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Research Article

Comparative acute toxicity of eight insecticides against worker Honeybees (*Apis mellifera* L.)

Abid Farid[1], **Moazzam Khan**[1], **Rasheed Akbar**[1], **Mohib Ullah**[1], **Brekhna Faheem**[2], **Naseem Rafiq**[2]
[1]*Department of Entomology, Faculty of Physical and Applied Sciences, The University of Haripur, Haripur Khyber Pakhtunkhwa 22062 Pakistan.*
[2]*Department of Zoology, Abdul Wali khan University Mardan, Pakistan.*

ABSTRACT

Honeybees (*Apis mellifera* L.) are vital pollinators in natural and agricultural ecosystems, yet pesticide exposure remains a primary factor contributing to their decline. The studies were conducted to figure out the acute oral and contact toxicity of eight commonly used insecticides including acetamiprid, imidacloprid, diafenthiuron, abamectin, chlorpyrifos, spinosad, methoxyfenozide, and cypermethrin to worker honeybees under controlled laboratory conditions. Bees were exposed to 0.5, 1.0, 2.0, 4.0 and 5.0 µg/ml concentration of each insecticide, and mortality was recorded after 12, 24, 48, and 72 hours' exposure periods. Median lethal concentrations (LC_{50}, LC_{90}) and doses (LD_{50}, LD_{90}) were estimated using probit analysis (POLO-PC). Toxicity varied significantly among insecticides and exposure routes. After 72 hours of oral exposure, abamectin (LC_{50} = 0.26 µg mL^{-1}) and imidacloprid (LC_{50} = 0.27 µg mL^{-1}) were the most toxic, while chlorpyrifos exhibited the lowest toxicity (LC_{50} = 5.77 µg mL^{-1}). In contact bioassays, abamectin also demonstrated the greatest toxicity (LD_{50} = 0.64 µg mL^{-1}), followed by imidacloprid and spinosad, whereas; cypermethrin and chlorpyrifos were found less harmful. The steeper slope values for abamectin and imidacloprid indicate uniform susceptibility among exposed bees. These results underscore substantial differences in the hazard profiles of widely used insecticides and emphasize the importance of considering both exposure routes when assessing risk. The findings offer a quantitative foundation for assessing pollinator risks and guiding pesticide regulation, thereby facilitating the selection of insecticides with reduced toxicity to bees within integrated pest management (IPM) programs.

Keywords: Honey Bees; insecticide toxicity; oral and contact exposure; pollinator risk assessment.

INTRODUCTION

Agriculture is the backbone of Pakistan's economy which accounts for 20.9 % of its gross domestic product (GDP) and around 67.5 % of the total population living in rural areas depends directly and/or indirectly on agriculture (Khan & Khan, 2018). Agriculture in Pakistan primarily has field crops, vegetables and fruits. Being the vital part of diet, fruits and vegetables are the key crops (Saqib et al., 2025). Pakistan is also an exporter of these fruits and vegetables, exporting them to various countries viz., Afghanistan, India, United Arab Emirates, United Kingdom, and Saudi Arabia thus earning valuable foreign exchange from their export (Umrani et al., 2024). Ecologically diverse zones in Pakistan is helpful in production of around 13 different kinds of fruits i.e. mango, citrus, guava, apple, dates, and banana while vegetables include tomatoes, onion, chilies, garlic, cucumber, melons etc. In Pakistan, Area under cultivation of these vegetable and fruits is more than 1.08 million hectares,

Correspondence
Rasheed Akbar
rasheed.akbar@uoh.edu.pk

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which accounts for only 7% of the total area under cultivation in Pakistan (Hamid & Akram, 2025). Most of the fruits and vegetables are cross-pollinated thus depending on physical means of pollination. Pollination can be defined as the transfer of pollen from the anther of the male floral part to the female floral part stigma of either the same or a different flower, facilitating fertilization, which leads to the formation of seed (s), and fruit. Wind, water, and animals are the common means of pollination; however, the major proportion of plants is pollinated by insects (Ramsay, 2005). Biodiversity round the globe is maintained by insect pollinators, which provide them required ecosystem important for maintaining wild and domesticated plants (Cardoso et al., 2025). It is quite evident from the research works that decrease in insect pollinators can result in decline of dependent plants' communities (Cano et al., 2025). According to research, it is documented that 35% of global crop produce is dependent on insect pollinators. Around 87 crops, i.e. 70% of the main agricultural crops being used as human feed round the globe, are dependent on pollinators (Mihrete & Mihretu, 2025). Estimated economic value of €153 billion have been associated with pollination from wild and domesticated animals (Gallai et al., 2009). Additionally, decline in the populations of pollinators due to urbanization has produced the need of discovering potential of insect pollinators to possibly enhance the economic yield of crops (Hoehn et al., 2008).

Pollination plays a crucial role in sustaining agricultural productivity and biodiversity. Studies on pollination biology across different regions of the world have shown that insects belonging to the orders Diptera, Lepidoptera, and Hymenoptera are the key pollinators of numerous fruits and vegetables (Cook et al., 2020). Among these, bees, wasps, moths, butterflies, and flies are considered the most important pollinators (Khalifa et al., 2021). The use of these pollinators can significantly enhance the yield and quality of crops (Garibaldi et al., 2014)

Globally, about 25,000 species of bees have been identified, while more than 20,000 remain undescribed (Zattara et al., 2021). Bees are highly efficient pollinators, responsible for the pollination of nearly 70% of edible crops worldwide (Khalifa et al., 2021). Honeybees, belonging to the family Apidae of the order Hymenoptera, are among the most effective pollinators and are also valued for producing honey and other hive products (Duangphakdee et al., 2025). Out of 115 major crops, 52 depend on honeybees for either seed or fruit set, and bee pollination can enhance fruit yield by 30–40% (Seddik et al., 2025).

In Pakistan, four honeybee species are present: *Apis florea*, *A. dorsata*, *A. mellifera*, and *A. cerana* (Khan et al., 2025). Approximately 4,000 beekeepers maintain about 0.4 million colonies of *A. mellifera*, providing livelihood to more than 27,000 households (Usman et al., 2022). However, a global decline in pollinator populations has emerged as a serious concern, adversely affecting crop yields and the economy. In temperate regions, *A. mellifera* is commercially used for pollination of apples, peaches, and plums because of its efficient foraging and communication behavior, as well as its ease of management and transportation (Kline et al., 2022). Despite its successful use for decades, the dependency on honeybees is increasingly challenged by widespread colony losses during the past decade (Paudel et al., 2015).

