

THE IMPACT OF HONEYBEE GUT BACTERIA ON THE SURVIVAL OF HONEYBEES EXPOSED TO INSECTICIDES

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ABSTRACT

The widespread use of insecticides poses a significant threat to the health and sustainability of honeybees (*Apis mellifera*). This study aimed to isolate, identify, and investigate the potential of honeybee gut bacterial strains in mitigating the detrimental effects of insecticides on honeybees and extending their lifespan. The efficacy of seven honeybee gut bacterial strains in reducing insecticide toxicity was evaluated. Through the identification of honeybee gut bacterial isolates using 16s rRNA, seven strains were identified, namely *Priestia endophytica*, *Bacillus subtilis*, *Bacillus pumilus*, *Peribacillus frigiditolerans*, *B. subtilis*, *Bacillus pumilus*, and *Bacillus tequilensis*. The experimental results revealed that bees treated with these gut bacteria significantly reduced oxidative stress markers and detoxification enzyme levels compared to untreated bees. Moreover, the treated bees demonstrated enhanced immune responses. The bees treated with deltamethrin + intestinal bacteria showed an increase in bees' lifespan to 6.33 days compared to a lifespan of 4 days with deltamethrin only. Similarly, bees treated with acetamiprid intestinal bacteria had further extended to 10.33 days compared to a lifespan of 7.67 days with acetamiprid. These findings suggest that using honeybee gut bacterial strains may serve as a sustainable strategy to mitigate the harmful impacts of insecticides on honeybees, thereby promoting their overall health and contributing to the preservation of pollination services and apiculture. Further research is warranted to elucidate the underlying mechanisms and optimize the application of these natural products in honeybee management practices.

Keywords: Honeybee, Gut bacteria, Survival, Insecticides, Deltamethrin, Acetamiprid

INTRODUCTION

A semi-free-ranging species of *Apis mellifera* holds immense value worldwide due to its exceptional honey properties and its crucial role in the ecological process of plant reproduction and in pollinating numerous economic crops, making it indispensable for agricultural productivity and food security (Visick and Ratnieks, 2023). Beekeeping practices rooted in local knowledge not only ensure livelihood security but also contribute to poverty alleviation in both urban and rural communities (Leska et al., 2021). Colony Collapse Disorder (CCD) is a perplexing phenomenon characterized by an unexplained decline in bee populations observed in both Europe and the United States (Al-Solami et al., 2022; Ferrier et al., 2018). The winter losses of CCD have ranged from 21.9% to 35.8%, with an average decrease of 28.7% in bee colonies (Hamer and Scuse, 2010). These may be due to encompass habitat degradation, the potential sub-lethal effects of genetically modified organisms (GMOs) on bees, viral infections, the invasion of disruptive species like the *Aethina tumida* beetle and wasps, air pollution impeding flower-insect interactions, the impact of chemical compounds such as pesticides, antibiotics, and heavy metals, the influence of microorganisms, and the presence of parasitic mites (van der Sluijs et al., 2013; Meikle and Diaz, 2012; Highfield et al., 2009). These factors often interact in intricate ways, resulting in population instabilities that vary across regions.

While there has been a recent decline in CCD symptoms, it is crucial to recognize that various contributing factors, such as insufficient food supply, inadequate bee management practices, queen-related issues, parasitic infestations, and overall compromised bee health, play a role in bee mortality (Leska et al., 2021). Despite

the global expansion of managed bee hives, concerns regarding the dwindling bee numbers persist, given the indispensable role of pollinators in ensuring global food production.

Pesticides like fungicides, herbicides, insecticides, and rodenticides have substantial effects on both living organisms and the environment, resulting in adverse health outcomes, especially in body systems (Alewu and Nosiri, 2011; Golshani et al., 2022). Certain insecticides, like neonicotinoids, coumaphos, Fipronil, Spinosad, and chlorpyrifos, have detrimental impacts on bees (Leska et al., 2021) and lead to the decline of bee populations, as they contaminate nectar and pollen, leading to developmental delays, compromised immune systems, and reduced lifespans in bees (Feazel-Orr et al., 2016). Honey bees, with their limited genetic capacity for resisting insecticides, are especially susceptible (Claudianos et al., 2006). Pesticides can also disrupt bee reproductive processes and impact their movement, orientation, overall development, and immune function (Pettis et al., 2013). Exposure to pesticides can heighten vulnerability to parasites, potentially resulting in the extinction of entire bee colonies. The combination of pesticide use and loss of natural habitats reduces the diversity of pollinators and disrupts the intricate network of pollination (Park et al., 2015). Bees can also encounter harmful pesticides from sources other than the targeted crops. Several studies were carried out on the sublethal effects of pesticides like acetamiprid and deltamethrin on honeybees, which contribute to declines in honeybee populations worldwide (Pervez and Manzoor, 2023; Yang et al., 2023). The impacts of acetamiprid on honeybees reduced survival rates, decreased locomotor activity, and impaired olfactory learning in the exposed honeybees (Aliouane et al., 2009). also, the negative effects on honeybees' foraging behavior

