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Effect of insecticides on the Indian honey bees *Apis cerana indica* F (Hymenoptera: Apidae) in cotton and bhendi ecosystems

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Abstract
Honey bees are critical pollinators worldwide, yet it is increasingly vulnerable to biotic and abiotic stresses. Agrochemicals are the major cause of pollinator decrease around the world. Field studies were conducted to study the effect of insecticidal exposure on Indian hiney bees.It was found that the honeybee death rate in both cotton and bhendi crops was lowest when exposed to chlorantraniliprole (14.19) followed by fipronil (23.69), imidacloprid (25.81), dinotefuran (30.53). In terms of toxicity ranking, the insecticides were ordered from least to most toxic as follows: chlorantraniliprole < fipronil < imidacloprid < dinotefuran. The mean mortality rate was highest when exposed to dinotefuran (15.09 and 15.44), followed by imidacloprid (12.35 and 13.46), fipronil (12.02 and 11.67), and chlorantraniliprole (5.27 and 8.92), in cotton and bhendi ecosystems. These pesticides caused varied poisoning symptoms in bees, which could aid beekeepers in identifying the reason behind colony deaths. The findings offer valuable insights for choosing insecticides that minimize harm to foraging honey bees while effectively managing crop pests.

Keywords: *Apis cerana indica*, insecticides, field doses, cotton, bhendi, different pests, mortality

Introduction

Honey bees are significant pollinators that play a crucial ecological and functional role in maintaining native plant communities and agricultural productivity (Potts *et al*., 2010) [17]. *Apis cerana* is an important social honey bee species present at high elevations in the Asian tropics and northeastern Asia, and it is a key pollinator (Corlett, 2004) [6]. *A. cerana indica* is distributed in China, Japan, India, Bangladesh, Nepal, Papua New Guinea, and Malaysia (Jones & Bienefeld, 2016) [27]. They impact 75 per cent of the world's most important food crop and 35 per cent of agricultural crop production (IPBES, 2016) [2]. As the frequency of foraging by honeybees in crops increases, the pollination effectiveness also increases (Singh *et al*., 2006) [22]. While foraging, they are exposed to challenges like parasites, predators, diseases, and xenobiotics in the environment (Ollerton *et al*., 2011) [15] and thereby contaminated by either plant-derived chemicals or agrochemicals that are applied to the crop (Kumar *et al*., 2020) [12]. The increasing use of synthetic pesticides such as insecticides, herbicides, and fungicides in agriculture has direct effects like worker bee mortality (Johnson *et al*., 2010) [10]. The xenobiotics are also accumulated in pollen and nectar stored in the colony. This accumulation can contribute to Colony Collapse Disorder (CCD), a phenomenon linked to significant colony losses and potential threats to agricultural systems and biodiversity. Currently, a variety of insecticides belonging to different classes are used for pest management in crop ecosystems.

Cotton (*Gossypium hirsutum* L.) and bhendi (*Abelmoschus esculentus* L.) are two important crops pollinated by Indian honey bees (*Apis cerana indica* Fab). Both crops face numerous insect pests throughout their growth cycle, starting from germination and continuing until harvest (Naeem-Ullah, 2020; Sharma & Rao, 2012) [14, 20]. They are vulnerable to sucking pests in the early stages and borers in the reproductive stages, which leads to a significant yield loss, estimated at around 20-40% (Ahmad,1999) [1] in cotton and 69% (Rawat & Sahu, 1975; Mani *et al*., 2005) [18, 13] in okra. Infestation by these pests negatively impacts both the quality and quantity of the yield, ultimately resulting in reduced market prices. A wide range of chemical insecticides used in these crops to control various pests, persist throughout various plant parts for extended durations and impact the foraging habits of bees and other pollinators.

Prolonged exposure to these residues can disrupt their behaviour, posing risks to their survival and the ecosystems they support.Certain insecticides lack taste and odor which honey bees struggle to distinguish between treated and untreated crops (Kessler *et al*., 2015) [11]. Consequently, when these insecticides are applied to crops, honey bees are significantly attracted to them, resulting in both lethal and sub-lethal consequences for the bee population. Hence, investigations onthe potential threats posed by these insecticides on the Indian honey bee, *A. cerana indica* in cotton and bhendi ecosystems are essential.

