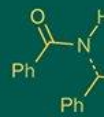


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Comparative contact toxicity of selected insecticides to European honeybee (*Apis mellifera* L.) under laboratory conditions

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Abstract

Aims: To assess the contact toxicity of selected insecticides to the European honey bee, *Apis mellifera*, under laboratory conditions and to compare their toxicity at recommended and sub-lethal doses.

Study Design: Laboratory evaluation of the contact toxicity of selected insecticides at recommended and sub-lethal doses using forage worker bees.

Place and Duration of Study: The laboratory experiment was conducted in the Department of Entomology, Agricultural College, Polasa, Jagtial, Telangana, India, during the *rabi* season of 2025-26. Healthy adult worker bees were obtained from disease-free *A. mellifera* colonies maintained under standard apicultural practices.

Methodology: Healthy adult worker bees were exposed to insecticide-treated glass jars using the dry-film method. Six insecticides from different chemical groups were tested: acephate 75 SP, fipronil 5 SC, spinosad 45 SC, imidacloprid 70 WS, chlorantraniliprole 18.5 SC, and *Bacillus thuringiensis* var. *kurstaki*. The insecticides were evaluated at recommended and sub-lethal dose (half of the recommended dose), and bee mortality was recorded at 2, 4, 6, 12, 24 and 48 h after exposure.

Results: Imidacloprid showed the highest initial contact toxicity, causing rapid mortality during the early exposure period. Acephate and fipronil also showed high toxicity, leading to complete mortality within 12-24 h at the recommended dose. Spinosad resulted in intermediate but delayed toxicity, reaching complete mortality after extended exposure. In contrast, chlorantraniliprole caused relatively lower mortality throughout the observation period, while *B. thuringiensis* var. *kurstaki* consistently showed the lowest mortality at both recommended and sub-lethal doses, indicating comparatively lower toxicity to honey bees.

Conclusion: The findings revealed significant differences in contact toxicity among the insecticides and emphasized the need to consider pollinator safety when selecting insecticides. The study supports the use of toxicity information for integrated pest and pollinator management while reducing negative impacts on *A. mellifera*.

Keywords: *Apis mellifera*, insecticides, mortality, sublethal, toxicity

1. Introduction

Honey bees are the key pollinators of oilseed and a few vegetable crops. Field studies from India have shown that managed bee pollination measurably improves seed set and seed yield in these crops (Free, 1970; Bhowmik *et al.*, 2014) [6, 5]. Pesticide sprays applied during the flowering period bring foraging bees into direct contact with residues on treated plant surfaces, and reconciling effective pest control with pollinator protection remains a central challenge in integrated pest management.

Pollinator populations have been declining worldwide because of multiple factors, including habitat loss, industrialization, deforestation, climate change, and pests and diseases, with the intensive and indiscriminate use of pesticides considered one of the major contributors to honeybee decline (Becher *et al.*, 2013) [4]. The severity of contact toxicity depends on several factors, including the physicochemical properties of the insecticide, formulation type, application rate, persistence on plant surfaces, cuticular penetration, metabolic detoxification capacity and the intrinsic susceptibility of the bee. Differences in cuticular absorption and metabolic rate partly explain why insecticides belonging to different chemical groups exhibit marked variation in acute contact toxicity to *A. mellifera* (Zaworra *et al.*, 2019) [17].

Conventional insecticides such as organophosphates and phenylpyrazoles have long been recognised for their high acute toxicity to honey bees, whereas the introduction of newer insecticides, including diamides and microbial biopesticides, has generated interest because of their comparatively selective activity against target pests (Johnson, 2015) ^[9]. Nevertheless, increasing evidence suggests that even several recently introduced insecticides considered relatively safe may cause significant contact toxicity depending on application dose, formulation and exposure duration (Tosi *et al.*, 2022) ^[16]. Therefore, assumptions regarding pollinator safety based solely on chemical class or mammalian toxicity may be misleading, highlighting the importance of empirical toxicity assessment.

Over the past decade, research on honey bee toxicology has expanded considerably. But comparatively fewer investigations have provided direct comparisons of the acute contact toxicity of insecticides that are currently recommended for crop protection under standardised laboratory conditions. Moreover, the continuous introduction of novel insecticide molecules and changing pesticide use patterns require periodic re-evaluation of their toxicity to non-target pollinators, since historical toxicity data may not adequately represent currently registered products or formulations (Siviter *et al.*, 2021) ^[15].