and navigational skills (Suchail *et al.*, 2001), on honeybee larvae lead to decreased brood viability and impeded colony expansion (Wu *et al.*, 2011), and on honeybees' learning and foraging abilities (El Hassani *et al.*, 2008). Sgolastra *et al.*, 2017, found that the impacts of deltamethrin also on beehive colonies encompass adverse effects on brood growth, foraging behavior, and overall colony performance. Bees have several mechanisms for detoxification to mitigate the harmful effects of insecticides and other toxins (Gong and Diao, 2017; Magesh *et al.*, 2017). These mechanisms consist of enzymatic detoxification (Zhu *et al.*, 2020), metabolic pathways (Rand *et al.*, 2015), and efflux pumps (Panini *et al.*, 2016). The makeup and variety of microorganisms in the gut of honeybees can impact their susceptibility to insecticides. Research indicates that honeybees with a richer and more varied community of gut bacteria generally display increased insecticide resilience compared to those with a less diverse microbiota (Hussain *et al.*, 2023). Bacteria have diverse mechanisms for breaking down pesticides, depending on the specific type of pesticide and the bacteria involved. For instance, organophosphate (OP) insecticides are broken down by enzymes called organophosphorus hydrolases (OPHs) produced by bacteria like *Pseudomonas*, *Flavobacterium*, and *Sphingobium* (Huang *et al.*, 2018; Singh and Walker, 2006; Johnsen *et al.*, 2001). Carbamate insecticides are degraded by enzymes called carbamoylases, which are synthesized by bacteria such as *Bacillus* and *Pseudomonas* (Huang *et al.*, 2018). Pyrethroid insecticides are typically broken down by enzymes like cytochrome P450 and esterases, which can be generated by bacteria like *Streptomyces*, *Brevibacterium* sp., and *Bacillus subtilis* (Soler, 2008). Neonicotinoid insecticides, on the other hand, are typically metabolized by enzymes called cytochrome P450s, which are produced by bacteria like *Rhodococcus* and *Sphingobium* (Ortiz-Hernández *et al.*, 2013; Soler, 2008). Understanding honeybees' detoxification mechanisms and their response to pesticides is crucial for effective bee management, particularly in the context of widespread insecticide use in agriculture (Mishukovskaya *et al.*, 2023). This study aimed to achieve several objectives, including the isolation and characterization of gut bacteria present in honeybees, measurement of sublethal concentrations of insecticides, and investigation of the impact of the isolated gut bacteria on honeybee survival.

MATERIALS AND METHODS

Sample collection

Honeybee samples were obtained from local honeybee colonies at the Bee and its Products Research Center, King Khalid University (KKU), Abha, Saudi Arabia (Figure 1). Using sterile forceps, worker bees of the *Apis mellifera jemenitica* (AMJ) species were collected from the hives. A single healthy colony was randomly chosen from the apiary, and worker bees were directly collected from this colony. The bees were then placed in a cage to facilitate their transport to the laboratory.



Figure 1 Illustrates the samples collected from the Bee's apiary and its Products Research Center.

Isolation of Gut bacteria

Bees were disinfected before gut dissection by immersing them in 70% ethanolic alcohol for 10 seconds to ensure the removal of external microbes, followed by rinsing three times in sterile purified water. The dissection of the entire digestive tract spanned from the ventricle to the rectum was performed on five bees within a laminar flow hood. The dissected intestines (n = 5) were homogenized using a pestle in 3 ml of normal saline (Figure 2). The modified version of the gut bacterial culture method was described by Khan *et al.*, (2017). Sterile wooden cotton applicators from Shanghai Channelmed Co., Ltd., China, were used to plate the homogenate onto fourteen nutrient agar media plates (Neogen® Culture Media). The plates were then incubated aerobically at 36°C and 80% relative humidity for 72 hours using an Incubator (MODEL: JSSI-100T, Desc: Compact Shaking Incubator, Serial No: 200106-83, KOREA). Bacterial colonies on the agar plates were selected based on their size (small, medium, and large), color (white, cream,

opaque, and yellow), and shape (round, irregular, filamentous). Selected colonies were individually streaked onto fresh agar plates to establish pure bacterial cultures (Figure 3).

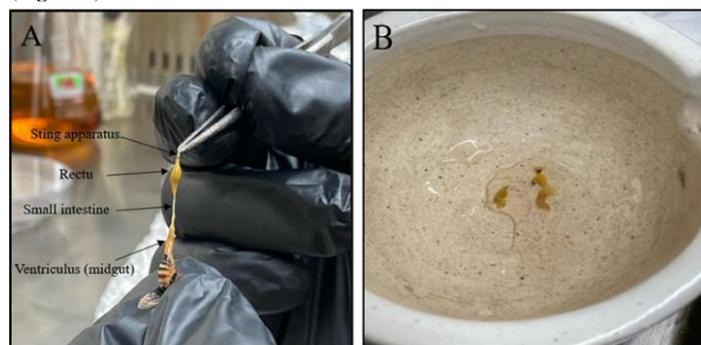


Figure 2 (A) Process remove the bee's intestines using sterile forceps (Dade, 2009). (B) Put the bee intestines in a dish containing normal saline, and then the isolation of bacteria from them.

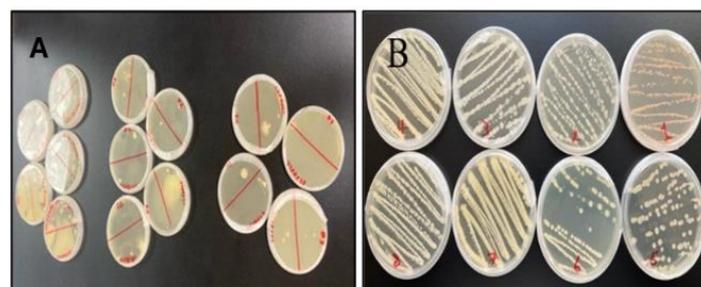


Figure 3 Displays the collection of gut bacterial species isolated in an impure form for initial identification (A) and the selected pure bacterial species utilized in the current study (B).

