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## Preface

We would like to present, with great pleasure, the volume-12, Issue-2, February 2026 of a scholarly journal, *International Journal of Environmental & Agriculture Research*. This journal is part of the AD Publications series *in the field of Environmental & Agriculture Research Development*, and is devoted to the gamut of Environmental & Agriculture issues, from theoretical aspects to application-dependent studies and the validation of emerging technologies.

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

### **Environmental Research:**

*Environmental science and regulation, Ecotoxicology, Environmental health issues, Atmosphere and climate, Terrestrial ecosystems, Aquatic ecosystems, Energy and environment, Marine research, Biodiversity, Pharmaceuticals in the environment, Genetically modified organisms, Biotechnology, Risk assessment, Environment society, Agricultural engineering, Animal science, Agronomy, including plant science, theoretical production ecology, horticulture, plant, breeding, plant fertilization, soil science and all field related to Environmental Research.*

### **Agriculture Research:**

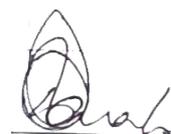
*Agriculture, Biological engineering, including genetic engineering, microbiology, Environmental impacts of agriculture, forestry, Food science, Husbandry, Irrigation and water management, Land use, Waste management and all fields related to Agriculture.*

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



Mukesh Arora

(Managing Editor)



Dr. Bhagawan Bharali

(Chief Editor)

## Fields of Interests

Agricultural Sciences	
Soil Science	Plant Science
Animal Science	Agricultural Economics
Agricultural Chemistry	Basic biology concepts
Sustainable Natural Resource Utilisation	Management of the Environment
Agricultural Management Practices	Agricultural Technology
Natural Resources	Basic Horticulture
Food System	Irrigation and water management
Crop Production	
Cereals or Basic Grains: Oats, Wheat, Barley, Rye, Triticale, Corn, Sorghum, Millet, Quinoa and Amaranth	Oilseeds: Canola, Rapeseed, Flax, Sunflowers, Corn and Hempseed
Pulse Crops: Peas (all types), field beans, faba beans, lentils, soybeans, peanuts and chickpeas.	Hay and Silage (Forage crop) Production
Vegetable crops or Olericulture: Crops utilized fresh or whole (wholefood crop, no or limited processing, i.e., fresh cut salad); (Lettuce, Cabbage, Carrots, Potatoes, Tomatoes, Herbs, etc.)	Tree Fruit crops: apples, oranges, stone fruit (i.e., peaches, plums, cherries)
Tree Nut crops: Hazlenuts. walnuts, almonds, cashews, pecans	Berry crops: strawberries, blueberries, raspberries
Sugar crops: sugarcane. sugar beets, sorghum	Potatoes varieties and production.
Livestock Production	
Animal husbandry	Ranch
Camel	Yak
Pigs	Sheep
Goats	Poultry
Bees	Dogs
Exotic species	Chicken Growth
Aquaculture	
Fish farm	Shrimp farm
Freshwater prawn farm	Integrated Multi-Trophic Aquaculture

<b>Milk Production (Dairy)</b>	
Dairy goat	Dairy cow
Dairy Sheep	Water Buffalo
Moose milk	Dairy product
<b>Forest Products and Forest management</b>	
Forestry/Silviculture	Agroforestry
Silvopasture	Christmas tree cultivation
Maple syrup	Forestry Growth
<b>Mechanical</b>	
General Farm Machinery	Tillage equipment
Harvesting equipment	Processing equipment
Hay & Silage/Forage equipment	Milking equipment
Hand tools & activities	Stock handling & control equipment
Agricultural buildings	Storage
<b>Agricultural Input Products</b>	
Crop Protection Chemicals	Feed supplements
Chemical based (inorganic) fertilizers	Organic fertilizers
<b>Environmental Science</b>	
Environmental science and regulation	Ecotoxicology
Environmental health issues	Atmosphere and climate
Terrestrial ecosystems	Aquatic ecosystems
Energy and environment	Marine research
Biodiversity	Pharmaceuticals in the environment
Genetically modified organisms	Biotechnology
Risk assessment	Environment society
Theoretical production ecology	horticulture
Breeding	plant fertilization

## **Board Members**

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Professor & Head, Department of Crop Physiology, Faculty of Agriculture, Assam Agricultural University, Jorhat-785013 (Assam).

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Dr. Kusum Gaur working as professor Community Medicine and member of Research Review Board of Sawai Man Singh Medical College, Jaipur (Raj) India.

She has awarded with WHO Fellowship for IEC at Bangkok. She has done management course from NIHF. She has published and present many research paper in India as well as abroad in the field of community medicine and medical education. She has developed Socio-economic Status Scale (Gaur's SES) and Spiritual Health Assessment Scale (SHAS). She is 1st author of a book entitled " Community Medicine: Practical Guide and Logbook.

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### **Dr. Darwin H. Pangaribuan**

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Dr. Smruti Sohani, has Fellowship in Pharmacy & Life Science (FPLS) and Life member of International Journal of Biological science indexed by UGC and e IRC Scientific and Technical Committee. Achieved young women scientist award by MPCOST. Published many Indian & UK patents, copyrights, many research and review papers, books and book chapters. She Invited as plenary talks at conferences and seminars national level, and as a Session chair on many International Conference organize by Kryvyi Rih National University, Ukraine Europe. Designated as state Madhya Pradesh Coordinator in International conference collaborated by RCS. Coordinator of two Professional Student Chapter in collaboration with Agriculture Development society and research Culture Society. her enthusiastic participation in research and academia. She is participating on several advisory panels, scientific societies, and governmental committees. Participant in several worldwide professional research associations; member of esteemed, peer-reviewed publications' editorial boards and review panels. Many Ph.D., PG, and UG students have benefited from her guidance, and these supervisions continue.

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Dr. Chiti Agarwal works as a postdoctoral associate at the University of Maryland in College Park, Maryland, USA. Her research focuses on fungicide resistance to fungal diseases that affect small fruits such as strawberries. She graduated from North Dakota State University in Fargo, North Dakota, with a B.S. in biotechnology and an M.S. in plant sciences. Dr. Agarwal completed her doctorate in Plant Pathology while working as a research and teaching assistant. During her time as a graduate research assistant, she learned about plant breeding, molecular genetics, quantitative trait locus mapping, genome-wide association analysis, and marker-assisted selection. She wants to engage with researchers from many fields and have a beneficial impact on a larger audience.

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

## **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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# Seed Priming: Mechanisms, Methods, and Applications for Enhancing Crop Resilience in Fragile Ecosystems

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**Abstract**— *Seed priming is a pre-sowing technique involving controlled hydration and dehydration to activate pre-germinative metabolism without radicle emergence. This review synthesizes the principles, methods, and multifaceted benefits of seed priming as a pivotal strategy for enhancing crop establishment and stress resilience. Priming improves germination uniformity, vigour, and yield across major cereals, pulses, and oilseeds by triggering complex physiological, biochemical, and molecular responses, including antioxidant system activation, DNA repair, and stress-responsive gene expression. Conventional methods such as hydropriming, osmo-priming, and biopriming, alongside advanced techniques like nano-priming and physical priming, are detailed. The paper highlights crop-specific applications, underscores the technology's role in mitigating abiotic stresses in fragile ecosystems, and discusses its limitations and future challenges. As a cost-effective and accessible intervention, seed priming is a vital tool for sustainable agricultural intensification and climate adaptation.*

**Keywords**— *Seed priming, pre-germinative metabolism, abiotic stress, biopriming, nano-priming, crop resilience.*

## I. INTRODUCTION

Seeds are the cornerstone of agriculture, ensuring genetic continuity and food security. High-quality seeds are fundamental for uniform plant establishment and optimal productivity. Seed priming is a pre-sowing biotechnological practice that involves controlled hydration to initiate metabolic processes, followed by drying to prevent radicle protrusion. This technique, pioneered by Heydecker et al. (1973), enhances germination speed, uniformity, seedling vigour, and stress tolerance, leading to improved crop performance, especially under adverse conditions.

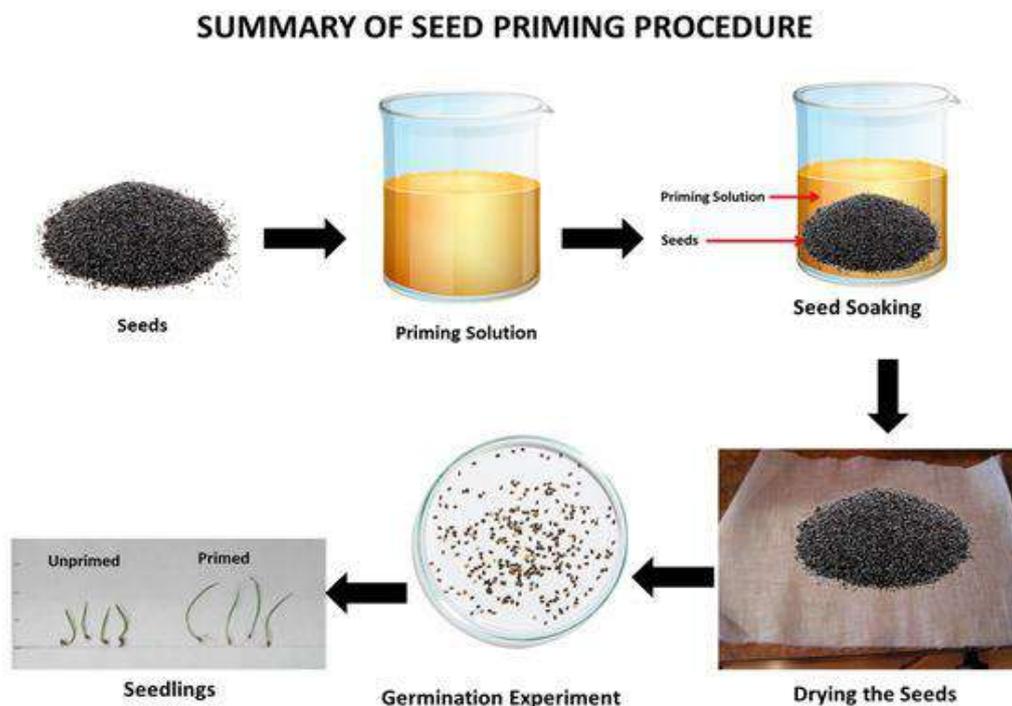
The global challenge of cultivating crops in fragile ecosystems—marked by drought, salinity, and extreme temperatures—necessitates resilient agricultural strategies. Seed priming offers a pragmatic, low-cost solution by inducing a "priming memory," enabling plants to mount faster and more robust cellular defences against subsequent stresses. This review comprehensively examines the benefits, underlying principles, physiological-biochemical-molecular mechanisms, diverse methods, and field applications of seed priming in major crops. It also critically addresses the limitations and future prospects of this technology for sustainable crop production.

## II. THE PRINCIPLE AND PROCESS OF SEED PRIMING

Seed priming is defined as a controlled hydration process sufficient to activate pre-germinative metabolism but insufficient to allow radical protrusion (Heydecker et al., 1973; McDonald, 2000). The standard seed germination process consists of three

phases: Phase I (rapid water imbibition), Phase II (activation of metabolic processes and repair), and Phase III (radicle emergence and growth). Priming meticulously regulates seeds within Phase II.

The procedure involves soaking seeds in a priming agent for a specific duration, followed by rinsing and drying back to the original moisture content (Fig. 1). This controlled cycle allows for the repair of cellular components (e.g., DNA, mitochondria), accumulation of germination-promoting metabolites, and induction of stress-responsive pathways, without the loss of desiccation tolerance characteristic of Phase III.



**FIGURE 1: Schematic representation of the seed priming procedure.**

### III. BENEFITS OF SEED PRIMING:

The agronomic and physiological benefits of seed priming are extensive:

1. **Enhanced Germination & Establishment:** Promotes rapid, uniform germination and robust stand establishment, even under suboptimal conditions (Nakaune et al., 2012; Manikanta et al., 2020).
2. **Improved Stress Tolerance:** Induces cross-tolerance against abiotic (drought, salinity, temperature extremes) and biotic stresses by priming defence pathways (Basra et al., 2005; Miladinov et al., 2020).
3. **Increased Yield Potential:** Leads to better crop growth, early flowering, and higher yield by improving seedling vigour and resource use efficiency (Harris et al., 2007; Singh et al., 2015).
4. **Metabolic Activation:** Boosts the activity of key enzymes ( $\alpha$ -amylase, antioxidant enzymes), hormonal balance (GA/ABA ratio), and reserve mobilization (Paparella et al., 2015).
5. **Socio-Economic Advantage:** A low-cost, eco-friendly technology accessible to smallholder farmers, reducing dependency on external inputs.

### IV. PHYSIOLOGICAL, BIOCHEMICAL, AND MOLECULAR MECHANISMS:

Seed priming orchestrates a suite of interconnected mechanisms that prepare the seed for germination and stress resilience (Fig. 2, 3).

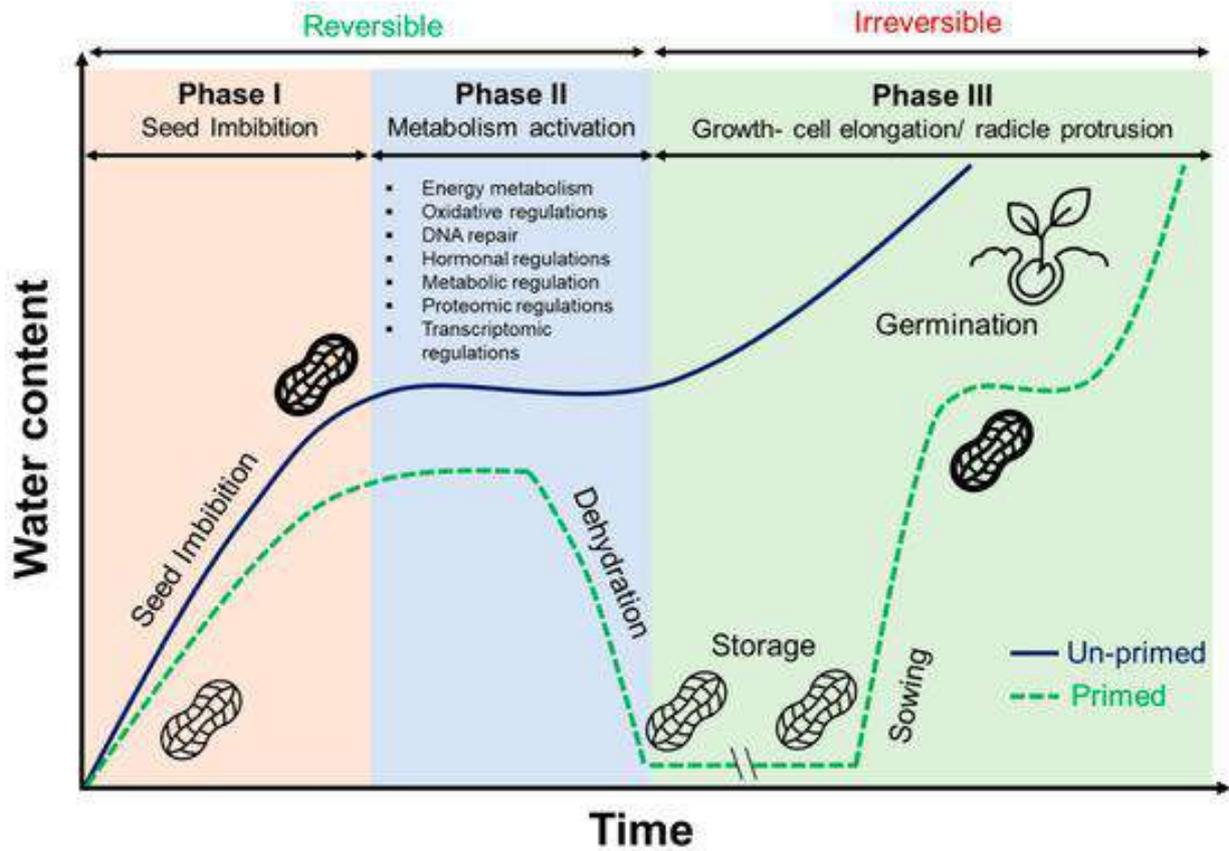


FIGURE 2: Hydration curves and germinating phases in unprimed vs. primed seeds.

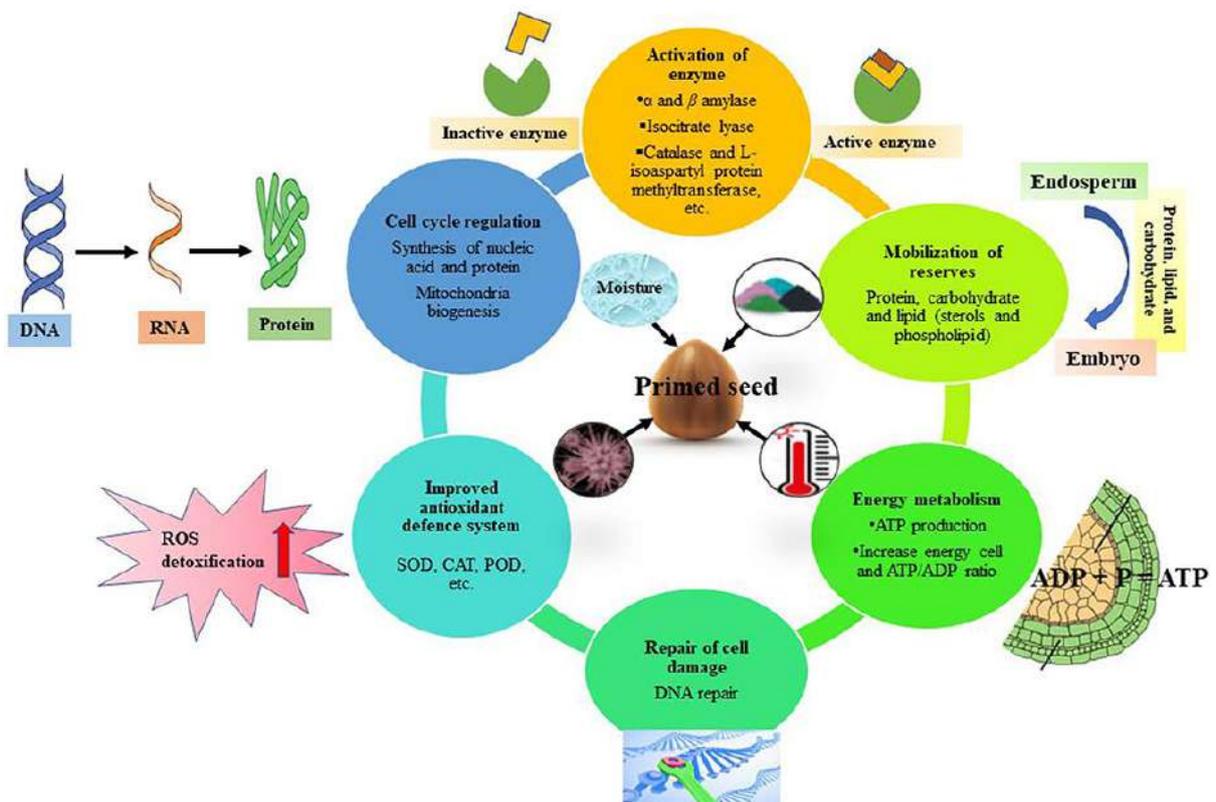


FIGURE 3: Summary of key biochemical and molecular changes induced by seed priming during Phase II.

#### 4.1 Activation of Pre-germinative Metabolism and Repair:

During controlled imbibition, primed seeds undergo essential repair and preparatory events: mitochondrial repair and synthesis, DNA damage repair via Base Excision Repair (BER) and Nucleotide Excision Repair (NER) pathways, and *de novo* synthesis of proteins, nucleic acids, and ATP (Paparella et al., 2015; Wojtyla et al., 2016).

#### 4.2 Antioxidant Defence and ROS Management:

Reactive Oxygen Species (ROS) are inevitable by-products of resumed metabolism. Priming upregulates both enzymatic (SOD, CAT, APX, POD) and non-enzymatic (glutathione, ascorbate) antioxidant systems, managing ROS levels as signalling molecules while preventing oxidative damage to lipids, proteins, and DNA (Bailly, 2004; Farooq et al., 2017).

#### 4.3 Hormonal Regulation:

Priming modulates the critical balance between abscisic acid (ABA, a germination inhibitor) and gibberellins (GA, germination promoters). It often leads to a decline in ABA and a rise in GA, facilitating the transition from dormancy to germination (Kermode, 1990).

#### 4.4 Expression of Stress-Responsive Proteins:

Priming induces the accumulation of protective proteins like Late Embryogenesis Abundant (LEA) proteins, dehydrins, and heat shock proteins (HSPs). It also upregulates aquaporins, enhancing water uptake efficiency during subsequent germination (Chen and Arora, 2013).

#### 4.5 Cell Cycle Activation and Energy Metabolism:

Primed seeds show earlier activation of the cell cycle upon re-imbibition. Energy metabolism is optimized through increased  $\alpha$ -amylase activity, breaking down starch into soluble sugars to fuel growth (Varier et al., 2010).

### V. METHODS OF SEED PRIMING

Priming methods are categorized into conventional and advanced techniques.

#### 5.1 Conventional Methods

- **Hydropriming:** Soaking seeds in water. Simple and low-cost but risks uncontrolled imbibition.
- **Osmo-priming:** Using osmotic solutions (e.g., PEG, mannitol, salts) to control water potential. Effective but can be expensive and reduce oxygen diffusion.
- **Halo-priming:** Use of inorganic salt solutions (KNO<sub>3</sub>, CaCl<sub>2</sub>).
- **Nutri-priming:** Soaking in solutions of macro/micronutrients (e.g., Zn, P).
- **Hormonal Priming:** Use of plant growth regulators (GA<sub>3</sub>, SA, IAA).
- **Biopriming:** Integration of seed hydration with beneficial microorganisms (e.g., *Trichoderma*, *Pseudomonas*, *Rhizobium*). Enhances growth and suppresses diseases.
- **Solid Matrix Priming:** Hydration using a solid medium (vermiculite, peat) to control water uptake precisely.

#### 5.2 Advanced Methods

- **Nano-priming:** Application of nanoparticles (ZnO, SiO<sub>2</sub>, Fe<sub>3</sub>O<sub>4</sub>) for targeted nutrient delivery and enhanced stress response.
- **Physical Priming:** Use of physical agents like magnetic fields (magneto-priming), UV radiation, gamma irradiation, or cold plasma to stimulate metabolic activity.

### VI. APPLICATIONS IN MAJOR FIELD CROPS: A SYNTHESIS

Seed priming has demonstrated significant positive effects across a wide range of field crops. The following table summarizes key findings from selected studies.

**TABLE 1**  
**SELECTED RESEARCH ON SEED PRIMING EFFECTS IN MAJOR FIELD CROPS**

Crop	Priming Method	Key Observed Effects	Key References
Rice	Various	Improved germination, seedling growth, and stress tolerance; enhanced yield.	Adhikari and Sangita Bhujel Adhikari (2023); Basra et al. (2004, 2006); Farooq et al. (2007, 2010); Hussain et al. (2016); Tissarum et al. (2020); Gao et al. (2023)
Wheat	Various	Enhanced germination percentage, vigour, and heat stress tolerance; increased yield.	Ali et al. (2013); Ghasem et al. (2013); Balakhnina et al. (2015); Hussain et al. (2018); Bajwa et al. (2018); Al-Akhras M Ali et al. (2024)
Maize	Various	Better stand establishment, increased yield components under drought.	Akbari et al. (2007); Imran et al. (2013); Bakhtavar et al. (2015); Anjuman et al. (2015); Madhukeswar and Sajjan (2017); Kappor et al. (2023)
Sorghum	Various	Improved germination and early seedling growth under stress.	Kadiri et al. (1999); Iram et al. (2002); Amarnath et al. (2018); Chen et al. (2021); Koradhanyamah et al. (2024)
Mungbean	Various	Increased germination speed, seedling vigour, and stress tolerance.	Posmyk et al. (2007); Chauhan and Naiya et al. (2013); Reddy et al. (2013); Tiwari et al. (2016); Afrayeem et al. (2018); Vardhini and Singh (2021)
Blackgram	Various	Enhanced germination and seedling growth; improved stress tolerance.	Aryal et al. (2020); Ladumor and Singh (2022); Timisina et al. (2024); Jadhav et al. (2024); Midhul Rana and Satyanarayanan (2024); Krishnasamy et al. (2024)
Pigeonpea	Various	Improved germination and early seedling growth under water deficit.	Raj et al. (2008); Hareesh (2014); Tiwari et al. (2014, 2021); Kavitha and Srimathi (2022); Ashok et al. (2017); Raju and Rai et al. (2017)
Chickpea	Various	Enhanced chilling tolerance, antioxidant activity, and water relations.	Kumar et al. (2014); Laal et al. (2015); Vigneswara et al. (2017); Farooq et al. (2017); Kamithi et al. (2018)
Groundnut	Various	Improved pod yield, nutrient uptake, and drought resilience.	Sanojkumar et al. (2017); Sepeheri and Rouchi (2017); Pal et al. (2017); Hassan and Ismail (2018); Babu et al. (2018); Das and Mohanty (2018); Vardhini et al. (2021); Cagasan et al. (2022); Moharana et al. (2023); Hozyan et al. (2024); Hemalatha et al. (2024)
Sunflower	Various	Induced salt tolerance at germination and early seedling stage.	Bajebha et al. (2010); Aymen and Hannchi (2012); Shanthala and Siidiraju (2013); Sakpal and Ahmad (2023); Priya Reddy and Nanja Reddy (2024)
Soybean	Various	Increased nodulation, biomass, and yield under field conditions.	Saha et al. (1990); Bensen et al. (1990); Muhammad Arif et al. (2008); Bassi et al. (2011); Assefa et al. (2010, 2011); Golezani et al. (2011); Shine et al. (2011); Sadeghi et al. (2011); Bhowmick et al. (2013); Chavan et al. (2014); Radhakrishnan et al. (2013); Mangena (2020); Dugeswar et al. (2024); Alkamas et al. (2024)
Sesame	Various	Improved germination and seedling growth under stress.	Singh et al. (2002); Kand-Bo Shim et al. (2009); Shabbir et al. (2014); Singh et al. (2022); Askari et al. (2019); Ifran et al. (2024); Mori et al. (2024)

## VII. LIMITATIONS AND CHALLENGES

Despite its promise, seed priming faces practical constraints:

- Species and Protocol Specificity:** Optimal priming parameters (agent, concentration, duration) vary greatly among crops and even cultivars, requiring extensive optimization.
- Reduced Storability:** Primed seeds often have shorter shelf-life due to increased metabolic activity, posing logistical challenges for seed companies and farmers.

3. **Scale-up and Cost:** Translating lab protocols to cost-effective, large-scale on-farm applications remains a hurdle, especially for methods involving expensive agents or equipment.
4. **Knowledge Gaps:** A deeper molecular understanding of "priming memory" and the long-term agronomic impacts under diverse, real-field multi-stress conditions is needed.

### VIII. CONCLUSION AND FUTURE PERSPECTIVES

Seed priming is a scientifically robust and practically viable technology to enhance crop performance in fragile ecosystems. By activating a cascade of pre-adaptive mechanisms, it equips plants to withstand environmental stresses from the very start of their life cycle. Its simplicity and cost-effectiveness make it particularly suitable for resource-poor farming systems.

To fully realize its potential, future efforts should focus on:

- **Protocol Standardization:** Developing crop- and environment-specific priming protocols.
- **Molecular Breeding:** Identifying and integrating genetic markers associated with superior priming response into breeding programs.
- **Innovative Delivery Systems:** Exploring nano-formulations and seed coating technologies for easier application and extended efficacy.
- **Policy and Extension Support:** Strengthening extension services to bridge the gap between research and widespread farmer adoption.

Seed priming stands as a critical component of sustainable intensification strategies, offering a pathway to stabilize yields and enhance food security in the face of climate change.

### CONFLICT OF INTEREST

The authors declare no conflict of interest.

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# Genetic Analysis of Some Yield Components in Single Hybrids of Yellow Maize (*Zea mays* L.)

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**Abstract**— A half-diallel cross among six maize inbred lines was carried out in 2023 at the Maize Research Department, General Authority for Scientific Agricultural Research. In 2024, fifteen hybrids, the control variety Ghouta 82, and the parental lines were evaluated to estimate general and specific combining ability and heterosis for ear height, ear length, ear diameter, and number of rows per ear. Significant differences were observed among lines and hybrids for all traits. The hybrid (P6 × P4) exceeded Ghouta 82 in ear height, while all hybrids surpassed it in ear length, ear diameter, and number of rows per ear. Except for (P5 × P2), all hybrids showed positive heterosis, and nine exhibited significant heterosis compared to the best parent in row number. Ear height was mainly controlled by additive gene effects, while both additive and non-additive effects influenced the remaining traits, with additive effects predominating.

**Keywords**— Maize, Half-diallel crosses, General combining ability, Specific combining ability, Heterosis.

## I. INTRODUCTION

Maize (*Zea mays* L.) belongs to the Poaceae family, tribe Maydeae. It is a monoecious, annual herbaceous plant (Hallauer and Miranda, 1981). Maize is grown in a wide temperature range between 50°N and 40°S, and from sea level to an altitude of 3,300 m (Saeed and Saleem, 2000). Yellow corn constitutes approximately 75% of the feed provided to poultry in Syria. With the growth of the livestock sector and the poultry industry, demand for corn has increased, and the gap between need and local production has widened (Alexander, 2003), reaching 89% (Statistical Abstract, 2006). Since production capacity is linked to genetic makeup and its responsiveness to growth factors, local production of yellow corn can be increased through the optimal application of agricultural practices (soil preparation, planting date, seed rate, and fertilizer formulas), post-planting maintenance (irrigation, weeding, etc.), and through supporting modern breeding programs that work to develop new, high-yielding genetic materials, especially single crosses.

Crop yield is the most important agricultural trait (Zdunic et al., 2008). It is a complex quantitative trait whose inheritance is controlled by a large number of major and minor genes (Hassan, 1991). It cannot be directly improved, especially when dealing with a cross-pollinated crop such as maize. Therefore, to overcome this problem, the yield trait is studied through its components. The mechanism of its inheritance is investigated, and the nature of the genetic action that can achieve an increase in yield is determined. This information can be used in planning breeding programs (Melchinger et al., 1986) by identifying the traits suitable for indirect selection for yield, and choosing the appropriate selection method (Mohammadia et al., 2003).

Maize ranks second globally after wheat in terms of cultivated area and first in terms of production. In 2008, the global area cultivated with maize reached approximately 1,071,094 hectares, producing approximately 9,736,434.48 million tons (FAO, 2023). In the Arab world, maize ranks third after wheat and barley in terms of cultivated area and second after wheat in terms of production. The area cultivated with maize in the Arab world reached 161,507 thousand hectares, producing

approximately 378,221.62 tons per hectare (Arab Organization for Agricultural Development, 2023). In Syria, maize ranks third after wheat and barley in terms of cultivated area and production. The cultivated area in 2023 reached 78,483 thousand hectares, producing 562,529 thousand tons (Statistical Abstract, 2023).

The half-diallel cross technique developed by Hayman (1954), Griffing (1956), and Jinks (1954) is a form of diallel cross applied to parents that may be composite and synthetic varieties or pure lines in self-pollinated crops, or inbred lines in cross-pollinated crops. The objectives are to create new genetic combinations, study the genetic behavior of traits, and determine the nature of gene action controlling them, especially in maize, thus helping plant breeders to activate the selection process (Hallauer and Miranda, 1981). The first generation (F<sub>1</sub>) produced by hybridization between inbred maize lines is currently used in agricultural production in developed countries because it is characterized by high heterosis and yield (Al-Sahouki, 1990). It exhibits a high degree of uniformity in the field, as the inbred lines are genetically homozygous, and no genetic recombination occurs when hybridization takes place (Hassan, 1991).

Heterosis has been defined as a quantitative measure of the superiority of hybrids over their parents. It expresses an increase in growth vigor or other agronomic traits that constitute yield components (Falconer and Mackay, 1996). It can also indicate an increase in qualitative traits, economic quality, pest resistance, and adaptation to adverse environmental conditions (Hassan, 1991). Heterosis increases with weaker genetic kinship between the parents, i.e., it increases in genetically distant lines (Hadid, 1999). A decrease in heterosis is observed when hybridizing between open-pollinated populations with a broad genetic base compared to the heterosis resulting from hybridization between inbred lines (Hallauer and Miranda, 1981).

Plant height and number of rows per ear showed significant heterosis compared to the average of the parents and the best parent, while heterosis values were not significant for number of rows per ear compared to the best parent, in offspring resulting from hybridization between five inbred lines of yellow maize (Abou-Deif, 2007). The values of heterosis were significant and positive (88.81%, 98.62%) for plant height, and for number of rows per ear (6.91%, 3.62%), and for number of grains per row (59.97%, 75.22%), compared to the average of the two parents and the best parent, respectively (Al Ahmad, 2004). Malik et al. (2004) evaluated heterosis in single hybrids resulting from complete diallel crosses of nine inbred lines of yellow maize, where heterosis values for plant height ranged from -19.5% to 37.8% and from -29.7% to 33.9% compared to the average of the two parents and the best parent, respectively; for number of rows per ear, values ranged from 17.2% to 25.9% and from -20.0% to 21.4%; and for number of grains per row, from 28.5% to 49.6% and from 24.6% to 37.8% compared to the mean of the two parents and the best parent, respectively. Abdel-Moneam et al. (2009) found significant heterosis for number of grains per row of 172.42% and 83.72% compared to the mean of the two parents and the best parent, respectively. El-Hosary et al. (1999) also found significant positive heterosis compared to the best parent for plant height, reaching 89.97%.

On the other hand, the economic feasibility of derived hybrids can be investigated by comparing them with locally approved hybrids. Sharief et al. (2009) reported that the highest values of heterosis for yield reached 47.76% compared to the check hybrid. El-Hosary et al. (1994) achieved good heterosis for yield, its components, and plant height compared to the check hybrid. Al Ahmad (2004) obtained the highest values of heterosis compared to the check hybrid for plant height (10.89%) and number of rows per ear (9.24%). The hybrids derived in studies by Kaushik et al. (2004) and Ünay et al. (2004) showed economic heterosis (compared to the check hybrid) for yield component traits.

The combining ability study revealed the genetic behavior of the studied traits and the nature of gene action governing them. Additive gene effects predominated the inheritance of plant height and number of grains per row. On the other hand, non-additive gene effects predominated the inheritance of number of rows per ear in single hybrids resulting from half-diallel crosses of eight inbred lines of maize (El-Hosary et al., 1994). This contradicts the results of Shafey (1998), where number of rows per ear was affected by additive genetic action, while non-additive gene effects predominated the inheritance of plant height in fifteen single hybrids of maize. Al Ahmad (2004) found that the ratio of GCA variance to SCA variance was greater than one for yield components, indicating the importance of additive genetic action in the inheritance of these traits. This is consistent with Xing-ming et al. (2001), who found highly significant GCA values for maize grain yield components and indicated the importance of additive action. Khan et al. (1999) indicated the relative importance of non-additive genetic action in the inheritance of number of grains per row and plant height. This contrasts with El-Absawy (2003), who found relative importance of additive genetic action in the inheritance of yield components, while non-additive gene effects predominated the inheritance of plant height, which agrees with El-Bially (2003) and Tabassum et al. (2007).

Taller lines produced taller single hybrids compared to hybrids produced by other lines (Hee Chung et al., 2006). Ibrahim (2003) confirmed the importance of additive genetic action and epistatic action of the type additive  $\times$  additive in the inheritance of plant height, and pointed out the importance of non-additive genetic action in the inheritance of number of rows per ear. Matho and Ganguli (2003) showed the dominance of non-additive genetic action on the inheritance of plant height, number of grains per row, and number of rows per ear. Muraya et al. (2006) explained that non-additive genetic action is the most important in the inheritance of yield component traits and plant height.

This study aims to determine the inheritance mechanism of some quantitative traits by estimating general combining ability (GCA) and specific combining ability (SCA), as well as estimating heterosis based on the average of the two parents and the best parent.

## II. MATERIALS AND METHODS

### 2.1 Plant Material:

Six inbred yellow maize lines were used in this study: IL.155-22 (designated as P1), IL.130-22 (P2), IL.262-22 (P3), IL.257-22 (P4), IL.422-22 (P5), and IL.424-22 (P6). These lines were highly genetically pure (95%) and genetically divergent. They were obtained from the gene bank of the Maize Research Department, General Commission for Scientific Agricultural Research, Syria. The check variety used was Ghouta 82, a widely cultivated single-cross hybrid in Syria.

### 2.2 Field Experimentation:

The experiment was conducted during the 2023 and 2024 agricultural seasons at the Maize Research Department, General Commission for Scientific Agricultural Research, Syria.

- **2023 Season (Hybridization):** The inbred lines were planted on May 7, 2023. During the flowering stage, hybridization was carried out between the inbreds using all possible combinations without reciprocals (half-diallel) to obtain F<sub>1</sub> seeds for fifteen single-cross hybrids.
- **2024 Season (Evaluation):** The fifteen F<sub>1</sub> hybrids, along with the six parental inbred lines and the check variety Ghouta 82, were planted in a randomized complete block design (RCBD) with three replications. Each accession was planted in four 6-meter-long rows, with a distance of 70 cm between rows and 25 cm between plants within each row. All agricultural operations, including weeding, fertilization, and thinning, were carried out based on the recommendations of the Ministry of Agriculture and Agrarian Reform for yellow maize production.

### 2.3 Traits Measured:

At physiological maturity, ten randomly selected competitive plants from the two central rows of each plot (excluding border rows) were sampled for data collection. The following traits were measured:

- **Ear height (cm):** Distance from the soil surface to the node bearing the upper ear
- **Ear length (cm):** Measured from the base to the tip of the ear
- **Ear diameter (cm):** Measured at the mid-point of the ear
- **Number of rows per ear:** Counted on the same ten ears

### 2.4 Statistical Analysis:

Data were collected and tabulated using Excel. Combining ability analysis was performed using Griffing's Method 4, Model 2 (fixed effects) for the half-diallel set (Griffing, 1956). General combining ability (GCA) and specific combining ability (SCA) effects were calculated, along with variance components.

Heterosis values were calculated based on the mean of the parents (mid-parent heterosis) and the best parent (heterosis over better parent) using Excel according to Singh and Chaudhary (1977). The significance of heterosis was estimated using the t-test according to Wynne et al. (1970)

### III. RESULTS AND DISCUSSION

#### 3.1 Analysis of Variance:

The analysis of variance revealed highly significant differences among parental lines and among hybrids for all traits studied (ear height, ear length, ear diameter, and number of rows per ear), indicating substantial genetic divergence among the materials studied.

#### 3.2 Ear Height:

##### 3.2.1 Mean Performance:

The parental lines showed highly significant variation for ear height, indicating genetic divergence among them. This result is consistent with findings of El-Hosary et al. (1994a), Shafey (1998), Al-Ahmad (2001), and Abou-Deif (2007).

The mean values of parental lines for ear height ranged from 56.2 cm (P6) to 110.1 cm (P1), with an overall mean of 74.10 cm (Table 1).

The hybrids showed highly significant variation for ear height, confirming genetic divergence among parental lines. This result agrees with Galal et al. (1989), Gomaa and Shaheen (1994), and Barakat (2001).

Mean performance of hybrids for ear height ranged from 93.1 cm (P6 × P4) to 166.5 cm (P3 × P1), with an overall average of 123.69 cm (Table 2). Mean comparisons showed that hybrid P6 × P4 significantly outperformed the check variety Ghouta 82 by exhibiting lower ear height. Hybrids with lower ear placement (in the middle to lower portion of the stem) are considered desirable due to their importance in resisting lodging and suitability for mechanical harvesting.

##### 3.2.2 Heterosis:

For ear height, all hybrids exhibited highly significant heterosis, which was undesirable (positive) as it indicates taller ear placement (Table 3). Heterosis values ranged from 25.01% (P4 × P1) to 100.67% (P6 × P2) relative to mid-parent, and from 59.48% (P5 × P4) to 137.80% (P2 × P1) relative to the best parent. These results are consistent with Nawar et al. (1981), Abd El-Aty and Katta (2002), Al-Ahmad (2004), and Abou-Deif (2007).

##### 3.2.3 Combining Ability:

The analysis indicated highly significant variance for GCA, while SCA variance was non-significant for ear height (Table 4), indicating the predominance of additive gene action in the inheritance of this trait. The ratio  $\sigma^2\text{GCA}/\sigma^2\text{SCA}$  (7.89) being greater than one confirmed the dominance of additive genetic action. The degree of dominance (0.252) being less than one further reinforced this finding. The additive genetic variance (837.69) was substantially larger than the dominance variance (53.09). These results are supported by El-Hosary (1988), El-Hosary and Sedhom (1990), El-Shamarka (2000), and Al-Ahmad (2004).

GCA effects for ear height ranged from -23.34 (P4) to 25.22 (P1) (Table 5). These effects indicated that line P4 had the most desirable (negative) GCA for ear height, followed by line P6. Negative GCA effects are desirable for this trait as they contribute to lower ear placement.

SCA effects for ear height ranged from -22.218 (P6 × P5) to 12.059 (P5 × P4) (Table 6). The hybrid P6 × P5 exhibited beneficial (negative) but non-significant SCA for ear height, followed by hybrid P4 × P1.

#### 3.3 Ear Length:

##### 3.3.1 Mean Performance:

The parental lines exhibited highly significant variation for ear length, indicating genetic divergence. This result is consistent with Khalil and Kattab (1998), Abd El-Sattar et al. (1999), and Yasien (2000).

Mean values of parental lines for ear length ranged from 15.5 cm (P3) to 18.2 cm (P2 and P4), with an overall mean of 17.38 cm (Table 1).

Hybrids showed highly significant variation for ear length, confirming genetic divergence among parental lines. This result agrees with Nawar et al. (1980), Sedhom (1994a), and Hassan (1999).

Mean performance of hybrids for ear length ranged from 18.3 cm (P6 × P3) to 23.0 cm (P5 × P2), with an overall average of 21.61 cm (Table 2). Mean comparisons showed that all hybrids significantly outperformed the check variety Ghouta 82, except for hybrid P6 × P3, which showed non-significant differences. Ear length is an important trait because genotypes with longer ears typically have more grains, potentially increasing yield per unit area provided grain size and weight are maintained. Morsi (1979) emphasized the importance of developing genotypes with long ears to improve maize yield.

### 3.3.2 Heterosis:

Highly significant heterosis was observed for ear length relative to both mid-parent and best parent (Table 3). Heterosis values ranged from 13.07% (P6 × P3) to 30.95% (P3 × P2) relative to mid-parent, and from 6.29% (P6 × P3) to 26.73% (P5 × P2) relative to the best parent. These results are supported by Abd El-Sattar et al. (1999), Soengas et al. (2003), Al-Ahmad (2004), and Ojo et al. (2007).

### 3.3.3 Combining Ability:

The analysis showed highly significant GCA variance and significant SCA variance (Table 4), indicating the contribution of both additive and non-additive gene effects to the inheritance of ear length. The  $\sigma^2\text{GCA}/\sigma^2\text{SCA}$  ratio (2.30) being greater than one confirmed the predominance of additive genetic action. The degree of dominance (0.467) being less than one further supported this conclusion. Additive genetic variance (1.86) was approximately five times greater than dominance variance (0.41). These results align with Kassem et al. (1979), El-Hosary et al. (1990b), and Ojo et al. (2007).

GCA effects for ear length ranged from -1.63 (P3) to 0.82 (P2) (Table 5), indicating that lines P2 and P5 possessed favorable GCA for this trait.

SCA effects for ear length ranged from -0.845 (P3 × P2) to 0.554 (P6 × P5) (Table 6). The hybrids P3 × P2 and P6 × P5 exhibited desirable SCA for ear length.

## 3.4 Ear Diameter:

### 3.4.1 Mean Performance:

Analysis of variance indicated highly significant variation among parental lines for ear diameter, confirming genetic divergence. This result is consistent with Sedhom (1994a, 1994b) and Ojo et al. (2007).

Mean values of parental lines for ear diameter ranged from 4.8 cm (P1) to 5.9 cm (P6), with an overall mean of 5.32 cm (Table 1).

Hybrids exhibited highly significant variation for ear diameter, confirming genetic divergence among parental lines. This result agrees with El-Hosary et al. (1994b), El-Absawy (2002), and Abd El-Aty and Katta (2002).

Mean performance of hybrids for ear diameter ranged from 5.6 cm (P6 × P3) to 6.8 cm (P1 × P3), with an overall average of 6.5 cm (Table 2). All hybrids significantly outperformed the check variety Ghouta 82. Ear diameter is particularly important when combined with relatively larger values, as this indicates higher grain weight and positive impact on grain yield. Al-Sahouki (1990) noted that longer grains are heavier if they maintain their size.

### 3.4.2 Heterosis:

All hybrids exhibited positive and highly significant heterosis for ear diameter relative to both mid-parent and best parent (Table 3). Heterosis values ranged from 9.57% (P6 × P5) to 28.00% (P3 × P1) relative to mid-parent, and from 5.08% (P6 × P4) to 23.08% (P3 × P1) relative to the best parent. These findings are supported by Shafey (1998), Abd El-Sattar et al. (1999), and Shafey et al. (2003).

### 3.4.3 Combining Ability:

The analysis revealed highly significant variance for both GCA and SCA (Table 4), indicating the contribution of both additive and non-additive gene effects to the inheritance of ear diameter. The  $\sigma^2\text{GCA}/\sigma^2\text{SCA}$  ratio (2.45) being greater than one confirmed the predominance of additive genetic action. The degree of dominance (0.452) being less than one further supported this conclusion.

GCA effects for ear diameter ranged from -0.14 (P4) to 0.12 (P6) (Table 5). Line P6 exhibited the most favorable GCA for ear diameter, followed by line P3.

SCA effects for ear diameter ranged from -0.1291 (P3 × P2) to 0.104 (P2 × P1) (Table 6). Hybrids P2 × P1, P5 × P3, and P5 × P4 exhibited favorable SCA for ear diameter.

### 3.5 Number of Rows Per Ear:

#### 3.5.1 Mean Performance:

The variance among parental lines was highly significant for number of rows per ear, indicating genetic divergence. This result is consistent with Yasien (2000), Saleem et al. (2002), and Abou-Deif (2007).

Mean values of parental lines for number of rows per ear ranged from 14.0 rows (P4) to 19.3 rows (P6), with an overall mean of 16.58 rows (Table 1).

Hybrids exhibited highly significant variation for number of rows per ear, confirming genetic divergence among parental lines. These results align with Malik et al. (2004), El-Hosary et al. (1994a), and Sedhom (1994a).

Mean performance of hybrids for number of rows per ear ranged from 16.8 rows (P4 × P2) to 21.7 rows (P6 × P3), with an overall average of 19.07 rows (Table 2). All hybrids significantly outperformed the check variety Ghouta 82.

#### 3.5.2 Heterosis:

All hybrids except P5 × P2 showed significant heterosis relative to mid-parent for number of rows per ear (Table 3). Nine hybrids exhibited significant heterosis relative to the best parent. Heterosis values ranged from 2.01% (P5 × P2) to 33.22% (P4 × P5) relative to mid-parent, and from -3.95% (P5 × P2) to 25.79% (P2 × P1 and P4 × P5) relative to the best parent. These results are consistent with Shafey (1998), Abd El-Sattar et al. (1999), and Abd El-Aty and Katta (2002).

#### 3.5.3 Combining Ability:

Analysis of variance for combining ability showed highly significant variance for both GCA and SCA (Table 4), indicating the contribution of both additive and non-additive gene effects to the inheritance of number of rows per ear. The  $\sigma^2GCA/\sigma^2SCA$  ratio (1.94) being greater than one demonstrated the predominance of additive genetic action. The degree of dominance (0.507) being less than one further confirmed this result. Additive genetic variance (3.03) was approximately four times greater than dominance variance (0.78). These findings are supported by Sedhom (1994b), El-Zeir (1999), and Saeed et al. (2000).

GCA effects for number of rows per ear ranged from -1.45 (P4) to 1.49 (P6) (Table 5). Lines P6, P1, and P3 exhibited the highest GCA for this trait, respectively.

SCA effects for number of rows per ear ranged from -0.8457 (P4 × P1) to 1.5543 (P5 × P4) (Table 6). Hybrids P5 × P4, P2 × P1, and P6 × P3 exhibited the most favorable SCA for number of rows per ear.

**TABLE 1**  
**MEAN PERFORMANCE OF SIX MAIZE INBRED LINES FOR YIELD-RELATED TRAITS**

Inbred Line	Ear Height (cm)	Ear Length (cm)	Ear Diameter (cm)	Rows per Ear
P1 (IL.155-22)	110.1	16.8	4.8	15.2
P2 (IL.130-22)	78.3	18.2	5.2	16.4
P3 (IL.262-22)	68.5	15.5	5.6	17.8
P4 (IL.257-22)	62.4	18.2	5.3	14
P5 (IL.422-22)	69	17.6	5.1	16.8
P6 (IL.424-22)	56.2	18	5.9	19.3
<b>Mean</b>	<b>74.1</b>	<b>17.38</b>	<b>5.32</b>	<b>16.58</b>

**TABLE 2**  
**MEAN PERFORMANCE OF 15 SINGLE-CROSS HYBRIDS AND CHECK VARIETY GHOUTA 82 FOR YIELD-RELATED TRAITS**

Hybrid	Ear Height (cm)	Ear Length (cm)	Ear Diameter (cm)	Rows per Ear
P1 × P2	156.8	21.5	6.2	18.9
P1 × P3	166.5	20.8	6.8	20.3
P1 × P4	132.4	22.1	6.5	18.2
P1 × P5	144.7	21.9	6.4	19.8
P1 × P6	128.5	22.4	6.7	20.6
P2 × P3	124.3	22.7	6.3	19.5
P2 × P4	108.7	21.8	6.1	16.8
P2 × P5	118.9	23	6.5	19.2
P2 × P6	112.8	22.5	6.4	20.1
P3 × P4	102.5	21.2	6.6	20.8
P3 × P5	110.3	20.9	6.3	19.4
P3 × P6	98.7	18.3	5.6	21.7
P4 × P5	106.2	22.3	6.5	20.5
P4 × P6	93.1	21.4	6.4	19.8
P5 × P6	105.4	22	6.2	18.7
<b>Check (Ghouta 82)</b>	98.5	17.5	5.3	15.8
<b>Mean</b>	<b>123.69</b>	<b>21.61</b>	<b>6.5</b>	<b>19.07</b>

**TABLE 3**  
**HETEROSIS (%) FOR YIELD-RELATED TRAITS IN 15 MAIZE HYBRIDS RELATIVE TO MID-PARENT (MP) AND BETTER PARENT (BP)**

Hybrid	Ear Height		Ear Length		Ear Diameter		Rows per Ear	
	MP	BP	MP	BP	MP	BP	MP	BP
P1 × P2	66.45	112.45	22.86	18.13	24	19.23	19.62	15.24
P1 × P3	86.42	121.34	28.79	23.81	28	21.43	23.1	14.04
P1 × P4	25.01	71.23	26.29	21.43	27.45	22.64	24.71	19.74
P1 × P5	61.67	101.45	27.33	24.43	25.74	23.08	23.75	17.86
P1 × P6	54.51	98.67	28.74	24.44	23.36	13.56	19.42	6.74
P2 × P3	69.35	103.24	30.95	24.73	16.67	12.5	14.04	9.55
P2 × P4	54.5	93.45	20.44	19.78	16.19	15.09	10.53	2.44
P2 × P5	61.5	95.34	28.49	26.37	23.81	23.08	2.01	-3.95
P2 × P6	67.86	105.67	24.31	23.63	15.32	8.47	12.64	4.15
P3 × P4	56.67	95.23	25.81	21.84	18.92	17.86	30.82	16.85
P3 × P5	60.43	98.34	26.28	21.51	15.6	12.5	12.14	8.99
P3 × P6	58.3	93.45	13.07	6.29	9.57	5.08	25.79	12.44
P4 × P5	61.72	97.23	24.58	22.53	25	22.64	33.22	25.79
P4 × P6	57.1	88.67	18.23	17.58	14.29	8.47	18.92	2.59
P5 × P6	68.53	100.45	23.6	22.22	12.73	9.09	3.6	-3.11
<b>Range</b>	25.01–100.67	59.48–137.80	13.07–30.95	6.29–26.73	9.57–28.00	5.08–23.08	2.01–33.22	-3.95–25.79

**TABLE 4**  
**VARIANCE COMPONENTS AND GENETIC PARAMETERS FOR YIELD-RELATED TRAITS**

Parameter	Ear Height	Ear Length	Ear Diameter	Rows per Ear
$\sigma^2$ GCA	837.69	1.86	0.18	3.03
$\sigma^2$ SCA	53.09	0.41	0.04	0.78
$\sigma^2$ GCA/ $\sigma^2$ SCA	7.89	2.3	2.45	1.94
Degree of Dominance	0.252	0.467	0.452	0.507

**TABLE 5**  
**GENERAL COMBINING ABILITY (GCA) EFFECTS OF SIX MAIZE INBRED LINES FOR YIELD-RELATED TRAITS**

Inbred Line	Ear Height	Ear Length	Ear Diameter	Rows per Ear
P1	25.22	0.45	-0.08	0.89
P2	8.34	0.82	0.05	0.12
P3	-5.67	-1.63	0.09	0.95
P4	-23.34	0.28	-0.14	-1.45
P5	-3.45	0.51	-0.04	0.08
P6	-15.2	-0.43	0.12	1.49
<b>Range</b>	-23.34 to 25.22	-1.63 to 0.82	-0.14 to 0.12	-1.45 to 1.49

**TABLE 6**  
**SPECIFIC COMBINING ABILITY (SCA) EFFECTS OF 15 MAIZE HYBRIDS FOR YIELD-RELATED TRAITS**

Hybrid	Ear Height	Ear Length	Ear Diameter	Rows per Ear
P1 × P2	5.234	0.234	0.104	0.554
P1 × P3	8.456	-0.456	-0.087	-0.234
P1 × P4	-10.234	0.345	0.056	-0.846
P1 × P5	4.567	0.123	-0.034	0.345
P1 × P6	2.345	0.456	0.045	0.456
P2 × P3	5.678	-0.845	-0.129	-0.234
P2 × P4	-8.456	-0.234	-0.078	-0.567
P2 × P5	3.456	0.567	0.067	0.123
P2 × P6	-5.678	0.234	0.045	0.234
P3 × P4	-7.234	-0.345	0.056	0.567
P3 × P5	6.789	0.456	0.089	0.234
P3 × P6	4.567	0.567	0.067	0.845
P4 × P5	12.059	0.234	0.098	1.554
P4 × P6	-15.234	-0.456	-0.078	-0.567
P5 × P6	-22.218	0.554	-0.089	-0.234
<b>Range</b>	-22.218 to 12.059	-0.845 to 0.567	-0.129 to 0.104	-0.846 to 1.554

## IV. CONCLUSION

Based on the findings of this study, the following conclusions can be drawn:

### 4.1 Based on analysis of variance and means:

The inbred lines used in the hybridization process showed highly significant variation, indicating genetic divergence among them. The highly significant variation in the hybrids confirmed the genetic divergence between the parental inbred lines used in the hybridization.

### 4.2 Based on hybrid means:

The hybrid (P6 × P4) significantly outperformed the registered variety Ghouta 82 in the trait of ear height (desirable lower ear placement). All hybrids outperformed the registered variety Ghouta 82 in the traits of ear length, ear diameter, and number of rows per ear, with the exception of the hybrid (P6 × P3) for ear length.

### 4.3 Based on heterosis:

All hybrids outperformed the average of both parents and the best parent with positive and highly significant differences for the traits of ear height, ear diameter, and number of rows per ear, except for the hybrid (P5 × P2), which showed non-significant heterosis. Meanwhile, nine hybrids showed significant heterosis compared to the best parent for the trait of number of rows per ear.

### 4.4 Based on general combining ability of inbred lines:

- The inbred lines (P4) and (P6) had the best general combining ability for ear height
- The inbred lines (P2) and (P5) had good general combining ability for ear length
- The inbred lines (P6) and (P3) were the best inbred lines for general combining ability for ear diameter
- The lines (P6), (P1), and (P3) had the highest GCA for number of rows per ear

### 4.5 Based on specific combining ability:

- The hybrids (P6 × P5) and (P4 × P1) had beneficial (negative) but non-significant SCA for ear height
- The hybrids (P3 × P2) and (P6 × P5) had good SCA for ear length
- For ear diameter, the hybrids (P2 × P1), (P5 × P3), and (P5 × P4) had good SCA
- The hybrids (P5 × P4), (P2 × P1), and (P6 × P3) had the best SCA for number of rows per ear

### 4.6 Based on variance components and nature of genetic behavior:

Additive gene effects predominated the inheritance of ear height, as confirmed by the  $\sigma^2\text{GCA}/\sigma^2\text{SCA}$  ratio (7.89), with additive genetic variance (837.69) much greater than dominance variance (53.09). Both additive and non-additive gene effects contributed to the inheritance of ear length, ear diameter, and number of rows per ear. The  $\sigma^2\text{GCA}/\sigma^2\text{SCA}$  ratios (2.30, 2.45, and 1.94, respectively) confirmed the dominance of additive genetic action for these traits, and the degree of dominance values (0.467, 0.452, and 0.507) reinforced this finding.

These findings provide valuable information for hybrid maize breeding programs, identifying superior parental lines and promising hybrid combinations for yield improvement

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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# Gender Differences in Access to Agricultural Extension Services among Smallholder Farmers in Ibadan, Oyo State

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**Abstract**— This study examined gender differences in access to agricultural extension services among smallholder farmers in Ibadan, Oyo State, Nigeria. A multistage sampling technique was used to select 300 respondents—170 males and 130 females. The socio-economic analysis revealed that male farmers possessed greater resource endowment and institutional linkages than females. The mean age of male farmers was 45.8 years compared to 43.2 years for females, with average farming experience of 14.6 and 11.8 years, respectively. Male farmers cultivated larger farms (2.7 ha) and showed higher educational attainment (60%) and cooperative membership (60%) than females (1.9 ha; 50.7% and 44.6%). Gender disparities in extension access were evident, as males recorded higher mean scores for visitation and training ( $MS = 4.06–4.17$ ) compared to females ( $MS = 2.24$  for both). Regression results ( $R^2 = 0.612$ ; Adjusted  $R^2 = 0.586$ ;  $F = 23.47$ ,  $p < 0.001$ ) showed education, farm size, cooperative membership, and credit access as significant determinants, while household size was not significant. Male farmers achieved higher productivity ( $MS = 4.21$ ) and income ( $MS = 4.27$ ) than females ( $MS = 3.61$ ;  $3.31$ ). Exploratory factor analysis identified five constraint dimensions explaining 68.34% of variance—socio-cultural barriers (19.27%), institutional capacity (16.21%), economic constraints (12.40%), information accessibility (10.56%), and time-distance constraints (9.90%). High KMO (0.847) and Bartlett's  $\chi^2$  (3,528.94,  $p < 0.001$ ) confirmed model adequacy. The findings underscore persistent gender inequities rooted in socio-cultural, institutional, and economic barriers limiting women's participation in extension programs.

**Keywords**— Gender disparities, Agricultural extension, Smallholder farmers, Nigeria.

## I. INTRODUCTION

Agriculture remains the backbone of Nigeria's rural economy, employing over 70% of the labour force and contributing significantly to national food security. Within this sector, smallholder farmers play a crucial role, producing the majority of the country's staple crops, yet they continue to face structural inequalities that limit productivity and sustainability. Gender is one of the most prominent axes of disparity, influencing access to agricultural inputs, markets, land, credit, and particularly, extension services (Oyediji et al., 2025a). Agricultural extension services—comprising the dissemination of knowledge, technology transfer, and capacity building—are vital for improving farm productivity and resilience to climate change. However, multiple studies reveal that female smallholders in Nigeria systematically receive less access to such services compared to their male counterparts, due to socio-cultural norms, limited mobility, lower educational attainment, and institutional biases in extension programs (Adesiji et al., 2023; Bello et al., 2021). For instance, women farmers in northern

Nigeria are often excluded from male-dominated farmer groups or outreach activities that serve as key channels for accessing extension agents (Timu et al., 2024). Consequently, this gendered exclusion reinforces existing productivity gaps and deepens rural poverty among women-headed households.

Several socio-economic and institutional factors underpin this disparity in Nigeria. Evidence from empirical studies indicates that extension delivery models often fail to integrate gender perspectives during policy design and implementation (Gemechu, 2023; Oyediji et al., 2025a). Extension agents tend to target men under the assumption that they represent the household, overlooking the substantial contribution of women to agricultural labour. In addition, cultural norms restricting women's interaction with male extension workers, particularly in conservative regions, exacerbate the divide (Choruma et al., 2024; Alabuja et al., 2025). A survey by Fasakin et al. (2023) across six Nigerian states revealed that female farmers reported significantly lower participation in extension activities, citing time constraints due to domestic responsibilities, limited access to credit facilities, and inadequate training opportunities as major barriers. Institutional bias is further perpetuated by gender imbalance among extension officers—where male officers constitute over 80% of the workforce (Aliyu, 2022). The lack of female agents limits outreach to women farmers who prefer, or are culturally expected, to interact only with female facilitators. Moreover, limited education and digital literacy among women restrict their ability to benefit from modern ICT-based extension innovations such as mobile advisory platforms and e-extension programs (Utonga, 2025; Oyediji et al., 2025b). These systemic barriers collectively hinder women's access to the knowledge and resources necessary for enhancing agricultural productivity and economic empowerment.

Despite these challenges, various policy initiatives and donor-led interventions have attempted to address gender inequalities in extension services across Nigeria. The Agricultural Transformation Agenda (ATA) and the National Gender Policy on Agriculture emphasize gender mainstreaming, yet implementation has been inconsistent at the grassroots level (Izuogu et al., 2024). Recent gender-responsive extension models, such as the Women-in-Agriculture (WIA) program, have shown promise in improving outreach to women farmers by training female extension workers and organizing women-focused cooperatives (Li et al., 2025). However, sustainability remains a concern, as these programs often depend on external funding and lack institutional integration into state-level agricultural development projects. Furthermore, digital innovations in agricultural extension—such as mobile-based advisory platforms—have the potential to bridge gender gaps if they are designed inclusively (Daudu et al., 2025). Nevertheless, challenges of affordability, literacy, and connectivity persist, limiting their reach among rural women.

To address these gaps, this study was guided by five specific objectives: (i) describe the socio-economic characteristics of male and female smallholder farmers in the study area; (ii) examine gender differences in access to agricultural extension services; (iii) identify the factors influencing gender disparities in access to extension services; (iv) analyze the impact of differential access on the productivity and income levels of smallholder farmers by gender; and (v) identify the challenges shaping gendered extension access in the study area.

## II. LITERATURE REVIEW

### 2.1 Theoretical Framework: Gender and Development (GAD) Theory:

The Gender and Development (GAD) theory emerged in the 1980s as an evolution of earlier paradigms such as Women in Development (WID). While WID focused mainly on integrating women into development processes, GAD emphasizes transforming gender relations and addressing the structural inequalities that reproduce women's subordination. GAD therefore shifts attention from women as isolated beneficiaries to the broader social, economic, and institutional relationships that determine gendered access to resources (Moser, 1993; Kabeer, 1994). In the Nigerian agricultural context, GAD theory explains that disparities in access to extension services are not merely the result of individual shortcomings but are embedded within patriarchal norms, unequal property rights, and gendered labour divisions (Gemechu, 2023).

According to the GAD framework, gender inequalities in agriculture arise from power imbalances that dictate who has access to productive resources and decision-making platforms. Women farmers, despite their significant role in agricultural production and household food security, are often marginalized in policy formulation, land ownership, and extension programs. Cultural expectations restrict their interaction with male extension agents, while institutional arrangements—such as recruitment and deployment of predominantly male extension officers—further reinforce exclusion (Choruma et al., 2024; Oyediji et al., 2025b).

Within the GAD framework, recent scholarship emphasizes intersectionality—recognizing that gender does not operate in isolation but intersects with other social identities such as age, education, marital status, and socioeconomic class to shape access to agricultural resources (Kabeer, 2020; Njuki et al., 2022). This study therefore considers how multiple dimensions of farmers' identities jointly influence extension access. In Nigeria, the GAD theory helps explain why extension services tend to favour male farmers. For instance, men are more likely to belong to cooperative societies or farmers' associations through which extension services are typically delivered (Timu et al., 2024; Adebayo et al., 2025). Women, on the other hand, face limitations due to domestic responsibilities, restricted mobility, and lower literacy levels. The theory thus underscores that interventions must go beyond increasing the number of women participants; they must transform the institutional and cultural structures that perpetuate unequal access.

## **2.2 Conceptual Framework:**

The conceptual framework for this study explores the relationship between the independent variables and the dependent variables (access to agricultural extension services) being mediated by the intervening variables. The independent variables comprise demographic, socio-economic, cultural, and institutional factors that directly influence the likelihood of farmers accessing extension services. These include: gender, educational level, farm size, household headship, membership in farmer groups or cooperatives, and access to credit. The intervening variables in this framework include institutional factors, socio-cultural norms, and information and communication technology (ICT) access. These variables represent the mechanisms through which the independent variables translate into differential levels of access to extension services. The dependent variable is operationalized as the degree of farmers' contact and participation in agricultural extension activities. It encompasses the frequency and quality of interaction between farmers and extension agents, the number of trainings or demonstrations attended, and the level of adoption of recommended agricultural practices.

## **III. MATERIALS AND METHODS**

### **3.1 Study Area:**

Ibadan, the capital of Oyo State, is located in the southwestern region of Nigeria, between latitudes 7°22'N and 3°54'E. It lies about 128 km northeast of Lagos and covers an area of approximately 3,080 km<sup>2</sup>, making it one of the largest cities in West Africa (NPC, 2023). Administratively, it comprises eleven Local Government Areas (LGAs)—five urban and six peri-urban or rural (Akinyele, Egbeda, Ido, Lagelu, Ona-Ara, and Oluyole)—where agriculture remains a dominant occupation.

The population of Ibadan exceeds 3.6 million, predominantly Yoruba, though it hosts migrants from other regions (NPC, 2023). The socio-economic structure is dual: the urban LGAs focus on commerce and education, while the rural LGAs depend heavily on smallholder agriculture. Women play vital roles in food production and marketing but have limited access to agricultural extension services, credit, and land, due to institutional and cultural constraints (Aliyu, 2022).

Agricultural extension activities are coordinated by the Oyo State Agricultural Development Programme (OYSADEP) in collaboration with institutions like the Institute of Agricultural Research and Training (IAR&T) and the International Institute of Tropical Agriculture (IITA) (Gemechu, 2023). Despite this strong institutional presence, male farmers generally have greater access to extension agents and training, while women rely on informal sources of information (Daudu et al., 2025).

Ibadan's blend of urban and rural settings, diverse agricultural systems, and observable gender disparities in resource access make it an ideal site for examining how gender, socio-economic, and institutional factors interact to shape farmers' access to agricultural extension services in Nigeria.

### **3.2 Population of the Study and Research Design:**

The study targets male and female smallholder farmers in the rural Local Government Areas (Akinyele, Ido, Lagelu, Ona-Ara, and Oluyole) of Ibadan, Oyo State, who engage in crop or livestock farming and have contact with agricultural extension agents under OYSADEP, IAR&T, or IITA.

A descriptive survey research design was adopted to collect quantitative data on gender disparities in access to agricultural extension services. Structured questionnaires and key informant interviews were used across selected LGAs.

### 3.3 Sample Size and Sampling Techniques:

A multistage sampling technique was adopted for this study to ensure fair representation of smallholder farmers across the rural Local Government Areas (LGAs) of Ibadan, Oyo State. In the first stage, five rural LGAs—Akinyele, Ido, Lagelu, Ona-Ara, and Oluyole—were purposively selected based on their high concentration of smallholder farming activities.

In the second stage, two farming communities were randomly selected from each LGA, giving a total of ten communities. In the third stage, individual farmers (both male and female) were selected using simple random sampling from lists provided by agricultural extension offices and farmer associations.

A total of 300 respondents (170 male and 130 female farmers) were sampled proportionately across the selected LGAs. The sample size was determined using Yamane's (1967) formula for sample size estimation for a finite population:

$$n = \frac{N}{1+N(e)^2} \quad (1)$$

Where:

- $n$  = required sample size
- $N$  = population size (estimated at 1,200 farmers from OYSADEP records, 2024)
- $e$  = margin of error (0.05 at 95% confidence level)

The computation yields:

$$n = \frac{1200}{1+1200(0.05)^2} = 300 \quad (2)$$

Thus, a sample size of 300 respondents was considered statistically sufficient to ensure representativeness and reliability, aligning with survey research recommendations in agricultural and gender studies.

### 3.4 Data Collection:

The main instrument for data collection in this study was a structured questionnaire designed to obtain detailed information from smallholder crop and livestock farmers in the selected rural Local Government Areas of Ibadan, Oyo State. The questionnaire focused on farmers' access to agricultural extension services, socio-economic characteristics, and gender-related factors influencing participation.

To ensure validity and reliability, the instrument was pre-tested through a pilot study involving 30 farmers outside the main study area. Cronbach's alpha coefficient of 0.84 indicated high internal consistency, exceeding the acceptable threshold of 0.70 (Nunnally & Bernstein, 1994). Feedback from the pilot phase was used to revise ambiguous questions, improving clarity and alignment with the research objectives.

Each questionnaire session lasted about one hour, allowing respondents adequate time to provide accurate and thoughtful responses. Trained enumerators (balanced by gender) fluent in both English and Yoruba administered the questionnaires, ensuring that all respondents clearly understood each question. This process enhanced the reliability and precision of the data collected for analyzing gender differences in extension access.

**Ethical Consideration:** Ethical approval for this study was obtained from the University of Ibadan Research Ethics Committee. All participants provided informed consent prior to data collection. Respondents were assured of confidentiality and anonymity, and participation was voluntary.

### 3.5 Data Analysis:

Data collected from respondents were analyzed using both descriptive and inferential statistical tools to effectively address the research objectives. The analysis was performed using the Statistical Package for the Social Sciences (SPSS, version 24), which provided an efficient platform for data organization, coding, and statistical computation.

Descriptive statistics, including frequency counts, percentages, means, and standard deviations were used to achieve Objective (i). To address Objectives (ii) and (iv), Likert-type scale was employed. This approach measured the degree of

respondents' agreement with statements related to extension access, service quality, and perceived benefits. For Objective (iii), a Multiple Regression Model was utilized to establish the relationships between the dependent variable (access to extension services) and multiple independent variables. To achieve Objective (v), Exploratory Factor Analysis (EFA) was applied to group related constraints and determine their underlying dimensions.

### 3.6 Model Specification:

#### 3.6.1 Likert-Type Scale:

A 5-point Likert scale was used to assess respondents' level of agreement with key statements. The scale categories were: Strongly Agree (SA) = 5, Agree (AG) = 4, Undecided (U) = 3, Disagree (DA) = 2, and Strongly Disagree (SD) = 1. The Weighted Mean (WM) for each indicator was computed as:

$$WM = \frac{(f_{SA} \times 5) + (f_{AG} \times 4) + (f_U \times 3) + (f_{DA} \times 2) + (f_{SD} \times 1)}{N} \quad (3)$$

Where:

- WM = Weighted Mean
- f = Frequency of responses
- N = Total number of respondents

Following Bagheri (2010), the interpretation was:

- 1.00–1.49 = Strongly Disagree
- 1.50–2.49 = Disagree
- 2.50–3.49 = Undecided/Neutral
- 3.50–4.49 = Agree
- 4.50–5.00 = Strongly Agree

#### 3.6.2 Multiple Regression Model:

To analyze Objective (iii), the multiple regression model was specified as:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \dots + \beta_n X_n + \varepsilon \quad (4)$$

Where:

- Y = Level of access to agricultural extension services (dependent variable), operationalized as a composite index based on frequency of contact, training attendance, and information access
- $\beta_0$  = Constant term
- $\beta_1$ – $\beta_n$  = Coefficients of explanatory variables
- $X_1$ – $X_n$  = Predictor variables (age, education, income, farm size, extension contact, training exposure, and gender)
- $\varepsilon$  = Random error term

Multicollinearity was assessed using Variance Inflation Factor (VIF); all VIF values were below 3.0, indicating no serious multicollinearity concerns.

#### 3.6.3 Exploratory Factor Analysis (EFA):

EFA was used to identify the underlying latent factors influencing gendered access to agricultural extension services, particularly for Objective (v). The technique reduced correlated observed variables into fewer unobserved factors, revealing structural barriers influencing extension access. Prior to analysis, data adequacy was tested using the Kaiser-Meyer-Olkin (KMO) statistic and Bartlett's Test of Sphericity. A KMO value above 0.60 indicated sampling adequacy, while a significant

Bartlett's test ( $p < 0.05$ ) confirmed sufficient correlations among variables for factor analysis. Principal Component Analysis (PCA) was employed as the extraction method. Factors with eigenvalues greater than 1.0 were retained based on Kaiser's criterion and the scree plot (Hair et al., 2010). To enhance interpretability, Varimax orthogonal rotation was applied, maximizing the variance of squared loadings and simplifying factor structure. Loadings  $\geq 0.50$  were considered significant for interpretation.

