

## **EXPLORING THE ROLE OF GUT MICROBIOTA IN DIFFERENT BEE SPECIES AND THE PESTICIDE PARADOX**

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### **ABSTRACT**

Bee populations worldwide are declining, posing a threat to biodiversity and food security. Bees including both *Apis* and non-*Apis* species frequently encounter various pesticides, including herbicides, fungicides, and insecticides during nectar and pollen foraging. Pesticide toxicity has been associated with a variety of health issues in bees, including metabolic diseases, immune system problems, neurotoxicity, and reproductive disorders. All bee species harbor microorganisms in their gut which play a significant role in their health and function, but can also be affected by environmental stressors including pesticides. Understanding the intricate relationship between bee gut microbiota and pesticide exposure is crucial for addressing pesticide toxicity crisis. This review explores the pivotal role of gut microbiota in bee's susceptibility to pesticides, highlighting the pesticide paradox - how chemicals intended to protect crops, can harm beneficial insects like bees. The impact of pesticides on gut microbiota composition and function, the synergetic role of detoxifying enzymes and gut microbiota in mitigating pesticide toxicity is discussed.

**Key words:** Detoxifying enzymes, gut microbes, bees, immunity, metabolism, pesticides.

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### **INTRODUCTION**

Pollinators are integral to ecosystem function, serving as key agents in the reproduction of plants and the maintenance of biodiversity (Klein *et al.*, 2007). Bees including both *Apis* and non-*Apis* species such as bumblebees and stingless bees are vital players in the natural world that ensure the stability of land-based ecosystems and boosts agricultural yields (Malathi *et al.*, 2025; Brittain *et al.*, 2013 ). Bees act as bio-monitor for the environmental pollution (Cunningham *et al.*, 2022). In recent years, bee populations have declined due to stressors such as reduced floral resources, agrochemicals, diseases, and climate change (Goulson *et al.*, 2015). Bees harbor a vibrant community of microorganisms, known as the microbiome which has an important role in their health and function (Engel *et al.*, 2016; Liu *et al.*, 2023; Motta and Moran, 2024). The bees gut microbiota has developed a compelling area of study for researchers aiming to unravel key aspects of gut microbiology. This interest stems from the microbiota's straight forward

community structure and its suitability for experimental research (Wang *et al.*, 2018). The bee gut microbiome contributes to metabolism, immune function, nutrition, reproduction, growth and development (Raymann and Moran, 2018; Li *et al.*, 2023a). Microbes provide nutritional benefits to their hosts, helping them to manage both temperature-related abiotic stress and biotic stress from natural enemies, serving a protective role (Gurung *et al.*, 2019). Symbiosis has significantly influenced the evolution of both bees and their gut microbes, emphasizing the critical role of mutualism in shaping their evolutionary trajectories (Kwong and Moran, 2015; Armitage *et al.*, 2022). Agrochemicals have been associated with a variety of health issues in honey bee, including metabolic diseases, immune system deficiency, neurotoxicity, and reproductive disorders. They also impact the gut microbiota, which play a crucial role in host defence, nutrition, and metabolism (Yuan *et al.*, 2019; Lee and Choi, 2020; Leska *et al.*, 2021). Exposure of bees to pesticides in agricultural environments not only raises mortality rates but also disturbs their innate

behavior and leads to various disorders (Henry *et al.*, 2012; Tome *et al.*, 2020). The intimate relationship between insect gut microbiota and insecticide metabolism in honey bees underscores a complex interplay between microbial communities and the ability of these insects to withstand the toxic effects of insecticides (Hotchkiss *et al.*, 2022; Wu *et al.*, 2020a). This review was based on publications retrieved from the Scopus and Google scholar using the keywords detoxifying enzymes, gut microbes, bees, immunity, metabolism and pesticides. Around 150 articles and book chapters were initially identified, and after screening titles and abstracts for relevance and removing duplicates, 113 publications were included. The inclusion criteria were: (i) publications between 2002 and 2025, (ii) original research, review papers or book chapters, and (iii) written in English. Studies unrelated to bee gut microbiota or pesticide exposure and duplicates were excluded. The reviewed literature collectively provides information into the significance of gut microbiota composition, the impact of pesticides on the gut, and the crucial role of detoxifying enzymes in this process.

**Gut microbial communities in bees:** Bees derive benefits from their gut microbiota in many ways. The microbial gut communities play a dual role by safeguarding against pathogens and contributing to nutritional aspects (Vojvodic *et al.*, 2013; Anjum *et al.*, 2018). Bees house distinctive bacterial communities that varies depending on the developmental stage, geographical location, species, weather and the diet consumed (Jones *et al.*, 2018; Nishanthini and Kanagarajan, 2023; Tuerlings *et al.*, 2023) (Table 1).