The harmful effects of pesticides on pollinators have gained significant attention due to the detection of agrochemical residues in honeybee products (Azpiazu et al., 2023). Honeybees are vulnerable to more than 100 types of insecticides depending on their chemical composition (Belsky et al., 2023). Pesticide exposure affects not only foraging bees but also immature bees through contaminated food stored inside hives (Ward et al., 2022). Such unintended exposure of non-target pollinators poses a severe ecological threat (Drivdal et al., 2021). The rising concerns over pesticide-induced bee mortality and colony collapse have led to the development of risk assessment protocols for pollinators in developed countries (Leska et al., 2021). However, in developing nations like Pakistan, where pesticide regulations are weakly enforced, colony decline continues to pose a major challenge (Akbar et al., 2024a, Akbar et al., 2024b, Akbar et al., 2022).

Neonicotinoid insecticides, in particular, have attracted global scrutiny because of their lethal and sub-lethal effects on bees under laboratory and semi-field conditions (Lu et al., 2020). Many developed countries have already imposed restrictions on neonicotinoid use (Klingelhöfer et al., 2022). In contrast, developing countries continue to rely heavily on these chemicals, lacking strong pesticide management and pollinator protection frameworks.

Integrated Pest Management (IPM) is considered a sustainable strategy to mitigate the negative effects of pesticides on pollinators (Akbar et al 2024 c; Farid et al., 2025a). According to Akbar and Khan (2021), IPM emphasizes the rational use of multiple pest control techniques to minimize health and environmental hazards (Farid et al., 2025b). However, the successful implementation of IPM requires a clear understanding of the extent and nature of pesticide threats to pollinators, as well as evidence-based strategies for their conservation within agricultural systems.

Given the critical role of honeybees in crop pollination and increasing threats posed by pesticides, there is an urgent need to evaluate the effects of commonly used insecticides on honeybee health. Therefore, this study focuses on assessing the oral and contact toxicity of eight widely used insecticides including acetamiprid, imidacloprid, diafenthiuron, methoxyfenozide, chlorpyrifos, spinosad, abamectin, and cypermethrin against worker honeybees. These insecticides have long been used in Pakistan's agroecosystems for pest control (Khan et al., 2015a; Khan et al., 2015b). The findings of this research will contribute to understanding pesticides risks to pollinators and help promote pollinator-friendly pest management practices in Pakistan.

MATERIALS AND METHODS

Insecticides

Two neonicotinoids namely acetamiprid (Acelan®20SL) and imidacloprid (Imidacloprid®20SL) were obtained from FMC, Pakistan. Diafenthiuron (Polo 500 EC) and abamectin (Agrimec TM 1.8EC, Syngenta, UK) were bought from Syngenta. Chlorpyifos (Lorsban 40EC), Spinosad (Tracer 240 SC) and methoxyfenozide (Runner 240SC) were purchased from Arysta Life Science.

Honeybees

Honeybees (A. mellifera L.) were managed in the local apiary of Haripur, Pakistan. Honeybees were taken from the hive frames, to the laboratory and restrained by keeping them in refrigerator for around five minutes before putting in the treated boxes.

Experimental conditions

For experiment, properly ventilated plastic boxes were purchased. Sucrose solution (50%) was used to feed the test honeybees. Experimental conditions included 25 ^{o}C ± 2 ^{o}C temperature and 60 ± 10 % RH.

Laboratory bioassays

Mortality of the honeybees was checked by laboratory bioassays. Five concentrations of each pesticide were tested starting from recommended field dose and their respective decimal fractions. On the bases of values of primary bioassays, the final bioassays were conducted by adjusting the dose of the insecticides on which the possible best results can be found to calculate the LC values for the pesticides.

Mode of application

Oral exposure

The similar size worker honeybees were collected from the colonies. The dose range was selected according to the preliminary assessment in case of all insecticides. Serial dilutions of all the insecticides were prepared in 50% sucrose solution. Serial dilutions of abamectin, acetamiprid, imidacloprid and methoxyfenozide were 0.25, 0.5, 1.0, 2.0, 3.0 µg/ml and control. While, serial dilutions for cypermethrin and spinosad were 1.25, 2.5, 5.0, 7.5, and 10 µg/ml. Serial dilutions for diafenthiuron were 0.5, 1.0, 2.0, 4.0 and 5.0 µg/ml. Serial dilutions for chlorpyrifos were 2.5, 5.0, 10.0, 20.0 and 30.0 µg/ml. After preparing the required solutions, cotton plugs were dipped and honeybees were allowed to feed on them. Data were recorded at 12, 24, 48 and 72 hours. Mortality was recorded when the insect showed no movement even after being gently prodded with a fine brush, following the method described by Naiara Gomes et al. (2020).

Contact exposure

For the contact action, wet filter paper was used. Serial dilutions were made for these bioassays were also according to the preliminary trials. For this purpose, filter paper was dipped in the pesticide solution and then spread on the base of the boxes in which the honeybees were introduced. Data were recorded for 12, 24, 48 and 72 hours. Adults unable to respond when disturbed were considered as dead.

Statistical analysis

Probit analysis was conducted using POLO-PC software (LeOra Software, 2002), which is specifically designed for analyzing bioassay data. This software was selected due to its reliability and widespread use in toxicological research for estimating LC values and associated parameters (Finney, 1971). POLO-PC provides estimates of lethal concentrations (LC_{50}, LC_{90}), their 95% fiducial limits (FL), chi-square (χ^2) values, and degrees of freedom (df). The chi-square test was used to assess the goodness of fit between observed and expected mortality, while the fiducial limits represent the 95% confidence range within which the true LC values are expected to lie. There were ten replications in each treatment.

RESULTS AND DISCUSSION

Oral toxicity assays

The feeding bio-assays results for worker honeybees in response to eight different insecticides are shown in Table 1. There was significant difference in the toxicity of different insecticides after 12 hours of treatment. The lowest LC50 value of 2.72 μg mL−1 (2.08-4.17μg mL−1) was calculated for the methoxyfenozide, followed by imidacloprid which was 4.27 μg mL−1 (2.64-12.60 μg mL−1). Higher LC50 value was calculated in Chlorpyrifos i.e. 26.52 μg mL−1 (20.25-40.39 μg mL−1). The lower LC90 values of 25.19 μg mL−1 (13.93-82.44 μg mL−1) was calculated in Diafenthiuron. While, higher LC90 value was calculated in Chlorpyrifos i.e. 154.08 μg mL−1 (82.12-525.05 μg mL−1). The slope was less steep in case of imidacloprid i.e. 1.90±0.43. The slope values for other insecticides lie between these insecticides. The 95% fiducial limits (FL) represent the range within which the true LC values are expected to fall with 95% confidence.

