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Evaluation of persistence of insecticide toxicity in honey bees (*Apis mellifera* L.)

V V Pashte[1]* & C S Patil[2]

[1]Department of Rural Technology and Social Development, School of Natural Resources, Guru Ghasidas Vishwavidyalaya, Koni, Bilaspur-495 009, Chhattisgarh, India
[2]Department of Entomology, Post Graduate Institute, Mahatma Phule Krishi Vidyapeeth, Rahuri, Ahmednagar-413 722, Maharashtra, India

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Study on persistence toxicity of different insecticides focusing newer compounds (imidacloprid, flipronil, and indoxacarb), conventional insecticides (dimethoate and cypermenthrin) and botanical insecticide (azadirachtin) to *Apis mellifera* was conducted on sunflower. Fipronil recorded higher residual toxicity to honey bees with a PT_{50} value of 5.83 days. It was followed by imidacloprid (5.74 days), cypermethrin (4.38 days), dimethoate (2.56 days) and indoxacarb (2.02 days). The order of relative persistence of insecticides was: fipronil 45 g a.i./ha > imidacloprid 20 g a.i./ha > cypermethrin 65 g a.i./ha > dimethoate 200 g a.i./ha > indoxacarb 44 g a.i./ha. Residues of all tested insecticides persisted after the application was highly toxic to *A. mellifera*. Findings focus on the indirect application hazards to the honey bees.

Keywords: *Apis mellifera*, Honey bees, Persistence, Residual toxicity

Bees and other pollinators not only provide services to the ecosystem but also to humans[1]. Honey bees are considered as the most efficient and reliable pollinators of varied agricultural crops[2,3]. The sunflower is important oilseed crop of most of the world. The sunflowers flowers produce a plentiful quantity of nectar and pollen which create a good foraging source for a large number of bees. In most of the crops we mostly seek honey bees and depend on them for pollination services[4]. However, there are many other insects especially the native bees which may play a significant role in pollination. Different insect visitors of the sunflower blossom were from order hymenoptera, diptera, lepidoptera, and coleoptera[4,5].

However, recent declines in pollinator populations have affected global agricultural production and impacted both food production and the economy[6]. Unfortunately, honeybee populations are in decline since the 1990s, possibly due to a combination of factors like pests, diseases, poor diet, and pesticides[7-10]. There is no clear single factor to date that clarifies colony loss in bees, but one factor anticipated is the extensive application of chemicals for the crop management[3-6,11]. Crop productivity is greatly influenced by pests, and use of poisonous pesticides has become inevitable in scientific farming. Agro-chemical use along with land use practices have been highlighted as a stress on pollinators[12,13]. Insecticides are the group of pesticides that pose the most direct risk to pollinators, and negative impacts of insecticides have been demonstrated for the honeybee *A. mellifera*[14-19] and several non-*Apis* bees[20-22].

The honey bee comes in contact with the applied insecticides during foraging and the chances of mortality of forager bees are obvious[23-25]. Field-based research of the responses of bee communities, in sites with carefully manipulated insecticide application management, could help to isolate the impact of insecticides from other management variables. Exposure of bees to insecticides can occur *via* the contact of treated plant parts (*i.e.* leaves, flowers, *etc.*). Depending on the application method and exposure to the weather factors, an insecticide could potentially persist up to several days. Multiple factors can be put forward to play a role in this mechanism including exposure to light, temperature differences, and the efficiency of translocation within the plant. This risk of exposure will differ with crop type and organisms.

However, while taking managerial decisions for sustaining crop productivity by employing pesticides, bee safety must be ensured. Pest management must

*Correspondence:
E-mail: pashte.vrushali@gmail.com

take into account judicious management of pollinators. Keeping this in view, the present investigation was undertaken to assess the relative toxicity of some commonly used insecticides to *A. mellifera*.

Materials and Methods

Experimental materials

Products

The different insecticides selected from different groups on the basis of their mode of action, which are recommended on sunflower and commonly used by the farmers. The six insecticides from (neonicotinoids and conventional insecticide groups) that we tested in this study together with their respective type of formulation and MFRC (Maximum Field Recommended Concentration) and the producing company name are listed in Table 1. The products were stored in accordance with the manufacturer's guidelines.

