be utilized effectively as biofertilizer in improving the vegetative growth in Spinach. The present study was carried out at Amity Institute of Organic Agriculture, Amity University Noida, UP to study the potential of seaweed extract, a bio stimulant on growth parameters in Spinach (Spinacia oleracea L.). Seaweed is an organic bio-stimulant, which is rich in vitamins, amino acids and phytohormones. The field experiment was conducted at the Amity Institute of Organic Agriculture in Randomized Block Design (RBD) with six treatments as 20, 40, 60, 80 and 100 ml were tested for its stimulative potential of plant growth.*

Keywords— Spinach, Seaweed extract, Biostimulant, Sustainable Agriculture.

I. INTRODUCTION

Spinach (*Spinacia oleracea* L.) is a leafy vegetable crop which is rich in nutrients and bioactive compounds like spinacetin, patuletin and coumaric acid derivatives with potent antioxidant activity (Bergman et al., 2001; Pandjaitan et al., 2005). Spinach being a nutritionally rich crop is also a substantial source of fiber (Antonia et al., 2001). Being a cool season crop, it is cultivated in a temperature range of 7-15 °C with adequate moisture conditions (Chitwood et al., 2017). Spinach which was originally indigenous to Central and Western Asia was later brought to India and then China in 7th century. As the agriculture is proceeding towards sustainability with an aim to utilize organic source for fertilizers for nutrition, more emphasis was made on organic compounds. Utilization of natural seaweed extracts as biofertilizers has thus, made it possible to partially replace the traditional synthetic fertilizers during recent years (Hong et al., 2007). Seaweed extracts are non-toxic, non-polluting, biodegradable which are safer for the environment and human (Khan et al. 2009). Different seaweed products are available that can be utilized as liquid extracts either applied as foliar spray or as soil drench that will act as soil conditioners (Thirumaran et al., 2009).

Among different organic amendments, seaweed is an organic extract that can be effectively utilized as a bio-stimulant by various researchers (Singh et al., 2016; Rouphael et al., 2017). Seaweed extract has been utilized effectively in various vegetable crops with benefits like higher yield and foliar biomass. The beneficial and economic effects of seaweed extracts on overall growth, yield and quality attributes with different horticultural crops have been reported (Kumar et al., 2020). Seaweed extracts are bio-stimulants that improve plant growth and stress tolerance. These extracts have been reported to increase plant tolerance to biotic and abiotic stress conditions (Guinan et al., 2013; Hariharan et al., 2024). The most common seaweed extract (*Ascophyllum nodosum*) positively affects the spinach germination, its vegetative growth as well as antioxidant activity under stressed conditions (Anjos et al., 2020). Seaweed extracts thereby improve the soil health by increasing the plant and soil microbial activity (Moore et al., 2004).

II. MATERIAL AND METHODS

2.1 Experimental location and climatic conditions:

The experimental trial was conducted at Experimental farm of Amity Institute of Organic Agriculture, Amity University Noida UP during the cropping season of 2025. The cropping area was situated at 200 meters above the mean sea level with geographic coordinates of 77°19′51.64″E longitude and 28°32′37.64″N latitude. The cropping area is under Trans-Gangetic plains having semi-arid, subtropical climate with dry summers and harsh winters having January as the ideal time for cultivation of spinach. Average rainfall of 700 mm followed by dry weather during December to February are ideal for the cultivation of spinach.

2.2 Land preparation and sowing:

Properly drained soil beds were prepared ensuring they are devoid of debris and weeds. The seed beds are prepared manually and sowing of spinach is done by broadcasting method to cover the whole area evenly. Later, the seeds are covered with thin layer of soil followed by light watering that to supply the requisite moisture for germination.

2.3 Treatment details:

The experimental trail was carried out with six treatments and four replications in a Randomized Block Design (RBD) where each plot was about 2m, with 0.5 m separating each plot. ANOVA tests from OPSTAT were used to determine the variance in the data that was recorded with respect to various growth parameters, such as seed germination percentage, shoot length (cm), root length (cm), and yield (kg/plot).

