

# Biopesticides and the Insect Gut Microbiome: Molecular Interactions, Immune Modulation, and Implications for Pest Control Efficiency

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## Abstract

## Review Article

Biopesticides including microbial, botanical, and biochemical agents represent sustainable alternatives to conventional synthetic pesticides. The insect gut microbiome plays a decisive role in determining biopesticide efficacy by modulating toxin activation, immune signaling, pathogen establishment, and detoxification pathways. Biopesticide-induced gut dysbiosis is a key driver of insect mortality, while certain microbial taxa confer resistance through enzymatic detoxification and immune priming. This review examines molecular interactions between biopesticides and the insect gut microbiome, discusses immune modulation mechanisms, and highlights emerging microbiome engineering strategies for advancing sustainable pest management.

**Keywords:** biopesticides, insect gut microbiome, *Bacillus thuringiensis*, immune modulation, biological control, microbial ecology, pest management, insect immunity.

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## 1. INTRODUCTION

Insect pests represent one of the most serious constraints to global agricultural productivity, with annual crop losses estimated between 20% and 40%, posing direct threats to food security worldwide (Douglas, 2015). For decades, synthetic chemical pesticides have served as the primary tool for controlling these pests. Despite their effectiveness, prolonged and widespread use has generated mounting environmental and ecological concerns, including soil degradation, water contamination, biodiversity decline, and the emergence of pesticide-resistant insect populations (Gupta and Nair, 2020). These challenges have accelerated the search for sustainable pest management alternatives.

Biopesticides have emerged as environmentally responsible options owing to their target specificity, biodegradability, and reduced impact on non-target organisms (Ding *et al.*, 2022). They encompass microbial agents such as *Bacillus thuringiensis* (Bt), entomopathogenic fungi, baculoviruses, and botanical compounds including azadirachtin and essential oils. Advances in next-generation sequencing have

simultaneously revealed that the insect gut microbiome once considered a passive bystander is in fact a critical determinant of biopesticide outcomes (Engel and Moran, 2013).

The gut microbiome contributes to insect nutrition, metabolism, immunity, reproduction, and environmental adaptation (Engel and Moran, 2013; Emery *et al.*, 2017). Resident microbial communities can alter pathogen colonization, toxin activation, immune responses, and detoxification pathways, collectively determining whether an insect survives or succumbs to a biological control agent (Broderick *et al.*, 2006). This review explores the molecular interactions between biopesticides and the insect gut microbiome, with emphasis on immune modulation and implications for pest control efficiency.

## 2. The Insect Gut Microbiome: Composition and Functional Significance

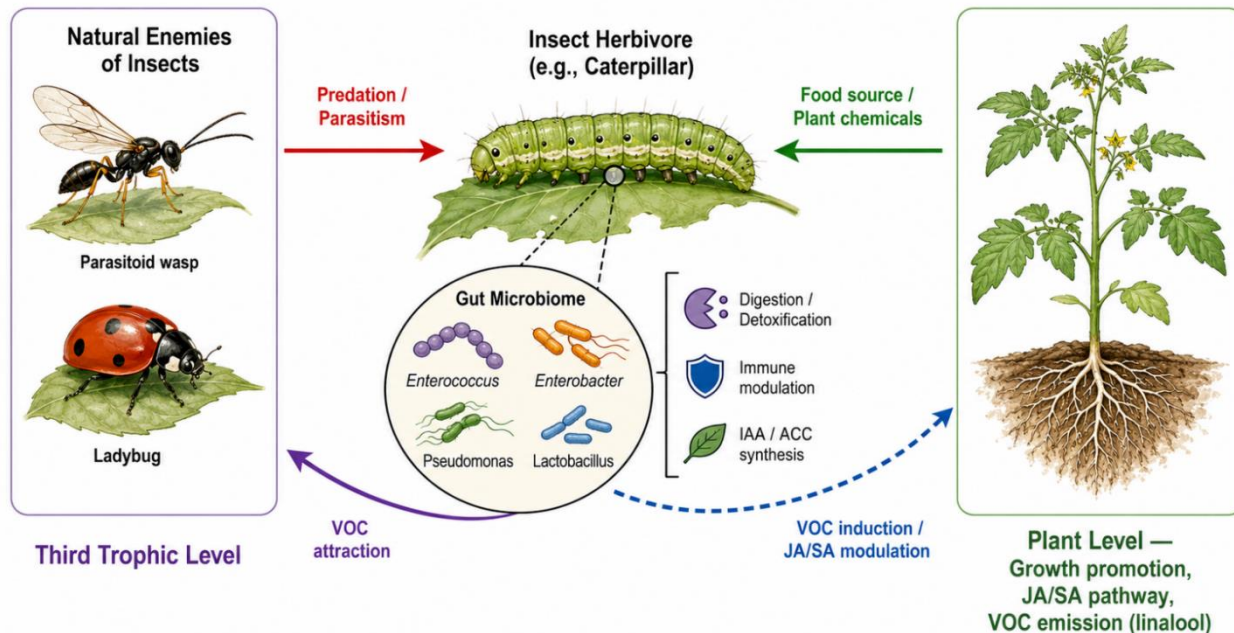
The insect digestive tract hosts a diverse and dynamic microbial ecosystem. Although microbial composition varies considerably among insect species, several bacterial genera consistently dominate gut