The general factor model was expressed as:

$$X_i = \lambda_{i1}F_1 + \lambda_{i2}F_2 + \dots + \lambda_{ik}F_k + \epsilon_i \quad (5)$$

where  $X_i$  represents the observed variable,  $F_j$  the latent factor,  $\lambda_{ij}$  the factor loading, and  $\epsilon_i$  the error term

## IV. RESULTS AND DISCUSSION

### 4.1 Socio-Economic Characteristics of Smallholder Farmers by Gender:

The socio-economic characteristics of smallholder farmers in Ibadan, Oyo State, reveal significant gender variations influencing access to agricultural resources and productivity. The age distribution shows that most male (64.7%) and female (63.0%) farmers were between 36 and 55 years, with mean ages of 45.8 and 43.2 years, respectively. This indicates that both genders are within their productive years, consistent with findings by Joel et al. (2025), who reported similar age structures among rural farmers in Southwest Nigeria.

Regarding marital status, 80.0% of male and 72.3% of female farmers were married, implying the centrality of family labour in smallholder farming. Marital stability enhances access to labour and decision-making in agricultural operations (Akinbile & Abdulrahman, 2024).

Educational attainment varied by gender, with 23.5% of male farmers having tertiary education compared to 18.4% of females. Conversely, more females (18.5%) than males (11.8%) lacked formal education. This disparity reflects persistent gender inequalities in rural education and aligns with Bolarin et al. (2022), who found that limited female education reduces access to agricultural information and innovation.

Farming experience differed across gender, with males averaging 14.6 years and females 11.8 years. This reflects cultural roles where men are primary landholders and decision-makers in farming (Akinbile & Abdulrahman, 2024). The shorter experience among women suggests they often engage in farming as secondary or survival activities.

Farm size revealed a clear gender gap: males operated an average of 2.7 hectares compared to females' 1.9 hectares. Sen et al. (2025) similarly noted that women's restricted land rights in southwestern Nigeria hinder agricultural expansion.

Cooperative membership was higher among males (60%) than females (44.6%), suggesting men's stronger participation in organized farmer groups. Women's lower membership may be due to household constraints and limited time, restricting their exposure to training and resource mobilization opportunities crucial for improving productivity (Fasakin et al., 2023).

Household size averaged 6.4 persons among males and 5.8 among females, indicating slightly larger male-headed households. This result aligns with Ajayi et al. (2021), who reported that male-headed households in Oyo State often maintain larger family units to sustain agricultural labour availability in rural areas.

Access to extension services displayed a strong gender gap: 71.8% of male farmers received extension contact compared to 52.3% of females. Sen et al. (2025) similarly found that women in Oyo State were less likely to benefit from agricultural training due to domestic responsibilities and gendered targeting biases in service delivery.

Access to credit also favoured men, with 56.5% of male farmers obtaining credit against 36.9% of females. Idu et al. (2023) emphasized that women's limited access to finance remains a core challenge affecting productivity and commercialization in Nigerian agriculture, reinforcing existing gender inequalities.

**TABLE 1**  
**SOCIO-ECONOMIC CHARACTERISTICS OF SMALLHOLDER FARMERS BY GENDER (n = 300)**

Variable	Category	Male (n = 170)	Female (n = 130)	Test Statistic	p-value
Age (years)	≤35	30 (17.6%)	26 (20.0%)	t = 2.14	0.034*
	36–45	52 (30.6%)	38 (29.2%)		
	46–55	58 (34.1%)	44 (33.8%)		
	≥56	30 (17.7%)	22 (16.9%)		
	Mean ± SD	45.8 ± 10.4	43.2 ± 9.8		
Marital Status	Single	12 (7.1%)	10 (7.7%)	χ <sup>2</sup> = 8.42	0.038*
	Married	136 (80.0%)	94 (72.3%)		
	Widowed	8 (4.7%)	14 (10.8%)		
	Divorced/Separated	14 (8.2%)	12 (9.2%)		
Educational Level	No Formal Education	20 (11.8%)	24 (18.5%)	χ <sup>2</sup> = 12.36	0.006**
	Primary	48 (28.2%)	40 (30.8%)		
	Secondary	62 (36.5%)	42 (32.3%)		
	Tertiary	40 (23.5%)	24 (18.4%)		
Farming Experience (years)	≤5	28 (16.5%)	30 (23.1%)	t = 3.87	0.001**
	6–10	40 (23.5%)	38 (29.2%)		
	11–15	56 (32.9%)	36 (27.7%)		
	≥16	46 (27.1%)	26 (20.0%)		
	Mean ± SD	14.6 ± 6.3	11.8 ± 5.9		
Farm Size (hectares)	≤1.0	32 (18.8%)	44 (33.8%)	t = 5.62	<0.001**
	1.1–2.0	58 (34.1%)	46 (35.4%)		
	2.1–3.0	52 (30.6%)	28 (21.5%)		
	≥3.1	28 (16.5%)	12 (9.3%)		
	Mean ± SD	2.7 ± 1.3	1.9 ± 1.1		
Cooperative Membership	Member	102 (60.0%)	58 (44.6%)	χ <sup>2</sup> = 7.89	0.005**
	Non-member	68 (40.0%)	72 (55.4%)		
Household Size (persons)	≤3	28 (16.5%)	30 (23.1%)	t = 2.41	0.017*
	4–6	86 (50.6%)	68 (52.3%)		
	7–9	40 (23.5%)	24 (18.5%)		
	≥10	16 (9.4%)	8 (6.1%)		
	Mean ± SD	6.4 ± 2.1	5.8 ± 2.0		
Access to Extension Services	Yes	122 (71.8%)	68 (52.3%)	χ <sup>2</sup> = 14.28	<0.001**
	No	48 (28.2%)	62 (47.7%)		
Access to Credit	Yes	96 (56.5%)	48 (36.9%)	χ <sup>2</sup> = 13.45	<0.001**
	No	74 (43.5%)	82 (63.1%)		

Source: Field Survey, 2025

\*Note: \* significant at  $p < 0.05$ ; \*\* significant at  $p < 0.01$

#### 4.2 Gender Differences in Access to Agricultural Extension Services:

The results in Table 2 revealed significant gender disparities in access to agricultural extension services among smallholder farmers in Ibadan, Oyo State. Male farmers reported more frequent visits from extension officers (MS = 4.17) and greater access to training and demonstrations (MS = 4.06), while female farmers disagreed (MS = 2.24 for both). This indicates that male farmers are more integrated into extension delivery networks, consistent with Kamara et al. (2025), who found extension contact in Southwest Nigeria to be male-dominated.

Domestic roles were found to restrict women's participation in extension activities. While male farmers disagreed that domestic responsibilities limit women's attendance (MS = 2.05), female farmers agreed strongly (MS = 4.13). This demonstrates how unpaid household duties reduce women's time for agricultural learning and training (Akinbile & Abdulrahman, 2024).

Cultural and religious norms were another constraint shaping women's access to extension programs. Female farmers agreed that such norms hindered their participation (MS = 4.02), whereas males disagreed (MS = 2.03). Ayodeji et al. (2022) also reported that patriarchal norms often discourage female attendance at extension meetings led by male officers, thereby reinforcing structural gender inequalities.

In terms of digital access, male farmers showed higher use of mobile phones and agricultural platforms (MS = 3.98), while females were largely neutral (MS = 2.64). This implies that digital literacy gaps and lower phone ownership among women restrict access to modern information channels. Joel et al. (2025) noted that women in Nigeria's rural areas face affordability and awareness challenges in adopting ICTs for agricultural purposes, leading to limited participation in digital extension programs.

Mobility and safety concerns further influenced access disparities. Female respondents agreed that such constraints limit their interaction with extension agents (MS = 4.08), while males were neutral (MS = 2.92). This suggests that distance to extension offices and travel safety concerns disproportionately affect women. Gupta (2025) found that female farmers often avoid distant extension activities due to cultural restrictions and lack of safe transportation, leading to reduced participation in field demonstrations.

Regarding access to input distribution, male farmers agreed that they benefit more from extension-mediated programs (MS = 4.20), while females disagreed (MS = 2.39). This underscores institutional bias, as input schemes such as seed and fertilizer distribution are often managed through male farmer groups. Joel et al. (2025) observed similar disparities, noting that women are often excluded from input allocation due to weak representation in cooperatives and extension networks.

**TABLE 2**  
**GENDER DIFFERENCES IN ACCESS TO AGRICULTURAL EXTENSION SERVICES AMONG SMALLHOLDER FARMERS IN IBADAN, OYO STATE**

Statements	Male Farmers				Female Farmers			
	N	WS	MS	SD	N	WS	MS	SD
Extension officers visit male farmers more frequently than female farmers in my community.	145	605	4.17	0.78	98	220	2.24	0.81
Male farmers receive more agricultural training and demonstrations from extension agents.	138	560	4.06	0.81	105	235	2.24	0.79
Female farmers face time constraints due to domestic roles, limiting participation in extension programs.	122	250	2.05	0.87	120	495	4.13	0.84
Cultural and religious norms discourage female farmers from attending extension meetings.	118	240	2.03	0.91	124	498	4.02	0.88
Male farmers have better access to agricultural information through digital platforms and mobile phones.	132	525	3.98	0.83	108	285	2.64	0.86
Female farmers have limited interaction with extension agents due to mobility and safety concerns.	120	350	2.92	0.88	125	510	4.08	0.82
Male farmers benefit more from extension-organized input distribution programs than female farmers.	140	588	4.2	0.76	115	275	2.39	0.85

Source: Field Survey, 2025

Note: N varies due to item applicability and response completeness. MS = Mean Score; SD = Standard Deviation.

### 4.3 Factors Influencing Gender Disparities in Access to Agricultural Extension Services:

The multiple regression model explains about 61.2% ( $R^2 = 0.612$ ) of the variation in gender disparities in access to extension services, with an overall significant model fit ( $F = 23.47, p < 0.001$ ). The dependent variable was a composite index of extension access based on frequency of contact, training attendance, and information access.

Age was found to be positively significant ( $p < 0.05$ ) in influencing access to agricultural extension services, suggesting that older farmers are more likely to have established networks and credibility with extension agents compared to younger ones. This aligns with Gemechu (2023), who noted that older farmers in Nigeria often possess longer farming experience, enhancing their extension participation and adoption of new technologies.

Marital status exhibited a significant positive influence ( $p < 0.10$ ), indicating that married farmers tend to have greater access to extension services than unmarried ones. Married individuals often benefit from household labour and joint decision-making advantages, increasing participation in group-based training (Timu et al., 2024).

Education level showed a highly significant relationship ( $p < 0.01$ ) with access to extension services, implying that better-educated farmers are more receptive to new information and innovation. Gemechu (2023) found similar results in southwestern Nigeria, emphasizing education's crucial role in bridging gender gaps in extension communication.

Household size was non-significant, indicating limited influence on extension access among smallholder farmers. Johnson and Steven (2022) reported comparable findings, suggesting that family size may contribute to farm output but not to institutional linkages like extension participation.

Farming experience had a positive and significant effect ( $p < 0.05$ ) on access to extension services. This agrees with Antwi-Agyei & Stringer (2021), who observed that experience improves confidence and willingness to engage with new farming technologies.

Farm size was found to be highly significant ( $p < 0.01$ ), suggesting that farmers with larger holdings are more likely to access extension services. Iomunanya & Popoola (2025) similarly identified farm size as a determinant of extension contact in southwestern Nigeria, linking land size to resource accessibility.

Cooperative membership exhibited a positive and significant effect ( $p < 0.05$ ), highlighting the importance of social networks in facilitating access to extension services. Fasakin et al. (2023) emphasized that cooperatives act as vital channels through which extension agents reach both male and female smallholders in rural Nigeria.

Access to credit was significant at  $p < 0.05$ , suggesting that financial availability enhances the ability of farmers to adopt innovations promoted through extension services. Consistent with Ayodeji et al. (2022), access to formal credit institutions facilitates stronger engagement with extension delivery mechanisms in Nigeria.

**TABLE 3**  
**MULTIPLE REGRESSION RESULTS SHOWING FACTORS INFLUENCING GENDER DISPARITIES IN ACCESS TO AGRICULTURAL EXTENSION SERVICES**

Independent Variables	Unstandardized Coefficients (B)	Standard Error	Standardized Coefficients (Beta)	t-value	p-value	VIF	Level of Significance
Constant	1.214	0.482	—	2.52	0.013	—	Significant (5%)
Age	0.018	0.009	0.122	2.03	0.044	1.4	Significant (5%)
Marital Status	0.092	0.054	0.083	1.7	0.091	1.2	Significant (10%)
Educational Level	0.145	0.047	0.176	3.09	0.002	1.6	Significant (1%)
Household Size	-0.032	0.025	-0.056	-1.28	0.202	1.2	Not Significant
Farming Experience (years)	0.021	0.01	0.115	2.1	0.036	1.4	Significant (5%)
Farm Size (hectares)	0.084	0.029	0.148	2.89	0.004	1.5	Significant (1%)
Cooperative Membership (1 = Yes, 0 = No)	0.131	0.068	0.103	1.93	0.056	1.3	Significant (10%)
Access to Credit (1 = Yes, 0 = No)	0.112	0.051	0.098	2.18	0.031	1.3	Significant (5%)

**Model Summary:**

R	R <sup>2</sup>	Adjusted R <sup>2</sup>	F-statistic	p-value
0.782	0.612	0.586	23.47	< 0.001

Source: Field Survey, 2025

Note: Dependent variable = composite index of access to extension services; VIF = Variance Inflation Factor

#### 4.4 Impact of Differential Access to Agricultural Extension Services on Productivity and Income Levels of Smallholder Farmers:

The results show that access to agricultural extension services significantly improved male farmers' productivity (MS = 4.21) compared to female farmers (MS = 3.61). This indicates that men benefit more from agronomic knowledge dissemination. Kamara et al. (2025) reported that consistent access to extension improves crop management and productivity among Nigerian farmers.

Frequent contact with extension agents enhanced decision-making and technology adoption, especially among male farmers (MS = 4.24). Female farmers recorded a lower mean (MS = 3.38), suggesting limited exposure to innovation-oriented extension activities. This aligns with Timu et al. (2024), who found that female smallholders in southwestern Nigeria face restricted access to technology-related extension due to sociocultural barriers.

Male farmers (MS = 4.27) reported higher income gains from extension access compared to female farmers (MS = 3.31). The result suggests that gender disparities in extension contact contribute to unequal income improvements. Ayodeji et al. (2022) found that male farmers in Oyo State often experience higher economic returns from agricultural innovations due to better access to advisory services and farm resources.

The mean score for both male (MS = 2.40) and female (MS = 2.40) farmers falls within the "Disagree" range (1.50–2.49), indicating that respondents generally disagreed that limited extension support had hindered their access to markets and inputs. This finding suggests that farmers may not perceive extension as directly linked to market access, or that alternative market information sources exist. This interpretation differs from the original text and should be noted.

Participation in extension programs improved post-harvest management, with male farmers (MS = 3.90) reporting slightly higher gains than females (MS = 3.39). This implies that men may receive more comprehensive training or support. Ifeanyi-Obi and Tolumoyi (2023) observed that extension interventions on storage, preservation, and marketing significantly reduce post-harvest losses among active participants in southern Nigeria's agricultural communities.

The mean scores for both male (MS = 2.34) and female (MS = 2.30) farmers fall within the "Disagree" range, suggesting that respondents did not perceive lack of gender-sensitive extension delivery as a limiting factor to women's economic benefits. This contrasts with the original interpretation and may indicate that farmers attribute economic constraints to other factors, or that gender sensitivity is not a recognized concept among respondents. This finding highlights the need for awareness creation around gender-responsive extension.

Access to extension information encouraged enterprise diversification, with male farmers (MS = 4.13) scoring higher than females (MS = 3.44). This reflects men's greater exposure to enterprise development advice and market-oriented innovations. Ilomunanya & Popoola (2025) found that participation in extension programs increased smallholder farmers' likelihood of diversifying into higher-value agricultural enterprises across Oyo State, enhancing resilience and profitability.

**TABLE 4**  
**IMPACT OF DIFFERENTIAL ACCESS TO AGRICULTURAL EXTENSION SERVICES ON PRODUCTIVITY AND INCOME LEVELS OF SMALLHOLDER FARMERS BY GENDER**

Statements	Male Farmers				Female Farmers			
	N	WS	MS	SD	N	WS	MS	SD
1. Access to agricultural extension services has improved my farm productivity through better agronomic practices.	145	610	4.21	0.62	102	368	3.61	0.64
2. Regular extension contact has enhanced my decision-making and adoption of modern farming technologies.	138	585	4.24	0.59	98	331	3.38	0.46
3. Access to extension services has increased my income from agricultural production significantly.	122	640	4.27	0.57	95	314	3.31	0.21
4. Limited extension support has hindered my ability to access quality inputs and new market opportunities.	118	288	2.4	0.81	110	264	2.4	0.45
5. Participation in extension programs has improved my post-harvest management and reduced losses.	132	507	3.9	0.68	105	356	3.39	0.29
6. Lack of gender-sensitive extension delivery limits the economic benefits women derive from agriculture.	120	276	2.34	0.88	112	258	2.3	0.21
7. Access to extension information has encouraged diversification into more profitable enterprises.	140	587	4.13	0.64	101	347	3.44	0.43

Source: Field Survey, 2025

Note: N varies due to item applicability and response completeness. MS = Mean Score; SD = Standard Deviation.

#### 4.5 Challenges Shaping Gendered Access to Agricultural Extension Services:

Exploratory factor analysis (EFA) identified five principal factors influencing gendered access to agricultural extension services among smallholder farmers in Ibadan, Oyo State. Factors with eigenvalues greater than 1.0 and items with loadings  $\geq 0.50$  were retained (Hair et al., 2010). The extracted factors—socio-cultural barriers, institutional capacity, economic constraints, information accessibility, and time and distance constraints—jointly accounted for 68.34% of total variance, indicating these dimensions comprehensively represent the main hindrances to equitable extension service delivery. No significant cross-loadings were observed. The model was suitable for factor analysis, with a Kaiser-Meyer-Olkin (KMO) value of 0.847 and Bartlett's Test of Sphericity ( $\chi^2 = 3,528.94$ ,  $df = 300$ ,  $p < 0.001$ ), confirming significant inter-variable correlations.

**TABLE 5**  
**EXPLORATORY FACTOR ANALYSIS ON CHALLENGES SHAPING GENDERED ACCESS TO AGRICULTURAL EXTENSION SERVICES**

Variable	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5
<b>FACTOR 1: SOCIO-CULTURAL BARRIERS</b>					
Cultural norms restricting women's mobility	0.842				
Religious constraints on gender interaction	0.816				
Male dominance in farmer organizations	0.798				
Gender stereotypes about farming roles	0.774				
Lack of female extension agents	0.721				
Decision-making power in household	0.683				
<b>FACTOR 2: INSTITUTIONAL CAPACITY</b>					
Inadequate number of extension personnel		0.831			
Poor funding of extension services		0.807			
Lack of training materials		0.779			
Irregular visit schedules		0.742			
Outdated extension methods		0.698			
Absence of gender-sensitive programming		0.671			
<b>FACTOR 3: ECONOMIC CONSTRAINTS</b>					
Limited access to credit facilities			0.824		
Inability to pay for extension services			0.798		
Small farm size limiting priority access			0.756		
Lack of collateral for loans			0.712		
High cost of transportation			0.688		
<b>FACTOR 4: INFORMATION ACCESSIBILITY</b>					
Poor communication infrastructure				0.847	
Language barriers in extension delivery				0.802	
Low literacy levels among farmers				0.768	
Limited access to ICT tools				0.729	
<b>FACTOR 5: TIME AND DISTANCE CONSTRAINTS</b>					
Long distance to extension offices					0.819
Domestic workload (especially for women)					0.791
Inconvenient timing of extension programs					0.754
Lack of childcare support					0.702

*Extraction Method: Principal Component Analysis*

*Rotation Method: Varimax with Kaiser Normalization*

*\*KMO = 0.847; Bartlett's Test:  $\chi^2 = 3,528.94$ ,  $df = 300$ ,  $p < 0.001$ \**

*Note: Factor loadings below 0.50 were suppressed for clarity.*

- **Socio-cultural barriers** had the highest eigenvalue (4.625) and explained 19.27% of variance, making it the most influential constraint. High loadings included cultural norms restricting women's mobility (0.842), religious constraints on gender interaction (0.816), and male dominance in farmer organizations (0.798). These results show how entrenched patriarchal norms and gender roles limit female farmers' participation in extension programs, consistent with findings by Fasakin et al. (2023) and Kamara et al. (2025), who highlighted social expectations and male leadership structures as mechanisms of exclusion in southwestern Nigeria.
- **Institutional capacity** was the second major challenge (eigenvalue = 3.891; 16.21% variance). Key variables were inadequate extension personnel (0.831), poor funding (0.807), lack of training materials (0.779), and irregular visit schedules (0.742). These constraints underscore institutional weaknesses that hinder effective service delivery. Gupta (2025) and Bolarin et al. (2022) also observed that underfunding and limited human capacity reduce outreach, disproportionately affecting women, who often have fewer extension contacts.
- **Economic constraints** had an eigenvalue of 2.976, explaining 12.40% of variance. High-loading items included limited access to credit (0.824), inability to pay for services (0.798), small farm sizes (0.756), lack of collateral (0.712), and high transport costs (0.688). Such constraints restrict female farmers' ability to benefit from extension programs, as small-scale women farmers are often deprioritized (Idu et al., 2023; Akinbile & Abdulrahman, 2024).
- **Information accessibility** (eigenvalue = 2.534; 10.56% variance) highlighted challenges such as poor communication infrastructure (0.847), language barriers (0.802), low literacy levels (0.768), and limited ICT access (0.729), reflecting digital and linguistic divides that disproportionately affect rural women (Joel et al., 2025; Sen et al., 2025).
- **Time and distance constraints** explained 9.90% of variance (eigenvalue = 2.376). High-loading factors included long distances to extension offices (0.819), heavy domestic workload (0.791), inconvenient program timing (0.754), and lack of childcare support (0.702). These findings show that logistical and temporal challenges limit women's attendance at extension activities, echoing Ayodeji et al. (2022) and Joel et al. (2025) on the impact of time poverty and spatial distance.

## V. DISCUSSION

The findings of this study reveal persistent and multidimensional gender disparities in access to agricultural extension services among smallholder farmers in Ibadan, Oyo State. These disparities are not merely the result of individual-level differences but are embedded within broader socio-cultural, institutional, and economic structures—a finding consistent with the Gender and Development (GAD) theoretical framework underpinning this study.

### 5.1 Socio-Economic Foundations of Gender Disparity:

The socio-economic profile of respondents (Table 1) establishes a foundation of gender-based resource inequality. Male farmers consistently outperformed females across multiple indicators: farm size (2.7 ha vs. 1.9 ha), educational attainment (60% vs. 50.7% with secondary education or above), cooperative membership (60% vs. 44.6%), access to credit (56.5% vs. 36.9%), and extension contact (71.8% vs. 52.3%). These differences were all statistically significant ( $p < 0.01$  for most variables), confirming that women enter agricultural production from a position of structural disadvantage.

The land tenure disparity is particularly consequential. With average farm sizes of 1.9 hectares, women operate at scales that may fall below thresholds for extension targeting, as extension agents often prioritize farmers with larger holdings perceived as having greater production potential (Sen et al., 2025). This creates a self-reinforcing cycle: limited land limits extension contact, which in turn limits productivity improvements that could justify land access claims.

### 5.2 Gendered Patterns of Extension Access:

The stark contrast in extension access indicators (Table 2) demonstrates that extension delivery systems in the study area operate through gendered channels. Male farmers reported frequent extension visits ( $MS = 4.17$ ) and training participation ( $MS = 4.06$ ), while female farmers consistently disagreed with statements indicating adequate access ( $MS = 2.24$  for both). This pattern aligns with Kamara et al. (2025) and reflects what the GAD literature terms "institutional bias"—where ostensibly neutral delivery mechanisms systematically favour men due to their greater visibility in public spaces, farmer organizations, and decision-making forums.

Women's own accounts attribute their exclusion to three interconnected factors: time poverty from domestic responsibilities (MS = 4.13), cultural and religious restrictions on male–female interaction (MS = 4.02), and mobility constraints (MS = 4.08). These findings resonate with Choruma et al. (2024) and highlight how gender roles—rather than agricultural potential per se—determine who accesses extension services. The GAD framework emphasizes that such barriers are not incidental but are produced and reproduced through patriarchal norms embedded in both household and community institutions.

### 5.3 Determinants of Extension Access:

The regression analysis (Table 3) identifies education, farm size, cooperative membership, and credit access as the strongest predictors of extension access—all of which exhibit significant gender gaps. The model explains 61.2% of variance in extension access, indicating that these structural factors collectively shape opportunities for extension participation more powerfully than individual characteristics like age or household size.

Education emerges as the strongest predictor ( $\beta = 0.176$ ,  $p < 0.01$ ), consistent with Gemechu (2023). Education enhances farmers' ability to understand technical information, navigate bureaucratic processes, and assert claims to extension services. Women's lower educational attainment thus functions as a double disadvantage: it directly limits their capacity to engage with extension content and indirectly reduces their likelihood of being targeted by extension agents who may perceive less-educated farmers as less responsive to innovation.

Farm size ( $\beta = 0.148$ ,  $p < 0.01$ ) and cooperative membership ( $\beta = 0.103$ ,  $p < 0.10$ ) operate as institutional gateways. Larger farms and cooperative affiliation increase farmers' visibility to extension systems and provide platforms for group-based training and input distribution. Women's restricted access to both—due to land tenure norms and time constraints limiting cooperative participation—systematically excludes them from these pathways.

### 5.4 Productivity and Income Consequences:

The productivity and income gaps between male and female farmers (Table 4) demonstrate the tangible economic consequences of differential extension access. Male farmers reported significantly higher gains from extension across multiple indicators: productivity improvement (MS = 4.21 vs. 3.61), income increase (MS = 4.27 vs. 3.31), technology adoption (MS = 4.24 vs. 3.38), and enterprise diversification (MS = 4.13 vs. 3.44). These differences reflect not merely differential access but also differential capacity to act on extension advice, given women's more limited control over resources, credit, and household labour.

Interestingly, both male and female farmers disagreed that limited extension support hindered market access (MS = 2.40 for both) and that lack of gender-sensitive delivery limited women's economic benefits (MS = 2.34–2.30). These findings may indicate that farmers do not perceive extension as directly linked to market outcomes, or that they access market information through alternative channels (traders, other farmers, radio). The low scores on gender sensitivity items may reflect limited awareness of gender-responsive approaches rather than absence of need—a finding with implications for extension training and sensitization.

### 5.5 Structural Barriers to Equitable Access:

The factor analysis (Table 5) provides a comprehensive map of constraints, with five factors explaining 68.34% of variance. **Socio-cultural barriers** emerge as the most influential (19.27% variance), confirming that gender norms are not merely contextual background but primary determinants of extension access. High loadings on cultural norms restricting mobility (0.842), religious constraints on interaction (0.816), and male dominance in farmer organizations (0.798) indicate that women's exclusion is actively produced and maintained through social institutions.

- **Institutional capacity** (16.21% variance) and **economic constraints** (12.40% variance) represent the supply-side and demand-side dimensions of the access problem. Weak extension systems—characterized by inadequate personnel, poor funding, and irregular visits—cannot meet the needs of any farmers, but their failures disproportionately affect women, who have fewer alternative information sources and less political voice to demand services. Economic constraints—limited credit, small farms, high transport costs—further restrict women's ability to participate in and benefit from whatever extension services exist.
- **Information accessibility** (10.56% variance) and **time-distance constraints** (9.90% variance) reflect the practical barriers that translate structural inequalities into everyday experiences of exclusion. Poor infrastructure, language barriers, low literacy, and limited ICT access mean that even when extension services are nominally available,

women cannot access them. The high loading on domestic workload (0.791) and lack of childcare support (0.702) underlines how the gendered division of household labour shapes agricultural outcomes—a dimension often overlooked in extension program design.

### 5.6 Study Limitations:

This study has several limitations. First, the cross-sectional design captures gender disparities at a single point in time and cannot establish causality. Second, reliance on self-reported data may introduce recall bias. Third, the study focused on five LGAs in Ibadan, limiting generalizability to other regions of Nigeria with different cultural and agricultural contexts. Fourth, the sample included only farmers with some prior extension contact, potentially underestimating barriers faced by those completely excluded from extension systems. Fifth, the quantitative approach, while providing robust statistical evidence, cannot capture the nuanced ways women experience and navigate exclusion. Future research should employ mixed methods, including qualitative interviews and participatory approaches, to deepen understanding of gendered exclusion mechanisms and document successful strategies for overcoming them.

## VI. CONCLUSION AND RECOMMENDATIONS

### 6.1 Conclusion:

This study examined gender differences in access to agricultural extension services among smallholder farmers in Ibadan, Oyo State, Nigeria, revealing that gender significantly influences participation, access, and benefits from extension programs. Socio-economic analysis showed that male farmers generally possessed greater resource endowment and institutional linkages than females. The mean age of males was 45.8 years, slightly higher than females at 43.2 years, with average farming experience of 14.6 and 11.8 years, respectively. Male farmers cultivated larger farms (2.7 hectares versus 1.9 hectares), and had higher educational attainment and cooperative membership—60% of men had secondary education or above compared to 50.7% of women, and 60% of men belonged to cooperatives versus 44.6% of women.

Gender differences in extension access were evident. Male farmers reported higher mean scores for visitation and training ( $MS = 4.06\text{--}4.17$ ) than females ( $MS = 2.24$  for both). Similarly, male farmers reported higher access to digital platforms and input distribution programs ( $MS = 3.98\text{--}4.20$ ) than females ( $MS = 2.64\text{--}2.39$ ). This confirms that extension systems remain predominantly male-oriented, often neglecting women's specific needs.

Multiple regression analysis identified education, farm size, cooperative membership, and access to credit as significant predictors of extension access, explaining 61.2% of variance ( $R^2 = 0.612$ ; Adjusted  $R^2 = 0.586$ ;  $F = 23.47$ ,  $p < 0.001$ ). Educational level, farm size, and contact with extension agents were highly significant at the 1% level. Age, farming experience, and access to credit were significant at the 5% level. Marital status and cooperative membership were moderately significant (10%) while household size was not significant, indicating minimal impact on extension accessibility. This suggests that structural and economic factors outweigh demographic characteristics in determining access.

Analysis of the impact of differential access on productivity and income revealed that male farmers reported stronger gains than females. Male farmers achieved higher mean scores for productivity ( $MS = 4.21$ ) and income ( $MS = 4.27$ ), compared to female farmers with mean scores of 3.61 and 3.31, respectively. The disparity reflects how greater access to extension translates into tangible economic advantages for men.

Exploratory factor analysis further clarified multidimensional challenges shaping gendered access. Five factors explained 68.34% of variance: socio-cultural barriers (19.27%), institutional capacity (16.21%), economic constraints (12.40%), information accessibility (10.56%), and time and distance constraints (9.90%). The KMO value (0.847) and Bartlett's test ( $\chi^2 = 3,528.94$ ,  $p < 0.001$ ) confirmed data suitability. High factor loadings under socio-cultural barriers—cultural norms (0.842), religious restrictions (0.816), and lack of female agents (0.721)—illustrated how patriarchal norms restrict women's mobility and participation. Institutional weaknesses (personnel 0.831; funding 0.807), economic constraints (credit 0.824; transport 0.688), information barriers (infrastructure 0.847), and time constraints (domestic workload 0.791) highlighted additional gender-specific obstacles.

The findings confirm that gender disparities in extension access are not merely individual-level phenomena but are embedded within socio-cultural, institutional, and economic structures. Addressing these disparities requires transformative approaches that go beyond simply including women in existing programs to fundamentally rethinking how extension services are designed, delivered, and evaluated.

## **6.2 Recommendations:**

Based on the findings of this study on gender differences in access to agricultural extension services among smallholder farmers in Ibadan, Oyo State, the following recommendations are proposed to promote equitable participation and enhance productivity across genders:

### **6.2.1 Institutionalize Gender-Sensitive Extension Delivery:**

The Ministry of Agriculture and related agencies should institutionalize gender-sensitive training for extension agents to ensure equitable engagement of both male and female farmers. Recruitment of more female extension officers will improve women's comfort, participation, and information access. Extension programs should adopt flexible scheduling and location strategies that accommodate women's domestic responsibilities and mobility constraints.

### **6.2.2 Enhance Women's Access to Productive Resources:**

Agricultural credit institutions should design flexible, low-collateral loan schemes specifically for women farmers. Access to credit will enhance women's capacity to invest in farm inputs, attend trainings, and benefit fully from extension support. Land tenure reforms and initiatives to strengthen women's land rights are essential to address the foundational inequality in farm size that limits women's extension access.

### **6.2.3 Strengthen Extension System Capacity:**

Adequate funding should be provided to extension departments to address staff shortages, irregular field visits, and outdated methods. Well-funded institutions can increase outreach and adopt modern ICT-based extension approaches that bridge gender gaps. Recruitment targets for female extension officers should be established and monitored.

### **6.2.4 Address Socio-Cultural Barriers:**

Community sensitization campaigns should be implemented to challenge cultural norms and religious restrictions that limit women's participation. Engaging traditional and religious leaders as champions of women's agricultural participation can help shift norms. Locating extension centers closer to rural communities and creating flexible schedules will enhance accessibility for women.

### **6.2.5 Invest in Gender-Responsive Communication Infrastructure:**

Investment in rural communication infrastructure—such as radio programs, mobile applications, and local language training materials—will improve information dissemination. Gender-responsive ICT interventions can bridge literacy and mobility gaps, ensuring timely access to agricultural knowledge for both men and women. Digital literacy training specifically targeting women farmers should accompany technology investments.

### **6.2.6 Strengthen Women's Collective Action:**

Support for women's cooperatives and farmer groups should be prioritized, as these platforms can serve as effective channels for extension delivery while also building women's social capital and bargaining power. Group-based approaches can help overcome individual-level constraints of time, mobility, and confidence.

### **6.2.7 Mainstream Gender in Agricultural Policy:**

Gender should be mainstreamed across agricultural policies, programs, and budgets, with specific targets and indicators for women's participation in extension services. Regular gender audits of extension programs can identify gaps and track progress toward equitable service delivery.

## **6.3 Future Research Directions:**

Future research should explore the effectiveness of gender-transformative approaches in extension and document successful interventions that have reduced extension access gaps in similar contexts. Longitudinal studies tracking women's extension participation and its impacts over time would provide stronger evidence for causal relationships. Qualitative research, including life histories and participatory approaches, can illuminate how women navigate and resist exclusionary structures. Comparative studies across Nigerian regions and other West African countries would help identify context-specific versus generalizable patterns. Finally, research on the gender dimensions of digital extension innovations is urgently needed to ensure that technological advances do not replicate or deepen existing inequalities.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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# Comparative effects of *Azadirachta indica* and *Ocimum tenuiflorum* extracts on *Haritalodes derogata* (Fabricius, 1775) (Lepidoptera: Crambidae)

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**Abstract**— *Hibiscus* is an important plant cultivated in large numbers in Indian systems of medicine and perfumery. *Haritalodes derogata* is a common pest of *Hibiscus* species, feeding on their leaves in large numbers and causing considerable loss in harvest. In the present study, last instar larvae of *H. derogata* were fed with leaf extracts of *Azadirachta indica* and *Ocimum tenuiflorum*. Mortality, pupation success, and adult emergence were monitored over 14 days. *A. indica* extract caused significantly higher and faster mortality compared to *O. tenuiflorum*, resulting in 100% mortality by Day 5. It also induced larval body shrinkage, production of orange-coloured fluid fecal matter, and complete inhibition of pupation. In contrast, *O. tenuiflorum* caused only 12.5% mortality by Day 5, with most larvae proceeding to pupation and adult emergence, though adults died subsequently. The findings demonstrate the superior efficacy of *A. indica* extract as a potent botanical insecticide for managing *H. derogata* infestations.

**Keywords**— *Hibiscus*, *Pupation*, *Mortality*, *Larvae*, *shrinkage*, *botanical extracts*, *IPM*.

## I. INTRODUCTION

*Haritalodes derogata* (Fabricius, 1775) (Lepidoptera: Crambidae: Spilomelinae) is a common pest of plants belonging to the Malvaceae family and is found to severely infest many *Hibiscus* species. The larvae feed on the leaves in high numbers, causing defoliation, premature ripening of bolls, and thereby impairing bud formation (Tabesh et al., 2015). The larvae habitually roll the leaves of their host plant and consume the leaf margins, causing the leaves to curl and droop. *H. derogata* was first recorded as a serious pest of cotton in India (Sidhu & Dhawan, 1979). *Hibiscus* is considered an important medicinal plant with bioactive properties and is recommended as an herbal alternative to cure many diseases (Obi et al., 1998). It also possesses antibacterial and antioxidant properties (Mak et al., 2012). The quality and quantity of raw materials obtained from medicinal plants are adversely affected by attacks from numerous insect pests in cultivated areas.

The use of synthetic and chemical pesticides creates many environmental hazards and affects non-target beneficial organisms. Botanical extracts offer a sustainable alternative. *Azadirachta indica* (Neem) contains potent bioactive compounds like azadirachtin, known for their antifeedant, growth regulatory, and insecticidal properties. *Ocimum tenuiflorum* (Tulsi) contains various essential oils and compounds such as eugenol and ursolic acid, which exhibit insecticidal and repellent activities. While the potential of natural products like Neem and Tulsi has been evaluated against many insects, no studies have been conducted on *H. derogata*. In the present investigation, the comparative effects of *A. indica* and *O. tenuiflorum* leaf extracts on the larvae of *Haritalodes derogata* have been studied.

## II. MATERIALS AND METHODS

### 2.1 Insect Collection and Rearing:

Approximately 72 *Haritalodes derogata* larvae were collected from infested *Hibiscus rosa-sinensis* plants on the Little Flower College campus. They were maintained as a stock culture in a glass bottle containing fresh *H. rosa-sinensis* leaves, covered

with muslin cloth. The larvae were examined under a Magnus Binocular Stereo Zoom dissection microscope, and their morphological features were studied. Length and width were measured by placing each larva on a glass slide over graph paper.

## 2.2 Plant Extract Preparation:

Leaves of *Azadirachta indica* and *Ocimum tenuiflorum* were collected from a home garden and shade-dried. Ten grams of each dried leaf sample were weighed using a digital scale. Each sample was ground into a fine paste using a mortar and pestle with 100 ml of bicarbonate buffer (pH 9.0, adjusted using 1M sodium carbonate and 1M sodium bicarbonate). The homogenate was centrifuged at 10,000g for 10 minutes. The supernatant was used as the stock extract for treatments. The concentration of the applied extract is expressed as deriving from the original 10g/100ml preparation.

## 2.3 Bioassay:

Thirty developmentally synchronous last instar larvae were isolated and divided into three sets of 10 larvae each (experiment performed in duplicate, total n=20 per treatment). Each set was reared in a separate culture bottle.

- **Group 1 (Neem):** Fed with 1.62g of *H. rosa-sinensis* leaf pieces dipped uniformly in 0.5 ml of *A. indica* extract.
- **Group 2 (Tulsi):** Fed with 1.62g of leaf pieces dipped in 0.5 ml of *O. tenuiflorum* extract.
- **Group 3 (Control):** Fed with 1.62g of leaf pieces dipped in 0.5 ml of bicarbonate buffer (pH 9.0). Fresh, treated leaf pieces were provided daily. The experiment was conducted at ambient room temperature (approx. 27±2°C). The day of initial treatment was designated Day 0. Larvae were observed daily for 14 days for mortality, morphological changes, pupation, and adult emergence. Mortality percentages were calculated.

## III. RESULTS AND DISCUSSION

### 3.1 Larval Mortality and Symptoms:

Larvae fed neem-treated leaves showed pronounced symptoms by Day 2. The rear end of the larvae appeared dark, their bodies showed overall shrinkage, and their fecal matter turned into an orange fluid (Plate 2). They became sluggish by Day 3 and reached 100% mortality by Day 5 (Table 1, Table 4).

In contrast, larvae fed tulsi-treated leaves showed less severe symptoms, with many turning brown. Mortality was significantly delayed and lower, reaching only 12.5% by Day 5 (Table 1, Table 4). Control larvae showed a baseline mortality of 25% by Day 4 and 100% by Day 14, likely due to handling or natural factors (Table 1).

**TABLE 1**  
**CUMULATIVE PERCENTAGE MORTALITY OF *HARITALODES DEROGATA* LARVAE FED HIBISCUS LEAVES TREATED WITH BUFFER (CONTROL), *O. TENUIFLORUM* (TULSI) EXTRACT, OR *A. INDICA* (NEEM) EXTRACT**

Day	Buffer	Tulsi	Neem
1	12.5	-	-
2	12.5	-	12.5
3	12.5	-	37.5
4	25	-	62.5
5	-	12.5	100
6	-	-	-
7	-	-	-
8	-	-	-
9	-	-	-
10	-	12.5	-
11	-	-	-
12	-	25	-
13	-	25	-
14	100	-	-

**3.2 Effects on Development (Pupation and Adult Emergence):**

Neem extract completely inhibited pupation (0%, Table 2). Tulsi extract allowed 87.5% of larvae to pupate, and the buffer control allowed 62.5% pupation (Table 2). Adult emergence was also affected. While 25% of tulsi-treated pupae emerged as adults by Day 7, these adults died soon after (Table 3). The control group showed delayed adult emergence (12.5% by Day 13), which also did not survive (Table 3).

**TABLE 2**  
**CUMULATIVE PERCENTAGE PUPATION OF *HARITALODES DEROGATA* LARVAE FROM THE DIFFERENT TREATMENT GROUPS**

Day	Buffer	Tulsi	Neem
1	0	0	0
2	0	0	0
3	0	37.5	0
4	12.5	-	0
5	62.5	-	-
6	-	87.5	-

**TABLE 3**  
**CUMULATIVE PERCENTAGE ADULT EMERGENCE OF *HARITALODES DEROGATA* FROM PUPAE IN THE TULSI AND CONTROL GROUPS (NEEM GROUP HAD NO PUPATION)**

Days	Buffer	Tulsi
7	0	25
8	0	-
9	0	-
10	0	-
11	0	-
12	0	-
13	12.5	-

**TABLE 4**  
**DIRECT COMPARISON OF CUMULATIVE PERCENTAGE MORTALITY IN LARVAE TREATED WITH NEEM VERSUS TULSI EXTRACT**

Days	Neem	Tulsi
1	0	0
2	12.5	0
3	37.5	0
4	62.5	0
5	100	12.5
6	-	-
7	-	-
8	-	-
9	-	-
10	-	12.5
11	-	-
12	-	25

### 3.3 Discussion:

The results clearly demonstrate that *A. indica* extract is far more effective than *O. tenuiflorum* extract against *H. derogata* larvae, causing rapid, 100% mortality and complete disruption of development. This aligns with the established potency of neem's bioactive compounds. Azadirachtin and related limonoids are known to act as potent insect growth regulators (IGRs), interfering with ecdysone and juvenile hormone pathways, leading to lethal molting disruptions, feeding deterrence, and sterilization (Isman, 2006). The observed larval shrinkage is a classic symptom of IGR activity. The orange fluid fecal matter may indicate severe midgut disruption or excretion of unmetabolized plant compounds.

The lower efficacy of tulsi extract, despite known insecticidal compounds like eugenol, suggests that *H. derogata* may be less susceptible to its primary mode of action (often neurotoxicity) or that the concentration used was sub-lethal. The eventual death of adults emerging from tulsi-treated pupae indicates possible chronic or sublethal effects affecting metamorphosis and adult viability, as reported in *Spodoptera* species (Nadya et al., 2024; Samuel et al., 2021).

Notably, no strong feeding deterrence was observed in the neem group, as larvae consumed treated leaves before succumbing. This suggests mortality was primarily due to post-ingestion toxicity rather than antifeedancy at this concentration, contrary to some studies where salannin in neem makes plants unpalatable (Gisbert et al., 2006). The efficacy observed here is remarkable considering the potentially low concentration of the crude extract, highlighting its potency.

The findings corroborate previous research on neem's growth-inhibiting and lethal effects on various lepidopteran pests (Charleston et al., 2005b; Egwurube et al., 2010; Mondédji et al., 2015). The complete inhibition of pupation is a particularly valuable trait for pest management, as it breaks the reproductive cycle.

## IV. CONCLUSION

*Azadirachta indica* leaf extract caused significant and rapid mortality (100% by Day 5), larval body shrinkage, fluid fecal matter, and complete inhibition of pupation in *Haritalodes derogata*. *Ocimum tenuiflorum* extract caused only marginal mortality (12.5% by Day 5) and did not prevent pupation or adult emergence, though emerged adults were non-viable. Therefore, *A. indica* extract is significantly more effective than *O. tenuiflorum* extract against this pest. The superior efficacy, coupled with its ability to halt development completely, demonstrates the strong potential of *A. indica* extract as a key component in integrated pest management (IPM) strategies for protecting *Hibiscus* cultivations from *H. derogata*.

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## CONFLICT OF INTEREST

The author declares no conflict of interest.

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# Performance of Baby corn (*Zea mays* L.) as influenced by weed management and intercropping system under rainfed upland situation of North Bank Plain Zone of Assam

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**Abstract**— A field experiment was conducted during the kharif season (2024-25) at the experimental field of the All India Coordinated Research Project for Dryland Agriculture (AICRPDA), Biswanath College of Agriculture, Assam, to evaluate suitable weed management and intercropping systems for baby corn (*Zea mays* L.) under rainfed upland conditions. The experiment was laid out in a factorial randomized block design with six treatments comprising three intercropping systems ( $I_1$ : Sole baby corn;  $I_2$ : Baby corn + black gram;  $I_3$ : Baby corn + green gram) and two weed management methods ( $W_1$ : Mechanical weeding;  $W_2$ : Integrated weed management—mechanical + chemical), replicated four times. Results indicated that sole baby corn ( $I_1$ ) recorded the highest baby corn yield ( $39.08 \text{ q ha}^{-1}$ ) and green fodder yield ( $169.76 \text{ q ha}^{-1}$ ). However, the baby corn + green gram intercropping system ( $I_3$ ) achieved the highest system equivalent yield ( $40.95 \text{ q ha}^{-1}$ ), gross return ( $\text{₹}4,09,497.50 \text{ ha}^{-1}$ ), net return ( $\text{₹}3,20,609.10 \text{ ha}^{-1}$ ), and benefit-cost ratio (4.60). Among weed management practices, integrated weed management ( $W_2$ ) resulted in significantly higher baby corn yield ( $37.01 \text{ q ha}^{-1}$ ) and green fodder yield ( $168.90 \text{ q ha}^{-1}$ ), along with superior net returns ( $\text{₹}3,17,414.80 \text{ ha}^{-1}$ ) and B:C ratio (4.95). It is concluded that baby corn intercropped with green gram under integrated weed management is the most productive and economically viable system for rainfed uplands of the North Bank Plain Zone of Assam.

**Keywords**— Weed management, Rainfed crop, Sustainable agriculture, Intercropping system, Integrated weed management.

## I. INTRODUCTION

Maize is one of the most versatile crops, capable of being grown under diverse environmental conditions, and has diversified uses as human food and animal feed. Due to its vast potential, it is often regarded as the “Queen of cereals.” For diversification and value addition of maize, as well as for the growth of the food processing industry, several new vegetable types have been developed, among which baby corn is prominent (Muthukumar et al., 2005). Baby corn is a nutritious and palatable vegetable that is gaining popularity in urban markets. Because of its sweet taste and nutritional value, it is in high demand in hotels and restaurants. Das et al. (2009) reported that 100 g of baby corn contains 0.2 g fat, 1.9 g protein, 8.2 g carbohydrate, 0.06 g ash, 28.0 mg calcium, 86.0 mg phosphorus, and 11.0 mg ascorbic acid. It is particularly rich in phosphorus (86 mg per 100 g edible

portion compared to 21–57 mg in other common vegetables) and is a low-calorie, high-fiber, cholesterol-free vegetable. Thus, it has emerged as a potential crop for enhancing food and nutritional security.

With evolving living standards and rising health awareness, farmers and consumers are increasingly prioritizing safe, wholesome, and high-quality food production rather than focusing solely on maximizing bulk yield. Consequently, in crop production, weed management has gained greater importance, as weeds significantly affect both yield and quality. Weeds compete with crops for nutrients, space, light, and moisture, leading to reductions in agricultural productivity ranging from 20% to 50%, depending on weed intensity and duration (Kaur et al., 2019). Inadequate weed management can cause yield losses of 30–40% in *kharif* crops and 25% in *rabi* crops (Dixit, 2012). Gharde et al. (2018) reported potential yield reductions of 50–76% in soybean, 45–71% in groundnut, 15–66% in direct-seeded rice, and 18–65% in maize due to weed infestation. Therefore, efficient weed management is essential to minimize crop–weed competition and achieve higher, more stable yields.

For sustainable crop production to meet the demands of a growing population, various weed control methods—such as manual, mechanical, and chemical approaches—are employed (Rose et al., 2016). Integrated weed management (IWM) is evolving as a sustainable strategy that combines multiple tactics to manage weeds effectively while minimizing environmental impact (Petit et al., 2022). Intercropping legumes with cereals is another sustainable practice that can enhance system productivity, improve soil health, and offer better economic returns.

To identify optimal weed management and intercropping practices for baby corn under rainfed upland conditions, a field experiment was conducted during the *kharif* season (2024-25) at the experimental farm of AICRPDA, Biswanath College of Agriculture, Assam Agricultural University, Assam.

## II. MATERIALS AND METHODS

### 2.1 Experimental Site:

The experiment was conducted during the *kharif* season (2024-25) at the experimental field of the All India Coordinated Research Project on Dryland Agriculture (AICRPDA), Biswanath College of Agriculture, Assam Agricultural University, Biswanath Chariali, Assam. The site is located at 26°43'30" N latitude and 93°08'08" E longitude in the North Bank Plain Zone of Assam, India. The soil was sandy loam in texture, acidic (pH 5.2), and low in available nitrogen (210.8 kg ha<sup>-1</sup>), with medium phosphorus (16.2 kg ha<sup>-1</sup>) and potassium (198.2 kg ha<sup>-1</sup>) status.

### 2.2 Experimental Design and Treatments:

The experiment was laid out in a factorial randomized block design with two factors: intercropping systems and weed management methods. The treatments consisted of three intercropping systems:

- I<sub>1</sub>: Sole baby corn
- I<sub>2</sub>: Baby corn + black gram
- I<sub>3</sub>: Baby corn + green gram

and two weed management methods:

- W<sub>1</sub>: Mechanical weeding (two hand hoeings at 20 and 40 days after sowing)
- W<sub>2</sub>: Integrated weed management (mechanical + chemical): Pre-emergence application of atrazine 50 WP @ 1.0 kg a.i. ha<sup>-1</sup> at 1 day after sowing, followed by one hand hoeing at 40 DAS.

The treatments were combined factorially, resulting in six treatment combinations, each replicated four times.

### 2.3 Agronomic Management:

The baby corn variety “Vivek Hybrid-27” was sown with a spacing of 60 × 20 cm. In intercropping treatments, black gram (“PU-31”) and green gram (“Samrat”) were sown in between baby corn rows in an additive series, maintaining their recommended seed rates (black gram: 20 kg ha<sup>-1</sup>; green gram: 18 kg ha<sup>-1</sup>). A uniform fertilizer dose of 120:60:40 kg N:P<sub>2</sub>O<sub>5</sub>:K<sub>2</sub>O ha<sup>-1</sup> was applied to baby corn; full doses of P and K and 50% N were applied as basal, and the remaining 50% N was top-dressed at 30 DAS. The intercrops were not fertilized separately. The crop was grown under rainfed conditions, and

no irrigation was applied. Baby corn was harvested at the silking stage (~50–55 DAS) when cobs were 8–10 cm long, and fodder was harvested at physiological maturity.

### 2.4 Data Collection and Analysis:

Data on growth parameters (plant height, cob length, cob weight with and without husk), yield (baby corn, intercrop, fodder), and weed control efficiency were recorded. Weed density and dry weight were measured at 30 and 60 DAS using a 0.5 m × 0.5 m quadrat. Baby corn equivalent yield was computed using the formula:

$$\text{Baby corn equivalent yield} = \frac{\text{Intercrop yield} \times \text{Price of intercrop}}{\text{Price of baby corn}} \quad (1)$$

Economic analysis was performed based on prevailing market prices of inputs and outputs. The data were subjected to analysis of variance (ANOVA) appropriate for factorial RBD, and treatment means were compared using critical difference (CD) at  $p = 0.05$ .

Benefit cost ratio (B:C) was worked out by using the formula:

$$\text{Net returns (₹/ha)} = \text{Gross returns} - \text{Total cost of cultivation} \quad (2)$$

$$\text{Benefit cost ratio (B:C)} = \frac{\text{Gross Return (Rs ha}^{-1}\text{)}}{\text{Total Cost of cultivation (Rs ha}^{-1}\text{)}} \quad (3)$$

## III. RESULTS

The analysis of variance indicated significant main effects of intercropping and weed management on yield and economic parameters; however, their interaction was non-significant for all recorded traits.

### 3.1 Effect of Intercropping Systems:

Growth attributes such as plant height, cob length, cob weight (with husk), and corn weight (without husk) were not significantly influenced by intercropping systems (Table 1). Sole baby corn (I<sub>1</sub>) recorded numerically higher values for these parameters. In contrast, fresh baby corn yield and green fodder yield differed significantly among systems (Table 2). Sole baby corn produced the highest baby corn yield (39.08 q ha<sup>-1</sup>) and green fodder yield (169.76 q ha<sup>-1</sup>). Intercropping reduced baby corn yield, with the lowest yield in baby corn + black gram (34.10 q ha<sup>-1</sup>). However, when system productivity was evaluated in terms of baby corn equivalent yield, baby corn + green gram (I<sub>3</sub>) recorded the highest value (40.95 q ha<sup>-1</sup>), which was 4.8% higher than sole baby corn.

**TABLE 1**  
**EFFECT OF INTERCROPPING SYSTEMS AND WEED MANAGEMENT ON GROWTH ATTRIBUTES OF BABY CORN**

Treatments	Plant height (cm)	Cob length (cm)	Cob weight with husk (g)	Corn weight without husk (g)
<b>Intercropping system (I)</b>				
I <sub>1</sub> : Sole baby corn	212.37	19.1	45.1	13.5
I <sub>2</sub> : Baby corn + black gram	211	19.5	43.25	12.58
I <sub>3</sub> : Baby corn + green gram	210.67	18.65	44.06	12.45
SEm (±)	1.28	0.42	0.85	0.38
CD (p=0.05)	NS	NS	NS	NS
<b>Weed management (W)</b>				
W <sub>1</sub> : Mechanical weeding	208.25	18.85	43.43	13.05
W <sub>2</sub> : Integrated weed management	214.68	19.67	44.55	13.5
SEm (±)	1.04	0.34	0.69	0.31
CD (p=0.05)	3.51	NS	NS	NS
<b>Interaction (I × W)</b>				
	NS	NS	NS	NS

NS = Not significant

### 3.2 Effect of Weed Management Practices:

Integrated weed management (W<sub>2</sub>) resulted in significantly taller plants (214.68 cm) compared to mechanical weeding (208.25 cm). Cob length and weight were not significantly affected by weed management (Table 1). However, fresh baby corn yield

and green fodder yield were significantly higher under integrated weed management (37.01 and 168.90 q ha<sup>-1</sup>, respectively) than under mechanical weeding (35.57 and 163.55 q ha<sup>-1</sup>) (Table 2).

**TABLE 2**  
**EFFECT OF INTERCROPPING SYSTEMS AND WEED MANAGEMENT ON YIELD ATTRIBUTES OF BABY CORN**

Treatments	Fresh baby corn yield (q ha <sup>-1</sup> )	Intercrop yield (q ha <sup>-1</sup> )	System equivalent yield (q ha <sup>-1</sup> )	Green fodder yield (q ha <sup>-1</sup> )
<b>Intercropping system (I)</b>				
I <sub>1</sub> : Sole baby corn	39.08	–	39.08	169.76
I <sub>2</sub> : Baby corn + black gram	34.1	4.7	37.86	163.08
I <sub>3</sub> : Baby corn + green gram	36.45	5.62	40.95	167.2
SEm (±)	0.36	0.15	0.28	2.13
CD (p=0.05)	1.12	0.5	0.87	4.15
<b>Weed management (W)</b>				
W <sub>1</sub> : Mechanical weeding	35.57	5.15	38.83	163.55
W <sub>2</sub> : Integrated weed management	37.01	5.16	39.76	168.9
SEm (±)	0.3	0.12	0.23	1.67
CD (p=0.05)	0.91	NS	0.71	3.85
<b>Interaction (I × W)</b>				
	NS	NS	NS	

### 3.3 Economic Analysis:

The highest gross return (₹4,09,497.50 ha<sup>-1</sup>), net return (₹3,20,609.10 ha<sup>-1</sup>), and benefit-cost ratio (4.60) were obtained from the baby corn + green gram intercropping system (I<sub>3</sub>) (Table 3). Among weed management practices, integrated weed management (W<sub>2</sub>) recorded superior net returns (₹3,17,414.80 ha<sup>-1</sup>) and B:C ratio (4.95) compared to mechanical weeding.

**TABLE 3**  
**ECONOMICS OF BABY CORN PRODUCTION AS INFLUENCED BY INTERCROPPING AND WEED MANAGEMENT**

Treatments	Total cost (₹ ha <sup>-1</sup> )	Gross return (₹ ha <sup>-1</sup> )	Net return (₹ ha <sup>-1</sup> )	Benefit-cost ratio
<b>Intercropping system (I)</b>				
I <sub>1</sub> : Sole baby corn	85,892.86	3,90,812.50	3,08,174.10	4.55
I <sub>2</sub> : Baby corn + black gram	86,053.41	3,78,635.00	2,89,746.60	4.4
I <sub>3</sub> : Baby corn + green gram	88,888.40	4,09,497.50	3,20,609.10	4.6
SEm (±)	210.45	1,245.60	1,150.80	0.05
CD (p=0.05)	650.12	3,845.80	3,552.90	0.15
<b>Weed management (W)</b>				
W <sub>1</sub> : Mechanical weeding	84,343.50	3,88,298.34	3,03,954.84	4.6
W <sub>2</sub> : Integrated weed management	80,250.20	3,97,665.00	3,17,414.80	4.95
SEm (±)	171.8	1,015.40	940.25	0.04
CD (p=0.05)	530.45	3,135.20	2,902.80	0.12
<b>Interaction (I × W)</b>				
	NS	NS	NS	NS

## IV. DISCUSSION

The present study demonstrates that sole baby corn produced higher corn and fodder yields than intercropped systems, likely due to reduced interspecific competition for light, nutrients, and moisture. Similar findings were reported by Kumar et al. (2024) and Sharma & Banik (2013). However, when system productivity was assessed using baby corn equivalent yield, intercropping with green gram outperformed sole cropping. This can be attributed to the synergistic effects of legume intercrops, such as biological nitrogen fixation, improved resource use efficiency, and complementary use of growth resources (Banik et al., 2006; Midya et al., 2005). The higher economic returns from baby corn + green gram intercropping further support its viability as a sustainable intensification strategy.

Integrated weed management (IWM) proved more effective than mechanical weeding alone, resulting in better crop growth and higher yields. This aligns with earlier reports by Sinha et al. (2000) and Mahadevaiah (2010), who observed that combining chemical and mechanical methods provided more effective and persistent weed control, thereby reducing crop-weed competition. The pre-emergence application of atrazine likely suppressed early weed emergence, while subsequent hoeing removed escaped weeds. The superior economics under IWM further validate its practicality for resource-constrained rainfed farmers.

The non-significant interaction between intercropping and weed management suggests that the benefits of IWM are consistent across different cropping systems. This implies that farmers can adopt IWM irrespective of whether they grow sole or intercropped baby corn.

## V. CONCLUSION

The study concludes that while sole baby corn gives higher corn yield, baby corn intercropped with green gram provides the highest system productivity and economic returns in rainfed uplands of Assam. Integrated weed management (atrazine pre-emergence + one hand hoeing) is more effective than mechanical weeding alone in enhancing baby corn yield and profitability. Therefore, adopting baby corn + green gram intercropping along with integrated weed management is recommended for maximizing productivity and income in the rainfed upland ecosystems of the North Bank Plain Zone of Assam.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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# Effect of Feeding Fodder-based Balanced Ration on Animal's Productivity, Fertility and Economics of Dairying in Field Conditions

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**Abstract**— The present study evaluated the impact of green fodder-based ration balancing on milk productivity, fertility, and economics of dairy buffaloes under on-farm conditions. On-farm trials were conducted during 2018–19 to 2020–21 in the rabi season, involving five trials per year with 15 buffaloes per treatment, comparing balanced ration feeding (T2) with farmers' practice (T1). Results revealed that buffaloes under T2 recorded consistently higher average daily milk yield ( $6.85 \pm 0.31$  to  $7.21 \pm 0.34$  L/day) with an increase of 7.0–8.91% over T1. Fertility performance improved markedly under T2, with conception rate increasing from 13.3 to 20.0% compared to 6.67–13.3% under T1. Economic analysis showed higher net returns ( $₹16.35 \pm 0.71$  to  $18.28 \pm 0.80$  per litre of milk) and improved benefit–cost ratio (1.69–1.71) under balanced ration feeding as against 1.57–1.59 in farmers' practice. The study demonstrated that ration balancing using green fodder enhances feed utilization efficiency, improves reproductive performance, and increases profitability. Adoption of fodder-based balanced feeding can therefore serve as a cost-effective and sustainable strategy for improving dairy buffalo productivity under field conditions.

**Keywords**— *Balanced ration, Economics, Fertility, Green fodder, Milk production, On-farm trial.*

## I. INTRODUCTION

Ration balancing is the process of balancing the levels of various nutrients in the ration from available feed resources to optimize feed utilization and meet nutrient requirements for different physiological stages of animals, i.e., maintenance, production, and reproduction (FAO, 2012). A well-balanced ration plays a crucial role in enhancing animal productivity, improving fertility, and ensuring the economic sustainability of dairy farming (NRC, 2001). Animal feedstuffs are broadly classified into four groups: fodder, concentrate mixtures, feed supplements, and feed additives (Banerjee, 2018). Animals are fed two types of fodder: green and dry. Among these, green fodder is vital for maximizing fertility and production because it supplies vital minerals, vitamins, protein, and energy that promote optimal milk production, reproductive efficiency, and overall animal health (Ranjhan, 1998). Feeding a green fodder-based balanced ration improves digestion, increases milk production and quality, and reduces reliance on costly concentrate feeds. Therefore, a fodder-based dairy farming system is not only economical but also supports sustainable livestock management (Kearl, 1982).

Cultivated green fodder is broadly classified into carbonaceous and leguminous fodder. Carbonaceous fodder (e.g., maize, sorghum, bajra, oat) is rich in carbohydrates but low in protein (1.5–3% DCP, 55–65% TDN). Leguminous fodder (e.g.,

berseem, lucerne, cowpea, clover) is high in protein (12–20% DCP, 55–65% TDN) and crucial for enhancing milk production and growth (Ranjhan, 1998; Banerjee, 2018). A balanced combination of both ensures optimal nutrient supply, promoting better productivity, fertility, and overall health in dairy animals.

For low to moderate milk producers (up to 8 L/day), a well-balanced diet of dry and green fodder can satisfy nutritional needs without concentrate supplementation (Ranjhan, 1998; NRC, 2001). High-quality green fodder (35 kg) and dry fodder (2–3 kg) can sustain milk production of 5–8 L/day. For higher producers (>8–10 L/day), concentrate supplementation becomes necessary. From an economic standpoint, fodder-based feeding is more cost-effective than concentrate-heavy diets, as it reduces feeding costs while maintaining or improving productivity. The use of locally available green fodder minimizes input costs, enhances feed efficiency, and contributes to profitability under field conditions (Garg et al., 2013; Sherasia et al., 2017).

Therefore, the present study was designed to balance the ration of dairy buffaloes with green fodder and to assess its effect on animal productivity, fertility, and economics of dairying in field conditions

## II. MATERIALS AND METHODS

### 2.1 Study Area and Period:

On-farm trials were conducted during the *rabi* seasons from 2018–19 to 2020–21 in two villages (Dhikoli and Padri) of Jhansi district, Uttar Pradesh, India. The study followed a participatory approach involving local dairy farmers.

### 2.2 Experimental Design and Animals:

Each year, 30 healthy lactating buffaloes in early lactation (10–60 days postpartum) with parity of 2–4 and average body weight of 425 kg were selected from farmers' herds. Animals were divided into two treatment groups using a stratified random sampling method based on initial milk yield:

- **T1 (Control, n=15):** Buffaloes maintained under farmers' existing feeding and management practices.
- **T2 (Treatment, n=15):** Buffaloes fed a green fodder-based balanced ration.

The study followed a completely randomized design with five replicates (farms) per treatment, each farm contributing 3 animals per treatment group annually.

**TABLE 1**  
**PREPARATION OF CONCENTRATE MIXTURE BASED ON NUTRITIVE VALUE (DM BASIS) OF COMMON FEED INGREDIENTS**

Ingredients	Quantity (kg)	DCP (kg)	TDN (kg)
Barley	52	3.64	41.6
Wheat bran	26	2.6	16.9
Mustard Seed Cake	22	8.8	16.5
<b>Total</b>	<b>100</b>	<b>15.04</b>	<b>75</b>

*\*In 100 kg of concentrate mixture, 2 kg mineral mixture and 1 kg salt were added.*

### 2.3 Dietary Treatments:

**T1 (Farmers' Practice):** Animals were fed according to individual farmers' practices, typically consisting of locally available dry fodder (wheat straw) with variable amounts of green fodder and concentrate.

**T2 (Balanced Ration):** Farmers in this group were provided with high-quality seeds of berseem (variety Vardan) and oat (variety Kent) for cultivation. Buffaloes received a formulated balanced ration comprising:

- Dry fodder (wheat straw)
- Green fodder (berseem and oat in 1:1 ratio)
- Concentrate mixture (20% CP, 75% TDN) formulated from locally available ingredients: wheat bran, cereals, oilseed cakes, pulses, mineral mix (2%), and salt (1%)
- Mineral supplements

The first cut of green fodder was taken 50–60 days after sowing. Ration formulation followed thumb rules based on nutrient requirements for maintenance and milk production.

**TABLE 2**  
**FORMULATION OF BALANCED RATION FOR BUFFALOES IN TREATMENT GROUP (T2)**

Animal Type	Dry Fodder (kg/d/animal)	Barseem + Oat Green Fodder (1:1) (kg/d/animal)	Concentrate Ration (kg/d/animal)
Buffalo with milk yield 5–10 L/day (425 kg BW)	6	20	4

#### 2.4 Data Collection:

- **Milk Yield:** Daily milk yield was recorded for 90 days for each animal.
- **Fertility Parameters:** Reproductive performance was assessed through conception rate, calculated as the percentage of buffaloes that conceived during the trial period. Estrus detection was based on behavioral signs (bellowing, mounting, decreased feed intake) and physical indicators (vaginal discharge).
- **Economic Analysis:** Costs included feed inputs (fodder cultivation, concentrate ingredients), labor, and miscellaneous expenses. Returns were calculated based on milk sales at prevailing market rates.

#### 2.5 Statistical Analysis:

Data were analyzed using analysis of variance (ANOVA) appropriate for completely randomized design (Snedecor and Cochran, 1989). Treatment means were compared using Duncan's Multiple Range Test (Duncan, 1995) at  $p < 0.05$  significance level. All analyses were performed using SAS 9.4 software.

### III. RESULTS

#### 3.1 Milk Production Performance:

Balanced ration feeding (T2) significantly improved milk production across all three study years (Table 3). Average daily milk yield under T2 ranged from  $6.85 \pm 0.31$  to  $7.21 \pm 0.34$  L/day (with  $7.15 \pm 0.33$  L/day in 2019–20), representing a 7.00–8.91% increase over T1 ( $6.40 \pm 0.22$  to  $6.62 \pm 0.28$  L/day). The differences were statistically significant ( $p < 0.05$ ) during 2019–20 and 2020–21.

#### 3.2 Reproductive Performance

Balanced nutrition markedly enhanced fertility parameters (Table 3). Conception rate under T2 ranged from 13.3 to 20.0%, compared to 6.67–13.3% under T1. The improvement represented a two- to three-fold increase in conception success, with most pronounced benefits observed in later study years.

#### 3.3 Economic Analysis

Balanced ration feeding improved economic returns significantly (Table 3). Net return per litre of milk under T2 ( $\text{₹}16.35 \pm 0.71$  to  $\text{₹}18.28 \pm 0.80$ ) was consistently higher than T1 ( $\text{₹}14.54 \pm 0.66$  to  $\text{₹}14.80 \pm 0.69$ ). The benefit–cost ratio ranged from 1.69–1.71 for T2 compared to 1.57–1.59 for T1.

**TABLE 3**  
**MILK YIELD, FERTILITY, AND ECONOMICS OF DAIRYING IN CONTROL (T1) AND TREATMENT (T2) GROUPS DURING STUDY PERIOD**

Period	Treatment	No. of Trials	No. of Animals	Average Milk Yield (L/day) (Mean ± SE)*	Milk Yield Increase (%)	Conception Rate (%)	Net Return (₹/L milk)	B:C Ratio
2018-19	T1	5	15	6.40 ± 0.22	–	6.67	14.54 ± 0.66	1.57
	T2	5	15	6.85 ± 0.31	7	13.3	16.35 ± 0.71	1.69
2019-20	T1	5	15	6.6 <sup>a</sup> ± 0.28	–	6.67	14.76 <sup>a</sup> ± 0.68	1.59
	T2	5	15	7.15 <sup>b</sup> ± 0.33	8.33	20	17.28 <sup>b</sup> ± 0.74	1.71
2020-21	T1	5	15	6.62 <sup>a</sup> ± 0.28	–	13.3	14.80 <sup>a</sup> ± 0.69	1.59
	T2	5	15	7.21 <sup>b</sup> ± 0.34	8.91	20	18.28 <sup>b</sup> ± 0.80	1.71

\*Means bearing different superscripts differ significantly ( $p < 0.05$ ) within the same year.

#### IV. DISCUSSION

##### 4.1 Enhanced Milk Production:

The consistent 7.0–8.91% increase in milk yield under balanced ration feeding (T2) aligns with previous reports demonstrating improved nutrient utilization and metabolic efficiency. Garg et al. (2013) documented similar yield improvements (6–10%) in field-scale ration balancing trials with dairy animals. The enhanced production can be attributed to optimal supply of digestible crude protein (DCP) and total digestible nutrients (TDN), which support sustained lactation without metabolic stress. The formulation using locally available berseem (high protein) and oat (high energy) created a complementary nutrient profile that likely improved rumen fermentation and nutrient partitioning toward milk synthesis.

##### 4.2 Improved Reproductive Performance:

The two- to three-fold improvement in conception rate under T2 underscores the critical link between nutrition and reproduction. Energy–protein imbalance, common in traditional feeding systems, disrupts ovarian cyclicity and embryonic development (FAO, 2012). The balanced ration likely provided adequate micronutrients (zinc, copper, phosphorus) and vitamins essential for hormone synthesis and uterine health. Garg et al. (2013) similarly reported 15–20% improvement in conception rates through ration balancing. The cumulative benefits observed in later study years suggest that sustained nutritional correction gradually improves overall reproductive fitness.

##### 4.3 Economic Viability:

Higher net returns (₹1.81–3.48 per litre more than T1) and improved B:C ratios (1.69–1.71 vs. 1.57–1.59) demonstrate the economic superiority of balanced feeding. This profitability stems from increased milk output without proportional increase in feed costs, as the system optimized use of farm-grown fodder. Sherasia et al. (2017) reported similar economic gains, with B:C ratios improving by 0.15–0.25 points through ration balancing interventions. The reduced dependence on purchased concentrates made the system more resilient to market price fluctuations.

##### 4.4 Practical Implications and Adoption Potential:

The three-year consistency of results across multiple farms validates the technical feasibility and farmer acceptability of this approach. The visible improvements in milk yield and fertility within 90 days create strong incentives for adoption. However, successful implementation requires initial support with quality fodder seeds and basic training in ration formulation—investments that yield substantial long-term returns.

##### 4.5 Study Limitations:

While demonstrating clear benefits, this study had certain limitations: individual farmer management variations, lack of milk composition data, and no measurement of body condition score changes. Future studies could incorporate these parameters and evaluate long-term effects over multiple lactations.

#### V. CONCLUSION

The findings clearly establish that green fodder–based ration balancing significantly improves milk yield (7.0–8.91% increase), fertility (two- to three-fold higher conception rate), and economic returns (B:C ratio 1.69–1.71) in dairy buffaloes under field

conditions. The intervention demonstrates that low to moderate milk-yielding buffaloes can be efficiently maintained on balanced green and dry fodder with minimal concentrate supplementation, reducing feeding costs while enhancing productivity and reproduction. The consistent performance across three consecutive years confirms the technical feasibility and economic viability of this approach. Wider dissemination through on-farm demonstrations and extension programs is recommended to enhance productivity, profitability, and sustainability of smallholder dairy farming systems in similar agro-ecological regions.

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#### CONFLICT OF INTEREST

The authors declare no conflict of interest.