Acetobacteraceae, *Lactobacillus kunkeei* and *Lactobacillus* sp. B. were isolated from the first and second instar larvae of European *Apis mellifera* Linnaeus, 1758 (Order: Hymenoptera; Family: Apidae) and Africanized (*A. mellifera scutellata*) honey bee (Vojvodic *et al.*, 2013). The 16S rRNA gut microbiota gene sequence of Asiatic honey bee, *Apis cerana* Fabricius, 1793 (Hymenoptera; Apidae) and the dwarf honey bee, *Apis florea* Fabricius, 1787 (Hymenoptera; Apidae) worker bees collected from Western Ghats of South India were detected with 16 and 12 bacterial phyla, respectively. The study revealed the predominance of Proteobacteria in *A. florea*, while *A. cerana indica* comprised of Firmicutes and Bacteroidetes other than Proteobacteria (Khan *et al.*, 2023).

*Apis dorsata* Fabricius, 1793 (Hymenoptera; Family: Apidae), the largest honey bee species primarily found in Southeast Asian forests (Kishan Tej *et al.*, 2017), exhibits a specific bacterial composition and diversity within its gut. It was found to contain 11 microbial phyla, with Firmicutes (95.8%), Proteobacteria (3.7%), and Actinobacteria (0.4%) being the most abundant. Bacilli, constituting 94.5% of the total bacterial

population, featured the dominant class, Bacillaceae (87.2%) stood out as the prevalent family, and *Bacillus* (87%) emerged as the dominant genus (Niode *et al.*, 2021), while the gut microbiota of the black banded honey bee, *Apis nigrocincta* Smith, 1858 (Hymenoptera; Apidae) showed dominance of Proteobacteria (58%), Firmicutes (29%), and Actinobacteria (8%). Major species within these phyla included *Enterobacteriaceae*, *Erwinia*, *Klebsiella*, *Lactococcus garvieae*, *Lactobacillus* spp., and *Bifidobacterium* spp (Lombogia *et al.*, 2020).

The gut of *A. cerana indica* workers at different stages of development showed significant variations with the relative abundance of Proteobacteria, Cyanobacteria, Planctomycetes, Spirochaetae, Bacteroidetes, Actinobacteria, Verrucomicrobia, Acidobacteria, Firmicutes, and Fibrobacteres. Bacteroidetes are of major and consistent importance in the workers gut as their respective abundance was low at 0 days post emergence (2.45%) and high at 30 days post emergence (28.07%) (Dong *et al.*, 2021). In *A. mellifera*, bacterial community's diversity in worker bees varied significantly across different ages. *Gilliamella*, *Frischella*, and *Snodgrassella*, occurred 1 day post-emergence (dpe), while *Lactobacillus*, *Bifidobacterium*, and *Commensalibacter* were abundant at 3 dpe. At 12 dpe, *Lactobacillus kunkeei* and *Bartonella* exhibited significant colonization. *Commensalibacter* and *Bifidobacterium* sustained until 25 dpe while the colonization by *Bacteroides*, *Escherichia shigella*, and *Porphyromonadaceae* were significantly reduced between 19 and 25 dpe (Dong *et al.*, 2020).

Anjum *et al.* (2018) reported 150 aerobic and facultative anaerobic strains of gut bacteria in *A. mellifera* worker bees from Pakistan apiaries. Gene sequencing studies of 100 bacterial isolates distinguished by colony morphology and other bacteriological characteristics revealed higher dominance of phylum Firmicutes than the other two phyla, Proteobacteria and Actinobacteria. The isolated bacteria of genera and families of *Staphylococcus*, *Bacillus*, *Enterococcus*, *Ochrobactrum*, *Sphingomonas*, *Ralstonia*, *Enterobacteriaceae*, *Corynebacterium* and *Micrococcineae* were capable of thriving in acidic environments and fermenting sugars and are considered valuable members of the gut, playing a role in sustaining a healthy microbiota.