communities, including *Enterococcus*, *Enterobacter*, *Pseudomonas*, *Serratia*, *Lactobacillus*, *Klebsiella*, and *Bacillus* (Yun *et al.*, 2014). Gut microbiome structure is shaped by a complex interplay of host genetics, developmental stage, environmental conditions, feeding behavior, and diet composition (Chen *et al.*, 2016). Herbivorous insects typically harbor microbial communities specialized in degrading plant material, whereas carnivorous insects associate predominantly with bacteria involved in protein digestion (Hansen and Moran, 2014).

A primary function of gut microbes is the enhancement of nutrient acquisition. Many insects consume nutritionally imbalanced diets deficient in essential amino acids or vitamins; symbiotic microorganisms compensate by synthesizing these nutrients and producing digestive enzymes that facilitate absorption (Douglas, 2015). Cellulolytic bacteria assist herbivorous insects in degrading plant cell walls to extract energy, while nitrogen-fixing microorganisms improve nitrogen availability in nutrient-poor environments (Sommer and Backhed, 2013). These symbiotic relationships collectively enhance insect growth, development, and reproductive success.

Gut microorganisms also provide protection against invading pathogens through colonization resistance, a process in which resident microbes occupy

ecological niches and compete for nutrients, limiting opportunities for pathogen establishment (Dillon and Dillon, 2004). Many gut bacteria additionally produce antimicrobial compounds that directly inhibit pathogenic microorganisms, and microbial communities stimulate basal immune activity that maintains the host in a state of immune readiness for rapid responses to infection (Zhang *et al.*, 2015). Gut symbionts also participate actively in multitrophic networks extending beyond the host insect (Fig. 1). Their activities include promoting plant growth, regulating key plant defense signaling pathways such as the salicylic acid (SA) and jasmonic acid (JA) pathways, and inducing plant volatile organic compounds (VOCs) (Gurung *et al.*, 2019). These interactions have been documented across diverse insect species including *Acyrtosiphon pisum*, *Helicoverpa zea*, *Diabrotica virgifera*, *Plutella xylostella*, *Leptinotarsa decemlineata*, and *Nilaparvata lugens*. In *Diabrotica virgifera*, resident gut bacteria produce lipases, proteases, and siderophores to aid host adaptation; in *Plutella xylostella*, gut bacteria synthesize growth-regulating molecules such as indole-3-acetic acid (IAA) and 1-aminocyclopropane-1-carboxylate (ACC); and in *Nilaparvata lugens*, microbial interactions drive production of defensive plant VOCs such as linalool, demonstrating that the microbiome can alter the surrounding plant canopy far beyond the host insect itself (Jones and Sanchez, 2020).



**Fig. 1: Gut bacteria mediate tri-trophic interactions across plant, insect herbivore, and natural enemy levels.**

Biopesticides are broadly categorized into microbial biopesticides, botanical biopesticides, and biochemical pesticides. In all cases, the biological efficacy of these agents is fundamentally shaped by the resident gut microbiota of the target pest (Crotti *et al.*, 2012).

