RESEARCH ARTICLE

Impact of Neonicotinoid Insecticides on the Foraging Activity of Indian Honey Bee, *Apiscerana indica* (Fab.) in the Cotton Ecosystem

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ABSTRACT

Indian honey bees are the important managed pollinators of several agricultural and horticultural crops in India. At present, bee colony decline is the biggest crisis amongbeekeepers. The use of neonicotinoid insecticides is considered the prime factor, and they were found to cause a direct impact on bees by mortality and indirectly impair the foraging behavior of bees. Hence, the study aimed to assess the impact of neonicotinoids on the foraging activity of Indian honey bees. The neonicotinoids *viz*., imidacloprid 17.8 SL, clothianidin 50 WDG, thiamethoxam 25 WG, and thiacloprid 21.7 SC, along with organophosphate dimethoate 30 EC (chemical check) and control (no spray) were sprayed at field recommended doses on cotton crop. Foraging activity of the bees,*viz.,*incoming foragers with nectar and pollen load and outgoing foragers were counted at the hive entrance during the morning (09.00-11.00), afternoon (13.00-15.00), and evening (16.00-18.00) hours of the day. The data were recorded at pre-treatment count and post-treatment count on different day intervals *viz*., 1DAS, 3DAS, 7DAS, and 15DAS. The mean number of incoming nectar, pollen foragers, and outgoing foragers were recorded to be maximum in control than neonicotinoid-treated plots. Hence, the reduction in foraging activity may lead to areduction in the food storage area and brood area ultimately lesseningthe overall colony growth. It's always better to avoid spraying cotton crop during the flowering period using neonicotinoids to dodge the residues even though cotton is not a food crop; meanwhile, it will safeguard the pollinators.

Keywords: *Indian honey bee; Pesticides; Neonicotinoids; Foraging activity; Cotton*

INTRODUCTION

Honey bees provide pollination services to several cultivated and wild species, thereby, maintaining biological diversity and also offering valuable hive products (Frankie *et al.,* 2009). Pollinator health is receiving increased attention as managed pollinators, and native pollinator populations are declining worldwide (Vanengelsdorp *et al.,* 2008; Kluser, 2010). The multiple factors that are suspected as responsible for colony loss, but are not limited to, including pesticides, electromagnetic waves, habitat fragmentation, use of genetically modified crops, climatic factors, the occurrence of pests and diseases (Alaux*et al.,* 2008). Among them, the most important cause is the use of various kinds of noxious pesticides, especially insecticides. Since some of the insecticides are tasteless and odorless compounds, bees are not able to differentiate between these treated and untreated crops (Kesseler *et al.,* 2015). After the application of such insecticides on crops, the honey bees are attracted largely which leads to lethal and sub-lethal problems. In intensive farming systems approach to guard sustained and enhanced crop yields, the use of chemical insecticides, which save about one-fifth of the crop yield, are vital and mandated to manage devastative and destructive insect pests infesting crops (Oerke, 2006). The insecticides used to suppress insect populations in farms can also affect non-target beneficial insects, including pollinators (Giri *et al.,* 2017).

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Among the different classes of chemical insecticides, the use of recently introduced neonicotinoid insecticides has been specifically pointed out as a key factor contributing to a sharp decline of both managed and wild bee populations (Goulson *et al.,* 2013). Neonicotinoids are the major class of insecticides that have outstanding potency and systemic action against sucking insect pests harboring crop plants. Among neonicotinoids, imidacloprid, introduced in 1991, is the most extensively used one (Yamamoto *et al.,* 1999), and that was followed subsequently by acetamiprid, nitenpyram, thiamethoxam, thiacloprid, clothianidin, and dinotefuran.

