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Many-fold less than the field recommended concentrations of neonicotinoids and malathion affect foraging of honeybee in three important crops in India

M.I. Olotu[1, 2] and G.T. Gujar[1*]

*[1]Division of Entomology, ICAR-Indian Agricultural Research Institute (IARI), New Delhi 110 012 (*Present address: Institute of Advanced Study in Science and Technology, Paschim Boragaon, Guwahati 781 035, India); [2]Mkwawa University College of Education (MUCE), P.O. Box 2513 Iringa, Tanzania. E-mail: gtgujar@yahoo.com*

ABSTRACT: Although insecticides effectively control the insect pests in different agro-ecosystems, they also reportedly affect the non-target insects including bee pollinators at the sub-lethal concentrations. A series of field experiments were conducted to evaluate the effects of two neonicotinoids and one organophosphate insecticide on the foraging activity of honeybee in three test crops at the sub-lethal concentration during flowering. The mean number of the dwarf honeybee (DHB), *Apis florea* (F.) (Hymenoptera: Apidae) recorded during the pre-spraying did not differ between treatments on each of the three crops. However, it differed significantly during the post-spraying except for malathion on inflorescences of the pearl millet. The DHB foraging time remained generally constant during the pre-spraying and varied greatly during the post-spraying on the three test crops and both groups of insecticides. The neonicotinoids and-malathion significantly reduced visits of the DHB on the inflorescences of the test crops, their foraging activities and time spent on the inflorescences at the concentration many-fold (5-50 fold) less than the field recommended concentration of the insecticide.

KEY WORDS: Neonicotinoids, imidacloprid, thiamethoxam, malathion, honeybee, *Apis florea*

INTRODUCTION

Insect pollination accounts for about 75 per cent of cultivated crops (Klein *et al.*, 2007) and 80 per cent of wild plant species (Potts *et al*., 2010) that help in production of seeds and fruits. Of the insects, honey bees are the important pollinators. Despite potential role of honey bees in maintaining agro-ecosystems, the insecticides which are needed for the control of harmful pests to enhance crop productivity threaten their role as pollinators even at sublethal concentrations (Desneux *et al.,* 2007, Feltham *et al.,* 2014). Insecticides are globally used for crop protection to the extent of about two million tons per year, of which 24 per cent is in the USA alone, 45 per cent in Europe and 25 per cent in the rest of the world including India (De *et al.,* 2014). Although their adverse effects on insect pollinators are suspected, the newly discovered neonicotinoids became a chief target in view of their high contact toxicity to bees and persistence in agro-ecosystems. In India, neonicotinoids such as imidacloprid, thiamethoxam, nitenpyram and the sulfoximine, sulfoxaflor are registered for pest control. Neonicotinoids are systemic insecticides. These are extensively applied by seed dressing (Halm *et al.*

* Author for correspondence

2006). Besides, these are also sprayed during crop growth. These are specifically used for the control of various sucking insect pests in India (Jeyalakshmi *et al.,* 2011, Mandal *et al.,* 2012, Gavkare *et al.,* 2013). It is reported that their residues are translocated to nectar and pollen, thereby affecting the pollinators coming in contact with the inflorescence. The neonicotinoids have the same target as neuron transmitter, acetycholine, and acting as agonist at nicotinic acetycholine receptor in the post-synapse during impulse transmission in an insect nerve, thus influencing neural behaviour (Schmuck *et al.,* 2003, Elbert *et al.,* 2008, Yang *et al.,* 2008).

Adverse effects of neonicotinoids reported as early 2001 are alleged to have caused bee decline (Jones *et al.,* 2006). This has led to either ban or restriction on their uses in some European countries (Kindemba 2009). For example, neonicotinoids affected waggle dance in the forage bees (Eiri and Nieh, 2012) and also decreased bee avoidance of predators (Tan *et al.,* 2014). Imidacloprid at the field-realistic concentrations decreased bee foraging activity (Decourtye *et al.,* 2004, Schneider *et al.,* 2012) and the ability of bees to successfully return to the nests (Desneux *et al.,* 2007, Henry *et al.,* 2012). Repellency of pollinators to the neonicotinoids at the field-realistic concentration was shown in the baited yellow pan traps tests (Easton and Goulson 2013). Contrary to these adverse reports, there are studies that contradict decline in honeybee population due to neonicotinoid use in the field crops (APVMA, 2014, Fairbrother *et al*., 2014).

Although studies have reported lethal and sublethal effects of neonicotinoids on different aspects of bees, there is little information on the effects of the neonicotinoids and organophosphate, malathion, on honeybee behavior exclusively at the sublethal concentration in the important crops under the field conditions in India. The present study therefore reports the effects of the two neonicotinoids *viz*., imidacloprid and thiamethoxam and organophosphate, malathion on three parameters, i) number of the dwarf honeybee (DHB) (*Apis florea* (F.)) (Hymenoptera: Apidae) visiting the test inflorescences, ii) foraging activities and iii) time spent on foraging during the observation period.

