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Effect of thiamethoxam 25 WG spray at pre-bloom on the foraging activity of *Apis mellifera* in mustard crop under open field condition

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Abstract
Thiamethoxam spray at mustard pre-bloom negatively affects the activity of *Apis mellifera* L. The average bee activity from 6 to 14 DAE was significantly less as compared to control. The data on *A. mellifera* foraging activity on different days and timing revealed that the activity of *A. mellifera* during 2018 and 2019 peaked 15.25 and 15.00 bees on 6 DAE at 1200h in thiamethoxam spray at pre-bloom; while in control the activity of *A. mellifera* was recorded on the peak (23.75 and 25.50 bees) on 8 DAE at 1200 h. The data on the interaction between treatment and time further revealed that average activity of *A. mellifera* in control was highest at 1200h (21.89 and 23.25 bees) significantly superior over 1000h (15.44 and 17.36 bees) and 1600h (9.81and 10.81 bees), while in thiamethoxam spray at pre-bloom the bee activity was highest at 1200h (5.83 and 7.58 bees) significantly superior over 1000h (4.44 and 6.11 bees) and 1600h (3.95 and 4.56 bees). Foraging activity during 2018 and 2019 irrespective of day and time was statistically higher in control (15.71 and 17.14 bees) in comparison to thiamethoxam spray at pre-bloom (4.74 and 6.08 bees), respectively. Therefore, it is evident from the experiment thiamethoxam 25 WG negatively affects the honey bee population. If the thiamethoxam 25 WG used continuously, the population of both managed as well as the wild honey bee will decline drastically in the near future. As a result of which total food production, particularly fruits and oilseed crops dependent on the honey bee for pollination will be decreased.

Keywords: thiamethoxam, neonicotinoids, foraging activity, *Apis mellifera* L

Introduction
Bees and mustard plants have a mutualistic relationship and coevolved during the long course of their evolutionary history. Since most of the oilseed crops are cross-pollinated, adequate pollination is vital for quality seed production. Mustard is also a cross-pollinated crop and requires sufficient pollinating agents for better pollination and seed production. The mustard flower attracts a wide range of insect species [31] especially pollen and nectar-feeders due to its bilateral, bright yellow flowers [1]. Insect pollination increases pollen deposition in mustard crop leading to expanded fruit set and seed production per plant and decreased variance of seed sets, enhanced quality of seeds, uniform ripening and plant vigour [22]. Nowadays the major problem is a constant decline in the population of pollinators and managed honey bee colonies. Factors that contribute to the decline of managed honey bee populations include the introduction of parasitic mites, pathogens, monoculture that results in malnutrition, genetically modified crops and the application of pesticides etc**.** Among them, the most important is the use of various kinds of pesticide on crops to which honey bees are attracted largely. To feed the fast-growing global population, synthetic insecticides are important for crop productivity in intensive farming systems where they preserve about one-fifth of the crop yield [25]. Insecticides that are used to suppress the insect pest population can affect non-target as well as and beneficial insects including pollinators [26]. Pesticide's effects on the honey bee are vital because of the need to control a wide variety of agricultural insect pests with insecticides [3] without hurting bees that accidentally come in contact with pesticides while foraging. In agricultural areas, a negative relationship was found between pesticide use and pollinator abundance, group richness, and diversity [27]. Foraging bees collect insecticide along with pollen and store it in the brood frames. Nurse bees feed the contaminated pollen to the developing brood, resulting in the total loss of the colony. Foraging bees are killed while collecting and transporting contaminated pollen, nurse bees while storing and feeding pollen and the brood by eating poisoned pollen [20].

Chronic toxicity by constant dietary exposure to residues found in pollen and honey affects the mortality of individual bees and the growth and reproduction of their colonies which include not only sub-lethal impairments but also delayed mortality [32]. If the neonicotinoid insecticides are used continuously, the population of both managed as well as the wild honey bee will decline drastically in the near future. As a result of which total food production, particularly fruits and oilseed crops dependent on the honey bee for pollination will be decreased. Hence it is necessary to find out the possible harmful effect of neonicotinoid compounds on the foraging activity of honey bee. Keeping in mind the above concern, the present investigation was undertaken to study the effect of thiamethoxam 25 WG on the foraging activity of *Apis mellifera* L.

