Sociobiology
An international journal on social insects

RESEARCH ARTICLE - BEES

Acute Toxicity of Selected Insecticides on *Apis mellifera* and *Ceratina smaragdula*: Laboratory and Field Assessments

Fayyaz Hussain[1], Mudssar Ali[1], Fawad Zafar Ahmad Khan[1,2], Ahmad Ibrahim Jalali[1]

1 - Department of Entomology, Muhammad Nawaz Shareef University of Agriculture Multan, Multan 60000, Pakistan
2 - Department of Outreach and Continuing Education, Muhammad Nawaz Shareef University of Agriculture Multan, Multan 60000, Pakistan

Article History

Edited by
Evandro Nascimento Silva, UEFS, Brazil
Received 03 August 2025
Initial acceptance 31 May 2026
Final acceptance 19 June 2026
Publication date 07August 2026

Keywords
Honey bee, solitary bee, pesticides, IPPM, safe chemistry.

Corresponding author
Dr. Mudssar Ali
Department of Entomology,
MNS University of Agriculture
Multan 60000, Pakistan.
E-Mail: mudssar.ali@mnsuam.edu.pk

Abstract

Pollinators are vital to agricultural productivity, yet their populations are declining due to anthropogenic disturbances, including pesticide use. This study evaluated the acute toxicity of selected insecticides to two bee species, the honey bee (*Apis mellifera*) and the small carpenter bee (*Ceratina smaragdula*), using laboratory and field experiments. Under laboratory conditions, emamectin benzoate exhibited the highest toxicity to *C. smaragdula*, with a 12-hour LC_{50} of 4.69 µg/mL. Moderate toxicity was observed for flubendiamide (LC_{50} = 60.57 µg/mL) and chlorantraniliprole (LC_{50} = 57.64 µg/mL), whereas the remaining insecticides showed higher LC_{50} values ranging from 141.41 to 846.77 µg/mL. After 24 hours, emamectin benzoate remained the most toxic (LC_{50} = 1.14 µg/mL), and similar toxicity patterns were observed for the other insecticides. In *A. mellifera*, emamectin benzoate was also the most toxic insecticide at both 12 hours (LC_{50} = 9.64 µg/mL) and 24 hours (LC_{50} = 5.10 µg/mL). Moderate toxicity was recorded for flubendiamide and chlorantraniliprole, while the other insecticides exhibited lower toxicity, with 24-hour LC_{50} values ranging from 56.35 to 589.92 µg/mL. Field applications resulted in significant differences in bee abundance between treated and untreated plots, with the highest abundance in untreated plots and the lowest in bifenthrin-treated plots. Overall, bee abundance increased over time, peaking 72 hours after insecticide application. These findings highlight the need for further investigation into the long-term effects of pesticides, particularly on understudied solitary bee species across diverse ecosystems.

Introduction

About 90% of flowering plant species are visited by animals (Tong et al., 2023), and among them, bees are key players (Klein et al., 2007), providing essential pollination services. Pollinators, particularly bees, play a crucial role in food security by providing pollination services to plants and enhancing crop yields (Anees et al., 2022; Q. Ali et al., 2024; Ejaz et al., 2025). Overall, bees provide roughly one-third of global crop pollination services (Garibaldi et al., 2013), yet declines in both managed and wild bee populations continue worldwide (Shi et al., 2024). Some of the reasons for bee decline include agricultural intensification, loss of diverse landscapes, and injudicious pesticide use (Nath et al., 2023; Pontarp et al., 2024). These declines are predicted to reduce the food production in pollinator-dependent crops and the ecosystem services, ultimately affecting food security (Devkota et al., 2024; Uwingabire & Gallai, 2024).

Among various factors contributing to the decline, agricultural intensification stands out as a crucial factor, as diverse landscapes are being rapidly converted into strict monocultures, ultimately affecting pollinator diversity and distribution (Proesmans et al., 2024). Most monocultures rely on pesticides for pest management and to ensure higher yields (Wagan et al., 2025; Zhang et al., 2023). Farmers routinely apply neonicotinoids, pyrethroids, organophosphates, and newer chemistries such as diamides, spinosyns, and macrocyclic lactones, each targeting distinct physiological systems (Sparks,

2025, Mode of Action|Insecticide Resistance Action Committee (IRAC) 2025). These insecticides primarily target nerves and muscles, as well as growth, respiration, the midgut, proteins, and unspecified regions of insect body systems (*Mode of Action|Insecticide Resistance Action Committee (IRAC)*, 2025). Systemic compounds may translocate into pollen and nectar, creating oral exposure routes for insect pollinators (Heller et al., 2020). Although protecting yield is the immediate priority of the grower, disregarding pollinator safety can impose hidden long-term economic costs through decreased ecosystem services (Nchang et al., 2023). This recognition highlights the emerging paradigm of integrated pest and pollinator management (IPPM) (Egan et al., 2020; Lundin et al., 2021; Merle et al., 2022).

To practice integrated pest and pollinator management, a broad understanding of the toxic effects of different insecticide groups on pollinators is needed. Most regulatory toxicology tests still rely on the European honey bee, *Apis mellifera* L. However, solitary species such as the small carpenter bee *Ceratina smaragdula* Smith often display greater sensitivity owing to their limited colony buffering, smaller body mass, and reduced diversity of detoxification enzymes. The effects of pesticides depend on whether bees live in large social colonies or nest alone, because social bees can share and buffer chemical exposure, whereas solitary bees face pesticide exposure individually without support from other bees (Sgolastra et al., 2019). *A. mellifera* colonies comprise 40,000 to 80,000 worker bees that share brood care and food stores, which potentially dilutes the individual pesticide loads. On the other hand, *C. smaragdula* females nest singly in pithy stems, provisioning each brood cell alone, and therefore, experience direct, uncompensated exposure to pesticide-exposed forage. Another example of the ground-nesting solitary bee, *Xenoglossa pruinose* (Say) (Hymenoptera: Apidae), demonstrated sensitivity to even a single pesticide application under field conditions (O'Reilly & Stanley, 2023; Rondeau & Raine, 2024). Understanding how social and solitary bees differ in vulnerability is essential for risk assessment.

Here, we selected several commonly used pesticides from different IRAC groups (Mode of Action | Insecticide Resistance Action Committee (IRAC), 2025) and tested their toxicity against both *A. mellifera* and *C. smaragdula*. Through laboratory feeding bioassays and field applications of the least-toxic pesticides, we assessed the effects on survival under laboratory conditions and on the abundance of these pollinators in sprayed plants in the field. The study aims to develop actionable recommendations for practicing an integrated pest and pollinator management approach.

Materials and Methods

Insecticides

A total of eleven insecticides were used; nine were tested against small carpenter bees, and all eleven were tested against *Apis mellifera*. Insecticide formulations (Table 1) were chosen after (i) interviewing 25 local farmers and pesticide dealers, ensuring representation of at least five IRAC mode-of-action groups. These insecticides were Rani® 20 SL (active ingredient: acetamiprid), Talstar® 10.8 EC (active ingredient: bifenthrin), Coragen® 20 SC (active ingredient: chlorantraniliprole), Pirate® 360g/1SC (active ingredient: chlorfenapyr), Polo® 50 SC (active ingredient: diafenthiuron), Timer® 1.9 EC (active ingredient: emamectin benzoate), Ulala® 50 DF (active ingredient: flonicamid), Hiflow® 10.8 EC (active ingredient: pyriproxyfen), Radiant® 120 SC (active ingredient: spinetoram), Belt 480 SC (active ingredient: fubendiamide), and Movento® 240 SC (active ingredient: spirotetramet). The insecticide solution was prepared by adding 20% sucrose to distilled water, and serial dilutions were made according to the recommended dose. Different concentrations of insecticides were used to test acute toxicity (Table 1).

Table 1. Serial concentrations and corresponding dose ranges (µg/mL) of insecticides used in laboratory bioassays.

Insecticide	Serial solution concentration (%)	Serial solutions range (µg mL^{-1})
Acetamiprid†	100 - 6.25	125 - 7.8125
Bifenthrin*	100 - 6.25	125 - 7.8125
Chlorantraniliprole	100 - 6.25	25 - 1.562
Chlorfenapyr	100 - 6.25	375 - 23.43
Difenthiuron*	100 - 6.25	400 - 25
Emamectin benzoate	100 - 6.25	19 - 1.19
Flonicamid*	100 - 6.25	125 - 7.8125
Pyriproxyfen*	100 - 6.25	250 - 6.25
Spinetoram*	100 - 6.25	360 - 22.5
Flubendiamide	100 - 6.25	25 - 1.562
Spirotetramet†	100 - 6.25	240 - 15

† Insecticides not used for solitary bees
* Insecticides used in the field

The concentrations of insecticides used in this study were selected to reflect both field-realistic exposure levels and higher-end concentrations that may occur in the field. While bees are not typically exposed to the full recommended application doses in nectar or pollen, residue studies have shown that insecticide levels in bee-consumed matrices can vary depending on application method, crop type, and environmental conditions (Sanchez-Bayo & Goka, 2014). Therefore, we tested a gradient of concentrations: from low (representing likely field exposure), to high (representing worst-case or acute exposure scenarios). This methodology allows for a more comprehensive understanding of both sublethal and lethal effects.

Ceratina smaragdula emergence and maintenance

We selected *C. smaragdula* as a model solitary bee species because it is a locally abundant and ecologically important species in the region, known for its role in pollinating

a wide range of crops (Abdullah et al., 2024; Rauf et al., 2021). Its manageable nesting in *Phragmites* reeds also makes it a practical candidate for convenient collection, laboratory rearing, and toxicity testing.

