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In vivo and *in vitro* studies of effect of different insecticides against Indian honey bees (*Apis cerana* Fab.)

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Abstract

The present study was conducted to record the effect of insecticides on foraging activity of honeybee (*Apis cerana* Fab.) on mango under field conditions during flowering season of the year 2018-2019 at National Agricultural Research Project, Regional Fruit Research Station, Ganeshkhind (Plain Zone). and toxicity of different insecticides against *Apis cerana* Fab. under laboratory condition at biocontrol laboratory, college of agriculture, Pune.

Among three sprays of insecticides maximum foraging activity observed in treatment T_6 N.S.E. followed by T_5 *Metarhizium anisopliae* and minimum activity observed in the treatment T_1 imidacloprid, T_3 thiamethoxam, T_4 lambda cyhalothrin and T_2 dimethoate respectively. Toxicity of insecticides under laboratory condition was observed as follows: Treatment T_1 Imidacloprid > treatment T_3 Thiamethoxam > treatment T_4 Lambda cyhalothrin > treatment T_2 Dimethoate > treatment T_6 N.S.E.+ treatment T_5 *Metarhizium anisopliae*.

Keywords: Mango, foraging activity, insecticides, toxicity, *Apis cerana* Fab

1. Introduction

Mango is an important fruit crop of Maharashtra and plays an important role in the economy of Maharashtra. Mango has been cultivated from last four thousand years back due to its hardy nature, low cost of cultivation and maintenance. Now, it is a commercially cultivated in subtropical region as a important fruit crop [1]. At the same time, the mango crop is heavily attacked by different sucking pests *viz.*, mango hoppers, mealy bugs, aphids and other insect species. There are several insecticides recommended for the control of mango pests. Several insecticides are broad spectrum in action and highly toxic in nature. Insecticides are important for ensuring both crop quality and quantity in todays integrated pests management programme. The use of insecticides is one of the most in control of mango pests but sublethal doses and repeated sprays of pesticides showing detrimental effects on the natural enemies as well as pollinators, its developments, foraging behavior and colony conditions during flowering period of mango which results in fruit set as well as yield of the crop. Honey bees mostly depends on the flowers for nectar and pollens and thus at risk endangering exposing to various levels of pesticides while they collecting nectar and pollen. The newest major groups of insecticides i.e. neonicotinoids, which include imidacloprid, acetamiprid, and thiamethoxam. The use of imidacloprid has been suspended because of concerns that it may have a drastic effect on bee populations, causing loss of honeybees and weakening hives. Acetamiprid and thiamethoxam are presented as potential alternatives to imidacloprid subject to proof that they are harmless to non-target species [2].

Honeybees are the social insects and having great importance because of production of honey, wax and also pollinates many crops and trees. It is due to bee pollination that crop yield increases. Utilization of pollinators especially honey bees is considered as one of the cheapest and ecofriendly approach in maximizing the yield of cross pollinated crops [3]. Many investigations have consistently confirmed that yield levels can be increased to an extent of 50 to 60 per cent in fruits and plantation crops. Insect pollination of crops is an essential crop management practice and should be utilized skillfully by harnessing the activity of wild and domestic honeybees. Therefore, it is necessary to spray the insecticides to control the sucking pests but due care must be taken while application of pesticides and safe for the pollinators during flowering of mango.

2. Materials and Methods

2.1 Effect of insecticides on foraging activity of honeybee (*Apis cerana* Fab) on mango under field condition.

Selecting 24 number of mango plants of 5 -6 year age of *keshar* variety at National Agricultural Research Project, Regional Fruit Research Station, Ganeshkhind, Pune.

2.1.1 Detail of insecticidal treatments and its application

Selected plants were tagged properly and randomly as per the treatment and insecticidal sprays applied after proper flowering and observing the hoppers population on mango flowers. The treatment 1 to 6 sprayed with respective four insecticides, one botanical insecticide and one biopesticide.

2.1.2 Preparation of insecticidal formulations:

The insecticidal formulations (Treatment 1-5) were prepared separately as per the dose for each and every insecticide treatment along with sticker. The 5 liters of water used for spraying and required quantity of insecticides for 5 liter of water was measured with measuring cylinder (10 ml size) for liquid insecticides while and weighing balance used for measuring granular insecticides. The insecticide was properly mixed in water by shaking the pump before spraying and sprayed on the tagged plants early in the morning as per the treatment. After application of each insecticide, the spray pump was cleaned by washing under tap water and used for next treatment spray.

