Original Research Article

" Pollination Peril: The Impact of Neonicotinoids' ~~Impact~~ on Foraging Behaviour of Indian Honey Bee, *Apis cerana* Fab. (Hymenoptera; Apidae) ~~Foraging~~ in Sunflower Fields"

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ABSTRACT

Honey bees are essential for sunflower pollination, which boosts crop yield and quality. The decline in bee populations, worsened by neonicotinoid insecticides, poses a significant threat to agriculture. This study ~~examin.es~~ examines the effects of neonicotinoids -specifically imidacloprid and thiamethoxam -on the foraging activity and health of *Apis cerana* Fabricius within sunflower crops. Sunflower seeds (RHA-92) were sown and managed according to standard agricultural practices, with the field divided into three sub-blocks (30 x 40 feet), each receiving one of three treatments: imidacloprid, thiamethoxam, or ~~an~~ untreated control. Insecticides were applied at recommended doses when 50 per cent of the plants were in the flowering stage. ~~To prevent external contamin.ation, the~~ The crops were covered with ~~a~~ nylon net before flowering to prevent external contamination. A colony of *Apis cerana*,~~,~~ was introduced into each plot one day after insecticide application. Foraging activity, bee behavior, and colony health were monitored daily for seven days post-application, with bee visits to flower heads recorded hourly. Results showed a significant decrease in foraging activity in treated plots, with imidacloprid causing the most pronounced reduction - from 5.40 bees/head/5 min. on day one to 2.13 bees/head/5 min. by day three. Thiamethoxam also led to reduced activity but to a lesser extent. The untreated control maintained stable foraging levels throughout the study. These findings underscore that neonicotinoid insecticides, particularly imidacloprid, severely impair the foraging behavior of *Apis cerana,* highlighting the need for careful use of these chemicals to mitigate their adverse effects on bee populations and ensure effective pollination in agriculture.

Keywords: *Apis cerana*,*Foraging activity, Imidacloprid, Sunflower, Thiamethoxam,*

1. INTRODUCTION

Honey bees are essential to global agriculture due to their pivotal role in pollinating crops, which enhances yield and quality (1). They are particularly vital for sunflower (*Helianthus annuus*), a major oilseed crop. The process of pollination by bees boosts seed set, weight, and oil content, underscoring their importance in sunflower cultivation (2). However, the decline in bee populations worldwide is a growing concern, with pesticide use -especially neonicotinoids - being a significant factor (3). Neonicotinoids, systemic insecticides that mimic nicotine, are widely used to manage pest insects but pose risks to beneficial pollinators like honey bees.

Neonicotinoids, including imidacloprid and thiamethoxam are insecticides ~~modeled~~modelled after nicotine, targeting the nervous systems of insects by binding to nicotinic acetylcholine receptors, causing paralysis and death (4). Imidacloprid, introduced in the 1990s, quickly gained popularity due to its effectiveness against sap-feeding and leaf-chewing pests (5). It is used as a foliar spray, soil treatment, or seed coating in various crops, including sunflowers. In sunflower ~~farmin.g~~farming, imidacloprid is often applied to control pests like aphids and flea beetles, which can severely damage plants and reduce yields. Its systemic nature allows it to be absorbed by plants, providing prolonged protection as the plant grows (6). However, this systemic property also leads to the accumulation of imidacloprid residues in pollen and nectar, exposing honey bees and other pollinators to the insecticide during foraging.

Honey bees are exposed to imidacloprid through various pathways in sunflower ~~agriculture~~farming. The most direct route is through the consumption of ~~contamin.ated~~contaminated pollen and nectar. Research has shown that neonicotinoids can be present in both at concentrations harmful to honey bees (7). Another significant exposure route occurs through dust released during the planting of treated seeds. When neonicotinoid-coated seeds are planted, dust containing the insecticide can be dispersed into the air and settle on nearby flowers, posing a risk to foraging bees (8-9). Additionally~~,~~ ~~can~~, can contamin~~.~~ate water sources such as puddles or dew, which honey bees may drink, further increasing their exposure.