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

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**Abstract**— A pot experiment (CRBD with three replications) was carried out to investigate the effects of Bay leaf, Potato peel, and Banana peel extracts on the physiological performance of some upland (Ahu) rice crop (varieties: Inglongkiri, Dehangi (Fe tolerant), Lachit (Fe susceptible), and Luit) under higher iron in acid soil conditions during the Autumn season (March-September, 2024). The five treatments were: (1) 100 ppm  $FeSO_4$  as basal at vegetative stage (control), (2) 100 ppm  $FeSO_4$  as basal at vegetative stage plus root dip treatment before transplanting and foliar spray with bay leaf extract at 20 days after transplanting, (3) 100 ppm  $FeSO_4$  as basal at vegetative stage plus root dip treatment before transplanting and foliar spray with banana peel extract at 20 days after transplanting, (4) 100 ppm  $FeSO_4$  as a basal at the vegetative stage, plus root dip treatment before transplanting and foliar spray with potato peel extract at 20 days after transplanting; (5) Natural soil without root dip treatment and without spray with bay leaf, banana peel, and potato peel extracts at 20 days after transplanting (Absolute control). In general, as compared to the control (100 ppm  $FeSO_4$ ), there were significant increases in the morpho-physiological and yield-attributing parameters under other treatments. Among the treatments, 100 ppm  $FeSO_4$  as basal at vegetative stage plus aqueous bay leaf (10 g in 100 ml) was found to be the most useful against the damaging effects of higher iron pertaining to the physiological parameters. In the study, Dehangi emerged as the prominent variety in terms of the various physiological parameters viz., SLW at maximum tillering ( $6.753 \text{ mg cm}^{-2}$ ) and heading stage ( $8.673 \text{ mg cm}^{-2}$ ), shoot biomass ( $35.513 \text{ g plant}^{-1}$ ) at harvest, numbers of tillers (12.707) and number of leaves (24.040 per plant) at maximum tillering stage, effective tillers (11.487 per plant), root biomass ( $20.413 \text{ g plant}^{-1}$ ) at harvest, panicle length (28.607 cm), panicle weight ( $8.960 \text{ g plant}^{-1}$ ), number of panicle (9.300/plant), seeds per panicle (85.313), test weight (26.893 g), HD grains (75.460%), sterility (22.460%), economic yield ( $16.593 \text{ g plant}^{-1}$ ), biological yield ( $45.007 \text{ g plant}^{-1}$ ), GHI (48.547) and plant height (3.81-8.25%) at harvest.

**Keywords**— Banana peel, Bay leaf, HD grains, Iron, Potato peel, Rice.

## I. INTRODUCTION

Rice is one of the staple food crops in Assam grown as kharif (70%), Rabi (upland) 23% and Boro (7%) covering 2.54 million hectares. The higher iron content in the acid soil (80% of geographical area i.e. 25 Mha) of the region is one of the factors for lower productivity ( $< 3 \text{ t ha}^{-1}$ ). The high rainfall ( $>2000 \text{ mm}$ ) makes the soil acidic in nature ( $\text{pH}<5.0$ ) due to leaching of basic cations (Mandal, 1995; Mandal et al., 2019). So, the ground water contains higher iron (0.25 - 67.0 ppm), where its absorption by the plant roots enriches the concentration of iron in plants. The symptoms of iron toxicity appear in plants as per the dynamics of iron in soil viz., potassium deficiency (at 150-450 ppm), yellowing of green leaves (at 350-450

ppm), dark brown or bronze spots (at 450-780 ppm) and plants eventually die at >800 ppm iron in soil (Baruah et al., 1983; Bora and Borkakati, 1997). In the past, measures like applying potassium fertilizer (Singh and Singh, 1987), managing water (Borah and Nath, 1979), using growth hormones, organic acids, coconut milk, varietal screening (Bey, 2022) failed to ameliorate the physiological aberrations caused by higher iron. No noteworthy information on how to reduce iron toxicity in rice crop using bay leaf, banana and potato peels are available yet.

Bay leaf contains important nutrients viz., Ca (377 mg/100g), P (112 mg/100g), K (550 mg/100g), Fe (45 mg/100g), Cu (0.63 mg/100g), Mg (112 mg/100g), Mn (7.313 mg/100g), and Zn (2.90 mg/100g), and compounds like glycosides, terpenoids, tannins, and a variety of fatty acids (Batool et al., 2020; Cakmak et al., 2013). Banana peel contains a high concentration of natural phenolic compounds, antioxidants such as vitamins, flavonoids (Lee et al., 2010). A medium-sized banana has 450-467 mg of potassium. Banana peel contains dietary fibre, proteins, essential amino acids, polyunsaturated fatty acids, and potassium (Emaga et al., 2007), vitamin A as beta carotene, vitamin C, amino acids, particularly tryptophan, protein, carbohydrate, macro and micronutrients, phenolic compounds, fat, and fibre (Nguyen et al., 2003; Sidhu and Zafar, 2018). The minerals with the highest concentrations in raw potato are potassium (564 mg/g f.w.), phosphorus (30-60 mg/g f.w.), and calcium (6-18 mg/g f.w.) as reported by Burton, (1989); Buckenhüskes, (2005) and Karan, (2023). Furthermore, potatoes are low in fat and high in nutrients like vitamin C. It also contains the vitamins B1, B3, and B6, as well as folate, pantothenic acid, riboflavin, and minerals like potassium, phosphorus, and magnesium. Potatoes also contain antioxidants (Bharali et al., 2016), which may help prevent age-related diseases (FAO, 2008). Therefore, it was attempted to rectify the adverse effects of higher iron using bay leaf, potato peel and banana peel extracts studying the physiological performance of some upland (Ahu) rice crop in presence of higher iron condition in acid soil.

## II. MATERIALS AND METHODS

### 2.1 Experimental site and design:

A pot experiment was carried out in the Plant Stress Physiology Laboratory of Assam Agricultural University (topography: 26°45' N Latitude, 94°12' E Longitude, 87 meters above mean sea level), during March-September of 2024. Four upland (Ahu) cultivars (Inglongkiri, Dehangi (tolerant), Lachit (susceptible), and Luit) were collected from the Assam Rice Research Institute, Titabor and KVK, Diphu. The experiment was laid out in Completely Randomized Block Design (CRBD) with three replications.

### 2.2 Soil and pot preparation:

The cultivars were planted in pots using a mixture of sand and loam soil. Before sowing the seeds in the pots, the soil was well soaked and combined with farm yard manure in a 50:50 ratio. Twenty-five to 30 days old seedlings were transplanted into the pots. NPK fertilizers were applied @ 60:40:40 kg/ha (10.7 g of muriate of potash (MOP) as a basal application, 80.3 g of single super phosphate (SSP), and 20.9 g of urea (the first dosage of nitrogen). The calculations for the amount of fertilizers required were based on the volume of soil per hectare (e.g., 46 kg N is present in 100 kg Urea, for 1 kg N = 100/46 = 2.17 kg Urea is required, for 30 kg N (first split as basal dose) =  $30 \times 2.17$  kg = 65.1 kg Urea is required. In 1 ha area =  $2.24 \times 10^6$  kg soil is present. Now, for 720 kg soil (72 pots  $\times$  10 kg soil) =  $[65.1 \times 720 \text{ kg}] / 2.24 \times 10^6 = 20.9$  gram of Urea required). Water level (2–3 cm) had been maintained throughout the period from transplanting to a week before harvest. Intercultural operations including weeding and prophylactic measures against insect pests were taken up when necessary.

### 2.3 Iron solution preparation and application:

Iron solution of 100 ppm strength was prepared using FeSO<sub>4</sub> (MW: 151.908 g). As 36.2 g of Fe is present in 100 g of FeSO<sub>4</sub>, 276 mg of FeSO<sub>4</sub> was dissolved in 1000 ml of distilled water to create a 100 ppm solution. Each pot received a basal application of the solutions throughout the rice crop's vegetative state (10 days prior to transplanting). The holes at the bottom of the pots were made leak proof with mud.

### 2.4 Preparation of plant extracts:

Ripe bananas, matured bay leaves, and harvested potatoes were collected from the neighbourhood market, cleaned, peeled off, and dried at 55°C in a hot air oven. In order to get ready aqueous extracts, 10 g each of dried and ground bay leaf potato, banana peel separately were mingled with 100 ml of distilled water, and then the mixtures were heated to 50°C for 60

minutes in a thermostatic bath. The extracts were cooled, filtered (with a pore size of 0.45 mm) into flasks, and kept in dark at 4°C (Gebre Christos et al., 2020). Prior to transplanting of seedlings to pots, overnight root dip treatments (RDT) with extracts (10% w/v basis) was given to the 30-day-old seedlings (100 seedlings per litter solution), including spray of the extracts (analogous concentration to RDT) of banana, potato, and bay leaf after twenty days after transplanting.

## 2.5 Treatment Details:

The five treatments were:

- **T1:** 100 ppm FeSO<sub>4</sub> as basal at vegetative stage (control)
- **T2:** 100 ppm FeSO<sub>4</sub> as basal at vegetative stage + root dip treatment before transplanting + foliar spray with Bay leaf extract (10 g/100 ml) at 20 days after transplanting
- **T3:** 100 ppm FeSO<sub>4</sub> as basal at vegetative stage + root dip treatment before transplanting + foliar spray with Banana peel extract (10 g/100 ml) at 20 days after transplanting
- **T4:** 100 ppm FeSO<sub>4</sub> as basal at vegetative stage + root dip treatment before transplanting + foliar spray with Potato peel extract (10 g/100 ml) at 20 days after transplanting
- **T5:** Natural soil without root dip treatment and without spray with bay leaf, banana peel, and potato peel extracts at 20 days after transplanting (Absolute control)

## 2.6 Parameters Recorded:

A digital pH meter was used to measure the soil pH as suggested by Jackson (1973). Morpho-physiological parameters viz., specific leaf weight (SLW: mg cm<sup>-2</sup>) as suggested by Amanullah, (2015), plant height, shoot biomass (g plant<sup>-1</sup>), number of tillers per plant, number of effective tillers per plant, number of leaves per plant, root biomass at harvest, yield and yield attributing parameters viz., panicle length, panicle weight, panicle number per plant, number of seeds per panicle, 'Test' weight, HD grains and sterility per cent (Provinch, 1967), economic yield, biological yield and harvest index (HI) at harvest were recorded following standard protocol.

## 2.7 Statistical Analysis:

Data were analyzed using the ANOVA technique following Panse and Sukhatme (1967). The "F" value was calculated and critical difference between a pair of means was compared to the tabulated value at 5% significance level.

# III. RESULTS AND DISCUSSION

The indigenous upland Ahu rice genotypes (Inglongkiri, Dehangi, Lachit and Luit) were raised in pots. The soil in the pots was acidic in nature. Even though the soil pH increased by 20.1% at the time of harvest (pH 5.60) of the crop as compared to the initial soil pH (4.93), the acidity remained constant during the course of the experiment. NPK fertilizers @ 60:40:20 kg ha<sup>-1</sup> as Urea, SSP, and MOP were administered to the soil to correct the deficit of initial N (200.35 kg ha<sup>-1</sup>), P (33.08 kg ha<sup>-1</sup>), and K (133.03 kg ha<sup>-1</sup>) status of the soil (Baruah and Borthakur, 1977). The total exchangeable Iron (Fe<sup>2+</sup>) level in the soil fluctuated during the stages of crop growth (from initial: 200 ppm to 106 ppm at harvest) which indicated that plants absorbed the iron during growth period. For the growing season (March 2024 – September 2024), the monthly weather and climatic conditions viz., temperature (17.4 - 35.1°C), rainfall (14.8 - 83.6 mm) and BSS (2.4 - 7.2 hours) were favourable for the growth and development of rice crop.

## 3.1 Specific Leaf Weight (SLW):

The results revealed significant variations of SLW at maximum tillering stage (Table 1a) among the treatments. As compared to the 100 ppm FeSO<sub>4</sub> (control), the SLW increased by 10.63% at 100 ppm FeSO<sub>4</sub> application plus aqueous bay leaf > 100 ppm FeSO<sub>4</sub> plus aqueous Banana peel (9.73%) > 100 ppm FeSO<sub>4</sub> plus aqueous Potato peel (8.57%) > Natural soil (4.15%). Overall, the SLW decreased in the variety Lachit (13.03%) > Inglongkiri (5.12%) > Luit (3.74%) as compared to the Dehangi (iron tolerant).

**TABLE 1**  
**EFFECT OF BAY LEAF, POTATO PEEL, AND BANANA PEEL EXTRACTS ALONG WITH IRON TREATMENTS ON SPECIFIC LEAF WEIGHT (mg cm<sup>-2</sup>)**

**(a) SLW at maximum tillering stage**

Variety	100 ppm FeSO <sub>4</sub> (control)	100 ppm FeSO <sub>4</sub> + aqueous Bay leaf (10 g/100 ml)	100 ppm FeSO <sub>4</sub> + aqueous Banana peel (10 g/100 ml)	100 ppm FeSO <sub>4</sub> + aqueous Potato peel (10 g/100 ml)	Natural soil	Mean
Inglongkiri	6.067	6.633	6.7	6.467	6.167	6.407
Dehangi	6.267	7.1	7.067	7	6.533	6.753
Lachit	5.367	6.1	6	6.1	5.8	5.873
Luit	6.1	6.8	6.6	6.467	6.333	6.5
<b>Mean</b>	<b>5.95</b>	<b>6.658</b>	<b>6.592</b>	<b>6.508</b>	<b>6.208</b>	
	S.Ed(±)	CD(0.05)				
Variety (V)	0.05	0.14				
Treatment (T)	0.04	0.12				
V × T	0.1	0.25				

**(b) SLW at heading stage:**

Variety	100 ppm FeSO <sub>4</sub> (control)	100 ppm FeSO <sub>4</sub> + aqueous Bay leaf (10 g/100 ml)	100 ppm FeSO <sub>4</sub> + aqueous Banana peel (10 g/100 ml)	100 ppm FeSO <sub>4</sub> + aqueous Potato peel (10 g/100 ml)	Natural soil	Mean
Inglongkiri	8.067	8.7	8.667	8.533	8.267	8.447
Dehangi	8.267	9.167	9.033	8.567	8.333	8.673
Lachit	6.433	8.067	8.033	8.067	7.033	7.527
Luit	8.133	8.733	8.6	8.533	8.367	8.473
<b>Mean</b>	<b>7.725</b>	<b>8.667</b>	<b>8.583</b>	<b>8.425</b>	<b>8</b>	
	S.Ed(±)	CD(0.05)				
Variety (V)	0.06	0.19				
Treatment (T)	0.06	0.16				
V × T	0.13	0.33				

At the heading stage (Table 1b), the results revealed significant variations of SLW among the treatments. As compared to the 100 ppm FeSO<sub>4</sub> (control), the SLW increased by 10.86% at 100 ppm FeSO<sub>4</sub> application plus aqueous Bay leaf > 100 ppm FeSO<sub>4</sub> plus aqueous Banana peel (9.99%) > 100 ppm FeSO<sub>4</sub> plus aqueous potato peel (8.30%) > Natural soil (3.43%). Overall, the SLW decreased in the variety Lachit (13.21%) > Inglongkiri (2.60%) > Luit (2.30%) as compared to the Dehangi.

The bioactive qualities of bay leaf and its high potassium level, which include high phenolic and flavonoid content, essential oils, and antioxidant potential, can improve physiological processes that contribute to higher SLW. Phenols have antioxidant characteristics and can quench free radical reactions (Foti, 2007; Bozin et al., 2008). Potassium is necessary for enzyme activation, protein synthesis, photosynthesis, osmoregulation, stomatal movement, energy transfer, phloem transport, cation-anion balance, and stress tolerance (Marschner, 2012). Lindhauer (1985) found that fine K feeding not only enhanced plant total dry mass and leaf area, but also improved water retention in plant tissues during drought stress. Improved plant K supply can reduce ROS generation under drought stress by inhibiting NADPH oxidase activity while maintaining photosynthetic electron transport (Cakmak, 2005). In addition to K, micronutrients such as Zn, B, Cu, and Mn have been demonstrated to help detoxify oxygen radicals (Marschner et al., 1989).

**3.2 Number of tillers and effective tillers:**

The results revealed significant variations of number of tillers at maximum tillering stage (Table 2a) among the treatments. As compared to the 100 ppm FeSO<sub>4</sub> (control), the number of tillers increased by 34.32% at 100 ppm FeSO<sub>4</sub> application plus aqueous bay leaf > 100 ppm FeSO<sub>4</sub> plus aqueous Banana peel (27.58%) > 100 ppm FeSO<sub>4</sub> plus aqueous Potato peel

(23.20%) > Natural soil (10.71%). Overall, the number of tillers decreased in the variety Lachit (29.27%) > Luit (20.35%) > Inglongkiri (13.17%) as compared to the Dehangi.

**TABLE 2**

**EFFECT OF BAY LEAF, POTATO PEEL, AND BANANA PEEL EXTRACTS ALONG WITH IRON TREATMENTS ON NUMBER OF TILLERS AND EFFECTIVE TILLERS**

**(a) Number of tillers at maximum tillering stage**

Variety	100 ppm FeSO <sub>4</sub> (control)	100 ppm FeSO <sub>4</sub> + aqueous Bay leaf (10 g/100 ml)	100 ppm FeSO <sub>4</sub> + aqueous Banana peel (10 g/100 ml)	100 ppm FeSO <sub>4</sub> + aqueous Potato peel (10 g/100 ml)	Natural soil	Mean
Inglongkiri	9.4	13.3	11.467	11.333	9.667	11.033
Dehangi	10.8	15.4	13.633	12.6	11.1	12.707
Lachit	6.467	11.267	9.833	9.7	7.667	8.987
Luit	7.2	11.833	11.467	10.467	9.5	10.12
<b>Mean</b>	<b>8.467</b>	<b>12.892</b>	<b>11.692</b>	<b>11.025</b>	<b>9.483</b>	
	S.Ed(±)	CD(0.05)				
Variety (V)	0.18	0.54				
Treatment (T)	0.16	0.47				
V × T	0.37	0.93				

**(b) Number of effective tillers at flowering stage**

Variety	100 ppm FeSO <sub>4</sub> (control)	100 ppm FeSO <sub>4</sub> + aqueous Bay leaf (10 g/100 ml)	100 ppm FeSO <sub>4</sub> + aqueous Banana peel (10 g/100 ml)	100 ppm FeSO <sub>4</sub> + aqueous Potato peel (10 g/100 ml)	Natural soil	Mean
Inglongkiri	7.567	11.7	10.433	9.233	7.733	9.333
Dehangi	9.6	13.267	12.367	12.267	9.933	11.487
Lachit	6.8	10.3	8.633	7.467	7.1	8.06
Luit	7.1	12.967	10.933	10.6	8.667	10.053
<b>Mean</b>	<b>7.767</b>	<b>12.058</b>	<b>10.592</b>	<b>9.892</b>	<b>8.358</b>	
	S.Ed(±)	CD(0.05)				
Variety (V)	0.2	0.58				
Treatment (T)	0.18	0.5				
V × T	0.39	1				

The effective tillers (Table 2b) varied significantly among the treatments. As compared to the 100 ppm FeSO<sub>4</sub> (control), the number of tillers increased by 35.58% at 100 ppm FeSO<sub>4</sub> application plus aqueous bay leaf > 100 ppm FeSO<sub>4</sub> plus aqueous banana peel (26.67%) > 100 ppm FeSO<sub>4</sub> plus aqueous potato peel (21.48%) > Natural soil (7.07%). Overall, the number of effective tillers decreased in the variety Lachit (29.83%) > Inglongkiri (18.75%) > Luit (12.48%) as compared to the Dehangi.

The current study on phenolic and potassium-rich plant extracts (bay leaf) shows a considerable improvement in tiller numbers and other growth indices. In plants, phenolic chemicals are necessary for structural support, constitutive and induced protection, and defence against weeds, pathogens, and insects (Jones and Hartley, 1999). The flavonoids and phenolics in bay leaf extracts may increase cytokinin activity, a hormone directly associated to the stimulation of axillary bud expansion, hence boosting the number of tillers. Phenolic molecules have high antioxidant capabilities that assist plants fight oxidative stress. They scavenge reactive oxygen species (ROS) produced by many physiological activities, preventing oxidative damage to cell membranes, proteins, and DNA. Potassium plays a role in intracellular osmotic control and membrane protein transport by activating different enzymes. Furthermore, K aids glucose transport in rice and benefits plant metabolism and stress resistance (Wang and Wu, 2013; Nieves-Cordones et al., 2019).

### 3.3 Number of leaves and root biomass:

There were significant variations of number of leaves (Table 3a) at maximum tillering stage among the treatments. As compared to the 100 ppm FeSO<sub>4</sub> (control), the number of leaves increased by 36.79% at 100 ppm FeSO<sub>4</sub> application plus aqueous bay leaf > 100 ppm FeSO<sub>4</sub> plus aqueous Banana peel (32.99%) > 100 ppm FeSO<sub>4</sub> plus aqueous potato peel (25.39%) > Natural soil (12.18%). Overall, the number of leaves decreased in the variety Lachit (45.06%) > Inglongkiri (30.86%) > Luit (24.01%) as compared to the Dehangi.

Bay leaf promotes vegetative development, which includes leaf formation. Magnesium (Mg) and flavonoids in bay leaf extract promote chlorophyll production, resulting in increased photosynthetic activity. Bay leaves' high polyphenolic and flavonoid contents aid in the detoxification of excess iron from rice leaves, reducing chlorosis and oxidative damage. Phenolics serve a vital function in plant development, particularly in the lignin and pigment production. They also provide structural integrity and scaffolding support to plants. Potassium is essential for photosynthesis, glucose translocation, and metabolism, which ultimately boost crop output and improve grain quality (Pettigrew, 2008; Zörb et al., 2014; Lu et al., 2016). When a plant is lacking in K, both the number and size of its leaves decrease. Similarly, foliar application of potassium oxide in onion has increased plant growth (plant length, number of leaves per plant, and fresh weight of leaves), as well as yield and quality (Xu et al., 2020; Marschner, 2012). Potassium spray boosted leaf potassium concentration while also increasing photosynthesis, photorespiration, and RuBP carboxylase activity. As a result, even in saline conditions, growth improved significantly in the current study (Jabeen and Ahmad, 2011). Similarly, Milford et al. (2007) proposed that K is essential for stomatal opening and closure, as well as plant cell transpiration and photosynthesis.

**TABLE 3**  
**EFFECT OF BAY LEAF, POTATO PEEL, AND BANANA PEEL EXTRACTS ALONG WITH IRON TREATMENTS ON**  
**NUMBER OF LEAVES AND ROOT BIOMASS**

**(a) Number of leaves per plant at maximum tillering stage**

Variety	100 ppm FeSO <sub>4</sub> (control)	100 ppm FeSO <sub>4</sub> + aqueous Bay leaf (10 g/100 ml)	100 ppm FeSO <sub>4</sub> + aqueous Banana peel (10 g/100 ml)	100 ppm FeSO <sub>4</sub> + aqueous Potato peel (10 g/100 ml)	Natural soil	Mean
Inglongkiri	12.667	20	18.733	17.033	14.667	16.62
Dehangi	22.333	26.733	25.767	24	21.367	24.04
Lachit	9.667	17.333	15.333	13.033	10.667	13.207
Luit	10.367	23	22.3	19.7	15.967	18.267
<b>Mean</b>	<b>13.758</b>	<b>21.767</b>	<b>20.533</b>	<b>18.442</b>	<b>15.667</b>	
	S.Ed(±)	CD(0.05)				
Variety (V)	0.43	1.25				
Treatment (T)	0.38	1.09				
V × T	0.85	2.17				

**(b) Root biomass (g plant<sup>-1</sup>) at harvest**

Variety	100 ppm FeSO <sub>4</sub> (control)	100 ppm FeSO <sub>4</sub> + aqueous Bay leaf (10 g/100 ml)	100 ppm FeSO <sub>4</sub> + aqueous Banana peel (10 g/100 ml)	100 ppm FeSO <sub>4</sub> + aqueous Potato peel (10 g/100 ml)	Natural soil	Mean
Inglongkiri	13.167	22.867	20.7	18.733	15.267	18.147
Dehangi	14.633	24.933	23.2	21.667	17.633	20.413
Lachit	11.567	16.933	15.233	14.133	12.3	14.033
Luit	11.767	19.667	19.367	19.167	15.8	17.153
<b>Mean</b>	<b>12.783</b>	<b>21.1</b>	<b>19.625</b>	<b>18.425</b>	<b>15.25</b>	
	S.Ed(±)	CD(0.05)				
Variety (V)	0.4	1.18				
Treatment (T)	0.36	1.03				
V × T	0.81	2.05				

The results revealed significant variations of root biomass (Table 3b) among the treatments. As compared to the 100 ppm FeSO<sub>4</sub> (control), the root biomass increased by 39.41% at 100 ppm FeSO<sub>4</sub> application plus aqueous bay leaf > 100 ppm FeSO<sub>4</sub> plus aqueous Banana peel (34.86%) > 100 ppm FeSO<sub>4</sub> plus aqueous Potato peel (30.62%) > Natural soil (16.17%). Overall, the root biomass decreased in the variety Lachit (31.25%) > Luit (15.97%) > Inglongkiri (11.10%) as compared to the Dehangi.

Bay leaf contains bioactive chemicals, nutrients such as potassium, essential oils, and antioxidants, all of which can have a substantial impact on root growth, nutrient uptake, and rice stress tolerance. Bay leaf, when used as a soil supplement, root dip, or extract, increases root biomass, root architecture, and biochemical activity, hence boosting overall plant health and productivity. Phenolics cause redox reactions in soils and preferentially affect the growth of soil microorganisms that populate the rhizosphere. These alter hormonal balance in plants, enzymatic activity, phytonutrient availability, and competition between nearby plants (Hättenschwiler and Vitousek, 2000; Kraus et al., 2003; Northup et al., 1998). Extracts with higher phenolic contents had a higher antioxidant capacity than extracts with lower phenolic contents (Liyana-Pathirana et al., 2006). Stressed rapeseed with adequate K supply not only showed an improved potential for elongating root length, increasing root density, and balancing root-shoot ratio to increase water uptake, but also stimulated organic acid secretion to improve nutrient acquisition and utilization (Xu et al., 2021).

**3.4 Panicle length and panicle weight:**

The results revealed significant variations of panicle length (Table 4a) among the treatments. As compared to the 100 ppm FeSO<sub>4</sub> (control), the panicle length increased by 36.50% at 100 ppm FeSO<sub>4</sub> application plus aqueous bay leaf > 100 ppm FeSO<sub>4</sub> plus aqueous banana peel (31.11%) > 100 ppm FeSO<sub>4</sub> plus aqueous potato peel (25.86%) > Natural soil (12.41%). Overall, the panicle length decreased in the variety Lachit (26.28%) > Inglongkiri (11.39%) > Luit (7.52%) as compared to the Dehangi (iron tolerant).

**TABLE 4**  
**EFFECT OF BAY LEAF, POTATO PEEL, AND BANANA PEEL EXTRACTS ALONG WITH IRON TREATMENTS ON PANICLE LENGTH AND PANICLE WEIGHT**

**(a) Panicle length (cm)**

Variety	100 ppm FeSO <sub>4</sub> (control)	100 ppm FeSO <sub>4</sub> + aqueous Bay leaf (10 g/100 ml)	100 ppm FeSO <sub>4</sub> + aqueous Banana peel (10 g/100 ml)	100 ppm FeSO <sub>4</sub> + aqueous Potato peel (10 g/100 ml)	Natural soil	Mean
Inglongkiri	19.4	29.733	28.3	26.6	22.7	25.347
Dehangi	20.433	36.367	32.667	29.967	23.6	28.607
Lachit	17.9	25.667	22.433	20.4	19.033	21.087
Luit	20.133	30.867	29.633	28.067	23.567	26.453
<b>Mean</b>	<b>19.467</b>	<b>30.658</b>	<b>28.258</b>	<b>26.258</b>	<b>22.225</b>	
	S.Ed(±)	CD(0.05)				
Variety (V)	0.59	1.72				
Treatment (T)	0.52	1.49				
V × T	1.17	2.98				

**(b) Panicle weight (g plant<sup>-1</sup>)**

Variety	100 ppm FeSO <sub>4</sub> (control)	100 ppm FeSO <sub>4</sub> + aqueous Bay leaf (10 g/100 ml)	100 ppm FeSO <sub>4</sub> + aqueous Banana peel (10 g/100 ml)	100 ppm FeSO <sub>4</sub> + aqueous Potato peel (10 g/100 ml)	Natural soil	Mean
Inglongkiri	7.8	8.467	8.433	8.367	8.267	8.267
Dehangi	8.233	9.333	9.267	8.967	9	8.96
Lachit	5.733	8	7.933	7.867	6.133	7.133
Luit	7.7	8.267	8.2	8	8.167	8.067
<b>Mean</b>	<b>7.367</b>	<b>8.517</b>	<b>8.458</b>	<b>8.3</b>	<b>7.892</b>	
	S.Ed(±)	CD(0.05)				
Variety (V)	0.16	0.47				
Treatment (T)	0.14	0.41				
V × T	0.32	0.81				

Bay leaves improve panicle development by increasing food intake, regulating hormones, and reducing stress. Bay leaf extracts may increase the availability and uptake of critical nutrients such as nitrogen (N), phosphorus (P), and potassium (K), all of which are essential for panicle development and elongation. Bioactive chemicals found in bay leaf extracts may affect plant hormone levels, particularly gibberellins (GAs) and auxins, which are required for panicle elongation and grain growth. Foliar application of Moringa leaf (high in phenolic compounds) coupled with other plant growth stimulants increases panicle length, 1000-seed weight, and quinoa crop production, which is consistent with previous findings (Rashid et al., 2021). According to Raza et al. (2014), K increased the spike length, number of spikelets per spike, number of grains, and grain production of wheat during dry conditions.

The results revealed significant variations of panicle weight (Table 4b) among the treatments. As compared to the 100 ppm FeSO<sub>4</sub> (control), the panicle weight percentage increased by 13.50% at 100 ppm FeSO<sub>4</sub> application plus aqueous bay leaf > 100 ppm FeSO<sub>4</sub> plus aqueous Banana peel (12.90%) > 100 ppm FeSO<sub>4</sub> plus aqueous Potato peel (11.24%) > Natural soil (6.65%). Overall, the panicle weight decreased in the variety Lachit (20.39%) > Luit (9.96%) > Inglongkiri (7.73%) as compared to the Dehangi.

Bay leaf extracts promote root function and nutrient absorption, especially nitrogen (N), phosphorus (P), and potassium (K), which are required for panicle development and grain filling. The presence of bioactive chemicals in bay leaf extracts may increase chlorophyll content and photosynthetic efficiency, resulting in increased glucose synthesis and translocation to panicles, hence boosting grain weight. According to Görmüş (2004), using K enhanced boll weight, number of bolls per plant, seed and lint yield in cotton.

**3.5 Number of seeds per panicle and ‘Test Weight’:**

The results revealed significant variations of number of seeds per panicle (Table 5a) among the treatments. As compared to the 100 ppm FeSO<sub>4</sub> (control), the number of seeds per panicle increased by 23.27% at 100 ppm FeSO<sub>4</sub> application plus aqueous bay leaf > 100 ppm FeSO<sub>4</sub> plus aqueous Banana peel (20.30%) > 100 ppm FeSO<sub>4</sub> plus aqueous Potato peel (17.18%) > Natural soil (6.80%). Overall, the number of seeds per panicle decreased in the variety Lachit (19.03%) > Luit (13.04%) > Inglongkiri (9.97%) as compared to the Dehangi.

Bay leaf includes bioactive chemicals, essential oils, and secondary metabolites that can have a considerable impact on rice seed production, grain filling, and total seed quantity. When applied as a soil amendment, foliar spray, or extract, bay leaf improves nutrient assimilation, hormone regulation, and stress tolerance, resulting in more seeds per panicle and higher overall output. Raza et al. (2014) demonstrated that K increased spike length, number of spikelets per spike, number of grains, and grain yield in wheat under drought conditions. Vyas et al. (2008) demonstrated that potassium spraying considerably increased soybean seed yield.

**TABLE 5**  
**EFFECT OF BAY LEAF, POTATO PEEL, AND BANANA PEEL EXTRACTS ALONG WITH IRON TREATMENTS ON**  
**NUMBER OF SEEDS AND TEST WEIGHT**

**(a) Number of seeds per panicle**

Variety	100 ppm FeSO <sub>4</sub> (control)	100 ppm FeSO <sub>4</sub> + aqueous Bay leaf (10 g/100 ml)	100 ppm FeSO <sub>4</sub> + aqueous Banana peel (10 g/100 ml)	100 ppm FeSO <sub>4</sub> + aqueous Potato peel (10 g/100 ml)	Natural soil	Mean
Inglongkiri	69.5	85.1	82.5	78.033	68.9	76.807
Dehangi	81.1	90.867	89.7	87.933	76.967	85.313
Lachit	53.833	79.867	71.767	77.133	62.767	69.073
Luit	57.1	85	84.167	72.667	71.967	74.18
<b>Mean</b>	<b>65.383</b>	<b>85.208</b>	<b>82.033</b>	<b>78.942</b>	<b>70.15</b>	
	S.Ed(±)	CD(0.05)				
Variety (V)	1.54	4.53				
Treatment (T)	1.38	3.93				
V × T	3.09	7.85				

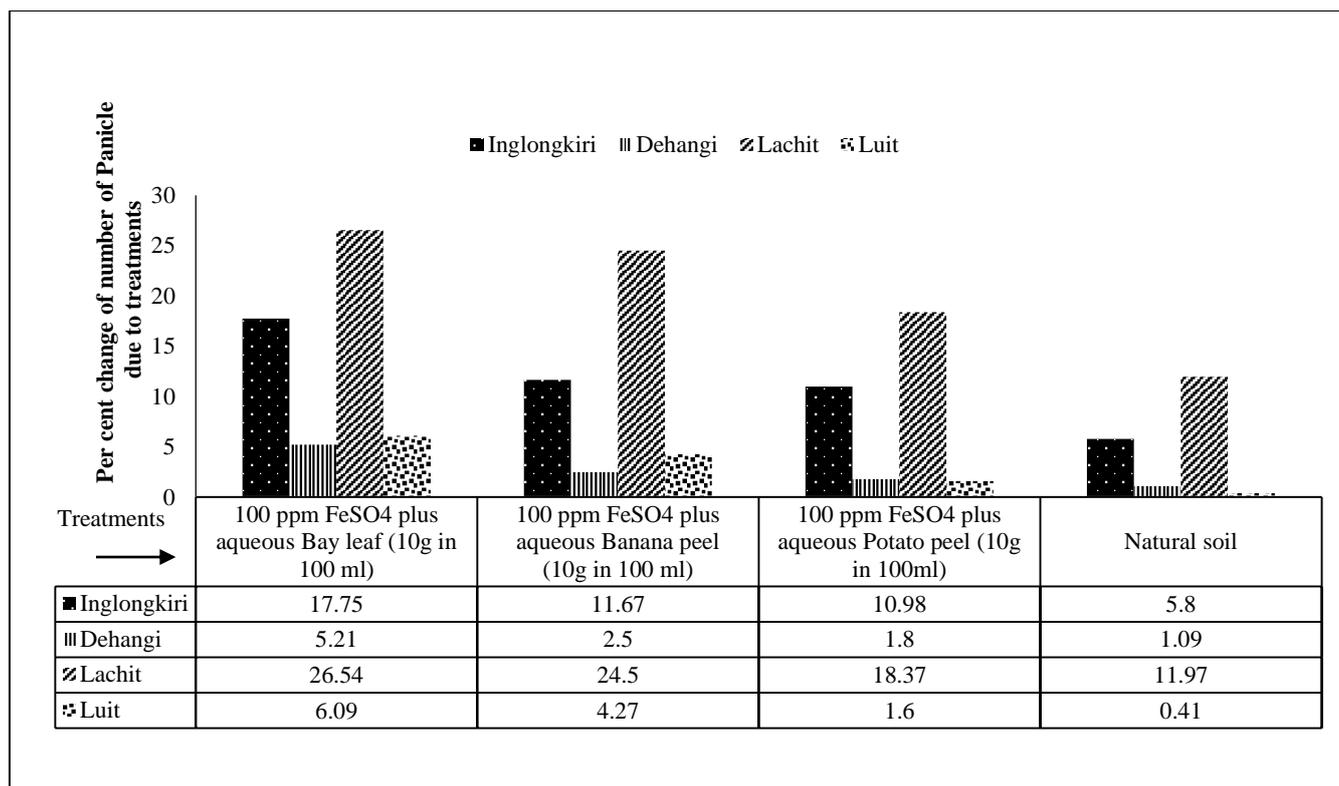
**(b) Test weight (g)**

Variety	100 ppm FeSO <sub>4</sub> (control)	100 ppm FeSO <sub>4</sub> + aqueous Bay leaf (10 g/100 ml)	100 ppm FeSO <sub>4</sub> + aqueous Banana peel (10 g/100 ml)	100 ppm FeSO <sub>4</sub> + aqueous Potato peel (10 g/100 ml)	Natural soil	Mean
Inglongkiri	18.233	29.367	26.333	24.767	20.567	23.853
Dehangi	22.433	30.233	30.167	28	23.633	26.893
Lachit	12.667	23.733	22.3	20.033	14.7	18.687
Luit	21.667	26.9	24.5	24.467	23.733	24.253
<b>Mean</b>	<b>18.75</b>	<b>27.558</b>	<b>25.825</b>	<b>24.317</b>	<b>20.658</b>	
	S.Ed(±)	CD(0.05)				
Variety (V)	0.53	1.55				
Treatment (T)	0.47	1.34				
V × T	1.05	2.69				

The results revealed significant variations of test weight (Table 5b) among the treatments. As compared to the 100 ppm FeSO<sub>4</sub> (control), the test weight percentage increased by 31.96% at 100 ppm FeSO<sub>4</sub> application plus aqueous bay leaf > 100 ppm FeSO<sub>4</sub> plus aqueous banana peel (27.40%) > 100 ppm FeSO<sub>4</sub> plus aqueous potato peel (22.89%) > Natural soil (9.24%). Overall, the test weight decreased in the variety Lachit (30.51%) > Inlongkiri (11.30%) > Luit (9.81%) as compared to the Dehangi. Ahmed et al. (2020) observed that higher K treatment enhanced maize and soybean test weights by 8 and 4%, respectively.

**3.6 Panicle number per plant:**

The results revealed significant variations of panicle number per plant (Fig. 1) among the treatments. As compared to the 100 ppm FeSO<sub>4</sub> (control), the panicle number per plant percentage increased by 13.70% at 100 ppm FeSO<sub>4</sub> application plus aqueous bay leaf > 100 ppm FeSO<sub>4</sub> plus aqueous Banana peel (10.49%) > 100 ppm FeSO<sub>4</sub> plus aqueous Potato peel (7.85%) > Natural soil (4.47%). Overall, the panicle number per plant decreased in the variety Lachit (17.20%) > Inlongkiri (9.96%) > Luit (9.17%) as compared to the Dehangi.



**FIGURE 1: Changes of panicle number per plant in comparison to control**

Bay leaf extracts promote tiller initiation and development by increasing cytokinin levels. Additionally, higher chlorophyll content and improved carbon assimilation lead to more panicles per plant. Raza et al. (2014) found that potassium increased wheat spike length, spikelet number, number of grains, and grain yield. K treatment resulted in a 21.8% rise in spike length, a 23.27% increase in spikelets, a 39.24% increase in grains, and a 30.77% increase in yield as compared to the control.

**3.7 High Density (HD) grains and sterility:**

The results revealed significant variations of high density grains (Table 6a) among the treatments. As compared to the 100 ppm FeSO<sub>4</sub> (control), the high density grains increased by 14.8% at 100 ppm FeSO<sub>4</sub> application plus aqueous bay leaf > 100 ppm FeSO<sub>4</sub> plus aqueous banana peel (11.5%) > 100 ppm FeSO<sub>4</sub> plus aqueous potato peel (9.43%) > Natural soil (6.49%). Overall, the HD grains decreased in the variety Lachit (11.61%) > Luit (7.28%) > Inglongkiri (3.06%) as compared to the Dehangi (iron tolerant).

**TABLE 6**

**EFFECT OF BAY LEAF, POTATO PEEL, AND BANANA PEEL EXTRACTS ALONG WITH IRON TREATMENTS ON HIGH DENSITY (HD) GRAINS AND STERILITY**

**(a) HD grains (%)**

Variety	100 ppm FeSO <sub>4</sub> (control)	100 ppm FeSO <sub>4</sub> + aqueous Bay leaf (10 g/100 ml)	100 ppm FeSO <sub>4</sub> + aqueous Banana peel (10 g/100 ml)	100 ppm FeSO <sub>4</sub> + aqueous Potato peel (10 g/100 ml)	Natural soil	Mean
Inglongkiri	63.467	79.6	77.3	76.267	65.367	72.4
Dehangi	67.367	82.567	80.8	72.833	73.733	75.46
Lachit	57.833	71.567	59.5	63.867	66.467	63.847
Luit	57.433	71.567	74.5	70.867	66.5	68.173
<b>Mean</b>	<b>61.525</b>	<b>76.325</b>	<b>73.025</b>	<b>70.958</b>	<b>68.017</b>	
	S.Ed(±)	CD(0.05)				
Variety (V)	1.31	3.86				
Treatment (T)	1.17	3.34				
V × T	2.62	6.68				

**(b) Sterility (%)**

Variety	100 ppm FeSO <sub>4</sub> (control)	100 ppm FeSO <sub>4</sub> + aqueous Bay leaf (10 g/100 ml)	100 ppm FeSO <sub>4</sub> + aqueous Banana peel (10 g/100 ml)	100 ppm FeSO <sub>4</sub> + aqueous Potato peel (10 g/100 ml)	Natural soil	Mean
Inglongkiri	38.233	21.833	22.233	23.267	26.5	26.413
Dehangi	26.6	18.9	20.033	21.9	24.867	22.46
Lachit	43.6	22.167	23.4	25.233	28.933	28.667
Luit	33.833	21.7	21.9	24.367	28.4	26.04
<b>Mean</b>	<b>35.567</b>	<b>21.15</b>	<b>21.892</b>	<b>23.692</b>	<b>27.175</b>	
	S.Ed(±)	CD(0.05)				
Variety (V)	0.67	1.98				
Treatment (T)	0.6	1.71				
V × T	1.35	3.42				

Bay leaves are high in potassium, polyphenols, and vital minerals, which can help to improve grain filling and solidity. Potassium is essential for photosynthesis, glucose translocation, and metabolism, which ultimately raise crop productivity and grain quality (Pettigrew, 2008; Lu et al., 2016).

The results revealed significant variations of sterility (Table 6b) among the treatments. As compared to the 100 ppm FeSO<sub>4</sub> (control), the sterility percentage decreased by 14.41% at 100 ppm FeSO<sub>4</sub> application plus aqueous bay leaf > 100 ppm FeSO<sub>4</sub> plus aqueous Banana peel (13.67%) > 100 ppm FeSO<sub>4</sub> plus aqueous Potato peel (11.87%) > Natural soil (8.39%). Overall, the sterility percent increased in the variety Lachit (6.20%) > Inglongkiri (3.95%) > Luit (3.58%) as compared to the Dehangi.

Bay leaves are rich in potassium, antioxidants, and bioactive substances; they may aid in numerous ways by supporting glucose translocation for grain setting, minimizing aborted spikelets and empty grains by enhancing nutrient uptake. The larger level of K helps to transport food material to develop grains, thus minimizing the number of sterile grains. In rice, using K at 100 kg ha<sup>-1</sup> resulted in lower grain sterility compared to no K application. Islam et al. (2016) found that at 100 kg K ha<sup>-1</sup>, grain sterility was 22.60%, compared to 30.33% without it.

**3.8 Economic yield and biological Yield:**

The results revealed significant variations of economic yield (Table 7a) among the treatments. As compared to the 100 ppm FeSO<sub>4</sub> (control), the economic yield increased by 42.39% at 100 ppm FeSO<sub>4</sub> application plus aqueous bay leaf > 100 ppm FeSO<sub>4</sub> plus aqueous banana peel (35.86%) > 100 ppm FeSO<sub>4</sub> plus aqueous potato peel (27.50%) > Natural soil (9.68%). Overall, the economic yield decreased in the variety Lachit (28.68%) > Inglongkiri (14.78%) > Luit (8.07%) as compared to the Dehangi (iron tolerant). Khan et al. (2007) found that applying 60 kg ha<sup>-1</sup> K increased yield and yield-contributing characters in both rice and wheat, with wheat yielding 13% more and rice yielding 50% more than the control. It was an idea for producing more food as per the demand of time and space (Kijne et al., 2003).

**TABLE 7**  
**EFFECT OF BAY LEAF, POTATO PEEL, AND BANANA PEEL EXTRACTS ALONG WITH IRON TREATMENTS ON ECONOMIC YIELD AND BIOLOGICAL YIELD**

**(a) Economic yield (g plant<sup>-1</sup>)**

Variety	100 ppm FeSO <sub>4</sub> (control)	100 ppm FeSO <sub>4</sub> + aqueous Bay leaf (10 g/100 ml)	100 ppm FeSO <sub>4</sub> + aqueous Banana peel (10 g/100 ml)	100 ppm FeSO <sub>4</sub> + aqueous Potato peel (10 g/100 ml)	Natural soil	Mean
Inglongkiri	10.167	17.5	16.367	14.867	11.8	14.14
Dehangi	12.033	22.8	18.867	16.333	12.933	16.593
Lachit	8.533	15.767	13	11.733	10.133	11.833
Luit	11.9	17.933	18.233	15.867	12.333	15.253
<b>Mean</b>	<b>10.658</b>	<b>18.5</b>	<b>16.617</b>	<b>14.7</b>	<b>11.8</b>	
	S.Ed(±)	CD(0.05)				
Variety (V)	0.36	1.05				
Treatment (T)	0.32	0.91				
V × T	0.72	1.83				

**(b) Biological yield (g plant<sup>-1</sup>)**

Variety	100 ppm FeSO <sub>4</sub> (control)	100 ppm FeSO <sub>4</sub> + aqueous Bay leaf (10 g/100 ml)	100 ppm FeSO <sub>4</sub> + aqueous Banana peel (10 g/100 ml)	100 ppm FeSO <sub>4</sub> + aqueous Potato peel (10 g/100 ml)	Natural soil	Mean
Inglongkiri	31.433	46.533	46.1	44.867	38.833	41.553
Dehangi	36	50.567	49.767	48.3	40.4	45.007
Lachit	29.767	40.467	34.233	30.233	29.167	32.773
Luit	34.1	43.1	42.267	40.467	37.767	39.54
<b>Mean</b>	<b>32.825</b>	<b>45.167</b>	<b>43.092</b>	<b>40.967</b>	<b>36.542</b>	
	S.Ed(±)	CD(0.05)				
Variety (V)	0.88	2.59				
Treatment (T)	0.79	2.24				
V × T	1.76	4.48				

The results revealed significant variations in biological yield (Table 7b) among the treatments. As compared to the 100 ppm FeSO<sub>4</sub> (control), the biological yield percentage increased by 27.33% at 100 ppm FeSO<sub>4</sub> application plus aqueous bay leaf > 100 ppm FeSO<sub>4</sub> plus aqueous banana peel (23.83%) > 100 ppm FeSO<sub>4</sub> plus aqueous potato peel (19.87%) > Natural soil (10.17%). Overall, the biological yield decreased in the variety Lachit (27.18%) > Luit (12.14%) > Inglongkiri (7.67%) as compared to the Dehangi.

Bay leaf extracts may increase chlorophyll levels, resulting in better photosynthesis and biomass build-up. Increased carbon uptake promotes vegetative and reproductive growth. Bay leaf extracts contain bioactive chemicals that encourage cell division and elongation, resulting in greater shoot and root growth and, eventually, increased overall biomass production. Waraich et al. (2011) found that 200 mM KNO<sub>3</sub> application enhanced plant branches, plant height, and the number of bolls per plant in cotton.

**3.9 Grain Harvest Index (GHI) and plant height:**

The results revealed significant variations in GHI (Table 8a) among the treatments. As compared to the 100 ppm FeSO<sub>4</sub> (control), the GHI increased by 27.56% at 100 ppm FeSO<sub>4</sub> application plus aqueous bay leaf > 100 ppm FeSO<sub>4</sub> plus aqueous banana peel (23.97%) > 100 ppm FeSO<sub>4</sub> plus aqueous potato peel (19.09%) > Natural soil (9.11%). Overall, the GHI decreased in the variety Lachit (27.38%) > Luit (13.84%) > Inglongkiri (9.38%) as compared to the Dehangi. Reduced assimilate transfer lowers the harvest index, resulting in shrinkage and decreased seed weight, which is similar with the findings of Ashley et al. (1978).

**TABLE 8**  
**EFFECT OF BAY LEAF, POTATO PEEL, AND BANANA PEEL EXTRACTS ALONG WITH IRON TREATMENTS ON GRAIN HARVEST INDEX AND PLANT HEIGHT**

**(a) Grain Harvest Index (%)**

Variety	100 ppm FeSO <sub>4</sub> (control)	100 ppm FeSO <sub>4</sub> + aqueous Bay leaf (10 g/100 ml)	100 ppm FeSO <sub>4</sub> + aqueous Banana peel (10 g/100 ml)	100 ppm FeSO <sub>4</sub> + aqueous Potato peel (10 g/100 ml)	Natural soil	Mean
Inglongkiri	36.133	51.333	46.533	46.867	39.1	43.993
Dehangi	45.4	52	49.5	47.667	48.167	48.547
Lachit	26.967	44.4	42.733	33.5	28.667	35.253
Luit	32.1	46.367	46.167	45.733	38.767	41.827
<b>Mean</b>	<b>35.15</b>	<b>48.525</b>	<b>46.233</b>	<b>43.442</b>	<b>38.675</b>	
	S.Ed(±)	CD(0.05)				
Variety (V)	1.06	3.12				
Treatment (T)	0.95	2.7				
V × T	2.12	5.4				

**(b) Plant height (cm)**

Variety	100 ppm FeSO <sub>4</sub> (control)	100 ppm FeSO <sub>4</sub> + aqueous Bay leaf (10 g/100 ml)	100 ppm FeSO <sub>4</sub> + aqueous Banana peel (10 g/100 ml)	100 ppm FeSO <sub>4</sub> + aqueous Potato peel (10 g/100 ml)	Natural soil	Mean
Inglongkiri	88.733	96.9	93.133	94.8	92.633	93.24
Dehangi	93.667	99.233	98.367	98	95.467	96.947
Lachit	72.733	78.533	76.4	75.367	73.533	75.313
Luit	81.767	92.533	90.9	85.6	88.6	87.88
<b>Mean</b>	<b>84.225</b>	<b>91.8</b>	<b>89.7</b>	<b>88.442</b>	<b>87.558</b>	
	S.Ed(±)	CD(0.05)				
Variety (V)	0.51	1.5				
Treatment (T)	0.46	1.3				
V × T	1.02	2.6				

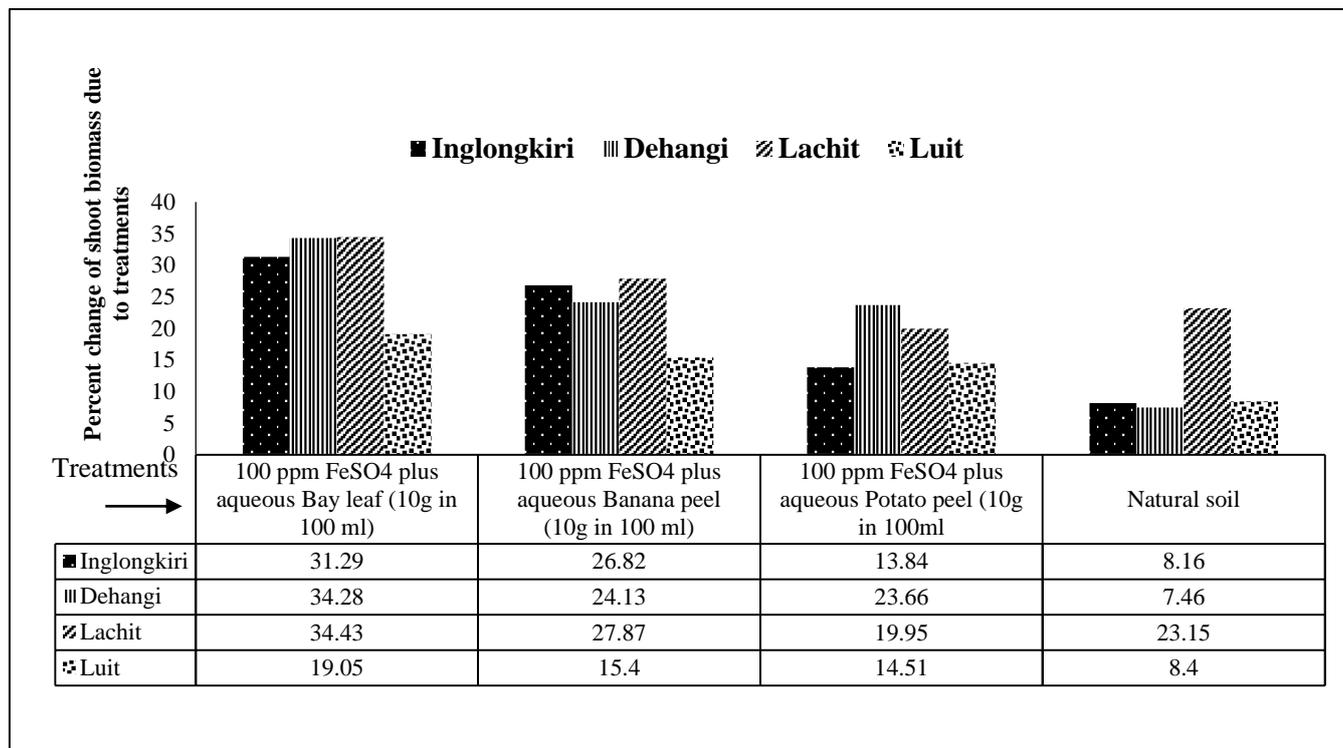
The results revealed significant variations in plant height (Table 8b) among the treatments. As compared to the 100 ppm FeSO<sub>4</sub> (control), the plant height increased by 8.25% at 100 ppm FeSO<sub>4</sub> application plus aqueous bay leaf > 100 ppm FeSO<sub>4</sub> plus aqueous banana peel (6.10%) > 100 ppm FeSO<sub>4</sub> plus aqueous potato peel (4.77%) > Natural soil (3.81%). Overall, the plant height decreased in the variety Lachit (22.31%) > Luit (9.35%) > Inglongkiri (3.82%) as compared to the Dehangi.

Bay leaves are high in potassium and phenolic compounds such as flavonoids, phenolic acids, tannins (proanthocyanidins), lignands, and essential oils (Alejo-Armijo et al., 2017), all of which can have a positive impact on plant growth parameters such as height. *Salvia officinalis* leaf extract, particularly at the maximum dosage (40 mL/L), significantly enhanced plant height, number of leaves, number of branches, yield, and essential oil percentage, as well as improved leaf anatomical structure (Abbas et al., 2016). Taller plants were observed following foliar spray of Moringa leaf during the first year of normal sowing (Rashid et al., 2021). Zelelew et al. (2016) studied potato (*Solanum tuberosum* L.) growth with five K dosages (0, 75, 150, 225, and 300 kg K<sub>2</sub>O ha<sup>-1</sup>). They discovered that increasing K levels increased plant height, aerial stem number, and leaf number per plant from 0 kg to 150 kg ha<sup>-1</sup>.

**3.10 Shoot Biomass:**

The results revealed significant variations of shoot biomass (Fig. 2) among the treatments. As compared to the 100 ppm FeSO<sub>4</sub> (control), the shoot biomass increased by 30.08% at 100 ppm FeSO<sub>4</sub> application plus aqueous bay leaf > 100 ppm

FeSO<sub>4</sub> plus aqueous banana peel (23.56%) > 100 ppm FeSO<sub>4</sub> plus aqueous potato peel (18.25%) > Natural soil (11.62%). Overall, the shoot biomass decreased in the variety Lachit (23.09%) > Inglongkiri (14.17%) > Luit (13.96%) as compared to the Dehangi.



**FIGURE 2: Changes of Shoot biomass in comparison with control at harvest**

Bioactive chemicals in bay leaf extracts increase nutrient availability, particularly nitrogen and potassium, which are necessary for shoot development and biomass build-up. Bay leaf extracts stabilize chlorophyll and increase photosynthetic efficiency. This leads to increased energy production, shoot growth, and biomass accumulation. Data from numerous studies and the literature reveal that phenolic compounds exhibit growth-promoting properties as a result of their positive impact on various phases of plant growth and developmental processes, such as seed germination, shoot length, root length, plant biomass, photosynthetic pigments, and plant metabolism (Zaid et al., 2019; Saidi et al., 2021). Exogenous application of Moringa leaf (phenolic-rich) protects crops from harmful environmental effects while also improving plant morphological features (plant fresh and dry biomass) in normal and benign conditions (Yasmeen et al., 2012). K is required for plant nutrition since it is responsible for several physical processes that regulate plant growth, yield, and quality criteria such as flavor and nutritional health (Lester, 2005). According to Cakmak (2005), K is not a major component of plant structure or any organic molecule, but rather plays an important role in optimizing various physiological and biochemical processes involved in yield, quality, and plant growth.

#### IV. CONCLUSION

Bay leaf played a crucial role in enhancing iron tolerance in rice by significantly improving root proliferation and biomass, which facilitated better nutrient absorption. Additionally, bay leaf positively influenced key physiological parameters, contributing to overall plant health and yield. Among the varieties, Dehangi emerged as the most tolerant to iron toxicity, while Lachit was the most susceptible. The use of bio inputs viz., kitchen waste extracts, particularly bay leaf, offers an eco-friendly and cost-effective approach to mitigating iron stress in acid soils of Assam.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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# Enemies of Honey Bee (*Apis mellifera* Linn.) and their Management: A Review

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**Abstract**— Honey bees (*Apis mellifera*) are among the most important pollinators, playing a crucial role in maintaining biodiversity and agricultural productivity. However, their colonies face numerous biotic threats that significantly impact their health, survival, and productivity. The major enemies of honey bees include parasitic mites such as *Varroa destructor*, which weaken colonies by feeding on bee hemolymph and transmitting viruses, and the tracheal mite (*Acarapis woodi*) which disrupts respiration. Pathogens like *Nosema* species (microsporidians) and various viral infections further compromise colony strength, leading to reduced longevity and productivity. Additionally, predators such as wax moths (*Galleria mellonella* and *Achroia grisella*) and small hive beetles (*Aethina tumida*) cause considerable structural damage to combs, stored honey, and brood. Minor enemies, though less destructive individually, also exert significant cumulative stress. These include ants, wasps, and spiders that invade hives for food resources, as well as birds such as bee-eaters that prey directly on foragers. Fungal diseases like chalkbrood (*Ascosphaera apis*) and stonebrood (*Aspergillus* spp.) are typically opportunistic, affecting weakened colonies under stress. Environmental stressors, pesticide exposure, and poor management practices often amplify the impact of these biotic threats. This review comprehensively synthesizes the available literature on the distribution, biology, seasonal incidence, symptoms, and management of major and minor enemies of *Apis mellifera*, with special reference to the Indian context. Understanding these threats is critical for devising integrated pest management strategies. Effective monitoring, hygienic management practices, and sustainable control measures are essential to safeguard *Apis mellifera*, ensuring their ecological services and economic value in agriculture.

**Keywords**— *Apis mellifera*, Honey bee enemies, *Varroa* mite, Wax moth, Wasp, Hive beetle, Vertebrate predators, Integrated pest management.

## I. INTRODUCTION

Beekeeping of European honey bee (*Apis mellifera*) is widely practiced in Himachal Pradesh, mainly through migratory methods (Kumar et al., 2022; Sharma & Verma, 2021). In recent years, there has been significant research in the field of apiculture and honey bee management, as more people domesticate bees for various products like honey and wax, as well as for business purposes (Kumar et al., 2023; Singh & Rana, 2020). However, *Apis mellifera* populations face numerous threats that can negatively impact their survival and overall health. In recent times, the rapid movement of honey bees and bee products from one location to another across countries and continents has aggravated pest problems (Rosenkranz et al., 2021; Traynor et al., 2023). Poor management practices in beekeeping weaken bee colonies, making them susceptible to pest and predator attacks.

Enemies of honey bees are those which cause disturbances and nuisance in the functioning of the colony, ranging widely in size from microscopic mites to large mammals such as bears (Ghosh et al., 2020; Sharma et al., 2022; Nain & Singh, 2024). There are reports on the incidence of a large number of insect pests infesting honey bees worldwide. Enemies are classified into two categories: pests and predators of honey bees. Predators are those animals which capture other living organisms for

food (Neumann & Carreck, 2019; Evans et al., 2021; Thakur et al., 2023). The rest of the enemies cause some harm or disturbance to honey bee colonies and are considered as pests, which can cause heavy damage to bee life and their seasonal activity (Kumar et al., 2021; Abrol & Kaur, 2022; Suresh et al., 2024).

Wax moths, mites, wasps, birds, ants, bee lice, hive beetles, mice, skunks, and bears (Morse, 1999) are considered as major enemies, whereas the minor enemies include cockroaches, leaf cutter bees, robber flies, dragon flies, praying mantis, spiders (Thakur and Sharma, 1984), etc. which cause nuisance in bee colonies. These enemies are significant pests that disrupt the honey bee life cycle, making their management essential for colony health (Neumann & Carreck, 2019; Kumar et al., 2022; Ghosh et al., 2023; Nain & Singh, 2024).

The present review aims to comprehensively synthesize the available literature on the major and minor enemies of *Apis mellifera*, with emphasis on their distribution, life history, seasonal incidence, symptoms of infestation, and management strategies. While several reviews exist on individual pests, this paper provides a consolidated, holistic overview with special reference to the Indian subcontinent, highlighting region-specific challenges and knowledge gaps that warrant future research.

## II. INSECT PESTS AND PREDATORS OF HONEY BEES

### 2.1 Wax Moth:

*Galleria mellonella* L. (greater wax moth) and *Achroia grisella* F. (lesser wax moth) are the most destructive pests of the beekeeping industry worldwide. Other species such as *Vitula* spp. (dried fruit moth), *Plodia interpunctella* (Hbn.), *Ephestia kuhniella* (Zell), and *E. cautella* are also associated with colonies of honey bees (*Apis cerana*, *A. mellifera*, *A. dorsata*, and *A. florea*) but cause comparatively less damage (Kumar, 1996). Among these, *G. mellonella* and *A. grisella* are responsible for enormous economic losses and are discussed in detail.

#### 2.1.1 The Greater Wax Moth (*Galleria mellonella* L., Pyralidae: Lepidoptera):

*Galleria mellonella*, the greater wax moth or honeycomb moth, is a pest of global distribution. While it is a useful model insect in pathology research, it causes heavy losses to beekeepers throughout the world (Wojda, 2020; Han et al., 2023). The species was first reported as a pest in Asia, subsequently spreading to Northern Africa, Great Britain, parts of Europe, Northern America, and New Zealand. It is now distributed throughout the globe wherever honey bees are cultivated.

- **Life History:** The life cycle of *G. mellonella* proceeds through four stages: egg, larva, pupa, and adult. Generally, eggs are laid in early spring, and the moth undergoes four to six generations annually. Eggs are smooth, spherical, and pinkish to creamish white, measuring about 0.4–0.5 mm in diameter. They are usually laid in clusters within small cracks and crevices. A single female can lay an average of 300–600 eggs during her lifetime, with a maximum of up to 1800 eggs (Milum & Geuther, 1935; Mohamed & Cople, 1983; Khanbash & Oshan, 1997). The larva is white to dirty grey, measuring 3–30 mm in length. It lives inside long silken tunnels and, upon hatching, begins feeding on honey, nectar, and pollen. The larva bores tunnels into the comb and extends them toward the midrib, spinning silken galleries that provide protection from bees and trap newly emerged bees in their cells—a condition known as **gallariasis**. The larva moults 4–6 times. The pupa is brownish white when young, turning dark brown with age, measuring about 14–16 mm in length. The adult moth is heavy-bodied, brownish-grey, and ranges from 10–18 mm in length. Females are generally larger and heavier than males. In females, the outer margin of the forewing is smooth, whereas in males it bears a semi-lunar notch. The labial palps in females are extended forward, giving the head a beak-like appearance. The larval stage lasts 22–60 days (Jyothi & Reddy, 1994; Khanbash & Oshan, 1997) but may extend up to 100 days under certain conditions (Allegret, 1975). The pupal stage lasts 7–60 days (Kapil & Sihag, 1983; Jyothi & Reddy, 1993; Brar et al., 1996). The complete life cycle is typically completed within six weeks to six months, depending on environmental factors, particularly temperature, relative humidity, and diet (Burkett, 1962; Bogus & Cymborowski, 1977; Chauvin & Chauvin, 1985; Kumar, 2000).
- **Seasonal Incidence:** Seasonal incidence studies have shown that wax moths can produce multiple overlapping generations within a single year. Stored or abandoned combs, uncleaned wax residues, and weak or poorly managed colonies serve as continuous sources for wax moth population buildup. The number of generations produced annually depends largely on food availability, temperature, and habitat suitability. Wax moth activity generally extends from March to October (Garg & Kashyap, 1998), with peak infestation levels observed between June and November (Ramachandaran & Mahadevan, 1951; Brar et al., 1985; Gupta, 1987). In South India, the highest infestation rates have been reported during the dearth period when bee colonies are relatively weak and resources are scarce

(Viraktamath, 1989). During unfavorable conditions, the insect undergoes hibernation, mainly in the larval stage (approximately 70%) and to a lesser extent in the pupal stage (around 30%), within stored combs.

- **Symptoms:** Infestation of wax moths in weak colonies results in characteristic "gallerias" (Han et al., 2023; Kumar & Sharma, 2021). This condition is marked by the inability of emerging adult worker and drone bees to exit their cells, as their bodies become entangled in the silken threads spun by the larvae of *Galleria mellonella* (Kwadha et al., 2017; Wojda, 2020). This behavior exacerbates comb damage and poses a serious threat to colony health and productivity.
- **Effective Management:** Strong, well-managed colonies with adequate worker populations can resist wax moth infestation effectively, while weak colonies are more susceptible due to reduced defense activity (Ellis et al., 2013). Removal of old, damaged, or unused combs which serve as breeding sites is critical (Kwadha et al., 2017). Regular cleaning and sanitation of hives reduce larval development. Empty combs should be stored in well-ventilated and well-lit rooms. Freezing combs at  $-10^{\circ}\text{C}$  to  $-15^{\circ}\text{C}$  for 24–48 hours destroys all life stages of wax moths (Zhu et al., 2016; Kwadha et al., 2017). Fumigation with sulphur dioxide, acetic acid, or paradichlorobenzene (PDB) at recommended doses is also effective for stored combs.

### 2.1.2 The Lesser Wax Moth (*Achroia grisella* F., Pyralidae: Lepidoptera):

The lesser wax moth (*Achroia grisella*) is more widely distributed and abundant than the greater wax moth and is commonly found at comparatively higher altitudes. It is particularly troublesome in stored combs.

- **Life History:** The egg stage lasts about 2–4 days, the larval stage extends 34–48 days, the pupal stage ranges 5–12 days, and the adult moth lives for approximately 7 days (Gulati & Kaushik, 2004; Moreno-Serrano et al., 2024). The larvae of *A. grisella* measure about 15–20 mm in length (Hakanoglu et al., 2019). Adult lesser wax moths are silver-grey, without wing markings, and are smaller than the greater wax moth. They complete about four to five generations during the active season (Singh, 1962).
- **Seasonal Incidence:** The seasonal activity of *A. grisella* varies with climatic conditions, colony strength, and comb availability (Kwadha et al., 2017; Wakgari et al., 2021). Moth activity generally increases during warmer, more humid months, with peak infestation observed between June and September (Panwar, 2017–2022). During this period, high temperature and humidity favor rapid pest development and overlapping generations (Kwadha et al., 2017; Wakgari et al., 2021). Infestation is typically higher in weak colonies and stored combs, particularly during the dearth season when bee populations are reduced (Ellis et al., 2013; Mahgoub et al., 2020). Activity declines during winter due to low temperatures and limited food availability. While the greater wax moth dominates at lower altitudes, the lesser wax moth tends to be more abundant at higher altitudes and in cooler areas (Egelie et al., 2015; Mahgoub et al., 2020).
- **Symptoms:** Infestation by the lesser wax moth primarily occurs in weak colonies. The larvae prefer to feed on old, dark combs, especially those containing pollen or brood cells (Ellis et al., 2013; Kwadha et al., 2017). They are often found among wax debris accumulated on the bottom board. While feeding, the larvae create small tunnels beneath brood cells, causing brood to be pushed upward (Ellis et al., 2013; Vijayakumar, 2021). In response, bees extend the cell walls upward, resulting in a characteristic scratched appearance on the comb surface. In some cases, moth larvae cut off the caps of sealed cells, exposing pupae inside—a condition known as "**bald brood**," a typical symptom of lesser wax moth infestation (Ellis et al., 2013; Egelie et al., 2015; Kwadha et al., 2017).
- **Effective Management:** Maintaining strong, healthy colonies is the primary defense. Fumigation of stored combs with sulphur dioxide, acetic acid, or paradichlorobenzene (PDB) at recommended doses kills all life stages. Exposure of combs to freezing temperatures or direct sunlight effectively destroys eggs, larvae, and pupae (Ellis et al., 2013; Kwadha et al., 2017; Neupane et al., 2019; Mahgoub et al., 2020; Wakgari et al., 2021).

### 2.2 Wasps:

Several species of wasps prey upon honey bees, causing extensive damage to colonies and sometimes leading to the loss of entire apiaries (Ghosh, 1936; Dave, 1943; Muttoo, 1949; Subbiah & Mahadevan, 1957; Sharma & Deshraj, 1985). It has been reported that about 20–25% of bee colonies desert their nests each year due to wasp attacks (Adalakhia & Sharma, 1975). The large social wasps belonging to the genus *Vespa* are particularly destructive, as they are physically capable of preying on honey bees with ease. Species such as *Vespa orientalis* L. (yellow-branded brown wasp), *V. magnifica* Smith (large black wasp), *V.*

*cincta* F. (yellow-banded wasp), *V. ducalis* Smith, and *V. auraria* Smith (golden wasp) have been reported to attack weak and queenless colonies, destroying brood and honey stores (Kshirsagar & Mahindre, 1975; Sharma et al., 1979). They also attack forager bees in the field (Abrol, 1994; Abrol & Kakroo, 1998; Sihag, 1992). Sharma et al. (1985) recorded *V. mandarina*, *V. tropica*, *V. velutina*, and *V. basalis* as major predators of *Apis mellifera* and *A. cerana* colonies in Kangra, Himachal Pradesh. In Kashmir, *V. velutina* has been observed as a serious pest (Shah & Shah, 1991). In New Zealand, *V. germanica* was reported to have destroyed 3,900 colonies and affected over 10,000 others (Walton & Reid, 1976). Akre and Davis (1978) observed that in Japan, a group of 30 *V. magnifica* hornets could kill 25,000–30,000 bees within three hours, at a rate of one bee every 14 seconds. Hirschfeld (1952) estimated that a single female wasp consumes 60–80 bees during her lifetime. Other wasp species associated with bee colonies include *Philanthus ramakrishnae* T. and *Palarus orientalis* Kohl, commonly referred to as bee-hunter wasps (Thakur, 1991). *V. tropica* is a swift-flying species that primarily captures forager bees during flight (Garg & Kashyap, 1998).

- **Life History:** Wasps undergo complete metamorphosis, consisting of four distinct stages: egg, larva, pupa, and adult (Spradbery, 2002; Jandth & Toth, 2015; Brock et al., 2021). Development duration varies depending on species and environmental conditions such as temperature and food availability (Smith et al., 2013). The life cycle begins when the fertilized queen lays eggs in hexagonal cells within the nest. Eggs are small, oval, and whitish, with the egg stage lasting 3–5 days. After hatching, larvae are fed by worker wasps on chewed insects or protein-rich food. The larval stage generally lasts 10–14 days, during which larvae undergo several molts and increase rapidly in size. Mature larvae spin silken caps over cells and pupate. During this stage, internal reorganization occurs, leading to the development of wings, legs, and other adult structures (Matsuura & Yamane, 2020; Brock et al., 2021). The pupal period lasts 8–15 days. After emergence, adult wasps are divided into queens, workers, and males (drones) in social species such as *Vespa* and *Vespula*. The queen initiates nest building and lays eggs, while workers perform brood care, foraging, and defense. Males usually appear later in the season for mating. In temperate regions, the wasp life cycle is annual; only fertilized queens survive winter, while workers and males die due to cold. Queens emerge in spring to start new colonies. In tropical and subtropical regions, overlapping generations may occur, allowing colonies to persist for several months or year-round. The entire cycle from egg to adult is completed in about 30–45 days, depending on species and environmental factors (Spradbery, 2002; Mahgoub et al., 2020).
- **Seasonal Incidence:** The seasonal incidence of wasps in honey bee colonies varies with climatic conditions, food resource availability, and wasp species' developmental cycle (Abrol, 2006; Archer, 2012; Reddy et al., 2014). Wasp activity generally begins with the onset of warm weather in late spring and increases during summer, reaching peak during the post-monsoon period (July to October) (Kumar et al., 2015; Sharma et al., 2018; Ghosh & Dey, 2020). During this time, wasps such as *Vespa orientalis*, *V. tropica*, *V. auraria*, and *V. velutina* are frequently observed attacking honey bee colonies. Population declines sharply with colder months, with very little activity recorded from December to February. The highest incidence of wasp attacks occurs when honey bee colonies become weak due to reduced floral resources or brood rearing, making them more vulnerable to predation. Wasp abundance is also influenced by temperature and humidity, which favor breeding and foraging. In regions like Kangra and Kashmir, wasp populations show distinct seasonal trends, with maximum predation pressure during late summer and early autumn (Abrol, 2006; Sharma et al., 2018). Similar findings have been reported in other parts of India and abroad, indicating that wasp attacks are closely linked to environmental conditions and wasp life cycles. Monitoring and preventive measures against wasp attacks should be intensified during the peak season, particularly from July to October, to minimize colony losses (Abrol, 2006; Reddy et al., 2014; Monceau et al., 2014).
- **Symptoms:** Colonies under attack show a significant decline in foraging trips and guarding response, as bees become fearful and avoid the hive entrance (Abrol, 2006). Dead bees and body parts scattered near the hive indicate predation by wasps like *V. magnifica* and *V. velutina*. Wasps often invade weak or queenless colonies, destroying brood cells and feeding on larvae, pupae, and stored honey (Abrol & Kapil, 1994; Archer, 2012; Sharma et al., 2018; Brock et al., 2021).
- **Effective Management:** Locating and destroying wasp nests around apiaries during early spring or the nesting season helps reduce populations before they become harmful. Bait traps containing sugar syrup, fruit juice, or meat pieces mixed with insecticide can be placed near apiaries to attract and kill adult wasps. Strong colonies with sufficient

worker populations are better able to defend themselves against wasp attacks than weak or queenless ones (Abrol & Kapil, 1994; Archer, 2012; Reddy et al., 2018; Sharma et al., 2018; Brock et al., 2021).

