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Monitoring on impact of insecticides on mortality of honey bees (*Apis mellifera* L.) in front of beehives

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Abstract: The present study investigated effect of pesticide usage and public awareness on honey bee mortality. The experiments were conducted at three different sites at Maharashtra, India with domesticated bee hives of *Apis mellifera* L. The maximum bee mortality during 51st week of 2012-13 (1559.10 bees/hive/week) clearly indicated towards the direct and indirect effect of insecticides in general at study site I (Case I). Similar experiments were repeated at other two different sites during 2013-14. Farmers (Case II and III) were aware of beekeeping and ill effects of pesticides. Farmers followed some precautionary measures to combat with the bad effect of insecticides on bees. As a result there was less mortality of bees. The experiments revealed that farmers should be aware of bee conservation and precautionary measures to combat with the bad effect of insecticides on bees.

Keywords: Bee mortality, Honey bee, Insecticide, Survey

INTRODUCTION

Wild as well as domesticated bees are major component of terrestrial ecosystem. Many plants would be unable to complete their development cycle without the intervention of pollinators
(Klein *et al.*, 2007). Bees act as a vital pollinator for many plants and have well known optimistic impact on the crop production due to their foraging activity (Pashte and Said, 2015; Pashte and Kulkarni, 2015). In addition honey bees have other positive impact, including the production of honey, propolis, royal jelly, maintain genetic diversity in plants and act as a bio-indicator (Porrini *et al.*, 2003). Bee heath is receiving increased attention as bee populations are declining worldwide (VanEgelsdorp *et al.*, 2008). Use of insecticides is being one of the promising causes for the bee deaths (Potts *et al.,* 2010). Concern about adverse effects of pesticides on honey bees have been the subject of research and debate. Many workers were recorded bee mortality near or in front of hive because of direct or residual pesticide toxicity (Mayer and Johansen, 1983; Delabie *et al.*, 1985; Celli *et al.,* 1989; Perez *et al*., 2001; Steen and Dinter, 2007). The main goal of this field study was to investigate the potential impact of insecticides usage which is widely used by the farmers for insect management on honey bee (*A. mellifera*) mortality.

MATERIALS AND METHODS

The experiments were conducted for two years. During the year 2012-2013, the experiment was conducted at horticultural division, Mahatma Phule Krishi Vidyapeeth (M. P. K. V.), Rahuri, Maharashtra, India (19.35°N and 74.65°E), where ten colonies of honey bees were kept under the supervision. The period of monitoring was from September 2012 to January 2013, the usual blooming period of most of the horticultural and field crops. In all, ten bee colonies with 5 m distance between two colonies were provided with dead bee tray in front of colonies. The plastic trays (40 X 30 cm) were used for this purpose. A thick band of grease was applied to the outer border of trays for protection of dead bees from ants. The trays were provided with a cover of metal mesh (mesh size-1 inch).
The count on dead bees observed in the tray kept in front of colonies was recorded thrice in each week during morning hrs (10.00-11.00 AM). Collected dead bees were disposed off after counting. All bee colonies were under supervision of trained person for the maintenance of bee colonies. Fortnightly observations were recorded on the pest and disease incidence and number of frames with bees.
Data on pesticide usage by different research projects in the periphery of 3 km from apiary (10 bee hives) were collected during the experimental period (Case I). Similar experiments were conducted at two other locations (Case II and III) during 2013-14. Details of location of the experimental site are given in Table 1. The observed bee mortality data were analysed by

using Kruskal-Wallis test (McDonald, 2014).

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

RESULTS AND DISCUSSION

Mortality of honey bees observed in front of colonies at Horticulture Division, Mahatma Phule Krishi Vidyapeeth, Rahuri (Case I): Foraging behaviour is the link between the bee colony and the environment. Besides collecting pollen, nectar, water and resin, this has significant importance for plant pollination. As such foraging activity is very important as a bioindicator for indirect studies of environmental contamination with insecticides. Studies were undertaken comprising case I with lack of coordination and case II and case III with proper coordination on farmer's field.

