Research Article

www.dergipark.org.tr/en/pub/mediterranean

MEDITERRANEAN AGRICULTURAL SCIENCES
(2025) 38(1): 15-20
DOI: 10.29136/mediterranean.1597263

Effects of different origins of two pesticides on several bacteria in the microbiota of *Bombus terrestris* L.

Asiye UZUN YIGIT[1], Ozan DEMIROZER[1], Serpil UGRAS[2], Ayhan GOSTERIT[3]

[1]Isparta Applied Science University, Faculty of Agriculture, Department of Plant Protection, 32260, Isparta
[2]University of Health Sciences, Hamidiye Faculty of Pharmacy, Department of Pharmaceutical Microbiology, 34668, Istanbul
[3]Isparta Applied Science University, Faculty of Agriculture, Department of Animal Science, 32260, Isparta

Corresponding author: A. Uzun Yigit, e-mail: asiyeuzun@isparta.edu.tr
Author(s) e-mail: ozandemirozer@isparta.edu.tr, serpil.ugras@sbu.edu.tr, ayhangosterit@isparta.edu.tr

ARTICLE INFO

Received: December 10, 2024
Received in revised form: February 4, 2025
Accepted: February 7, 2025

Keywords:

Bacillus subtilis
Thiamethoxam
Neonicotinoid
Biopesticide
Pesticide effects

ABSTRACT

This study aimed to evaluate the effects of pesticides from different origins on several bacteria in the microbiota of *Bombus terrestris*. In the experiments, recommended field doses (1/1) and sublethal doses (1/10, 1/100, 1/1000) of Subtilex Foliar (*Bacillus subtilis* MBI 600, Bioglobal©) and Pusula (Thiamethoxam, Hektaş©) were used. The experiments were performed with 5 replications (10 worker bees per replication). The worker bees were fed the sugar syrup + pesticide mixture prepared for 5 days in each replication. On the fifth day after applications, 5 random individuals from each trial were selected from the live worker bees and, after being mechanically euthanized, placed in sterilized falcon tubes labeled with experimental group details. The same procedure was applied to the microbiota analysis of the control group as the experimental groups. These individuals were then analyzed to determine microorganism changes. According to the results obtained, the colony development of two different species of bacteria, *Staphylococcus xylosus* and *S. gallinarum*, was determined in the control group. *Staphylococcus xylosus* and *S. gallinarum* were completely inhibited in 1/1 and 1/10 dose applications of *B. subtilis* MBI 600, while the dominant species was *B. subtilis* MBI 600. *Staphylococcus xylosus* in the control group became dominant after the application of 1/100 dose of *B. subtilis* MBI 600, and after the application of 1/1000 dose, *B. subtilis* MBI 600 could not colonize. When Thiamethoxam applications were compared with the control group, *Staphylococcus gallinarum* colonies and *Pseudomonas* sp. colonies became dominant in individuals who received 1/1 dose application. The results show that the different origins of the pesticides used cause significant changes in the microbiota of worker bees for life.

1. Introduction

Bumblebees are of significant global ecological importance due to their role as pollinators for a considerable number of plant species (Klein et al. 2007; Garibaldi et al. 2013). They have high commercial value as they can be used to pollinate a variety of agriculturally grown plants in field environments and are raised for commercial use in greenhouse environments (Goulson 2003; Velthuis and Van Doorn 2006; Nayak et al. 2020; Weinhold et al. 2024). The bumblebee has a simple but specific gut microbiota that plays an important role in food digestion (Kwong et al. 2013; Killer et al. 2014; Praet et al. 2017) and absorption, enhances immunity, and resists pathogen infection (Li et al. 2015; Kwong et al. 2017). For both honeybees and bumblebees, the native gut microbiota has been shown to support the development and health of adult workers (Engel et al. 2016; Zheng et al. 2018). It has been reported that the gut microbiota in healthy individuals increases the production of bee-encoded P450 enzymes (Wu et al. 2020) that can neutralize toxins in food but do not protect from commonly used insecticides (Raymann et al. 2018). However, it has been observed that some pesticides that reach the bee gut are detoxified by the gut microbiota, and in this context, the microbiota causes a decrease in pesticide toxicity for the bee (Zhang et al. 2024). However, the unequal distribution of microbiomes in the bee gut (low concentrations in the foregut and midgut, and abundance in the hindgut) may hinder their ability to detoxify pesticides encountered through oral exposure (Kwong and Moran 2016; Kesnerova et al. 2017). Apart from unequal distribution, the detoxification capacity of the microbiota can be affected by many factors, such as pesticide type and concentration, exposure time, and colony seasonality (Hotchkiss et al. 2022). In current agricultural practice, a range of pesticides are commonly utilized to protect plants against pests. However, the elevated levels of these pesticides in soil, water, and pollen have adversely affected the health of non-target organisms, particularly pollinators such as bumblebees. The scientific evidence indicates that neonicotinoids may have adverse effects on non-target organisms, particularly honeybees and other wild pollinators such as bumblebees (Laycock et al. 2012; Bonmatin et al. 2015; Dussaubat et al. 2016; Zhang et al. 2022). Neonicotinoids have been widely used in agricultural settings since their introduction in the 1990s, and their use has continued to steadily increase globally (Simon-Delso et al. 2015). Neonicotinoid pesticides are suggested to be one of the causes of population declines in bumblebees and other pollinators due to their widespread use, specificity to the nervous system of