C. smaragdula was collected from Kot Addu, a district of Punjab, Pakistan. Reeds of *Phragmites* sp. containing *C. smaragdula* pupae were taken from natural nesting sites in the field. To reduce stress during collection, reeds containing pupae were carefully cut with a sharp blade and placed in a ventilated container in the shade to prevent overheating or physical disturbance. Later, the container was transferred to an air-conditioned vehicle and transported to the Bee Laboratory, MNS University of Agriculture, Multan, Pakistan. These reeds were maintained under controlled laboratory conditions (29 ± 1 °C, ~60% relative humidity, and a 16:8 h light:dark photoperiod) until adult emergence. Emerged bees were monitored daily, and newly emerged adults were identified by their soft exoskeletons, lighter coloration, and absence of wing wear, indicating they had not yet taken flight. Individuals were collected using gentle aspiration within 12 hours of emergence and transferred into 60 cm^3 labeled holding cages to prevent escape and for use in toxicity assays.

Apis mellifera emergence and maintenance

Worker honey bees were selected for laboratory experiments involving different insecticides. Newly emerged *Apis mellifera* workers were identified and collected from healthy, queen-right colonies maintained at the agricultural farm of MNS University of Agriculture. Brood frames containing sealed worker pupae were removed from the hives and placed in a glass cabinet at 29 ± 1 °C, ~60% relative humidity, and 16:8 h light:dark photoperiod. Frames were inspected every few hours, and bees emerging from sealed cells were immediately collected using soft forceps. Only bees that emerged within the last 2–3 hours were used to ensure uniform age and reduce variability in susceptibility. These bees had not yet engaged in foraging or hive tasks and showed soft cuticle and light coloration, confirming their newly emerged status.

A. mellifera colonies were managed according to standard field practices. For insecticide exposure risk assessment, worker honey bees have been considered the most environmentally exposed caste (Picard-Nizou et al., 1995). Worker honey bees start foraging 20 days after they emerge from the brood (Winston, 1991). According to farm records, no obvious diseases were reported, and no hive treatments involving pesticides were applied. Emerged worker bees kept in containers were maintained at a temperature of 32 ± 2 ºC, 65 ± 5% relative humidity, and a 12:12 h light:dark photoperiod, and were fed a 50% (w/v) sucrose solution (Williams et al., 2013). The sucrose solution was supplied in identical feeders for all experimental groups and replenished daily to prevent depletion. Because nutrition was not an experimental factor, the volume of sucrose solution consumed per group was not measured.

Experimental trials

The sugar solution was prepared by mixing 50 g of sugar with 50 ml of distilled water for the feeding bioassay. A micropipette was used to measure the quantity of insecticide required for the mother solution. A beaker was used to measure the volume of the stock solution. Glass cups were used for the food bioassay, with tissue paper soaked in the insecticide-treated stock solution placed inside each cup. For each trial, 18 cups were used. Goggles and gloves were worn to prevent contamination from exposure to insecticides. Experiments were conducted in a climate-controlled growth chamber with the following parameters: 32 ± 2 °C, 65 ± 5% relative humidity, and a 12:12 light-dark photoperiod (Badawy et al., 2015).

Feeding Bioassays

The toxicity of insecticides to social and solitary bees was evaluated by determining LC50 values. The susceptibility of both bee types to insecticides was assessed via oral toxicity assays. The methodology was based on the standardized protocol described by (Medrzycki et al., 2010), with necessary adaptations to accommodate the physiological differences between social and solitary bee species.

Different concentrations of insecticides were used for each treatment (Table 1). For insecticide application, we used glass boxes with proper ventilation, kept in an air-conditioned laboratory at 25 ± 2 °C and 60–70% relative humidity. Bee mortality assessments were recorded at different hours of the day to track time-dependent effects. Both social and solitary bees received various insecticide doses via food (a 50% w/v sucrose solution) in plastic feeding cups. A total of eleven insecticides were tested against *Apis mellifera*, while only nine were tested against small carpenter bees because reeds containing *Ceratina* populations were not available for further insecticide testing.

Before the experiment, bees were randomly assigned to treatment and control groups to avoid bias. The research was conducted using synthetic insecticides at different concentrations that reflect environmentally realistic exposure levels. Bees were deprived of food for two hours prior to the trial to encourage feeding behavior.

Each treatment group received a sucrose solution spiked with a specific insecticide concentration after the fasting period, whereas the control group received an insecticide-free sucrose solution. All treatments were replicated at least three times, with each replicate consisting of at least 10 bees kept in separate test units.

Oral toxicity was assessed using the above-described feeding method, and the survival time of bees post-exposure was monitored. The mortality rate of bees was recorded at 12 and 24 hours after exposure.

Insecticides for the field

After lab trials, only the less toxic insecticides with low mortality (higher LC_{50} values) were selected for field

application to assess their toxicity to bees. Bifenthrin, diafenthiuron, flonicamid, pyriproxyfen, and spinetoram were used for field trials at the recommended dose.

Field toxicity

The canola crop was selected to evaluate the field toxicity of selected insecticides because it hosts multiple pollinators and is cultivated over a large area during winter in Multan, Pakistan. In this study, we selected the five least-toxic insecticides identified through laboratory experiments, along with a control. One acre of canola was selected for insecticide application. A randomized complete block design (RCBD) was used in this experiment. A total of 25 plots were selected, each measuring 10 feet wide by 20 feet long. The spacing between plots was the same as that between treatment plots. For the insecticide application, a knapsack sprayer was used. To control insecticide drift into surrounding plots, plastic sheets were used with dimensions matching each plot. Insecticide was mixed into 1 liter of water for application to each plot, and the same volume of water was used for the control without insecticide (Stanley et al., 2010). Insecticide application data were recorded on cross-pollinated plants. The toxicity of insecticides on social and solitary bees was assessed using the LC_{50}. Bee abundance was recorded on clear, sunny days by conducting direct visual counts within 1 m² quadrats placed randomly across the field. Observations were made over 5 minutes per quadrat, between 9:00 AM and 11:00 AM, when bee foraging activity was at its peak (Ali et al., 2011).

Statistical analysis

Polo Plus software was used for lab data analysis, in which we analyzed the LC50 and LC90 values to check the toxicity effect on bees. We measured the degree of freedom and slope. Statistix 8.1 software was used to analyze field data. We used Tukey's HSD test for all pairwise comparisons to assess the abundance of social and solitary bees in experimental plots after insecticide application.

Results

Laboratory Bioassay (*C. smaragdula*)

Nine insecticides were used to assess their toxicity to *C. smaragdula*. After 12 hours of treatment, emamectin benzoate was more toxic than other insecticides (Fig 1). The LC_{50} value for emamectin benzoate was 4.69 µg $mL^{-1,}$ with no overlap to cause higher mortality than other insecticides. On the other hand, moderate toxicity was observed in flubendiamide (LC_{50} value = 60.57 µg mL^{-1}) and chlorantraniliprole (LC_{50} value = 57.64 µg mL^{-1}) based on overlapping fiducial limits. Other insecticides, such as flonicamid, chlorfenapyr, spinetoram, bifenthrin, pyriproxyfen, and diafenthiuron, were least toxic to *C. smaragdula*, with LC50 values ranging from 141.41 to 846.77 µg mL^{-1}. The LC_{90} value for emamectin benzoate was 31.25 µg mL^{-1}, with no overlap, indicating no higher mortality than other insecticides. On the other hand, moderate toxicity was observed for flubendiamide (LC90 = 407.07 µg mL^{-1}) and chlorantraniliprole (LC90 = 383.12 µg mL^{-1}), as indicated by overlapping fiducial limits. Other insecticides such as flonicamid, chlorfenapyr, spinetoram, bifenthrin, pyriproxyfen, and diafenthiuron were the least toxic to *C. smaragdula*, with LC_{90} values ranging from 1066.8 to 18663 µg mL^{-1}. The slope of diafenthiuron was lower than that of the other insecticides, while the slope of emamectin benzoate was higher than that of the other insecticides. The slopes for flubendiamide and chlorantraniliprole were moderate compared with other insecticides (Table 2).

Table 2. Acute oral toxicity (LC_{50} and LC_{90}) of different insecticides against *Ceratina smaragdula* at 12 hours post-treatment. Values are expressed in µg/mL with 95% fiducial limits (FL). Different letters within a column indicate significant differences between treatments (based on non-overlapping 95% FL). Slope ± SE, chi-square (χ^2), and degrees of freedom (df) are from probit regression analysis.

