

Research Article

Evaluation of Lethal Toxicity of Commonly Used Pesticides on Three Species of Honeybees in the Tehsil Taunsa, Punjab Pakistan

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Abstract | Insecticides are critical in modern agriculture to effectively curb insect pests that harm ornamental plants and agricultural crops. However, the use of insecticides is believed to be one of the primary causes of decline in honeybee populations worldwide that may result in decreased yield and quality in crop pollination and honey. There is a dire need to conduct region-specific toxicity studies on insecticides that can inform pollinator conservation strategies and promote safer, sustainable agricultural practices. This study assessed the fatal effects of various pesticides on forager workers of *Apis florea*, *A. mellifera*, and *A. dorsata*. The experiment followed a completely randomized design with three replications. The pesticides were diluted in water in accordance with the recommended field doses listed on their labels in order to assess their indirect toxicity. The filter paper was then placed inside petri dishes after being submerged in the insecticidal solution and given time to air dry. Every plate had fourteen honeybees in it. Four time intervals (3, 6, 12, and 24 h) were used to record the honeybee death rate. All three honeybee species exhibited low mortality rate at 3 h of exposure to insecticides, and high death rate at 24 h. After a 24 h exposure, Imidacloprid caused the greatest mortality rate among *A. florea* (97.75%), followed by *A. mellifera* (88.86%) and *A. dorsata* (82.2%). Talstar® and X-Tall® also showed significant toxicity towards honeybees. Lancer®, Decis Super®, Response®, Big hit®, Checkworm®, and Admiral® exhibited moderate toxicity, whereas Bunch® displayed the lowest toxicity and was less detrimental to honeybees in the study. The findings of this study are important for pollinators conservation and sustainable agriculture, thereby discouraging unsustainable agricultural practices and excessive pesticide use.

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Keywords | Honey bees, Insecticides, Mortality rate, Pesticide, lethal Toxicity, Time interval



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Introduction

Apiculture, commonly referred to as beekeeping, involves the careful management and cultivation of bees with the aim of producing various bee-related products such as honey, beeswax, and royal jelly. Today, apiculture is an important industry that provides a variety of products and services, including pollination for crops, honey production, and the sale of beeswax and other bee-related products (Razik, 2019).

Apiculture can be practiced on a small or large scale, and it requires a certain level of knowledge and skill to be successful. Beekeepers must understand the behavior of bees, the management of hives, and the techniques for harvesting honey and other products (Abdu-Allah and Pittendrigh, 2018). Honey production is another important aspect of beekeeping. Bees obtain nectar from flowers to produce honey. The process involves the bees regurgitating the nectar and then evaporating the water content, resulting in a thick, sweet liquid (honey) that is stored in the hive. Beekeepers can harvest honey by removing frames from the hive and extracting the honey using a centrifugal extractor. Beeswax, propolis, royal jelly, and pollen are other products that can be harvested from the hive (Abrol and Anil, 2009).

In recent years, there has been growing concern about the health of bee populations worldwide. Factors such as habitat loss, pesticide use, and disease have contributed to declines in bee populations, which could have significant impacts on agriculture and the environment. As a result, there has been increased interest in sustainable and organic beekeeping practices that prioritize the health of the bees and their habitat (Abrol and Anil, 2009). By managing hives near crops, beekeepers can help to ensure that crops are pollinated, resulting in higher yields and better quality produce (Abdu-Allah and Pittendrigh, 2018).

Bees are essential pollinators for many crops and managed hives can be used to increase pollination rates and improve crop yields. This is particularly important in areas where wild or native bee populations have declined, as managed hives can help to fill the gap and ensure that crops are pollinated (Abdu-Allah and Pittendrigh, 2018).

The decline in honeybee populations is a growing

concern worldwide. In recent years, there has been a significant decrease in the number of honeybee colonies, with some regions experiencing losses of up to 30% or more. While there are many factors contributing to this decline, the use of insecticides is believed to be one of the primary causes (Carvalho *et al.*, 2009).

Pesticide use has significantly increased as a need to intensify agriculture to meet the growing demand for food and to protect crops (Tariq *et al.*, 2024). Insecticides are used to control insect pests that can cause significant damage to crops, resulting in lower yields and reduced quality to produce. However, the use of insecticides also has unintended consequences, particularly for beneficial insects, pollinators such as honeybees (Bonabeau *et al.*, 1997).

