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Impact of Neonicotinoid Insecticides on the Foraging Preference of Indian Honey Bee, *Apis cerana indica* (Fab.) (Hymenoptera, Apidae) Visiting Sunflower *Helianthus annus* L. Crop

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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

Aim: Forage preference of Indian honey bee on neonicotinoid insecticides treated sunflower
Study Design: Randomized Block Design (RBD)
Place and Duration of Study: Insectary, Department of Agricultural Entomology, Tamil Nadu Agricultural University, Coimbatore between August 2021 to December 2021 during the Adipattam season
Methodology: Bee activity was recorded by *in situ* counting method. Seven treatments with three replications (5flowers/replication) in Randomized Block Design were followed. Different treatments *viz*., Imidacloprid 17.8 SL @ 280µl/l, Clothianidin 50 WDG @ 80µg/l, Thiamethoxam 25 WG @ 250µg/l, Thiacloprid 21.7 SC @ 1100/µl, Dimethoate 30 EC @ 1400µl/l (chemical check), Water control (Water spray) and Absolute control (no spray) were given at 50% flowering period using

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Knapsack sprayer. The number of bees visiting 5 flowers per 5 minutes was observed during morning (09.00-11.00), afternoon (13.00-15.00) and evening (16.00-18.00) hours of the day for seven days after spray (DAS). DAS. Statistical Analysis Software (SAS) academics was used to statistically analyse the data.
Results: The mean population of *Apis cerana indica* Fab. was more in absolute control (5.97) followed by water control (4.77), imidacloprid (4.10), clothianidin (3.40), thiacloprid (3.21), thiamethoxam (3.14) and dimethoate (1.90) during morning hours (09.00-11.00) of the day. The mean bee visitation rate was high in control (3.40), followed by water control (2.65), imidacloprid (1.77), clothianidin (1.52), thiacloprid (1.55), thiamethoxam (1.40) and dimethoate (1.17) during afternoon hours (13.00-15.00) of the day. In the evening hours (16.00-18.00), mean bee activity was high in control (4.95) followed by water control (4.27), imidacloprid (3.40), clothianidin (3.13), thiamethoxam (2.75), thiacloprid (2.53) and dimethoate (1.88). Among these different hours, morning forage activity of the bees are high in morning hours of the day followed by evening and very less in afternoon hours.
Conclusion: The present study revealed that Indian honey bees preferred to forage both on neonicotinoid treated, however at a reduced rate, and untreated flowers. Since, neonicotinoids are odourless, tasteless compounds that increase the risk of pesticide exposure for the bee colony owing to less capability for segregation of insecticide treated surfaces.

Keywords: Apis cerana indica; forage preference; bee activity; sunflower; neonicotinoid.

1. INTRODUCTION

Honey bees provide pollination services to agricultural and horticultural crops as well as wild plants [1]. Worldwide, declines of various pollinators in recent-day have been reported [2,3]. The annual honey bee colony losses are suspected due to several factors. Among them, pesticide usage is considered as the major anthropogenic factor rather than other important issues such as climate change, habitat loss, occurrence of pest and diseases, heavy metals poisoning *etc*. [4]. Hence, bee activity on insecticide treated crops is needed to be explored to understand the direct and indirect effects of pesticide poisoning. The impact of pesticide exposure, particularly from neonicotinoid insecticides, has received substantial recent research attention [5,6] due to the fastest bee declines happened after the introduction of neonicotinoids for agricultural insect management. It is vital that while managing agricultural pests, there should be no harming to the pollinators during foraging on pesticide treated crops.

For pollinators, the hazards of insecticide application on flowering crops include direct mortality, sublethal effects, repellent effects and contamination of insecticide residues on various floral parts and nectar [7]. A prolonged repellent effect may deprive flowers of the pollination benefits of insect visits. At the same time, a short repellence will deter the insect pollinators for a brief period but thereafter allow them to resume foraging activities (with minimal residual hazards due to degradation of treated pesticides over time bought out of repellence nature) without compromising the yield potential of the crop [8]. The effect of insecticides as a repellent on honey bees has already been documented by earlier works [9,10]. However, there is a lack of data regarding bee abundance and foraging preference on insecticide treated and untreated crops especially for neonicotinoids, which has a cognizable size of share among the agria bigger molecule. Taking that background, in this study, the adverse effect of neonicotinoid insecticides on the foraging activity of bees was tested on sunflower (*Helianthus annus* L.; Family: Compositae) crop, an excellent foraging source for honey bees using *Apis cerana indica* Fab.