invertebrates, and toxicity to bees (Campa and Lehmann 2021). The majority of the study on the effects of neonicotinoids on bumblebee health was focused on imidacloprid, while it has been reported that wild populations are increasingly exposed to alternative neonicotinoids, such as Thiamethoxam (Laycock et al. 2014).

This study aimed to determine the relationship between the bacteria in the gut microbiota (competition) when *B. terrestris* worker bees are exposed orally to *B. subtilis* MBI 600, which is a biopesticide with a high probability of exposure, especially in greenhouses and open production areas, and Thiamethoxam, an insecticide in the neonicotinoids group, at the recommended field dose and sublethal doses with sugar water.

2. Materials and Methods

In the study, *Bombus terrestris* L. worker bees (8-12 day-old), biopesticide *Bacillus subtilis* MBI 600 ($2x10^{11}$ cfu g^{-1}, Subtilex Foliar, Bioglobal©, 50 g 100 L^{-1}), and an insecticide Thiamethoxam (Pusula, Hektaş Company©, 350 g 100 L^{-1}) from the neonicotinoid group were used.

2.1. Application of pesticides to worker bees

Bacillus subtilis MBI 600 (Subtilex Foliar, Bioglobal©, 50 g 100 L^{-1}) and Thiamethoxam (Pusula Hektaş©, 350 g 100 L^{-1}) sugar syrup were mixed in 4 different doses, namely recommended maximum field dose (1/1) and sublethal doses (1/10, 1/100, 1/1000), and worker bees were fed orally (Table 1). Five replications were given for each pesticide dose, and 10 *B. terrestris* worker bees were given the sugar syrup+pesticide mixture as food for 5 days in each replication. The experiments were carried out with a randomized plot design. *Bombus terrestris* colonies were produced in growth chambers (27-28°C, 50-60% relative humidity). Worker bees within the colony were randomly selected, and the experiments were conducted with 5 replications. 10 *B. terrestris* worker bees were given the sugar syrup+pesticide mixture as food for 5 days in each replication. In the control group, worker bees were given sugar water (50 brix) without pesticide for the same period. On the fifth day, 5 individuals from each dose were randomly selected from the living worker bees and taken into falcon tubes. These individuals were mechanically euthanized. These individuals were then analyzed to determine microorganism changes.

Table 1. Pesticides used in the study and their application doses

Pesticides	Doses (g 100 L^{-1} water)			
	1/1	1/10	1/100	1/1000
Pusula (Thiamethoxam)	350	35	3.5	0.35
Subtilex Foliar (*Bacillus subtilis* MBI 600)	50	5	0.5	0.05

2.2. Isolation of bacteria from worker bees

Following the observation of the health status of *B. terrestris* worker bees exposed to varying doses of biopesticide and chemical pesticide, the bees were subsequently killed by mechanical means and placed in specially designed falcons that had been previously sterilized and transported to the microbiology laboratory. Here, two different bee samples from each trial were randomly selected from the trials and surface sterilized with alcohol (70%). Then, the insects were cleaned of alcohol (70%) to remove possible contaminants, and were homogenized in a Nutrient Broth (NB) by using a sterilized glass tissue grinder. Homogenization was performed to isolate microorganisms associated with the insect. Homogenization was repeated three times on different occasions. After homogenization, the samples were filtered and diluted with NB at different rates (10^3, 10^4, 10^5). 100 µL of the final dilutions were taken, and the samples were spread onto Nutrient Agar (NA) using sterile swabs. The inoculated petri dishes were incubated at 30°C for 16-18 hours (Poinar and Thomas 1978). The entire study was performed in duplicate.