The microbiota of non-*Apis* bees has also been explored. Stingless bees, like honey bees, are eusocial and collect nectar and pollen, but they also gather resin, enhancing their role as vital ecosystem service providers (Shanahan and Spivak, 2021). Their gut flora, dominated by Lactobacillaceae, Bifidobacteriaceae, and Proteobacteria, supports nutrient metabolism, antimicrobial activity, and pathogen defense (Ramirez-Ahuja *et al.*, 2025). While functionally comparable to honey bees, the microbial composition of stingless bees is

more variable across species and regions, reflecting ecological adaptation (Sarton-Loheac *et al.*, 2023; Ramirez-Ahuja *et al.*, 2025). Researchers in Thailand have unveiled the information on bacteria living inside stingless bee, *Tetragonula pagdeni*, Cockerell, 1919 (Hymenoptera; Apidae). Taxonomic profiling revealed *Lactobacillaceae*, *Halomonadaceae*, and *Bifidobacteriaceae* as the dominant bacterial families which has the potential to biosynthesize amino acids and antimicrobial compounds. The isolation of *Weissella*, *Leuconostoc*, and *Bacillus*, the core functional groups of Thai stingless bees suggests their potential pivotal role in sustaining bee health (Sinpoo *et al.*, 2023).

The phylum Proteobacteria exhibited the highest relative abundance compared to Firmicutes and Actinobacteria in stingless bees, *Lepidotrigona terminata* Smith, 1857 (Hymenoptera; Apidae), *L. ventralis*, and *T. pagdeni* (Tang *et al.*, 2021). In contrast, core microbial taxa such as *Snodgrassella*, *Gilliamella*, *Lactobacillus* Firm-4, and *Lactobacillus* Firm-5 are consistently associated with honey bees but are rarely detected in non-*Apis* carpenter bees, *Xylocopa* spp. (Hymenoptera; Apidae), except for occasional detection of *Gilliamella* (Kawasaki *et al.*, 2023).

Gut microbiota have been characterized in diverse non-*Apis* bee groups, including leafcutter bees, carpenter bees, solitary bees, and the European wool carder bee (Arbaciauskienė *et al.*, 2019; Subta *et al.*, 2020; Tuerlings *et al.*, 2023). For instance, in *Megachile sculpturalis* Smith, 1853 (Hymenoptera; Megachilidae) collected in Japan, *Moniliella* and *Kosakonia* were found prevalent in the native range, whereas in invaded regions *Brevibacillus* dominated, followed by *Enterobacteriaceae*, *Bacillus*, *Acidocella*, and *Lactococcus*. In the European wool carder bee, *Anthidium florentinum* Fabricius, 1775 (Hymenoptera; Megachilidae) gut bacterial community composed of *Acinetobacter*, *Weissella*, *Brevibacillus*, and *Carnimonas*, while fungi were mainly represented by *Metschnikowia*, with occasional detection of *Starmerella* and *Saccharomycetales incertae sedis*. Similarly, the solitary bee *Halictus scabiosae* Rossi, 1790 (Hymenoptera; Halictidae) harbored bacterial taxa (*Apilactobacillus* and *Lactococcus*) along with fungal species (*Metschnikowia gruessii*, *Metschnikowia reukaufii*, and *Starmerella*) (Tuerlings *et al.*, 2023). These examples highlight that the gut microbiota of *Apis* and non-*Apis*, varies with dietary resources and nesting materials, which range from plants to wood and soil (Table 1).

### Role of gut microbiota

**Learning and memory:** The gut microbiota plays a role in controlling the activity of brain genes associated with honey bee learning and memory. Learning and memory exert significant influence over labor division, feeding

organization, kin recognition, and mating processes (Galizia *et al.*, 2011; Zhang *et al.*, 2022a).

Microbiota free bees fed with sucrose and tryptophan shows better response in memory test than microbiota free bees supplemented with sucrose alone. Microbiota free bees with *Lactobacillus* W8172 (firm 5) and tryptophan showed significant response in memory test compared to microbiota free bees colonised with *Lactobacillus* firm 5 alone. These observations could be due to *Lactobacillus* firm 5 producing indole derivatives at high Tryptophan levels (Zhang *et al.*, 2022a).

A comparison study between honey bees exposed to imidacloprid (0.02 ng/μl) and a control group for 11 days of chronic oral exposure revealed a weaker learning capacity of treated bees than control (Li *et al.*, 2019). The learning performance declined in bumble bees fed with pure glyphosate (66.1 %) and commercial herbicide (79.0 %) but increased when fed with sugar water (81.0 %) while the memory test showed the greatest decline with a commercial herbicide (18.9%), followed by sugar water (13.8%) and pure glyphosate (10.5%) (Kaakinen *et al.*, 2024). Supplementation of bumble bees with *Lactobacillus apis* result in improved long-term memory retention compared to bees supplemented with *Snograsella alvi* and *Gilliamella apicola* (Li *et al.*, 2021). Bees exposed to acute or chronic thiacloprid exhibits reduced learning abilities, which didn't affect the short-term memory but disrupted the medium term and long term memory (Li *et al.*, 2023b; Tison *et al.*, 2017). These effects can hinder their foraging behavior and their ability to return to the hive.