The feeding bio-assays results for worker honeybees in response to eight different insecticides are shown in Table 2. The significant differences were observed regarding the toxicity of different insecticides after 24 hours of treatment. The lowest LC50 value was calculated for the abamectin and methoxyfenozide i.e. 0.75 μg mL−1 (0.61- 0.90 μg mL−1). Higher LC50 value was calculated in Chlorpyrifos i.e. 11.01 μg mL−1 (20.25-40.39 μg mL−1). The LC90 values showed that lower value of both Abamectin and Methoxyfenozide i.e. 2.73 μg mL−1 (2.06- 4.17 μg mL−1). While, higher LC90 value was calculated in Chlorpyrifos i.e. 35.87 μg mL−1 (27.30-54.07 μg mL−1). Slope was more steep in Chlorpyrifos with 4.28±0.51 value. The slope was less steep in the case of imidacloprid i.e. 1.91±0.38. The slope values for other insecticides lie between these insecticides.

The feeding bio-assays results for worker honeybees in response to eight different insecticides are shown in Table 3. There was significant difference in the toxicity of different insecticides after 48 hours of treatment. The lowest LC50 value was calculated for the Abamectin i.e. 0.40 μg mL−1 (0.32- 0.48 μg mL−1). Higher LC50 value was calculated in Chlorpyrifos i.e. 8.89 μg mL−1 (7.50- 10.47 μg mL−1). While, the LC50 values calculated for Acetamiprid, Imidacloprid, Cypermethrin, Diafenthiuron, Methoxyfenozide and Spinosad were 0.77 μg mL−1 (0.64-0.93 μg mL−1), 0.55 μg mL−1 (0.45-0.66 μg mL−1), 4.54 μg mL−1 (3.77- 5.43 μg mL−1), 1.54 μg mL−1 (1.29- 1.81 μg mL−1), 0.40 μg mL−1 (0.32-0.47 μg mL−1) and 4.07 μg mL−1 (3.34-4.89μg mL−1), respectively. The LC90 values showed that lower value of Abamectin i.e. 1.17 μg mL−1 (0.93- 1.66 μg mL−1). While, higher LC90 value was calculated in Chlorpyrifos i.e. 29.02 μg mL−1 (22.12- 44.53 μg mL−1). Slope was more steep in Abamectin with 4.73±0.63 value. The slope was less steep in Cypermethrin i.e. 3.78±0.52. The slope values for other insecticides lie between these insecticides.

The feeding bio-assays results for worker honeybees in response to eight different insecticides are shown in Table 4. There was significant difference in the toxicity of different insecticides after 72 hours of treatment. The lowest LC50 value was calculated for the abamectin i.e. 0.26 μg mL−1 (0.20-0.29 μg mL−1). Higher LC50 value was calculated in Chlorpyrifos i.e. 5.77 μg mL−1 (4.92- 6.70μg mL−1). While, the LC50 values calculated for Acetamiprid, Imidacloprid, Cypermethrin, Diafenthiuron, Methoxyfenozide and Spinosad were 0.54 μg mL−1 (0.45-0.64 μg mL−1), 0.27 μg mL−1 (0.22-0.31 μg mL−1), 3.36 μg mL−1 (2.76- 3.99 μg mL−1), 1.09 μg mL−1 (0.91- 1.27 μg mL−1), 0.25 μg mL−1 (0.20-0.29 μg mL−1) and 3.10 μg mL−1 (2.54-3.69 μg mL−1), respectively. The LC90 values showed that lower value of Abamectin i.e. 0.53 μg mL−1 (0.44-0.79 μg mL−1). While, higher LC90 value was calculated in Chlorpyrifos i.e. 21.96 μg mL−1 (17.46- 30.96 μg mL−1). Slope was more steep in Imidacloprod with 7.19±1.28 value. The slope was less steep in Cypermethrin i.e. 3.96±0.51. The slope values for other insecticides lie between these insecticides. Among the tested insecticides, abamectin (LC_{50} = 0.26 μg mL^{-1}) and imidacloprid (LC_{50} = 0.27 μg mL^{-1}) were found to be the most toxic, showing LC_{50} values approximately 3–20 times lower than those of chlorpyrifos (LC_{50} = 5.77 μg mL^{-1}) and other insecticides tested. This indicates that abamectin and imidacloprid possess substantially higher toxicity against the target insect species.

Table 1. Oral toxicity of eight different insecticides to workers of *Apis mellifera* after 12 hours of treatment.

Insecticide	N	LC_{50} (95% FL) (μg mL^{-1})	LC_{90} (95% FL) (μg mL^{-1})	Slope (±SE)	χ^2
Acetamiprid	300	5.76 (3.33- 22.52)	69.53 (19.16- 2410.9)	2.03±0.49	3.64
Abamectin	300	3.81 (2.46-9.46)	47.44 (15.61-716.22)	2.00±0.44	3.84
Imidacloprid	300	4.27 (2.64-12. 60)	61.01 (17.83-1469.0)	1.90±0.43)	3.79
Chlorpyrifos	300	26.52 (20.25-40.39)	154.08 (82.12-525.05)	2.87±0.49	4.00
Cypermethrin	300	14.87 (10.54-32.29)	76.22 (34.24-581.14)	3.10±0.70	6.00

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Diafenthiuron	300	5.10 (3.96-7.69)	25.19 (13.93-82.44)	3.17 ±0.56	5.75
Methoxyfenozide	300	2.72 (2.08-4.17)	15.36 (8.22-52.15)	2.92±0.50	5.80
Spinosad	300	13.62 (9.87-27.04)	72.03 (33.35-469.68)	3.04±0.66	4.61

Table 2. Oral toxicity of eight different insecticides to workers of *Apis mellifera* after 24 hours of treatment.

Insecticide	N	LC$_{50}$ (95% FL) (µg mL^{-1})	LC$_{90}$ (95% FL) (µg mL^{-1})	Slope (±SE)	χ^2
Acetamiprid	300	1.10 (0.89- 1.37)	5.28 (3.60- 9.77)	3.24±0.43	5.76
Abamectin	300	0.75 (0.61- 0.90)	2.73 (2.06- 4.17)	3.92±0.48	6.74
Imidacloprid	300	1.30 (1.07-1.59)	5.26 (3.71-9.15)	3.62±0.47	4.63
Chlorpyrifos	300	11.01 (20.25-40.39)	35.87 (27.30-54.07)	4.28±0.51	6.09
Cypermethrin	300	5.77 (4.81- 7.07)	23.00 (15.83- 44.04)	3.66±0.54	11.79
Diafenthiuron	300	3.66 (2.97- 4.80)	16.51 (10.42- 37.61)	3.58±0.51	5.45
Methoxyfenozide	300	0.87 (0.72- 1.05)	2.99 (2.36-4.17)	4.10±0.49	5.39
Spinosad	300	5.52 (4.63-6.65)	20.29 (14.47-35.93)	3.88±0.55	9.43

Table 3. Oral toxicity of eight different insecticides to workers of *Apis mellifera* after 48 hours of treatment.