In India, information comparing the contact toxicity of commonly recommended insecticides representing different modes of action is still limited, particularly for pollinator-dependent crops such as mustard, sunflower, sesame, etc., where insecticide applications often coincide with the flowering period. Such comparative data are essential for identifying insecticides that are compatible with pollinator conservation and for developing IPM strategies that balance effective pest management with pollinator safety. In view of these considerations, the present investigation was

undertaken to assess the contact toxicity of selected insecticides to *A. mellifera* under laboratory conditions.

2. Materials and Methods

2.1. Experimental site

The laboratory experiment was conducted during the *rabi* season of 2025-26 in the Department of Entomology, Agricultural College, Polasa, Jagtial, Telangana, India, to evaluate the contact toxicity of selected insecticides against the European honey bee, *Apis mellifera*

2.2 Honey bee colonies

Healthy adult worker bees of *A. mellifera* were obtained from four disease-free nucleus colonies maintained under standard apicultural practices. The colonies were procured from beekeepers of Vikarabad district, Telangana. Throughout the experimental period, the colonies were regularly inspected and maintained under hygienic conditions. During periods of nectar scarcity, colonies were supplemented with 1:1 (w/v) sugar syrup to maintain colony strength. Only healthy worker bees collected from active foraging colonies were used for the bioassays.

2.3 Collection of worker bees

Adult worker bees were collected from the hive entrance using a cone-type muslin hand net (30 cm diameter). The collected bees were transferred into ventilated containers and immobilized by chilling in a refrigerator for approximately 5 min following the procedure described by Sharma and Abrol (2005) ^[14]. The bees were allowed to recover completely before initiation of the bioassays.

2.4 Insecticides evaluated

Six insecticides representing different chemical groups were evaluated at their recommended field doses (Table 1). Water served as the untreated control.

Table 1: Insecticides evaluated for contact toxicity against *Apis mellifera*

Treatment	Insecticide	Recommended dose
T ₁	Acephate 75 SP	1.5 g L ⁻¹
T ₂	Fipronil 5 SC	2.0 mL L ⁻¹
T ₃	Spinosad 45 SC	0.3 mL L ⁻¹
T ₄	Imidacloprid 70 WS	0.3 g L ⁻¹
T ₅	Chlorantraniliprole 18.5 SC	0.3 mL L ⁻¹
T ₆	<i>Bacillus thuringiensis</i> var. <i>kurstaki</i> WP	2.0 g L ⁻¹
T ₇	Control	—

2.5 Contact toxicity bioassay

The contact toxicity of the selected insecticides was evaluated using the dry-film method described by Reddy and Reddy (2006). The insecticides were prepared at their respective recommended field concentrations using distilled water. One millilitre of each insecticide solution was dispensed into a clean glass rearing jar (10 × 7 cm). The jars were gently rotated to ensure uniform coating of the inner surface and allowed to air dry at room temperature until a thin, uniform insecticide film was formed.

After complete drying, a cotton pad soaked in 20% sucrose solution was placed inside each jar as a food source. Ten adult worker bees were introduced into each treated jar,

which was then covered with muslin cloth to provide adequate ventilation. Water-treated jars served as the untreated control. Each treatment consisted of three independent replicates arranged in a Completely Randomised Design (CRD).

2.6 Mortality assessment

Bee mortality was recorded at 2, 4, 6, 12, 24 and 48 h after exposure. A bee was considered dead when it showed no movement or response after gentle stimulation with a soft brush for approximately thirty seconds. Mortality observed in the untreated control was used to correct treatment mortality using Abbott's formula (Abbott, 1925) ^[12]:

$$\text{Percent corrected mortality} = \frac{\text{Percent mortality in treatment} - \text{percent mortality in control}}{100 - \text{percent mortality in control}} \times 100$$

2.7 Statistical analysis

The experiment was conducted in a Completely Randomized Design with three replications. Percentage mortality was corrected using Abbott's formula and subjected to angular transformation. Analysis of variance (ANOVA) was performed to determine the significance of treatment effects, and treatment means were separated using the least significant difference (LSD) test at the 5% probability level. Original (untransformed) means are

presented in the tables, whereas transformed values were used for statistical comparisons.