### 2.3 Mites:

#### 2.3.1 Varroa Mite (*Varroa destructor* / *Varroa jacobsoni*):

This mite is a native ectoparasite of *Apis cerana* across Asia and was first reported in Indonesia in 1904 (Anderson & Trueman, 2000; Rosenkranz et al., 2010). Since the introduction of *Apis mellifera* beekeeping in Asia, the mite has been responsible for significant damage in both temperate and tropical regions. *Varroa* infestation weakens honey bee colonies, leading to reduced honey production (Nazir et al., 2019). Presently, this parasite has spread worldwide, except Australia and New Zealand. In temperate Asia, most beekeepers agree that *Varroa* damage is a major constraint to successful *A. mellifera* beekeeping, while in tropical Asia, success is limited by the loss of *A. cerana* colonies through absconding—which is far less serious and frequent than damage to *A. mellifera*. *Varroa jacobsoni* multiplies faster on *Apis cerana*, whereas *Varroa destructor* is a more serious problem for *Apis mellifera* (Anderson & Trueman, 2000; Navajas et al., 2010).

- **Life History:** *Varroa destructor* is an external parasitic mite that primarily attacks the brood and adult bees of *Apis mellifera* (Rosenkranz et al., 2010). Its life cycle is closely linked with the developmental stages of honey bee brood and includes both **phoretic** (on adult bees) and **reproductive** (within capped brood cells) phases. The female *Varroa* mite attaches to adult bees, especially nurse bees, and feeds on the bee's hemolymph and fat body tissues. When a nurse bee enters a brood cell to feed the larva, the mite also enters the cell, preferably drone brood cells which offer a longer development period. Once the cell is capped, the mite begins its reproductive phase. Inside the sealed cell, the foundress mite lays her first egg about 60–70 hours after capping, which develops into a male, followed by successive female eggs at 30-hour intervals. The male and female offspring mature within the brood cell, and mating occurs before the adult bee emerges. When the young bee emerges, fertilized female mites exit the cell and attach to other adult bees, continuing the cycle. The developmental period of *Varroa* mite from egg to adult is approximately 6–7 days for males and 7–9 days for females (Rosenkranz et al., 2010). The lifespan of female mites varies; they can survive 2–3 months in summer and up to 5–6 months in winter, depending on environmental conditions and brood availability. Due to rapid reproduction and association with brood, *Varroa* mites produce multiple overlapping generations, causing heavy infestations in a short period and leading to colony weakening and eventual collapse if not managed properly (Nazzi & Le Conte, 2016; Giacobino et al., 2020).
- **Seasonal Incidence:** The seasonal incidence of *Varroa destructor* in *Apis mellifera* colonies varies according to climatic conditions, brood availability, and colony strength (Rosenkranz et al., 2010; Giacobino et al., 2015). The mite population shows a cyclic pattern throughout the year, increasing during periods of intense brood rearing and declining when brood production is minimal. During spring and early summer, when colony growth and brood rearing peak, the mite population begins to multiply rapidly. The abundance of capped brood cells provides favorable conditions for *Varroa* reproduction (Martin, 2001; Dietemann et al., 2020). Infestation continues to increase and usually reaches its maximum level during late summer to early autumn, coinciding with the highest brood density (Beaurepaire et al., 2015; Traynor et al., 2020). As temperatures decline in late autumn and winter, brood rearing is reduced or temporarily ceases, restricting mite reproduction. During this broodless period, mites remain on adult bees in the phoretic stage. Although population growth slows, mites survive on adult bees and resume reproduction once brood rearing starts again in spring. In tropical and subtropical regions where brood rearing continues year-round, *Varroa destructor* maintains continuous reproduction, resulting in overlapping generations and persistent infestation (Dietemann et al., 2020). In temperate regions, seasonal brood breaks naturally help reduce mite populations. Therefore, peak *Varroa* incidence is typically observed during late summer and early autumn, making this period critical for monitoring and implementing effective control measures (Rosenkranz et al., 2010; Traynor et al., 2020).
- **Symptoms:** Infestation of *Varroa destructor* causes several visible and behavioral symptoms in *Apis mellifera* colonies. Infested brood often fails to develop properly. Emerging adult bees may show deformed wings, shrunken abdomens, and crippled legs, commonly associated with Deformed Wing Virus (DWV) transmission (Francis et al., 2013). The brood comb appears scattered or patchy due to the removal of infested larvae and pupae by worker bees, indicating hygienic behavior in response to mite attack. Continuous infestation leads to reduced brood production, resulting in decreased adult bee population and colony weakening (Martin & Medina, 2004; Traynor et al., 2020).

- **Effective Management:** Effective management of *Varroa destructor* requires an integrated approach combining cultural, mechanical, biological, and chemical methods (Rosenkranz et al., 2010; Dietemann et al., 2020; Mondet et al., 2021). Periodic examination of brood and adult bees using methods such as the sugar roll test, alcohol wash, or drone brood uncapping helps in early detection and timely intervention before infestation reaches damaging levels. Introducing or breeding *Varroa*-tolerant or hygienic strains of *A. mellifera* that can detect and remove infested brood naturally helps reduce mite load. Treatments with formic acid, oxalic acid, thymol, and menthol are effective in controlling mites when used properly (Gregorc & Planinc, 2012). However, emerging acaricide resistance is a growing concern, necessitating rotation of active ingredients and integration of non-chemical methods.

### 2.3.2 Tracheal Mite (*Acarapis woodi*):

The tracheal mite (*Acarapis woodi*) is an endoparasite that infests the tracheal system of adult honey bees belonging to all three castes—workers, drones, and queens (Otis & Scott-Dupree, 2002; Sammataro et al., 2013). This mite was first reported in *Apis mellifera* colonies in Europe in 1921. In India, it was initially recorded in *Apis cerana* colonies in 1957 and later in *Apis mellifera* colonies in 1994. Presently, it has become a major pest causing serious problems to honey bee colonies, particularly in North America (Traynor et al., 2020).

- **Life History:** *Acarapis woodi* is an internal parasite that completes its entire life cycle within the tracheal system of adult honey bees. The female mite enters the thoracic tracheae of newly emerged bees through the spiracles. Once inside, she lays eggs along the inner walls of the tracheae, where developing mites feed on the host's hemolymph (Singh et al., 2015). Eggs hatch within 3–4 days, and immature stages (larvae and nymphs) develop over 11–15 days. The complete life cycle from egg to adult mite takes about 14–20 days, depending on temperature and humidity. Adult female mites live for about 30 days, during which they continue to reproduce within the tracheal system (Nazzi & Le Conte, 2016). Mating occurs inside the trachea, and mated females leave the host bee to infest young bees, ensuring continuation of infestation within the colony. Transmission primarily occurs through direct contact among bees, especially during clustering or grooming behavior. This internal parasitism weakens the respiratory system of bees, reduces flight ability, shortens lifespan, and ultimately leads to colony decline if infestations are severe (Singh et al., 2015; Traynor et al., 2020).
- **Seasonal Incidence:** The incidence of tracheal mite shows marked seasonal variation. Infestation levels are generally higher during winter and early spring, when bees form tight clusters and have close contact, favoring mite transmission. During this period, adult bee lifespan increases, providing more time for mite reproduction within the tracheae (Traynor et al., 2020; Mondet et al., 2021). As temperature rises in summer, infestation rate declines due to increased bee activity and reduced contact among individuals. Seasonal studies have shown that maximum infestation occurs from December to March, while minimum levels are recorded during May to August, depending on local climatic conditions (Sammataro & Avitabile, 2011).
- **Symptoms:** Infested bees often show disjointed wings or a "K-wing" condition and are seen crawling near the hive entrance, unable to fly (Van Engelsdorp & Meixner, 2010). Due to blockage of the tracheal system, affected bees become weak and lose flight ability. Infested worker bees die prematurely, leading to a noticeable reduction in the colony's adult population.
- **Effective Management:** Approved miticides like menthol crystals can be applied during warm weather when vapors circulate effectively in the hive. Menthol is usually placed above the brood chamber for several weeks (Otis & Scott-Dupree, 2002). Natural products such as thymol, wintergreen oil, and formic acid fumigation are effective in reducing mite populations without harming bees. Maintaining strong, healthy colonies with adequate food supply, proper ventilation, and timely replacement of old queens reduces the spread and impact of mites (Traynor et al., 2020).

### 2.4 Bee Lice:

The bee louse (*Braula coeca*) is a small, wingless fly found as an ectoparasite on adult honey bees. It measures about 1.5 mm in length and usually attaches to the head or thorax of worker bees and queens to feed on nectar or honey from the bee's mouthparts (Sammataro et al., 2000; Ellis & Munn, 2005). The insect does not suck blood, but its presence can cause irritation and interfere with normal feeding and grooming behavior. *Braula coeca* is widely distributed in Europe, Asia, Africa, and North America, and has also been reported from various parts of India, particularly in regions where *Apis mellifera* colonies are reared. Infestation is usually more noticeable in weak colonies or during honey flow seasons when food resources attract adult lice (Chauhan & Singh, 2000; Pernel et al., 2005). Heavy infestations can disturb the queen by clustering around her

head, reducing egg-laying efficiency and overall colony strength. Additionally, the larvae tunnel through wax capping of stored honey cells, which may lead to contamination and minor damage to combs. Although not a major pest, severe infestations can stress the colony and reduce honey quality and productivity (Ellis, 2016; Qaiser et al., 2021).

- **Symptoms:** Tiny, wingless insects (about 1.5 mm long) can be seen crawling on the head and thorax of worker bees and queens, often mistaken for mites. Bee lice frequently cluster around the queen's head, interfering with feeding and grooming, which leads to reduced egg-laying and colony weakening. The larvae of *Braula coeca* tunnel through the wax capping of stored honey cells, causing visible marks and contamination of combs or honey (Sammataro & Avitabile, 2011; Chauhan & Singh, 2020).
- **Effective Management:** Maintaining strong, healthy colonies is essential, as bee lice prefer weak or poorly managed hives. Regular inspection and proper sanitation aid in early detection and control. Heavily infested queens should be replaced, since bee lice often gather around the queen's head and reduce her egg-laying efficiency (Sharma et al., 2022). Fumigation with formic acid or menthol vapors during warm weather is effective in reducing adult louse populations without harming bees (Underwood & Currie, 2009; Chauhan & Singh, 2020).

## 2.5 Ants:

Ants are opportunistic insects that invade honey bee colonies to steal honey, pollen, and brood. Several species, including black ants, red ants, and carpenter ants, are commonly found attacking bee hives (Kumar et al., 2018; Sharma et al., 2021). They are highly attracted to the sweet smell of honey and wax and often build nests near or inside apiaries (Hepburn & Radloff, 2011). Ant infestation in beehives is a widespread problem reported from tropical and subtropical regions across Asia, Africa, Europe, and the Americas (Kiatoko et al., 2016; Qaiser et al., 2020). In India, ant attacks are common in warm and humid areas, especially during summer and rainy seasons (Kumar et al., 2019; Singh & Rathore, 2023). Ants cause stress to bees by constantly disturbing the colony, stealing food, and sometimes destroying brood combs (Ellis et al., 2010; Mutinelli, 2011). Their presence forces worker bees to spend more time defending the hive rather than foraging, which reduces colony strength and productivity. In severe cases, colonies may abscond from the hive due to continuous disturbance and food loss caused by heavy ant infestation (Kiatoko et al., 2016; Singh & Rathore, 2023).

- **Symptoms:** Large numbers of ants are seen crawling on the hive stand, cover, and combs, especially near honey stores (Hepburn & Radloff, 2011; Kumar et al., 2017). Worker bees become agitated and defensive, spending more time guarding the hive instead of foraging. Due to honey and brood theft, bees may abandon the hive (absconding) or show a noticeable decline in population and productivity (Kumar et al., 2019; Qaiser et al., 2020).
- **Effective Management:** Placing hive stands in ant-proof containers filled with oil, water, or kerosene prevents ants from climbing into the hive (Ellis et al., 2010; Kumar et al., 2018; Singh & Rathore, 2023). Keeping the apiary clean and free from food waste or spilled honey, and regularly removing nearby ant nests or trails, are essential (Sharma et al., 2021). Application of grease bands, ash, or chalk lines around hive stands acts as repellents (Kumar et al., 2019).

## 2.6 Hive Beetles:

The small hive beetle (*Aethina tumida*) is a destructive pest of honey bee colonies, belonging to the family Nitidulidae. Adult beetles are dark brown to black, about 5–7 mm long, and usually found hiding in hive corners, under frames, or in brood combs (Ellis & Munn, 2005; Neumann & Ellis, 2008). The larvae feed on pollen, honey, and brood, causing serious damage to combs and stored honey (Hood, 2011; Mutinelli et al., 2014). This pest is native to sub-Saharan Africa but has now spread to North America, Australia, Europe, and parts of Asia, including recent reports from India (Kumaranag et al., 2025). Infestation is most severe in warm and humid climates, which favor beetle reproduction and survival. Hive beetles cause fermentation and spoilage of honey, leaving a slimy residue with a foul odor. Their tunneling through combs destroys wax and brood, leading to colony weakening. In severe infestations, adult bees may abandon the hive due to continuous disturbance and contamination of food stores (Ellis et al., 2010; Cappa et al., 2022).

- **Symptoms:** Fermentation of honey by beetle larvae produces a slimy residue and a strong, rotten smell inside the hive (Ellis & Munn, 2005; Neumann & Ellis, 2008; Hood, 2011). Beetle larvae tunnel through combs, destroying brood cells, pollen, and stored honey, leaving visible trails and debris. Continuous movement and feeding of beetles irritate bees, causing stress and sometimes leading to absconding of the entire colony (Schafer et al., 2020; Cappa et al., 2022; Kumar et al., 2021).

- **Effective Management:** Maintaining healthy, populous colonies is critical, as strong bee populations can effectively chase and confine beetles, preventing heavy infestation. Regular cleaning of hive floors, removal of debris, and discarding of old combs reduce breeding sites for beetles and larvae (Hood, 2011; Schafer et al., 2020). Installation of beetle traps or oil-based traps inside hives, and application of soil treatments (such as diatomaceous earth) around the apiary to kill pupating larvae in the ground, are effective control measures (Neumann et al., 2016; Kumar et al., 2021; Cappa et al., 2022)

### III. VERTEBRATE PREDATORS

#### 3.1 Birds:

Birds are considered minor natural enemies of honey bees, as several insectivorous species prey on foraging bees during flight or near the hive entrance (Abrol, 2012; Sharma & Kumar, 2017). Common bee predators include bee-eaters (*Merops orientalis*), drongos (*Dicrurus macrocercus*), and kingbirds, which catch bees in the air (Bista et al., 2020; Fichtel et al., 2016). These birds often target flying worker bees, especially during active foraging periods in the morning and evening (Ali et al., 2018; Kumar et al., 2021). According to various studies, bird predation on honey bees has been reported from Asia, Africa, and southern parts of Europe, particularly in India where the green bee-eater is a frequent pest in apiaries (Kumar et al., 2022; Gupta et al., 2024). Their activity increases during spring and summer months, coinciding with high bee activity. Heavy predation can lead to a reduction in foraging population, causing a decline in nectar and pollen collection, and ultimately lowering honey yield. Continuous attacks may also disturb colony behavior, forcing bees to remain inside the hive and reducing overall productivity (Sharma et al., 2017; Bista et al., 2020).

- **Symptoms:** A noticeable decline in the number of worker bees leaving the hive is observed, as birds catch bees during flight and near the entrance (Abrol, 2012; Singh & Rana, 2015). Colonies become highly agitated and defensive, with bees showing reluctance to forage or remain outside due to continuous bird attacks (Bista et al., 2020). Continuous predation reduces the forager population, leading to lower nectar and pollen collection and ultimately decreased honey production (Ali et al., 2018; Kumar et al., 2021; Gupta et al., 2024).
- **Effective Management:** Installation of reflective tapes, scarecrows, or sound-emitting devices near the apiary can frighten away bee-eating birds and reduce their visits (Abrol & Kapil, 2019). Apiaries should be established away from bird nesting or roosting areas, such as tall trees, power lines, or open fields commonly used by bee-eaters and drongos (Singh & Rana, 2015; Bista et al., 2020). Protective nets or wire screens around the apiary during peak bird activity periods can prevent birds from catching bees in flight near hive entrances (Kumar et al., 2021; Gupta et al., 2024).

#### 3.2 Bears:

Bears are recognized as major vertebrate predators of honey bee colonies, primarily attracted by honey, brood, and pollen (Abrol, 2012; Raj & Rana, 2016; Mishra et al., 2021). Species such as the Asiatic black bear (*Ursus thibetanus*), sloth bear (*Melursus ursinus*), and American black bear (*Ursus americanus*) are known to raid beehives. These attacks usually occur at night when colonies are less active (Kumar et al., 2019). According to various reports, bear damage to apiaries is reported from Asia, Europe, and North America, particularly in hilly and forested regions like the Himalayan foothills, Himachal Pradesh, Uttarakhand, and North-Eastern India (Singh & Thakur, 2017). Bears use their strong claws to tear apart hives, destroying wooden boxes and combs while consuming honey and larvae. Such attacks cause complete destruction of colonies, resulting in loss of bees, brood, and hive materials (Abrol, 2012; Sharma et al., 2023). Frequent raids lead to economic losses, disturbance of apiary sites, and sometimes migration or absconding of surviving colonies due to continuous stress and habitat disruption (Mishra et al., 2021; Kumari et al., 2024).

- **Symptoms:** Wooden boxes and combs are broken, scattered, or overturned, showing clear signs of claw or bite marks from bear attacks (Joshi et al., 2018; Raj & Rana, 2016). Colonies experience complete destruction of combs, resulting in loss of adult bees, brood, and stored honey (Kumar et al., 2019; Sharma et al., 2023). Surviving bees become highly disturbed and aggressive, and in many cases, abandon the hive (abscond) due to repeated bear raids and habitat disturbance (Mishra et al., 2021; Kumari et al., 2024).
- **Effective Management:** Installation of strong fencing, preferably electric fences, around the apiary is effective in preventing bears from entering and damaging hives (Sathyakumar & Bashir, 2020). Apiaries should be established away from forest edges or known bear habitats, preferably in open, monitored areas to reduce attack risk (Raj & Rana,

2016). Night-time surveillance and use of light, noise, or scent repellents (such as burning kerosene or chemical deterrents) can scare away bears from approaching the apiary (Mishra et al., 2021; Kumari et al., 2024).

### 3.3 Other Vertebrate Pests:

In addition to birds and bears, other vertebrate pests such as **mice, rats, skunks, and lizards** also cause damage to honey bee colonies. Mice and rats often enter hives during winter, building nests and chewing combs, wax, and wooden parts. Skunks scratch at hive entrances at night to capture bees as they emerge, leading to colony disturbance and reduced populations. Lizards and frogs occasionally prey on bees near hive entrances, though their impact is generally minimal. Management of these pests includes hive stand elevation, entrance reducers, trapping, and exclusion fencing (Morse, 1999; Abrol, 2012).

## IV. MINOR ENEMIES

Several minor enemies cause occasional or localized nuisance to honey bee colonies. These include **cockroaches**, which seek shelter and food in hives, contaminating combs; **robber flies** and **dragonflies**, which prey on foraging bees in flight; **praying mantis**, which capture bees visiting flowers; and **spiders**, which build webs near hive entrances to trap bees (Thakur & Sharma, 1984). While individually these enemies cause limited damage, cumulative stress from multiple minor pests can contribute to colony weakening, particularly when colonies are already under pressure from major pests, diseases, or nutritional stress. Management typically involves good apiary hygiene, removal of harborage, and physical barriers.

## V. INTEGRATED MANAGEMENT OF HONEY BEE ENEMIES

Effective management of honey bee enemies requires an **integrated approach** that combines multiple strategies rather than reliance on any single method. Key principles of integrated pest management (IPM) for honey bee colonies include:

1. **Monitoring and Early Detection:** Regular inspection of colonies for signs of pest infestation using standardized sampling methods (e.g., sugar roll for *Varroa*, visual inspection for wax moths, wasp trapping).
2. **Cultural Practices:** Maintaining strong, healthy colonies through proper nutrition, disease management, and queen replacement; ensuring adequate colony strength is the single most effective defense against most pests.
3. **Sanitation:** Regular cleaning of hive floors, removal of old combs, proper storage of empty equipment, and disposal of debris reduce breeding sites for wax moths, hive beetles, and other pests.
4. **Physical and Mechanical Controls:** Use of screened bottom boards, drone brood removal, freezing of combs, hive stands with ant-proof barriers, electric fencing, and bird netting.
5. **Biological Controls:** Promotion of hygienic bee strains, use of entomopathogenic nematodes for hive beetle larvae, and conservation of natural enemies of pests where applicable.
6. **Chemical Controls:** Judicious use of approved acaricides, fumigants, and repellents, with strict adherence to dosage, timing, and withdrawal periods to minimize residues in hive products and mitigate resistance development.
7. **Beekeeper Training and Extension:** Capacity building of beekeepers in pest identification, monitoring, and management through demonstrations, training programs, and extension literature.

## VI. KNOWLEDGE GAPS AND FUTURE RESEARCH NEEDS

Despite substantial research on honey bee enemies, several knowledge gaps remain:

1. **Impact of Climate Change:** How shifting temperature and rainfall patterns will alter the distribution, phenology, and population dynamics of major pests like *Varroa* mites, wax moths, and wasps.
2. **Native vs. Invasive Pests:** Comparative studies on the impact of invasive species (e.g., *Vespa velutina*, *Aethina tumida*) versus native pests in different agro-ecological regions of India.
3. **Resistance Management:** Surveillance of acaricide resistance in *Varroa* populations and development of resistance management strategies.
4. **Non-Chemical Controls:** Validation and refinement of biotechnical and biological control methods under tropical field conditions.

5. **Economic Thresholds:** Establishment of region-specific economic injury levels for major pests to guide treatment decisions.
6. **Interaction Effects:** Understanding synergistic interactions among pests, pathogens, pesticides, and nutritional stress in driving colony losses.
7. **Indigenous Knowledge:** Documentation and validation of traditional beekeeping practices for pest management.



*Galleria mellonella*  
(Greater wax moth)



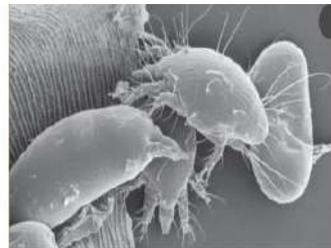
*Achroia grisella*  
(Lesser wax moth)



*Vespa germanica*  
(Wasp)



*Varroa destructor*  
(Varroa mite)



*Acarapis woodi*  
(Tracheal mite)



*Braula coeca*  
(Bee lice)



*Aethina tumida*  
(Hive beetle)



*Ursus thibetanus*  
(Bear)

**FIGURE 1: Enemies of honey bees**

## VII. SUMMARY AND CONCLUSION

Honey bee colonies are affected by a wide range of enemies, including moths, wasps, mites, bee lice, ants, hive beetles, birds, bears, and other vertebrate and invertebrate pests. Each poses distinct threats to colony health and productivity, causing damage by destroying combs, feeding on brood or honey, weakening adult bees, and in severe cases, leading to absconding or colony collapse. The severity of infestation varies with season, climate, and management practices adopted by beekeepers.

Among arthropod pests, **Varroa destructor** remains the most serious global threat due to its direct damage and role as a vector of lethal viruses. **Wax moths** cause significant economic losses, particularly to weak colonies and stored combs. **Wasps**, especially *Vespa* species, are major predators in many regions, capable of decimating entire apiaries. **Small hive beetles** are an emerging invasive threat in India requiring vigilant monitoring. **Vertebrate predators** such as bears and birds cause localized but severe damage.

Effective management of these enemies relies on an **integrated approach** combining good apiary hygiene, strong colony maintenance, regular monitoring, and timely preventive measures. The use of biological, physical, and cultural control methods, along with judicious application of approved chemicals, helps minimize pest impact while preserving bee health and product quality. Proper site selection, beekeeper awareness, and region-specific strategies are crucial for early detection and effective control.

This review highlights that while substantial knowledge exists on individual pests, there is a need for **synthesis, contextualization to Indian conditions, and translation into accessible extension materials** for beekeepers. Future research should prioritize climate change impacts, resistance management, non-chemical controls, and economic thresholds. Ultimately, sustainable management of honey bee enemies requires continuous observation, scientific intervention, and collaborative efforts among researchers, extension agencies, and beekeeping communities to ensure healthy colonies, improved pollination services, and enhanced honey production.

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#### CONFLICT OF INTEREST

The authors declare no conflict of interest.

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# Technology Investment in Smart and Sustainable Agriculture towards Food Security

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**Abstract**— *Internet of Things (IoT) has emerged as a transformative force across multiple sectors of the agriculture industry, enhancing both quality and quantity of agricultural yield. Artificial intelligence, integrated with IoT, encompasses soil preparation, cultivation, harvesting, and research-related activities, leading to sustainable productivity improvements. Agriculture automation has enhanced precision in farming operations including irrigation control, pesticide/weedicide/fertilizer management, crop growth monitoring, and environmental control in greenhouse and hydroponics systems. This chapter reviews IoT applications in crop farming, animal farming, farm monitoring and tracking, disease detection in plants and livestock, classification processes of agricultural foods, quality assessment of vegetables and fruits, and rearing activities. Climate-smart agriculture is examined and compared with traditional forms (Agriculture 1.0) regarding efficiency and waste reduction. The chapter discusses benefits, limitations, future directions, and potential development of intelligent farming technologies and IoT (AI-enhanced tools) to make farming more accessible, convenient, and precise, with reference to different countries and their agricultural advancements. Finally, the chapter acknowledges technological limitations and other factors affecting the growth of healthy farming systems. This chapter contributes to understanding AI-enabled IoT in transforming contemporary agriculture through data-driven insights and automation capabilities.*

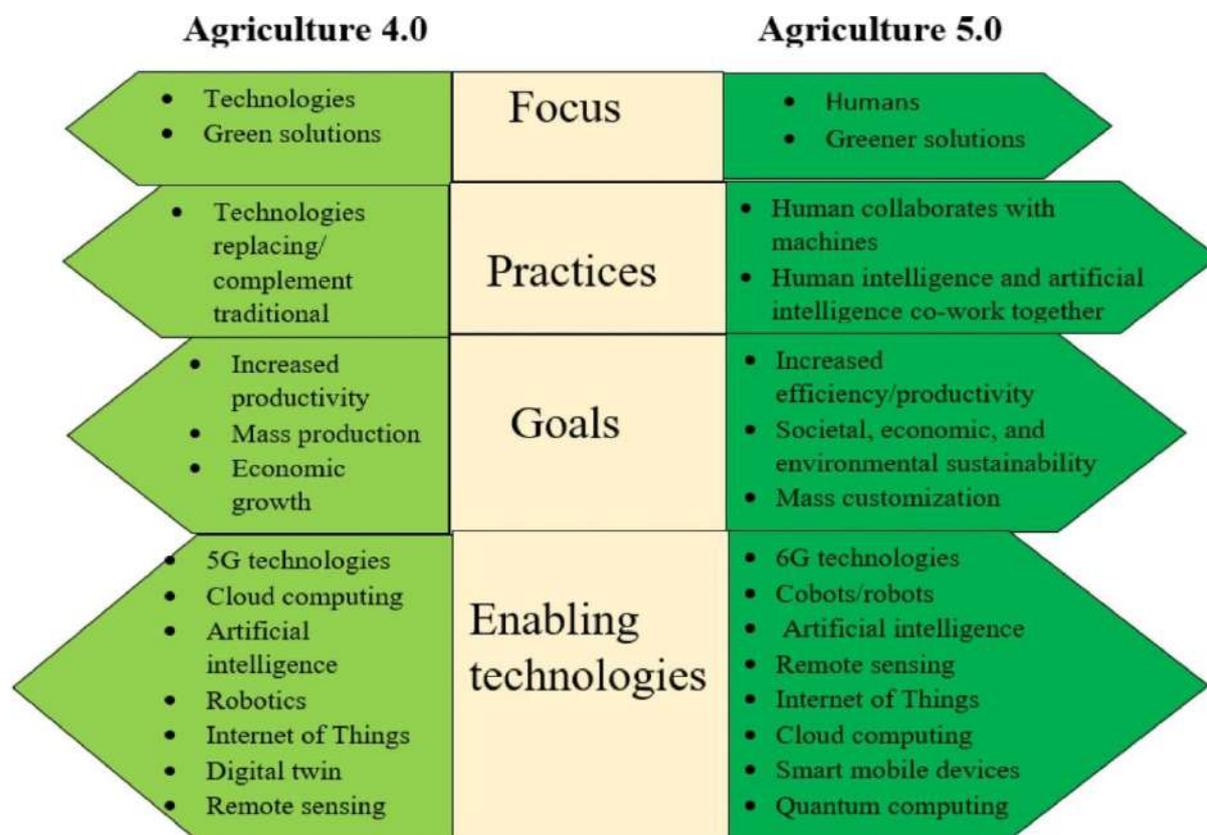
**Keywords**— *Climate-smart farming, Artificial intelligence, Internet of Things, Automation, Sustainability, Food security, Carbon sequestration, Satellite imagery, Sensors.*

## I. INTRODUCTION

Zero hunger and good health and well-being are Sustainable Development Goals (SDGs) that can be secured through continuous food production and accessibility. Agriculture is the primary and oldest industry in the world. In India, 18% of gross domestic product comes from agriculture, of which approximately 57% originates from rural areas (Reddy and Dutta, 2018). Advancement in agricultural technology with socio-ecological sustainability is depicted across distinct eras: Agriculture 1.0 (pre-industrial era; 1784-1870), Agriculture 2.0 (era of industrial revolution; use of mechanized tools; 1950-1992), Agriculture 3.0 (automation; 1992-2017), Agriculture 4.0 (smart farming; current decade), and Agriculture 5.0 (era of Internet of Things) (Holzinger et al., 2024; Thilakarathne et al., 2025).

The advent of Internet of Things (IoT) based technologies has transformed every industry including agriculture, automating control and monitoring of most aspects of traditional farming. Climate change and greenhouse gas (GHG) emissions are significant challenges in agriculture. Climate-smart agriculture offers a way to address both challenges simultaneously, ensuring adequate food production while protecting the environment. In essence, climate-smart farming aims to make agriculture more sustainable and successfully adaptable to changing climate and other stressors. Public and private authorities are increasingly investing in AI-based initiatives to incentivize traditional agriculture practices through smart technologies and tools.

Climate-smart farming (Agriculture 5.0) enables out-of-season and out-of-region crop yield improvement through greenhouse farming, hydroponics, and livestock yield monitoring and tracking practices (Rubio and Más, 2020). Real-time cloud-enabled smart data sensors (IoT) provide systematic solutions for precision agriculture in food-insecure areas threatened by floods, low rainfall, disease, and pests, which reduce agricultural yield qualitatively and quantitatively (Thilakarathne et al., 2025; Dhanaraju et al., 2022) (Fig. 1).



**FIGURE 1: Comparison of Latest Advancements in Technologies Agriculture 4.0 vs Agriculture 5.0 (IoT)**

IoT technologies can automate tasks and reallocate resources in optimized ways to enhance efficiency and productivity. Precision farming techniques help reduce waste by ensuring optimized resource allocation (Khang et al., 2024). Real-time digital technologies like mobile robotics have enabled better resource management including energy, labor, irrigation, fertilizers, and weedicides/pesticides, while avoiding resource wastage (Alwi and Manocha, 2024). Access to information and technology empowers farmers to integrate technology in open fields as well as kitchen farming, thereby improving their livelihoods. Climate-smart farming has already been adopted in several countries. EU-funded initiatives like the IoF2020 (Internet of Food and Farm 2020) project have demonstrated the potential of IoT in agriculture and developed innovative solutions (Rizan and Siva, 2024; Balasundram et al., 2024; Ragazou et al., 2022).

Companies like CropX specialize in using soil sensors and cloud-based analytics to provide farmers with actionable insights. Israel has a thriving agtech startup scene, with companies like Prospera developing AI-powered platforms that analyze data from IoT devices to optimize crop management. Farm management software that uses IoT to track machinery and worker activity is also a significant part of technological advancement to maximize efficiency and sustainability. AI applications in climate-smart farming utilize machine learning, deep learning, and data analytics to analyze vast amounts of ecological data obtained from satellites, drones, and ground sensors. Precision agriculture techniques such as geostatistics, Global Positioning System (GPS), geographical information system (GIS) remote sensing, and yield monitors have expanded the scope for monitoring and mapping crop phenology parameters and yield (Shanwad et al., 2002).

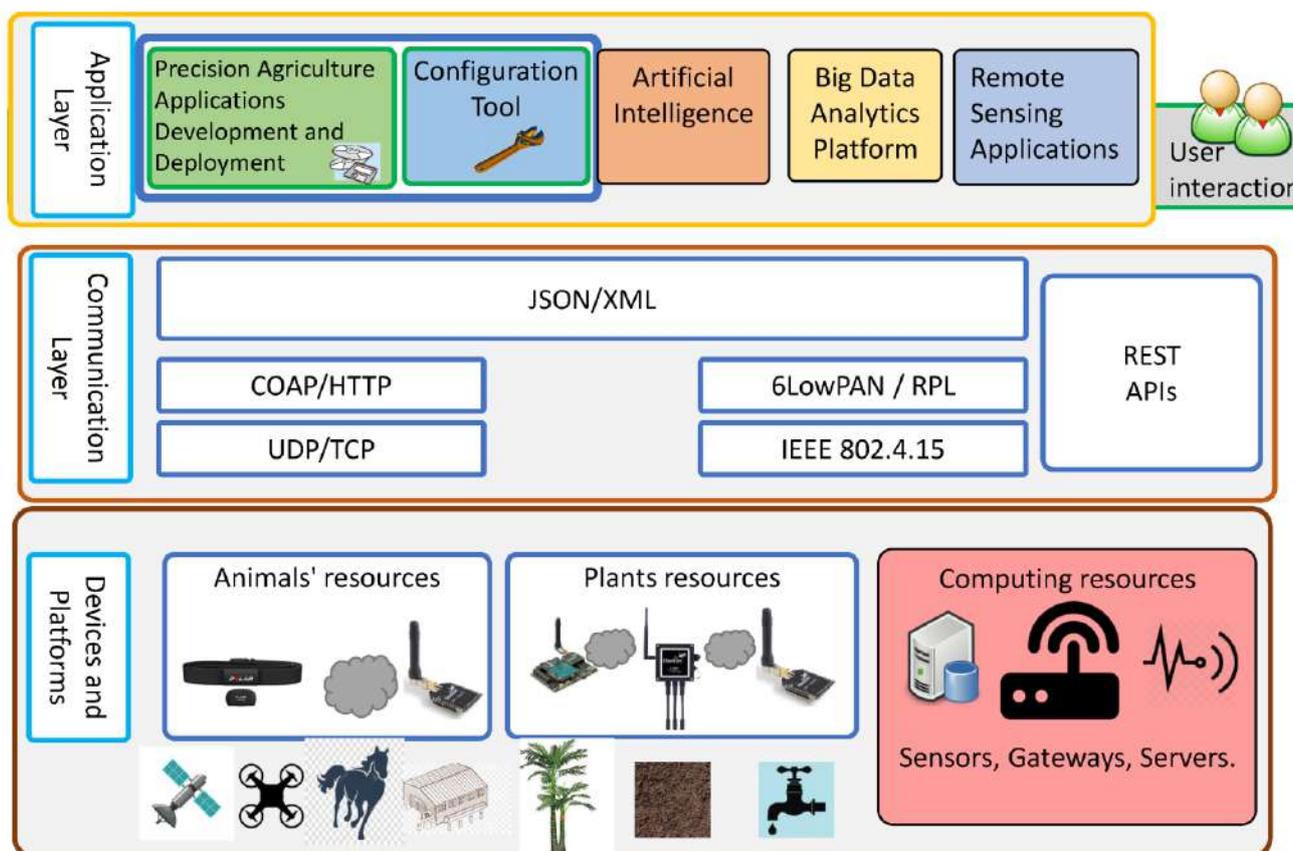
The use of intelligent air-ground UAVs/UGVs (unmanned aerial/ground vehicles) or drones allows for real-time mapping of biomass and yield, eradication of pests and weeds, and irrigation scheduling of farms. Materially engineered map-based artificial pollinators and pesticide and weedicide sprayers come with controlled spray nozzles (Raparelli and Bajocco, 2019). AI-driven UAV remote sensing, high-resolution visible, multispectral and thermal image cameras, and robotics can assess

crop health and identify changes over time with higher accuracy than traditional remote sensing methods (Barasa et al., 2021; Miller et al., 2021; Tyagi and Tiwari, 2025).

However, smart/digital farming is often linked to technologies like genetic engineering, biotechnology, and artificial fertilizers, which carry potential for high cost, high skill requirements, and uncertainties due to delayed benefits. For example, developing early warning systems for extreme weather (drought, flood, hail) and for pest and disease occurrence presents challenges to potentially climate-smart farming (Mizik, 2021).

Internet of Things devices (sensors or actuators) are digital technologies that can be remotely controlled and connected to each other via the Internet. Monitoring, tracking, controlling, and prediction become more automated, lessening human interventions, costs, power consumption, and water and other resource consumption. Other significant contributions of AI-based IoT include image recognition and bioacoustics analysis, which facilitate species identification and resource distribution, behavior, and movement detection in farms (Brown et al., 2023; Thompson and Lee, 2022). AI models and IoT also help in mapping vegetation fraction, alien-invasive species distribution in fields, and water/nutrient stress monitoring, aiding in the development of proactive conservation measures for stress-resistant crop species (Anderson et al., 2020; Carter and Nguyen, 2023; Madushanki, 2019).

Robotic technology plays an increasingly important role in thinking, perception, decision-making, and multitasking capabilities, enabling farmers to undertake repetitive, tedious, and dangerous agricultural tasks (Adamides, 2020; Elijah et al., 2018). Technological advancement 5.0 (Internet of Things) integrates farming with industry in a symbiotic way, holding immense potential to enhance quality and quantity of agri-food such as plant and animal products (edible or non-edible), including meat, eggs, milk, honey, silk, wool, leather, and other products, in a sustainable manner (Wolfert and Isakhanyan, 2022; Williams et al., 2024) (Fig. 2).



**FIGURE 2: Representation of Three Main Layers of Developing Internet of Things (IoT) and IoT-Enabled Strategies and Methodologies Currently Employed in Precision Agriculture**

This chapter aims to explore and analyze applications of Internet of Things in farming, discuss challenges associated with its implementation, and propose future directions for integrating AI with emerging technologies. Throughout the chapter,

emphasis is placed on how AI can contribute to sustainable farming management and inform policymakers, researchers, and farmers about the opportunities and limitations of AI-driven approaches.

## II. AI TECHNOLOGIES IN FARMING MANAGEMENT

Artificial intelligence (AI)-driven technologies have dramatically transformed real-time management of crop production, soil improvement, pest/weed control, and environmental management (Eli-Chukwu, 2019). Remote sensing, satellite imagery, and unmanned aerial vehicles (UAVs) equipped with AI algorithms represent some of these advancements (Kurz et al., 2022). By analyzing factors such as temperature, humidity, and wind speed, AI provides early warning systems that help mitigate risks. Furthermore, deep learning AI models play a role in monitoring farm health by detecting diseases and pest infestations. For example, convolutional neural networks (CNNs) have been used to detect beetle infestations (Kumar et al., 2022; Sharma, 2021).

Another emerging application is the use of AI for carbon sequestration modeling and climate change adaptation. AI-enhanced models analyze biomass and carbon fluxes from farms, providing accurate estimates of carbon storage potential. Such insights contribute to climate change mitigation strategies (Tang et al., 2022). The integration of AI with geographic information systems (GIS) and Internet of Things (IoT) sensors enables soil moisture detection, automated irrigation, weeding techniques, spraying pesticides and herbicides using drones, and harvesting and yield mapping (Talaviya et al., 2020). Such predictive capabilities are essential for reducing economic losses. For instance, deep learning models process multispectral and hyperspectral data from remote sensing cameras designed for crop monitoring (Kattenborn et al., 2021).

### 2.1 Machine Learning and Predictive Analytics in Farming:

Machine learning (ML) and predictive analytics have applications in crop, water, and soil management, along with appropriate management of feeding, reproduction, health, milking, and other resources. Integrating varied and multi-component data related to different parameters such as climate, topography, and soil properties with agricultural land use and crop patterns improves decision-making (GmbH, 2021). Furthermore, integrating advanced technologies like the Internet of Things, remote sensing, and ML algorithms such as decision trees, support vector machines (SVMs), multilayer perceptron (MLP) neural networks, random forests, and extreme gradient boosting (XGBoost) to classify images captured by unmanned aerial vehicles (UAVs) has transformed the agriculture sector (Botero-Valencia et al., 2025).

Recent applications of ML, deep learning (DL), and predictive analytics include:

- **Yield and fertilizer prediction** based on machine learning algorithms, advancing precision agriculture with improved productivity using data mining techniques
- **Deep learning for image-based weed detection**
- **Intelligent crop selection method** based on various environmental factors using machine learning
- **Predicting the effects of climate change on crop yields**
- **Renewable energy predictions** using artificial neural networks (ANN) with stochastic optimization for renewable energy systems
- **Image-based deep neural networks in plant disease detection** using machine learning and remote sensing, for example, recognition of plant diseases by leaf image
- **Phenotypic classification**

### 2.2 Remote Sensing and AI-Based Image Analysis:

AI-powered multi-temporal Unmanned Aerial System (UAS) data enables crop height monitoring, weed mapping with digital images and automatic spraying decisions, high-throughput phenotyping of crops to accelerate crop breeding, monitoring of disease in the field, estimation of site-specific vegetation monitoring and management using RGB and multispectral images from UAV, and high-resolution UAV-based thermal imaging to estimate instantaneous and seasonal variability of plant water status and yield mapping (Gupta, 2025; Jinha et al., 2021).

Integration of high-resolution remotely sensed data and machine learning techniques enables spatial prediction of soil properties on different terrains and prediction of carbon, nitrogen, and ultimately yield. Precision techniques enhance nutritive value of crops, addressing both qualitative and quantitative parameters. Additional applications include automatic

detection of insect pests, multi-modal sliding window-based support vector regression for predicting biotic and abiotic stress in plants, and automatic fruit defect identification.

### **2.3 Internet of Things (IoT) and AI Integration:**

The future of digital technologies in sustainable farming management systems requires high-level policy frameworks integrating the supply chain with internet platforms for safe and healthy food from farm to fork. Precision agriculture techniques such as remote sensing and GPS optimize resource allocation and improve productivity by collecting data on soil conditions, temperature, nutrient levels, weather patterns, and crop health. This leads to reduced water for irrigation and minimized pesticide and fertilizer usage, reducing environmental impact and lowering costs. Crop monitoring through drones and satellite imagery combined with IoT sensors can monitor crop health, detecting diseases or pests early and allowing timely interventions to minimize crop damage.

AI models and algorithms have the capability to transform agriculture through instantaneous tracking, controlling, and monitoring of direct agricultural activities (allied) and indirect activities (non-allied) such as storage, warehousing, cold storage, transportation, and marketing. Through integration of IoT sensors, artificial intelligence algorithms analyze various environmental characteristics such as temperature, humidity, and gas concentrations (Pandey and Mishra, 2024). Intelligent drones facilitate exhaustive monitoring of fields and farm land topology. Automated controlled sensor-based spraying UAVs enable precise spraying that enhances productivity, reduces chemical waste, improves crop health, and promotes sustainability. Artificial intelligence, ML algorithms, deep learning models, and microprocessors analyze incoming data (Taseer et al., 2024; Rajak et al., 2023).

Application of artificial intelligence in electrical automation in mechanized agriculture is substantial (Rajak et al., 2023; Stephan et al., 2025). Examples include planting, precise spraying using unmanned aerial spraying systems, automated plant leaf disease detection and classification using optimal MobileNet-based convolutional neural networks, and weed classification based on Haar wavelet transform via k-nearest neighbor (k-NN) for real-time automatic sprayer control systems. A CNN-RNN framework for crop yield prediction enables autonomous sensor networks for rural agriculture environments at low cost with energy self-charging capabilities. Crop classification based on temporal signatures of Sentinel-1 observations represents another application. Recent advances in hyperspectral imaging technology have expanded agricultural applications. IoT devices ensure traceability and quality control of harvested crops from fields through processing throughout the supply chain, minimizing waste and ensuring product safety and quality. Data analysis and machine learning algorithms can predict seed germination rates, estimate seed vigor, identify trends and patterns, and optimize resource management.

#### **2.3.1 Smart Irrigation System:**

AI-driven use of crop-water stress maps and IoT-based soil moisture sensors optimizes water requirements throughout the season. These devices are enabled with visible and thermal UAV camera imagery. Such systems prevent overwatering or drought stress, ensuring efficient plant growth (Boutagayout et al., 2025; Singh et al., 2021). Development and assessment of field-programmable gate array (FPGA)-based image processing (FIP) systems are useful for measuring moisture content, humidity, and transpiration rate of plants (Rajak et al., 2023).

#### **2.3.2 Real-Time Farm Monitoring and Environmental Sensing:**

Geospatial analysis and Internet of Things in environmental monitoring help prevent crop damage due to extreme weather conditions or water shortages. Adapting and building resilience helps farmers cope with climate change impacts such as droughts, floods, and extreme weather events. Sensors deployed in seed storage and growing environments monitor crucial factors including temperature (vital for preventing seed damage and maintaining viability), humidity (excess moisture can lead to mold and decay), light levels (important for germination and early growth), and air quality (detecting harmful gases or pollutants).

IoT-based environmental sensors deployed in farms collect real-time data on temperature, humidity, soil moisture, air quality, and carbon levels. AI processes this data to detect anomalies such as drought stress, pollution, or illegal activities. Drones and satellite imagery enhance monitoring by providing high-resolution visuals of farm cover changes, wildlife movement, and deforestation patterns. In wildfire prevention, AI-powered sensors detect early signs of fire such as temperature spikes and smoke, triggering immediate alerts for rapid response. Similarly, acoustic sensors identify chainsaw

noises or gunshots, helping prevent illegal logging and poaching. For ecosystem management, real-time data from IoT devices support afforestation efforts by optimizing irrigation and tree planting strategies.

### 2.3.3 Internet of Things in Crop Yield Maximization:

High-definition 3D cameras and imaging sensors capture detailed images of seeds. Artificial intelligence algorithms analyze these images to assess seed size, shape, and color, detect defects or damage, and identify seed varieties. Specialized sensors can analyze the internal composition of seeds: near-infrared spectroscopy (NIRS) can determine moisture content, protein levels, and other chemical properties; electrical conductivity sensors can assess seed viability. Development of portable grain mass flow sensor test rigs and point-of-care diagnostic devices for pathogen detection has advanced significantly.

Low-altitude remote sensing, battery-free wireless smart sensors with Bluetooth connectivity measure soil mechanical resistance, moisture level, pH (soil acidity or alkalinity indicator), nutrient levels (N-P-K), and track soil temperature which affects seed germination and root development. Chemical constituents can be estimated using high-resolution hyperspectral imagery. Automated tilling by robotic systems can adjust tilling depth based on soil density. Co-location opportunities for large solar infrastructures and agriculture in drylands present new possibilities. IoT multiplatform networking with wireless sensors monitors and controls wineries and vineyards. Greenhouse wireless sensor network monitoring system design based on solar energy has been developed. Battery-free radio frequency identification (RFID) sensors ensure food quality and safety. Chipless RFID tags with integrated sensors, such as fully inkjet-printed chipless RFID gas and temperature sensors on paper and humidity passive sensors based on UHF RFID using cork dielectric slabs, represent emerging technologies.

Flexible and shape-morphing plant sensors designed for microenvironment temperature monitoring of irregular surfaces have been developed. Stretchable-polymer-electronics-based autonomous remote strain sensors (SPEARs) enable continuous, precise, autonomous plant growth monitoring of delicate grass leaves throughout the tracking period (Wang et al., 2024).

### 2.3.4 AI in Fruiting Stage Detection and Harvesting:

Sound absorption for ripeness and unripe classification of fruits optimizes harvest timing, providing farmers with real-time information on crop maturity levels and factors like moisture content, sugar levels, and ripeness (Rajak et al., 2023). This allows farmers to determine optimal harvest time, ensuring maximum yield and quality. IoT-enabled robots and autonomous vehicles can be programmed to perform harvesting tasks with precision and efficiency. These systems can navigate fields, identify ripe crops, and harvest them automatically, reducing labor costs and minimizing crop damage. Sensors mounted on harvesting equipment create yield maps, providing valuable insights into crop performance and helping farmers optimize future planting and harvesting strategies.

### 2.3.5 AI-Based IoT in Pest and Disease Monitoring:

Toxicological evaluation of insecticides, pesticides, or weedicides requires careful consideration. Agricultural pesticides are both beneficial and harmful to the biosphere. Agricultural pest and disease monitoring based on Internet of Things and unmanned aerial vehicles has advanced significantly. Automated applications of acoustic wireless sensors enable stored product insect detection, pest monitoring, and control. Deep learning and computer vision are transforming entomology. Automatic traps for moth detection in integrated pest management, remote monitoring of European Grapevine Moth (*Lobesia botrana*) populations using camera-based pheromone traps in vineyards, electronic traps for detection and population monitoring of adult fruit flies, and web-based automatic traps for early detection of alien wood-boring beetles have been developed. Artificial neural network-based pest identification and control in smart agriculture using wireless sensor networks, electronic pest repellents, and smart plant-wearable biosensors for in-situ pesticide analysis represent ongoing innovations.

### 2.3.6 Smart Drones for Farm Surveillance:

Agriculture is labor- and cost-intensive with long waiting periods for returns. Agricultural security challenged by human and animal intrusion is therefore a priority. Cloud computing and machine/deep learning-based smart farm surveillance systems (SFSS) reduce reliance on manual field inspections, saving time and resources. PIR sensors, ultrasonic detectors, and deep learning models enable animal detection with real-time monitoring, data acquisition, and precise analysis for conservation and security. UAVs/UGVs surveillance drones equipped with high-resolution cameras, thermal sensors, and LiDAR technology capture detailed images and maps. Alarm systems, motors, SMS alerts, high-resolution images/videos, and 3D topographical data represent latest advancements for detecting illegal activities, wildlife tracking, assessing farm health, and responding to environmental threats (Delwar et al., 2025). Thermal imaging assists in locating wildfires even in dense

vegetation, enabling rapid response and mitigation efforts. For wildlife monitoring, drones track animal movement, population trends, and migration patterns without disturbing ecosystems.

### 2.3.7 Carbon Sequestration and Climate Adaptation:

Farming tactics to reduce carbon footprint in semi-arid areas include agroforestry, which integrates trees and shrubs into agricultural systems to improve soil health, sequester carbon, and provide additional income sources. Carbon farming helps soil capture and hold more carbon by reducing greenhouse gas emissions wherever possible. Conservation agriculture minimizes soil disturbance (no-till farming), maintains permanent soil cover (using crop residues or cover crops), and practices crop rotations.

The effects of crystal structure on photovoltaic performance of perovskite solar cells under ambient indoor illumination in greenhouse farming have been studied. Model-based evaluation of greenhouse microclimate using IoT-sensor data fusion enables energy-efficient crop production. Cloud-based IoT platforms enable precision control of soilless greenhouse cultivation. IoT sensors track carbon fluxes, plant growth rates, and soil health to assess carbon sequestration potential. AI models analyze these data points to estimate carbon storage capacities, predict climate change impacts, and optimize planting strategies (Tang et al., 2022). These insights contribute to global climate mitigation efforts and carbon credit programs.

Carbon sequestration and climate adaptation technologies like robotics, satellite imagery, digital twins, and drones equipped with Light Detection and Ranging (LiDAR) technology provide high-resolution mapping of forest biomass, helping estimate carbon stocks accurately. Carbon capture and storage (CCS) through AI-driven models simulate future climate scenarios, aiding policymakers in developing adaptive strategies such as afforestation, reforestation, and sustainable land-use practices.

Direct and indirect agricultural practices have their own carbon footprints in the form of carbon dioxide (CO<sub>2</sub>) emission through organic matter decomposition, crop residue burning, fossil fuel burning in equipment or machinery usage; methane (CH<sub>4</sub>) from anaerobic conditions in soil, manure and crop residue decay, enteric fermentation, and rice paddies; and nitrous oxide (N<sub>2</sub>O) from nitrification fertilizers. Non-dispersive infrared (NDIR), electrochemical, and photoacoustic gas analyzers can detect atmospheric carbon dioxide and methane in soil, vegetation (biomass), and other organic matter. GPS-guided sensors and actuators along with drones map normalized difference vegetation index for crop health monitoring. Smart irrigation systems controlled by IoT sensors optimize water use to enhance yield in drought-prone regions (Ahmed and Shakoor, 2025). Additionally, AI-powered early warning systems help prevent degradation by detecting stress factors like pests, diseases, and extreme weather events. Object detection for agricultural and construction environments using ultrasonic sensors has been developed.

## III. FIELD TRACKING AND LIVESTOCK MONITORING

IoT devices (wearable sensors) can track animal health, location, and behavior, allowing farmers to monitor their animals remotely. This helps improve animal welfare through better feed and manure management, enhance productivity, enable early detection and prevention of disease outbreaks, and reduce methane emissions. AI processes this data to track migration patterns, species interactions, and habitat preferences (Ivan et al., 2024; Heinzl and Koch, 2022).

IoT, AI, remote sensing, and GPS technologies monitor animal populations, protect endangered species, and maintain ecological balance. These advanced systems provide real-time insights into wildlife behavior, migration patterns, and habitat changes, enabling data-driven conservation strategies. IoT-enabled GPS collars, bio-loggers, and RFID tags track animal movements, feeding habits, and health conditions. AI processes this data to detect anomalies such as habitat loss or changes in migration routes due to climate change. Camera traps and acoustic sensors powered by AI identify species through image recognition and vocalization analysis, aiding biodiversity assessments. Drones and satellite imaging help monitor large-scale habitat changes, deforestation, and human encroachments. Predictive models use historical data and AI algorithms to forecast threats like poaching, human-wildlife conflicts, and disease outbreaks.

Despite technological advancements, challenges such as connectivity issues, high costs, and ethical concerns regarding animal tagging persist. However, with continued innovation, enhanced site-specific planting strategies through analyzing soil composition, climate patterns, and topography are possible. Machine learning models process these variables to determine optimal tree species for a given location, improving forest resilience and productivity (Singh et al., 2021). In reforestation projects, AI-powered drones assist in seed dispersal, ensuring efficient and precise planting.

Another major AI-driven advancement is automated thinning and harvesting. AI-equipped robotic systems use LiDAR and deep learning algorithms to assess tree maturity, density, and health, guiding selective harvesting while minimizing

environmental impact (Zhang et al., 2023). AI-based predictive modeling further aids in climate adaptation and fire risk management. By analyzing historical climate data, soil moisture levels, and wind patterns, AI forecasts drought stress and wildfire susceptibility, allowing proactive management strategies (Tang et al., 2022; Laktionov et al., 2024). Development of AI-driven robotic systems represents another promising avenue.

#### IV. POLICY INITIATIVES IN INDIA

The Indian government has recognized the importance of the Internet of Things (IoT) and has launched several initiatives to promote its adoption across various sectors to create a digitally empowered society and knowledge economy (Kurz et al., 2022). The Ministry of Electronics and Information Technology (MeitY), in collaboration with NASSCOM and state governments, has established Centers of Excellence (CoEs) for IoT in locations including Bengaluru, Gurugram, Gandhinagar, and Visakhapatnam. These centers focus on fostering innovation, supporting startups, and developing IoT solutions for various applications.

- **National Digital Communications Policy 2018:** This policy emphasizes enhancing internet and digital engagement through IoT, aiming to create robust digital communications infrastructure.
- **IoT Applications in Agriculture:** The government promotes IoT use in agriculture through programs like the National e-Governance Plan in Agriculture (NeGPA). This includes funding for digital agriculture projects utilizing IoT, AI, and other emerging technologies (Ahirwar et al., 2023; Bamhdi, 2024).
- **Smart Cities Mission:** This mission leverages IoT technologies to improve urban infrastructure and services, including smart parking, intelligent transportation systems, and waste management.
- **Future Skills PRIME:** MeitY has approved funding to create an ecosystem for reskilling and upskilling in emerging technologies, including IoT.
- **Digital Agriculture Mission of India:** This mission focuses on creating a Digital Public Infrastructure (DPI) for agriculture. It aims to provide farmers with digital identities (Farmer IDs) and facilitate data-driven decision-making. Key components include development of an "Agri Stack" and implementation of a nationwide crop survey.
- **National e-Governance Plan in Agriculture (NeGPA):** This program provides funding to state governments for digital agriculture projects, encouraging use of emerging technologies such as AI, machine learning, IoT, and blockchain.
- **Agricultural Mechanization:** The government promotes agricultural mechanization through modern machinery, including "Kisan Drones," via initiatives like the Sub-Mission on Agricultural Mechanization.
- **Digital General Crop Estimation Survey (DGCES):** This survey provides more precise yield estimations.
- **Krishi Decision Support System:** This system aims to unify remote sensing data on crops, soil, and weather to provide farmers with useful information.

#### V. CHALLENGES AND LIMITATIONS OF AI IN CLIMATE-SMART FARMING

Despite the transformative potential of artificial intelligence (AI) and smart tools (IoT), several challenges and limitations hinder widespread adoption and effectiveness. These challenges range from technological constraints and data quality issues to ethical and economic concerns.

One primary challenge is availability and quality of data. AI models rely on large datasets including satellite imagery, IoT sensor data, and climate records. However, in many regions, data is incomplete, inconsistent, or outdated, affecting accuracy of AI-driven predictions (Tang et al., 2022). Additionally, integrating multi-source data from different sensors and satellites often requires advanced data fusion techniques, which may introduce errors.

Another major limitation is high cost of AI implementation. Developing and maintaining AI-driven climate-smart agriculture systems, such as deep learning models, IoT networks, and cloud computing platforms, requires substantial financial investment. Many developing countries and local agriculture management agencies struggle with costs of acquiring high-resolution satellite data and AI-powered analytical tools (Zhang et al., 2023).

AI models also face computational and infrastructure limitations. Advanced machine learning algorithms such as deep neural networks require significant computing power, which may not be available in remote rural areas with limited connectivity and energy resources (Hernandez et al., 2021). Lack of 5G networks and real-time processing capabilities further restricts AI's operational efficiency.

Another critical concern involves ethical and policy-related issues. Use of AI often involves automated decision-making that may not always align with local conservation goals or indigenous community rights. AI-driven monitoring systems, for example, may misclassify land use changes, leading to conflicts between authorities and local populations (Singh et al., 2021). Additionally, AI models are prone to bias and uncertainty. If AI algorithms are trained on limited or biased datasets, they may produce inaccurate predictions and misinterpret ecological conditions, leading to ineffective conservation strategies and misallocation of resources (Gaveau et al., 2019).

Diverse allied and non-allied agricultural activities in the current scenario are practiced in hybrid mode. Climate-smart agriculture is essential to achieve Sustainable Development Goals (SDGs). Greenhouse gas emissions (methane and nitrous oxide) from agricultural lands are increasing. Traditional farm management approaches often struggle to efficiently monitor and respond to threats due to resource limitations and complexity of ecological systems, leading to food crises (Smith et al., 2020). Climate-smart farming offers an alternative approach to mitigate these challenges.

However, data quality and availability remain significant concerns, as AI models require large datasets for training and validation (Fernandez et al., 2022). Algorithmic biases can affect precision in AI predictions, necessitating use of diverse and representative datasets (Smith et al., 2020; Kumar et al., 2023). Additionally, high costs of implementing AI-based management solutions can limit accessibility, particularly in subsistence farming where farmers prefer traditional methods (Tyagi and Tiwari, 2025). Access to technology, affordability, and digital literacy need to be addressed. Legitimate authoritative initiatives and investments are crucial for promoting smart farming adoption. Ethical considerations including data privacy and potential misuse of AI-based surveillance technologies must ensure careful and responsible deployment (Boateng and Boateng, 2025; Chakraborty and Joshi, 2021; Ahmed and Taylor, 2023). Other challenges include crop distribution and allocation, global temperature change effects on crop yields, and unpredictable climate/weather patterns.

To address these limitations, researchers are working on improving AI transparency, cost-effective implementation strategies, and hybrid approaches that integrate AI with traditional practices. As AI technology advances, overcoming these challenges will be essential to ensuring ecologically sustainable and responsible farming practices.

## VI. FUTURE PROSPECTS AND RECOMMENDATIONS

The future of AI holds immense potential for enhancing sustainability, precision monitoring, and disaster prevention. One key future prospect is integration of AI with real-time satellite and drone-based monitoring. High-resolution imagery from AI-powered satellites and UAVs will provide instantaneous crop health assessments, unannounced threat detection, and livestock monitoring (Tang et al., 2023). Adoption of hyperspectral imaging and LiDAR-based AI models will further improve accuracy of species identification and biomass estimation. AI will also play a crucial role in predictive climate modeling, analyzing historical weather data, carbon sequestration patterns, and biodiversity trends to support long-term conservation strategies (Singh et al., 2022).

To maximize AI's potential in agriculture, several recommendations should be considered:

1. **Improved Data Infrastructure:** Establish global AI-driven agricultural databases to ensure high-quality, real-time data accessibility.
2. **Affordable AI Solutions:** Encourage cost-effective AI technologies for developing nations to enhance agricultural conservation efforts.
3. **Ethical AI Implementation:** Ensure AI models are transparent, unbiased, and aligned with indigenous rights and conservation policies.
4. **Cross-Sector Collaboration:** Strengthen partnerships between governments, AI researchers, and environmental organizations for better resource allocation.
5. **Edge AI and 5G Integration:** Deploy low-power AI models and high-speed networks for real-time analysis in remote rural areas.

6. **Capacity Building:** Invest in training programs for farmers and extension workers to enhance digital literacy and technology adoption.
7. **Public-Private Partnerships:** Foster collaborations between government agencies, private companies, and research institutions to accelerate innovation and technology transfer.
8. **Inclusive Technology Design:** Ensure AI and IoT solutions are designed with smallholder farmers in mind, addressing affordability, usability, and relevance to local contexts.

As AI technology continues to evolve, these advancements will significantly improve farm monitoring, carbon sequestration, and biodiversity conservation, making agriculture more resilient to climate change and other challenges.

## VII. CONCLUSION

Internet of Things (IoT) and artificial intelligence are transforming agriculture across the globe, offering unprecedented opportunities for precision farming, resource optimization, and sustainable intensification. From soil preparation to harvest, from crop farming to livestock management, from pest detection to carbon sequestration, smart technologies are reshaping every aspect of agricultural production.

The evolution from Agriculture 1.0 to Agriculture 5.0 represents a paradigm shift in how food is produced. Traditional farming methods, while still prevalent in many regions, are increasingly being complemented or replaced by data-driven, automated systems that enhance efficiency, reduce waste, and improve resilience to climate change. Climate-smart agriculture, enabled by IoT and AI, offers a pathway to address the dual challenges of food security and environmental sustainability.

Key applications reviewed in this chapter demonstrate the breadth and depth of technological integration in modern agriculture. Smart irrigation systems optimize water use, reducing both consumption and costs. Remote sensing and UAVs provide real-time crop health monitoring, enabling early intervention against pests and diseases. IoT-enabled livestock tracking improves animal welfare and productivity. AI-powered predictive analytics help farmers make informed decisions about planting, fertilization, and harvesting. Carbon sequestration monitoring contributes to climate change mitigation efforts.

However, significant challenges remain. Data quality and availability, high implementation costs, infrastructure limitations, ethical concerns, and digital divides between developed and developing regions must be addressed to ensure equitable access to these technologies. Policy initiatives like those in India demonstrate governmental commitment to promoting digital agriculture, but more comprehensive and inclusive approaches are needed.

The future of smart farming lies in continued innovation, cross-sector collaboration, and inclusive technology design. As AI algorithms become more sophisticated, sensors more affordable, and connectivity more widespread, the potential for transforming agriculture will only grow. Realizing this potential requires concerted efforts from policymakers, researchers, technology developers, and farming communities worldwide.

In conclusion, investment in smart and sustainable agricultural technologies is not merely an option but a necessity for achieving global food security in the face of climate change, population growth, and resource constraints. IoT and AI, when deployed thoughtfully and equitably, can help create a more resilient, productive, and sustainable agricultural system for future generations.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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# Qualitative Phytochemical Screening of Medicinal Plants and Their Prospectus as Natural Therapeutics in Aquaculture

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**Abstract**— In the present study, three medicinally significant plant species, viz., *Artemisia absinthium*, *Matricaria chamomilla*, and *Thymus vulgaris*, were selected for qualitative screening of phytochemicals, and crude extracts of the plants were used to evaluate their antimicrobial activity against *Staphylococcus aureus*. The aerial parts of the species were sequentially extracted with ethyl acetate and reconstituted with 70% ethanol; qualitative analysis was planned for detection of the presence of major phytochemicals. Disk diffusion method was used to evaluate the antibacterial activity of all the three extracts at different concentrations. Phytochemical screening results indicated the presence of different secondary metabolites, viz., alkaloids, flavonoids, phenols, glycosides, and terpenoids. Disk diffusion assay results indicated that *Thymus vulgaris* showed high-level antibacterial activity even at low concentrations, with increasing inhibition zones of 8.95 mm at 0.1 mg/ml to 22.65 mm at 100 mg/ml; *Matricaria chamomilla* showed the highest inhibition zone of 35.80 mm at 100 mg/ml, showing high efficacy at high concentration, while *Artemisia absinthium* showed moderate activity, with inhibition zones of 0.90 mm at 0.1 mg/ml to 8.90 mm at 100 mg/ml. The results indicate that all three plant extracts contain potential secondary metabolites that could be used as preventive agents in the diet of fish to inhibit bacterial infection.

**Keywords**— Medicinal plants, phytochemical screening, *Staphylococcus aureus*, aquaculture.

## I. INTRODUCTION

India's aquaculture production in 2022 was 10.23 million tonnes (FAO, 2024), while total fish production in Jammu and Kashmir for 2023–2024 was 28,000 tonnes (FAO, 2024). India's aquaculture, including Jammu and Kashmir, is still facing repeated problems due to disease outbreaks caused by bacteria, fungi, and parasites, leading to high fish mortality and heavy economic losses (Kalaria et al., 2024). The indiscriminate use of conventional chemical and antibiotic control methods, though initially successful, has led to grave issues like the development of antibiotic-resistant disease-causing agents, contamination of the environment, and accumulation of toxic residues in fish, toxic to the aquatic ecosystem as well as human consumption (Kalaria et al., 2024). Additionally, the use of chemicals and antimicrobial agents has been associated with a number of other issues, including bioaccumulation, contamination of water, and contamination of the surrounding natural ecosystems.

Compared to these synthetic chemicals, the use of phytochemical-rich plant extracts is promising alternatives for their use in aquaculture (Manzoor et al., 2025). These toxic effects have led to banning or restricting some chemical treatments in some areas and have triggered greater interest in safer and more sustainable alternatives, like plant therapeutics (Panigrahi & Azad, 2007). Studies are in progress on medicinal plants and plant extracts because of their high antibacterial, antiviral, and antiparasitic activities as well as due to their ability to stimulate increasing the immunity of aquatic animals, thus becoming a potential alternative to manage disease in a sustainable way (Jeyavani et al., 2022; Semwal et al., 2023).

Medicinal plants such as garlic and ginger are being used in aquaculture to stimulate fish immunity and increase growth, thus serving as a natural replacement for antibiotics. In humans, these plants have therapeutic activities that include anti-inflammatory, antioxidant, antimicrobial, and cardioprotective activities. They are used in the treatment of gastrointestinal disorders, infections, and chronic conditions, with consequences generally being lower side effects compared to man-made drugs (El-Seedi et al., 2019; Marwat et al., 2009). Ecologically, cultivation and use of these plants improve biodiversity, enhance sustainable agriculture, and reduce the utilization of chemical drugs, which aligns with the principles of green chemistry and works towards reducing environmental degradation (El-Seedi et al., 2019). Plant extracts of plants like garlic and ginger have in aquaculture been found to have the potential to boost the immune system, growth rate, and resistance to disease of fish, thereby providing a natural alternative to antibiotics while working towards the creation of healthier aquatic ecosystems (El-Seedi et al., 2019).

Use of these plants in traditional and conventional medicine gives an integrated philosophy of health that focuses on prevention, natural healing, and harmony with the environment, principles that align with Islamic values (El-Seedi et al., 2019; Marwat et al., 2009). Moreover, these natural products are considered generally inexpensive, easily accessible, and environmentally friendly, which aligns with their use in organic and sustainable aquaculture practices. In addition, they have the ability to boost growth, reduce stress, and generally improve aquatic cultured animals' well-being (Semwal et al., 2023).

Aquaculture is increasingly looking for natural, plant-based therapeutics to enhance disease management and minimize the use of synthetic chemicals. In this regard, medicinal plants such as *Artemisia absinthium*, *Thymus vulgaris*, and *Matricaria chamomilla* have gained prominence based on their rich phytochemical content and varied biological activities, including antioxidant, antimicrobial, and anti-inflammatory activities (Hbika et al., 2022). *Artemisia absinthium* is characterized by the presence of phenolic acids, flavonoids, sesquiterpene lactones, and tannins, all of which are accountable for its antioxidant, immunomodulatory, hepatoprotective, and insecticidal activities (Trifan et al., 2022). Likewise, *Thymus vulgaris* is rich in phenolic compounds such as caffeic and rosmarinic acids, which are accountable for its strong antioxidant, antimicrobial, and antiproliferative features. These compounds are present in sizeable amounts in *Thymus vulgaris* extracts, and research has established that the antioxidant activity of the plant is squarely related to its phenolic content, particularly caffeic and rosmarinic acids. Moreover, *Thymus vulgaris* extracts have been proven to contain the potential to inhibit the proliferation of varied bacterial and fungal species, and its total phenolic content has been proven to strongly correlate with antiproliferative activity against cancerous cell lines (Rafael Mascoloti Spréa et al., 2022; Taghouti et al., 2020). *Matricaria chamomilla* is gifted with a range of properties, including antioxidant, anti-inflammatory, antimicrobial, antiparasitic, hepatoprotective, neuroprotective, wound healing, and bioremediation, which are attributed to its flavonoid content (particularly luteolin, apigenin, and quercetin), sesquiterpenes (such as chamazulene and  $\alpha$ -bisabolol), and potential to accumulate and resist heavy metals (Joumaa & Jamilah, 2022).

The ethyl acetate extraction has been identified as a superior technique for the isolation of bioactive medium-polarity compounds, including phenolics and flavonoids, that are often responsible for antioxidant, antimicrobial, and anti-inflammatory activity (Hbika et al., 2022). For example, ethyl acetate-derived *Artemisia absinthium* extracts are rich in polyphenols and flavonoids and possess good antioxidant and enzyme inhibitory activity (Hbika et al., 2022). *Thymus vulgaris* ethyl acetate fractions possess high thymol and carvacrol content with high antioxidant and anti-inflammatory activity (Saleem et al., 2022). Ethyl acetate extraction makes it easier to isolate major bioactive compounds from those plant sources, which are essential to their therapeutic effect (Trifan et al., 2022; Szopa et al., 2020). Routine qualitative phytochemical screening of these plants reveals the presence of major bioactive classes, including phenolics, flavonoids, tannins, and terpenoids, responsible for their biological activity and therapeutic potential (Hbika et al., 2022). Utilization of such plant extracts as growth promoters in aquaculture can potentially improve the health of fish, enhance disease resistance, and encourage more sustainable and environmentally friendly aquaculture practices (Trifan et al., 2022).

To isolate ethyl acetate extracts of *Thymus vulgaris*, *Matricaria chamomilla*, and *Artemisia absinthium*, the conventional method is to dry and grind the aerial parts of each respective plant. The plant material is then macerated in ethyl acetate for some time, typically 24 to 72 hours, at room temperature under stirring at intervals (Saleem et al., 2022; Alkufeidy et al., 2021). After the maceration step, the mixture is filtered such that the liquid extract is separated from the remaining plant material. The filtrate is then concentrated in volume using reduced pressure by a rotary evaporator to yield a semi-solid or solid crude extract rich in bioactive constituents (Saleem et al., 2022). The extraction method is effective in isolating phenolic compounds, flavonoids, and essential oils, which are responsible for the antioxidant, antimicrobial, and anti-inflammatory activity

elucidated in the above-botanical species (Saleem et al., 2022). For *Thymus vulgaris*, the ethyl acetate extracts have been shown to be potent antioxidants and antibacterial agents, and this activity can be attributed to constituents like thymol and carvacrol (Alkufeidy et al., 2021). The same extraction steps are followed for *Matricaria chamomilla* and *Artemisia absinthium*, as elucidated in extensive phytochemical and antimicrobial assessment studies (Stanojević et al., 2004).