Molecular identification

Seven types of bacterial samples isolated from the gut of a honeybee were sent to Macrogen10F, 254, Beotkkot-ro, Geumcheon-gu, Seoul (Gasan-dong, World Meridian D), for DNA extraction, PCR, and DNA sequencing. PCR using universal primers (27F 5' AGAGTTTGATCMTGGCTCAG-3') and (1492R 5' TACGGYTACCTTGTTACGACTT-3') to amplify the 16S rRNA gene. Chromatograms of the DNA sequences were visually inspected, edited with Bio Edit (Hall, 1999), and aligned with MEGA version 11. Further, the sequences were compared to previously deposited sequences in the NCBI GenBank database using BLAST-N. Sequences were deposited to National Center for Biotechnology Information –NCBI to get the accession numbers.

Analysis of Phylogenetic

The diversity and evolutionary background of gut bacteria were investigated using phylogenetic analysis. Partially related 16S rRNA sequences were obtained using BLAST-N from the National Center for Biotechnology Information (NCBI) database. In Bio Edit, many sequence alignments are created and modified by hand (Hall, 1999). MEGA version 11 (Tamura *et al.*, 2021) was used to perform phylogenetic and molecular evolutionary analyses of the sequences acquired from GenBank and those produced in this investigation. The neighbor-joining approach was employed, with several bootstrap repeats.

Preparation of bacterial species

After isolating seven distinct types of bacteria from the gut of local honeybees and obtaining pure cultures of each on Nutrient Agar, they were individually transferred into the nutrient broth. Subsequently, the seven bacterial types were placed in separate tubes and incubated at 32 ± 2 °C for 24 hours. Following incubation, the tubes were stored in the refrigerator until their final application and use.

Measuring the sublethal concentration of insecticides

Experimental cages

The wooden cages were utilized measuring 15 x 15 x 5 cm, which featured a glass panel on one side to allow visibility while the other side with an iron grid to facilitate airflow. On the top of the cage two holes, one hole fitted with a 20 mL syringe containing a 50% (w/v) sucrose solution, and another hole designated for the treatment Khan and Ghranh, (2022) (Figure 4).

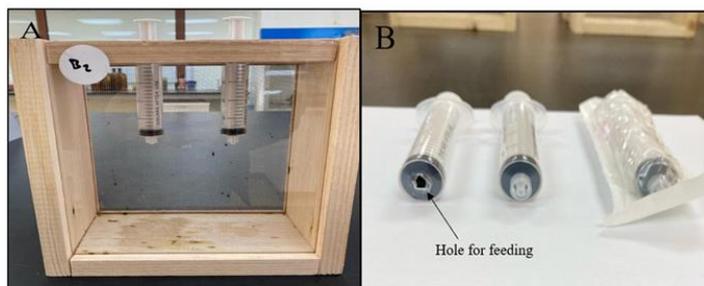


Figure 4 (A); The cage used in the experiment, (B); the syringes used to feed the bees, showing the holes in them.

Collection of nurse bee (*Apis mellifera*)

Nurse honeybees, sourced from the Bee Research Center, (*A. mellifera jemenitica*) hives. The nurse honeybees were carefully collected and housed in individual cages, each cage accommodating 20 bees.

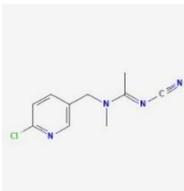
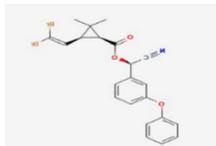
Insecticides

Deltamethrin, an insecticide belonging to the group of pyrethroid pesticides with an active substance concentration of 2.5%, was selected for the study. Additionally, Acetamiprid, a systemic pesticide from the neonicotinoid group with an active substance concentration of 20%, was chosen (Figure 5). Molecular formula and chemical structure for each acetamiprid and deltamethrin are mentioned in (Table 1) according to National Center for Biotechnology Information (2024).



Figure 5 The insecticides used in the experiment (A); Acetamiprid and (B); Deltamethrin

Table 1 The molecular formula and chemical structure for each insecticide

Insecticide	Molecular formula	Chemical structure
Acetamiprid	C ₁₀ H ₁₁ ClN ₄	
Deltamethrin	C ₂₂ H ₁₉ Br ₂ NO ₃	

Preliminary bioassay of insecticides

A no-choice bioassay was conducted to determine the sublethal doses of insecticides, following the methodology outlined by Laurino with some potential modifications (Laurino et al., 2011). The objective was to identify the concentrations of insecticides that cause mortality rates higher than 0% but lower than 100%. Four different concentrations of each insecticide were prepared: 9 ppm, 6 ppm, 3 ppm, and 1 ppm. These concentrations were incorporated into a 50% sugar solution. Multiple cages were employed in the experiment, with each cage containing 20 healthy individuals of *Apis mellifera*. Each cage was equipped with two 20 ml syringes, one containing a 50% sucrose solution and the other containing the tested insecticide concentration in a. Data were recorded daily following the initial exposure. Deceased bees were regularly removed from the cages and

counted. Bees that showed no movement upon being touched with a needle were also considered dead (Figure 6).

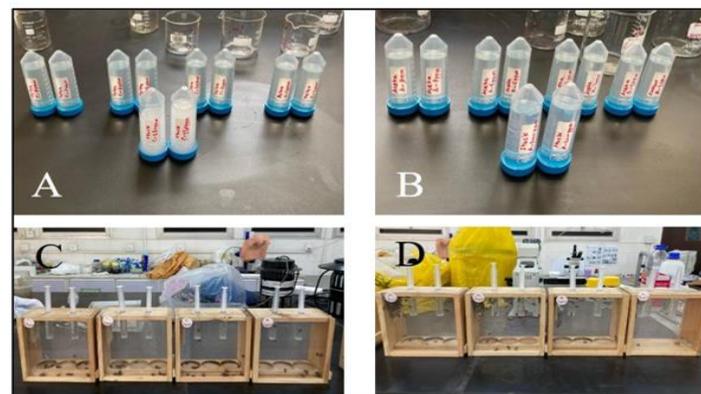


Figure 6 The preparation of insecticides where (A); the stock and different concentrations of deltamethrin, (B); acetamiprid, (C); application of the concentrations of deltamethrin on honeybees in the cages, (D); application of acetamiprid.