### 3.1 Microbial Biopesticides

Microbial biopesticides consist of living microorganisms capable of infecting or killing insect pests. *Bacillus thuringiensis* (Bt) is the most widely used microbial biopesticide globally. During sporulation, Bt produces crystalline proteins known as Cry toxins, which are activated within the alkaline midgut environment

upon ingestion by susceptible insects. Activated toxins bind to specific receptors on epithelial cells, causing membrane disruption and cell death (Caccia *et al.*, 2016). Destruction of gut epithelial tissue compromises intestinal integrity, allowing commensal bacteria to enter the hemocoel and induce systemic septicemia (Broderick *et al.*, 2006).

Entomopathogenic fungi such as *Beauveria bassiana* and *Metarhizium anisopliae* infect insects by directly penetrating the cuticle; fungal spores germinate and proliferate throughout host tissues, producing toxins that cause death (Wei *et al.*, 2017). Unlike Bt, fungal pathogens do not require ingestion, making them effective against insects with diverse feeding behaviors. Baculoviruses (including NPV, GV, and CPV) infect insects through ingestion and replicate in gut epithelial cells before spreading systemically, causing extensive tissue damage and mortality. Other biological control agents include protozoa such as *Nosema* spp. And entomopathogenic nematodes including *Steinernema carpocapsae* and *Heterorhabditis bacteriophora* (Gurung *et al.*, 2019).

### 3.2 Botanical and Biochemical Biopesticides

Botanical pesticides include azadirachtin (derived from neem), pyrethrum, pongamia extract, garlic extract, and rotenone. Biochemical strategies also encompass semiochemicals such as allelochemicals (e.g., gossypol, cucurbitacin) and behavior-disrupting pheromones, including sex pheromones such as Helilure and Spodolure and aggregation pheromones such as Grandlure, utilized across Lepidoptera and Coleoptera (Gupta and Nair, 2020; Zhao *et al.*, 2019).

### 3.3 Transgenic Approaches and Classical Biological Control

Plant-incorporated protectants (PIPs) are genetically modified crops that express insecticidal proteins directly within plant tissues, such as Bt cotton, Bt corn, and Bt brinjal. Classical biological control leverages natural enemies' predators including coccinellid beetles and lacewings, and parasitoids including hymenopteran wasps and dipteran flies to suppress pest populations (Hammer *et al.*, 2017). The efficacy of all these biological interventions is fundamentally modulated by the composition and stability of the pest's resident gut microbiota.