Monitoring studies were carried out in the M.P.K.V. premises, focusing on the mortality of honey bees in front of the hives. The foraging activity of the honey bee colonies at the test site was not directly measured. It was assumed, based on the honey bee biology, that bee placed in the flowering orchards forage as nearly as possible (*A. mellifera* foraging range: 2-3 km) and that the M.P.K.V. was rich source of flowering plants attractive to bees. The consideration of the meteorological data also supported the hypothesis about the bee activity.

The mortality of *A. mellifera* observed from 35th week to 45th week was normal [(Normal mortality for a colony with 60000 bees/colony: <100 bees/day), Abrol, 2009]. During 46th and 47th week, there was marginal increase in mortality as compared to earlier two months as 46.40 bees/hive/week and 56.00 bees/hive/week, respectively. However there was significant mortality (level of significance- 0.05%) of *A. mellifera* in front of bee hives (A to J) during 48th week of the year 2012 (Table 2). Subsequently, bee mortality increased leading to more bee population loss during 49th to 50th week as 960.70 and 887.80 bees/hive/week, respectively. The maximum bee (*Apis mellifera* L.) mortality during 51st week (1159.10 bees/hive/week).

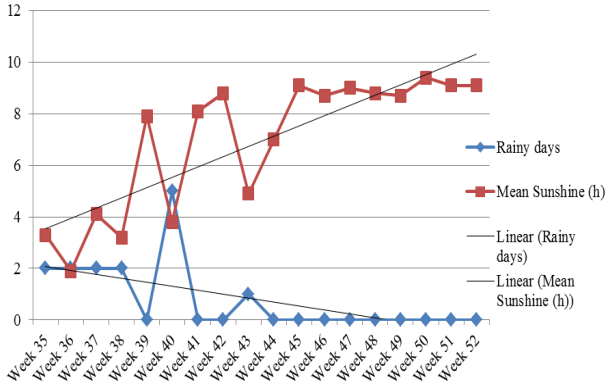

Fig. 1. *Status of rainfall and sunshine hours during the observation period (Case I).*

During 52nd week and 53rd week, the mean bee mortality was declined due to less population inside the hives. More than 90 per cent of dead bees showed abnormal symptoms.

While monitoring bee deaths, the population inside the hives was observed for the presence of bee disease incidence and population density by recording number of frames with bee cluster. During 35th to 37th week, all the bee colonies were with healthy bee population of 9 -8 frames/hive. However, during 49th to 53rd week the drastic reduction was observed in the bee population. During 49th and 51st week, the mean number of frames with bees/hive were 4.50 and 1.30, respectively. During 53rd week (1st week of 2013) all the bee hives were empty.

The meteorological data indicated that there was no rainfall but increase in sunshine hours after 44th week (Fig.1). The favourable environmental conditions supported more foraging hours and more exposure of bees to the different crops. The environmental factors such as temperature, humidity, rainfall and wind greatly influenced foraging activity of honey bees (Abou-Shaara, 2014). At ambient temperatures of about 20-30 °C, the highest foraging activity was recorded (Tan *et al*., 2012), while the low-est foraging activity was found at 43°C (Blazyte-Cereskiene *et al.,* 2010) as well as at or below 10°C (Joshiand Joshi, 2010). Higher humidity, rainfall, wind and low temperature had negative impact on bee visits (Puskadija *et al.*, 2007). However, the exact foraging activity of honey bees

Table 1. Details of honey bee colonies with locations observed for bee mortality.

S. N.	Name of Location	Owner/Farmer	Bee species	Foraging crop and area	Period of observation
1	Horticultural Farm, M.P.K.V., Taluka-Rahuri, Ahmednagar (M.S.)	Directorate of Beekeeping, Mahabaleshwar, KVIC (M.S.)	*A. mellifera*	Multiflora* (30 ha)	August 2012-January 2013
2.	Chanda, Taluka- Nevasa, Ahmednagar (M.S.)	Mr. Dahatonde V.	*A. mellifera*	Pomegranate (6 ha)	October 2013-December 2013
3.	Aalefata, Taluka- Narayangao, Pune (M.S.)	Mr. Kuhrade S. G.	*A. mellifera*	Pomegranate (10 ha)	November 2013-February 2014

Multiflora*= Grape, Guava, Pomegranate, Custard Apple, Ber, Anola, Pigeon Pea, Chick Pea, Cotton, Sorghum, Vegetables: Bhendi, Wal, Brinjal, Chilli, Bitter Gourd, Tomato, Garlic, Cabbage, Cauliflower, Sponge gourd, Green Pea, Onion

Table 2. Mortality of honey bees observed in front of colonies at Horticulture Division (M. P. K. V., Rahuri)(Case I).

