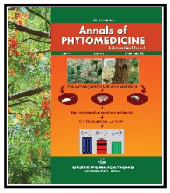
Annals of Phytomedicine 11(1): 694-699, 2022

DOI: http://dx.doi.org/10.54085/ap.2022.11.1.83

Annals of Phytomedicine: An International Journal

http://www.ukaazpublications.com/publications/index.php

Print ISSN : 2278-9839 Online ISSN : 2393-9885



Original Article : Open Access

Response of neonicotinoid (thiamethoxam 25 WG) spray at pre-bloom on the abundance of *Apis mellifera* in *Brassica rapa* L. crop under caged environment

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Article Info

Article history

Received 3 January 2022

Revised 21 February 2022

Accepted 22 February 2022

Published Online 30 June 2022

Keywords

Thiamethoxam

Mustard preflowering

Neonicotinoids

Abstract

Abundance of *Apis mellifera* was remarkably less as compared to control from 6 to 14 days after spraying during 2018-19 and 2019-20. Population of *A. mellifera* on day 6 was recorded to be 30.83 bees/m^2/5 min. This data were remarkably low in comparison to untreated field population (38.08 bees/m^2/5 min). Thenceforward on 7, 8, 9, 10 and 11 days after exposure, a reducing trend (23.75, 19.17, 16.42, 14.67 and 14.17 bees/m^2/5 min) was observed. This data were also remarkably low in comparison to control (38.50, 35.75, 38.00, 36.58 and 34.92 bees/m^2/5 min). Here after on 12, 13 and 14 days after spraying, a significant rebound in population (18.33, 22.00 and 24.00 bees/m^2/5 min) was registered which was significantly less in comparison to control (38.25, 36.00 and 35.58 bees/m^2/5 min).

1. Introduction

The relationship between bees and mustard plants has always been mutualistic and it has coevolved throughout the long course of their biological process history. Bee pollinators play important role in pollination and standard seed manufactured by sufficient pollination. For higher seed production, adequate pollinating agents play important role and additionally mustard is a cross pollinated crop (Stanley *et al.*, 2015). Transmission of pollen from one flower to another flower is done by different pollinators and these pollinators increase the fructification of grain in almost 88% of blooming plants (Ollerton *et al*., 2011), whereas various diversity of kingdom animalia responsible for pollination in different flowering plants (Ollerton, 2017). Among of these, honey bees play major performance in procreation (Willmer *et al*., 2017) and it is main pollinator attributed to *Brassica* species crop because as we know that the mustard crop flower is greatly charming and these flowers gave the nectar and pollen to different pollinators. As we know that the major pest of *Brassica rapa* L. is *erysimi* and for the management of *Lipaphis erysimi*, farmers used different type of neonicotinoid isecticides for example, thiamethoxam, clothianidin and imidacloprid, *etc.* These insecticides showed negative effect on the honey bee population directly (Desneux *et al*., 2007; Schafer *et al*., 2012; Lambert *et al*., 2013; Matsumoto, 2013). Because it affects the population of honey bee, due to this the production of mustard crop is also affected qualitatively and quantitatively. Among all groups of the insecticides, thirty per cent insect killing chemicals are neonicotinoids (Simon-Delso *et al*., 2015). In USA and European countries, some neonicotinoids are banned; therefore, assuming that these insecticides used regularly then neonicotinoids, affect the population of honey bee fastly in the negative way (Gill *et al*., 2012; Decourtye *et al*., 2003; El-Hassani *et al*., 2008; Aliouane *et al*., 2009; Alix *et al*., 2009; Thompson, 2010; Blacquiere *et al*., 2012). Finally, the production of fruits, oilseed crop and various different crops will be negatively affected. Keeping in mind the above concern, the present study “Effect of thiamethoxam on abundance of *A. mellifera* on mustard crop under caged condition” was planned.

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2. Materials and Methods

2.1 Experimental location

The cage studies were conducted at Norman E. Borlaug Crop Research Centre, G.B. Pant University of Agriculture and Technology, Pantnagar-263145, Udham Singh Nagar, UK, India; to studies on the response of neonicotinoid (thiamethoxam 25 WG = Actara) spray at before flowering on the abundance of Italian honey bee in *B. rapa* inside caged condition during Rabi season of 2018 and 2019.