2.1.3 Preparation of Neem Seed Extract (NSE):

For the treatment T_6 NSE was prepared just a day before the application. For Preparation of Neem Seed Extract, need seeds were collected and dried under shade. Dried neem seeds were ground into fine course powder using grinder and then soaked in water in plastic bucket and kept overnight. The soaked material was then filtered through muslin cloth and filtrate was dissolved in desired quantity of water before spraying

2.1.4 Insecticidal spray

The first insecticide spray given when 50 percent of flowers opened and maximum number of honey bees seen foraging in mango flowers. The foraging activities of *A. cerana* were recorded with number of visits of *A. cerana* honeybees per panicle in 5 minutes period at 10.00 to 11.00 am when the 50 percent flowers opened. The five panicles were selected from each treatment for recording the effect of insecticides on foraging behavior of honey bee in mango.

2.2 Toxicity of different insecticides against *Apis cerana* Fab. under laboratory conditions

The laboratory experiment was conducted at 22-24^{0}C temperature and 70- 80 per cent relative humidity.The insecticidal formulations were prepared as per the treatments. The plastic jars of 1.5 kg capacity were used for this work. The insecticide spray was given to inner surface of jar with the help of ganesh sprayer and dried under shade for one hour and the mango flowers also sprayed with same chemicals and kept in respective jars. The honey bees (*A. cerana*) visiting to mango flowers were collected with the help of insect collection net early in the morning before application of insecticides. After application of insecticides 30 number of worker bees/treatment were released in each jar and covered with muslin net and rubber band. All the jars were kept at 22-24 ^{0}C temperature and 70- 80 per cent relative humidity under laboratory condition. The observations on mortality of honeybees *A. Cerana* were recorded at 5, 10 and 15 hours after spray and percent corrected mortality was calculated.

2.3 Statistical analysis of the data

The data recorded on peak foraging activity of honey bees was transformed in to $\sqrt{x + 0.5}$ and arcsin values as per the statistical methods and used for analysis and interpretation [4].

3. Results

3.1 Effect of different insecticides on foraging activity of *Apis cerana* Fab. on mango

Effect of insecticides on foraging activity of honeybee (*A. cerana*) on mango after first spray are presented in Table 1.

3.1.1 After first spray

The pre-count data regarding foraging activity of honeybee *A. cerana* on mango was found non-significant. A day before first spray, the number of bee visiting to mango flowers ranged from 7.64 to 9.53 bees/panicle/5 min. and did not differ significantly among the treatments. The foraging activity of honeybee, *A. cerana* on mango was significantly affected after first spray due to spraying of all chemical insecticides during the research. Lowest foraging activity of bee was observed on mango flowers i.e. 6.13, 7.13 and 7.67 bee/panicle/5 min. due to spraying of Imidacloprid 17.8 SL and it was at par with Thiamethoxam 25 WG with 6.80, 6.87 and 7.40 on mango trees bee/panicle/5 min. at 1,5 and 10 days after first spraying, respectively. The foraging activity was also affected and less in number due to spraying of Lambda cyhalothrin 5 EC and Dimethoate 30 EC. 7.47, 7.07 and 9.40 bee/panicle/5 min. in Lambda cyhalothrin while it was 7.93, 7.47 and 8.13 bee/panicle/5 min. were recorded in the treatment Dimethoate 30 EC at 1, 5 and 10 days after spray. The foraging activity of bees was not affected due to spraying N. S. E. @ 5 per cent amd *M. anisoplae* @ 5 g per litre of water and it was at par with untreated control after first spray. The foraging activity was 8.87, 8.27 and 9.60 bee/panicle/5 min. in the treatment *M. anisoplae* and 9.60, 9.00 and 9.47 bee/panicle/5 min. in the treatment N. S. E. @ 5 per cent at 1, 5 and 10 days after first spray.