Honey bee Hive/ colony observed	Weeks																		
	35	**36**	**37**	**38**	**39**	**40**	**41**	**42**	**43**	**44**	**45**	**46**	**47**	**48**	**49**	**50**	**51**	**52**	**53**
Colony A	10		16		11	25	16	45	32	52	64	47	50	338	1558	493	671	73	6
Colony B	33		41		13	20	20	13	58	36	34	57	82	1125	2885	546	1920	84	0
Colony C	7		10		3	3	2	4	3	65	74	44	101	1134	964	1526	871	75	2
Colony D	3		6		5	7	15	9	17	19	14	23	34	564	584	2550	2007	56	1
Colony E	9	Rain-fall	8	Rainfall	3	8	5	3	8	44	31	47	36	335	641	658	471	39	0
Colony F	12		5		22	13	9	7	5	28	22	64	32	278	1262	261	775	54	3
Colony G	2		7		0	14	56	25	26	34	5	69	72	832	376	941	1742	56	0
Colony H	0		0		8	3	21	7	9	38	11	38	37	245	758	505	2553	59	7
Colony I	8		4		0	3	16	8	7	18	17	36	25	258	313	221	351	-	-
Colony J	16		3		2	10	4	7	12	45	11	39	91	643	266	1177	230	52	0
Mean observed mortality	10.0	-	10.0	-	6.70	10.6	16.4	12.8	17.7	37.9	28.3	46.40	56.0	575.20	960.70	887.80	1159.10	60.89	2.11

* Colony I absconded in week 52, Almost empty bee hives during week 53, Week 35-52: weeks of year 2012, Week 53 = 1st week of 2013, Foraging crop range: Pigeon Pea, Chick Pea, Cotton, Sorghum, Fruits: Grapes, Mango, Pomegranate, Sapota, Guava , Vegetables: Tomato, Bhendi, Wal, Brinjal, Chilli, Bitter Gourd, Garlic, Cabbage, Cauliflower, Sponge gourd, Green Pea, Onion

was not monitored during the present experimental period.

The data regarding pesticide usage of various research projects at M. P. K. V., Rahuri was collected for the experimental period (Sept. 2012- Dec. 2012). The data revealed that organophosphorus insecticides ranked first with 38.85 per cent followed by newer insecticides excluding neonicotinoids (26.62 %), synthetic pyrethroids (16.55 %), neonicotinoids (13.67 %) and carbamates (4.32 %) (Table 3).

The diverse cropping ecosystem supported more foraging hours and more exposure of bees to the different crops. However, being an experimental crop area M. P. K. V, Rahuri, pesticides were frequently used to manage pests. In the present investigation, drastic reduction in bee population was due to mortality of bees in front of colonies which could be attributed to peak period of pesticide spray interventions on different foraging flowering plants. This clearly indicated the direct and indirect effect of pesticides in general. However, bee mortality in front of colonies could not be attributed to any specific pesticide.

The pesticides have detrimental effects on managed honey bee colonies and their productivity (Schenbayoand Goka, 2016). The evidence of bee deaths in front of colonies was a result of pesticidal poisoning, which was in conformity with earlier reports (Pongthep, 1990; Radunz *et al.*, 1996; Abrol, 2009; Hoven *et al*., 2013, Anonymous, 2013). Some authors observed abnormal dead bees due to pesticidal toxicity (Abrol, 2009, Mayer *et al.*, 1999; Muthuraman, 2000, Suhail *et al.*, 2001).