Insect

Apis mellifera (L.) was selected as target organism for experimental purpose as it can be easily domesticated, suitable for wide range of climate and easy to handle for bioassay studies as compared to wild bees.

Honey bees were collected from hives maintained at central campus, Mahatma Phule Krishi Vidyapeeth, Rahuri (Maharashtra), India which is situated at 19°20'31"N latitude and 74°38'46"E Longitude and at an elevation of 800 m above sea level. The hives were observed for the presence of diseases and pests during routine colony maintenance practices as described by Abrol[26]. It was observed that throughout the experiment, the colonies were free from diseases and pests. Therefore, no hive treatment of any chemical was conducted prior and during the studies.

Cages for conducting bioassay experiment

The cages used for experiment were prepared by using thin metal wire with a cylindrical shape to hold the flower in position. Each cage (40 cm height × 30 cm diameter) was covered muslin bag and open at the lower side to facilitate the release of bees.

Collection and inactivation of bees

Adult workers of honey bees were collected from the frame which contained honey and pollen (apart from brood frame for avoiding chances of nurse bees) during morning hours[27]. The bees were shaken from the frames into a big muslin cloth bag (90×60 cm). The opening of bag was covered with a rubber band and the bees were transported immediately to the laboratory. They were preconditioned for 2h and anaesthetised by chilling for 5 min to facilitate easy

Table 1 — Details of insecticide evaluated for honey bee toxicity

Sr. No.	Common Name	Chemical Name	Trade Name	Formulation	Source
1	Azadirachtin	Dimethyl (2a*R*,3*S*,4*S*,*R*,*S*,7a*S*,8*S*,10*R*,10a*S*,10b*R*)- 10-(acetyloxy)-3,5-dihydroxy- 4-[(1*S*,2*S*,6*S*,8*S*,9*R*,11*S*)-2-hydroxy- 11-methyl- 5,7,10-trioxatetracyclo [6.3.1.0^{2,6}.0^{9,11}]dodec- 3-en- 9-yl]- 4-methyl- 8-{[(2*E*)-2-methylbut-2-enoyl] oxy} octahydro- 1*H*-furo[3',4':4,4a] naphtho[1,8-*bc*] furan- 5,10a (8*H*)-dicarboxylate	NEEMRAJ	0.15%	M/S. Khandekeshwar Oil Mills Pvt Ltd, J-1/8, MIDC Chikhalthana, Aurangabad, Maharashtra, India
2	Dimethoate	*O*,*O*-dimethyl *S*-[2-(methylamino)-2-oxoethyl] dithiophosphate	TATA TAFGOR®	30% SC	M/S. Rallis India Ltd. 156/157, Nariman point, Mumbai-400021, India
3	Cypermethrin	[Cyano-(3-phenoxyphenyl)methyl] 3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropane-1-carboxylate	CYPER PLUS	10% EC	M/S. Cheminova India Ltd., Dehradun, India
4	Fipronil	(*RS*)-5-amino-1-[2,6-dichloro-4-(trifluoromethyl) phenyl]-4-(trifluoromethylsulfinyl)-1H-pyrazole-3-carbonitrile	DEVIGENT PLUS™	5% SC	M/S. Devidayal Agro chemicals Ltd. Tulsiram Gupt mills Estate, Reay Road, Mumbai-400010, India
5	Imidacloprid	*N*-{1-[(6-Chloro-3-pyridyl) methyl]-4,5-dihydroimidazol-2-yl}nitramide	TRISHUL	17.8% SL	M/S. Advanced esticide, G. No.152/2/1 Brahamnwada, Tal-Sinnar, Dist-Nashik, Maharashtra, India
6	Indoxacarb	Methyl 7-chloro-2,5-dihydro-2-[[(methoxycarbonyl)[4-(trifluoromethoxy) phenyl]amino] carbonyl] indeno [1,2-e][1,3,4] oxadiazine-4a (3*H*)carboxylate	INDEX	14.5% SC	M/S. Devidayal Agro chemicals Ltd., Tulsiram Gupta mills Estate, Reay Road, Mumbai-400010, India

handling. The chilling method used with slight modifications as recommended by Thomas and Phadke[28] and Human *et al.*[29]. Before the start of bioassay experiment, the mortality and activation period was observed for different periods of exposure at low temperature (0 to ±4°C). It was observed that 5 min chilling period was sufficient to make bees inactive for handling. Newly emerged workers with light yellow setae on the thorax were discarded[30].