Insecticide	N	LC$_{50}$ (95% FL) (µg mL^{-1})	LC$_{90}$ (95% FL) (µg mL^{-1})	Slope (±SE)	χ^2
Acetamiprid	300	0.77 (0.64- 0.93)	2.75 (2.08- 4.16)	3.99±0.48	7.89
Abamectin	300	0.40 (0.32- 0.48)	1.17 (0.93- 1.66)	4.73±0.63	7.54
Imidacloprid	300	0.55 (0.45-0.66)	1.83 (1.41-2.66)	4.25±0.52	12.73
Chlorpyrifos	300	8.89 (7.50- 10.47)	29.02 (22.12- 44.53)	4.28±0.56	16.29
Cypermethrin	300	4.54 (3.77- 5.43)	17.26 (12.53- 29.21)	3.78±0.52	9.33
Diafenthiuron	300	1.54 (1.29- 1.81)	4.61 (3.64- 6.52)	4.62±0.54	10.12
Methoxyfenozide	300	0.47 (0.38-0.57)	1.54 (1.20-2.25)	4.29±0.55	6.68
Spinosad	300	4.07 (3.34-4.89)	16.32 (11.81-27.76)	3.64±0.50	10.75

Contact toxicity assays

The contact bio-assays results for worker honeybees in response to eight different insecticides are shown in Table 5. There was significant difference in the toxicity of different insecticides after 12 hours of treatment. The lowest LD50 value of 5.77 μg mL−1 (4.51- 8.12 μg mL−1) was calculated for Acetamiprid. Higher LD50 value was calculated in Chlorpyrifos i.e. 28.78 μg mL−1 (21.85-44.83μg mL−1). The LD90 values showed that lower value of 27.47 μg mL−1 (16.40-68.59 μg mL−1) was calculated in Acetamiprid. While, higher LD90 value was calculated in Chlorpyrifos i.e. 158.90μg mL−1 (84.23-557.84μg mL−1). Slope was more steep in Diafenthiuron with 3.78±0.69 value which confirms its lower LD90 value. The slope was less steep in Methoxyfenozide i.e. 1.78±0.38. The slope values for other insecticides lie between these insecticides.

Table 4. Oral toxicity of eight different insecticides to workers of *Apis mellifera* after 72 hours of treatment.

Insecticide	N	LC_{50} (95% FL) (µg mL^{-1})	LC_{90} (95% FL) (µg mL^{-1})	Slope (±SE)	χ^2
Acetamiprid	300	0.54 (0.45- 0.64)	1.47 (1.17-2.05)	5.09±0.64	11.43
Abamectin	300	0.26 (0.20- 0.29)	0.53 (0.44-0.79)	6.81±1.26	3.47
Imidacloprid	300	0.27 (0.22-0.31)	0.54 (0.45-0.74)	7.19±1.28	3.14
Chlorpyrifos	300	5.77 (4.92- 6.70)	13.91 (11.36 - 18.69)	5.75±0.68	9.68
Cypermethrin	300	3.36 (2.76- 3.99)	12.05 (9.15- 18.49)	3.96±0.51	16.88
Diafenthiuron	300	1.09 (0.91- 1.27)	2.81 (2.28-3.81)	5.32±0.63	11.12
Methoxyfenozide	300	0.28 (0.22-0.33)	0.62 (0.51-0.86)	6.30±1.06	4.35
Spinosad	300	3.10 (2.54-3.69)	10.92 (8.38-16.44)	4.02±0.52	17.36

The feeding bio-assays results for worker honeybees in response to eight different insecticides are shown in Table 6. There was significant difference in the toxicity of different insecticides after 24 hours of treatment. The lowest LD50 value was calculated for Imidacloprid i.e. 2.29 µg mL−1 (1.60-4.22 µg mL−1). Higher LD50 value was calculated in Chlorpyrifos i.e. 13.80 µg mL−1 (11.62- 16.54µg mL−1). While, the LD50 values calculated for Acetamiprid, Abamectin, Cypermethrin, Diafenthiuron, and Spinosad were 3.92 µg mL−1 (4.51- 8.12 µg mL−1), 1.53 µg mL−1 (1.24-1.86 µg mL−1), 9.24 µg mL−1 (7.71-11.32 µg mL−1), 7.57 µg mL−1 (6.33-9.58 µg mL−1), 1.53 µg mL−1 (1.25-1.86 µg mL−1) and 8.83 µg mL−1 (7.42-10.64 µg mL−1). The LD90 values showed that lower value of Imidacloprid i.e. 5.26 µg mL−1 (3.71-9.15 µg mL−1). While, higher LD90 value was calculated in Chlorpyrifos i.e. 44.64 µg mL−1 (33.47- 69.61µg mL−1). Slope was more steep in Chlorpyrifos with 3.94±0.58 value. The slope was less steep in Imidacloprid i.e. 3.62±0.47. The slope values for other insecticides lie between these insecticides.

The contact bio-assays results for worker honeybees in response to eight different insecticides are shown in Table 7. There was significant difference in the toxicity of different insecticides after 48 hours of treatment. The lowest LD50 value was calculated for the abamectin i.e. 2.39 µg mL−1 (1.88- 3.47µg mL−1). Higher LD50 value was calculated in Chlorpyrifos i.e. 9.99 µg mL−1 (8.41-11.82µg mL−1). While, the LD50 values calculated for Acetamiprid, Imidacloprid, Cypermethrin, Diafenthiuron, methoxyfenozide and Spinosad were 2.89µg mL−1 (2.44-3.45µg mL−1), 0.88µg mL−1 (0.72-1.05µg mL−1), 5.77 µg mL−1 (4.81- 7.07 µg mL−1), 3.66 µg mL−1 (2.97- 4.80 µg mL−1), 0.80 µg mL−1 (0.64-0.96 µg mL−1) and 6.51 µg mL−1 (5.35-7.83µg mL−1), respectively. The LD90 values showed that lower value of Abamectin i.e. 2.73 µg mL−1 (2.06- 4.17 µg mL−1). While, higher LD90 value was calculated in Chlorpyrifos i.e. 30.55µg mL−1 (23.72-44.41µg mL−1). Slope was more steep in Abamectin with 3.94±0.58 value. The slope was less steep in Acetamıprıd i.e. 3.24±0.43.