The effects of neonicotinoids on honey bees are well documented. At lethal doses, imidacloprid causes direct mortality. However, even at sublethal doses - those that do not cause immediate death these insecticides can significantly impair honey bee behaviour and physiology (10). For example, sublethal exposure has been shown to reduce honey bees' ability to forage effectively (11), leading to decreased nectar and pollen collection (12-13). This reduction can result in dimin~~.~~ished food availability for the colony (14), affecting its health and survival.

In addition to impaired foraging, neonicotinoids has been found to disrupt honey bees' navigation abilities. Honey bees rely on spatial memory and orientation skills to locate food sources and return to the hive (15). Studies indicate that imidacloprid exposure can cause disorientation, increasing the likelihood of bees getting lost and failing to return to the hive (16). This disorientation is linked to Colony Collapse Disorder (CCD), a syndrome where most worker bees disappear from a colony, leaving behind the queen and a few remaining bees (17). While CCD is multifactorial, with contributions from pathogens, habitat loss, and climate change, neonicotinoid exposure is considered a significant factor (18). Similarly, thiamethoxam is also known to cause negative effects on homin~~.~~g flights in honey bee foragers (12). Furthermore, thiamethoxam and its metabolite clothianidin lead to a significant reduction in foraging activity and longer foraging trips in exposed foragers (19), inhibit the honey bee immune system and detoxification genes.

The decline of honey bee populations due to neonicotinoids exposure has significant implications for agricultural productivity and biodiversity (20). Honey bees are essential for sunflower pollination, and their reduced populations can lead to lower pollination rates, decreased yields, and reduced seed quality (2). This decline not only impacts sunflower farmers economically but also poses broader ecological risks. Honey bees contribute to the pollination of many wild plants that are crucial for maintaining biodiversity and providing food and habitat for other wildlife (21). The loss of honey bees could trigger cascading effects on ecosystems, reducing plant diversity and threatening species that depend on these plants.

In India, research studies on impact of neonicotinoids toxicity on Indian honey bees are minimal~~is min.imal~~. Hence, ~~The~~the present investigations were focused on the impact of neonicotinoids particularly on the foraging activity and colony performance of *Apis cerana* Fabricius (Hymenoptera: Apidae*)* when bees forage on plants sprayed with imidacloprid and thiamethoxam*.*

2. MATERIAL AND METHODS

2.1. Experimental Setup

The study aimed to assess the impact of neonicotinoid insecticides on honey bee colony health in sunflower crops, which are primarily pollinated by honey bees. Sunflower seeds of the variety RHA-92 were sown~~,~~ and all crop management practices were followed according to the guidelines from the University of Agricultural Sciences (UAS), Bangalore.

2.2. Treatments Details

The sunflower field was divided into three sub-blocks, each measuring 30 x 40 feet. Three different treatments were applied: Imidacloprid 17.8 SL ®, Thiamethoxam 25 WG ®, and an untreated control. To prevent cross-contamin~~-~~ation and external foraging, the crop was covered with a nylon net (2 mm mesh) before flowering commenced. Insecticides were sprayed when 50% of the plants reached the flowering stage (62-65 days after sowing). Two commonly used neonicotinoid insecticides ~~-~~ *viz.,* imidacloprid and thiamethoxam - were applied at field-recommended doses using a knapsack sprayer in separate blocks. The control block received only water. Table 1 provides details of the insecticides and their concentrations used in the experiment. One colony of honey bees (*Apis cerana*) consisting of five frames was placed inside the net in each experimental plot one day after insecticide application.

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Table 1: Details of treatment imposed during field assessment of effect of neonicotinoids on honey bees.

Sl.No	Treatments	Formulation	Dosage (g ai/ha)
1	Imidacloprid	17.8 SL	27 g *a.i.*/ha
2	Thiamethoxam	25 WG	38 g *a.i.*/ha
3	Untreated control (Water)	-	-

2.3. Observations

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Foraging activity, bee behaviour, and colony health were monitored until the end of the flowering stage. In each experimental plot, five flower heads (capitula) were randomly selected before the treatment. Honey bee activity on these flower heads was recorded for five min~~.~~utes at hourly intervals between 7:00 AM and 6:00 PM. The peak period of honey bee activity was noted for each plot. Observations of honey bee visits to the selected flower heads were conducted daily for seven days post-spraying, with counts expressed as visits per flower per five ~~min.utes~~minutes.