Sunflower is the world's fourth important oilseed crop that improves the human diet, prevents malnutrition [11]. In India, sunflower is majorly cultivated in Karnataka, Orissa, Haryana, Maharashtra, Bihar and Tami Nadu (APEDA, 2019-2020). The flowering period of sunflower varies in different varieties and ranges from two to four weeks [12]. Since its self-incompatible nature, especially floral arrangement and flower opening sequence attract more insect pollinators [13]. It produces both nectar and pollen that encourages bee visits and other pollinators numerously [14]. Pollination in sunflowers is carried out mainly by both *Apis* and non-*Apis* sp. of insects, whose behaviour and efficiency are largely dependent on weather parameters [15]. The honey bees are the important insect pollinators that increase both seed (30%) and oil content (6%) of sunflower. The Indian honey bee, *A. c. indica* is one of the most important bee pollinators in sunflower ecosystem [16]. So, we need to rely on honey bee-based sunflower pollination, to increase the seed set, yield and quality improvement. On sunflower, infestation of sucking insect pests including aphid (*Aphis gossypii* (Glov.)), leaf hopper (*Amrasca devastans* (Dist.)), whitefly (*Bemisia tabaci* (Genn.)) and thrips (*Thrips tabaci* (Lind.)) leads to considerable yield losses [17] and therefore, warrants spraying interventions with insecticides.

Neonicotinoids are systemic, neuro-active insecticides used for seed and soil treatments to manage soil-dwelling arthropods, seed and seedling feeders and sucking insect pests [18]. Among neonicotinoids, imidacloprid, thiamethoxam and clothianidin are widely used to manage these kinds of pests in maize, oilseed rape and sunflower [19]. As they are systemic pesticides, may get translocated to various parts of treated plants including nectar and pollen of crops [18]. Bees are exposed to these pesticides, while foraging such treated crops which had shown some direct and indirect negative effects too [20].

Neonicotinoids can persist in the environment within the pollen and nectar of treated crops, or as soil residues which are degraded slowly (half-life: 148-6900 days) [21]. This can contaminate nearby non-treated wildflowers too [22]. Previously, most of the impact studies assaying pesticides were carried out under laboratory or semi-field settings rather than in the field and used pesticide-treated foods containing unrealistically high dosages [23]. The present study was conducted as an organised field experiment to explore Indian honey bee, *A. c. indica* foraging preference to neonicotinoid treated and untreated sunflower crops.

2. MATERIALS AND METHODS

2.1 Sunflower Field

The present study was carried out at Insectary farm, Department of Agricultural Entomology, Tamil Nadu Agricultural University, Coimbatore from August 2021 to December 2021. The experimental site is situated at 11^{o} 59'N latitude, 76^{o} 47'E longitude and at an altitude of 152m above mean sea level. The experimental farm is characterized by a tropical climate with good rainfall during both monsoon and the soil type is sandy clay loam in texture. The TNAU sunflower hybrid CO2 was selected because of its distinguishing morphological characters *viz*., height is 160-175cm, medium-sized head, flat to convex in shape, mature at 85 – 90 days [24]. The sunflower crop was raised at a spacing of 60 x 30 cm by following recommended agronomic practices. During the experiment, several sucking pests were documented and to manage the pests, pesticide spray was given.

2.2 Pesticide Spray

The foliar spray of different insecticides was given during blooming (after 50% flowering) of sunflower crop *viz*., Imidacloprid 17.8 SL @ 280µl/l, Clothianidin 50 WDG @ 80µg/l, Thiamethoxam 25 WG @ 250µg/l, Thiacloprid 21.7 SC @ 1100/µl, Dimethoate 30 EC @ 1400µl/l (chemical check), Water control (Water spray) and Absolute control (no spray) with recommended doses [25] (Table 1) at the respective dilutions using hand operated knapsack sprayer (VBD09: 33.5 x 14.0 x 47.0cm). Among these neonicotinoids, imidacloprid, clothianidin and thiamethoxam were belongs to the nitro-substitution group while thiacloprid belongs to cyano-substitution. The organophosphate insecticide dimethoate was taken as a chemical check since it was a standard check used for any toxicity analysis study on honey bees [26]. The experiment was laid out in a Randomized Block Design (RBD) with seven treatments and three replications. The individual plot size was 2m x 6m and each plot was maintained with one meter isolation distance to avoid the pesticide drift effect while spraying. In water control, only water spray was given and absolute control was kept without any spray treatments. The data were recorded in pre-treatment (PTC-Pre-Treatment Count) and post-treatment (DAS-Days After Spray) to study the direct effect of neonicotinoid spray. The post-treatment count was again divided into two parts *viz*., early spray count (1-2 DAS) and late spray count (3-7 DAS) to assess the immediate effect of pesticide's negative effects. The observations were taken during the morning (09.00-11.00), afternoon (13.00-15.00) and evening (16.00-18.00) hours of a day for one week period. The observations were taken based on *in situ* counting method. The frequent *A. c. indica* visitors to the sunflower head were recorded daily on five randomly selected plants for five minutes and expressed as the mean number of pollinators/5 plants/5 mins.