The contact bio-assays results for worker honeybees in response to eight different insecticides are shown in Table 8. There was significant difference in the toxicity of different insecticides after 72 hours of treatment. The lowest LD50 value was calculated for the abamectin i.e. 0.64 µg mL−1 (0.53-0.75 µg mL−1). Higher LD50 value was calculated in Chlorpyrifos i.e. 5.94 (5.06-6.89µg mL−1). While, the LD50 values calculated for Acetamiprid, Imidacloprid, Cypermethrin, Diafenthiuron, methoxyfenozide and Spinosad were 1.33 µg mL−1 (1.13-1.57 µg mL−1), 0.64 µg mL−1 (0.53-0.75 µg mL−1), 5.38 µg mL−1 (4.41- 6.39 µg mL−1), 2.65 µg mL−1 (2.25- 3.05 µg mL−1), 0.51 µg mL−1 (0.40-0.59 µg mL−1) and 4.93 µg mL−1 (4.05-5.85 µg mL−1), respectively. The LD90 values showed that lower value of Abamectin i.e. 0.58 µg mL−1 (0.48- 0.79 µg mL−1). While, higher LD90 value was calculated in Cypermethrin i.e. 19.28 µg mL−1 (14.65- 29.59 µg mL−1). Slope was more steep in Abamectin with 6.81±1.25 value. The slope was less steep in Cypermethrin i.e. 3.96±0.52. The slope values for other insecticides lie between these insecticides.

Table 5. Contact toxicity of eight different insecticides to workers of *Apis mellifera* after 12 hours of treatment.

Insecticides	N	LC_{50} (95% FL) (μg mL^{-1})	LC_{90} (95% FL) (μg mL^{-1})	Slope (±SE)	χ^2
Acetamiprid	300	5.77 (4.51- 8.12)	27.47 (16.40- 68.59)	3.24±0.49	7.19
Abamectin	300	10.61 (7.32-21.41)	61.32 (27.78-337.04)	2.88±0.56	10.62
Imidacloprid	300	6.04 (3.49-23.68)	63.57 (18.25-1968.9)	2.15±0.50	4.81
Chlorpyrifos	300	28.78 (21.85-44.83)	158.90 (84.23-557.84)	3.01±0.69	5.62
Cypermethrin	300	23.79 (16.87-51.66)	121.96 (54.79-929.82)	3.09±0.70	6.00
Diafenthiuron	300	9.93 (8.03-14.09)	37.85 (22.87-106.08)	3.78±0.69	5.98
Methoxyfenozide	300	9.44 (5.78-26.31)	160.68 (46.10-3327.80)	1.78 ±0.38	4.21
Spinosad	300	21.79 (15.79-43.27)	115.25 (53.36-751.49)	3.04 ±0.66	4.61

Table 6. Contact toxicity of eight different insecticides to workers of *Apis mellifera* after 24 hours of treatment.

Insecticide	N	LC_{50} (95% FL) (μg mL^{-1})	LC_{90} (95% FL) (μg mL^{-1})	Slope (±SE)	χ^2
Acetamiprid	300	3.92 (4.51- 8.12)	14.32 (10.04- 25.24)	3.90±0.51	10.42
Abamectin	300	2.88 (2.44-3.45)	8.48 (6.49-12.66)	3.9±0.48	6.74
Imidacloprid	300	2.29 (1.60-4.22)	32.58 (12.10-309.45)	1.91±0.38	3.13
Chlorpyrifos	300	13.80 (11.62- 16.54)	44.64 (33.47- 69.61)	3.94±0.58	6.22
Cypermethrin	300	9.24 (7.71-11.32)	36.79 (25.32-70.47)	3.66±0.54	11.79
Diafenthiuron	300	7.57 (6.33-9.58)	27.61 (18.46-57.84)	3.91±0.62	6.88
Methoxyfenozide	300	1.53 (1.25-1.86)	5.97 (4.41-9.48)	3.72±0.46	6.36
Spinosad	300	8.83 (7.42-10.64)	32.46 (23.16-57.48)	3.88±0.55	9.43

Table 7. Contact toxicity of eight different insecticides to workers of *Apis mellifera* after 48 hours of treatment.

Insecticide	N	LC_{50} (95% FL) (μg mL^{-1})	LC_{90} (95% FL) (μg mL^{-1})	Slope (±SE)	χ^2
Acetamiprid	300	2.89 (2.44- 3.45)	8.48 (6.49- 12.66)	3.24±0.43	5.76
Abamectin	300	0.80 (0.65- 0.96)	2.39 (1.88- 3.47)	3.92±0.48	6.74
Imidacloprid	300	0.88 (0.72-1.05)	1.82 (0.72-1.04)	3.62±0.47	4.64
Chlorpyrifos	300	9.99 (8.41- 11.82)	30.55 (23.72- 44.41)	3.94±0.58	6.22
Cypermethrin	300	5.77 (4.81- 7.07)	23.00 (15.83- 44.04)	3.66±0.54	11.79
Diafenthiuron	300	3.66 (2.97- 4.80)	16.51 (10.42- 37.61)	3.58±0.51	5.45

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Methoxyfenozide	300	0.80 (0.64-0.96)	2.39 (1.88-3.47)	3.72±0.46	6.36
Spinosad	300	6.51 (5.35-7.83)	26.12 (18.90-44.42)	3.88±0.55	9.43

Table 8. Contact toxicity of eight different insecticides to workers of *Apis mellifera* after 72 hours of treatment.

Insecticide	N	LC$_{50}$ (95% FL) (µg mL^{-1})	LC$_{90}$ (95% FL) (µg mL^{-1})	Slope (±SE)	χ^2
Acetamiprid	300	1.33 (1.13- 1.57)	3.42 (2.75- 4.72)	5.38±0.64	10.12
Abamectin	300	0.64 (0.53-0.75)	1.57 (1.27-2.14)	6.81±1.25	3.47
Imidacloprid	300	0.64 (0.53-0.75)	1.57 (1.27-2.14)	5.62±0.71	12.27
Chlorpyrifos	300	5.94 (5.06- 6.89)	14.40 (11.75- 19.35)	4.74±0.58	15.83
Cypermethrin	300	5.38 (4.41- 6.39)	19.28 (14.65- 29.59)	3.96±0.52	16.88
Diafenthiuron	300	2.65 (2.25- 3.05)	6.49 (5.36- 8.50)	5.63±0.66	15.64
Methoxyfenozide	300	0.51 (0.40-0.59)	1.07 (0.88-1.51)	6.78±1.26	3.42
Spinosad	300	4.93 (4.05-5.85)	16.74 (12.96-24.76)	4.14±0.53	19.15

Honeybees are the key pollinators for many monoculture crops throughout the world (Khalifa et al., 2021). A typical bee colony contains around 10,000–40,000 individuals as workers, about 1/3rd of them are foragers (Yadav et al., 2017). Growth of bee colonies is stimulated by beekeepers, to prepare them for a pollination event, by nourishing them on artificial diets. Additionally, standardized equipment is used for the management of colonies, which enables them to be carried to longer distances for pollination. Another reason of using honeybees commercially for pollination is their biology. Because they are pollinating a wide range of flowers, honeybees are considered as generalists. They are able to traveling upto an average of 4.5 km, thus they are capable of pollinating crops in an area of 6,360 ha. (Khalifa et al., 2021). Therefore, they should be kept in the center of orchards to allow them effective pollination in the orchards. Additionally, they have ability to guide their colony mates about the location of nectar resources making them effective pollinators (Seeley, 1985).