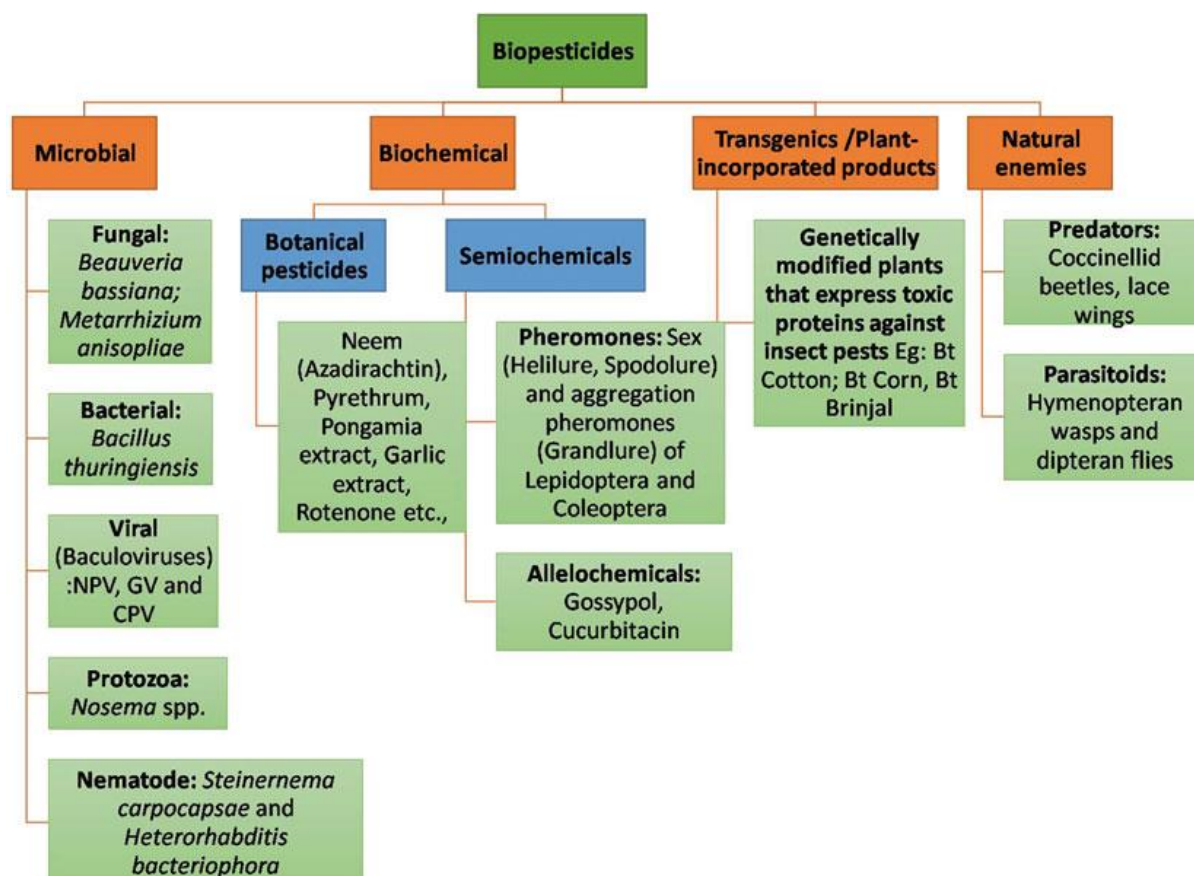


Fig. 2: Classification of biopesticides — microbial, botanical, biochemical, transgenic, and classical biological control categories

## 4. Molecular Interactions Between Biopesticides and the Gut Microbiome

The insect gut is the primary site where microbial biopesticides encounter resident microbial

communities, and these interactions profoundly affect pest mortality. The relationship between Bt and gut bacteria has been the most extensively characterized. Broderick *et al.*,(2006) demonstrated that antibiotic-

treated larvae with depleted gut bacteria exhibited markedly reduced susceptibility to Bt toxins, establishing that indigenous gut microorganisms actively contribute to Bt-induced mortality. Following Cry toxin activation and epithelial disruption, commensal bacteria translocate across the gut barrier into the hemocoel, where they proliferate rapidly and cause septicemia. Consequently, insect death typically results from a combination of toxin-mediated tissue damage and bacterial infection rather than toxin activity alone (Raymond *et al.*, 2009).

Several bacterial genera enhance Bt virulence. *Enterococcus* and *Serratia* promote inflammation, accelerate epithelial degradation, and amplify immune responses that contribute to host mortality (Mason *et al.*, 2011). Paramasiva *et al.*, (2014) further confirmed that commensal gut bacteria are indispensable cofactors in Bt efficacy, with their removal substantially diminishing Cry toxin lethality. Bt exposure frequently induces gut dysbiosis characterized by reduced microbial diversity and expansion of opportunistic pathogens, which further compromises gut integrity and increases susceptibility to infection (Shao *et al.*, 2017).

Entomopathogenic fungi interact with gut microbiota through distinct but related mechanisms. *Beauveria bassiana* infection alters gut bacterial composition in ways that suppress antifungal immune responses, facilitating fungal proliferation and accelerating host mortality (Brumin *et al.*, 2011; Wei *et al.*, 2017). Similarly, botanical pesticides such as azadirachtin disrupt microbial homeostasis; however, certain gut bacterial communities can enzymatically degrade these compounds, reduce their *in vivo* toxicity and confer measurable resistance at the field level (Xia *et al.*, 2018; Zhao *et al.*, 2019).