Materials and Methods

Site of the experiment

The cotton experimental field was located at Thondamuthur, in the Coimbatore district of Tamil Nadu (10˚ 35΄N latitude, 76˚ 46΄E longitude) which experiences a tropical climate with ample rainfall during both the monsoon seasons. The cotton hybrid RCH 659was raised at a spacing of 45 x 30 cm during 2023 by following recommended agronomic practices. Bhendi experimental field was located at Oddanchatram, in the Dindigul district of Tamil Nadu (10˚ 28΄N latitude, 77˚ 44΄E longitude). The bhendi hybrid Vikram was raised at a spacing of 15 × 30 cm during 2023 by following recommended agronomic practices.

Spraying of insecticides

During the experiment, the following insecticides were administered as foliar sprays at 50% flowering stage in both cotton and bhendi. All the insecticides were used at field-recommended doses as per the CIBRC guidelines, 2021. Water spray was used as control.

Table 1: List of insecticides used in the cotton and bhendi fields

S. No	Insecticides	Recommended Dose / ha	Quantity/ litre	Company
1	Dinotefuran 20% WG	125- 150 g	0.25 g	Indofil Industries Ltd
2	Fipronil 5% SC	750 ml	1.5 ml	Bayer crop science
3	Chlorantraniliprole 18.5% SC	150 ml	0.3ml	FMC India private ltd
4	Imidacloprid 17.8% SL (Chemical check)	150 ml	0.3 ml	Rallis India ltd

These insecticides were applied using a hand-operated knapsack sprayer. The experiment was conducted in Randomized Block Design (RBD), with five treatments and four replications. Each individual plot was measured 10 x 5 m^2, with a three-meter isolation distance between plots to prevent the drift of pesticides during spraying. To prevent the escape of bees during the experiment, nylon nets were placed around the individual plots. These nets had dimensions of 10 meters in length, 5 meters in width, and 2 meters in height, with a mesh size of 1 mm (Fig 1). Within each plot, Indian bee hives with equal strength, sufficient food storage, and queens of the same age were placed. These hives were well-maintained with regular water supply to prevent overheating and forage space was confined using netting to ensure that bees remained within the experimental area.