Materials and Methods

The experiment was conducted under open field condition at Norman E. Borlaug Crop Research Centre, G. B. Pant University of Agriculture and Technology, Pantnagar-263145, Udham Singh Nagar, Uttarakhand, India. Thiamethoxam spray at mustard pre-bloom and control with an isolation distance of 3 km to evaluate the effects of neonicotinoid insecticides on *A. mellifera* abundance in the mustard ecosystem during Rabi season of 2018 and 2019.

Plant-plant and row-row spacing for *Brassica juncea* were taken 20 and 30 cm, respectively. Mustard sowing for the first trial was done on 12 November 2018 and sowing for 2nd trial was done on 18 November 2019. Spraying of thiamethoxam 25% WG was done (@ 100 g/ha) at pre-bloom of mustard crop with the help of knapsack sprayer. The spraying was done during the late evening to avoid maximum hazards to the bees. Abundance (number of bees/m^2/5 minute) of different pollinators visiting on mustard flower-heads were recorded at 1000h, 1200h and 1600h. Abundance of pollinators was recorded by taking direct count on flower heads of the plants covering an area of one sq m in each replication to study the relative abundance. Four such places were selected randomly for taking insect counts. Different observations were taken on day 6, 7, 8, 9, 10, 11, 12, 13 and 14. The data collected from field experiments were subjected to the analysis of variance following 3-factorial Randomized Block Design.

Results

The data enumerated in table 1 pertaining to the effect of thiamethoxam spray at pre-bloom on the foraging activity of *A. mellifera*. The bee foraging activity from 6 to 14 DAE was significantly less as compared to control. Bee foraging abundance on 6 DAE was found to be 11.25 bees being significantly less as compared to control (14.67 bees). Furthermore on 7 and 8 DAE a decrease (5.42 and 3.75 bees) was recorded, which was significantly less as compared to control (15.25 and 16.50 bees). Thenceforward on 9 and 10 DAE the bee foraging activity was slightly increased to 4.17 and 4.25 bees being significantly less as compared to control (15.58 and 14.83 bees). From that point forward on 11, 12 and 13 DAE a subtle decrease (3.83, 3.69 and 2.83 bees) was recorded being significantly less as compared to control (3.83, 3.69 and 2.83 bees). Hereafter on 14 DAE a slight increase was observed to 3.50 bees which were significantly less as compared to control (16.25 bees).

The data on *A. mellifera* foraging activity on different days and timing revealed that the activity of *A. mellifera* peaked 15.25 bees on 6 DAE in thiamethoxam spray at pre- bloom; while in control the activity of *A. mellifera* was recorded on the peak (23.75 bees) on 8 DAE at 1200 h.

The data on the interaction between treatment and time further revealed that average activity of *A. mellifera* in control was highest at 1200h (21.89 bees) significantly superior over 1000h (15.44 bees) and 1600h (9.81 bees), while in thiamethoxam spray at pre-bloom the bee activity was highest at 1200h (5.83 bees) significantly superior over 1000h (4.44 bees) and 1600h (3.95 bees).

The data further revealed that foraging rate irrespective of day and time was statistically higher in control (15.71 bees) in comparison to thiamethoxam spray at pre-bloom (4.74 bees).

Table 1: Effect of thiamethoxam spray at pre-bloom on the abundance of *Apis mellifera* on mustard under open field condition during 2018-19

No. Of flowers visited by single bee/ minute at different day after exposure								
DAE	**Thiamethoxam spray at Pre-bloom**				**Control**			
	1000h	**1200h**	**1600h**	**MEAN**	**1000h**	**1200h**	**1600h**	**MEAN**
6	10.50	15.25	8.00	11.25	12.25	22.75	9.00	14.67
7	5.75	5.50	5.00	5.42	13.75	22.00	10.00	15.25
8	4.00	4.75	2.50	3.75	17.00	23.75	8.75	16.50
9	3.25	5.25	4.00	4.17	15.00	23.00	8.75	15.58
10	4.50	4.75	3.50	4.25	14.25	21.00	9.25	14.83
11	3.75	5.00	2.75	3.83	16.25	22.00	10.50	16.25
12	3.00	3.50	4.58	3.69	17.00	22.00	10.00	16.33
13	2.50	4.00	2.00	2.83	17.50	19.25	10.50	15.75
14	2.75	4.50	3.25	3.50	16.00	21.25	11.50	16.25
MEAN	4.44	5.83	3.95	4.74	15.44	21.89	9.81	15.71
	SE(m)±				LSD/CD			
A	0.243				0.677			
B	0.514				1.437			
A×B	0.728				2.032			
C	0.297				0.83			
A×C	0.42				1.173			
B×C	0.891				NS			
A×B×C	1.26				NS			

