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Comparative Assessment of Oral Insecticide Toxicity in the Indian Honey Bee and a Stingless Bee

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Abstract

Pollinators, particularly honeybees, are essential for agricultural productivity and ecological balance. However, the indiscriminate use of insecticides poses a significant threat to these vital species. The present study evaluated the oral toxicity of commonly used insecticides, including neonicotinoids, pyrethroids, and organophosphates, on two important pollinators, *Apis cerana indica* and *Tetragonula iridipennis*. Test insects were orally exposed to varying concentrations of thiamethoxam, carbosulfan, lambda-cyhalothrin, imidacloprid, profenofos, and chlorpyrifos. Mortality, behavioral changes, and physiological effects were recorded 24 hours after treatment (HAT). All tested insecticides exhibited extreme toxicity, resulting in 80 – 100% mortality in both bee species within 24 HAT. In contrast, buprofezin, an insect growth regulator, exhibited significantly lower toxicity, causing 25.28% mortality in *A. cerana indica* and 21.48% mortality in *T. iridipennis* at 24 HAT. These findings show the importance of considering species-specific sensitivity when formulating pesticide regulations and promoting bee-safe agricultural practices.

Introduction

Pollination in plants depends heavily on insects, particularly bees from the superfamily *Apoidea*, which comprise over 20,000 species. Honey bees, responsible for more than 90% of global pollination across 85% of cross-pollinated plant species (Ostiguy et al., 2019), are essential for agricultural productivity and ecosystem stability. Beyond honey production, they play a pivotal role in pollinating horticultural and agricultural crops, significantly influencing global economies (Forsgren, 2009). Recent research further emphasized that pollination services are not limited to honey bees, as stingless bees and other non-Apis bees also contribute substantially to crop pollination, particularly in tropical and subtropical agro-ecosystems (Raine & Rundlof, 2024).

Insecticides, designed to eliminate pests, inadvertently threaten pollinators. Systemic insecticides contaminate pollen and nectar, which foraging worker bees collect and transport to colonies, exposing developing broods to toxic residues (Wu et al., 2011). Contemporary studies demonstrate that such exposure results in chronic, colony-wide contamination, as pesticide residues accumulate in stored food, wax, and brood food, leading to prolonged exposure rather than isolated individual effects. Exposure to insecticides, even at environmentally relevant sub-lethal concentrations, can impair respiration, induce behavioral alteration, and disrupt learning ability in honey bees by affecting antioxidant defenses, immune responses, and gene expression, ultimately reducing their resilience to additional environmental stressors (Karise & Mand, 2015; Moreira et al., 2025). Environmental contamination from pesticides further endangers bee colonies by contaminating pollen, wax, and brood food (Chauzat et al., 2006).

At the molecular level, transcriptomic studies have shown that insecticides such as fipronil can alter genes associated with circadian rhythms and hormonal regulation, which are critical for normal foraging behaviour, development, and colony organization in honey bees (Astolfi et al., 2025).

Cashew production in India faces intense pest pressure, with over 180 insect pests reported. Consequently, 81.4% of cashew farmers rely on chemical pesticides as the primary crop protection method. This high pesticide usage increases the likelihood of pollinator exposure and associated risks. Sub-lethal exposure to insecticides disrupts honey bee behaviors, including communication dances, return flights, orientation, and foraging efficiency during floral visits (Van Dame et al., 1995). Most recent evidence confirms that such behavioral impairments extend beyond honey bees, affecting flight capacity and foraging in other pollinators, including stingless bees, thereby directly influencing pollination efficiency (Costa et al., 2024; Raine & Rundlof, 2024). Direct mortality, repellent effects, and residual toxicity on floral parts further exacerbate declines in bee populations (Desneux et al., 2007). Given the critical role of honey bees and their heightened sensitivity to insecticides, the present study provides a comparative assessment of oral insecticide toxicity in the Indian honey bee and the stingless bee, aiming to generate pollinator-specific toxicity data to support the development of safer and more sustainable pest management strategies.

Materials and Methods

The experiment was carried out under laboratory conditions at the Department of Agricultural College and Research Institute, Madurai, Tamil Nadu, during 2024, and the design adopted was a Completely Randomized Design. There were eight treatments and three replications. The insecticides used for the toxicity studies were as follows: T_1- Thiamethoxam 25% WG @ 0.6g/l, T_2- Carbosulfan 25% EC @ 1ml/l, T_3 - Buprofezin 25% SC @ 1ml/l, T_4- Lambda-cyhalothrin 5% EC @ 0.6 ml/l, T_5- Imidacloprid 17.8% SL @ 0.6ml/l, T_6 - Chlorpyrifos 20% EC @1.5ml/l, and T_7 - Profenofos 50% EC @1.5ml/l. They were compared with the untreated control, 50% sucrose. These insecticides were selected to reflect common cashew pest management practices in South India, to encompass diverse modes of action, and to account for known variation in pollinator toxicity.