Insecticides harm honeybees by disrupting their ability to forage and find food. Honeybees rely on their sense of smell to locate flowers and other sources of nectar and pollen. Insecticides can interfere with this sense of smell, making it more difficult for honeybees to find food and have a significant impact on the health of the hive, as bees that cannot find enough food may become weak and more susceptible to disease (Bos *et al.*, 2007). In Pakistan, integrated pest management practices remain limited among farmers and over-reliance on insecticides has also caused problems such as environmental contamination and pest resistance (Abbas *et al.*, 2023).

This study's goal was to investigate whether exposure to widely used insecticides have a deadly effect on foraging behavior of three different species of honeybees: *A. florea*, *A. mellifera* and *A. dorsata*. The study aimed to explore the lethal efficacy of different insecticides on worker bees, as worker bees are essential for the functioning of the hive, include caring for the hive, feeding larvae, and foraging for food and to identify the most effective and least harmful insecticidal options for pest control in crops adjacent to honeybee colonies (Muzammal *et al.*, 2022). This research offers a thorough investigation of the potential risks and benefits of different pest control strategies on honeybee colonies.

Methods and Materials

The experimental conditions

The experimental work was carried out in Tehsil

Taunsa District 2023, Dera Ghazi Khan (27.2046° N, 77.4977° E), Punjab, Pakistan with a temperature of 25±2°C and a relative humidity (R.H.) of 70±5%.

Study area

The apiary was used to gather *A. florea*, *A. mellifera* and *A. dorsata* forager workers, from the fields near the beehives at Union Counsel Lakhani Tehsil Taunsa District Dera Ghazi Khan, Punjab Pakistan. Three medium-sized plates filled with sugar solution were set within plastic cages for this reason. These cages were positioned in three separate spots close to the beehives. Many forager workers were foraging inside and outside the cages after 30 minutes. These cages were brought into the laboratory and covered with muslin material.

Table 1: Insecticides with their active ingredients, trade names, formulations, and the recommended field dose per hectare

Trade Name	Active ingredient	Formulation	(Dose/hectare)
Bunch	Acetamiprid	25% SL	260 ml
Admiral	Pyriproxyfen	15% EC	210 ml
Check-worm	Emamectin Benzoate	10% EC	410 ml
Big hit	Flonicamid+Abamectin	10.10 WG	310 g
Response	Nitenpyrum	12% SL	110 ml
Decis super	Deltamethrin	12% EC	110 ml
Lancer	Lambda-cyhalothrin	20% EC	90 ml
X Tall	Triazophos+Lufenuron+Indoxacarb	30% EC	1010 ml
Talstar	Bifenthrin	12% EC	260 ml
Confidor	Imidacloprid	25% SL	130 ml

Experimental material

The experiment involved the utilization of different insecticides: X-Tall® (triazophos + lufenuron + Indoxacarb 30% EC), Checkworm® (emamectin benzoate 10% EC), Admiral® (pyriproxyfen 15% EC), Decis Super® (deltamethrin 12% EC), Lancer® (lambda-cyhalothrin 20% EC), Talstar® (bifenthrin 12% EC), Confidor® (imidacloprid 25% SL), Bunch® (acetamiprid 25% SL), Response® (nitenpyrum 12% SL), and Bighit® (flonicamid + abamectin 10.10% WG) (Table 1). The investigation also involved the use of various materials, including syringes, microscopes, tube, glass, bottle, water, Petri plate, papers napkin, dropper, indicator, netting, plastic cages, rubber, as well as workers of *A. florea*, *A. mellifera* and *A. dorsata*.

The insecticidal solution

The insecticides were mixed with their recommended solvents, which in this case was water, at the appropriate concentrations. The prescribed dose of each insecticide was measured using a cup, poured into a glass, and then mixed with the corresponding amount of clean water. The solution was thoroughly shaken and stored in bottles for future application.

