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FRONTIERS IN ENTOMOLOGICAL RESEARCH

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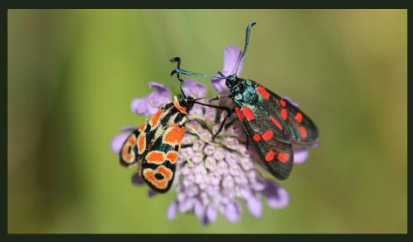

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Frontiers in Entomological Research

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PREFACE

The field of entomology continues to evolve as a cornerstone of biological and environmental sciences, bridging ecology, genetics, physiology, and biotechnology. The book *Frontiers in Entomological Research* explores the dynamic world of insects, emphasizing their ecological importance, adaptive evolution, and the innovations driving current research. Insects, with their extraordinary diversity and complex behaviors, play pivotal roles in pollination, nutrient cycling, pest management, and environmental monitoring. Understanding their biology and interactions is crucial not only for biodiversity conservation but also for sustainable agricultural and ecological practices.

This volume compiles scholarly contributions from researchers across various branches of entomology, focusing on emerging trends such as insect–microbe symbiosis, vector biology, climate change impacts, integrated pest management, molecular entomology, and bioinformatics applications. Each chapter reflects both fundamental insights and applied perspectives, highlighting how modern tools and interdisciplinary approaches are reshaping entomological studies. The discussions also underline the necessity of conserving beneficial insects while developing eco-friendly strategies to mitigate pest-related challenges.

In the context of global environmental change and food security concerns, entomological research assumes greater relevance than ever before. The contributors to this book provide an integrated understanding of insect diversity and their ecological services, showcasing innovative methodologies, analytical techniques, and experimental designs that enrich the field. This compilation is designed to serve as a valuable resource for students, educators, and researchers seeking to explore both traditional and cutting-edge aspects of entomology.

We express sincere gratitude to all contributors for their dedication and intellectual input, which have collectively shaped this work. It is our hope that *Frontiers in Entomological Research* inspires future studies, fosters collaboration, and deepens appreciation for the indispensable role of insects in maintaining ecological balance and advancing scientific frontiers.

- Editors

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DIGITAL ENTOMOLOGY: TRANSFORMING INSECT SCIENCE WITH ARTIFICIAL INTELLIGENCE

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Abstract:
The integration of artificial intelligence (AI) into entomological research is revolutionizing the study, monitoring, and management of insects. Techniques such as deep learning, computer vision, and neural networks enhance species identification, behavioral analysis, and habitat modeling, outperforming traditional morphological approaches. In agriculture, AI enables real-time pest detection and precision pest management using smart traps, UAVs, and image-based monitoring systems, achieving classification accuracies above 93% and precision up to 96%. Image-based models like InsectNet facilitate large-scale biodiversity assessments, invasive species monitoring, and pollinator activity quantification. AI also advances medical and forensic entomology by supporting vector surveillance, disease risk prediction, and life-stage classification. Despite challenges related to data requirements, computational resources, technical expertise, and ethical considerations, AI integration with IoT, robotics, and big data analytics holds promise for real-time ecological monitoring, predictive pest management, and conservation planning. Digital entomology, combining traditional methods with AI-driven tools, offers innovative, scalable, and sustainable solutions for agriculture, public health, and ecosystem conservation.

Keywords: Artificial Intelligence, Digital Entomology, Deep Learning, Computer Vision, Pest Management, Biodiversity Assessment, Precision Agriculture

Introduction:
The integration of artificial intelligence (AI) into entomological research has gained significant attention, transforming the study, monitoring, and management of insects. AI methods, particularly deep learning, computer vision, and neural networks, are being applied across multiple subfields including behavioural biology, biodiversity assessment, climate change studies, pest management, and disease vector control. AI-based species identification has demonstrated higher accuracy and speed compared to traditional morphological approaches, while AI-driven tracking systems facilitate detailed analysis of insect behavior and movement patterns. Additionally, habitat and population modeling have improved using AI, allowing for accurate predictions of insect distribution and abundance under changing environmental conditions.

In agriculture, AI has revolutionized pest management by enabling the real-time detection and identification of pest species using smart traps, UAVs (drones), and image-based monitoring systems. For example, the EfficientNetB7 architecture with transfer learning has been deployed to develop resource-efficient pest detection systems, achieving classification accuracies above 93%. Similarly, UAV-based models, optimized with YOLOv5s and enhanced attention modules, have identified pests such as ants, grasshoppers, palm weevils, shield bugs, and wasps, achieving an average precision of 96% and mAP of 95%. These approaches enable precision agriculture, reducing unnecessary pesticide use and supporting sustainable farming practices.

Image-based monitoring also addresses global concerns over declining insect populations. Large annotated datasets of insects—including bees, hoverflies, butterflies, and beetles—facilitate training of deep learning models such as YOLOv5 and InsectNet. These models accurately detect, classify, and monitor hundreds to thousands of agriculturally and ecologically relevant insect species, including pollinators, predators, parasitoids, and pests. Notably, InsectNet combines self-supervised learning, citizen science datasets, and streamlined workflows to deliver >96% identification accuracy, supporting invasive species monitoring and biodiversity assessments.

AI is also transforming medical and forensic entomology. Automated insect identification supports accurate vector monitoring and early prediction of vector-borne disease risks, aiding public health strategies. Deep learning models applied to datasets like IP102 can classify insects across life stages—egg, larva, pupa, and adult—enhancing pest management interventions while reducing reliance on expert evaluation. AI applications extend to pollination studies and beneficial insect monitoring, where computer vision and sensor networks quantify pollinator activity, evaluate habitat quality, and optimize agricultural yields.

Despite these advancements, challenges remain. AI requires large, high-quality datasets, computational resources, and technical expertise. Model interpretability, algorithmic bias, and ethical considerations—including disturbance to insect populations and reliance on automated systems over traditional taxonomy—must be addressed. Future research should focus on integrating AI with IoT, robotics, and big data analytics to enable real-time ecological monitoring, predictive pest management, and conservation planning. Collaborative efforts between entomologists and AI specialists are essential to ensure ethical, accurate, and scalable applications.

In conclusion, AI has immense potential to revolutionize entomology by enabling rapid species identification, behavioural analysis, precision pest management, biodiversity monitoring, and vector control. By complementing traditional methods with advanced computational tools, digital entomology offers innovative, sustainable solutions for agriculture, public health, and ecosystem conservation.

Definition

Digital Entomology is the application of digital technologies, computational tools, and artificial intelligence (AI) to the study, monitoring, and management of insects.
It integrates computer vision, machine learning, bioinformatics, remote sensing, robotics, and digital databases to enhance traditional entomology by providing faster, more accurate, and large-scale insect detection, identification, and ecological analysis.

Scope of Digital Entomology

The scope is broad and multidisciplinary, covering research, agriculture, environment, and society**:**

1. Insect Identification and Taxonomy

Insect identification and taxonomy form the foundation of entomology, helping classify the vast diversity of insects into a systematic framework. Identification involves recognizing insects based on morphological, anatomical, molecular, and behavioral traits**,** while taxonomy provides their nomenclature, classification, and evolutionary relationships**.** Traditional methods relied on morphology and dichotomous keys, but modern tools like DNA barcoding, molecular phylogenetic, and AI-based image recognition have made identification faster and more accurate. Together, they support research, biodiversity studies, and practical applications like pest management and conservation.

2. Pest Surveillance and Management

Pest surveillance is the systematic monitoring of pest populations, their natural enemies, and crop conditions to assess potential risks and outbreak trends. It provides early warning signals that guide farmers and researchers in making informed decisions. Pest management involves applying suitable strategies—cultural, mechanical, biological, and chemical control, often integrated under Integrated Pest Management (IPM)**.** Modern approaches use GIS, remote sensing, predictive models, and AI-driven smart traps for real-time monitoring and targeted interventions. Together, surveillance and management ensure effective pest control, reduced crop losses, and sustainable agricultural practices**.**

3. Biodiversity and Conservation

Biodiversity refers to the variety and variability of living organisms, including insects that play vital roles in pollination, decomposition, food webs, and ecosystem balance. Insects are indicators of environmental health and contribute significantly to agriculture and natural ecosystems. Conservation focuses on protecting this diversity through habitat preservation, sustainable practices, legal frameworks, and awareness programs**.** With threats like habitat loss, climate change, and pesticide overuse, biodiversity conservation is essential to maintain ecosystem services and food security. Modern tools such as DNA barcoding, digital databases, remote sensing, and AI-based monitoring are increasingly used to assess, track, and conserve insect diversity.

4. Entomological Research

Entomological research is the scientific study of insects covering their morphology, physiology, ecology, taxonomy, behavior, genetics, and applied aspects. It provides insights into insect roles as pollinators, decomposers, vectors, and pests, contributing to agriculture, forestry, medicine, and environmental management. Traditional research relied on field observations and microscopy, while modern approaches integrate molecular biology, genomics, biotechnology, GIS, remote sensing, and AI-based tools for deeper understanding and precision. Such research supports pest management, biodiversity conservation, climate change studies, and development of eco-friendly technologies, making it vital for food security and sustainable ecosystems.

5. Forensic and Medical Entomology

Forensic entomology is the use of insect evidence in legal investigations, mainly to estimate the post-mortem interval (PMI) by studying insect succession on decomposing bodies. It also aids in cases of neglect, abuse, and wildlife crimes. Medical entomology focuses on insects and arthropods of medical importance, particularly those that act as vectors of diseases such as malaria, dengue, chikungunya, and filariasis. Research in this field includes vector biology, pathogen transmission, epidemiology, and control strategies. Modern tools like DNA barcoding, molecular diagnostics, GIS mapping, and AI-based surveillance enhance both forensic and medical entomology, supporting public health, crime investigation, and disease management.

6. Agri-business and Industry

Insects play a vital role in agri-business and industry through their use in pollination, silk, lac, and honey production**,** as well as in emerging sectors like insect-based protein, biopesticides, and waste management**.** Industrial entomology focuses on harnessing beneficial insects for commercial purposes, while reducing losses caused by pests through integrated pest management, AI-driven surveillance, and eco-friendly control methods**.** With the rise of sustainable farming and circular economies, insect-based products are gaining importance in food, feed, pharmaceuticals, and cosmetics industries**,** making entomology a key driver of innovation in agri-business and bio-industrial sectors.

7. Image-Based Insect Recognition (Deep Learning, Computer Vision)

Image-based Insect Recognition uses computer vision and deep learning to automate insect identification from images or videos. High-resolution images of insects are processed using convolutional neural networks (CNNs) and other deep learning models to detect morphological features and classify species accurately. This approach reduces reliance on manual taxonomy, enabling rapid identification of pests, vectors, or biodiversity specimens. Coupled with digital databases, AI models can learn from thousands of annotated images, improving accuracy over time. Applications include precision agriculture, ecological monitoring, forensic entomology, and citizen science, making insect research scalable, fast, and accessible globally

8. Automated Taxonomy and Species Classification

Automated taxonomy refers to the use of computational tools, AI, and bioinformatics to classify insects into species, genera, and higher taxonomic

Automated taxonomy and species classification involve the use of digital tools, artificial intelligence (AI), machine learning (ML), and bioinformatics to identify, classify, and organize insects with greater speed and accuracy than traditional methods. Conventional taxonomy relied heavily on morphological keys and expert judgment, which were time-consuming and prone to human error. Modern approaches integrate image recognition systems, DNA barcoding, genomic databases, and AI-driven algorithms that can rapidly analyze large datasets of insect images, sounds, or genetic sequences to distinguish species. Automated classification not only accelerates biodiversity assessments and ecological research but also supports real-time pest detection, conservation biology, and digitization of museum collections, making insect taxonomy more standardized, accessible, and globally collaborative

AI applications in Pest Surveillance

Automated Pest Detection and Identification

Automated pest detection and identification represent a major advancement in modern entomology and agricultural management, integrating artificial intelligence (AI), machine learning (ML), digital sensors, image processing, and IoT-based technologies to monitor and manage pest populations efficiently. Traditionally, pest detection relied on manual surveys, field scouting, and visual inspections, which were labor-intensive, time-consuming, and prone to human error, especially for large-scale farming or when pests were morphologically similar. Automated systems use smart traps, digital cameras, acoustic sensors, and spectral imaging to continuously capture data on pest presence, abundance, and behavior. AI and ML algorithms process these data to identify pest species, quantify population density, and detect early signs of infestation, often in real time. This enables proactive pest management, allowing farmers and researchers to implement targeted interventions, optimize pesticide use, and reduce crop losses. Integration with decision support systems and predictive modeling further enhances accuracy, scalability, and efficiency. Overall, automated pest detection transforms pest management from reactive manual approaches to precision, data-driven, and sustainable strategies, supporting modern agriculture, ecological monitoring, and food security initiatives.

Precision Agriculture and Pest Management

Precision agriculture (PA) represents a paradigm shift in modern farming, integrating digital technologies, remote sensing, geographic information systems (GIS), Internet of Things (IoT) devices, and artificial intelligence (AI) to optimize crop production, resource use, and pest management. Unlike traditional agriculture, which applies uniform management practices across entire fields, PA emphasizes site-specific interventions based on real-time data about soil conditions, crop health, weather, and pest populations.

In the context of pest management, precision agriculture enables high-resolution monitoring of pest dynamics. Smart traps, cameras, drones, and sensor networks collect continuous data on pest presence, abundance, behavior, and crop damage. These datasets are processed using AI and machine learning algorithms to identify pest species, quantify infestation levels, and predict outbreaks before significant damage occurs. For instance, AI-driven image recognition can differentiate between morphologically similar pests, while predictive models can forecast population surges based on climatic variables, crop phenology, and historical trends.

Precision pest management also incorporates site-specific interventions, such as targeted pesticide application, biological control releases, or habitat manipulation, reducing unnecessary chemical use and environmental impact. Integration with decision support systems (DSS) allows farmers and agronomists to make data-driven, timely, and cost-effective decisions, optimizing crop protection while conserving beneficial insects and biodiversity.

AI in Pollination and Beneficial Insects

AI is proving transformative for the study and management of pollinators and other beneficial insects by enabling more efficient monitoring, behavioral analysis, and conservation strategies. AI-powered systems provide detailed, real-time insights that were previously difficult or impossible to obtain through manual methods.

Monitoring pollination efficiency

Traditional methods for assessing pollination, such as manual observation or pan trapping, are labor-intensive and not scalable across large areas. AI provides a non-invasive, automated solution.

Video and Computer Vision: Camera traps, often placed near flowers, record video footage of visiting insects. AI models, particularly deep learning with convolutional neural networks (CNNs), analyze the video to accurately identify the insects and track their movements. This data reveals which insect species visit which flowers, how frequently, and for how long.

Precision Pollination Management: This granular data allows farmers to make informed decisions to boost crop yield. For example, growers can add or remove beehives based on real-time pollination levels, adjust attractant flowers, or modify greenhouse conditions to encourage pollinator activity.

AI in Beekeeping

AI is enhancing the practice of beekeeping, helping to protect honeybee colonies from major threats like disease and pesticides.

Predicting Bee Health: An AI-based app can analyze the sound of buzzing bees to detect whether a colony is suffering from diseases or on the verge of collapsing. A neural network is trained to recognize sound patterns associated with different hive problems, providing an early warning system for beekeepers.

Toxicovigilance: Using individually tagged bees with RFID, AI models can analyze flight activity data. A study by INRAE showed that AI could classify bee flight performance with 99% accuracy to determine if they had been exposed to neurotoxic pesticides.

Remote Hive Monitoring: IoT devices in "smart hives" can use AI to analyze data from sensors, including hive weight, temperature, and humidity. This helps beekeepers manage their colonies remotely, predicting honey yields and identifying issues before they lead to colony loss.

Wild Pollinator and Beneficial Insect Monitoring

AI is also crucial for tracking wild insect populations, which are vital for ecosystem health and crop production.

Wild Pollinator Health: AI systems can track the health of native and wild bees using camera data or citizen science contributions. By analyzing this data, scientists can identify where conservation interventions are most needed to protect these critical species from pesticide exposure or habitat loss.

Biodiversity Indicators: Because moths are important pollinators and indicators of environmental change, AI systems are being developed to monitor them. The Autonomous Monitoring of Insects (AMI) system uses a light trap and an AI pipeline to identify moth species and track their movement, offering a standardized, long-term monitoring solution.

Protecting Beneficial Insects During Pest Control: AI-driven precision agriculture, which uses drones and computer vision to target pesticide application, can protect beneficial insects by limiting insecticide exposure to specific problem areas. This contrasts with "blanket" spraying, which indiscriminately harms both pests and beneficial insects.

Alternative Pollination Methods

In response to declining pollinator populations, AI is assisting in the development of artificial pollination solutions.

Pollination Robots: Researchers are developing AI-enhanced robots and drones that can autonomously identify flowers and execute precise pollination. Some systems use computer vision to navigate and apply pollen directly, while others use air pulses to mimic a bumblebee's vibrations.

Digital Twins: Scientists are creating virtual representations ("digital twins") of pollination processes to understand and optimize them. These AI-powered simulations provide insights into ideal pollination timings and patterns, which can guide strategies for maximizing crop yields.

Real-Time Ecological Monitoring:

AI systems, integrated with sensors, provide continuous, non-invasive monitoring of insects in diverse habitats.

IoT Sensor Networks: Arrays of smart traps equipped with cameras, microphones, and environmental sensors are deployed across fields and forests. These devices automatically collect

data on insect activity, which AI then processes to identify species and analyze population trends in real-time.

Acoustic Monitoring: AI models are trained to identify insect species based on their unique sounds, such as wingbeat frequencies or chirps. Acoustic sensors can be deployed in a habitat to monitor a wide range of species simultaneously, which is particularly useful for tracking cryptic or nocturnal insects.

Automated Camera Traps: AI-equipped cameras can capture and analyze images of insects attracted to light or pheromones. This provides continuous, high-volume data on insect presence and abundance, even in remote or difficult-to-access areas.

Comprehensive Biodiversity Assessment

AI is helping scientists overcome the sheer scale and complexity of assessing insect biodiversity, which constitutes a vast and often undescribed portion of life on Earth.

Digital Collection Databases: AI is a powerful tool for analyzing digitized natural history collections. By processing vast image and metadata datasets from museum specimens, AI can help identify new or understudied species and reveal historical trends in insect diversity.

Dark Diversity Discovery: AI workflows, in combination with DNA analysis, can help identify and confirm previously unknown species from image libraries derived from field cameras or digitized collections.

Biodiversity Loss Prediction: AI models can integrate data from various sources, such as satellite imagery, remote sensors, and ecological surveys, to track changes in habitats and predict how environmental shifts may affect insect biodiversity. This helps forecast ecological outcomes and prioritize conservation efforts.

Conclusion:

The integration of artificial intelligence (AI) into entomology is rapidly transforming the study, monitoring, and management of insects, with applications across behavioral biology, biodiversity assessment, climate change studies, pest management, and vector control. AI techniques such as deep learning, computer vision, and neural networks enable faster and more accurate species identification than traditional morphological methods, while AI-driven tracking and habitat modeling improve understanding of insect behavior, distribution, and population dynamics. In agriculture, AI supports real-time pest detection through smart traps, drones, and image-based monitoring systems, using architectures like EfficientNetB7 and YOLOv5s to achieve classification accuracies above 93% and average precision up to 96%, facilitating precision farming and reducing pesticide use. Image-based monitoring of pollinators, predators, parasitoids, and pests, aided by large annotated datasets and models like InsectNet, enhances biodiversity assessments, invasive species detection, and conservation planning. AI also advances medical and forensic entomology by supporting vector monitoring, disease risk prediction, and life-stage classification, while enabling real-time quantification of pollinator

activity to optimize agricultural yields. Despite challenges such as the need for extensive datasets, computational resources, technical expertise, algorithm interpretability, and ethical considerations, integrating AI with IoT, robotics, and big data analytics promises real-time ecological monitoring, predictive pest management, and effective conservation strategies. Overall, AI complements traditional methods to offer innovative, sustainable solutions in agriculture, public health, and ecosystem conservation, marking a new era in digital entomology.

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INTEGRATING NANOBIOTECHNOLOGY IN SERICULTURE: ENHANCING SILKWORM PHYSIOLOGY, SILK BIOMATERIALS, AND SUSTAINABLE PRODUCTION SYSTEM

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Abstract:
The application of nanobiotechnology in sericulture represents a transformative approach to enhancing silk production by integrating engineered nanoparticles (ENPs) into the silkworm's physiological processes and silk biomaterial synthesis. This chapter delves into the multifaceted interactions between silkworms (Bombyx mori) and ENPs such as silver (Ag), zinc oxide (ZnO), titanium dioxide (TiO_2), and chitosan, administered through fortified mulberry leaves. These nano-supplements have been shown to modulate digestive enzyme activities, improve gut microbiota balance, and influence endocrine pathways, leading to optimized growth and development. Furthermore, ENPs impact the expression of genes involved in fibroin and sericin synthesis, enhancing silk quality and yield. The chapter also explores the green synthesis of ENPs using botanical extracts, emphasizing eco-friendly approaches that align with sustainable sericulture practices. By bridging the disciplines of entomology, nanotechnology, and materials science, this work provides a comprehensive framework for advancing sericulture through precision nanobiotic interventions.

Keywords: Sericulture, Nanobiotechnology, Silkworm Physiology, Engineered Nanoparticles, Silk Biomaterials, Green Synthesis.

Introduction:
Sericulture, the age-old practice of silk production, has witnessed significant advancements through the integration of nanobiotechnology, offering innovative pathways to enhance silkworm health and silk quality. The introduction of engineered nanoparticles (ENPs) into the silkworm's diet, particularly via fortified mulberry leaves, has emerged as a promising strategy to modulate

physiological processes and improve silk production outcomes. These nano-supplements influence various aspects of silkworm biology, including digestive enzyme activity, gut microbiota composition, and hormonal regulation, leading to optimized growth and development. Additionally, ENPs affect the molecular pathways governing fibroin and sericin synthesis, resulting in superior silk quality characterized by enhanced tensile strength and lustrous appearance. The green synthesis of ENPs using botanical extracts further aligns with sustainable practices, minimizing environmental impact and promoting eco-friendly sericulture. This chapter delves into the intricate interactions between silkworms and ENPs, providing a comprehensive understanding of how nanobiotechnology can revolutionize sericulture by enhancing silkworm physiology, silk biomaterials, and sustainable production systems.

Nantoniotic Interface of Mulberry Silkworms

Advances in nanotechnology have opened up unprecedented possibilities across diverse biological domains, ranging from agriculture and biotechnology to sericulture (Hazarika, *et al.,* 2024**)**. Among the emerging frontiers, the application of nanomaterials within the biological systems of economically valuable insects, such as the mulberry silkworm (*Bombyx mori*), stands out as particularly promising. This species, the sole producer of natural silk, plays a crucial role in the sericulture sector and significantly supports rural livelihoods and the textile industry, especially in countries such as India, China, and Thailand (Fometu *et al.,* 2021). Nevertheless, persistent challenges—such as vulnerability to environmental stress, infectious diseases, and genetic limitations—continue to hinder efforts to optimize growth, strengthen immunity, and improve silk quality.

Nanotechnology has emerged as a transformative approach for enhancing physiological functions in organisms of agricultural and economic importance. In the case of *B. mori*, sericulture's long-standing traditions face modern demands for improved health, productivity, and fiber quality. However, these insects remain highly sensitive to pathogens, dietary fluctuations, and climatic changes, which can negatively affect yield and fiber properties. Recent innovations in engineered nanoparticles (ENPs) have introduced novel strategies for improving invertebrate performance **(**Dukare *et al.,* 2024**)**. Due to their nanoscale dimensions, high surface-to-volume ratio, and unique reactivity, ENPs can interact with biological systems at both cellular and molecular levels, thereby stimulating or inhibiting specific physiological processes. The concept of a *nanobiotic interface*—described as the deliberately engineered contact zone between nanomaterials and living systems—offers an advanced platform for targeted manipulation of insect biology (Biswas *et al.,* 2025).

When carefully applied, such nanoparticles can function as growth enhancers, immune modulators, and silk-quality improvers. Within silkworm biology, the nanobiotic interface

concept provides an opportunity to regulate essential traits, including larval development rate, immune defense mechanisms, and silk phenotype (Zhang *et al.,* 2024).

The present interdisciplinary work investigates how various engineered nanoparticles—such as silver, zinc oxide, titanium dioxide, and chitosan-based nanostructures—affect *B. mori* when delivered through diet or environmental exposure. The study aims to evaluate their potential in advancing growth performance, boosting immunity, and improving silk production traits.

1. Mulberry and its Role in Sericulture

Mulberry (*Morus alba*) is the exclusive food plant of the mulberry silkworm (*Bombyx mori*), and its nutritional quality directly influences silkworm health, cocoon yield, and silk quality (Sori and Bhaskar, 2012). The biochemical composition of mulberry leaves—rich in proteins, carbohydrates, vitamins, and minerals—is a key determinant in larval development and silk gland metabolism. Enhancing mulberry leaf quality through biological and feed-based interventions has emerged as a sustainable and non-invasive strategy to improve silk production without genetic modification of the silkworm itself.

2. Nutritional Influence of Mulberry on Silkworm Physiology

Research consistently demonstrates that leaf quality in terms of moisture content, nitrogen, crude protein, and mineral balance affects larval growth rate, silk gland size, cocoon weight and shell ratio, filament length, and denier (Datta, 2002).

3. Biological Interventions in Mulberry Cultivation

A. Biofertilizers and Organic Amendments

The use of *Azotobacter*, *Azospirillum*, phosphate-solubilising bacteria (PSB), and mycorrhizal fungi improves nitrogen fixation, phosphorus availability, and overall leaf quality. Organic manures (Kumbar, 2016), such as FYM and vermicompost, enhance leaf nutrient profiles and reduce chemical residues, thereby improving digestibility by larvae (Chaitanya *et al.,* 2014).

B. Microbial Foliar Sprays

Microbial sprays containing *Bacillus subtilis*, *Pseudomonas fluorescens*, or fungal biocontrol agents act as both plant growth promoters and stress protectants (Hajam, 2024). These treatments help increase chlorophyll content and bioactive metabolites in leaves.

4. Feed-Based Interventions: Leaf Fortification for Larvae

A growing body of research focuses on fortifying mulberry leaves with additives or coating agents to enhance silkworm performance.

A. Nutritional Sprays

Fortification with amino acids (lysine, methionine), vitamins (B-complex, E), and minerals (Mg, Zn) directly impacts fibroin synthesis (Rahman *et al.,* 2025).

B. Herbal and Botanical Extracts

Neem, turmeric, tulsi, and aloe vera extracts improve immunity and reduce pathogen load in larvae (Hassan, 2025).

C. Prebiotics and Probiotics

Supplementation with beneficial microbes or oligosaccharides enhances gut flora, improving digestion and silk protein conversion (Gheorghe *et al.,* 2024; Priyadharshini *et al.,* 2021).

5. Nanobiotic Integration with Mulberry Leaves

A novel approach in sericulture involves applying nanoparticles directly onto mulberry leaves to enhance nutrient absorption and strengthen the immune system of silkworms.

- **Silver nanoparticles (AgNPs)**: Enhance antimicrobial defence (Elsaffany *et al.,* 2025).
- **Zinc oxide nanoparticles**: Aid in enzyme activity and stress resistance (Zhang *et al.,* 2023).
- **Chitosan nanoparticles**: Improve digestion and promote silk gland health (Villiers *et al.,* 2009)

This strategy creates a nanobiotic interface prior to larval ingestion, effectively priming their physiological systems for improved performance (Suresh *et al.,* 2023).

6. Outcomes on Silk Phenotype and Production

Reported improvements following such interventions include:

- **Cocoon weight**: +10–15%
- **Shell ratio**: +12–18%
- **Filament length**: +20%
- **Silk strength and luster**: Noticeably enhanced

Source: Compiled from multiple studies in Journal of Sericulture Technology, 2018–2024.

Future Prospects:

Biological and feed-based interventions in mulberry cultivation and leaf preparation represent cost-effective, eco-friendly, and scalable strategies to enhance silk productivity. By focusing on leaf biochemistry and its interaction with larval physiology, researchers can unlock new opportunities for silkworm performance enhancement.

Integrating precision nanotechnology with plant–microbe interactions holds strong potential for shaping the next era of sustainable sericulture. This research also seeks to explore nanoparticle modes of action—including oxidative stress regulation, hormone mimicry, and epigenetic modifications—while assessing both beneficial and potentially adverse effects.