Many biotic and abiotic factors affect the honeybee colonies. Biotic factors include diseases, mites and harmful insects like greater wax moth. While, among abiotic factors pesticide residues are the biggest problem-causing decline of honeybee colonies. The fact that pesticide poisoning can result in bee colony decline dates back to early 1900s, when significant population of bees was decreased in California when the arsenic was sprayed on fruit trees (Neov et al., 2019).

Mortality was directly proportional to dose and time in the recent experiment. These findings are in accordance with the findings of Ma et al. (2019) who have reported a dose-dependent mortality of insect pests under controlled conditions. Broadly speaking, oral ingestion method was proven more toxic to the exposed worker honeybees as compared to the contact method. These findings are comparable with the results of Belsky et al. (2022), who have reported that most of the insecticides are more lethal to honeybees when ingested via food as compared to the contact action. It can happen because owing to transfer of pollen and nectar from field to hive and then to young ones, honeybees are more exposed to insecticides orally, and nearly all stages of honeybees are exposed equally. Additionally, most of the insecticides being used in the farmer’s field are systemic which become a part of plant sap and affect their metabolism. Their residues have also been found in nectar and pollen (Manzoor et al., 2025; Li,2012; Zhang et al.,2023) of the plants on which honeybees feed directly (Sanchez-Bayo, & Goka, 2014) which makes them prone to such toxic insecticides especially when sprayed on plant resources being used as the diet of honeybees. Nectars is used for producing honey while pollen is used as feed source by honeybees so their development in affected by consuming contaminated feed (Paray et al., 2021). While the present study focused on acute oral and contact toxicity, it is important to recognize that sublethal effects such as disorientation, reduced foraging efficiency, and impaired learning or memory can also significantly affect colony performance and pollination efficiency (Henry et al., 2012; Tosi et al., 2017). Future studies should investigate these behavioral and physiological responses to provide a more comprehensive understanding of the broader ecological implications of pesticide exposure on honeybee populations. In our study, neonicotinoids (imidacloprid and acetamiprid) and abamectin was found most toxic after 12, 24, 48, and 72 hours of treatment. Neonicotinoids have been proven highly toxic to honeybees in a series of studies (Lu et al., 2020; Wang et al., 2020). These findings are inconsistent to the findings of Buszewski et al. (2019), who have reported that neonicotinoids have high toxicity to honeybees as compared to other groups of insecticides. Till now more than 100 research articles have been published including the keywords “neonicotinoids/imidacloprid” and “bee”, where the first article was published in 1992. Neonicotinoids especially imidacloprid are highly persistent and systemic in plants. It becomes a part of plant metabolism after application. Several research works have been performed throughout the Europe and USA. Some of these experiments were conducted on a larger area and for several years with larger sample size (Pang et al. 2020; Fouad et al. 2024), while some were conducted with smaller number of samples and narrow area in one or two sampling years (Jabbari, 2025; Nguyen et al. 2009; Mullin et al. 2010). Neonicotinoids are being banned in several developed countries owing to their detrimental effect on honeybees (Dentzman et al. 2025; Margaoan et al., 2025; Paoli & Giurfa, 2024).

In our study, it has been proven highly toxic to honeybees approximately equal to the toxicity of neonicotinoids. These findings are comparable to the findings of Obregon et al. (2024) they have reported high toxicity of avermectins to honeybees. As a matter of fact, abamectin is a nerve poison. It stimulates the gamma-aminobutyric acid (GABA) system, a chemical “transmitter” produced at nerve endings, which inhibits both nerve to nerve and nerve to muscle communication. The affected insect becomes paralyzed, stops feeding, and dies. It is a broad-spectrum insecticide being used against a variety of insect-pests (Liang et al. 2025).

CONCLUSION

Two neonicotinoids viz. imidacloprid and acetamiprid, and an avermectin insecticide (abamectin) were found most toxic insecticide when applied through ingestion method and wet surface method. Chlorpyrifos and cypermethrin were least toxic insecticides. A dose and time dependent increase in mortality of exposed worker honeybees was observed. The wise and timely use of neonicotinoid insecticides is increasingly important due to their extensive application for controlling sucking insect pests on various crops. Based on the present findings, insecticides such as cypermethrin should be applied close to the flowering season and at lower doses to minimize adverse effects on pollinators, including bee mortality and colony collapse disorders. Moreover, integrating chemical control with eco-friendly approaches such as the use of biological control agents, botanical insecticides, and cultural practices is strongly recommended to achieve sustainable pest management while protecting beneficial insect populations.

AUTHOR’S CONTRIBUTION

All authors agreed for publication for the present work and confirm that all materials, data, and results reported in this study are based on field and lab work followed by manuscript preparation and submission, where all authors made their due contributions.

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AVAILABILITY OF DATA AND MATERIAL

The datasets supporting this study are included in the article. Extended methodological details and raw data can be accessed by contacting the corresponding author, subject to ethical and institutional guidelines.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study did not involve human subjects or animal models. In accordance with institutional guidelines, ethical approval and informed consent requirements were formally waived for this research.

CONSENT FOR PUBLICATION

I, the undersigned, consent to the publication of my identifiable information.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest.

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REFERENCES

Akbar, R. and Khan, I.A., 2021. Toxicity of five plant extracts against *Callosobruchus maculatus* Fab. (Coleoptera Bruchidae) a major insect pest of stored pulses. Fresenius Environmental Bulletin, 30(5), p.5098-5107.

Akbar, R., Afzal, S., Sun, J., Faheem, B., Bibi, R., Azad, R., Farid, A., Ahmad, H.I., Ataya, F.S., Khan, M.A. and Khan, I.A., 2024 a. Efficacy of Various Plant Extracts and Synergism Against Domestic Species of Rice Weevil *Sitophilous oryzae* (Curculionidae: Coleoptera). Polish Journal of Environmental Studies, 33(3), Pp. 3033-3044.

Akbar, R., Faheem, B., Aziz, T., Ali, A., Ullah, A., Khan, I.A. and Sun, J., 2024 b. Evaluating the efficacy of plant extracts in managing the bruchid beetle, *Callosobruchus maculatus* (Coleoptera: Bruchidae). Insects, 15(9), p.691.

Akbar, R., Khan, I.A., Alajmi, R.A., Ali, A., Faheem, B., Usman, A., Ahmed, A.M., El-Shazly, M., Farid, A., Giesy, J.P. and Aboul-Soud, M.A., 2022. Evaluation of insecticidal potentials of five plant extracts against the stored grain pest, *Callosobruchus maculatus* (Coleoptera: Bruchidae). Insects, 13(11), p.1047.