Treatment with insecticidal solution

To assess the lethal effects of insecticides on honeybees, petri dishes and glass bottles were utilized. The experimental materials, including the petri dishes, were completely washed using sodium hypochlorite and boiled water, and then allowed to dry. Netting pieces were cut to fit the petri dishes conveniently. Filter papers were soaked in the insecticidal solution, air-dried for approximately 20-25 minutes, and placed inside the petri plate. Using a syringe, 5 mL of the prepared solution was put on top side of the petri plate, ensuring that the insecticidal mixture reached all corners. The lab dishes were left to air-dry. Subsequently, the filter paper was placed in each petri dish, and the worker bees were released for subsequent observations. In the control dishes, dH₂O was added to the filter paper instead of insecticidal solution.

Data recording

The insecticides' deadly effects were recorded 3, 5, 12 and 24 h after the honeybees were added to the petri dishes. While the remaining honeybees were examined under a stereoscope, those that were actively moving were put back into the vial. A needle was placed into the motionless bodies of honeybees to determine that they were dead. The proportion of mortality was noted in the data sheet after the total number of dead bees was counted.

Statistical analysis

Data analysis was performed by employing the ANOVA technique and Least Significant Difference (LSD) test. Error bars in Figure 1A, B, C, and D show variability (± standard error of mean, SEM), and various lowercase letters above the bars denote statistical groupings at 5 % probability level.

Results

The lethal toxicity of various insecticidal solutions was evaluated against 3 honeybee species, *A. florea*, *A. mellifera*, and *A. dorsata* using the petri plate technique

at various time duration (3 h, 6 h, 12 h and 24 h) (Figure 1).

Effect of Bunch® (acetamiprid)

The proximate toxic effect of Bunch® was recorded against *A. florea*, *A. mellifera*, and *A. dorsata* using the petri plate technique at various time intervals. Bunch® exhibited low toxicity as an insecticide against *A. florea*, *A. mellifera*, and *A. dorsata*. Initially, the death rate was

quite minimal after 3 h of exposure, with maximum mortality experienced after 24 h. Acetamiprid was less toxic to honeybees which is consistent with earlier research (Iwasa *et al.*, 2004) and exhibited lower levels of toxicity towards honeybees (Tison *et al.*, 2017).

Effect of admiral® (pyriproxyfen)

After 3 h of exposure, it caused mortality rates of 14.46% in *A. florea*, 13.36% in *A. mellifera*, and

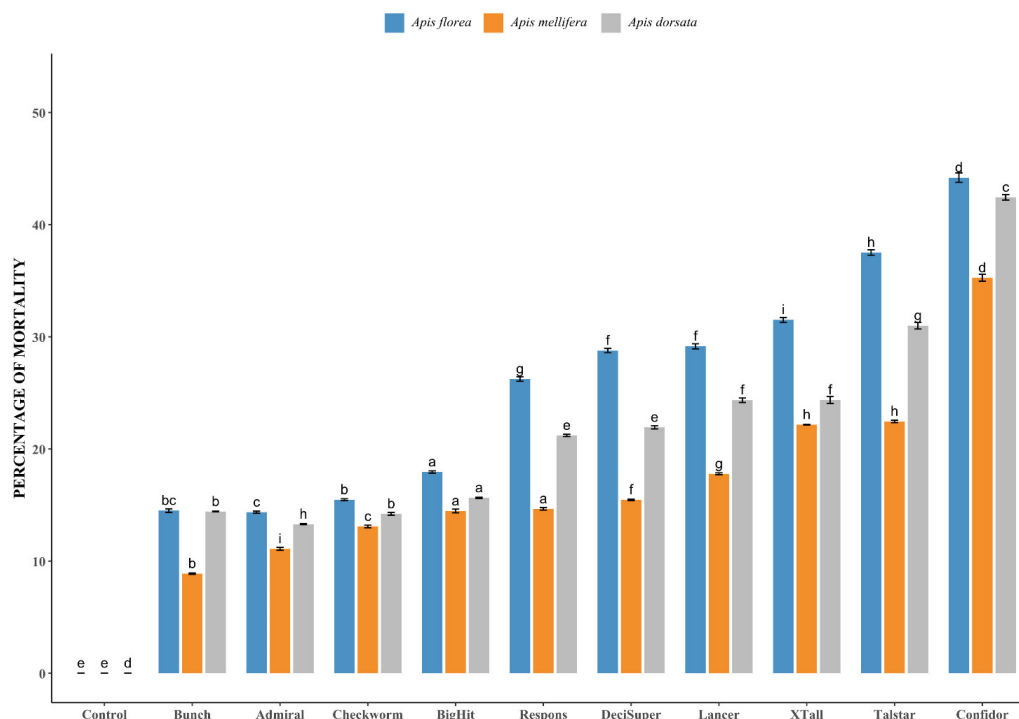


Figure 1A: Comparative assessment of lethal toxic effect of various insecticides against 3 honeybee species after 3 h time period of exposure. $p \leq 0.05$

