Indian Journal of Entomology 86(1): 118-122 (2024) DoI. No.: 10.55446/IJE.2024.1467

EFFECT OF THIACLOPRID EXPOSURE ON HONEY BEES *APIS MELLIFERA* F.

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ABSTRACT

An evaluation of the effect of thiacloprid (21.7% SC), an N-cyanoamidine neonicotinoid exposure on honey bees *Apis mellifera* F. was done under field conditions. Study on foraging behaviour, mortality and food stores were carried out with 500, 325, 250, 125, 62.50, and 31.25 ppm, while 0 ppm concentration served as control. Of these, a significant effect was observed with 500 and 325 ppm as could be observed from the foraging behaviour. Thus, thiacloprid is found to be safe at lower concentrations <250 ppm.

Key words: *Apis mellifera*, thiacloprid, neonicotinoid, foraging, behaviour, mortality, honey stores, pollen stores, exposure, 250 ppm, safety

Honey bees account for 35% of the global food production and hence are important for food security (Genersch, 2010). In the United States alone, *Apis mellifera* F. pollinates $15 to $20 billion worth of crops and around $200 billion worldwide (Van Engelsdorp and Meixner, 2010). A serious ailment of honey bees is Colony Collapse Disorder (CCD) in which the worker bees fly off leaving the colony starve to death leading to serious losses (Underwood and Van Engelsdorp, 2007; Dainat et al., 2012). In less than 20 years, neonicotinoids have become the most widely used class of insecticides with a global market share of more than 25%. But these have shown indirect or sublethal effects on honey bees which may include effect on behaviour like foraging, learning, cleaning, navigation. Being symptomatic such as uncoordinated movements of bees, tremors; these effects greatly impact the overall growth of the colonies (Buszewski et al., 2019). Neonicotionoids have shown a wide range of effects detected through their acute toxicity tests as shown by Cresswell (2011), and on gut microbiota by Alauxet al (2010). High rate of mortality from *Nosema* infections was caused as a result of imidacloprid and thiacloprid exposure (Vidau et al., 2011); and detrimental effect on behaviour, learning and memory as studied by Aliouane et al. (2008). Thiacloprid has profound effect on sucking or biting insects and even nematodes like *Meloidogyne incognita*. The LD_{50} value of thiacloprid was found to be as low as 14600 ng/ bee (quite less) as against imidacloprid, clothianidin, thiamethoxam, dinotefuran, and nitenpyram (Iwasa et al., 2004) Residues of various neonicotinoids are found in bee products like honey, propolis, pollen, nectar (Tanner and Czerwenka, 2011). Residue analysis revealed, the presence of thiacloprid in the most of honey samples when compared with the other three neonicotinoids, acetamiprid, thiamethoxam and imidacloprid (Birdi et al., 2018). In addition, thiacloprid has an effect on the flight pattern of honey bees with a slowed down flight speed compared to imidacloprid and clothianidin treated honey bees (Fischer et al., 2014). Queen failure and subsequent colony loss is another disturbing case (Brandt et al., 2017); and thiacloprid and clothianidin lead to the failure of immune defense response. Jammu and Kashmir holds a high potential for beekeeping industry in India (Mutto, 1952). In Jammu division of J&K, beekeeping is practiced on traditional as well as commercial level, with 81,000 bee colonies (*A. mellifera*) with honey production of 860 mt. Information on the impact of thiacloprid on bee health is limited, and hence this study was taken up to study the effects of thiacloprid on behaviour and food stores.

MATERIALS AND METHODS

Study was conducted during February-March, 2021 for which 21 full frame hives uniform in terms of adult, brood and food stores of the bees were selected to account for seven treatments with three replications including control. Various concentrations of thiacloprid 21.7% SC were prepared. Sugar candies were prepared using 100 g sugar and 10 ml of the treatment concentration. For control, candies were prepared using sugar powder and distilled water (0 ppm of thiacloprid). A total of three feedings were given at weekly intervals. The day 2nd was chosen for the observations because thiacloprid has quite a fast rate of metabolism and the waiting period is of five days in brinjal. For recording number of foraging bees getting in and out from the entrance of the hive for a period for five min was done. Mortality on one hr basis was recorded by counting the number of dead bees fallen 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 5th day after every exposure. For this a transparent sheet was divided into squares of equal size using a black marker and placed over the frame and the area having honey and pollen was quantified by counting the number squares on the sheet covered (Delaplane et al., 2012) and % reduction over control was calculated. The means of data were analysed after square root transformation with statistical tests performed with SPSS16.0 for MS Windows.

RESULTS AND DISCUSSION

The results for foraging behaviour showed a significant difference between the number of incoming and outgoing *A. mellifera* only with the higher concentrations, namely 500 and 325 ppm (Table 1). On 2nd day after first feeding, the outgoing bees outnumbered the incoming bees in both 500 and 325 ppm concentrations. This may be attributed to the loss of memory, orientation (Yang et al, 2008; Henry et al., 2012). For the rest of the concentrations, the difference between the incoming and outgoing bees was insignificant, as thiacloprid, being a cyanoamidine neonicotinoid is safer (Tison et al., 2016). A slight increase in the difference between incoming and outgoing was observed, with an overall decrease in both incoming and outgoing bees after 2nd and 3rd feeding. This may be due to the cumulative effect of feedings one after the other but again significant difference was only seen with 500 and 325 ppm concentration. Pyke (2022) showed that consumption of neonics impaired ability to navigate and thus time spent foraging. Yang et al. (2008) while working with different concentrations of imidacloprid, viz., 50, 100, 200, 400, 600, 800 and 1200 μg/ l observed abnormal behaviour only with concentrations >50 μg/ l. The effect of nitro substituted neonics was much adverse than thiacloprid on honey bee foragers (Marimuthu et al., 2022). Long-term exposure to thiacloprid showed greater effect on learning and memory abilities than a single administration (Shi et al., 2023).