3. Results and Discussion

3.1. Contact toxicity of selected insecticides to *Apis mellifera* L. at the recommended dose

Mortality of *A. mellifera* workers exposed through contact toxicity to the test insecticides at the recommended dose is presented in Table 2.

Table 2: Contact toxicity of selected insecticides to *Apis mellifera* at the recommended dose

Treatments	Dose (ml or g/l)	Mean corrected mortality (%)					
		2 h	4 h	6 h	12 h	24 h	48 h
T ₁	Acephate 75 SP	33.23 (35.20) ^b	36.57 (37.21) ^b	76.78 (61.20) ^a	100.00 (90.00) ^a	100.00 (90.00) ^a	100.00 (90.00) ^a
T ₂	Fipronil 5 SC	31.62 (34.22) ^b	31.62 (34.22) ^b	76.78 (61.20) ^a	100.00 (90.00) ^a	100.00 (90.00) ^a	100.00 (90.00) ^a
T ₃	Spinosad 45 SC	18.26 (25.30) ^c	18.26 (25.30) ^c	56.66 (48.83) ^c	70.30 (56.98) ^b	100.00 (90.00) ^a	100.00 (90.00) ^a
T ₄	Imidacloprid 70 WS	61.65 (51.73) ^a	66.65 (54.73) ^a	66.71 (54.76) ^b	100.00 (90.00) ^a	100.00 (90.00) ^a	100.00 (90.00) ^a
T ₅	Chlorantraniliprole 18.5 SC	9.99 (18.43) ^d	16.64 (23.85) ^c	18.64 (25.58) ^d	24.98 (29.99) ^c	40.70 (39.64) ^b	51.45 (45.83) ^b
T ₆	<i>Bacillus thuringiensis</i> var. <i>kurstaki</i>	0.00 (0.00) ^e	0.00 (0.00) ^d	13.00 (21.14) ^d	16.34 (23.85) ^d	24.03 (29.36) ^c	27.01 (31.32) ^c
T ₇	Control	0.00 (0.00) ^e	0.00 (0.00) ^d	0.00 (0.00) ^e	0.00 (0.00) ^e	9.99 (18.43) ^d	9.99 (18.43) ^d
	S.Em±	1.04	1.47	1.89	1.72	0.33	0.28
	C.D (0.05)	3.20	4.49	5.80	5.27	1.02	0.85

Figures in parentheses are angular transformed values. Means followed by the same alphabet do not differ significantly by DMRT (P = 0.05%)

After 2 hours of exposure, imidacloprid recorded the highest mortality (61.65%), followed by acephate (33.23%) and fipronil (31.62%), which were statistically on par with each other. Spinosad recorded 18.26% mortality, and chlorantraniliprole caused the lowest mortality (9.99%) among the active treatments. No mortality was recorded in *Bt* var. *kurstaki* (0.00%). The order of toxicity was imidacloprid > acephate > fipronil > spinosad > chlorantraniliprole > *Bt* var. *kurstaki* = control. After 4 hours, imidacloprid mortality increased to 66.65%, followed by acephate (36.57%), which was significantly higher than fipronil (31.62%). Both differed significantly from spinosad (18.26%) and chlorantraniliprole (16.64%), which were on par with each other. *Bt* recorded no mortality. The toxicity order remained similar to that observed at 2 h. At 6 h, acephate (76.78%) and fipronil (76.78%) recorded the highest mortality and were on par with each other. Imidacloprid (66.71%) was significantly lower than these two, followed by spinosad (56.66%); chlorantraniliprole recorded 18.64% mortality. *Bt* recorded lower mortality (13.00%), on par with chlorantraniliprole. The order of toxicity was acephate = fipronil > imidacloprid > spinosad > chlorantraniliprole > *Bt*. After 12 h, 100 percent mortality was observed with acephate, fipronil and imidacloprid. Spinosad and chlorantraniliprole recorded 70.30% and 24.98% mortality, respectively, while *Bt* recorded the lowest mortality (16.34%). The order of toxicity was acephate = fipronil = imidacloprid > spinosad > chlorantraniliprole > *Bt*. After 24 h, acephate, fipronil, spinosad and imidacloprid recorded 100.00 percent mortality. Chlorantraniliprole recorded 40.70% mortality, and *Bacillus thuringiensis* (24.03%) was significantly lower than all other treatments. After 48 h, acephate, fipronil, spinosad and imidacloprid