2.3 Statistical Analysis

The values, after square root transformation, were analysed by using a one-way analysis of variance (ANOVA) [27] and PROC GLM in Statistical Analysis Software programme (SAS academics) [28]. The means, when significant were separated by using Tukey's studentized range (honestly significant difference) test procedure (*P*<0.05).

3. RESULTS AND DISCUSSION

3.1 Insect Visitors in Sunflower Crop

The observations had shown that the sunflower crop was visited by different insects. A total of twelve insects *viz*., *Apis cerana indica* (Fab.), *A. mellifera* (L.), *A. dorsata* (Fab.), *A. florea* (Fab.), *Tetragonula iridipennis* (Smith), *Amegilla zonata* (L.), *Xylocopa* sp., *Polistes* sp., *Vespa* sp., *Papilio polytes* (L.), *Pieris rapae* (L.) and *Mylabris pustulata* (Thumb.) had visited sunflower head (Table 2) (Plate 1). The predominant insect order was Hymenoptera (83.33 %)-followed by Lepidoptera (16.67 %) and Coleoptera (8.33 %). Earlier report of [29] also stated that the majority of the insect pollinators in sunflower belonged to Hymenoptera, Lepidoptera, Diptera and Coleoptera, since, sunflower supplies both nectar and pollen that attracts more pollinators. Further, *A. mellifera* was reported to be the most frequent insect pollinator visiting sunflower at normal field condition (without insecticide spray) and helped increasing yield than others [29-31]. However, all honey bees were reported to be involved in increasing sunflower hybrid seed production by improving seed set ratio, 100 seed weight, number of filled seed per head and seed yield per head [32,33]. As like previous results *A. mellifera* was also the predominant pollinator next to *A. c. indica* in the sunflower ecosystem. This foraging preference study was conducted with *A. cerana indica* since it is the most prevailing native bee in Southeast Asia.

Table 1. Neonicotinoids and other insecticide along with their dose used for spraying in the sunflower field to assess the forage preference of *A. c. indica*

Insecticide formulation	Insecticide group	Dose (g ai/ha)	Dose (ai/l)
Imidacloprid 17.8 SL	Neonicotinoid	25	280µl/l
Clothianidin 50 WDG	Neonicotinoid	20	0.08mg/l
Thiamethoxam 25 WG	Neonicotinoid	30	0.25mg/l
Thiacloprid 21.7 SC	Neonicotinoid	120	1100µl/l
Dimethoate 30 EC (Chemical Check)	Organophosphate	200	1400µl/l
Water Control	-	-	-
Absolute Control (No Spray)	-	-	-

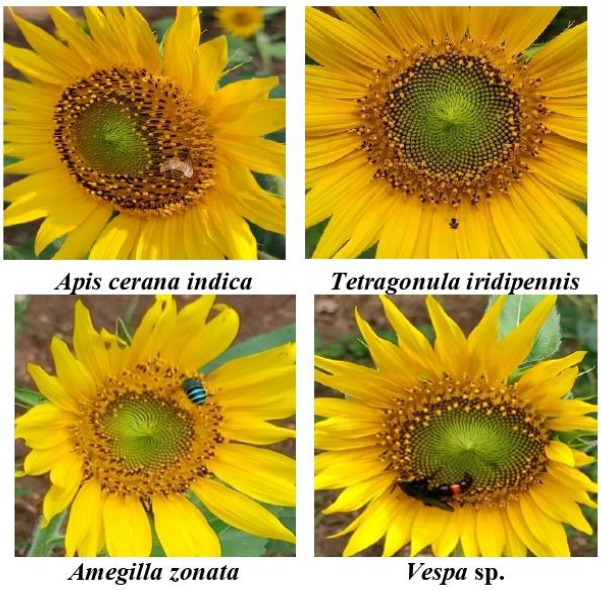

Plate 1. A few pollinating insect visitors at major proportions observed in sunflower crop during the study

Table 2. Floral visitors recorded in sunflower crop during pre-treatment period of the study