Baculovirus infection also disrupts gut epithelial homeostasis in ways modulated by the resident microbiome. Wang *et al.*, (2011) observed that gut microbial dynamics influence the speed and completeness of viral replication cycles, with diverse, stable microbiomes providing partial buffering against viral pathogen establishment. These findings collectively demonstrate that biopesticide-microbiome interactions are not incidental but mechanistically central to biological pest control outcomes (Huang *et al.*, 2019; Kim *et al.*, 2015).

## 5. Immune Modulation by Gut Microbiota During Biopesticide Exposure

The insect immune system is closely integrated with gut microbial dynamics. The gut microbiome actively shapes immune signaling pathways that determine the outcome of biopesticide exposure, operating primarily through three major cascades: the Toll pathway, the Immune Deficiency (IMD) pathway, and the JAK/STAT pathway.

The Toll signaling pathway is primarily activated against Gram-positive bacteria and fungal pathogens. Upon activation, it induces transcription of antimicrobial peptides (AMPs) such as defensins and drosomycin, which reduce microbial load and regulate infection progression (Zhang *et al.*, 2015). During entomopathogenic fungal infection, gut microbes can either enhance or suppress Toll pathway activation. Some symbiotic bacteria prime the host immune system, increasing resistance, while others suppress immune signaling to maintain microbial homeostasis, indirectly increasing susceptibility to fungal biopesticides (Salvucci, 2016).

The IMD pathway governs defense against Gram-negative bacteria, regulating production of AMPs such as dipterin and cecropins. Gut microbiota maintain a delicate balance of IMD activation; overactivation leads to chronic inflammation and tissue damage, while suppression increases susceptibility to opportunistic infections following Bt exposure (Jing *et al.*, 2020). Wang GH *et al.*, (2014) demonstrated that the composition of gut microbiota directly influences IMD pathway tone, with certain commensal genera acting as persistent IMD modulators that either sensitize or desensitize the insect to subsequent pathogen challenge.

The JAK/STAT pathway plays a central role in antiviral defense, gut regeneration, and stress responses. Following biopesticide-induced epithelial damage, JAK/STAT signaling promotes tissue repair; however, microbial dysbiosis can impair this regenerative response, prevent gut recovery and increase insect mortality (Zheng *et al.*, 2017). Wang J *et al.*, (2017) further showed that specific gut bacterial communities modulate JAK/STAT-driven regeneration in a composition-dependent manner, creating inter-individual variation in recovery capacity within the same insect species.

Reactive oxygen species (ROS) regulation represents a fourth critical immune-microbiome axis. ROS are generated rapidly in the gut during microbial invasion; low to moderate levels eliminate pathogens, but excessive production causes oxidative stress and tissue damage (Oliveira *et al.*, 2011). Gut microbes regulate ROS homeostasis by modulating antioxidant enzyme activity. Disruption of this balance during biopesticide exposure exacerbates oxidative damage and accelerates insect death. Zha *et al.*, (2021) provided molecular evidence that specific microbial taxa directly regulate reactive oxygen metabolism through enzymatic and signaling pathways, implicating the microbiome as a proximal regulator of oxidative immune tone.

## 6. Gut Dysbiosis and Microbiome-Mediated Resistance Mechanisms

A growing body of evidence identifies gut dysbiosis as a central mechanism in biopesticide-induced mortality. Dysbiosis the disruption of normal microbial

community structure characterized by reduced diversity and overgrowth of pathogenic bacteria weakens nutrient absorption, reduces detoxification capacity, and impairs immune regulation, creating a physiological state that favors pathogen establishment (Sommer and Backhed, 2013). Exposure to Bt, entomopathogenic fungi, or botanical pesticides consistently produces a decrease in beneficial symbionts, expansion of opportunistic pathogens, altered microbial metabolic activity, and breakdown of gut epithelial integrity. Shao *et al.*, (2017) documented a marked increase in Enterobacteriaceae populations in Bt-exposed larvae, an abundance shift strongly correlated with elevated mortality rates. Guégan *et al.*, (2018) similarly observed biopesticide-associated dysbiotic shifts in mosquito gut communities, with consequent changes in vector fitness and susceptibility.