Evaluation of the oral toxicity of insecticides to Indian bees and stingless bees was conducted using the methodology of Abdel Razik (2019) with slight modifications. The worker bees required for the study were obtained from cultures maintained at the Apiary unit of the Insectary, Department of Agricultural Entomology, Agricultural College and Research Institute (TNAU), Madurai. Field recommended doses of different insecticides and their corresponding $1/10^{th}$ field doses were prepared using a 50% sucrose solution for oral toxicity evaluation. The control treatment includes a 50% sucrose solution without insecticides. Plastic containers of 250 ml capacity were used for this experiment. 2 ml of different insecticides was applied to folded cotton wool bits measuring 3.5 cm × 3.5 cm, which were tied with a thread and hung inside the plastic containers. Indian bees and stingless bees were immobilized by refrigerating them for 5 minutes, then released into the plastic container at 10/container and covered with a muslin cloth to provide proper aeration.A preliminary study was conducted to determine the adequate time required for feeding honey bees. Based on this, honey bees were allowed to feed for 20 minutes, then transferred to polyethylene bags and provided with 50% sucrose solution in cotton wool. The mortality of bees was recorded at 6, 12, 18, and 24 hours after treatment, and the percentage mortality was worked out.

Statistical analysis

The percentage mortality data obtained under laboratory conditions using a Completely Randomized Design were arcsine transformed prior to analysis. The transformed data were subjected to three-way analysis of variance to test the overall significance among treatments. When the ANOVA indicated significant differences, treatment means were separated using Duncan's Multiple Range Test at the 5% significance level. All statistical analyses were performed using SPSS software.

Results

Oral toxicity at 24 h showed clear differences between *Apis cerana indica* and *Tetragonula iridipennis*, as well as among insecticide groups and dose levels (Tables 1–4). Three-way ANOVA revealed that cumulative mortality at 24 h was significantly influenced by bee species, insecticide dose, and insecticide type (Table 5).

At the recommended dose, *A. cerana indica* exhibited higher overall mortality than *T. iridipennis*, indicating greater species sensitivity to insecticide exposure. In *A. cerana indica*, buprofezin showed the lowest toxicity, causing only 25.28% mortality at 24 h, whereas lambda-cyhalothrin induced moderate mortality (80.93%). Neonicotinoids such as thiamethoxam (92.96%) and imidacloprid (95.83%) caused high mortality, while the organophosphates chlorpyrifos (100%) and profenofos (100%) were the most toxic at 24 h (Table 1).

At one-tenth of the recommended dose, mortality in *A. cerana indica* declined for most insecticides; however, chlorpyrifos (100%) and profenofos (96.30%) continued to cause very high mortality, indicating strong toxicity even at reduced doses. In contrast, buprofezin remained the least toxic insecticide, causing only 10.37% mortality at 24 h (Table 2). These patterns are consistent with the significant main effect of dose detected in the three-way ANOVA ($F_{1,56} = 118.53$, $p < 0.001$).

A similar toxicity pattern was observed in *T. iridipennis* at 24 h (Tables 3 and 4). At the recommended dose, buprofezin again exhibited the lowest toxicity (21.48%), whereas chlorpyrifos (96.30%) and profenofos (93.33%) caused the highest mortality. Neonicotinoids, including thiamethoxam (89.63%) and imidacloprid (92.59%), resulted in high mortality, while lambda-cyhalothrin induced moderate toxicity (60.37%). At one-tenth of the recommended dose, mortality in *T. iridipennis* declined for most insecticides; however, chlorpyrifos (96.67%)

Table 1. Oral toxicity of different insecticides (recommended dose) to *A. cerana indica.*