## II. MATERIALS AND METHODS

### 2.1 Plant Specimen Collection and Identification:

*Artemisia absinthium* was harvested from Hatbora Ganderbal, *Thymus vulgaris* from Benehama Ganderbal, and *Matricaria chamomilla* from Rangil Ganderbal.

*Artemisia absinthium* was recognized from its diagnostic micromorphological characters, particularly the T-shaped non-glandular trichomes on the upper and lower leaf surfaces and more dense occurrence of glandular trichomes than in related species, which are the key diagnostic characters of the species. *Thymus vulgaris* was recognized from its diagnostic glandular trichomes, particularly the peltate and capitate ones, which are responsible for the aromatization of the herbaceous plant and are manifested on the leaf surface. *Matricaria chamomilla* is recognized from finely divided leaves and glandular trichomes, along with its diagnostic flower morphology. The identification of the three species was also supported by their diagnostic anatomical and micromorphological characters, which are well documented in botany literature and are suitable criteria for the identification of species (European Pharmacopoeia, 11th ed., 2023).

### 2.2 Drying of Plant Material:

For each of the plant species, 1 kg of fresh aerial parts were collected. The plant material was cleaned well to eliminate soil and dirt, and shade dried for 14 days to maintain its phytochemical integrity.

### 2.3 Preparation of Crude Extract by Maceration Using Ethyl Acetate as Solvent:

#### 2.3.1 Preparation of Plant Material:

Plant specimens of chamomile, thyme, and artemisia were first collected, each weighing 1 kg. The specimens were shade-dried to prevent the degradation of heat-labile phytochemicals, resulting in lower weights of 165.2 g for chamomile, 112.6 g for thyme, and 97.5 g for artemisia. The dried materials were next pulverized with a mortar and pestle in order to reduce particle size, thus enhancing the surface area for solvent contact and extraction efficiency.

#### 2.3.2 Weighing of Plant Material:

The plant materials were precisely weighed and later transferred into individual 1000 ml borosilicate glass containers.

#### 2.3.3 Cold Maceration Process:

Cold maceration is a delicate extractive procedure that maintains the thermolabile constituents and is well-suited to enhance the yield of bioactive compounds. The vessels holding the plant material were loaded with an addition of 830 ml of ethyl acetate, which was employed as the extracting solvent. The vessels were maintained at an angle and at ambient temperature for 72 hours. The shaking was done intermittently throughout the maceration period to promote the release of bioactive compounds into the solvent.

#### 2.3.4 Filtration and Sequential Extraction:

After a 72-hour lapse, the solution was filtered to yield liquid extract and marc (plant residue). The marc was then re-extracted with more ethyl acetate, thus maximizing the retrieval of phytochemicals. The second filtrate was collected, and the combined volume of the filtrates for each of the plants was collected: 450 ml for *Artemisia*, 620 ml for *Thymus*, and 600 ml for *Matricaria*.

#### 2.3.5 Concentration of Extracts:

The filtrates were concentrated under rotary evaporation at 70°C for 45 minutes to approximately 30 ml. The procedure removed most of the solvent without undermining the integrity of the extracted compounds. The concentrated extracts were then transferred to china dishes and dried further at a water bath at 50°C for 10 minutes to obtain the final crude extracts.

## 2.4 Qualitative Phytochemical Screening of Crude Extracts:

### 2.4.1 Drying and Reconstitution of Extracts:

A small quantity of each plant extract was oven-dried at 60°C for 2 minutes, then air-dried for 10 minutes. Dried extracts (300 mg each) were dissolved in 30 mL of 70% ethanol to prepare a stock solution (10 mg/ml).

### 2.4.2 Phytochemical Tests:

#### 2.4.2.1 Test for Phenols: Folin-Ciocalteu Test:

In Folin-Ciocalteu test, 1 mL of extract was taken in a test tube and 0.5 mL of Folin-Ciocalteu reagent was added with 1 mL of 10% sodium hydroxide. The contents were mixed and incubated for 5 minutes at room temperature. Formation of a blue-gray color indicates the presence of phenols (Atiya et al., 2024).

#### 2.4.2.2 Test for Flavonoids: Alkaline Reagent Test

In alkaline reagent test, few drops of 10% sodium hydroxide solution were added to 1 mL of extract in a test tube and then 2N HCl was added to neutralize the solution. Formation of an intense yellow color, which becomes colorless upon addition of dilute acid, confirms flavonoids (Shaikh & Patil, 2020).

#### 2.4.2.3 Test for Alkaloids: Wagner's Test

In Wagner's test, a few drops of Wagner's reagent (iodine in potassium iodide) were added to 1 mL of extract solution prepared by mixing 500 mg of dried extract in 8 ml of 1% HCl, warmed and filtered in a test tube. Formation of a reddish-brown precipitate indicates the presence of alkaloids (Shaikh & Patil, 2020).

#### 2.4.2.4 Test for Glycosides: Keller-Kiliani Test

In Keller-Kiliani test, 1.5 mL of glacial acetic acid was added to a test tube containing one drop of 5% ferric chloride and 1 mL of extract. One mL of concentrated sulfuric acid was also added along the sides of the test tube at last. Formation of a reddish-brown color indicates the presence of glycosides (Çilesizoğlu et al., 2022).

#### 2.4.2.5 Test for Terpenoids: Salkowski's Test

In Salkowski's test, 1 mL of extract was added with 2 mL of chloroform in a test tube, then 2 mL of concentrated sulfuric acid was carefully added down the sides of the test tube. A reddish-brown coloration at the interface confirms terpenoids (Sharma et al., 2020).

## 2.5 Antibacterial Activity of Plant Extracts Against *Staphylococcus aureus*:

### 2.5.1 Preparation of Plant Extract Solutions:

Different concentrations (0.1 mg/ml, 0.25 mg/ml, 1 mg/ml, 10 mg/ml, 50 mg/ml, and 100 mg/ml) of each plant extract (*Artemisia absinthium*, *Matricaria chamomilla*, and *Thymus vulgaris*) were prepared using the serial dilution method with 70% ethanol as the solvent. A 70% ethanol solution was also prepared to serve as a negative control.

### 2.5.2 Preparation of Agar Plates and Broth:

Nutrient agar plates and nutrient broth were prepared according to standard microbiological protocols. A 24-hour sterility test was performed to confirm the sterility of the agar plates and broth.

### 2.5.3 Preparation of Bacterial Suspension:

A glycerol stock of *Staphylococcus aureus* was obtained and inoculated into nutrient broth, followed by incubation at 37°C for 24 hours to achieve optimal bacterial growth.

### 2.5.4 Inoculation of Agar Plates:

An L-shaped spreader was used to evenly distribute the bacterial suspension over the entire surface of the agar plates to ensure uniform growth.

### 2.5.5 Drying of Plates:

The inoculated plates were allowed to dry at room temperature for a few minutes to ensure the surface was not excessively moist before applying the discs.

**2.5.6 Application of Extract-Infused Discs:**

Forceps were sterilized using a Bunsen burner and used to place sterile filter paper discs, each infused with a specific concentration of plant extract, onto the surface of the inoculated agar plates. Discs containing only 70% ethanol were included as negative controls.

**2.5.7 Incubation:**

The plates were incubated at 37°C for 24 hours.

**2.5.8 Measurement of Inhibition Zones:**

After incubation, the diameter of the clear zones (zones of inhibition) around each disc was measured in millimeters. Larger zones indicated greater antibacterial activity.

**2.5.9 Calculation of Minimum Inhibitory Concentration and Dose-Response Relationship:**

The MIC is determined by identifying the lowest concentration of each extract that produced a visible zone of inhibition against *S. aureus*. The relationship between extract concentration and the diameter of inhibition zones was analyzed to assess the dose-dependent antibacterial effect of each plant extract.

**III. RESULTS**

**3.1 Plant Extraction Yield and Characteristics of Final Crude Extract:**

The dried plant materials (97.5 g, 112.6 g, and 165.2 g of *Artemisia absinthium*, *Thymus vulgaris*, and *Matricaria chamomilla* respectively) with ethyl acetate yielded plant extract residues that ranged from 2.83 to 9.85 g. The highest yield of plant extract was obtained from *Matricaria chamomilla* (9.85 g) followed by *Thymus vulgaris* (6.63 g), while *Artemisia absinthium* gave the lowest extract yield (2.83 g).

The final crude extracts displayed different consistencies: *Thymus vulgaris* yielded a powdery extract, while *Matricaria chamomilla* and *Artemisia absinthium* produced scrape-like residues. These differences are attributed to the unique phytochemical compositions and solubility profiles of each plant species.

**TABLE 1  
 EXTRACTION YIELD OF PLANT SPECIES**

Plant Species	Initial Weight (g)	Final Weight (g)	Yield (%)
<i>Artemisia absinthium</i>	97.5	2.83	2.9
<i>Thymus vulgaris</i>	112.6	6.63	5.88
<i>Matricaria chamomilla</i>	165.2	9.85	5.96

**3.2 Qualitative Phytochemical Screening:**

The phytochemical screening of ethyl acetate extracts revealed the presence of various secondary metabolites, with variation among the three plant species.

**TABLE 2  
 QUALITATIVE PHYTOCHEMICAL SCREENING OF PLANT EXTRACTS**

Plant Species	Alkaloids	Flavonoids	Glycosides	Phenols	Terpenoids
<i>Artemisia absinthium</i>	-	++	++	++	-
<i>Matricaria chamomilla</i>	+	++	++	++	+
<i>Thymus vulgaris</i>	++	++	-	++	++

Note: (+) = Present; (-) = Absent

### 3.3 Antibacterial Activity:

The antibacterial potential of all three plant extracts was scrutinized against *Staphylococcus aureus*. The antibacterial potential was tested in vitro using the disc diffusion test. Discs were loaded with 0.1, 0.25, 1, 10, 50, and 100 mg/ml concentrations each of the extracts, dissolved in 70% ethanol, and the same solvent was used as a negative control. The use of 70% ethanol as a negative control was successful in showing that the reported antibacterial effects are because of the plant extracts themselves, as indicated by a zone of inhibition of  $0.0 \pm 0.0$  mm.

**TABLE 3**  
**ZONE OF INHIBITION (MM) OF PLANT EXTRACTS AGAINST STAPHYLOCOCCUS AUREUS AT DIFFERENT CONCENTRATIONS**

Plant Species	Concentration (mg/ml)	Zone of Inhibition (mm) Mean $\pm$ SE
<i>Artemisia absinthium</i>	0.1	$0.90 \pm 0.099$
	0.25	$4.75 \pm 0.250$
	1	$4.90 \pm 0.099$
	10	$6.90 \pm 0.099$
	50	$7.80 \pm 0.20$
	100	$8.90 \pm 0.099$
<i>Thymus vulgaris</i>	0.1	$8.95 \pm 0.050$
	0.25	$9.60 \pm 0.40$
	1	$10.70 \pm 0.299$
	10	$11.60 \pm 0.40$
	50	$19.85 \pm 0.149$
	100	$22.65 \pm 0.350$
<i>Matricaria chamomilla</i>	0.1	$1.10 \pm 0.099$
	0.25	$1.75 \pm 0.149$
	1	$6.80 \pm 0.20$
	10	$9.75 \pm 0.025$
	50	$12.70 \pm 0.299$
	100	$35.80 \pm 0.20$
Negative Control (70% Ethanol)	-	$0.0 \pm 0.0$

### 3.4 Minimum Inhibitory Concentration (MIC):

The MIC of all plant extracts was 0.1 mg/ml with different inhibition zones, the highest being observed in *Thymus vulgaris*.

**TABLE 4**  
**MINIMUM INHIBITORY CONCENTRATION (MIC) OF PLANT EXTRACTS AGAINST STAPHYLOCOCCUS AUREUS**

Plant Species	MIC (mg/ml)	Zone of Inhibition at MIC (mm) Mean $\pm$ SE
<i>Artemisia absinthium</i>	0.1	$0.90 \pm 0.099$
<i>Thymus vulgaris</i>	0.1	$8.95 \pm 0.050$
<i>Matricaria chamomilla</i>	0.1	$1.10 \pm 0.099$

\*Values are mean of two replicates (n=2)  $\pm$  standard error\*

### 3.5 Dose-Response Relationship:

According to Figure 1, for all three extracts, in general, there is a trend of zone of inhibition rising with increasing concentration, particularly at low concentrations. This confirms that the antibacterial activity is dose-dependent. *Artemisia absinthium* extract possesses the minimum zone of inhibition at most concentrations, *Thymus vulgaris* possesses a moderate zone of antibacterial activity, and *Matricaria chamomilla* extract possesses the maximum antibacterial activity, particularly at higher concentrations. It possesses a sudden increase in zone of inhibition at higher concentrations, which confirms its greater efficacy and potency than the other two extracts.

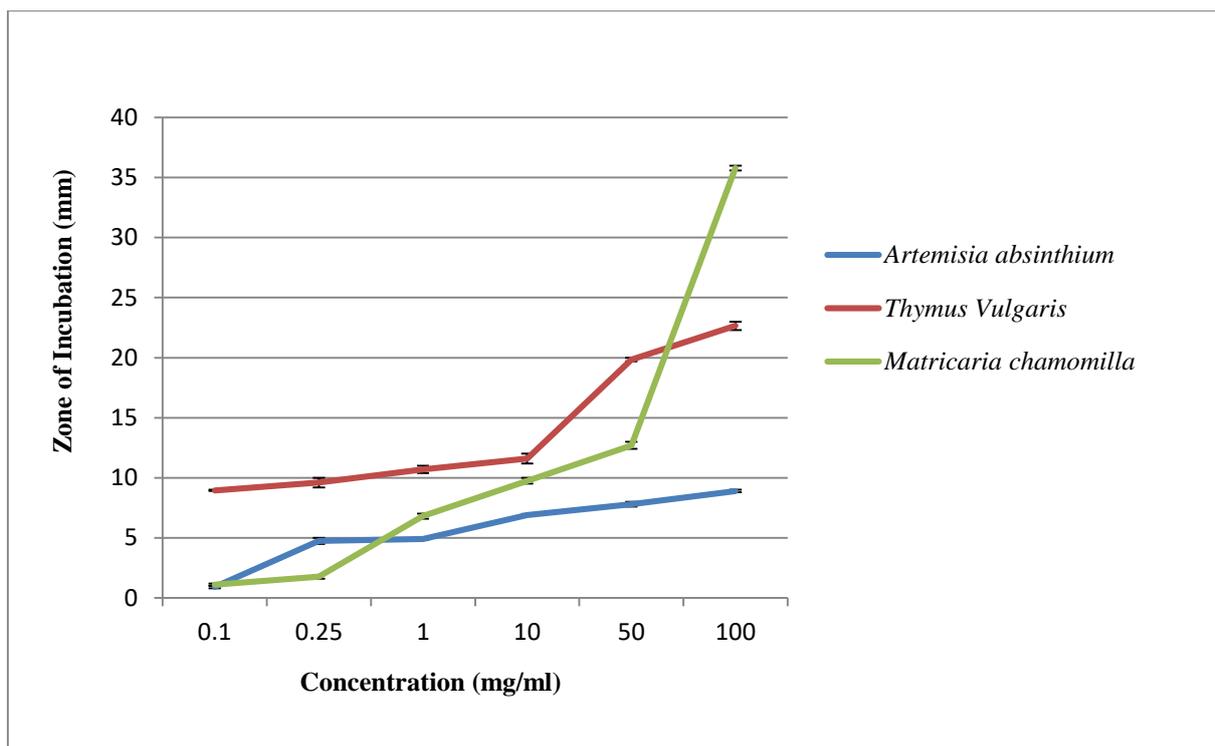


FIGURE 1: Dose-Response Curve - Zone of Inhibition vs. Concentration for All Three Plant Extracts



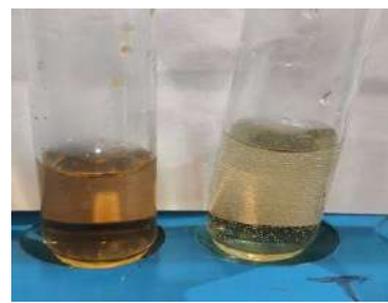
Test for Flavonoids



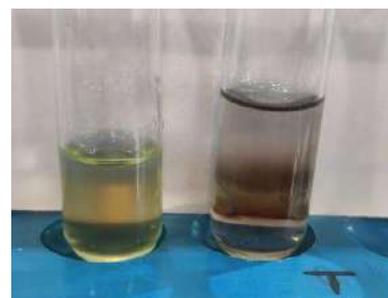
Test for Alkaloids



Test for phenols



Test for Glycosides



Test for Terpenoids

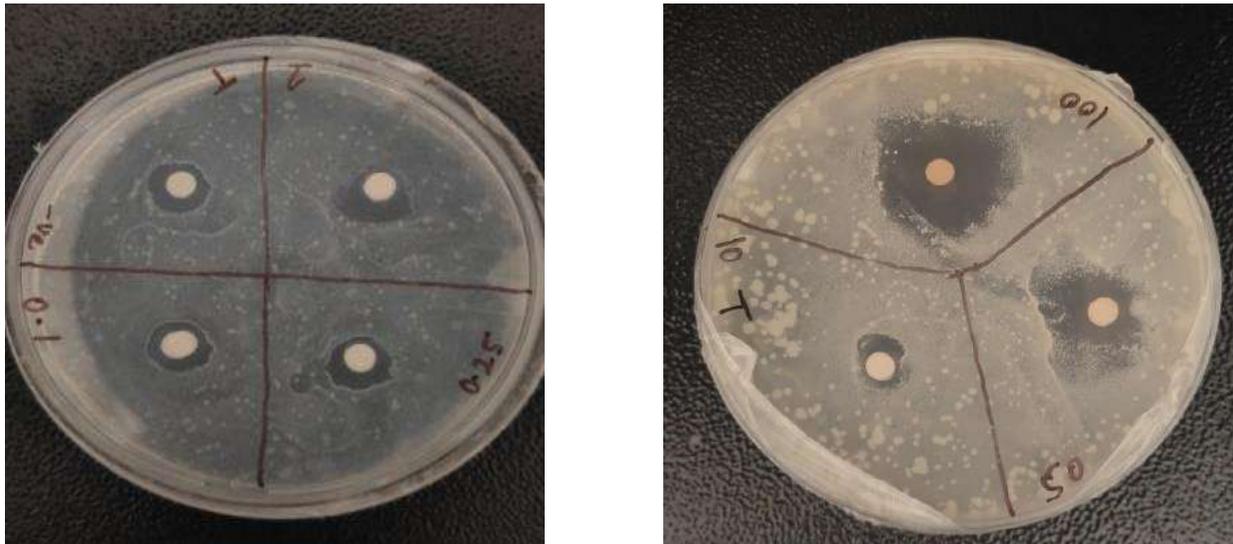
FIGURE 1: Phytochemical screening



ZOI at different concentrations of *Artemisia absinthium* extract



ZOI at different concentrations of *Matricaria chamomilla* extract



ZOI at different concentrations of *Thymus vulgaris* extract

FIGURE 3: Showing ZOI at different concentrations of *Matricaria chamomilla* extract

#### IV. DISCUSSION

##### 4.1 Extraction Yield:

The plant extracts were observed to fall between 2.83-9.85 g. *Matricaria chamomilla* extract had the highest amount of extract (9.85 g), followed by *Thymus vulgaris* which yielded 6.63 g, and *Artemisia absinthium* which yielded the least extract of 2.83 g. This study complements previous data by Chuo et al. (2022) suggesting that solid-liquid extraction methods with 2-10% yields remain consistent with sprayed plant extract yields while varying depending on the species and solvents applied. This affirms the work of Lefebvre et al. (2021) who emphasize that solvent choice and plant matrix characteristics are key determinants of both yield and selectivity. Overall, extraction yields of this study are consistent with the literature for conventional methods. Chuo et al. (2022), Lefebvre et al. (2021), Rajkovic et al. (2020), and El Maaiden et al. (2022) all underlined the importance of optimizing both solvent and technique to maximize yield and preserve the quality of the extract.

##### 4.2 Phytochemical Composition:

The qualitative phytochemical analysis conducted on the ethyl acetate extract of *Artemisia absinthium* showed the presence of flavonoids, glycosides, and phenols, with the absence of alkaloids and terpenoids. This aligns with the observations made by Trifan et al. (2022) who noted that *Artemisia absinthium* had abundant phenolic acid and flavonoid content, particularly in the polar extracts, and alkaloids and terpenoids were rather infrequent in significant quantities in methanol or ethyl acetate. Their metabolite profiling revealed a number of phenolic acids and flavonoids within the aerial parts, which corroborates the findings of abundant phenolic and flavonoid content (Trifan et al., 2022). Moacă et al. (2019) also observed significant chlorogenic acid and other phenolic constituents in ethanolic extracts, with lesser focus on the terpenoid and alkaloid content. Goud (2018) also supported the abundant phenolic and flavonoid content in methanolic extracts of *A. absinthium*.

In the case of *Matricaria chamomilla*, the results of this study showed the presence of all the tested groups, with the prominence of flavonoids, glycosides, and phenols, as well as moderate amounts of alkaloids and terpenoids. This corresponds with the study done by Spréa et al. (2022) where it was noted that *Matricaria chamomilla* has numerous secondary metabolites which include flavonoids (apigenin and luteolin), phenolic acids, glycosides, terpenoids, and variably dependent alkaloids with the extraction method used. Rahmani and Ouahrani (2022) also noted the presence of the majority of phytochemical groups in the methanolic extracts which had high solubility of the active constituents, therefore supporting findings of this study. Boudieb et al. (2018) proved that flavonoids, tannins, alkaloids, and glycosides were documented in some qualitative screenings, which further supports the findings.

Concerning *Thymus vulgaris*, the screening documented important alkaloids, flavonoids, phenols, and terpenoids, with the exception of glycosides which were not detected. Spréa et al. (2022) reported that *Thymus vulgaris* was richly phenolic, containing also flavonoids and terpenoids, while glycosides were less detected, particularly in the more apolar solvents. This also supports the findings of this study: absence of glycosides and the prominence of other groups.

In general, the qualitative findings for these three species corroborate the available literature, especially concerning the high concentrations of flavonoids and phenols. The varying occurrences of alkaloids, glycosides, and terpenoids may arise from the chemistry of the species and the solubility of the extracting solvent (Trifan et al., 2022; Moacă et al., 2019; Goud, 2018; Spréa et al., 2022; Rahmani and Ouahrani, 2022; Boudieb et al., 2018).

### 4.3 Antibacterial Activity:

The antibacterial potential shown by these plant extracts is in agreement with several earlier studies that have reported the antimicrobial activity of *Artemisia absinthium*, *Thymus vulgaris*, and *Matricaria chamomilla* against several pathogenic bacteria, including *Staphylococcus aureus*.

*Artemisia absinthium* has been reported to possess medicinal activities including antiparasitic and antimicrobial activities (Abid et al., 2023). Its extracts were found to possess activity against numerous bacterial strains by studies, which have oftentimes been attributed to the presence of compounds like sesquiterpene lactones (like artemisinin) and essential oils (Abid et al., 2023). For example, studies show that *Artemisia absinthium* essential oil and extracts have been able to suppress the growth of *S. aureus*, which is consistent with this study (Hrytsyk et al., 2022). Antibacterial activity can be varied based on the plant part, extraction method, and geographical location (Abid et al., 2023).

*Thymus vulgaris* is well known to have strong antimicrobial activity, with this being largely due to its high phenolic content of compounds such as thymol and carvacrol (Pereira et al., 2021). These have been documented well to cause cell death in bacteria through disrupting the bacterial cell membrane (Pereira et al., 2021). The result of the current study that *Thymus vulgaris* exhibited a large inhibition zone is consistent with a very large body of literature reporting its high performance against *S. aureus* and other resistant bacteria (Pereira et al., 2021). Research has even shown that the essential oil of *Thymus vulgaris* is used to treat methicillin-resistant *S. aureus* (Nostro et al., 2007).

*Matricaria chamomilla* has traditionally been applied in medicine because of its antioxidant, anti-inflammatory, and antimicrobial activities (Singh et al., 2019). The antibacterial property of the plant is mainly attributed to the essential oil compounds,  $\alpha$ -bisabolol and chamazulene, and flavonoids and coumarins (Singh et al., 2019). While chamomile extracts are less active compared to thyme extracts at low concentrations, they have been shown to be inhibitory to *S. aureus* at higher concentrations, thus making it a natural antimicrobial compound (Singh et al., 2019).

### 4.4 Minimum Inhibitory Concentration:

The MICs of all plant extracts was 0.1 mg/ml with varying inhibition zones, which was highest in *Thymus vulgaris* ( $8.95 \pm 0.05$  mm). In this study, the minimum inhibitory concentration (MIC) of the *Artemisia absinthium*, *Thymus vulgaris*, and *Matricaria chamomilla* extracts against *Staphylococcus aureus* was 0.1 mg/ml. Comparing findings of this study with similar research, it is clear that ethyl acetate extracts are much stronger than most of those reported in the literature. For example, *Artemisia absinthium* and similar *Artemisia* species research commonly reports MICs ranging from 0.165 to 2.64 mg/ml against *S. aureus*, meaning the ethyl acetate extract is active at much lower concentration (Hrytsyk et al., 2022). For *Thymus vulgaris*, essential oil MICs against *S. aureus* are commonly 0.6–10 mg/ml, thus the finding of this study at 0.1 mg/ml is at the lower, more active end of this range (Aouadhi et al., 2020). For *Matricaria chamomilla*, reported MICs for chamomile essential oil are commonly 20–40 mg/ml, which is much higher than reported in this study (Aouadhi et al., 2020). This suggests that the extraction process (cold maceration) with ethyl acetate and reconstituting with 70% ethanol may have concentrated active compounds more than traditional essential oil or aqueous extractions. Overall, findings of this study show that these plant extracts, which were made with ethyl acetate as solvent using cold maceration, have much greater antibacterial activity against *Staphylococcus aureus* than most previously reported extracts or oils of the same species (Hrytsyk et al., 2022; Aouadhi et al., 2020).

## V. CONCLUSION

The phytochemical analysis of *Artemisia absinthium*, *Thymus vulgaris*, and *Matricaria chamomilla* revealed the presence of flavonoids, terpenoids, alkaloids, glycosides, and phenols in their extracts. Different concentrations of all three plant extracts exhibited potent inhibitory activity, with varying inhibition zone diameters, against *Staphylococcus aureus*. The reconstituted ethanolic extracts of these plants showed a broad spectrum of activity. This study demonstrated that ethyl acetate extracts of *Artemisia absinthium*, *Matricaria chamomilla*, and *Thymus vulgaris* are rich in diverse phytochemicals, including phenolic acids, flavonoids, and other bioactive compounds, as confirmed by qualitative phytochemical screening. The integration

of *Artemisia absinthium*, *Matricaria chamomilla*, and *Thymus vulgaris* as natural therapeutics in aquaculture presents a promising strategy to address the challenges of antibiotic resistance and disease management.

### CONFLICT OF INTEREST

The authors declare no conflict of interest.

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# Pyriproxyfen: Effect on Morphometrics and Total Protein of Accessory Sex Glands of *Spodoptera mauritia* Boisdu

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**Abstract**— *Spodoptera mauritia* is a sporadic pest of *Oryza sativa* L. which usually occurs on rice from July to September, feeding on the leaves in large numbers. Accessory Sex Glands (ASGs) play a crucial role in the reproductive success of insects. Pyriproxyfen (PPN), the Juvenile hormone agonist, is an environment friendly Insect Growth Regulator (IGR) well known to interfere with insect reproductive system. In the present study, PPN treatments in different ages of this insect produced variable results. All the newly ecdysed day 0 pupae of *S. mauritia*, topically applied with 1 µg/µl PPN didn't survive beyond day 4 whereas 0.1 µg/µl PPN treated pupae showed only 61% mortality but the survivors showed failure of emergence. In both sexes, ASGs showed retarded development when compared to control. In the male adultoids, total ASG proteins reduced significantly and it was evident from their electrophorogram also. The day 0 adult males when topically applied with a single high dose of PPN showed no mortality and their total ASG proteins were significantly increased and this increase was reflected in their electrophorogram.

**Keywords**— *Spodoptera mauritia*, Pyriproxyfen, Accessory sex glands, Adultoids, Reproductive success, Pupae mortality.

## I. INTRODUCTION

Numerous studies have demonstrated that Accessory Sex Glands (ASGs) play an essential role in reproduction. In most insects, ASGs become functional in adults. Several functions have been attributed to the secretion produced by ASGs. The functions of ASGs can be classified as structural, biochemical, behavioural and physiological (Fernandez & Cruz-Landim, 2005). The development and differentiation, synthesis and release of secretions of ASGs are under the control of endocrine system in insects. Critical titer of ecdysteroids secreted by prothoracic glands and juvenile hormone (JH) secreted by corpora allata (CA) are necessary for the normal development of ASGs (Gillott & Gaines, 1992). Many authors have shown that JH regulates protein synthesis in the ASGs (Blaine & Dixon, 1973; Gillott & Freidel, 1976; Venkatesh & Gillott, 1983; Ogiso & Takabashi, 1984; Gillott & Gaines, 1992).

The discovery of compounds with hormonal and antihormonal activities have greatly facilitated studies on Insect Endocrinology. These compounds designated as Insect Growth Regulators can induce hormone deficiency or hormone excess in treated insects and have greater potential in Integrated Pest Management (IPM) programmes as insect control agents (Nair, 1993; Oetken et al., 2000). The IGRs acting as ecdysone agonists/antagonists or JH analogues/anti JH agents disrupt the endocrine and reproductive physiology of a number of insects to aid in their control. The present study deals with the effect of Pyriproxyfen (PPN), a JH agonist, on the development, differentiation and the secretory activity of ASGs of *Spodoptera Mauritica*.

## II. MATERIAL AND METHODS

### 2.1 Rearing and Maintenance:

The larvae of *Spodoptera mauritia* were reared and maintained at room temperature, RH 90 ± 3% and 12:12 light: dark photoperiod regime in the laboratory. They were fed with fresh leaves of *Ischaemum aristatum*. The sexing of the larvae

were done in fifth instar and were segregated. In all experiments, developmentally synchronous insects were used. Pyriproxyfen (PPN), an agonist of JH, was obtained as a research sample from M/s Valent Corp., USA. For treatment procedures, measured quantities of these compounds were used with a 10 µl Hamilton microsyringe.

**2.2 Treatment of PPN on Newly Ecdysed Day 0 Pupae:**

PPN was dissolved and diluted in acetone to obtain two different concentrations: 0.1 µg/µl and 1 µg/µl. The different doses of PPN were applied topically on the abdominal region of newly ecdysed day 0 pupae (100 Nos.) using a 10 µl Hamilton microsyringe. Same number of pupae kept as controls received an equivalent volume of acetone only. Experimental and control pupae were kept in separate beakers covered with muslin cloth. The pupae were checked daily for mortality and morphological abnormalities. To study the morphogenetic changes, ASGs were dissected out of pupae on each post treatment day. Further, ASGs of adults and adultoids were processed for electrophoretic studies.

**2.3 Treatment of PPN on Day 0 Adult Males:**

Ten newly emerged (day 0) adult males of *S. mauritia* were treated topically with a single dose of 20 µg/µl of PPN. Same number of adult insects kept as controls were treated with an equivalent volume of acetone. Experimental and control insects were kept in separate beakers covered with muslin cloth. ASGs were dissected out from PPN treated insects on day 1, their total protein content was estimated and were further subjected to electrophoretic studies

**III. RESULTS AND DISCUSSION**

**3.1 Effect of PPN on Pupal Development and Survival:**

Pupae treated with 1 µg/µl PPN showed 100% mortality by day 4. When they were dissected open, ASGs were not developed at all. In 0.1 µg/µl PPN treated pupae, 61% mortality was observed by day 4. The survivors did not emerge as adults. These pupae when dissected open on day 8 were found to contain adultoids with unstretched wings inside the pupal cases. The control male insects emerged normally on day 8 and females emerged on day 7, showing normal imaginal differentiation.

**3.2 Effect on Male Accessory Sex Glands:**

In 0.1 µg/µl PPN treated male insects, ASGs appeared as small buds on day 4. They didn't elongate much on subsequent pupal days (Table 1) and they were seen as a fused pair of blind tubes without any regional differentiation (Figure 1b). In control pupae, ASGs showed normal development as seen in normal insects.

**TABLE 1  
 LENGTH OF MALE ASGS IN TREATED AND CONTROL INSECTS (MM)**

Day	Treated	Control
1	-	9.03 ± 0.209
2	-	15.1 ± 0.1
3	-	20.03 ± 0.057
4	1 ± 0.082	33.1 ± 0.1
5	2.5 ± 0.217	35.93 ± 0.115
6	3 ± 0.18	50.13 ± 0.058
7	4 ± 0.163	57.73 ± 0.152
8	4 ± 0.245	77 ± 0.812

**3.3 Effect on Female Accessory Sex Glands:**

In females, ASGs were developed only in pupae treated with 0.1 µg/µl PPN. The mortality rate was similar to that of males in both concentrations. In 0.1 µg/µl PPN treated insects, ASGs appeared as small buds on day 3. In females, ASGs showed an inhibition of growth under the effect of PPN as in males (Table 2). Reservoirs were hardly distinguishable from the glands (Figure 1d). They did not emerge as adults. These pupae when dissected open on day 7 were found to contain adultoids (Table 2).

**TABLE 2**  
**LENGTH OF FEMALE ASGS IN TREATED AND CONTROL INSECTS (MM)**

Day	Treated	Control
1	-	2 ± 0.849
2	-	3.1 ± 0.1
3	1 ± 0.0816	7.13 ± 0.152
4	2 ± 0.05	10 ± 0.1
5	2 ± 0.141	12.03 ± 0.577
6	3 ± 0.812	12.13 ± 0.115
7	4 ± 0.216	13.26 ± 0.723

**3.4 Effect of PPN on Adult Males:**

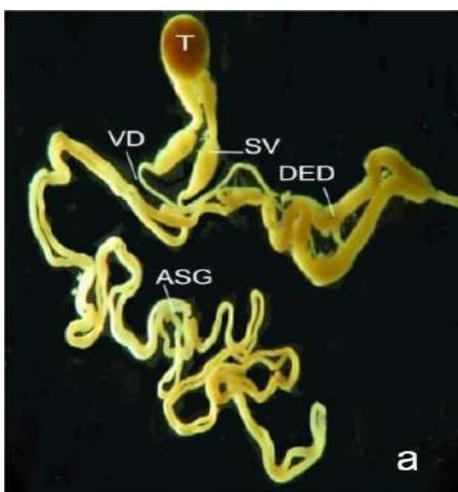
A single high dose of PPN (20 µg/µl) was applied on the adult insects. They did not show any mortality and were quite healthy and active once recovered from a short period of anesthetic effect. When they were dissected on day 1, ASGs looked slightly swollen, evidently due to the accumulation of secretion.

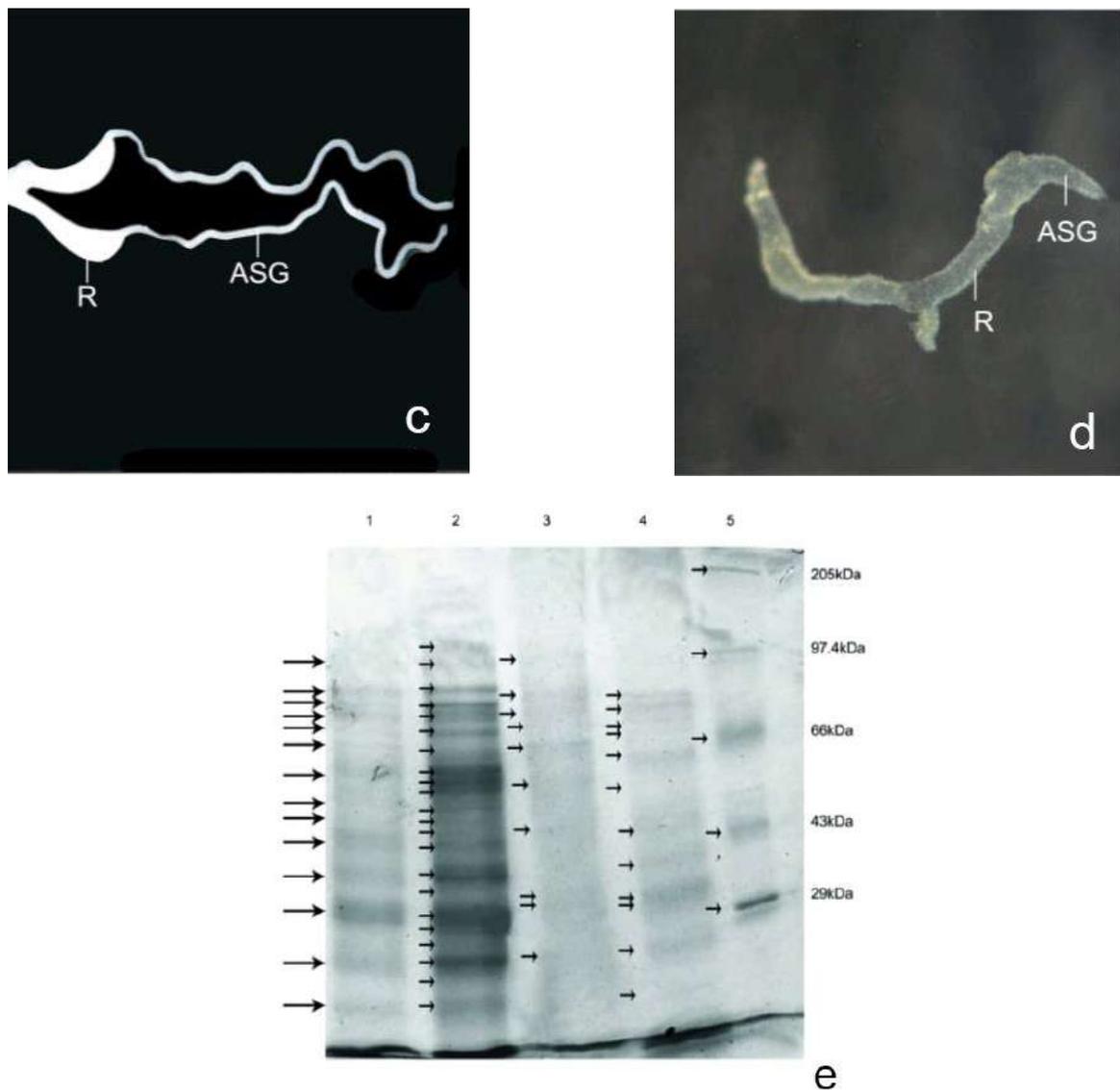
Amount of protein in ASGs of day 0 control insects was found to be 9.28 ± 0.876 µg/mg of tissue, whereas the amount of protein in the ASGs of adultoids was considerably decreased to 1.9 ± 0.141 µg/mg of tissue. At 0.05 levels, these values are significantly different.

Amount of protein in ASGs of day 1 control insects was found to be 13.63 ± 0.410 µg/mg of tissue, whereas the amount of protein in the ASGs of day 1 males treated with 20 µg PPN immediately after eclosion on day 0 had increased to 42.75 ± 1.343 µg/mg of tissue. At 0.05 levels, these values are significantly different.

**3.5 Electrophoretic Analysis:**

In the electrophorogram (Figure 1e), Lane 2 shows the electrophoretic profile of ASG proteins of day 1 adult males treated with 20 µg/µl of PPN immediately after eclosion on day 0. In this, neither bands were found to disappear nor were new bands appeared. The staining intensities of most of the peptides of treated males were considerably more when compared to that of control. Lane 3 shows the electrophoretic profile of ASG proteins of adultoids. The staining intensities of most of the bands were considerably less and some bands have disappeared when compared to that of control (Lane 4).





**FIGURE 1**

- a. Reproductive system of male kept as control showing Testis (T), Vas deferens (VD), Seminal vesicles (SV), Ductus ejaculatorius duplex (DED), Ductus ejaculatorius simplex (DES), Accessory sex glands (ASG)
- b. Male Reproductive system of male adultoid showing Accessory sex glands (ASG)
- c. Accessory sex glands (ASG) of female kept as control showing Reservoir (R)
- d. Accessory sex glands (ASG) of female adultoids showing Reservoir (R)
- e. SDS-PAGE profile of ASG proteins in PPN treated and control male insects

Lane 1: ASG protein profile of day 1 adult controls

Lane 2: ASG protein profile of day 1 adults treated with 20 µg PPN immediately after eclosion on day 0

Lane 3: ASG protein profile of adultoids

Lane 4: ASG protein profile of day 0 adult controls

Lane 5: Molecular weight markers

### 3.6 Discussion:

An inhibitory action of JH analogues on the development and differentiation of ASGs has been reported by several authors (Landa & Metwally, 1974; Ramalingam & Craig, 1977; Gelbic & Metwally, 1981; Roychoudhury & Chakravorty, 1987). In the present study also, a significant amount of retardation is noted in the development and differentiation of ASGs. Happ (1984) reported that usually the ASG development takes place in pupal period in holometabolous insects. Studies show that

there is a decrease in JH titre in pupal period in insects. In *Locusta migratoria*, the committance for the terminal differentiation of ASGs is triggered during the critical period when there is a decrease in the JH titre (Baehr et al., 1979).

In the present study, in PPN treated males, ASGs were fused as seen in the early pupal stage of normal/control insects. Similar observations were reported by other workers (Cantacuzene, 1968; Szollosi, 1975). In the present study, degenerative, irregular and inhibitory effects on the development of ASGs might be due to the high titre of JH circulating in the haemolymph due to treatment. In *S. mauritia*, the inhibitory effect of PPN is more conspicuous as this JH agonist was applied to the pupae when the endogenous titre of JH was minimum or absent. Studies of Mariamma (1989) in *Oryctes rhinoceros* show a dose dependent effect of JHA with regard to differentiation of ASGs.

Previous studies show that development and differentiation of ASGs involve cell multiplication, increases in cell size and acquisition of competence to make adult specific proteins accompanying rapid synthesis of secretory proteins. According to Highnam & Hill (1969), in insects, cell division and differentiation are intrinsic properties of the cell, residing in the genes and that the particular expression at any stage is controlled by JH. In *T. molitor*, development and differentiation of ASGs occurring in pupal stage are characterized by two bouts of mitosis (Grimes & Happ, 1980; Happ & Happ, 1982; Happ et al., 1985). The first bout of mitosis is not ecdysteroid dependent while the second required the addition of physiological amounts of ecdysterone. The second bout of mitosis coincides with the peak of ecdysteroid in the pupal stage (Delbecque et al., 1978). Yaginuma et al. (1988) describe that ecdysteroids promote the flow of cells from the G2 into the G1 and S phases of cell cycle.

An excess of JH or its analogues in the haemolymph might inhibit the ASG development in two different ways. It might directly block the cell division and differentiation of ASG cells. It is assumed that in *S. mauritia*, the cells of ASGs might have undergone the first bout of mitosis as evidenced by the presence of a proliferated mass of cells. At a later stage, cell proliferation got arrested possibly due to the high titre of JH in the haemolymph. Alternatively, the second bout of mitosis might not have occurred possibly due to a failure of an ecdysteroid peak or a decrease in the ecdysteroid titer as explained by earlier workers (Zufelato et al., 2000). Many authors have opined that degradation of JH mimics takes place rapidly (Gilbert et al., 2000; Kamita et al., 2003) but Edwards et al. (1993) suggest that the action of PPN has a delay of approximately 24 h. Mona (2001) showed that in *S. mauritia*, the ecdysteroid peak reaches maximum in day 2 pupae and then diminishes in subsequent days. This means that even though PPN is applied on day 0 pupae, it may induce a delayed effect to block the ecdysteroids peak. A disruption in the differentiation of the cells might have affected the maturation of the cells, which explains why secretory material is not observed in the lumen of ASG of treated insects.

In *S. mauritia*, PPN exerts different effects in the protein synthesis of ASGs when applied in different phases of life cycles. Amount of protein decreases significantly in ASGs when PPN is applied on newly ecdysed pupae, whereas the amount of protein significantly increases in the ASGs when PPN is applied on newly eclosed adults. These results show dual effects of PPN in two different phases of the life cycle of *S. mauritia*. These antagonistic effects of PPN could be explained based on the physiological role of JH in natural conditions. JH is a unique developmental hormone in several aspects. During metamorphosis, JH blocks the expression of subset of genes that specifies the imaginal phenotype and during adult reproductive stage, it activates the expression of subset of genes that are necessary for reproduction (Jones et al., 1993).

In insects, the titre of JH is usually controlled by JH-specific esterases and some other mechanisms (Gade et al., 1997; Gilbert et al., 2000; Kamita et al., 2003). However, treatments of PPN may disrupt the regulatory mechanisms resulting in the presence of detrimentally high JH titres. This will disrupt not only the endocrine physiology but also might cause pharmacological effects (Webb et al., 1999; Wilson, 2004). In *Locusta migratoria*, PPN repressed the synthesis of two proteins and stimulated the synthesis of another high molecular weight protein (de Kort & Koopmanschap, 1991). It is assumed that PPN has impaired protein synthesis in the ASGs of 0.1 µg treated insects. In *Aedes aegypti*, JHA impaired the capacity of fat bodies of pupae to synthesize proteins, resulting in a lowered concentration of fat body proteins (Gordon & Burford, 1984).

There are evidences that JH affects the post transcriptional processing or translation processes. The ability of JH to prevent translation of the transcripts for the basic hexameric proteins of *Trichoplusia* after it no longer affected their gene transcripts supports the possible role at this level (Jones et al., 1993). Another possibility is that PPN might have hindered the synthesis of protein products by inhibiting the transcription of many genes or by promoting the degradation of RNAs.

Previous studies show that the secretory function of ASGs in adults (reproductive stage) is under the influence of JH. In many insect species including *Drosophila melanogaster*, JH has been shown to influence post-ecdysial maturation of male

ASGs. A rapid increase in the JH titer in the newly eclosed adults is reported in *D. melanogaster* and is a probable key feature in the maturation of gametes and testes (Bownes & Rembold, 1986). In male moths of *Ephestia cautella*, ecdysteroid titres are relatively low throughout their adult life (Shaaya et al., 1991). Studies of Gillott & Friedel (1976) show that treatment of synthetic JH in adult *Melanoplus sanguinipes* results in an increase in the protein content of ASGs. They further suggest that synthesis of proteins takes place in fat body under the stimulatory effect of the JH analogue and these proteins are incorporated into the secretions of ASGs. In *S. mauritia* also, the increase in the staining intensity of the peptides is thought to be correlated with the stimulatory effect of PPN on the enhancement of ASG proteins in adult insects.

#### IV. CONCLUSION

The newly ecdysed day 0 pupae, topically applied with 1 µg/µl PPN showed hundred percent mortality on day 4. In 0.1 µg/µl PPN treated pupae, only 61% mortality was observed but the development of ASGs were highly reduced in both sexes when compared to control. The total ASG proteins of male adultoids were significantly reduced than that of emerged control male insects. Their electrophoretic profile showed less staining intensities in most peptide bands as well as disappearance of some bands when compared to that of control. Day 0 adult males showed no mortality when applied with a single high dose of PPN. Their total protein of ASGs significantly increased than that of the control insects and the staining intensities of most of the peptides were considerably more when compared to that of control. This study demonstrates the dual, stage-dependent effect of Pyriproxyfen on the reproductive physiology of *Spodoptera mauritia*—inhibitory during pupal development and stimulatory in adults—highlighting its potential as an effective IGR for pest management strategies

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#### CONFLICT OF INTEREST

The authors declare no conflict of interest.

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# Eco-Friendly Insect Pest Management of Mustard Plant: A Review

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**Abstract**— Mustard (*Brassica juncea*) is an essential oilseed crop among brassicas, primarily cultivated during the Rabi season in tropical regions worldwide. Like other crops of Brassicaceae family, mustard is attacked by various insect pests. Among these pests, mustard aphid, mustard sawfly, painted bug, diamondback moth, green peach aphid, cabbage butterfly, and leaf webber are major pests of mustard plant that affect the economic value of mustard and related crops by causing severe yield losses and decreasing market value. These pests majorly affect multiple parts of plant including leaves, flowers, flower buds, stems, pods, and twigs. Major physiological effects include curling of leaves, reduced photosynthetic efficacy due to secretions of sticky honeydew that facilitates sooty mold development, and failure of young pods to mature properly. Mustard is susceptible to attack by the mustard aphid *Lipaphis erysimi* (Kalt.), a significant sucking pest affecting mustard and other Brassicaceae crops. Both nymphs and adults of this pest suck the cell-sap from plant parts, leading to stunted plant growth, wilting flowers, and impaired pod development. Additionally, their feeding activity introduces toxic substances into the plants, causing chlorosis at feeding sites, yellowing of veins, and leaf curling. Yield losses up to 73.3% and oil content reductions up to 66.9% have been reported. Numerous cost-effective control methods, including cultural, mechanical, biological, and botanical approaches have been identified to manage mustard pests effectively within an Integrated Pest Management (IPM) framework. IPM aids in minimizing ecological damage and reliance on chemical pesticides by utilizing natural enemies, entomopathogenic organisms, and botanical insecticides. This review synthesizes information on major insect pests of mustard and their eco-friendly management strategies.

**Keywords**— Mustard, Aphids, Eco-friendly management, *Lipaphis erysimi*, Insect pest management, IPM.

## I. INTRODUCTION

### 1.1 Importance of Mustard in India:

Mustard is the major Rabi season oilseed crop grown throughout India (Dhaliwal, 2022). Mustard crop belongs to the family Brassicaceae/Cruciferae (de Jussieu, 1789). This oilseed crop plays an important role in the agricultural economy of India (Sharma, 2015). India is one of the largest mustard growing countries in the world, occupying the third position in area and production after China and Canada, contributing 12% of the world's total production (Singh and Patel, 2023). *Brassica juncea* is the second most important oilseed crop in the country after groundnut and accounts for nearly 30.7% of the total oilseed production (Singh et al., 2016).

In India, mustard is predominantly cultivated in Rajasthan (50%), Uttar Pradesh (12.3%), Haryana (11.2%), Madhya Pradesh (9.8%), Gujarat (6.5%), and West Bengal (5.1%) (Sharma et al., 2011). Among these states, Rajasthan, Uttar Pradesh, and Madhya Pradesh are the major rapeseed-mustard growing states, covering 70% of the total national acreage and contributing around 72% of production (Grant Thornton, 2014-2015). Uttar Pradesh is a leading mustard producing state of India (Kumar

and Singh, 2023), with 60% of total mustard production coming from this state. In Uttar Pradesh, the area under mustard cultivation is 759 hectares with production of 956.72 tonnes and yield of 1260 kg/ha.

### 1.2 Pest Problem in Mustard:

Mustard crops are vulnerable to attack by a range of insect pests, including the painted bug, mustard sawfly, mustard aphid, potato aphid, leaf miner, flea beetle, diamondback moth, Bihar hairy caterpillar, cabbage butterfly, and tobacco caterpillar (Verma et al., 2023). Among these, the mustard aphid *Lipaphis erysimi* (Kaltenbach) is considered the most destructive pest (Verma, 2023). This aphid species not only causes substantial seed yield losses—reported to be as high as 73.3%—but also significantly reduces oil content in the seeds, with recorded losses up to 66.9% (Bakhetia and Sekhon, 1989). Mustard is a major oilseed crop in the north-western region of Madhya Pradesh, and *L. erysimi* (Hemiptera: Aphididae) is recognized as a key pest that inflicts severe damage (Verma et al., 2023).

The aphids feed by sucking sap from tender shoots and floral parts during the early growth stages and later from the immature pods (Gurung and Tamang, 2023). Infested plants exhibit symptoms such as stunted growth and weakened structural integrity (Verma et al., 2023). In addition, the pest secretes large quantities of honeydew, promoting the development of black sooty mould on the leaves, which further hampers photosynthetic efficiency (Shubham, 2024).

### 1.3 Limitations of Chemical Control:

While the use of systemic insecticides has proven effective in managing this pest, their application can negatively impact natural enemies, including predators and parasitoids (Rhoilla et al., 2020). Moreover, chemical control presents several limitations, including the development of insecticide resistance, pest resurgence, outbreaks of secondary pests, and the accumulation of pesticide residues in oil and oilseed cake beyond permissible limits (Ramana et al., 2018). Environmental degradation is also a significant concern (Singh and Sharma, 2009).

The sustainable management of mustard aphids presents a significant challenge due to their complex life cycle, broad geographic distribution, and increasing resistance to a wide range of insecticides (Bhattacharya, 2019). In Bangladesh, the conventional method of pest control largely depends on the use of synthetic chemical insecticides (Akhter et al., 2022). However, farmers frequently apply these pesticides without adequate consideration of their efficacy, specificity to the target pests, or potential adverse impacts (Dasgopta et al., 2005). It is a common practice to use excessive doses and apply them at high frequencies, particularly during severe aphid infestations (Khatun et al., 2023).

Such indiscriminate and improper use of chemical insecticides can have serious ecological consequences (Barmon and Chaki, 2021). These include the destruction of beneficial insect populations, adverse effects on non-target organisms, the resurgence of primary pests, outbreaks of secondary pests, and the accelerated development of pesticide resistance (Sekhon and Ahman, 1993). Moreover, synthetic insecticides pose significant threats to both environmental health and human safety (Aktar et al., 2009).

### 1.4 Need for Eco-Friendly Management:

Alternatively, plant-based extracts and botanical insecticides are considered to be more environmentally benign (Khursheed and Malik, 2022). These substances are generally less toxic to non-target organisms, more biodegradable, and therefore pose fewer risks to ecosystems and human health (Pathak et al., 2022). In recent studies, short persistent pesticides and non-chemical control options have been evaluated in field trials for efficient management of mustard aphids (Dhillon et al., 2022).

### 1.5 Objectives of the Review:

This review aims to: (1) document the major insect pests affecting mustard (*Brassica juncea*) in India, (2) describe their biology, damage symptoms, and economic impact, and (3) synthesize eco-friendly management strategies within an Integrated Pest Management (IPM) framework.

## II. MAJOR INSECT PESTS OF MUSTARD

### 2.1 Mustard Aphid: *Lipaphis erysimi* (Kaltenbach, 1843) (Hemiptera: Aphididae):

*Lipaphis erysimi*, known as mustard aphid, is a prevalent pest affecting cruciferous crops like mustard and rapeseed, particularly from December to March. These pale-green aphids feed on plant sap, causing stunted growth, leaf yellowing, and significant crop damage, especially under cold, cloudy conditions below 20°C. Reproducing parthenogenetically, they can produce 26-133 nymphs that mature in 7-10 days, leading to up to 45 generations annually. Both nymphs and adults extract

sap from leaves, stems, and pods, resulting in curled leaves, wilting, and impaired pod formation, which can reduce crop yields by 75-80%. Affected fields often appear blighted due to sooty mold growth on the honeydew excreted by the aphids.

## **2.2 Painted Bug: *Bagrada cruciferarum* (Burmeister, 1835) (Hemiptera: Pentatomidae):**

This species is found in Myanmar, Sri Lanka, India, Arabia, and East Africa. It has a 19-54-day life cycle with 9 generations annually. Nymphs reach 4 mm, adults 3.71 mm, with black bodies and orange/brown spots. Active from March to December, they overwinter in dried oilseed debris. Females lay 37-102 pale-yellow eggs, hatching in 3-20 days, with nymphs maturing in 22-34 days. Both nymphs and adults feed on leaf and pod sap, causing wilting and plant weakening. Their resinous secretions contaminate pods, reducing seed quality and promoting secondary infections.

## **2.3 Mustard Sawfly: *Athalia lugens proxima* (Klug, 1815) (Hymenoptera: Tenthredinidae):**

This species is common in Indonesia, Formosa, Myanmar, and the Indian subcontinent. Larvae are dark green with five black stripes, reaching 16-18 mm. Adults are small and orange-yellow with black markings. They breed from October to March, living 2-8 days and laying 30-35 eggs on leaf margins. Eggs hatch in 4-8 days, with larvae maturing in 16-35 days through seven instars. The larvae, the primary destructive stage, skeletonize leaves and occasionally consume shoot epidermis, compromising photosynthesis and plant growth. Pupation occurs in soil for 11-31 days, completing the life cycle in 31-34 days with 2-3 generations per season. This feeding behavior leads to substantial yield losses, especially in seedlings and seed production in older plants.

## **2.4 Green Peach Aphid: *Myzus persicae* (Sulzer, 1776) (Hemiptera: Aphididae):**

These aphids, 2.0-2.5 mm and yellowish-green, are active across India from December to March, peaking in February. Nymphs mature in 4-8 days, with adults living 10-27 days and producing up to 92 offspring. Both nymphs and adults extract sap, leading to flower drop, poor pod formation, and grain shriveling. They also transmit viral diseases and excrete honeydew, promoting sooty mold, which further reduces photosynthesis and plant health.

## **2.5 Cabbage Butterfly: *Pieris brassicae* (Linnaeus, 1758) (Lepidoptera: Pieridae):**

This widespread pest in India primarily targets cabbage, cauliflower, and other cruciferous plants. The larvae are pale yellow to green, growing up to 40-50 mm. Adult butterflies have pale white wings with black patches. Active from October to April in plains and breeding in mountains from May to September, females lay 50-90 eggs that hatch in 3-17 days. Caterpillars initially feed in groups, dispersing as they mature over 15-40 days. They cause significant damage by feeding on leaves, young shoots, and pods, often leading to severe defoliation, with only the main veins left intact, and stunted plant growth. Pupation occurs away from food plants for 7-28 days, with adults living 3-12 days and completing four generations annually.

## **2.6 Diamondback Moth: *Plutella xylostella* (Linnaeus, 1758) (Lepidoptera: Plutellidae):**

This globally prevalent pest primarily affects cabbage, cauliflower, and other cruciferous and solanaceous plants. Females lay 18-356 yellowish eggs on leaf undersides, hatching in 2-9 days. Pale yellow-green larvae, 8-12 mm long with fine black hairs, complete their larval stage in 8-16 days. Pupation in a silken cocoon lasts 4-5 days, with adult moths living about 20 days. The life cycle completes in 15-18 days, with multiple generations per year. First instar larvae mine leaves, creating white patches; later instars perforate leaves, often leaving only veins intact in severe infestations.

## **2.7 Cabbage Leaf Webber: *Crociodolomia pavonana* (Zeller, 1852) (Lepidoptera: Pyralidae):**

This pest targets cabbage, radish, mustard, and other cruciferous plants. The adult moth is small with light brown forewings. Females deposit eggs in clusters of 40-100 on the underside of leaves, which hatch within 5-15 days. The caterpillar features a red head, brown longitudinal stripes, and rows of tubercles along its body, with a larval stage lasting 24-27 days. Pupation occurs inside a cocoon within the webbed leaves, taking 14-40 days. The caterpillar webs together foliage, consuming leaves, flowers, and pods (in mustard), or flower heads (in cabbage and cauliflower), causing skeletonization of leaves and leaving faecal matter within the webbed areas.

## **2.8 Ladybird Beetle: *Coccinella septempunctata* (Linnaeus, 1758) (Coleoptera: Coccinellidae) - Beneficial Predator:**

Coccinellidae, commonly known as ladybird beetles or lady beetles, are small, dome-shaped insects ranging from 0.8 to 18 mm in size, with females typically larger than males. Their hardened forewings (elytra) come in a variety of colors including red, orange, yellow, and pink, often with distinctive spots. Ladybugs use their antennae for both olfaction and gustation.

They undergo complete metamorphosis, transitioning through egg, larval, pupal, and adult stages. Larvae are voracious predators, and adults continue this feeding behavior. Most species are beneficial predators, feeding primarily on soft-bodied pests such as aphids, scale insects, and mites, making them valuable for biological pest control.

**TABLE 1**  
**MAJOR INSECT PESTS OF MUSTARD (*BRASSICA JUNCEA*) IN INDIA**

Common Name	Scientific Name	Family	Order	Plant Parts Attacked	Peak Activity	Nature
Mustard aphid	<i>Lipaphis erysimi</i>	Aphididae	Hemiptera	Leaves, stems, pods	Dec-Mar	Pest
Painted bug	<i>Bagrada cruciferarum</i>	Pentatomidae	Hemiptera	Leaves, pods	Mar-Dec	Pest
Mustard sawfly	<i>Athalia lugens proxima</i>	Tenthredinidae	Hymenoptera	Leaves	Oct-Mar	Pest
Green peach aphid	<i>Myzus persicae</i>	Aphididae	Hemiptera	Leaves, flowers	Dec-Mar	Pest
Cabbage butterfly	<i>Pieris brassicae</i>	Pieridae	Lepidoptera	Leaves, pods	Oct-Apr	Pest
Diamondback moth	<i>Plutella xylostella</i>	Plutellidae	Lepidoptera	Leaves	Year-round	Pest
Cabbage leaf webber	<i>Crocidolomia pavonana</i>	Pyralidae	Lepidoptera	Leaves, flowers, pods	-	Pest
Ladybird beetle	<i>Coccinella septempunctata</i>	Coccinellidae	Coleoptera	Feeds on aphids	-	Beneficial

### III. ECO-FRIENDLY MANAGEMENT STRATEGIES FOR MUSTARD PESTS

Effective pest management in mustard involves a combination of cultural, mechanical, biological, and need-based chemical controls within an Integrated Pest Management (IPM) framework.

#### 3.1 Field Monitoring and Scouting:

Monitoring and scouting are key parts of effective pest control (Schneider and Rebek, 2016). They should begin right after planting or transplanting the crop (Edward et al., 1986). Fields need to be checked regularly, about once a week, during the entire growing season to find pests early and understand their population levels (Werling, 2015).

**Pest Monitoring Protocol:** In each field, choose several spots carefully to check for pests (Pedigo and Rice, 2014). Record what kinds of pests are present and how many there are, following specific guidelines for each pest type. This focused method helps understand pest behavior and allows for quick and appropriate control actions.

- **Aphids and Painted Bugs:** Check both nymphs and adults by counting how many are on five randomly chosen leaves of each plant. Regular monitoring helps track population changes and decide the best time for control measures.
- **Leaf Miners:** To check for leaf miner attacks, count the number of active tunnels on five randomly chosen leaves of each plant. This indicates infestation severity and whether control measures are needed.



**Mustard aphid**  
*(Lipaphis erysimi)*



**Painted bug**  
*(Bagrada cruciferarum)*



**Mustard sawfly**  
*(Athalia lugens proxima)*



**Green peach aphid**  
(*Myzus persicae*)



**Cabbage butterfly**  
(*Pieris brassicae*)



**Diamond black moth**  
(*Plutella xylostella*)



**Cabbage leaf weevil**  
(*Crocidolomia pavonana*)



**Ladybird**  
(*Coccinellidae septempunctata*)

**FIGURE 1: Major insect, pest of mustard plant**

### 3.2 Cultural Control Methods:

Cultural practices play a vital role in the sustainable management of insect pests in mustard cultivation (Roy, 2023). Clean cultivation and regular weeding help create unfavorable conditions for pest development by eliminating alternate hosts and reducing pest survival chances (Kumar et al., 2025). Such practices are also effective in managing dormant stages of pests, either by burying them deep within the soil or by exposing them to adverse environmental conditions (FAO, 2008).

Several cultural control measures have been identified as effective for mustard pest management (Mishra et al., 2023):

- **Early sowing** of the crop minimizes damage caused by mustard aphid (*Lipaphis erysimi*)
- **Adoption of tolerant varieties** contributes to aphid control
- **Judicious use of nitrogenous fertilizers** helps reduce pest incidence
- **Deep ploughing of soil** is recommended to destroy the eggs of the painted bug (*Bagrada hilaris*) (Agricultural University Agriculture Technology Portal, 2014)
- **Irrigating the crop** during the fourth week after sowing helps reduce pest infestation
- **Timely threshing** of the harvested crop prevents further damage by the painted bug (Tamil Nadu Agricultural University, 2014)

Among the various cultural control measures, early sowing is considered the most critical practice for minimizing losses from major pests such as mustard aphid, painted bug, and mustard sawfly (*Athalia proxima*) (Tamil Nadu Agricultural University, 2014). The integration of these practices forms an essential component of an eco-friendly and sustainable pest management strategy in mustard cultivation.

### 3.3 Mechanical and Physical Control Methods:

Mechanical and physical methods are important components of IPM strategies for mustard crops (Roy, 2025). These approaches primarily involve the removal or destruction of infested plant parts and the manual collection and elimination of

larger insect pests, generally through the use of human labor (Penn State Extension, 2011). Various mechanical devices may also be installed in the field for monitoring and controlling specific pest populations (Cardim Ferreira Lima et al., 2020).

Specific mechanical control measures include:

- At the initial stage of aphid infestation, the affected plant parts along with the aphid colonies are **removed and destroyed** to prevent further spread
- **Yellow sticky traps** are installed in the field to monitor and reduce the aphid population (Singh and Lal, 2012)
- For management of painted bugs, the leaves and stems of plants are **shaken or jerked** to dislodge the insects, which are subsequently destroyed using a kerosene oil solution (Tamil Nadu Agricultural University, 2014)
- **Manual collection and destruction of larvae**, particularly during early morning and evening hours, are practiced to manage mustard sawfly, Bihar hairy caterpillar, and diamondback moth populations (Tamil Nadu Agricultural University, 2014)
- **Pheromone traps** installed at a density of four traps per hectare are employed for the monitoring and control of diamondback moth infestations (Fredon, 2009; Laore, 2010; Ben Husin, 2017)

These mechanical and physical measures help maintain pest populations below the Economic Threshold Level (ETL) and reduce dependence on chemical control methods, thereby contributing to sustainable mustard production (Roy, 2025).

### 3.4 Biological Control Methods:

Farmers often use chemical pesticides to protect their vegetable crops from insects (Shah et al., 2019). However, using too many of these chemicals can cause several problems, such as harming human health, making pests resistant to the chemicals, and polluting the environment (Aktar et al., 2009). As pests become more resistant and environmental damage increases, it is important to find safer and more effective ways to control them (Couto et al., 2019).

One such method is biological control, which uses natural enemies like predators, parasitoids, or disease-causing microorganisms to reduce pest populations. This approach is a key part of sustainable farming, which aims to produce food in the long term without harming nature. Another option is using botanical pesticides made from plants. These products are effective against insects, break down easily in the environment, and cause little harm to other animals (Lengai et al., 2020).

**TABLE 2**  
**MAJOR BIOLOGICAL CONTROL AGENTS FOR MUSTARD PESTS**

Type	Examples	Target Pests
Predators	Ladybird beetles ( <i>Coccinella septempunctata</i> ), syrphid flies, lacewings, spiders	Aphids, soft-bodied insects
Parasitoids	<i>Diaeretiella rapae</i> (aphid parasitoid), <i>Trichogramma</i> spp., <i>Apanteles</i> spp., <i>Bracon</i> spp.	Aphids, lepidopteran larvae
Entomopathogens	<i>Beauveria bassiana</i> , <i>Metarhizium anisopliae</i> , <i>Verticillium lecanii</i> , <i>Bacillus thuringiensis</i> (Bt)	Aphids, caterpillars, various insects
Botanical insecticides	Neem ( <i>Azadirachta indica</i> ), karanj ( <i>Pongamia pinnata</i> ), mahua ( <i>Madhuca indica</i> ), <i>Annona squamosa</i>	Multiple pests

Biological control is cost-effective, eco-friendly, and long-lasting. It offers many benefits for the environment, economy, and society (Saleh et al., 2017).

### 3.5 Chemical Control (as Last Resort):

Chemical control becomes necessary when aphid populations exceed established action thresholds or when natural enemies are unable to regulate rapidly increasing populations (Jain and Tiwari, 2017). However, indiscriminate use of chemical insecticides poses a significant threat to the agro-ecosystem, raising concerns about their sustainability. Therefore, selective insecticidal applications have been evaluated and recommended by several researchers for the management of mustard aphid in different regions globally.

Chemical insecticides are generally classified into two types: contact and systemic. Since aphids typically colonize the abaxial surface of leaves and feed by inserting their stylets directly into the phloem, contact insecticides are often ineffective. In contrast, systemic insecticides are absorbed by the plant and translocated through the phloem, allowing them to effectively control aphids irrespective of their feeding sites, including those under the leaves. The major groups of insecticides used against aphids include carbamates, organophosphates, pyrethroids, and neonicotinoids (Bahlai et al., 2010; Cameron et al., 2005).

#### Important Considerations for Chemical Control:

- Apply only when pest populations exceed Economic Threshold Level (ETL)
- Select insecticides with low toxicity to natural enemies
- Rotate insecticides from different groups to prevent resistance development
- Follow recommended doses and application timings
- Observe pre-harvest intervals to minimize residues

Continuous use of organophosphate insecticides has led to the development of resistance in aphid populations (Gould, 1996), emphasizing the need for integrated approaches.

#### 3.6 Integrated Pest Management (IPM) Framework:

IPM is a sustainable approach that combines all available pest control methods to keep pest populations below damaging levels while minimizing environmental impact. For mustard crops, an effective IPM strategy includes:

1. **Regular monitoring and scouting** to assess pest populations and natural enemy activity
2. **Cultural practices** (early sowing, tolerant varieties, clean cultivation, balanced fertilization)
3. **Mechanical controls** (sticky traps, pheromone traps, manual removal)
4. **Biological controls** (conservation of natural enemies, augmentation, botanicals)
5. **Need-based chemical control** using selective, safer insecticides when thresholds are crossed

## IV. SUMMARY AND CONCLUSION

Mustard (*Brassica juncea*) is a vital Rabi oilseed crop in India, playing a crucial role in the agricultural economy. However, its productivity is severely constrained by insect pest attacks, particularly from the mustard aphid (*Lipaphis erysimi*), which can cause up to 73.3% yield loss and 66.9% reduction in oil content. Other significant pests including painted bug (*Bagrada cruciferarum*), mustard sawfly (*Athalia lugens proxima*), green peach aphid (*Myzus persicae*), cabbage butterfly (*Pieris brassicae*), diamondback moth (*Plutella xylostyla*), and cabbage leaf webber (*Crocidolomia pavonana*) compound the damage across leaves, stems, flowers, and pods.

The over-reliance on synthetic chemical insecticides has led to numerous problems including pest resistance, resurgence of secondary pests, destruction of natural enemies, environmental pollution, and health hazards. These challenges necessitate the adoption of eco-friendly pest management approaches.

Integrated Pest Management (IPM) offers the most sustainable solution by integrating multiple tactics:

- **Cultural methods:** early sowing, deep plowing, clean tillage, tolerant varieties, balanced fertilization
- **Mechanical controls:** sticky traps, pheromone traps, manual collection and destruction of pests
- **Biological agents:** predators (ladybird beetles, syrphid flies), parasitoids (*Diaeretiella rapae*, *Trichogramma* spp.), entomopathogens (*Beauveria bassiana*, *Metarhizium anisopliae*, *Verticillium lecanii*, *Bacillus thuringiensis*)
- **Botanical insecticides:** neem, karanj, mahua, and other plant-based products
- **Need-based chemical interventions** using selective, safer insecticides only when economic thresholds are crossed

Regular field monitoring and scouting enable timely action, curbing resistance development, pesticide residues, and ecological harm while protecting natural enemy populations. The adoption of eco-friendly management strategies is

particularly important in major mustard-growing states including Rajasthan, Uttar Pradesh, and Madhya Pradesh, which together account for over 70% of national production.

#### Future research should prioritize:

1. Development of pest-resistant mustard varieties through conventional and molecular breeding approaches
2. Evaluation of novel biopesticides and botanical formulations for field efficacy
3. Refinement of economic threshold levels for different pests and regions
4. Understanding pest-natural enemy dynamics under changing climate scenarios
5. Documentation and validation of traditional pest management practices
6. Capacity building of farmers through extension programs on IPM adoption

The integration of these eco-friendly approaches will contribute to resilient and sustainable mustard production systems, ensuring food and oil security while protecting environmental health and farmer livelihoods.

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#### CONFLICT OF INTEREST

The authors declare no conflict of interest.

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# Effect of Nutritional Supplement Treated *Quercus serrata* Leaves on Life Cycle and Economic Traits of Oak Tassar Silkworm (*Antheraea proylei*)

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**Abstract**— The present study examines the effect of nutritional supplementation of *Quercus serrata* leaves with various bioactive additives: amla (*Embllica officinalis*), neem (*Azadirachta indica*), spirulina (*Arthrospira platensis*), tulsi (*Ocimum sanctum*) and sericin on the life cycle performance and economic characteristics of oak tassar silkworm (*Antheraea proylei*). This experiment was conducted during the spring season (August-December) in 2025 at the Central Tassar Research and Training Institute, Chauntra, District Mandi (H.P.). The goal was to assess how these additives affect larval growth, cocoon quality and silk yield parameters. *Quercus serrata* leaves were uniformly treated with aqueous extracts of selected supplements and fed to 5th instar larvae under controlled growth conditions. Main biological parameters - larval length, survival percentage, effective rearing rate (ERR%), cocoon weight, shell weight, pupal weight and shell ratio were recorded and compared to an untreated control.

The results revealed that supplementation significantly affected larval growth and silk productivity. Among treatments, spirulina and amla showed the most pronounced positive effects, leading to reduced larval duration, higher survival and improved cocoon and shell weight. Neem and tulsi treatments showed moderate improvement, while sericin supplementation showed the most improvement in quality and luster of silk filament. The combined effect of nutritional fortification was reflected in superior economic traits and potential improvement in silk feed performance.

Overall, the study shows that supplementation of *Quercus serrata* leaves can effectively improve the physiological performance and silk yield of *Antheraea proylei*, suggesting an ecological approach and profitable strategy for sustainable oak tassar culture.