2.2 Experimental trial

The soil of experimental field was prepared by preparatory tillage operations. *B. rapa* broadcast for 1st trial was on 3 Dec 2018 for semi-field condition. Sowing for second trial was done on 15 Dec 2019 for semi-field.

2.3 Preparation of cages

The framework of the cages was made up of bamboos which were further covered by nylon mesh to prevent any trespassing of the pollinators. Each structure maintained a dimension of 12 x 7 x 4 cubic meters.

2.4 Materials used

Mustard seed	Variety PR 20
Honey Bee colony	*Apis mellifera* Colony (12 nos.)
Nylon mesh cage	Mesh size: 1.2 mm (0.047 inch)
Bamboo sticks	12 m length, 7 m width, 4.5 m height
Insecticides	Thiamethoxam (Actara 25 WG)
Stopwatch	
Knapsack sprayer	

2.5 Abundance of *A. mellifera* arriving the *B. rapa* under caged condition

Abundance of honey bee population was calculated by bee/m^2/5 min. Randomly 4 places were selected and each selected place was covering the 1 square meter area. In this area, observed the population of honey bee for 5 min. In this experiment, one treatment and 1 control were taken and each treatment has 4 replication. Observations were taken after flowering of mustard crop. Spray of thiamethoxam has been done on before-flowering of mustard and 6 days after spraying flowers on the mustard was emerged and observations were taken from 6 days after spraying, After that, 1 days interval regular observations was taken up to 14 days after spraying. The raw observations were analyzed with the help of SPSS 16 following the 3- factorial randomized block design. The similar observation taken by this method by several authors, *viz.* (Giri, 2017; Budhiram, 2018; Shakeel and Mian Inayatullah, 2015).

3. Results

3.1 Response of actara 25 WG sprinkle at before-flowering on abundance of *A. mellifera* in *B. rapa* crop under caged environment in the year 2018-19

The statistics showing the response of actara sprinkle at before-flowering on population of *A. mellifera* under caged environment (Table 1). Abundance of *A. mellifera* from six to fourteen days after spraying was remarkably low in comparison to untreated field population. *A. mellifera* activity on day 6 was observed 29.33 individuals/m^2/5 min, on 6^{th} day activity was remarkably low in comparison to untreated field population (37.00 individuals /m^2/ 5 min). Thenceforth on 7, 8, 9, 10 and 11 days, foraging activity of *A. mellifera* was continuously decreased, *viz.*, 22.00, 17.42, 14.92, 13.17 and 12.50 individuals/m^2/5 min, on that day activity was low in comparison to untreated field population, *viz.*, 37.00, 34.58, 36.42, 35.33 and 33.08 bees/m^2/5 min. Thereafter, on 12, 13 and 14 days after spraying, a rebound in bee population was observed, *viz.*, 16.92, 20.50 and 22.75 bees/m^2/5 min, which was remarkably less as compared to control, *viz.*, 36.50, 34.25 and 34.92 bees/m^2/5 min.

Table 1: Response of thiamethoxam spray at pre-bloom on foraging activity of *A. mellifera* on mustard under caged condition during 2018-19

Abundance of *A. mellifera* (no. of bees/m^2/5 min)								
DAS	Thiamethoxam spray at pre-bloom				Control			
	10.00 AM	12.00 PM	4.00 PM	Mean	10.00 AM	12.00 PM	4.00 PM	Mean
6	28.25	36.75	23.00	**29.33**	38.00	41.50	31.50	**37.00**
7	20.75	28.75	16.50	**22.00**	36.50	41.50	33.00	**37.00**
8	15.00	23.75	13.50	**17.42**	34.25	38.25	31.25	**34.58**
9	13.25	17.50	14.00	**14.92**	36.50	40.00	32.75	**36.42**
10	10.75	19.25	9.50	**13.17**	37.00	41.00	28.00	**35.33**
11	10.50	18.25	8.75	**12.50**	32.50	38.75	28.00	**33.08**
12	15.25	19.75	15.75	**16.92**	37.75	41.75	30.00	**36.50**
13	20.75	22.50	18.25	**20.50**	34.75	38.50	29.50	**34.25**
14	23.00	24.50	20.75	**22.75**	34.50	39.25	31.00	**34.92**
Mean	**17.50**	**23.44**	**15.56**	**18.83**	**35.75**	**40.06**	**30.56**	**35.45**
	± SEM				**CD**			
A	**0.259**				**0.723**			
B	**0.549**				**1.534**			
A × B	**0.776**				**2.169**			
C	**0.317**				**0.885**			
A × C	**0.448**				**1.252**			
B × C	**0.951**				**2.656**			
A × B × C	**1.345**				**NS**			

A = Treatment, B = Days, C = Time interval, BE = Before exposure, DAE = Days after exposure.