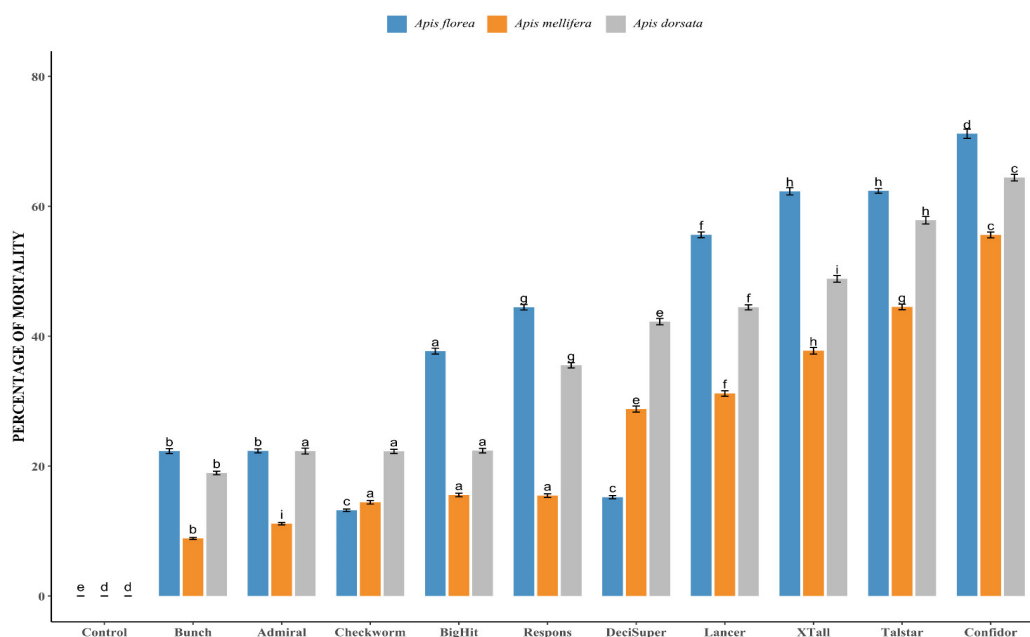


Figure 1B: Comparative assessment of lethal toxic effect of various insecticides against 3 honeybee species after 6 h time period of exposure. $p \leq 0.05$

11.12% in *A. dorsata*. Following 6 h of exposure, *A. florea* 22.24% and *A. mellifera* exhibited mortality rates of 22.24%, while *A. dorsata* showed a mortality rate of 11.11%. After 12 h, Admiral® caused mortality rates of 44.42% in *A. florea*, 37.75% in *A. mellifera*, and 15.57% in *A. dorsata*. Upon 24 h of exposure, the mortality rates increased to 64.46% in *A. florea*, 48.82% in *A. mellifera*, and 24.46% in *A. dorsata*. In the control group, all forager workers of honeybees remained alive (Table 2).

Effect of Big hit® (flonicamid + abamectin)

A. florea exhibited moderate mortality rates to Big hit®, followed by *A. mellifera* and *A. dorsata*. Initially, the death ratio was initially low after a 3 h exposure period, with the maximum mortality after 24 h of exposure to Big Hit®.

Effect of Response® (nitenpyrum)

During short-term exposure, Response® demonstrated lower toxicity to honeybees with mortality rates of 26.64% in *A. florea*, 21% in *A. mellifera*, and 14.42% in *A. dorsata*.