Neonicotinoids are translocated into pollen and nectar, the principal food source for bees (Ghosh *et al.,* 2014). Foraging bees used to collect insecticide-contaminated pollen from treated crops and store it in the brood frames. Nurse bees feed the contaminated pollen and nectar to the developing brood. This resulted in the total loss of the colony while foraging bees are getting killed when involved with the collection and transportation of contaminated pollen, whereas nurse bees are killed while storing and feeding pollen to colony members and the broods are destroyed by consuming unassumingly poisoned pollen and nectar. Moreover, after applying insecticides to crops, some of the compounds are present in the environment (soil, water, treated plants) and degraded slowly, leading to residual poisoning (Iwasa*et al.,* 2004).

By strongly binding to nicotinic acetylcholine receptors (nAChRs) present in the central nervous system of insects, neonicotinoids cause receptor blockage, paralysis, and death (Tomizawa and Casida, 2009; Uragayala*et al.,* 2015). The sucking pests, including aphids, whiteflies, planthoppers, scale insects, and a few soil-inhabiting insects, are managed by these neonicotinoids ((Zhang *et al.,* 2010). Today approximately 60% of all neonicotinoid applications are as soil/seed treatments and most spray applications are directed against pests attacking crops such as cereals, vegetables, fibre crops, fruits, flowers and ornamental plants (Jeschke*et al.,* 2011).

Cotton (*Gossypium hirsutum* L.) is an important cash crop that is grown for its fibre and seed oil worldwide (Gunasekaran *et al.,* 2020). Nectar is secreted by floral nectaries found inside the flower and by extrafloral nectaries on the outer or sub-bracteal, foliar, and unipapillate areas on the flower peduncles and young leaf petioles (McGregor, 1976). Since the presence of copious nectar secreting sites and the production of more pollen, cotton attracts more honey bees as foragers (Rhodes, 2002). After the introduction of Bt cotton, the importance of sucking pest damage had increased (Kranthi and Stone, 2020) due to the withdrawal of insecticide umbrella spread for bollworm complex management. Due to phenomenal success achieved in suppressing sucking insects on cotton with the introduction of imidacloprid and other neonicotinoids, farmers were indiscriminately applying them through seed treatment and foliar spray (Murugesan and Kaitha, 2009). The neonicotinoids as residues are also present all over the plant parts for a prolonged period and the foraging behaviour of bees and other pollinators was affected when they were exposed to those residues. With this background, the present study was conducted to recognize the neonicotinoid impact on the foraging activity of the Indian honey bee, *Apis cerana indica* Fab. *Template Specifications*

MATRIALS AND METHODS

The current study was carried out at the cotton farm, Department of Cotton, Tamil Nadu Agricultural University, Coimbatore from September 2019 to January 2020. The experimental site was situated at 11° 02'N latitude, 76° 92'E longitude, and at an altitude of 152m above mean sea level. The experimental farm was characterized by a tropical climate with good rainfall during monsoons and the soil type was clay loam in texture. The cotton variety, CO 17 was selected for the study because of the distinguishing morphological characteristics *viz*., medium height (100cm), sparse stem hairiness, flowering at 53 days, medium-sized boll, 35% ginning per cent, mature at 135 days, and yields 2361 Kgha^{-1} seed cotton (Gunasekaran *et al.,* 2020). It was raised at a 60 x 20 cm spacing by adopting all recommended agronomic practices. Several sucking pests were documented and necessarily warranted insecticides spraying as an intervention during the experiment.

Spray of insecticides

The foliar spray of different insecticides was given at once during blooming (after 50% flowering) of cotton crop with neonicotinoids *viz*., imidacloprid 17.8 SL @ 280µL/L, clothianidin 50 WDG @ 80µG/L, thiamethoxam 25 WG @ 250µG/L, and thiacloprid 21.7 SC @ 1100µL/L, organophosphorus dimethoate 30 EC @ 1400µL/L (chemical check) and control (no spray) at recommended doses (CIBRC, 2021) (Table 1) with respective dilutions using hand-operated knapsack sprayer (VBD09: 33.5 x 14.0 x 47.0cm). Among these neonicotinoids, imidacloprid, clothianidin, and thiamethoxam belong