Pollinators*	Systematic position (Order: Family)	Role (N/P/N+P)
Apis cerana indica	Hymenoptera: Apidae	N+P
A. mellifera	Hymenoptera: Apidae	N+P
A. dorsata	Hymenoptera: Apidae	N+P
A. florea	Hymenoptera: Apidae	N+P
Tetragonula iridipennis	Hymenoptera: Apidae	N+P
Amegilla zonata	Hymenoptera: Apidae	N+P
Xylocopa sp.	Hymenoptera: Apidae	N+P
Polistes sp.	Hymenoptera: Vespidae	N
Vespa sp.	Hymenoptera: Vespidae	N
Papilio polytes	Lepidoptera: Papilionidae	N
Pieris rapae	Lepidoptera: Pieridae	N
Mylabris pustulata	Coleoptera: Meloidae	P

N- Collects nectar only; P-Collects pollen only; N+P- Collects both nectar and pollen

The sucking pests including, *Amrasca biguttula biguttula* (Ishida), *Bemisia tabaci* (Genn.), *Scirtothrips dorsalis* (Hood) and *Phenacoccus solenopsis* (Tinsley) were identified during the experimental period on sunflower (Plate 2). The management of these pests was done by spraying different neonicotinoids and the impact of these neonicotinoids on the foraging preference of honey bees was studied.

3.2 Foraging Preference of *Apis cerana indica* During Different Hours of the Day

The forage activity of Indian honey bees was recorded during different hours of the day. The maximum foraging was observed during morning (09.00-11.00) and evening hours (16.00-18.00) whereas, the minimum was at afternoon hours (13.00-15.00) of the day. At morning hours (09.00-11.00) of the day, the mean population of *A. c. indica* was more in absolute control (5.97±1.34/5flowers/5mins) followed by water control (4.77±0.76), imidacloprid (4.10±2.00), clothianidin (3.40±1.47), thiacloprid (3.21±1.50), thiamethoxam (3.14±1.74) and dimethoate (1.90±1.31) (F= 1901.10; df=12; P=<0.0001) (Table 3). The bee forage activity had not differed between treatments in the pre-treatment count, whereas, early spray count (1-2 DAS) had indicated that bee forage activity was only recorded in control than other treatments (Fig. 1). Initial deterrence was observed both in dimethoate and neonicotinoids especially imidacloprid and clothianidin spray treatments. However, bee visitation was restored in neonicotinoids spray treatment plants as indicated by late spray count (3-7 DAS). At 7 DAS, both control with imidacloprid and water control with clothianidin were on par with each other (F= 65.70; df=12; P=<0.0001). The results indicated that there was initial deterrence of bees towards pesticide sprayed plants, however, it was restored later in the morning hours. Since neonicotinoids are odourless and tasteless compounds, *A. c. indica* might have no ability to differentiate both treated and untreated flowers.

During afternoon hours (13.00-15.00) of the day, mean bee visitation rate was significantly high in absolute control (3.40±0.72), followed by water control (2.65±0.61), imidacloprid (1.77±0.88), clothianidin (1.52±0.82), thiacloprid (1.55±0.67), thiamethoxam (1.40±0.83) and dimethoate (1.17±0.75) (F= 644.82; df=12; P=<0.0001) (Table 4). Like morning hours, bee activity was initially deterred and then, it was restored at 3 DAS in afternoon hours of the day also. Compared to the morning hours, the afternoon hours of the day were less preferred to forage by bees. The reason may be due to the weather factors like high temperature, low relative humidity and wind speed. At 1 DAS, for afternoon hours, more visitation was observed in absolute control, while low visitation was observed in imidacloprid, thiamethoxam and dimethoate sprayed plants. However, at 7 DAS, both absolute control and imidacloprid treatments showed highly significant difference (F= 511.60; df=12; P=<0.0001) while, clothianidin, thiamethoxam and thiacloprid were on par with each other.

As same as previous results, in the evening hours (16.00-18.00) of the day, mean bee forage activity was significantly high in absolute control (4.95±0.62) followed by water control (4.27±0.41), imidacloprid (3.40±1.07), clothianidin (3.13±1.27), thiamethoxam (2.75±1.13), thiacloprid (2.53±0.96) and dimethoate (1.88±0.94) (F= 1893.21; df=12; P=<0.0001) (Table 5) with highly significant differences among the treatments. During the early spray count, less bee activity was observed in all the neonicotinoids and dimethoate sprayed plants. The bee visitation rate had increased from 3 DAS, here both water control and imidacloprid were on par with each other (F= 70.67; df=12; P=<0.0001). At 7 DAS, all the treatments were highly significantly different (F= 180.39; df=12; P=<0.0001).