Silk Chemistry

1. Introduction to Silk Chemistry

Silk is a valued natural biopolymer renowned for its tensile strength, elasticity, biocompatibility, and sheen. Produced primarily by the mulberry silkworm (*Bombyx mori*), silk consists of two main proteins: fibroin (the core structure) and sericin (the outer coating). The amino acid

composition, secondary structure, and molecular interactions of these proteins determine the physical and functional traits of both raw and processed silk.

2. Molecular Composition of Silk

A. Fibroin

- Accounts for ~70–80% of silk fiber.
- Dominated by β-sheet structures, which give strength.
- Main amino acids: Glycine (~45%), Alanine (~30%), Serine (~12%).
- The repeating sequence *Gly–Ala–Gly–Ala–Gly–Ser* allows tight molecular packing (Zhou *et al.,* 2001).

B. Sericin

- Constitutes ~20–30% of silk.
- Hydrophilic, serine-rich polypeptide that binds fibroin filaments into a cocoon.
- Protects fibroin from environmental damage (Kundu *et al.,* 2008).

3. Structural Hierarchy of Silk

- **Primary structure**: Repetitive amino acid sequences.
- **Secondary structure**: Predominantly β-sheet crystals interspersed with amorphous regions.
- **Tertiary/quaternary structure**: Supercoiling and fiber bundling.

4. Factors Affecting Silk Chemistry

- **Nutrition and Feed Additives**: Extra amino acids can boost fibroin production; nanoparticle supplementation can alter silk-related gene expression.
- **Genetics and Hybrids**: Different silkworm strains produce silk with distinct fibroin molecular weights and crystalline ratios.
- **Environment**: Temperature and humidity during rearing influence β-sheet formation, moisture content, and viscosity.

5. Physicochemical Properties of Silk

- **Tensile strength**: Comparable to steel (400–600 MPa).
- **Elasticity**: Recovers from ~20–30% deformation.
- **Thermal stability**: Stable up to 180°C; decomposes at ~250–280°C.
- **Water interaction**: Hygroscopic; swells in high humidity.
- **Biodegradability**: Fully biodegradable under natural conditions (Altman *et al.,* 2003; Reizabal López-Para, 2020).

6. Analytical Techniques in Silk Chemistry

- **FTIR**: Analyzes β-sheet and random coil structures (Haliloğlu, 2023; Orlandi, 2021).
- **XRD**: Measures crystallinity and molecular alignment (Mhuka, 2014).
- **TGA and DSC**: Assess thermal degradation and melting transitions (Baena and Guerrero-Álvarez, 2022).

- **Amino Acid Profiling**: Quantifies major silk amino acids (Kabir *et al.,* 2024).

7. Modifications and Applications of Silk Proteins

A. Biomedical Uses: Silk fibroin scaffolds are used in tissue engineering, wound healing, and drug delivery (Orlandi, 2021; Nair, 2015). Silk sericin, rich in hydrophilic amino acids, shows antioxidant and antimicrobial properties, making it valuable in cosmetics and therapeutics (Kabir *et al.,* 2024).

B. Material Engineering: Blends of silk with polymers produce films, hydrogels, and nanofibers for applications in packaging, filtration, and electronics (Xue, 2020).

8. Impact of Nanoparticles on Silk Chemistry

Feeding silkworms engineered nanoparticles via mulberry leaves or artificial diets can modify fibroin/sericin gene expression, increase crystallinity, and enhance tensile strength, luster, and dye uptake (Sreenivas *et al.,* 2022).

Conclusion:

Silk chemistry is the result of interactions between genetics, nutrition, and environmental conditions. Advances in biotechnology and nanoscience now allow precise tuning of silk's molecular structure for targeted performance outcomes (Reizabal López-Para, 2020; Orlandi, 2021).

Nanoparticle–Silkworm Interface: Immune System, Enzyme Activity and Toxicity Balance

The integration of engineered nanoparticles (ENPs) into sericulture represents an advanced approach for enhancing the health, productivity, and disease resistance of the mulberry silkworm (*Bombyx mori*). Due to their nanoscale dimensions and high surface reactivity, ENPs are capable of interacting with silkworm biological systems at both cellular and molecular levels. These interactions influence key physiological parameters, including the insect's immune function, metabolic enzyme activity, and overall homeostasis. While the benefits of ENPs include improved growth performance and pathogen resistance, potential risks such as bioaccumulation, oxidative stress, and tissue damage necessitate precise determination of safe exposure levels (Lavine and Strand, 2002). This section explores the dual role of nanoparticles in modulating immunity, altering enzymatic activity, and maintaining the balance between beneficial stimulation and toxic effects.

Silkworm Innate Immune System

Unlike vertebrates, *B. mori* depends solely on innate immune defenses, which encompass both humoral and cellular components. Humoral immunity includes antimicrobial peptides (AMPs), the phenoloxidase cascade, and lysozyme production, whereas cellular immunity is mediated by hemocytes, which participate in processes such as phagocytosis, encapsulation, and nodulation. Activation of these pathways becomes critical during pathogen exposure or physiological stress, including that induced by nanoparticle ingestion (Lavine and Strand, 2002).

Nanoparticles as Immuno-modulators

Experimental evidence indicates that nanoparticles such as silver (AgNPs), zinc oxide (ZnO-NPs), and chitosan-based formulations can act as potent immunomodulators. These nanomaterials upregulate AMP genes, including *cecropin*, *attacin*, and *lysozyme*, and stimulate the prophenoloxidase (proPO) system, which enhances melanization and pathogen clearance (Prasad *et al.,* 2021). Furthermore, ZnO and TiO_2 nanoparticle exposure has been associated with increased hemocyte counts, improved phagocytic efficiency, and heightened encapsulation responses, suggesting a primed immune state.

Enzymatic Responses to Nanoparticle Exposure

Silicon dioxide nanoparticles ($nSiO_2$) are widely used, raising potential ecological and health concerns. Dietary exposure in silkworms (*Bombyx mori*) caused midgut damage, reduced larval growth, and lower cocoon production, while inducing antioxidant enzyme activity. Multi-omics analyses revealed disruptions in gene expression, gut microbiota, and metabolic pathways. These findings highlight $nSiO_2$'s potential to cause gut dysbiosis and metabolic and genetic alterations, providing a multi-dimensional assessment of its toxicity.

Various nanoparticles (NPs) have been studied for their effects on silkworm physiology, growth, and silk production. Silver nanoparticles (Ag NPs), tested at concentrations of 25, 50, 75, and 100 mg/L using white rot fungi, were found to improve feed efficiency in silkworms (Gudikandula *et al.,* 2019). Gold nanoparticles (Au NPs) with sizes ranging from 86–150 nm enhanced silk protein content and increased cocoon weight (Patil *et al.,* 2017). Titanium dioxide (TiO_2 NPs) at 1000 mg/L were applied to investigate gene expression related to fat metabolism and nutrition, resulting in boosted fibroin production (Li at al., 2020). Carbon nanotubes (CNTs), sized 6–8 nm, were utilized to mass-produce silk fibers with enhanced mechanical strength, allowing for directly improved silk harvests (Tian *et al.,* 2016). Zinc nanoparticles (Zn NPs, 98.8%, 50 ± 10 nm) were assessed for their toxicity and accumulation in the silk gland and midgut; studies reported activation of antioxidant enzymes in midgut cells, indicating a protective catalytic effect (Wang *et al.,* 2016). Lastly, chitosan (CS) solution at 5 mg/mL demonstrated strong antibacterial properties, significantly reducing bacterial infections in silkworms and providing protection to the larvae (Doddananjaiah *et al.,* 2024).

Oxidative Stress and Toxicity Thresholds

While moderate nanoparticle doses may promote beneficial physiological responses, chronic or excessive exposure can disrupt homeostasis. Overproduction of reactive oxygen species (ROS) can overwhelm antioxidant defenses, resulting in lipid peroxidation, protein oxidation, and DNA damage (Zhao *et al.,* 2020). Histological observations reveal degeneration of midgut epithelial cells, abnormalities in silk gland morphology, and increased hemocyte apoptosis under toxic

conditions. Elevated lipid peroxidation levels, disrupted gut architecture, and reduced cocoon quality serve as biomarkers of nanoparticle-induced toxicity.

Optimizing Safe Doses and Biocompatibility

To ensure safe application in sericulture, researchers are focusing on establishing dose-dependent safety profiles, including determination of the No Observed Adverse Effect Level (NOAEL). Biocompatible coatings, such as chitosan or polyethylene glycol (PEG), are being investigated to mitigate nanoparticle toxicity while maintaining functional activity. Additionally, synchronization of exposure with specific larval stages—particularly the early fourth instar—has been shown to optimize beneficial outcomes (Sharma *et al.,* 2022).

Conclusion:

Nanoparticle integration into silkworm rearing strategies presents both opportunities and risks. At optimal concentrations, ENPs can stimulate immunity and enhance metabolism, whereas overexposure leads to oxidative damage and physiological decline. Understanding the intricate balance between immune enhancement and nanoparticle toxicity will enable the development of precision nanobiotic interventions for sustainable sericulture.

Green Synthesis of Nanoparticles

In recent years, green synthesis of nanoparticles has emerged as a sustainable alternative to conventional chemical and physical production methods. Unlike traditional synthesis routes, which often require hazardous reagents and high-energy processes, green synthesis employs biological agents such as plant extracts, microorganisms, algae, and biopolymers as reducing and stabilizing agents (Ahmed *et al.,* 2016). This approach is not only eco-friendly and cost-effective but also produces biocompatible nanoparticles that are suitable for applications in sensitive biological systems, including silkworm rearing.

Principles of Green Nanoparticle Synthesis

The process is primarily driven by redox reactions facilitated by biomolecules such as polyphenols, flavonoids, peptides, and polysaccharides. These compounds act dually as reducing agents—converting metal salts like $AgNO_3$ into elemental nanoparticles—and as capping agents that stabilize particles against aggregation. The dual role of these biomolecules results in nanoparticles with tunable sizes, shapes, and surface functionalities (Ahmed *et al.,* 2016).

Biological Sources for Green Synthesis

Among the biological sources, plant extracts remain the most common due to their abundance of secondary metabolites like tannins, alkaloids, and organic acids. Examples include neem (*Azadirachta indica*) leaves for AgNP synthesis, *Ocimum sanctum* and *Aloe vera* for ZnO-NPs, and tea leaf extracts for AuNPs (Iravani, 2011). Microbial synthesis, involving bacteria, fungi, and actinomycetes, offers precise control but requires sterile conditions and longer reaction times. Algae and cyanobacteria, rich in pigments and reducing sugars, are utilized for large-scale

aqueous synthesis of metal oxide nanoparticles. Biopolymers such as chitosan, gelatin, and starch can either synthesize or encapsulate nanoparticles, improving their stability and biocompatibility.

Example Protocol – Silver Nanoparticles via Neem Extract

A typical green synthesis procedure involves preparing a plant extract by boiling fresh neem leaves in distilled water, filtering the extract, and mixing it with an aqueous $AgNO_3$ solution. The reaction mixture is stirred at moderate temperatures until a color change—from pale yellow to brown—indicates nanoparticle formation. The resulting nanoparticles are purified through centrifugation and washing, then air-dried or lyophilized for storage.

Characterization of Green-Synthesized Nanoparticles

Characterization techniques such as UV–Vis spectroscopy confirm nanoparticle formation via surface plasmon resonance, while X-ray diffraction (XRD) provides information on crystallinity. Fourier transform infrared (FTIR) spectroscopy identifies functional groups involved in reduction and capping, and electron microscopy (TEM/SEM) determines particle morphology and size distribution (Elsaffan, 2025).

Advantages and Challenges

Green synthesis offers several advantages, including the absence of toxic reducing agents, compatibility with biological applications, scalability, and minimal environmental impact. However, variability in biological extract composition can lead to inconsistent particle characteristics, and achieving high purity may require additional purification steps.

Conclusion:

Green nanoparticle synthesis presents an environmentally responsible and biologically safe method for producing nanomaterials suited for integration into sericulture. Understanding the underlying biochemical mechanisms of nanoparticle formation and stabilization is key to tailoring materials for specific applications, including immune modulation and silk production enhancement in *B. mori*.

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TECHNOLOGICAL ADVANCEMENTS IN ENTOMOLOGY RESEARCH

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Abstract:
Frontiers in entomological research are being shaped by technology and new approaches to insect management, such as using AI and drones for monitoring and targeted pest control. Other key areas include the study of social insect behavior, evolutionary ecology, insect health and pathology, and the development of sustainable, plant-based pesticides to reduce the environmental impact of traditional chemicals.
It explores the biological, physiological, and ecological roles of insects using modern technologies to address global challenges.
Keywords: Entomology, IPM, Insect Health and Pathology.

Technological advancements

- **Artificial Intelligence (AI) and machine learning:**

Automating the identification of insects, analyzing behaviors, and processing large datasets from sensor networks. AI is transforming entomological research by automating tasks like species identification, behavior analysis, and pest detection through computer vision, machine learning, and data analysis. This allows for more efficient, large-scale, and real-time monitoring of insects for applications in agriculture, public health, and conservation. Other applications include creating accurate habitat models, analyzing genomic data, and integrating with citizen science initiatives and robotics.

- **Drones (UAVs):**

Used for rapid, large-scale monitoring of pest infestations and for precisely applying pesticides or releasing natural enemies. Drones are a transformative tool in entomological research, used for everything from pest monitoring and spraying to bio-sampling and behavioral studies. They offer advantages in precision agriculture for tasks like identifying pests with advanced sensors, applying pesticides and beneficial insects, and collecting data in remote or hard-to-reach areas. Recent research focuses on integrating drones with AI and other technologies to create more sophisticated systems for forecasting and pest management.

Monitoring and Surveillance:

- **Pest detection:** Drones equipped with high-resolution cameras (including multispectral and hyperspectral) can identify pest infestations or diseases with high accuracy.

- **Population estimation:** They can be used for large-scale surveys, such as monitoring the swarming front of insects like rice planthoppers to predict outbreaks.
- **Behavioral studies:** Researchers use drones to observe insect behavior, such as the response of species to drone overflights, which can inform monitoring strategies and minimize disturbance
- **Remote sensing and IoT:** Utilizing sensor networks and "smart traps" to continuously gather and transmit data on insect populations and their habitats. Sustainable pest management
- **Plant-based pesticides:** Plant-based pesticides, also called botanical pesticides, are natural pest control agents derived from plants that are used as an alternative to synthetic pesticides. They contain bioactive compounds like neem oil, pyrethrins, and rotenone that target pests by disrupting their growth or acting as repellents. These pesticides are biodegradable, often more specific in their action, and safer for the environment and non-target organisms compared to their synthetic counterparts. Research is focused on finding effective alternatives to synthetic pesticides, such as developing insecticidal and repellent formulas from essential oils from plants like Aegle marmelos and Mentha arvensis.
- **Integrated Pest Management (IPM):** Integrated Pest Management (IPM) is a sustainable approach to pest control that uses a combination of biological, cultural, and chemical methods to minimize the use of pesticides. It prioritizes prevention through practices like crop rotation and monitoring to keep pest populations below economically damaging levels. Chemical pesticides are used only as a last resort when other methods fail. Combining various strategies like biological controls (releasing natural enemies) and targeted chemical applications to minimize environmental impact.

Broader Research Areas

- **Social insect behavior:**

  Investigating the complex communication, division of labor, and collective decision-making in social insects like ants and bees, with potential applications in areas like swarm intelligence for robotics and AI.
- **Insect health and pathology:**

  Focusing on insect diseases, their causes (entomopathogens), and their associated parasites, with a goal of advancing knowledge and supporting the "Life on Land" Sustainable Development Goal.

  This field has two main applied purposes: developing pathogens as a form of biological control to manage pest insects and protecting beneficial insects like bees from diseases.

- **Evolution and systematics:**
  Studying insect evolution and classification to understand biodiversity and the spread of invasive species.
- **Insect neurobiology and physiology:**
  Insect health and pathology is the study of insect diseases, including the pathogens that cause them and the insects' responses to these illnesses. This field investigates the basic science of how pathogens infect and harm insects and applies this knowledge to practical purposes like controlling pest insects through biological control and protecting beneficial insects like honeybees. Key areas include identifying pathogens (like viruses, bacteria, fungi, and nematodes), understanding their virulence, and using this information to develop sustainable pest management strategies.

Exploring the nervous systems and overall functions of insects to better understand their roles in ecosystems and their interactions with their environment.

Conclusion:

Recent entomology research highlights a shift towards advanced, interdisciplinary, and sustainable approaches to address global challenges like food security and disease control. The field increasingly integrates genetic engineering, bio-inspired robotics, and molecular genetics to gain insights into insect biology and develop solutions for pest management and public health.

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ROLE OF STEROLS IN INSECT NUTRITION, PHYSIOLOGY AND ECOLOGY

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Introduction:

Sterols are crucial for insect growth and development. Sterols are used in the moulting process, reproduction, and other physiological processes. Sterol can't be synthesized on its own by insects, it should be obtained through their diet. Cholesterol is the main sterol used by insects. Sterol said to be a lipid possess multiple rings of carbon atoms. Since the structure of sterol is complex it posses at least one double bond (C5), with additional double bonds occurring at other places such as C7, C22, and/or C24. Cholesterol, phytosterols, and ergosterol are the primary sterols in animals, plants, and fungus. It consists of three six-member cyclohexane ring and one five-member cyclopentane ring. Sterol are widely distributed in animals and plant. Most common sterol are zoosterol, phytosterol, mycosterol, where Zoosterol *is found* in animals *as* a cholesterol**.** Phytosterol is found plants as a stigmasterol. Mycosterol is found in fungi as a ergosterol (Jing and Behmer, 2020).

Uses of Sterol:

The main uses of sterol are as a structure for cell membranes and as precursors for various hormones and signalling molecules in insect development and metabolism. Insect behaviour and ecology are influenced by sterols. Sterols are used for the development of insects and as a precursor to moulting hormone (Janson *et al.,* 2009).

1.Types of Sterol

1.1 Plant Sterols

Orthoptera, Phasmida, Hemiptera, Coleoptera and Lepidoptera are known to feed on a wide range of plant tissue and parts. The sterol structure varies depending on position and side chain unsaturation. The primary source of sterol is cholesterol in insects. Plants produce small quantities of cholesterol (Jing *et al.,* 2014*)*. Plants, on the other hand, contain phytosterols, like sitosterol and stigmasterol. In herbivorous insects, the most plant-feeding insects convert sitosterol into cholesterol. However, not all insects can convert sitosterol to cholesterol. Grasshoppers, for example, cannot convert stigmasterol into cholesterol. When grasshoppers are fed a diet with stigmasterol as the sole dietary sterol, they fail to complete development. Some insects can also use phytosterols in place of cholesterol it varies among species. (Behmer and Elias, 2000). Cholesterol was lacking in two dipteran gall-formers but found in extremely low amounts in a beetle. Cholesterol concentration was significantly varied in three hemipteran

phloem feeders; even species from the same genus showed significant variability in their cholesterol contents (Janson *et al.,* 2009).

1.1.2 Campesterol:

Campesterol is a precursor for many polyhydroxysteroid, which occurs in minute quantities as a growth hormone. It was detected in brassica, and it was used as a cell elongation, division, and differentiation. Brassinosteroids (BRs) a group of phytohormones that are essential for plant growth and development (Chmur and Bajguz, 2021).

1.1.3 Sitosterol and Stigmasterol:

Major role in maintaining the structure and function of the cell membrane and regulating the activity of the Na+/K+-ATPase plant cell. Stigmasterol is required for cell differentiation and proliferation. Sitosterol and stigmasterol, two common phytosterols, each contains an ethyl group on the side-chain (at the C24-position). Stigmasterol also contains a double bond at the C22-position (Behmer *et al.,* 2011).

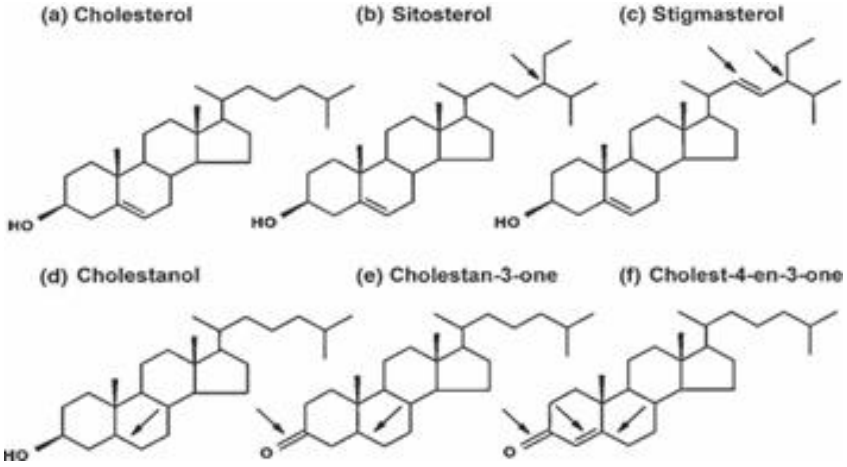

Figure 1: Types of sterol

2. Lichen Sterol:

Lichens are a symbiotic relationship between algae and fungi, rare among insects seen in Plecoptera and Psocoptera. The majority of aquatic insects consume algae, while collembola consume a mixture of algae and lichen. Several hundred species of algae contain sterol content. Fresh water algae eaten by insects contain two dominant sterols, ethylcholesterol and isofucosterol (Behmer and Nes, 2003).

3. Fungal Sterol:

The fungus-eating insect orders include Coleoptera, Diptera, Thysanoptera, and Hymenoptera. For example, leaf cutting ants are the main fungus feeders**.** There are three major groups of fungi such as Zygomycota, Ascomycota and Basidiomycota. For example, the ambrosia beetle is a well-known example of fungi eating insects. The role of fungi is important because it provides enzymes for digestion, essential amino acids, vitamins, and sterols. It helps to detoxify toxic plant metabolites in the host (Weber, 1973).

3.1.1 Mycetocyte Symbiots:

Mycetocytes are found in cockroaches, aphids, and weevils, as well as in specialized somatic cells of insects. Mycetome is a discrete organ found in insects, which is present in the midgut. Symbiont and host share a mutual relationship. The symbiont provides vitamins, sterols, and amino acids to the host, where the insect provides shelter and protection. Hemipterans are not the sole insects that possess microorganisms with a nutritional role in mycetocytes. The occurrence of mycetocyte symbioses has occurred through multiple instances of evolution. The mycetocyte symbiont can be transmitted from one generation to the next. The biomass of a symbiont varies according to age, sex, and weight. Beetles get ergosterol and related steroids from their fungi partner located in gut caeca. They then convert fungi sterol to dominant sterol (Douglas, 1989).

3.1.2 Yeast Like Fungi Symboints

Yeast-like endosymbionts (YLSs) of insects are frequently limited to certain hosts and are critical to the host's survival. For example, in planthoppers (Homoptera: Delphacidae). Some fungi associated with insects are said to be yeast-like fungi symbionts. They are dimorphic, seen between the yeast phase and the hyphal phase in environmental conditions. The role of yeast is important because it provides enzymes for digestion, essential amino acids, vitamins, and sterols. It helps to detoxify toxic plant metabolites in the host. Yeast plays a nutritional role and provides sterols. They are present intracellularly and extracellularly in insects. Ascomycete and Saccharomycetes are considered true yeasts, as they lack a sexual organ, instead containing sexual spores. *Sogatella furcifera*, *Nilaparvata lugens*, *Laodelphax striatellus*, and rice planthoppers all have intracellular yeast-like symbiotes (YLS) in their fat bodies. Transovarially inherited, the YLS are obligatory endosymbiotes (Noda *et al.,* 1995).

4. Bacteriocyte Symbionts:

These bacterial or yeast intracellular symbionts are frequently limited to specific host cells known as bacteriocytes. Bacteriophage symbionts are well integrated into the biology and genetics of their host cells and have smaller genomes. Bacteriocytes are similar to mycetocytes, which are specialized adipocytes seen in aphids and weevils. Bacteriocyte-associated symbionts can improve insect fitness by supplying needed nutrients (Douglas 2015). A sterol is not synthesised directly by bacteriocytes, it helps the insects indirectly. The aphid contain endosymbiotic bacteria, which can be transmitted to offspring. Functions of bacteriocytes, it helps in digestion of woods and give food to insects. Bacterial symbionts cannot produce sterols because they lack the ability to synthesize them. Symbiotic bacteria do not contribute directly to the sterol requirements of insects (Koga *et al.,* 2011). Additionally, the bacteriocytes are found in the *Sitophilus* cereal weevil's intestines (Maire *et al.,* 2020).

5. Animal Sterol:

Cholesterol is a water-soluble and fat-soluble substance. It is called an amphipathic. Cholesterol is composed of three regions, a hydrocarbon tail. a ring structured with four hydrocarbon rings, hydroxyl group. The main source of cholesterol for insects can be obtained from diet. Cholesterol vital role in the nutrition and ecology of these insects. Beetles have low cholesterol content, while hemipteran insects have higher cholesterol content. Even species from the same genus can have differences in sterol content. The minimum amount of cholesterol is required for the vital role in the house fly**.** Except for Zoraptera and Phasmida, all insects are zoophagous and saprophagous (Southwood, 1973). The cholesterol is abundant sterol that persists free and forms the plasma membrane and myelin of nervous tissue. In phytophagous insects, cholesterol is the dominant sterol, even though phytosterol is also present.

5. Sterol Absorption:

Sterol absorption takes place in the midgut of the insect, particularly the phytophagous species**.** A region of the midgut that is designated for the purpose of sterol absorption in phytophagous insect. The primary site of cholesterol in carpenter ant and diving beetle is the foregut (Joshi and Agarwal, 1977).

6. Sterol In Reproduction Physiology:

Cholesterol is important for the development of an embryo, and lowering cholesterol levels affects the development of the embryo. Ecdysone is important for growth and development. Cholesterol is needed as a precursor to ecdysone (Jing and Behmer, 2020). No egg development occurs if the ecdysone level falls below 70-80%. Below 50% normal embryonic development but swelling of the egg. Below 40-50% normal egg hatched, but larvae were malformed. Below 40% of embryogenesis and normal larvae. A lack of cholestrol affects the egg of an adult female (Behmer and Nes, 2003).

7. Sterol In Insect Ecology:

The requirement for sterol has a significant impact on the food uptake behavior of predaceous insects. The sterol may reflect adaptation to local abiotic conditions. The host range of insect herbivores describes an insect sterol utilization ability. The phytosterol acts as a defence mechanism in the host plant, thereby preventing the feeding of insects. Different habitats can cause different sterol compounds to be formed (Svoboda, 2020).

8. Role of Sterol in Insects

8.1 Aphids

The aphids transform sitosterol into cholesterol. The aphid has a low survival rate and reproduces more. Ketonesteroid may interfere with cholesterol uptake from the insect's gut. The pea aphid is composed of three sterols, namely cholesterol, stigmasterol, and sitosterol (Campbell and Nes, 1983).

8.2 Silkworm

Silkworms are phytophagous insects that feed on mulberry leaves. Silkworms have a diet that contains phytosterol but lacks cholesterol. After reaching the intestinal tract, phytosterol is converted into demosterol through several enzymatic steps. Cholesterol is made by turning desmosterol into cholesterol. From the midgut cell to the haemolymph, cholesterol is transferred to the main insect lipoprotein, lipophorin. Major dietary sterol, phytosterol are sitosterol, campesterol, stigmasterol. Brain and prothoracic glands are cholesterol rich **so** disruption of sterol homeostasis causes malfunctioning (Ito *et al.,* 1984).

8.3 Honey Bee

Pollen supplies cholesterol to honey bees. Oogenesis and fecundity are reduced by lack of dietary sterol and insufficient cholesterol ingestion during the larval stage. Pollen and nectar constitute the major food sources for most bee species. pollen contain proteins, amino acids, vitamins, and lipids. Among lipids, sterols are key nutrients extracted from pollen (Furse *et al.,* 2023).

8.4 Mosquito

Mosquito cannot develop beyond 2rdinstar without sterol. Diets must contain cholesterol, so only the good adult stage occurs. The supply of arachidonic acid in lipids is essential for the survival of newly emerged adults in the context of flight. Aside from arachidonic acid, the lipid supplement contains antioxidants (Perera and Wijerathna, 2019).

8.5 Ambrosia Beetle

The ambrosia beetle has a mutually beneficial relationship with fungi that provide nutrients and sterols to the beetle. The ambrosia beetle is surrounded by fungi. Ambrosia beetles are one of three insect lineages that practice agriculture, along with termites and fungus-farming ants. They produce their own food fungi within tunnel systems in wood (Juarez *et al.,* 2020).