Sr. No.	Insecticides	Population	LC_{50} (95% FL) (µg mL^{-1})	LC_{90} (95% FL) (µg mL^{-1})	Slope ± SE	χ^2	df
1	Bifenthrin	180	141.41 (79.29-589.19) a	1927.3 (499.91-99914.) a	1.13 ± 0.28	7.01	13
2	Chlorantraniliprole	180	57.64 (41.38-92.10) b	383.12 (192.92-1549.15) b	1.55 ± 0.28	11.23	13
3	Chlorfenapyr	180	387.85 (191.49-6823.29) a	5822.4 (1087.3-0.229) a	1.08 ± 0.37	8.30	13
4	Diafenthiuron	180	846.77 (362.74-17791.01) a	18663.0 (2700.90- 0.42728) a	0.95 ± 0.29	6.23	13
5	Emamectin Benzoate	180	4.69 (2.07- 8.29) c	31.25 (14.50-501.34) c	1.55 ± 0.38	16.76	13
6	Flonicamid	180	217.00 (104.59-3749.54) a	2613.2 (521.53-0.41) a	1.18 ± 0.39	11.84	13
7	Pyriproxyfen	180	296.96 (212.49-584.23) a	1066.8 (553.77-5280.5) a	2.30 ± 0.51	6.90	13
8	Spinetoram	180	174.93 (104.26-471.13) a	2487.5 (742.40-0.12) a	1.11 ± 0.26	14.80	13
9	Flubendiamide	180	60.57 (38.65-125.12) b	407.07 (171.76-5461.19) b	1.54 ± 0.34	15.34	13

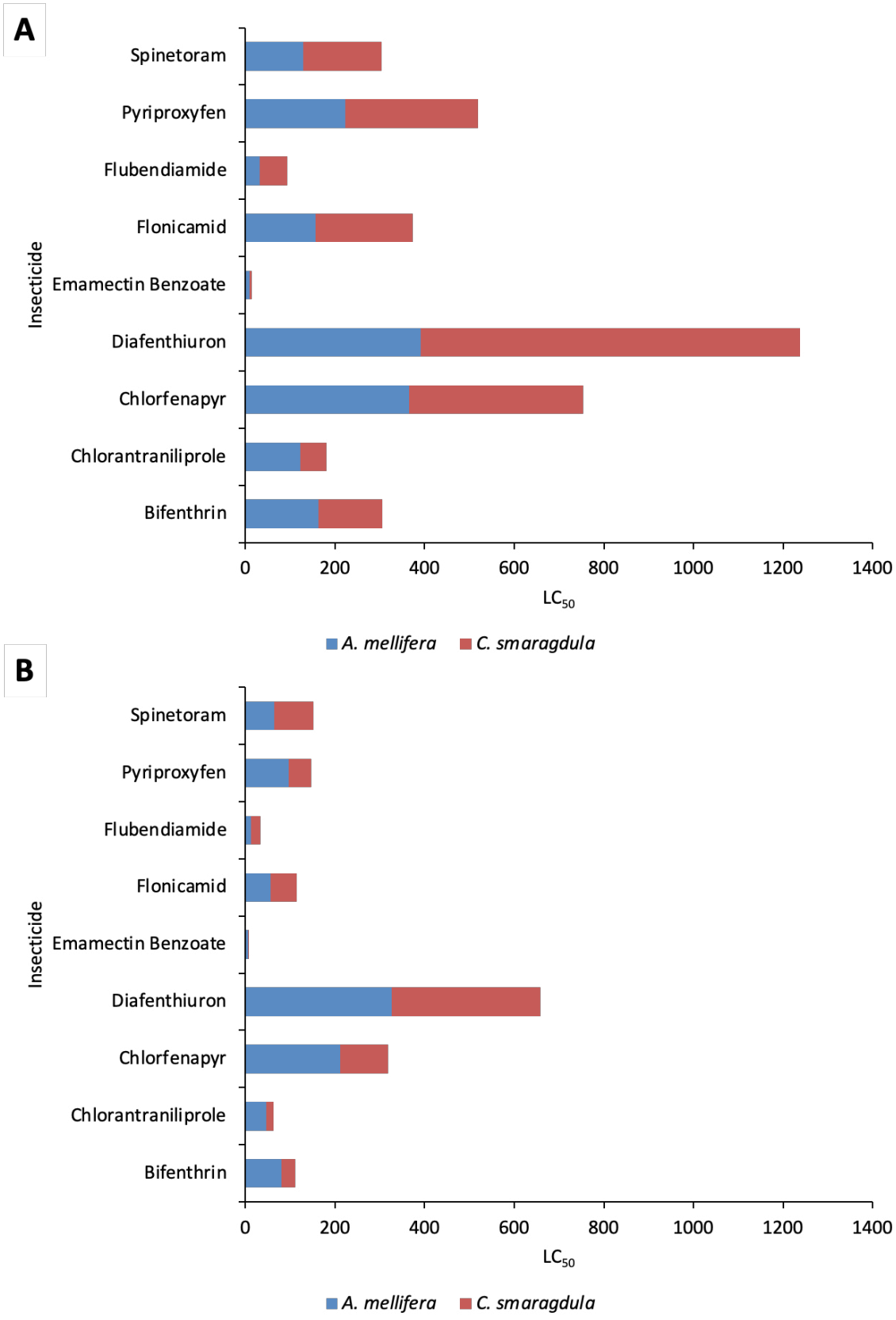

Fig 1. Median lethal concentration (LC_{50}) values of different insecticides against *Apis mellifera* and *Ceratina smaragdula* at two exposure intervals: (A) 12 hours and (B) 24 hours post-treatment. Blue bars represent *A. mellifera*; red bars represent *C. smaragdula*. The figure highlights species-specific differences in insecticide susceptibility over time.

After 24 hours of treatment, emamectin benzoate was found to be more toxic than the other insecticides. The LC50 value for emamectin benzoate was 1.14 µg mL^{-1}, with no overlap with other insecticides to cause higher mortality. On the other hand, moderate toxicity was observed in flubendiamide (LC_{50} value = 20.88 µg mL^{-1}) and chlorantraniliprole (LC_{50} value = 15.83 µg mL^{-1}) based on overlapping fiducial limits. Other insecticides, such as flonicamid, chlorfenapyr, spinetoram, bifenthrin, pyriproxyfen, and diafenthiuron, were least toxic to *C. smaragdula*, with LC50 values ranging from 30.07 to 332.28 µg mL^{-1}. The LC_{90} value for emamectin benzoate was 8.81 µg mL^{-1}, with no overlap, indicating it was not more toxic than other insecticides. On the other hand, moderate toxicity was observed in flubendiamide (LC_{90} value = 104.39 µg mL^{-1}) and chlorantraniliprole (LC_{90} value = 70.56 µg mL^{-1}) based on overlapping fiducial limits. Other insecticides like flonicamid, chlorfenapyr, spinetoram, bifenthrin, pyriproxyfen, and diafenthiuron were least toxic to *C. smaragdula*, with LC90 values ranging from 122.53 to 2221.9 µg mL^{-1}. Diafenthiuron showed a lower slope of toxicity than other insecticides. The slope of emamectin benzoate showed it was more toxic than other insecticides. The slopes of flubendiamide and chlorantraniliprole were moderate compared to other insecticides (Table 3).

Laboratory Bioassay (*Apis mellifera*)

In this experiment, 11 insecticides were tested to check their toxicity against *A. mellifera*. After 12 hours of treatment, emamectin benzoate showed the highest toxicity (Fig 1). The LC_{50} value for emamectin benzoate was 9.64 µg mL^{-1}, with no overlap to cause higher mortality than other insecticides. On the other hand, moderate toxicity was observed in flubendiamide (LC_{50} value = 32.24 µg mL^{-1}) and chlorantraniliprole (LC_{50} value = 122.55 µg mL^{-1}) based on overlapping fiducial limits. Other insecticides such as flonicamid, chlorfenapyr, spinetoram, bifenthrin, pyriproxyfen, and diafenthiuron were less toxic to *A. mellifera*, with LC_{50} values ranging from 129.18 to 589.92 µg mL^{-1}. The LC_{90} value for emamectin benzoate was 18.21 µg mL^{-1}, with no overlap with other insecticides, indicating higher mortality. On the other hand, moderate toxicity was observed for flubendiamide (LC90 = 867.09 µg mL^{-1}) and chlorantraniliprole (LC90 = 450.36 µg mL^{-1}), based on overlapping fiducial limits. Other insecticides, such as flonicamid, acetamipride, spirotetramat, chlorfenapyr, spinetoram, bifenthrin, pyriproxyfen, and diafenthiuron, were less toxic to *A. mellifera*, with LC90 values ranging from 953.43 to 12234 µg mL^{-1}. Diafenthiuron showed lower toxicity, while emamectin benzoate showed the highest toxicity (Table 4).

Table 3. Acute oral toxicity (LC_{50} and LC_{90}) of different insecticides against *Ceratina smaragdula* at 24 hours post-treatment. Values are expressed in µg/mL with 95% fiducial limits (FL). Different letters within a column indicate statistically significant differences between treatments based on non-overlapping 95% FL. Slope ± SE, chi-square (χ^2), and degrees of freedom (df) are from probit regression analysis.

Sr. No.	Insecticides	Population	LC_{50} (95% FL) (µg mL^{-1})	LC_{90} (95% FL) (µg mL^{-1})	Slope ± SE	χ^2	df
1	Bifenthrin	180	30.07 (20.82-41.90) a	122.53 (77.90-297.66) a	2.10 ± 0.34	14.48	13
2	Chlorantraniliprole	180	15.83 (10.48-21.29) b	70.56 (48.13-140.49) b	1.97± 0.35	9.99	13
3	Chlorfenapyr	180	105.42 (71.50-154.60) a	472.92 (273.08-1785.89) a	1.96 ± 0.45	11.71	13
4	Diafenthiuron	180	332.28 (223.59-688.09) a	2221.9 (953.67-151220.00) a	0.22 ± 0.10	11.33	13
5	Emamectin Benzoate	180	1.14 (0.27-1.95) c	8.81 (5.19-33.90) c	1.44 ± 0.32	15.84	13
6	Flonicamid	180	58.06 (38.05-106.62) a	365.17 (166.96-3091.3) a	1.60 ± 0.34	14.49	13
7	Pyriproxyfen	180	51.23 (37.40-68.76) a	156.53 (108.13-304.10) a	2.64 ± 0.35	18.57	13
8	Spinetoram	180	88.06 (53.19-131.19) a	502.68 (281.97-1933.01) a	1.69 ± 0.38	12.31	13
9	Flubendiamide	180	20.88 (13.37-29.46) b	104.39 (64.55-276.40) b	1.83 ± 0.32	13.49	13

Table 4. Acute oral toxicity (LC_{50} and LC_{90}) of different insecticides against *Apis mellifera* at 12 hours post-treatment. Values are expressed in µg/mL with 95% fiducial limits (FL). Different letters within a column indicate statistically significant differences between treatments based on non-overlapping 95% FL. Slope ± SE, chi-square (χ^2), and degrees of freedom (df) are from probit regression analysis.