Table 1. List of different insecticides used for spraying in the cotton field

Treatment	Dose (g ai/ha)	Dose (ai/l)
Imidacloprid 17.8 SL	25	280µL/L
Clothianidin 50 WDG	20	0.08µG/L
Thiamethoxam 25 WG	30	0.250µG/L
Thiacloprid 21.7 SC	120	1100µL/L
Dimethoate 30 EC (Chemical Check)	200	1400µL/L
Control	-	-

to the nitro-substitution group while thiacloprid belongs to the cyano-substitution group. Dimethoate served as a standard chemical check used in any toxicity analysis study involving honey bees (Gough *et al.,* 1994). The experiment was laid out in a Randomized Block Design (RBD) with six treatments and four replications. The individual plot was sized 10m x 5m and each three meters isolation distance between plots was maintained to avoid the pesticide drift effects while spraying. The individual plots were confined in nylon net (Length: 10m, Width: 5m, Height: 2m, and Mesh size: 1mm) immediately after 20% flowering of cotton. These nets were used to avoid the escape of bees during the experiment. Indian bee hives with equal strength and abundant food storage and same-aged queen were selected and were kept inside the plot at one hive per plot and were well maintained with frequent water supply to avoid overheating and forage space confinement with netting.

Differences in foraging activity of honey bee

Foraging activity of the bees, *viz.,* incoming nectar, and pollen foragers and outgoing foragers were counted by *in situ* counting method. The observations were taken in front of the hive entrance during the morning (09.00-11.00), afternoon (13.00-15.00), and evening (16.00-18.00) hours of the day in each plot. The bees with pollen load were considered pollen foragers while without pollen load were considered nectar foragers. Likewise, the bees departing the colony were considered an outgoing foragers. The number of incoming and outgoing foragers was counted for 1 min at each hourly interval and expressed as mean foragers per 1 min. The data were recorded at pre-treatment count (PTC) followed by post-treatment count at different day intervals *viz*., 1DAS, 3DAS, 7DAS, and 15DAS (Days After Spray).

Statistical analysis

The values, after square root transformation, were analyzed by using a one-way analysis of variance (ANOVA) (Panse and Sukhatme, 1954) and PROC GLM in the Statistical Analysis Software programme (SAS academics) (SAS Institute, 1985). The means, when significant, were separated by using Tukey's studentized range (honestly significant difference) test procedure (P<0.05).

RESULTS AND DISCUSSION

The negative impact of neonicotinoids on the bee's foraging activity was recorded at specific time intervals *viz*., 1 DAS, 3 DAS, 7 DAS, and 15 DAS. The mean incoming nectar foragers were significantly maximum in control with 8.34 foragers/1min and was followed by dimethoate (5.02), thiacloprid (4.33), imidacloprid (3.97), thiamethoxam (3.65) and clothianidin (3.37) (F=25.48, df=15, P=<0.0001) (Table 2). In control, the forager's activity was regular as observed throughout the study period than in other treatments. The organophosphate insecticide dimethoate was considered highly toxic to bees (NPIC fact sheet, 2022) but, it had recorded significantly less interference effect on the foraging activity of bees than neonicotinoids. Even at 15 DAS, the forager's activity remained significantly different and with regular activity being noticed in control (9.06), while it was significantly depressed in dimethoate (4.00), and however, was steeply reduced in thiacloprid (2.17), thiamethoxam (1.19), imidacloprid (1.17) and clothianidin (0.78) and across the DAS (1 DAS to 15 DAS), the foraging activity did not significantly differ (F=21.14, df=15, P=<0.0001). Among all the treatments, clothianidin registered a highly significant negative impact at different intervals *viz*., 1 DAS (5.00), 3 DAS (4.22), 7 DAS (1.17), and 15 DAS (0.78). This indirectly might influence the food storage abilities of the colony due to reduced incoming nectar foragers.