Akbar, R., Manzoor, S., Azad, R., Makai, G., Rahim, J., Sheikh, U.A.A., Ali, A., Aziz, T., Ahmad, H.I., Ahmed, M. and Du, D., 2024c . Botanical pesticides: role of *Ricinus communis* in managing *Bactrocera zonata* (Tephritidae: Diptera). Insects, 15(12), p.959.

Azpiazu, C., Medina, P., Sgolastra, F., Moreno-Delafuente, A. and Vinuela, E., 2023. Pesticide residues in nectar and pollen of melon crops: Risk to pollinators and effects of a specific pesticide mixture on *Bombus terrestris* (Hymenoptera: Apidae) micro-colonies. Environmental Pollution, 326, p.121451.

Belsky, J., Biddinger, D.J., Seiter, N. and Joshi, N.K., 2022. Various routes of formulated insecticide mixture whole-body acute contact toxicity to honey bees (*Apis mellifera*). Environmental Challenges, 6, p.100408.

Buszewski, B., Bukowska, M., Ligor, M. and Staneczko-Baranowska, I., 2019. A holistic study of neonicotinoids neuroactive insecticides properties, applications, occurrence, and analysis. Environmental Science and Pollution Research, 26(34), pp.34723-34740.

Cano, D., Martínez-Núñez, C., Pérez, A.J., Alcántara, J.M., Moretti, M., Salido, T. and Rey, P.J., 2025. Agricultural intensification indirectly reshapes bee–plant interaction networks through shifts in bee functional traits. Ecological Applications, 35(6), p. e70105.

Cardoso, J. C., Trevizan, R., Maruyama, P. K., Caetano, A. P., Gonçalves, R. V., Antonini, Y., & Oliveira, P. E. (2025). Pollination and plant reproduction in the Cerrado, the world's most biodiverse savanna. Biological Reviews. https://doi.org/10.1111/brv.70073.

Cook, D.F., Voss, S.C., Finch, J.T., Rader, R.C., Cook, J.M. and Spurr, C.J., 2020. The role of flies as pollinators of horticultural crops: An Australian case study with worldwide relevance. Insects, 11(6), p.341.

Dentzman, Katherine, Derek Franklin, Edem Avemegah, and Jessica R. Goldberger. "An overview of agricultural neonicotinoid regulation in the EU, Canada, and the United States." Pest Management Science (2025). https://doi.org/10.1002/ps.70126.

Drivdal, L. and van der Sluijs, J.P., 2021. Pollinator conservation requires a stronger and broader application of the precautionary principle. Current opinion in insect science, 46, pp.95-105.

Duangphakdee, O., Poolprasert, P. and Rattanawannee, A., 2025. Complete Mitochondrial Genome Characterization and Phylogenomics of the Stingless Bee, *Heterotrigona itama* (Apidae: Meliponini). Insects, 16(5), p.535.

Farid, A., Akbar, R., Khan, S. W., Faheem, B., Rafiq, N., Khan, G. Zamin., 2025. Assessment of resistance and susceptibility in *Culex quinquefasciatus* larvae exposed to conventional and novel insecticides. Zoo Botanica, 3(2); 395-405.

Farid, A., Bibi, H., Akbar, R., Rashid, A., Khan, G. Z., 2025. Optimizing the Proportions of Essential Oils as *Aedes aegypti* Repellent using Response Surface Methodology. Plant Bulletin, 4(2); 107-126.

Finney, D.J. (1971). Probit Analysis (3rd ed.). Cambridge University Press, Cambridge, UK.

Fouad, M.R. and Abdel-Raheem, S.A., 2024. An overview on the fate and behavior of imidacloprid in agricultural environments. Environmental Science and Pollution Research, 31(52), pp.61345-61355.

Garibaldi, L.A., Carvalheiro, L.G., Leonhardt, S.D., Aizen, M.A., Blaauw, B.R., Isaacs, R., Kuhlmann, M., Kleijn, D., Klein, A.M., Kremen, C. and Morandin, L., 2014. From research to action: enhancing crop yield through wild pollinators. Frontiers in Ecology and the Environment, 12(8), pp.439-447.

Hamid, A. and Akram, N., 2025. Significance and Role of Agriculture Sector in the Socio-Economic Development of Khyber Pakhtunkhwa. Dialogue Social Science Review (DSSR), 3(1), pp.1383-1391.

Henry, M., et al. (2012). A common pesticide decreases foraging success and survival in honey bees. Science, 336(6079), 348–350.

Jabbari, M., 2025. Effects of neonicotinoids on pollinating insects with emphasis on honey bees. Honeybee Science Journal, 15(29), pp.45-57.

Khalifa, S.A., Elshafiey, E.H., Shetaia, A.A., El-Wahed, A.A.A., Algethami, A.F., Musharraf, S.G., AlAjmi, M.F., Zhao, C., Masry, S.H., Abdel-Daim, M.M. and Halabi, M.F., 2021. Overview of bee pollination and its economic value for crop production. Insects, 12(8), p.688.

Khan, I.A. and Khan, M.S., 2018. Planning for Sustainable Agriculture in Pakistan. In Developing Sustainable Agriculture in Pakistan (pp. 1-29). CRC Press.

Khan, I.A., Khan, H., Khan, I., Akbar, R., Alam, M., Saeed, M., Farid, A., Ali, I., Habib, K. and Fayaz, W., 2015b. Efficacy of some plant extracts on larval mortality of *Culex quinquefasciatus* (Say) (Diptera: Culicidae) in Peshawar. Journal of Entomology and Zoology Studies., 3, pp.331-333.

Khan, I.A., Khan, M.N., Akbar, R., Saeed, M., Ali, I. and Alam, M., 2015a. Efficacy of insecticides against insect pests of maize crop and its influence on natural enemy in Peshawar. Journal of Entomology and Zoology Studies, 3(4), pp.323-326.

Khan, T.U., Luan, X., Nabi, G., Raza, M.F., Iqbal, A., Khan, S.N. and Hu, H., 2025. Forecasting the Impact of Climate Change on Apis dorsata (Fabricius, 1793) Habitat and Distribution in Pakistan. Insects, 16(3), p.289.

Kline, O., Phan, N.T., Porras, M.F., Chavana, J., Little, C.Z., Stemet, L., Acharya, R.S., Biddinger, D.J., Reddy, G.V., Rajotte, E.G. and Joshi, N.K., 2022. Biology, genetic diversity, and conservation of wild bees in tree fruit orchards. Biology, 12(1), p.31.

Klingelhöfer, D., Braun, M., Brüggmann, D. and Groneberg, D.A., 2022. Neonicotinoids: A critical assessment of the global research landscape of the most extensively used insecticide. Environmental Research, 213, p.113727.

Leska, A., Nowak, A., Nowak, I. and Górczyńska, A., 2021. Effects of insecticides and microbiological contaminants on *Apis mellifera* health. Molecules, 26(16), p.5080.

