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Determining the Effect of Thiacloprid Exposure on Honey Bees (*Apis mellifera*)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The effect of thiacloprid (21.7% SC), an N-cyanoamidine neonicotinoid exposure on honey bees, *Apis mellifera* was evaluated under field conditions. A study on foraging behaviour, hygienic behaviour, mortality, and food stores was carried out using different concentrations of thiacloprid *viz*., 500 ppm, 325 ppm, 250 ppm, 125 ppm, 62.50 ppm, 31.25 ppm, and 0 ppm. A significant effect was observed from the use of concentrations of 500 and 325 ppm only. This was evident for all the behaviours studied. This indicated that thiacloprid remained safer for the honey bees at lower concentrations of 250, 125, 62.50, and 31.25 ppm as the effect of these concentrations was not significantly different from that of the control. Thus, an impact to a great extent on the behaviour of honey bees was possible with the higher concentrations of thiacloprid only.

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Keywords: Apis mellifera; thiacloprid; cyanoamidine neonicotinoid; behaviour; mortality; food stores.

1. INTRODUCTION

Honeybees account for 35% of global food production and hence are important for food security [1]. In the United States alone, the American honeybee, *Apis mellifera* L. pollinates $15 billion to $20 billion worth of crops and around $200 billion worldwide [2]. A serious ailment of honey bees is Colony Collapse Disorder or CCD in which the worker bees fly off. As they never return, they leave the remaining bees to starve to death in the hive. First seen in several areas of the United States in 2006-2007 leading to a loss of 651,000-875,000 out of 2.4 million colonies [3] and outside the US in 2009 when the Swiss Bee Research Centre inspected colony loss of a local beekeeper [4] and later on in Asia. Several indications point CCD to being induced by pesticides other than pathogens, nutritional deficits, and environmental stresses. In less than 20 years, neonicotinoids have become the most widely used class of insecticides with a global market share of more than 25%. More than direct or lethal effects, neonicotinoids have shown indirect or sublethal effects on honey bees which may include effects on behavior like foraging, learning, cleaning, and navigation. Being symptomatic such as uncoordinated movements of bees, and tremors; these effects greatly impact the overall growth of the colonies [5,6,7]. Neonicotionoids have shown a wide range of effects detected through their acute toxicity tests when Cresswell [8] found out contact LD_{50} values for imidacloprid were higher than that of oral LD_{50} values; effect on gut microbiota as reported by Alaux et al. [9], when a high rate of mortality from *Nosema* infections was caused as a result of imidacloprid exposure and due to thiacloprid exposure as reported by Vidau et al. [10] effect on behavior, learning, and memory as studied by Aliouane et al. [11].

Like other neonicotinoids, the use of thiacloprid is profound be it for sucking or biting insects and even nematodes like *Meloidogyne incognita*. Thiacloprid [3-{(6-chloro-3-pyridinyl) methyl-2-thiazolidinylidene} cyanamide] is a chloronicotinyl insecticide chemically and its systemic nature stands out. Under the Insecticides Act, 1968, the formulation that is registered for use in India is 21.7% SC. The LD_{50} value of thiacloprid was found to be 14600 ng/bee (quite less) as against imidacloprid, clothianidin, thiamethoxam, dinotefuran, and nitenpyram with LD_{50} values of 18 ng/bee, 22 ng/bee, 30 ng/bee, 75 ng/bee, and 138 ng/bee respectively [12]. Residues of various neonicotinoids are found in bee products like honey, propolis, pollen, and nectar [13]. In another study on residue analysis by Bridi et al. [14], thiacloprid was found to be in most honey samples when compared with the other three neonicotinoids, acetamiprid, thiamethoxam, and imidacloprid. In addition to this, thiacloprid has an effect on the flight pattern of honeybees with a slowed down flight speed whereas it was not that lower with imidacloprid and clothianidin-treated honeybees [15]. Queen failure and subsequent colony loss is another disturbing case that has been mentioned in a lot of studies [16] where thiacloprid and clothianidin led to the failure of honeybees' immune defense response. Thus, not just in terms of mortality but also having an impact on the behaviour patterns of honeybees, neonicotinoids have proved to be significant. The information on the impact of thiacloprid on bee health is limited. Keeping in view the importance of neonicotinoids, thiacloprid in particular, and its safety to honey bees, this study was taken up to study the effects of thiacloprid on the behaviour and food stores of honey bees.

2. MATERIALS AND METHODS

The study was conducted during February-March, 2023 for which 21 full-frame hives uniform in terms of adult, brood, and food stores of the bees were selected to account for 7 treatments and 3 replications including control. Various concentrations of thiacloprid 21.7% SC were prepared (Table 1).