The mean incoming pollen foragers were maximum in control (5.90), followed by clothianidin (3.66), thiamethoxam (3.41), dimethoate (3.17), thiacloprid (3.12) and imidacloprid (2.90) (F=15.39, df=15, P=<0.0001) (Table 3). Here also the forager's activity was constant and high in control for all the intervals *viz*., 1 DAS (6.00), 3 DAS (6.47), 7 DAS (6.33) and 15 DAS (6.72). At 15 DAS, the maximum was observed in control (6.72), followed by dimethoate (3.00), thiacloprid (1.89), clothianidin (1.36), imidacloprid (0.97) and thiamethoxam (0.92) (F=8.48, df=15, P=0.0006). Very low activity was observed in imidacloprid at 1 DAS (3.69), 3 DAS (3.61), 7 DAS (3.53) and 15 DAS (2.69).

The results indicated that pollen foragers were not preferred to forage on imidacloprid treated flowers than nectar foragers which probably leads to a reduction in the pollen storage area. Hence, this food shortage primes to lessening of brood area followed by declining overall colony growth and performance.

The mean outgoing foragers were maximum in control (10.75) followed by dimethoate (6.77), imidacloprid (6.08), thiacloprid (5.48), thiamethoxam (5.42), and clothianidin (5.07) (F=17.59, df=15, P=<0.0001) (Table 4). Moreover, increased forage activity was observed in control due to the increment of incoming nectar and pollen foragers for all the intervals *viz*., 1 DAS (11.14), 3 DAS (11.47), 7 DAS (11.17), and 15 DAS (11.42). At 15 DAS, the maximum bee forage activity was observed in the same as control (11.42), followed by dimethoate (5.17), thiacloprid (3.50), imidacloprid (2.06), clothianidin (2.00) and thiamethoxam (1.83) (F=8.89, df=15, P=0.0004). Very low activity was observed in clothianidin at 1 DAS (6.47), 3 DAS (6.97), 7 DAS (3.11), and 15 DAS (2.00). The overall results indicated that the falling of outgoing foragers in clothianidin might result from the mortality of the foraged bees not returned to the colony. The overall incoming nectar, pollen, and outgoing foragers rates were high in control throughout the study period (Figure 1). At the same time, all the insecticides have shown reduced foraging activity, including dimethoate. It clearly visualizes the harmful effect of insecticides on honey bees. Since these neonicotinoids are targeting AChR, foragers might lose their memory to return to the home. Decreasing outgoing foragers indirectly paves a way for dropping off the incoming foragers, leading to overall colony loss.

The difference in the activity of incoming and outgoing foragers showed the harmful effect of neonicotinoids on bees foraging behavior, which led to destruction of the foraging behavior that is most essential to maintain the colony in good strength. A high dose of pesticides caused extreme levels of death of bees, while sub-lethal doses resulted in behavioral changes like loss of navigation and communication ability, followed by homing failure (Desneux *et al*., 2007; Sanford,2011). In this study, we found that there was reduced foragers activity in the overall experimental period after exposure to the field recommended dose of insecticides than control. The low concentrated pesticides were considered safe for honey bees but influenced foraging behavior drastically (Mommaerts *et al.,* 2010). Colin *et al.* (2004) also reported 70 times low LD50 of deltamethrin (LD50 = 67ng/bee) had shown disorientation of foraging bees. Tison*et al.* (2016) studied a sublethal dose of thiacloprid on *Apis mellifera carnica* that impaired foraging behavior, homing success, navigation performance, and social communication. In our study, all the neonicotinoid-treated plots recorded less foraging activity than dimethoate treated and control plots. This had evidenced the negative impact of neonicotinoids especially impaired foraging behavior in bees.

Gill *et al.* (2012) monitored colonies after exposure to imidacloprid, L-cyhalothrin, and their mix, and found reduced worker foraging performance, particularly pollen collecting efficiency, forager recruitment, worker losses, and overall worker productivity. Consequently, they found that a high rate of workers was getting lost in colonies only in imidacloprid treatment either alone (50%) or in mix (55%) application than control (3.1%). In our results, both incoming nectar and pollen foragers' activity was reduced in neonicotinoid exposed bee colonies (Table 2&3). They also reported that imidacloprid-exposed foragers returned with smaller pollen loads, increased foraging duration, and reduced foraging bout. It showed that imidacloprid-exposed workers were less efficient at collecting pollen in the field. The same results were observed in our study that, very less pollen (0.97), nectar foragers (1.17), and outgoing foragers (2.06) were found in imidacloprid treated hives at 15 DAS (Tables 2-4). The study not only focused on imidacloprid, but also on other neonicotinoids residue in pollen and nectar, which had caused impairment to nectar and pollen foraging efficiency, leading to increased demand for food that leads to colony loss too. Hameed and Singh (1998) also indicated that pollinating bees were directly exposed to insecticidal sprays and the left-over insecticides on crops resulting in reduced foraging thereafter for a few weeks.