S. No.	Treatment	Dose	Cumulative Mortality (%)*			
			6 HAT	12 HAT	18 HAT	24 HAT
T1	Thiamethoxam 25% WG	0.6 g/l	56.67 (48.83)^{bc}	76.67 (61.12)^{cd}	83.33 (65.91)^{cd}	92.96 (74.62)^{cd}
T2	Carbosulfan 25% EC	1 ml/l	53.33 (46.91)^{b}	70.00 (56.79)^{c}	80.00 (63.43)^{cd}	92.13 (73.71)^{cd}
T3	Buprofezin 25% SC	1 ml/l	16.67 (24.09)^{a}	20.00 (26.57)^{b}	20.00 (26.57)^{b}	25.28 (30.18)^{b}
T4	Lambda-cyhalothrin 5% EC	0.6 ml/l	56.67 (48.83)^{bc}	73.33 (58.91)^{cd}	76.67 (61.12)^{c}	80.93 (64.10)^{c}
T5	Imidacloprid 17.8% SL	0.6 ml/l	73.33 (58.91)^{bcd}	76.67 (61.12)^{cd}	90.00 (71.57)^{d}	95.83 (78.22)^{d}
T6	Chlorpyriphos 20% EC	1.5 ml/l	83.33 (65.91)^{cd}	86.67 (68.58)^{de}	100.00**(89.09)^{e}	100.00 (89.09)^{d}
T7	Profenofos 50% EC	1.5 ml/l	90.00 (71.57)^{d}	96.67 (79.48)^{e}	96.67 (79.48)^{e}	100.00 (89.09)^{d}
T8	Untreated check	50% Sucrose	0.00**(0.91)^{a}	0.00 (0.91)^{a}	0.00 (0.91)^{a}	0.00 (0.91)^{a}
	Sed		9.21	6.34	3.84	6.14

*Mean of three replications

**Figures in parentheses are arcsine transformed with formulae: 1/4n for 0% and 100-1/4n for 100%. Values followed by same letter(s) do not differ significantly at p = 0.05 (DMRT), HAT-Hours after treatment.

Table 2. Oral toxicity of insecticides (1/10th of the recommended dose) to *A. cerana indica.*

S. No.	Treatment	Dose	Cumulative Mortality (%)*			
			6 HAT	12 HAT	18 HAT	24 HAT
T1	Thiamethoxam 25% WG	0.06 g/l	53.33 (46.91)^{bc}	70.00 (56.79)^{bc}	80.00 (63.43)^{c}	89.63 (71.21)^{d}
T2	Carbosulfan 25% EC	0.1 ml/l	40.00 (39.23)^{b}	66.67 (54.74)^{b}	73.33 (58.91)^{c}	82.59 (65.34)^{cd}
T3	Buprofezin 25% SC	0.1 ml/l	0.00**(0.91)^{a}	6.67 (14.96)^{a}	10.00 (18.43)^{b}	10.37 (18.79)^{b}
T4	Lambda-cyhalothrin 5% EC	0.06 ml/l	56.67 (48.83)^{c}	60.00 (50.77)^{b}	76.67 (61.11)^{c}	79.26 (62.91)^{c}
T5	Imidacloprid 17.8% SL	0.06 ml/l	60.00 (50.77)^{cd}	66.67 (54.74)^{b}	76.67 (61.11)^{c}	89.63 (71.21)^{d}
T6	Chlorpyrifos 20% EC	0.15 ml/l	73.33 (58.91)^{e}	86.67 (68.58)^{c}	100.00** (89.09)^{d}	100.00(89.09)^{e}
T7	Profenofos 50% EC	0.15 ml/l	66.67 (54.74)^{cd}	70.00 (56.79)^{bc}	96.67 (79.48)^{d}	96.30 (78.90)^{e}
T8	Untreated check	50% Sucrose	0.00 (0.91)^{a}	0.00 (0.91)^{a}	0.00 (0.91)^{a}	0.00 (0.91)^{a}
	Sed		3.84	6.87	5.47	3.26

*Mean of three replications

** Figures in parentheses are arcsine transformed with formulae: 1/4n for 0% and 100-1/4n for 100%. Values followed by same letter(s) do not differ significantly at p = 0.05 (DMRT), HAT-Hours after treatment.

Table 3. Oral toxicity of different insecticides (recommended dose) to *T. iridipennis*.