3.1.2 After second spray

The effect of second spray of different insecticides on foraging activity of *Apis cerana* Fab. on mango after second spray shows that there was significant difference amongst the treatments. Reduction in the foraging activity of *A. cerana* on mango after second spray indicated that all four chemical insecticidal sprays viz., Imidacloprid @17.8% SL, Thiamethoxam 25 WG, Lambda cyhalothrin 5 EC and Dimethoate 30 EC shown negative effect on foraging activity of bees in mango after second spray.

The foraging activity of bees significantly reduced after spraying in the treatment T_1 (Imidacloprid @17.8% SL) with minimum foraging activity viz., 5.00, 5.53 and 6.40 bee/panicle/5 min. at 1,5 and 10 days. The treatment T_3 Thiamethoxam 25 WG was second chemical insecticide which was found harmful for foraging to bees on mango with 5.87, 6.13and 7.00 bee/panicle/5 min. and which was at par with T_3 Lambda cyhalothrin 5 EC with 6.07, 6.20 and 6.80 bee/panicle/5 min. and treatment T_2 Dimethoate 30 EC with 6.33, 6.80 and 7.07 bee/panicle/5 min. at 1, 5 and 10 days after second spray, respectively. The foraging activity of bees not affected in the treatment T_5 *Metarhizium anisoplae* and T_6

N.S.E. after second spray and the activity at par with the treatment T_7 Untreated Control.

3.1.3 After third spray

The sequential third spray of different insecticides has shown statistically significant effect on foraging activity of *A. cerana* on mango and there was significant difference amongst the treatments. The application of chemical insecticides significantly reduction in the foraging activity of *A. cerana* on mango after third spray and indicated that all four chemical insecticidal sprays viz., Imidacloprid @17.8% SL, Thiamethoxam 25 WG and Lambda cyhalothrin 5 EC were highly toxic to *A. cerana* for foraging on mango flowers after spray. The foraging activity of bees significantly affected and reduced due to spraying of the treatment T_1 (Imidacloprid @17.8% SL) with foraging activity viz., 3.73, 3.00 and 2.97bee/panicle⁄5min. at 1, 5 and 10 days and which was at par with the treatment T_3 Thiamethoxam 25 WG with 3.67, 4.27 and 3.00 bee/panicle/5min. at 1, 5 1nd 10 days after spraying of insecticides.

While the treatment and the treatment T_4 Lambda cyhalothrin 5 EC with 4.20, 5.47 and 4.60 bee/panicle/5min. and T_2 Dimethoate 30 EC were observed to be moderately toxic to *A. cerana* for foraging on mango flowers after third spray. 4.60, 5.27 and 4.73 bee/panicle/5min. after 1, 5 and 10.

Table 1: Effect of insecticides on foraging activity of honeybee (*Apis cerana* Fab.) on mangoafter first spray

Tr. No.	Treatment Details	Dose (g or ml)	Pre count	After first spray Post count (DAS**)			After second spray Post count (DAS**)			After third spray Post count (DAS**)		
				1 DAS	5 DAS	10 DAS	1 DAS	5 DAS	10 DAS	1 DAS	5 DAS	10 DAS
			(Bees/panicle/5 min.)				(Bees/panicle/5 min.)			(Bees/panicle/5 min.)		
T_1	Imidacloprid @17.8% SL	0.40	8.93 (3.07)	6.13 (2.58)	7.13 (2.76)	7.67 (2.86)	5.00 (2.35)	5.53 (2.46)	6.40 (2.63)	3.73 (2.06)	3.00 (1.87)	2.97 (1.86)
T_2	Dimethoate 30 EC	2.00	8.33 (2.97)	7.93 (2.90)	7.47 (2.82)	8.13 (2.94)	6.33 (2.61)	6.80 (2.70)	7.07 (2.75)	4.60 (2.26)	5.27 (2.40)	4.73 (2.29)
T_3	Thiamethoxam 25 WG	1.00	9.53 (3.17)	6.80 (2.70)	6.87 (2.71)	7.40 (2.81)	5.87 (2.52)	6.13 (2.58)	7.00 (2.74)	3.67 (2.04)	4.27 (2.18)	3.00 (1.87)
T_4	Lambda cyhalothrin 5 EC	1.00	8.47 (2.99)	7.47 (2.82)	7.07 (2.75)	9.40 (3.15)	6.07 (2.56)	6.20 (2.59)	6.80 (2.70)	4.20 (2.17)	5.47 (2.44)	4.60 (2.26)
T_5	*Metarhizium anisopliae*	5.00	8.47 (2.99)	8.87 (3.06)	8.27 (2.96)	9.60 (3.18)	9.53 (3.17)	8.73 (3.04)	9.60 (3.18)	6.27 (2.60)	5.73 (2.50)	6.67 (2.68)
T_6	N.S.E.*	5%	7.67 (2.86)	9.60 (3.18)	9.00 (3.08)	9.47 (3.16)	10.13 (3.26)	10.33 (3.29)	10.73 (3.35)	7.60 (2.85)	6.60 (2.66)	5.33 (2.42)
T_7	Control	-	7.87 (2.89)	9.47 (3.16)	9.07 (3.09)	9.53 (3.17)	10.60 (3.33)	8.40 (2.98)	10.40 (3.30)	6.67 (2.68)	5.60 (2.47)	4.27 (2.18)
	SE ±		N.S.	0.09	0.09	0.09	0.05	0.07	0.07	0.07	0.06	0.16
	CD at 5%		-	0.27	0.27	0.28	0.15	0.22	0.21	0.21	0.17	0.51
	CV		-	5.12	5.25	5.25	3.06	4.44	4.01	5.01	4.00	12.89