Mortality of honey bees observed in front of colonies at Chanda (Case II): An experiment was repeated at Chanda during the year 2013-14, where pomegranate grower maintained *A. mellifera* colonies. The data was recorded on bee mortality during flowering period of pomegranate from 14-10-2013 to 11-12-2013. The mean mortality of *A. mellifera* bees per day ranged between 0-32.50 dead bees/day. The pomegranate orchard was sprayed twice with azadirachtin 0.03 % @ 5ml/lit on 31-10-2014 and 4-11-12014. Also the flowering orchard was subjected to spray of chlorpyriphos 20 % EC+ metalaxyl 8 % + mancozeb (Twin) on 26-11-2014 (Table 4). However, there was meagre mortality on subsequent days. The observed number of frames with bee cluster ranging between 7.50 to 8.00 indicated healthy population of *A. mellifera* bees inside the hives. Further, there was no incidence of any pest and disease throughout the monitoring period.

Farmer at the location Chanda was well aware of beekeeping management. All the insecticidal sprays mentioned above were given at dusk (late evening). Also he restricted to minimum (three) sprays to maintain the bee fauna during flowering period of pomegranate. Preventive care was also taken to close the bee colony gates for the period of 24 hrs after spray interventions.

Table 3. Insecticides used at M.P.K.V., Rahuri during experimental period (OP/SP carbamates/neonicotinoids/ newer compounds other than neonicotinoids).

	Insecticide	Total no. of sprays	Per cent use among group	Per cent use among total use insecticides
OP compounds	Triazophos	6	11.11	4.32
	Monocrotophos	3	5.56	2.16
	Acephate	1	1.85	0.72
	Quinalphos	7	12.96	5.04
	Chlorpyriphos	12	22.22	8.63
	Malathion	5	9.26	3.60
	Dimethoate	14	25.93	10.07
	Dichlorvos	2	3.70	1.44
	Profenophos	4	7.41	2.88
SP compounds	Lambda-cyhalothrin	4	17.39	2.88
	Deltamethrin	10	43.48	7.19
	Cypermethrin	8	34.78	5.76
	Fenpropathrin	1	4.35	0.72
Carbamates	Thiodicarb	1	16.67	0.72
	Carbosulfan	5	83.33	3.60
Neonicotinoids	Clothianidin	1	5.26	0.72
	Acetamiprid	1	5.26	0.72
	Thiamethoxam	9	47.37	6.47
	Imidacloprid	8	42.11	5.76
Newer compounds other than neonicotinoids	Flubendiamide	1	2.70	0.72
	Lufenuron	1	2.70	0.72
	Emamectin benzoate	4	10.81	2.88
	Indoxacarb	3	8.11	2.16
	Spinosad	6	16.22	4.32
	Buprofenzin	1	2.70	0.72
	Fipronil	17	45.95	12.23
	Fenpyroximate	1	2.70	0.72
	Rynaxypyr	1	2.70	0.72
	Novaluron	2	5.41	1.44

Mortality of honey bees observed in front of colonies at Aalefata (Case III): Similar experiment was also conducted on the pomegranate orchard at Aalefata during 23-11-2013 to 28-02-2014. The data collected revealed that, the mean mortality of *A. mellifera* per day ranged between 0- 28.33 dead bees/day.

The pomegranate orchard was sprayed six times as: Acetamiprid 20 % SP) (Tata manik) on 24-11-2013; Carbosulfan 25 % EC) + (Acephate 75 % SP) + (Carbendazim + Mancozeb75 % WP) [Marshal + Asataf + Saaf] on 28-11-2013; Monocrotophos + Bavistin (Chetak + Bavistin) on 17-12-2013; (Dichlorvos 76 % EC + Mancozeb 75 % WP) (Nuvan + M-45) on 4-01-2014; (Carbosulfan 25 % EC) + (Acephate 75 % SP) + (Carbendazim 50 % DF) [Marshal + Asataf + Bavistin] on 02-02-2014; (Carbosulfan 25 % EC) + (Acephate 75 % SP) + (Carbendazim 50 % DF) (Table 5).