Conversely, the gut microbiome plays a significant role in conferring resistance to biopesticides. Certain gut bacteria produce esterases, proteases, and oxidoreductases capable of degrading or modifying toxic compounds, thereby detoxifying Bt Cry proteins or plant secondary metabolites before they reach target tissues (Li *et al.*, 2007). Long-term association with specific microbial communities primes insect immune systems, enabling faster and stronger responses to pathogens that reduce the effectiveness of microbial biopesticides (Kim *et al.*, 2015). Some microbial taxa metabolize botanical pesticides such as azadirachtin, reducing their *in vivo* toxicity (Xia *et al.*, 2018). Highly stable microbiomes can resist disruption by external agents, allowing insects to maintain homeostasis even under biopesticide pressure and contributing measurably to field-level resistance (Zhao *et al.*, 2019; Gurung *et al.*, 2019).

### 7. Strategies for Enhancing Biopesticide Efficiency Through Microbiome Manipulation

Recent advances in microbial ecology have opened new avenues for improving pest control efficiency by targeting the insect gut microbiome. Probiotic-based pest management introduces beneficial or competitive microbes to alter gut community composition, increase gut permeability, or enhance toxin activation (Crotti *et al.*, 2012). While experimental antibiotic pre-treatment studies demonstrate that depleting gut bacteria increases Bt susceptibility, this approach is not viable in field settings due to practical and environmental constraints. It serves nevertheless as a proof of concept for microbiome-mediated susceptibility.

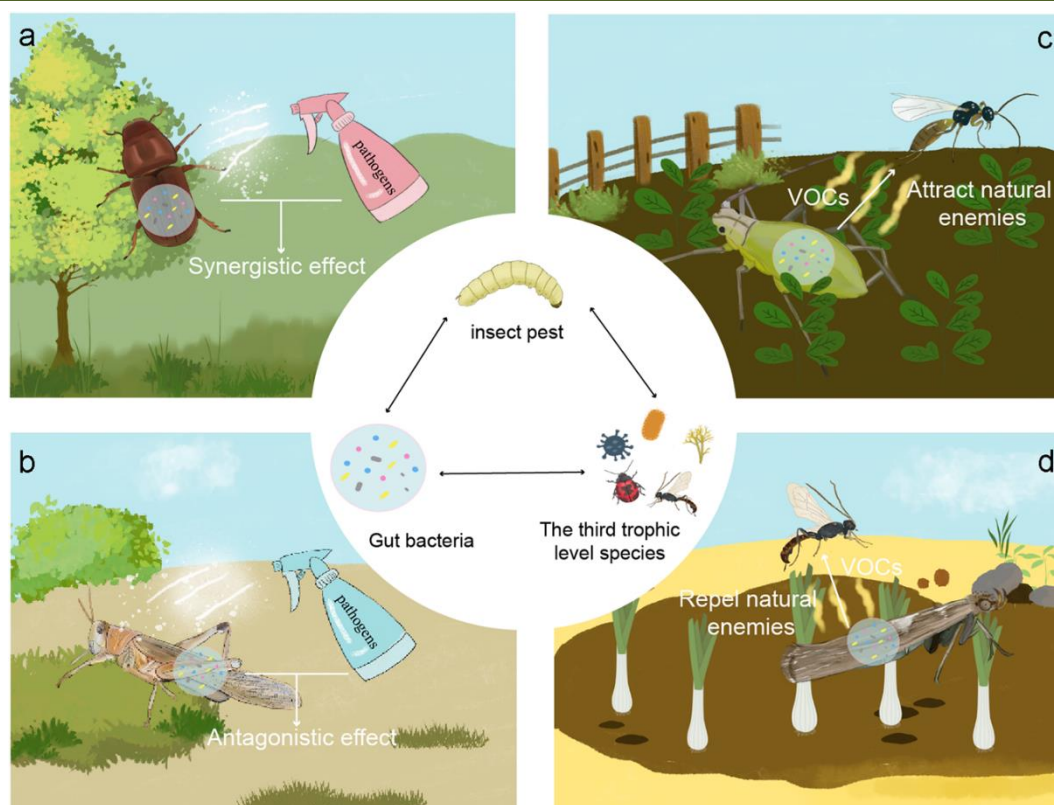
Genetically modified symbiotic bacteria represent a more promising avenue. Engineered symbionts can be designed to produce insecticidal toxins,