Sublethal concentration study

After preliminary bioassay and calculation of sublethal concentrations, the bees were studied the LC₅₀ and LC₉₀ of insecticide solution. For this purpose, about 20 nurse bees were subjected to each concentration. Three different replications were made to achieve more accuracy. The exposure time was only 72 hrs. and after that. The survival of workers exposed to insecticide solution was compared with those in the control group (without insecticide exposure and given only sugar solution). The data was calculated until 72 hrs.

Application of treatments

The study aimed to investigate the impact of gut bacteria on the toxicity of pesticides to honeybees and their lifespan. Each treatment was administered to nurse honeybees (n=25) in specially designed cages. Within the cages, two 20 ml syringes were provided. One syringe contained the treatments, which consisted of bacteria isolated from the gut of *Apis mellifera jemenitica* (AMJ). For each bacterial species (total of seven species), 200 µl was taken from the respective tube, placed in 500 ml of pure nutrient broth, then incubated for 24 hrs. Subsequently, 10 ml of the bacterial mixture in nutrient broth was combined with 1 L of a solution containing 50% sugar and water. The second syringe in the cage contained a concentration of 3 ppm of the pesticide deltamethrin or acetamiprid, dissolved in a solution of water and 50% sugar. This setup served as the positive control, with different treatments applied alongside deltamethrin or acetamiprid. Additionally, there was a positive control group for each pesticide, consisting of the respective pesticide (3 ppm) dissolved in water solution and a syringe with only water. The negative control group comprised a syringe with a solution of water and 50% sugar and another syringe containing only water. (Table 2) provides a summary of the treatments.

The study encompassed three repetitions of each treatment, utilizing 15 cages. Among these, 6 cages contained the pesticide deltamethrin at a concentration of 3 ppm along with gut bacteria (3 cages), three cages served as the positive control group for deltamethrin, and the other 6 cages contained the pesticide acetamiprid at a concentration of 3 ppm with bacteria (3 cages), three cages formed the positive control group for acetamiprid. Additionally, three cages comprised the negative control group.

Table 2 Application of treatments with acetamiprid and deltamethrin

Treatment	Feeder-1	Feeder-2
Gut bacteria	10 ml of the bacteria mixture in nutrient broth /	3 ppm acetamiprid + H ₂ O + 50% sugar solution
	1 liter of H ₂ O + 50% sugar solution	3 ppm deltamethrin + H ₂ O + 50% sugar solution
Positive control	H ₂ O	3 ppm acetamiprid + H ₂ O + 50% sugar solution
		3 ppm deltamethrin + H ₂ O + 50% sugar solution
Negative control	H ₂ O + 50% sugar solution	H ₂ O

Statistical analysis

Data analysis was subjected to statistical analysis; one-way ANOVA, to compare the datasets, and the differences between means seemed to be significant at P < 0.05. All of these analyses were performed using SPSS software (version 26).

Additionally, the survival curve was analyzed using the Kaplan-Meier method. For toxicological and ecological studies, the Ldp-line software was utilized to conduct probit analysis. This analysis was employed to illustrate the relationship between the stimulus and response in the context of the honeybees' exposure to insecticides. Furthermore, the dose-response regression line was utilized to depict this relationship accurately.

RESULTS

Isolation and characterization

The study yielded seven bacterial isolates, subjected to molecular characterization based on their 16s rRNA partial sequences. The analysis revealed that these isolates corresponded to seven distinct bacterial species: *Priestia endophytica*, two *Bacillus subtilis*, two *Bacillus pumilus*, *Peribacillus frigoritolerans*, and *Bacillus tequilensis*. Further details regarding these species can be found in (Table 3), including information obtained from the NCBI blast.

Phylogenetic analysis

A phylogenetic tree was constructed for gut bacteria isolated from the local honeybees collected from Abha, Saudi Arabia. The partial 16S rRNA gene sequence obtained in this study and 35 sequences from previous studies conducted in different countries obtained from NCBI were used to construct a phylogenetic tree by MEGA 11 (Figure 7).

Table 3 Identification of gut bacteria isolated from *Apis mellifera jemenitica*

Isolated Code	Accession No. (Current study)	Closest bacterial species in GenBank	Accession No. Blast-N	Identity (%)
GB-1	PP346328	<i>Priestia endophytica</i>	MN252910	99.79
GB-2	PP346329	<i>Bacillus subtilis</i>	OP986262	100
GB-4	PP346330	<i>Bacillus pumilus</i>	JN210909	99.93
GB-5	PP346331	<i>Peribacillus frigoritolerans</i>	MK318217	100
GB-6	PP346332	<i>Bacillus subtilis</i>	MF136610	100
GB-7	PP346333	<i>Bacillus pumilus</i>	KF158227	100
GB-8	PP346334	<i>Bacillus tequilensis</i>	OQ405609	99.93