MATERIALS AND METHODS

(i) Experimental Sites

Experiments were conducted in the fields planted with pearl millet (*Pennisetum typhoides*), Indian plum (*ber*) (*Ziziphus mauritiana*) and mustard (*Brassica juncea*) at Indian Agricultural Research Institute (IARI), New Delhi, India. The pearl millet field was located between latitude N 26° 37.9' and longitude E 77° 09.3' and about 224.94 m.a.s.l and the Indian plum field between latitude N 28° 38.8' and longitude E 77° 09.2' and 198.12 m.a.s.l and the mustard field between 28° 38.8' and longitude E 77° 08.2' and 207 m.a.s.l. The mean temperature recorded at the IARI weather station ranged between minimum of 6.7-24.0 ºC and the maximum of 20.4-34.4ÚC from September to December, 2014. Sunrise time varied from 6:05 to 7:05 h Indian standard time (IST) and sunset varied from 17:30 to 18:26 h IST.

The neonicotinoid insecticides tested were imidacloprid17.8% SL, Confidor® (Bayer CropScience Limited, manufactured by Saraswati Agro Chemicals Pvt. Ltd, Jammu and Kashmir, India) and thiamethoxam25% WG, Tagxone™ (Tropical Agrosystem Pvt. Ltd, Chennai, India). Organophosphate insecticide tested was malathion50% EC, Suthion (manufactured by Super Ford Insecticide Limited, Secunderabad, India).

(ii) Evaluation of imidacloprid and malathion in pearl millet

The study was conducted during flowering of the pearl millet (Pusa composite 612) between the 27th September and the 10th October, 2014. The Pearl millet flowering is protogynous, stigmas mature first and its emergence begins near the tip and progress to the base. Its flowering period has been observed to coincide with high incidence of bees visiting inflorescences. Imidacloprid was applied at the selected inflorescences of the pearl millet in a

randomized block design. The effect of the neonicotinoid was determined on three parameters stated previously. The experimental site was divided into five plots, having pearl millet inflorescences of similar in size and flowering intensity. In each plot, five pearl millet inflorescences were selected randomly for the experimentation. One inflorescence at the middle was treated with 5 ppm of imidacloprid at a rate of 100 ml per inflorescence and four neighboring inflorescences were untreated and acted as control treatments (control one to four). The four neighbouring inflorescences were sprayed with 1% emulsifier solution (Triton X-100) and covered by polythene plastic bags to avoid drift of insecticide treatment. The spraying was done by using a 1.5 l Pneumatic Hand Sprayer (ASPEE Agro Equipments Pvt. Ltd, Mumbai, India). The spraying of imidacloprid was done late in the evening from 05:45 h IST. At this time the bee foraging activities were greatly reduced thereby avoiding direct contact with the imidacloprid during the spraying.

Abundance of bees visiting the inflorescences was determined twice per day between 08:00-10:00 and 16:00-18:00 h IST for six consecutive days, three days pre-spraying and three days post-spraying. Five minute observation period was made for each replicate and three evaluation parameters were monitored during the observation period. Six foraging activities were frequently exhibited by the bees. These included i) tasting inflorescence ii) picking nectar from flowers, iii) rasping legs on flowers, iv) transferring pollen to a basket leg (pumping legs), v) collecting pollen by abdomen hairs and vi) rasping flower with mouth parts.

(iii) Evaluation of imidacloprid and malathion in Indian plum

The study was also conducted during flowering of the Indian plum (variety, Umran) between the 26th October and the 8th November, 2014. Unlike the pearl millet, the Indian plum flowering is protandrous, having the anthers come to maturity before the stigmas. The spraying of imidacloprid and malathion was done at the randomly selected branches of Indian plum trees. Thirty branches of Indian plum tree similar in size and flowering intensity were selected randomly. In each treatment, 10 branches of similar size, one from each tree were treated with 5 ppm of imidacloprid at a rate of 200 ml/ branch; another 10 branches were treated with 5 ppm of malathion at the rate of 200 ml/ branch. Further, 10 branches were sprayed with 1% emulsifier solution which acted as control. The spraying time and techniques was similar to the previous study conducted in the pearl millet.

The effect of imidacloprid and malathion was also determined on the three parameters by using the same experimental protocol as before for the pearl millet except for reduction in the observation period to three minutes for each replicate.

(iv) Evaluation of malathion and thiamethoxam in mustard

The study was conducted during the mass flowering of mustard (Pusa mustard-28, 2012) from the 18th November to the 5th December, 2014. Like the pearl millet, the mustard flowering is protogynous, having the stigmas come to maturity before the anthers. The experiment protocol was similar to the previous study carried out in the Indian plum. However, in this field experiment, imidacloprid was replaced by thiamethoxam. The application of malathion and thiamethoxam was done at the floral parts of randomly selected mustard crop plants using the same field spraying protocol used in the previous experiment.