Acute or chronic effects of thiamethoxam on the foraging ability of foragers were studied using foraging mills (Tosi *et al.* 2017). At acute sublethal dose (1.34ng/bee) of thiamethoxam, excitation, and increase in foraging duration (+78%) and distance (+72%) and at the same time in chronic exposure, decrease in foraging duration (−54%), distance (−56%) were noted. However, increased foraging duration and distance were not beneficial due to the development of foraging disorientation (Fischer *et al.,* 2014). These results indicated that acute or chronic exposure to a neonicotinoid can alter bee foraging and impair foraging and homing aspects. Studies of foraging behavior in bumble bees using a low concentration of neonicotinoids had shown impairment in homing behavior (Henry *et al.,* 2012), foraging behaviour (Schneider *et al.,* 2012), reduced colony growth and queen production as well (Whitehorn *et al.,* 2012; Gill *et al.,* 2012). In the previous study, we documented bees prefer to forage on neonicotinoid treated sunflowers with a little bit of early aversion to open field conditions (Sowmiya *et al.,* 2022). This initial day repellence was due to high concentration of insecticides whereas, after degradation at later period which led to more bee activity. However, bees were not able to differentiate neonicotinoids treated and untreated crops which leads to increased negative effect. But, in dimethoate, that aversion was prolonged throughout the blooming period which might be due to the presence of an unpleasant odour on the treated flowers.

In the present study, an acute dose of neonicotinoids at a recommended dose was applied, which resulted in reduced foraging activity than the control. Because of insensitivity towards neonicotinoids rather than brood care, the loss of foragers seemed to affect brood development, resulting in reduced worker production that leads to an entire colony decline. Honey bees play a major role in the pollination of cotton and colonies can be placed near cotton fields at the time of flowering to enhance seed cotton yield. But, the high insecticide sprays in the cotton ecosystem would cause pollinators decline (Sinduja *et al.,* 2016), and the same was understood for the use of neonicotinoids in cotton ecosystem through the present study.