Effect of decis Super® (deltamethrin)

Decis Super® resulted in moderate mortality in *A. florea*, followed by *A. mellifera* and *A. dorsata*. Initially, the death rate was *A. florea*, 14.46%, *A. mellifera*, 14.48% and *A. dorsata* 8.84% after 3 h of exposure, with highest mortality being observed after 24 h (*A. florea* 97.75%, *A. mellifera* 88.86% and *A. dorsata* 82.20%).

Effect of Lancer® (lambda-cyhalothrin)

In *A. florea*, Lancer® showed the highest mortality rate, followed by *A. mellifera* and *A. dorsata*. Additionally, after 3 h of exposure, the honeybee death rate was modest; however, it gradually rose with time, reaching its maximum after 24 h.

Effect of X-Tall (triazophos+andoxacarb)

A. florea exhibited the highest susceptibility to X-Tall®, followed by *A. mellifera* and *A. dorsata*, respectively. Furthermore, the mortality rate of honeybees was minimum after a 3 h exposure, with a 24 h exposure showing the highest mortality.

Effect of talstar® (bifenthrin)

Talstar® exhibited lethal effects on all three honeybee species. Notably, the toxicity of Talstar® intensified over time, with the highest level of mortality observed

after 24 h.

Table 2: Overall comparative mortality (%) of three different honeybee species after 24 h exposure to various insecticides.

Insecticides	<i>Apis florea</i>	<i>Apis mellifera</i>	<i>Apis dorsata</i>	Control
Bunch	57.72%	48.62%	42.24%	0.00%
Admiral	64.46%	48.82%	24.46%	0.00%
Checkworm	55.56%	51.11%	22.22%	0.00%
Big hit	64.42%	57.55%	51.11%	0.00%
Response	71.11%	71.11%	62.20%	0.00%
Decis super	75.54%	75.54%	68.86%	0.00%
Lancer	82.20%	77.75%	75.53%	0.00%
X Tall	84.42%	82.20%	77.75%	0.00%
Talstar	88.86%	86.64%	77.75%	0.00%
Confidor	97.75%	88.86%	82.20%	0.00%

Effect of confidor (imidacloprid)

Confidor® resulted in the highest mortality rate in *A. florea*, followed by *A. mellifera* and *A. dorsata*. The initial death rate of honeybees after 3 h of subjection was relatively less, but it progressively high over rhythm. Consequently, the death toll was observed following 24 h when filter paper used with Confidor® was exposed to vagrant workers.

Assessing the effectiveness of insecticides after a 3 h exposure period

Following a 3 h exposure, most insecticides demonstrated low levels of toxicity to honeybees, except for Confidor® and Talstar® which exhibited moderate toxicity. Therefore, except for Confidor® and Talstar®, the remaining insecticides were deemed safe for honeybees during short-term exposure (Figure 1A).

The effects of insecticides with reduced toxicity on *A. florea*, *A. mellifera*, and *A. dorsata* following a 6 h exposure period

The findings indicated that after a 6-h exposure, Bunch®, Admiral®, Checkworm®, and Bighit® demonstrated lower toxicity towards honeybees. Response® and Decis Super® exhibited moderate toxicity towards *A. florea* and *A. mellifera*, but were deemed safe for *A. dorsata*. On the other hand, Lancer® and X-Tall® displayed moderate toxicity towards *A. dorsata* and high toxicity towards *A. florea* and *A. mellifera*. Talstar® and Confidor® caused the highest mortality among *A. florea* and *A. mellifera*, while resulting