recorded 100.00% mortality and were on par. Chlorantraniliprole caused 51.45% mortality and *Bacillus thuringiensis* showed 27.01% mortality. The control (9.99%) remained significantly lower than all treatments. Overall, imidacloprid exhibited rapid and significantly higher toxicity at early intervals (2-4 h), followed by acephate and fipronil, which reached 100 percent mortality by 12 h. Spinosad showed intermediate, delayed toxicity, eventually reaching 100% mortality at 24 h. Chlorantraniliprole was moderately toxic, while *Bt* was comparatively safer to honeybees, showing negligible effects up to 6-12 h and only moderate mortality after 24-48 h.

These results are in agreement with the findings of Tosi *et al.* (2022) ^[16] and Hristov *et al.* (2020) ^[8], who reported that imidacloprid exhibits rapid and high toxicity, causing significant mortality even at early exposure intervals. Ratnakar *et al.* (2009) ^[12] similarly concluded that acephate shows strong toxicity that increases with exposure time. Abbassy *et al.* (2020) ^[1] reported that chlorantraniliprole exhibits comparatively lower toxicity to honey bees than neonicotinoids under contact exposure. The lowest toxicity was observed here with *Bacillus thuringiensis* var. *kurstaki*, which has negligible mortality during early exposure and relatively low mortality at later intervals. This is also consistent with the findings of Glare and O'Callaghan (2000) ^[7].

3.2. Contact toxicity of selected insecticides to *Apis mellifera* L. at sub-lethal dose

Mortality of *A. mellifera* workers exposed through contact toxicity to the test insecticides at sub-lethal (half-recommended) dose are presented in Table 3.

Table 3: Contact toxicity of selected insecticides to *Apis mellifera* at the sub lethal dose

Treatments		Dose (ml or g/l)	Mean corrected mortality (%)					
			2 h	4 h	6 h	12 h	24 h	48 h
T ₁	Acephate 75 SP	0.75 g/l	26.64 (31.07) ^c	29.98 (33.20) ^b	69.98 (56.77) ^a	93.36 (75.07) ^a	93.68 (75.45) ^a	94.42 (76.33) ^a
T ₂	Fipronil 5SC	1.0 ml/l	28.31 (32.15) ^b	28.31 (32.15) ^c	69.98 (56.77) ^a	93.36 (75.07) ^a	93.68 (75.45) ^a	94.42 (76.33) ^a
T ₃	Spinosad 45SC	0.15 ml/l	13.32 (21.41) ^d	14.98 (22.77) ^d	44.97 (42.11) ^c	63.30 (52.71) ^b	88.92 (70.56) ^b	88.92 (70.56) ^b
T ₄	Imidacloprid 70 WS	0.15 ml/l	56.64 (48.81) ^a	57.30 (49.20) ^a	59.96 (50.75) ^b	93.36 (75.07) ^a	93.68 (75.45) ^a	94.80 (76.82) ^a
T ₅	Chlorantraniliprole 18.5 SC	0.15 ml/l	7.32 (15.70) ^e	13.31 (21.40) ^e	14.65 (22.51) ^d	21.64 (34.23) ^c	32.19 (34.57) ^c	47.75 (43.71) ^c
T ₆	<i>Bacillus thuringiensis</i> var. <i>kurstaki</i>	1.0 g/l	0.00 (0.00) ^f	0.00 (0.00) ^f	11.65 (19.96) ^e	14.30 (22.22) ^d	20.35 (26.82) ^d	22.20 (28.11) ^d
T ₇	Control		0.00 (0.00) ^f	0.00 (0.00) ^f	0.00 (0.00) ^f	0.00 (0.00) ^e	9.99 (18.43) ^e	9.99 (18.43) ^e
	S.Em±		0.35	0.33	0.36	0.79	0.56	0.53
	C.D (0.05)		1.06	1.00	1.11	2.43	1.71	1.62

Figures in parentheses are angular transformed values. Means followed by the same alphabet do not differ significantly by DMRT (P = 0.05%)