The effect of malathion and thiamethoxam was also determined in the mustard field twice a day for ten consecutive days, three days pre-treatment and seven days post-treatment. Similar to the Indian plum field experiment, three minute observation period was made for each replicate following three evaluation parameters used in the previous experiment.

(v) Determination of potential bee pollinators

To assess the specificity of bee pollinators, sweep net (with lightweight aluminium frame, approx.30 cm diameter and a 0.6 m handle) was used to

sample flower visitors on each crop. A total of 10 sweepings were made in the randomly selected inflorescences in each field studied. The sampled bees were identified to species level and their relative abundance was quantified to determine the most frequent bee species in each field. Identification of bees was done in the Division of Entomology, IARI, New Delhi and voucher specimens were also kept at the same institute.

(vi) Data Analysis

Data were analyzed using SAS 9.3 software. Generalized linear model procedure (GLM) was used for the analysis. Analysis of variance (ANOVA) was used to compare the mean difference between treatments, day and time and their interactions. The parameters of the study were tested separately for each insecticide applied in the pearl millet field and their means were compared between treated inflorescences against each of the untreated inflorescences. The parameters were also tested separately for the three treatments in the Indian plum field (*i.e.* control, imidacloprid and malathion) and in the mustard field (control, malathion and thiamethoxam). Bonferroni correction was used to adjust for multiple mean comparisons. It is the most common way to control the family-wise error rate (SAS Institute Inc. 2008).

RESULTS

(i) Flower visitors

The DHB, *A. florea* was the most abundant flower visitors in all three crops tested. The mean percentage of this species accounts for about 82.67 per cent of all pollinators sampled. It has outnumbered other three species of the family Apidae *viz., Apis dorsata* (F.), *Apis mellifera* (L.) and *Tetragonula iridipennis* (Smith) (Table 1). Beside bees, there were other pollinators visiting the inflorescences of the test crops.

(ii) Abundance of bees on the inflorescences of the test crops

Pearl millet

The frequencies of DHB which visiting the

Table 1: Abundance (%) of the four species of bees sampled from three different experimental sites in IARI, New Delhi

Test crop	Species of bees	No. of individuals	% per crop
Pearl millet	*Apis florea* (F.)	53	77.94
	Apis dorsata (F.)	6	8.82
	Tetragonula iridipennis (Smith)	7	10.29
	Apis mellifera (L.)	2	2.94
Indian plum	*Apis florea* (F.)	50	83.33
	Apis dorsata (F.)	5	8.33
	Tetragonula iridipennis (Smith)	1	1.67
	Apis mellifera (L.)	4	6.67
Mustard	*Apis florea* (F.)	72	86.75
	Apis dorsata (F.)	5	6.02
	Tetragonula iridipennis (Smith)	2	2.41
	Apis mellifera (L.)	4	4.82

inflorescences were not significantly different between treatments prior to the spraying of the two insecticides, *viz.*, imidacloprid and malathion at the test inflorescences (Table 2 and 3). Following the spraying of these insecticides, the mean numbers of DHB did not differ significantly between malathion treated and untreated inflorescences neither morning nor evening (Table 2). The only significance difference in the mean numbers of DHB was between imidacloprid treated and untreated inflorescences ($F_{(1,59)} = 22.26$; $P < 0.0001$). However, there was no significant difference in their interactions: treatment, day and time during the pre-spraying of malathion (Table 2) and imidacloprid (Table 3). A similar trend was also recorded during the post-spraying of malathion and imidacloprid as indicated in the respective tables 2 and 3.

Indian plum

The mean numbers of DHB visiting the *ber* inflorescences was not significantly different between treatments and time during the pre-spraying of both imidacloprid and malathion (Table 4). Following the spraying of these insecticides, the numbers of DHB generally remained similar in the untreated inflorescences throughout the experimental period, but declined significantly in all the treated inflorescences ($F_{(2,179)} = 30.33$; $P<0.0001$) (Table 4). Similar to the Pearl millet experiment, the mean numbers of DHB did not differ significantly in the overall interactions during the pre-spraying and post-spraying of these insecticides (Table 4).

Mustard

There was also no significant difference in the mean numbers of DHB between treatment during the pre-spraying of both malathion and thiamethoxam at the mustard inflorescence. However, the mean numbers of DHB differed significantly between time (Table 5). Following the spraying of these insecticides, the mean numbers of DHB generally remained similar in the untreated inflorescences, but declined significantly in all the treated inflorescences ($F_{(2,179)} = 70.59$; $P<0.0001$). The mean numbers of DHB did not differ significantly in the overall interactions during the pre-spraying and the post-spraying (Table 5).