Granulated crystal sugar was powdered very finely using a mixer grinder and separated into 21 batches of 100 g each after weighing using balance. Each candy was prepared using 100 g sugar and 10 ml of the treatment concentration and allowed to solidify for one day in the lower bottom of the Petri plate. For control, candies were prepared using sugar powder and distilled water. Each time, fresh candies were prepared for feeding following the same steps as discussed earlier were served on the Petri plate itself, and were kept on the left side bottom of the hive. A total of 3 feedings were given at weekly intervals.

Table 1. Preparation of thiacloprid concentrations

Concentration name	Parts of solute	Parts of solvent	Concentrations
A	Stock solution	-	1000ppm
B	1 of A	1	500ppm
C	0.325 of A	0.675	325ppm
D	1 of B	1	250ppm
E	1 of D	1	125ppm
F	1 of E	1	62.50ppm
G	1 of F	1	31.25ppm

The day 2nd was chosen for the observations because thiacloprid has quite a fast rate of metabolism and the waiting period is 5 days in the case of the brinjal crop according to which the whole study was carried out.

Foraging behavior was recorded every 2nd day after each exposure of treatments. This involved counting the number of bees getting in and coming out from the entrance of the hive for a period of 5 minutes. The hygienic behavior of bees was recorded by counting the number of dead bees thrown out in 15 minutes as a measure of frequency on 2nd day after every exposure to treatments. For recording the mortality of honeybees, the top cover of the honeybee hive was placed below the entrance of the hive, and mortality was recorded forenoon by counting the number of dead bees fallen on the top cover. Mortality on one-hour basis was recorded by counting the number of dead bees that fell on the top cover kept below the entrance of the hives every 2nd day after the exposure to treatments.

Food stores (honey and pollen) were evaluated by quantifying the area of the frame. This was done on the 5th day after every exposure of honey bees to the insecticide. For this, a transparent sheet was used as a reference which was cut out as per the size of the frame of the hive. Then this transparent sheet was divided into squares of equal size using a black permanent marker. For evaluating food stores, both sides of two frames from each hive were selected. All the bees were dislodged and the frame was made free of bees. The transparent sheet was placed of over the frame and the area having honey and pollen was quantified by counting the number squares on the sheet covered. Later, the number of squares was converted into percentages. After this, per cent reduction over control was calculated for each treatment as per the formulae:

Per cent reduction over control

$$= \frac{\text{\% honey per pollen stores in control} - \text{\% honey per pollen stores in treatment}}{\text{\% honey per pollen stores in control}} \times 100$$

The data were averaged out for all three replications. The data on foraging behavior were square root transformed and analyzed using a paired-*t* test. Data on mortality and hygienic behavior were square root transformed and were analyzed using one-way analysis of variance (ANOVA) followed by post hoc-Tukey test. The data on food stores were arc-sine transformed and analyzed using one-way analysis of variance (ANOVA) followed by post hoc- Tukey test. All the statistical tests were performed with SPSS 16.0 for MS Windows.

3. RESULTS

Data on foraging behavior after 1st feeding showed that there was a significant difference between incoming and outgoing honey bees at 500 and 325ppm whereas at 250, 125, 62.50, 31.25, and 0ppm concentrations, the number of incoming and outgoing bees at these concentrations were at par with each other. The same trend was seen for 2nd and 3rd feedings, although a slight increase in the difference was seen between the number of incoming and outgoing bees. The number of bees incoming and outgoing also decreased after the 2nd and 3rd feedings.

The data showed that mortality in 1 hour was maximum at a concentration of 500 ppm followed by 325 ppm. These data were found to be significantly different from that of the rest of the concentrations but were at par with each other. The mortality data in 1 hour at concentrations of 250, 125, 62.50, and 31.25 ppm were at par with each other and with control (0 ppm) also. A similar trend was seen after the 2nd and 3rd feedings.

The data on percent reduction over control in honey and pollen stores for 500 and 325 ppm was significantly different from that for 250 ppm, 125 ppm, 62.50 ppm, and 31.25ppm.

Table 2. Effect of thiacloprid on the foraging behaviour of *Apis mellifera* on the 2nd day after feeding