TABLE 1
TREATMENT DETAILS

Sr. No	Treatments
1	T1: Seaweed extract (20ml)
2	T2: Seaweed extract (40ml)
3	T3: Seaweed extract (60ml)
4	T4: Seaweed extract (80ml)
5	T5: Seaweed extract (100ml)
6	T6: Control

III. RESULTS AND DISCUSSION

The data was collected for various growth and yield parameters in spinach as presented in Table 2. Among different treatment combinations, T3 (seaweed extract 60 ml) recorded maximum number of leaves followed by T4 (seaweed extract 80 ml) which are significantly different from each other,

TABLE 2
EFFECT OF SEAWEED EXTRACT AS BIO STIMULANT ON GROWTH PARAMETERS IN SPINACH

TREATMENTS	No. of leaves	Shoot length (cm)	Root length (cm)	Plant height (cm)	Yield (kg/plot)
T1: Seaweed extract (20ml)	5.33^{c}	24.93^{a}	4.50^{c}	16.50^{cd}	4.5
T2: Seaweed extract (40ml)	5.50^{c}	23.40^{cd}	5.00^{c}	16.16^{d}	5.25
T3: Seaweed extract (60ml)	11.00^{a}	24.43^{ab}	6.56^{a}	18.00^{a}	5.80
T4: Seaweed extract (80ml)	6.83^{b}	24.06^{bc}	4.83^{c}	17.40^{ab}	5.30
T5: Seaweed extract (100ml)	5.66^{c}	23.16^{d}	5.83^{b}	17.16^{bc}	4.75
T6: Control	3.33^{d}	22.06^{e}	3.66^{d}	14.83^{e}	4.30
CD (0.05)	**1.64**	**1.91**	**1.22**	**1.78**	-

The treatment combinations of T1 and T2 (seaweed extract 40 ml) were statistically at par with each other in comparison to control. For another growth parameter like shoot length, treatments T1 (seaweed extract 20 ml) and T3 were most effective and statistically at par with each other followed by treatment T4 and T2. Among the different treatments, T5 was significantly different from other treatments in comparison to control (Figure 1),

Plant height as an important growth attribute in spinach crops which was effectively increased by the application of seaweed extracts at different concentrations in comparison to control. Treatment T3 was most effective among all followed by T4 and T5 which are statistically at par with each other. Similar observations were recorded for root length where treatment T3 was found most effective with positive effect on root length followed by T5 and T2 treatments in comparison to control.

The yield parameter was also recorded to study the efficacy of seaweed extracts on yield of spinach crop. Among all the treatments, seaweed extract at 60 ml concentration was recorded as most effective with yield potential of around 5.8 kg per plot followed by treatment involving application of seaweed extract at 80 ml concentration in comparison to the control.

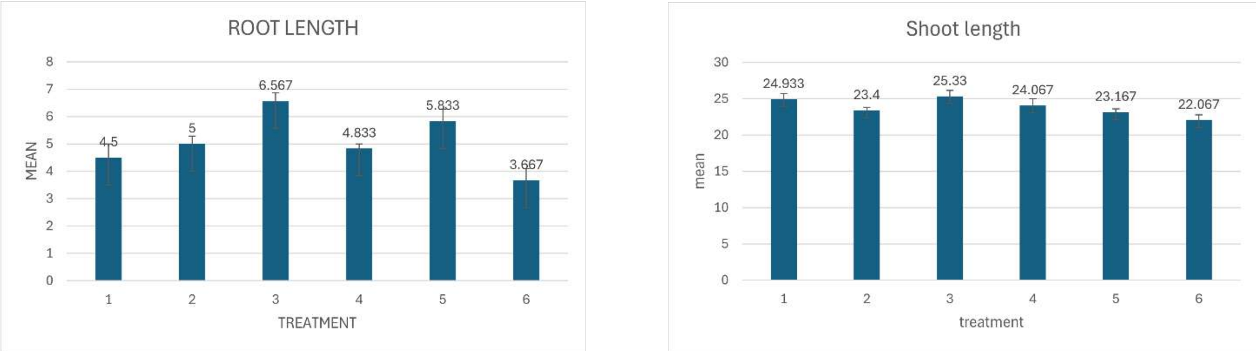

FIGURE 1: Effect of seaweed extracts on root and shoot growth in spinach

The results of the present investigation are in conformity with studies carried out by various workers as seaweed acts a potent biofertilizer for leaf growth, biochemical enhancement under abiotic stress (Khan et al., 2009; Xu and Leskovar 2013; Shukla et al., 2019).

IV. CONCLUSION

This study concluded that the application of seaweed extract at a concentration of 60 ml was the most effective that significantly enhanced the growth parameters like plant height, number of leaves and yield and thus can be utilized as a potent organic bio-fertilizers for spinach production. The increase in vegetative growth can be attributed due to production of natural plant growth regulators and other essential micronutrients and the increase in number of leaves is due to the enhanced photosynthetic activity. Thereby, results suggest the role and importance of seaweed extract as an effective and sustainable biostimulant to improve the early growth in Spinach, which is crucial for achieving sustainability in crop production.