(iii) Bee foraging activities at the inflorescences of the test crops

Pearl millet

The mean numbers of DHB foraging activities during the pre-spraying was not significantly different between treatments and time at the test inflorescences (Table 2 and 3). The only significant difference in the mean numbers of bee foraging activities between the treated and untreated inflorescences was for imidacloprid, ($F_{(1,59)} = 31.87$; $P<0.0001$). The mean numbers of DHB foraging activities did not differ significantly in the overall interactions during the pre-spraying and the post-spraying of malathion and imidacloprid (Table 2 and 3).

Indian plum

The mean numbers of DHB foraging activities during the pre-spraying of Indian plum inflorescence with imidacloprid and malathion was not significantly different. The high mean numbers of DHB foraging activities was recorded in the untreated inflorescences than in treated inflorescences during the post-spraying experimental period. It varied significantly between the treated and untreated inflorescences ($F_{(2,179)} = 23.81$; $P<0.0001$) and the treatments (*i.e.* control, imidacloprid and malathion) as shown in table 4b. The overall interactions of the number of DHB foraging activities were not significant both during the pre-spraying and the post-spraying (Table 4).

Mustard

Although the mean numbers of DHB foraging activities was significantly different between morning and evening, it was insignificant between treatments during the pre-spraying of mustard with malathion and thiamethoxam (Table 5). The high mean numbers of DHB foraging activities was recorded in the untreated inflorescences than in treated inflorescences during the post-spraying

Table 2: Abundance of bees, foraging activities, time spent (X±SE) and inferential statistics (F and P-values) in pearl millet

	X±SE bees visiting each inflorescence for *5 minutes* duration						
Treatment	Day1[1]	Day1[2]	Day2[1]	Day2[2]	Day3[1]	Day3[2]	F&P-values: Treatment
	Pre-treatment						
Control 1	2.6±0.5a	1.6±0.3a	2.6±0.8a	2.0±0.3a	2.6±0.9a	1.6±0.5a	F(1,59)=1.59;P=0.21
Malathion	3.2±0.4a	2.0±0.3a	3.0±0.3a	2.6±0.3a	2.4±0.5a	1.8±0.4a	
F&P-values: Treat*day*time	F(2,59)=0.11;P=0.90						
Post-treatment							
Control 1	1.2±0.4a	1.6±0.7a	1.6±0.4a	2.0±0.5a	1.8±0.2a	1.0±0.3a	F(1,59)=0.07; P=0.79
Malathion	1.4±0.4a	1.4±0.4a	1.6±0.5a	1.6±0.5a	1.6±0.4a	1.2±0.5a	
F&P-values: Treat*day*time	F(2,59)=0.28; P=0.76						
Treatment	X±SE bees' activities recorded in each inflorescence for *5 minutes* duration						F&P-values: Treatment
	Pre-treatment						
Control 1	4.8±.04a	3.8±0.4a	4.6±0.5a	5.0±0.3a	3.6±0.9a	3.6±1.0a	F(1,59)=1.59;P=0.21
Malathion	5.2±0.3a	5.0±0.3a	5.0±0.2a	4.0±0.6a	4.2±0.4a	4.6±0.3a	
F&P-values: Treat*day*time	F(2,59)=1.29;P=0.28						
Post-treatment							
Control 1	2.2±1.0a	2.8±0.8a	3.0±0.9a	3.8±0.5a	4.2±0.2a	3.6±0.9a	F(1,59)=1.34;P=0.25
Malathion	4.0±0.8a	3.6±0.9a	4.2±1.1a	4.0±1.1a	4.0±0.3a	3.2±0.9a	
F&P-values: Treat*day*time	F(2,59)=0.07;P=0.93						
Treatment	X±SE time spent by bees visiting each inflorescence during 5 *minutes* duration						F&P-values: Treatment
	Pre-treatment						
Control 1	236.6±38.1a	146.0±19.4a	194.6±48.2a	212.2±44.9a	169.4±54.9a	96.2±29.9a	F(1,59)=2.26;P=0.14
Malathion	234.4±11.1a	161.8±17.9a	221.8±29.4a	207.0±29.6a	213.0±21.1a	180.2±26.4a	
F&P-values: Treat*day*time	F(2,59)=0.35;P=0.70						
Post-treatment							
Control 1	160.4±49.9a	123.4±33.4a	136.2±38.9a	198.8±14.1a	191.4±10.2a	135.4±35.4a	F(1,59)=7.63;P=0.01
Malathion	105.4±37.0b	97.2±31.7b	107.6±29.0b	117.6±33.4b	120.2±34.1b	84.2±33.1b	
F&P-values: Treat*day*time	F(2,59)=0.47;P=0.63						

[1&2] Denotes morning and evening, respectively; figures followed by the different letters in a column show significant difference at P<0.05 inflorescences pre-and post-malathion treatment at the division of Agronomy, IARI, New Delhi.