DAE= Days After Exposure, A= Treatment, B= Days, C= Time interval

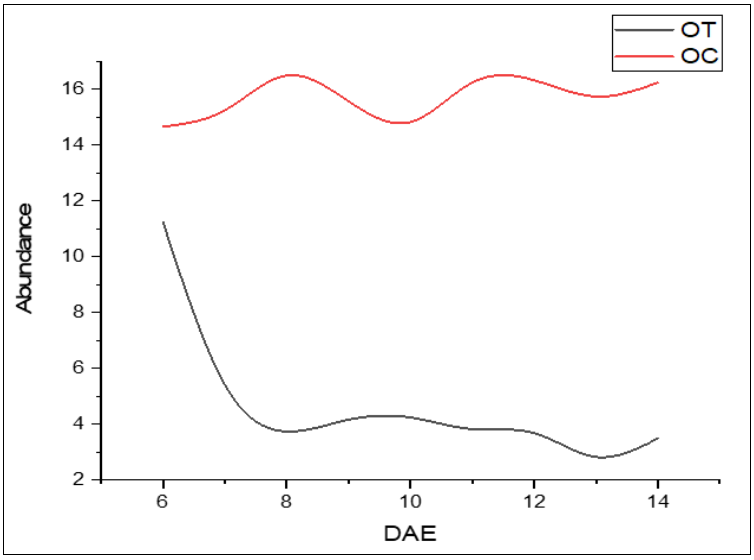

Fig 1(A): Average foraging activity of *A. mellifera* under open field condition in thiamethoxam spray at pre-bloom during 2018-19

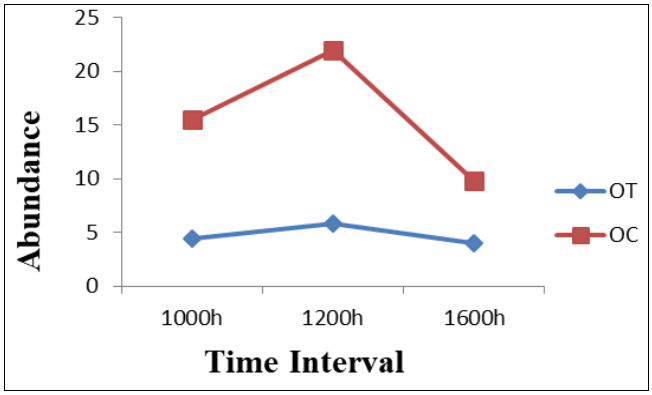

Fig 1(B): Average Foraging activity of *A. mellifera* at different time interval under open field condition in thiamethoxam spray at pre-bloom during 2018-19
OT= Thiamethoxam spray at pre-bloom under open field condition, OC= Control under open field condition

A perusal of data in table 2 pertaining to the effect of thiamethoxam spray at pre-bloom on the foraging activity of *A. mellifera* under open field condition during 2019-20. Bee population from 6 to 14 DAE was found to be significantly less in comparison to control. Bee count on day 2 was found to be 12.08 which was significantly less as compared to control (16.25 bees). Furthermore, on 7 and 8 DAE the bee activity was recorded to decrease (6.50 and 5.17 bees) being significantly less as compared to control (16.75 and 18.08 bees). Hereafter on 9 DAE a subtle increase (5.83 bees) was registered to be significantly less as compared to control (16.92 bees). From that point on 10, 11, 12, 13 and 14 DAE a decreasing trend (5.67, 5.08, 4.92, 4.75 and 4.75 bees) was noticed being significantly less as compared to control (16.50, 18.00, 17.25, 17.17 and 17.33 bees).

The data on *A. mellifera* foraging activity on different days and timing revealed that the activity of *A. mellifera* peaked 15.00 bees on 6 DAE at 1200h in thiamethoxam spray at pre-bloom; while in control the activity of *A. mellifera* was recorded on the peak (25.50 bees) on 8 DAE at 1200 h.

The data on the interaction between treatment and time further revealed that average activity of *A. mellifera* in control was highest at 1200h (23.25 bees) significantly superior over 1000h (17.36 bees) and 1600h (10.81 bees), while in thiamethoxam spray at pre-bloom the bee activity was highest at 1200h (7.58 bees) significantly superior over 1000h (6.11 bees) and 1600h (4.56 bees).