Sr. No.	Insecticides	Population	LC_{50} (95% FL) (µg mL^{-1})	LC_{90} (95% FL) (µg mL^{-1})	Slope ± SE	χ^2	DF
1	Acetamiprid	180	176.49 (88.55-3674.49) a	1605.2(364.50-5021800.00) a	1.33 ± 0.38	16.34	13
2	Bifenthrin	180	163.60 (89.70-1030.45) a	953.43 (299.53- 6742.00) a	1.67 ± 0.37	19.39	13
3	Chlorantraniliprole	180	122.55 (84.49-260.65) b	450.36(224.90-2679.9) b	2.26 ± 0.44	15.20	13
4	Chlorfenapyr	180	365.69 (188.68-2862.54) a	5434.5(1153.0-16846000.00) a	1.09 ± 0.32	12.67	13
5	Diafenthiuron	180	390.51(205.81-2654.54) a	6508.6(1382.2-238500) a	1.04 ± 0.27	14.18	13
6	Emamectin Benzoate	180	9.64 (6.59-15.66) c	18.21 (12.32-65.90) c	4.63 ± 0.70	46.44	13
7	Flonicamid	180	156.04 (93.28-662.46) a	1121.2(362.83- 53511.00) a	1.49 ± 0.42	11.63	13
8	Pyriproxyfen	180	222.70 (113.81-2739.61) a	5399.6(908.87-687970.00) a	0.92 ± 0.32	11.14	13
9	Spinetoram	180	129.18 (74.17-278.20) a	1103.8(424.45-26538.00) a	1.37 ± 0.28	20.67	13
10	Flubendiamide	180	32.24 (12.87-87.87) b	867.09 (197.95-2942800.00) b	0.89 ± 0.26	16.34	13
11	Spirotetramet	180	589.92 (242.09-15994) a	12234(1712.4-371200.00) a	0.97 ± 0.30	12.52	13

While evaluating the results 24 hours post-treatment on *A. mellifera*, emamectin benzoate proved to be more toxic than other insecticides. The LC_{50} values for emamectin benzoate were 5.10 µg mL^{-1}. While moderate toxicity was observed in flubendiamide and chlorantraniliprole, at concentrations of 12.73 µg mL^{-1} and 46.74 µg mL^{-1}, respectively, based on the overlapping fiducial limits. Other insecticides, such as flonicamid, acetamiprid, spirotetramet, chlorfenapyr, spinetoram, bifenthrin, pyriproxyfen, and diafenthiuron, were less toxic to *A. mellifera* with LC_{50} values ranging from 56.35 to 326.06 µg/mL^{-1}. The LC_{90} value for emamectin benzoate was 16.01 µg mL^{-1}. On the other hand, moderate toxicity was observed in flubendiamide and chlorantraniliprole, 142.29 µg/mL^{-1} and 209.21 µg/mL^{-1}, respectively. Other insecticides like that flonicamid, acetamiprid, spirotetramet, chlorfenapyr, spinetoram, bifenthrin, pyriproxyfen, and diafenthiuron were less toxic to *A. mellifera* with LC_{90} values ranging 239.89-13428.0µg mL^{-1}. Slope of diafenthiuron showed the least toxicity, while emamectin benzoate was more toxic than the other tested insecticides (Table 5).

Field Assay

The abundance of *C. smaragdula* differed significantly among insecticides at 24, 48, and 72 hours after spraying. It was highest in control plots, while the lowest was recorded in bifenthrin-treated plots (Fig 2). A significant difference was observed in the average abundance of *C. smaragdula* across different time intervals after insecticide spraying. Highest abundance of this bee species was recorded after 72 hours while it was lowest after 24 hours of spray (Fig 3). Average abundance of *A. mellifera* was significantly higher in control plot where no insecticide application was done while lowest abundance was recorded in the plot where bifenthrin was applied (Fig 4). There was a significant difference in average abundance of *A. mellifera* after different time intervals of spray. The highest abundance was recorded after 72 hours, while it was lowest after 24 hours of spray (Fig 5). The average abundance of *C. smaragdula* and *A. mellifera* was significantly different. *Apis mellifera* was higher than *C. smaragdula* (Fig 6).

Table 5. Acute oral toxicity (LC_{50} and LC_{90}) of different insecticides against *Apis mellifera* at 24 hours post-treatment. Values are expressed in µg/mL with 95% fiducial limits (FL). Different letters within a column indicate statistically significant differences between treatments based on non-overlapping 95% FL. Slope ± SE, chi-square (χ^2), and degrees of freedom (df) are from probit regression analysis.

Sr. No.	Insecticides	Population	LC_{50} (95% FL) (µg mL^{-1})	LC_{90} (95% FL) (µg mL^{-1})	Slope ± SE	χ^2	DF
1	Acetamiprid	180	56.35 (36.79-99.09) a	345.17 (177.55-1925.4) b	2.25(±0.44)	22.58	13
2	Bifenthrin	180	80.48 (44.02-428.56) a	935.29 (245.35-40637) a	1.20(±0.29)	19.46	13
3	Chlorantraniliprole	180	46.74 (24.77-72.77) b	209.21 (112.70-1545.48) b	1.47(±0.32)	13.59	13
4	Chlorfenapyr	180	212.27 (119.17-990.02) d	3819 (875.93-959660) d	1.02(±0.30)	11.37	13
5	Diafenthiuron	180	326.06 (160.36-5128.35) d	13428.0 (1761.3-13356) d	0.79(±0.28)	10.14	13
6	Emamectin Benzoate	180	5.10 (3.04-7.40) c	16.01 (10.23-52.12) c	2.58(±0.48)	22.05	13
7	Flonicamid	180	55.68 (40.29-80.86) a	239.89 (141.14-760.41) a	2.02(±0.41)	12.91	13
8	Pyriproxyfen	180	95.80 (2765.85-593.52) d	2765.8 (495.78-3233200) d	0.87(±0.27)	6.73	13
9	Spinetoram	180	63.73 (27.86-110.23) a	653.49 (278.83-10389) a	1.26(±0.27)	18.81	13
10	Flubendiamide	180	12.73 (2.81-22.97) b	142.29 (63.20-2952.38) b	1.22(±0.28)	20.68	13
11	Spirotetramet	180	280.48 (149.27-1957.27) d	3943 (885.79-1233100) d	1.11(±0.34)	10.83	13

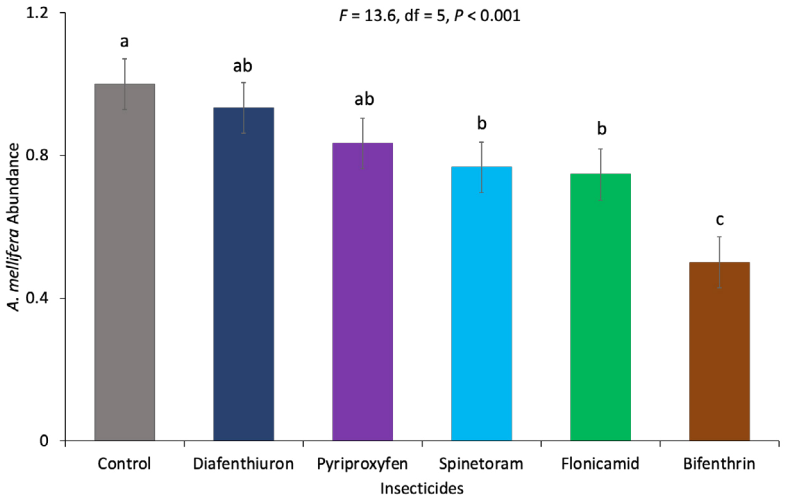

Fig 2. Average abundance (mean ± SE) of *Ceratina smaragdula* recorded on insecticide-treated plots at 24-, 48-, and 72-hours post-application. Bars represent different insecticides; differences in bee abundance reflect species response to residual toxicity and repellent effects of treatments over time.

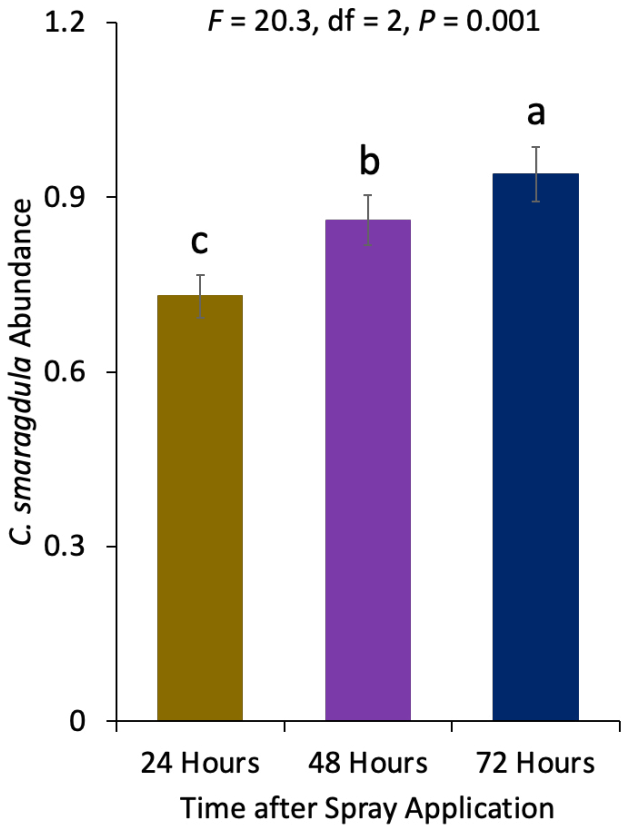

Fig 3. Average abundance (mean ± SE) of *Ceratina smaragdula* observed across all insecticide-treated plots at 24-, 48-, and 72-hours post-spray application. The bars show the increased visitation post-pesticide treatment.