The overall bee forage activity in the pre-treatment count indicated that there is no significant difference between the treatments. The bee activity results confirmed that the Indian honey bees preferred to forage both on neonicotinoid treated and untreated flowers. However, the post-treatment count revealed interesting results. In the early spray count (1-2 DAS), bees preferred to forage on untreated sunflowers than insecticide treated flowers (Fig. 1). This result indicated that there is a presence of initial deterrence or repellence effect of the insecticides including dimethoate and neonicotinoids. But, at late spray count (3-7 DAS), bee activity was restored in neonicotinoid treated crops with less reduction. The overall foraging activity during the late spray count indicated that mean bee activity was significantly high in absolute control and was followed by water control, imidacloprid, clothianidin, thiacloprid, thiamethoxam and dimethoate (Fig. 1). Among different neonicotinoids, the honey bees preferred to forage on the nitro-substituted neonicotinoids including imidacloprid and clothianidin rather than cyano-substituted thiacloprid. Since, dimethoate is being an organo-phosphorous insecticide, there is least bee activity noticed on plants treated with dimethoate in the overall experimental period.

In yet another case, [34] reported that a high dose of fipronil (phenyl pyrazole chemical family), reduces the number of foraging trips continuously from the first day of exposure. Interestingly, after 4 days, in a low fipronil dose treatment, bees made more foraging trips per day. It showed that initial day repellence was due to more concentration of pesticides whereas, in late spray count, degradation of pesticides might have led to more bee activity. Findings by [35], showed the toxicity effects of imidacloprid on the foraging behavior of *A. mellifera* using artificial flowers fed with imidacloprid contaminated sugar solution, wherein it was reported that the foragers would continue to visit imidacloprid treated crops, making regular trips to and from the hive, but only at lower rate and the forager’s return rate and foraging trips were reported declined with increased imidacloprid dose.

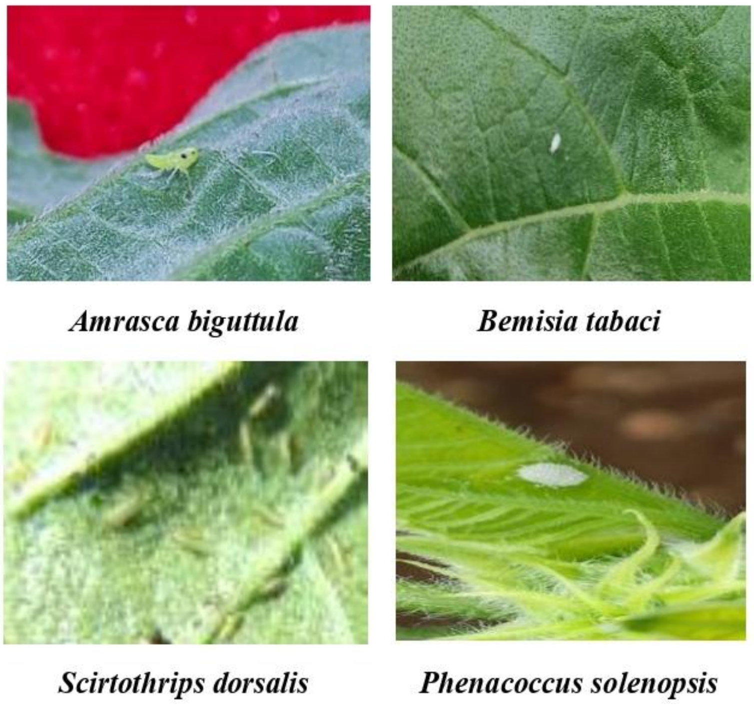

Plate 2. Major sucking insect pests observed in sunflower crop during the study

Table 3. Indian honeybee, *A. c. indica* activity recorded during morning hours of a day after spraying of insecticides on sunflower crop in the field