2.3. Isolation and identification of bacterial cultures

After incubation, the bacterial isolates obtained were carefully examined and pure cultures were obtained by streaking colonies on NA medium from colonies that were found to be morphologically different from each other. Pure cultures were stored in sterile glycerol (30%) at -20°C to be used in subsequent studies. To identify the isolates, the gram properties of the pure cultures were first elucidated (Suslow et al. 1982). Then, the isolates were streaked on Mueller Hinton Agar (MHA), left for 24 hours of incubation, and identified with the VITEK 2 device (Verweij et al. 1999; Pincus 2002).

In this study, bacteria living together with insects were determined. Worker bees kept in the same conditions without any application were evaluated as control applications. Microorganism developments in Petri dishes were presented in terms of microorganism diversity and inhibition of worker bees kept in the same conditions with different doses of pesticides compared to the control application. In addition, bacterial development experiments were performed in 3 replicates.

3. Results

According to the results, in the control intensive colonization was observed in the nutrient agar (NA) medium under aerobic conditions at 30°C, and these dominant colonies were first evaluated morphologically as white and orange colonies (Figure 1a). Then, the gram properties of the purely cultured colonies were determined and the isolates were determined to be gram (+). This bacterial was identified as the white colony of *Staphylococcus xylosus* and an orange colony *Staphylococcus gallinarum* at the species level (100% rate) by VITEK. However, there were serious changes in the microbiota of the worker bees treated with *B. subtilis* MBI 600 compared to the control group depending on the biopesticide concentration acquired to their guts. The dominant *S. xylosus* and *S. gallinarum* bacteria in the control group were completely inhibited as a result of the treatment with 1/1 and 1/10 doses of *B. subtilis* MBI 600 and the dominant species was *B. subtilis* MBI 600 (Figure 1b and c). The white colony *S. xylosus* bacteria in the control group became dominant as a result of the treatment with 1/100 dose of *B. subtilis* MBI 600, however, *B. subtilis* MBI 600 colonies were still dominant in the environment (Figure 1d). After the application 1/1000 dose of *B. subtilis* MBI 600, *S. xylosus* and *S. gallinarum* which are the same colonies as the control were detected in the microbiota, it was observed that *B. subtilis* MBI 600 could not colonize (Figure 1e). Higher doses of *B. subtilis* MBI 600 (1/1, 1/10) led to a dominance of *B. subtilis*, whereas lower doses (1/1000) allowed recolonization by native bacteria such as *S. xylosus* and *S. gallinarum*. When the results of the microbiota obtained in line with the nutrient medium, temperature, and aerobic conditions used in this study were evaluated, it was seen that the treatment with high levels of *B. subtilis* MBI 600 changed the microbiota of the bee and became the dominant species. In addition, it was determined that the dominant species in the control group became dominant again

due to the *B. subtilis* MBI 600 treatment at decreasing application rates.

The evaluation of the Pusula applications revealed that the orange colonies of *S. gallinarum* bacteria and *Pseudomonas* sp. colonies exhibited a dominant growth pattern in individuals administered with the 1/1 dose, in comparison to the control group. However, it was observed that the white colonies of *S. xylosus* were inhibited (Figure 2b). In other applications, all bacterial colonies were inhibited and a different microorganism not defined in this study became dominant (Figure 2c, d, and e). The insecticide Pusula, which includes the active ingredient Thiamethoxam, was determined to be a highly effective molecule with a low dosage of 1/1000. It was observed that this molecule causes significant bacterial inhibition and the growth of a single type of microorganism in the context of the conditions studied in *B. terrestris* bees.