**Social communication:** Bacterial populations are highly communicative and combats environmental threats through a cell-to-cell communication mechanism called quorum sensing. Quorum sensing is pivotal for bacterial adaptation to environmental stress as it is a key part of the gene expression (Williams *et al.*, 2007). This communication system is mediated by autoinducer-2 (AI-2), which controls a wide range of behaviors: virulence, motility, nutrient acquisition, and biofilm formation. The AI-2 signalling molecule is enzymatically synthesized by LuxS gene in honey bees. Both *Gilliamella* and *Bifidobacteria* species produces AI-2 but *Gilliamella* produces AI-2 only in matured honey bee and not in newly emerged bees. The communication between functionally significant members of the honey bee microbiota during their association with the host may involve a potential mechanism, suggesting that autoinducer-2 could regulate density-dependent behaviors, including biofilm formation (Miller *et al.*, 2018). The presence of specific gut microbial communities may drive consistent cues for nestmate recognition among the bees, as the gut microbiome produces chemical signals indicating group membership in honey bee colonies (Vernier *et al.*, 2020).

Table 1. Core gut bacterial phylotypes of *Apis* and non - *Apis* bees

Host species	Stages	Geographical distribution	Climatic conditions	Core gut phylotypes	Functions	References
<i>Apis cerana</i>	Adult worker	Vietnam	Subtropical monsoon	<i>Gilliamella</i>	Sugar fermentation, pectin degradation, biofilm formation, detoxification, gut protection	Duong <i>et al.</i> , 2020; Engel <i>et al.</i> , 2012
				<i>Enterobacter</i>	Lactose fermentation	Duong <i>et al.</i> , 2020; Cabral, 2010
				<i>Snodgrassella</i>	Consumes oxygen, maintains gut anaerobic environment	Duong <i>et al.</i> , 2020; Kwong and Moran, 2013
				<i>Bifidobacterium</i>	Short chain fatty acid production and carbohydrate fermentation	Duong <i>et al.</i> , 2020; Wang <i>et al.</i> , 2020
				<i>Lactobacillus</i>	Antimicrobial activity and carbohydrate fermentation	Duong <i>et al.</i> , 2020; Kwong <i>et al.</i> , 2017a
<i>A. mellifera</i>	Adult worker	Kenya	Tropical to subtropical	<i>Apibacter</i>	Consumes simple sugars, avoids complex polysaccharides, colonizes gut wall	Duong <i>et al.</i> , 2020; Kwong <i>et al.</i> , 2018
				<i>Gilliamella</i>	Sugar fermentation, pectin degradation, biofilm formation, detoxification, gut protection	Tola <i>et al.</i> , 2020; Engel <i>et al.</i> , 2012
				<i>Snodgrassella</i>	Consumes oxygen, maintains gut anaerobic environment	Tola <i>et al.</i> , 2020; Kwong and Moran, 2013
				<i>Lactobacillus (Firm-4 and Firm-5)</i>	Antimicrobial activity, digest pollen compounds, and ferment sugars into organic acids	Tola <i>et al.</i> , 2020; Kwong <i>et al.</i> , 2017a; Ellegaard <i>et al.</i> , 2015
				<i>Bifidobacterium</i>	Short chain fatty acid production and carbohydrate fermentation	Tola <i>et al.</i> , 2020; Wang <i>et al.</i> , 2020
				<i>Frischella</i>	Stimulates gut defense via melanization, forming visible scabs	Tola <i>et al.</i> , 2020; Emery <i>et al.</i> , 2017; Engel <i>et al.</i> , 2015
				<i>Commensalibacter</i>	Sugar metabolism	Tola <i>et al.</i> , 2020; Siozios <i>et al.</i> , 2019
				<i>Bombella</i>	Sugar metabolism	Tola <i>et al.</i> , 2020; Kwong <i>et al.</i> , 2017a
				<i>Bartonella</i>	Sugar metabolism	Tola <i>et al.</i> , 2020; Kesnerova <i>et al.</i> , 2016
				<i>Apibacter</i>	Digests simple sugars and protects the host gut from parasites	Tola <i>et al.</i> , 2020; Mockler <i>et al.</i> , 2018
<i>A. florea</i>	Adult worker	India	Tropical	<i>Enterobacter</i>	Lactose fermentation	Ganeshprasad <i>et al.</i> , 2022; Cabral, 2010
				<i>Lactobacillus</i>	Antimicrobial activity and carbohydrate fermentation	Kwong <i>et al.</i> , 2017a
				<i>Massilia</i>	Secretes enzymes and secondary metabolites	Ganeshprasad <i>et al.</i> , 2022; Yang <i>et al.</i> , 2019a
				<i>Klebsiella</i>	Probiotic role and dietary Component Breakdown	Ganeshprasad <i>et al.</i> , 2022; Anderson <i>et al.</i> , 2011
				<i>Escherichia-Shigella, Citrobacter, Serratia, Rhodococcus</i>	-	Ganeshprasad <i>et al.</i> , 2022
				<i>Pantoea</i>	Dietary component breakdown	Ganeshprasad <i>et al.</i> , 2022; Dillon and