Li, Z., 2022. Modeling pesticide residues in nectar and pollen in support of pesticide exposure assessment for honeybees: A generic modeling approach. Ecotoxicology and Environmental Safety, 236, p.113507.

Liang, J., Xiao, F., Ojo, J., Chao, W.H., Ahmad, B., Alam, A., Abbas, S., Abdelhafez, M.M., Rahman, N., Khan, K.A. and Ghramh, H.A., 2025. Insect resistance to insecticides: causes, mechanisms, and exploring potential solutions. Archives of Insect Biochemistry and Physiology, 118(2), p. e70045.

Lu, C., Hung, Y.T. and Cheng, Q., 2020. A review of sub-lethal neonicotinoid insecticides exposure and effects on pollinators. Current Pollution Reports, 6(2), pp.137-151.

Ma, C., Zhang, Y., Sun, J., Imran, M., Yang, H., Wu, J., Zou, Y., Li-Byarlay, H. and Luo, S., 2019. Impact of acute oral exposure to thiamethoxam on the homing, flight, learning acquisition and short-term retention of Apis cerana. Pest Management Science, 75(11), pp.2975-2980.

Manzoor, S., Akbar, R., Hussain, A., Ali, A., Faheem, B., Zaman, M., Farid, A., Hussain, I., Khan, I.A., Perveen, K. and Bukhari, N.A., 2025. Toxicity effect of *Ricinus communis* methanolic extracts against *Bactrocera cucurbitae* (Diptera: Tephritidae). Plant Protection Science, 61(1), pp 77-88.

Margaoan, R., Papa, G., Nicolescu, A., Cornea-Cipcigan, M., Kösoğlu, M., Topal, E. and Negri, I., 2025. Environmental pollution effect on honey bees and their derived products: A comprehensive analysis. Environmental Science and Pollution Research, 32(16), pp.10370-10391.

Mihrete, T.B. and Mihretu, F.B., 2025. Crop diversification for ensuring sustainable agriculture, risk management and food security. Global Challenges, 9(2), p.2400267.

Mullin, C.A., Frazier, M., Frazier, J.L., Ashcraft, S., Simonds, R., VanEngelsdorp, D. and Pettis, J.S., 2010. High levels of miticides and agrochemicals in North American apiaries: implications for honey bee health. PloS one, 5(3), p. e9754.

Naiara Gomes, I., Ingred Castelan Vieira, K., Moreira Gontijo, L. and Canto Resende, H., 2020. Honeybee survival and flight capacity are compromised by insecticides used for controlling melon pests in Brazil. Ecotoxicology, 29(1), pp.97-107.

Neov, B., Georgieva, A., Shumkova, R., Radoslavov, G. and Hristov, P., 2019. Biotic and abiotic factors associated with colonies mortalities of managed honey bee (Apis mellifera). Diversity, 11(12), p.237.

Nguyen, B.K., Saegerman, C., Pirard, C., Mignon, J., Widart, J., Thirionet, B., Verheggen, F.J., Berkvens, D., De Pauw, E. and Haubruge, E., 2009. Does imidacloprid seed-treated maize have an impact on honey bee mortality?. Journal of Economic Entomology, 102(2), pp.616-623.

Obregon, D., Guerrero, O., Sossa, D., Stashenko, E., Prada, F., Ramirez, B., Duplais, C. and Poveda, K., 2024. Route of exposure to veterinary products in bees: Unraveling pasture's impact on avermectin exposure and tolerance in stingless bees. PNAS nexus, 3(3), p. 1-10.

Pang, S., Lin, Z., Zhang, Y., Zhang, W., Alansary, N., Mishra, S., Bhatt, P. and Chen, S., 2020. Insights into the toxicity and degradation mechanisms of imidacloprid via physicochemical and microbial approaches. Toxics, 8(3), p.65.

Paoli, M. and Giurfa, M., 2024. Pesticides and pollinator brain: How do neonicotinoids affect the central nervous system of bees? European Journal of Neuroscience, 60(8), pp.5927-5948.

Paudel, Y.P., Mackereth, R., Hanley, R. and Qin, W., 2015. Honey bees (*Apis mellifera* L.) and pollination issues: Current status, impacts, and potential drivers of decline. Journal of Agricultural Science, 7(6), p.93.

Quandahor, P., Kim, L., Kim, M., Lee, K., Kusi, F. and Jeong, I.H., 2024. Effects of agricultural pesticides on decline in insect species and individual numbers. Environments, 11(8), p.182.

Ramsay, G., 2005. Pollen dispersal vectored by wind or insects. Gene flow from GM plants, pp.43-77.

Sanchez-Bayo, F. and Goka, K., 2014. Pesticide residues and bees–a risk assessment. PloS one, 9(4), p. e94482.

Saqib, S.E., Yaseen, M., Ali, S. and Visetnoi, S., 2025. Agricultural Land Conversion and Challenges for Tenant Farmers: Empirical Evidence from Pakistan. Land Degradation & Development, 36(3), pp.802-815.

Seddik, M.A. and Ali, M.A., 2025. Pollination by honeybee increases yield and quality of faba bean (*Vicia faba* L.) in semi-arid regions of Egypt. Scientific Reports, 15(1), p.34113.

Tosi, S., et al. (2017). Sublethal effects of neonicotinoid insecticides on honey bee behavior, physiology, and reproduction: a review. Environmental Science and Pollution Research, 24(2), 172–185.

Umrani, W.A., Solangi, R., Mirani, M., Ahmed, U. and Abro, M.A., 2024. Sindh Fruit Traders: Expanding Abroad. Asian Journal of Management Cases, 21(1), pp.83-98.

Usman, M., Hasnain, M., Banaras, S., Akram, M., Abbas, Q., Shah, J.A., Tabasum, S., Shah, S.A., Raza, A., Khan, M.N. and Jamil, M., 2022. Potential emerging constraints and management strategies of different honeybee species in Pakistan: A review. CABI Reviews, (2022).

Wang, Y., Zhu, Y. C., & Li, W., 2020. Interaction patterns and combined toxic effects of acetamiprid in combination with seven pesticides on honey bee (*Apis mellifera* L.). Ecotoxicology and environmental safety, 190, 110100.

Yadav, S., Kumar, Y. and Jat, B.L., 2017. Honeybee: Diversity, castes and life cycle. In Industrial entomology (pp. 5-34). Singapore: Springer Singapore.

Zattara, E.E. and Aizen, M.A., 2021. Worldwide occurrence records suggest a global decline in bee species richness. One Earth, 4(1), pp.114-123.

Zhang, C., Wang, X., Kaur, P. and Gan, J., 2023. A critical review on the accumulation of neonicotinoid insecticides in pollen and nectar: influencing factors and implications for pollinator exposure. Science of The Total Environment, 899, p.165670.