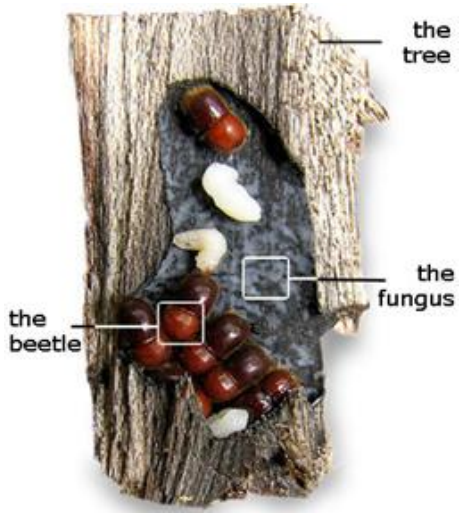

Figure 2: Ambrosia beetle symbiosis with fungus

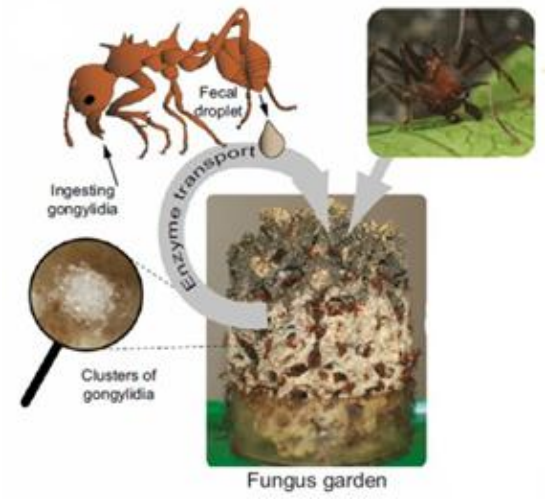

Figure 3: Leaf cutter ant symbiosis with fungus

8.6 Leaf Cutting Ant

The occurrence of mycetocyte symbioses has occurred through multiple instances of evolution. Possess these distinctive fungal sterols. *Acromyrmex octospinosus*, a leaf-cutting ant, coexists with a fungus that it grows on leaf fragments. Ants prefer to eat the hyphal apical swellings known as "gongylidia," which are formed by the symbiote, over the mycelium. (Maurer *et al.,* 1992).

8.7 Corn Earworm

The corn earworm is incapable of synthesizing sterol and its uptake by diet. Sterol absorption was connected to sterol utilization. Insects absorbed 27-66 μg of the most efficient sterols, while the least effective sterols ranged from 0.6 to 6 μg per insect (Nes *et al.,* 1997)

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EXPLORING ARTIFICIAL INTELLIGENCE IN SCIENTIFIC BEEKEEPING

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Abstract:
The utilization of Artificial Intelligence (AI) in apiculture presents transformative opportunities for improving colony health, production and sustainability. The integration of AI with Machine Learning (ML), the Internet of Things (IoT) and remote sensing facilitate continuous, non-invasive surveillance of hive characteristics including temperature, humidity, vibration and sound. Advanced algorithms, such as Convolutional Neural Networks (CNNs) and Support Vector Machines (SVMs), enable the early identification of honey bee swarming, queen loss and pest infestations, allowing prompt management actions. Building on these capabilities, AI-driven prediction algorithms further enhance apicultural practices by facilitating honey yield forecasting, product authentication and the diagnosis of diseases such as *Varroa destructor* infection. Remote sensing and IoT also enhance the mapping of floral resources and the evaluation of environmental factors that affect foraging and colony vitality. Even though there are problems with data quality, cost, and ethics, AI-enabled precision beekeeping is a big step toward managing honey bee populations in a way that is good for the environment and food security in the long term.

Keywords: Honey Bee, Artificial Intelligence, Internet of Things, Remote Sensing

1. Introduction:

Bees are vital pollinators essential for ecological balance and global food security. Approximately 75% of leading food crops rely on animal pollination, with bees contributing to nearly 35% of global agricultural production (Klein *et al.,* 2007; IPBES, 2016). The annual economic value of bee pollination is estimated at USD 235–577 billion, underscoring their indispensable role in food supply chains (FAO, 2018). Among thousands of bee species, managed bees like *Apis mellifera* and *Apis cerana* are crucial for pollinating fruits, vegetables, oilseeds, and nuts (Potts *et al.,* 2016). Beyond pollination, beekeeping economies produce honey, wax, royal jelly, pollen, and propolis, supporting livelihoods worldwide (vanEngelsdorp and Meixner, 2010).

Despite their importance, bee populations are declining due to multiple threats, including Colony Collapse Disorder (CCD), pesticide exposure, habitat loss, climate change, and pathogens like the Varroa destructor mite (Goulson *et al.,* 2015). Agrochemicals, particularly neonicotinoids,

impair bee navigation and foraging, while climate variability disrupts floral cycles, leading to nutritional stress. These challenges highlight the urgent need for innovative, technology-driven solutions to safeguard bee populations and optimize apiculture management.

2. Introduction to AI and machine learning (ML)

Artificial Intelligence

Artificial Intelligence (AI) refers to systems that mimic human intelligence to perform tasks and improve through experience. Advances in neural networks, machine learning, and computer vision have significantly expanded AI's capabilities (Abreu *et al.,* 2021).

AI systems are often categorized by their functionality, ranging from reactive machines with no memory to theoretical future systems capable of self-awareness. Key enabling technologies include artificial neural networks, modelled on the human brain for self-learning, and computer vision, which allows machines to interpret visual information.

Machine Learning

Machine Learning (ML), a subfield of AI, enables computers to learn from data without explicit programming. ML algorithms identify patterns and relationships within datasets, allowing them to make predictions or decisions based on new information. Their performance improves with more data (Jordan and Mitchell, 2015).

Machine learning is broadly categorized into three types:

i. Supervised learning uses labelled data for classification (e.g., identifying diseased brood) or regression tasks (e.g., predicting honey yield).
ii. Unsupervised learning works with unlabelled data to uncover hidden patterns, such as grouping bees by foraging behaviour.
iii. Reinforcement learning uses a trial-and-error system with rewards and penalties to optimize decisions, like automated hive management.

In apiculture, ML transforms hive monitoring by integrating data from sensors, images, and acoustic recordings. For example, computer vision can count bees or monitor pollen loads, while acoustic analysis can detect signs of queen loss or swarming. ML's adaptability allows it to be tailored to different species, climates, and management styles, making it a powerful tool for precision beekeeping, especially when combined with IoT for real-time insights (Kulyukin *et al.,* 2020).

3. Role of IoT and Remote Sensing in Beekeeping

Internet of Things

The Internet of Things **(**IoT) is a network of physical objects or devices embedded with sensors, software, and communication technologies that enable them to collect, exchange, and process data over the internet without direct human intervention. The term *Internet of Things* was first coined by Kevin Ashton in 1999 while working at the Massachusetts Institute of Technology

(MIT) in the context of radio-frequency identification (RFID) and supply chain optimization (Ashton, 2009). The main idea behind IoT is to connect the physical world with digital systems, enabling smart monitoring, automation, and decision-making.

The core principle of IoT is the continuous collection, communication, and processing of data to enable intelligent actions. Its workflow includes: sensing environmental or physical parameters (temperature, humidity, movement), transmitting data via wired or wireless networks, analyzing it locally or on the cloud and triggering automated actions, alerts, or recommendations. IoT has applications across domains of smart homes, healthcare, industry, and urban infrastructure. Now IoT is being introduced in agriculture and apiculture as well.

The Internet of Things (IoT) in beekeeping employs connected sensors and smart devices to collect real-time data inside and outside the hive. Critical parameters such as hive temperature, humidity, weight, and acoustic signatures are measured and transmitted wirelessly to a cloud-based platform for centralized analysis. This allows for proactive maintenance and targeted intervention in situations like disease outbreaks, food shortages, or hazardous climate conditions.

Major start-ups like BeeHero and ApisProtect are pioneering this approach, offering platforms that combine advanced analytics and real-time alerts, leading to 20–30% improvements in hive productivity and a significant reduction in colony losses. IoT-enabled hive networks are cost-effective and support scalability, allowing large apiaries to monitor numerous hives simultaneously with minimal human intervention. The application of low-power edge computing (TinyML) on IoT devices for scalable, real-time monitoring in remote apiaries, addressing constraints like connectivity and power consumption. This approach integrates sensor data for hive condition tracking, pest and disease detection, and swarming prediction (Sucipto *et. al* 2025.)

Remote Sensing

Remote sensing is the science of obtaining information about objects or phenomena without making direct contact with them. It involves the detection and measurement of reflected or emitted electromagnetic radiation from the Earth's surface using sensors mounted on satellites, aircraft, or unmanned aerial vehicles (UAVs). The data collected across visible, infrared and microwave spectral bands enable large-scale monitoring of land use, vegetation health, temperature, and other environmental parameters (Campbell and Wynne, 2011).

The concept originated with aerial photography. By the 1900s–1930s, aerial photography using airplanes became increasingly common for mapping, military reconnaissance, and land surveys (Campbell and Wynne, 2011). In the 1940s–1960s, multispectral aerial surveys were introduced, allowing analysis of reflected radiation beyond the visible spectrum, which significantly enhanced vegetation and land-use studies (Jensen, 2007). The launch of the first Earth observation satellite, Landsat 1, in 1972, by NASA and the U.S. Geological Survey marked the

beginning of spaceborne remote sensing for systematic environmental monitoring. Since then, national and international space agencies have contributed to the rapid development and global application of remote sensing technologies in agriculture, forestry, environmental monitoring, and climate studies (Lillesand *et al.,* 2015).

Remote sensing provides insights into environmental conditions, floral resources and climate patterns around apiaries. Remote sensing combined with GIS and machine learning helps evaluate landscape suitability for bee foraging on large scales, analyzing vegetation cover, diversity, and crop types. Studies have shown that landscapes with forested and diverse vegetation improve honey bee colony survival and health, whereas monoculture crops may reduce survival rates (Vadnais, 2024). SmartBeeKeep is an advanced beekeeping platform that combines remote sensing, AI, and GIS technologies to support efficient and sustainable apiary management. It offers both a web and a mobile app facilitating comprehensive mapping of beekeeping flora using satellite data and user input, enabling beekeepers to identify and monitor flowering periods and forage availability around their apiaries. Additionally, SmartBeeKeep supports an e-marketplace for beekeepers to manage product listings, sales, and crowdfunding campaigns (Grammalidis, 2023).

Integration for Apiculture

The convergence of AI, ML, IoT, and remote sensing offers unprecedented opportunities for beekeeping. Remote sensing provides insights into floral resources and climate, while IoT enables continuous hive monitoring. When analyzed by AI and ML, these data streams can predict colony health, honey yield, and risks like disease. This integrated, data-driven approach enhances decision-making, reduces human error, and minimizes stress on bees, paving the way for a new era of sustainable, intelligent apiculture.

4. Application of Artificial Intelligence in Beekeeping

The AI workflow begins with data acquisition from sensors, images, or audio. This is followed by data preprocessing to clean and organize the information. Next, feature extraction identifies meaningful patterns, such as acoustic or visual traits. During model training and validation, algorithms like Convolutional Neural Networks (CNNs) learn to recognize relationships between inputs and outcomes. Finally, the model is deployed for real-time analysis, continuously refining its performance through feedback (Astuti *et al.,* 2024). The various applications and models on the market represent iterative improvements, each enhancing detection accuracy and precision by integrating data from more parameters. The key differences often lie in their learning algorithms and their access to extensive, real-world datasets.

a. Colony Monitoring and Management

Artificial Intelligence enables continuous, non-invasive colony monitoring by analyzing multi-modal data from hive-embedded sensors. Machine learning algorithms like Support Vector

Machines (SVMs) and Deep Neural Networks (DNNs) process data on temperature, humidity, vibration, sound, and visual activity to identify patterns and anomalies.

Table 1: AI tools employed in colony management and behaviour and activity recognition in beekeeping

AI Tool / Algorithm / Platform	Application in Beekeeping	References
Semi-supervised model integrating temperature, humidity, pressure	Disease detection with 78% accuracy	Zhang, 2021
Frequency-domain analysis (spectral features)	Distinguish queenright vs queenless colonies	Robles-Guerrero *et al.,* 2019
Machine learning algorithms on vibroacoustic features	Early detection of swarming, queen failure, environmental stress	Qandour *et al.,* 2014
Deep learning (LSTM, CNN)	Queen detection, swarming prediction, colony health assessment	Abdollahi *et al.,* 2025
Support Vector Machines (SVMs)	Bumblebee species classification, acoustic behavior detection	Abdollahi *et al.,* 2025
Apivox Smart Monitor (2015)	Swarm detection, brood assessment, pest identification	Cecchi *et al.,* 2020
Bee Health Guru (2019)	Swarm detection, brood assessment, pest identification	Cecchi *et al.,* 2020
Commercial platforms (Arnia, Nectar Technologies Inc.)	Hive diagnostics integrating acoustic, temperature, humidity sensors	Cecchi *et al.,* 2020
UrBAN dataset (3,000+ hours of hive audio + sensor data)	Predict colony strength, detect stress or disease, provide real-time, non-invasive insights	Abdollahi *et al.,* 2025
YOLOv8m (object detection)	Detect bees entering/exiting hives; entrance monitoring	Lei *et al.,* 2024
OC-SORT (tracking algorithm)	Multiple Object Tracking (MOT) for individual bee movements	Lei *et al.,* 2024
Box method for counting bees	Counting bee traffic at hive entrances	Lei *et al.,* 2024
Deep learning with pose estimation	Detecting key points of bees (head, abdomen, tail) to monitor bee activity and entrance counts	Padubidri *et al.,* 2024
Hungarian algorithm–based tracker	Tracking bee movements across frames for accurate counting	Padubidri *et al.,* 2024
Computer vision + CNN / object detection	Monitoring foraging activity, counting bees in flight and on flowers	Rodriguez, 2019

Temperature and humidity data help evaluate brood development and hive thermoregulation. Acoustic analysis can detect queenlessness, swarming, or general colony stress. Vibration and motion sensors identify unusual activity patterns. Video-based AI monitors bee traffic and movement at the hive entrance. This monitoring is enhanced by predictive modelling, which uses historical and real-time data to forecast colony strength, honey yield, or potential queen failure. This allows beekeepers to make proactive decisions, such as adjusting hive conditions or providing supplemental feeding, without disruptive manual inspections, thereby enhancing colony resilience (Kulyukin *et al.,* 2020). Table 1 shows a list of AI models used for colony monitoring and management and behaviour, activity recognition.

b. Behaviour and Activity Recognition

AI analyzes honey bee behaviour by processing data from cameras, microphones, and vibration sensors. Computer vision systems, using models like Convolutional Neural Networks (CNNs) and object detection algorithms like YOLO (You Only Look Once), track foraging activity, count bees at the entrance, and analyze flight patterns. This provides accurate metrics on activity levels, foraging intensity, and overall colony strength (Abdollahi *et al.,* 2025).

For specific behaviours, AI integrates multiple data streams to recognize subtle actions like hygienic behaviour (uncapping and removing diseased brood) or defensive responses. Machine learning models are trained on labelled datasets to automatically classify these behaviours, identifying patterns often missed by human observation. This non-invasive, real-time assessment supports selective breeding programs and informs critical management decisions (Kulyukin *et al.,* 2020).

c. AI in Disease and Pest Detection

While manual monitoring is feasible for small apiaries, it becomes exhaustive for commercial operations, where delays in control can devastate a colony. Artificial intelligence enables automated, remote hive monitoring, allowing for the immediate tracking of any deviation in colony parameters. Numerous AI algorithms have been developed to identify existing pest infestations or even forecast upcoming attacks (Table 2).

i. AI in Automated Detection and Monitoring

AI systems are now adept at classifying honey bee colony conditions with high precision. Berkaya *et al.* (2021) used Deep Neural Networks (DNNs) and Support Vector Machines (SVMs) to differentiate between healthy bees, pollen-bearing individuals, and those with abnormalities like ant or small hive beetle infestations, robber bees, and Varroa mites. Other modern approaches use video and image-based detection to identify mites. These systems segment video into image patches and use statistical learning and decision tree models, achieving over 70% classification accuracy while reducing monitoring time by more than 50% compared to manual methods (Schurischuster *et al.,* 2018; Batz *et al.,* 2022).

Table 2: Application of different AI technology in honey bee pest management

Technology / AI Method	Application / Function	Pest Targeted / Focus Area	Reference
Deep Learning (DNNs) and SVM	Image-based classification of bee conditions and detection of abnormalities	Varroa mites, small hive beetles, robber bees, ants	Berkaya *et al.,* 2021
Maximum Entropy (MaxEnt) Modeling	Predictive modeling of pest dispersal based on climatic and environmental variables	*Galleria mellonella*, *Aethina tumida*, *Varroa destructor*	Hosni *et al.,* 2022; Astuti *et al.,* 2024
Bayesian and Decision Tree Models	Image patch analysis for early detection of parasites from bee behaviour videos	Varroa mites	Schurischuster *et al.,* 2018; Batz *et al.,* 2022
IoT + CNN	Real-time video/image monitoring and pest detection with automated alerts	Varroa mites (within cells)	Mrozek *et al.,* 2021; Wachowicz *et al.,* 2022
Multi-sensor Hive Monitoring Systems	Continuous monitoring of hive environment (temperature, humidity, sound) to detect abnormal conditions indirectly	Varroa mites, hive stress factors	Astuti *et al.,* 2024
Acoustic Data Analysis + ML (KNN, NN, SVM)	Analyzing hive sound to detect abnormal conditions indicating stress or pest presence	Colony stress, Varroa-related weakening	Robles-Guerrero *et al.,* 2023
Mobile App with ML (Apizoom)	Image upload and automated Varroa count from bottom boards	Varroa mites	Rahman and Ravi, 2022
Commercial AI Platforms (BeeHero)	Continuous monitoring of hive activity and environment to detect anomalies related to pest infestations	Varroa mites, hive congestion	BeeHero, 2025
Big Data + ML Image Analysis	Larval disease detection through image classification	Mite infestations, brood health	USDA, 2021

This technology is becoming increasingly accessible. The Apizoom App, for instance, allows users to upload images of hive bottom boards, where its machine learning algorithm automatically counts Varroa mites and provides management alerts (Rahman and Ravi, 2022). Commercial firms are integrating AI into comprehensive hive management. BeeHero's system uses environmental and activity sensors, with machine learning algorithms analyzing the data to

identify patterns indicating mite infestations or nutritional deficiencies (BeeHero, 2025). Similarly, USDA research has leveraged big data and machine learning to diagnose larval health from high-resolution images, enabling faster and more accurate disease identification than traditional methods (USDA, 2021).

ii. Pest Predictive Modelling

Beyond detection, AI can forecast pest distribution. Environmental and climatic factors heavily influence pest prevalence. Hosni *et al.* (2022) applied the Maximum Entropy (MaxEnt) model with 19 bioclimatic variables to predict the distribution of *Galleria mellonella*. This approach was extended to forecast the spread of *Aethina tumida*, *Galleria mellonella*, *Oplostomus haroldi*, and *Varroa destructor* in Kenya and Tanzania (Astuti *et al.,* 2024). The models revealed expanding suitable habitats for these pests, demonstrating AI's power to inform proactive, large-scale pest management by identifying future surveillance hotspots.

iii. Real-Time Monitoring Using IoT

Integrating the Internet of Things (IoT) with AI has advanced precision beekeeping. Wachowicz *et al.* (2022) developed a real-time system combining camera-based IoT devices with Convolutional Neural Networks (CNNs), achieving 90% accuracy in detecting Varroa mites and transmitting alerts via a cloud platform. Similarly, Mrozek *et al.* (2021) used IoT devices at hive entrances, analyzing image frames with CNN models to achieve 90% sensitivity and 70% precision in Varroa detection. Multi-sensor platforms like IndusBee 4.0 and the BeePi system further provide a holistic view by combining environmental, image, and acoustic data (Astuti *et al*, 2024).

iv. Acoustic Data Analysis for Hive Health Assessment

Acoustic analysis is a powerful, non-invasive tool for early pest and stress detection, such as queenlessness or reduced foraging. Robles-Guerrero *et al.* (2023) compared five machine learning algorithms to classify colony health from sound recordings. Neural Networks (NN) and Support Vector Machines (SVM) offered the best balance of accuracy and computational efficiency for real-time use. Systems like Beeasy also leverage sound and hive data to detect early signs of disease or pest infestation (Astuti *et al.,* 2024).

d. AI in Honey Production and Quality Analysis

The apiculture sector faces challenges from climate change, disease, and demands for product authenticity. Artificial Intelligence is introducing data-driven precision to enhance honey yield and verify quality, securing the industry's future.

AI reshapes hive management through predictive, precision-based approaches. Non-invasive sensors monitor internal temperature, weight, and acoustic patterns. Machine learning models interpret this data, providing early warnings for swarming or pests, allowing for timely interventions. Furthermore, AI systems leverage historical harvest data and real-time

environmental factors to forecast the optimal time for honey extraction. A study by Ramirez-Diaz *et al.* (2022) demonstrated that the eXtreme Gradient Boosting (XGBoost) algorithm achieved the lowest prediction error for total honey harvest, enabling beekeepers to maximize yield while minimizing disruptive inspections.

Automation extends within the hive. Software like DeepBee© uses convolutional neural networks (CNNs) to automatically analyze honeycomb images, classifying contents like eggs, larvae, pollen, nectar, and ripened honey for an instant assessment of brood health and food stores. This ensures honey is harvested only when ample is available. AI also integrates with other technologies; for instance, Random Forest algorithms can process UAV imagery to map floral cover across landscapes, allowing for large-scale prediction of nectar flow and potential honey yield (Astuti *et al.,* 2024).

Beyond production, AI is crucial for guaranteeing honey quality, authenticity, and traceability. Monofloral honeys command premium prices, making adulteration a significant problem. Machine learning can classify honey based on its physicochemical profile to authenticate its botanical origin. Mateo *et al.* (2021) classified seven Spanish unifloral honey types using parameters like electrical conductivity and pH. Their Penalized Discriminant Analysis (PDA) model achieved the highest accuracy, proving AI can reliably protect against fraudulent labelling.

Similarly, AI verifies geographical origin. A study in Colombia by Zuluaga-Dominguez *et al.* (2018) analyzed 236 samples for physicochemical and aroma profiles. Using a genetic algorithm to identify key variables, an artificial neural network successfully classified honey by geographical origin with an 81% success rate, providing a powerful tool for certifying provenance.

e. AI in managing Colony Collapse Disorder

Colony Collapse Disorder (CCD), the sudden disappearance of worker bees, is linked to a complex web of stressors including parasites, pesticides, and poor nutrition. AI offers sophisticated, data-driven solutions for early detection and management of these contributing factors. Acoustic analysis technology is available in mobile apps that allow a beekeeper to analyze their hive's sound and receive instant health notifications (Abreu, 2021).

AI is also vital for monitoring pesticide exposure. A collaborative study created a toxicovigilance tool using RFID-tagged bees and hive-mounted detectors. AI modeling can successfully classify whether bees had been exposed to pesticides based on their foraging behaviour. The most robust systems are multimodal. An Attention-based Multimodal Neural Network (AMNN) can processes both video and audio data and can pinpoint specific stressors like ant issues, a missing queen, or pesticide exposure (Liang, 2024). Organizations like BeeOdiversity use AI-powered platforms with satellite imagery to analyze land use across thousands of acres, estimating the

presence of pesticides and plants with about 80% accuracy to provide a macro-level view of environmental risks (Microsoft, 2023). For hive-specific management, AI-powered sensors can collect data on hive activity and environment, providing data-driven recommendations on hive management. Furthermore, smart monitoring systems with AI can now automatically track bee flight activity and identify pollen-bearing foragers, offering deep insights into colony health and the impacts of sublethal pesticide doses (Wang *et al.,* 2024).

5. Challenges and Ethical Consideration

Technical Limitations and Data Quality: Reliable operation of IoT sensors and remote sensing platforms in field conditions is often challenged by environmental factors, power constraints, and connectivity issues. Incomplete or biased data can lead to inaccurate analyses and decisions impacting bee health.

Scalability and Cost: While IoT solutions enable scalable hive monitoring, the initial costs of deploying sensor networks and maintaining hardware can be prohibitive, particularly for small-scale or subsistence beekeepers.

Privacy and Security Risks: IoT systems inherently collect extensive environmental and operational data, raising concerns about unauthorized access, data breaches, and misuse of sensitive information such as the precise location of apiaries which could be exploited.

Fairness and Bias: AI algorithms depend on underlying data. If training data inadvertently reflects geographic or species-specific biases, the resulting AI models could unfairly favor certain bee populations or regions, leading to inequitable resource allocation and support.

Transparency and Explainability: Many AI decisions in hive management come from complex models that can be opaque. Ethical practice demands that beekeepers and stakeholders understand how AI-driven recommendations or alerts are generated to foster trust and accountability.

Environmental Impact: Deployment of electronic devices for remote sensing and IoT contributes to electronic waste and energy use. Ethical stewardship involves choosing sustainable materials and minimizing ecological footprint during the design and deployment phases.

Respect for Local and Indigenous Knowledge: The use of AI and technology in beekeeping should complement rather than replace traditional beekeeper expertise. Inclusive approaches that respect local knowledge systems help ensure culturally appropriate technology adoption.

6. Future Direction

AI in beekeeping emphasizes integration of real-time sensor data, advanced analytics, and predictive modelling to enhance hive health and productivity. Upcoming AI systems will focus on continuous, non-invasive monitoring of hive conditions such as temperature, humidity, acoustics, and bee behaviour to detect diseases and stressors early. Combining multimodal data (audio, visual, environmental) with machine learning will improve accuracy in diagnosing hive

issues and forecasting colony collapse. Furthermore, AI will support sustainable practices by optimizing foraging resource management and automating routine inspections, ultimately advancing precision apiculture while balancing technology and ecological preservation.

Conclusion:

The integration of Artificial Intelligence into apiculture marks a paradigm shift towards precision beekeeping. By leveraging machine learning, IoT sensors, and remote sensing, AI enables continuous, non-invasive monitoring of hive health and bee behaviour. This facilitates the early detection of critical issues such as swarming, queen loss, and infestations by pests, allowing for proactive management. Furthermore, AI applications extend to forecasting honey yields, authenticating product quality, and mapping floral resources, thereby enhancing both productivity and sustainability. For AI to realize its full potential, future efforts must focus on developing affordable, transparent, and equitable systems that complement, rather than replace, the invaluable knowledge of beekeepers. Ultimately, AI-powered beekeeping presents a powerful, data-driven pathway to safeguarding pollinator populations, ensuring global food security, and securing the future of this vital industry.

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NANOTECHNOLOGY IN IPM

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Introduction:

Agriculture is vital to national economies and global food security. Rising population and environmental stress demand greater productivity (Godfray *et al.,* 2010; McClung, 2014). The Green Revolution introduced synthetic pesticides and fertilizers that reduced pest losses from over 80% to around 30% (Oerke and Dehne, 2004).

However, excessive pesticide use over two million tons annually has led to pest resistance and ecological harm (Dawkar *et al.,* 2013; Kohler and Triebskorn, 2013). This calls for sustainable and eco-friendly pest management solutions. Genetically engineered crops and natural compounds show potential, but regulatory and practical limitations restrict their use.

Advances in material science now offer new opportunities through nano and microstructures that enable precise delivery, enhanced stability, and reduced chemical loss (Glare *et al.,* 2012). Integrating nanotechnology with sustainable pest management can revolutionize modern agriculture by improving efficacy while minimizing environmental impact.

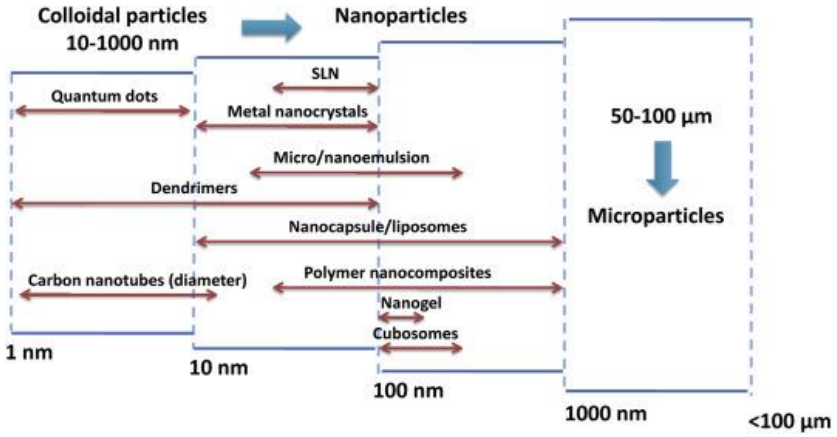

Figure 1: Representation of size range of different types of micro and nanoparticles (Source: Romeis *et al.*, 2006)

1. Interaction of NPs with Insect Physiological Status:

The use of nano-biotechnological approaches in pest management offers remarkable potential for sustainable agriculture. The most destructive crop pests belong mainly to Lepidoptera and Coleoptera, causing major losses in field and stored products. Herbivorous insects are classified as generalists, which feed on multiple plant species, or specialists, which feed on a single host. These feeding strategies influence insect physiology and biochemical adaptation. Some species

can alter their metabolism to detoxify plant defense compounds and optimize nutrient utilization (Dawkar *et al.,* 2013; Stam *et al.,* 2014).