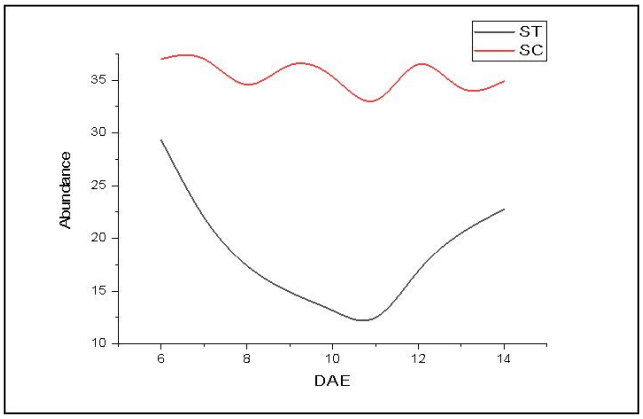

Figure 1(A): Figure showing at which days the highest activity was found (2018-19).

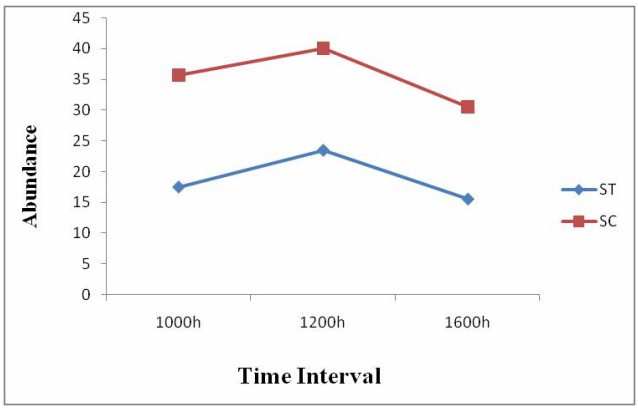

Figure 1(B): Figure showing at which time activity of *A. mellifera* was highest.

ST = Thiamethoxam spray at before-flowering under caged environment.

SC = Untreated field under caged environment.

The population of bees spiked 36.75 bees/m^2/5 min on 6 DAS at 12.00-2.00 PM in thiamethoxam spray at before-flowering; whereas in untreated field, the population of bees was found to be spiked (41.75 bees) on 12 DAS at 12.00-2.00 PM (Figure 1 A).

Mean abundance of *A. mellifera* in untreated plot was maximum at 12.00-2.00 PM (40.06 bees/m^2/5 min), remarkably superior over 10-12 PM (35.75 bees/m^2/5 min) and 2.00-4.00 PM (30.56 bees/m^2/5 min). Whereas in thiamethoxam spray at preflowering was maximal at 12.00-2 PM (23.44 bees/m^2/5 min), remarkably superior over 10-12 AM (17.50 bees/m^2/5 min) and 2-4 PM (15.56 bees/m^2/5 min) (Figure 1 B).

3.2 Response of actara 25 WG sprinkle at before-flowering on abundance of *A. mellifera* in *B. rapa* crop under caged environment in the year 2019-20

The statistics referring to response of actara sprinkle at before flowering on population of honey bee under caged environment. *A. mellifera* foraging activity was notably less as compared to control from 6 to 14 days after spraying. Population of *A. mellifera* on day 6 was recorded to be 30.83 bees/m^2/5 min, on 6th day the population was remarkably low in comparison to untreated field population (38.08 individuals/m^2/5 min). Thenceforward from 7, 8, 9, 10 and 11 days after spraying, a reducing trend (23.75, 19.17, 16.42, 14.67 and 14.17 individuals/m^2/5 min) were observed, on these days, the population was remarkably low in comparison to untreated field population (38.50, 35.75, 38.00, 36.58 and 34.92 bees/m^2/5 min). Here after on 12, 13 and 14 days after spraying a significant rebound (18.33, 22.00 and 24.00 bees/m^2/5 min) was registered being remarkably less in comparison to control (38.25, 36.00 and 35.58 bees/m^2/5 min).