Table 3: Abundance of bees, foraging activities and time spent (X±SE) and inferential statistics (F and P-values) visiting pearl millet inflorescences pre-and post-imidacloprid treatment at the Division of Agronomy, IARI

	X±SE bees visiting each inflorescence for *5 minutes* duration						
Treatment	Day1[1]	Day1[2]	Day2[1]	Day2[2]	Day3[1]	Day3[2]	F&P-values: Treatment
	Pre-treatment						
Control 1	2.8±0.4a	1.6±0.7a	2.2±0.5a	2.2±0.05a	2.0±0.6a	2.2±0.6a	F(1,59)=0.95;P=0.34
Imidacloprid	3.0±0.7a	2.0±0.6a	2.4±0.4a	2.4±0.5a	3.2±0.4a	1.8±0.6a	
F&P-values: Treat*day*time	F(2,59)=0.85;P=0.43						
Post-treatment							
Control 1	2.6±0.4a	2.0±0.3a	2.4±0.3a	1.6±0.2a	2.4±0.5a	1.8±0.4a	F(1,59)=22.26;P<0.0001
Imidacloprid	1.0±0.5b	1.0±0.4b	1.6±0.3a	0.8±0.2b	1.4±0.4b	1.2±0.2b	
F&P-values: Treat*day*time	F(2,59)=0.19;P=0.83						
Treatment	X±SE bees' activities recorded in each inflorescence for *5 minutes* duration						F&P-values: Treatment
	Pre-treatment						
Control 1	4.2±0.6a	2.2±0.9a	4.2±1.1a	4.2±0.7a	4.0±1.0a	4.0±0.7a	F(1,59)=0.66;P=0.42
Imidacloprid	4.4±0.4a	3.8±0.6a	5.0±0.5a	3.0±0.9a	4.4±0.3a	4.2±0.8a	
F&P-values: Treat*day*time	F(2,59)=1.42;P=0.25						
Post-treatment							
Control 1	4.4±0.3a	3.8±0.5a	3.8±0.4a	3.4±0.5a	3.6±0.9a	3.4±0.9a	F(1,59)=31.87;P<0.0001
Imidacloprid	1.6±0.8b	1.0±0.5b	1.8±06b	1.6±0.4b	2.2±0.7b	2.0±0.6b	
F&P-values: Treat*day*time	F(2,59)=0.01;P=0.99						
Treatment	X±SE time spent by bees visiting each inflorescence during 5 *minutes* duration						F&P-values: Treatment
	Pre-treatment						
Control 1	216.6±28.5a	109.6±55.7a	179.8±18.6a	174.8±39.0a	180.2±47.2a	185.2±22.6a	F(1,59)=0.08;P=0.78
Imidacloprid	197.6±27.0a	166±33.1a	210.6±19.1a	148.4±37.4a	207.2±19.4a	149.0±24.6a	
F&P-values: Treat*day*time	F(2,59)=1.41;P=0.26						
Post-treatment							
Control 1	202.8±6.8a	169.6±13.9a	170.6±24.9a	129.4±16.3a	178.8±15.2a	111.0±18.5a	F(1,59)=94.05;P<0.0001
Imidacloprid	42.0±19.6b	39.6±16.8b	89.6±11.1b	37.2±11.1b	89.6±28.0b	59.0±19.4b	
F&P-values: Treat*day*time	F(2,59)=0.53;P=0.59						

[1&2] Denotes morning and evening, respectively; figures followed by the different letters in a column show significant difference at P<0.05

Table 4: Abundance of bees, foraging activities and time spent (X±SE) and inferential statistics (F and P-values) visiting *Indian plum* inflorescences pre-and post-imidacloprid and malathion treatments at the Division of Horticulture, IARI