<i>A. dorsata</i>	Adult worker	Thailand	Tropical	<i>Morganella</i>	Minor role in carbohydrate metabolism	Charnley, 2002 Ganeshprasad <i>et al.</i> , 2022; Lyapunov <i>et al.</i> , 2008 Gruneck <i>et al.</i> , 2021; Engel <i>et al.</i> , 2012 Gruneck <i>et al.</i> , 2021; Wang <i>et al.</i> , 2020 Gruneck <i>et al.</i> , 2021; Kwong <i>et al.</i> , 2017a Gruneck <i>et al.</i> , 2021; Kwong and Moran, 2013 Hosokawa <i>et al.</i> , 2010; Hedges <i>et al.</i> , 2008 Arbaciauskienė <i>et al.</i> , 2019; Guo <i>et al.</i> , 2019 Arbaciauskienė <i>et al.</i> , 2019
<i>Megachile centuncularis</i>	Adult	Lithuania	Temperate continental	<i>Wolbachia</i> <i>Arthrobacter</i>	Sugar fermentation, pectin degradation, biofilm formation, detoxification, gut protection Short chain fatty acid production and carbohydrate fermentation Antimicrobial activity and carbohydrate fermentation Consumes oxygen, maintains gut anaerobic environment Reproduction and immune modulation Complex compound degradation, antimicrobial support	
<i>Xylocopa tenuiscapa</i>	Adult	Thailand	Tropical	<i>Microbacterium, Erwinia</i> Lactobacillales Enterobacteriaceae <i>Bacteroides</i> <i>Porphyromonas, Flavobacteriaceae</i> <i>Dysgonomonas</i> <i>Bifidobacterium</i> <i>Gilliamella</i>	Facilitates digestion, nutrient absorption, and immune protection Carbohydrate fermentation and nitrogen fixation Short chain fatty acid production and carbohydrate fermentation - Lignocellulose degradation Facilitates digestion, nutrient absorption, and immune protection Sugar fermentation, pectin degradation, biofilm formation, detoxification, gut protection	Subta <i>et al.</i> , 2020 Subta <i>et al.</i> , 2020; Rizzi <i>et al.</i> , 2013 Subta <i>et al.</i> , 2020; Wang <i>et al.</i> , 2020 Subta <i>et al.</i> , 2020 Subta <i>et al.</i> , 2020; Luo <i>et al.</i> , 2019 Subta <i>et al.</i> , 2020 Subta <i>et al.</i> , 2020; Engel <i>et al.</i> , 2012

**Metabolism:** The role of nutrition in *A. mellifera* is crucial because it not only provides a source of energy and nutrients but it also plays a vital role in caste determination and socially influenced behavioral adaptability. The metabolic pathways in bees and their microbiota are driven by their dietary focus on honey and pollen feeding, as reflected in their enzyme composition (Kunieda *et al.*, 2006). Phyla such as  $\gamma$  - Proteobacteria, Bacilli, and Actinobacteria help in breakdown of macromolecules present in carbon rich diet as well as in protein and carbohydrate synthesis, membrane transport, RNA metabolism and virulence (Lee *et al.*, 2015). Host and microbiota participate in the metabolism of certain dietary plant metabolites. For example, Glycoside hydrolase (GH) is produced in the bee head gland and catalyzes the degradation of xenobiotics while the products of GH are further metabolized by the midgut core gut microbiota (Motta *et al.*, 2022). Diet also affects the metabolic capability of bees; pollen biomass induces the production of  $\beta$ -galactosidase, which has the ability to break down sugars through hydrolysis (Ricigliano *et al.*, 2017).