After chilling, ten bees were separated into glass test tube and the opening was closed with muslin cloth piece by using rubber a band (Plate 1). The bees containing test tubes were immediately shifted to the field.

Field assay

The experiment was conducted on sunflower crop with the foliar treatment of insecticides (Table 1). The sunflower crop (var. Raviraj) grown at a spacing of 60×45 cm in the plots (4×5 m) by following recommended agronomic practices. The experiment was laid out in a completely randomised block design with three replications at PGI Farm. One meter distance was maintained between the replications. The complete batch of sunflowers used in the entire experiment were exposed to insecticides in one go.

In the field, the blooming sunflower crop (50% flowering) was sprayed with the recommended dose of each formulated insecticide [Azadirachtin 0.15% (5mL/L), Dimethoate 30% EC (200 g a.i./ha), Cypermethrin10% EC (65 g a.i./ha), Fipronil 5% SC (45 g a.i./ha), Imidacloprid 17.8% SL (20 g a.i./ha) and Indoxacarb 14.5% SC (44 g a.i./ha)] care was taken to avoid drift (Plate 2). The insecticides doses used for Azadirachtin, Dimethoate, Cypermethrin, Fipronil, Imidacloprid and Indoxacarb were 0.500%, 0.100%, 0.100%, 0.200%, 0.020% and 0.060%, respectively. The control plots were sprayed with water only. Each cage with the cloth bag was tied over the flower such that the opening was towards the ground (Plate 3). A batch of ten bees was released inside the cage by gentle tapping of the test tube (Plate 4). A swab of cotton soaked with sugar solution was provided before closing the cage. The bees were starved for 2 h and released in the cages holding the sunflower treated with insecticide. An Experiment was conducted with three replications (Plate 5). Each replication was with a batch of 10 bees released over the sunflower.

The mortality was recorded 24 h after worker bees released over the flowers. The bees were released 2 h after the spray. Subsequent releases of bees were done after 1, 3, 5, 7 and 9 days after spray. Sunflower exposed once for bees was not used again. The sunflowers in the untreated crop (sprayed with water) served as control. It was observed that *A. mellifera* was not naturally foraging in the experimental sunflower field. There was meagre mortality at nearby the colony. Unexposed bees were used for the subsequent persistent test.

Nature of season during experimental period

The metrological data on important weather parameters during the experimental period was recorded at the meteorological observatory of the Mahatma Phule Krishi Vidyapeeth, Rahuri, Maharashtra, India.

Statistical analysis

Data obtained on mortality of test bees was converted into percentage mortality and was corrected by Abbott's formula[31]. The residual toxicity in terms of persistent toxicity and PT_{50} (Time required to give 50% mortality) was worked out according to Pradhan[32].

The PT_{50} calculations based on the day- mortality response were done by using the on the probit analysis method by Finney[33] and Kim Vincent[34]. To reduce the calculation errors, these indices were calculated using Microsoft office Excel 2007, instead of doing manual calculations.

Results

The data on the residual toxicity of insecticides to *A. mellifera* revealed that azadirachtin 0.15%, 5 mL/L. was least toxic with 22.22% initial mortality of bees and persisted upto 3 days. Indoxacarb 44 g a.i./ha was next in the order which exhibited 71.43 % initial mortality (0 day) but persisted upto 9 DAT to cause 10.71% mortality of bees. Dimethoate 200 g a.i./ha recorded 85.71% initial mortality and retained its toxicity upto 9 DAT to cause 7.14% mortality of bees. Cypermethrin 65 g a.i./ha caused 82.14 initial mortality and persisted upto 9 days with 21.43% mortality. Imidacloprid 20 g a.i./ha showed higher initial mortality of 89.43% but gradually declined with the advancement of time. It, however, persisted upto 9 DAT with 35.71% mortality. Fipronil 45 g a.i./ha exhibited highest (96.43%) initial mortality of bees and declined gradually with 28.57% mortality at 9 DAT.