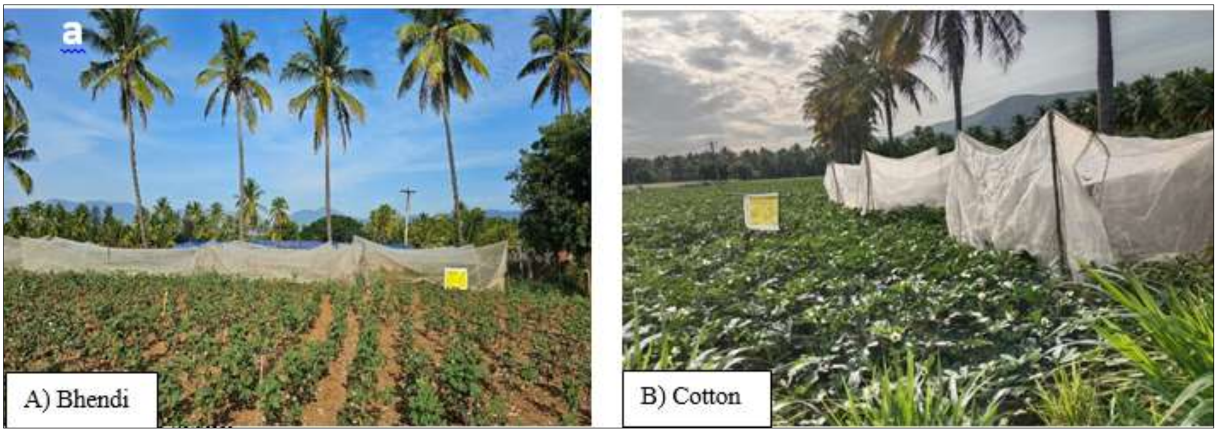

Fig 1: Field covered with net for the experiment

Experimental setup

The experimental setup involved the use of an Indian honey bee hive and a khada cloth. This same setup was employed for both cotton and bhendi fields. Marthandam type of Indian honey bee hive with 27cm x 23cm x 24cm dimension was chosen for the study. This hive contained a healthy queen, approximately 2500 to 3,000 bees, and three full frames containing an average amount of food and brood storage. A khada cloth, measuring 100cm in length and 25cm in width, was placed around the hive (Faucon *et al*., 2005) [8]. To assess the impact of insecticide spray, the number of dead adult bees in front of the hive was monitored (Fig 2). The khada cloth was emptied on a daily basis throughout the exposure period. The count of dead bees was recorded at various intervals, including 1 day after spray (DAS), 2 DAS, 3 DAS, and 4 DAS, to analyze the immediate effects of insecticide application. Dead bees that were collected and counted were disposed of at specific intervals during the experiment.

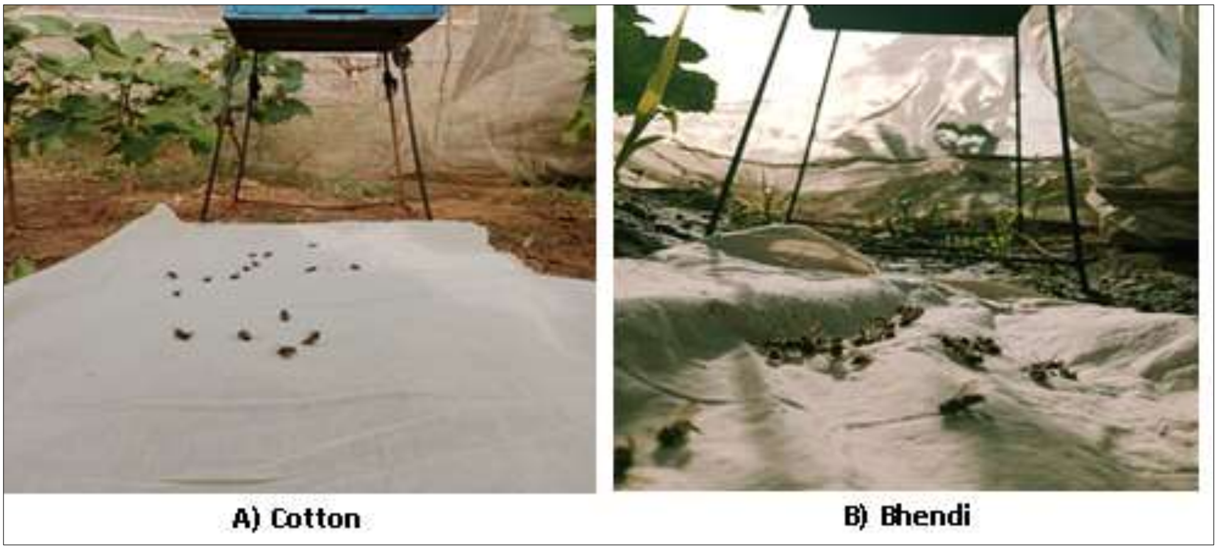

Fig 2: Dead bees at the entrance of the hive

Statistical analysis

The values, after square root transformation, were analyzed by using a one-way analysis of variance (ANOVA) in the Statistical Analysis Software programme (SAS academics) (SAS Institute, 1985). When significant, the means were separated using Tukey's test ($p<0.05$).

Results and Discussion

Bees that ingested food contaminated with insecticides displayed signs of dying. As part of their hygienic behavior, dead bees were removed and collected on the khada cloth, and some dead bees were also observed in the area around the net. Forager bees returning to the hive experienced mortality and fell onto the khada cloth placed near the hives. A differential rate of mortality with different insecticidal exposure was observed in both cotton and bhendi crops. The mean mortality rate was highest when exposed to dinotefuran (15.09 and 15.44 bees), followed by imidacloprid (12.35 and 13.46 bees), fipronil (12.02 and 11.67 bees), and chlorantraniliprole (5.27 and 8.92 bees), in cotton and bhendi ecosystems. In terms of toxicity ranking, the insecticides were ordered from least to most toxic as follows: chlorantraniliprole < fipronil < imidacloprid < dinotefuran. Among all the insecticides tested, diamide (chlorantraniliprole) safest for the bees. Neonicotinoids like dinotefuran and imidacloprid caused higher mortality in honeybees compared to pyrazoles like fipronil. (Carrillo *et al*., 2013; Yasuda *et al*., 2017) [4, 28]