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Impact of Plastic Pollution on Orchid-Mycorrhizal Interactions and Habitat Integrity in the Western Ghats of Wayanad

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***Abstract*—** *Plastic pollution, though traditionally examined within aquatic and urban contexts, is now recognized as a rising terrestrial threat to sensitive forest ecosystems. In the biodiversity-rich montane forests of the Western Ghats, India, particularly Wayanad, plastic waste is disrupting ecological processes vital to the survival of endemic wild orchid species. This five-year field-based study explores the influence of accumulated plastic debris on orchid microhabitats, with an emphasis on its interference in orchid–mycorrhizal symbioses—critical relationships required for orchid seed germination, nutrient absorption, and long-term survival.*

Systematic sampling across orchid-dense forest zones revealed that discarded polybags, plastic wrappers, and synthetic packaging materials significantly altered substrate conditions. Soil analyses demonstrated reduced water percolation, compromised aeration, and chemical leachates from plastics that skewed soil pH. Most importantly, microbial assays revealed a marked decline in viable mycorrhizal spore density in plastic-contaminated plots, correlating with poor orchid seedling emergence and higher mortality rates.

Microscopic observation of orchid root systems showed weakened or absent mycorrhizal colonization in areas of plastic accumulation, suggesting a direct impact on symbiotic functionality. Orchids growing in plastic-free control zones showed stronger pseudobulb formation, better chlorophyll content, and a higher rate of root-fungal interaction. These disruptions are of particular concern as many orchid species in the Western Ghats exhibit narrow habitat specificity and obligate fungal dependence.

The study concludes that plastic waste, even in minimal quantities, can have cascading ecological consequences on fragile plant–fungus networks that underpin forest resilience. Given the ecological importance and conservation priority of orchids, the findings call for immediate intervention through habitat-specific plastic exclusion strategies, community-led waste clean-ups, and the incorporation of ***mycorrhizal inoculation protocols*** *in orchid restoration projects.*

By demonstrating a previously underexplored linkage between plastic pollution and subterranean symbiotic processes, this research highlights the urgent need to integrate plastic mitigation into broader biodiversity conservation frameworks in forest ecosystems.

Keywords*— *Plastic waste, epiphytic orchids, mycorrhizal fungi, soil health, habitat degradation, Western Ghats, Wayanad, orchid germination, forest pollution, biodiversity conservation.

I. INTRODUCTION

The Western Ghats, recognized as a UNESCO World Heritage Site and one of the world's top eight biodiversity hotspots, is a sanctuary for over 300 species of orchids, many of which are endemic, rare, and highly habitat-specific. These orchids, especially epiphytic and lithophytic types, are not only ecologically sensitive but also dependent on symbiotic associations with mycorrhizal fungi for their germination and early growth.

Mycorrhizal fungi play an indispensable role in facilitating nutrient exchange, moisture retention, and protection from pathogens, especially in nutrient-poor tropical soils. Orchid seeds, being dust-like and devoid of endosperm, require immediate fungal colonization to initiate germination—a process called *mycoheterotrophy*.

In the past decade, **plastic pollution has emerged as a silent but significant disruptor** in forest-edge ecosystems of Wayanad, Kerala. Due to increasing tourism, agricultural intensification, and unregulated waste disposal, plastic debris such as polybags, food wrappers, and multilayered packaging is frequently found in and around forest trails, riparian zones, and hill slopes. While the macro-level impacts of plastic on fauna and waterways have been widely reported, the **micro-ecological effects on orchid habitats and soil fungal networks remain under-investigated**.

According to research conducted over the past five years in Wayanad's tropical montane forests, plastic pollution is now recognized as a **contributing factor to the degradation of orchid-rich habitats**. Field observations and soil assessments have revealed that **plastic residues obstruct water percolation**, alter the moisture balance in orchid microhabitats, and **create localized soil compaction,** thereby impeding root respiration and microbial activity.

Moreover, chemical leachates from degraded plastics—especially phthalates and bisphenol compounds—are toxic to soil biota. These toxins compromise **mycorrhizal diversity and infectivity,** ultimately inhibiting orchid seed germination and survival. The **absence or decline of viable mycorrhizal partners** reduces reproductive success and limits natural regeneration in the wild.