4. Discussion and Conclusion

Bumblebees are important pollinators in natural and agricultural ecosystems and also play an important role in maintaining plant diversity and ecological balance (Gallai et al. 2009; Williams and Osborne 2009). The bumblebee gut hosts a relatively simple yet specialized microbiota (Koch et al. 2012; Martinson et al. 2011; Li et al. 2021). The gut microbiota of various social insects belonging to the genera *Apis mellifera* L. (Hymenoptera: Apidae) and *B. terrestris* (Hymenoptera: Apidae) plays an important role in their health, ability to absorb nutrients, and defense against pathogens (Engel et al. 2016; Zheng et al. 2016; Mondal et al. 2023). Previous studies have focused on the lethal and sub-lethal effects of pesticides on *B. terrestris*. The widespread use of commercial pesticides poses a problem for bumblebees due to the sublethal effects of exposure to field doses (Cameron and Sadd 2020). Exposure to neonicotinoid pesticides such as Thiamethoxam and Imidacloprid can disrupt the social networks, foraging behaviors, learning and memory abilities of bumblebees, leading to colony failures (Gill et al. 2012; Feltham et al. 2014; Gill and Raine 2014; Stanley et al. 2015; Crall et al. 2018; Smith et al. 2020; Richman et al. 2021). It has been supported by different studies that non-lethal doses of Thiamethoxam negatively affect the hemocytes and morphological structure of the brain of *B. terrestris* worker bees (Çakıcı et al. 2023a, b). It has been reported that under laboratory conditions, *B. subtilis* QST713 (Serenade) on *B. terrestris* caused 100% mortality in worker bees after 72 hours of weekly exposure to sugar water (maximum recommended field dose, 7.5×10^9 CFU L^{-1}) for 11 weeks, and 79% mortality occurred at 1/10 recommended field dose (Mommaerts et al. 2009). Previous studies have determined that neonicotinoid and bacterial pesticides have lethal and sub-lethal effects on *B. terrestris* worker bees. It has also been reported that exposure to low-dose pesticides poses a threat to beneficial pollinators by reducing immune function and increasing susceptibility to infection (Chmiel et al. 2019). Pesticides affect not only bees but also their resident gut microbial communities, which are linked in a fundamental symbiosis (Hotchkiss et al. 2022). The decline of bumblebee populations, especially due to pesticides, and the agricultural importance of bumblebees necessitate a better understanding of factors related to bumblebee health, such as the gut microbiota and their health-related functions (Wang et al. 2019).

As a result of this study, no previous study has been found on the presence of *S. xylosus* and *S. gallinarum*, the two dominant bacterial species obtained from *B. terrestris* individuals in the control group without any application. However, it has been reported that *S. gallinarum* was isolated from the midgut of *S. littoralis* larvae, and *S. xylosus* was found in the *Lymantria dispar* microbiota (Johnston and Crickmore 2009; Ragab et al. 2022). In addition, it has been determined that *Staphylococcus* spp. in the insect microbiota are related to insect development and the effectiveness of insecticides. It has been reported that *Staphylococcus* spp. in the *Drosophila melanogaster* microbiota detoxify imidacloprid, which is in the neonicotinoid group (Chmiel et al. 2019). It has been determined that *S. gallinarum* strain SWGB 7 positively supports the development of *Bombyx mori* larvae and pupae (Saranya et al. 2019). In this study, especially with the application of high doses of *B. subtilis* MBI 600 (1/1, 1/10), *S. xylosus* and *S. gallinarum* in the *B. terrestris* microbiota were inhibited, while in the control application of *B. subtilis* MBI 600 at a dose of 1/1000, *S. xylosus* and *S. gallinarum* were colonized and *B. subtilis* MBI 600 was not found. These results suggest that *S. xylosus* and *S. gallinarum* in the *B. terrestris* microbiota exposed to low doses of the biopesticide can tolerate *B. subtilis* MBI 600.

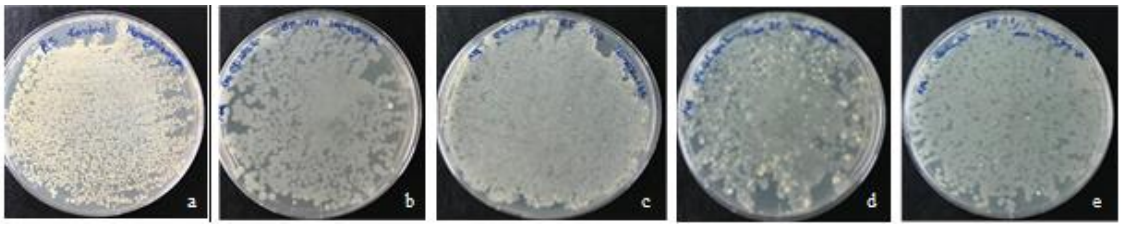

Figure 1. Microbiota changes observed in *Bombus terrestris* bees after the application of different doses of *Bacillus subtilis* MBI 600. **(a)** Control, **(b)** 1/1 biopesticide application, **(c)** 1/10 biopesticide application, **(d)** 1/100 biopesticide application, **(e)** 1/1000 biopesticide application.

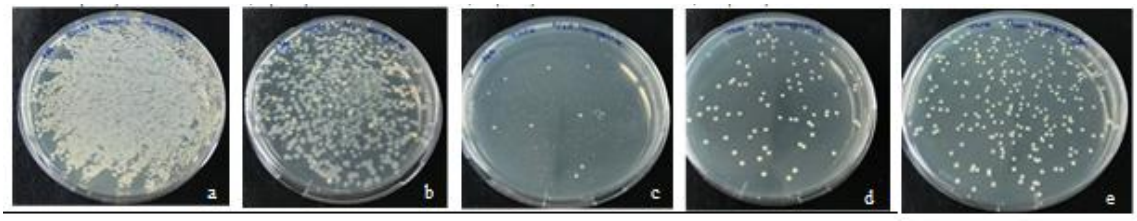

Figure 2. Microbiota changes observed in *Bombus terrestris* bees after the application of different doses of Thiamethoxam. **(a)** Control, **(b)** 1/1 pesticide application, **(c)** 1/10 pesticide application, **(d)** 1/100 pesticide application, **(e)** 1/1000 pesticide application.