	X±SE bees visiting each inflorescence for *3 minutes* duration						
Treatment	Day1[1]	Day1[2]	Day2[1]	Day2[2]	Day3[1]	Day3[2]	F&P-values: Treatment
	Pre-treatment						
Control	3.9±0.3a	3.4±0.2a	4.0±0.3a	3.0±0.3a	3.9±0.2a	2.9±0.2a	
Imidacloprid	3.7±0.3a	3.5±0.2a	3.6±0.3a	3.2±0.3a	4.0±0.3a	3.0±0.2a	F(2,179)=0.02;P=0.98
Malathion	3.8±0.3a	3.3±0.3a	3.5±0.2a	3.4±0.4a	4.2±0.3a	3.0±0.3a	
F&P-values: Treat*day*time	F(4,179)=0.59;P=0.67						
Post-treatment							
Control	3.6±0.2a	3.0±0.3a	3.3±0.3a	3.2±0.2a	2.9±0.3a	3.2±0.3a	
Imidacloprid	2.3±0.3b	1.9±0.2b	2.4±0.3b	2.0±0.2b	1.7±0.3b	1.9±0.3b	F(2,179)=30.33;P<0.0001
Malathion	2.4±0.3b	2.1±0.2b	2.3±0.3b	2.5±0.3b	2.0±0.2b	2.1±0.2b	
F&P-values: Treat*day*time	F(4,179)=0.30;P=0.88						
Treatment	X±SE bees' activities recorded in each inflorescence for *3 minutes* duration						F&P-values: Treatment
	Pre-treatment						
Control	4.2±0.2a	3.8±0.3a	3.9±0.2a	3.8±0.3a	4.0±0.2a	3.9±0.2a	
Imidacloprid	4.1±0.2a	3.5±0.3a	3.8±0.2a	3.6±0.3a	3.8±0.2a	3.6±0.2a	F(2,179)=1.12;P=0.33
Malathion	4.2±0.2a	3.7±0.2a	3.9±0.2a	3.7±0.2a	4.1±0.2a	3.5±0.3a	
F&P-values: Treat*day*time	F(4,179)=0.19;P=0.94						
Post-treatment							
Control	3.9±0.3a	3.9±0.3a	4.4±0.3a	3.8±0.1a	4.1±0.3a	3.8±0.3a	
Imidacloprid	2.8±0.3b	2.9±0.2b	3.0±0.2b	3.0±0.3b	2.7±0.4b	3.3±0.4b	F(2,179)=23.81;P<0.0001
Malathion	3.1±0.3c	3.5±0.2c	3.1±0.2b	3.4±0.2c	3.3±0.2c	3.5±0.2c	
F&P-values: Treat*day*time	F(4,179)=0.48;P=0.75						
Treatment	X±SE time spent by bees visiting each inflorescence during *3 minutes* duration						F&P-values: Treatment
	Pre-treatment						
Control	83.7±5.3a	78.5±3.5a	79.1±7.6a	84.5±4.0a	81.6±6.5a	78.2±3.2a	
Imidacloprid	84.2±5.0a	79.3±5.9a	83.6±2.8a	83.4±2.7a	82.0±3.5a	80.2±2.9a	F(2,179)=0.48;P=0.62
Malathion	84.8±4.1a	81.3±5.2a	85.0±3.2a	80.9±3.0a	86.0±4.6a	82.7±4.1a	
F&P-values: Treat*day*time	F(4,179)=0.25;P=0.91						
Post-treatment							
Control	84.3±2.2a	76.3±3.0a	79.4±2.5a	78.7±3.9a	76.8±2.5a	76.7±2.3a	
Imidacloprid	60.4±4.4b	53.9±3.0b	63.5±2.4b	51.1±3.2b	51.2±6.6b	55.0±7.0b	F(2,179)=55.12;P<0.0001
Malathion	70.4±5.4c	65.2±2.5c	74.8±4.1c	76.5±4.6c	62.4±1.6c	63.7±2.4c	
F&P-values: Treat*day*time	F(4,179)=0.86;P=0.49						

[1&2] Denotes morning and evening, respectively; figures followed by the different letters in a column show significant difference at P<0.05

Table 5: Abundance of bees, foraging activities and time spent (X±SE) and inferential statistics (F and P-values) visiting mustard inflorescences pre-and post-malathion and thiamethoxam treatments at the Division of Genetics, IARI