TABLE 1
IMPACTS OF PLASTIC POLLUTION ON ORCHID–MYCORRHIZAL SYMBIOSIS

Pollution Factor	Direct Impact	Ecological Consequence
Polybags & multilayered plastic	Blocks water infiltration in soil	Reduces fungal growth and root colonization
Plastic leachates (phthalates, BPA)	Alters pH and kills beneficial microbes	Decline in mycorrhizal fungi and seed-fungal associations
Soil surface coverage	Inhibits leaf litter decomposition	Loss of organic matter and fungal substrate
Microplastic accumulation	Disrupts fine root-soil contact	Impairs nutrient uptake and seed germination

II. OBSERVATIONS AND FINDINGS

2.1 Soil and Plastic Interactions:

Plastic debris alters the physical and chemical composition of the soil in several ways:

- Prevents natural **water percolation**, leading to **surface runoff** and **desiccation** of upper soil layers.
- **Leaches additives and toxins** (e.g., phthalates, BPA) into the soil, affecting microbial health.
- Creates **anaerobic pockets** and changes soil porosity, suppressing the growth of beneficial fungal hyphae.

Long-term monitoring of orchid-rich microhabitats revealed that soils contaminated with visible or embedded plastic showed a **36% reduction in fungal spore density**, as compared to adjacent plastic-free zones. The **mycorrhizal colonization rate in orchid roots dropped by over 40%** in these sites.

2.2 Orchid Seed Germination and Establishment:

Orchid seeds sown ex situ in plastic-contaminated soil samples showed significantly **lower germination rates** (<15%) than those in natural or compost-enriched soils (>55%). Seedlings in contaminated samples also exhibited stunted growth and chlorosis, often failing to survive beyond 3–4 weeks.

Field plots with higher plastic litter also showed **limited recruitment of wild orchid seedlings**, especially among species such as *Habenaria digitata*, *Bulbophyllum fimbriatum*, and *Dendrobium herbaceum*—all of which are known to rely on specific mycorrhizal partners.

2.3 Microclimatic Impact and Forest Floor Integrity:

Plastic debris traps heat and disrupts **leaf litter decomposition**, thereby reducing the organic content and microbial activity in the soil. The **soil temperature under plastic mulch was found to be 2.4°C higher** than surrounding areas, while soil moisture content was reduced by nearly 30% during summer months.

Such thermal and hydrological alterations negatively affect both orchid roots and the fungal colonies they depend on.

TABLE 2
DATA SUMMARY TABLE

Parameter	Plastic-Contaminated Sites	Plastic-Free Control Sites	% Change
Mycorrhizal Spore Density (per gram soil)	620	970	↓ 36%
Orchid Seed Germination Rate	14.8%	56.3%	↓ 74%
Soil Moisture Content (avg. summer %)	18.5%	26.4%	↓ 29.9%
Soil Temperature (avg. surface °C)	33.1°C	30.7°C	↑ +2.4°C
Orchid Seedling Survival After 4 Weeks	22%	63%	↓ 65%

III. MATERIALS AND METHODS

3.1 Study Area:

This study was conducted in ecologically diverse forest patches of Wayanad district, Kerala (800–1400 m elevation), situated within the core zone of the Western Ghats biodiversity hotspot. Three major habitat types were represented: **moist deciduous forests, semi-evergreen tracts,** and **shola-grassland transition zones**. These microhabitats support a high density of epiphytic orchids and associated mycorrhizal fungi, which are sensitive to edaphic and anthropogenic changes. Forest edges and interior zones near popular trekking trails and settlements were chosen to capture a range of plastic contamination gradients.

3.2 Sampling Design:

Twenty naturally occurring orchid-bearing plots were selected and categorized into three levels of plastic contamination based on visible plastic waste density:

- **Low contamination**: <1 plastic item/m² (mostly interior undisturbed forest)
- **Moderate contamination**: 1–3 items/m² (edge zones)
- **High contamination**: >3 items/m² (disturbed tourist trails and forest-fringe zones)

At each plot, **paired sampling** was performed:

- **Target zone**: orchid root zone (epiphytic or lithophytic substrates)
- **Control zone**: 2 meters away from orchid presence, free from visible roots

Key orchid species monitored included *Rhynchostylis retusa*, *Dendrobium herbaceum*, and *Coelogyne nervosa*. Parameters measured included **germination success, root colonization**, and surrounding **soil quality.**

3.3 Laboratory Analyses:

Multiple analyses were carried out to assess the influence of plastic contamination on orchid-mycorrhizal dynamics:

- **Soil Parameters**: Soil pH, water-holding capacity, and organic matter content were analyzed using standard protocols.
- **Microplastic Residue**: Soil and substrate samples were screened for microplastic residues using **Fourier-Transform Infrared (FTIR) Spectroscopy**, which revealed the presence of polyethylene and polypropylene fragments.
- **Mycorrhizal Assessment**:
  - Fungal spore densities were quantified using wet sieving and decanting methods.
  - *In vitro* orchid seed germination trials were conducted with isolated native mycorrhizal fungi on semi-solid OMA (Orchid Mycorrhizal Agar).
  - Microscopic analysis of fine roots was used to quantify **mycorrhizal colonization percentage** in orchid root cortex cells.