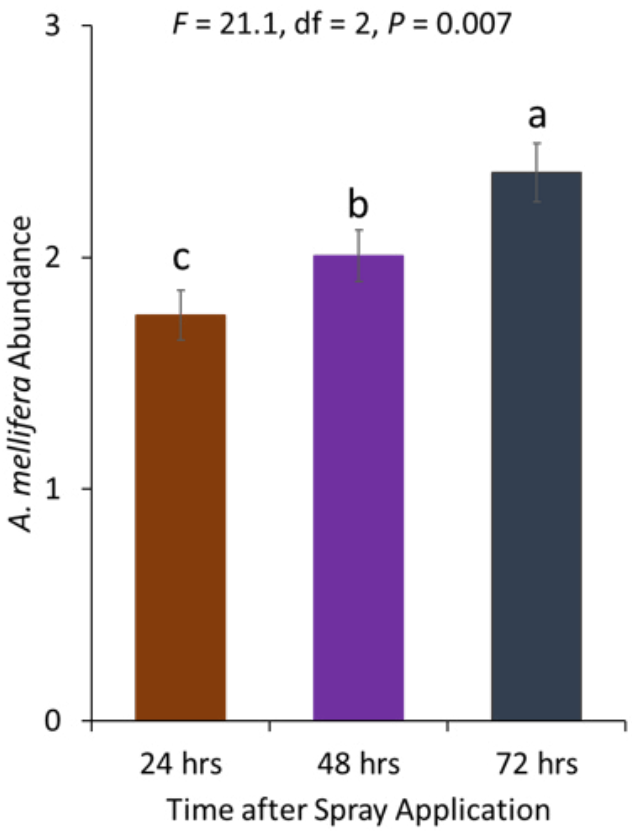

Fig 5. Average abundance (mean ± SE) of *Apis mellifera* recorded across all insecticide-treated plots at 24-, 48-, and 72-hours post-spray application. The trend reflects temporal changes in honey bee activity and potential recovery or sustained avoidance following exposure.

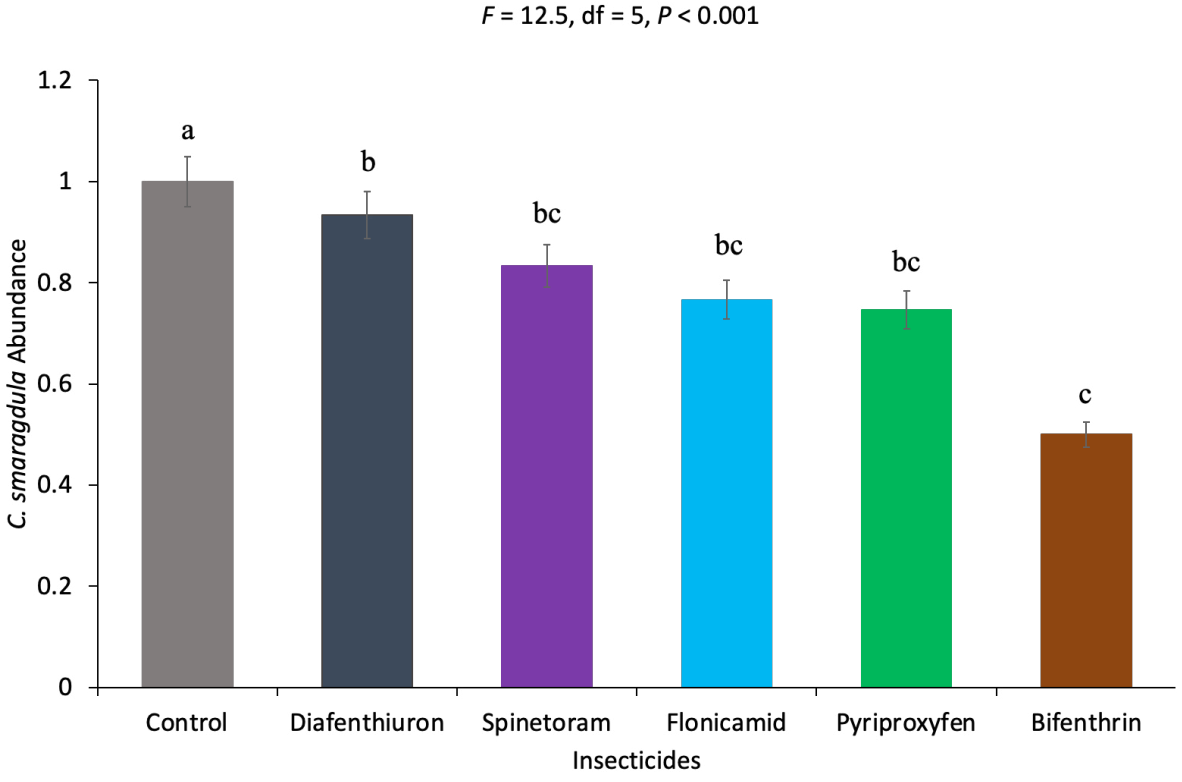

Fig 4. Average abundance (mean ± SE) of *Apis mellifera* observed on insecticide-treated plots at 24, 48, and 72 hours after spray application. The bar graph compares the impact of different insecticides on honey bee foraging activity over time, reflecting differences in repellent or toxic effects.

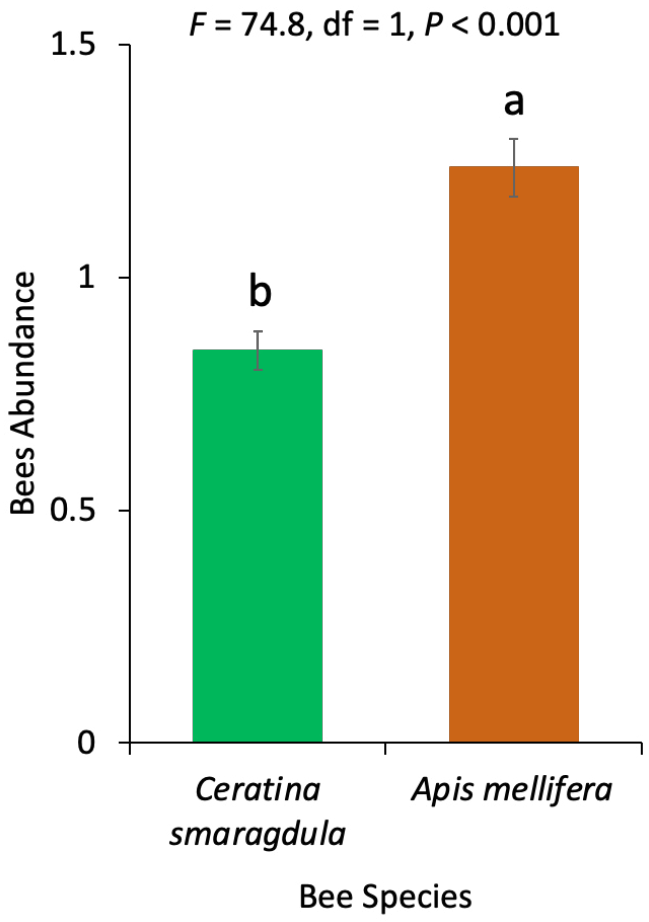

Fig 6. Average abundance (mean ± SE) of *Ceratina smaragdula* and *Apis mellifera* recorded across all insecticide-treated plots. The comparison illustrates overall differences in foraging activity between the solitary and social bee species under identical field conditions.

Discussion

The current study demonstrates the toxicity of insecticides with different modes of action in two bee species. Most of the reported research focuses on the managed bee, i.e., *A. mellifera*. However, there has been little research on solitary bees. The ecotoxicological assessment methods, including acute testing of insecticides, are limited for solitary bees, mainly due to their seasonal occurrence and the lack of standard rearing protocols (Leonard & Harmon-Threatt, 2019). On the other hand, standard toxicity protocols for honey bees and bumble bees have been well established and tested (Kueh Tai et al., 2022). Bees are among the few insect species whose pesticide-related mortality is not adequately addressed. Bees serve as indicators of environmental contamination due to their population decline, the presence of pesticide residues in their bodies, and enzyme inhibition in their tissues when exposed to toxic concentrations of pollutants (Hyne & Maher, 2003).

Emamectin benzoate is a relatively recent and extensively used pesticide that could create harmful health consequences through oxidative stress (El-Sheikh & Galal, 2015). In our study, *C. smaragdula* LC_{50} values for emamectin benzoate were 4.69 µg mL^{-1} and 1.14 µg mL^{-1} after 12 and 24 hours, respectively. Similarly, the LC_{50} values for emamectin benzoate in *A. mellifera* were 9.64 µg mL^{-1} and 1.14 µg mL^{-1} after 12 and 24 hours, respectively. A previous study has also reported high toxicity of emamectin benzoate against *A. mellifera* (Abdu-Allah & Pittendrigh, 2018). Emamectin benzoate induces oxidative stress, or an imbalance in antioxidant status, which causes the oxidation of lipids, proteins, and DNA in cells, resulting in tissue damage. Emamectin benzoate primarily reduces intracellular antioxidant levels, leading to lipid peroxidation, changes in membrane permeability, DNA damage, and various biochemical and histological abnormalities (Sies, 1997). This effect can be attributed to the fact that emamectin benzoate has effects on the bees' population. Additionally, increased exposure of adult bees to insecticides can lead to the development of resistance to those chemicals. Increased exposure of adult bees to insecticides can lead to resistance through natural selection and physiological changes. When bees are repeatedly exposed to low or long-term doses of insecticides, those with traits that help them survive, such as stronger detoxification systems or less sensitive target sites, are more likely to live and reproduce. Over time, these traits become more common in the population, making bees less sensitive to the insecticide (Bass et al., 2015).