Additional observations included monitoring behavioural changes, uncoordinated movements, and overall colony health over the seven-day period following insecticide application.The study aimed to assess the impact of neonicotinoid insecticides on the health of honey bee colonies within a sunflower crop, which relies heavily on pollination by honey bees. The combined observations from these experimental plots provided critical insights into the short-term effects of neonicotinoid insecticides on honey bee colonies, specifically their foraging patterns, behaviour, and health when exposed to treated sunflower crops.

2.4. Statistical analysis

The data related to the foraging activity of bees was analysed for 't' test statistical software SPSS® (version 25). Here, the foraging activity of bees across the treatments at different days ~~–~~were compared. The figure was drawn using Tableau Desktop® 2022.1 to represent the foraging activity of honey bees at different days after exposure of treated plots

3. RESULTS AND DISCUSSION

Following the spraying of insecticides on sunflower heads, bee foraging activity decreased significantly, particularly in imidacloprid-treated plots. One day after spraying, imidacloprid plots had the lowest bee activity (5.40 bees/head/5 min.), followed by thiamethoxam (5.86 bees/head/5 min.), while the untreated control recorded 6.00 bees/head/5 min.~~.~~ By day two, bee activity further decreased in imidacloprid (4.67 bees/head/5 min.) and thiamethoxam (5.20 bees/head/5 min.) plots, with the control remaining stable (6.53 bees/head/5 min.). The decline was most pronounced on day three, with imidacloprid showing 2.13 bees/head/5 min. and thiamethoxam 3.20 bees/head/5 min., compared to the control (6.67 bees/head/5 min.). This trend continued with min~~.~~imal recovery by day eight, where foraging in treated plots (imidacloprid: 3.67; thiamethoxam: 4.67 bees/head/5 min.) remained significantly lower than the untreated control (7.33 bees/head/5 min.).

Among different treatments, imidacloprid spray on sunflower plants registered ~~caused~~ maximum reduction (3.38 bees/head/5 min.) in foraging activity of *Apis cerana* bees and ~~was~~ significantly differed from thiamethoxam (3.95 bees/head/5 min.). However, significantly higher activity was recorded in control plot (6.48 bees/head/5 min.)

In the present study, a significant reduction in foraging activity of *A. cerana* bees on sunflower was noticed in neonicotinoids insecticide treated plots as compared to the untreated control. Further, across the different days after spraying of neonicotinoids, foraging activity of bees reduced significantly from 3rd to 6th day after spraying. Despite there was increase in foraging activity of bees on sunflower heads, extent of bee activity was low in sprayed plots until six days after spraying.Additionally un co-ordinated movements such as trembling, abdomen upside down, wing vibrations and paralysis of bees ~~was~~ were also recorded.

The results of our study align with findings from Chandrakumar *et al*. (22), ~~which~~ who observed a reduction in bee foraging activity up to five days after insecticide application, followed by a gradual recovery to near-normal levels. Although our study also noted an increase in foraging activity post-spray, bee populations did not reach the levels recorded in untreated plots. Previous research similarly reported a decrease in foraging activity in response to insecticides like imidacloprid within 24 hours of application, with significant recovery observed after three days and normalization by seven days under field conditions (23). Giri *et al.* (24) also documented a notable decline in foraging activity of *Apis mellifera* up to seven days after thiamethoxam application on mustard blooms. Studies have indicated that pesticide exposure impairs pollen collection efficiency, with significant reductions in foraging activity and prolonged foraging bouts in honey bees exposed to imidacloprid or clothianidin (19). This inhibition is consistent with findings from semi-field studies, which suggest that honey bees exhibit a general reduction in foraging activity, extending even to untreated food sources, rather than a specific aversion to neonicotinoids (25). Sharma et al. (26) reported significant bee mortality at 1, 2, and 3 days following thiamethoxam (0.1 g/lit) and imidacloprid (0.3 ml/lit) sprays. Furthermore, Matre *et al*. (27) found that imidacloprid application affected foraging behaviour, with higher bee visits at a half dose compared to a full dose, while Pashte and Patil (14) observed normal foraging activity resuming by the third day post-spray. Chandrakumar *et al.* (22) noted restoration of bee activity from five days after spraying, reaching levels close to pre-spray conditions by the seventh day. Thiamethoxam has been reported to negatively impact foraging activity for 3 to 4 days following application (28-29). Conversely, Pilling *et al.* (30) reported similar foraging activity in thiamethoxam-treated and control fields, suggesting a negligible impact of the insecticide on bee foraging behaviour.