Abundance of *A. mellifera* acknowledged that the population of honey bee spiked 38.25 individuals /m^2/5 min on six days after spraying at 12.00-2.00 PM in actara sprinkle at before flowering; whereas within untreated plot population of honey bee was found to be on mountain (43.75 individuals/m^2/5 min) on 12 days after spraying at 12.00-2.00 PM (Figure 2 A).

The statistics on interaction between treatment and time acknowledged that mean abundance of honey bee in control was maximum at 12.00-2.00 PM (41.53 bees/m^2/5 min), remarkably superior over 10.00-12.00 AM (37.36 bees/m^2/5 min) and 2.00-4.00 PM (31.67 bees/m^2/5 min). Whereas in actara spray at before flowering population of individuals was spiked at 12.00-2.00 PM (24.69 bees/m^2/5 min), notably superior over 10.00-12.00 AM (19.22 individuals /m^2/5 min) and 2.00-4.00 PM (17.19 individuals/m^2/5 min) (Figure 2 B).

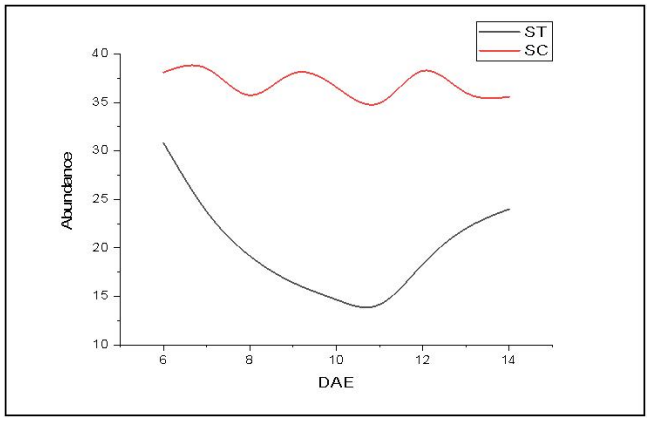

Figure 2(A): Mean abundance of *A. mellifera* under caged environment in thiamethoxam spray at before flowering in the year 2019-20.

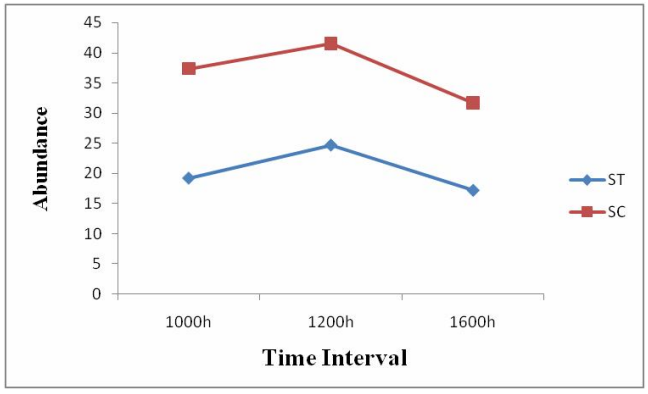

Figure 2(B): Figure showing at which time activity of *A. mellifera* was highest.

Table 2: Response of thiamethoxam spray at pre-bloom on foraging activity of *A. mellifera* on mustard under caged condition during 2019-20

Abundance of *A. mellifera* (no. of bees/m²/5 min)								
DAS	Thiamethoxam spray at pre-bloom				Control			
	10.00 AM	12.00 PM	4.00 PM	Mean	10.00 AM	12.00 PM	4.00 PM	Mean
6	30.00	38.25	24.25	**30.83**	39.50	42.75	32.00	**38.08**
7	22.75	30.25	18.25	**23.75**	38.25	43.25	34.00	**38.50**
8	17.00	25.00	15.50	**19.17**	35.25	39.50	32.50	**35.75**
9	15.25	18.75	15.25	**16.42**	38.25	41.75	34.00	**38.00**
10	12.00	20.50	11.50	**14.67**	38.75	41.25	29.75	**36.58**
11	12.00	19.75	10.75	**14.17**	34.50	40.50	29.75	**34.92**
12	16.75	20.75	17.50	**18.33**	39.25	43.75	31.75	**38.25**
13	22.50	23.50	20.00	**22.00**	36.50	40.25	31.25	**36.00**
14	24.75	25.50	21.75	**24.00**	36.00	40.75	30.00	**35.58**
MEAN	**19.22**	**24.69**	**17.19**	**20.37**	**37.36**	**41.53**	**31.67**	**36.85**
	± SEM				CD			
A	**0.251**				**0.701**			
B	**0.533**				**1.488**			
A × B	**0.753**				**2.104**			
C	**0.308**				**0.859**			
A × C	**0.435**				**1.215**			
B × C	**0.923**				**2.577**			
A × B × C	**1.305**				**3.645**			