The susceptibility of European honey bee *Apis mellifera* (Brar *et al.*, 2022) [3] and Indian bees *Apis cerana* (Chandrasekar *et al.*, 2022 & Sowmiya *et al*., 2022) [5, 24] to various groups of insecticides was earlier demonstrated by many scientists. Prolonged exposure to imidacloprid increases imidacloprid toxicity to honey bees. The higher toxicity of the neonicotinoids to honey bees could be attributed to the fact that neonicotinoids accumulate in pollen (Suchail *et al*., 2000) [25]. High worker mortality was observed when honeybees consumed imidacloprid-contaminated pollen and sugar syrup (Decourtye *et al*., 2003; Iwasa *et al*., 2004) [7, 9]. The field-realistic levels of neonicotinoid residues in pollen pose substantial risks to honeybees and bumblebees (Sanchez-Bayo & Goka, 2014) [19]. Nitro-containing neonicotinoids like dinotefuran and imidacloprid are known for their high toxicity compared to the cyano-containing neonicotinoids like acetamiprid and thiacloprid to bees (Simon-Delso *et al*., 2015) [21]. Fipronil, part of the phenyl pyrazole family, also acts systemically in plants. Both neonicotinoids and fipronil are particularly toxic when ingested by honeybees (Suchail *et al*., 2001) [26]

When bees interact with flowers, they often come into contact with the floral parts, which differ from crop to crop. Additionally, variability may also result from differences in the environmental conditions during testing and any natural disparities in the condition of the bees being studied. Variation in the effect of thiamethoxam on honey bees in maize and oilseed rape ecosystem was already reported (Pilling *et al*., 2013) [16].

Initially, the presence of pesticide residues resulted in heightened toxicity to the bees, leading to a significant increase in the mortality rate (Sowmiya, Unpublished data 2022) [23]. However, over the subsequent days, the mortality rate decreased significantly. In cotton, the initial mortality rate was highest on 1 DAS (26.22 to 8.46 bees), followed by 2 DAS (16.65 to 6.70 bees), 3 DAS (10.75 to 3.00 bees), and 4 DAS (6.75 to 2.90 bees). A similar trend was observed in bhendi and it was 23.25 to 14.10 bees on 1 DAS followed by 20.53 to 11.51 bees on2 DAS, 12.00 to 7.25 bees on 3 DAS, and 6.00 to 2.50 bees on 4 DAS.

Comparing the mortality of bees in both cotton and bhendi, the overall mean mortality of bhendi is slightly higher than cotton to all the insecticides tested *viz*., dinotefuran, imidacloprid, fipronil, and chlorantraniliprole. (Fig 3).