The order of persistent toxicity based on the PT index was: fipronil 45 g a.i./ha (575.02) >

Table 2 — Residual toxicity of insecticides to honey bees (*A. mellifera*)

Insecticide	Dose (g. a.i/ha)	Corrected Per cent Mortality – Days After Treatment						P	T	PT	ORT	PT_{50}	RP	ORP
		0	1	3	5	7	9							
Azadirachtin 0.15%	5 mL/L	22.22	18.52	10.71	0.00	0.00	0.00	3	8.58	25.73	6	-	-	-
Dimethoate 30% EC	200	85.71	81.48	57.14	25.00	13.79	7.14	9	45.05	405.41	4	2.56	1.27	4
Cypermethrin 10% EC	65	82.14	77.78	75.00	50.00	31.03	21.43	9	56.23	506.08	3	4.38	2.17	3
Fipronil 5% SC	45	96.43	88.89	60.71	57.14	55.17	28.57	9	63.89	575.02	1	5.83	2.89	1
Imidacloprid 17.8% SL	20	89.29	85.19	71.43	50.00	44.83	35.71	9	62.76	564.86	2	5.74	2.84	2
Indoxacarb 14.5% SC	44	71.43	66.67	64.29	21.43	17.24	10.71	9	41.96	377.65	5	2.02	1.00	5

*P- Period of observations in days; T- Average percentage mortality; PT- Persistent toxicity; ORT- Order of relative toxicity; RP- Relative Persistence; ORP- Order of relative persistence; Mean of three replications

imidacloprid 20 g a.i./ha (564.86) > cypermethrin 65 g a.i./ha (506.8) > dimethoate 200 g a.i./ha (405.41) > indoxacarb 44 g a.i./ha (377.65) > azadirachtin 0.15% 5mL/L (25.73). The persistence of the insecticides as evaluated from PT_{50} values suggests that fipronil 45 g a.i./ha recorded higher residual toxicity to honey bees with PT_{50} value 5.83 days (Table 2). It was followed by imidacloprid 20 a.i./ha (5.74 days), cypermethrin 65 g a.i./ha (4.38 days), dimethoate 200 g a.i./ha (2.56 days) and indoxacarb 44 g a.i./ha (2.02 days). The order of relative persistence of insecticides was: fipronil 45 g a.i./ha > imidacloprid 20 g a.i./ha > cypermethrin 65 g a.i./ha > dimethoate 200 g a.i./ha > indoxacarb 44 g a.i./ha.

The average weather conditions recorded during the experimental period were: Temperature (max: 29.9°C, min: 10.75°C), humidity (morning: 54.2%, evening: 23.5%), wind velocity (2.5 km/h), Evaporation (5.85 mm) and rainfall (nil).

Discussion

Insecticides should be reasonably persistent for the effective control of pest but should not be highly persistent to pose hazards to beneficial insects. Residual toxicity of insecticides to honey bees has been reported by many workers[35-38]. From the present observations, it may be inferred that all the tested insecticides exhibit considerable indirect toxic effects on the honey bees. The present finding on persistence toxicity of fipronil is contradictory to results of Kim *et al.*[39] who reported that fipronil was toxic to bees upto 28 days. Gulati *et al.*[25] stated that imidacloprid became safer for *A. mellifera* after 48h of their application on the crop which was contradictory to our results which represent the PT_{50} value of 5.74 days for *A. mellifera*. It may because of difference in the forage crop, insecticide formulation or weather condition during the experimental period.

The present finding on persistence toxicity of cypermethrin could not be discussed due to lack of literature. The finding of the persistence of dimethoate was in agreement with Sharma *et al.*[24] who recorded toxicity of dimethoate to bees up to 96h after spray. However, Kumar[40] and Thakur[36] observed residual toxicity of dimethoate for 7 days.

During the present investigation, azadirachtin proved to be least persistent with the order of relative persistence as the last position. The safety of azadirachtin to honey bees has been documented by several workers[36,41-42]. The ongoing discussion clearly indicated the toxic effect of tested insecticides to *A. mellifera.*

Present studies conducted using domesticated *A. mellifera* may not adequately reflect the risk posed by insecticides to wild bees because of their differential susceptibility and biology. Results of the present studies indicate that all the insecticides were highly toxic to *A. mellifera*. It is therefore suggested that these insecticides must be used only with greatest care as they destroy bees including non target insects that are essential for pollination. In the view of the great importance of the service of insect pollinators provide to the natural vegetation and crops, they require some protection. Therefore the experimental studies based on field-realistic doses in field condition helps to focus on risk to the bees as well as other non-target pollinators.

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