Metallic nanoparticles (MNPs) have gained attention for pest control due to their tunable physicochemical properties and potential for precise, targeted action. However, large-scale field application remains limited. Successful implementation requires evaluating both physical factors such as light, temperature, and humidity—and biological factors including insect feeding habits, motility, and detoxification ability. Moreover, the movement and interaction of nanoparticles within insect systems are still poorly understood. Further studies on nanoparticle transport, release, and fate in relation to insect physiology are essential for optimizing their safe and effective use in pest management.

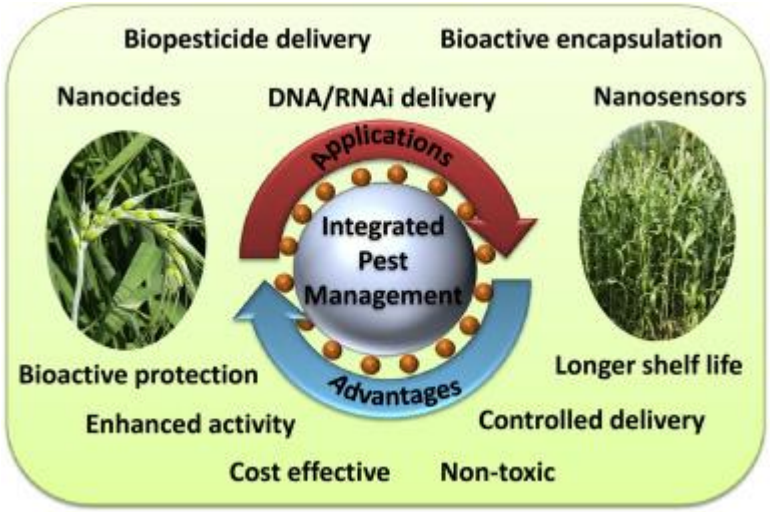

Figure 2: Applications and advantages of micro/nano systems in IPM

In invertebrate systems, the gut pH of herbivorous insects ranges from 6 to 11, highlighting the need for designing pH-responsive nanomaterials for effective bioactive delivery. Nano-encapsulated compounds must withstand varying pH gradients from the plant surface to the insect gut. For example, Khandelwal *et al.* (2015) demonstrated that defensive proteins immobilized on silica nanospheres can effectively target the alkaline digestive tract of insects. Advances in chemical and molecular engineering now allow tailoring of such nanomaterials to specific biological conditions.

Gut redox potential also varies among insects, influencing the oxidative or reducing environment. Plant allelochemicals can induce oxidative stress, while insects like Manduca sexta mitigate this by maintaining a reducing midgut environment through antioxidant secretion (Chapman *et al.,* 2013). Exposure to metallic nanoparticles such as silver (Ag) and gold (Au) which are themselves redox-active can affect both nanoparticle and protein stability. Silver nanoparticles, for instance, induce oxidative stress in insects, prompting elevated activity of antioxidant enzymes such as superoxide dismutase, peroxidases, and catalases (Yasur and Rani, 2015).

Nanoparticles also interact with biological macromolecules by adsorbing a dynamic layer of proteins known as a protein corona. High-affinity proteins may replace abundant ones, altering nanoparticle translocation, uptake, and fate in insect systems (Nel *et al.* 2009). Upon ingestion, nanoparticles encounter diverse biochemical environments within insect mouthparts and gut. Lepidopteran insects predominantly utilize serine proteases active in alkaline pH, while coleopterans rely on cysteine proteases functioning under mildly acidic conditions. Understanding these protein-nanoparticle interactions is crucial for designing stable and effective nanoformulations in pest control.

2. Rationales for Micro and Nano-Biotechnological Approach Inpest Management:

In recent decades, there has been a growing emphasis on biological methods for pest control. Research has focused on the characteristics of living organisms, plants, microbes, and their products to develop effective biopesticides. Their commercial success depends on factors such as effectiveness, safety, cost, shelf life, and non-target organism safety. To achieve these goals, it's important to design stable materials that preserve the effectiveness of bioactive agents. Combining these materials with biological ingredients can create formulations that perform consistently in the field. Additionally, biopesticides often provide a synergistic effect with multiple modes of action, reducing the risk of pest resistance compared to synthetic pesticides, which typically rely on a single mode of action (Hubbard *et al.,* 2014).

2.1. Microbe-Derived Bioactives:

Microbial biocontrol agents, such as Trichoderma, Neotyphodium, Beauveria, and Metarhizium, target specific pests and reduce crop losses. Some Neotyphodium endophytes and Bt-based products are commercially used for their insecticidal properties (Glare *et al.,* 2012; St Leger and Wang, 2010). Genetic engineering can enhance microbial stability and efficacy by boosting metabolite production or introducing insecticidal genes (St Leger *et al.,* 1996).

Field performance is improved using adjuvants that enhance uptake, spreading, and resistance to UV or rain (Hynes and Boyetchko, 2006). Examples include Tinopal LPW reducing LD50 for Spodoptera exigua (Murillo *et al.,* 2003), magnetic gellan gum-Fe3O4 composites (Wang *et al.* 2007), and double-shelled avermectin microcapsules for controlled release (He *et al.,* 2013a; He *et al.,* 2013b). Interest in Bt-nanopesticides is growing for targeted crop protection (Mahadeva Swamy and Asokan, 2013).

2.2. Plant Derived Products and Their Application:

Plant secondary metabolites, including alkaloids, terpenoids, and phenolics, act as repellents, toxins, or antinutritional agents against insects (Mishra *et al.,* 2015; Ibanez *et al.,* 2012). For example, Ocimum species exhibit strong insecticidal activity against Helicoverpa armigera due to compounds like camphor and limonene (Singh *et al.,* 2014), while caffeic acid inhibits lepidopteran growth (Joshi *et al.,* 2014). Wound-inducible plant proteinase inhibitors interfere

with insect digestion, reducing growth and reproductive success in H. armigera (Tamhane *et al.,* 2005; Tamhane *et al.,* 2007; Mishra *et al.,* 2010).

Despite promising results, few plant-based products are commercially available, including neem seed extract, azadirachtins, and pyrethrin (Isman *et al.,* 1990). Azadirachtin, for instance, disrupts embryonic development in *Spodoptera frugiperda,* and *H. armigera* is highly susceptible to these natural insecticides (Correia *et al.,* 2013).

Table 1: Selected examples of plant defensive compounds applied through conjugation on nanoparticles or prepared as formulations against insect pests

Class	**Plant insecticidal compounds**	**Mode of action**	**Effective against**	**References**
Monoterpenoids	α-pinene linalool	Antifeedant	*Spodoptera litura, Achaea janata* (Lepidopteran)	Rani *et al.,* 2014;
	Limonene	Neurotoxic	*Pseudococcus longispinus Aleurodicus disperses* (Hemipterans)	Hollingsworth, 2005
Tetranor triterpenoids	Azadirachtin	Molt disruptant Antifeedant	*Plutella xylostella* (Lepidoptera)	Forim *et al.,* 2013
Alkaloids	Nicotine	Neurotoxic mode of action, blocks ca++ ion channels	Aphids (Hemiptera)	Casanova *et al.,* 2002
Wound inducible Pin-II	Proteinase inhibitor	Digestive enzyme inhibition Antibiosis	*Helicoverpa armigera* (Lepidoptera)	Tamhane *et al.,* 2012, Khandel wal *et al.,* 2015
Essential oils	Garlic	Antifeedant	*Tribolium castaneum*	Yang *et al.,* 2009

3. Reinforcing Nano or Micro Based Pesticides: Techniques and Applications:

Small materials, particularly magnetic nanoparticles (MNPs) under 100 nm, play a critical role in agricultural applications. While carbon nanotubes and quantum dots are well-studied in medicine, their use in agrochemical delivery is limited. Nanoparticle size and surface chemistry

influence the loading and stability of bioactive ingredients, which is essential for effective pest control.

Nano- and micro-based formulations, such as emulsions, nanocapsules, and MNPs, enhance solubility, thermal stability, and biodegradability. Effective formulations improve activity, spreading, stability, and handling. Poorly soluble pesticides can be microencapsulated or delivered via nanoemulsions to increase bioavailability. For instance, two-step milling produces nano-suspensions that enhance dissolution and efficacy at lower concentrations, ensuring safer and more efficient pest management.

3.1. Micro/Nanoemulsion Formulations

Emulsions are formed by dispersing one liquid phase into another, such as oil-in-water or water-in-oil, and are stabilized with surfactants (Sathishkumar *et al.,*2008). Micro- and nanoemulsions are particularly effective in delivering high payloads of bioactives with long-term stability and small droplet sizes, enhancing solubilization and efficacy against pests (Wang *et al.,* 2007). For example, a microemulsion containing a plant proteinase inhibitor effectively controlled Helicoverpa armigera while improving protein retention on leaves without damaging plant surfaces (Tamhane *et al.,* 2012).

Although promising, agricultural use remains limited. Formulations with cyhalothrin or β-cypermethrin have improved wetting, spreading, and penetration (Zhao *et al.,* 2009; Wang *et al.,* 2007). Environmental safety requires careful evaluation of potential phytotoxicity, with long-term testing necessary to ensure sustainable application.

3.2. Nanoparticles as Advanced Delivery Systems:

Nanoscience, evolving into nanotechnology, offers advanced targeted delivery systems for bioactive molecules. Both organic and inorganic materials have been explored, with nanosystems capable of loading cargo of varying sizes via conjugation (Khandare et al.,2012). Common components include biopolymers such as sodium alginate and chitosan, and inorganic materials like zeolites, iron oxide, and nanoporous silica, enabling versatile applications. Multifunctional components allow controlled release of payloads (Ghormade *et al.,* 2011), while adsorption on surfaces is a widely used approach (Asfaram *et al.,* 2015; Khandelwal *et al.,* 2015). Chemical conjugation can affect delivery efficiency, so careful selection of materials is essential to optimize interactions between bioactives and nanostructures for effective delivery.

3.3. Delivery via Encapsulation:

Protecting bioactive compounds from environmental factors such as UV, evaporation, and temperature fluctuations is critical for efficacy. Ideal encapsulating materials safeguard sensitive molecules while enabling controlled, sustained release. Azadirachtin, a potent insecticide, is particularly vulnerable, but sodium alginate nanocarriers combined with coatings like natural rubber, starch, or polyethylene glycol (PEG) achieve over 80% encapsulation efficiency (Riyajan

and Sakdapipanich, 2009; Jerobin *et al.,* 2012). Encapsulation systems typically include a nanocarrier or coating agent with a reticulating agent to control release rates.

Plant-derived products, including essential oils (EOs), are gaining prominence in agriculture and organic food production. PEG-coated garlic EO, for example, maintained over 80% efficacy against Tribolium castaneum for five months, compared to 11% for free oil (Yang *et al.,* 2009). PEG nanoformulations also enable gradual release of plant secondary metabolites, enhancing contact toxicity against storage pests (Gonzalez *et al.,* 2014). Solid-lipid nanoparticles and alginate beads have stabilized EOs like Artemisia arborescens, reducing degradation and extending shelf life (Lai *et al.,* 2006). These results highlight the potential of nano- and micro-systems for controlled, efficient, and sustainable pesticide delivery using both organic and inorganic materials.

3.5. Interaction via Surface Functionalization:

Covalent attachment of structural moieties to surfaces is crucial for effectively adsorbing or coupling active ingredients. The surface chemistry of nanomaterials enables specific modifications that significantly impact their interactions with biomolecules. In insect pest management, suitable surface chemistry enhances efficacy, stability, and reduces ecotoxicity through targeted conjugation of chemical moieties. For instance, issues with hydrophobic pesticide distribution can be resolved by encapsulating active ingredients in carriers with hydrophilic surfaces, improving their distribution. Dendrimers have been effectively used for this purpose after being modified with amine groups to enhance hydration (Liu *et al.,* 2015). Silica nanoparticles can also be functionalized with organic groups due to the presence of silanol groups, enhancing their effectiveness against pests (Debnath *et al.,* 2012). Moreover, modifying surface chemistry can optimize the release profiles of bioactive compounds, preventing rapid outbursts and ensuring sustained release through selective coatings like polyethylene glycol (PEG).

3.6. Inorganic Micro and Nano-Pesticide Delivery Systems:

Concerns over excessive pesticide use have prompted the exploration of micro- and nanotechnology (MNT) for targeted delivery, reducing environmental exposure and dosages. Inorganic nanoparticles, particularly silica, are favored for their stability, chemical versatility, tunable pore sizes, and high surface area, making them effective carriers for bioactive molecules. Functionalized silica nanospheres and rods immobilized with proteinase inhibitor peptides enable pH-triggered release, achieving a 62% peptide loading capacity and demonstrating higher efficacy than bulk silica, partly through insect dehydration mechanisms.

Silica nanoparticles also protect sensitive pesticides like avermectin from UV degradation while providing controlled release. Other inorganic materials, such as clay nanoparticles, are being explored for their interactive surfaces and delivery potential. These innovations offer significant opportunities to enhance the efficiency, stability, and sustainability of pest management systems.

3.7. Advance Polymeric Delivery Systems in Pest Management:

Polymer-based nanocarriers are increasingly used for pesticidal delivery, though clinical regulations and toxicity concerns can slow progress. Biodegradable polymers, such as chitosan and alginates, enable sustained release of bioactive compounds with high biocompatibility and low cytotoxicity (Yasur and Rani, 2015). Sodium alginate, an acid-resistant polysaccharide, facilitates release through sodium-calcium exchange, achieving over 98% entrapment efficiency with imidacloprid (Bhagat *et al.,* 2013), while calcium alginate dissolves under alkaline conditions (Kumar *et al.,* 2014). Starch-alginate matrices and silver nanoparticles have successfully encapsulated chlorpyrifos and dichlorvos, attaining over 95% entrapment and controlled release (Baohua, 2013; Roy *et al.,* 2009; Ihegwuagu *et al.,* 2016).

Hydrophobic pesticides, like bifenthrin, benefit from stable formulations via flash nanoprecipitation (Liu *et al.,* 2008). Polymeric gels and pheromone nanogels have also been applied for eco-friendly control of Bactrocera dorsalis (Bhagat *et al.,* 2013). Dendrimers, traditionally used in drug delivery, show potential for nanoparticle-mediated gene delivery in pest management (Khandare and Minko, 2006; Liu *et al.,* 2015).

4. Other applications:

4.1. Detection of Pesticide Residue:

Sustainable agriculture requires judicious pesticide use, as excessive applications can contaminate soil and groundwater, making residue monitoring in harvested foods essential (Barry *et al.,* 2009). Traditional immunological assays are limited, often necessitating multiple tests. Advances in micro- and nano-technologies have enabled microanalytical biosensors for rapid, sensitive, and simultaneous detection of pesticide residues. Magnetic nanoparticles (MNPs) combined with biomolecules allow colorimetric and fluorometric biorecognition of contaminants.

Biosensors for organophosphate (OP) and carbamate pesticides often rely on cholinesterase (ChE) inhibition, with Au-Pt nanoparticles or quantum dots enhancing sensitivity for compounds like trichlorfon and carbaryl (Sassolas *et al.,* 2012). Zirconia nanoparticles on gold electrodes facilitate portable electrochemical OP detection (Liu and Lin, 2005), while enzyme-based nano-biosensors demonstrate high sensitivity and low sample requirements for paraoxon detection (Khaksarinejad *et al.,* 2015).

4.2. Pesticide Degradation:

To reduce the potential exposure of pesticides in the environment, their removal via nano-based degradation methods were explored. As many conventional approaches are time-consuming and incur a high cost, contaminant removal by nanomaterials promises rapid action with low cost. Photocatalytic degradation of hexachlorobenzene, cypermethrin and dicofol pesticides using titanium oxide NPs was demonstrated. At the interface of pesticide and TiO2 NP, a series of electron transfer reactions takes place along with the production of peroxide. Presence

of hydroxyl radicals further accelerates photodegradation. Dispersion of TiO2 over a porous nanosilica was found to be effective in degradation of commercial pesticidal preparation of imidacloprid and phosphamidon. Degradation of various chemical classes was also evaluated though are not discussed in this review considering its limited scope.

4.3 Beneficial Enhancements of Nano Pesticides to Control Insect Pests:

Nano-agriculture involves using nano-sized particles to enhance crop production (Batsmanova *et al.,* 2013; Scott and Chen, 2013). Nano-encapsulation offers benefits like reduced human exposure, controlled release, and improved efficacy of pesticides (Hack *et al.,* 2012). This technology modernizes pesticide application by decreasing the necessary doses for effective pest control, using a process where the active ingredient is coated and reduced to nanoscale. Nanotechnology significantly improves all aspects of agricultural production, particularly through the development of slow-release pesticides, which are more efficient than traditional delivery methods (Harish *et al.,* 2022). Regulated delivery systems aim to release precise quantities of agrochemicals over time, enhancing biological effectiveness while minimizing losses and adverse effects (Shojaei *et al.,* 2019). Compared to traditional formulations, nanoparticles ensure continuous release of active ingredients, increasing the solubility and reducing pesticide usage (John *et al.,* 2017; Anjali *et al.,* 2012; Yu *et al.,* 2017). This approach also protects ecosystem biodiversity and promotes plant growth, with carbon nanotubes increasingly used as growth regulators (Zheng *et al.,* 2005; Khodakovskaya *et al.,* 2013).

5. Future of Nanopesticides and Pest Control:

The excessive use of conventional pesticides and fertilizers has caused environmental degradation and health hazards due to inefficient application and chemical losses. Nanotechnology offers a sustainable alternative by enabling precise, controlled delivery of active ingredients to target pests and crops. Nanopesticides and nanofertilizers improve solubility, stability, and nutrient or toxin release, thereby minimizing leaching, runoff, and non-target effects.

Nanoparticles such as silver, copper, gold, and zinc oxides have shown strong insecticidal and antifungal properties, providing efficient pest control with reduced chemical inputs. For example, silver nanoparticles can suppress nematode populations by up to 99%, while copper nanoparticles exhibit excellent antifungal efficacy.

As global food demand rises, the integration of nanotechnology into IPM offers a promising path toward sustainable agriculture—combining precision, efficiency, and environmental safety to redefine future pest management practices.

Conclusion:

Nano-pesticides play a transformative role in Integrated Pest Management (IPM) by enhancing pest control efficiency and reducing environmental and health risks. Their targeted delivery and controlled release improve pesticide stability, prolong effectiveness, and reduce application

frequency. By enhancing solubility, bioavailability, and compatibility with biopesticides, nano-formulations support sustainable, eco-friendly pest management. Overall, nanotechnology offers a promising path toward more precise, effective, and environmentally responsible IPM strategies.

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NANOBIOTECHNOLOGY IN SERICULTURE: APPLIED ENTOMOLOGICAL INSIGHTS FROM SILKWORM BIOLOGY TO SILK MATERIAL INNOVATION

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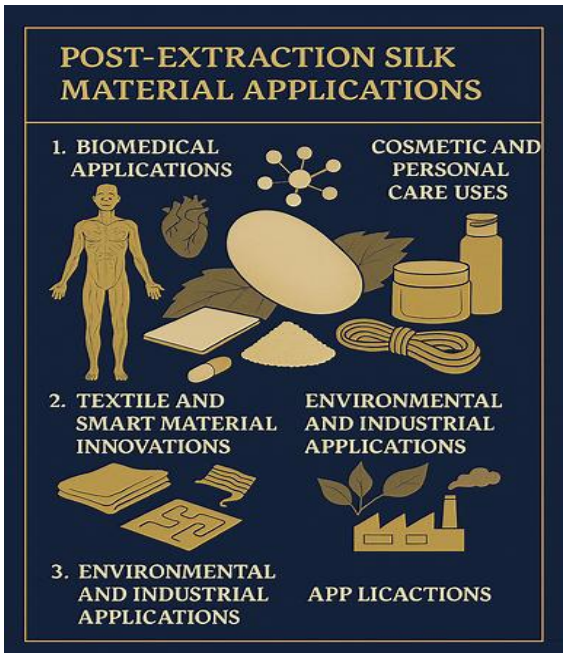
Abstract:
Sericulture, a specialized branch of applied entomology, integrates silkworm biology with advanced material science to produce high-value silk and silk-derived biomaterials. Recent nanobiotechnological interventions have expanded the scope of sericulture, enabling targeted modulation of Bombyx mori physiology, immunity, and hormonal regulation, while simultaneously enhancing silk yield and quality. Engineered nanoparticles—such as silver, zinc oxide, titanium dioxide, and iron oxide—when administered via mulberry leaf supplementation or foliar applications, influence larval growth, digestive enzyme activity, nutrient assimilation, oxidative stress responses, and endocrine pathways. These interventions can improve cocoon architecture, fibroin and sericin gene expression, and β-sheet crystallinity, resulting in stronger, more lustrous silk fibers. Post-extraction, silk proteins undergo processing to yield fibroin- and sericin-based materials in forms including films, hydrogels, fibers, powders, and scaffolds. These biomaterials exhibit tunable mechanical properties, biodegradability, biocompatibility, and chemical modifiability, supporting applications in tissue engineering, controlled drug delivery, cosmetics, smart textiles, and sustainable packaging. The integration of nanoparticle-mediated silkworm modulation with post-extraction silk material innovation provides a holistic framework for advancing sericulture as both a biological and technological discipline. By bridging insect physiology, nanobiotechnology, and materials science, this approach enables sustainable, high-performance silk production and positions silk as a versatile platform for next-generation biomaterials and industrial applications.

Keywords: Sericulture, Applied Entomology, *Bombyx mori*, Nanobiotechnology, Engineered Nanoparticles.

Introduction:

Silk, historically prized as a luxury textile, has emerged as a multifunctional biomaterial bridging biology and technology. Modern sericulture integrates applied entomology with nanobiotechnology, exploring both the physiology of silkworms and the versatile applications of post-extraction silk proteins—fibroin and sericin. Engineered nanoparticles can modulate growth, immunity, and hormonal regulation in Bombyx mori, enhancing cocoon quality and fiber properties, while processed silk materials serve as films, hydrogels, fibers, and scaffolds for biomedical, cosmetic, textile, and environmental applications. This chapter examines these complementary dimensions, highlighting the synergy between silkworm biology and advanced material innovation in sustainable silk production.

1. Post-Extraction Silk Material Applications



Traditionally valued as a luxury textile fiber, silk has expanded its role into multiple high-value applications in biomedicine, cosmetics, environmental remediation, and advanced materials engineering. Following extraction from *Bombyx mori* cocoons, the two primary silk proteins—fibroin and sericin—can be processed into diverse material forms such as films, hydrogels, powders, fibers, and scaffolds. These biomaterials are characterized by exceptional mechanical strength, tunable biodegradability, biocompatibility, and chemical modifiability, making them suitable for numerous industrial and medical uses.

Post-Extraction Processing of Silk Proteins

After degumming, which removes sericin, silk fibroin is typically dissolved in solvents such as lithium bromide or ternary calcium chloride/ethanol/water systems. The fibroin solution is then dialyzed to remove salts before being cast or spun into films, fibers, gels, or porous scaffolds. Sericin extraction is usually performed by boiling in water or through mild alkaline treatment,

followed by drying to yield powder, films, or emulsions suitable for cosmetic and pharmaceutical formulations.

Biomedical Applications

Silk fibroin has been widely used in tissue engineering due to its capacity to form scaffolds that mimic the extracellular matrix. These scaffolds support cell adhesion, proliferation, and differentiation in applications such as bone, cartilage, skin, vascular, and neural tissue engineering (Wang *et al.,* 2006). Sericin, on the other hand, demonstrates bioactivity in wound healing by promoting fibroblast proliferation, collagen synthesis, and antioxidant protection. Silk-based wound dressings, including composite hydrogels and electrospun mats, maintain optimal moisture levels while reducing infection risk (Aramwit *et al.,* 2012). In drug delivery, silk fibroin nanoparticles, hydrogels, and films act as controlled-release carriers for therapeutic compounds, offering stability and biodegradability suitable for cancer therapy, ocular treatments, and transdermal delivery (Seib *et al.,* 2013).

Cosmetic and Personal Care Uses

Silk proteins are widely used in cosmetic formulations. Sericin is incorporated into moisturizers, anti-aging creams, and shampoos for its hydrating, UV-protective, and collagen-stimulating effects. Silk powders are used in facial products to enhance smoothness and provide a natural sheen, while the antioxidant properties of both fibroin and sericin support skin regeneration (Zhaorigetu *et al.,* 2001).

Textile and Smart Material Innovations

Regenerated silk fibroin is often blended with synthetic or natural fibers to produce fabrics with enhanced strength, breathability, and functional properties. Functionalization with antibacterial agents, dyes, or nanoparticles enables the production of smart textiles with UV-responsiveness, antimicrobial action, or conductivity (Tao *et al.,* 2012). Emerging composite materials, such as silk–graphene and silk–gold hybrids, are being developed for wearable sensors, bioelectronic fabrics, and flexible energy storage devices.

Environmental and Industrial Applications

Silk proteins have shown potential as adsorbents for heavy metals such as Pb^{2+}, Cu^{2+}, and Cr^{6+}, due to the chelating capacity of amino and hydroxyl groups on their molecular structure. In addition, silk-based biodegradable films are being explored as sustainable packaging materials with strong oxygen barrier properties, mechanical integrity, and edibility. These films have applications in food preservation, such as fruit and meat coating, as well as in developing eco-friendly plastics (Guo *et al.,* 2020).

Future Prospects

Advances in nanotechnology and green processing methods have expanded the functionalization possibilities for silk proteins, enabling their incorporation into personalized medical devices,

bioelectronics, and agricultural materials. The versatility of fibroin and sericin, combined with their renewable origin, positions silk as a cornerstone in the development of next-generation biomaterials.

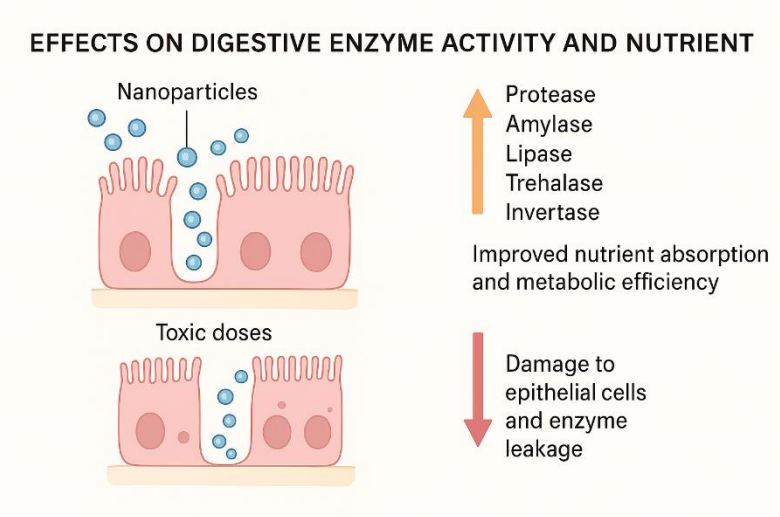
2. Nanoparticles in Silkworm Growth, Immunity, and Hormonal Regulation

Influence on Growth and Development

Engineered nanoparticles—including AgNPs, ZnO, TiO_2, and Fe_3O_4—have been integrated into mulberry leaf feeding regimes or applied as foliar sprays to improve *B. mori* performance. Positive outcomes include accelerated larval weight gain, shortened developmental duration, and increased cocoon mass. However, excessive nanoparticle concentrations can cause delayed pupation, reduced reproductive fitness, and tissue-level oxidative stress (Raja *et al.,* 2018).

Effects on Digestive Enzyme Activity and Nutrient Uptake

Nanoparticles can interact with the midgut epithelium, enhancing digestive enzyme activities such as protease, amylase, and lipase, as well as carbohydrate-processing enzymes like trehalase and invertase. These effects contribute to improved nutrient absorption and metabolic efficiency. Morphological changes, including increased villus height and microvilli density, have been reported following nanoparticle supplementation. At toxic doses, however, damage to epithelial cells and enzyme leakage can occur (Raja *et al.,* 2018).