After 2 hours of exposure, imidacloprid recorded the highest mortality (56.64%), followed by fipronil (28.31%) and acephate (26.64%). Spinosad recorded 13.32% mortality, and chlorantraniliprole caused the lowest mortality (7.32%). No mortality was recorded in *Bt* var. *kurstaki*. The order of toxicity was imidacloprid > fipronil > acephate > spinosad > chlorantraniliprole > *Bt* var. *kurstaki*. After 4 hours, imidacloprid mortality increased to 57.30%, followed by acephate (29.98%), which was significantly higher than fipronil (28.31%). Both differed significantly from spinosad (14.98%) and chlorantraniliprole (13.31%). *Bt* recorded no mortality. At 6 h, acephate (69.98%) and fipronil (69.98%) recorded the highest mortality and were on par with each other. Imidacloprid (59.96%) was significantly lower, followed by spinosad (44.97%); chlorantraniliprole recorded 14.65% mortality. *Bt* recorded lower mortality (11.65%), on par with chlorantraniliprole. After 12 h, acephate, fipronil and imidacloprid recorded 93.36 percent mortality. Spinosad and chlorantraniliprole recorded 63.30% and 21.64% mortality, respectively, while *Bt* recorded the lowest mortality (14.30%). After 24 h, acephate, fipronil and imidacloprid recorded 93.68% mortality. Spinosad exhibited 88.92% mortality, chlorantraniliprole recorded 32.19%, and *Bacillus thuringiensis* (20.35%) remained significantly lower than all other treatments. After 48 h, imidacloprid recorded the highest mortality (94.80%), followed by acephate and fipronil (94.42%, on par with each other). Spinosad caused 88.92% mortality, chlorantraniliprole caused 47.75%, and *Bacillus thuringiensis* showed 22.20% mortality. The control (9.99%) remained significantly lower than all treatments.

Overall, even at sub-lethal (half-recommended) doses, imidacloprid exhibited rapid and significantly higher toxicity at early intervals (2-4 h), followed by acephate and fipronil. Spinosad showed intermediate, delayed toxicity, eventually reaching higher mortality by 24 h. Chlorantraniliprole remained moderately toxic, while *Bt* continued to be relatively safer to honeybees, showing negligible effects up to 6-12 h and the least mortality even after 24-48 h.

These results are similar to the findings of Ahsan *et al.* (2025) [3], who reported that sub-lethal exposure to neonicotinoids, even at reduced concentrations, adversely

affects honey bee survival. Li *et al.* (2024) [10] concluded that although chlorantraniliprole is considered relatively safe compared with many conventional insecticides, prolonged exposure at field-relevant concentrations can still reduce honey bee survival. Libardoni *et al.* (2021) [11] reported that *B. thuringiensis* formulations did not significantly affect the survival and behaviour of adult honey bees, a finding consistent with the present study.

4. Conclusion

Among the insecticides evaluated, imidacloprid, acephate and fipronil proved highly toxic to *A. mellifera* workers under contact exposure, reaching complete mortality within 12-24 hours even at sub-lethal doses. Chlorantraniliprole showed comparatively moderate toxicity and *Bacillus thuringiensis* var. *kurstaki* was reported to have the lowest contact toxicity among the insecticide treatments evaluated. These findings indicate that conventional neonicotinoid and organophosphate/phenylpyrazole insecticides pose a higher acute contact risk to foraging honeybees than the tested diamide and microbial alternatives. Consequently, chlorantraniliprole and *Bt*-based formulations may be preferred over imidacloprid, acephate and fipronil when insecticidal intervention coincides with the flowering period of certain crops to minimise pollinator mortality without compromising pest management. These results provide a basis for incorporating bee-safety considerations into integrated pest-cum-pollinator management recommendations for the cropping system.

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6. Competing Interests

Authors have declared that no competing interests exist.

7. Author's Contributions

K. Narendra conducted the study, performed the statistical analysis and prepared the first draft of the manuscript. V. Ratnakar designed the study, supervised the study and contributed to the interpretation of the results. V. Sunitha also designed the study, interpretation of results. Bharati Narayan Bhat contributed to supervision and critical revision of the manuscript. All authors read and approved the final manuscript.

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