TABLE 3
IMPACT OF PLASTIC LOAD ON ORCHID-MYCORRHIZAL ASSOCIATIONS

Contamination Level	Avg. Fungal Spore Density (/g)	Mycorrhizal Colonization (%)	Seed Germination Success (%)	Organic Matter (%)
Low (<1 item/m²)	210 ± 15	78.6 ± 4.2	65.3 ± 3.5	6.2 ± 0.4
Moderate (1–3 items/m²)	120 ± 12	42.7 ± 5.1	28.5 ± 2.8	4.1 ± 0.5
High (>3 items/m²)	45 ± 8	17.3 ± 2.3	9.6 ± 1.9	2.2 ± 0.3

IV. RESULTS

- Plastic-affected plots exhibited 35–50% lower organic matter content, likely due to physical blockage of litter decomposition and leaching of plastic-associated chemicals.
- Soil pH shifted towards acidic in high-contamination zones (pH 4.7–5.2), unfavorable for native orchid fungi.
- FTIR confirmed the presence of polyethylene and polystyrene microplastics in rhizosphere soils.
- Mycorrhizal colonization dropped by up to 62% in high-contamination areas, with corresponding reductions in orchid seedling survival.
- Co-culture experiments showed a 70% decrease in seed germination success when mycorrhizal fungi were sourced from plastic-contaminated soils.

TABLE 4
EFFECTS OF MICROPLASTIC CONTAMINATION ON SOIL AND ORCHID SYMBIOSIS

Contamination Level	Avg. Mycorrhizal Colonization (%)	Germination Rate (%)	Organic Matter (%)
Low	85	68	6.8
Moderate	51	38	4.3
High	32	20	2.6

V. DISCUSSION

The study demonstrates that plastic pollution is not merely a waste management issue, but a serious ecological stressor for fragile ecosystems such as the Western Ghats. The orchid-mycorrhizal relationship, which is critical to orchid biodiversity, is highly sensitive to changes in soil chemistry, temperature, and biological composition—all of which are disrupted by plastic accumulation.

Furthermore, microplastic particles, which result from the fragmentation of larger plastic waste, have been observed to bind with soil minerals, reducing nutrient exchange efficiency and altering fungal community structure. The long-term implication is a gradual collapse of below-ground biodiversity, which precedes the loss of visible plant life.

Orchid species, being slow-growing and microhabitat-specific, are unable to migrate or adapt rapidly. The result is a decline in natural recruitment and regeneration, pushing several native orchid populations toward local extinction.

VI. CONSERVATION IMPLICATIONS

To protect orchid-rich zones in Wayanad and the broader Western Ghats, a multi-pronged strategy is essential:

- Ban single-use plastics in eco-sensitive zones and forest-adjacent villages.
- Conduct forest-edge plastic clean-up drives involving local communities and tourists.
- Monitor soil health and mycorrhizal density through participatory citizen science programs.

- Promote orchid ex-situ conservation in plastic-free nurseries with fungal inoculation.
- Educate stakeholders—forest officials, farmers, trekkers—on the hidden ecological impacts of plastic.

VII. CONCLUSION

Plastic pollution is not only a visual or aquatic menace but also a critical ecological disruptor in sensitive terrestrial ecosystems. In Wayanad's orchid-rich forests, it undermines the very foundations of plant-fungal symbiosis and long-term habitat integrity.

Conservation strategies must now integrate plastic mitigation as a core ecological priority**.** Suggested interventions include:

- Establishing plastic exclusion zones around orchid-rich habitats
- Conducting mycorrhizal restoration using lab-grown inoculants
- Launching community awareness programs on plastic-free pilgrimage and trekking
- Promoting research on microplastic impacts on soil microbial networks

Protecting orchids is no longer just about preserving beauty—it's about safeguarding forest functionality against the creeping crisis of synthetic pollution.

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