Treatment	PTC	DAS1	DAS2	DAS3	DAS4	DAS5	DAS6	DAS7	Mean
Imidacloprid	4.80^{a}(2.30)	0.67^{d}(1.08)	1.33^{c}(1.35)	5.60^{b}(2.47)	5.40^{b}(2.43)	5.20^{b}(2.39)	4.00^{b}(2.12)	5.80^{ab}(2.51)	4.10^{c}(2.08)
Clothianidin	4.74^{a}(2.29)	1.00^{c}(1.22)	1.50^{c}(1.42)	4.20^{c}(2.17)	3.00^{c}(1.87)	4.20^{c}(2.17)	3.60^{b}(2.02)	5.00^{bc}(2.34)	3.40^{d}(1.94)
Thiamethoxam	4.80^{a}(2.30)	0.50^{d}(1.00)	1.00^{d}(1.22)	3.40^{d}(1.97)	2.40^{cd}(1.70)	5.40^{b}(2.43)	3.40^{b}(1.97)	4.20^{cd}(2.17)	3.14^{f}(1.85)
Thiacloprid	4.56^{a}(2.25)	1.00^{c}(1.22)	1.50^{c}(1.41)	4.00^{cd}(2.12)	5.00^{b}(2.34)	4.00^{c}(2.12)	2.00^{c}(1.58)	3.60^{d}(2.02)	3.21^{e}(1.88)
Dimethoate	4.79^{a}(2.30)	1.00^{c}(1.22)	1.00^{d}(1.22)	1.00^{e}(1.22)	2.00^{d}(1.58)	1.80^{d}(1.52)	1.00^{d}(1.22)	2.60^{e}(1.76)	1.90^{g}(1.51)
Water Control	4.59^{a}(2.26)	4.20^{b}(2.17)	4.60^{b}(2.26)	5.40^{b}(2.43)	5.80^{b}(2.51)	5.60^{b}(2.47)	3.60^{b}(2.02)	4.40^{c}(2.21)	4.77^{b}(2.29)
Absolute Control	4.55^{a}(2.25)	4.80^{a}(2.30)	5.00^{a}(2.35)	7.80^{a}(2.88)	7.60^{a}(2.84)	6.80^{a}(2.70)	4.80^{a}(2.30)	6.40^{a}(2.63)	5.97^{a}(2.53)
SE	NS	0.06	0.05	0.11	0.18	0.11	0.10	0.14	0.50
CD (P=0.05)	NS	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

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

Table 4. Indian honeybee, *A. c. indica* activity recorded during afternoon hours of a day after spraying of insecticides on sunflower crop in the field

Treatment	PTC	DAS1	DAS2	DAS3	DAS4	DAS5	DAS6	DAS7	Mean
Imidacloprid	2.53^{a}(1.74)	0.20^{e}(0.84)	0.60^{e}(1.05)	2.00^{c}(1.58)	2.00^{abc}(1.58)	2.00^{c}(1.58)	2.40^{bc}(1.70)	2.40^{c}(1.70)	1.77^{c}(1.47)
Clothianidin	2.80^{a}(1.82)	0.40^{d}(0.95)	0.40^{f}(0.95)	1.40^{d}(1.38)	1.80^{bc}(1.52)	1.40^{d}(1.38)	2.00^{cd}(1.58)	2.00^{d}(1.58)	1.52^{de}(1.39)
Thiamethoxam	3.00^{a}(1.87)	0.20^{e}(0.84)	0.80^{d}(1.14)	1.00^{e}(1.22)	1.60^{bc}(1.45)	1.20^{d}(1.30)	1.60^{d}(1.45)	1.80^{d}(1.52)	1.40^{e}(1.35)
Thiacloprid	2.40^{a}(1.70)	0.60^{c}(1.05)	0.60^{e}(1.05)	2.00^{c}(1.58)	1.18^{c}(1.29)	1.80^{c}(1.52)	2.00^{cd}(1.58)	1.80^{d}(1.52)	1.55^{d}(1.41)
Dimethoate	2.80^{a}(1.81)	0.20^{e}(0.84)	1.20^{c}(1.30)	0.60^{f}(1.05)	1.15^{c}(1.28)	1.20^{d}(1.30)	1.20^{e}(1.30)	1.00^{e}(1.22)	1.17^{f}(1.26)
Water Control	2.40^{a}(1.70)	1.60^{b}(1.45)	2.00^{b}(1.58)	3.00^{b}(1.87)	2.80^{ab}(1.82)	3.20^{b}(1.92)	2.80^{b}(1.82)	3.40^{b}(1.97)	2.65^{b}(1.77)
Absolute Control	2.60^{a}(1.76)	2.80^{a}(1.82)	2.60^{a}(1.76)	4.20^{a}(2.17)	3.20^{a}(1.92)	4.00^{a}(2.12)	3.40^{a}(1.97)	4.40^{a}(2.21)	3.40^{a}(1.97)
SE	NS	0.02	0.03	0.04	0.09	0.05	0.08	0.04	0.30
CD (P=0.05)	NS	<0.001	<0.001	0.003	<0.001	<0.001	<0.001	<0.001	<0.001

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

Table 5. Indian honeybee, *A. c. indica* activity recorded during evening hours of a day after spraying of insecticides on sunflower crop in the field