The observed reduction in foraging activity of *Apis cerana* bees after the application of neonicotinoids, particularly imidacloprid, could be attributed to several factors. Neonicotinoids, such as imidacloprid and thiamethoxam, are known to exert sublethal effects on bees, impairing their cognitive and motor functions. These effects can disrupt navigation, reduce foraging efficiency, and ultimately impact colony health, as suggested by Sluijs *et al.* (31). Furthermore, the confinement of bees under net-covered cropped areas may have exacerbated the negative impact by limiting the bees' ability to disperse, increasing their exposure to the insecticides. This prolonged exposure could result in slower recovery of foraging activity compared to bees in open-field conditions, where foraging behaviour may normalize more quickly due to wider dispersion and less concentrated exposure.

This study confirms the significant negative impact of neonicotinoid insecticides on the foraging activity of *A. cerana* bees. The results highlight the sublethal effects of neonicotinoids, suggesting the need for cautious use of these chemicals to ~~min.imize~~minimize their adverse effects on bee populations and ensure healthy pollination services in agricultural ecosystem.

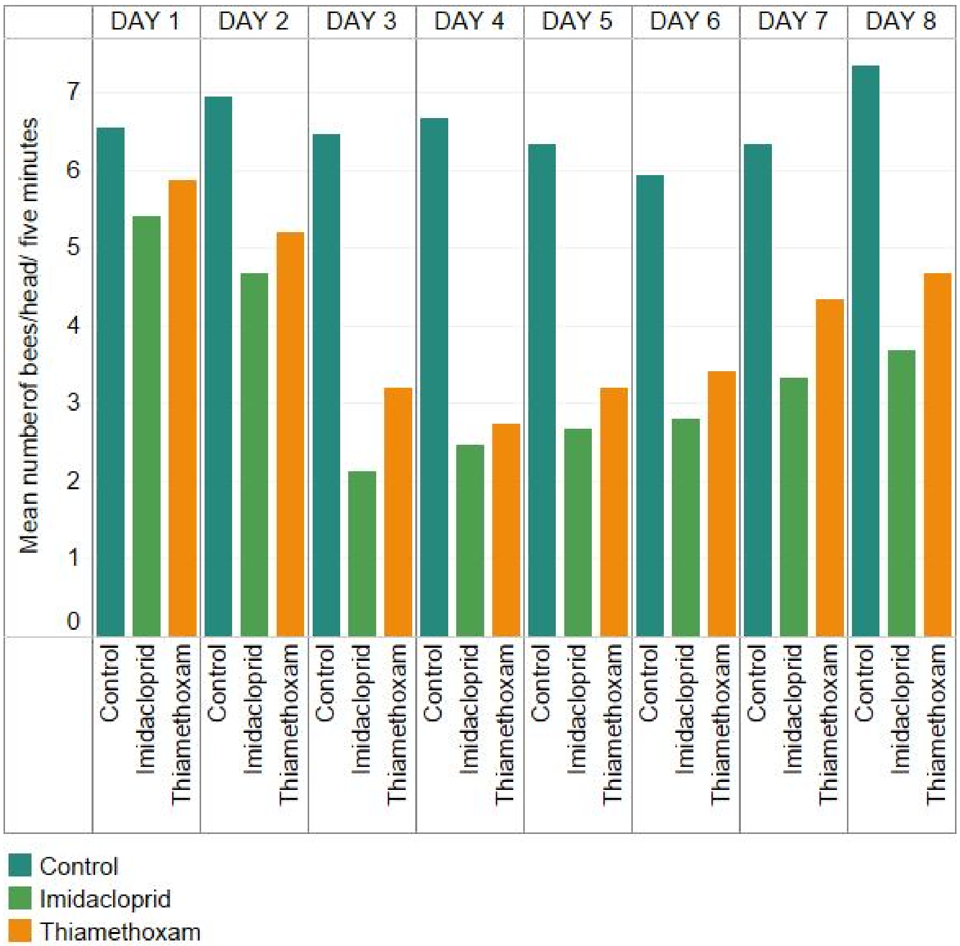

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Fig. 1: Effect of foliar spray with recommended dose of neonicotinoids on foraging activity of *Apis cerana.*

4. CONCLUSION

In conclusion, the impact of neonicotinoids on honey bees, particularly in sunflower agriculture, presents a complex challenge. While these chemicals effectively control pests, their sublethal effects—such as impaired foraging behavioor, cognitive dysfunction, and immune suppression—pose significant threats to pollinator health and ecosystem services. The ongoing debate highlights the need for sustainable agricultural practices that min.imize pesticide use and protect pollinator populations. Collaboration among policymakers, farmers, and scientists is essential to develop strategies that balance crop yields with the preservation of crucial pollinators.

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REFERENCES