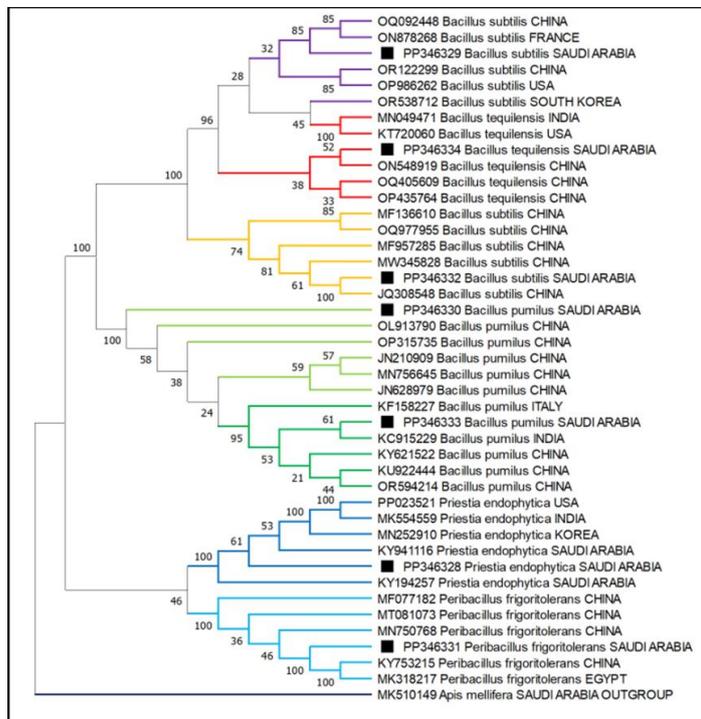


Figure 7 A phylogenetic tree was constructed using closely similar sequences acquired from the NCBI database and the 16S rRNA gene sequences of gut bacteria isolated from the native Saudi Arabian honeybee (*Apis mellifera jemenitica*).

Impact of gut bacteria on the longevity of A.m. Jemenitica

The study investigated the impact of gut bacteria on the longevity of honeybees exposed to deltamethrin and acetamiprid. The presence of gut bacteria was found to have a significant positive effect on the lifespan of worker honeybees compared

to the positive control treatments where only deltamethrin or acetamiprid was applied.

In the case of honeybees exposed to deltamethrin, the presence of gut bacteria resulted in the highest lifespan, with an average of 6.33±1.4528 days (Figures 8-10). Conversely, honeybees treated with the positive control (deltamethrin only) had the lowest lifespan. The negative control group (non-treatment) exhibited an average lifespan of 23.67±0.6667 days. These findings evidence that gut bacteria enhance the bees' immunity against toxicity, leading to an increased life expectancy as nurse honeybees.

Similarly, for honeybees exposed to acetamiprid, the presence of gut bacteria also longevity. The highest lifespan, averaging 10.33±1.2019 days, was observed when gut bacteria were present (Figures 8-10). Conversely, honeybees treated with the positive control (acetamiprid only) had a lower average lifespan of 7.67±0.3333 days. The negative control group displayed an average lifespan of 23.67±0.6667 days. These results indicate that gut bacteria enhance the bees' immunity against the neonicotinoid systematic insecticide acetamiprid, thereby increasing their life expectancy as nurse honeybees.

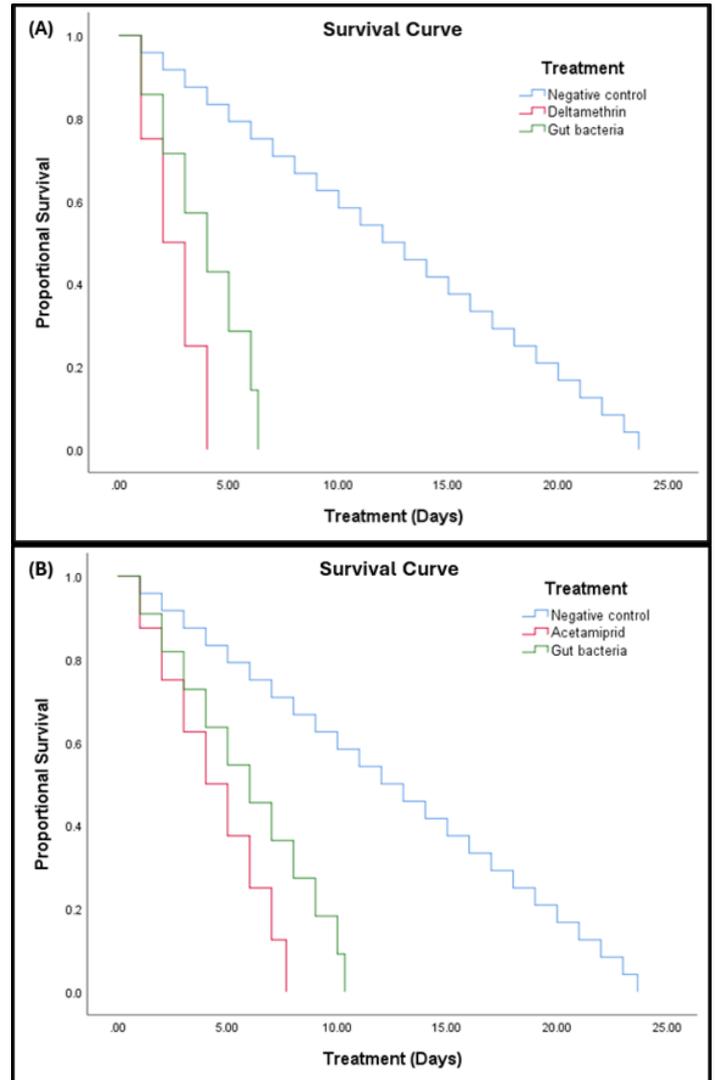


Figure 8 Kaplan-Meier Survival curve of nurse bees in each experimental group during the 25-day cage bee experiment. The y-axis represents the Kaplan-Meier estimates of the survival probabilities. The x-axis represents the survival days until the last bee. A comparison of cumulative survival between nurse bees under (A) deltamethrin and (B) acetamiprid insecticide exposure (positive control), 50% sugar solution only (negative control), and deltamethrin with honeybee gut bacteria (treatments).