Antioxidant and Oxidative Stress Responses

Moderate nanoparticle exposure can upregulate antioxidant defense enzymes such as SOD, CAT, glutathione-S-transferase (GST), and peroxidases, potentially enhancing tissue resilience. However, chronic or high-dose exposure can lead to ROS accumulation, lipid peroxidation, and protein oxidation, ultimately impairing cellular function (Muralidharan *et al.,* 2020).

Modulation of the Immune System

Nanoparticle exposure stimulates both cellular and humoral immunity in silkworms. This includes elevated hemocyte counts, enhanced phagocytic activity, and the upregulation of antimicrobial peptide genes such as *cecropin*, *defensin*, and *attacin*. The prophenoloxidase

cascade, critical for pathogen encapsulation and melanization, is also activated by nanoparticle feeding (Selvi *et al.,* 2021).

Hormonal Regulation and Molting

Nanoparticles can influence molting cycles by modulating ecdysteroid and juvenile hormone pathways. Depending on the dose and type, nanoparticles may accelerate or delay molting, shorten inter-instar periods, or cause incomplete ecdysis. At the molecular level, nanoparticles have been shown to alter the expression of genes such as *ecdysone receptor* (EcR) and *broad-complex* (Br-C), impacting cuticle formation and pupal development (Zhang *et al.,* 2022).

Cocoon Quality and Silk Strength

Nano-treated silkworms often produce cocoons with improved architecture, higher shell ratios, and stronger, more lustrous filaments. Mechanisms include upregulation of fibroin and sericin gene expression and improved fibroin β-sheet crystallinity. Certain treatments, such as nano-chitosan supplementation, have been linked to a ~20% increase in raw silk reeling efficiency and enhanced tensile properties (Patil *et al.,* 2021).

Conclusion:

Nanoparticle supplementation in sericulture offers a powerful method for improving silkworm growth, immunity, and silk quality. However, realizing these benefits requires precise control over nanoparticle dose, exposure timing, and biocompatibility to avoid toxicity.

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HEMOCYTES OF INSECTS: STRUCTURE, FUNCTION, AND ROLE IN IMMUNITY AND TOXICITY STUDIES

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Abstract:
Hemocytes are the primary immune cells circulating in the hemolymph of insects, playing a vital role in their innate immune system, which operates in the absence of adaptive immunity. These cells are responsible for cellular defense mechanisms such as phagocytosis, encapsulation, and nodulation, as well as for the secretion of antimicrobial peptides and signaling molecules that coordinate immune responses. Morphologically, hemocytes exhibit considerable diversity among insect taxa, including distinct types such as prohemocytes, plasmatocytes, granulocytes, spherulocytes, and oenocytoids, each specialized for particular immune and physiological functions. Functionally, they participate not only in immune defense but also in processes like wound healing, coagulation, and tissue remodeling. Beyond their biological role, hemocytes serve as effective biomarkers in ecotoxicology, providing insights into the physiological impacts of environmental stressors, pollutants, and xenobiotics. Their responses to pesticides and biopesticides are also increasingly utilized in pest management research, contributing to the development of sustainable and eco-friendly control strategies.

Keywords: Hemocytes, Insect Immunity, Cellular Defense, Phagocytosis, Encapsulation, Antimicrobial Peptides, Ecotoxicology, Pesticide Toxicity, Biopesticides, Pest Management

1. Introduction:

Insects, like all multicellular animals, defend themselves against infectious agents through immune mechanisms. However, unlike vertebrates, insects lack an acquired immune system and rely solely on innate immunity, which provides rapid and efficient protection against a broad range of microbial and multicellular pathogens (Fearon, 1997). The insect innate immune system comprises both physical and physiological barriers, as well as cellular and humoral components that work in concert to recognize, neutralize, and eliminate invading organisms (Muta and Iwanaga, 1996; Gillespie *et al.,* 1997).

The cuticle represents the first line of defense, serving as a robust physical barrier that prevents pathogen entry. It also extends to the tracheal system, foregut, and hindgut, forming a continuous protective layer. The midgut, a primary site of digestion, contributes actively to immunity by

producing Reactive Oxygen Species (ROS) and other defense molecules capable of damaging microbial membranes (Vass and Nappi, 2001). Internally, the fat body, functionally analogous to the mammalian liver, synthesizes and secretes a wide range of Antimicrobial Peptides (AMPs) and immune proteins into the hemolymph, which circulates throughout the hemocoel (Meister *et al.,* 2000; Lowenberger, 2001).

Hemolymph serves as the medium for systemic immune responses and consists of plasma and hemocytes, the immune cells of insects. Hemocytes are the key effectors of cellular immunity, executing functions such as phagocytosis, nodulation, and encapsulation of invading pathogens (Strand and Pech, 1995; Lavine and Strand, 2002). In addition, hemocytes secrete defense molecules, including AMPs, and participate in coagulation and melanization, processes critical for wound healing and pathogen containment (Cornelis and Soderhall, 2004; Theopold *et al.,* 2004). These cells also express various receptors, opsonins, and cytokines that facilitate pathogen recognition and intercellular communication (Irving *et al.,* 2005).

While insect immunity is traditionally divided into humoral and cellular components, this distinction is largely functional rather than absolute, since many humoral factors regulate hemocyte activity, and hemocytes themselves are important sources of humoral effectors (Lavine and Strand, 2002; Irving *et al.,* 2005). The dynamic interplay between these two systems ensures a coordinated and efficient immune response. For example, recognition of microbial cell wall components by Pattern Recognition Receptors (PRRs) triggers signaling cascades that activate both AMP production and hemocyte-mediated responses (Blandin and Levashina, 2004; Imler and Bulet, 2005).

Recent advances in molecular and cellular techniques have greatly expanded our understanding of hemocyte development, diversity, and function. Studies in model insects such as *Drosophila melanogaster*, *Manduca sexta*, and various mosquito species have revealed that hemocytes are not only involved in immunity but also in physiological processes such as development, wound repair, and homeostasis (Strand, 2008). Furthermore, hemocytes have gained increasing attention in environmental toxicology and pest management, serving as bioindicators for pesticide exposure and as tools for evaluating the immunotoxic effects of biopesticides (Eleftherianos *et al.,* 2021).

Overall, hemocytes form the cornerstone of insect immunity, bridging cellular defense and systemic regulation. Their multifunctional roles make them indispensable for understanding insect physiology, host–pathogen interactions, and the ecological implications of immune modulation in response to environmental stressors. Continued research on hemocytes will not only deepen our insight into invertebrate immunobiology but also facilitate the development of innovative and sustainable strategies for pest and disease control. In this review, the diverse types, functions, and regulatory mechanisms of insect hemocytes were emphazied by

highlighting their pivotal role in immunity, development, and environmental adaptation. A comprehensive understanding of hemocyte biology can contribute to advance in insect research and broaden our knowledge in evolution of insect immune system.

2. Classification and Types of Hemocytes

Insect hemocytes have been classified based on their morphological, histochemical, and functional characteristics (Gupta, 1985; Brehelin and Zachary, 1986). In recent years, these conventional criteria have been complemented by the application of antigenic and molecular markers for more precise identification (Willott *et al.,* 1994; Gardiner and Strand, 1999; Lanot *et al.,* 2001; Jung *et al.,* 2005). The major hemocyte types commonly recognized across insect taxa include prohemocytes, granulocytes (granular cells), plasmatocytes, spherulocytes (spherule cells), and oenocytoids. These cell types have been documented in numerous insect orders such as Lepidoptera, Diptera, Orthoptera, Blattaria, Coleoptera, Hymenoptera, Hemiptera, and Collembola, demonstrating their broad taxonomic distribution (Ahmad, 1988; Azambuja *et al.,* 1991; Ahmad, 1992; Luckhart *et al.,* 1992; Fenoglio *et al.,* 1993; Sonawane and More, 1993; Butt and Shields, 1996; Joshi and Lambdin, 1996; Hernandez *et al.,* 1999; de Silva *et al.,* 2000a).

Table 1: Morphology of different hemocytes and their function

Hemocyte Type	Morphology	Main Functions
Prohemocytes	Small (5–10 µm), spherical, high nucleus-to-cytoplasm ratio, scanty basophilic cytoplasm	Stem-cell precursors of all hemocytes, hemocyte renewal, maintain hemolymph homeostasis
Plasmatocytes	Spindle-shaped, oval, or irregular; cytoplasmic extensions (filopodia/pseudopodia), moderately basophilic cytoplasm with organelles	Phagocytosis, encapsulation, wound healing, tissue repair, extracellular matrix secretion
Granulocytes	Round or oval, dense cytoplasm packed with electron-dense granules, central nucleus	Phagocytosis, encapsulation, melanization, release of cytotoxic enzymes and antimicrobial peptides
Spherulocytes	Large (15–30 µm), spherical, cytoplasm filled with refractile spherules, small eccentric nucleus	Transport and secretion of cuticular precursors, enzymes, and pigments, support molting and tissue repair
Oenocytoids	Large (15–25 µm), oval/spherical, large eccentric nucleus, homogeneous cytoplasm	Non-phagocytic, prophenoloxidase activation, melanization, wound healing and encapsulation
Adipohemocytes	Large, round/oval, cytoplasm contains numerous lipid droplets, small eccentric nucleus	Lipid and carbohydrate storage and transport; energy regulation; detoxification; metabolic homeostasis

a. Prohemocytes

Prohemocytes are small, spherical, undifferentiated insect hemocytes regarded as stem-cell precursors of all other blood cell types. They typically measure 5–10 μm in diameter, possess a large centrally located nucleus with scanty basophilic cytoplasm, and exhibit a high nucleus-to-cytoplasm ratio. Originating from embryonic mesodermal or post-embryonic hematopoietic tissues such as lymph glands or hemopoietic nodules, prohemocytes actively proliferate and differentiate into plasmatocytes, granulocytes, or oenocytoids under hormonal and immune regulation (Lavine and Strand, 2002; Hillyer and Strand, 2014). Though not directly involved in defense, they play a key role in maintaining hemocyte renewal and hemolymph homeostasis. Prohemocytes form about 10–20% of total hemocytes in larvae of *Bombyx mori*, though this proportion varies among insect species (Nakahara *et al.,* 2009; Boulanger *et al.,* 2001). These cells are therefore critical for sustaining hematopoiesis and immune competence in insects.

b. Plasmatocytes

Plasmatocytes are the most abundant and versatile insect hemocytes, primarily responsible for cellular immune responses such as phagocytosis of pathogens, encapsulation of larger invaders, and wound healing (Buchon *et al.,* 2014). Morphologically, they are typically spindle-shaped, oval, or irregular, often exhibiting cytoplasmic extensions like filopodia or pseudopodia that aid in adhesion and interaction with foreign particles. Their cytoplasm is moderately basophilic and contains numerous organelles, including mitochondria, lysosomes, and rough endoplasmic reticulum, reflecting high metabolic and immune activity. Plasmatocytes originate from prohemocytes through mitotic differentiation and are functionally analogous to vertebrate macrophages. Besides immunity, they also contribute to tissue repair and extracellular matrix secretion, supporting hemocyte differentiation and hemolymph homeostasis (Gupta, 1979; Ribeiro and Brehelin, 2006). Plasmatocytes typically comprise 30–60% of total hemocytes in larvae of *Bombyx mori*, though proportions vary among species and developmental stages (Zhang *et al.,* 2021).

c. Granulocytes

Granulocytes, also referred to as granular cells, are an important type of insect hemocyte primarily involved in innate immune defense, including phagocytosis, encapsulation, and release of cytotoxic substances. They are typically round or oval with a centrally located nucleus and a cytoplasm densely packed with electron-dense granules containing enzymes, antimicrobial peptides, and other immune effectors (Stephenson *et al.,* 2022). Granulocytes are derived from prohemocytes and often work in conjunction with plasmatocytes during immune responses, particularly in recognizing and neutralizing invading microorganisms. These cells are highly motile and can adhere to foreign surfaces or pathogens, releasing their granule contents to mediate microbial killing and melanization (Ghosh *et al.,* 2021). Granulocytes constitute a

smaller proportion of total hemocytes around 10–20%, compared to plasmatocytes, but their abundance can increase during immune challenges.

d. Spherulocytes

Spherulocytes are large, spherical insect hemocytes primarily involved in the transport and secretion of macromolecules, particularly cuticular components and pigments essential for integument formation and repair. They typically measure 15–30 µm in diameter and are characterized by the presence of numerous cytoplasmic spherules or granules that stain variably depending on their chemical composition. The nucleus is small, eccentric, and compact, while the cytoplasm appears filled with refractile spherules containing proteins, lipids, and mucopolysaccharides (Sheehan, 2020). These cells are non-phagocytic and are mainly responsible for the secretion and transport of cuticular precursors, enzymes, and pigments to the integument, especially during molting and wound healing. Ultrastructural studies reveal that spherulocytes possess a well-developed rough endoplasmic reticulum and Golgi complex, consistent with their secretory function (Moyetta *et al.,* 2021). They are relatively less abundant compared to plasmatocytes and granulocytes, generally constituting 5–10% of the total hemocyte population, with abundance varying among species and developmental stages.

e. Oenocytoids

Oenocytoids are large, oval to spherical insect hemocytes primarily involved in melanization and phenoloxidase activity during immune responses. They typically measure 15–25 µm in diameter and are characterized by a large eccentric nucleus and a homogeneous, dense cytoplasm that often stains lightly with Giemsa or Wright's stain (Hillyer *et al.,* 2003). Unlike plasmatocytes or granulocytes, oenocytoids are non-phagocytic but play a crucial role in the prophenoloxidase (proPO) activation cascade, leading to melanin synthesis for wound healing and encapsulation of pathogens (Ghosh *et al.,* 2021). Oenocytoids are believed to originate from prohemocytes or directly from hematopoietic tissues, depending on the insect species and developmental stage (Lanot *et al.,* 2001; Grigorian and Hartenstein 2013). Similar to spherulocytes, their number in hemolymph constitutes 5–10% of the total hemocyte population.

f. Adipohemocytes

Adipohemocytes are specialized insect hemocytes associated with lipid metabolism, storage, and transport, as well as detoxification and energy regulation. They are generally large, round or oval cells containing numerous refractile lipid droplets dispersed throughout the cytoplasm, which give them a characteristic foamy appearance under light microscopy. The nucleus is usually small, eccentric, and condensed, while the cytoplasm stains lightly due to its lipid-rich content. Adipohemocytes are non-phagocytic and function primarily in metabolic and homeostatic roles, including the synthesis and mobilization of lipids and carbohydrates during molting, metamorphosis, and starvation (Castillo *et al.,* 2006). They also participate in detoxification processes, possibly by sequestering harmful substances or metabolic by-products, thereby

maintaining hemolymph stability. Though less abundant than plasmatocytes or granulocytes, adipohemocytes are occasionally reported in several insect orders, including Lepidoptera, Orthoptera, and Coleoptera, reflecting their importance in insect physiology beyond immunity (Holz *et al.,* 2003).

2.1. Hemocyte Development and Hematopoiesis

Insect hemocytes originate from hematopoietic organs or the embryonic mesoderm, reflecting a highly conserved developmental process in the insect taxa (Nardi *et al.,* 2003). In embryonic stages, hemocytes differentiate from mesodermal progenitors, giving rise to the first circulating hemocytes, including prohemocytes, plasmatocytes, and granulocytes. Post-embryonically, hematopoiesis occurs in specialised hematopoietic tissues such as lymph glands, hematopoietic nodules, or sessile hemocyte clusters, which serve as reservoirs for hemocyte production throughout the insect's life (Gardiner and Strand, 2000). Prohemocytes act as stem-like cells that proliferate and differentiate into various hemocyte types. Hemocyte differentiation is influenced by hormonal signals, immune challenges, and environmental cues, ensuring adaptive immune and physiological responses. During molting and metamorphosis, hematopoietic tissues enable partial renewal of hemocytes to maintain cellular balance. Hematopoiesis occurs in lymph glands, hematopoietic nodules, and the embryonic mesoderm, while immune activation involves plasmatocytes and granulocytes that respond to infections and tissue damage. The developmental stages of hemocyte through hematopoiesis were demonstrated in the figure 1.

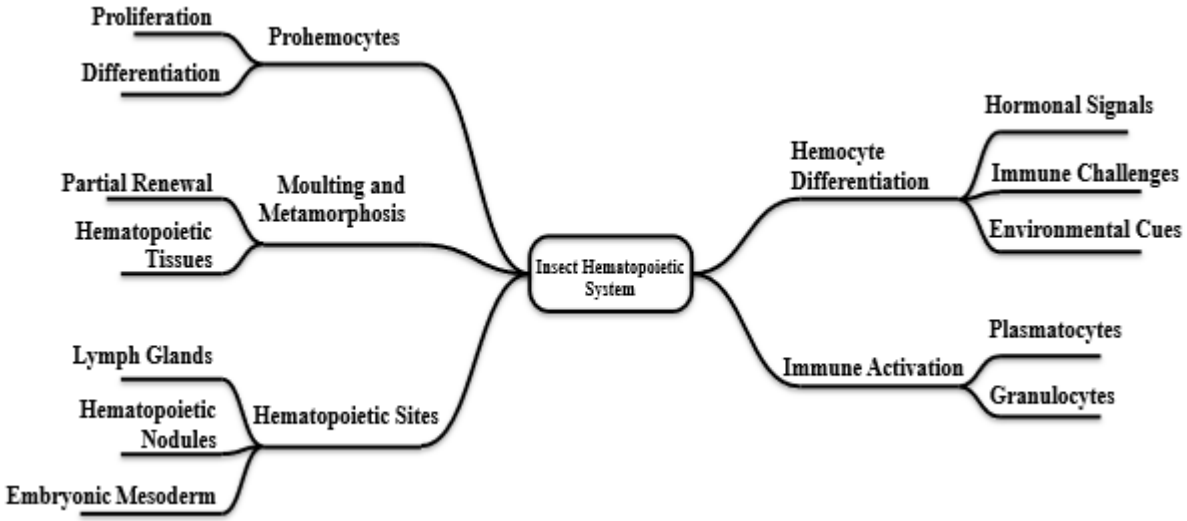

Figure 1: Insect Hematopoietic system

2.2. Functional Roles of Hemocytes

Insect hemocytes are central effectors of innate immunity, performing diverse functions that protect the host from pathogens, aid in tissue repair, and maintain hemolymph homeostasis (Gold and Bruckner, 2015; Tikhe and Dimopoulos, 2021). The important functions of hemocytes are described below.

a. Phagocytosis

Phagocytosis is the engulfment and intracellular digestion of microbes, apoptotic cells, or debris. Plasmatocytes and granulocytes are the primary phagocytic cells, extending pseudopodia to surround and internalize targets, which are then degraded in lysosomes (Yokoo *et al.,* 1995; Rommelaere *et al.,* 2024). This is an essential first line of defense against bacteria, fungi, and smaller parasites.

b. Encapsulation

Encapsulation involves surrounding large pathogens or foreign bodies (e.g., parasitoid eggs) that are too big to be phagocytosed. Hemocytes, primarily plasmatocytes, form multilayered capsules around the invader, which are then melanized through the prophenoloxidase (proPO) cascade, leading to pathogen neutralization (Ribeiro and Brehelin, 2006).

c. Nodule Formation

Nodulation is a collective cellular immune response in which hemocytes aggregate around clusters of microbes, forming nodules that immobilize and kill pathogens. Granulocytes release cytotoxic granules while plasmatocytes stabilize the nodule structure. This mechanism is particularly important in hemocoelic bacterial infections.

d. Coagulation

Hemocytes contribute to hemolymph clotting, which prevents pathogen entry and limits hemolymph loss at wound sites. Granulocytes and spherulocytes participate in forming clot matrices by secreting coagulation factors, adhesive proteins, and extracellular enzymes, facilitating rapid wound repair (Gold and Bruckner, 2015).

e. Apoptosis

Hemocytes are also involved in programmed cell death to remove damaged, senescent, or infected cells, maintaining tissue and hemolymph homeostasis. Plasmatocytes can recognize and phagocytose apoptotic cells, preventing secondary infections and inflammation.

2.3. Hemocyte Response in Immunity

Hemocytes play a vital role in insect immunity by integrating cellular and humoral responses to combat pathogens. They directly eliminate invaders through phagocytosis, encapsulation, and nodulation while also regulating systemic defense by producing signaling molecules that stimulate AMPs secretion from the fat body and releasing clotting factors that aid in pathogen containment (Tikhe and Dimopoulos, 2021). Oenocytoids store prophenoloxidase (proPO), which activates melanin synthesis to encapsulate and kill microbes, while granulocytes release proPO activators, linking cellular and humoral immunity. These immune activities are coordinated by conserved signalling pathways: the Toll pathway responds to Gram-positive bacteria and fungi; the Imd pathway targets Gram-negative bacteria; and the JAK/STAT pathway governs hemocyte proliferation, differentiation, and stress responses (Minakhina *et al.,* 2011). In

model insects such as *Drosophila melanogaster*, *Bombyx mori*, and *Galleria mellonella*, hemocytes demonstrate these interactions through AMP induction, melanization, encapsulation, and nodulation, highlighting their role as key sentinels and effectors in maintaining a robust and integrated immune defense system (Kavanagh and Reeves, 2004; Evans *et al.,* 2014).

2.4. Hemocyte Alterations Under Stress and Toxic Exposure

Environmental stressors, including insecticides, plant-based compounds, and nanoparticles, significantly affect insect hemocytes, altering their numbers, structure, and function. Sublethal doses of insecticides such as acephate, azadirachtin, and spinosad have been reported to reduce total hemocyte counts, disrupt plasmatocyte and crystal cell ratios, and impair immune responses in species like *Drosophila melanogaster* and *Melipona quadrifasciata* (Ragab *et al.,* 2011). Plant-derived compounds, such as neem-based insecticides, can similarly decrease hemocyte populations and cause structural disintegration, as observed in *Danaus chrysippus* (Ribeiro and Brehelin, 2006; Ghosh *et al.,* 2021). Nanoparticles, including zinc oxide (ZnO) and silica (SiO_2), induce oxidative stress, genotoxicity, and apoptosis in hemocytes, compromising cellular immunity in insects such as *Bombyx mori* (Hillyer and Strand, 2014). Due to their sensitivity to chemical and physical stressors, hemocytes serve as effective biomarkers in toxicological studies, where changes in cell count, morphology, and apoptosis provide early indicators of environmental toxicity and help evaluate ecological risks (Westlake *et al.,* 2024; Estevez-Lao *et al.,* 2020).

2.5. Techniques and Methods for Hemocyte Study

Hemocyte studies involve several standard techniques to analyze their morphology, function, and response to physiological and experimental conditions. Hemolymph is collected from insect body sites such as the proleg, dorsal vessel and intersegmental membranes using a capillary tube containing an anticoagulant buffer or phenylthiourea to prevent coagulation and melanization. The Total Hemocyte Count (THC) is performed with a hemocytometer under a light microscope, while Differential Hemocyte Count (DHC) involves staining hemocyte smears with Giemsa or Wright stains to identify and quantify different cell types based on morphology. Cell viability is assessed using Trypan blue or propidium iodide staining to distinguish live and dead cells. The microscopic techniques, including light microscopy, Transmission Electron Microscopy (TEM), and Scanning Electron Microscopy (SEM), are employed to examine cellular details, surface features, and immune-related structures such as pseudopodia and granules. The data from hemocyte studies are statistically analyzed, expressing THC as cells per microliter, DHC as a percentage of total cells, and viability as percent live cells. Results are presented as mean ± SD or SE, and analyzed using statistical tests like ANOVA or t-tests to determine significant variations between treatments, developmental stages, or experimental conditions.

2.6. Applications of Hemocytes in Research

Hemocytes play an important role in insect research as key indicators of immune and physiological status. They are widely used to evaluate the effects of bioinsecticides, plant-based compounds, and nanoparticles through assays assessing cytotoxicity, viability, and immune modulation (Kinoshita *et al.,* 2024). Their responses, such as phagocytosis, encapsulation, and nodulation, are valuable for developing immune-based pest control strategies (Mowlds *et al.,* 2020). The hemocytes serve as useful models in comparative immunology and nanotoxicology, helping to understand cellular immunity, stress responses, and potential biomarkers for environmental or toxicological studies (Dolezal, 2023; Vanha-aho *et al.,* 2016).

Conclusion:

The hemocyte research is expected to integrate with omics approaches, such as proteomics and transcriptomics, to provide a comprehensive understanding of hemocyte functions, differentiation, and immune responses at the molecular level. Hemocytes will also play an increasing role in nanotoxicology and immune modulation studies, helping to evaluate the effects of nanoparticles and novel bioactive compounds on insect immunity. Likewise, due to their sensitivity to environmental stressors and toxins, hemocytes hold great potential as early bioindicators of physiological stress, offering valuable tools for ecological monitoring, environmental toxicology, and the development of sustainable pest management strategies.

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INTEGRATING NANOTECHNOLOGY INTO MODERN PEST MANAGEMENT STRATEGIES

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Abstract:
Stored grain pests cause significant losses in quantity and quality of grains, affecting both economic stability and food security. Understanding the major insect pests and their impact is essential for developing effective control strategies. Nanotechnology has emerged as a promising tool in pest management, offering innovative solutions that can be integrated with traditional approaches. The application of nanoparticles in grain protection includes various types and synthesis methods, providing advantages over conventional chemical insecticides such as higher stability, targeted action, and reduced environmental impact. Nanoparticles act through physical and biochemical interactions, affecting insect physiology and behavior, while advances in nano-encapsulation and controlled release enhance their efficacy. In the future, integration of nanotechnology into Integrated Pest Management (IPM) offers sustainable approaches to reduce post-harvest losses, improve food security, and minimize ecological risks. Therefore, the present study aimed to assemble the application of nanotechnology in the field of stored grain insect pests, and analyze its mode of action on insect pests and amalgamate this advanced technology in IPM for sustainable pest management.

Keywords: Stored Grain Pests, Economic Stability, Food Security, Nanotechnology, Pest Management, Traditional Approaches, Chemical Insecticides, Insect Physiology, Integrated Pest Management, Post-Harvest Losses

Introduction:
Stored grain pests pose a significant hazard to agricultural commodities, causing both quantitative and qualitative losses (Taddese *et al.* (2020); Berhe *et al.* (2022). These insects feed on stored commodities, reducing grain weight and viability (Kumar *et al.* (2020) and leading to significant qualitative deterioration that makes produce unsuitable for human consumption or commercial trade (Ahmedani *et al.* (2011). Infestation can occur at any stage of storage, processing, or transport, resulting in reduced nutritional value, discoloration, and contamination by insect fragments and excreta. Among the various insect groups associated with stored products, several beetle and moth species are particularly notorious for their destructive impact

on stored grains and their derivatives (Demis *et al.* (2022). The most common insect pests infesting stored cereals include weevils (*Sitophilus* spp.), the lesser grain borer (*Rhyzopertha dominica*), rust-red flour beetle (*Tribolium* spp.), sawtoothed grain beetle (*Oryzaephilus* spp.), grain beetle (*Cryptolestes* spp.), Indian meal moth (*Plodia interpunctella*), and Angoumois grain moth (*Sitotroga cerealella*). In stored oilseeds, major pests included flour beetles, sawtoothed beetles, flat grain beetles, and moths, while bruchid beetles (*Callosobruchus chinensis*) were common in pulses. Overall, cereals and pulses are attacked by different storage pests, mainly belonging to the insect orders Coleoptera, Lepidoptera, and Psocoptera, with Coleoptera and Lepidoptera accommodates most of the economically important insect pests such as rice weevil, maize weevil, lesser grain borer, granary weevil, red flour beetle, and pulse beetles (Berhe *et al.* (2022). In recent years, nanotechnology has emerged as a promising and sustainable solution to reduce losses caused by pests. By using nanoscale materials and delivery systems such as nanoparticle-based formulations, encapsulated essential oils, and nanostructured dusts researchers are working to improve the effectiveness of insecticides while minimizing chemical residues and environmental pollution. The controlled release and targeted action offered by nanomaterials open up new possibilities for protecting stored grains, providing greater stability, longer-lasting effects, and better integration with pest management programs. As a result, nanotechnology stands out as a cutting-edge innovation that could transform post-harvest protection, ensuring safer and more eco-friendly storage of agricultural products compared to traditional methods.

Overview of Major Insect Pests of Stored Grains

Stored grains are commonly attacked by beetles, weevils, and moths, which can cause significant damage and economic losses during storage and transport. Although over a thousand insect species are known to be associated with stored grains, only a few are responsible for most of the serious damage. These pests reduce grain quality, contribute to post-harvest losses, and pose a major threat to global food security. They thrive in warm and humid environments, where they reproduce quickly and feed on whole kernels as well as processed grain products.