1. Kumar S, MC K, Kumar V, Singh T, Maity A, Yadav VK. Diurnal and temporal activity of pronubial insects on berseem flowers in a subtropical environment. J Apic Res. 2021; 1–6
2. Klein AM, Vaissie BE, Cane JH, Dewenter I, Cunningham SA, Kremen C, Tscharntke T. Importance of pollinators in changing landscapes for world crops. Proc R Soc B Biol Sci. 2007; 274(1608):303–313
3. Sanchez-Bayo F, Goka K. Pesticide residues and bees–a risk assessment. PloS one. 2014;9;9(4):94482.
4. Godfray HC, Blacquiere T, Field LM, Hails RS, Potts SG, Raine NE, Vanbergen AJ, McLean AR. A restatement of recent advances in the natural science evidence base concerning neonicotinoid insecticides and insect pollinators. Proc Roy Soc London Ser B BiolSci.. 2015; 7;282(1818):20151821.
5. Brown MJ, Paxton RJ. The conservation of bees: a global perspective. Apidologie. 2009 1;40(3):410-6.
6. Vanbergen AJ, Insect Pollinators Initiative. Threats to an ecosystem service: pressures on pollinators. Front Ecol Environ. 2013;11(5):251-9.
7. Goulson D, Stout JC. Homin.g ability of the bumblebee *Bombus terrestris* (Hymenoptera: Apidae). Apidologie. 2001; 1;32(1):105-11.
8. Blacquiere T, Smagghe G, Van Gestel CA, Mommaerts V. Neonicotinoids in bees: a review on concentrations, side-effects and risk assessment. Ecotox. 2012;21:973-92.
9. Dively GP, Kamel A. Insecticide residues in pollen and nectar of a cucurbit crop and their potential exposure to pollinators. J Agric Food Chem. 2012; 9;60(18):4449-56.
10. Palmer MJ, Moffat C, Saranzewa N, Harvey J, Wright GA, Connolly CN. Cholinergic pesticides cause mushroom body neuronal inactivation in honeybees. Nat. Commun. 2013; 27;4(1):1634.
11. Gill RJ, Raine NE. Chronic impairment of bumblebee natural foraging behaviour induced by sublethal pesticide exposure. Funct Ecol. 2014;28(6):1459-71.
12. Henry M, Beguin M, Requier F, Rollin O, Odoux JF, Aupinel P, Aptel J, Tchamitchian S, Decourtye A. A common pesticide decreases foraging success and survival in honey bees. Science. 2012;20;336(6079):348-50.
13. Stanley DA, Smith KE, Raine NE. Bumblebee learning and memory is impaired by chronic exposure to a neonicotinoid pesticide. Sci. Rep. 2015;16;5(1):16508.
14. Pashte VV, Patil CS Impact of different insecticides on the activity of bees on sunflower. Res on Crops. 2017;18(1):153–156.