Concentrations (ppm)	Number of honey bees					
	1st feeding		2nd feeding		3rd feeding	
	Incoming	Outgoing	Incoming	Outgoing	Incoming	Outgoing
500	175.00 (13.22±0.30)	206.33 (14.35±0.43)	155.33 (12.45±0.20)	187.67 (13.69±0.13)	135.33 (11.63±0.11)	169.00 (12.99±0.13)
325	185.67 (13.62±0.16)	217.00 (14.72±0.23)	170.33 (13.04±0.39)	200.33 (14.14±0.32)	143.67 (11.98±0.15)	175.00 (13.22±0.13)
250	267.00 (16.33±0.10)	273.00 (16.52±0.06)	252.33 (15.88±0.21)	258.33 (16.07±0.17)	229.33 (15.14±0.19)	235.33 (15.33±0.24)
125	273.00 (16.52±0.04)	279.00 (16.70±0.10)	272.67 (16.51±0.11)	279.00 (16.70±0.05)	231.33 (15.20±0.18)	237.67 (15.41±0.12)
62.50	288.00 (16.96±0.10)	294.00 (17.14±0.17)	273.00 (16.52±0.06)	279.00 (16.70±0.05)	245.67 (15.67±0.09)	251.67 (15.86±0.05)
31.25	298.00 (17.26±0.10)	304.00 (17.43±0.05)	279.33 (16.71±0.17)	285.33 (16.88±0.22)	247.00 (15.71±0.06)	253.33 (15.91±0.05)
Control	299.00 (17.29±0.07)	306.00 (17.49±0.03)	285.56 (16.90±0.05)	292.00 (17.08±0.05)	250.33 (15.82±0.06)	255.67 (15.98±0.01)

Data are average of three replications
Figures in parenthesis are square root transformed values ± SE

Table 3. Effect of thiacloprid on mortality of *Apis mellifera* in one hour on the 2nd day after feeding

Concentrations (ppm)	Number of dead honey bees		
	1st feeding	2nd feeding	3rd feeding
500	106.67 (10.32±0.08)	109.17 (10.44 ±0.10)	111.67 (10.56±0.10)
325	105.00 (10.24±0.07)	105.83 (10.28±0.04)	106.67 (10.32±0.04)
250	73.33 (8.56±0.09)	76.67 (8.75±0.09)	78.33 (8.84±0.09)
125	71.66 (8.46±0.09)	76.66 (8.75±0.09)	76.67 (8.75±0.09)
62.50	71.00 (8.42±0.12)	73.33 (8.56±0.09)	76.67 (8.75±0.09)
31.25	67.50 (8.20±0.22)	70.00 (8.36±0.11)	71.66 (8.46±0.09)
Control	66.67 (8.15±0.25)	68.33 (8.26±0.10)	71.66 (8.46±0.07)

Data are average of three replications
Figures in parenthesis are square root transformed values± SE

Table 4. Per cent reduction over control in the honey and pollen stores of *Apis mellifera* due to thiacloprid on the 5th day after feeding

Concentrations (ppm)	Honey			Pollen		
	1st feeding	2nd feeding	3rd feeding	1st feeding	2nd feeding	3rd feeding
500	30.98 (0.31±0.005)	32.01 (0.32±0.003)	37.97 (0.39±0.005)	32.89 (0.33±0.004)	39.27 (0.40±0.004)	44.36 (0.46±0.002)
325	30.25 (0.30±0.001)	31.63 (0.32±0.005)	37.11 (0.38±0.007)	31.65 (0.32±0.004)	38.93 (0.39±0.003)	43.68 (0.45±0.002)
250	15.12 (0.15±0.023)	16.52 (0.16±0.019)	17.07 (0.17±0.026)	16.89 (0.16±0.010)	17.50 (0.17±0.003)	19.02 (0.19±0.026)
125	12.83 (0.12±0.009)	15.47 (0.15±0.01)	16.11 (0.16±0.008)	14.85 (0.14±0.025)	16.62 (0.16±0.008)	17.61 (0.17±0.003)
62.50	10.42 (0.10±0.009)	11.45 (0.11±0.01)	12.78 (0.12±0.010)	14.19 (0.14±0.021)	15.71 (0.15±0.008)	16.49 (0.16±0.008)
31.25	10.00 (0.10±0.023)	10.07 (0.10±0.01)	10.26 (0.10±0.01)	12.04 (0.12±0.021)	12.94 (0.13±0.02)	15.71 (0.15±0.008)

Data are average of three replications
Figures in parenthesis are arc sine transformed values ± SE

4. DISCUSSION

The results after the paired-*t* test with the number of incoming and outgoing bees as the two variables showed a significant difference between the number of incoming and outgoing honey bees only in the case of the higher concentrations, namely 500 and 325 ppm. On 2nd day after the first feeding, the outgoing bees outnumbered the incoming bees in both 500 and 325ppm concentrations. This may be attributed to the loss of memory, and orientation [17]. At concentrations of 250, 125, 62.50, 31.25, and 0ppm, the difference between the incoming and outgoing bees was not significant since thiacloprid, being a cyanoamidine neonicotinoid is towards the safer side to honey bees [18]. In a study by Henry et al. [19] with thiamethoxam exposure to honey bees, the failure in the homing behavior in the forager was found due to which they could not return to their respective hives. Moreover, a slight increase in the difference was seen in the number of incoming and outgoing honey bees and an overall decrease in the number of both incoming and outgoing bees when observed after 2nd and 3rd feeding which may be due to the cumulative effect of feedings one after the other but again significant difference was only seen with 500 and 325ppm concentration and not with 250, 125, 62.50, 31.25 and 0ppm concentrations. The study by Yang et al. [17] had similar findings while working with different concentrations of imidacloprid, *viz*., 50, 100, 200, 400, 600, 800, and 1200 µg/litre and found abnormal behavior only with the honey bees which were treated with concentrations higher than 50 µg/litre. Similar result was reported by ke et al. [20] in which thiacloprid exposure had a detrimental impact on honeybee learning and memory.