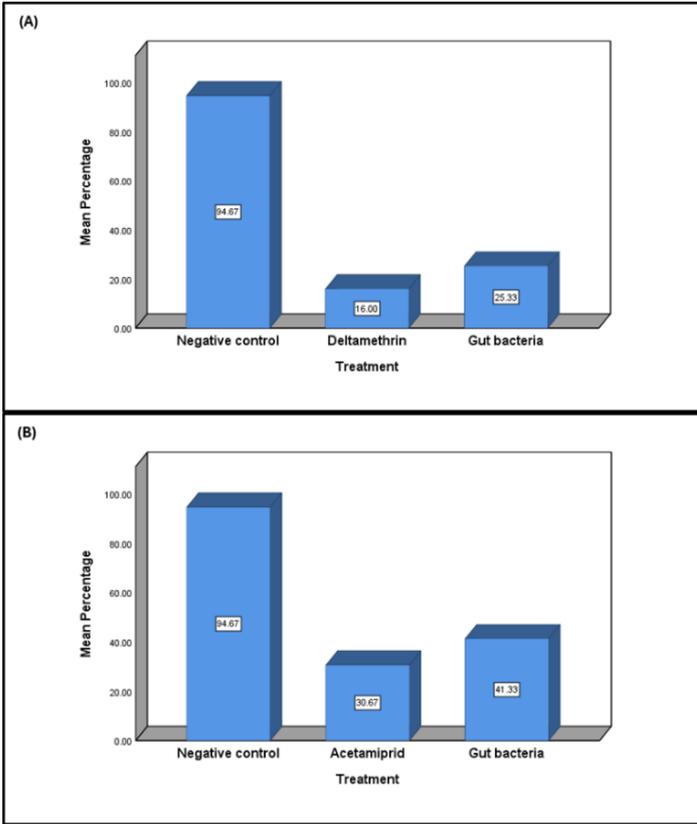


Figure 9 The mean of the percentage days of honeybee survival exposed to (A) deltamethrin and (B) acetamiprid.

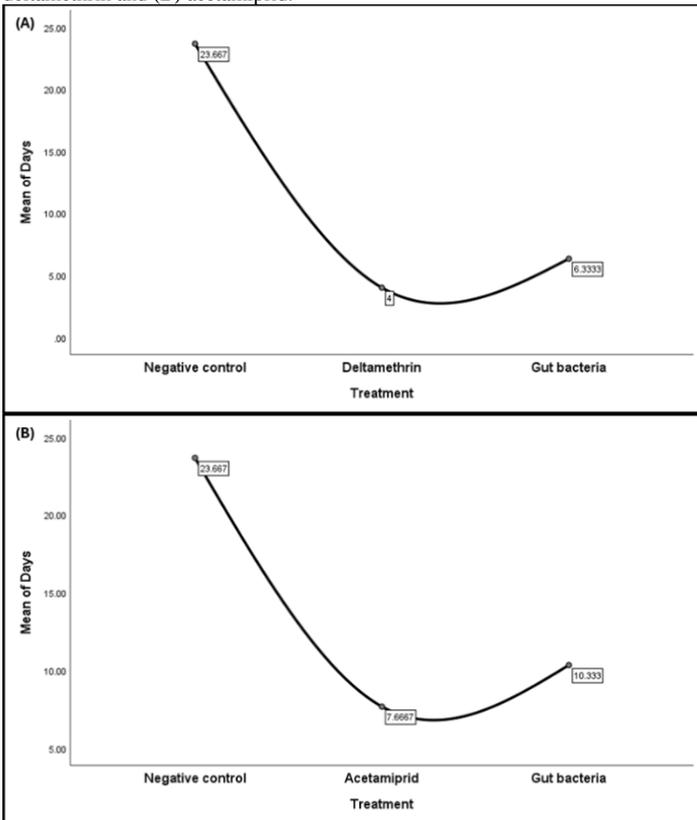


Figure 10 The mean of days of honeybee survival exposed to (A) deltamethrin and (B) acetamiprid.