*NSE: Neem Seed Extract *Figures in parenthesis are $\sqrt{x+0.5}$ transformed values. **DAS: Days after spray

days after third spray. While significantly more number of forager activity were found in the treatment T_5 (*Metarhizium anisoplae*) and T_6 (N.S.E.) as compared to untreated control.

The maximum forager activity i.e. 6.67 bee/panicle/5min.was recorded in the treatment T_5 (*Metarhizium anisoplae*) and it was at par with the treatment T_6 (N.S.E.) with 5.33 bee/panicle/5min. at 10 days after 3rd spray on mango. In the untreated control, the forager activity was 4.27 and which was less as compared to T_5 (*Metarhizium anisoplae*), T_6 (N.S.E.), T_2 Dimethoate 30 EC and the treatment T_4 Lambda cyhalothrin 5 EC.

Among three sprays of insecticides maximum foraging activity observed in treatment N.S.E. followed by *Metarhizium anisoplae* and minimum activity observed in imidacloprid, thiamethoxam, lambda cyhalothrin and dimethoate respectively.

Neonicotinoids, a relatively new class of insecticides affect honey bees ability to forage, learn and remember navigation routes to and from food sources [5]. Exposure of two pesticides (neonicotinoids and pyrethroids) on bumblebees, impairs natural foraging behavior of honey bees [6]. The maximum foraging activity of honey bees observed on neem product treated plant [7].

Thus, the observations of the earlier workers in respect of adverse effects of chemicals on foraging behaviour of honey bees are in agreement with present findings

3.2 Toxicity of different insecticides against *Apis cerana* Fab. under laboratory condition

The data revealing to the corrected percent mortality of bee *A. cerana* at 5, 10 and 15 hours after treatment presented in the Table 2.

3.2.1 After 5 hours

The data regarding the toxicity of different insecticides against honey bee *A. cerana* under laboratory condition after 5 hours was found to be statistically non-significant. The treatment T_2 was found highly toxic to the treated bee (10.00% mortality) which was followed by the treatment T_1 imidacloprid @ 17.8 SL (6.67% mortality) after 5 hours.

In case of the treatment T_4 Lambda cyhalothrin 5 EC, the percent mortality was 5.56 while in the treatment T_3, it was 3.33 percent after 5 hours while mortality percent of bee was 2.22 percent in the treatment T_5 i.e. *Metarhizium anisoplae* and 1.11 percent mortality recorded in the treatment T_6 as well as in untreated control after 5 hours period.