The results on mortality of *A. mellifera* in 1 hour revealed maximum mortality with 500ppm concentration followed by 325ppm, both being at par with each other. The mortality at 325ppm (recommended) may be attributed to the direct feeding of thiacloprid as treated sugar candy. However, mortality with concentrations on the lower side of 325ppm, *viz*., 250, 125, 62.50, 31.25ppm was not significantly different from the control, owing to its safer nature towards bees. Our findings with mortality are in line with that of the laboratory study done by Laurino et al. [21] which showed that thiacloprid could not cause mortality at the recommended dose and the concentrations which were lower than this even after 3 days. However, our study shows mortality at the recommended concentration of 325ppm also which is significantly different from that of the control. This contrast might be due to the difference in the experimental conditions where our study was conducted in the field conditions and also due to different observation times where observations of our study were taken on 2nd day of feeding the treated candy. Earlier, Siede et al. [22] conducted a study in which sublethal doses of thiacloprid administered to honey bees could not cause any significant effect on their mortality even after exposure for 2 long years, thus agreeing with our study where except 500 and 325 ppm, there was no effect seen on the survival of the honey bees. Also, our results are in line with those of Tison et al. [23], in which no mortality was seen with the concentrations starting from 0.5, 5, 50 ppm up to 200ng/bee which were later labelled as the sub lethal ones. The experiments done by Liu et al. [24] revealed that significant mortality was observed when honey bees were fed with sucrose solution mixed with different concentrations of thiacloprid *viz*., 0, 0.2, 0.6, and 2.0 mg/l for about 2 weeks. A contrast with this study might be due to the different experimental conditions *i.e*., laboratory versus field.

Since thiacloprid impacted the foraging activity of the honey bees, a reduction in the food stores was also recorded. Maximum reduction over control of food (honey and pollen) stores in terms of percentage was seen at 500 ppm followed by 325 ppm both being at par with each other but significantly different from the data on per cent reduction over control for concentrations of 250, 125, 62.50 and 31.25ppm. The data on the per cent reduction over control at 250, 125, 62.50, and 31.25ppm did not differ significantly from each other, thus depicting that as there was not much difference in the number of incoming and outgoing bees at these concentrations, there is no marked influence on the reduction of the food (honey and pollen) stores and that the bees treated with these concentrations continued with their normal storage activity. Further, on the 5th day after the 2nd and 3rd feeding, a slight increase in the per cent reduction over control was seen synchronizing with the cumulative effect of thiacloprid on the foraging activity. But again, the per cent reduction over control at concentrations 500 and 325 ppm was found to be quite similar to each other but significantly different from the per cent reduction over control at concentrations of 250, 125, 62.50, and 31.25 ppm. This is in accordance with the study carried out by Rumkee et al. [25] where lower doses of pesticides were received by larvae when there were less number of foraging bees and higher doses of pesticides were received by the developing larvae when the number of foraging bees entering the hive increased. Also, our study is in confirmity with Wu-Smart and Spivak [26] in which the number of cells containing honey and pollen was significantly reduced in the hives which received higher concentrations of imidacloprid treatment, to the tune of 20, 50, and 100 ppb as against 10 ppb where no reduction in the number of cells containing honey and pollen was observed. Similar finding was reported by Chen et al. (2023) in which they found that chronic larval exposure to thiacloprid may diminish foraging abilities of honeybees. However, a contrast in the concentrations responsible for bringing this effect is seen. This might be due to the difference in the neonicotinoid used.

5. CONCLUSION

Thiacloprid was towards the safer side to honey bees at lower concentrations of 250, 125, 62.50, and 31.25ppm, unlike the two higher concentrations to the tune of 325 and 500ppm at which thiacloprid brought significant effect. The recommended concentration of 325ppm showed an effect which was not significantly different from the effect due to 500ppm and this is attributed to the fact that thiacloprid was fed directly to the honey bees. Also, several studies on honey bees have been carried out in the Jammu region to evaluate the effects of various neonicotinoids other than thiacloprid. Hence, our findings would be a step for further investigations with thiacloprid not only on honey bees but with other crop insect pests as well.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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