In *A. mellifera* and bumble bees (*Bombus griseocollis* and *Bombus impatiens*), the carbohydrate mannose, which is toxic to bees, is digested by mannose-6-phosphate (MPI) produced by gut microbe, *Gilliamella apicola* (Zheng *et al.*, 2016). The gut microbiota regulates amino acid metabolic pathways, such as tryptophan metabolism. Aromatic amino acid aminotransferase in *Lactobacillus* strains promote tryptophan metabolism and inhibit Tryptophan dioxygenase in Kynurenine pathway (Zhang *et al.*, 2022a). *Bifidobacterium* spp. represent a fundamental component of the honey bee's gut microbiota, contributing significantly to the overall stability and functionality of the gut ecosystem. *Bifidobacterium asteroides* was found to stimulate the production of host hormone known to influence bee development (Kesnerova *et al.*, 2017; Chen *et al.*, 2021).

**Immunity:** Antimicrobial peptides serve as vital components of the innate immune system, acting as the frontline defenders against a wide array of pathogens in bees (Wu *et al.*, 2018). In *A. mellifera*, gene expression of antimicrobial peptides including apidaecin and hymenoptaecin, significantly increased in bees fed with *Snodgrassella alvi* and hive microbiota. *Lactobacillus Firm-5* and *Bifidobacterium* exhibited strong resistance to both apidaecin and hymenoptaecin, whereas *Snodgrassella alvi* was more susceptible to hymenoptaecin (Kwong *et al.*, 2017b). The presence of gut bacteria in *A. cerana* led to a notable increase in the expression of apidaecin, abaecin, and hymenoptaecin, along with activation of the JNK (c-Jun N-terminal kinase) pathway. The presence of gut bacteria suppressed the growth of *Nosema ceranae* (Wu *et al.*, 2020b) while in their absence, mortality rate of *A. cerana* and *A. mellifera* was higher when infected with *N. ceranae* compared with *N. apis* (Sinpoo *et al.*, 2018). The variation could be attributed to the differing

virulence of *N. ceranae*, influenced by its geographical origin, leading to diverse characteristics observed across global regions (Paris *et al.*, 2018).

An experimentally established normal gut microbiota was also shown to influence the virulence of a major bee viral pathogen, deformed wing virus (DWV), leading to increased survival rates compared to bees with perturbed microbiota due to environmental stress (Dosch *et al.*, 2021). Expression of immunity related genes was significantly reduced in bees treated with *N. ceranae* or insecticide alone and combination of both but did not result in notable alterations in the expression of genes associated with detoxification (Aufauvre *et al.*, 2014). The probiotic, *Lactobacillus brevis* B50 enhanced the immunity response in bee colonies and resulted in a marked upregulation of genes encoding antimicrobial peptides (abaecin, defensin-1) as well as pattern recognition receptors (toll-like receptor, peptidoglycan recognition proteins) thereby increasing resilience against infectious diseases and stressful conditions (Maruscakova *et al.*, 2020). *Gilliamella apicola* W8136 and *Lactobacillus apis* W8172 activated the bee immune system against the pathogen *Hafnia alvei*. Gene expression of surface layer protein found in *L. apis* strain appears to mediate Toll signalling pathway activation and the production of antimicrobial protein (Lang *et al.*, 2022). A comparison between gammaproteobacterium, *Frischella perrara* (FP) colonised and non-colonised bees showed that only in FP colonised bees, there was strong activation of the host immune system with the increased production of the antimicrobial peptides apidaecin type 14, apidaecin type 73, abaecin and defensin 1 by upregulating PRRs (Pattern Recognition Receptors):  $\beta$ -1, 3-glucan recognition protein, the peptidoglycan recognition protein S2 (PGRP-S2, 6x) and the peptidoglycan recognition protein S3 (PGRP-S3, 1.5x) (Emery *et al.*, 2017).

**Impact of pesticides on gut microbiota:** The gut microbiota of social bees plays crucial roles in maintaining the health and performance of host. Unfortunately, however these micro-organisms can be disturbed by pesticides that applied to agricultural systems to protect crops. Pesticide concentration, duration of exposure, season and concurrent stressors influence the effect on the bee gut microbiota (Motta and Moran, 2024). Naggar and Wubet (2024) noted the scarcity of studies investigating the effects of pesticides on both fungal and bacterial communities, whereas most studies had solely reported on bacterial communities. However, examining the cross kingdom characteristics of bee gut microbiota is crucial in assessing the impact of pesticides on its abundance and richness. A comparative collection of data regarding the impact of pesticides on the microbial community in bees is given below.