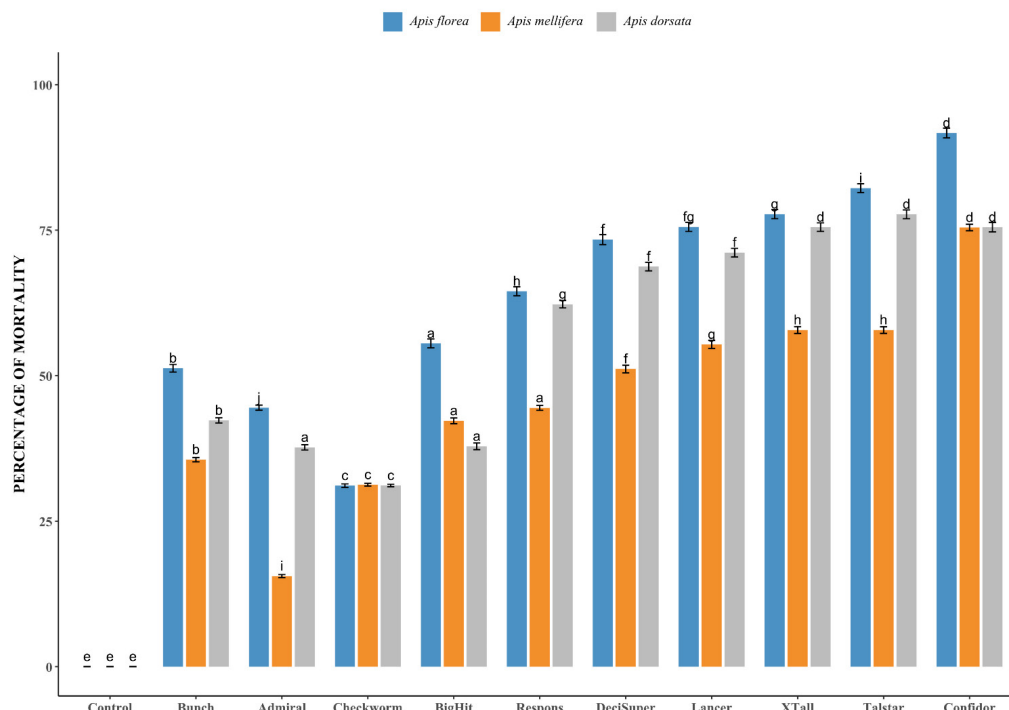


Figure 1C: Comparative assessment of lethal toxic effect of various insecticides against 3 honeybee species after 12 h time period of exposure. $p \leq 0.05$

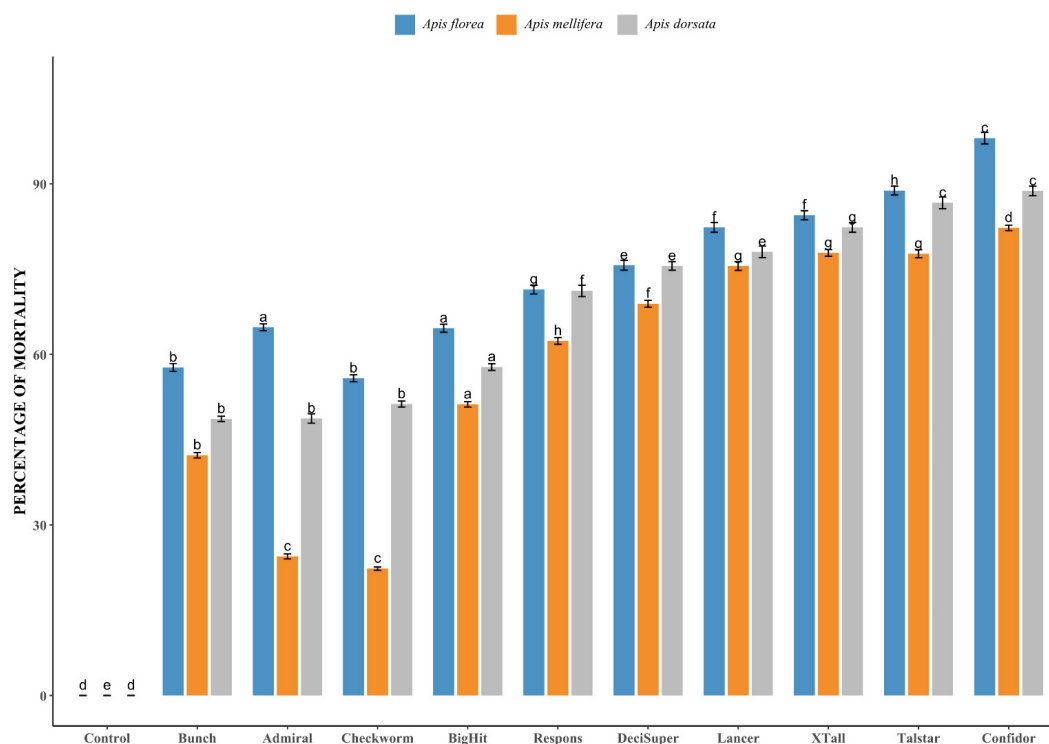


Figure 1D: Comparative assessment of lethal toxic effect of various insecticides against 3 honeybee species after 24 h time period of exposure. $p \leq 0.05$

in moderate mortality for *A. dorsata* (Figure 1B). The control group did not show any mortality (Table 2).