It has been reported that pesticides can disrupt gut microbiota by directly affecting the growth of microbes and impairing the health of the host or by altering the gut environment (Daisley et al. 2020). Thiamethoxam is thought to indirectly disrupt bee gut microbial taxa by weakening host health (Rouzé et al. 2019; Hotchkiss et al. 2022). Thiamethoxam was concluded to be a highly potent molecule against the *B. terrestris* microbiota (including the 1/1000 dose) and causes severe bacterial inhibition. In particular, it should be emphasized that after having exposed *B. terrestris* bees to these pesticides orally for 5 days, the workers were evaluated before dying to observe the change in microbiota and to obtain healthy results. It was observed that different microorganisms became dominant in the gut microbiota of *B. terrestris* worker bees thanks to *B. subtilis* MBI 600 and Thiamethoxam and that it did not cause death in the short term.

The length of pesticide exposure is one of the factors that play an important role in pesticide-induced bee gut microbiota changes. However, while long-term exposure to a pesticide does not necessarily indicate that the gut microbiota will be disrupted (Wintermantel et al. 2018; Yang et al. 2019), it is also known that short-term pesticide exposure causes disruptions in the bee gut microbiota (Motta et al. 2018; Motta et al. 2020). Similarly, it has been revealed that even short-term and low-dose Thiamethoxam application can cause serious changes in the microbiota. These changes need to be investigated in the long term, and the results obtained from this study have shown that changes in the microbiota may also be effective in bee deaths. However, high dosages of *B. subtilis* MBI 600 used as a biopesticide also appear to cause serious changes in the microbiota. Again, this situation can be tolerated in the short term. Nevertheless, it is thought that exposure of *B. terrestris* bees to high doses for a long time may cause dramatic changes in the microbiota. Microbiota dynamics are quite variable and depending on the environments exposed (nectar source or pollen availability, flower diversity, etc.) (Weinhold et al. 2024), existing species may become dominant or inhibited. This preliminary study uses limited environments (NA, temperature, aerobic environment, etc.). However, the results show us that the different origins of pesticides used cause dramatic changes in the microbiota of such species that are important for life. It has been observed that these changes in microbiota are especially dependent on the dosage. Changes in the microbiota adversely affect the health of the living being and are associated with death. Sensitivity regarding application doses of pesticides needs to be increased. Subsequent studies plan to include selective environments to detect and control the change in the entire microbiota.

References

Bonmatin JM, Giorio C, Girolami V, Goulson D, Kreutzweiser DP, Krupke C, Liess M, Long E, Marzaro M, Mitchell EAD, Noome DA, Simon-Delso N, Tapparo A (2015) Environmental fate and exposure; neonicotinoids and fipronil. Environmental Science and Pollution Research 22: 35-67.

Cameron SA, Sadd BM (2020) Global trends in bumble bee health. Annual Review of Entomology 65: 209-232.

Campa AA, Lehmann DM (2021) Impacts of neonicotinoids on the bumble bees *Bombus terrestris* and *Bombus impatiens* Examined through the lens of an adverse outcome pathway framework. Environmental Toxicology and Chemistry 40(2): 309-322.

Chmiel JA, Daisley BA, Burton JP, Reid G (2019) Deleterious effects of neonicotinoid pesticides on *Drosophila melanogaster* immune pathways. doi: 10.1128/mBio.01395-19.

Crall JD, Switzer CM, Oppenheimer RL, Ford Versypt AN, Dey B, Brown A, Eyster M, Guérin C, Pierce NE, Combes SA, de Bivort BL (2018) Neonicotinoid exposure disrupts bumblebee nest behavior, social networks, and thermoregulation. Science 362: 683-686.

Çakıcı Ö, Uysal M, Demirözer O, Gösterit A (2023a) Sublethal effects of thiamethoxam on immune system cells in the workers of *Bombus terrestris* (Hymenoptera: Apidae). Springer Science and Business Media LLC 30(37): 87424-87432.