DAE = Days after exposure, BE = Before exposure, A = Treatment, B = Days, C = Time interval.

4. Discussion

Already invention that nAcR are localized in several areas of the *A. mellifera* cerebrum (Bicker, 1999; Kreissl and Bicker, 1989). nAchR helps in the learning behaviour (Canao *et al*., 1996; Canao *et al*., 2001). Here, the observations acknowledged differences in the abundance behaviour inside the colony under caged conditions. *A. mellifera* remained hindered inside the hive up to 14 DAS under caged conditions. It is therefore illustrated that less abundance in exposed field may be because of shunning of honey bee arriving the destination flora wherever it had been habituated and it is in obedience with the work of Decourtye *et al.* (2004), who noticed evasion action in *A. mellifera* when come in contact to imidacloprid, same findings were recorded by Tremolada and Mazzoleni (2010). They have recorded remarkably less population of bee individuals in the thiamethoxam exposed field in comparison to control field. The current research findings are also similar with the findings of some former scientists (Mayer and Lunden, 1997; Kirchner, 1999; Colin *et al*., 2000). They observed abundance of *A. mellifera* and were low in syrup exposed with imidacloprid under caged conditions.

The low activity of the bees in the insecticidal treated plots may be because of evasion of foraging on insecticide exposed plots and it was reported as similar with Cresswell (2011), who reported that *A. mellifera* exposed to neonicotinoid consumed less contaminated syrup and lack of consumption may have been due to lower foraging activity

under semi-field conditions. Personal observations during the present investigation recorded large population of *A. mellifera* foragers remaining confined inside and little activity was observed at the hive entrance in thiamethoxam and imidacloprid treated crop.

Pollinators activity reduced in the pesticide treated field (Bryden *et al*., 2013). Yang *et al*. (2008) noticed that abundance of *A. mellifera* reduced when feeding site exposed with sub-lethal doses of imidacloprid. Similar findings are reported by Schneider *et al*. (2012), who reported that *A. mellifera* population was reduced when come in contact with clothianidin and imidacloprid exposed field. Sandrock *et al*. (2014) also reported the negative response of actara and clothianidin on the population of honey bee. Cresswell (2011) noticed that under caged condition when honey bee come in contact to neonicotinoid, they forage reduced amount of adulterated syrup.

Abundance of *A. mellifera* was reduced after 24 h of imidacloprid treatment. They also observed that after 3 and 7 days, the population of *A. mellifera* was recovered (Sharma and Abrol, 2014). Similar data observed by Giri (2017), who observed that the population of *A. mellifera* was declined up to 7 days after spraying under caged condition. After that, the population of bee activity was normal.

5. Conclusion

Thiamethoxam spray at pre-bloom showed negative effect on *A. mellifera* population. Abundance of *A. mellifera* irrespective of days and time interval was significantly less in thiamethoxam spray at pre-bloom in comparison to control. The population of *A. mellifera* was reduced up to 11 days after spraying of thiamethoxam. Thenceforth, the population of bee individual was regain and showed the normal activity of *A. mellifera* population. As we know that farmers injudiciously use neonicotinoids for the management of aphids, in mustard. If farmers used continuously thiamethoxam for the management of aphid, then it is harmful for the *A. mellifera* and other pollinators due to this the productivity of the mustard crop will be directly affected.

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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Citation **Ravindra Kumar, Pramod Mall and Renu Pandey (2022). Response of neonicotinoid (thiamethoxam 25 WG) spray at pre-bloom on the abundance of *Apis mellifera* in *Brassica rapa* L. crop under caged environment. Ann. Phytomed., 11(1):694-699. http://dx.doi.org/10.54085/ap.2022.11.1.83.**