Treatment	PTC	DAS1	DAS2	DAS3	DAS4	DAS5	DAS6	DAS7	Mean
Imidacloprid	4.20 ^{a}(2.17)	1.60 ^{d}(1.45)	2.00 ^{c}(1.58)	4.00 ^{bc}(2.12)	3.80 ^{b}(2.07)	4.60 ^{b}(2.26)	3.20 ^{c}(1.92)	3.80 ^{c}(2.07)	3.40 ^{c}(1.96)
Clothianidin	5.00 ^{a}(2.34)	1.00 ^{e}(1.22)	1.80 ^{c}(1.52)	3.80 ^{cd}(2.07)	3.60 ^{b}(2.02)	4.00 ^{bc}(2.12)	2.80 ^{c}(1.82)	3.00 ^{d}(1.87)	3.13 ^{d}(1.87)
Thiamethoxam	5.20 ^{a}(2.39)	2.00 ^{c}(1.58)	1.60 ^{d}(1.45)	2.80 ^{e}(1.82)	2.40 ^{c}(1.70)	3.40 ^{cd}(1.97)	2.20 ^{d}(1.64)	2.40 ^{e}(1.70)	2.75 ^{e}(1.78)
Thiacloprid	4.20 ^{a}(2.17)	1.40 ^{d}(1.38)	1.40 ^{e}(1.38)	3.20 ^{de}(1.92)	2.80 ^{c}(1.82)	3.00 ^{d}(1.87)	2.00 ^{d}(1.58)	2.20 ^{e}(1.64)	2.53 ^{f}(1.72)
Dimethoate	4.00 ^{a}(2.12)	1.00 ^{e}(1.22)	1.20 ^{f}(1.30)	2.00 ^{f}(1.58)	1.60 ^{d}(1.45)	2.20 ^{e}(1.64)	1.60 ^{e}(1.45)	1.40 ^{f}(1.38)	1.88 ^{g}(1.52)
Water Control	4.40 ^{a}(2.21)	3.60 ^{b}(2.02)	3.80 ^{b}(2.07)	4.60 ^{ab}(2.26)	4.20 ^{b}(2.17)	4.80 ^{ab}(2.30)	4.60 ^{b}(2.26)	4.20 ^{b}(2.17)	4.27 ^{b}(2.18)
Absolute Control	4.20 ^{a}(2.17)	4.20 ^{a}(2.17)	4.40 ^{a}(2.21)	5.20 ^{a}(2.39)	5.80 ^{a}(2.51)	5.60 ^{a}(2.47)	5.20 ^{a}(2.39)	5.00 ^{a}(2.34)	4.95 ^{a}(2.33)
SE	NS	0.04	0.04	0.11	0.11	0.15	0.08	0.12	0.40
CD (P=0.05)	NS	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

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

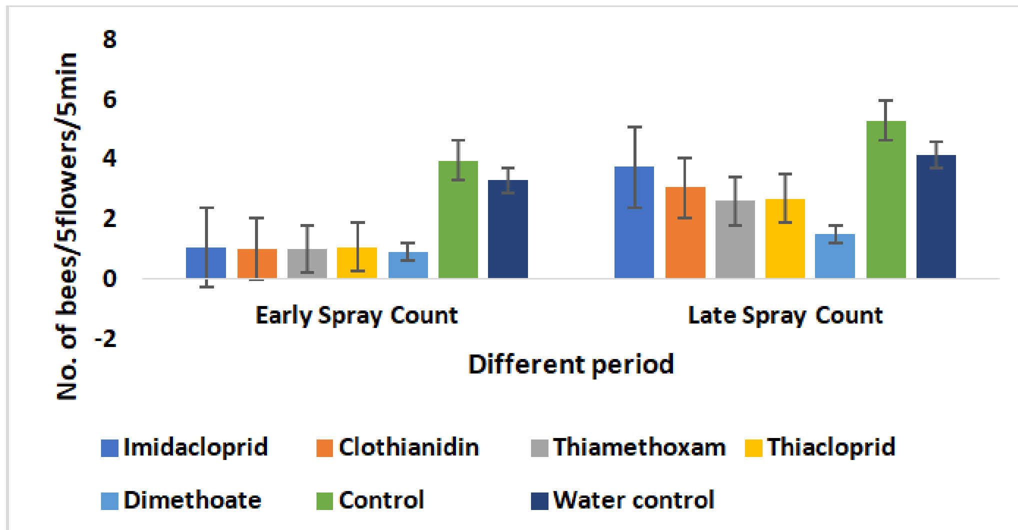

Fig. 1. Mean* number of Indian honey bee, *A. c. indica* foragers recorded in sunflower field at early (1-2) and late (3-7) days after spraying (DAS)

**Mean values obtained after averaging recorded Indian honey bee foragers at morning, afternoon and evening hours of a day*

The bumblebees (*Bombus terrestris* L.) foraging preference against thiamethoxam treated surfaces at different concentrations (0, 2 and 11 ppb) was studied by [36]. theses authors found that a proportion of visits to thiamethoxam laced feeders resulted in greater consumption relative to untreated sucrose and as such increasing preference for consuming neonicotinoid-treated food, therefore, increased the risk of exposure for the colony during prolonged pesticide exposure. It seems bees preferred to forage at low concentrations of thiamethoxam than high at initial application. The results by [36] implied that the use of thiamethoxam on flowering crops might result in the treated crops becoming disproportionately attractive to foraging bumble bees, and might further increase the risk of dietary exposure to these insecticides in wild bees [37]. Positional change of the laced feeders, resulted in bees being adjusted with their behaviour to continue preferentially feeding on thiamethoxam-treated sucrose indicating that bumble bees possess a sensory mechanism that can detect thiamethoxam.