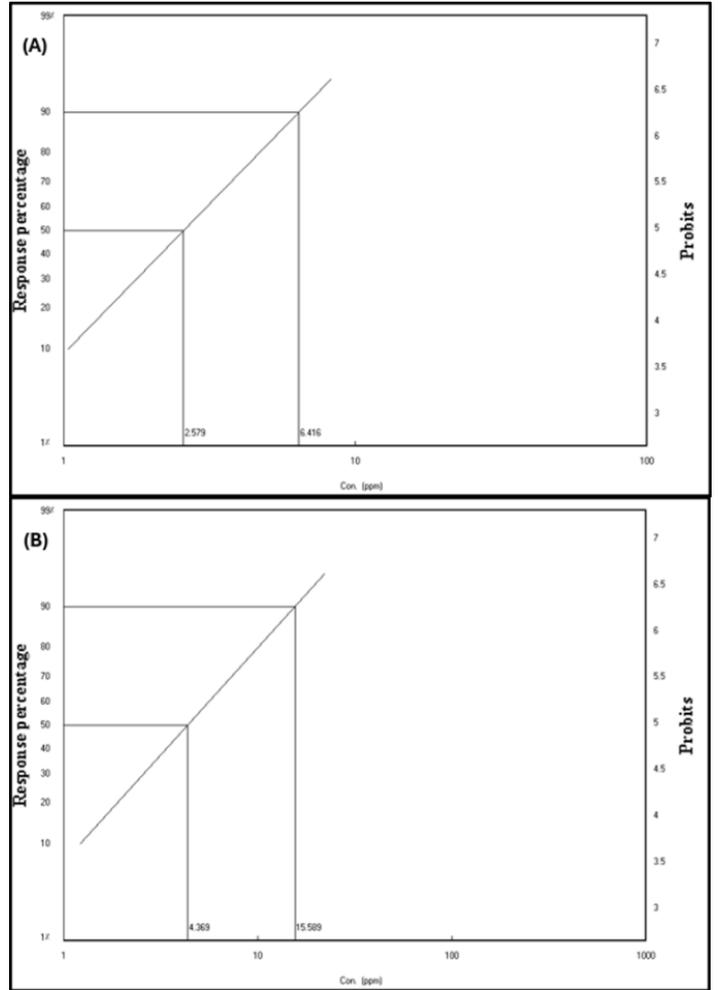


Figure 11 The relationship between concentrations of (A) deltamethrin, (B) acetamiprid, and mortality percentage of honeybee.

LC50, and LC90 Concentrations

To quantify the LC50 and LC90 concentrations, the honeybees were subjected to various concentrations of acetamiprid (1 ppm, 3 ppm, 6 ppm, and 9 ppm), resulting in concentration-dependent mortality percentages of 10%, 30%, 55%, and 85%, respectively. In comparison, the control group exhibited a mortality rate of 5% (Table 3). The calculated LC50 value for acetamiprid was determined to be 4.369 ppm, indicating the concentration at which 50% of the honeybees were expected to perish, while the LC90 value was found to be 15.589 ppm (Figure 11). Similarly, the honeybees were exposed to different concentrations of deltamethrin (1 ppm, 3 ppm, and 6 ppm), demonstrating mortality percentages of 10%, 55%, and 99.9%, respectively. In contrast, the control group displayed a mortality rate of 5%. The estimated LC50 value for deltamethrin was 2.579 ppm, indicating the concentration where 50% of the honeybees were expected to perish, while the LC90 value was determined to be 6.416 ppm (Figure 11).

Resistance Ratio (RR)

The LC50 values represent the concentration at which 50% of the test organisms, such as honeybees, are expected to succumb to the insecticide's toxicity. In this study, the LC50 value for deltamethrin was determined as 2.579, while for acetamiprid, it was found to be 4.369. Also, the resistance ratio (RR) of honeybees was determined by comparing the pesticides acetamiprid and deltamethrin, resulting in an RR value of 1.694 (Figure 12).

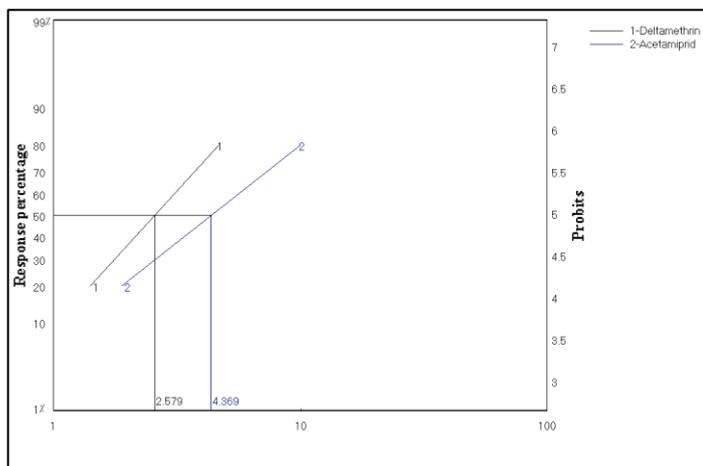


Figure 12 The relationship between LC50 concentrations of insecticides and mortality percentage of honeybees

DISCUSSION

Bees play a significant role as pollinators, making them economically valuable for agriculture globally (Leska et al., 2021). The bee-derived products possess numerous properties, such as antioxidant (Shakoori et al., 2024), antimicrobial (Al-Masaudi et al., 2020, Al-Masaudi and Al-Maaqar, 2020, Al-Maaqar, 2020), anti-inflammatory, antiproliferative, and anticancer activity (Zein et al., 2024). Bee colony health is threatened by insecticides (Johnson and Corn, 2015). The extensive use of pesticides, including Deltamethrin and Acetamiprid, in Saudi Arabia poses a significant threat to the health and survival of honey bees, putting them at risk of extinction (Ali and Selem, 2012). Several studies highlight the detrimental effects of acetamiprid and deltamethrin on honeybees, emphasizing the risks they pose to bee health, colony dynamics, and overall pollination processes (Sgolastra et al., 2017; Wu et al., 2011; Aliouane et al., 2009). Understanding the differential impacts of these insecticides can aid in developing appropriate strategies to mitigate their negative effects and promote the conservation of honeybee populations.

Bees have developed detoxification mechanisms to counteract the effects of insecticides. The diversity of gut bacteria in honeybees has been found to impact their vulnerability to these chemicals, as bees with a more diverse microbiota show greater resilience (Hussain et al., 2023). These findings emphasize the significance of gut bacteria in the survival of honeybees when exposed to pesticides.