**Apis bees:** Application of the miticide coumaphos to *A. mellifera* bee colonies for a duration of six weeks led to increased abundance of *Bifidobacteriales* (Kakumanu *et al.*, 2016; Premrov

Bajuk *et al.*, 2017) while studies on the effect of the neonicotinoid insecticide imidacloprid reported diverse results. No impact on gut microbiota was observed by Yang *et al.* (2019b), while Alberoni *et al.* (2021) reported decreases in *Lactobacillus* spp. in colonies fed syrup containing imidacloprid. The 16S rDNA gene sequencing and linear discriminate analysis in *A. mellifera* newly emerged adults showed that exposure to the fungicide chlorothalonil impacted *Pseudomonadales* and *Burkholderiales* (Wu *et al.*, 2022). Naggar and Wubet. (2024) reported that exposure to two novel insecticides flupyradifurone and sulfoxaflor, and to the fungicide azoxystrobin resulted in dysbiosis of bee gut microbiota, leading to sudden hike in the relative abundance of the opportunistic pathogens such as *Serratia marcescens*. In *A. cerana* exposed to acetamiprid, the abundance of *Lactobacillus* and *Apibacter* was decreased and in those exposed to the fungicide difenoconazole, the relative abundance of *Lactobacillus*, *Candidatus* and *Gilliamella* were increased whereas *Lactobacillus* and *Gilliamella* abundance was increased in combination treatment of difenoconazole + acetamiprid (Han *et al.*, 2023).

The study by Tsvetkov *et al.* (2017) revealed that neonicotinoids weakens the bee capacity to maintain social immunity posing significant threats to bee populations and ecosystems. Exposure of *A. mellifera* to thiacloprid (0.2 to 2.0 mg/L) for a duration of up to 13 days resulted in a reduction of absolute abundance of *Lactobacillus*, a gram-positive bacterium (Liu *et al.*, 2020). Likewise, exposure of *A. mellifera* to nitenpyram at a concentration of 300 µg/L for 14 days led to reduction of *Gilliamella apicola* levels and an increase in *Lactobacillus helsingborgensis* and *Bifidobacterium* spp. (Zhu *et al.*, 2020). Beehives adjacent to oilseed rape fields treated with thiamethoxam resulted in an increase of *Lactobacillus* levels and a decline in Proteobacteria in the gut (Jones *et al.*, 2018). Exposure of Africanized honey bees to imidacloprid decreased the abundance of *Snodgrassella alvi*, *Serratia* sp., and *Lactobacillus* Firm-5 and increased the abundance of Enterobacteriaceae (Balbuena *et al.*, 2023).

**Non *Apis* bees:** Pesticide exposure not only affects the managed bees, but also impacts some of the efficient non-*Apis* pollinators of crops (Motta and Moron, 2023). Both *Apis* and non-*Apis* bees share core gut microbiota that are vulnerable to pesticide exposure. In *Apis* bees, pesticides often cause direct compositional shifts in core bacteria such as *Lactobacillus* and *Bifidobacterium* (Alberoni *et al.*, 2021). By contrast, non-*Apis* bees generally retain stability in their core communities but exhibit fungal shifts and metabolic disruptions such as carbohydrate metabolism, membrane transport, weakens nucleotide metabolism and translation pathways (Zhang *et al.*, 2022b). These effects are context-dependent, varying with pesticide type, concentration, and exposure duration (Tang *et al.*, 2023). Exposure of the eastern

bumble bee, *Bombus impatiens* Cresson, 1863 (Order: Hymenoptera; Family: Apidae), to glyphosate and spinosad, reduced the relative abundance of *Snodgrassella* (Motta and Moron, 2023). In Stingless bee, *Partamona helleri* Friese, 1900 (Hymenoptera; Apidae) treated with glyphosate and spinosad, only glyphosate reduced the levels of *Gilliamella* spp. and *Bifidobacterium* spp., while spinosad had no significant effect on the gut microbiota (Botina *et al.*, 2023). In contrast, glyphosate, increased the abundance of glyphosate-sensitive *Candidatus Schmidhempelia* in bumble bees (Helander *et al.*, 2023). The larvae of solitary bee, *Osmia cornifrons* Radoszkowski, 1887 (Hymenoptera; Megachilidae), carried significantly lower bacterial diversity and abundance when fed on mancozeb-treated pollen (Porrás *et al.*, 2024).