Primary and Secondary Pests

- Primary pests, such as the rice weevil (Sitophilus oryzae), lesser grain borer (Rhyzopertha dominica), and khapra beetle (Trogoderma granarium), attack whole kernels, laying eggs inside grains where larvae develop and feed unseen. Damage from these pests is often internal and difficult to detect until grains are hollowed or riddled with exit holes.
- Secondary pests, including rust-red flour beetle (*Tribolium castaneum*) and saw-toothed grain beetle (*Oryzaephilus surinamensis*), typically feed on broken kernels, grain dust, or previously damaged grain surfaces, compounding losses initiated by primary pests.

The most prevalent stored grain insect pests that infests the grains are discussed as follows.

Rice Weevil, *Sitophilus oryzae* (Coleoptera: Curculionidae)

The rice weevil is one of the most widespread and damaging pests of stored cereals, especially rice, wheat, and maize. Adult weevils are small, reddish-brown beetles with a characteristic long snout. Female weevils bore tiny holes into grains to lay their eggs, and the larvae develop inside the kernels, feeding on the grain's interior. As a result, the grains become hollow and powdery residues. Because infestations begin inside the grain, they often go unnoticed until serious damage and, making the rice weevil as a major challenge in long-term grain storage. (Bhargude *et al.* (2022)

Red Flour Beetle, *Tribolium castaneum* (Coleoptera: Tenebrionidae)

The red flour beetle is a common secondary pest that mainly infests processed or damaged grain products such as flour, bran, and cereals. Both adults and larvae feed externally, resulting in contaminated and foul-smelling products due to secreted quinones (Kharel *et al.* (2022). Its ability to reproduce quickly in warm and humid environments makes it a persistent problem in flour mills and grain storage facilities (Duarte *et al.* (2022). *Tribolium castaneum*, better known as the red flour beetle, is a tiny insect pest commonly found in stored food items such as grains, flour, cereals, and other dry products. It is a member of the Tenebrionidae family under the Coleoptera order. This beetle is typically 3–4 mm long and has a smooth, reddish-brown body with a shiny surface. Although it can fly short distances, it usually spreads through infested food materials. Its life cycle includes four stages such as egg, larva, pupa, and adult, and it can complete this cycle in roughly a month under warm conditions. The red flour beetle mainly feeds on damaged or processed grains and is unable to attack whole kernels. Infestations lead to economic damage by contaminating and degrading stored foods, making them less suitable for consumption or sale. Beyond being a major pest, *T. castaneum* is also widely used as a model organism in scientific studies, especially in areas like genetics, physiology, and pest management, owing to its fully sequenced genome and ease of laboratory cultivation.

Lesser Grain Borer, *Rhyzopertha dominica* (Coleoptera: Bostrychidae)

The lesser grain borer is one of the most destructive primary pests of stored grains and also a notorious internal feeder known for its ability to bore whole grains, particularly wheat, barley, and rice. Both adults and larvae feed inside the kernels, causing extensive internal damage that leads to weight loss and reduced grain quality. The adult beetles are small, cylindrical, dark brown in colour and capable of flying long distances, which helps them spread rapidly between storage sites found powdery grains after infestation and emit a distinctive, unpleasant odor (Edde *et al.* (2006). Due to its high reproductive rate and tolerance to low moisture conditions, *R. dominica* is considered a major pest in warm, dry storage environments around the world (Koehler *et al.* (2006).

Pulse Beetle *Callosobruchus maculatus* (Coleoptera: Bruchidae)

Bruchids are, commonly called as pulse beetles, pea beetles, or bean beetles. The pulse beetle is a significant pest affecting stored pulses like chickpeas, lentil, mung bean, green gram, cowpeas and other pulses both in the field and storage. They are found throughout tropical and subtropical regions Females lay eggs on the seed surface, and upon hatching, larvae bore into the seed and complete their development inside. Infested seeds show distinct round exit holes and become unsuitable for either eating or planting. In tropical and subtropical areas, *C. maculatus* can repeatedly breed in storage conditions, eventually causing complete seed damage if not controlled (Hosamani *et al,* (2018).

Saw Toothed Grain Beetle, *Oryzaephilus surinamensis* (Coleoptera: Silvanidae)

The saw-toothed grain beetle *Oryzaephilus surinamensis L.* (Coleoptera: Silvanidae) is a serious secondary insect pest of postharvest grains, since it can infest only damaged or broken grains (Awadalla *et al.* (2023). Therefore, it typically occurs alongside primary pests that first cause the initial damage. Both the adults and larvae consume the outer portions of broken or damaged grains, causing contamination of stored products rather than complete kernel destruction. With its fast reproductive rate and adaptability to diverse temperature and humidity conditions, *O. surinamensis* has become a widespread contaminant in food storage and processing facilities worldwide (Armitage *et al.* (1987); Abdel-Banat *et al.* (2024).

Economic and Food Security Impact

Nearly 1,000 insect species are associated with stored grain products worldwide, and the Coleopteran and Lepidopteran pests have serious implications for both economic stability and food security, making their control a critical area in postharvest management. Losses during storage can occur due to a range of factors. After harvest, grains continue to face losses caused by inadequate storage techniques insects, mites, rodents, and microorganisms, with insect pests being the most destructive. These pests not only reduce the quantity of stored grains through direct feeding but also lower their quality by causing contamination, heating, and mold growth. Contamination from insect excreta, body parts, and secretions further degrades grain hygiene and marketability, lowering its economic value. Such damage leads to considerable economic losses for farmers, traders, and consumers along the supply chain. However, insect pests are the predominant cause of harm to stored food commodities. Hence, early detection of infestations is essential for farmers and storage operators to reduce losses and preserve the quality of stored grains. In addition to direct damage, stored grain pests increase operational costs associated with fumigation, pest control, and infrastructure maintenance. The combined impact of these factors leads to severe economic losses at both national and global levels, threatening food security and trade quality standards (Keerthana *et al.* (2025). Guru *et al.* (2022) have stated that in developing countries, where storage facilities are often inadequate, post-harvest losses caused by insect

infestation can range from 10% to 40%, directly affecting food availability and income generation. Moreover, the degradation of grain quality impacts market value, seed viability, nutritional content, national food reserves and trade potential. Therefore, managing stored grain pests is vital to ensuring food security, maintaining grain quality, and supporting sustainable agricultural development.

Overview of Nanotechnology

Nanotechnology is chosen for pest management because it offers innovative solutions to the limitations of conventional chemical control methods, such as low solubility, rapid degradation, non-target toxicity, and pest resistance. By utilizing nanoscale materials like nanoparticles, nanocapsules, and nanoemulsions, active ingredients can be encapsulated and delivered in a controlled and targeted manner, enhancing insecticidal efficacy while reducing environmental contamination and health risks. These nanomaterials improve the stability, persistence, and bioavailability of active compounds by protecting them from environmental degradation and facilitating better adhesion and penetration through insect cuticles and plant surfaces. Furthermore, nanotechnology supports the principles of integrated pest management (IPM) by enabling precision delivery, reducing pesticide use, and promoting the development of eco-friendly, biodegradable, and green formulations. Thus, it represents a sustainable and efficient approach to addressing key challenges such as pest resistance, chemical overuse, and food safety in modern agricultural systems.

Definition and Historical Background

Silva (2004) defined nanotechnology as the design, synthesis, characterization, and application of materials and devices that operate at the nanoscale. The word nano is derived from the Greek term meaning "dwarf" and denotes something extremely small, specifically one billionth of a unit (10^{-9}). Hulla *et al.* (2015) reported that modern nanotechnology originated from the visionary ideas of Richard Feynman, the 1965 Nobel Prize winner in Physics. In 1959, during a lecture titled “There’s Plenty of Room at the Bottom” at the American Physical Society meeting held at Caltech, Feynman introduced the groundbreaking concept of manipulating and controlling matter at the atomic scale. His innovative perspective opened new possibilities for scientific exploration, and many of his predictions have since been validated. Hence, Feynman is widely recognized as father of modern nanotechnology. Nearly fifteen years after Feynman’s lecture, Japanese researcher Norio Taniguchi became the first to introduce the term “nanotechnology.” The 1980s represented the golden era of nanotechnology, characterized by the discovery of fullerenes by Kroto, Smalley, and Curl, along with the significant contributions of Eric Drexler from the Massachusetts Institute of Technology (MIT). Building on Feynman’s ideas from “There’s Plenty of Room at the Bottom” and Taniguchi’s concept of nanotechnology, Drexler published his influential 1986 book “Engines of Creation: The Coming Era of

Nanotechnology". Drexler's concept later became widely recognized as molecular nanotechnology (Bayda *et al.* (2019). Raju *et al.* (2016) reported that in the early 2000s, this field gained scientific, political, and commercial attention, which led to both controversy and progress. At the start of the 21st century, interest in the rapidly developing disciplines of nanoscience and nanotechnology grew significantly (Krukemeyer *et al.* (2015). Kumar *et al.* (2023) state that nanotechnology is regarded as one of the most promising and revolutionary technologies of this century. It involves transforming the principles of nanoscience into practical applications by observing, measuring, manipulating, assembling, and controlling matter at the nanometer scale (1–100 nm). In recent years, nanotechnology has transformed the creation of advanced materials, enabling a wide range of applications in agriculture, engineering, medicine, environmental science, food technology, biotechnology, and analytical chemistry (Tirunagaru *et al.* (2024). With in agriculture, nanomaterials have shown great promise in controlling stored-grain pests. The use of nanoformulated insecticides and nanocarriers has improved the accuracy and effectiveness of pest management while reducing harmful chemical residues. Ragaei and Sabry (2014) investigated how the nanomaterials play a vital role in developing sensors, medical tools, catalysts, pesticide coatings, electrical conductors, and semiconductors, thereby enhancing efficiency, sustainability, and overall performance in numerous human-centered applications for stored grain pest. Therefore, nanotechnology would provide green and efficient alternatives for the management of insect pests in agriculture without harming the nature.

Integration of Nanotechnology with Traditional Pest Management Strategies

Many farmers depend on traditional grain storage methods such as earthen pots, jute or gunny bags, steel drums, and bamboo baskets. Unfortunately, most of them are not familiar with modern hermetic storage technologies that can effectively regulate moisture levels and prevent insect infestations without relying on chemical pesticides. In contrast to metal silos or Purdue Improved Crop Storage (PICS) bags, these traditional methods fail to protect stored grains from issues like self-heating, microbial and fungal growth, mycotoxin formation, and nutrient loss. As a result, the grains' shelf life shortens, leading to higher rates of spoilage. In addition, the lack of continuous monitoring and real-time information on key storage conditions makes it difficult to take timely actions to maintain grain quality (Tushar *et al.* (2023). The integration of nanotechnology with traditional pest management offers a modern and eco-friendly approach to protecting crops and stored grains. Conventional methods such as chemical pesticides, botanical extracts, and biological control agents often face challenges like reduced efficacy, pest resistance, and environmental contamination. Synthesis of nanoparticles for pest control is classified through physical, chemical, and biological (green) method (Khan *et al.* (2023). Physical methods such as ball milling, laser ablation, and evaporation condensation create highly pure nanoparticles but it requires significant energy consumption. Chemical techniques,

including sol–gel, co-precipitation, and chemical reduction, allow precise control over particle dimensions and morphology but often depend on hazardous reagents that may introduce toxicity. Alternatively, green synthesis methods utilize plant extract, biomolecules, microorganisms, or agricultural byproducts as reducing and stabilizing agents. This environmentally friendly approach offers cost-efficiency, scalability, minimizes toxicity and enhanced biocompatibility, producing nanoparticles well suited for integrated pest management (IPM) applications (Sharma and Thakur, (2025). Nanotechnology helps to overcome these drawbacks by improving the stability, delivery, and controlled release of active ingredients. For example, encapsulating plant-based essential oils or natural insecticides in nanoparticles enhances their persistence and reduces volatilization losses. Similarly, nano-formulated pesticides can target pests more precisely, minimizing harm to beneficial organisms and lowering the required dosage. When applied alongside traditional integrated pest management (IPM) practices, such as crop rotation and biological control, these nano-based innovations create a synergistic effect that increases efficiency and sustainability (Sakthiyavathi *et al.* (2025). Ultimately, combining nanotechnology with established pest management techniques represents a shift toward smarter, safer, and more sustainable agricultural protection systems (Izuafa *et al.* (2025).

Applications of Nanoparticle in Grain Protection

Types and Role of Nanoparticle in Stored Grain Pest Management

Nanoparticles have emerged as a promising tool for protecting stored grains from insect pests, microbial infections, and oxidative deterioration. Due to their extremely small size, large surface area, and adjustable properties, they allow precise and controlled delivery of active compounds. This ensures long-lasting protection while maintaining the quality and safety of the grains. Nanoparticles used in pest management are generally classified according to their composition, source material, and functional role such as metal and metal oxide nanoparticles (Ag, ZnO, CuO, TiO_2, and Fe_2O_3), silica-based particles, carbon nanostructures (including nanotubes and fullerenes), and polymeric or biopolymeric types like chitosan and polylactic acid. Each group exhibits distinct physicochemical characteristics that determine its efficiency and suitability for targeting different pests. Silver, zinc oxide, and silica exhibit strong insecticidal and antimicrobial activities (Gopal *et al.* (2011). El-Samahy *et al.* (2015) have states that silica (SiO_2), zinc oxide (ZnO), and aluminum oxide (Al_2O_3) have shown significant potential in controlling major stored-grain pests including *Sitophilus oryzae, Tribolium castaneum, and Rhyzopertha dominica*. Copper (Cu), and ZnO, silver (Ag) exhibits antimicrobial and antifungal activity that suppress spoilage organisms like Aspergillus spp. and Fusarium spp (Van Nguyen *et al.* (2022). These nanomaterials inhibit spore germination and mycelial growth, helping to prevent grain mold and mycotoxin contamination during storage. while nanoencapsulation techniques allow controlled and sustained release of bioactive compounds such as essential oils

and botanical insecticides. Incorporating conventional agrochemicals into nanoparticle carriers such as nanosilica, chitosan, or liposomes allows for controlled release, reduces chemical dosage, and minimizes environmental side effects. Studies have shown that nanosilica-based formulations loaded with deltamethrin or pyriproxyfen are highly effective against *Trogoderma granarium* larvae, with their efficacy influenced by factors such as the surface type and duration of exposure (Raduw *et al.* (2020). Biopolymer-based nanocoatings infused with ZnO or TiO_2 nanoparticles act as effective protective barriers that reduce moisture absorption, microbial growth, and oxidative spoilage in stored grains.

Mode of Action of Nanoparticles Against Insects

Nanoparticles exert their insecticidal effects through a combination of physical disruption and biochemical interference, affecting multiple vital systems within insects. Their nanoscale size, high surface activity, and ability to interact at the molecular level interactions allow them to penetrate barriers such as the insect cuticle and cell membranes, leading to critical physiological disturbances that result in death (Shahzad and Manzoor, (2021)

Physical Interactions

Nanoparticles such as silica (SiO_2) and alumina (Al_2O_3) physically interact with insects by adhering the cuticle, removing the protective wax layers and disrupting epicuticular lipids. This process causes excessive water loss, leading to desiccation and death, as these nanoparticles penetrate deeply into the body wall and spiracles, further disrupting respiration and mobility (Gamal, (2018). Futhermore, nanoparticles accumulate around the spiracles, blocking gas exchange and accelerating dehydration, making them efficient alternatives to traditional insecticides. In formulations where nanoparticles serve as carriers (for example, chitosan or liposome-based systems), they enable controlled and sustained release of insecticidal compounds, prolonging exposure and increasing insect mortality while minimizing chemical residues in stored products. (Arjunan *et al.* (2024)

Biochemical Interactions

Silver (Ag), Zinc oxide (ZnO), and Titanium dioxide (TiO_2), exert biochemical toxicity against insects and induce oxidative stress within insect cells through the production of reactive oxygen species (ROS). These ROS damage vital cellular components such as proteins, lipids, and DNA, disrupting enzyme activity, cell signalling, and mitochondrial function, which ultimately leads to oxidative cell death. (Mohmed (2025). When combined with bioactive compounds or pesticides, nanoparticles enhance uptake and targeted delivery, increasing their efficacy at lower doses. Nanoparticles also interfere with critical detoxification enzymes, including glutathione S-transferase (GST), cytochrome P450 monooxygenases, and acetylcholinesterase (AChE), impairing metabolic processes and neural activity, potentially causing paralysis or death (Jafir *et al.* (2023). Moreover, they can alter gene expression related to reproduction, stress responses,

and cuticle formation, resulting in developmental delays, sterility, and moulting problems. Metal nanoparticles additionally bind to sulfur and phosphorus groups in proteins and nucleic acids, compromising membrane integrity, denaturing enzymes, and disrupting organelle function, leading to cytoplasmic leakage, metabolic failure, and programmed cell death. Gold and silver nanoparticles can also trigger caspase-mediated apoptotic pathways, while combinations such as Ag–TiO_2 composites enhance toxicity synergistically by increasing ROS generation and enzyme inhibition, amplifying stress responses and mortality in insects. Silica nanoparticles, synthesized via sol–gel or green methods, cause mortality by disrupting insect cuticles and impairing respiration when applied as dust or fumigant coatings (Jasrotia *et al.* (2023). Combining SiO_2 nanoparticles with botanicals such as neem extract reduces egg laying and enhances pest deterrence (Saw *et al.* (2023).

Effects on Insect Physiology and Behavior

Nanoparticles can significantly impact insects' physiology and behavior. When nanoparticles interact with insect tissues, they can cause oxidative stress and enzyme inhibition, disrupting essential functions like breathing, digestion, and reproduction. This can lead to suppressed growth, deformities, and increased mortality rates. Insects exposed to nanoparticles may also experience hormonal imbalances, affecting their development and fertility. Moreover, nanoparticles can alter insects' behavior, making them less likely to feed or lay eggs on treated surfaces (Zhang *et al.* (2019). Some nanoparticles can even interfere with their nervous system, causing disorientation, sluggish movement, and coordination problems. These effects can disrupt communication and mating behaviors, ultimately reducing insect populations and making nanoparticles a promising tool for sustainable pest control. By understanding how nanoparticles interact with insects, we can develop more effective and environmentally friendly solutions for managing pests (Ansari *et al.* (2025).

Advances in Nano-Encapsulation and Controlled Release

Recent advances in nano-encapsulation and controlled release technologies have transformed the development of more efficient, safer, and sustainable pest control formulations. Nano-encapsulation involves enclosing biologically active compounds such as essential oils, or plant extracts within nanocarriers designed to protect them from premature degradation and to enable gradual release under specific environmental or physiological conditions (Suparna *et al.* (2022). These nanocarriers can be composed of materials like biodegradable polymers (e.g., chitosan, alginate, polyethylene glycol) or natural proteins (e.g., zein), which ensure compatibility with ecosystems and minimize residue persistence in soil and water. In order to synchronize the release of active chemicals with pest activity or environmental stimuli like temperature, pH, or the presence of enzymes, controlled release mechanisms control the diffusion or disintegration of the encapsulating material. For example, it has been demonstrated that pH-controlled carriers and

temperature-responsive micelle systems only release insecticides when they reach the midgut of insects or at stages of the pest life cycle. These "smart release" technologies increase efficiency, sustain long-term efficient pest control, and extend the bioavailability of the active ingredient. The advantages of nano-encapsulation go beyond efficiency. By stabilizing volatile or photosensitive compounds such as essential oils or botanical insecticides (for example, azadirachtin), nanocarriers prevent rapid degradation due to UV exposure or oxidative stress, allowing for a consistent and sustained insecticidal effect. Moreover, encapsulated formulations reduce the required frequency and dosage of pesticide applications, which not only decreases production costs but also mitigates environmental contamination and non-target toxicity. Co-encapsulation techniques where multiple active agents such as neem oil and garlic extract are combined in a single nanocarrier further improve pest mortality by providing synergistic effects and multi-target action (Manjul *et al.* (2025).

Comparative Performance with Conventional Insecticides

Nanoparticles have shown promising potential as safer and more effective alternatives to conventional insecticides in stored grain protection. Unlike traditional chemicals that often degrade rapidly and require frequent reapplication, nanoparticle-based formulations exhibit greater stability and longer residual activity. Their small particle size allows better adhesion to grain surfaces and enhanced penetration into insect cuticles, resulting in improved contact toxicity and sustained pest control efficiency. In comparison, conventional insecticides may lead to problems such as pest resistance, chemical residues, and adverse effects on non-target organisms. Nanoparticles, on the other hand, offer controlled release and targeted action, reducing the amount of active ingredient needed for effective pest suppression. Moreover, they minimize environmental contamination and human health risks, making them a sustainable component in modern integrated pest management (IPM) programs (Kale *et al.* (2021).

Future Prospects and Sustainable Approaches

Integration of nanotechnology into IPM (Integrated Pest Management)

The integration of nanotechnology into Integrated Pest Management (IPM) offers innovative and sustainable strategies for controlling stored grain pests. Nanoparticles and nanoformulations can be effectively combined with traditional IPM components such as biological control agents, pheromone traps, and hermetic storage to enhance pest suppression while minimizing chemical use. The controlled release, target-specific delivery, and low environmental persistence of nanopesticides make them ideal tools for maintaining pest populations below economic thresholds without disturbing ecological balance (Albuquerque *et al.* (2022). Future prospects include the development of biodegradable and eco-friendly nanomaterials derived from plant-based or polymeric sources to ensure environmental safety and compatibility with food systems. Advances in nano-encapsulation, biosensing, and precision delivery systems will enable real-

time pest monitoring and site-specific application, reducing overuse of insecticides and post-harvest losses. By integrating nanotechnology with IPM principles, sustainable pest management can be achieved with improved efficacy, reduced resistance, and enhanced food security (Hafez *et al.* (2024).

Conclusion:

Stored grain pests continue to pose significant threats to global food security and the economy, causing both quantitative and qualitative losses. Traditional pest management strategies, although partially effective, often rely on chemical insecticides that can lead to the development of resistance, environmental contamination, and health concerns. The emergence of nanotechnology offers a promising alternative, providing innovative tools for sustainable pest control. Nanoparticles, through their unique physical and biochemical interactions, can effectively target insect pests, alter their physiology and behavior while minimizing adverse effects on non-target organisms. Advances in nanoparticle synthesis, nano-encapsulation, and controlled release have enhanced the efficacy, stability, and safety of pest control agents, demonstrating clear advantages over conventional insecticides. Furthermore, the integration of nanotechnology into Integrated Pest Management (IPM) frameworks holds substantial potential for developing environmentally friendly, efficient, and sustainable storage protection strategies. Overall, the convergence of nanotechnology with traditional pest management represents a forward-looking approach to reducing post-harvest losses, ensuring food safety, and sustainably supporting global food security.

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SPICES AS BIOACTIVE ALLIES FOR PROTECTING STORED GRAINS IN SUSTAINABLE PEST MANAGEMENT

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Abstract:
The preservation of stored grains represents a critical challenge to global food security, with postharvest insect infestations causing extensive economic and nutritional losses. Conventional reliance on synthetic pesticides has led to increasing concerns over resistance development, human toxicity, and environmental degradation. This chapter explores the emerging potential of spices as bioactive allies in sustainable pest management for stored grains. Drawing upon recent advances in entomology, phytochemistry, and formulation science, it synthesizes evidence on the insecticidal, repellent, and anti-feeding properties of key spices such as clove, cinnamon, garlic, neem, and turmeric. The discussion encompasses the mechanisms of action of their active metabolites including eugenol, cinnamaldehyde, and allicin, their synergistic roles in Integrated Pest Management (IPM), and technological innovations such as nanoencapsulation and biopolymer-based delivery systems. The chapter also examines socio-economic and regulatory dimensions affecting the commercialization of spice-based protectants, identifying opportunities for scaling within smallholder systems and global supply chains. By addressing existing research gaps in efficacy validation, formulation stability, and policy integration, this work positions spices as a realistic, safe, and sustainable alternative to synthetic grain protectants, advancing the broader transition toward ecologically sound pest management.

Keywords: Spice-Based Pesticides, Stored Grain Protection, Sustainable Pest Management, Botanical Insecticides, Integrated Pest Management, Bioactive Phytochemicals, Postharvest Loss Prevention, Eco-Friendly Pest Control, Grain Storage Biosecurity, Food Safety and Sustainability

Introduction:

The concept of food security has evolved from simply ensuring sufficient caloric supply to encompassing the quality, safety, and sustainability of food systems. Within this framework, postharvest protection of grains represents a vital but often neglected component of global food security. Each year, an estimated 20–30 % of grains produced in developing regions are lost to storage pests, moisture, and microbial contamination (FAO, 2021). While much attention has

been paid to agricultural productivity, fewer resources have been directed toward protecting harvested grain during storage, a period when the crop remains biologically vulnerable yet receives limited scientific scrutiny. In recent years, the discourse on sustainable pest management has expanded beyond synthetic pesticides to embrace botanical and bio-based alternatives. Among these, spices occupy a unique niche because of their historical familiarity, culinary safety, and reservoir of bioactive compounds with demonstrable pesticidal potential.

Insect pests such as *Sitophilus oryzae*, *Rhyzopertha dominica*, and *Tribolium castaneum* are responsible for severe quantitative and qualitative losses in stored grains. Traditional chemical control has relied on fumigants like phosphine and contact insecticides such as organophosphates and pyrethroids (Arthur and Campbell, 2019). However, the continued dependence on these chemicals has led to multiple problems, insecticide resistance, residue accumulation in food, and ecosystem disruption. Moreover, regulatory frameworks have increasingly restricted the use of several synthetic compounds because of environmental and human-health concerns (Machuca-Mesa *et al.,* 2024). The search for safe, cost-effective, and biodegradable alternatives has thus become a research imperative. Botanicals, especially those derived from spices, have gained prominence as natural pest protectants. Many spices traditionally used for culinary and medicinal purposes contain essential oils rich in terpenoids, alkaloids, and phenolics with insecticidal, repellent, and anti-feeding activities (Isman, 2020).

Several reviews and meta-analyses have explored the insecticidal potential of plant-derived products, including essential oils, crude extracts, and powders. Studies Isman (2020) and Rajashekhar *et al.,* 2012) systematically catalogued botanical species in stored-product pest management. More recent syntheses by Ayllón-Gutiérrez *et al.,* 2024) and Chaudhary *et al.,* 2017), emphasized encapsulation technologies and formulation stability. However, these reviews often aggregated data across all botanicals, offering limited discussion specific to spices and their unique phytochemical profiles. Many focused-on laboratory efficacy but lacked insights into sensory impacts, regulatory hurdles, and integration within Integrated Pest Management (IPM) frameworks. Furthermore, quantitative comparisons across commodities and pest species remain sparse, hindering the translation of findings into scalable interventions.

The current body of knowledge demonstrates the promise of spice-derived bioactives but still leaves several critical aspects insufficiently explored. There remains limited understanding of which specific compounds exhibit consistent and reproducible efficacy across different pest taxa and storage conditions. The influence of formulation techniques and delivery systems on the persistence, safety, and bioavailability of these compounds also requires deeper investigation. Moreover, socio-economic and policy mechanisms necessary for the responsible commercialization and large-scale adoption of spice-based protectants have yet to be adequately developed. This chapter addresses these gaps through an integrative review that weaves together

entomological, chemical, and socio-economic perspectives, moving beyond descriptive summaries to evaluate how spices can serve as realistic, sustainable, and culturally acceptable protectants for stored grains within modern pest-management paradigms.

The Postharvest Challenge of Insect Pests in Stored Grains

Stored-product insects exploit the stable microclimate and nutritional richness of stored commodities. Their activity generates metabolic heat, moisture, and carbon dioxide, creating conditions conducive to mold growth and toxin formation (Nayak and Daglish, 2018). Beyond physical loss, infestation lowers seed viability, alters grain color and odor, and introduces contaminants unacceptable to consumers and trade authorities (Bisheko *et al.,* 2023). Historically, fumigants such as methyl bromide offered broad-spectrum control but were phased out under the Montreal Protocol due to ozone-depletion concerns (UNEP, 2014). Phosphine subsequently became the mainstay fumigant, yet resistant populations of *S. oryzae* and *R. dominica* have emerged globally (Collins *et al.,* 2016)**.** Contact insecticides, though effective initially, raise toxicity and residue issues. The economic burden of chemical inputs and the risk of resistance underscore the urgency of developing alternative methods aligned with sustainability goals (Fields and White, 2019).