S. No.	Treatment	Dose	Cumulative Mortality (%)*			
			6 HAT	12 HAT	18 HAT	24 HAT$^{\#}$
T_1	Thiamethoxam 25% WG	0.6 g/l	53.33 (46.91)^{bc}	66.67 (54.74)^{c}	79.26 (62.91)^{cd}	89.63 (71.21)^{d}
T_2	Carbosulfan 25% EC	1 ml/l	46.67 (43.09)^{b}	63.33 (52.73)^{c}	75.56 (60.37)^{cd}	89.26 (70.87)^{d}
T_3	Buprofezin 25% SC	1 ml/l	13.33 (21.42)^{a}	16.67 (24.09)^{b}	17.41 (24.65)^{b}	21.48 (27.61)^{b}
T_4	Lambda-cyhalothrin 5% EC	0.6 ml/l	46.67 (43.09)^{b}	60.00 (50.77)^{c}	61.48 (51.64)^{c}	60.37 (50.99)^{c}
T_5	Imidacloprid 17.8% SL	0.6 ml/l	70.00 (56.79)^{bc}	80.00 (63.43)^{cd}	82.96 (65.62)^{cd}	92.59 (74.21)^{d}
T_6	Chlorpyriphos 20% EC	1.5 ml/l	80.00 (63.43)^{c}	90.00 (71.57)^{d}	89.63 (71.21)^{de}	96.30 (78.90)^{d}
T_7	Profenofos 50% EC	1.5 ml/l	80.00 (63.43)^{bc}	86.67 (68.58)^{d}	93.33 (75.04)^{e}	93.33 (75.04)^{d}
T_8	Untreated check	50%Sucrose	0.00**(0.91)^{a}	0.00 (0.91)^{a}	0.00 (0.91)^{a}	0.00 (0.91)^{a}
	Sed		9.57	7.86	6.38	9.44

*Mean of three replications

** Figures in parentheses are arcsine transformed with formulae: 1/4n for 0%. Values followed by the same letter(s) do not differ significantly at p = 0.05 (DMRT). #Corrected Mortality, HAT-Hours after treatment.

and profenofos (90.00%) remained highly toxic. Buprofezin continued to show the lowest toxicity (16.67%), whereas neonicotinoids and lambda-cyhalothrin caused intermediate mortality levels (Table 4).

Overall, the three-way ANOVA confirmed significant differences in mortality between *A. cerana indica* and *T. iridipennis* ($F_{1,56} = 158.13$, $p < 0.001$), demonstrating species-specific sensitivity to insecticides. Insecticide type exerted the strongest influence on mortality ($F_{6,56} = 1033.65$, $p < 0.001$), and significant interactions among species, dose, and insecticide indicated differential responses of bee species under varying dose regimes.

Table 4. Oral toxicity of insecticides (1/10th of the recommended dose) to *T. iridipennis*.

S. No.	Treatment	Dose	Cumulative Mortality (%)*			
			6 HAT	12 HAT	18 HAT	24 HAT
T_1	Thiamethoxam 25% WG	0.06 g/l	50.00 (45.00)^{d}	63.33 (52.73)^{d}	73.33 (58.91)^{d}	83.33 (65.91)^{de}
T_2	Carbosulfan 25% EC	0.1 ml/l	26.67 (31.09)^{c}	46.67 (43.09)^{c}	56.67 (48.83)^{c}	70.00 (56.79)^{cd}
T_3	Buprofezin 25% SC	0.1 ml/l	6.67 (14.96)^{b}	10.00 (18.43)^{b}	13.33 (21.42)^{b}	16.67 (24.09)^{b}
T_4	Lambda-cyhalothrin 5% EC	0.06 ml/l	43.33 (41.17)^{d}	53.33 (46.91)^{c}	53.33 (46.91)^{c}	63.33 (52.73)^{c}
T_5	Imidacloprid 17.8% SL	0.06 ml/l	56.67 (48.83)^{de}	70.00 (56.79)^{de}	83.33 (65.91)^{de}	86.67 (68.58)^{de}
T_6	Chlorpyriphos 20% EC	0.15 ml/l	73.33 (58.91)^{f}	76.67 (61.12)^{e}	90.00 (71.57)^{e}	96.67 (79.48)^{f}
T_7	Profenofos 50% EC	0.15 ml/l	66.67 (54.74)^{ef}	76.67 (61.12)^{e}	80.00 (63.43)^{d}	90.00 (71.57)^{ef}
T_8	Untreated check	50% sucrose	0.00** (0.91)^{a}	0.00 (0.91)^{a}	0.00 (0.91)^{a}	0.00 (0.91)^{a}
	Sed		3.85	2.44	4.48	5.62

*Mean of three replications

** Figures in parentheses are arcsine transformed with formulae: 1/4n for 0%. Values followed by same letter(s) do not differ significantly at p = 0.05 (DMRT), HAT-Hours after treatment.

Table 5. Three-way ANOVA showing the effects of bee species (*Apis cerana* indica and *Tetragonula iridipennis*), dose (recommended and one-tenth of the recommended dose), insecticide, and their interactions on cumulative mortality at 24 h after treatment.