**The protective role of detoxifying enzymes:** The bee gut microbiota plays a crucial role in detoxification. Recent studies showed that the gut microbiota contributes to xenobiotic metabolism and produces metabolites that can modulate host detox gene expression, including cytochrome P450 monooxygenases (CYP450s) and glutathione S-transferases (GSTs) (Liu *et al.*, 2024; Wu *et al.*, 2020a). Cytochrome P450 monooxygenases are key enzymes in bee xenobiotic detoxification, converting harmful chemicals into less toxic forms (Berenbaum and Johnson, 2015). In *A. mellifera*, the gut microbiota enhances detoxification by upregulating several midgut P450 genes, including CYP6AS1, CYP6AS3, CYP6AS4, CYP6AS10, CYP9Q1, CYP9Q2, and CYP9Q3. Among these, CYP9Q3 is highly efficient in metabolizing thiacloprid, while CYP9Q1, CYP9Q2, and CYP9Q3 efficiently metabolize tau-fluvalinate (Manjon *et al.*, 2018; Mao *et al.*, 2011). When gut microbiota are absent, P450 expression drops, pesticide residues increase, and mortality rises-100% for thiacloprid and 40% for tau-fluvalinate. Bees with a healthy microbiota survive much better, highlighting the critical role of gut microbes in P450-mediated detoxification in *A. mellifera* (Wu *et al.*, 2020a).

Glutathione S-transferases (GSTs) are key detoxification enzymes in bees, neutralizing harmful chemicals by conjugating them with glutathione to facilitate their elimination (Berenbaum and Johnson, 2015). Exposure to pesticides such as flumethrin reduces GST activity in the bee head and disrupts core gut microbial taxa (Liu *et al.*, 2024). It is possible that pesticide-induced dysbiosis weakens GST function, while reduced GST activity may further exacerbate microbial imbalance and pesticide toxicity, leading to a dual impairment of detoxification capacity and gut microbial health in bees under pesticide stress. Exposure to the flumethrin affects both the gut microbiota and the host's detoxification and immune systems in *A. mellifera* larvae. At low concentrations, the relative abundance of gut microbes initially declines, while the host's immune and detoxification enzymes, such as GSTs remain largely unchanged, suggesting that gut microbes may play a primary role in

metabolizing the pesticide (Yu *et al.*, 2021; Ramya *et al.*, 2016). As the flumethrin concentration increases, the diversity and abundance of gut microbiota gradually rise, accompanied by the activation of host detoxification GST genes, indicating a cooperative response between the microbiota and the host (Yu *et al.*, 2021). These findings suggest that the gut microbiota contributes to the regulation and enhancement of host GST activity, helping mitigate pesticide stress, although the precise mechanisms of these microbe-host interactions require further investigation.

**Future prospects:** Since bees are crucial pollinators of agroecosystems and are well known model organism for studying behavioural and social activities, it is necessary to profile the gut obligatory and facultative symbionts, which positively influence bees to respond and acclimatize various biotic and abiotic stress factors. Additionally, growing evidence suggests that the gut microbiota, which differs between bee species, influences bee health, immunity and behavior to great extent. However, the role of microbiota in modifying host physiology is yet to be explored in detail which opens an avenue and challenges scientists to further investigate their functional role in bees. There is lack of sufficient research on transcriptomic and metabolomic studies on gut microbiota in bees. Hence, the future research should focus on detailed understanding on the mechanism of pesticide detoxification in synergism with bee gut microbiomes through metagenomic sequencing, transcriptomics, enzymatic assays and conformational studies. An advent of gene editing tools such as CRISPR/cas9 and RNAi can be employed as a functional validation tool to characterize the activity of novel candidate genes by silencing or knockout to investigate their critical role in bee health and metabolism including pesticide detoxification.

**Conclusion:** While *Apis* and non-*Apis* bees play an important role in pollination, they are all exposed to pesticides aimed to protect crops from harmful pests. This exposure affects their health and the microbial communities. The disruption of bee gut microbiomes can weaken the bee's immunity, lifecycle, physiology, and affect individual and colony survival. To overcome this issue, and preserve our vital pollinators, the inclusion of biorational alternatives as part of integrated pest management approaches is needed. Increased communication with farmers regarding this issue is crucial to protect pollinators. Additionally, interventions aiming to increase the pesticide detoxification capabilities of managed bees may be encouraged by protecting or altering the gut microbiota with supplementary food sources enriched with probiotics such as *Lactobacillus*, *Saccharomyces*, *Escherichia*, although further research in this area is required. Ultimately, protecting pollinator bees and their microbiome will have a great impact on the livelihood of beekeepers and the sustainability of agricultural production.

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