	X±SE bees visiting each inflorescence for *3 minutes* duration						
Treatment	Day1[1]	Day1[2]	Day2[1]	Day2[2]	Day3[1]	Day3[2]	F&P-values: Treatment
	Pre-treatment						
Control	5.7±0.2a	1.5±0.3a	5.5±0.3a	1.6±0.2a	5.6±0.2a	1.5±0.3a	F(2,179)=0.68;P=0.51
Malathion	5.4±0.2a	1.7±0.3a	5.1±0.3a	1.5±0.3a	5.3±0.3a	1.6±0.2a	
Thiamethoxam	5.6±0.2a	1.3±0.3a	5.3±0.2a	1.4±0.2a	5.3±0.3a	1.5±0.3a	
F&P-values: Treat*day*time	F(4,179)=0.14;P=0.97						
Post-treatment							
Control	5.4±0.3a	1.6±0.2a	5.4±0.2a	1.7±0.2a	5.2±0.2a	1.1±0.2a	F(2,179)=70.59;P<0.0001
Malathion	3.3±0.2b	0.9±0.2b	3.5±0.2b	1.3±0.2b	4.4±0.3b	0.8±0.2a	
Thiamethoxam	2.7±0.2c	1.0±0.2b	3.1±0.2b	1.2±0.3b	3.5±0.2c	0.9±0.2a	
F&P-values: Treat*day*time	F(4,179)=1.04;P=0.39						
Treatment	X±SE bees' activities recorded in each inflorescence for *3 minutes* duration						F&P-values: Treatment
	Pre-treatment						
Control	4.2±0.2a	2.5±0.5a	4.3±0.2a	3.3±0.2a	4.5±0.2a	2.8±0.5a	F(2,179)=0.08;P=0.93
Malathion	4.1±0.2a	3.1±0.4a	4.1±0.2a	3.2±0.4a	4.2±0.2a	3.2±0.2a	
Thiamethoxam	4.5±0.2a	2.5±0.5a	4.5±0.2a	3.2±0.4a	4.5±0.2a	2.8±0.5a	
F&P-values: Treat*day*time	F(4,179)=0.21;P=0.93						
Post-treatment							
Control	4.6±0.2a	3.9±0.2a	4.6±0.2a	4.2±0.2a	4.4±0.2a	3.1±0.6a	F(2,179)=35.09;P<0.0001
Malathion	3.1±0.2b	2.7±0.5b	3.6±0.2b	3.6±0.2b	3.5±0.2b	2.3±0.5b	
Thiamethoxam	2.5±0.2c	2.2±0.3c	3.4±0.2b	2.5±0.5c	3.4±0.2b	2.4±0.5b	
F&P-values: Treat*day*time	F(4,179)=0.60;P=0.67						
Treatment	X±SE time (sec) spent by bees visiting each inflorescence during *3 minutes* duration						F&P-values: Treatment
	Pre-treatment						
Control	79.8±4.1a	44.7±7.5a	76.8±3.6a	54.8±1.3a	80.7±3.8a	45.3±7.7a	F(2,179)=0.26;P=0.77
Malathion	77.8±2.6a	53.8±6.3a	77.9±2.6a	50.7±5.8a	75.0±2.9a	54.8±2.1a	
Thiamethoxam	79.0±4.2a	46.0±7.8a	78.2±4.1a	50.2±5.7a	78.9±4.3a	46.5±8.0a	
F&P-values: Treat*day*time	F(4,179)=0.69;P=0.60						
Post-treatment							
Control	79.1±3.0a	52.9±2.9a	77.0±3.6a	53.5±3.1a	78.5±3.5a	40.6±6.9a	F(2,179)=87.59;P<0.0001
Malathion	48.3±2.5b	35.2±6.0b	54.4±3.2b	42.0±2.8b	56.9±2.9b	30.4±6.7b	
Thiamethoxam	36.3±2.8c	32.6±4.0b	43.2±2.9c	29.9±5.1c	46.9±2.7c	26.9±5.9b	
F&P-values: Treat*day*time	F(4,179)=0.41;P=0.80						

[1&2] Denotes morning and evening, respectively; figures followed by the different letters in a column show significant difference at P<0.05

period ($F_{(2,179)}$ = 35.09; P<0.0001). There was also significant difference in the foraging activities between the treatments (Table 5). The mean numbers of DHB foraging activities did not differ significantly in the overall interactions both during the pre-spraying and the post-spraying (Table 5).

(iv) Bee foraging time recorded in the test crops

Pearl millet

Apart from the DHB foraging activities, their mean foraging time/ observation period was also recorded to determine time spent on the test inflorescences. There was no significant difference in the mean foraging time (seconds) spent by DHB on the Pearl millet inflorescences prior to the spraying of the imidacloprid and malathion (Table 2 and 3). Following the spraying of these insecticides, the mean DHB foraging time varied significantly between malathion treated and untreated inflorescences ($F_{(1,59)}$ = 7.63; P = 0.01). A similar trend was also recorded between imidacloprid treated and untreated inflorescences ($F_{(1,59)}$ = 94.05; P < 0.0001). The overall interactions of the mean time spent foraging were also not significantly different both during the pre-spraying and the post-spraying trial (Table 2 and 3).

Indian plum

There were also no significant differences in the mean foraging time spent by bees on the Indian plum inflorescences prior to the spraying of both imidacloprid and malathion (Table 4). Following the spraying of these insecticides, the mean foraging time declined significantly in the treated inflorescences ($F_{(2,179)}$ = 55.12; P < 0.0001). The mean foraging time was also significant different between the three treatments as indicated in table 4c. However, the overall interactions of the time foraging were also not significantly different both during the pre-spraying and the post-spraying (Table 4).

Mustard

Similar to the mean numbers of DHB foraging activities, the mean time foraging was also significantly different between morning and evening. It was significant between treatments during the pre-spraying of the malathion and thiamethoxam (Table 5). Much more time was spent on the untreated than in treated inflorescences of mustard during the post-spraying period ($F_{(2,179)}$ = 35.09; P<0.0001). There was also a significant difference in the mean foraging time between the treatments (Table 5). Similarly, the mean foraging time did not differ significantly in the overall interactions both during the pre-spraying and the post-spraying (Table 5).