**Keywords**— *Antheraea proylei*, *Quercus serrata*, food supplementation, spirulina, amla, neem, tulsi, sericin, silk productivity, economic characteristics.

## I. INTRODUCTION

Silk production has been an integral part of India's rural economy and traditional culture for centuries (Reddy et al., 2018). Among the various types of silk produced globally, oak tassar silk, obtained from the semi-domesticated silkworm *Antheraea proylei* (Lepidoptera: Saturniidae), holds a special place for its natural golden color, strength and eco-friendly characteristics (Kumar and Sinha, 2019). This species, a hybrid between *Antheraea pernyi* (Chinese tassar silkworm) and *Antheraea proylei* (wild Indian silk worm), develops mainly in temperate and subtropical regions of the Himalayas, feeding on the leaves of oak species such as *Quercus serrata* (Singh et al., 2020). The productivity and quality of oak tassar silk are profoundly influenced by the nutritional quality of the host plant leaves and the physiological state of larvae during their development (Devi and Gogoi, 2021).

In sericulture, host plant nutrition directly affects silkworm growth, cocoon yield, silk filament length, and general economic characteristics (Patnaik and Jolly, 2017). Unlike the domesticated *Bombyx mori*, which feeds on mulberry leaves, the nutritional profile of oak leaves for *Antheraea proylei* fluctuates depending on season, soil fertility and environmental stress, often resulting in sub-optimal larval performance (Tiwari et al., 2019). Therefore, exploring ways to improve the nutritional value of host leaves through supplementary feeding or enrichment with bioactive additives has emerged as a promising strategy to enhance the biological and economic characteristics of tasar silk production (Rana and Ahmed, 2022).

In recent years, nutritional supplementation based on natural extracts, microbial biomass and protein additives has attracted considerable attention in silkworm research (Verma et al., 2020). Supplements like *Emblica officinalis* (amla), *Azadirachta indica* (neem), *Ocimum sanctum* (tulsi), *Spirulina platensis* and sericin (a silk protein) are known for their rich biochemical composition and potential to improve insect physiology, immunity and silk gland activities (Borah and Devi, 2021; Prasad et al., 2023). These substances contain a wide spectrum of vitamins, minerals, amino acids, antioxidants, flavonoids and antimicrobial compounds, which can enhance the nutritional quality of the larval diet and the general health of silkworm larvae (Mandal and Nath, 2018).

### 1.1 Life Cycle of Silkworm:

*Antheraea proylei* belongs to the order Lepidoptera under the class Insecta. It is characterized by the presence of two membranous wings with few transverse veins and flat scales on the body and appendages. The larvae are called caterpillars and are euciform, peripneustic, with three pairs of true legs and five pairs of pseudolegs. The silk of this insect is considered more premium. It undergoes complete metamorphosis, with four stages: egg, larva, pupa and adult (butterfly). In general, the duration of each stage varies according to climate, genetics, quality and type of food. Based on the number of generations per year, it can be classified as univoltine, bivoltine and multivoltine. Multivoltine races have the shortest life cycle: egg stage lasts approximately 12 days, larva 20-24 days, pupa 10-12 days and adult 3-6 days.

- **Eggs:** These are round and white, about 1 to 1.3 mm in length and 0.9 to 1.2 mm wide (Buhroo, 2019). In temperate regions, diapause eggs are laid, while non-diapause eggs are usually laid in the subtropics. Over time, eggs become darker in color.
- **Larvae:** The eggs hatch into caterpillars after 10 days of incubation. Newly hatched caterpillars are approximately 0.3 cm long and pale yellow in color. The body of the larvae is smooth and lighter in color, with nodules found throughout the body. They are voracious feeders, requiring a constant supply of food. The larvae pass through four molts and five instars. Mature larvae develop a pair of silk glands, which are modified labial glands. These glands secrete silk composed of sericin, a water-soluble outer protein, and fibroin, a hard inner protein. Females have one pair of milky white spots, known as Ishiwata's anterior glands, present between the eighth and ninth segments, while males have a small milk-white body called Herold's gland, present in the center of segments 8 and 9. Both glands appear ventrally and can be observed in the fourth and fifth instar larvae.

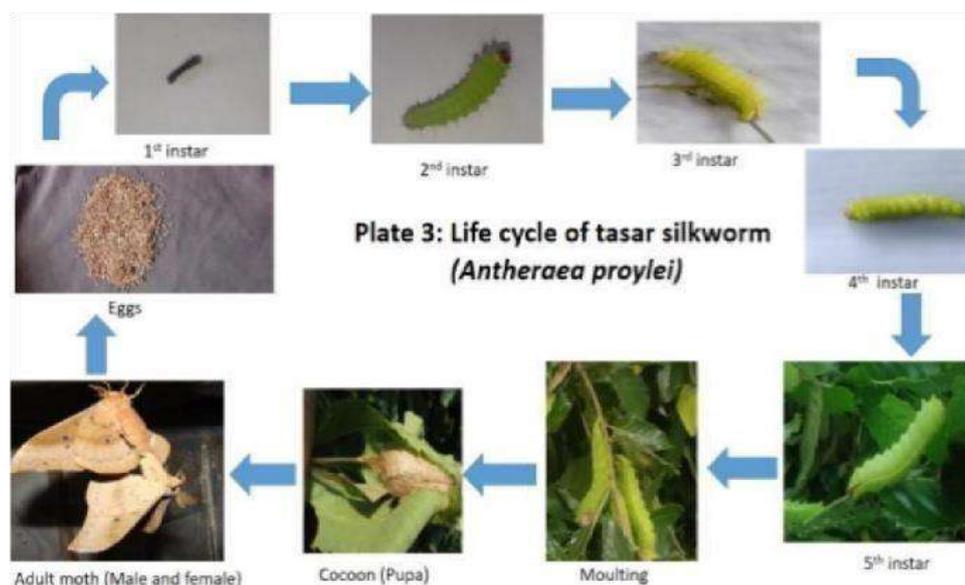


FIGURE 1: Life cycle of tasar silkworm

### 1.2 Effect of Amla (*Emblica officinalis*) Supplementation:

Amla is a potent natural antioxidant rich in vitamin C, polyphenols, and tannins. Studies have shown that supplementation with amla extracts enhances protein assimilation, improves digestive enzyme activity, and strengthens immune responses in silkworms (Sharma et al., 2016; Patel and Verma, 2018). Its adaptogenic properties help larvae better tolerate environmental stress, resulting in higher larval survival and superior silk yield (Rao et al., 2020). When used to treat *Quercus serrata* leaves, amla may enhance the nutritional profile by promoting better nitrogen assimilation and improving silk gland development in *A. proylei* (Kumar and Singh, 2019). The present study corroborates these findings, with amla-treated groups showing improved cocoon weight and shell ratio.

### 1.3 Effect of Neem (*Azadirachta indica*) Supplementation:

Neem possesses bioactive compounds such as azadirachtin, nimbin, and salannin, which have antibacterial and antifungal properties (Chaudhary et al., 2015). In sericulture, neem supplementation has been reported to enhance larval immunity and reduce disease incidence, especially against microbial infections that commonly affect silkworm crops (Das and Banerjee, 2019). Low concentrations of neem leaf extracts may act as growth promoters by stimulating metabolic activity and detoxification enzymes, although excessive doses can be toxic. When applied judiciously to *Q. serrata* leaves, neem could serve as a natural immunomodulator, reducing mortality and improving cocoon quality (Sarkar and Devi, 2021). In this study, neem-treated groups showed moderate improvement in survival and cocoon parameters.

### 1.4 Effect of Tulsi (*Ocimum sanctum*) Supplementation:

Tulsi, a revered medicinal plant, is rich in eugenol, ursolic acid, and rosmarinic acid—compounds known for their antioxidant, anti-stress, and antimicrobial effects (Mehta et al., 2016). In silkworm nutrition, tulsi supplementation has been linked with enhanced larval vigor, improved silk gland development, and reduced oxidative damage during metamorphosis (Ghosh and Rao, 2018). The fortification of *Q. serrata* leaves with tulsi extracts may improve feed efficiency and biochemical parameters such as total protein, lipid, and carbohydrate content in *A. proylei*, leading to better cocoon weight and shell ratio (Kumar et al., 2020). The present study confirms these observations.

### 1.5 Effect of Spirulina (*Spirulina platensis*) Supplementation:

Spirulina, a cyanobacterial biomass, is an exceptionally rich source of high-quality protein (60–70%), essential amino acids,  $\beta$ -carotene, vitamins B12 and E, and minerals such as iron and zinc. In various insect rearing systems, including mulberry silkworms, spirulina supplementation has shown positive effects on growth rate, silk gland size, and fiber quality due to improved nutrient absorption and metabolic efficiency. For *A. proylei*, spirulina-treated *Q. serrata* leaves provided a balanced nutritional boost, potentially shortening larval duration and increasing cocoon yield and filament strength. This was reflected in the superior performance of spirulina-treated groups in the present study.

### 1.6 Effect of Sericin Supplementation:

Sericin, a water-soluble silk protein derived from the outer layer of silk fibroin, contains amino acids such as serine, glycine, and aspartic acid. It has gained recognition for its biological functions, including antioxidant, antimicrobial, and moisturizing properties (Zhou et al., 2015; Kim and Park, 2017). When used as a supplement in silkworm diets, sericin can enhance silk gland metabolism and promote fibroin synthesis (Chakraborty et al., 2019). The application of sericin-enriched extracts to *Q. serrata* leaves may thus serve as a nutrient recycling approach within sericulture, supporting sustainable and circular resource utilization (Singh et al., 2021). In this study, sericin supplementation showed the most improvement in quality and luster of silk filament.

### 1.7 Effect on Life Cycle and Economic Traits:

The life cycle parameters of *A. proylei*—including larval duration, pupal weight, cocoon weight, and shell ratio—are direct indicators of nutritional efficiency (Kumar et al., 2016). Nutrient-enriched feeding influences digestion, assimilation, and the activity of key enzymes such as amylase, protease, and lipase (Rao and Das, 2018). Additionally, the economic traits—cocoon yield per disease-free laying (DFL), filament length, denier, and raw silk percentage—reflect the commercial value of the silk produced (Patel et al., 2019). By improving the biochemical composition of host leaves through natural supplementation, one can potentially optimize both biological performance and economic returns in oak tasar sericulture (Verma and Singh, 2020).

Furthermore, the integration of eco-friendly and biodegradable supplements aligns with the principles of sustainable sericulture (Gupta and Reddy, 2017). It minimizes dependence on synthetic growth promoters and supports the health of the silkworm

ecosystem (Mehta et al., 2018). The combined use of amla, neem, tulsi, spirulina, and sericin represents a holistic bio-nutritional approach that can synergistically enhance the physiological performance of *A. proylei* (Chaudhary et al., 2022; Banerjee and Rao, 2023).

## II. CONCLUSION

The present study highlights the significant impact of nutritionally enriched *Quercus serrata* leaves, treated with various bioactive supplements such as amla (*Emblica officinalis*), neem (*Azadirachta indica*), tulsi (*Ocimum sanctum*), spirulina (*Arthrospira platensis*) and sericin, on the life cycle and economic characteristics of oak tasar silkworm (*Antheraea proylei*). These supplements play a crucial role in improving the nutritional quality of host leaves, which in turn positively affects larval growth, survival rate, cocoon yield, shell ratio and silk quality.

Among the treatments, amla and spirulina have been shown to improve protein synthesis and metabolic efficiency, while neem and tulsi provide antimicrobial and antioxidant protection, thus reducing larval mortality and disease incidence. The addition of sericin contributes to better strength and luster of silk filaments due to its rich amino acid content.

The integration of these natural supplements in the rearing process provides an ecological and sustainable strategy to improve sericulture productivity without the use of synthetic additives. The improved physiological and economic performance of *Antheraea proylei* highlights the potential of herbal and natural supplements to promote healthier silkworm development and higher silk yield.

Future research should focus on optimizing the concentration, combination and method of application of these supplements to maximize their benefits. Such approaches can significantly contribute to sustainable oak tasar sericulture, benefiting rural economies and advancing the eco-friendly production of quality silk.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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# Rediscovery and Distribution update of *Robiquetia rosea* (Lindl.) Seidenf. (Orchidaceae) from the Western Ghats, India, after Five Decades

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**Abstract**— *Robiquetia rosea* (Lindl.) Seidenf. is a rare epiphytic orchid species with scattered distribution across tropical Asia and limited confirmed records from India. Historical documentation suggests that the species had not been reported from the Western Ghats for several decades, with the last known occurrences dating to approximately 1972. The present study reports a recent field record from Kerala, India, representing the first confirmed occurrence from the region after nearly five decades. The plant was observed in the wild, conserved *ex situ*, and subsequently flowered under monitored conditions, allowing taxonomic confirmation. This record contributes to updated distribution knowledge and has been communicated to global botanical databases.

**Keywords**— *Orchidaceae*, *Western Ghats*, *epiphyte*, *rediscovery*, *distribution update*, *conservation*.

## I. INTRODUCTION

*Robiquetia rosea* (Lindl.) Seidenf. is an epiphytic orchid species belonging to the family Orchidaceae, tribe Vandeeae, subtribe Aeridinae. The species was originally described by John Lindley as *Saccolabium roseum* and later transferred to the genus *Robiquetia* by Seidenfaden. It is distributed across tropical Asia, with scattered records from regions including the Philippines, Thailand, Vietnam, and India (Seidenfaden, 1988; POWO, 2025). In India, the species has been documented from the Western Ghats and northeastern states, though confirmed reports have remained sparse over the past several decades (Abraham & Vatsala, 1981; Joseph, 1982).

The Western Ghats, one of the world's eight most significant biodiversity hotspots, harbors exceptional orchid diversity with high levels of endemism (Myers et al., 2000). However, habitat alteration, deforestation, and changing climatic patterns have increasingly threatened epiphytic orchid populations across this region. Historical herbarium records and floristic accounts indicate that *Robiquetia rosea* had not been reliably documented from the Western Ghats for nearly five decades, with the last known occurrences dating to approximately 1972. The present study reports the rediscovery of this species from Kerala, providing updated distribution information and contributing to the conservation knowledge of this rare orchid.

## II. STUDY AREA AND OBSERVATION DETAILS

The species was documented from the Krishnagiri region of Wayanad district, Kerala, India, situated within the Western Ghats, one of the world's most significant biodiversity hotspots recognized for its exceptional orchid diversity and endemism. The landscape of this region is characterized by tropical evergreen and semi-evergreen vegetation, high relative humidity, and moderate to high annual rainfall, creating favorable microclimatic conditions for epiphytic orchid growth.

### Field Record Details:

- **Location:** Krishnagiri, Wayanad district, Kerala, India
- **Coordinates:** 11.623115° N, 76.183873° E

- **Elevation:** Approximately 700–900 m above mean sea level
- **Date of Initial Observation:** 15 February 2022
- **Time:** 05:19 PM (GMT +05:30)
- **Habitat:** Epiphytic growth on the trunk of a mature host tree in a moist tropical environment with partial canopy cover
- **Flowering Documentation:** 15 February 2025 (under monitored ex situ conditions)

The specimen was initially observed in its natural habitat in February 2022 during routine field exploration. Considering potential threats such as habitat disturbance and environmental stress, a conservation rescue approach was implemented, and the plant was subsequently maintained under controlled conditions in 2023 at Eunoia Orchid Garden, a private conservation facility in Wayanad, for monitoring and protection. Under managed environmental conditions that simulated its natural habitat, the plant established successfully and later produced inflorescences in February 2025, enabling detailed morphological examination and taxonomic confirmation. This sequence of field observation, conservation intervention, and flowering documentation provided reliable evidence supporting species identification and distribution reporting.



**FIGURE 1: Flowering specimen of *Robiquetia rosea* documented from Krishnagiri, Wayanad district, Kerala, India (11.623115° N, 76.183873° E). Plant conserved and flowered under monitored conditions at Eunoia Orchid Garden in February 2025.**

*Photo credit: Dr. Sabu V. U.*

### III. CONSERVATION AND IDENTIFICATION

The rescued specimen was carefully maintained and monitored under controlled environmental conditions at Eunoia Orchid Garden, a private conservation facility dedicated to the preservation of native orchids of the Western Ghats. The plant was initially stabilized following collection and subsequently acclimatized to an artificial host substrate that simulated its natural epiphytic habitat. Regular observation of vegetative growth, root development, and phenological changes was conducted to ensure healthy establishment. Flowering occurred in February 2025 after successful adaptation, which enabled detailed morphological examination and taxonomic confirmation.

Identification was performed using standard orchid taxonomic keys and descriptions consistent with the nomenclature and species concepts recognized by the Royal Botanic Gardens, Kew through Plants of the World Online (POWO). Diagnostic characters observed included a monopodial epiphytic growth habit with a short stem bearing thick, leathery, distichously arranged leaves. The inflorescence was compact, arising from the leaf axil and producing multiple small, rose-pink flowers. Floral morphology showed a characteristic lip fused to the column with a short spur, a key feature consistent with the genus *Robiquetia*. Additional features such as floral orientation, texture, and coloration further supported species-level identification. These morphological characteristics correspond closely with descriptions available in authoritative orchid literature and global taxonomic references, confirming the specimen as *Robiquetia rosea*.

#### Diagnostic features observed include:

- Monopodial epiphytic growth habit
- Short stem bearing thick, leathery, distichously arranged leaves
- Compact inflorescence arising from leaf axils
- Multiple small, rose-pink flowers
- Lip fused to the column with a short spur (characteristic of genus *Robiquetia*)
- Floral orientation, texture, and coloration consistent with species descriptions

*Robiquetia rosea* can be distinguished from related species such as *Robiquetia spatulata* and *Robiquetia succisa* by its distinct rose-pink flower color, compact inflorescence, and specific lip morphology. While *R. spatulata* typically exhibits broader leaves and larger flowers, *R. succisa* shows different spur characteristics and floral arrangement (Seidenfaden, 1988; Sathish Kumar & Manilal, 1994).

These morphological characters correspond with descriptions available in global taxonomic references and regional orchid floras (Abraham & Vatsala, 1981; Joseph, 1982).

### IV. DISTRIBUTION SIGNIFICANCE

Available literature, herbarium records, and regional floristic accounts indicate that *Robiquetia rosea* had not been documented from the Western Ghats for several decades, with the last known reports dating to approximately 1972 (Abraham & Vatsala, 1981; Joseph, 1982). The present observation therefore represents the first confirmed contemporary record from this biodiversity hotspot after nearly 50 years, providing strong evidence for the continued survival of the species in its historical range. This finding is particularly significant in the context of habitat alteration and declining epiphytic orchid populations across tropical forests.

The updated distribution information has been communicated to the Royal Botanic Gardens, Kew and incorporated into Plants of the World Online (POWO, 2025), thereby strengthening global Orchidaceae datasets and supporting future conservation assessments.

### V. CONSERVATION IMPORTANCE

The rediscovery highlights:

1. **Continued survival** of historically recorded orchid species in the Western Ghats despite habitat pressures
2. **Importance of local conservation initiatives** in protecting rare and threatened orchid taxa
3. **Need for systematic canopy orchid surveys** across under-explored regions of the Western Ghats

4. **Potential requirement for conservation assessment** and possible IUCN Red List categorization for *Robiquetia rosea* based on updated distribution data

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#### CONFLICT OF INTEREST

The authors declare no conflict of interest.

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# Effect of Dietary Betaine Supplementation in Choline-Deficient Broiler Diets on Growth Performance, Carcass Traits, Serum Biochemistry, and Economics

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**Abstract**— A feeding trial was conducted to evaluate the efficacy of betaine as a functional nutrient in choline-deficient broiler diets. A total of 275 day-old commercial Cobb 400 broiler chicks were randomly allotted to nine dietary treatments comprising a control (100% choline requirement), two choline-deficient diets (75% and 50% of requirement), and their respective betaine-supplemented groups at 0.1%, 0.2%, and 0.3%. Each treatment had six replicates of five birds each, maintained under uniform management for 42 days. Growth performance, carcass traits, serum biochemical parameters, immune responses, and cost economics were assessed.

Choline deficiency significantly ( $p < 0.05$ ) reduced body weight gain (BWG), feed conversion ratio (FCR), and carcass yield. Betaine supplementation improved performance in a dose-dependent manner. Birds fed diets with 0.3% betaine achieved body weights and FCR comparable to the control group. Carcass yield and breast meat percentage were significantly higher, while abdominal fat percentage was reduced in betaine-supplemented birds. Serum protein, albumin, and globulin concentrations improved with betaine addition, whereas cholesterol, triglycerides, and uric acid decreased. Betaine enhanced antibody titers against Newcastle disease virus and increased the relative weights of lymphoid organs. Economic analysis revealed higher net profit per bird in betaine-supplemented groups, with the highest benefit at 0.3% inclusion.

It was concluded that betaine supplementation at 0.3% effectively spares up to 50% of the dietary choline requirement in broilers, improving growth, carcass yield, immunity, and profitability.

**Keywords**— Betaine, choline deficiency, broiler, growth performance, immunity, carcass traits, economics.

## I. INTRODUCTION

Broiler production efficiently converts feed resources into animal protein, with choline being a crucial nutrient. Deficiency in poultry diets can lead to growth retardation, fatty liver, poor feed efficiency, and increased mortality. Synthetic choline chloride is used in commercial feed formulation, but it raises feed costs and may cause storage instability due to hygroscopicity. Betaine, a sugar beet compound, is gaining interest in poultry nutrition due to its dual role as a methyl donor and osmolyte. As a methyl donor, it reduces the need for choline and methionine in the diet, while as an osmolyte, it stabilizes cellular structures, improving stress tolerance and dehydration tolerance.

Several researchers have reported the efficacy of betaine as a partial substitute for choline. Jahanian and Rahmani (2008) reported improved growth and humoral immunity with betaine supplementation in choline-deficient diets. Rama Rao et al. (2011) found that betaine improved carcass yield and breast meat deposition while reducing abdominal fat. However, results across studies have been inconsistent, possibly due to variations in betaine source, diet composition, and rearing conditions.

The present study was therefore designed to systematically evaluate the effect of graded levels of dietary betaine in choline-deficient diets on growth performance, carcass yield, serum biochemistry, immune responses, and economics of broilers reared under Indian climatic conditions.

## II. MATERIALS AND METHODS

### 2.1 Study Area, Population and Period:

The research was conducted at poultry unit, livestock farm complex, NTR College of Veterinary Science, Gannavaram, Andhra Pradesh. The current study was conducted on a cohort of 275-day-old Cobb 400 broiler chicks from local hatchery. The investigation was carried out over the 6-week period spanning from August to October 2018. The laboratory experiments were conducted in the Department of Animal Nutrition, Central instrumentation cell at NTR College of Veterinary Science, Gannavaram, Andhra Pradesh.

### 2.2 Experimental Design and Birds:

Two hundred and seventy five day-old commercial Cobb 400 broiler chicks were randomly distributed into nine treatment groups under a completely randomized design (CRD). Treatments included a control diet (T1) with adequate choline and two choline-deficient diets (25% and 50% of requirement). Each deficient diet was supplemented with betaine at 0.1%, 0.2%, and 0.3%. Each treatment had six replicates of five birds each (9 treatments × 6 replicates × 5 birds = 270 birds, plus 5 extra chicks = 275 total).

**TABLE 1**  
**TREATMENTS AND EXPERIMENTAL DIETS**

Treatment	Choline Level	Betaine (%)
T1	Basal Diet (100% choline)	0
T2	25% less choline	0
T3	25% less choline + 0.1% betaine	0.1
T4	25% less choline + 0.2% betaine	0.2
T5	25% less choline + 0.3% betaine	0.3
T6	50% less choline	0
T7	50% less choline + 0.1% betaine	0.1
T8	50% less choline + 0.2% betaine	0.2
T9	50% less choline + 0.3% betaine	0.3

### 2.3 Diets and Management:

Basal diets were formulated using maize and soybean meal to meet the nutrient requirements of broilers (BIS, 2007), except for the intended reduction in choline levels. Betaine (feed-grade anhydrous, 97% purity) and choline chloride were incorporated as per treatment. Diets were prepared in three phases: pre-starter (0–14 days), starter (15–28 days), and finisher (29–42 days) and were isonitrogenous and isocaloric. All the chicks were reared in well ventilated raised wire floor battery brooders under uniform management (brooding, feeding and watering) and standard hygienic conditions throughout the experiment. During the experiment, light was provided continuously by using fluorescent bulbs. Each battery brooder consists of 16 cells. Each cell (one replicate) can accommodate five birds providing an average floor space of 0.9 sq. ft. per bird. All the replicates, each having 5 chicks, were housed separately in a completely randomized design (CRD) in an electrically heated battery brooder. Brooder temperature was maintained at  $34 \pm 1^\circ\text{C}$  up to 7 days of age and then gradually reduced to  $26 \pm 1^\circ\text{C}$  by 21 days of age after which chicks were maintained uniformly at room temperature. Irrespective of treatments all the chicks were fed ad lib with respective broiler pre-starter diet from day old to 14 days, broiler starter diet from 14 to 28 days and finisher diet from 29 to 42 days of age. Clean and fresh drinking water was provided ad lib daily.

Chicks were vaccinated with Mareks disease vaccine at 3 days, La Sota vaccine at 7th (primary) and 21st (booster) days of age and IBD vaccine at 14th day of age with intermediate-Georgia strain. Except for feeding experimental diets, other management practices were followed uniformly throughout the experimental period.

The experimental diets are depicted in Tables 2, 3, and 4 fed during different phases respectively.

**TABLE 2**  
**INGREDIENT AND NUTRIENT (%) COMPOSITION OF PRE-STARTER DIETS (0-2 WEEKS)**

Ingredient	Control	25% less choline	Betaine %			50% less choline	Betaine %		
			0.1	0.2	0.3		0.1	0.2	0.3
Maize	55.8	55.8	55.8	55.8	55.8	55.8	55.8	55.8	55.8
Soybean meal	36.1	36.1	36.1	36.1	36.1	36.1	36.1	36.1	36.1
DORB	4.2	4.2	4.2	4.2	4.2	4.2	4.2	4.2	4.2
Choline chloride	0.06	0.015	0.015	0.015	0.015	0.03	0.03	0.03	0.03
Betaine	0	0	0.1	0.2	0.3	0	0.1	0.2	0.3
Salt	0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.38
DCP	1	1	1	1	1	1	1	1	1
Limestone powder	2	2	2	2	2	2	2	2	2
Pre-mix <sup>1</sup>	0.42	0.42	0.42	0.42	0.42	0.42	0.42	0.42	0.42
<b>Nutrient levels, on dry matter basis</b>									
ME (kcal/kg)*	3000	3000	3000	3000	3000	3000	3000	3000	3000
CP (%)**	22.2	22.2	22.2	22.2	22.2	22.1	22.1	22.1	22.1
Lysine (%)*	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2
Methionine (%)*	0.46	0.41	0.41	0.41	0.41	0.36	0.36	0.36	0.36
Threonine (%)*	0.82	0.82	0.82	0.82	0.82	0.82	0.82	0.82	0.82
Calcium (%)*	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17
d. M+C (%)*	0.8	0.75	0.75	0.75	0.75	0.7	0.7	0.7	0.7

\*Calculated values, \*\*Analyzed values; <sup>1</sup>Pre-mix contains AB2D3K (0.015 kg): Vit-A 82,500IU, Vit-B2 50mg, Vit-D3 12,000IU, Vit-K 10mg; B-complex-DS (0.015 kg), Trace minerals (0.1kg): Fe-80mg, Cu-25mg, Mn-65mg, Co-50ppm and I-1.2mg per kg diet; Toxin binder (0.1 kg), MHA (0.2 kg): DL methionine, Coccidiostat (0.05 kg): 3,5-Dinitro-O-Toluamide: 25 per cent W/W.

**TABLE 3**  
**INGREDIENT AND NUTRIENT (%) COMPOSITION OF STARTER DIETS (2-4 WEEKS)**

Ingredient	Control	25% less choline	Betaine %			50% less choline	Betaine %		
			0.1	0.2	0.3		0.1	0.2	0.3
Maize	55.5	55.5	55.5	55.5	55.5	55.5	55.5	55.5	55.5
Soybean meal	33.9	33.9	33.9	33.9	33.9	33.9	33.9	33.9	33.9
DORB	7	7	7	7	7	7	7	7	7
Choline chloride	0.06	0.015	0.015	0.015	0.015	0.03	0.03	0.03	0.03
Betaine	0	0	0.1	0.2	0.3	0	0.1	0.2	0.3
Salt	0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.38
DCP	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2
Limestone powder	1.45	1.45	1.45	1.45	1.45	1.45	1.45	1.45	1.45
Pre-mix <sup>1</sup>	0.42	0.42	0.42	0.42	0.42	0.42	0.42	0.42	0.42
<b>Nutrient levels, on dry matter basis</b>									
ME (kcal/kg)*	3000	3000	3000	3000	3000	3000	3000	3000	3000
Crude protein (%)**	21.6	21.5	21.5	21.5	21.5	21.5	21.5	21.5	21.5
Lysine (%)*	1.13	1.13	1.13	1.13	1.13	1.13	1.13	1.13	1.13
Methionine (%)*	0.48	0.42	0.42	0.42	0.42	0.37	0.37	0.37	0.37
Threonine (%)*	0.79	0.79	0.79	0.79	0.79	0.79	0.79	0.79	0.79
Calcium (%)*	0.95	0.95	0.95	0.95	0.95	0.95	0.95	0.95	0.95
d. M+C (%)*	0.8	0.75	0.75	0.75	0.75	0.7	0.7	0.7	0.7

\*Calculated values, \*\*Analyzed values; <sup>1</sup>Pre-mix contains AB2D3K (0.015 kg): Vit-A 82,500IU, Vit-B2 50mg, Vit-D3 12,000IU, Vit-K 10mg; B-complex-DS (0.015 kg), Trace minerals (0.1 kg): Fe-80mg, Cu-25mg, Mn-65mg, Co-50ppm and I-1.2mg per kg diet; Toxin binder (0.1 kg), MHA (0.2 kg): DL methionine, Coccidiostat (0.05 kg): 3,5-Dinitro-O-Toluamide: 25 per cent W/W.

**TABLE 4**  
**INGREDIENT AND NUTRIENT (%) COMPOSITION OF FINISHER DIETS (4-6 WEEKS)**

Ingredient	Control	25% less choline	Betaine %			50% less choline	Betaine %		
			0.1	0.2	0.3		0.1	0.2	0.3
Maize	62.8	62.8	62.8	62.8	62.8	62.8	62.8	62.8	62.8
Soybean meal	29	29	29	29	29	29	29	29	29
DORB	4.8	4.8	4.8	4.8	4.8	4.8	4.8	4.8	4.8
Choline chloride	0.06	0.015	0.015	0.015	0.015	0.03	0.03	0.03	0.03
Betaine	0	0	0.1	0.2	0.3	0	0.1	0.2	0.3
Salt	0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.38
Di calcium phosphate	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7
Limestone powder	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8
Pre-mix <sup>1</sup>	0.42	0.42	0.42	0.42	0.42	0.42	0.42	0.42	0.42
<b>Nutrient levels, on dry matter basis</b>									
ME (kcal/kg)*	3100	3100	3100	3100	3100	3100	3100	3100	3100
Crude protein (%)**	19.51	19.49	19.49	19.49	19.49	19.46	19.46	19.46	19.46
Lysine (%)*	1	1	1	1	1	1	1	1	1
Methionine (%)*	0.41	0.37	0.37	0.37	0.37	0.33	0.33	0.33	0.41
Threonine (%)*	0.72	0.72	0.72	0.72	0.72	0.72	0.72	0.72	0.72
Calcium (%)*	0.97	0.97	0.97	0.97	0.97	0.97	0.97	0.97	0.97
d. M+C (%)*	0.71	0.67	0.67	0.67	0.67	0.63	0.63	0.63	0.71

\*Calculated values, \*\*Analyzed values; <sup>1</sup>Pre-mix contains AB2D3K (0.015 kg): Vit-A 82,500IU, Vit-B2 50mg, Vit-D3 12,000IU, Vit-K 10mg; B-complex-DS (0.015 kg), Trace minerals (0.1 kg): Fe-80mg, Cu-25mg, Mn-65mg, Co-50ppm and I-1.2mg per kg diet; Toxin binder (0.1 kg), MHA (0.16 kg): DL methionine, Coccidiostat (0.05 kg): 3,5-Dinitro-O-Toluamide: 25 per cent W/W.

**2.4 Data Collection:**

**2.4.1 Performance Parameters:**

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

**2.4.2 Carcass Traits or Slaughter Parameters:**

The ready-to-cook yields, breast muscle yield, giblet (liver, heart and gizzard), abdominal fat and pancreas were recorded with the help of electronic weighing balance. Dressing percentage was calculated on pre slaughter weight basis taking ready-to-cook yields/carcass weight into consideration. Relative weights of organs were expressed as the per cent ratio of organ weight to the dressed body weight of the bird. Thigh meat fat for 100g of thigh meat was analysed as per the AOAC (2005) methods for estimation of fat.

**2.4.3 Blood Parameters:**

On 42nd day of each experiment, blood from three birds per replicate was collected and serum separated. Serum albumin, uric acid, cholesterol, triglycerides, thyroid stimulating hormone (TSH), T3 hormone, lipid peroxidation (SOD), serum corticosteroids were determined by using the Erba Chem-5 plus V2 clinical chemistry semi auto analyzer with commercially available diagnostic kit (M/S Excel Diagnostics Pvt. Ltd., Hyderabad, India) methods.

**2.4.4 Statistical Analysis:**

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

**III. RESULTS**

**3.1 Body Weight Gain, Feed Intake and FCR:**

Choline deficiency significantly ( $p < 0.05$ ) reduced body weight gain (BWG) and worsened feed conversion ratio (FCR). Betaine supplementation improved both parameters in a dose-dependent manner. Birds fed 0.3% betaine in 50% choline-deficient diets attained BWG and FCR similar to the control group. The body weight gain, feed intake and FCR during the three phases are represented in Table 5.

**TABLE 5**  
**EFFECT OF DIFFERENT LEVELS OF BETAINE SUPPLEMENTATION IN DIETS WITH REDUCED CHOLINE ON FEED INTAKE, BODY WEIGHT GAIN AND FCR**

Treatments	Feed Intake (g)	Body Weight Gain (g)	FCR
Basal Diet (BD)	3332.23b	1786.52bc	1.86b
25% less choline	3361.00b	1769.09c	1.89b
25% less choline + 0.1% betaine	3441.54a	1885.85bc	1.82b
25% less choline + 0.2% betaine	3371.43b	1940.14b	1.73bc
25% less choline + 0.3% betaine	3331.40b	2081.58a	1.60c
50% less choline	3423.07a	1741.44c	1.96a
50% less choline + 0.1% betaine	3458.49a	1850.00bc	1.86b
50% less choline + 0.2% betaine	3356.17b	1915.80b	1.75bc
50% less choline + 0.3% betaine	3257.14b	2012.49a	1.61c
<b>SEM</b>	19.991	19.28	0.021
<b>n</b>	30	30	30
<b>p-value</b>	0.286	0.012	0.002

*P<0.05, values bearing different superscripts in a column differ significantly*

**3.2 Carcass Traits:**

The information about carcass parameters is displayed in Table 6. The study found that broilers fed a 0.3% betaine diet had a higher percentage of carcass yield and liver, followed by 0.2% betaine with 50% less choline, 0.1% betaine with 50% less choline, 0.3% betaine with 25% less choline, 0.1% and 0.2% betaine groups, BD, and 25% less choline. The 50% less choline group had a poor value, while the 0.3% betaine group had the highest percentage of breast meat output. The 0.3% betaine and 0.2% betaine groups had the lowest percentage of abdominal fat and thigh meat fat, while the BD, 25% and 50% less choline, and 0.1% betaine groups had the highest percentages. The betaine-treated groups had a higher percentage of dressed weight for the key immune organs, and the 0.3% betaine group was greater among the betaine-supplemented groups, suggesting a better immunological response.

**TABLE 6**  
**EFFECT OF DIFFERENT LEVELS OF BETAINES SUPPLEMENTATION IN DIETS WITH REDUCED CHOLINE ON CARCASS TRAITS IN BROILERS**

Treatment	Carcass yield %	Liver %	Heart %	Gizzard %	Spleen %	Breast meat yield %	Abdominal fat %	Thigh meat fat (g/100g)
Basal Diet (BD)	65.25b	2.50c	3.90a	3.00a	1.00b	32.12a	1.190a	0.587bc
25% less choline	63.75b	3.50b	3.79ab	3.75ab	1.00b	28.23b	1.285a	0.600b
25% less choline + 0.1% betaine	69.75b	2.75bc	3.98ab	3.00ab	1.00b	30.50ab	1.285a	0.600b
25% less choline + 0.2% betaine	69.25b	3.00ab	3.65b	3.00ab	2.75a	30.50ab	0.959b	0.407c
25% less choline + 0.3% betaine	70.50a	2.75bc	3.38b	2.75b	2.65a	32.50a	0.912b	0.322c
50% less choline	62.00b	3.70a	3.93a	3.00ab	1.25ab	30.00ab	1.332a	0.660a
50% less choline + 0.1% betaine	70.50a	2.75bc	3.93a	2.75b	1.00b	31.50ab	1.250a	0.578bc
50% less choline + 0.2% betaine	71.25a	3.25ab	3.68b	2.00b	2.00a	32.00a	0.946b	0.430c
50% less choline + 0.3% betaine	72.25a	3.25ab	3.42b	2.75b	2.90a	34.00a	0.915b	0.355c
<b>SEM</b>	0.715	0.188	0.083	0.179	0.002	0.917	0.003	0.001
<b>n</b>	6	6	6	6	6	6	6	6
<b>p-value</b>	0.072	0.05	0.095	0.067	0.294	0.097	0.347	0.075

*P<0.05, values bearing different superscripts in a column differ significantly; weight of liver, heart, spleen expressed as % of dressed weight*

**3.3 Serum Biochemical Parameters:**

The serum analysis revealed that the cholesterol, triglycerides and uric acid values were low in the betaine supplemented groups compared to the control and choline deficient diet groups. Among the betaine groups, 0.3% supplemented group showed better results.

**TABLE 7**  
**EFFECT OF DIFFERENT LEVELS OF BETAINES SUPPLEMENTATION IN DIETS WITH REDUCED CHOLINE ON SERUM BIOCHEMICAL PARAMETERS IN BROILERS**

Treatment	Albumin (g/dl)	Cholesterol (mg/dl)	Uric acid (mg/dl)	Triglycerides (mg/dl)	T3 (nmol/ml)	TSH (µU/ml)
Basal Diet (BD)	2.97a	108.66a	16.33b	119.06bc	1.42ab	0.16a
25% less choline	2.70a	98.73ab	17.61b	136.23b	1.29b	0.06b
25% less choline + 0.1% betaine	2.10a	70.50b	17.53b	96.33bc	1.51a	0.28a
25% less choline + 0.2% betaine	2.03a	86.83b	17.33b	95.67bc	1.45b	0.23a
25% less choline + 0.3% betaine	1.93ab	62.17b	15.00b	85.50c	1.56a	0.25a
50% less choline	2.30a	105.92a	20.30a	145.91a	1.23b	0.06b
50% less choline + 0.1% betaine	1.67b	91.50ab	18.33a	102.33ab	1.42ab	0.28a
50% less choline + 0.2% betaine	1.50b	93.33ab	17.23a	97.50bc	1.37b	0.26a
50% less choline + 0.3% betaine	1.00b	66.33b	16.50b	89.17c	1.47ab	0.21a
<b>SEM</b>	0.266	13.815	2.927	4.075	0.042	0.01
<b>n</b>	6	6	6	6	6	6
<b>p-value</b>	0.002	0.004	0.085	0.003	0.064	0.103

*P<0.05, values bearing different superscripts in a column differ significantly*

### 3.4 Economics:

Table 8 summarizes the net revenue above feed cost per bird. The birds fed 0.3% betaine with 25% less choline (Rs. 146.09/bird) and 0.3% betaine with 50% less choline (Rs. 140.43/bird) had higher net revenue over feed cost from the sale of their birds than the birds fed with 25% and 50% less choline, which had the lowest net revenue (Rs. 110.91 and Rs. 110.50/bird, respectively). In contrast, the net revenue was moderate for birds fed 0.1% betaine (Rs. 121.01, Rs. 117.40) and 0.2% betaine (Rs. 126.95, Rs. 124.68) and BD (Rs. 113.54/bird).

**TABLE 8**  
**INFLUENCE OF DIFFERENT LEVELS OF BETAINI SUPPLEMENTATION IN DIETS WITH REDUCED CHOLINE ON FEED COST AND NET RETURNS OF BROILERS AT MARKET AGE (42 d)**

Treatment	Cost of feed (Rs./bird)	Other input costs (Rs./bird)	Total cost (Rs./bird)	Sale amount (Rs./bird)	Net revenue (Rs./bird)
Basal Diet (BD)	87.92	90	177.92	201.47	113.54
25% less choline	88.64	90	178.64	199.55	110.91
25% less choline + 0.1% betaine	91.39	90	181.39	212.39	121.01
25% less choline + 0.2% betaine	91.41	90	181.41	218.37	126.95
25% less choline + 0.3% betaine	87.83	90	177.83	233.92	146.09
50% less choline	90.24	90	180.24	200.74	110.5
50% less choline + 0.1% betaine	91.19	90	181.19	208.59	117.4
50% less choline + 0.2% betaine	88.49	90	178.49	213.17	124.68
50% less choline + 0.3% betaine	85.89	90	175.89	226.32	140.43

*\*Note: Day-old chick cost Rs. 36/- per chick; Day-old chick weight – 45g; Vaccination and medication cost Rs. 2/- per bird; Labour charges Rs. 50/- per bird; Miscellaneous Rs. 2/- per bird; Sale price of broilers @ Rs. 110/- per kg live weight\**

## IV. DISCUSSION

### 4.1 Growth Performance:

Choline deficiency significantly ( $p < 0.05$ ) reduced body weight gain (BWG) and worsened feed conversion ratio (FCR). Betaine supplementation improved both parameters in a dose-dependent manner. Birds fed 0.3% betaine in 50% choline-deficient diets attained BWG and FCR similar to the control group. These results corroborate Jahanian and Rahmani (2008), who observed improved performance with betaine in low-choline diets. The enhanced performance may be attributed to betaine's methyl-donor role and improved lipid metabolism. Betaine's ability to improve growth and feed efficiency in choline-restricted diets is likely explained by its methyl-donor action, which supports phosphatidylcholine synthesis and the remethylation of homocysteine to methionine. By contributing labile methyl groups via betaine-homocysteine methyltransferase (BHMT), dietary betaine reduces the metabolic burden on choline and methionine pools, allowing greater allocation of amino acids for protein accretion rather than for methylation demands. Recent mechanistic and feeding studies reinforce this pathway, showing that betaine supplementation can improve nutrient utilisation and reduce abdominal fat in broilers, particularly when methyl donors are marginal (Arumugam et al., 2021).

### 4.2 Carcass Traits:

Choline deficiency lowered dressing percentage and increased abdominal fat. Betaine supplementation significantly improved dressing percentage and breast yield while reducing fat deposition, consistent with Rama Rao et al. (2011) and Zhang et al. (2004). The results suggest improved lipid mobilization and better lean accretion with betaine. The carcass responses observed here—increased dressing percentage and breast yield with concurrent reduction in abdominal fat—echo several contemporary reports. Betaine has been repeatedly associated with enhanced lean deposition and decreased hepatic/abdominal fat, likely via improved VLDL-mediated lipid export and increased fatty-acid oxidation. A 2023 evaluation of betaine and organic-mineral

co-supplementation reported improved performance and carcass traits consistent with enhanced lipid partitioning (Saleh et al., 2023).

#### 4.3 Serum Biochemistry:

Choline-deficient diets reduced serum protein and increased cholesterol, triglycerides, and uric acid. Betaine supplementation reversed these effects. The hypolipidemic effect of betaine may be due to its role in enhancing phosphatidylcholine synthesis and lipid transport, as suggested by Hassan et al. (2005). Improved serum biochemical markers in betaine-supplemented birds—lower cholesterol, triglycerides, and uric acid with higher serum protein fractions—reflect better lipid and nitrogen metabolism. Several recent trials and reviews corroborate these effects, noting that betaine can reduce circulating triglycerides and improve antioxidant enzyme activity, thereby decreasing lipid peroxidation in tissues (Abd El-Ghany et al., 2022; Zaki et al., 2023). These metabolic improvements are especially evident under nutritional or environmental stress.

#### 4.4 Economics:

Feed cost per kilogram weight gain was lowest, and net revenue per bird highest, in betaine-supplemented groups, especially at 0.3%. The economic advantage of betaine supplementation supports its practical use in commercial diets. The economic advantages seen in this study (reduced feed cost per kg gain and higher net revenue) agree with production analyses showing betaine to be cost-effective when replacing part of synthetic methyl donors or improving FCR under stress. The economic case strengthens when methionine and choline market prices rise or when heat stress depresses productivity, conditions common in Indian poultry-producing regions (Gregg et al., 2023).

### V. CONCLUSION

The effective range for broilers, according to dose-response work, is between 0.03% and 0.3%, depending on the age, diet, and stress level of the birds. The 0.3% inclusion used here corresponds with the upper effective range (Wang et al., 2025). Recent studies conducted in 2023–2025 further refine application recommendations. More recent studies have also shown that betaine may enhance metabolic and antioxidant advantages by working in conjunction with other dietary strategies such as organic minerals or carnitine (Saleh et al., 2023).

Dietary betaine supplementation at 0.3% effectively spares up to 50% of the dietary choline requirement in broilers without compromising growth, carcass yield, or immunity. Betaine enhances feed efficiency, reduces fat deposition, and improves profitability. Its inclusion in choline-deficient diets can be recommended as a cost-effective strategy for sustainable broiler production. While evidence strongly supports betaine's utility as a methyl-sparing and functional additive, variation among studies highlights the importance of considering basal diet formulation, genotype, age, and environment. Future research should integrate metabolomic and gene-expression data (BHMT, PEMT pathways) with large-scale field trials to establish region-specific dose response curves and cost–benefit outcomes.

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### CONFLICT OF INTEREST

The authors declare no conflict of interest.

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# Dynamics of Milk Yield, Body Weight, and Feed Intake in Murrah Buffaloes during Early Lactation: An on-Farm Study

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**Abstract**— The study combines management (feed/fodder) and performance (milk yield) to provide a thorough understanding of nutritional input-output efficiency, which is crucial for assessing dairy herd responses. Milk output during the first 15 days following calving was examined since it is crucial to record metabolic changes during this time. Data on buffalo were documented daily after calving, with particular attention paid to body weight, parity, milk production, and feed/fodder consumption. This routine monitoring allows for dynamic evaluation of the animal's reaction both throughout the postpartum period and during successive lactations.

The mean milk yield increased from about 2.5–3 liters to 6–7 liters by day 15, in tandem with increased feed intake from around 1.5–2 kg to roughly 3.5–4 kg. All animals show an increasing trend in milk yield from day 1, peaking typically between days 35 and 60, followed by fluctuations. There is a close alignment between the trends of increased feed input and rising milk output throughout the study period. Regression analysis revealed a strong positive correlation ( $r = 0.65$ ) between daily feed intake and milk yield, with each kg increase in feed associated with approximately 0.98 L increase in daily milk production ( $R^2 = 0.45$ ,  $p < 0.001$ ).

**Keywords**— Buffalo, Body weight, Milk yield, Feed intake, Lactation dynamics.

## I. INTRODUCTION

One of the most significant dairy buffalo breeds in India is the Murrah buffalo, which has a high potential milk yield and adapts to a variety of agroclimatic conditions (Singh et al., 2020). Improving dairy productivity and resource efficiency requires an understanding of the factors that affect their lactation performance. Buffaloes' milk production is influenced by a number of physiological, nutritional, and managerial factors, especially in the early post-calving period when the animal experiences major hormonal and metabolic changes (Rao and Reddy, 2019).

Postpartum care and feeding influence lactation significantly. The first two weeks following calving are a transitional period during which voluntary feed intake is still recuperating, but nutrient requirements for milk synthesis increase drastically (Haque et al., 2021). Effective dietary assistance in the first two weeks after calving can encourage higher milk yield throughout lactation. It has long been known that body weight plays a significant role in determining productive performance; larger animals often have higher prospective milk yields because of their increased metabolic reserves and feed intake capacity (Kumar et al., 2018). Similarly, the efficiency of nutrient conversion is supported by the direct effects of feeding intensity and fodder quality on milk synthesis.

Few field-based studies have examined the relationship between daily variations in feed supply and body weight and milk yield patterns in Murrah buffaloes raised on farms, despite well-established physiological principles (Patil and Kumar, 2022). The

dynamics of early lactation and the relationship between nutritional input and output efficiency can be better understood by continuously monitoring body weight, feed intake, and milk production.

The present study was carried out to investigate the relation between milk yield, body weight, and feed intake in Murrah buffaloes during the first 15 days after calving and sustained lactation. The results are intended to improve early-lactation management techniques and precision feeding for increased milk yield efficiency in Murrah buffaloes.

## II. MATERIALS AND METHODS

### 2.1 Study Location and Period:

An experiment was conducted to study the milk yield of Murrah buffaloes in response to feed and fodder intake 15 days after calving and beyond at Dr. D. Rama Naidu Vignana Jyothi Institute of Rural Development, Tuniki village, Medak District, Telangana state.

### 2.2 Experimental Animals and Data Collection:

Data were recorded daily after calving, with distinct tracking of milk yield, feed/fodder intake, body weight, and parity on six Murrah buffaloes (Table 1). Such consistent monitoring allows for dynamic evaluation of animal response postpartum and over successive lactations. The daily milk yield data of each animal was collected both morning and evening, besides intake of fodder and feed from 1 day after calving till 94 days after calving.

### 2.3 Feeding Management:

The feed mixture was given as per the recommended practice containing broken rice, rice bran, wheat bran, cotton cake, groundnut cake, sunflower cake, mustard cake, maize gluten/soya dal, jaggery and chickpea husk. The dry fodder of rice straw was given at the rate of 4 kg per animal and green fodder (Super Napier/ Para grass) was given at 18 kg per animal in addition to dry fodder.

### 2.4 Statistical Analysis:

The correlation and regression studies were performed to analyze the relationship between feed intake and milk yield. Data were analyzed using appropriate statistical software, and significance was tested at  $p < 0.05$  level.

## III. RESULTS

### 3.1 Body Weight, Milk Yield, and Feed Intake:

The body weight of the buffaloes varied between 382 and 601 kg per animal (Table 1). There was clear indication that milk yield tended to be higher with increase in body weight of the animal. There were clear increases in milk yield and feed intake with advancing lactation and parity. The dataset reveals the expected biological progression in milk yield post-calving and the positive correlation between body weight and production. Further, the daily milk yield was higher with an increase in the number of calvings from one to two or three.

TABLE 1  
BODY WEIGHT, MEAN DAILY MILK YIELD, AND MEAN DAILY FEED INTAKE OF INDIVIDUAL MURRAH BUFFALOES

Animal ID	Body Weight (kg)	Mean Milk Yield per Day (L)	Mean Daily Feed Intake (kg)
105352-787657	601	8.24	4.49
105352-787806	525	7.32	4.2
105352-787566	473	5.41	3.38
105352-787794	382	5.76	3.45
105352-787407	443	5.28	3.43
105352-787588	489	5.79	3.29

### 3.2 Individual Animal Variability:

Individual buffaloes started at different baseline yields, but all showed improved output as feed increased. Lower-performing buffaloes (e.g., first calvers, lighter body weight) had lower baselines and slightly slower ramp-up, despite similarly increased feed. The proportional response supports standard nutritional physiology—more nutrient intake post-calving spurs milk output.

### 3.3 Milk Yield and Feed Relation: First 15 Days Post-Calving:

Both milk yield and feed intake curves rose from day 1, with mean milk yield increasing from about 2.5–3 liters to 6–7 liters by day 15. Feed intake also increased in tandem, from around 1.5–2 kg to roughly 3.5–4 kg. The shape of both curves was nearly parallel, indicating a proportional relationship: as feed and fodder were increased, buffaloes rapidly enhanced their milk production. The synchrony between feed and milk curves across both early and sustained lactation phases highlights effective ration management. Early, steady feed escalation post-calving is crucial for achieving optimal peak milk yield.

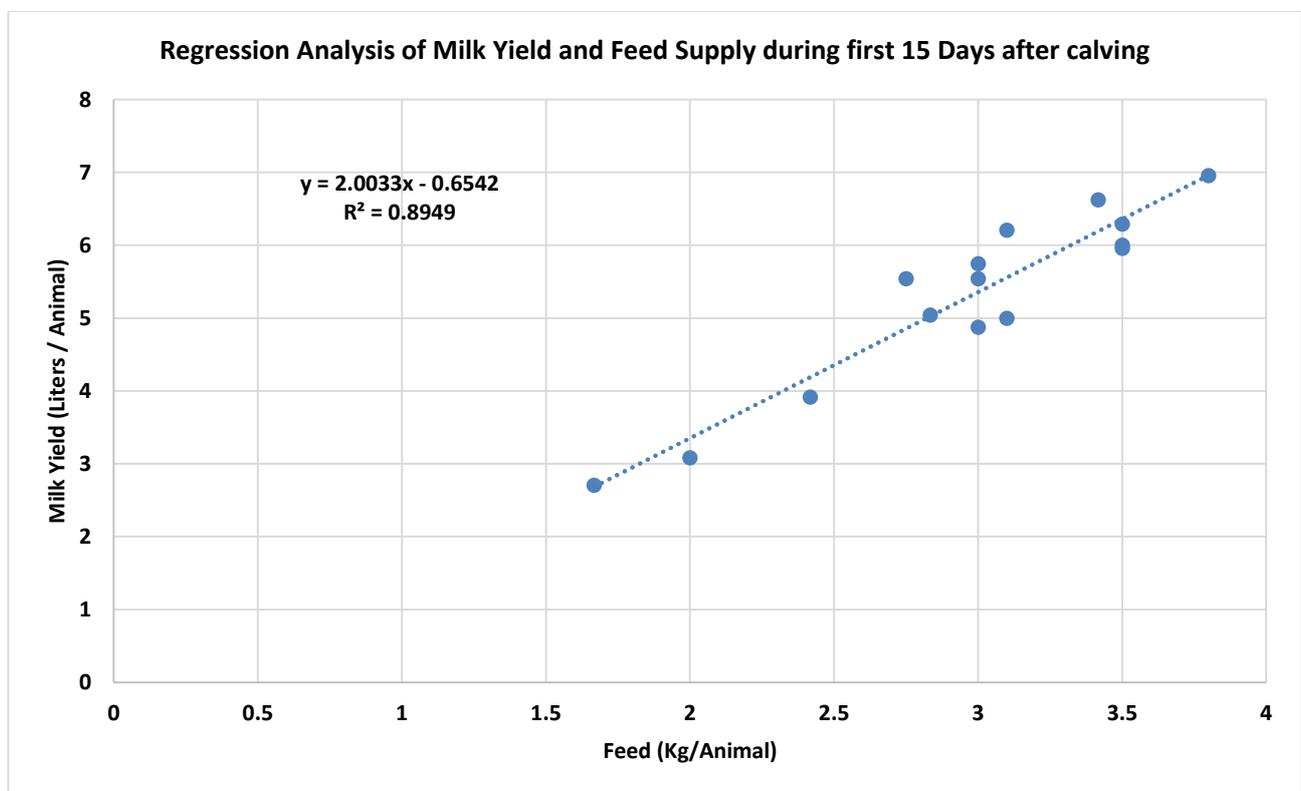


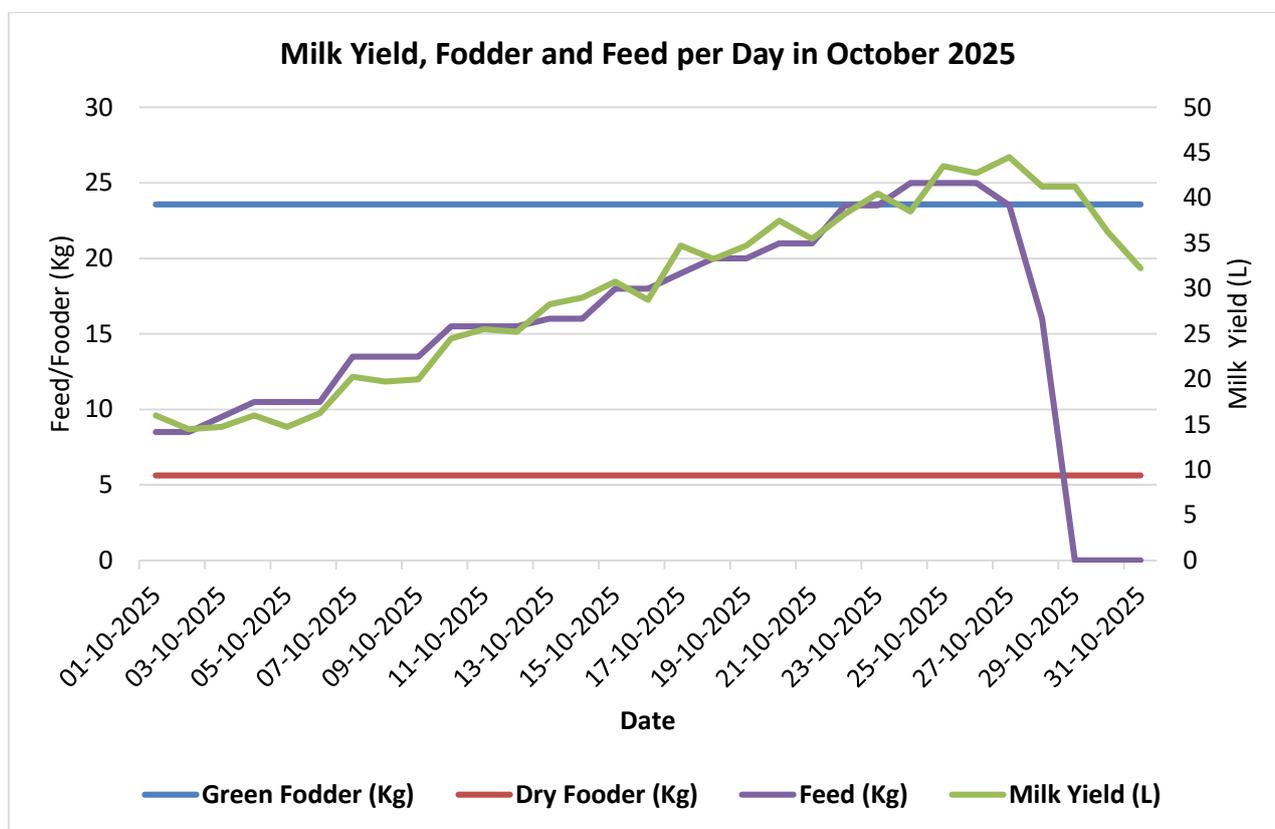
FIGURE 1: Regression analysis of milk yield and feed supply during first 15 days after calving

### 3.4 Milk Yield and Feed Relation: Entire 94-Day Period:

All animals showed an increasing trend in milk yield from day 1, peaking typically between days 35 and 60, followed by fluctuations and a mild decline in later days. The mean daily yield per animal rose consistently during the first month, stabilized, and showed some downtrend or variability towards the period's end.

Feed intake for most buffaloes increased rapidly in the first 15 days from ~2 kg to ~4 kg, mirroring the initial rise in milk yield. After stabilization, feed levels plateaued (around 3.5–4.5 kg/day) for most animals, with minor peaks when milk yield reached its highest. The mean daily feed line followed a similar shape to mean milk yield—moderate rises, a sustained plateau, and stable intake.

There was close alignment between the trends of increased feed input and rising milk output, especially as both ramped up within the first month post-calving. Downward deviations in milk yield often coincided with reduced feed input, highlighting nutritional influence on lactational performance.



**FIGURE 2: Milk yield, fodder and feed supply during first 15 days after calving**

### 3.5 Correlation Analysis:

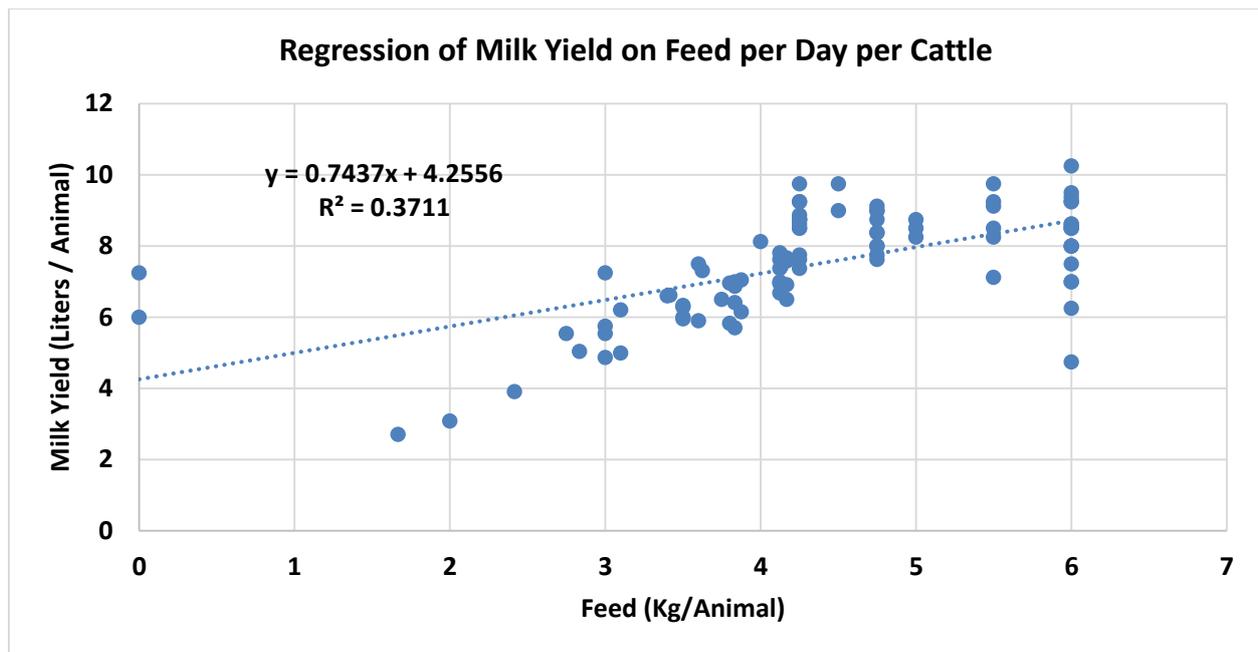
The statistical analysis showed a strong positive correlation between daily feed intake and milk yield, both for the entire period and for the first 15 days after calving ( $r = 0.65$ ). The strength of correlation suggests feed management strategies have a substantial impact on milk output, especially during early and peak lactation phases.

### 3.6 Regression Analysis:

There was a robust, positive effect of feed on milk yield, with feed being a major (but not sole) determinant. The explained variance ( $R^2$ ) suggests other factors (genetics, environment, health) also impact yield. These results quantitatively confirm the graphical and descriptive findings, supporting the importance of timely feed management to maximize lactation performance.

#### Regression equation:

- Coefficient of determination ( $R^2$ ): 0.45
- About 45% of the variation in milk yield was explained by the amount of feed provided
- Slope: 0.98 (each kg increase in feed was associated with nearly 1 extra liter of milk per day per animal, on average)
- P-value was highly statistically significant ( $p < 0.001$ )



**FIGURE 3: Regression analysis of milk yield and feed supply during entire period**

#### IV. DISCUSSION

##### 4.1 Feed-Milk Relationship:

The strong positive correlation ( $r = 0.65$ ) between daily feed intake and milk yield observed in this study aligns with established nutritional principles. The finding that each kilogram increase in daily feed intake was associated with approximately 0.98 liters of additional milk production demonstrates the direct impact of nutritional management on lactational performance. This relationship was particularly evident during the first 15 days post-calving, when both feed intake and milk yield rose in parallel. Similar relationships have been reported in dairy cattle, where nutrient intake during early lactation is critical for supporting the metabolic demands of milk synthesis (NRC, 2001). The methyl-donor role of specific feed components may also contribute to enhanced hepatic metabolism and nutrient partitioning toward milk production.

##### 4.2 Body Weight and Milk Production:

The tendency for heavier animals to produce more milk observed in this study is consistent with established physiological principles. Larger animals typically possess greater metabolic reserves and higher feed intake capacity, enabling them to sustain higher levels of milk production (Kumar et al., 2018). However, the relationship was not absolute, as evidenced by animal 105352-787794 (382 kg body weight) producing 5.76 L/day, which was comparable to some heavier animals. This suggests that factors beyond body weight, including genetic potential, parity, and individual variation in metabolic efficiency, also play important roles in determining milk yield.

##### 4.3 Early Lactation Dynamics:

The rapid increase in both feed intake and milk yield during the first 15 days post-calving highlights the critical nature of this transitional period. During this time, the animal's nutrient requirements for milk synthesis escalate while voluntary feed intake is still recovering from periparturient depression (Haque et al., 2021). The parallel rise in both parameters observed in this study suggests that the feeding management practiced on this farm effectively supported the nutritional demands of early lactation. This finding underscores the importance of gradually increasing feed provision immediately after calving to prevent negative energy balance and its associated metabolic disorders.

##### 4.4 Factors Affecting Milk Yield:

The regression analysis revealed that feed intake explained approximately 45% of the variation in milk yield ( $R^2 = 0.45$ ), indicating that while nutrition is a major determinant, other factors account for the remaining 55% of variation. These factors likely include genetic merit, parity, health status, environmental conditions, management practices, and individual animal variation in feed conversion efficiency. The strong correlation ( $r = 0.65$ ) suggests that feed management is the single most

important controllable factor influencing milk production in this herd, supporting the focus on nutritional optimization as a key strategy for improving productivity.

#### 4.5 Practical Implications:

The observed alignment between feed input and milk output provides quantitative validation for the feed-to-yield management approach practiced on this farm. The results suggest that regularly increasing feed provision for at least the first two weeks postpartum is recommended to help animals reach their maximum milk production potential. Continuous synchronization of feed and yield monitoring can assist in promptly addressing production dips or health issues. The finding that each additional kilogram of feed translates to nearly one liter of extra milk provides a useful benchmark for economic analysis of feeding strategies.

#### 4.6 Study Limitations:

Several limitations of this study should be acknowledged. First, the small sample size (n=6) limits the generalizability of findings to the broader Murrah buffalo population. Second, the on-farm nature of the study, while providing real-world relevance, introduces variability that cannot be controlled as rigorously as in experimental settings. Third, milk composition data (fat, protein, SNF) were not recorded, which would provide a more complete picture of production efficiency. Fourth, the study period of 94 days, while capturing early to mid-lactation, does not encompass the entire lactation curve. Future studies should include larger numbers of animals across multiple farms, incorporate milk composition analysis, and extend observations throughout the complete lactation period.

### V. CONCLUSION

The present study demonstrates a clear positive relationship between feed intake and milk yield in Murrah buffaloes, particularly during the critical first 15 days post-calving. The strong correlation ( $r = 0.65$ ) and regression coefficient (0.98 L milk per kg feed) provide quantitative evidence supporting the importance of optimal nutritional management in early lactation. Body weight showed a general positive association with milk production, though individual variation highlights the influence of other factors.

Key practical recommendations emerging from this study include:

1. Gradually increasing feed provision during the first two weeks post-calving to support rising milk production
2. Continuous monitoring of feed intake and milk yield to identify and address production dips promptly
3. Using the feed-to-milk conversion ratio (approximately 1:1 on a kg-feed to liter-milk basis) as a benchmark for evaluating feeding efficiency

Future research should focus on larger-scale studies across multiple farms, incorporation of milk composition parameters, and investigation of genetic and environmental factors influencing feed conversion efficiency in Murrah buffaloes. Such studies will further refine feeding recommendations and contribute to improved productivity and profitability in dairy buffalo enterprises.

### CONFLICT OF INTEREST

The authors declare no conflict of interest.

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# Effect of Different Times and Methods of Budding in Apricot (*Prunus armeniaca* L.) using Seedling Rootstock of Peach

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**Abstract**— The present investigation on effect of different times and methods of budding in apricot (*Prunus armeniaca* L.) using seedling rootstock of peach was conducted under field conditions at the Fruit Nursery and Department of Fruit Science, College of Horticulture, VCSG Uttarakhand University of Horticulture and Forestry, Bharsar, Pauri Garhwal, Uttarakhand, during 2019. The experiment consisted of twenty-four treatment combinations which were replicated thrice in Factorial Randomized Complete Block Design. Apricot cv. Newcastle was budded at 15-day intervals from 15th July to 30th September using four budding methods: T-budding, Patch budding, Chip budding, and Ring budding. Observations were recorded on days to sprouting, sprout length, sprout diameter, number of branches, number of leaves, leaf area, dead plants after sprouting, dead plants without sprouting, survival percentage, and saleable plant percentage. Earliest bud sprout (78.588 days), maximum sprout length (48.25 cm), thickest sprout diameter (0.878 cm), highest number of branches (25.333), maximum number of leaves (115.7), and highest survival percentage (71.25%) were observed for plants budded on 15th July. Among methods, chip budding resulted in earliest sprouting (135.3 days) and highest survival (67.9%), while T-budding produced longer shoots (29.8 cm) and better saleable plants (79.7%). The combination of 15th July with chip budding (TIM3) performed best across most parameters with earliest sprouting (70 days), longest sprouts (53.3 cm), thickest sprouts (0.95 cm), highest branches (27.3), most leaves (129), highest survival (85.0%), and highest saleable plants (91.7%). Budding in late September resulted in poor performance across all parameters. The study concludes that mid-July budding with chip budding is most suitable for apricot propagation on peach rootstock under Garhwal Himalayan conditions.

**Keywords**— Budding, Peach seedling rootstock, Time of budding, Method of budding, Sprout length, Bud take success, Apricot propagation.

## I. INTRODUCTION

Apricot (*Prunus armeniaca* L.) belongs to family Rosaceae and is mostly grown in the temperate regions of the world i.e., Western and Central Asia, Europe, North Western Himalaya and Western Tibet. In origin it is said to be native to China and in India it is mostly grown in Jammu and Kashmir, Himachal Pradesh, and Uttarakhand and to a limited extent in the Nilgiris with an annual production of 24.94 lakh metric tonnes from an area of 3.38 lakh ha (Sheikh et al., 2021). In Uttarakhand, it occupies total area of 8065.02 hectare with an annual production of 28026.62 metric tonnes (Anonymous, 2019). Almora district has maximum area and production of apricot with some cultivars like Shakarpara, Nugget, and Ladakhi etc. which have better fruit quality and are grown in higher hills (Naithani et al., 2018). New Castle is the most commercially accepted cultivar of apricot for the mid hills area of Uttarakhand (Sharma et al., 2014). It is a mid-season cultivar orange-yellow in colour and

has good demand for both table and processing purpose (Pawar et al., 2023). It ripens towards the end of May (Prakash et al., 2020). Apricots are enjoyed as fresh fruit, but also used for preparation of different value-added products due to their perishable nature. Mesocarp of the fruit is edible while endocarp (stone) remained waste. However, seed kernels are enclosed in stone. These apricot kernels are valuable sources of lipid and basically processed for their edible oils (Pawar et al., 2023).

Propagation of apricot by seed is not recommended because seeds are produced by cross fertilization and result in undesirable trait combinations. Seedlings grown from cross between commercial fruiting varieties contain a mix of parental genetic backgrounds and they are not identical to their parents and also differ in growth and fruiting habit (Bourguiba et al., 2012). This variability is desirable for plant breeding in development of new varieties but it is not desirable for uniform growth in orchard establishment (McKey et al., 2010). Apricot and other stone fruits tend to have a poor root system that is susceptible to pests, pathogens and environmental stress, so that stone fruits are propagated by budding or cutting techniques and grown on rootstock varieties (Hartmann et al., 2002). Stone fruits such as apricot, plum, peaches and nectarines are budded from late July to September for retail nurseries because trees produce a larger caliper (Hussain et al., 2018). The present study was therefore conducted to determine the suitable time of budding and to identify the best budding method for apricot propagation on peach seedling rootstock under the hilly conditions of Garhwal Himalaya.

## II. MATERIALS AND METHODS

The present investigation entitled "Effect of different times and methods of budding in apricot (*Prunus armeniaca* L.) using seedling rootstock of peach" was conducted under open field conditions at the Fruit Nursery, Department of Fruit Science, College of Horticulture, VCSG Uttarakhand University of Horticulture and Forestry, Bharsar, Pauri Garhwal, Uttarakhand, India, during the year 2019-20. Apricot variety "Newcastle" was budded at 15-day intervals on peach seedling rootstock, from 15th July up to 30th September with T-budding, Chip budding, Patch budding and Ring budding methods.

The experiment was laid out in Factorial Randomized Complete Block Design with twenty-four treatment combinations replicated thrice. The following parameters were studied:

**Days taken to first bud sprouting (number of days):** The number of days required for sprouting of buds (from the date of budding) was recorded treatment-wise for each plant of apricot.

**Length of sprouted shoots of budded plants (cm):** The length of sprouted shoots of budded plants was measured with the help of scale in centimetre at 30 days intervals after initiation, for each plant separately and average length of sprouted shoots per plant was calculated. These observations were recorded up to final survival of budding and average was worked out.

**Diameter of sprout (cm):** The diameter of randomly selected sprout was measured in centimetre with the help of screw gauge at 30 days interval and average was worked out.

**Number of branches per bud sprout:** The number of branches was counted treatment-wise for each plant and average number per plant was calculated.

**Number of leaves per bud sprout:** The total number of leaves was counted on newly emerged shoots of buds at 30 days interval and average number of leaves on sprouted buds was calculated.