Evaluation of less toxic insecticides against A. florea, A. mellifera and A. dorsata after 12 h exposure

Bunch® exhibited moderate toxicity towards *A. dorsata*,

but more toxic towards *A. florea* and *A. mellifera*. Admiral® was safe for *A. dorsata*, but moderately toxic for *A. florea* and *A. mellifera*. Checkworm® displayed high toxicity towards *A. florea*, and moderately toxic towards *A. mellifera* and *A. dorsata*. Bighit® displayed high toxicity towards *A. florea*, and medium toxicity

towards *A. mellifera* and *A. dorsata*. Response[®] exhibited high toxicity towards *A. florea* and *A. mellifera*, while demonstrating moderate toxicity towards *A. dorsata*. Lancer[®] and X-Tall[®] showed high toxicity towards honeybees. Talstar[®] and Confidor[®] were lethal for honeybees (Figure 1C).

Assessing the Effectiveness of Low-Toxicity Insecticides on A. florea, A. mellifera, and A. dorsata following 24 h of Exposure

Bunch[®] exhibited high toxicity towards *A. florea* and *A. mellifera*, while demonstrating medium toxicity towards *A. dorsata* after a 24-h exposure period. Admiral[®] displayed high toxicity towards *A. florea*, medium toxicity towards *A. mellifera* and lower towards *A. dorsata*. Checkworm[®] demonstrated high toxicity towards *A. florea* and *A. mellifera*, while exhibiting low toxicity towards *A. dorsata*. Big Hit[®], Response[®], and Decis Super[®] resulted in high mortality rates for all honeybee species. The very high levels of poisonous effects on all three bee species were found with Confidor[®] and Talstar[®] (Figure 1D).

Discussion

Since the beginning of this century, Pakistan has seen a 12-fold increase in the use of pesticides. The following is a list of the main pesticides used in the nation: pyrethroids > organophosphates > organochlorines > carbamates (Rashid *et al.*, 2022). Moreover, various neonicotinoids are still used in the country (Pervez and Manzoor, 2021), despite being prohibited in the EU due to their detrimental impact on the health of honey bees (EUROPA, 2013). Pollinators are affected by pesticides in both deadly and sublethal ways, either directly or indirectly through their impacts on habitat, nesting, and forage resources. According to Gill *et al.* (2012), Feltham *et al.* (2014) and Balbuena *et al.* (2015), sub-lethal levels of pesticide exposure can hinder foraging bees' ability to find nectar and pollen resources and return them to the nest, whereas high levels of exposure can induce acute mortality. Additionally, the longevity of adult bees and their capacity for foraging may be diminished if developing brood are exposed to pesticides (Tsvetkov *et al.*, 2017). Neurotoxic substances like organophosphates, carbamates, pyrethroids, phenylpyrazoles, and neonicotinoids are the most widely used insecticides. Research suggest that Acetamiprid may have negative impacts on honeybee populations, highlighting the importance of considering its potential consequences

and using it responsibly to protect honeybee health and well-being (Desneux *et al.*, 2007). Additionally, Neonicotinoids, which are one of the most commonly used insecticides worldwide, include acetamiprid. The memory and learning of honey bees may be negatively impacted by sublethal levels of neonicotinoids. Lu *et al.* (2020) compromised important activities like foraging behavior (Henry *et al.*, 2012; Tison *et al.*, 2019), and affect the organ morphology of adults and larvae (Domingues *et al.*, 2017; Wu *et al.*, 2017; Tavares *et al.*, 2019). Thiacloprid and acetamiprid can negatively impact honeybees' cellular response, memory function and survival (Tison *et al.*, 2017; Shi *et al.*, 2019; Shi *et al.*, 2020; Wang *et al.*, 2020).