DISCUSSION

The three test crops used in the present study are predominantly cultivated in the States of Rajasthan, Gujarat, Uttar Pradesh and Haryana. Pearl millet is cultivated in about 9.3 m ha and mustard in about 6 m ha. The estimated country-wide area for Indian plum is about 22,000 ha (Radha and Mathew, 2007). The neonicotinoids are highly recommended for the control of insect sucking pests in different crops in India. But these are also found effective against other insect pests. For example, imidacloprid is widely used against shootfly and termites in pearl millet and Indian plum fruitfly, while thiamethoxam is used for the control of mustard aphids (Gavkare *et al.,* 2013, www.cibrc.nic.in). Further, malathion is frequently used for the control of aphid and sawfly in mustard, Indian plum fruitfly and earhead midge in sorghum, which is closely related to pearl millet on the basis of crops group concept (Mandal *et al.,* 2012, www.cibrc.nic.in).

Besides their insecticidal activity, no adverse effects were reported in field studies (Blacquiére *et al.,* 2012, APVMA, 2014). Yet, these are also implicated for adverse effects on the pollinators including honeybees. Neonicotinoids have high contact toxicity to honey bees (Suchail *et al.,* 2001, Iwasa *et al.,* 2004, Bonmatin *et al.,* 2005). Hence, it is expected that these will also affect foraging activity as neonicotinoids are persistent. Decourtye *et al*. (2003) reported chronic and sublethal concentrations of neonicotinoids impairing foraging and learning activities of bees. Similar studies were

also carried out by Aliouane *et. al*. (2008). Similarly, field studies have also reported low visitation rate of bumblebee on the inflorescences of an ornamental shrub, *Rhododendron catawbiense* (L.) (Ericales: Ericaceae) treated with imidacloprid than in the untreated shrub (Maus *et al.,* 2006). Even, dead bees were seen in treated plots but not untreated plots (Maus *et al.,* 2007). Lethal toxicity to honeybee in hive treated with imidacloprid, at dosages reflecting residue levels in the environment was also reported during the *in situ* study conducted in the central Massachusetts, USA (Lu *et al.,* 2012). Easton and Goulson (2013) also reported adverse effects of neonicotinoids on attraction of pollinators towards water using the baited pan traps.

The concern over the adverse effect of neonicotinoids on the pollinators including honey bees was also raised by the Government of India through Department of Agriculture and Cooperation (No. 13001/2013-PP-I, 8th July, 2013) to constitute an expert committee to examine the use of the registered neonicotinoids in the country (Anonymous 2013). These were deliberated and the expert committee recommended studies on the toxicity and foraging activities of the native bees in the different crops which are approved for the application of neonicotinoid insecticides (Anonymous 2014).

The present study was carried out on the adverse effects of imidacloprid and that of malathion at the concentration which was eight-time less than recommended foliar concentration of 40 ppm on pearl millet and 50-time less than the recommended concentration of 250 ppm on Indian plum. Similarly, the adverse effect of thiamethoxam on DHB was studied at the concentration which was five times less than the foliar concentration of 25 ppm recommended on mustard plants (www.cibrc.nic.in). Our study showed decline in visits of DHB to the treated flowers/inflorescences, less foraging activity and time spent on all the treated crops. Creswell (2010) also reported reduced honey bee behaviour between 6 and 20 per cent on sunflower and canola flowers containing low levels of 0.7 and 10 ppb of imidacloprid, respectively for the crops treated at the time of seed sowing. Our results are also in agreement with Feltham *et al.* (2014) who found that foraging ability of bumblebee workers was substantially affected at low residual levels (6 ppb in pollen) in flowers of domestic gardens in Central-belt, Scotland.

Although, present studies indicate adverse effects of neonicotinoids, results strengthen our knowledge base concerning the on-going debate of overall utility of these pesticides. The neonicotinoids are invariably used for seed coating of hybrids of some crops, notably Bt cotton cultivated over the large area of about 11 m ha annually. Similarly, seed coating with neonicotinoids is recommended for other crops like soybean, mustard. However, extent of seed coating in the test crops is limited. Foliar sprays of neonicotinoids and other insecticides are more common in mustard than other two crops. Hence, it appears that foliar/floral sprays of insecticides may have adverse impact on bee pollinators which are found in abundance during flowering in mustard than in any other two test crops. These short-term effects of neonicotinoids on DHB may not impair their long-term ability to pollinate the crops in the agro-ecosystems, as these crops are often cultivated on small farms, with wide temporal distribution of flowering and with infrequent use of insecticides as per needs. Hence, foliar or floral sprays may also provide ample scope for bees to visit nearby fields. At the same time, a precaution is needed that foliar sprays of insecticides may be avoided during intense foraging activities, provided at the same time, harmful pests do not cause substantial loss of productivity. It is essential to conduct a large scale ecosystem-wise analysis of these neonicotinoids on pollination services before arriving at a final conclusion, especially in the Indian context.

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