Çakıcı Ö, Uysal M, Demirözer O, Gösterit A (2023b) Effects of thiamethoxam on brain structure of *Bombus terrestris* (Hymenoptera: Apidae) workers. doi: 10.1016/j.chemosphere.2023.139595.

Daisley BA, Chmiel JA, Pitek AP, Thompson GJ, Reid G (2020) Missing microbes in bees: how systematic depletion of key symbionts erodes immunity. doi: 10.1016/j.tim.2020.06.006.

Dussaubat C, Maisonnasse A, Crauser D, Tchamitchian S, Bonnet M, Cousin M, Kretzschmar A, Brunet JL, Le Conte Y (2016) Combined neonicotinoid pesticide and parasite stress alter honeybee queens' physiology and survival. Scientific Reports 6: 1-7.

Engel P, Kwong WK, McFrederick Q, Anderson KE, Barribeau SM, Chandler JA, Cornman RS, Dainat J, De Miranda JR, Doublet V, Emery O, Evans JD, Farinelli L, Flenniken ML, Granberg F, Grasis JA, Gauthier L, Hayer J, Koch H, Kocher S Martinson VG, Moran N, Munoz-Torres M, Newton I, Paxton RJ, Powell E, Sadd BM, Schmid-Hempel P, Schmid-Hempel R, Song SJ, Schwarz RS, van Engelsdorpa D, Dain B (2016) The bee microbiome: Impact on bee health and model for evolution and ecology of host-microbe interactions. mBio 7: e02164-15.

Feltham H, Park K, Goulson D (2014) Field realistic doses of pesticide imidacloprid reduce bumblebee pollen foraging efficiency. Ecotoxicology 23: 317-323.

Gallai N, Salles JM, Settele J (2009) Economic valuation of the vulnerability of world agriculture confronted with pollinator decline. Ecological Economics 68: 810-821.

Garibaldi LA, Steffan-Dewenter I, Winfree R, Aizen MA, Bommarco R, Cunningham SA, Kremen C, Carvalheiro LG, Afik O, Bartomeus I, Benjamin F, Boreux V, Cariveau D, Chacoff NP, Dudenhöffer JH, Freitas BM, Greenleaf S, Hipólito J, Holzschuh A, Howlett B, Isaacs R, Javorek SK, Kennedy CM, Krewenka KM, Krishnan S, Mandelik Y, Mayfield MM, Motzke I, Munyuli T, Nault BA, Otieno M, Petersen J, Pisanty G, Potts SG, Rader R, Ricketts TH, Rundlöf M, Seymour CL, Schüepp C, Szentgyörgyi H, Taki H, Tscharntke T, Vergara CH, Viana BF, Wanger TC, Westphal C, Williams N, Klein AM (2013) Wild pollinators enhance fruit set of crops regardless of honey bee abundance. Science 339(6127): 1608-1611.

Gill RJ, Ramos-Rodriguez O, Raine NE (2012) Combined pesticide exposure severely affects individual- and colony-level traits in bees. Nature 491: 105-108.

Gill RJ, Raine NE (2014) Chronic impairment of bumblebee natural foraging behaviour induced by sublethal pesticide exposure. Functional Ecology 28: 1459-1471.

Goulson D (2003) Bumblebees: their behavior and ecology. Oxford University Press, New York.

Hotchkiss MZ, Poulain AJ, Forrest JRK (2022) Pesticide-induced disturbances of bee gut microbiotas. FEMS Microbiology Reviews 46(2): fuab056.

Johnston PR, Crickmore N (2009) Gut bacteria are not required for the insecticidal activity of *Bacillus thuringiensis* toward the tobacco hornworm, *Manduca sexta*. Applied and Environmental Microbiology 75: 5094-5099.

Kesnerova L, Mars RA, Ellegaard KM, Troilo M, Sauer U, Engel P (2017) Disentangling metabolic functions of bacteria in the honey bee gut. PLOS Biology 15(12): e2003467.

Killer J, Votavová A, Valterová I, Vlková E, Rada V, Hroncová Z (2014) *Lactobacillus bombi* sp. nov., from the digestive tract of laboratory-

reared bumblebee queens (*Bombus terrestris*). International Journal of Systematic and Evolutionary Microbiology 64: 2611-2617.

Klein AM, Vaissière BE, Cane JH, Steffan-Dewenter I, Cunningham SA, Kremen C, Tscharntke T (2007) Importance of pollinators in changing landscapes for World crops. doi: 10.1098/rspb.2006.3721.

Koch H, Cisarovsky G, Schmid-Hempel P (2012) Ecological effects on gut bacterial communities in wild bumblebee colonies. Journal of Animal Ecology 81(6): 1202-1210.