The data further revealed that foraging activity irrespective of day and time was statistically higher in control (17.14 bees) in comparison to thiamethoxam spray at pre-bloom (6.08 bees).

Table 2: Effect of thiamethoxam spray at pre-bloom on the abundance of *Apis mellifera* on mustard under open field condition during 2019-20

No. Of flowers visited by single bee/ minute on different day after exposure								
DAE	**Thiamethoxam spray at Pre-bloom**				**Control**			
	1000h	**1200h**	**1600h**	**MEAN**	**1000h**	**1200h**	**1600h**	**MEAN**
6	12.50	15.00	8.75	12.08	14.25	24.25	10.25	16.25
7	7.75	7.50	4.25	6.50	15.75	23.25	11.25	16.75
8	5.25	6.75	3.50	5.17	19.00	25.50	9.75	18.08
9	4.50	7.25	5.75	5.83	16.75	24.25	9.75	16.92
10	6.00	6.75	4.25	5.67	16.25	23.00	10.25	16.50
11	5.25	7.00	3.00	5.08	18.00	24.00	12.00	18.00
12	4.75	5.50	4.50	4.92	19.00	22.00	10.75	17.25
13	4.50	6.00	3.75	4.75	19.50	21.00	11.00	17.17
14	4.50	6.50	3.25	4.75	17.75	22.00	12.25	17.33
MEAN	6.11	7.58	4.56	6.08	17.36	23.25	10.81	17.14
	SE(m)±				LSD/CD			
A	0.243				0.68			
B	0.516				1.443			
A×B	0.73				2.04			
C	0.298				0.833			

A×C	0.422	1.178
B×C	0.895	NS
A×B×C	1.265	NS

DAE= Days After exposure, A= Treatment, B= Days, C= Time interval

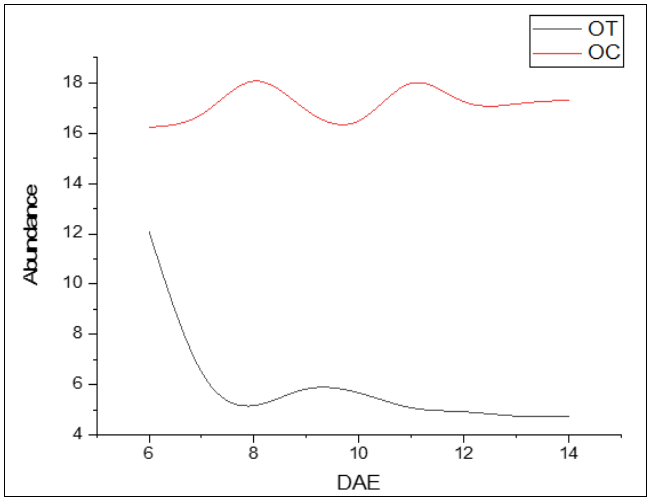

Fig 2(A): Average Foraging activity of *A. mellifera* under open field condition in thiamethoxam spray at pre-bloom during 2019-20

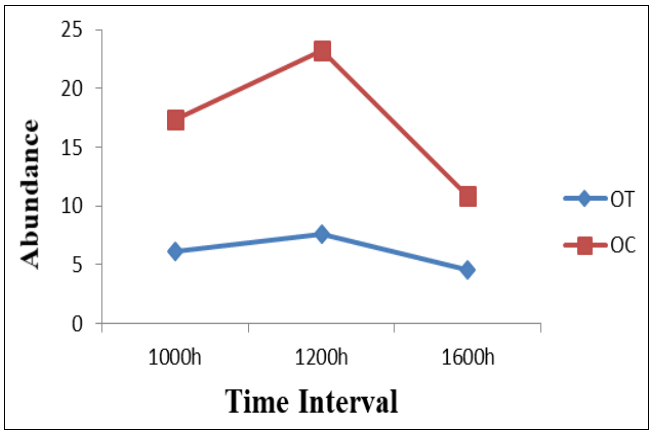

Fig 2(B): Average foraging activity of *A. mellifera* at different time interval under open field condition in thiamethoxam spray at pre-bloom during 2019-20

OT= Thiamethoxam spray at pre-bloom under open field condition, OC= Control under open field condition