Spices as Sources of Bioactive Phytochemicals

Spices are remarkable repositories of volatile and non-volatile secondary metabolites evolved for plant defense. Their phytochemical complexity contributes to multifaceted insecticidal and repellent actions (Akbar *et al.,* 2023). Key examples include:

- **Clove (*Syzygium aromaticum*)**: Rich in eugenol, a phenolic compound with potent fumigant and contact toxicity. Eugenol interferes with octopaminergic neurotransmission and disrupts cellular respiration (Ibrahim *et al.,* 2023).
- **Cinnamon (*Cinnamomum zeylanicum*)**: Contains cinnamaldehyde and eugenol analogs exhibiting neurotoxic and anti-feeding activities (Liñán-Atero *et al.,* 2022).
- **Garlic (*Allium sativum*)**: Produces allicin and diallyl sulfides that inactivate thiol-dependent enzymes in insects, resulting in oxidative stress and mortality (Plata-Rueda *et al.,* 2017).
- **Neem (*Azadirachta indica*)**: Though not strictly a spice, neem's azadirachtin limonoids act as ecdysone antagonists that suppress molting and reproduction (Chaudhary *et al.,* 2017).
- **Turmeric (*Curcuma longa*)**: Yields curcuminoids and ar-turmerone that deter feeding and interfere with larval development (Akbar *et al.,* 2023).

These compounds collectively illustrate the biochemical diversity of spices. Their activity spans multiple pathways from neural signaling disruption to endocrine interference rendering them less prone to single-mechanism resistance (Isman and Grieneisen, 2014). Moreover, many possess

antimicrobial properties that mitigate fungal growth, offering dual benefits in grain preservation (Saad *et al.* 2020).

Mechanisms of Insecticidal Action of Spice-Derived Compounds

Understanding how spice-derived compounds affect insects is critical for rational application. The major mechanisms include neurotoxicity, metabolic inhibition, endocrine disruption, and behavioral modification.

- **Neurotoxic and neuromodulatory effects:** Phenylpropanoids like eugenol and cinnamaldehyde target octopamine and GABAergic receptor sites, producing hyperexcitation followed by paralysis (Ding *et al.,* 2023). Electrophysiological assays reveal that eugenol modulates calcium ion influx and interferes with acetylcholinesterase activity (Harmatha and Nowrat, 2016).
- **Metabolic inhibition and oxidative stress:** Organosulfur compounds in garlic induce oxidative imbalance by generating reactive oxygen species that damage mitochondrial membranes (Plata-Rueda *et al.,* 2017). Similarly, terpenoids in turmeric impede cytochrome P450-mediated detoxification, thereby amplifying toxicity (Koul *et al.,* 2020).
- **Endocrine disruption:** Azadirachtin affects hormonal pathways, reducing chitin synthesis and causing larval–pupal deformities. Such slow-acting physiological effects are advantageous for resistance management because they act at multiple developmental stages (Saxena *et al.,* 2018).
- **Behavioral repellency and oviposition deterrence:** Volatile compounds in clove and cinnamon alter olfactory receptor neuron firing, leading to avoidance behavior in *Tribolium* and *Callosobruchus* species (Kavallieratos *et al.,* 2023). By deterring colonization rather than killing outright, these compounds reduce selection pressure for resistance.

Laboratory and Semi-Field Evidence of Efficacy

A growing corpus of empirical data validates the pesticidal efficacy of spice-derived products. In controlled fumigation assays, eugenol nanoemulsions achieved over 90 % mortality in *S. oryzae* within 48 hours, surpassing crude oil performance (Ibrahim *et al.,* 2023). Garlic essential oil showed LC_{50} values of 0.23 mg cm^{-2} against *Tenebrio molitor*, confirming potent contact toxicity (Plata-Rueda *et al.,* 2017). Cinnamon oil and cinnamaldehyde demonstrate strong repellency and suppression of oviposition, while turmeric powders mixed with wheat grains significantly reduced *Tribolium* emergence after 90 (Akbar *et al.,* 2023).

Semi-field studies, though fewer, provide encouraging insights. In warehouse-scale trials, sachets containing encapsulated clove and cinnamon oilsmaintained *S. zeamais* mortality above 70 % for three months without altering grain flavor (Liñán-Atero*et al.,* 2022). Neem-garlic blends applied as surface coatings showed synergistic effects, extending protection duration (Chaudhary *et al.,*

2017). Nevertheless, efficacy varies with grain type, environmental conditions, and formulation, highlighting the importance of context-specific optimization (Rajashekhar *et al.,* 2012).

Advances in Formulation and Delivery Systems

Although the bioefficacy of spice-derived essential oils is well established, their practical use has been constrained by volatility, photodegradation, and limited persistence on grain surfaces. Formulation science has therefore emerged as a cornerstone of translational research. Nanoemulsions, microcapsules, and polymeric films have shown promise in stabilizing and controlling the release of volatile components (Ayllón-Gutiérrez *et al.,* 2024). These delivery systems not only improve persistence but also minimize sensory alteration in treated grains a crucial factor for consumer acceptance.

Recent advances in nanoemulsion technology have enabled the creation of droplet sizes below 200 nm, which increases surface area and enhances contact efficacy against insect cuticles (Kavallieratos *et al.,* 2023). Biodegradable surfactants, such as lecithin and chitosan, have further improved emulsification stability while ensuring food safety (Kumar *et al.,* 2025). Microencapsulation using natural polymers like alginate or gelatin creates a protective matrix that slows evaporation, maintaining fumigant action over extended periods (Maurya *et al.,* 2024). Moreover, biopolymer films impregnated with cinnamon or clove oil can serve as packaging materials that provide passive, long-term repellency (Sanches-Silva *et al.,* 2019). These innovations address two long-standing constraints, short residual life and odor intensity that previously limited adoption. Nonetheless, scalability remains a challenge, as encapsulation and nano-formulation require specialized equipment and quality control to maintain uniformity across production batches (Liñán-Atero*et al.,* 2022). Research into cost-effective encapsulation using agro-waste derivatives may therefore offer a sustainable path forward (Chowdhury *et al.,* 2021).

Integration into Sustainable and Integrated Pest Management Frameworks

For botanical protectants to achieve real-world relevance, they must function as complementary components within IPM rather than as stand-alone interventions. IPM combines preventive measures such as sanitation, hermetic storage, and temperature regulation with curative controls, applying botanicals or synthetic insecticides judiciously when thresholds are reached (Arthur and Campbell, 2019).

Spice-derived compounds are well suited to preventive roles due to their repellent and anti-ovipositional properties. In hermetic storage systems, for instance, sachets of clove or cinnamon essential oils can create a volatile barrier that deters entry of insects while maintaining safe oxygen levels (Yewle *et al.,* 2023). In community-level warehouses, spice formulations could complement biological control agents such as parasitoid wasps (*Theocolax elegans*) or entomopathogenic fungi (*Beauveria bassiana*), since sublethal doses of spice volatiles generally show minimal negative effects on beneficial species (Isman, 2020). However, the successful

incorporation of spice-derived formulations within integrated pest management frameworks necessitates comprehensive compatibility assessments. These studies are essential to confirm that the chemical volatiles emitted by spices complement, rather than compromise, the activity of microbial and biological control agents. Empirical validation of such synergistic or antagonistic interactions is critical, since some compounds may inadvertently suppress spore germination or influence parasitoid foraging and reproductive behavior (Koul *et al.,* 2020). Farmer training and extension programs are also vital to disseminate proper dosage, timing, and application methods, ensuring that adoption remains both safe and effective (Bisheko *et al.,* 2023).

Regulatory, Commercial, and Socio-Economic Perspectives

Despite increasing evidence of efficacy, regulatory frameworks for botanical pesticides remain fragmented. Many spice-based products are classified ambiguously between food additives and pesticides, complicating their registration and trade (Machuca-Mesa *et al.,* 2024). Harmonizing global standards for botanical pesticide registration including toxicological, residue, and efficacy data is crucial to mainstream adoption (EPA, 2023).

Commercialization efforts are emerging and startups in India, Brazil, and Kenya have piloted small-scale production of clove and neem-based grain protectants using locally sourced biomass (Kumar *et al.,* 2020). These ventures highlight the socio-economic value chain potential from spice farmers to small manufacturing units creating livelihood opportunities while reducing reliance on imported synthetic chemicals. The cost-benefit ratio often favors botanicals when considering health and environmental externalities, though up-front production costs can be higher due to formulation complexity (Chaudharida *et al.,* 2021).

Consumer perception also influences market viability. Since spices are associated with food and culture, their application in pest control elicits less resistance than “chemical” insecticides. Transparent labeling, demonstration projects, and farmer cooperatives could further enhance acceptance (Yewle *et al.,* 2023). Policymakers must recognize that promoting local production of spice-based biopesticides aligns with circular-economy and bio-innovation objectives, particularly within the global south.

Research Priorities and Future Directions

While the potential of spice-derived protectants for stored grain management is increasingly recognized, their translation from laboratory innovation to field-scale application remains limited. The path toward broader adoption requires a concerted effort to refine scientific understanding, improve formulation technologies, and establish enabling regulatory and socio-economic frameworks. As research in entomology, material science, and food safety converges, identifying key priorities will be essential to accelerate progress and ensure that spice-based interventions achieve both efficacy and sustainability in real-world storage systems. The following section outlines the critical research directions that can bridge existing gaps and guide the next generation of innovations in this promising field**.**

- **Standardization of Phytochemical Profiles:** Variability in spice oil composition due to climate, soil, and processing methods complicates reproducibility (Isman and Grieneisen (2014). Establishing chemotype-specific reference standards would enable consistent formulation and efficacy testing.
- **Mechanistic and Omics-Level Insights:** Genomic and proteomic analyses of insect responses to spice actives can elucidate detoxification pathways, guiding rational combination strategies ((Harmatha and Nowrat (2002).
- **Formulation Optimization:** Continued innovation in nanoencapsulation, biodegradable carriers, and controlled-release systems is essential to balance efficacy, cost, and safety (Ayllón-Gutiérrez *et al.,* 2024).
- **Field Validation and Sensory Analysis:** Large-scale warehouse and farmer-level trials must quantify long-term performance, while parallel studies evaluate impacts on taste, odor, and consumer acceptance (Liñán-Atero *et al.,* 2022).
- **Regulatory and Policy Research:** Comparative analyses of pesticide registration systems could inform streamlined approval pathways for botanical products across regions (EPA (2023).
- **Socio-Economic Assessment**: Participatory approaches involving farmers, traders, and policymakers will ensure that technology development aligns with user needs and local contexts (Bisheko *et al.,* 2023).

Through such coordinated efforts, the promise of spice-derived protectants can evolve from isolated laboratory successes into a mainstream pillar of sustainable pest management.

Conclusion:

Spices offer a compelling convergence of tradition and innovation in the quest for sustainable pest management. Their rich phytochemical diversity confers multi-target insecticidal activity, while their cultural familiarity and biodegradability align with global sustainability goals. Although challenges persist, particularly concerning standardization, persistence, and regulation, the rapid progress in formulation science and growing policy interest in green pesticides signal a transformative shift. As research deepens and cross-sector partnerships strengthen, spices may soon attain a transition from kitchen staples to critical agents to safeguard the world's grain reserves.

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SEASONAL VARIATION IN PHYSICOCHEMICAL PARAMETERS AND THEIR INFLUENCE ON AQUATIC INSECT ASSEMBLAGES IN A WESTERN MAHARASHTRA LAKE

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Abstract:

This study investigates the seasonal dynamics of aquatic insect communities in relation to water quality parameters at Sir Piraji Rao Lake, Murgud, Kolhapur District, Maharashtra, from June 2022 to May 2023. Monthly sampling was carried out using standard littoral and benthic collection methods, while physicochemical parameters of the lake were analyzed following APHA (2017) protocols. The aquatic insect fauna recorded during the study period belonged to Coleoptera, Hemiptera, Odonata, Ephemeroptera, Diptera, and Trichoptera, with marked variation in species composition and abundance across seasons. Diversity indices indicated higher species richness and community evenness during the post-monsoon season, whereas comparatively lower diversity during the pre-monsoon period corresponded with increased temperature and reduced dissolved oxygen levels. Correlation analysis revealed that Shannon diversity and evenness indices were negatively associated with temperature and BOD but positively associated with dissolved oxygen, suggesting that oxygen availability is a key factor shaping community structure. The overall findings emphasize that seasonal shifts in physicochemical conditions significantly influence aquatic insect assemblages and reaffirm the value of these organisms as sensitive bioindicators of freshwater ecosystem health. The study provides baseline ecological data that may guide future monitoring, conservation, and management strategies in freshwater habitats of the Kolhapur region.

Keywords: Aquatic Insects, Bioindicators, Diversity Indices, Physicochemical Parameters, Seasonal Variation, Sir Piraji Rao Lake.

Introduction:

Freshwater ecosystems such as lakes, reservoirs, ponds, and wetlands represent some of the most dynamic and productive habitats on Earth, supporting a wide array of biological communities and providing essential ecosystem services including water supply, fisheries, nutrient cycling, and climate regulation (Wetzel, 2001). Aquatic insects constitute a significant component of

these systems, playing crucial roles in food web dynamics, energy transfer, and nutrient processing (Contreras-Ramos, 2010). Their sensitivity to environmental changes makes them effective bioindicators for assessing ecological integrity and monitoring anthropogenic disturbances (Lenat, 1993).

In India, freshwater reservoirs contribute substantially to biodiversity conservation and community livelihoods, yet many are increasingly vulnerable to eutrophication, agricultural runoff, waste discharge, and hydrological alterations (Kubendran and Ramesh 2016). Seasonal variation, particularly in tropical regions, exerts a strong influence on aquatic insect assemblages, as fluctuations in rainfall, temperature, and water chemistry directly shape habitat structure and resource availability (Subramanian and Sivaramakrishnan, 2007). Understanding these seasonal dynamics is therefore essential for evaluating ecosystem stability and developing sustainable conservation strategies.

Maharashtra hosts numerous medium and minor reservoirs that support rich aquatic fauna, but comprehensive ecological assessments remain limited, especially in rural landscapes where local communities depend on these water bodies for agriculture, fisheries, and domestic needs (Sangve, 2020). Within Kolhapur district, Sir Piraji Rao Lake at Murgud represents a significant freshwater system influenced by monsoonal hydrology and surrounding land-use patterns. Despite its ecological and socio-economic importance, baseline ecological data on aquatic insect diversity and water quality from this lake are scarce, creating a gap in scientific understanding and management planning.

Given the strong link between aquatic insect diversity and environmental gradients, integrating biological assessment with physicochemical analysis can provide a more holistic evaluation of lake ecosystem health (Bonada *et al.,* 2006). The present study addresses this need by investigating seasonal variation in aquatic insect communities and major water quality parameters at Sir Piraji Rao Lake, Murgud. The findings aim to contribute to ecological monitoring frameworks and support the development of region-specific freshwater conservation measures.

Materials and Methods:

A. Study Area

The present study was conducted at Sir Piraji Rao Lake, situated Murgud in Kagal Taluka of Kolhapur District, Maharashtra, India (Fig.1). The lake is a medium-sized freshwater ecosystem primarily sustained by monsoonal rainfall and local catchment inflow. It supports diverse aquatic biota and is influenced by agricultural runoff and domestic activities in the adjoining areas. Sampling sites were selected from different zones of the lake to represent variation in habitat characteristics such as depth, vegetation, and anthropogenic disturbance.

B. Study Period

The study was carried out from June 2022 to May 2023, covering three major seasons: Pre-monsoon (February–May), Monsoon (June–September), and Post-monsoon (October–January). Seasonal sampling facilitated the assessment of changes in aquatic insect community structure and water quality dynamics.

C. Collection of Aquatic Insects

Aquatic insects were collected monthly during the study period using a D-frame aquatic net (mesh size 500–800 µm), kick sampling, and hand-picking methods to cover both littoral and benthic habitats. The collected specimens were sorted on-site in white enamel trays and preserved in 70% ethanol. Each collection was properly labelled with details of location, date, and habitat type.

D. Identification of Aquatic Insects

In the laboratory, specimens were examined under a stereo-zoom binocular microscope and identified to the lowest possible taxonomic level using standard taxonomic references. The principal identification keys included *Aquatic Insects of North America* (Contreras-Ramos, 2010), *Aquatic Insects of India* (Subramanian and Sivaramakrishnan, 2007), *Freshwater Biology* (Schwoerbel, 2016) and *Freshwater Invertebrates of the United States* (Smith, 2001). Diagnostic morphological features such as body segmentation, wing venation, antennae, gills, and leg structures were used for accurate classification.

E. Water Quality Analysis

Water samples were collected fortnightly with insect sampling from surface water (0–30 cm depth) using pre-cleaned polyethylene bottles. On-site measurements included temperature (°C) using a mercury thermometer, pH using a portable digital meter, and transparency using a Secchi disc. Laboratory analysis included Dissolved Oxygen (DO) by Winkler's method, Biological Oxygen Demand (BOD) by the 5-day incubation method, Total Dissolved Solids (TDS) using a digital meter, and Nitrate and Phosphate concentrations using spectrophotometric techniques. All analyses followed APHA (2017) Standard Methods for the Examination of Water and Wastewater.

F. Data Analysis

Quantitative data were used to compute community structure indices including Shannon–Wiener Diversity Index (H'), Simpson's Diversity Index (1–D), Margalef Richness Index (d), and Pielou's Evenness Index (J'). Correlation matrices were established between physicochemical parameters and diversity indices to assess relationships among variables. All statistical analyses were performed using PAST software version 4.03 (Hammer *et al.,* 2001).

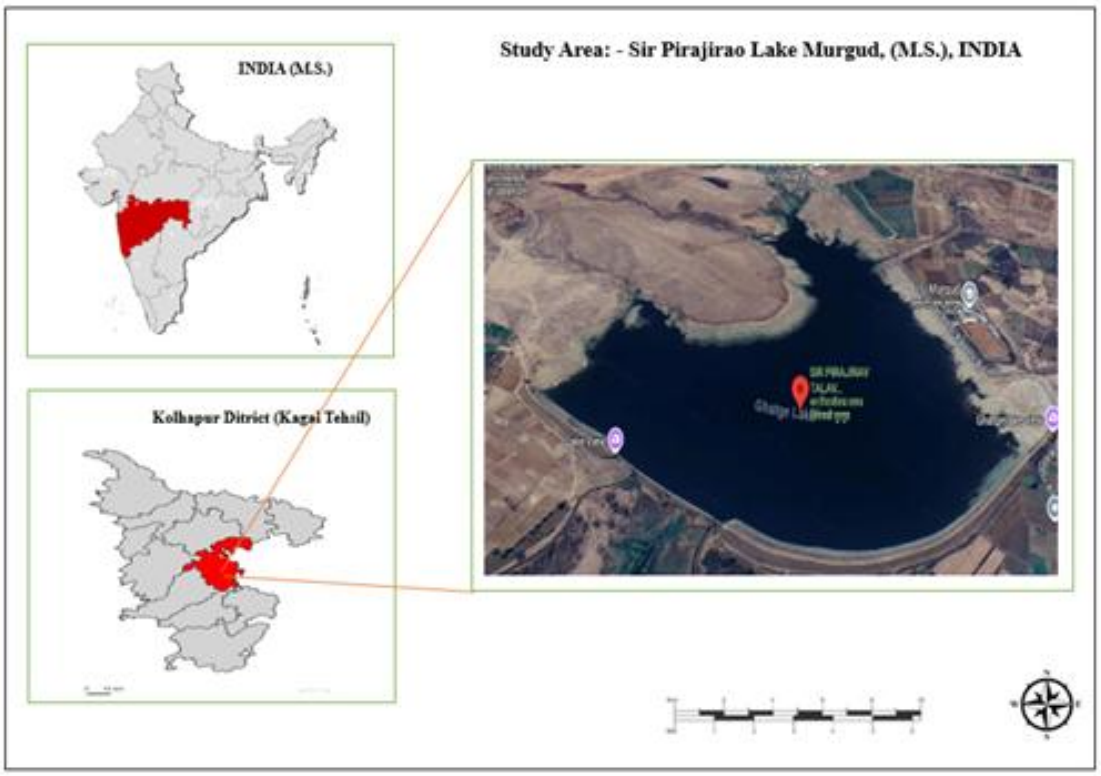

Figure 1: Location map of Sir Pirajirao Lake, Murgud, Kolhapur District, Maharashtra

Results:

Table 1 presents the seasonal fluctuation in the physicochemical properties of Sir Piraji Rao Lake, Murgud, showing distinct variations influenced by climatic conditions. Water temperature showed a marked rise during summer (31.7 ± 1.5°C) compared to winter (21.3 ± 1.2°C) and monsoon (26.5 ± 1.3°C), which is likely due to increased solar radiation and reduced water volume. The pH remained slightly alkaline in summer (8.3 ± 0.4), while winter (7.2 ± 0.3) and monsoon (6.9 ± 0.2) recorded comparatively lower values, indicating seasonal shifts in buffering capacity. Transparency was highest in winter (92.6 ± 3.1 cm) and lowest during the monsoon (42.3 ± 2.1 cm), reflecting the influence of suspended particles brought by rainwater runoff. Dissolved oxygen (DO) reached its maximum in monsoon (8.6 ± 0.7 mg/L), while biological oxygen demand (BOD) peaked in summer (4.7 ± 0.4 mg/L), suggesting higher biological activity and organic load during this season. Similarly, TDS and total hardness values were elevated in summer, possibly due to evaporation and concentration effects. Nutrient levels showed seasonal enrichment, with nitrate and phosphate concentrations increasing notably during the monsoon, likely as a result of agricultural runoff and watershed inflow. The obtained F-values and significance levels indicate that most parameters varied significantly across seasons, confirming that seasonal dynamics play a crucial role in regulating the lake's water quality.

Table 1: Seasonal Variation in Physicochemical Parameters of Sir Piraji Rao Lake, Murgud

Parameter	Unit	Winter (Mean ± SD)	Summer (Mean ± SD)	Monsoon (Mean ± SD)	F-value	Significance
Temperature	°C	21.3 ± 1.2	31.7 ± 1.5	26.5 ± 1.3	6.42	$p < 0.05$
pH	–	7.2 ± 0.3	8.3 ± 0.4	6.9 ± 0.2	4.56	$p < 0.05$
Transparency	cm	92.6 ± 3.1	67.4 ± 2.9	42.3 ± 2.1	11.83	$p < 0.01$
DO	mg/L	7.4 ± 0.6	4.2 ± 0.4	8.6 ± 0.7	9.28	$p < 0.01$
BOD	mg/L	2.3 ± 0.3	4.7 ± 0.4	1.9 ± 0.2	7.35	$p < 0.05$
TDS	mg/L	184 ± 8.4	238 ± 12.6	169 ± 9.2	8.72	$p < 0.05$
Total Hardness	mg/L	212 ± 10.8	276 ± 15.2	194 ± 11.5	5.46	$p < 0.05$
Nitrate	mg/L	0.41 ± 0.03	0.28 ± 0.02	0.73 ± 0.04	10.18	$p < 0.01$
Phosphate	mg/L	0.21 ± 0.02	0.14 ± 0.02	0.47 ± 0.03	8.04	$p < 0.01$

A total of 39 aquatic insect taxa representing the orders Coleoptera, Hemiptera, Odonata (dragonflies and damselflies), Ephemeroptera, Diptera, and Trichoptera were recorded from Sir Piraji Rao Lake across the three seasons (Table 2). Coleopteran species such as *Callosobruchus maculatus* (Fabricius, 1775), *C. chinensis* (Linnaeus, 1758), and *Zabrotes subfasciatus* (Boheman, 1833) were observed to be moderate to common during the monsoon, indicating favorable humidity and vegetation conditions. Similarly, cerambycid beetles including *Olenecamptus bilobus* (Fabricius, 1801) and *Stibara nigricornis* (Fabricius, 1781) showed increased abundance during monsoon and post-monsoon. Hemipteran reduviids such as *Acanthaspis fulvipes* (Dallas, 1850), *Acanthaspis sexguttata* (Fabricius, 1775) and *Coranus fuscipennis* Reuter, 1881 exhibited seasonal fluctuations, with several species showing higher abundance during monsoon due to increased prey availability. Odonates were prominent throughout the study, particularly dragonflies of the family Libellulidae, where species like *Orthetrum sabina* (Drury, 1770), *Crocothemis servilia* (Drury, 1770), *Diplacodes trivialis* (Rambur, 1842) and *Pantala flavescens* (Fabricius, 1798) remained common in almost all seasons, reflecting their ecological adaptability. Damselflies including *Ceriagrion coromandelianum* (Fabricius, 1798) and *Agriocnemis pygmaea* (Rambur, 1842) were more abundant in monsoon and post-monsoon, corresponding to emergent aquatic vegetation growth. Ephemeropteran taxa such as *Labiobaetis soldani* Kubendran *et al.,* 2014 and *Baetis* spp. showed increased abundance after the monsoon, likely due to reduced water turbidity. Dipteran vectors *Culex quinquefasciatus* Say, 1823 and *Aedes aegypti* (Linnaeus, 1762) were common during monsoon and post-monsoon, indicating the presence of suitable breeding microhabitats.

Trichopteran caddisflies like *Hydropsyche rizali* Banks, 1937 and *Cheumatopsyche apicalis* (Banks, 1903) showed similar post-monsoon increases, suggesting improved water clarity and substrate stability. Overall, the observed seasonal patterns demonstrate that hydrological conditions, nutrient influx, and habitat structure strongly influence the composition and abundance of aquatic insect communities in the lake ecosystem.

Table 2: Taxonomic Composition and Seasonal Abundance of Aquatic Insects at Sir Piraji Rao Lake

Sr.	Order	Family	Scientific Name (with Author and Year)	Pre-Monsoon	Monsoon	Post - Monsoon
1	Coleoptera	Chrysomelidae	*Callosobruchus maculatus* (Fabricius, 1775)	M	C	M
2		Chrysomelidae	*Callosobruchus chinensis* (Linnaeus, 1758)	M	C	R
3		Chrysomelidae	*Callosobruchus analis* (Fabricius, 1781)	R	M	R
4		Chrysomelidae	*Zabrotes subfasciatus* (Boheman, 1833)	M	M	M
5		Bruchidae	*Bruchus pisorum* (Linnaeus, 1758)	R	M	R
6		Cerambycidae	*Olenecamptus bilobus* (Fabricius, 1801)	R	M	M
7		Cerambycidae	*Stibara nigricornis* (Fabricius, 1781)	R	R	M
8		Cerambycidae	*Glenea spilota* (Thomson, 1860)	R	R	M
9		Scarabaeidae	*Onthophagus ludio* Boucomont, 1914	M	C	M

10		Elateridae	*Melanoxanthus anticus* (Candèze, 1892)	M	M	C
11		Chrysomelidae	*Chaeridiona metallica* (Baly, 1869)	R	M	C
12		Tenebrionidae	*Spathomeles anceps* Gorham, 1895	R	M	M
13	Hemiptera	Reduviidae	*Acanthaspis fulvipes* (Dallas, 1850)	R	M	C
14		Reduviidae	*Acanthaspis rugulosa* Stål, 1863	R	R	M
15		Fulgoridae	*Pyrops delessertii* (Guérin-Ménéville, 1840)	R	R	C
16		Aleyrodidae	*Paraleyrodes minei* Iaccarino, 1971	M	C	M
17		Reduviidae	*Coranus fuscipennis* Reuter, 1881	R	M	R
18		Reduviidae	*Ectomocoris cordiger* Stål, 1866	R	R	M
19		Reduviidae	*Acanthaspis sexguttata* (Fabricius, 1775)	R	R	M
20		Reduviidae	*Acanthaspis flavipes* Stål, 1855	R	M	M
21	Odonata (Dragonfly)	Libellulidae	*Orthetrum sabina* (Drury, 1770)	M	C	C

22		Libellulidae	*Orthetrum pruinosum* (Burmeister, 1839)	M	M	C
23		Libellulidae	*Crocothemis servilia* (Drury, 1770)	M	C	C
24		Libellulidae	*Diplacodes trivialis* (Rambur, 1842)	M	C	M
25		Libellulidae	*Pantala flavescens* (Fabricius, 1798)	C	C	C
26		Libellulidae	*Brachydiplax chalybea* (Brauer, 1868)	M	M	C
27		Libellulidae	*Neurothemis fulvia* (Drury, 1773)	M	M	C
28	Odonata (Damselfly)	Coenagrionidae	*Ceriagrion coromandelianum* (Fabricius, 1798)	M	M	C
29		Coenagrionidae	*Ischnura elegans* (Vander Linden, 1820)	R	M	M
30		Coenagrionidae	*Agriocnemis pygmaea* (Rambur, 1842)	M	C	C
31		Coenagrionidae	*Pseudagrion microcephalum* (Rambur, 1842)	M	M	C
32		Lestidae	*Lestes elatus* (Hagen, 1862)	R	M	C
33	Ephemeroptera	Baetidae	*Labiobaetis soldani* Kubendran *et al.,* 2014	M	R	C
34		Baetidae	*Baetis* spp.	M	M	C

35	Diptera	Muscidae	*Musca domestica* Linnaeus, 1758	C	C	M
36		Culicidae	*Culex quinquefasciatus* Say, 1823	M	C	C
37		Culicidae	*Aedes aegypti* (Linnaeus, 1762)	M	C	C
38	Trichoptera	Hydropsychidae	*Hydropsyche rizali* Banks, 1937	M	R	C
39		Hydropsychidae	*Cheumatopsyche apicalis* (Banks, 1903)	M	R	C

(**C** = Common, **M** = Moderate, **R** = Rare)

Table 3: Seasonal Diversity Indices of Aquatic Insects at Sir Piraji Rao Lake, Murgud

Season	Shannon Diversity (H')	Simpson Diversity (1–D)	Margalef Richness (d)	Evenness (J')	Total Relative Abundance
Pre-Monsoon	3.60	0.97	9.14	0.98	64
Monsoon	3.60	0.97	8.65	0.98	81
Post-Monsoon	3.62	0.97	8.36	0.99	94

The seasonal variation in diversity indices of aquatic insects at Sir Piraji Rao Lake as per shown in table 3. The Shannon diversity index (H') remained consistently high across all seasons, with values ranging from 3.60 to 3.62, indicating a well-structured and stable insect community. Simpson's diversity index (1–D) also showed very high values (0.97 in all seasons), suggesting low dominance and a balanced distribution of species without a single taxon overwhelmingly prevailing. Margalef's richness index (d) showed a slight decline from pre-monsoon (9.14) to post-monsoon (8.36), indicating that although species richness was highest before monsoon, a relatively large number of species continued to persist throughout the year. Evenness (J') values were close to 1.0 in all seasons, reflecting that species were uniformly distributed in their relative abundance. Total relative abundance increased steadily from pre-monsoon (64) to monsoon (81) and was highest during the post-monsoon period (94), which may be attributed to favorable habitat conditions and increased availability of food resources following rainfall. Overall, the indices demonstrate a diverse, evenly structured, and seasonally responsive aquatic insect community in the lake ecosystem.