3.2.2 After 10 hours

The data regarding the toxicity of different insecticides to *A. cerana* under laboratory condition after 10 hrs of treatment was found statistically significant. All the four chemical insecticides were toxic to honeybee after 10 hours of period after spray. The treatment T_1 Imidacloprid @17.8% SL was showed high toxicity with highest rate of mortality of bees

(44.44%) and which was at par with the treatment T_3 Thiamethoxam 25 WG (41.11%)and treatment T_4 Lambda cyhalothrin 5 EC (38.89%) after 5 hours.
While in thetreatment T_2 Dimethoate 30 EC was found moderately toxic (34.44% mortality) to honeybee. In case of treatment T_5 *Metarhizium anisoplae* only 12.22per cent mortality was recorded while 10 per cent mortality recorded in the treatment T_6 and untreated control.

3.2.3. After 15 hours
The data presented in the table revealed that the chemical insecticidal treatments viz., T_1 Imidacloprid @17.8% SL and which was at par with the treatment T_3 Thiamethoxam 25 WG with 73.33 and 70.00 percent mortality of honeybees after 15 hours of spray under laboratory condition. The treatment T_4 Lambda cyhalothrin 5 EC (57.78%) and the treatment T_2 Dimethoate 30 EC (34.44% mortality) were found moderately toxic to honeybee after 15 hours. The treatment T_5 *Metarhizium anisoplae* and N.S.E. were showed less mortality i.e. 18.89 each and proved to safer to honeybee. The least mortality (14.44%) was recorded in untreated control after 15 hours.Toxicity of insecticides under laboratory condition was observed as follows: Imidacloprid > Thiamethoxam > Lambda cyhalothrin > Dimethoate > N.S.E., *Metarhizium anisopliae*.
The present finding are in close conformity with study the neonicotinoids toxicity over honey bees and founded that neonicotinoids is very toxic to pollinating agent and particular to honey bees [8]. Lethal and sublethal effects of neonicotinoids toxicity on honeybees, under laboratory studies and neonicotinoids effects on the foraging behavior, and learning and memory abilities of bees [9]. Intrinsic toxicity of insecticides (LC_{50}) and in the order as imidacloprid > fipronil > indoxicarb> cypermethrin > dimethoate to honey bees [10] Acephate, cypermethrin and dimethoate highly toxic while dichlorvos and neem oil were least toxic to honeybee, *A. mellifera* when released into treated jars at different intervals i.e., 0, 12 and 24 hours by dry film method [11]
Thus, the observations of the earlier workers in respect of toxic effect of insecticides on honey bees are in agreement with present findings.

Table 2: Toxicity of different insecticides against *Apis cerana* under laboratory conditions

Tr. No.	Treatment Details	Dose (g or ml)	Bee/Rep/Trt.	Hours after spray		
				5	10	15
T_1	Imidacloprid @17.8% SL	0.40	30	6.67 (14.64)	44.44 (41.78)	73.33 (59.03)
T_2	Dimethoate 30 EC	2.00	30	3.33 (8.49)	34.44 (35.86)	52.22 (46.28)
T_3	Thiamethoxam 25 WG	1.00	30	10.00 (18.01)	41.11 (39.87)	70.00 (57.00)
T_4	Lambda cyhalothrin 5 EC	1.00	30	5.56 (13.16)	38.89 (38.03)	57.78 (49.61)
T_5	*Metarhizium anisoplae*	5.00	30	2.22 (7.01)	12.22 (20.42)	18.89 (25.69)
T_6	N.S.E.*	5%	30	1.11 (3.51)	10.00 (18.01)	18.89 (25.74)
T_7	Untreated Control	-	30	1.11 (3.51)	10.00 (18.44)	14.44 (22.21)
T_8	Plant covered with shade net	-	-	-	-	-
	SE ±			N.S.	2.36	3.13
	CD at 5%			7.58	7.28	9.64
	CV			13.4	13.1	16.3

*Figures in parenthesis are arc sin transformed values.

4. Conclusion
Among three sprays of insecticides maximum foraging activity observed in treatment T_6 N.S.E. followed by treatment T_5 *Metarhizium anisopliae* and minimum activity observed in treatment.
T_1 imidacloprid, treatment T_3 thiamethoxam, treatment T_4 lambda cyhalothrin and treatment T_2 dimethoate respectively.
Toxicity of insecticides under laboratory condition was observed as follows: Treatment T_1 Imidacloprid > T_3 Thiamethoxam > T_4 Lambda cyhalothrin > T_2 Dimethoate > T_6 N.S.E.+ T_5 *Metarrhizium anisopliae*.

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