Discussion

Personal observations revealed different behaviour within the colony under open field conditions. *A. mellifera* activity at hive entrance was normal in field condition and bees instead of confinement to the hive started foraging on the alternate flowering area around the colony. This was also evident from personal observations where incoming *A. mellifera* were seen with pollen loads during the period of low activity in the treated plots. It is thus indicated that reduced bee activity in the treated field might be due to avoidance of *A. mellifera* visiting the target crop where it was conditioned and it is in conformity with the work of Decourtye *et al.* (2004) [9], who observed avoidance behaviour in honey bees when exposed to imidacloprid, similar results were observed by Tremolada *et al*., 2010 [33], who have reported a significantly lower number of foraging bees in the neonicotinoids (thiamethoxam in particular) treated field as compared to the untreated field. The present investigations are also in line with the finding of some earlier authors [31, 21, 6] who reported reduced foraging bees to visit the syrup contaminated with imidacloprid under field and semi-field conditions. Bryden *et al.* (2013) [5] reported that pesticide exposure decreases pollination services. Yang *et al* (2008) [35] studied foraging behaviour of bees and reported delay in bee's visit to the feeding site when treated with Sub-lethal doses of imidacloprid. Elston *et al.* (2013) [13]. found altered foraging behaviour of bumblebees in field conditions after doses from field-realistic levels of thiamethoxam were given. Schneider *et al.* (2012) [29] investigated the same type of result, honey bees exposed to clothianidin and imidacloprid showed a reduction in foraging activity. Van der Slujis *et al.* (2013) [34] reported a wide range of adverse Sub-lethal effects of neonicotinoids through field-realistic doses in the honey bee. Gels *et al.* (2002) [16] found a significant reduction in foraging activity of *Bombus impatiens* colonies on non-irrigated imidacloprid-treated weed. Sandrock *et al.* (2014) [28] observed negative effect of two environmentally relevant concentrations of thiamethoxam and clothianidin, during chronic exposure on foraging efficiency of honey bee over two brood cycles. Cure *et al.* (2001) [8] observed a temporary reduction or interruption in foraging activity of bee at 100 ppb concentration of imidacloprid. Based on these result, it's obvious that neonicotinoids interfere with bee's foraging activity. Similarly, Cresswell (2011) [7] under semi-field conditions observed that when *Apis mellifera* was exposed to neonicotinoid, it consumed less contaminated syrup which reduced the foraging activity of bees. Sharma and Abrol (2014) [30] reported that reduction in the number of foraging in *A. mellifera* after 24 hours of insecticidal treatments with imidacloprid. They also recorded considerable recovery and normal activity after 3 days and 7 days, respectively of spray under field conditions. Similarly, Giri (2017) [17] reported a significant decline in foraging activity of *A. mellifera* up to 7th day after spraying of thiamethoxam on the mustard bloom under field and semi-field condition. In semi or open field condition, several workers reported that foraging bees reduced their visits to a syrup feeder when it was contaminated with 2mg/kg (Mayer and Lunden, 1997) [24], 100 mg/kg (Kirchner, 1999) [21] and 50mg/kg of imidacloprid (Colin *et al*., 2000) [6]. The field treated with deltamethrin showed a decrease in foraging activity of *A. mellifera* foragers (Bocquet *et al*., 1980 [4]; Faucon *et al*., 1985; Florclli *et al*., 1987) [4, 14, 15]. Decourtye *et al.* (2001) [12] reported a negative effect of lower concentration of imidacloprid (50 ppb) on the learning performance of honey bees. A concentration of 1000 ppb of imidacloprid affected foraging activity, but a concentration of 100 ppb showed no negative effect on bee's activity (Guez *et al*., 2001) [18]. Decourtye *et al.* (2005) reviewed the work of earlier researchers (Decourtye *et al*., 2003; Lambin *et al*., 2001 and Guez *et al*., 2001) [11, 23, 18] and reported altered foraging activity as the negative sub-lethal effects of thiamethoxam. On the contrary, Ambolet *et al*., 1999 [2] studied the effect of the neonicotinoid on honey bees as a seed dressing in 3 tunnels and 8 field trials in France. He found no adverse effect of the neonicotinoid on the foraging activity of honey bees. While Henry *et al* (2012) [19] have been reported that sub-lethal doses of the neonicotinoid such as thiamethoxam and imidacloprid have a negative impact on honey bee foraging success.

Conclusion

Thiamethoxam 25 WG spray at pre-bloom had an adverse effect on the foraging activity of *A. mellifera*, which is evident from significant reduction in the quantum of bee activity from 6 to 14 DAE in thiamethoxam sprayed crop.

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