Other insecticides identified as moderately or less toxic in the present study have nevertheless been reported to cause sublethal effects on honey bees and other solitary bees in previous investigations. Sublethal exposure to the pyrethroid bifenthrin reduced fecundity and delayed larval development in *Apis mellifera*, indicating negative impacts on growth and reproduction without causing immediate mortality (Dai et al., 2009). Pyriproxyfen, a juvenile hormone analog, has been shown to interfere with development, emergence, and reproductive processes in *A. mellifera* and other non-target insects, suggesting potential risks to solitary bees such as *Ceratina* spp. (Devillers & Devillers, 2020). Spinosyn insecticides, including spinetoram, have been reported to alter behavior, feeding activity, and physiological processes in bees at sublethal concentrations, potentially affecting foraging efficiency and colony performance (Biondi et al., 2012). Diamide insecticides such as chlorantraniliprole and flubendiamide are generally considered less acutely toxic to bees; however, chronic exposure has been associated with sublethal behavioral effects, including reduced locomotor activity and impaired responsiveness in *A. mellifera* (Williams et al., 2015). Although data on flonicamid and chlorfenapyr in bees are limited, studies on other insects indicate that low-dose exposure can affect metabolism and physiological performance, highlighting the need for further evaluation of their sublethal effects on honey bees and solitary bee species.

The distinct toxicological responses observed between *C. smaragdula* and *A. mellifera* might be attributable to key interspecific differences in physiology, behavior, and routes of exposure. Physiologically, honey bees have more developed detoxification systems, including cytochrome P450 enzymes, which enhance their ability to metabolize and

excrete toxic compounds (Claudianos et al., 2006). By contrast, solitary bees generally lack these enzyme families or possess fewer detoxification genes, making them more vulnerable to pesticide exposure – a gap highlighted in recent scoping reviews on solitary bee ecotoxicology (Lehmann & Camp, 2021). Behaviorally, *C. smaragdula* forages over shorter distances and nests in plant stems, which increases the likelihood of direct contact with pesticide residues on treated surfaces. In contrast, *Apis mellifera* workers collect food as a colony rather than individually, so foraging decisions and resources are coordinated through a centralized system. This colony-level organization means that individual bees may be less directly exposed to toxic substances, as the distribution of food and tasks spreads risk across the entire colony (Sponsler & Johnson, 2016). Additionally, exposure routes differ as solitary bees may face greater contact exposure through nesting substrates and unprocessed food provisions, whereas *A. mellifera* primarily experiences oral exposure through processed nectar and pollen stores. These differences show that honey bee-based toxicity thresholds may not accurately reflect risks to solitary bees, underlining the need for species-specific ecotoxicological assessments.

In the field, bifenthrin proved to be the most toxic among the insecticides tested. Due to the field application, a low abundance of both social and solitary bees was recorded. The LC_{50} values of pesticides indicated that bifenthrin was highly toxic to honeybees. The results of the present study can be attributed to pesticide exposure of bees in the field. The recommended concentration of bifenthrin for agricultural pest control is approximately 20-100 mg/L, a level that is highly toxic to bees in the field. In each plot, a low abundance of social and solitary bees was observed due to bifenthrin in the field. Few bees visited bifenthrin-treated plots due to its repellency. Bifenthrin belongs to the pyrethroid group, which is repellent in nature. Cyclopropane carboxylate and phenylacetate-ester, both pyrethroids, repel insects due to their mode of action (Delabie et al., 1985; Rieth & Levin, 1988). Pyrethroids are known to repel insects not only because of their toxic effects but also due to sublethal impacts on the insect's body. When insects come into contact with pyrethroids, their tarsi can be affected, reducing their ability to walk or cling to treated surfaces. Additionally, pyrethroids can affect the digestive system, which may reduce feeding or cause discomfort, further discouraging insects from staying on or consuming treated material (Mamood & Waller, 1990). Like *A. mellifera, Osmia lignaria* was five times more susceptible to bifenthrin (Peterson et al., 2021). Bifenthrin is known to reduce neuronal excitability in the honeybee brain by inhibiting sodium currents (Zhou et al., 2011). Pyrethroids are extremely harmful insecticides when they come into close contact with bees. In field conditions, honey bee foragers may encounter insecticides in ways that are unpredictable compared to controlled laboratory experiments. The repellent properties of pyrethroids and similar chemicals can cause foragers to avoid treated flowers or hive areas, reducing direct contact with toxic residues. This avoidance behavior under variable field conditions helps protect individual bees from mortality, allowing the colony to maintain foraging activity even when pesticides are present (Rieth & Levin, 1988). Pyrethroids function as both insecticides and repellents against target insect pests as well as non-target organisms. Although they are highly toxic to bees when applied directly (Smart & Stevenson, 1982), their toxicity under field conditions is generally lower and largely depends on the application rate used on the crop (Rieth & Levin, 1988). It has also been found that residues of these insecticides are present in honey bee hives (Mullin et al., 2010; Pettis et al., 2013), indicating that these insecticides may not completely repel foraging bees. Honeybees are highly susceptible to bifenthrin, and exposure can be fatal (Qualls et al., 2010). Furthermore, bifenthrin has been shown to have deleterious effects on bees, reducing fertility and growth and prolonging immature life stages (Dai et al., 2009).

Multiple studies have reported relative sensitivities of insecticides on managed bees and solitary bees. For example, a meta-analysis compared the comparative sensitivity of different bee species and reported that solitary and bumble bees are more sensitive to insecticide application than honey bees. Moreover, the safety levels defined for honey bees should be divided by 10 to be considered as safe for all other bee species (Arena & Sgolastra, 2014). A recent study revealed that in honey bees and bumble bees, a cytochrome P450 enzyme belonging to the CYP9Q subfamily is responsible for the detoxification of neonicotinoids (Manjon et al., 2018). However, solitary bees, such as *O. bicornis* and *M. rotundata*, lack these detoxifying enzymes (Beadle et al., 2019). In a previous study, imidacloprid was tested against ground nesting and social bees, revealing that its contact toxicity was equally sensitive to honey bee (*A. mellifera*) as well as ground nesting bee (*N. melanderi*) (Stark et al., 1995), while Tai et al. (2022) reported that the sensitivity of *L. paahaumaa* was 194 times greater than that of *A. mellifera*.

Pesticides have been shown to negatively affect the foraging performance of both social and solitary bees (Hesselbach et al., 2020). Pesticide exposure reduces pollination service, biodiversity, and crop yields. Insecticides also affect the survival rate of social and solitary bees (Boff et al., 2021). Emamectin benzoate is more toxic than other insecticides, and we should use less toxic insecticides. In the evening, spraying is better than in the morning for the conservation of natural pollinators and the bee population. Development of those insecticides is required that can affect the target insect pest but not other beneficial insects. IPM strategies for insect pest control should be used instead of direct insecticide application. We need to use biopesticides because they are safer for natural pollinators and highly effective against insect pests. Those insecticides should be used that are less toxic to the bee population and other natural pollinators. Public awareness and education about toxic insecticides are required to promote the habitat of natural pollinators.

Moreover, multiple factors affect the bees' ability to resist insecticide exposure in their surroundings. These include the environmental variability in different seasons around the hive, temperature fluctuation, age of bees, biological factors including disease spread in the colony, pests and parasites attack, and the food resources present in their habitat (Wahl & Ulm, 1983; Johansen & Mayer, 1990; Johnson et al., 2009; Vidau et al., 2011). These changes can affect their degree of intoxication and, consequently, how they behave after being exposed to pesticides. To understand the effects of pesticides on bees, future research should investigate how different biotic and abiotic pressures influence bee behavior across seasons.

Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' Contributions

F.H.: Methodology, resources, writing-original draft.
M.A.: Conceptualization, methodology, software, formal analysis, data curation, writing-review and editing, visualization, project administration, funding acquisition.
F.Z.A.K.: Investigation, visualization, funding acquisition, writing-review and editing.
A.I.J.: Investigation, validation, supervision, writing-review & editing.

Acknowledgements

This research was funded by PARB Project No. 20-276, Government of Punjab, Pakistan, available to M.A. The authors thank the farm management team at the MNS University of Agriculture, Multan, Pakistan. The authors are thankful to the three anonymous reviewers for their valuable contributions to improving the manuscript.