Source of variation	df	Mean square	F value	p value	Partial η^2
Species	1	680.496	158.13	<0.001	0.738
Dose	1	510.097	118.53	<0.001	0.679
Insecticide	6	4448.217	1033.65	<0.001	0.991
Species × Dose	1	9.779	2.27	0.137	0.039
Species × Insecticide	6	50.924	11.83	<0.001	0.559
Dose × Insecticide	6	53.402	12.41	<0.001	0.571
Species × Dose × Insecticide	6	14.414	3.35	0.007	0.264
Error	56	4.303			

df = degrees of freedom; F values were obtained from three-way ANOVA assessing the effects of bee species (*Apis cerana indica* and *Tetragonula iridipennis*), insecticide dose (recommended dose and one-tenth of the recommended dose), insecticide type, and their interactions on cumulative mortality at 24 h after treatment. Partial η^2 indicates effect size, representing the proportion of variance explained by each factor. Significant effects are indicated at $p < 0.05$.

Discussion

Laboratory investigations in the present study clearly demonstrated that buprofezin was the least toxic insecticide to both *Apis cerana indica* and *Tetragonula iridipennis*. This reduced toxicity is consistent with its mode of action as an insect growth regulator that primarily disrupts chitin synthesis rather than causing acute neurotoxicity. Similar findings were reported by Alexander et al. (2013), who observed low oral toxicity of buprofezin to *A. cerana indica*, with an LC_{50} of 304.96 ppm at 12 HAT.

In contrast, chlorpyrifos and profenofos exhibited extremely high oral toxicity to both bee species, resulting in severe mortality even at one-tenth of the recommended dose. Organophosphate insecticides inhibit acetylcholinesterase activity, leading to rapid neurophysiological disruption, which likely explains their pronounced toxicity. These findings agree with Leite et al. (2021), who recorded 100% mortality in *Tetragonisca angustula* within 3 hours of oral exposure to chlorpyrifos, and with Dorneles et al. (2017), who reported low LC_{50} values for chlorpyrifos in *Scaptotrigona bipunctata* and *Tetragonisca fiebrigi*.

Neonicotinoids such as imidacloprid and thiamethoxam caused consistently high mortality (>85%) in both *A. cerana indica* and *T. iridipennis* at recommended and reduced doses, highlighting their strong oral toxicity across bee taxa. This elevated toxicity is attributed to their agonistic action on nicotinic acetylcholine receptors, resulting in prolonged neural excitation. Comparable toxicity patterns have been documented in stingless bees by Quiroga-Murcia et al. (2017) and Jacob et al. (2019), who reported extremely low LD_{50} and LC_{50} values for these compounds.

Lambda-cyhalothrin exhibited moderate toxicity in the present study, with mortality ranging from approximately 60–80% at 24 h, depending on species. Although previous studies have reported complete mortality in *Apis mellifera* following oral exposure (Melisie et al., 2015; Yeebyo et al., 2020), the comparatively lower mortality observed here may reflect interspecific differences in susceptibility, dose levels, or formulation effects. Notably, profenofos induced rapid mortality at shorter exposure periods, whereas lambda-cyhalothrin required longer exposure to exert lethal effects, suggesting differences in toxicokinetics.

Conclusions

The present study clearly demonstrates that the oral toxicity of insecticides to *Apis cerana indica* and *Tetragonula iridipennis* is strongly influenced by chemical class and mode of action. Organophosphate insecticides, particularly chlorpyrifos and profenofos, were extremely toxic to both species, causing complete mortality, while neonicotinoids such as imidacloprid and thiamethoxam also resulted in high levels of mortality. Lambda-cyhalothrin exhibited pronounced toxicity, leading to substantial mortality with delayed lethal effects. In contrast, buprofezin showed comparatively low toxicity, causing minimal mortality in both bee species and indicating its relative safety under laboratory conditions These findings emphasize the need for judicious insecticide selection and support the inclusion of safer alternatives such as insect growth regulators within integrated pest management strategies to minimize risks to pollinators and ensure the sustainability of essential pollination services.

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Author's Contribution

KS: Conceptualization, methodology, writing-original draft.
ND, BU: Supervision, writing-review & editing, data curation.
BSV: Validation, writing-review & editing, supervision.
All authors have gone through and approved the manuscript.

Consent to participate

Informed consent was obtained from all individual participants included in the study.

Compliance with ethical standards

This article does not contain any studies with human participants performed by any of the authors.

Conflict of interest

The authors declare that they have no conflicts of interest.

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