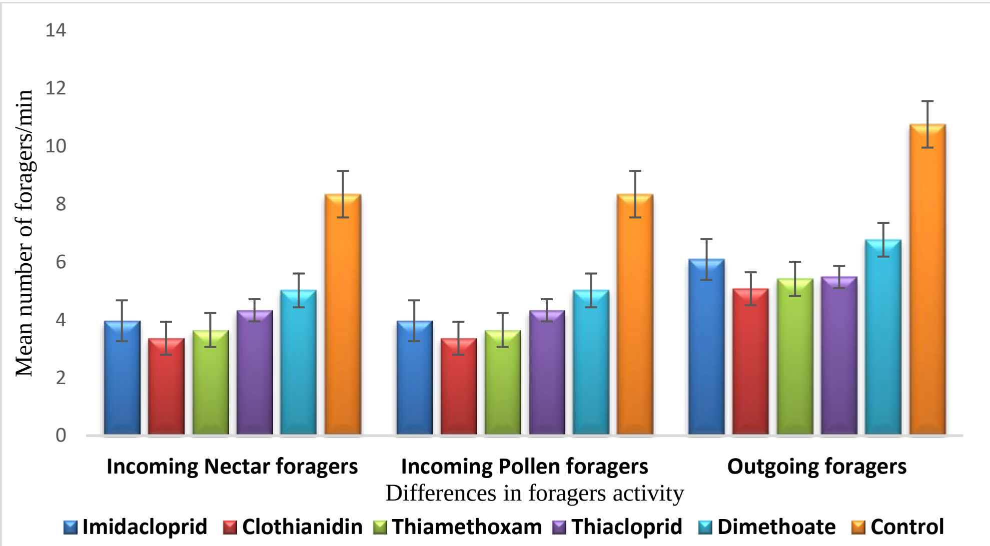

Figure 1. Differences in mean foraging activity, *viz*., incoming nectar and pollen foragers and outgoing foragers of Indian honey bees after exposure to different insecticides during different hourly intervals of morning, afternoon and evening time of a day at different days after spraying

Table 4. Effect of neonicotinoid insecticides against Indian honey bee, *A. c. indica* foraging activity as reflected in outgoing foraging bees recorded during different hourly intervals of morning, afternoon and evening time of a day on different days after spraying

	Mean number of outgoing foragers/min					
Treatment	PTC	1 DAS	3 DAS	7 DAS	15 DAS	Mean
Imidacloprid	8.53 [a] (3.00)	7.78 [b] (2.87)	6.78 [b] (2.70)	5.28 [b] (2.40)	2.06 [b] (1.46)	6.08 [b] (2.49)
Clothianidin	6.81 [a] (2.70)	6.47 [b] (2.64)	6.97 [b] (2.73)	3.11 [b] (1.80)	2.00 [b] (1.44)	5.07 [b] (2.26)
Thiamethoxam	7.97 [a] (2.91)	7.14 [b] (2.76)	6.22 [b] (2.59)	3.92 [b] (2.10)	1.83 [b] (1.46)	5.42 [b] (2.36)
Thiacloprid	7.33 [a] (2.80)	6.75 [b] (2.69)	5.67 [b] (2.48)	4.14 [b] (2.15)	3.50 [b] (1.99)	5.48 [b] (2.42)
Dimethoate	8.67 [a] (3.02)	9.33 [ab] (3.12)	5.67 [b] (2.48)	5.00 [b] (2.33)	5.17 [ab] (2.38)	6.77 [b] (2.67)
Control	8.56 [a] (3.01)	11.14 [a] (3.41)	11.47 [a] (3.45)	11.17 [a] (3.41)	11.42 [a] (3.45)	10.75 [a] (3.35)
SE	NS	0.74	0.90	1.19	1.51	0.87
CD (P=0.05)	NS	0.0001	0.0001	0.0002	0.0004	<0.0001

Table 2. Effect of neonicotinoid insecticides against Indian honey bee, *A. c. indica* foraging activity as reflected in incoming nectar foraging bees recorded during different hourly intervals of morning, afternoon and evening time of a day on different days after spraying

	Mean number of incoming nectar foragers/min					
Treatment	PTC	1 DAS	3 DAS	7 DAS	15 DAS	Mean
Imidacloprid	6.08 [a] (2.56)	5.17 [b] (2.38)	4.39 [b] (2.21)	3.03 [b] (1.86)	1.17 [c] (1.22)	3.97 [bc] (2.05)
Clothianidin	5.67 [a] (2.48)	5.00 [b] (2.34)	4.22 [b] (2.17)	1.17 [c] (1.24)	0.78 [c] (1.10)	3.37 [c] (1.86)
Thiamethoxam	6.17 [a] (2.58)	4.53 [b] (2.24)	4.19 [b] (2.16)	2.17 [bc] (1.62)	1.19 [c] (1.26)	3.65 [c] (1.97)
Thiacloprid	6.61 [a] (2.67)	5.08 [b] (2.36)	4.50 [b] (2.23)	3.28 [b] (1.93)	2.17 [bc] (1.62)	4.33 [bc] (2.16)
Dimethoate	6.92 [a] (2.72)	5.50 [b] (2.45)	4.67 [b] (2.27)	4.00 [b] (2.12)	4.00 [b] (2.12)	5.02 [b] (2.34)
Control	6.78 [a] (2.69)	8.94 [a] (3.07)	9.28 [a] (3.12)	7.64 [a] (2.85)	9.06 [a] (3.09)	8.34 [a] (2.97)
SE	NS	0.66	0.82	0.91	1.29	0.75
CD (P=0.05)	NS	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

PTC-Pre-treatment count, DAS–Day after spray. NS: Not Significant.
Figures in parentheses are √(x+0.5) transformed values. Mean values followed by the same superscript (s) in the columns do not differ significantly by Tukey at P=0.05 level.