Kwong WK, Moran NA (2013) Cultivation and characterization of the gut symbionts of honey bees and bumble bees: description of *Snodgrassella alvi* gen. nov., sp. nov., a member of the family Neisseriaceae of the Betaproteobacteria, and *Gilliamella apicola* gen. nov., sp. nov., a member of Orbaceae fam. nov., Orbales ord. nov., a sister taxon to the order 'Enterobacteriales' of the Gammaproteobacteria. International Journal of Systematic and Evolutionary Microbiology 63: 2008-2018.

Kwong WK, Moran NA (2016) Gut microbial communities of social bees. Nature Reviews Microbiology 14: 374-384.

Kwong WK, Mancenido AL, Moran NA (2017) Immune system stimulation by the native gut microbiota of honeybees. Royal Society Open Science 4: 170003.

Laycock I, Lenthall KM, Barratt AT, Cresswell JE (2012) Effects of imidacloprid, a neonicotinoid pesticide, on reproduction in worker bumble bees (*Bombus terrestris*). Ecotoxicology 21: 1937-1945.

Laycock I, Cotterell KC, O'Shea-Wheller TA, Cresswell JE (2014) Effects of the neonicotinoid pesticide thiamethoxam at field-realistic levels on micro colonies of *Bombus terrestris* worker bumblebees. Ecotoxicology and Environmental Safety 100: 153-158.

Li JL, Powell JE, Guo J, Evans JD, Wu J, Williams P, Lin Q, Moran NA, Zhang, ZG (2015) Two gut community enterotypes recur in diverse bumblebee species. Current Biology 25: R652-653.

Li K, Wang L, Zhang Z, Guo Y, Guo J, Amjad N, Shaikh HM, Wu J, Li J (2021) Isolation of Bacillus from the gut of *Bombus terrestris* and its correlation in queen mating. Journal of Agricultural Science 13(9): 155-162.

Martinson VG, Danforth BN, Minckley RL, Rueppell O, Tingek S, Moran NA (2011) A simple and distinctive microbiota associated with honey bees and bumble bees. Molecular Ecology 20: 619-628.

Mommaerts V, Sterk G, Hofmann L and Smagghe G (2009) A laboratory evaluation to determine the compatibility of microbiological control agents with the pollinator *Bombus terrestris*. Pest Management Science 65: 949-955.

Mondal S, Somani J, Roy S, Babu A, Pandey AK (2023) Insect Microbial Symbionts: Ecology, Interactions, and Biological Significance. Microorganisms 11(2665): 1-27.

Motta EVS, Raymann K, Moran NA (2018) Glyphosate perturbs the gut microbiota of honey bees. doi: 10.1073/pnas.1803880115.

Motta EVS, Mak M, De Jong TK, Powell JE, O'Donnell A, Suhr KJ, Riddington IM, Moran NA (2020) Oral or topical exposure to glyphosate in herbicide formulation impacts the gut microbiota and survival rates of honey bees. doi: 10.1128/AEM.01150-20.

Nayak RK, Rana K, Bairwa VK, Singh P, Bharthi VD (2020) A review on role of bumblebee pollination in fruits and vegetables. Journal of Pharmacognosy and Phytochemistry 9: 1328-1334.

Pincus DH (2002) Microbial identification using the biomérıeux Vitek 2 system, Biomérıeux Inc., Hazelwood, MO, USA.

Poinar GO, Thomas GM (1978) Diagnostic Manual for the Identification of Insect Pathogens. USA: Plenum Press, New York.

Praet J, Cnockaert M, Meeus I, Smagghe G, Vandamme P (2017) *Gilliamella intestini* sp. nov., *Gilliamella bombicola* sp. nov., *Gilliamella bombi* sp. nov. and *Gilliamella mensalis* sp. nov.: Four novel *Gilliamella* species isolated from the bumblebee gut. Systematic and Applied Microbiology 40(4): 199-204.

Ragab A, Abd-ElAziz MF, Esmael A, Baz MM (2022) Gut microbial diversity and immunological effects of antibiotics on *Spodoptera littoralis* feeding on different diets. Benha Journal of Applied Sciences 7(12): 53-61.

Raymann K, Motta EVS, Girard C, Riddington IM, Dinser JA, Moran NA (2018) Imidacloprid decreases honey bee survival rates but does not affect the gut microbiome. doi: 10.1128/AEM.00545-18.

Richman SK, Muth F, Leonard AS (2021) Measuring foraging preferences in bumble bees: a comparison of popular laboratory methods and a test for sucrose preferences following neonicotinoid exposure. Oecologia 196: 963-976.