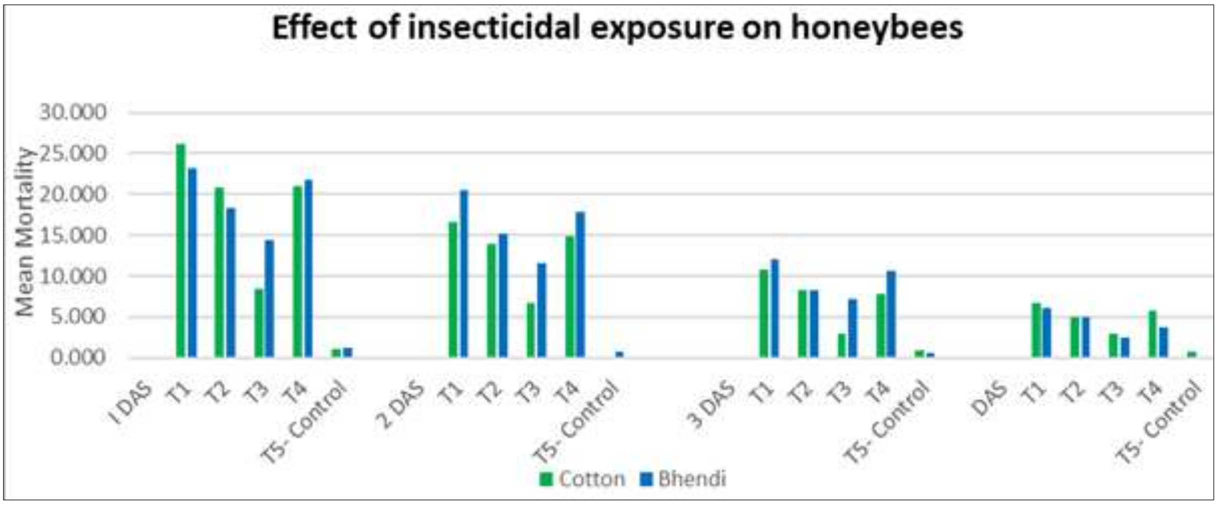

Fig 3: Comparison of mean mortality of bees in cotton and bhendi ecosystems

Table 2: Effect of insecticides on the Indian honeybees *(A. cerana indica)* in the cotton and bhendi ecosystems

Treatment	Mean no. of dead bees recorded at indicated DAS									
	Cotton					Bhendi				
	1 DAS	2 DAS	3 DAS	4 DAS	Mean	1 DAS	2 DAS	3 DAS	4 DAS	Mean
Dinotefuran	26.22^{a} (5.22)	16.65^{a} (4.15)	10.75^{a} (3.48)	6.75^{a} (2.66)	15.09 (3.87)	23.25^{a} (4.85)	20.53^{a} (4.59)	12.00^{a} (3.42)	6.00^{a} (2.53)	15.44 (3.84)
Fipronil	20.85^{b} (4.61)	13.98^{c} (3.80)	8.25^{b} (2.95)	5.00^{ab} (2.32)	12.02 (3.42)	18.25^{b} (4.32)	15.19^{c} (3.96)	8.25^{bc} (2.95)	5.00^{ab} (2.29)	11.67 (3.13)
Chlorantraniliprole	8.46^{c} (2.98)	6.70^{d} (2.67)	3.00^{c} (1.86)	2.90^{b} (1.80)	5.27 (2.33)	14.40^{c} (3.85)	11.51^{d} (3.47)	7.25^{c} (2.76)	2.50^{bc} (1.70)	8.92 (2.95)
Imidacloprid	21.00^{b} (4.63)	14.93^{ab} (3.92)	7.75^{b} (2.86)	5.75^{a} (2.49)	12.35 (3.48)	21.75^{a} (4.72)	17.81^{b} (4.27)	10.54^{ab} (3.31)	3.75^{ab} (2.05)	13.46 (3.58)
Control	1.00^{d} (1.22)	0.00^{e} (0.71)	0.88^{c} (1.17)	0.69^{c} (1.09)	0.64 (1.05)	1.25^{d} (1.31)	0.76^{e} (1.12)	0.62^{d} (1.05)	0.00^{c} (0.71)	0.65 (1.04)
SE	0.121	0.102	0.118	0.215	0.139	0.11	0.12	0.18	0.25	0.165
CD(P=0.05)	0.264	0.222	0.257	0.469	0.303	0.251	0.262	0.408	0.558	0.369

DAS- Days after spraying
Figures in parentheses are $\sqrt{(x+0.5)}$ transformed values.
Mean values followed by the same superscript alphabet (s) in the columns do not differ significantly by Tukey at P=0.05 level

Conclusion

A. cerana indica offers significant economic prospects for both small and large-scale beekeeping operations. Sustained and improved beekeeping with *A. cerana indica* is crucial to species preservation (Jones & Bienefeld, 2016) [27]. Exposure of bees to such insecticides has the potential to reduce the overall health and fitness of bee colonies, thereby impacting their sustainability. Additional research is required to assess the safety of applying these chemical insecticides in bee foraging areas. This should include the persistence of systemic insecticides that remain in the environment and affect plants that attract bees. It's crucial to understand that exposure of insect pollinators to hazardous chemicals like dinotefuran, imidacloprid, etc., even a small amount can disrupt the regular functions and performance of the bees, and cause mortality. The use of these insecticides must be restricted in bee-foraging crops that might endanger bees through contamination. Furthermore, these highly toxic chemicals should be replaced with insecticides which have lower toxicity to pollinators.

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