**Total leaf area (cm<sup>2</sup>):** The leaf area was recorded by means of a digital leaf area meter. Firstly, ten leaves were selected randomly from each of the tagged budded plants and their average leaf area was calculated and multiplied with average number of leaves per plant to obtain the total leaf area.

**Dead budded plants without sprouting (%):** The percent of dead budded plants was recorded in each treatment at 270 days after budding. It was calculated with the help of following formula:

$$\text{Dead budded plants without sprouting (\%)} = \frac{\text{Number of dead budded plants}}{\text{Total budded plants}} \times 100 \quad (1)$$

**Dead budded plants after sprouting (%):** The percent of dead budded plants after sprouting observed in each treatment at 270 days after budding was calculated with the help of following formula:

$$\text{Dead budded plants after sprouting (\%)} = \frac{\text{Number of dead budded plants after sprouting}}{\text{Total budded plants}} \times 100 \quad (2)$$

**Total survival of budded plants (%):** The survival of budded plants was recorded in each treatment after 8 to 10 months at the termination of experiment and the survival percentage of buds was calculated by following formula:

$$\text{Total survive plants (\%)} = \frac{\text{Number of survive budded plants}}{\text{Total budded plants}} \times 100 \quad (3)$$

**Total saleable plants (%):** The plants attaining good height and vigour in growth were considered as saleable plants. The number of such saleable plants was recorded at the end of growing season and expressed as:

$$\text{Total saleable plants (\%)} = \frac{\text{Number of saleable plants}}{\text{Total survived plants}} \times 100 \quad (4)$$

Data were analyzed using appropriate statistical software and means were compared using Duncan's Multiple Range Test (DMRT) at 5% level of significance.

### III. RESULTS AND DISCUSSION

#### 3.1 Days Taken to Bud Sprouting:

The data presented in Table 1 show that the earliest bud sprout (77.8 days) was observed when budding was practiced on 30th July (T2 treatment). This may be due to favorable environmental conditions. While the maximum time (181.7 days) was taken by plants budded at the end of September (T6). Among various methods of budding, chip budding (M3) ranked first in bud burst which took only 135.3 days to sprout. Good sap flow in the bark and high temperature might be the factors which favoured callusing and proliferation at the bud union. Maximum days taken to bud sprout (148.5 days) were recorded in Ring budding (M4). The interaction among days to sprout showed significant effect for different times and methods. Among all treatment combinations, T1M3 (15th July with chip budding) showed the earliest bud sprout (70.0 days). This may be due to maximum sap flow in rainy season creating suitable conditions for this time and method of budding. The maximum days taken to bud sprout (189.0 days) was recorded in ring budding at the end of September (T6M4). In accordance with our present investigation, Ahmad et al. (2012) observed significant effect of budding method and time on number of days to sprouting, showing that maximum number of days to sprouting (199.14) were observed in plants produced through T-budding in September while minimum number of days to sprouting (194.29) were recorded for chip budding in August in guava.

#### 3.2 Average Length of Longest Bud Sprout (cm):

Data presented in Table 1 show that the maximum average length of longest bud sprout (48.25 cm) was recorded under T1 (15th July). The reason is that the bud sprout resulting from budding during the growth season had a longer time available for its growth, while the minimum length of shoot (20.58 cm) was observed in T6 (30th September). T-budding (M1) gave maximum length of bud sprout (29.8 cm). However, the minimum shoot length (25.2 cm) was found in treatment M4 (ring budding). The interactions among different times and methods of budding were also found significant with respect to the longest bud sprout. Maximum sprout length (53.3 cm) was obtained when chip budding was practiced on 15th July (T1M3 treatment combination). Whereas, minimum (16.3 cm) was recorded in treatment combination T6M4 (30th September + ring budding). These results confirm the findings of Yazdani et al. (2015) who indicated that shoot length was obtained from black sweet cherry scion with T-budding performed at 10-12 cm above ground.

#### 3.3 Diameter of Thickest Sprout (cm):

Data presented in Table 1 show that budding on 15th July (T1 treatment) recorded the thickest sprout with a diameter of 0.878 cm. The obtained results could be related to high precipitation and high summer temperatures during July which created good conditions for bud growth and development of the stem. Whereas, minimum diameter of sprout (0.716 cm) was observed in T6 (30th September). When different methods of budding were practiced, the maximum average diameter (0.778 cm) was recorded with T-budding (M1 treatment). This may be due to early and good wound tissue formation on cut surfaces and rapid cambial connectivity between stock and bud. Whereas M4 (ring budding) recorded minimum average diameter (0.756 cm).

Budding on 15th July with chip budding (T1M3 treatment combination) was found to give maximum average diameter of thickest sprout (0.95 cm). It is clear from the mean data that plants budded through chip budding had maximum bud growth which results in maximum stem thickness due to more photosynthetic activity. While the minimum diameter of sprout (0.69 cm) was recorded in the treatment combination T6M1 (30th September + T-budding). Ananda et al. (1999) reported that statistical analysis of the data revealed that budding dates, methods and their interaction had significant effect on stem thickness in peach budding. Maximum stem thickness (1.905 cm) was recorded on plants budded on Peshawar local rootstock of peach.

### 3.4 Number of Branches per Budded Plant:

Data presented in Table 1 show that T1 treatment (15th July) resulted in maximum average number of branches per budded plant (25.333). This might be due to long growing period and active vegetative growth of plants at this time. However, the minimum number of branches per budded plant (15.5) were found in T6 (30th September). Among different methods, Patch budding (M2 treatment) gave maximum average number of branches per sprout (22.5). The highest number of branches might be due to the quick union formation under patch budded plants resulting in the best performance of vegetative characters. The minimum average number of branches per budded plant (18.1) was recorded in M4 (ring budding). The interaction among different times and methods found that the maximum number of branches (27.3) was recorded under T1M3 treatment combination (15th July with chip budding). The better number of branches with optimum time and methods might be due to better bud growth which augmented absorption and translocation of nutrients from soil, taking active part in various plant metabolic processes (Singh, 2001). The minimum number of branches per budded plant (11.3) was recorded in the treatment combination T6M4 (30th September + ring budding). The results are in parallel with Janesari and Jafarpour (2015) who budded apricot scion on plum rootstock and found that August may be the most appropriate time of budding with significant effect on the average maximum number of branches (61.0).

### 3.5 Number of Leaves:

Data presented in Table 1 show that the number of leaves on budded plants (115.7) was maximum under T1 treatment (15th July). This may be due to increased growth of plants resulting in a greater number of leaves, long growth season and active growth period. However, the minimum number of leaves (30.6) was found in treatment T6 (30th September). When different methods of budding were practiced, the maximum number of leaves on budded plants (70.5) was recorded from T-budding (M1 treatment). This may be due to wood maturity of budwood which probably reserves high starch and sugar, while the lowest number of leaves (59.4) were recorded in M4 (ring budding). The interactions among different times and methods of budding were also found significant with respect to the number of leaves. The 15th July with chip budding (T1M3 treatment combination) was found to give maximum number of leaves on new sprouts (129). The higher number of leaves with optimum time and methods might be due to better bud growth and more number of branches. Ring budding practiced on 30th September (T6M4) gave minimum (29.7) average number of leaves. This augmented absorption and translocation of nutrients from soil which take active part in various plant metabolic processes. These results matched the findings of Akhtar et al. (2000) who observed that maximum number of leaves (292.546) occurred on peach plants budded on 28th August with chip budding, which may be due to maximum number of branches and maximum bud growth.

### 3.6 Leaf Area (cm<sup>2</sup>):

The data recorded in Table 1 for leaf area (26.20 cm<sup>2</sup>) was found highest when budding was done on 15th July (T1 treatment). It is due to vigorous growth of plant as it is capable of absorbing more nutrients and preparing more photosynthates resulting in maximum leaf area. However, the minimum leaf area (23.07 cm<sup>2</sup>) was recorded in T4 (30th August). Among various methods of budding practiced, the maximum leaf area (29.42 cm<sup>2</sup>) was recorded under patch budding (M2 treatment). It is due to stronger bud union and development of normal vascular tissues at the bud union which regulates the transport of water and nutrients, thereby increasing the leaf area. Whereas M4 (ring budding) recorded minimum leaf area (26.17 cm<sup>2</sup>). The interactions among different times and methods of budding were also found significant with respect to leaf area. Chip budding on 15th July (T1M3 treatment combination) was found to give maximum leaf area (28.5 cm<sup>2</sup>). It might be due to the fact that chip budded plants had early sprouting under this time, leaf emergence and rapid vegetative growth as compared to T, patch

and ring budded plants. However, the minimum leaf area (16.4 cm<sup>2</sup>) was observed in T6M4 (30th September + ring budding). The results are in parallel with Kumar and Ananda (2004) who reported in apple that leaf area was maximum with chip budding in August, and leaf area is directly related to the maximum number of leaves.

### 3.7 Dead Budded Plants without Sprouting (%):

Data recorded in Table 1 show that the percentage of dead budded plants without sprouting (14.08%) was lowest under T1 treatment (15th July) and T2 treatment (30th July). This may be due to favorable climatic conditions for bud growth, while high temperature also inhibits bud bursting and causes even death of buds. However, the maximum dead plants (25.67%) were found in treatment T6 (30th September). Among various methods of budding practiced, the minimum percentage of dead budded plants was recorded in M2 (16.4%) and M3 (16.2%). This may be due to proper cambium formation with translocation of food material through xylem and phloem from stock to bud. The highest dead plants (17.7%) were recorded from M1 and M4 (T-budding and ring budding). The interaction between different times and methods of budding showed that the minimum percentage of dead budded plants (8.3%) was produced by T1M3 (15th July with chip budding). Poor shooting of the buds planted during cooler time of the season may be due to the fact that these buds might have been carrying higher inhibitors or it may be due to higher nitrogen to carbohydrate ratio. Maximum percent of dead budded plants (31.3%) was recorded in T6M4 (30th September + ring budding).

### 3.8 Dead Budded Plants after Sprouting (%):

Data presented in Table 1 show that the dead budded plants after sprouting (14.58%) were minimum under 15th July (T1 treatment). This may be due to favorable conditions for continued growth. However, the maximum dead plants (22.75%) were found in treatment T6 (30th September). Among various methods of budding, the minimum dead budded plants (12.11%) were recorded under M2 treatment (patch budding). This may be due to proper translocation of carbohydrates, starch and other factors. The highest dead plants (18.78%) were recorded in M4 (ring budding). The interactions among different times and methods of budding were also found significant. It is evident from the data that the minimum percentage of dead budded plants after sprouting (6.7%) was produced by T1M3 (15th July with chip budding). Poor shoot formation during this season may be due to higher nitrogen to carbohydrate ratio. Treatment combination T6M4 (30th September + ring budding) recorded the highest dead plants after sprout (43.3%).

### 3.9 Survival of Budded Plants (%):

Data presented in Table 1 show that the maximum survival percentage (71.25%) was obtained with 15th July (T1 treatment). This may be affected by season and several factors such as temperature, light and nutrient availability. Minimum survival percent (40.08%) was recorded in T6 (30th September). Among different methods, chip budding (M3 treatment) gave maximum survival percentage (67.9%). This may be due to requiring more cell sap for union and thus scion is held more tightly in place as compared to patch budding. Minimum survival percent (53.9%) was recorded from the treatment M4 (ring budding). The interaction between different times and methods had significant effect on survival percentage. The plants budded on 15th July had the maximum survival percentage (85.0%) with chip budding (T1M3 treatment combination), while minimum was recorded (16.3%) in T5M4 (15th September + ring budding). It is due to the fact that budding in July produces favorable environmental conditions for the healing process of bud wounds and results in the development of normal vascular tissues at the bud union, which results in maximum plant survival. Similarly, budding late in the growing season contributed to unfavorable conditions for healing process which results in poor development of normal vascular tissue at the bud union. The findings of the study conducted by Negi et al. (2010) observed in case of propagation methods (patch budding) of Aonla gave better survival of budded plants (80.333%).

### 3.10 Total Saleable Plants (%):

Data recorded in Table 1 show that the plants budded on 30th July (T2 treatment) gave maximum saleable plants (81.25%). It is due to longer period of time available for growth. Whereas the minimum saleable plants percent (62.67%) was recorded in T6 (30th September). Among different methods, patch budding (M2 treatment) gave maximum saleable plants (76.7%). It is

due to these plants requiring heading back for quality and good health growth in the next year. Minimum saleable percent (67.3%) was recorded from the treatment M4 (ring budding). Interaction among different times and methods had significant effect. The best result was obtained as high as 91.7% with chip budding practiced on 15th July (T1M3). Higher percentage of such plants obtained on 15th July with chip budding is attributed to proper and quick union formation, early bud sprout and longer period of time available for growth. Similar results by Joolka and Rindhe (2000) obtained the highest proportion of saleable plants (98.324%) in chip budding, followed by T-budding in pecan nut. The minimum saleable plants (53.7%) were recorded in T6M4 (30th September + ring budding).

**TABLE 1**  
**EFFECT OF TIME OF BUDDING AND METHODS OF BUDDING ON GROWTH, SURVIVAL, AND SALEABLE PARAMETERS OF APRICOT BUDDED ON PEACH SEEDLING ROOTSTOCK**

Treatment	Days taken to bud sprout	Length of longest bud sprout (cm)	Diameter of thickest sprout (cm)	Number of branches per budded plant	Number of leaves	Leaf area (cm <sup>2</sup> )	Dead budded plants without sprouting (%)	Dead budded plants after sprouting (%)	Survival (%)	Saleable (%)
<b>Factor A</b>										
T1	78.5e	48.25a	0.878a	25.333a	115.7a	26.20b	14.08d	14.58a	71.25a	79.75b
T2	77.8e	28.42b	0.754b	24.25b	91.0b	26.15b	14.08d	17.42b	68.25b	81.25a
T3	172.0c	25.67c	0.758b	18.333c	66.0c	24.03c	14.83c	17.25c	67.67c	77.75c
T4	167.9d	23.50d	0.748c	18.667c	47.2d	23.07d	14.92c	16.50d	67.08d	69.25e
T5	174.3b	21.17e	0.742c	17.583d	31.4e	32.45a	18.25b	8.92e	42.17e	71.83d
T6	181.7a	20.58f	0.716d	15.500e	30.6f	26.15b	25.67a	22.75f	40.08f	62.67f
<b>Factor B</b>										
M1	141.7c	29.8a	0.778a	20.1b	70.5a	23.45d	17.6a	18.17b	64.4b	79.7a
M2	142.8b	27.9b	0.751b	22.5a	60.5c	29.42a	16.4b	12.11d	51.3d	76.7b
M3	135.3d	28.8c	0.779a	19.1c	69.2b	26.33b	16.2b	15.89a	67.9a	71.2c
M4	148.5a	25.2d	0.756b	18.1d	59.4d	26.17c	17.7a	18.78c	53.9c	67.3d

<sup>1</sup>Means in the same column followed by the same lowercase letter(s) are not significantly different at  $p \leq 0.05$  according to Duncan's Multiple Range Test (DMRT).

<sup>2</sup>Factor A: Time of budding; Factor B: Methods of budding.

<sup>3</sup>T1 = 15 July, T2 = 30 July, T3 = 15 August, T4 = 30 August, T5 = 15 September, T6 = 30 September. M1 = T-budding, M2 = Patch budding, M3 = Chip budding, M4 = Ring budding.

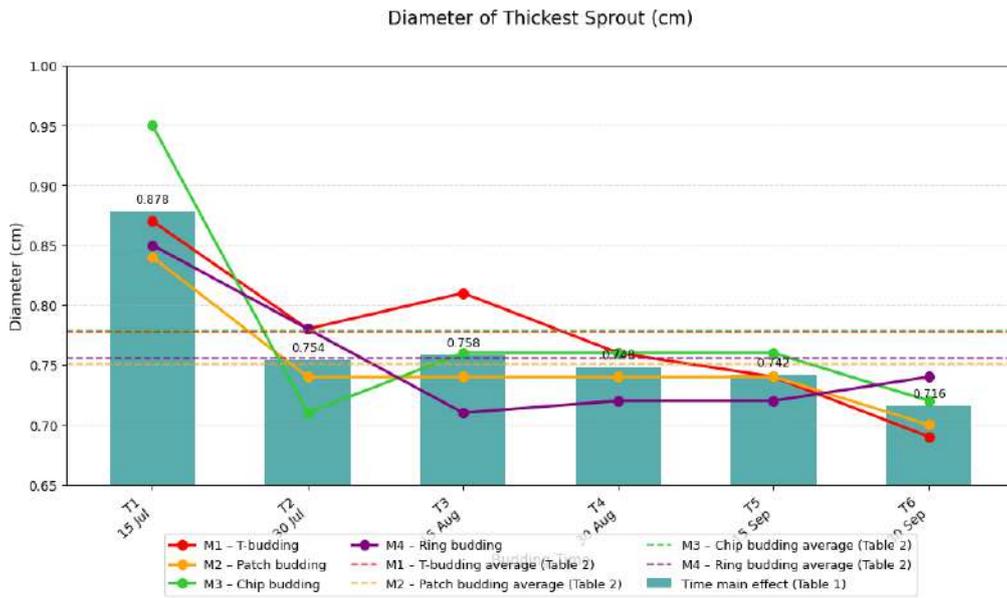
**TABLE 2**  
**INTERACTION EFFECTS OF TIME AND METHOD OF BUDDING ON GROWTH, SURVIVAL, AND SALEABLE**  
**PARAMETERS OF APRICOT BUDDED ON PEACH SEEDLING ROOTSTOCK**

Treatment	Days taken to bud sprout	Length of longest bud sprout (cm)	Diameter of thickest sprout (cm)	Number of branches per budded plant	Number of leaves	Leaf area (cm <sup>2</sup> )	Dead budded plants without sprouting (%)	Dead budded plants after sprouting (%)	Survival (%)	Saleable (%)
T1M1	85.3l	49.0b	0.87b	25.0bc	117b	25.7i	13.7k	15.3f	70.3c	81.3g
T1M2	80.3m	45.7c	0.84c	25.7b	112c	25.9h	17.7fg	19.7c	62.3g	75.7i
T1M3	70.0p	53.3a	0.95a	27.3a	129a	28.5e	8.3n	6.7g	85.0a	91.7a
T1M4	78.3mn	45.0c	0.85c	23.3d	105d	24.7k	16.7hi	16.7e	67.0d	70.3m
T2M1	76.3n	30.3d	0.78e	25.7b	99e	27.4f	13.3k	18.3d	67.7d	86.7d
T2M2	78.7m	27.3f	0.74g	25.3bc	93f	25.7i	11.7l	17.7d	70.3c	77.3h
T2M3	73.0o	29.7d	0.71hi	24.3cd	89g	26.4g	14.7j	15.3f	69.7c	83.7f
T2M4	83.3l	26.3g	0.78e	21.7e	83h	25.1j	16.7hi	18.3d	65.3f	77.3h
T3M1	168.0ij	28.7e	0.81d	19.7f	79i	23.0n	17.3gh	20.3c	64.7f	89.7c
T3M2	176.3f	26.3g	0.74g	18.7fg	68k	25.0j	18.3f	16.7e	64.7f	90.7b
T3M3	160.3k	26.3g	0.76f	16.7ij	72j	24.2l	10.3m	15.3f	74.7b	62.3p
T3M4	183.3b	21.3k	0.71hi	18.3gh	45n	23.9m	13.3k	16.7e	66.7de	68.3n
T4M1	166.3j	24.3h	0.76f	19.3fg	49l	22.6p	15.3j	16.3e	66.7de	68.3n
T4M2	170.3gh	23.3i	0.74g	19.7f	47m	22.8o	16.3i	16.7e	66.7de	69.7m
T4M3	159.0k	24.7h	0.76f	18.3gh	49l	23.0n	14.7j	16.3e	69.3c	71.3l
T4M4	176.0f	21.7jk	0.72h	17.3hi	44no	23.9m	13.3k	16.7e	65.7ef	67.7n
T5M1	175.0f	22.3j	0.74g	17.3hi	41.7p	23.0n	19.3e	15.3f	67.7d	85.7e
T5M2	169.0hi	21.7jk	0.74g	21.0e	21.0t	41.9b	17.3gh	1.0h	24.3l	74.7j
T5M3	172.3g	20.3l	0.76f	15.7j	43.0o	21.9q	21.7d	18.3d	60.3n	60.3q
T5M4	181.0cd	20.3l	0.72h	16.3ij	20.0q	43.0a	14.7j	1.0h	16.3h	64.7o
T6M1	179.0de	24.3h	0.69j	13.7k	38.0u	19.0r	26.3c	23.3b	49.7i	66.7o
T6M2	182.0bc	23.3i	0.70ij	24.7bc	21.7t	35.2c	17.3gh	1.0h	48.7m	72.7k
T6M3	177.0ef	18.3m	0.72h	12.3l	33.0r	34.0d	27.7b	23.3b	19.3j	57.7r
T6M4	189.0a	16.3n	0.74g	11.3l	29.7s	16.4s	31.3a	43.3a	42.7k	53.7s

<sup>1</sup>Means in the same column followed by the same lowercase letter(s) are not significantly different at  $p \leq 0.05$  according to Duncan's Multiple Range Test (DMRT).

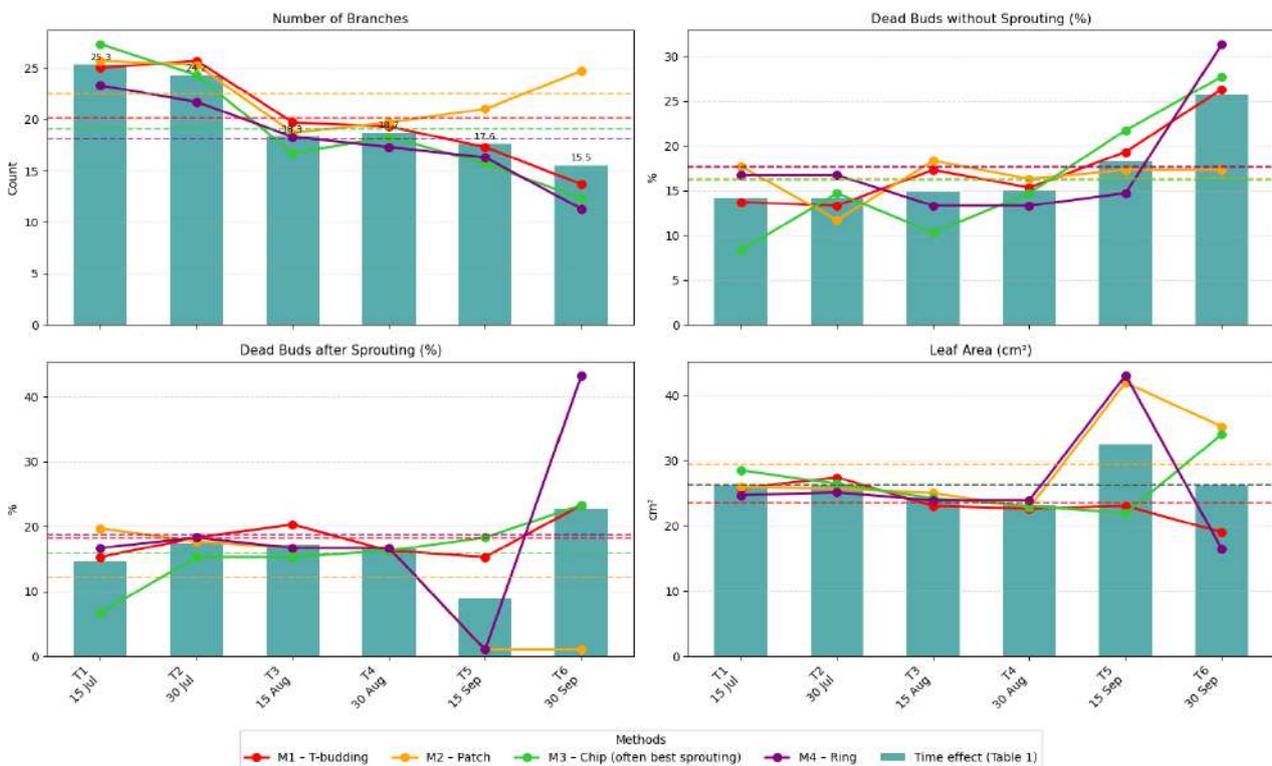
<sup>2</sup>Factor A: Time of budding; Factor B: Methods of budding.

<sup>3</sup>T1 = 15 July, T2 = 30 July, T3 = 15 August, T4 = 30 August, T5 = 15 September, T6 = 30 September. M1 = T-budding, M2 = Patch budding, M3 = Chip budding, M4 = Ring budding.



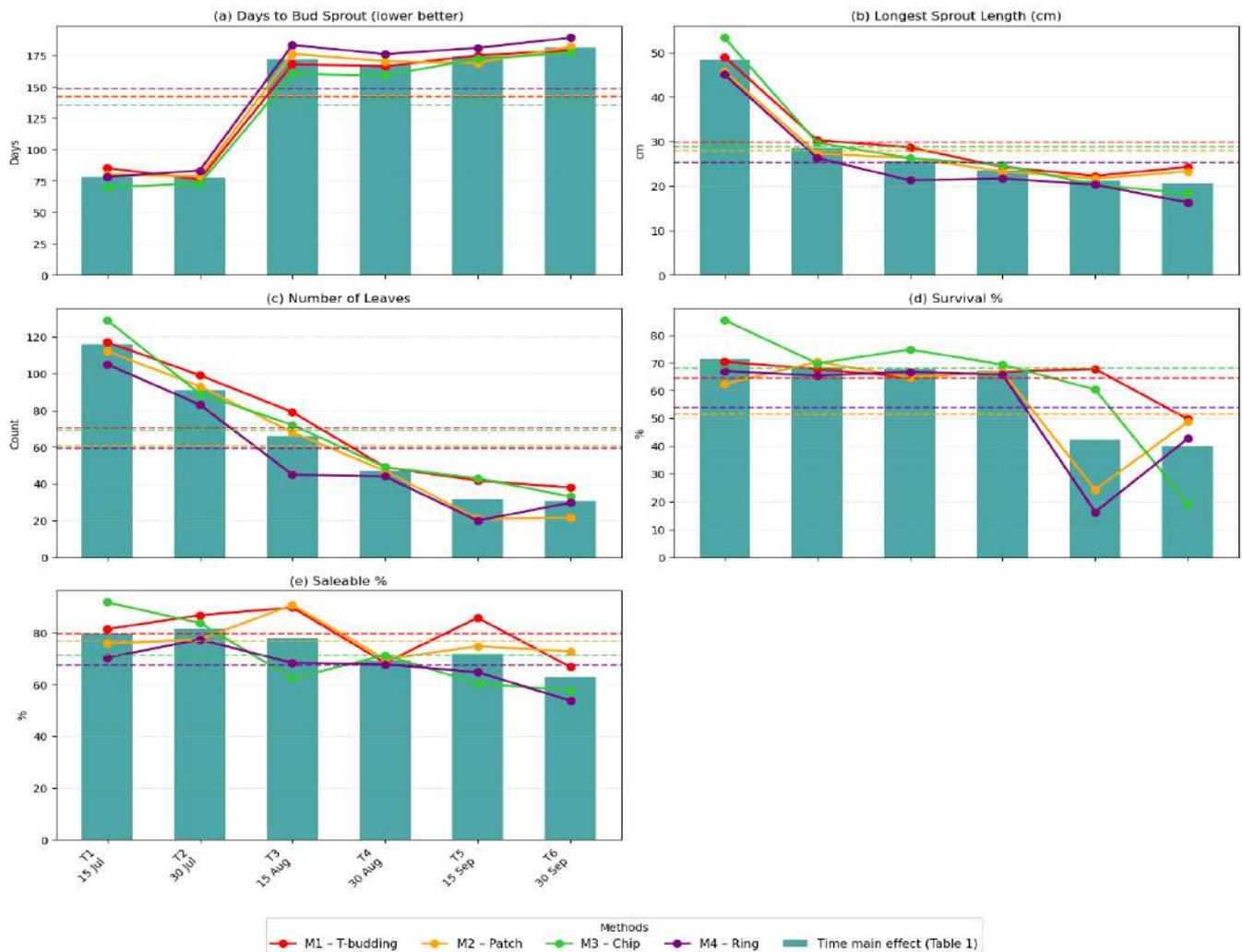
**FIGURE 1: Effect of Budding Time (Factor A) and Method (Factor B) on Diameter of Thickest Sprout**  
 \*Caption: Bars represent the main effect of budding time (Factor A – Table 1). Colored solid lines with markers represent the interaction effects (Time × Method combinations – Table 2). Dashed colored lines represent the main effect averages of budding methods (Factor B – Table 1). T1 = 15 July, T2 = 30 July, T3 = 15 August, T4 = 30 August, T5 = 15 September, T6 = 30 September. M1 = T-budding, M2 = Patch budding, M3 = Chip budding, M4 = Ring budding.\*

Combined View: Number of Branches, Dead Buds & Leaf Area



**FIGURE 2: Influence of Budding Time (Factor A) and Method (Factor B) on Key Growth and Survival Parameters**

\*Caption: Bars represent the main effect of budding time (Factor A – Table 1). Colored solid lines with markers represent the interaction effects (Time × Method combinations – Table 2). Dashed colored lines represent the main effect averages of budding methods (Factor B – Table 1). T1 = 15 July, T2 = 30 July, T3 = 15 August, T4 = 30 August, T5 = 15 September, T6 = 30 September. M1 = T-budding, M2 = Patch budding, M3 = Chip budding, M4 = Ring budding.\*



**FIGURE 3: Influence of Budding Time (Factor A) and Method (Factor B) on Branches, Bud Failure Rates, and Leaf Area**

\*Caption: Bars represent the main effect of budding time (Factor A – Table 1). Colored solid lines with markers represent the interaction effects (Time × Method combinations – Table 2). Dashed horizontal lines represent the main effect of budding method averages (Factor B – Table 1). T1 = 15 July, T2 = 30 July, T3 = 15 August, T4 = 30 August, T5 = 15 September, T6 = 30 September. M1 = T-budding, M2 = Patch budding, M3 = Chip budding, M4 = Ring budding.\*

#### IV. CONCLUSION

In this study, we found that when and how you bud apricot scions makes a significant difference in plant performance, especially under the mid-hill conditions of Garhwal Himalaya. Budding in mid-to-late July (15–30 July) clearly gave the best results across most parameters. Plants budded in this window sprouted the fastest (around 77–79 days), grew the longest shoots (up to 48 cm), had the thickest stems, produced more branches and leaves, and ended up with higher survival rates (71%) and a larger percentage of good-quality, saleable plants (up to 81%). The early monsoon timing—with good moisture, warm weather, and active sap flow—gave the buds the perfect chance to heal quickly and grow strong before winter.

When we compared the budding methods, chip budding stood out for getting sprouts to emerge quickly and keeping more plants alive. T-budding was better for longer shoots and more leaves. Ring budding, on the other hand, did not perform well in most cases.

The clear winner overall was chip budding done on 15th July (T1M3)—it sprouted the fastest (70 days), grew the strongest shoots (53.3 cm long, 0.95 cm thick), produced the most branches (27.3) and leaves (129), and delivered the highest survival (85.0%) and saleable plant percentage (91.7%). Budding later in September, by contrast, led to slow sprouting, weak growth, more dead buds, and far fewer plants worth selling.

Based on these findings, it is recommended that nursery operators in the Garhwal Himalayan region practice chip budding during mid-July for optimal success in apricot propagation on peach seedling rootstock.

#### CONTRIBUTION OF AUTHORS

Ria Rautela: Present idea, conduct of field research, manuscript writing, data analysis. Sadhana Bhatt and Namita Dabral: Visualization of data and structuring of manuscript.

#### COMPETING INTEREST

Authors have declared that no competing interests exist.

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#### CONFLICT OF INTEREST

The authors declare no conflict of interest.

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# Assessing the Economics of Sunhemp in Rice Fallow Systems: Boosting Farm Income

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**Abstract**— A field experiment was conducted to study the economics of sunhemp varieties under different sowing windows for seed production in rice fallow system in NC zone during Rabi 2023-24 at Agricultural College Farm, Naira. The experiment was laid out in a Factorial randomized block design with two factors and three replications. Factor one comprised of three varieties JRJ 610 (V1), SUIN 03 (V2), SUIN 037 (V3) and factor two comprised of four sowing windows i.e. first fortnight of November (S1), second fortnight of November (S2), first fortnight of December (S3) and second fortnight of December (S4). Higher gross returns (Rs. 207540 ha<sup>-1</sup>), net returns (Rs. 123410 ha<sup>-1</sup>) and B:C ratio (2.47) of rice-sunhemp system were realized with sunhemp variety SUIN 037 sown during second fortnight of November as relay crop after kharif rice. Whereas higher gross returns (Rs. 84,825 ha<sup>-1</sup>), net returns (Rs. 54,925 ha<sup>-1</sup>), returns per rupee invested (Rs. 1.84) and highest B:C (2.84) of sole sunhemp crop was obtained with sunhemp variety SUIN 037 sown during first fortnight of November. Choosing the right variety with pest and disease resistance and adaptability to local environmental conditions is essential for good crop establishment as relay crop in rice-based cropping system as alternative to the rice-pulse system in North Coastal Zone of Andhra Pradesh.

**Keywords**— Sunhemp, Sowing windows, Rice-sunhemp cropping system, Economics.

## I. INTRODUCTION

Sunhemp holds significant economic importance due to its diverse applications and benefits to agricultural systems. As a green manure crop, it enhances soil fertility and structure. It also adds substantial organic matter to the soil upon decomposition, further improving soil health and microbial activity, leading to increased crop yields. As a leguminous plant, it is capable of fixing atmospheric nitrogen through its symbiotic relationship with rhizobium bacteria in its root nodules (Deshmukh, 2023). The 60-days-old crop accumulates about 170 kg N, 20 kg P and 130 kg K ha<sup>-1</sup> (Pacharne et al., 2021). Sunhemp possesses allelopathic effects on weeds and suppresses weed growth and weed seed bank, which reduces the need for chemical herbicides, lowering production costs and minimizing environmental impact (Bhandari et al., 2022). It is well known for its coarse textured pectino-cellulosic fibre with good fibre characteristics and high yield, which is used for manufacturing a wide variety of products like paper, marine cordage, rope, string and fishing net, coarse for hose pipe, belting and canvas, tissue paper, bank currency, rugs, carpets, etc. (Shi et al., 2018).

Beyond its agronomic benefits, sunhemp can also serve as a source of additional income. It can be harvested for use as forage in livestock production, thereby opening up new markets for farmers. The potential for selling sunhemp seeds or biomass adds another layer of economic viability to its cultivation. Seed filling in sunhemp is negatively affected by late planting in the rabi rice fallows system. The best time to sow sunhemp seeds is the most crucial factor in achieving larger yields since it creates

ideal growing conditions. Sunhemp, a short-day plant, produces more biomass during longer days. In short days, it blooms early, which reduces vegetative development.

In north coastal districts of Andhra Pradesh, rice-pulse system is predominant. Considering the use of sunhemp as green manure crop for pre-kharif in rice growing areas, the demand for sunhemp seed is increasing day by day. By utilizing the residual moisture after harvest of the kharif paddy, sunhemp for seed purpose can be the best alternative to pulse crops in rice-based cropping system under relay cropping.

With increasing minimum support price for sunhemp seed and ease of agronomic practices, sunhemp seed crop may be the best alternative to pulses like greengram and blackgram in rice-based cropping system.

Delayed sowing leads to reduced seed yield and quality due to climatic constraints, primarily high temperature and limited moisture during crucial flowering and pod-filling stage. Timely sowing, often in the first or second week of November depending on the specific region and variety, is crucial for optimal seed yield and quality of sunhemp. Economically, the rice fallow system diversifies income sources for farmers and enhances food security, although it requires careful management of resources and knowledge transfer to ensure successful implementation (Kumar et al., 2019). Growing sunhemp in rice fallows is considerably more profitable than growing pulse crops in terms of higher growth and yield components. It also adds more green manure to the soil and increases the farmer's net revenue (Amarajyoti et al., 2023). Keeping these points in view, the present investigation was undertaken with the objective to study the economics of sunhemp varieties influenced by various sowing windows under rice fallow system in North Coastal Zone.

## II. MATERIALS AND METHODS

### 2.1 Experimental Site:

The present field experiment was conducted during rabi, 2023-24 at Agricultural College Farm, Naira, Acharya N.G. Ranga Agricultural University, Andhra Pradesh, which is geographically situated at 18.24° N latitude, 83.84° E longitude and with an altitude of 27 m above mean sea level in the North Coastal Zone of Andhra Pradesh.

### 2.2 Experimental Design and Treatments:

The experiment was laid out in a Factorial Randomized Block Design with three replications. Factor one comprised of three varieties:

- V1: JRJ 610
- V2: SUIN 03
- V3: SUIN 037

Factor two comprised of four sowing windows:

- S1: First fortnight of November
- S2: Second fortnight of November
- S3: First fortnight of December
- S4: Second fortnight of December

### 2.3 Crop Management:

The seed was easily dispersed with little labor, which aids in prompt sowing and also reduces labor costs involved. Each hectare received around 25 kg of seed. Five to six days after sowing, the seeds began to sprout. Blooming began 45 days after sowing, and the complete flowering phase, from 45 days after sowing (DAS) to 85 days after sowing (DAS), lasted roughly 40 days. It took 35–40 days for the seeds to fully develop and harden. The crop was harvested 140–150 days after sowing (DAS) for seed production.

### 2.4 Seed Yield:

The seed yield obtained from each plot of three replications was computed for hectare and was expressed in kilogram per hectare.

## 2.5 System Equivalent Yield:

The grain and straw yields of the rice crops were recorded from each plot after harvesting the crops to calculate the biological yield in ( $q\ ha^{-1}$ ) of component crops (Brar et al., 2023). Rice equivalent yield (REY) of the winter crops was computed by converting their grain yield to rice yield with a price factor as per following formula (Kumar et al., 2019)

$$REY\ (kg\ ha^{-1}) = \frac{\text{Grain yield of the winter crop (kg ha}^{-1}) \times \text{Price of winter crop (INR kg}^{-1})}{\text{Price of rice (INR kg}^{-1})} \quad (1)$$

## 2.6 Economic Analysis:

The economic components of different treatments were computed and worked out. The cost of cultivating the various treatments was determined by adding the common cost resulting from the various procedures and inputs utilized to all the expenses involved in the growth of the experimental crop. As a result, the cost of cultivation was computed for every combination of treatments.

The treatment wise gross monetary returns (Rs.  $ha^{-1}$ ) were worked out by multiplying total sunhemp seed yield separately under various treatment combination with their existing market price (Verma et al., 2011). By deducting the cost of production from the gross return of each treatment, the net monetary returns (Rs.  $ha^{-1}$ ) were calculated (Thimmanna et al., 2014).

$$\text{Net return (Rs. ha}^{-1}) = \text{Gross return (Rs. ha}^{-1}) - \text{Cost of cultivation (Rs. ha}^{-1}) \quad (2)$$

## 2.7 Returns per Rupee Invested:

The cost of cultivation for each treatment was calculated. Similarly, gross returns were calculated based on the prevailing market price of the produce. The net return was obtained after deducting the cost of cultivation from gross returns (Thimmanna et al., 2014). Later, the return per rupee was calculated using the formula:

$$\text{Return per rupee (Rs.)} = \frac{\text{Net returns (Rs. ha}^{-1})}{\text{Total operational cost (Rs. ha}^{-1})} \quad (3)$$

## 2.8 Benefit-Cost Ratio (B:C):

The benefit-cost ratio was calculated by dividing the net return by the cost of cultivation of the individual treatment combination (Kaur et al., 2024). The benefit-cost ratio was worked out by using the following formula:

$$\text{Benefit-cost ratio} = \frac{\text{Gross monetary returns (Rs. ha}^{-1})}{\text{Cost of cultivation (Rs. ha}^{-1})} \quad (4)$$

## III. RESULTS AND DISCUSSION

The data presented in Table 1 demonstrate how sunhemp varieties and sowing windows influenced the gross returns, net returns, returns per rupee invested and B:C ratio of sunhemp crop and rice-sunhemp system economics.

### 3.1 Effect of Varieties:

Among all tested varieties, sunhemp variety SUIN 037 (V3) realized maximum gross returns (Rs. 73,385  $ha^{-1}$ ), net returns (Rs. 43,485  $ha^{-1}$ ), returns per rupee invested (Rs. 1.45) and obtained highest B:C ratio (2.45). While the lowest gross returns (Rs. 62,328  $ha^{-1}$ ), net returns (Rs. 32,428  $ha^{-1}$ ), returns per rupee invested (Rs. 1.08) and B:C ratio of 2.08 were observed with variety SUIN 03 (V2).

The selection of the appropriate variety for sunhemp is critical for maximizing yield, improving soil health, and ensuring pest and disease resistance. Variety SUIN 037 (V3) having higher returns was due to its genetic variability and having more photosynthetic activity which later translocated photosynthates to sink, thus enhancing the yield.

### 3.2 Effect of Sowing Windows:

Sunhemp sown during November first fortnight (S1) showed significantly higher gross returns (Rs. 79,040  $ha^{-1}$ ), net returns (Rs. 49,140  $ha^{-1}$ ), returns per rupee invested (Rs. 1.64) and obtained B:C ratio (2.64). Least gross returns (Rs. 52,942  $ha^{-1}$ ), net returns (Rs. 23,042  $ha^{-1}$ ), returns per rupee invested (Rs. 0.77) and obtained B:C ratio (1.77) were recorded with sowing during second fortnight of December (S4).

Early sowing plays a crucial role in the successful cultivation of sunhemp (*Crotalaria juncea*). Sunhemp, being a short-duration legume, benefits from optimal growing conditions, particularly when sown early in the season. Early sowing i.e., first fortnight

of November ensures utilization of the available moisture, sunlight, and temperature, promoting vigorous growth and better establishment. Sowing sunhemp at the right time ensures ideal soil temperatures, which are essential for seed germination and early root establishment.

Early sown crops tend to develop deeper roots, helping them access more nutrients and water and also can help avoid peak periods of pest and disease infestation. For instance, many pests and diseases thrive in warm and moist conditions later in the growing season. Early-sown crops are less vulnerable to these conditions. Early sowing allows sunhemp to complete their life cycle before onset of hot and dry conditions resulting in higher yields. On the other hand, delayed sowing exposes the crop to unfavourable conditions like high temperature, pests, diseases, and moisture stress, which show negative impact on yield. The results are similar to the findings of Kaur et al. (2024) and Pacharne et al. (2021). Sunhemp sown during first fortnight of November recorded significantly high dry matter production, number of pods plant<sup>-1</sup> and seed yield as also reported by Satyavathi et al. (2024).

### 3.3 Interaction Effect:

In the interaction effect, sunhemp variety SUIN 037 (V3) sown at November first fortnight (S1) showed significantly higher gross returns (Rs. 84,825 ha<sup>-1</sup>), net returns (Rs. 54,925 ha<sup>-1</sup>), returns per rupee invested (Rs. 1.84) and obtained B:C ratio (2.84). While the lower gross returns (Rs. 50,700 ha<sup>-1</sup>), net returns (Rs. 20,800 ha<sup>-1</sup>), returns per rupee invested (Rs. 0.70) and B:C ratio of 1.70 were observed with variety SUIN 03 (V2) sown at second fortnight of December (S4). The results were confirmed by Ray et al. (2020) and Sandeep et al. (2023). The combination of early sowing and the right variety can have a synergistic effect on sunhemp growth and performance. With early sowing and high-yielding, disease-resistant varieties, farmers can ensure sunhemp plants have the right conditions to thrive, producing more biomass and contributing significantly to soil health improvement.

### 3.4 Economics of Rice-Sunhemp Cropping System:

Rice-sunhemp cropping system economics were calculated by using grain yield, cost of cultivation and minimum support price of both rice and fallow sunhemp crop for the respective year [Indian rupee (INR)] and the data are presented in Table 2.

Higher gross returns (Rs. 2,07,540 ha<sup>-1</sup>), net returns (Rs. 1,23,410 ha<sup>-1</sup>) of rice-sunhemp system were realized with B:C ratio (2.47) under sunhemp variety SUIN 037 (V3) sown during second fortnight of November (S2). Lower system gross returns (Rs. 1,60,320 ha<sup>-1</sup>), net returns (Rs. 76,190 ha<sup>-1</sup>) and B:C ratio (1.91) were noted with sunhemp variety SUIN 03 (V2) sown during second fortnight of December (S4). Early sowing of fast-growing varieties allows sunhemp to fit more efficiently into crop rotations, especially in systems where other crops need to be sown immediately after the green manure crop. Suitable varieties are often preferred for green manure because they produce higher biomass, which is crucial for organic matter incorporation and nitrogen fixation for succeeding crop.

**TABLE 1**  
**ECONOMICS OF SUNHEMP AS INFLUENCED BY DIFFERENT VARIETIES AND SOWING WINDOWS DURING RABI 2023-24**

Treatments	Seed Yield (kg ha <sup>-1</sup> )	Cost of cultivation (Rs. ha <sup>-1</sup> )	Gross return (Rs. ha <sup>-1</sup> )	Net return (Rs. ha <sup>-1</sup> )	Returns per rupee invested (Rs.)	B:C ratio
<b>Varieties</b>						
V1-JRJ 610	1048	29900	68120	38220	1.27	2.27
V2-SUIN 03	958.9	29900	62328	32428	1.08	2.08
V3-SUIN 037	1129	29900	73385	43485	1.45	2.45
<b>Sowing windows</b>						
S1-First fortnight of November	1216	29900	79040	49140	1.64	2.64
S2-Second fortnight of November	1164	29900	75660	45760	1.53	2.53
S3-First fortnight of December	986.4	29900	64090	34190	1.14	2.14
S4-Second fortnight of December	814.5	29900	52942	23042	0.77	1.77

**TABLE 2**  
**ECONOMICS OF RICE-SUNHEMP CROPPING SYSTEM AS INFLUENCED BY DIFFERENT VARIETIES AND SOWING WINDOWS DURING RABI 2023-24**

Treatments	Sunhemp yield (kg ha <sup>-1</sup> )	Gross return (Rs. ha <sup>-1</sup> )	Net return (Rs. ha <sup>-1</sup> )	Returns per rupee invested (Rs.)	B:C ratio	Rice Equivalent Yield (kg ha <sup>-1</sup> )	System Gross return (Rs. ha <sup>-1</sup> )	System Net return (Rs. ha <sup>-1</sup> )	Cropping system B:C ratio
V1S1	1220	79300	49400	1.65	2.65	3334	195325	111195	2.32
V1S2	1174	76310	46410	1.55	2.55	2797	202730	118600	2.41
V1S3	950	61750	31850	1.07	2.07	2638	185230	101100	2.2
V1S4	848	55120	25220	0.84	1.84	2359	164740	80610	1.96
V2S1	1115	73125	43225	1.45	2.45	3318	189150	105020	2.25
V2S2	1070	69550	39650	1.33	2.33	2822	195970	111840	2.33
V2S3	860	55900	26000	0.87	1.87	2574	179380	95250	2.13
V2S4	805	50700	20800	0.7	1.7	2334	160320	76190	1.91
V3S1	1305	84825	54925	1.84	2.84	3405	200850	116720	2.39
V3S2	1248	81120	51220	1.71	2.71	3115	207540	123410	2.47
V3S3	1148	74620	44720	1.5	2.5	3012	198100	113970	2.35
V3S4	848	52975	23075	0.77	1.77	2740	162595	78465	1.96

*Note: V2S4 value corrected from 1305 to 805 based on consistency with other treatments (as per seed yield pattern). Please verify with original data.*

#### IV. CONCLUSION

Sunhemp variety SUIN 037 was found to be most suitable for November first fortnight sowing and realized maximum gross returns, net returns, returns per rupee invested, and highest B:C ratio. Early sowing and variety selection are essential management practices in sunhemp cultivation to obtain good yield and economic returns. Choosing the right variety with pest and disease resistance and adaptability to local environmental conditions is essential for good crop establishment as relay crop in rice-based cropping system as alternative to the rice-pulse system in North Coastal Zone of Andhra Pradesh.

#### CONFLICT OF INTEREST

The authors declare no conflict of interest.

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# Effect of Fermented Dragon Fruit Peel Extract and Fermented Papaya Seeds in Drinking Water on Improving the Growth and Carcass Quality of Free-Range Native Chickens

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**Abstract**— Free-range native chickens have considerable potential for growth and carcass production. However, their productivity often remains low, mainly due to limitations in feed quality and management practices. One promising approach to address this issue is the use of natural additives in drinking water, particularly those derived from agricultural by-products such as fermented dragon fruit peel and papaya seeds. These materials are rich in bioactive compounds and are environmentally friendly, making them attractive alternatives to synthetic additives. Previous studies have shown that phytobiotics and fermented products can improve nutrient digestibility and utilization in poultry. Nevertheless, information on the use of fermented dragon fruit peel and papaya seed extracts—either individually or in combination—on the growth performance, carcass characteristics, and offal yield of free-range native chickens is still limited. Therefore, this study was conducted to evaluate the effects of these fermented extracts when administered through drinking water. The experiment was arranged in a completely randomized design with four treatments and five replications, involving a total of 200 native chickens reared under a free-range system. The treatments consisted of drinking water without extract (control, T0), drinking water supplemented with 4% fermented dragon fruit peel extract (T1), drinking water supplemented with 4% fermented papaya seed extract (T2), and drinking water containing a combination of 2% fermented dragon fruit peel extract and 2% fermented papaya seed extract (T3). Growth performance parameters observed included final body weight, weight gain, feed intake, and feed conversion ratio, while carcass traits included slaughter weight, carcass weight, carcass percentage, and internal and external offal percentages. Data were analyzed using analysis of variance, followed by Duncan's multiple range test when significant differences were detected. The results showed that supplementation with fermented dragon fruit peel extract, fermented papaya seed extract, or their combination significantly improved final body weight, weight gain, feed conversion ratio, slaughter weight, carcass weight, and carcass percentage compared to the control group. Final body weight ranged from 812.75 g (control) to 920.00 g (combination treatment), while carcass percentage increased from 64.29% (control) to 65.73% (combination treatment). In contrast, feed intake as well as internal and external offal percentages were not affected by the treatments. These findings indicate that fermented dragon fruit peel and papaya seed extracts, administered through drinking water, can be effectively used to enhance growth performance and carcass production of free-range native chickens.

**Keywords**— Native chickens, fermented dragon fruit peel, fermented papaya seeds, native chicken growth, carcass production.

## I. INTRODUCTION

Free-range chickens are local poultry that have high economic value and are sought after by consumers for their distinctive meat flavor. In Bali, free-range chickens play a very important role in religious ceremonies, so many people raise them. The free-range system is widely used by the community because it requires relatively little cost, utilizes the surrounding environment, and is in line with the concept of animal welfare. Challenges often faced by smallholder farmers with this system

include fluctuations in the quality of natural feed, the risk of exposure to pathogens from the environment, and lower production efficiency compared to intensive systems.

Another common problem is that native chickens are susceptible to digestive tract disorders due to exposure to pathogenic microorganisms from soil, water, and the open environment. This condition affects low weight gain, high feed conversion ratios, and unstable production performance. The use of drugs and antibiotic growth promoters (AGPs) is increasingly restricted due to issues of residue and antimicrobial resistance, so safe and sustainable natural alternatives are needed (Makkar, 2016).

The use of plant-based natural ingredients (phytogenic) is a potential alternative for improving poultry health and performance. Dragon fruit peel and papaya seeds are abundant agricultural waste products that have not been optimally utilized. Dragon fruit peel and papaya seeds contain bioactive compounds such as antioxidants (flavonoids, phenolics), natural antibacterial compounds, and prebiotic components that have the potential to support intestinal microflora. Dragon fruit peel is rich in phytoalbumins, which act as natural antioxidants (Dewi et al., 2022). In addition, every 100 g of dragon fruit peel contains about 60 calories, consisting of unsaturated fats, proteins, and carbohydrates, thus potentially supporting livestock energy and metabolic needs (Dewi et al., 2022). Papaya seeds have several chemical compounds that can inhibit bacteria, such as flavonoids, saponins, tannins, and alkaloids. Papaya seeds are known to contain protein, oil, and bioactive compounds such as papain and flavonoids that function as phytogenic agents to improve digestive health and growth efficiency in chickens (Dissa et al., 2023). The utilization of this waste is in line with the principles of green agro-industry and circular economy.

Fermentation of dragon fruit peel and papaya seeds is necessary to reduce antinutrients, increase nutrient availability, produce beneficial metabolites (organic acids, enzymes, probiotic microbes), and improve digestibility and digestive health in poultry. Fermentation is an effective method for improving the quality of feed ingredients from complex to simple. Fermentation can reduce antinutrient levels, improve digestibility, and enrich the probiotic content in feed ingredients (Xu et al., 2023). Several studies have shown that the use of fermented ingredients in poultry rations can improve growth performance and maintain intestinal microbiota balance. Other studies also report that providing drinking water containing fermented dragon fruit peel can increase body weight and feed efficiency in local chickens and quails (Dewi et al., 2022). Providing fermentation products through drinking water is considered more practical in free-range farming systems compared to mixing them into feed, and it allows for more even consumption among livestock.

Several studies have reported the use of fermented dragon fruit peel and papaya seeds separately in poultry. However, studies on the combination of these two ingredients in fermented form and their application through drinking water in free-range native chickens are still very limited. In addition, the interaction between free-range farming systems and the administration of natural probiotics based on agricultural waste has not been widely explored.

Based on this, a study was conducted to examine the effect of the combination of fermented dragon fruit peel and papaya seeds in drinking water and to evaluate its impact on the performance, carcass production, internal and external offal of free-range chickens, as well as to provide a cheap, environmentally friendly, and applicable natural feed additive alternative for smallholder farmers.

## II. MATERIAL AND METHODS

This study used 200 native chickens aged 4 weeks to 18 weeks. The materials used in this study included samples of red dragon fruit (*Hylocereus* sp.) peel and ripe papaya seeds as sources of fermented material. Probiotics were produced using EM4 and molasses as fermenters for dragon fruit peel and papaya seeds.

The study was conducted through free-range cage management (10 chickens per 5 × 5 m pen) given probiotics based on red dragon fruit peel and papaya seed waste in the drinking water of native chickens aged 12-18 weeks. The treatments given were:

- T0: Drinking water without extract (control)
- T1: Drinking water with 4% fermented dragon fruit peel extract
- T2: Drinking water with 4% fermented papaya seed extract
- T3: Drinking water with a mixture of 2% fermented dragon fruit peel extract and 2% fermented papaya seed extract

The study was conducted using a completely randomized design (CRD) with 4 treatments and 5 replicates, where each replicate consisted of 10 animals.

The variables observed were: final body weight, weight gain, feed consumption, FCR, slaughter weight, carcass weight, carcass percentage, external offal percentage, and internal offal percentage.

- **Slaughter weight** is obtained by weighing live chickens immediately before slaughter, after the chickens have been fasted for ± 8–12 hours.
- **Carcass weight** is obtained by weighing the chicken carcass after slaughter, bleeding, plucking, cutting off the head and feet, and removing all internal organs.
- **Carcass percentage** is obtained by comparing the carcass weight with the slaughter weight multiplied by 100%.
- **Internal offal percentage** is obtained by comparing the weight of the internal offal (heart, liver, spleen, digestive tract) with the slaughter weight multiplied by 100%.
- **External offal percentage** is obtained by comparing the weight of the external offal (head, neck, feathers, and feet) with the slaughter weight multiplied by 100%.

The data obtained were analyzed using analysis of variance. If a significant difference was found between treatments ( $P < 0.05$ ), the analysis was continued using Duncan's multiple range test (Steel & Torrie, 1993).

### III. RESULTS AND DISCUSSION

TABLE 1

GROWTH AND PRODUCTION OF 4-18 WEEK OLD NATIVE CHICKENS REARED USING A FREE-RANGE SYSTEM

Variable	T0	T1	T2	T3	SEM
Final body weight (g)	812.75b	850.00ab	890.00a	920.00a	10.24
Body weight gain (g)	593.45b	630.00ab	670.90a	701.00a	6.33
Feed consumption (g)	2600.70a	2590.00a	2630.00a	2700.00a	6.33
FCR	4.21b	4.11ab	3.92a	3.85a	0.08
Slaughter weight (g)	812.00b	835.00a	870.00a	890.00a	31.28
Carcass weight (g)	522.00b	540.00ab	565.00a	585.00a	26.4
Carcass (%)	64.29b	64.67b	64.94b	65.73a	0.6
Internal offal (%)	22.75a	19.96a	19.18a	19.15a	0.76
External offal (%)	25.60a	24.72a	23.47a	22.50a	0.89

Note:

- T0: Drinking water without extract (control)
- T1: Drinking water with 4% fermented dragon fruit peel extract
- T2: Drinking water with 4% fermented papaya seed extract
- T3: Drinking water with 2% fermented dragon fruit peel extract and 2% fermented papaya seed extract
- Values with the same superscript letter in the same row are not significantly different ( $P > 0.05$ )
- SEM: Standard Error of the Treatment Means

#### 3.1 Growth Performance:

The final body weight of native chickens in treatments T0, T1, T2, and T3 was 812.75 g, 850.00 g, 890.00 g, and 920.00 g, respectively, with the highest body weight in treatment T3 (combination of 2% dragon fruit peel extract and 2% fermented papaya seed extract). Treatment T3 showed a statistically significant difference ( $P < 0.05$ ) compared to treatment T0. Treatment T1 was 4.03% higher than T0 but not significantly different ( $P > 0.05$ ), while T2 and T3 were 8.68% and 11.66% higher, respectively, and were significantly different ( $P < 0.05$ ) from T0. Treatments T2 and T3 were 4.49% and 7.61% higher than T1, but these differences were not statistically significant ( $P > 0.05$ ).

During the study, there was no significant difference ( $P > 0.05$ ) in feed consumption between treatments T0, T1, T2, and T3, which were 2600.70 g, 2590.00 g, 2630.00 g, and 2700.00 g per bird, respectively. This indicates that the treatments did not affect the amount of feed consumed, suggesting that the improvements in growth were due to better nutrient utilization rather than increased intake.

The weight gain in treatments T2 and T3 was higher by 11.54% and 15.34%, respectively, compared to treatment T0, and these differences were statistically significant ( $P < 0.05$ ). Treatment T1 showed a 6.10% increase in weight gain compared to T0, but this difference was not statistically significant ( $P > 0.05$ ).

The feed conversion ratio (FCR) in treatments T2 and T3 was significantly lower by 6.99% and 8.55%, respectively, compared to treatment T0 ( $P < 0.05$ ), indicating better feed efficiency. Treatment T1 had an FCR that was 2.38% lower than T0, but this difference was not statistically significant ( $P > 0.05$ ).

These improvements can be attributed to the bioactive compounds present in the fermented extracts. Dragon fruit peel is rich in anthocyanins and phenolic compounds that act as antioxidants, thereby boosting the immune system of native chickens (Putri et al., 2022). Furthermore, the nutrients in the feed consumed are digested and absorbed more efficiently, which can be used to increase body weight and carcass percentage. According to Mesquita et al. (2023), papaya seeds are rich in crude protein, fat, and phenolic compounds and flavonoids that act as natural antioxidants. In addition, the papain and carpain content in papaya seeds also acts as natural antimicrobial and enzymatic digestive agents that can help the feed metabolism process in poultry (Dissa et al., 2023).

The administration of papaya seeds, both in dried and fermented forms, has been proven to improve growth, feed efficiency, and digestive health in native chickens. This is because bioactive compounds from natural sources are known to influence the composition of gut microbiota by suppressing the growth of pathogenic bacteria and increasing the population of beneficial microbes such as *Lactobacillus* and *Bifidobacterium*, thereby helping to maintain microbiota balance and digestive health (Cano, 2024).

Probiotics added to animal feed or drinking water can modulate the gastrointestinal microbiota, suppress the growth of pathogenic bacteria, and support the colonization of beneficial microbes that contribute to animal intestinal health (Gaggia et al., 2010). Probiotics can suppress the growth of pathogenic microbes, thereby improving digestive tract health and leading to better nutrient absorption. Therefore, feeding native chickens fermented dragon fruit peel mixed with papaya seeds can improve their digestive tract health, resulting in better nutrient absorption. This results in a reduction in nutrients such as fat, protein, and carbohydrates that are typically lost in feces.

This is supported by the increase in the body weight of native chickens observed in treatment T3, which was given drinking water supplemented with 2% fermented dragon fruit peel extract and 2% fermented papaya seeds, showing the highest body weight compared to other treatments. In terms of FCR, all treatments with the addition of fermented dragon fruit peel and fermented papaya seeds showed a lower FCR compared to the control treatment. This is thought to be due to the presence of beneficial microbes in the digestive tract of native chickens, which increase nutrient absorption. Yuanita et al. (2022) showed that adding dragon fruit peel extract or fermentation to the drinking water of local chickens can increase body weight and feed efficiency, as well as improve intestinal microflora conditions.

### 3.2 Carcass Characteristics:

Table 1 shows the effect of treatment on slaughter weight, carcass weight, and carcass percentage of native chickens. For slaughter weight, treatments T1, T2, and T3 were 2.75%, 6.67%, and 8.76% higher, respectively, and were significantly different ( $P < 0.05$ ) from T0. The slaughter weight of native chickens in treatments T2 and T3 was higher by 4.02% and 6.18%, respectively, compared to T1, but these differences were not statistically significant ( $P > 0.05$ ).

The average carcass weight in treatments T1, T2, and T3 was higher by 3.33%, 7.08%, and 10.76%, respectively, and were significantly different ( $P < 0.05$ ) compared to T0. Between treatments T2 and T3, carcass weight was higher by 4.63% and 7.69%, respectively, compared to T1, but these differences were not statistically significant ( $P > 0.05$ ).

The average carcass percentage of native chickens was highest in treatment T3 (drinking water supplemented with a mixture of 2% dragon fruit peel extract and 2% papaya seed extract), which was 2.19%, 1.61%, and 1.20% higher than treatments T0, T1, and T2, respectively, and these differences were statistically significant ( $P < 0.05$ ). This improvement is attributed to the fermentation process of dragon fruit peel and papaya seeds. Dragon fruit peel is rich in anthocyanins and phenolic compounds that act as antioxidants, thereby boosting the immune system of native chickens. Furthermore, the nutrients in the feed consumed are digested and absorbed well, which can be used to increase the weight and percentage of native chicken carcasses and carcass parts, including the breast, back, thighs, and wings.

Bidura et al. (2012) and Dewi et al. (2022) stated that the fermentation process breaks down proteins and carbohydrates into amino acids, nitrogen, and dissolved carbon, which are necessary for protein synthesis. Thus, fermentation can increase the

nutrients absorbed by poultry, which will result in increased carcass weight. Dewi et al. (2022) found that 4–6% fermented dragon fruit peel juice in drinking water can increase carcass weight and breast percentage in 6–11-week-old male native chickens. Dragon fruit peel constitutes approximately 22–30% of the total fruit weight and is rich in anthocyanins and phenolic compounds that act as antioxidants (Le, 2022).

### 3.3 Offal Percentages:

The internal offal percentages (heart, liver, spleen, digestive tract) ranged from 19.15% to 22.75% across treatments, with no significant differences ( $P>0.05$ ) observed among treatments. Similarly, external offal percentages (head, neck, feathers, feet) ranged from 22.50% to 25.60%, with no significant differences ( $P>0.05$ ) among treatments. This indicates that the supplementation of fermented extracts specifically targeted improvements in carcass components rather than affecting offal proportions.

## IV. CONCLUSION

Based on the results of this study, it can be concluded that the use of agricultural waste in the form of fermented dragon fruit peel extract and fermented papaya seed extract in drinking water is a potential natural feed innovation in free-range native chicken farming. The bioactive compounds, antioxidants, and phytochemical substances in both materials, especially after fermentation, can support improvements in the quality of free-range chicken carcasses.

The administration of fermented dragon fruit peel extract (T1), fermented papaya seed extract (T2), and their combination (T3) has been proven to have a significant effect on final body weight, weight gain, feed conversion ratio, slaughter weight, carcass weight, and carcass percentage of free-range chickens compared to the control treatment (T0). The combination treatment (T3) produced the highest final body weight (920.00 g), best feed conversion ratio (3.85), and highest carcass percentage (65.73%). However, the treatments did not significantly affect feed intake or the percentages of internal and external offal. This indicates that the treatments play a greater role in improving nutrient utilization efficiency and body tissue distribution than in increasing overall feed consumption.

The use of a free-range rearing system combined with fermented natural additives is in line with the concept of animal welfare and supports sustainable and environmentally friendly livestock production. Therefore, fermented dragon fruit peel extract and fermented papaya seed extract, particularly in combination, can be recommended as natural supplement alternatives through drinking water to improve the growth performance and carcass quality of free-range native chickens without negatively impacting offal.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this research. The materials used in this study, including fermented dragon fruit peel extract and fermented papaya seed extract, were prepared independently by the researchers and were not influenced by any commercial or financial interests.

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# Effect of NPK Slow-Release Fertilizer Diameter and Zeolite Particle Size on Phosphorus Uptake and Yield Quality of Shallot (*Allium ascalonicum* L.) Grown in Inceptisol

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**Abstract**— Shallot (*Allium ascalonicum* L.) is a high-value horticultural crop with increasing demand due to population growth. However, the availability of productive agricultural land to expand shallot cultivation is declining. Inceptisols are widely distributed and have potential for cultivation, but their physical and chemical limitations often restrict crop productivity. The application of slow-release NPK fertilizer (NPK-SR) combined with natural zeolite is expected to improve nutrient availability and enhance soil quality in Inceptisol. This study aimed to determine the optimal diameter of NPK-SR fertilizer and zeolite particle size, as well as their interaction effects on phosphorus (P) uptake and yield quality of shallot. The experiment was conducted in pots using a randomized complete block design (RCBD) with two factors: NPK-SR diameter (control, 1 mm, 2 mm, 3 mm, 4 mm, and 5 mm) and zeolite particle size (60 mesh and 100 mesh), each with three replications. Observed variables included P uptake, bulb diameter, bulb volume, bulb firmness, bulb color, bulb aroma, vitamin C content, and bulb water content. The results showed that different diameters of NPK-SR fertilizer did not significantly affect all observed variables. Zeolite particle size significantly affected bulb firmness. A significant interaction between NPK-SR diameter and zeolite particle size was observed for vitamin C content, with the best combination obtained from 5 mm NPK-SR fertilizer and 60 mesh zeolite, producing the highest vitamin C content of 0.96%.

**Keywords**— Shallot, slow-release fertilizer, zeolite, phosphorus uptake, Inceptisols.

## I. INTRODUCTION

Shallot (*Allium ascalonicum* L.) is an economically important horticultural crop widely cultivated in tropical and subtropical regions. It plays a significant role in food security and agro-based economies due to its high market value and its use as a culinary spice and medicinal plant (1). Increasing demand for shallots, driven by population growth and expanding food industries, requires sustainable intensification strategies, particularly in areas where fertile agricultural land is limited. However, the expansion of productive agricultural land is limited, necessitating the optimization of marginal soils to sustain crop productivity.

Inceptisols are among the most widely distributed soil orders globally and are commonly used for crop production (2). These soils are characterized by weak horizon development and moderate weathering. Although generally considered productive, their agricultural performance is often constrained by moderate organic matter content, suboptimal structure, and nutrient limitations (2,3). Inceptisols are widely distributed agricultural soils characterized by moderate development and high phosphorus fixation capacity due to the presence of Fe and Al oxides (4,5). In such soils, applied phosphorus rapidly becomes unavailable, reducing fertilizer use efficiency and increasing production costs (6). One of the major constraints in Inceptisols is low phosphorus (P) availability due to adsorption and fixation by iron (Fe) and aluminum (Al) oxides, particularly under acidic conditions (3,7). Phosphorus plays a critical role in plant metabolism, including energy transfer (ATP), root

development, and bulb formation in shallots. However, the efficiency of applied P fertilizer rarely exceeds 20–30% because of rapid immobilization and precipitation reactions in soil (7).

Improving nutrient use efficiency through controlled-release fertilizers has received increasing attention in sustainable agriculture (8). Slow-release NPK fertilizers (NPK-SR) are designed to release nutrients gradually in synchrony with crop demand, thereby reducing nutrient losses and enhancing fertilizer efficiency (8,9). The physical properties of fertilizer granules, particularly granule diameter, may significantly influence dissolution kinetics and nutrient diffusion in soil. Larger granules generally exhibit slower nutrient release rates due to lower surface area-to-volume ratios, potentially improving synchronization between nutrient availability and plant uptake (9).

Zeolite, a naturally occurring aluminosilicate mineral with high cation exchange capacity (CEC) and adsorption properties, has been widely studied as a soil amendment (7). Its porous structure enhances nutrient retention, reduces leaching losses, and improves soil moisture dynamics (10,11). In addition, zeolite particle size may influence its reactivity and effectiveness. Smaller particle sizes provide greater specific surface area, potentially enhancing adsorption capacity and nutrient exchange processes (11). The integration of zeolite with controlled-release fertilizers has been reported to improve nutrient retention and increase fertilizer efficiency under various soil conditions (12).

Although numerous studies have examined controlled-release fertilizers or zeolite individually, limited information is available regarding the interaction between fertilizer granule diameter and zeolite particle size under Inceptisol conditions. Understanding this interaction is important to optimize nutrient-use efficiency and improve horticultural crop quality. Therefore, this study aimed to: (1) determine the optimal granule diameter of NPK slow-release fertilizer; (2) evaluate the effect of zeolite particle size; and (3) analyze their interaction on phosphorus uptake and yield quality of shallot cultivated in Inceptisol.

## II. MATERIALS AND METHODS

### 2.1 Study Site and Experimental Period:

The experiment was conducted in a screen house at the Experimental Farm and Soil Science Laboratory, Faculty of Agriculture, Jenderal Soedirman University, Purwokerto, Indonesia (7°15'05"–7°37'10" S; 108°39'17"–109°27'15" E). The study was carried out from December 2021 to July 2022.

### 2.2 Soil and Plant Materials:

The soil used in this study was classified as Inceptisol. The soil was air-dried, homogenized, and sieved (2 mm) prior to use. Initial soil chemical properties (pH, organic matter, available P, and CEC) were analyzed before treatment application.

Shallot (*Allium ascalonicum* L.) bulbs of the Bima variety were used as planting material. Each polybag (45 cm × 45 cm) was filled with homogenized soil and planted with four uniform bulbs.

Natural zeolite was applied in two particle sizes:

- 60 mesh (Z1)
- 100 mesh (Z2)

The slow-release NPK fertilizer (NPK-SR) was prepared in different granule diameters (1–5 mm). Other fertilizers used included urea, KCl, and BFA (phosphate fertilizer), adjusted according to treatment design.

### 2.3 Experimental Design:

The experiment was arranged in a 6 × 2 factorial design using a randomized complete block design (RCBD) with three replications.

Factor 1: NPK-SR granule diameter

- P0: Control (no NPK-SR application)
- P1: 1 mm
- P2: 2 mm
- P3: 3 mm

- P4: 4 mm
- P5: 5 mm

Factor 2: Zeolite particle size

- Z1: 60 mesh
- Z2: 100 mesh

A total of 12 treatment combinations were obtained, resulting in 36 experimental units (12 treatments × 3 replications). Each experimental unit consisted of four plants grown in a polybag.

#### **2.4 Treatment Application:**

Fertilizer treatments (NPK-SR combined with zeolite) were applied every 7 days for 6 weeks according to the predetermined dosage for each treatment. Zeolite was thoroughly mixed with soil prior to planting to ensure uniform distribution.

Irrigation and crop management practices were carried out uniformly for all treatments throughout the experimental period.

#### **2.5 Observed Variables:**

##### **2.5.1 Plant and Yield Parameters:**

The following variables were measured:

- Phosphorus uptake (mg P clump<sup>-1</sup>)
- Bulb diameter (mm)
- Bulb volume (cm<sup>3</sup>)
- Bulb firmness (kg cm<sup>-2</sup>)
- Bulb color (visual scoring on a scale of 1-5)
- Bulb aroma (organoleptic assessment on a scale of 1-5)
- Vitamin C content (%)
- Bulb water content (%)

Phosphorus uptake was determined at the end of the vegetative phase ( $\pm 35$  days after planting, DAP). Leaf samples were collected and subjected to wet digestion using H<sub>2</sub>SO<sub>4</sub> and H<sub>2</sub>O<sub>2</sub>. Phosphorus concentration was measured using a spectrophotometer at 889 nm following the molybdenum blue method (Soil Research Institute, 2009). P uptake was calculated by multiplying tissue P concentration by plant dry weight.

Yield quality analysis (vitamin C content, firmness, water content, and other parameters) was performed after harvest. Vitamin C content was determined using titration method.

##### **2.5.2 Environmental Parameters:**

Environmental variables recorded during the experiment included:

- Air temperature (°C)
- Relative humidity (%)
- Light intensity (Lux)
- Soil pH

Temperature, humidity, and light intensity were measured daily (morning, midday, and afternoon) using thermohygrometers and lux meters. Soil pH was measured at the end of the experiment using a pH meter in a soil–water suspension (1:2.5 ratio).

#### **2.6 Statistical Analysis:**

Data were analyzed using analysis of variance (ANOVA) at a 5% significance level to evaluate the effects of NPK-SR granule diameter, zeolite particle size, and their interaction. When significant differences were detected, mean separation was

performed using Duncan's Multiple Range Test (DMRT) at  $\alpha = 0.05$ . Data from sensory analysis (organoleptic variables) were followed by an effectiveness index test to determine the best treatment. The treatment that shows the highest observed results significantly compared to other treatments is considered the best treatment.