Honeybee intestines host vital bacteria, such as Lactobacillus strains known as Firm-5 and Lactobacillus Firm-4, along with Bifidobacterium spp., Gilliamella apicola, and Snodgrassella alvi. Furthermore, Frischella perrara, Bartonella apis, Apibacter adventoris, and Parasaccharibacter apium may also be found, albeit in fluctuating quantities, and considered the core of gut bacteria in honeybees (Zheng et al., 2018).

In our current study, seven bacterial species were isolated from the intestines of local honeybee workers, *Apis mellifera jemenitica*, from the Abha region. These species include *Priestia endophytica*, two strains of *Bacillus subtilis*, two strains of *Bacillus pumilus*, *Peribacillus frigoritolerans*, and *Bacillus tequilensis*. The microbial communities of foraging honey bees (*Apis mellifera jemenitica*) were studied in the Al-Baha and Riyadh regions, where numerous types of bacteria were identified, including *Bacillus subtilis*, which aligns with our study (Khan et al., 2017 & Al-Ghamdi et al., 2020) showed that *Bacillus* strains obtained from the gut of *A. mellifera* can suppress chalkbrood pathogens (Al-Ghamdi et al., 2020). 87% of the *Bacillus* species were found in the intestines of the Asian honeybee *Apis dorsata* (Niode et al., 2021). In a study conducted in Turkey on *Apis mellifera*, it was found that newly emerging queens and workers of bees contain bacteria, including *Bacillus subtilis* and *Bacillus pumilus*, which are consistent with the two types observed in our study (Rustemoglu, 2023). *Bacillus subtilis* and *Bacillus pumilus* have been isolated from bee food (pollen and bee bread), providing evidence that bees acquire non-essential bacterial species from various sources (Gilliam, 1979).

As for the other species isolated in this study, they may indeed be newly acquired species for honeybees: *Bacillus tequilensis*, *Peribacillus frigoritolerans*, and *Priestia endophytica*. According to our information, no one has yet proven their isolation from the intestines of *Apis mellifera jemenitica*. *Peribacillus frigoritolerans* is a rod-shaped, Gram-positive bacterium belonging to the family Bacillaceae, originally classified as *Brevibacterium frigoritolerans* (Montecillo and Bae, 2022).

The evolutionary history was inferred using the UPGMA method (Sneath, 1973). The bootstrap consensus tree inferred from 500 replicates (Felsenstein, 1985) is taken to represent the evolutionary history of the taxa analyzed (Felsenstein, 1985). Branches corresponding to partitions reproduced in less than 50% of bootstrap replicates are collapsed. The percentage of replicate trees in which the

associated taxa clustered together in the bootstrap test (500 replicates) are shown above the branches (Felsenstein, 1985). The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura et al., 2004), and are in the units of the number of base substitutions per site. This analysis involved 43 nucleotide sequences. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There were a total of 1627 positions in the final dataset. Evolutionary analyses were conducted in MEGA11 (Tamura et al., 2021).

In our study, it was found that the LC50 of the pesticide deltamethrin on nurse bees of *A. m. jemenitica* was 2,579 ppm, and for LC90, it was 6,416 ppm, while the lethal concentration of acetamiprid for LC50 was 4,369 ppm and for LC90, it was 15,589 ppm. The mortality rate of the bees at the lowest concentration of 1 ppm was 10% for both pesticides, while at the highest concentration of deltamethrin (6 ppm), it was 99.9%, and the mortality rate at the highest concentration of acetamiprid (9 ppm) was 85%. Symptoms of honeybee poisoning can vary, but a primary sign is a notable increase in dead bees. According to FAO guidelines, a colony typically sees about 100 bee deaths/day under normal circumstances. However, pesticide poisoning can cause this number to rise significantly. For example, if there are 200-400 dead bees, it may indicate a low level of poisoning, while 500-1000 dead bees could suggest a medium level. If over 1000 dead bees are present, it indicates a high level of poisoning (Akranakul, 1990). A study was conducted on the toxicity of the pesticide deltamethrin on the lifespan of foraging bees of *Apis mellifera jemenitica*. It concluded that the LC50 was 32.53 ppm, and the LC90 was 115.11 ppm by oral administration. Additionally, the concentration of 250 ppm (the highest concentration in the study) and 25 ppm (the lowest concentration in the study) caused a mortality rate of 75% and 22.5%, respectively, after 4 hours of exposure (Abuagla et al., 2023). A study concluded that exposing newly emerged *Apis mellifera* workers to a dose higher than 0.5 µg/bee of acetamiprid affects honey bee survival and memory (Shi et al., 2019). A study demonstrated that deltamethrin is more toxic than acetamiprid after exposing *Apis mellifera* larvae for 72 hrs. Deltamethrin's LC50 was 1.79 mg/l, and its LD50 was 0.05 µg/larva, whereas acetamiprid's LC50 was 188.49 mg/l, and its LD50 was 5.65 µg/larva (Yang et al., 2020).

CONCLUSION

The study findings revealed a significant positive impact of bacteria isolated from honeybee intestines on the survival of honeybees exposed to pesticides. Notably, the results demonstrated that bee gut bacteria exhibited higher efficacy against acetamiprid than deltamethrin. This discrepancy may be attributed to the severe toxicity associated with deltamethrin. To enhance the survival of honeybees exposed to pesticides, it is recommended to increase the concentration of both bee intestinal bacteria in bee food, thus yielding improved outcomes. Additionally, instead of utilizing a mixture of bacterial cells, employing individual bacterial strains isolated from bee intestines may prove to be beneficial. Further research is highly recommended to explore effective methods of safeguarding honeybees against pesticide toxicity and harm. Additionally, studying the mechanisms by which bee gut bacteria break down and mitigate the toxicity of pesticides, thereby enhancing honeybees' resistance and immunity, is crucial.

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