Bumblebees were more attracted to visiting feeders containing low levels of nicotine, however such effect disappeared when the concentration of nicotine was high [38]. It is plausible that neonicotinoids, a compound more related to nicotine could stimulate similar effects on the foraging activity of bees. A decrease in the consumption of neonicotinoid-laced sucrose with increase in concentration was noticed by [39] involving in *B. terrestris.* Further, it is interesting to note that neonicotinoids excite the nicotinic acetylcholine receptors associated with learning and memory [40]. Therefore, it is possible that low concentrations of neonicotinoids acted in a manner similar to low doses of naturally occurring alkaloids, like caffeine and nicotine, to provide a memorable psychoactive signal, thus, acting as a post-ingestive stimulant that can encourage bees to remain faithful to contaminated food sources [41]. Most of the forage preference studies reported were done on *B. terrestris* and *A. mellifera*, and hence it is important to know the pesticide impact with reference to *A. c. Indica* also. There will be species or colony-level differences in sensitivity to pesticides, and the speed at which they metabolize neonicotinoids [42].

The ability of both honeybees (*A. mellifera*) and bumblebees (*B. terrestris*) were studied by [37] to taste the three most commonly used neo-nicotinoids *viz*., clothianidin, imidacloprid and thiamethoxam. When hungry worker bees could choose to collect from feeders containing either a solution of neonicotinoid-treated sugar water or an untreated solution, neither species avoided the treated food. Surprisingly, the bees in fact preferred the treated solution in the imidacloprid and thiamethoxam tests, which may due to the pharmacological action of these insecticides on receptors in the bee’s brains. In contrast, [43] found that diazinon, lambda-cyhalothrin, deltamethrin, malathion and profenophos were more repellent and there were fewer visiting honey bees than in the control plot. In onion seed production plot, [44] found that fipronil-sprayed plots had fewer honey bee visits (0.80 bees/ 5 umbels/min) than control.

The same forage preference was studied by [45] on *A. mellifera* with insecticides *viz*., azadirachtin, dimethoate, cypermethrin, fipronil, imidacloprid and indoxacarb. Fewer bees visited cypermethrin, imidacloprid and fipronil treatments while Azadirachtin posed less/no repellence on the treated crop blossom. The contrasting results of [46] mentioned no evidence of forager’s preferences to consume neonicotinoid containing solutions, finding effects on feeding motivation and locomotor activity. Bees were repelled from dimethoate treated sunflowers due to the presence of a strong chemical smell (garlicky odour) [47], however, dimethoate was not found to alter either bee activity or repel them [48, 49].

3.3 Bee Floral Constancy

In general, honey bees are following the behaviour of floral constancy which means that bees visit a kind of flower until it is exhausted and flower constancy was not affected by imidacloprid doses while increased sugar concentration [35]. It is a risk that if the bees are visiting insecticide treated flowers, there is a chance of getting more exposure to the insecticide. It will lead to sub-lethal effects like interference in regular functions like cognition, behaviour and physiology in bees [50].

4. CONCLUSION

The present study revealed that Indian honey bees, *A. c. indica* preferred to forage both on neonicotinoid treated and untreated flowers however, significantly on the later and at the same time least on dimethoate at field recommended dose. The variations among the findings so far reported may be attributed to the richness of floral source, differences among the formulations and doses used at the field level. Further, masking of floral odour due to strong chemical smells from the formulated pesticides may not be ignored. The climatic factors may also influence repellence effect of insecticides. Some insecticides may be regarded as safe when they repel bees, although as noticed with some instances, however, the attractiveness of food may be overriding the repellent effect. Since neonicotinoids are odourless and tasteless compounds, *A. c. indica* is unable to differentiate both treated and untreated flowers. Increasing preference by the Indian bees to neonicotinoids, especially nitro-substituted ones including imidacloprid and clothianidin, as found with the present study, increases the risk of pesticide exposure for the bee colony. Also, farmers should avoid spraying during the flowering period of sunflowers. This is the need of hour to develop pro-insecticides, that target only pest *vis-a-vis* safeguard the pollinators. Among these different hours, morning forage activity of the bees are high in morning hours of the day followed by evening and very less in afternoon hours.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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