Rouzé R, Moné A, Delbac F, Belzunces L, Blot N (2019) The honeybee gut microbiota is altered after chronic exposure to different families of insecticides and infection by *Nosema ceranae*. doi:10.1264/jsme2.ME18169.

Saranya M, Krishnamoorthy S, Murugesh K (2019) Fortification of mulberry leaves with indigenous probiotic bacteria on larval growth and economic traits of silkworm (*Bombyx mori*, L.). Journal of Entomology and Zoology Studies 7: 780-784.

Simon-Delso N, Amaral-Rogers V, Belzunces LP, Bonmatin JM, Chagnon M, Downs C, Furlan L, Gibbons DW, Giorio C, Girolami V, Goulson D, Kreutzweiser DP, Krupke CH, Liess M, Long E, McField M, MIneau P, Mitchell EAD, Morrissey CA, Noome DA, Pisa L, Settele J, Stark JD, Tapparo A, Van Dyck H, Van Praagh J, Van der Sluijs JP, Whitehorn PR, Wiemers M (2015) Systemic insecticides (neonicotinoids and fipronil): Trends, uses, mode of action and metabolites. Environmental Science and Pollution Research 22: 5-34.

Smith DB, Arce AN, Rodrigues AR, Bischoff PH, Burris D, Ahmed F, Gill RJ (2020) Insecticide exposure during brood or early-adult development reduces brain growth and impairs adult learning in bumblebees. Proceedings of the Royal Society B: Biological Sciences 287: 20192442.

Stanley DA, Smith KE, Raine NE (2015) Bumblebee learning and memory is impaired by chronic exposure to a neonicotinoid pesticide. Scientific Reports 5: 16508.

Suslow TV, Schroth MN, Isaka M (1982) Application of a rapid method for gram differentiation of plant pathogenic and saprophytic bacteria without staining. Phytopathology 72: 917-918.

Velthuis HHW, Van Doorn A (2006) A century of advances in bumblebee domestication and the economic and environmental aspects of its commercialization for pollination. Apidologie 37: 421-451.

Verweij PE, Breuker IM, Rijs AJ, Meiss JF (1999) Comparative study of seven commercial yeast identification systems. Journal of Clinical Pathology 52: 271-273.

Wang L, Wu J, Li K, Sadd BM, Guo Y, Zhuang D, Zhang Z, Chen Y, Evans JD, Guo J, Zhang Z, Lia J (2019) Dynamic Changes of Gut Microbial Communities of Bumble Bee Queens through Important Life Stages. mSystems 4: e00631-19.

Weinhold A, Grüner E, Keller A (2024) Bumble bee microbiota shows temporal succession and increase of lactic acid bacteria when exposed to outdoor environments. doi: 10.3389/fcimb.2024.1342781.

Williams PH, Osborne JL (2009) Bumblebee vulnerability and conservation world-wide. Apidologie 40: 367-387.

Wintermantel D, Locke B, Andersson GKS, Semberg E, Forsgren E, Osterman J, Pedersen TR, Bommarco R, Smith HG, Rundlöf M, de Miranda JR (2018) Field-level clothianidin exposure affects bumblebees but generally not their pathogens. doi: 10.1038/s41467-018-07914-3.

Wu Y, Zheng Y, Chen Y, Wang S, Chen Y, Hu F, Zheng H (2020) Honey bee (*Apis mellifera*) gut microbiota promotes host endogenous detoxification capability via regulation of P450 gene expression in the digestive tract. doi: 10.1111/1751-7915.13579.

Yang Y, Ma S, Yan Z, Liu F, Diao Q, Dai P (2019) Effects of three common pesticides on survival, food consumption, and midgut bacterial communities of adult workers *Apis cerana* and *Apis mellifera*. doi: 10.1016/j.envpol.2019.03.077.

Zhang ZJ, Zheng H (2022) Bumblebees with the socially transmitted microbiome: A novel model organism for gut microbiota research. Insect Science 29: 958-976.

Zhang G, Dilday S, Kuesel RW, Hopkins B (2024) Phytochemicals, probiotics, recombinant proteins: enzymatic remedies to pesticide poisonings in bees. Environmental Science & Technology Journal 58: 54-62.

Zheng H, Nishida A, Kwong WK, Koch H, Engel P, Steele MI, Moran NA (2016) Metabolism of toxic sugars by strains of the bee gut symbiont *Gilliamella apicola*. mBio 7: e01326-16.

Zheng H, Steele MI, Leonard SP, Motta EVS, Moran NA (2018) Honey bees as models for gut microbiota research. doi:10.1038/ s41684-018-0173-x.