The results on mortality of *A. mellifera* in 1 hr revealed maximum mortality at 500 ppm followed by 325 ppm, both being at par with each other (Table 1); mortality at 325 ppm (recommended dose) may be attributed to the direct feeding of thiacloprid as treated sugar candy. These present observations agree with those obtained in the laboratory study done by Laurino et al. (2011). However, the present study shows mortality at the recommended concentration of 325 ppm also which is significantly different from that of the control. This contrast might be due to the difference in the experimental conditions and observation times, the present study is a field study, with observations taken on 2nd day of feeding the treated candy. Woodcock et al. (2017) observed that neonicotinoids interacting with environmental factors can amplify the losses of worker bees. Also, even the field realistic concentrations of thiamethoxam and clothianidin reduced the honey bee drone survival by 51% (Straub et al., 2020). Siede et al. (2017) observed that sublethal doses of thiacloprid administered to honey bees could not cause any significant effect on their mortality even after an exposure for two long years; these results are in agreement with present study, where except 500 and 325 ppm, there was no effect seen on the survival. Also, present results are in line with those of Tison et al. (2017), in which no mortality was seen with the concentrations starting from 0.5, 5, 50 ppm up to 200 ng/ bee which were later labelled as the sublethal. Liu et al. (2019) 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.

Since thiacloprid impacted foraging activity, a reduction in the food stores of was also recorded; maximum was seen at 500 ppm followed by 325 ppm (Table 1). These results are in line with those of Ohlinger et al. (2021) on imidacloprid. Further, on 5th day after 2nd and 3rd feeding, a slight increase in % reduction was seen which can be attributed to the cumulative effect of thiacloprid on the foraging activity; reduction with 500 and 325 ppm were found to be at par, but significantly different from rest of the concentrations. Rumkee et al. (2017) observed that with lower doses of pesticides were received by larvae when there were less number of foraging bees and higher doses were received with foraging bees increasing. Wu-Smart and Spivak (2016) observed that number of cells containing honey and pollen were significantly reduced in the hives which received higher concentrations of imidacloprid- > 20 ppb as against 10 ppb. Meikle et al. (2022) found that food stores in colonies decreased significantly in spite of

Table 1. Effect of thiacloprid on foraging, mortality, honey and pollen stores of *Apis mellifera*

Foraging behaviour and mortality on 2nd day after feeding

Conc. (ppm)	No. of bees (foraging behaviour)						No. of dead bees (mortality)		
	1st feeding		2nd feeding		3rd feeding		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)	106.67 (10.32± 0.08)	109.17 (10.44± 0.10)	111.67 (10.56± 0.10)
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)	105.00 (10.24± 0.07)	105.83 (10.28± 0.04)	106.67 (10.32± 0.04)
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)	73.33 (8.56± 0.09)	76.67 (8.75± 0.09)	78.33 (8.84± 0.09)
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)	71.66 (8.46± 0.09)	76.66 (8.75± 0.09)	76.67 (8.75± 0.09)
62.5	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)	71.00 (8.42± 0.12)	73.33 (8.56± 0.09)	76.67 (8.75± 0.09)
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)	67.50 (8.20± 0.22)	70.00 (8.36± 0.11)	71.66 (8.46± 0.09)
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)	66.67 (8.15± 0.25)	68.33 (8.26± 0.10)	71.66 (8.46± 0.07)

Reduction over control in honey and pollen stores after 5th day after feeding

Concentration (ppm)	% reduction over control					
	Honey stores			Pollen stores		
	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 (0.13± 0.021)	12.94 (0.13± 0.02)	15.71 (0.15± 0.008)

Figures in parenthesis arc sine transformed values ± SE.

higher brood production when exposed to imidacloprid. Thus, it can be concluded that thiacloprid is safe to honey bees at lower concentrations. The recommended concentration of 325 ppm showed an effect which was not significantly different from the effect due to 500 ppm. This is in conformity with the study of Cabirol and Haase (2019) which showed that field realistic doses vary from place to place and have different uptake pathways.

ACKNOWLEDGEMENTS

The authors acknowledge the Department of Entomology, SKUAST of Jammu for providing facilities.

FINANCIAL SUPPORT

No external funds.

AUTHOR CONTRIBUTION STATEMENT

Pratibha Angral and R S Bandral conceived and designed research. Pratibha Angral conducted experiments, analyzed data and wrote the manuscript.

CONFLICT OF INTEREST

No conflict of interest.

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(Manuscript Received: July, 2023; Revised: October, 2023;
Accepted: November, 2023; Online Published: January, 2024)
Online First in www.entosocindia.org and indianentomology.org Ref. No. e23467