References

Abdu-Allah, G.A.M. & Pittendrigh, B.R. (2018). Lethal and sub-lethal effects of select macrocyclic lactones insecticides on forager worker honey bees under laboratory experimental conditions. Ecotoxicology, 27: 81-88. https://doi.org/10.1007/s10646-017-1872-6

Abdullah, S., Ali, M., Khan, F. Z. A., Sajjad, A., Qayyum, M. A. & Ahmad, N. (2024). Solitary Bees Are More Efficient Pollinators of Sponge Gourd than Giant honeybees and Syrphid Flies. Sociobiology, 71: e10279. https://doi.org/10.13102/sociobiology.v71i3.10279

Ali, M., Saeed, S., Sajjad, A. & Whittington, A. (2011). In search of the best pollinators for canola (*Brassica napus* L.) production in Pakistan. Applied Entomology and Zoology, 46: 353-361. https://doi.org/10.1007/s13355-011-0051-0

Ali, Q., Ali, M., Khan, F. Z. A., Noureldeen, A., Alghamdi, A., Darwish, H., Fatima, A., Jalali, A. I., Prendergast, K. & Saeed, S. (2024). Water Deprivation and Sowing Times Alter Plant-Pollination Interactions and Seed Yield in Sunflower, *Helianthus annuus* L. (Asteraceae). Plants, 13: 3194. https://doi.org/10.3390/plants13223194

Anees, M., Ali, M., Ghramh, H. A., Sajjad, A., Ali Khan, K., Saeed, S. & Razzaq, K. (2022). Impact of Bee and Fly Pollination on Physical and Biochemical Properties of Strawberry Fruit. Horticulturae, 8: 1072. https://doi.org/10.3390/horticulturae8111072

Arena, M. & Sgolastra, F. (2014). A meta-analysis comparing the sensitivity of bees to pesticides. Ecotoxicology, 23: 324-334. https://doi.org/10.1007/s10646-014-1190-1

Badawy, M. E. I., Nasr, H. M. & Rabea, E. I. (2015). Toxicity and biochemical changes in the honey bee *Apis mellifera* exposed to four insecticides under laboratory conditions. Apidologie, 46: 177-193. https://doi.org/10.1007/s13592-014-0315-0

Bass, C., Denholm, I., Williamson, M. S. & Nauen, R. (2015). The global status of insect resistance to neonicotinoid insecticides. Pesticide Biochemistry and Physiology, 121: 78-87. https://doi.org/10.1016/j.pestbp.2015.04.004

Beadle, K., Singh, K. S., Troczka, B. J., Randall, E., Zaworra, M., Zimmer, C. T., Hayward, A., Reid, R., Kor, L., Kohler, M., Buer, B., Nelson, D. R., Williamson, M. S., Davies, T. G. E., Field, L. M., Nauen, R. & Bass, C. (2019). Genomic insights into neonicotinoid sensitivity in the solitary bee *Osmia bicornis*. PLOS Genetics, 15: e1007903. https://doi.org/10.1371/journal.pgen.1007903

Biondi, A., Mommaerts, V., Smagghe, G., Viñuela, E., Zappalà, L. & Desneux, N. (2012). The non-target impact of spinosyns on beneficial arthropods. Pest Management Science, 68: 1523-1536. https://doi.org/10.1002/ps.3396

Boff, S., Scheiner, R., Raizer, J. & Lupi, D. (2021). Survival rate and changes in foraging performances of solitary bees exposed to a novel insecticide. Ecotoxicology and Environmental Safety, 211: 111869. https://doi.org/10.1016/j.ecoenv.2020.111869

Claudianos, C., Ranson, H., Johnson, R. M., Biswas, S., Schuler, M. A., Berenbaum, M. R., Feyereisen, R. & Oakeshott, J. G. (2006). A deficit of detoxification enzymes: Pesticide sensitivity and environmental response in the honeybee. Insect Molecular Biology, 15: 615-636. https://doi.org/10.1111/j.1365-2583.2006.00672.x

Dai, P.-L., Wang, Q., Sun, J.-H., Liu, F., Wang, X., Wu, Y.-Y. & Zhou, T. (2009). Effects of sublethal concentrations of bifenthrin and deltamethrin on fecundity, growth, and development of the honeybee *Apis mellifera ligustica*. Environmental Toxicology and Chemistry, 29: 644-649. https://doi.org/10.1002/etc.67

Delabie, J., Bos, C., Fonta, C. & Masson, C. (1985). Toxic and repellent effects of cypermethrin on the honeybee: Laboratory, glasshouse and field experiments. Pesticide Science, 16: 409-415. https://doi.org/10.1002/ps.2780160417

Devillers, J. & Devillers, H. (2020). Lethal and Sublethal Effects of Pyriproxyfen on *Apis* and Non-Apis Bees. Toxics, 8: 104. https://doi.org/10.3390/toxics8040104

Devkota, K., Dos Santos, C. F., Ferreira, A. B. & Timberlake, T. P. (2024). Assessing the economic and nutritional value of pollination services in Nepal. Scientific Reports, 14: 24037. https://doi.org/10.1038/s41598-024-75584-x

Egan, P.A., Dicks, L.V., Hokkanen, H M.T. & Stenberg, J.A. (2020). Delivering Integrated Pest and Pollinator Management (IPPM). Trends in Plant Science, 25: 577-589. https://doi.org/10.1016/j.tplants.2020.01.006

Ejaz, K., Ali, M., Khan, F. Z. A. & Mozūratis, R. (2025). Enhancing Alfalfa (*Medicago sativa*) Seed Yield: The Effect of Honey Bee (*Apis mellifera*) Supplementation and Efficiency of Other Pollinators. Biology, 14: 599. https://doi.org/10.3390/biology14060599

El-Sheikh, E.-S. A. & Galal, A. A. A. (2015). Toxic effects of sub-chronic exposure of male albino rats to emamectin benzoate and possible ameliorative role of *Foeniculum vulgare* essential oil. Environmental Toxicology and Pharmacology, 39: 1177-1188. https://doi.org/10.1016/j.etap.2015.04.008

Garibaldi, L. A., Steffan-Dewenter, I., Winfree, R., Aizen, M. A., Bommarco, R., Cunningham, S. A., Kremen, C., Carvalheiro, L. G., Harder, L. D., Afik, O., Bartomeus, I., Benjamin, F., Boreux, V., Cariveau, D., Chacoff, N. P., Dudenhöffer, J. H., Freitas, B. M., Ghazoul, J., Greenleaf, S., … Klein, A. M. (2013). Wild Pollinators Enhance Fruit Set of Crops Regardless of Honey Bee Abundance. Science, 339: 1608-1611. https://doi.org/10.1126/science.1230200

Heller, S., Joshi, N. K., Chen, J., Rajotte, E. G., Mullin, C. & Biddinger, D. J. (2020). Pollinator exposure to systemic insecticides and fungicides applied in the previous fall and pre-bloom period in apple orchards. Environmental Pollution, 265: 114589. https://doi.org/10.1016/j.envpol.2020.114589

Hesselbach, H., Seeger, J., Schilcher, F., Ankenbrand, M. & Scheiner, R. (2020). Chronic exposure to the pesticide flupyradifurone can lead to premature onset of foraging in honeybees *Apis mellifera*. Journal of Applied Ecology, 57: 609-618. https://doi.org/10.1111/1365-2664.13555

Hyne, R. V. & Maher, W. A. (2003). Invertebrate biomarkers: Links to toxicosis that predict population decline. Ecotoxicology and Environmental Safety, 54: 366-374. https://doi.org/10.1016/s0147-6513(02)00119-7

Johansen, C. A. & Mayer, D. F. (1990). Pollinator protection: A bee & pesticide handbook. Wicwas Press.

Johnson, R. M., Pollock, H. S. & Berenbaum, M. R. (2009). Synergistic Interactions Between In-Hive Miticides in *Apis mellifera*. Journal of Economic Entomology, 102: 474-479. https://doi.org/10.1603/029.102.0202

Klein, A.-M., Vaissière, B. E., Cane, J. H., Steffan-Dewenter, I., Cunningham, S. A., Kremen, C. & Tscharntke, T. (2007). Importance of pollinators in changing landscapes for world crops. Proceedings of the Royal Society B: Biological Sciences, 274: 303-313. https://doi.org/10.1098/rspb.2006.3721

Kueh Tai, F., Pattemore, D. E., Jochym, M., Beggs, J. R., Northcott, G. L. & Mortensen, A. N. (2022). Honey bee toxicological responses do not accurately predict environmental risk of imidacloprid to a solitary ground-nesting bee species. Science of The Total Environment, 839: 156398. https://doi.org/10.1016/j.scitotenv.2022.156398

Lehmann, D.M. & Camp, A.A. (2021). A systematic scoping review of the methodological approaches and effects of pesticide exposure on solitary bees. PLOS ONE, 16: e0251197. https://doi.org/10.1371/journal.pone.0251197

Leonard, R. J. & Harmon-Threatt, A. N. (2019). Methods for rearing ground-nesting bees under laboratory conditions. Apidologie, 50: 689-703. https://doi.org/10.1007/s13592-019-00679-8

Lundin, O., Rundlöf, M., Jonsson, M., Bommarco, R. & Williams, N.M. (2021). Integrated pest and pollinator management – expanding the concept. Frontiers in Ecology and the Environment, 19: 283-291. https://doi.org/10.1002/fee.2325

Mamood, A.N. & Waller, G.D. (1990). Recovery of learning responses by honeybees following a sublethal exposure to permethrin. Physiological Entomology, 15: 55-60. https://doi.org/10.1111/j.1365-3032.1990.tb00492.x

Manjon, C., Troczka, B. J., Zaworra, M., Beadle, K., Randall, E., Hertlein, G., Singh, K. S., Zimmer, C. T., Homem, R. A., Lueke, B., Reid, R., Kor, L., Kohler, M., Benting, J., Williamson, M. S., Davies, T. G. E., Field, L. M., Bass, C. & Nauen, R. (2018). Unravelling the Molecular Determinants of Bee Sensitivity to Neonicotinoid Insecticides. Current Biology, 28: 1137-1143.e5. https://doi.org/10.1016/j.cub.2018.02.045

Medrzycki, P., Sgolastra, F., Bortolotti, L., Bogo, G., Tosi, S., Padovani, E., Porrini, C. & Sabatini, A. G. (2010). Influence of brood rearing temperature on honey bee development and susceptibility to poisoning by pesticides. Journal of Apicultural Research, 49: 52-59. https://doi.org/10.3896/ibra.1.49.1.07