15. Zhou T, Song HL, Wang Q, Dai PL, Wu YY, Sun JH. Effects of imidacloprid on the distribution of nicotine acetylcholine receptors in the brain of adult honeybee (*Apis melliferaligustica*). Acta Entomol Sinica. 2014; 56(11):1258–1266
16. Fischer J, Müller T, Spatz AK, Greggers U, Grünewald B. Neonicotinoids Interfere with Specific Components of Navigation in. honeybees. PLoS ONE. 2014;9, 91364
17. Menzel, R. The honeybee as a model for understanding the basis of cognition. Nat Rev Neurosci. 2012; 13, 758–768.
18. Tison L, Hahn ML, Holtz S, Rößner A, Greggers U, Bischoff G, Menzel R. Honey bees' behavior is impaired by chronic exposure to the neonicotinoid thiacloprid in the field. . Environ Sci Technol. 2016; 5;50(13):7218-27. .
19. Schneider CW, Tautz J, Grünewald B, Fuchs S. RFID tracking of sublethal effects of two neonicotinoid insecticides on the foraging behavior of *Apis mellifera*. PloS one. 2012;11;7(1):e30023.
20. Schmuck R, Schöning R, Stork A, Schramel O. Risk posed to honeybees (Apis mellifera L, Hymenoptera) by an imidacloprid seed dressing of sunflowers. Pest Manag Sci: formerly Pesticide Science. 2001 Mar;57(3):225-38.
21. Bryden J, Gill RJ, Mitton RA, Raine NE, Jansen VA. Chronic sublethal stress causes bee colony failure. Ecol Lett. 2013;16(12):1463-9.
22. Chandrakumara K, Muralimohan K, Gundoju S, Belavadi VV, Ramanappa TM. Seed treatment with neonicotinoid insecticides does not affect the foraging behavior of honey bees. Apidologie. 2023 Jun;54(3):29.
23. Sharma D, Abrol DP. Effect of insecticides on foraging behaviour and pollination role of *Apis mellifera*L.(Hymenoptera: Apidae) on toria*(Brassica campestris* var. toria) crop. Egypt J Biol 2014;19;16:79-86.
24. Giri GS, Mall P, Pandey R. Effect of thiamethoxam on colony development of *Apis mellifera* L. J EntomolZool Stud. 2017;5:177-9.
25. Yang EC, Chuang YC, Chen YL, Chang LH. Abnormal foraging behavior induced by sublethal dosage of imidacloprid in the honey bee (Hymenoptera: Apidae). J EconEntomol 2008; 1;101(6):1743-8.
26. Sharma HK, Ram B, Rana K, Thakur M. Effect of neonicotinoids on *Apis mellifera* under field and semifield conditions. J PharmacognPhytochem. 2018;7(6):59-63.
27. Matre YB, Telangre AH, Latpate CB, Zanwar PR. Effect of neonicotinoids ie imidacloprid 17.8% SL on foraging behaviour of honey bee on safflower (*Carthamus tinctorius* L.). Int J Chem Stud. 2018;6(5):5-8.
28. Tremolada P, Mazzoleni M, Saliu F, Colombo M, Vighi M. Field trial for evaluating the effects on honeybees of corn sown using Cruiser® and Celest xl® treated seeds. Bull Environ ContamToxicol. 2010;85:229-34.
29. Giri GS, Tiwari S, Mall P, Pandey R. Thiamethoxam had negative impact on Apis mellifera, hence yield of Brassica. J PharmacognPhytochem. 20187(3);2812-2813.

30. Pilling E, Campbell P, Coulson M, Ruddle N, Tornier I. A four-year field program investigating long-term effects of repeated exposure of honey bee colonies to flowering crops treated with thiamethoxam. PLoS one. 2013;23;8(10):e77193.
31. Van der Sluijs JP, Simon-Delso N, Goulson D, Maxim L, Bonmatin JM, Belzunces LP. Neonicotinoids, bee disorders and the sustainability of pollinator services. Curr. Opin. Environ. Sustain. 2013;1;5(3-4):293-305.