While evaluating the toxicity of different insecticides, including Pyriproxyfen, on melon crops (Costa *et al.*, 2014) reported that Pyriproxyfen exhibited lower toxicity towards *A. mellifera*. With Big Hit[®] after 6 h exposure, the mortality rates were at 15.54% for *A. dorsata*. Our findings partially support previous studies that Flonicamid has been considered as safe insecticide for pollinators, particularly honeybees (Thomazoni *et al.*, 2009). The highest level of toxicity was from Decis Super[®] and Lancer[®], after 24 h of exposure, resulting in a mortality rate of 75.54% in both *A. florea* and *A. mellifera*, where Deltamethrin and Karate 5EC (lambda-cyhalothrin) exhibited moderate toxicity towards honeybees, resulting in alterations in their behavior (Bailey *et al.*, 2005; Ramirez-Romero *et al.*, 2005). Triazophos, revealed toxicity towards honey bees which is in line with findings of a previous study (Gökce *et al.*, 2022).

Variation in honey bee death rates can be caused by differences in pesticide formulations, insecticide potency and concentration, genetic variation in bee species, and physiological conditions (Poquet *et al.*, 2016; Jacob *et al.*, 2019; Laurino *et al.*, 2013).

Conclusions and Recommendations

The present study aimed to identify insecticides that are comparatively less toxic to honeybees through screening processes. *A. florea* and *A. dorsata* forager workers were wild harvested from the field for the study, whereas *A. mellifera* forager workers were obtained from a domestic hive.

Ten treatments, including a control group, and five biological replications were used in the completely

randomised design of the study. Twenty forager worker honeybees were kept in each petri dish, and data was collected at predetermined intervals (3, 6, 12, and 24 h).

All the tested insecticides exhibited lethality towards honeybees (*A. florea*, *A. mellifera*, and *A. dorsata*). However, Bunch® (acetamiprid) demonstrated relatively lower toxicity compared to other insecticides, making it the least toxic. Lancer® (lambda-cyhalothrin), Decis Super® (deltamethrin), Response® (nitenpyrum), Big hit® (flonicamid + abamectin), Checkworm® (emamectin benzoate), and Admiral® (pyriproxyfen) were categorized as moderately harmful to honeybees. On the other hand, Confidor® (imidacloprid) exhibited the highest mortality rate among the tested insecticides, indicating it is harmful to honeybees. Talstar® (bifenthrin) and X-tall® (triazophos + lufenuron + indoxacarb) also demonstrated significant toxicity towards honeybee. All the insecticides evaluated in this study demonstrated toxic effects on honeybees. Confidor® appeared as highly toxic and Bunch® as the least toxic to honeybees. These findings can serve as important guidelines for identifying the potential toxicity of insecticides towards honeybees. Hence, proper mechanisms that reduce pesticide use should be adopted to facilitate honeybee conservation. To prevent insect pest populations from falling below the economic threshold, insecticides should only be applied when absolutely necessary. Furthermore, this study provides valuable information for sustainable agriculture by promoting the appropriate and responsible use of insecticides.

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Novelty Statement

This study is the first to compare the toxicity of field-recommended pesticide doses on three key Asian honeybee species (*Apis florea*, *A. mellifera*, and *A. dorsata*), revealing that *A. florea* is the most vulnerable to Imidacloprid. Unlike previous research focusing solely on *A. mellifera*, our findings demonstrate species-specific pesticide risks, urging tailored

pollinator protection strategies. These data challenge the assumption that pesticide impacts are uniform across bee species, providing critical data for region-specific agricultural policies and conservation efforts.

Authors' Contribution

Muhammad Tauseef: Conceptualization, Methodology, Writing – Original Draft
Hadia Gul: Supervision, Writing – Original Draft
Nasr Ullah Khan: Formal analysis
Douglas Law: Validation, Funding acquisition
Salma Shaheen: Investigation, Software
Muhammad Mohibullah: Validation and data visualization
Muhammad Shakeel: Resources
Daniel K.Y. Tan: Writing – Review & Editing
Muhammad Yasin: Data curation, Writing – Review & Editing, Funding acquisition

Generative AI and AI-assisted technology statement

The authors declare that no generative AI and AI assisted technology was used in the creation of this manuscript.

Conflict of interest

There is no conflict of interest among the authors of the manuscript.

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