disrupt immune signaling, or interfere with gut homeostasis in target pests (Zhang *et al.*, 2023). Wang GH *et al.*, (2014) demonstrated that naturally occurring gut bacteria can be engineered to express heterologous insecticidal factors without disrupting their symbiotic integration, providing a proof-of-concept for symbiont-based delivery systems. RNA interference (RNAi) technology combined with microbiome-targeted delivery can silence genes involved in immune regulation or detoxification, significantly enhancing pest mortality (Zhang *et al.*, 2023). Future pest management frameworks are expected to integrate these microbiome-based approaches with traditional biological control to create more precise, durable, and ecologically sustainable strategies (Wang Q *et al.*, 2024; Emery *et al.*, 2017).

### 8. Ecological Implications, Trophic Dynamics, and Future Perspectives

The interaction between biopesticides and insect gut microbiomes carries significant implications for agriculture and ecosystem management. Variability in gut microbiota composition partly explains why the same biopesticide product can exhibit markedly different efficacy across insect populations or geographic locations; environmental factors such as diet, temperature, and habitat strongly influence these microbial communities (Jones and Sanchez, 2020). Microbiome-targeted strategies hold potential to reduce the frequency of pesticide applications, lowering production costs and minimizing ecological disruption. However, unintended consequences including disruption of non-target insect microbiomes and cascading effects on pollinators and natural enemies must be carefully evaluated before large-scale deployment (Hammer *et al.*, 2017; Gurung *et al.*, 2019).

These ecological interactions extend into higher trophic levels (Fig. 3). In some scenarios, applied pathogens and resident gut bacteria act synergistically to compromise pest fitness (panel a); in others, specific microbial profiles shield the pest and suppress pathogen efficacy (panel b). Gut bacteria also modulate multi-trophic communication by inducing plant volatile organic compounds (VOCs) that can attract natural enemies such as predators and parasitoids to enhance biological control (panel c), or alternatively repel natural enemies or alter herbivore behavioral choices (panel d) (Jones and Sanchez, 2020; Huang *et al.*, 2019). This tripartite interaction among gut bacteria, insect pests, and third trophic-level species confirms that microbiome manipulation has community-wide ecological consequences that must be integrated into pest management design.



**Fig. 3: Gut bacteria participating in plant-insect interactions and influencing third trophic-level species; panels (a-d) illustrate synergistic, antagonistic, attractive, and repellent microbiome-mediated outcome**

Despite significant progress, important research gaps remain. These include limited mechanistic understanding of microbe-toxin interactions at the molecular level, insufficient field-scale validation of microbiome-based pest control strategies, inadequate characterization of viral and fungal interactions with gut microbiota, and poor understanding of microbiome evolutionary dynamics under sustained biopesticide pressure. Future studies integrating metagenomics, metabolomics, and transcriptomics will be essential for advancing this field (Zha *et al.*, 2021; Zhang *et al.*, 2023). Development of predictive models linking microbiome composition to pest susceptibility holds particular promise for transforming biological control strategies (Wang Q *et al.*, 2024).

## 9. CONCLUSION

The insect gut microbiome is a central determinant of biopesticide efficacy. Through modulation of immune responses, detoxification pathways, microbial metabolism, and gut epithelial integrity, resident microbial communities profoundly shape insect susceptibility to biological control agents. Biopesticide-induced gut dysbiosis frequently acts as a pivotal mechanism driving insect mortality, while specific microbial taxa confer measurable resistance through enzymatic detoxification and immune priming. These findings necessitate a microbiome-integrated framework for understanding and designing pest management systems.

Future research must prioritize the molecular characterization of microbe-toxin interactions, field validation of microbiome manipulation strategies, and the development of ecological risk assessment frameworks that account for multi-trophic microbiome effects. The integration of microbiome engineering with biopesticide technology holds strong potential for achieving the precision, durability, and ecological sustainability that next-generation pest management demands.

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