Merle, I., Hipólito, J. & Requier, F. (2022). Towards integrated pest and pollinator management in tropical crops. Current Opinion in Insect Science, 50: 100866.

https://doi.org/10.1016/j.cois.2021.12.006

Mode of Action | Insecticide Resistance Action Committee (IRAC). (2025, January 20). Insecticide Resistance Action Committee. https://irac-online.org/mode-of-action/

Nath, R., Singh, H. & Mukherjee, S. (2023). Insect pollinators decline: An emerging concern of Anthropocene epoch. Journal of Apicultural Research, 62: 23-38. https://doi.org/10.1080/00218839.2022.2088931

Nchang, E. C., Nkontcheu, D. B. K., Taboue, G. C. T., Bonwen, F. R. & Fokam, E. B. (2023). Pesticides drive patterns of insect visitors and pollination-related attributes of four crops in Buea, Southwest Cameroon. International Journal of Tropical Insect Science, 44: 117-128. https://doi.org/10.1007/s42690-023-01138-9

O'Reilly, A. D. & Stanley, D. A. (2023). Solitary bee behaviour and pollination service delivery is differentially impacted by neonicotinoid and pyrethroid insecticides. Science of The Total Environment, 894: 164399. https://doi.org/10.1016/j.scitotenv.2023.164399

Peterson, E. M., Green, F. B. & Smith, P. N. (2021). Toxic responses of blue orchard mason bees (*Osmia lignaria*) following contact exposure to neonicotinoids, macrocyclic lactones, and pyrethroids. Ecotoxicology and Environmental Safety, 208: 111681. https://doi.org/10.1016/j.ecoenv.2020.111681

Picard-Nizou, A. L., Pham-Delègue, M. H., Kerguelen, V., Douault, P., Marilleau, R., Olsen, L., Grison, R., Toppan, A. & Masson, C. (1995). Foraging behaviour of honey bees (*Apis mellifera* L.) on transgenic oilseed rape (*Brassica napus* L.var. Oleifera). Transgenic Research, 4: 270-276. https://doi.org/10.1007/bf01969121

Pontarp, M., Runemark, A., Friberg, M., Opedal, Ø. H., Persson, A. S., Wang, L. & Smith, H. G. (2024). Evolutionary plant-pollinator responses to anthropogenic land-use change: Impacts on ecosystem services. Biological Reviews, 99: 372-389. https://doi.org/10.1111/brv.13026

Proesmans, W., Felten, E., Laurent, E., Albrecht, M., Cyrille, N., Labonté, A., Maurer, C., Paxton, R., Schweiger, O., Szentgyörgyi, H. & Vanbergen, A. J. (2024). Urbanisation and agricultural intensification modulate plant-pollinator network structure and robustness. Functional Ecology, 38: 628-641. https://doi.org/10.1111/1365-2435.14503

Qualls, W. A., Xue, R.-D. & Zhong, H. (2010). Impact of Bifenthrin on Honeybees and *Culex quinquefasciatus*. Journal of the American Mosquito Control Association, 26: 223-225. https://doi.org/10.2987/09-5956.1

Rauf, A., Saeed, S., Ali, M. & Nadeem Tahir, M. H. (2021). Comparative Efficiency of Native Insect Pollinators in Reproductive Performance of *Medicago sativa* L. in Pakistan. Insects, 12: 1029. https://doi.org/10.3390/insects12111029

Rieth, J. P. & Levin, M. D. (1988). The repellent effect of two pyrethroid insecticides on the honey bee. Physiological Entomology, 13: 213-218. https://doi.org/10.1111/j.1365-3032.1988.tb00925.x

Rondeau, S. & Raine, N. E. (2024). Single and combined exposure to 'bee safe' pesticides alter behaviour and offspring production in a ground-nesting solitary bee (*Xenoglossa pruinosa*). Proceedings of the Royal Society B: Biological Sciences, 291: 20232939. https://doi.org/10.1098/rspb.2023.2939

Sanchez-Bayo, F. & Goka, K. (2014). Pesticide Residues and Bees – A Risk Assessment. PLoS ONE, 9: e94482. https://doi.org/10.1371/journal.pone.0094482

Sgolastra, F., Hinarejos, S., Pitts-Singer, T. L., Boyle, N. K., Joseph, T., Lūckmann, J., Raine, N. E., Singh, R., Williams, N. M. & Bosch, J. (2019). Pesticide Exposure Assessment Paradigm for Solitary Bees. Environmental Entomology, 48: 22-35. https://doi.org/10.1093/ee/nvy105

Shi, X., Ma, C., De Kraker, J., Gong, S., Hodgson, J. A., Luo, S., Van Der Steen, J. J. M., Xiao, H., Wang, F., Tie, X., Chen, Z. & Zou, Y. (2024). Influence of agricultural intensification on pollinator pesticide exposure, food acquisition and diversity. Journal of Applied Ecology, 61: 1905-1917. https://doi.org/10.1111/1365-2664.14701

Sies, H. (1997). Oxidative stress: Oxidants and antioxidants. Experimental Physiology, 82: 291-295. https://doi.org/10.1113/expphysiol.1997.sp004024

Smart, L.E. & Stevenson, J.H. (1982). Laboratory Estimation of Toxicity of Pyrethroid Insecticides to Honeybees: Relevance to Hazard in the Field. Bee World, 63: 150-152. https://doi.org/10.1080/0005772x.1982.11097888

Sparks, T. C. (2025). Insecticide mixtures – Uses, benefits and considerations. Pest Management Science, 81: 1137-1144. https://doi.org/10.1002/ps.7980

Sponsler, D. B. & Johnson, R. M. (2016). Mechanistic modeling of pesticide exposure: The missing keystone of honey bee toxicology. Environmental Toxicology and Chemistry, 36: 871-881. https://doi.org/10.1002/etc.3661

Stanley, J., Chandrasekaran, S., Preetha, G., & Kuttalam, S. (2010). Toxicity of diafenthiuron to honey bees in laboratory, semi-field and field conditions: Toxicity of diafenthiuron to bees under different conditions. Pest Management Science, 66: 505–510. https://doi.org/10.1002/ps.1900

Stark, J. D., Jepson, P. C. & Mayer, D. F. (1995). Limitations to Use of Topical Toxicity Data for Predictions of Pesticide Side Effects in the Field. Journal of Economic Entomology, *88*: 1081-1088. https://doi.org/10.1093/jee/88.5.1081

Tong, Z.-Y., Wu, L.-Y., Feng, H.-H., Zhang, M., Armbruster, W. S., Renner, S. S. & Huang, S.-Q. (2023). New calculations indicate that 90% of flowering plant species are animal-pollinated. National Science Review, 10: nwad219. https://doi.org/10.1093/nsr/nwad219

Uwingabire, Z. & Gallai, N. (2024). Impacts of degraded pollination ecosystem services on global food security and

nutrition. Ecological Economics, 217: 108068. https://doi.org/10.1016/j.ecolecon.2023.108068

Vidau, C., Diogon, M., Aufauvre, J., Fontbonne, R., Viguès, B., Brunet, J.-L., Texier, C., Biron, D. G., Blot, N., El Alaoui, H., Belzunces, L. P. & Delbac, F. (2011). Exposure to Sublethal Doses of Fipronil and Thiacloprid Highly Increases Mortality of Honeybees Previously Infected by Nosema ceranae. PLoS ONE, 6: e21550. https://doi.org/10.1371/journal.pone.0021550

Wagan, S. A., Memon, Q. U. A., Tan, Y., Damalas, C. A., Memon, A., Sheikh, M. J. & Khushk, G. M. (2025). Farmers' knowledge of pests and decision for pesticide selection in cotton: Impact on production efficiency levels in southern Pakistan. International Journal of Pest Management, 71: 343-352. https://doi.org/10.1080/09670874.2023.2209041

Wahl, O. & Ulm, K. (1983). Influence of pollen feeding and physiological condition on pesticide sensitivity of the honey bee *Apis mellifera carnica.* Oecologia, 59: 106-128. https://doi.org/10.1007/bf00388082

Williams, G.R., Alaux, C., Costa, C., Csáki, T., Doublet, V., Eisenhardt, D., Fries, I., Kuhn, R., McMahon, D.P., Medrzycki, P., Murray, T.E., Natsopoulou, M. E., Neumann, P., Oliver, R., Paxton, R.J., Pernal, S. F., Shutler, D., Tanner, G., Van Der Steen, J.J.M. & Brodschneider, R. (2013). Standard methods for maintaining adult *Apis mellifera* in cages under *in vitro* laboratory conditions. Journal of Apicultural Research, 52: 1-36. https://doi.org/10.3896/IBRA.1.52.1.04

Williams, G. R., Troxler, A., Retschnig, G., Roth, K., Yañez, O., Shutler, D., Neumann, P. & Gauthier, L. (2015). Neonicotinoid pesticides severely affect honey bee queens. Scientific Reports, 5: 14621. https://doi.org/10.1038/srep14621

Winston, M. L. (1991). The biology of the honey bee (1. Harvard Univ. Press paperback ed). Harvard University Press.

Zhang, Z., Huang, J., Yao, Y., Peters, G., Macdonald, B., La Rosa, A. D., Wang, Z. & Scherer, L. (2023). Environmental impacts of cotton and opportunities for improvement. Nature Reviews Earth & Environment, 4: 703-715. https://doi.org/10.1038/s43017-023-00476-z

Zhou, T., Zhou, W., Wang, Q., Dai, P.-L., Liu, F., Zhang, Y.-L. & Sun, J.-H. (2011). Effects of pyrethroids on neuronal excitability of adult honeybees *Apis mellifera.* Pesticide Biochemistry and Physiology, 100: 35-40. https://doi.org/10.1016/j.pestbp.2011.02.001