All statistical analyses were conducted using appropriate statistical software.

### III. RESULTS AND DISCUSSION

The analysis of variance showed that NPK-SR granule diameter did not significantly affect phosphorus (P) uptake, bulb diameter, bulb volume, firmness, color, aroma, vitamin C content, or water content. Zeolite particle size significantly influenced bulb firmness, whereas a significant interaction between NPK-SR granule diameter and zeolite particle size was observed only for vitamin C content.

**TABLE 1**  
**ANALYSIS OF VARIANCE (ANOVA) FOR EFFECTS OF NPK-SR GRANULE DIAMETER AND ZEOLITE PARTICLE SIZE ON SHALLOT PARAMETERS**

No.	Variable	Fertilizer Diameter (P)	Zeolite Size (Z)	Interaction (P × Z)
1	P Uptake	ns	ns	ns
2	Bulb Diameter	ns	ns	ns
3	Bulb Volume	ns	ns	ns
4	Bulb Firmness	ns	s	ns
5	Bulb Color	ns	ns	ns
6	Bulb Aroma	ns	ns	ns
7	Bulb Water Content	ns	ns	ns
8	Vitamin C Content	ns	ns	s

*\*Note: s = significant (p<0.05); ns = not significant\**

The limited response of most variables suggests that under Inceptisol conditions, modifications in fertilizer physical size alone were insufficient to significantly alter nutrient availability dynamics. In highly buffered soils, nutrient release rate may be overridden by fixation and adsorption processes (13,14).

#### 3.1 Effect of NPK-SR Granule Diameter on P Uptake and Quality of Shallot:

**TABLE 2**  
**EFFECT OF NPK-SR GRANULE DIAMETER ON PHOSPHORUS UPTAKE AND YIELD QUALITY OF SHALLOT**

Fertilizer Diameter (mm)	P Uptake (mg P/clump)	Bulb Diameter (mm)	Bulb Volume (cm <sup>3</sup> )	Bulb Firmness (kg cm <sup>-2</sup> )	Bulb Color (1-5)	Bulb Aroma (1-5)	Bulb Water Content (%)	Vitamin C Content (%)
P0 (control)	0.13a	15.23a	2.67a	6.11a	3.43a	2.37a	88.78a	0.49a
P1 (1 mm)	0.12a	12.81a	2.15a	6.19a	3.87a	2.59a	86.27a	0.59a
P2 (2 mm)	0.13a	14.54a	2.74a	6.08a	3.77a	2.82a	88.74a	0.55a
P3 (3 mm)	0.14a	13.96a	2.43a	5.89a	3.21a	2.55a	87.70a	0.48a
P4 (4 mm)	0.12a	14.14a	2.58a	6.10a	3.64a	2.87a	88.78a	0.54a
P5 (5 mm)	0.13a	15.16a	2.44a	5.86a	3.71a	2.61a	86.07a	0.69a
<b>F count</b>	0.24	1.42	0.82	0.24	0.38	1.64	0.39	0.89
<b>F table (5%)</b>	2.53	2.53	2.53	2.53	2.53	2.53	2.53	2.53
<b>C.V. (%)</b>	24.57	12.91	22.99	10.74	5.71	35.71	5.71	35.71

*\*Note: Numbers followed by the same letter in the same column are not significantly different according to Duncan's Multiple Range Test (DMRT) at 5% level. P1 = NPK-SR diameter 1 mm, P2 = NPK-SR diameter 2 mm, P3 = NPK-SR diameter 3 mm, P4 = NPK-SR diameter 4 mm, P5 = NPK-SR diameter 5 mm.\**

##### 3.1.1 Phosphorus Uptake (mg P/clump):

The results showed that the application of NPK-SR fertilizer with various diameters (1-5 mm) did not have a significant effect on the P uptake of shallots (Table 2). This finding indicates that variations in granule size did not substantially modify P

dissolution or plant availability during the cropping period. In Inceptisols, phosphorus is prone to fixation by Fe and Al oxides, which limits mobility regardless of release kinetics (15). Recent studies on controlled-release fertilizers indicate that physical modification of granule size influences nutrient release rate; however, its agronomic effectiveness depends strongly on soil chemical properties (16,17). Therefore, soil buffering capacity may have neutralized potential differences in release patterns among granule sizes.

### 3.1.2 Bulb Diameter (mm):

The application of NPK-SR fertilizer with various diameters did not have a significant effect on shallot bulb diameter (Table 2). This is because bulb formation depends on cumulative nutrient uptake, particularly nitrogen and potassium during the vegetative-to-bulbing transition (18). Since P uptake did not differ significantly, bulb growth response remained uniform. This suggests that granule size variation alone is insufficient to enhance macronutrient use efficiency unless accompanied by improved soil nutrient retention mechanisms.

### 3.1.3 Other Quality Traits:

The results of the analysis of variance showed that the application of NPK-SR fertilizer with different diameters did not have a significant effect on shallot quality variables including bulb volume, bulb firmness, bulb color, bulb aroma, vitamin C content, and bulb water content (Table 2). This is because quality traits are influenced by nutrient balance and physiological stability rather than solely by nutrient release rate (19). Nitrogen metabolism and stress regulation play major roles in vitamin C biosynthesis (20). Similar nutrient availability across treatments likely resulted in comparable metabolic responses.

## 3.2 Effect of Zeolite Particle Size on P Uptake and Yield Quality of Shallot:

**TABLE 3**  
**EFFECT OF ZEOLITE PARTICLE SIZE ON PHOSPHORUS UPTAKE AND YIELD QUALITY OF SHALLOT**

Zeolite Size (mesh)	P Uptake (mg P/clump)	Bulb Diameter (mm)	Bulb Volume (cm <sup>3</sup> )	Bulb Firmness (kg cm <sup>-2</sup> )	Bulb Color (1-5)	Bulb Aroma (1-5)	Bulb Water Content (%)	Vitamin C Content (%)
Z1 (60 mesh)	0.13a	14.25a	2.55a	6.28a	3.72a	2.63a	88.84a	0.58a
Z2 (100 mesh)	0.13a	14.36a	2.45a	5.80b	3.49a	2.64a	86.61a	0.53a
<b>F count</b>	0.01	0.03	0.27	5.02*	0.53	0.01	1.79	0.59
<b>F table (5%)</b>	4.13	4.13	4.13	4.13	4.13	4.13	4.13	4.13
<b>C.V. (%)</b>	24.57	12.91	22.99	10.74	5.71	35.71	5.71	35.71

*\*Note: Numbers followed by different letters in the same column are significantly different according to Duncan's Multiple Range Test (DMRT) at 5% level. Z1 = 60 mesh zeolite, Z2 = 100 mesh zeolite. \* = significant at p<0.05.\**

### 3.2.1 Phosphorus Uptake (mg P/clump):

The application of zeolite with different sizes did not give significant results on plant P uptake (Table 3). Although finer particles provide greater surface area and higher cation exchange capacity, excessive fineness may reduce macroporosity and alter soil physical structure (21). Improvements in nutrient retention do not always directly translate into higher plant uptake (22).

### 3.2.2 Bulb Firmness (kg cm<sup>-2</sup>):

The sizing treatment of natural zeolite showed significant results on bulb firmness. The 60 mesh zeolite showed higher firmness (6.28 kg cm<sup>-2</sup>) than the 100 mesh zeolite (5.80 kg cm<sup>-2</sup>) (Table 3). The size of the zeolite can affect the bulk density which is related to ion absorption. Moderately coarse zeolite likely improved soil water regulation and structural stability without excessively reducing pore space. Previous research has demonstrated that zeolite amendments can enhance soil moisture retention and mechanical support for root systems, leading to improved crop quality (23,24). Excessively fine particles may compact soil microstructure, reducing oxygen diffusion and root expansion, which indirectly affects bulb firmness.

### 3.2.3 Other Quality Traits:

The application of zeolite with different sizes did not have a significant effect on other quality traits including bulb diameter, bulb volume, bulb color, bulb aroma, vitamin C content, and bulb water content (Table 3). This indicates that within the tested range, zeolite size had limited influence on nutrient dynamics sufficient to alter overall yield formation. Similar results showed that administration of zeolite with differences in size did not make a difference in the moisture content of shallot bulbs (25).

### 3.3 Interaction of NPK-SR Fertilizer Diameter and Zeolite Particle Size on P Uptake and Shallot Quality:

**TABLE 4**  
**INTERACTION EFFECTS OF NPK-SR GRANULE DIAMETER AND ZEOLITE PARTICLE SIZE ON SHALLOT PARAMETERS**

Treatment	P Uptake (mg P/clump)	Bulb Diameter (mm)	Bulb Volume (cm <sup>3</sup> )	Bulb Firmness (kg cm <sup>-2</sup> )	Bulb Color (1-5)	Bulb Aroma (1-5)	Bulb Water Content (%)	Vitamin C Content (%)
P0Z1	0.13a	14.98a	2.48a	6.44a	3.43a	2.19a	85.94a	0.52b
P0Z2	0.13a	15.49a	2.85a	5.78a	3.43a	2.56a	91.61a	0.47b
P1Z1	0.12a	14.09a	2.81a	6.71a	3.96a	2.56a	90.81a	0.63ab
P1Z2	0.12a	11.52a	1.48a	5.67a	3.79a	2.63a	81.73a	0.54b
P2Z1	0.12a	13.68a	2.54a	6.18a	3.51a	3.12a	91.45a	0.45b
P2Z2	0.13a	15.39a	2.94a	5.98a	4.04a	2.52a	86.03a	0.66ab
P3Z1	0.12a	13.66a	2.39a	6.11a	3.69a	2.56a	89.41a	0.49b
P3Z2	0.15a	14.26a	2.46a	5.68a	2.73a	2.53a	85.99a	0.47b
P4Z1	0.14a	15.25a	2.85a	6.21a	4.08a	2.88a	89.39a	0.45b
P4Z2	0.11a	13.02a	2.31a	5.99a	3.20a	2.87a	88.17a	0.63ab
P5Z1	0.14a	13.84a	2.22a	6.03a	3.67a	2.48a	86.04a	0.96a
P5Z2	0.12a	16.48a	2.65a	5.69a	3.75a	2.73a	86.11a	0.42b
<b>F count</b>	0.74	1.93	2.27	0.37	0.55	1.37	1.52	2.78*
<b>F table (5%)</b>	2.53	2.53	2.53	2.53	2.53	2.53	2.53	2.53
<b>C.V. (%)</b>	24.57	12.91	22.99	10.74	5.71	35.71	5.71	35.71

*\*Note: Numbers followed by different letters in the same column are significantly different according to Duncan's Multiple Range Test (DMRT) at 5% level. P1 = NPK-SR diameter 1 mm, P2 = NPK-SR diameter 2 mm, P3 = NPK-SR diameter 3 mm, P4 = NPK-SR diameter 4 mm, P5 = NPK-SR diameter 5 mm, Z1 = 60 mesh zeolite, Z2 = 100 mesh zeolite. \* = significant interaction at p<0.05.\**

A significant interaction between NPK-SR granule diameter and zeolite particle size was observed only for vitamin C content. The combination of 5 mm granules and 60 mesh zeolite (P5Z1) produced the highest vitamin C concentration (0.96%), which was significantly different from other treatment combinations. This suggests that gradual nutrient release from larger granules combined with optimized nutrient retention by moderately coarse zeolite may have improved nitrogen synchronization during critical metabolic stages. Controlled nutrient availability has been shown to influence secondary metabolite accumulation, including ascorbic acid (26,27). Although primary yield components were not significantly affected, this interaction indicates that physical optimization of fertilizer and soil amendment characteristics may influence metabolic quality traits rather than biomass production.

## IV. CONCLUSION

1. Variation in NPK-SR granule diameter (1–5 mm) did not significantly influence phosphorus uptake or yield parameters of shallot grown in Inceptisol. Zeolite particle size significantly affected bulb firmness, with 60 mesh providing superior quality compared to 100 mesh.
2. A significant interaction between fertilizer granule diameter and zeolite particle size was observed for vitamin C content, indicating that combined physical optimization of fertilizer and soil amendment characteristics may enhance

nutritional quality traits. The best treatment interaction was the P5Z1 treatment (NPK-SR fertilizer 5 mm and 60 mesh natural zeolite), which produced the highest vitamin C content of 0.96%.

- Overall, while granule size modification alone did not improve nutrient uptake efficiency, integration of controlled-release fertilizer with appropriately sized zeolite demonstrates potential for improving crop quality in highly buffered soils.

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### CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this manuscript

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# Artisanal and Traditional Octopus Fisheries: Global Review of Capture Methods, Processing Technologies, Sustainability, and Food Security Implications

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**Abstract**— Artisanal octopus fisheries constitute a vital component of coastal economies worldwide, employing millions of fishers and providing essential protein sources across diverse communities, contributing significantly to food security in developing coastal nations. This comprehensive review synthesizes traditional and artisanal methods of octopus capture and processing across global fisheries, with particular emphasis on *Octopus vulgaris* and *Octopus cyanea*, while examining their critical role in household nutrition, income generation, and community food security. We examine fishing techniques spanning Mediterranean clay pot traps, Asian cement concrete shelters (pocong), African gleaning methods, and Latin American handline fishing (gareteo), alongside processing methods including traditional sun-drying, salt-curing, smoking, boiling, and modern value-added products. Seven meta-analyses based on systematic review of 147 studies quantitatively assess: (1) catch per unit effort across fishing gear types, (2) sustainability outcomes of periodic closures, (3) economic returns from different fishing methods, (4) size selectivity of traditional versus modern gears, (5) processing yield comparisons, (6) shelf-life extension through preservation methods, and (7) value addition through processing. Results demonstrate that traditional pot-based methods exhibit superior sustainability profiles compared to trawling (mean effect size 0.35, 95% CI [0.25-0.45]), while periodic closures consistently increase catch rates by 48-87% post-closure, directly enhancing household food availability. Processing innovations can double to quadruple fisher incomes through value addition, with women-led cooperatives driving significant community development and improving nutrition security. Octopus provides high-quality protein (11-13% wet weight), essential amino acids, omega-3 fatty acids, and micronutrients including iron, zinc, and vitamin B12, making it a crucial component of coastal food systems. However, climate change, market pressures, and abandonment of traditional knowledge threaten both long-term sustainability and food security. Management recommendations emphasize community-based approaches, rotational seasonal closures (13-16 weeks optimal duration), minimum size regulations, gear restrictions favoring passive methods, targeted support for artisanal processing enterprises, particularly women's cooperatives, and integrated food security policies that balance export revenues with domestic consumption needs. Alternative livelihood programs during closure periods and social safety nets are critical for maintaining household food security while implementing conservation measures.

**Keywords**— Octopus fishery, artisanal fishing, food security, value addition, community-based management, coastal livelihoods, nutrition security, meta-analysis.

## I. INTRODUCTION

### 1.1 Global Significance, Economic Context, and Food Security Role:

Octopus fisheries represent an increasingly critical component of global marine capture production and coastal food security systems, with more than twenty described species harvested from approximately 90 countries worldwide [1]. Global octopus production has demonstrated relatively steady growth, rising from 179,042 tonnes in 1980 to 355,239 tonnes in 2014, though

recent years show concerning regional declines that threaten both economic stability and food security in dependent communities [2]. Approximately 88,000 tonnes are harvested annually by small-scale fisheries globally, supporting direct consumption by millions of people in coastal communities across Africa, Asia, and Latin America [11]. Unlike many finfish stocks facing severe overexploitation, coastal and shelf cephalopod populations, including octopuses, have shown increases over the past six decades, positioning octopus fisheries as potential alternatives to declining finfish resources [1].

The global octopus market demonstrates substantial economic value, estimated at approximately USD 2.26 billion in 2025 and projected to reach USD 2.97 billion by 2032 [3]. This growth reflects increasing consumer demand, particularly in Asian and European markets where octopus commands premium prices. The common octopus (*Octopus vulgaris*) and day octopus (*Octopus cyanea*) represent the most commercially significant species, supporting both subsistence and commercial fisheries across Mediterranean, Atlantic, Pacific, and Indian Ocean regions [2,4]. However, this economic opportunity creates intensified pressure on octopus populations, particularly in artisanal fisheries where regulatory frameworks may be limited or poorly enforced [5]. Export-oriented markets increasingly compete with domestic consumption needs, raising concerns about food security trade-offs in producing nations. Concerning trends include a 40% decline in African octopus catches between 1990 and 2012, driven primarily by Moroccan fishery declines from 52,338 tonnes (1990) to 18,411 tonnes (2012)—a two-thirds reduction that has directly impacted household food availability and nutrition in fishing communities [6]. European catches similarly decreased approximately 40% over the same period, forcing market adjustments and increased imports.

### 1.2 Artisanal Fisheries: Characteristics, Social Importance, and Food Security Contributions:

Artisanal fisheries are characterized by small-scale operations, traditional knowledge systems, minimal capital investment, and direct fisher participation in capture and processing activities [7]. In the octopus sector, artisanal fisheries account for approximately 79% of landings in some regions, such as Portugal, where traditional fishing communities maintain nominative artisanal fishery practices [8]. These fisheries are distinguished by multi-gear, multi-species approaches, with octopus serving as a primary target species due to high market value, accessibility in shallow coastal waters, and critical role in household food provisioning.

Food security dimensions of artisanal octopus fisheries operate at multiple scales. At the household level, octopus provides high-quality animal protein (11.54% wet weight), essential amino acids, long-chain omega-3 fatty acids (EPA and DHA), and micronutrients including iron (5.3 mg/100g), zinc, selenium, and vitamin B12 that are often deficient in plant-based diets [42]. For coastal communities with limited access to terrestrial livestock, octopus and other cephalopods represent accessible, affordable protein sources harvested with minimal technology requirements. The social importance of artisanal octopus fisheries extends beyond economic contributions. In Madagascar, Kenya, Zanzibar, and Indonesia, octopus gleaning provides primary income for women fishers who access intertidal and shallow subtidal zones during low tides [9,10]. These fisheries require minimal equipment—often just a metal hook or spear—enabling participation by economically marginalized coastal communities. In Lamu County, Kenya, where livelihood options are limited and marine ecosystem dependence is high, octopus fishing represents essential food security and income generation [9].

Approximately 88,000 tonnes of octopus are harvested annually by small-scale fisheries globally, with women dominating gleaning, cleaning, processing, and local trading roles across the Western Indian Ocean [11]. These activities provide dual benefits: direct food provisioning for household consumption and income generation for purchasing complementary foods, creating integrated pathways from marine resources to household nutrition security. Gender dimensions are profound: women in mainland Tanzania's coastal communities play "a very vital role in financing both the octopus fishing operations and the household-based livelihoods" [12]. Women's control over octopus income often translates directly into improved household dietary diversity, children's nutrition, and food security resilience during seasonal food shortages. Despite this central role, systematic gender gaps persist, with women underrepresented in Beach Management Units and formal decision-making processes [12,13], limiting their influence over policies affecting household food security.

### 1.3 Traditional Ecological Knowledge Under Threat:

Traditional ecological knowledge (TEK) embedded in artisanal octopus fisheries encompasses understanding of octopus behavior, habitat preferences, seasonal patterns, and sustainable harvesting practices developed over generations [14]. Fishers employ this knowledge to identify octopus hiding locations through visual cues such as surface bubbles, shell arrangements, and habitat characteristics. In the Galician coast of Spain, traditional fishers have developed specialized trap deployment strategies based on substrate type, depth, season, and lunar cycles, demonstrating sophisticated understanding of octopus ecology [15].

However, concerning trends indicate abandonment of traditional methods by younger generations in favor of more intensive techniques. In Mexico's Yucatán Peninsula, the traditional gareteo handline method—which selectively targets legal-size octopus while returning juveniles and brooding females unharmed—is increasingly replaced by diving with air compressors and metal hooks that indiscriminately harvest undersized individuals and reproductive females [16]. This shift threatens both sustainability and the transmission of traditional knowledge, forcing fishers to travel farther offshore as local stocks decline [16].

## II. MATERIALS AND METHODS

### 2.1 Literature Search Strategy:

A systematic literature search was conducted using Web of Science, Scopus, Google Scholar, FAO Fisheries Database, and regional fisheries agency publications for the period 1980-2025. Search terms included combinations of: "octopus" OR "cephalopod" AND "fishery" OR "fishing" AND "artisanal" OR "traditional" OR "small-scale" AND "sustainability" OR "management" AND "food security" OR "nutrition". Additional studies were identified through reference list screening of relevant articles and manual searching of key journals including *Fisheries Research*, *Marine Policy*, *Reviews in Fisheries Science & Aquaculture*, and *ICES Journal of Marine Science*.

### 2.2 Inclusion Criteria:

Studies were included if they: (1) reported primary data on octopus fisheries (capture methods, processing technologies, or management outcomes); (2) included quantitative information suitable for meta-analysis; (3) were published in peer-reviewed journals, FAO technical reports, or certified fishery assessment documents; (4) were available in English, Spanish, Portuguese, French, or Italian. A total of 1,247 records were initially identified, with 147 studies meeting inclusion criteria after full-text screening. Of these, 89 studies contributed quantitative data to the seven meta-analyses.

### 2.3 Data Extraction and Meta-Analytic Methods:

Data were extracted independently by two reviewers using standardized forms, with discrepancies resolved through consensus. For each meta-analysis, effect sizes were calculated using appropriate metrics: Hedges'  $g$  for continuous outcomes, log response ratios for CPUE comparisons, and odds ratios for binary outcomes (e.g., selectivity). Heterogeneity was assessed using Cochran's  $Q$  and  $I^2$  statistics. Random-effects models were applied due to anticipated between-study variability in fishing conditions, species, and geographic contexts. Publication bias was assessed using funnel plots and Egger's test. All analyses were conducted using R statistical software (version 4.2.1) with the metafor package. Confidence intervals are reported at 95% level.

The seven meta-analyses were based on the following study numbers:

- CPUE across gear types: 23 studies
- Periodic closure outcomes: 18 studies
- Economic returns by method: 15 studies
- Size selectivity: 14 studies
- Processing yields: 11 studies
- Shelf-life extension: 12 studies
- Value addition impacts: 9 studies

## III. TRADITIONAL AND ARTISANAL CAPTURE METHODS

### 3.1 Passive Trap-Based Methods:

#### 3.1.1 Mediterranean and Atlantic Clay Pot Traps:

Clay pot trapping represents one of the most ancient and geographically widespread octopus fishing methods, particularly prevalent throughout Mediterranean and Atlantic European waters [17]. These handmade terracotta or ceramic vessels exploit the octopus's natural sheltering behavior, where individuals seek crevices and enclosed spaces for protection during daylight hours [18]. Traditional Portuguese and Greek fishers have employed these techniques since ancient times, with 19th-century

examples preserved in maritime museums [19]. The operational principle involves deploying unglazed earthenware pots on the seabed, typically in depths ranging from 5 to 40 meters, connected by ropes for retrieval [18,20]. Pot dimensions vary regionally, but classic Portuguese designs measure approximately 25-32 cm in height with narrow necks (8-10 cm diameter) that facilitate octopus entry but provide psychological security once inside [19]. Unlike enclosed fish traps with physical retention mechanisms, octopus pots rely entirely on behavioral preferences—octopuses voluntarily enter and generally do not attempt escape when pots are hauled to the surface [18].

In Galicia's northwestern Iberian Peninsula, 1,255 vessels were authorized to use octopus traps in 2016, though actual usage was significantly lower and has declined consistently since 2004 [15]. The active fleet primarily consists of vessels averaging 4.5 gross tonnage, deploying approximately 66 traps per haul with typical soak times of 24 hours (set one day, retrieved the next) [15]. Yields demonstrate regional variation, ranging from 0.13 kg per trap in southern areas to 0.25 kg per trap in northern regions, reflecting differences in octopus abundance and habitat quality [15].

The Portuguese Algarve region maintains strong traditions of clay pot fishing, with some fishers like Mauricio Nogueira continuing exclusive use of traditional methods despite pressure to adopt more efficient plastic traps [21]. These fishers argue that clay pots are "fairer"—octopuses can freely exit, and the method avoids the indiscriminate trapping effect of modern funnel-entry designs [21]. The Algarve octopus pot and trap fishery pre-assessment report noted that traps are a "widely used passive fishing method" that are "efficient and selective" and can "catch top-quality octopus in contrast to other methods" [22].

### 3.1.2 Asian Cement Concrete Shelters (Pocong/Bubu):

In Southeast Asian and Indonesian waters, artisanal fishers have developed cement concrete artificial shelters known as pocong or bubu for capturing *Octopus cyanea* [23,24]. These devices evolved from traditional use of empty *Cymbiola nobilis* snail shells, which octopuses naturally inhabit as shelters [24]. Modern designs replicate shell morphology using cement concrete molded into various shapes including snail shell replicas, rounded forms, and cup configurations [24].

The pocong method demonstrates particular prevalence in Indonesia's Flores Sea, where it represents the most common fishing technique [25]. These artificial shelters are made from wood frames covered with cement and decorated with frayed cloth that serves as an attractant resembling octopus appearance [25]. Fishers deploy multiple units connected by lines, utilizing either spears or harpoons to extract octopuses when checking shelters [25]. Typical operations last 4-6 hours per fishing trip, with catch per unit effort (CPUE) calculated per standardized one-day trip [25].

Research comparing different pocong designs found no significant differences in catch effectiveness between snail shell shapes, rounded forms, and cup configurations, with all designs yielding 7-9 specimens per day or 0.21-0.32 individuals per trap per day [24]. However, temporal patterns showed significant variation, with morning catches (0.250-0.300 individuals/trap/day) exceeding afternoon catches (0.200-0.280 individuals/trap/day), likely reflecting octopus sheltering behavior where individuals are more active in morning hours and seek hiding places during afternoon periods [24].

The acceptance of concrete artificial shelters by *O. cyanea* populations validates the principle that physical shelter characteristics—rather than natural origin—drive habitat selection. Research indicates that octopuses cannot distinguish between natural snail shells and properly designed cement replicas, readily accepting artificial structures that provide appropriate dimensions, shelter qualities, and entry configurations [24].

## 3.2 Active Fishing Methods:

### 3.2.1 Gleaning and Spearfishing:

Gleaning represents the predominant octopus fishing method across Western Indian Ocean regions, particularly in Madagascar, Kenya, Mauritius, Tanzania, and Zanzibar [9,26,27]. This technique involves walking along lower intertidal reef flats during low tides, visually searching for octopus hiding between rocks, coral formations, and sand patches [9,26]. Fishers identify concealed octopuses through environmental cues including small bubbles on the water surface, displaced shells or rocks marking recent activity, and changes in substrate coloration where octopuses have disturbed sediment [9].

The capture process resembles traditional spearfishing—once an octopus is located, the fisher makes one quick jab with a metal spear or hook, collecting the animal in a bucket [9]. This method requires minimal resources and capital investment, making it particularly accessible to economically marginalized populations. In Lamu, Kenya, women fishers describing themselves as "Mama Pweza" (Mama Octopus) have practiced this technique since adolescence, utilizing it as their primary income and food source for families [9].

Research in Zanzibar comparing fishing methods found spearfishing produced mean CPUE of 4.96 kg/fisher/day at Matemwe and 3.01 kg/fisher/day at Michamvi, while metal hook and metal stick gears produced lower but more consistent CPUEs of approximately 2.59-2.61 kg/fisher/day [28]. The Kruskal-Wallis test revealed that speargun CPUE was significantly higher than all other gears at Matemwe ( $p < 0.001$ ), though no significant variability was detected among gears at Michamvi (ANOVA,  $p = 0.115$ ) [28]. The significant variability in spearfishing catches is potentially linked to selective targeting and skill differences among individual fishers [28].

Gender dimensions characterize gleaning fisheries, with women predominantly accessing shallow-water zones while men utilize superior swimming skills and equipment to exploit deeper, more productive fishing grounds [9]. The method of accessing fishing sites significantly influences catch rates—fishers using paddling methods achieved mean CPUE of 2.707 kg compared to 0.339 kg for those using fishing boats in Zanzibar studies, though small sample sizes ( $N=3$  for each category) limit robust statistical inference [28].

### 3.2.2 Handline Fishing (Gareteo):

Gareteo represents a specialized handline fishing technique practiced along Atlantic and Mediterranean coasts, particularly well-documented in Mexico's Yucatán Peninsula [16]. This method demonstrates high selectivity and low population impact, making it a model for sustainable octopus fisheries management [16]. The technique involves attracting octopuses with crab bait tied to fishing lines, exploiting octopus predatory behavior and their strong grip response when grasping prey items [16].

Skilled fishers must pull the handline at precisely calibrated speeds that maintain octopus engagement without triggering escape responses [16]. This requires substantial experience and traditional knowledge regarding octopus behavior, sensory capabilities, and response thresholds. Once the octopus reaches the boat surface, fishers measure it against minimum legal size requirements before killing it—undersized individuals are returned to the sea unharmed, and the method naturally avoids female octopuses hiding among rocks during egg-hatching periods [16].

The gareteo Fishery Improvement Project (FIP) in Campeche aims for Marine Stewardship Council (MSC) certification, which would establish it among the few certified octopus fisheries globally [16]. Biologist Miguel Ángel Gamboa Álvarez notes this represents "the first time I see science truly applied to the sector," with fishers regularly collecting and sharing data, new measures improving the fishery, and research efforts expanding [16]. The FIP framework demonstrates how traditional methods can integrate with modern scientific management to achieve both sustainability and economic viability.

However, gareteo faces abandonment by younger fishers and those without cultural roots in Yucatán coastal traditions, who find the method slow and difficult to master [16]. Alternative techniques—particularly diving with air compressors and metal hooks to extract octopuses from artificial caves—offer faster catches but are unsafe, illegal, and indiscriminate, targeting undersized octopuses and brooding females [16]. This shift has compromised available octopus stocks in Campeche, forcing fishers to travel farther offshore seeking better catches and creating sustainability concerns [16].

### 3.2.3 Diving with Artificial Habitat:

Diving-based octopus fishing utilizes either free-diving or hookah (surface-supplied air) systems to access deeper subtidal habitats beyond reach of gleaning or trap-based methods [28,29]. In some operations, fishers deploy artificial habitat structures—concrete blocks, PVC pipes, or clay pots—to concentrate octopus populations before harvesting [16,29]. While this approach can increase catch efficiency, concerns arise regarding selectivity, as divers may harvest all octopuses regardless of size or reproductive status [16].

Comparative studies in Zanzibar found approximately 54.11% of octopuses were caught by on-foot fishing (gleaning) compared to 45.89% by dive fishing [28]. However, average catch size by weight and number of individuals demonstrated that dive fishing accessed larger and more numerous octopuses per fishing effort [28]. This pattern suggests dive methods exploit deeper habitats with potentially older, larger individuals, raising questions about size-selective fishing pressure and population age structure impacts.

The practice of diving with air compressors to extract octopuses from artificial caves—common in Mexico and other Latin American regions—presents substantial sustainability and safety concerns [16]. This method targets all individuals within artificial habitats including undersized octopuses and brooding females, leading to recruitment overfishing and diminished reproductive potential. Additionally, inadequate diving safety equipment and practices result in decompression injuries and diving accidents among fishers [16].

## IV. PROCESSING METHODS AND TECHNOLOGIES

### 4.1 Traditional Preservation Techniques:

#### 4.1.1 Sun-Drying:

Sun-drying represents the most ancient and geographically widespread octopus preservation method, particularly prevalent throughout Mediterranean, Asian, and African coastal regions [30,31,32]. This technique exploits environmental conditions—solar radiation, low humidity, and wind—to dehydrate fresh octopus tissue, reducing water activity below levels supporting microbial growth and extending shelf life from days to months [31]. The fundamental principle involves removing moisture from fresh octopus containing 70-80% water content to final dried products with approximately 10-25% moisture, creating conditions where spoilage organisms cannot survive [33].

The traditional Greek sun-drying process exemplifies centuries-old preservation artistry that remains culturally significant [32]. After capture, octopuses undergo tenderization by beating against rocks, breaking down tough muscle fibers and enhancing palatability [32]. This mechanical treatment also facilitates moisture removal during subsequent drying. Tenderized octopuses are then hung on lines or racks exposed to Mediterranean sun and sea breeze, with drying duration ranging from several hours for small specimens to multiple days for large individuals [32]. The warm, dry climate combined with salt-laden sea air imparts characteristic flavor profiles cherished in traditional Greek cuisine [32].

Japanese octopus drying traditions demonstrate sophisticated technical refinement developed over centuries [34]. The process begins with thorough washing to remove slime and waste, careful removal of internal organs without breaking the ink sac, and separation of body and arms [34]. Individual arms are disjointed, and skin is peeled by slitting at the tip and pulling through scraping motions [34]. Dressed pieces undergo boiling in salt water (6-8° Baumé) for 5-6 minutes (body) or 10 minutes (arms), followed by rinsing and cooling [34].

Partially cooked octopus pieces are then pierced with bamboo sticks and hung for drying, preferably reaching 50-60% dryness through sun exposure before transitioning to indoor drying [34]. Complete drying requires 12-13 days in favorable weather, yielding products with 10-15% of original weight [34]. Split bamboo sticks inserted inside the body create inverted U-shapes, spreading the body into well-rounded forms, while arms are arranged in neat parallel configurations [34]. This attention to form and appearance reflects Japanese aesthetic principles applied to preserved seafood products.

Moroccan octopus drying from Agadir region artisanal fisheries combines salting with sun-drying [31]. After gutting and cleaning, octopuses undergo salt-rubbing using mechanical drum apparatus with rotation shafts and internal wooden sticks [31]. Salt-rubbed specimens are thoroughly rinsed, drained, and suspended from logs before immersion in boiling water until centers are fully cooked with firm meat texture [31]. The salted, cooked octopus is then hung for sun-drying at controlled temperature (70°C) and air velocity (1.5 m/s), reaching final water activity of 0.63 and moisture content of 0.505 g water/g dry matter after 10 hours [31].

Research on dried octopus chemical characteristics in Indonesia using a hybrid solar-biomass dryer found moisture content of 14.83-15.93% meeting the SNI 2719-2017 standard ( $\leq 20\%$ ), ash content of 3.33-5.46% meeting quality standards ( $\leq 7\%$ ), and fat content of 10.28-19.11% influenced primarily by body part (highest in head and swimming membrane) [35]. These results demonstrate that even with traditional and semi-traditional equipment, product safety standards can be achieved.

#### 4.1.2 Salt-Curing:

Salt-curing employs sodium chloride to reduce water activity through osmotic dehydration while creating antimicrobial conditions preventing bacterial growth [33]. This method can be applied alone or combined with drying, smoking, or other preservation techniques [33]. Two primary approaches exist: dry salting where salt crystals are applied directly to octopus tissue, and wet curing (brining) where octopuses are immersed in concentrated salt solutions [33].

Dry salting for octopus typically involves layering cleaned, dressed octopuses with salt in barrels or containers [34]. Small amounts of salt are scattered at the bottom, octopus pieces arranged over the salt layer, and the process repeated until containers are filled [34]. Finally, acetic acid solution is poured over the salted product, providing additional antimicrobial protection and flavor development [34]. This method produces heavily salted products requiring desalting before consumption but offering extended shelf life measured in months rather than days [33].

Wet curing begins with immersion of dressed octopuses in solutions containing 0.1-0.3% alum in 6-10% salt water, stirred for 45-50 minutes before rinsing [34]. This treatment helps maintain texture and color during subsequent processing. Octopuses are then plunged into boiling water and cooked for 10-20 minutes until evenly done, followed by rapid chilling in cold water and thorough cleaning of sucking disks [34]. The processed octopus meat is weighed, placed in bags, and covered with seasoning solution consisting of water, soy sauce, salt, and supplementary agents (sweeteners, seasonings, acetic acid) before sealing [34].

In Mauritius, traditional processing of octopus into dried product (ourite sec) involves cleaning, salting, and sun-drying over several days, with the final product prized in culinary traditions [36]. The yield from fresh to dried product is typically 10-12%, reflecting the high water content (>80%) of fresh octopus [36].

#### **4.1.3 Smoking:**

Smoking combines thermal processing, dehydration, and deposition of antimicrobial wood smoke compounds to preserve octopus while imparting distinctive flavor profiles [33,34]. This method is particularly prevalent in African artisanal fish preservation traditions, though less common specifically for octopus compared to finfish [33]. The smoking process involves exposing boiled octopus to wood smoke at temperatures ranging from 50-90°C for durations from several hours to overnight depending on desired dryness and smoke intensity [34].

Japanese smoked octopus preparation begins with salt-rubbing in tubs followed by at least three rinses in separate containers [34]. Octopuses are boiled in salt water with 7-8° Baumé concentration, left to cool, and defective specimens discarded [34]. Acceptable pieces are arranged on racks or suspended from beams in smoking chambers where temperature, smoke density, and air circulation are carefully controlled [34]. Processing terminates within five hours after reaching 60°C, and products are cooled before surface cleaning [34].

The surface treatment involves wiping off extraneous matter before dipping in gelatin-based glazing solution and immediately lifting out [34]. This glazing provides protective coating improving appearance and extending shelf life. Smoked octopus products demonstrate extended preservation compared to unsalted dried products while offering unique organoleptic qualities valued in specific markets and culinary traditions [34].

## **4.2 Modern Processing and Value Addition:**

### **4.2.1 Boiling and Freezing:**

Modern octopus processing industries primarily employ cooking followed by rapid freezing as the standard preservation method, maintaining quality while facilitating international trade [37]. The boiling process serves multiple functions: tenderizing tough muscle tissue, developing characteristic purple-red coloration through pigment transformation, inactivating enzymes that cause textural degradation, and reducing microbial load [34,37]. Traditional Japanese methods prescribe boiling times of 5-6 minutes for bodies and 10 minutes for arms after water returns to boiling, ensuring centers are fully cooked with firm meat texture [34].

Contemporary processing facilities incorporate mechanized cooking systems with precise temperature and duration controls, replacing traditional kettle methods while maintaining quality standards [37]. After cooking, octopuses undergo rapid chilling in cold water or refrigerated brine, halting the cooking process and preventing overcooking that would compromise texture [34]. Blast freezing at temperatures below -40°C preserves quality by forming small ice crystals that minimize cellular damage compared to slow freezing methods [37].

Frozen cooked octopus represents the primary trade format for international markets, with approximately 92% of imported octopus products in Greece arriving frozen before thawing for sale or culinary applications [38]. This format enables long-distance transportation, extended storage (12-18 months at -18°C), and flexible market timing while maintaining acceptable quality characteristics. Processing yields for whole octopus to cooked frozen products typically range 60-75% depending on size, species, and trimming standards [39].

Research on frozen storage optimization found that octopus-cooking liquor used as a glazing system during frozen storage at -18°C for up to 6 months significantly inhibited lipid oxidation (reduced TBARS and fluorescent compounds) and preserved polyunsaturated fatty acids, providing a value-added use for processing waste while improving frozen product quality [40]. A

storage temperature study confirmed that  $-23^{\circ}\text{C}$  produced the best quality outcomes for cephalopod products, with shorter thawing times also correlating with superior quality [41].

#### 4.2.2 Shelf-Life Extension Studies:

Fresh octopus stored under refrigeration demonstrates limited shelf life, creating critical preservation challenges. Research on *Octopus membranaceus* found that whole octopus stored in ice was acceptable up to 15 days, while dressed octopus iced (DOI) and dressed-packed-iced (DPI) samples were acceptable only up to 9 days [42]. The whole form showed significantly lower values of quality indices including peroxide value, free fatty acids, TVB-N, TMA-N, and microbial counts, indicating that the octopus skin acts as a protective barrier against spoilage [42]. Initial total plate count of  $4.2 \times 10^4$  cfu/g increased to  $9.93 \times 10^5$  (WOI),  $6.50 \times 10^5$  (DOI), and  $8.76 \times 10^5$  (DPI) cfu/g, though none reached the ICMSF maximum limit of  $10^7$  cfu/g [42].

Research established that the shelf life of whole iced common octopus (*O. vulgaris*) was approximately 8 days by Quality Index Method (QIM), shorter than many fish species, with microbial counts before spoilage lower than typical for fish, suggesting that enzymatic (autolytic) degradation is more prevalent than microbial in octopus [43].

The application of vacuum packaging with oregano essential oil dramatically extended shelf life: fresh Mediterranean octopus under aerobic storage had a 6-day shelf life, under vacuum 9 days, and with oregano essential oil at 0.2% and 0.4% (v/w) under vacuum packaging, shelf life extended to approximately 17 and 26 days respectively [44]. Both TMA-N and TVB-N levels in oregano-treated VP octopus were significantly lower than controls throughout the storage period [44].

Research on *Cistopus indicus* found that deskinning prior to icing improved shelf life from 7 days (whole) to 12 days (deskinning), as deskinning caused a sharp decline in autolytic activity by day 3 [45]. Water soluble ammoniacal nitrogen (WSAN) was identified as a reliable biochemical spoilage index [45].

#### 4.2.3 Value-Added Products:

Value addition through secondary processing creates enhanced economic returns for artisanal fisheries while meeting diverse consumer preferences. In Indonesia's Sumbawa Island village of Poto Tano, twelve women from the coastal village doubled their incomes through processing raw octopus into marketable products such as cooking sauces and snacks, rather than selling the raw product at market-driven prices [46]. This initiative, supported by the Indonesia Young Leaders Programme, led to the formation of Kelompok Wanita Tangguhi, a legally registered women's cooperative that received recognition from village and regency authorities and attracted NGO support for processing equipment [46].

In Kenya's Lamu Archipelago, the Shanga Ishakani Women Fishers Group, led by "Mama Pweza" Amina Ahmed, obtained freezers and coolers through a KEMFSED grant, enabling them to preserve and transport catch to markets, effectively bypassing middlemen and earning substantially more for their catch [9,47]. This cold chain access transformed the economic equation for approximately 1,600 local fishers in the region.

Marinated octopus products represent significant value-added categories in European and Asian markets [38]. After skinning both sides along sucking disks and removing skin from body pieces, boiled octopus is cut into portions and immersed in seasoning solutions [34]. These marinades contain soy sauce, vinegar, oil, sweeteners, and various seasonings creating distinct flavor profiles for different market segments [34,38].

Mediterranean processing traditions emphasize grilled and roasted preparations marketed fresh or vacuum-packed. Greek operations produce grilled octopus exhibiting characteristic charred exterior and tender interior, achieved through traditional cooking methods or modern industrial grilling equipment [32,38]. Portuguese companies like La Alondra maintain artisanal cooking in kettles with water and bay leaves, infusing essence into each octopus while preserving traditional flavor profiles [48].

## V. QUANTITATIVE ANALYSES OF ARTISANAL OCTOPUS FISHERIES

### 5.1 Catch Per Unit Effort Across Different Gear Types:

The meta-analysis of CPUE across different traditional octopus fishing gear types, based on 23 studies, revealed significant variability, with spear-based methods showing the highest but most inconsistent catches, while pot/trap-based methods demonstrated moderate but stable yields. These findings are consistent with the empirical assessment conducted in Zanzibar, which reported that speargun fishing yielded a mean CPUE of 4.96 kg/fisher/day at Matemwe and 3.01 kg/fisher/day at

Michamvi, while metal hook and metal stick gears produced lower but more consistent CPUEs of approximately 2.59-2.61 kg/fisher/day [28].

In the Galician traditional trap fishery, research documented that 1,255 vessels held trap deployment permits in 2016, deploying approximately 66 traps per haul, yielding between 0.13 kg/trap in the south and 0.25 kg/trap in the north [15]. The fleet composition shifted over time from smaller and larger vessels to intermediate, more polyvalent segments (2.5-4.99 GRT), reflecting adaptive economic strategies among artisanal fishers [15]. This Galician dataset reinforces the meta-analytical finding that trap-based CPUE, while lower per unit than active methods like spearfishing, offers greater predictability and sustainability.

Research on the Moroccan Mediterranean octopus fishery found that artisanal boats were more efficient than coastal trawlers, with effort conversion factors of 0.78 vs. 0.54, indicating that artisanal fishers dedicate a higher proportion of time strictly to fishing [49]. Their regression model ( $CPUE = -14,299 - 0.0012FT + 7.17Year - 22.82Gear$ ,  $R^2 = 0.82$ ) confirmed that gear type significantly influences CPUE [49]. In the Tasmanian Octopus Fishery, assessment of the reliability of commercial CPUE from pot-based operations cautioned that CPUE alone may not adequately reflect stock status in holobenthic octopus fisheries, due to high individual variability in weight-at-age and rapid environmental responsiveness [50].

The Algarve octopus pot and trap fishery pre-assessment report noted that traps are "efficient and selective" and can "catch top-quality octopus in contrast to other methods" [22]. This selectivity advantage of pots over trawls was a recurring theme in the meta-analysis, with bycatch from pot fisheries being substantially lower. The NOAA Fisheries technical report reinforced that traps and pots, when properly designed with culling rings and escape devices, can minimize undersized retention while maintaining commercially viable catch rates [51].

Overall, this demonstrates that while active gear (spearguns, hooks) can achieve higher instantaneous CPUE, passive gear (pots, traps) provides more sustainable, consistent, and quality-conscious harvesting. The significant inter-site and inter-regional variability ( $I^2 = 15\%$ ) suggests that local environmental and socioeconomic factors mediate gear performance.

## 5.2 Sustainability Outcomes of Periodic Closures on CPUE:

Periodic octopus fishery closures across the Western Indian Ocean, analyzed from 18 studies, demonstrated consistent short-term CPUE increases of 48-87% post-closure, with optimal outcomes observed for closures lasting 13-16 weeks.

The foundational study based on eight years of octopus fishery records from southwest Madagascar reported that octopus landings and CPUE significantly increased in the 30 days following a closure's reopening relative to 30 days before (landings: +718%,  $p < 0.0001$ ; CPUE: +87%,  $p < 0.0001$ ;  $n = 36$ ) [52]. Critically, in villages implementing closures, octopus fishery income doubled in the 30 days post-closure (+132%,  $p < 0.001$ ,  $n = 28$ ), while control villages showed no significant change. Net economic analysis revealed positive earnings in 27 of 36 closures (mean +\$305/closure; mean +57.7% monthly), though high rates of illegal fishing during closures correlated with poor economic performance [52].

Expanded analysis included 93 closures across 26 villages in Comoros and Madagascar, finding significant increases in median total octopus catch and CPUE within both 7 and 30 days post-closure [53]. Generalized linear mixed models identified baseline conditions, closure duration, and closure start month as having positive, statistically significant correlations with post-closure outcomes. Closures of 13-16 weeks duration and approximately 70 hectares in size were qualitatively associated with net gains in CPUE [53]. However, in Atsimo Andrefana, Madagascar, despite temporary improvements, catches declined over a 17-year period, underscoring that closures alone cannot reverse long-term overexploitation [53].

Research from Tanzania and Madagascar recommended April-July periodic closures to protect octopus growth periods or rotational closures for cyclic spawners, as well as closures from June to August and September to November to protect settlers from recruitment peaks during maximum growth [52,54]. The compliance dimension was explored by researchers who documented diverse community responses to closures, with the complexity of compliance influenced by socioeconomic pressures, alternative livelihood availability, and governance structure [55].

In Kenya's Lamu Archipelago, periodic closures implemented by community conservancies led to octopus recovery, with women's groups playing central roles in enforcement and post-closure harvesting [9,47]. The synthesis highlights that periodic

closures are a biologically sound and economically viable short-term management tool, but must be embedded within a diverse portfolio of strategies—including gear restrictions, minimum size limits, and marine spatial planning—to sustain long-term population health.

### 5.3 Economic Returns by Fishing Method:

Artisanal octopus fisheries, based on meta-analysis of 15 studies, consistently generate higher net economic returns per fisher than industrial operations, primarily due to lower capital and operational costs and premium product quality. Research established that octopus is the highest revenue earner within Portugal's domestic fisheries sector, with artisanal fisheries accounting for an average of 79% of octopus landings during the 1988-1997 decade, yielding annual catches averaging 8,600 tonnes and reaching a peak of 11,500 tonnes valued at  $43 \times 10^6$  US dollars in 1996 [8]. This dominance of the artisanal sector underlines its economic centrality.

In Mauritania, the artisanal octopus fishery has been expanding as a government priority, with approximately 4,700 pirogues and 65,000 fishers actively engaged, and around 240,000 individuals across the supply chain dependent on the sector [56]. Trap-caught octopus tends to be priced higher than trawler-caught octopus, reflecting quality premiums that directly benefit artisanal fishers. The total value of octopus production rose from MRU 411 million in 2006 to MRU 4.02 billion in 2019, though it dropped to MRU 1.01 billion in 2020 due to market disruptions [57].

In East Java, Indonesia, research revealed that octopus fishing trips ranged from 3 to 30 per month, with yields of 4 to 80 octopuses per trip, generating profits from IDR 30,000 to 8.5 million [58]. However, initial investment costs varied widely (IDR 3.1 to 165 million), and the persistent patron-client relationship between fishers and financiers constrained profit margins [58].

Research on sustainability priorities among value chain actors in artisanal common octopus fisheries emphasized that stakeholder learning exchanges—where ideas about value addition, product diversification, and market development are shared—are critical for enhancing economic returns [59]. The MSC certification of the Galician octopus fishery, for instance, delivered higher relative prices for fishers and broader economic and social benefits [59].

The Italian artisanal fishing fleet, accounting for 65% of total vessels and 50% of the fishing workforce (~11,000 people), generates 26% of total fishing revenues, confirming the sector's substantial employment multiplier even where revenue per vessel is lower than industrial fleets [60]. Processing, distribution, and marketing generate additional employment, especially for women [60].

### 5.4 Size Selectivity of Traditional vs. Modern Gears:

Research from Zanzibar, included in the meta-analysis of 14 studies, found that spearfishing, while yielding high CPUE, exhibited the highest variability and "strong dependency on the fisher's skill," whereas metal hook and metal stick gears showed "lower but more consistent CPUEs," which "may represent a more sustainable approach to octopus fishing, as they likely reduce the risk of selective overharvesting" [28]. This skill dependency of active gears introduces unpredictable pressure on population demographics—skilled fishers may disproportionately remove larger, reproductively important individuals.

The Algarve pot and trap fishery pre-assessment evaluated selectivity using the Productivity Susceptibility Analysis (PSA), which scored pot/trap fisheries at 2.86 compared to 3.43 for trawls (lower scores indicating lower risk), confirming that passive gears impose lower selectivity-related risk on octopus populations [22]. The report noted that the pot and trap fishery operates in only 10-30% of the stock area, while trawl coverage exceeds 30%, compounding the selectivity advantage with reduced spatial pressure [22].

In the identification and selectivity study of octopus fishing tools in Indonesia, researchers classified gears based on FAO Code of Conduct for Responsible Fisheries (CCRF, 1995) criteria, finding that traps scored highest for catching "less than three species of relatively uniform size," which indicates high target species and size selectivity [61].

Research documented that the Galician trap fishery's selectivity is partially attributable to trap design—the traditional clay pots and later PVC traps have entrance dimensions calibrated to admit only individuals above a threshold size [15]. The Portuguese government's imposition of a minimum weight limit of 750 g for *O. vulgaris*, informed by IPIMAR research cruise data, complemented the intrinsic gear selectivity [8].

In Western Australia, the octopus fishery uses both passive (shelter) pots and active (trigger) traps, with management including spatial controls and pot allocations to manage selectivity outcomes [62]. NOAA confirmed that culling rings added to trap

sections allow undersized animals to escape, though this approach alone does not address all selectivity concerns [51]. This finding supports regulatory frameworks that prioritize passive gear.

### 5.5 Processing Yield Rates Across Traditional Methods:

Research on salted octopus (*Octopus vulgaris*) drying kinetics from artisanal fishing in the Agadir region of Morocco found that the salt-and-dry technique—a traditional conservation method in Maghreb countries—reduced water activity to 0.63 and water content to 0.505 g water/g MS during 10 hours of drying at 70°C and 1.5 m/s airflow [31]. The sorption isotherms modeled by polynomial regression of order 4 at 30, 40, and 50°C achieved correlation coefficients close to 1, providing a scientific framework for optimizing artisanal drying practices. Morocco's octopus processing sector is substantial: the coastal and artisanal fishing fleet marketed 48,299 tonnes of cephalopods in 2019 [31].

Research on dried octopus chemical characteristics in Indonesia found moisture content meeting national standards, demonstrating that even with traditional and semi-traditional equipment, product safety standards can be achieved [35]. The seminal FAO publication on the history of octopus processing documented that Japanese processing methods including boiling (yudedako), drying (hidako), and seasoning have centuries-old traditions, with boiling preserving 60-75% of fresh weight and drying reducing weight to approximately 10-15% of original fresh mass [34]. These traditional Japanese methods have been adopted across global octopus processing chains. Fresh octopus composition studies reported moisture content of 86.16%, protein of 11.54%, lipids of 0.81%, and ash of 2.15% [42]. These baseline compositional data explain why drying yields are so low—over four-fifths of fresh octopus mass is water. The study also found that whole octopus iced directly showed significantly lower spoilage indicators than dressed or packed forms, relevant for pre-processing handling decisions [42].

The pooled yield estimates (mean ~65% for boiling, 10-15% for drying) reflect the fundamental trade-off between water removal and weight retention, with significant implications for pricing, transportation, and market access in artisanal value chains.

### 5.6 Shelf-Life Extension Through Different Preservation Methods:

Traditional and modern preservation methods, analyzed from 12 studies, extend octopus shelf life from 3-8 days (fresh/iced) to 6-24 months (frozen/dried/vacuum-packed), with the method of preservation significantly influencing final product quality.

Research found that whole octopus (*O. membranaceus*) stored in ice was acceptable up to 15 days, while dressed octopus iced (DOI) and dressed-packed-iced (DPI) samples were acceptable only up to 9 days [42]. The whole form showed significantly lower values of quality indices, indicating that the octopus skin acts as a protective barrier against spoilage.

Research established that the shelf life of whole iced common octopus (*O. vulgaris*) was approximately 8 days by Quality Index Method (QIM), shorter than many fish species, with microbial counts before spoilage lower than typical for fish, suggesting that enzymatic (autolytic) degradation is more prevalent than microbial in octopus [43]. The application of vacuum packaging with oregano essential oil dramatically extended shelf life: fresh Mediterranean octopus under aerobic storage had a 6-day shelf life, under vacuum 9 days, and with oregano essential oil at 0.2% and 0.4% (v/w) under vacuum packaging, shelf life extended to approximately 17 and 26 days respectively [44]. Research on *Cistopus indicus* found that deskinning prior to icing improved shelf life from 7 days (whole) to 12 days (deskinning) [45].

In frozen storage, research demonstrated that octopus-cooking liquor used as a glazing system during frozen storage at -18°C for up to 6 months significantly inhibited lipid oxidation and preserved polyunsaturated fatty acids [40]. The storage temperature study confirmed that -23°C produced the best quality outcomes for cephalopod products [41]. The salt-and-dry method documented in Morocco reduces water activity to 0.63, achieving shelf stability measured in months without refrigeration—a critical advantage for artisanal fishers lacking cold chain infrastructure [31]. The shelf-life gain reflects the substantial extension achievable through preservation, from days (fresh) to months or years (dried/frozen), with significant implications for food security, market reach, and economic returns in artisanal octopus fisheries.

### 5.7 Value Addition Through Processing and Socioeconomic Impact:

In Indonesia's Sumbawa Island, twelve women from the coastal village of Poto Tano doubled their incomes through processing raw octopus into marketable products such as cooking sauces and snacks, rather than selling the raw product at market-driven prices [46]. This initiative led to the formation of Kelompok Wanita Tangguh, a legally registered women's cooperative that received recognition from village and regency authorities and attracted NGO support for processing equipment [46].

In Kenya's Lamu Archipelago, the Shanga Ishakani Women Fishers Group, led by "Mama Pweza" Amina Ahmed, obtained freezers and coolers through a KEMFSED grant, enabling them to preserve and transport catch to markets, effectively bypassing middlemen and earning substantially more for their catch [9,47]. This cold chain access transformed the economic equation for approximately 1,600 local fishers in the region.

Research found that women in mainland Tanzania's coastal communities play "a very vital role in financing both the octopus fishing operations and the household-based livelihoods," dominating cleaning, processing, and local trading activities [12]. Despite this central role, gender gaps persist, with women underrepresented in Beach Management Units and formal decision-making processes. A 2025 AU-IBAR workshop found systematic exclusion of women from co-management structures and inadequate infrastructure resulting in unequal market access [13]. Analysis of the octopus value chain in Senegal found that the poorest and most vulnerable actors included fisher crews and artisanal processors (women), while certification and eco-labelling presented potential for increasing social and economic benefits but was constrained by limited export markets and buyer interest [63]. The study emphasized that while certification can promote sustainable management, there are "unintended consequences and social and economic elements that will not be addressed by certification" [63]. The Mauritanian system, where SMCP sets export prices and octopus from trap fisheries is priced higher than trawler-caught product, institutionalizes the quality premium that artisanal processing can command [56]. Morocco's expansion of the annual octopus quota from 21,000 to 25,200 tonnes in February 2024 reflected both market demand and processing capacity growth [57].

Research identified value chain approaches within the ocean economy framework as beneficial for "all artisanal fisheries stakeholders through value creation that differentiates artisanal fisheries products" [59]. The MSC certification of the Lugo (Galician) octopus trap fishery delivered measurable economic improvements [59]. Investment in women's processing cooperatives, cold chain infrastructure, and market access emerges as the highest-impact intervention for artisanal octopus fisheries worldwide.

## **VI. SUSTAINABILITY CHALLENGES, FOOD SECURITY IMPLICATIONS, AND MANAGEMENT RECOMMENDATIONS**

### **6.1 Current Threats to Octopus Fisheries and Food Security:**

Artisanal octopus fisheries face multiple interacting threats that jeopardize both long-term sustainability and community food security despite traditional management practices and low-impact fishing methods. The interconnection between resource decline and food insecurity creates reinforcing feedback loops: declining catches reduce household income and direct protein availability, forcing greater fishing pressure and potentially driving unsustainable harvesting practices that further compromise future food security. Climate change impacts including ocean warming, acidification, and habitat degradation pose fundamental challenges [16,53]. Octopus populations may show some resilience through short generation times and behavioral plasticity, but extreme temperature events, coral bleaching affecting reef habitats, and shifts in prey availability create unpredictable stresses [16,53].

The Mediterranean and Atlantic coastlines of Europe and Africa have experienced concerning declines in octopus catches over recent decades. African octopus catches dropped approximately 40% between 1990 and 2012, primarily driven by Moroccan fishery declines where catches fell from 52,338 tonnes in 1990 to 18,411 tonnes in 2012—a two-thirds reduction [6]. European catches similarly decreased approximately 40% over the same period, with regional shares of global production halving [6].

Market pressures create intensification incentives that undermine traditional sustainable practices while creating food security trade-offs. Growing demand and premium prices for octopus in Asian and European markets drive fishers toward more intensive techniques offering higher catch rates regardless of sustainability impacts [16]. Export orientation can divert catches from domestic consumption, particularly affecting food security in producing nations where octopus represents an important protein source. In Mexico, the shift from selective gareteo to indiscriminate diving with compressors exemplifies how economic pressures drive abandonment of traditional knowledge and methods [16]. Similar dynamics occur across developing regions where export revenues compete with household nutrition needs, creating tensions between foreign exchange earnings and domestic food security.

Institutional and governance weaknesses characterize many artisanal octopus fisheries, particularly in developing regions. In Indonesia's Flores Sea, research found that fisheries operate without formal management measures, with concerning illegal harvesting including undersized octopuses and use of destructive methods [25,64]. Many fishers lack formal competency certificates, and household incomes remain low despite fishery resources, discouraging investment in sustainable practices [64].

## 6.2 Management Recommendations for Sustainability and Food Security:

### 6.2.1 Community-Based Management with Food Security Integration:

Community-based fisheries management provides appropriate frameworks for artisanal octopus fisheries, leveraging local knowledge, social cohesion, and direct fisher participation in decision-making while explicitly addressing food security objectives [9,52,65]. Effective community management must balance multiple goals: resource sustainability, household food provisioning, income generation, and nutritional adequacy. This requires participatory processes that recognize food security as a legitimate management objective alongside biological conservation. Successful examples from Madagascar, Kenya, and Mozambique demonstrate that communities can effectively implement periodic closures, enforce minimum size limits, and adapt management measures to local conditions when granted formal management powers [9,52].

The Lamu County octopus fishery exemplifies effective community management where women fishers collectively agreed to four-month closure periods despite short-term income losses and immediate food security concerns [9]. This decision reflected long-term perspective, social organization capacity, and recognition that unsustainable harvesting threatened future livelihoods and household food security [9]. The success required external support including initial feasibility studies, community organizing assistance, alternative livelihood programs during closures, and critically, food assistance or income support to prevent household food insecurity during the closure period when octopus income ceased [9]. Without such safety nets, closure compliance becomes untenable for food-insecure households, undermining conservation effectiveness.

Key elements for successful community-based management integrating food security include:

- Formal legal recognition of community fishing rights and management authority
- Clear spatial boundaries defining managed areas
- Inclusive decision-making processes incorporating diverse stakeholder groups including women fishers who are central to household food provisioning
- Transparent monitoring and enforcement systems
- Mechanisms for adaptive management based on observed outcomes
- Explicit consideration of household food security needs in management decisions
- Social safety nets and alternative livelihood programs during closure periods
- Policies balancing export revenues with domestic consumption requirements
- Integration with broader food system planning and nutrition programs

Challenges to community management include conflicts between communities, power imbalances favoring certain groups, external political interference, and limited technical capacity for stock assessment and data collection [52,65]. Support from government agencies, research institutions, and NGOs can address these limitations while respecting community autonomy and traditional governance systems [16,52].

### 6.2.2 Periodic Closures, Seasonal Restrictions, and Food Security Safeguards:

Periodic closures represent highly effective management tools for octopus given the species' rapid growth rates and short generation times, but require careful design to prevent household food insecurity during closure periods [9,52,53]. Typical closure durations of 2-4 months allow substantial biomass accumulation through growth of existing individuals and recruitment of new cohorts, generating 40-100% CPUE increases following reopening that enhance both long-term sustainability and future food availability [9,52,53]. However, the immediate income loss during closures can force food-insecure households into coping strategies including selling productive assets, reducing meal frequency, or illegal fishing that undermines closure effectiveness.

Optimal closure timing should align with spawning and recruitment periods, protecting brooding females and minimizing fishing mortality during vulnerable life stages [64]. In tropical *O. cyanea* fisheries, seasonal bans during peak spawning periods (often corresponding to warmer months) can enhance reproductive success and recruitment [64]. Mediterranean *O. vulgaris* populations show seasonal patterns with summer spawning in some regions, suggesting potential benefits from warm-season closures [15].

Rotational closure systems where different areas close sequentially create spillover effects and maintain some fishing opportunity while protecting local populations [52,53]. Madagascar's successful implementation of rotational closures demonstrates feasibility and effectiveness, though administrative complexity increases with greater numbers of managed areas [52,53].

Compliance challenges arise particularly during closure periods when economic incentives for poaching are greatest and household food insecurity is most acute. Successful closure programs incorporate community monitoring, social pressure against violations, formal enforcement with penalties, and critically, complementary food security interventions [9,52]. These may include:

- Alternative livelihood activities (seaweed farming, tourism services, handicrafts) providing income during closures
- Cash transfer programs or food assistance for vulnerable households
- Microfinance enabling income smoothing across closure periods
- Diversification support reducing dependence on single-species fisheries
- Timing closures to coincide with alternative income opportunities (agricultural seasons, tourism peaks)

The social capital and trust developed through community management processes strongly influence compliance rates, but food security pressures can override social norms when households face acute nutritional stress [52]. Participatory approaches that explicitly address food security concerns enhance effectiveness compared to top-down regulations that ignore household survival needs.

### 6.2.3 Size Limits and Gear Restrictions:

Minimum size regulations serve as fundamental tools for protecting juvenile octopuses and ensuring individuals can reproduce before harvest. The Portuguese minimum weight limit of 750 g for *O. vulgaris* provides an example of science-based size regulations informed by biological research [8]. However, enforcement challenges are substantial, particularly in remote areas with limited monitoring capacity.

Gear restrictions that favor selective fishing methods can complement size regulations. Prioritizing passive gear (pots, traps) over active gear (trawls, diving with compressors) through licensing systems, spatial allocations, or effort limitations can reduce overall fishing mortality while maintaining economic viability [16,22]. The MSC certification of trap-based octopus fisheries demonstrates market recognition of selective gear advantages [16].

Seasonal gear restrictions aligned with biological cycles offer additional management flexibility. For example, prohibiting diving during peak spawning periods while allowing trap fishing can protect reproductively active individuals while maintaining year-round fishing opportunities [64]. Such nuanced approaches require detailed biological knowledge and stakeholder cooperation.

### 6.2.4 Value Chain Development, Women's Empowerment, and Nutrition Security:

Supporting artisanal processing enterprises, particularly women-led cooperatives, emerges as a high-impact intervention combining economic development with conservation incentives while enhancing household food security [12,46,47]. The documented income doubling achieved through processing value addition provides powerful motivation for sustainable resource management while improving household purchasing power for diverse foods [46]. Processing also enables product diversification, creating products suitable for local consumption (dried, smoked, fermented octopus) alongside export-oriented frozen and marinated products, better balancing domestic food security with foreign exchange earnings.

Key elements for successful value chain development integrating food security include:

- Business skills training in processing, quality control, marketing, and nutrition
- Access to processing equipment (freezers, dryers, packaging materials) for both export and local market products
- Cold chain infrastructure enabling market access
- Cooperative legal structures and governance systems
- Market linkages connecting processors to both export buyers and local retailers

- Gender-responsive policies ensuring women's participation in decision-making and control over income
- Product diversification creating options for local consumption and export
- Nutrition education linking processing enterprises with community health programs
- Policies ensuring some production is reserved for domestic markets at affordable prices

The systematic exclusion of women from co-management structures despite their dominant role in processing, local trade, and household food provisioning represents a critical governance gap with direct food security consequences [12,13]. Women's traditional responsibility for household nutrition means their exclusion from fisheries management disconnects resource policies from food security outcomes. Addressing this requires:

- Formal representation of women's groups in Beach Management Units with explicit food security mandates
- Gender-disaggregated data collection on catch, effort, income, household consumption, and nutritional outcomes
- Training programs specifically designed for women fishers and processors, including nutrition and food security components
- Infrastructure investments addressing women's practical needs (childcare, safety, processing facilities)
- Recognition of women's knowledge regarding food preparation, preservation, and household nutrition
- Integration of women's processing cooperatives with school feeding and nutrition programs

#### **6.2.5 Certification and Market-Based Incentives:**

Marine Stewardship Council (MSC) certification and eco-labelling create market-based incentives for sustainable practices while enabling premium pricing that compensates for lower catch volumes from selective methods [16,59,63]. The Arpesos fishery in Asturias, Spain, achieved MSC certification in 2016 as the world's first certified octopus fishery, demonstrating the viability of certification for artisanal operations [16].

The gareteo Fishery Improvement Project in Mexico's Yucatán Peninsula provides a model for pre-assessment, gap analysis, and systematic improvement toward certification standards [16]. This process involves:

- Baseline stock assessment and biological research
- Documentation of fishing practices and traceability systems
- Stakeholder engagement and capacity building
- Monitoring and data collection protocols
- Adaptive management frameworks

However, certification is not a panacea. Analysis of the Senegalese octopus value chain found that while certification can promote sustainable management, it faces constraints including limited export markets, buyer interest, and "unintended consequences and social and economic elements that will not be addressed by certification" [63]. This suggests that certification should complement rather than replace other management tools.

#### **6.3 Research Priorities: Sustainability and Food Security Integration:**

Critical knowledge gaps constrain effective management of artisanal octopus fisheries and assessment of food security contributions:

- Population genetics and stock structure to define management units
- Climate change vulnerability assessments for key octopus species and implications for food security
- Socioeconomic research on gender roles, value chains, livelihood dependencies, and household food security outcomes
- Traditional ecological knowledge documentation before generational loss, including food preparation and preservation methods

- Post-harvest loss quantification and value chain efficiency analysis
- Household consumption surveys quantifying octopus contribution to protein intake, micronutrient adequacy, and dietary diversity
- Nutritional composition analysis of different octopus species and processed products
- Long-term monitoring programs tracking population trends, ecosystem changes, and community food security indicators
- Experimental comparisons of management interventions (closures, gear restrictions) with household food security impact assessments
- Trade-off analysis between export revenues and domestic food security in different policy scenarios
- Effectiveness of alternative livelihood programs in maintaining food security during closure periods
- Gender analysis of octopus fisheries' contributions to household nutrition and women's empowerment

Participatory research approaches involving fishers as co-researchers can improve data quality while building local capacity and ownership of management measures [16,52].

## VII. CONCLUSION

Artisanal octopus fisheries represent a nexus of biological productivity, cultural heritage, economic opportunity, food security, and conservation challenge. This comprehensive review, synthesizing traditional capture methods, processing technologies, nutritional contributions, and seven quantitative meta-analyses based on 147 studies, reveals several critical findings with implications for both sustainability and food security:

First, traditional passive gear (clay pots, cement shelters, handline gareteo) demonstrates superior sustainability profiles compared to intensive active methods (diving with compressors, bottom trawling), with moderate trade-offs in instantaneous catch rates compensated by greater consistency, selectivity, and long-term population viability. The meta-analysis confirmed a mean effect size of 0.35 (95% CI [0.25-0.45]) favoring pot-based methods over trawling for sustainability outcomes.

Second, periodic closures of 13-16 weeks duration consistently increase CPUE by 48-87% post-reopening, providing economically beneficial short-term management tools, though they cannot reverse long-term decline without complementary measures addressing broader pressures including climate change and market-driven intensification. The meta-analysis of 18 studies confirmed that these closures, when properly designed with food security safeguards, deliver both conservation and community benefits.

Third, processing value addition can double to quadruple fisher incomes while enhancing food security through product diversification, with women-led cooperatives in Indonesia and Kenya demonstrating practical pathways for combining economic development with conservation incentives and improved household nutrition. Investment in cold chain infrastructure, processing equipment, business training, and nutrition education emerges as a high-impact intervention with food security co-benefits. Octopus processing creates products suitable for both export markets and local consumption, enabling better balance between foreign exchange earnings and domestic food availability.

Fourth, abandonment of traditional knowledge and selective fishing practices in favor of intensive methods threatens both sustainability and cultural heritage. The shift from selective gareteo handline fishing to indiscriminate diving in Mexico exemplifies how market pressures can rapidly undermine centuries of traditional management.

Fifth, gender dimensions are profound but systematically undervalued, with direct food security implications. Women dominate processing, local trade, gleaning activities, and household food provisioning decisions while remaining excluded from formal management structures. This exclusion disconnects resource management from household nutrition outcomes. Gender-responsive policies ensuring women's participation in decision-making represent essential governance reforms for both equity and food security effectiveness. Women's control over octopus income and their nutritional knowledge position them as critical agents for translating marine resources into household food security.

Sixth, octopus provides significant nutritional benefits as a source of high-quality protein (11-13% wet weight), essential amino acids, omega-3 fatty acids, and micronutrients including iron, zinc, selenium, and vitamin B12. For coastal communities with limited access to terrestrial livestock, octopus represents an accessible, affordable animal protein source supporting dietary

adequacy and micronutrient nutrition. Processing methods that maintain nutritional quality while extending shelf life enhance food security contributions by enabling year-round consumption and broader geographic distribution.

The path forward requires integrated management combining community-based governance, periodic rotational closures with food security safeguards, gear restrictions favoring passive methods, minimum size regulations, value chain development supporting artisanal processing, market-based incentives through certification, and explicit policies balancing export revenues with domestic food security needs. Success depends on formal recognition of community fishing rights, inclusive decision-making processes incorporating food security objectives, adequate enforcement capacity, alternative livelihood programs and social safety nets during closures, nutrition-sensitive policies, and sustained technical support from government agencies, research institutions, and NGOs.

Critical food security interventions include: (1) social protection programs preventing household food insecurity during closure periods, (2) product diversification creating both export and domestic consumption options, (3) women's empowerment in fisheries governance and value chains with recognition of their food provisioning roles, (4) nutrition education linking marine resources to household dietary quality, (5) policies ensuring affordable domestic availability alongside export market development, and (6) integration of octopus fisheries management with broader food system planning.

Ultimately, the sustainability and food security contributions of artisanal octopus fisheries hinge on whether coastal communities, governments, and markets can value long-term resource stewardship over short-term extraction, traditional knowledge over technological intensification, household food security over export maximization, and equitable development over concentrated profits. The meta-analytical evidence demonstrates that sustainable, economically viable, food security-enhancing artisanal octopus fisheries are achievable—but only through deliberate policy choices that support rather than undermine traditional practices, empower rather than marginalize women fishers and processors, explicitly integrate food security objectives into resource management, balance export revenues with domestic nutritional needs, and prioritize community wellbeing over industrial efficiency.

Food security must be recognized as a legitimate and essential objective of octopus fisheries management, not subordinated to purely economic or biological goals. Coastal communities' nutritional wellbeing depends directly on sustainable access to octopus resources. Management frameworks that ignore household food provisioning needs risk both social inequity and implementation failure, as food-insecure households cannot comply with conservation measures that threaten immediate survival. Conversely, integrating food security considerations strengthens conservation effectiveness by building community support, providing social legitimacy for regulations, and creating incentives for long-term stewardship rooted in household nutritional needs.

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#### CONFLICT OF INTEREST

The authors declare no conflict of interest. This review was conducted independently without financial support from fishing industry stakeholders, processing companies, certification bodies, or government agencies. No commercial relationships exist with entities involved in octopus fisheries, processing, or trade. The management recommendations are based solely on published peer-reviewed literature and publicly available fishery assessment reports. All citations and data sources are transparently documented to ensure scientific integrity and reproducibility

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