Table 3. Effect of neonicotinoid insecticides against Indian honey bee, *A. c. indica* foraging activity as reflected in incoming pollen foraging bees recorded during different hourly intervals of morning, afternoon and evening time of a day at different days after spraying

	Mean number of incoming pollen foragers/min					
Treatment	PTC	1 DAS	3 DAS	7 DAS	15 DAS	Mean
Imidacloprid	3.69 [a] (2.04)	3.61 [b] (2.02)	3.53 [c] (2.00)	2.69 [b] (1.79)	0.97 [b] (1.16)	2.90 [b] (1.80)
Clothianidin	3.36 [a] (1.96)	4.17 [b] (2.16)	5.50 [a] (2.45)	3.89 [b] (2.08)	1.36 [b] (1.19)	3.66 [b] (1.97)
Thiamethoxam	4.17 [a] (2.16)	4.06 [b] (2.13)	5.33 [ab] (2.41)	2.56 [b] (1.73)	0.92 [b] (1.16)	3.41 [b] (1.92)
Thiacloprid	3.94 [a] (2.11)	3.86 [b] (2.09)	3.61 [bc] (2.03)	2.28 [b] (1.66)	1.89 [b] (1.53)	3.12 [b] (1.88)
Dimethoate	3.17 [a] (1.91)	2.92 [b] (1.84)	3.50 [c] (2.00)	3.25 [b] (1.93)	3.00 [b] (1.87)	3.17 [b] (1.91)
Control	3.97 [a] (2.11)	6.00 [a] (2.54)	6.47 [a] (2.63)	6.33 [a] (2.61)	6.72 [a] (2.69)	5.90 [a] (2.52)
SE	NS	0.42	0.52	0.61	0.91	0.45
CD (P=0.05)	NS	0.0003	0.0002	0.0002	0.0006	<0.0001

PTC-Pre-treatment count, DAS–Day after spray. NS: Not Significant.
Figures in parentheses are √(x+0.5) transformed values. Mean values followed by the same superscript (s) in the columns do not differ significantly by Tukey at P=0.05 level.

CONCLUSION

The negative impact of neonicotinoids on the foraging activity of Indian honey bees visiting cotton crop during blooming was established in the present study. There was a reduced number of incoming nectar and pollen foragers and outgoing foragers in the neonicotinoid sprayed plots throughout the study period compared to control. The chemical check dimethoate created aversion due to noxious odour which the bees were able to sense and that sensing disappeared with neonicotinoids treated surfaces when days post spraying time progressed. Reduction in nectar and pollen foragers reduces honey and pollen storage area in colonies and is followed by reduced brood area that ultimately reduces the overall colony growth. Hence, the food and brood storage area loss rapidly led to colony loss. These findings also confirmed that exposure specifically to nitro-substituted neonicotinoids including imidacloprid, clothianidin, and thiamethoxam caused a heightened adverse effect on foraging behaviour of honey bees. Our observations show that farmers should avoid spraying insecticides, especially nitro-substituted neonicotinoids during the cotton flowering period even though it was not a food crop.

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Ethics statement

No specific permits were required for the described field studies because no human or animal subjects were involved in this research.

Consent for publication

All the authors agreed to publish the content.

Competing interests

There was no conflict of interest in the publication of this content

Data availability

All the data of this manuscript are included in the MS. No separate external data source is required.

Author contributions

Research work-CS, Idea conceptualization-MM, CS, Experiments-CS, PAS, Guidance–MM, PAS,AS,KB,JM,SN, writing original draft –CS,MM,PAS, Writing- reviewing &editing-CS,MM.

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