Table 4: Correlation Matrix: Diversity Indices vs. Major Water Quality Parameters

Parameter	H’	1–D	d	J’	Temp.	DO	BOD	Nitrate	Phosphate
Shannon (H’)	1	0.99	**–0.70**	1.00	**–0.80**	0.14	–0.27	–0.35	–0.42
Simpson (1–D)	0.99	1	**–0.78**	0.99	**–0.87**	0.26	–0.38	–0.24	–0.31
Margalef (d)	**–0.70**	**–0.78**	1	**–0.70**	**0.99**	**–0.80**	**0.88**	–0.42	–0.35
Evenness (J’)	1.00	0.99	**–0.70**	1	**–0.80**	0.14	–0.27	–0.35	–0.42
Temperature	**–0.80**	**–0.87**	**0.99**	**–0.80**	1	–0.70	**0.79**	–0.28	–0.20
DO	0.14	0.26	**–0.80**	0.14	–0.70	1	**–0.99**	**0.88**	**0.84**
BOD	–0.27	–0.38	**0.88**	–0.27	**0.79**	**–0.99**	1	**–0.81**	**–0.76**
Nitrate	–0.35	–0.24	–0.42	–0.35	–0.28	**0.88**	**–0.81**	1	**0.99**
Phosphate	–0.42	–0.31	–0.35	–0.42	–0.20	**0.84**	**–0.76**	**0.99**	1

Table 4 presents the correlation relationships between aquatic insect diversity indices and key physicochemical parameters of Sir Piraji Rao Lake. Shannon diversity (H’) and evenness (J’) showed a strong positive relationship with Simpson’s diversity index (1–D), indicating that the community structure remained uniform and balanced across sampling periods. A negative correlation was observed between H’, 1–D and temperature, suggesting that higher temperatures were associated with a decrease in overall diversity. Conversely, Margalef’s richness index (d) showed a strong positive correlation with temperature and BOD, indicating that warmer conditions and increased organic load supported a higher number of species, even if distribution among species became less even. Dissolved oxygen demonstrated a positive relationship with H’ and 1–D but a strong negative correlation with BOD, reflecting typical ecological oxygen-demand dynamics. Nitrate and phosphate levels showed moderate negative correlations with diversity indices but strong positive correlations with each other, suggesting linked nutrient influx.

The results of the present study demonstrate that both water quality parameters and seasonal hydrological changes play a significant role in shaping the composition, abundance, and diversity of aquatic insect communities at Sir Piraji Rao Lake, Murgud. The physicochemical characteristics of the lake varied distinctly across seasons, and these variations were reflected in the distribution patterns of different insect taxa. Diversity indices indicated a well-structured and stable community with high species richness and evenness throughout the year, although abundance increased notably during the post-monsoon period. Correlation analysis further confirmed that temperature, dissolved oxygen, and nutrient levels are critical determinants influencing species richness and community balance. These findings emphasize the sensitivity of aquatic insects to environmental fluctuations and highlight their value as effective biological indicators for assessing freshwater ecosystem health.

Discussion:

The present study reveals that the aquatic insect communities at Sir Piraji Rao Lake in Murgud are clearly influenced by seasonal changes in water quality parameters. The strong negative correlation between diversity indices such as Shannon's H' and temperature suggests that increased thermal stress may reduce community evenness and favour dominance by a few tolerant taxa (Ghosh, 2015). Similarly, the positive correlation between marginal richness (d) and temperature in our data echoes findings by Mandape and Kamdi (2022) in Maharashtra, where warmer summer conditions increased species richness though often lowered evenness.

Dissolved oxygen (DO) and biological oxygen demand (BOD) also emerged as key predictors of community structure: higher BOD and lower DO coincided with reduced diversity and increased dominance of tolerant taxa, a pattern widely observed in biomonitoring literature (Barman and Gupta 2015). Our nutrient-diversity relationships negative correlations of nitrate and phosphate with diversity indices mirror the outcomes from a hilly-river study in northeast India that documented nutrient spikes post-monsoon linked to reduced taxonomic evenness (Chakravarty and Gupta, 2024). These results align with global findings that nutrient enrichment and organic loading shift macroinvertebrate assemblages toward fewer, more tolerant species (Omoigberale *et al.,* 2020).

Seasonal abundance increases post-monsoon in our study correspond with patterns observed in other Indian freshwater bodies. A lake survey in Karnataka reported higher insect abundance after rainfall, attributing this to increased habitat complexity and emergent vegetation (Abhilash, 2023). In Maharashtra, studies in Chandrapur recorded species peaking in post-monsoon and linked those patterns to habitat renewal and stable water levels (Tekade and Telkhade, 2024). These parallels suggest that the seasonal hydrological regime particularly the monsoon and its aftermath plays a major role in structuring insect communities in tropical lentic systems.

At the regional scale, Maharashtra studies (Pauni, Bhandara) recorded 25 species of aquatic insects across five orders and found temperature, pH, DO, BOD, and TDS to influence insect diversity (Mandape and Kamdi, 2022). In the Kolhapur area, though quantitative data are fewer, odonate surveys at Rankala Lake show that emergent vegetation and stable littoral habitats support higher dragonfly richness across seasons, consistent with our observation of persistent Libellulidae dominance despite seasonal change. Thus, the present lake's insect assemblage appears to follow similar regional ecological gradients while adding specific insight into diversity water quality coupling.

Globally, stream and lake studies have long established that hydrology, water chemistry, and habitat structure jointly determine macroinvertebrate diversity and community composition (Wallace and Webster, 1996; Bogan and Lytle, 2007). Our correlation matrix further supports the mechanistic link between abiotic filters (temperature, nutrients, oxygen) and biotic responses,

reinforcing the value of integrative monitoring approaches that combine physicochemical and biological metrics (Wright *et al.,* 2007).

From a management perspective, the results highlight the need to monitor catchment nutrient inputs, maintain littoral habitat complexity (emergent vegetation, substrate heterogeneity), and preserve seasonal hydrological dynamics. In particular, controlling organic load and nutrient enrichment may help maintain evenness and resist dominance by tolerant taxa a strategy endorsed in national guidelines for freshwater biomonitoring (Chutia and Kardong, 2022). Further, since our study is based on one annual cycle, longer-term monitoring will be essential to detect trends arising from climate change or land-use alteration.

Conclusion:

The present study revealed that the aquatic insect community of Sir Piraji Rao Lake shows clear seasonal variation influenced by changes in water quality. Species richness and abundance were highest in the post-monsoon period, when habitat conditions were more favorable, while lower diversity in the pre-monsoon season corresponded with higher temperature and reduced dissolved oxygen. The observed correlations between diversity indices and key parameters such as temperature, DO, BOD, nitrates, and phosphates confirm that aquatic insects serve as effective indicators of ecological health. These findings emphasize the need to maintain water quality and protect shoreline vegetation to preserve the ecological balance of the lake and provide useful baseline data for future monitoring and conservation efforts in the region.

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INTEGRATED MANAGEMENT OF SORGHUM SHOOT FLY

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Introduction:

Sorghum is part of major millet among all the millet, and it is originated in Sudan (Anonymous) country, and its domestication has started in East Central Africa around 3000 BC. Most of the sorghum cultivation can be seen in tropical and sub-tropical regions. It is the world's fifth-most important cereal crop after rice, wheat, maize, and barley (Awika *et al*., 2011).

In India, the sorghum producing states are Maharashtra, Karnataka, Madhya Pradesh, Andhra Pradesh, Telangana, Tamil Nadu, Gujarat, Uttar Pradesh, Rajasthan and Haryana. Among these Maharashtra, Gujarat, Rajasthan and Tamil Nadu are the major sorghum growing states. In India, sorghum is important growing major millet crop, with an annual production in the year 2024-25 with area including (*kharif, rabi and* summer) 40,76,000 ha, production 47,37,000 tonnes and productivity 1162 kg/ha. In *kharif* (2024-25) the production details of sorghum in India as area 19.19 lakh ha, production 21.96 lakh tonnes and productivity 1144 kg/ha. In Gujarat, the production details including all the seasons (*kharif, rabi* and summer) area 42,660 ha, production 66,130 tonnes, and productivity 1550 kg/ha and the area under sorghum production includes 4.52 lakh ha, production 5.53 lakh tonnes and productivity of 1144 kg/ha (Anon., 2024).

Sorghum is important fodder and food crop in the world and used as fodder to feed millions of animals providing milk and meat for human beings. Sorghum grain is an important raw material for the starch industry and is also used as cattle and poultry feed and used in the production of starch, biscuits, sugar and alcohol. This grain is principal source of alcoholic beverages in many countries, and it is a very nutritious. Sorghum fodder contains more than 50 per cent digestible nutrients with 8 per cent protein, 2.5 per cent fat and 45 per cent nitrogen free extract. The demand for sorghum as a staple food is growing in recent years.

About 150 insect species have been recorded on sorghum including both in field as well as stored condition. Out of which 31 species are economically important. Damage inflicted by these cause serious losses (Daware *et al*., 2011). In India, shoot fly and stem borer significantly reduce the yield, at times as high as 100% in grain and crop stand (Padmaja *et al*., 2010). In northern dry zone of Gujarat, the insect pests namely, shoot fly: *Atherigona soccata* Rondani (Diptera: Muscidae), Sorghum stemborer: *Chilo partellus* Swinhoe (crambidae: leidoptera), Ear head worm: *Helicoverpa armigera* (Hubner) and aphids: *Melanaphis sacchari* (Zehntner) are the important regular pests.

The sorghum shoot fly, *A. soccata* causes severe damage in the early stage and lasts up to four weeks causing severe reduction in plant population. The shoot fly, *A. soccata* gets attracted from second to seventh leaf period of seedling and placed cigar shaped white eggs singly on the lower surface of the leaves. The maggot of shoot fly after hatching, crawl to the plant whorl and then cut the growing point/tissue and then feed on decaying leaf tissues. As a result, central shoot becomes pale yellow and subsequently form dead hearts. Tillers may be formed in about two-week-old seedlings; characteristic damage symptoms of shoot fly damage can be identified by the presence of subsidory tillering and emitting stinky odour from the dead hearts. The losses due to shoot fly alone were to the tune of 22.11 to 83.94 per cent (Jotwani and Sukhani, 1971; Mote *et. al*., 1981 and 1982). The shoot fly incidence is greater in late sown crop in rainy and post rainy seasons in India.

Morphology

The shoot fly completes its life cycle by passing through four stages *viz*., egg, maggot, pupa and adult in this the egg stage is sessile, and it is microscopic later the most important damaging stage is maggot stage, and the adult stage is free living and only lays eggs. The description of various stages as follows:

Egg

The eggs are white, and cigar shaped flattened at distal end and they are laid along the mid rib mostly underside of the leaves and takes 1-5 days incubation period for hatching and the freshly hatches maggots will enter the central growing shoot for further infestation and mostly early sown crop is infested and nearly 1-3 eggs can be seen per plant.

Maggot

The maggots of shoot fly are very sluggish, apodous and dirty white to brown in colour fully grown larvae measures 6mm long with total larval period of 8-10 days with four larval instars and this stage is damaging stage and cause the economic damage to the crop and cause the dead hearts in the growing central shoot and also subsidiary tillering can be observed once the main central shoot is affected the effected central shoot initially pulls out easily from the plant and stinky odour can be also emitted from the infected shoot and at later stage of infection as the

infestation progresses the dead may become dry and the plant remains at same height which may incur economic loss.

Pupa

Chitinous pupa which is brown coloured cylindrical in shape laid in soil or at the base of the stem with a pupal period of 8-10 days it is resting stage of shoot fly life cycle. The pupal period varies during seasons based on the season and favourable conditions prevailing there.

Adult

The adults are whitish-grey in colour and resemble house flies, although they differ in size. The female uses a microscopic ovipositor to deposit eggs on the underside of the leaves, and her abdomen is wider than the male's.

When the temperature stays between -25 and 30 degrees Celsius, they swiftly finish their life cycle. Although there were inconsistent rainfall and a relative humidity of over 60%, the frequency of pests was reduced by continuous rainfall.

The genus *Atherigona* spp attacking potential host plants (Bhattacharya, 2024)

Sr. No.	Name of the species	Host	Damage percentage
1	*A. soccata* Rondani	Maize, Wheat, Sorghum, Ragi in India, Kodo in Nepal	84-91%
2	*A. approximata* Malloch	Pearl Millet, Finger Millet	33-42%
3	*A. simplex* Thompson	Proso millet, Kodo Millet	56-61%
4	*A. atripalpis* Malloch	Fox tail Millet, Korle in AP	26-34%
5	*A. falcata* Thompson	Barnyard Millet, Sorghum, Pearl Millet, Finger Millet, Kutki, Shavan, Buckwheat millet	78-86%
6	*A. pulla* Wiedmann	Little Millet, Kodo Millet	16-20%
7	*A. punctate* Karl	Proso Millet, Graminaceous wild hosts	9-13%
8	*A. oryzae* Karl	Paddy, Wheat, Sorghum	15-23%
9	*A. reversura*	Other Graminaceous wild hosts.	-
10	*A. reversura*	Bamboo Shoot fly feeding on mosquito larvae	-
11	*A. reversura*	Pepper, Damaged plant parts, Faeces	15-23%

Integrated management of shoot fly:

Cultural control:

i) Different dates of sowing or planting time:

It is a crucial procedure for controlling the occurrence of shot flies. Because planting time manipulation helps to minimize pest damage by creating a synchrony between the host plant and peak pest occurrence at that particular time, it is advised that the early sowing crop has experienced the lower incidence of shoot flies after receiving the South West or North East monsoon. According to experimental findings conducted in CCS HAU, Hisar during *kharif*, 2021, Among the four distinct sowing dates, which were July 21 to August 11 with a seven-day gap between them, it was found that the sowing that occurred on August 11 had the highest dead heart percentage (29.51%), while the first/early date of sowing had the lowest dead heart percentage (15.49%) (Kumar *et al*., 2024). Similarly, at the Main Sorghum Research Station, Navsari Agricultural University, Surat, during the kharif seasons of 2014, 2015, and 2016, four evaluations of shoot fly incidence at different dates of sowing were chosen: D1: Onset of monsoon, D2: 15 days after the first date of sowing, D3: 30 days after the first date of sowing, and D4: 45 days after the first date of sowing. A total of four varieties, namely GJ 38, GJ 42, SR 2872, and SR 1904, were tested, and it was found that sowing sorghum at the onset of monsoon (treatment D1) was excellent, recording the least amount of shoot fly infestation. Treatment D4 had the largest infestation of shoot flies 45 days after the first date of sowing (24.67 and 37.66% SFDH at 14 DAE and 28 DAE, respectively), with 18.22 and 27.07% SFDH at 14 DAE and 28 DAE, respectively. Variety SR 1904 has been shown to have a lower level of dead heart infection, while variety GJ 38 exhibits a higher incidence (Saini *et al*., 2018). Additional research was conducted at the Main Agricultural Research Station, University of Agricultural Sciences, Dharwad, Karnataka, in wheat for the purpose of controlling shoot flies during Rabi 2012. Out of all the evaluated treatments, four distinct treatments were chosen for shoot fly control: the first fortnight of October, the second fortnight of October, the first fortnight of November, and the second fortnight of November, 2012. The October II-FN had the lowest mean dead heart damage of 7.67 percent, whereas the December I-FN had the greatest dead heart percentage of 19.57 percent (Belagalla *et al*., 2021). Similarly, it is noted that among the five distinct dates of sowing (I FN of September, II FN of September, I FN of October, II FN of October, and I FN of November) chosen for the shoot fly control treatment at the Regional Agricultural Research Station, Vijayapura, Dharwad, Karnataka The October IFN had the lowest infection rate of 1.51% dead hearts, whereas the September IFN had the greatest infestation rate of 2.14% dead hearts at 45 DAE.

ii) Plant Spacing:

It is advised to adhere to standard spacing guidelines, which include 45 cm between rows and 15 cm between plants. This is because plant spacing also affects pest abundance and pest dispersal from one location to another. Additionally, it affects the microenvironment of the crop and pest and contributes to the growth of pest populations by preventing adequate ventilation and compromising the vigor and strength of crop stands.

iii) Seed Rate:

Using of higher seed rate (12.5 kg/ha) than the normal ensure the proper plant stand with the shoot fly incidence with optimum spacing and good plant canopy to deter shoot fly.

iv) Using resistant cultivars:

At the Research Farm of Forage Section, Department of Genetics and Plant Breeding, CCS Haryana Agricultural University, Hisar, several genotypes were screened against shoot fly during kharif 2018 under natural field conditions. It was discovered that SPH 1906, SPV 2581, SPV 2586, SPV 2594, SPV 2530, SPV 2531, SPV 2597, SPV 2598, SPV 2604, SPV 2521, AKSV 425, RBSV 34, RBSV 36, SFRM 2, SFRM 3, and SFRM 4 exhibited resistance against shoot fly, as these genotypes produced less than 45% of dead hearts caused by A. Soccata. But it was discovered that the SFRM 1 genotype was resistant to both of the pests that produced (Jakhar *et al*., 2021). Similar findings were made when twenty genotypes were examined against A. soccata. Of the genotypes screened, genotype SPH1905 showed 17.8% dead hearts compared to the resistant check, while genotype SPH 1840 showed 63.9% dead hearts and was classified as susceptible (Atri *et al*., 2021). Furthermore, out of the 35 genotypes evaluated, genotype DJ 6514 recorded 90.70% dead hearts, and genotype IS 18551 recorded 33.60%. These two genotypes were shown to be resistant. (Subbarayudu *et al*., 2011). Furthermore, during the 2019 kharif season, 46 sorghum genotypes were screened for resistance against shoot fly at the Forage section of the Department of Genetics and Plant Breeding, CCS Haryana Agricultural University, Hisar, along with resistant, susceptible, and local checks. It was discovered that, in natural field conditions, the percentage of dead hearts caused by shoot fly was recorded at 28 and 45 days after emergence, respectively. In terms of the least percentage of dead hearts produced by A. soccata, nine genotypes—CSH 40F, CSV 21F, SPV 2704, SPV 2591, SPV 2582, SPV 2587, SPV 2581, SPV 2584, and SPV 2593—showed resistance, ranging from 19.6% to 30.7% (Sharma *et al*., 2020). Additionally, three testers and twelve lines that are screened for shoot fly resistance revealed that the genotype IS18551 had 0.20% dead hearts, while genotype B55301 had 0.38% dead hearts, indicating a higher infestation (Aruna *et al*., 2009). SPH 1838, SPV 2444, SPV 2388, SPV 2387, SPV 2522, SPV 2521, SPH 1895, SPV 2530, SPV 2533, SPV 2532, SPV 2383, and SPV 2385 were among the fourteen genotypes that were screened for shoot fly resistance. These genotypes also included commercial checks, SSG 59-3 and CSV 30F, which

were found to have multiple resistance against A. soccata under natural field conditions (Kumar *et al*., 2019). Additionally, out of the twelve genotypes that underwent susceptible and resistant examinations, genotype SR 2872 was found to be susceptible with a dead heart percentage of 29.41, whereas genotype GJ 42 showed a low dead heart infestation of 22.69% and was determined to be resistant (Patel *et al*., 2016). Furthermore, 25 genotypes were examined at RVSKVV College of Agriculture, Research Field, Indore (M.P.), India. The results showed that genotype CSV-14 had 78.82% dead hearts and genotype IS-2312 had a low infestation with 18.48 percent dead hearts (Thakur *et al*., 2019). Additionally, a total of 18 genotypes were examined, and the results showed that genotype IS-2205 had a low level of infestation (27.11%) and genotype DJ 6514 had a higher dead heart percentage (56.91) (Radadiya *et al*., 2022). The screening of 105 genotypes was conducted based on the percentage of dead heart at 28- and 45-days post-emergence against shot flies. With the exception of the resistant checks, the results showed that several genotypes were extremely resistant to shot flies. Multiple resistance to shot flies has been demonstrated by the following genotypes: SPV 2768, SPV 2669, SPV 2587, SPV 2593, SPV 2584, SPV 2704, CSV 21F, SPV 2809, SPV 2800, SPV 2808, SPV 2790, SPV 2604, and SPV 2529 (Sharma *et al*., 2023). Additionally, during kharif 2007, 2008, and 2009, an experiment was carried out at AICSIP centers in Surat, Udaipur, Indore, Coimbatore, and Parbhani. It was discovered that seven entries—JJ1041, PVK 809, CO 28(S), CSV 15, SPV 1616, and CSV 17—were moderately resistant to shoot flies, while three entries—IS 2312, IS 18551, and IS 2205 (resistant checks)—were resistant (Bhagwat *et al*., 2011). Furthermore, a total of eighteen lines were screened for shoot fly resistance at Akola in kharif 2022. It was discovered that, in comparison to the susceptible check Swarna (74.69%), the resistant checks IS 2205 (RC) and IS 18551 (RC) had the least amount of shoot fly damage, with 21.78 and 28.66 percent dead hearts damage, while test line SPH2006 had the highest dead hearts, at 80.49 percent, followed by test lines SPV2884 (76.97%) and SPV2885 (76.48%) (Sonalkar *et al*., 2024).

iv) Crop Rotation:

The practice of monocropping of cereal crops, will helps to building of the pest and incese the chance of resistance against the pest, crop rotation of cereals with different pulses and oilseed crops and vegetables crops will fetch an advantage in breaking the pest life cycle, and provides less favourable conditions for shoot fly survival during the off season.

Mechanical Control

- Elimination of damaged seedlings caused by shoot flies when thinning or preparing healthy seedlings for transplantation
- Observing the infected shoot that is home to the shoot fly puparium.
- When thinning and gap filling, remove and eliminate any plants that exhibit dead hearts.

- Installing the inexpensive TNAU fish meal trap at a rate of 12 per hectare until the crop is 30 days old.

Biological control

Order/Family	Parasitoid	Host stage	Country	Reference
Trichogrammatidae	*T. simmondsi* Nagaraja	Egg	India	AICSIP,1986,1987, 1989
Trichogrammatidae	*Trichogramma spp.*	Egg	India	Pont,1972; Taley& Thakare,1979
Muscidae	*Acritochaeta orientalis* Schiner	larva	Nigeria	Adesiyun,1981
Pteromalidae	*Callitula spp.*	Larva	India	Kundu etal.,1971b; Kishore *et al.*,1977a; Jotwani,1978
Eulophidae	*Neotrichoporoides nyemitawus*	Larva pupa	Burkina Faso	Zongo,1992; Zongo Rohwer *et al*.,1993c
Eulophidae	Crataepiella sp.	Larva-Pupa	India	Kishoreetal.,1977a, b; Jotwani,1978
Braconidae	*Alysia* sp.	Pupa	Burkina Faso	Zongo *et al*.,1993a
Encyrtidae	*Exoristobia deeming* Subbarao	Pupa	Nigeria	Deeming,1971

Chemical Control

The chemical control is very effective in managing the shoot fly because it is an internal maggot that is causing economic damage to the crop. Here are some case studies that show that, out of all the insecticides tested at different doses, the chemical Thiamethoxam 70 WS @ 0.80% seed treatment has recorded a very low dead heart percentage compared to the control, which is 48.96%, and the chemical Profenofos 40% + cypermethrin 4% @ 0.08% recorded the lowest egg/plant of shoot flies, 1.12%, proving to be a better chemical for shoot flies (Aghav *et al*., 2007). Similarly, when eight compounds, including botanicals, were evaluated against shoot fly chemicals, Imidacloprid 70 WS @ 5 g/kg and Chlorpyriphos 20 EC (RPP) 5 ml/kg, (Biradar *et al*. 2018) at Dharwad found that the dead heart percentage was the lowest (0.73%) compared to the control 2.00%. Additionally, Carbofuron 3 G @30 kg/ha had the lowest dead heart percentage (7.90%) out of the 15 chemicals tested against shoot flies, compared to the control (27.6%) (Ambarish *et al*., 2017). Similarly, (Balikai *et al*., 2009) When various integrated pest management components were tested against shoot flies at Dharwad, intercropping with

chickpea (2:2) resulted in a higher dead heart percentage (33.1%) than the control (33.50%). (Sridhar *et al.,* 2016) Imidacloprid 70WS @ 0.01% (w/w) recorded the lowest dead heart percentage (20.00%) over control 45.80% in the first location during the evaluation of various seed dressing chemicals for shoot fly management at two different locations (Chinnamatham Palayam and Idikarai) in Coimbatore. The second location has a low infestation, and imidacloprid was shown to be a superior chemical. The dead heart percentage was the lowest, with a damage percentage variance of 11.0 percent over the control of 33.50%. (Radadiya *et al.,* 2023) carried out the seed treatment with Thiamethoxam 30 FS @ 3g/kg seed + whorl application with Phorate 10 CG @ 1.0 kg/ha recorded the lowest dead heart percentage (19.97) over control (33.81%), according to an evaluation of insecticide and botanical combinations for the management of sorghum shoot flies. The assessment of bio-intensive integrated pest management modules against rabi sorghum pests was also conducted by (Karabhantanal *et al*., 2023) and reported that seed treatment with imidacloprid 48% FS @ 12ml/kg- cypermethrin @ 2ml/l spray at 45DAE was recorded lowest dead heart percentage 13.70 over the farmers control 23.19%. Further, Effect of New Molecules of Insecticides on Shoot Fly (*Atherigona soccata*) (Rondani) Incidence and Grain Yield of Sorghum was performed by (Patil *et al*., 2017) and discovered that following foliar spraying at 10 and 20 DAE, Deltamethrin 2.8 EC @ 1 ml/l recorded a lower dead heart percentage 10.84% among the control compared to 17.01% among the control. Quinalphos 25% EC @ 20 ml/10 lit water two sprays, out of the nine treatments tested for the control of Shoot Fly, *Atherigona soccata* (Diptera: Muscidae) on kharif sorghum in Vidarbha, reported the lowest dead heart percentage 10.39% over the control 12.89%. The impact of applying various pesticides on the prevalence of the shoot fly *Atherigona soccata* (Rondani) on rabi sorghum was investigated by (Yashwant *et al*., 2022) and found that Thiamethoxam 30 foliar spray (seed treatment) followed by Chlorantraniliprole-18.5% SC @10 ml/kg followed by 3 ml/10 l recorded lowest dead heart percentage 24.24% over the control 46.66%.

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