The bee mortality was meagre on subsequent days. The observed number of frames with bee cluster ranged between 7.00 to 8.00, indicating healthy population of *A. mellifera* inside the hives. Further, there was no disease incidence throughout the monitoring period.

Pomegranate grower was aware of bee pollination and beekeeping management. Since the farmer was owner of all three colonies of *A. mellifera*. All the insecticidal sprays were made at dusk period (late evening). Also he tried to reduce unnecessary sprays to maintain the bee flora during flowering of pomegranate. All colony gates were closed for the period of 24 hrs. The internal feeding with artificial diet before spray also supported the reduction of bee exposure on the sprayed crop which minimized the hazards of pesticides. The managed shifts in the flowering of pomegranate also ensured the food resource for bees.

The precautionary measures were taken by the farmer to reduce the pesticidal poisoning to honey bees at case II and III. This has been reflected in the meagre mortality of bees under the case II and III compared to case I. This clearly indicated that there should be cooperation between the growers and beekeepers regarding management of honey bee colonies and pesticide use from the point of view bee safety. The present findings are in conformity with the earlier suggestions i.e clear and regular communication between the grower and beekeeper is the best way to avoid the pesticide hazards with bees (Ellis *et al*., 2014). Many bee poisoning problems could be prevented by better communication and cooperation among the grower, pesticide applicator and the beekeeper (Krupke *et al*., 2014).Some workers mentioned different hazards and safeguards to bees in

Table 4. Mortality of honey bees observed in front of colonies at Chanda (Case II).

Interval in days	A	B	Mean	Mortality / day	Mean number of frames with bee cluster	Date of spray	Pesticide sprayed (Active ingredi-ent)	Diseases observed
14-10-2013 to 16-10-2013	18	25	21.50	7.17	8			Nil
17-10-2013	3	2	2.50	2.50	8			Nil
18-10-2013 to 23-10-2015	46	19	32.50	5.42	8			Nil
24-10-2013	4	0	2.00	2.00	8			Nil
25-10-2013 to 30-10-2017	17	6	11.50	1.92	8			Nil
31-10-2013	4	10	7.00	7.00	8	31-10-2013	Nimbecidine (Azadirachtin 0.03%)	Nil
1-11-2013 to 2-11-2013	15	13	14.00	7.00	8			Nil
3-11-2013 to 5-11-2020	17	21	19.00	6.33	8	04-11-2013	(Nimbecidine) Azadirachtin 0.03%	Nil
06-11-2013	0	1	0.50	0.50	8			Nil
7-11-2013 to 8-11-2013	3	0	1.50	0.75	8			Nil
09-11-2013	3	3	3.00	3.00	8			Nil
10-11-2013 to 11-11-2013	10	6	8.00	4.00	8			Nil
12-11-2013	3	2	2.50	2.50	8			Nil
13-11-2013 to 14-11-2013	3	2	2.50	1.25	8			Nil
15-11-2013	5	2	3.50	3.50	8			Nil
16-11-2013 to 17-11-2013	6	4	5.00	2.50	8			Nil
18-11-2013	3	0	1.50	1.50	8			Nil
19-11-2013 to 21-11-2013	22	4	13.00	4.33	8			Nil
22-11-2013	5	0	2.50	2.50	8			Nil
23-11-2013	4	5	4.50	4.50	8			Nil
24-11-2013	1	1	1.00	1.00	8			Nil
25-11-2013	0	0	0	0.00	8			Nil
26-11-2013	9	1	5.00	5.00	8	26-11-2013	Twin (Chlorpyriphos 20%EC+ metalax-yl 8%+ Mancozeb)	Nil
27-11-2013	0	2	1.00	1.00	8			Nil
28-11-2013	4	1	2.50	2.50	8			Nil
29-11-2013	7	6	6.50	6.50	8			Nil
30-11-2013	3	1	2.00	2.00	8			Nil
01-12-2013	5	3	4.00	4.00	8			Nil
02-12-2013	5	4	4.50	4.50	8			Nil
03-12-2013	2	1	1.50	1.50	7.5			Nil
04-12-2013	3	0	1.50	1.50	7.5			Nil
05-12-2013	9	3	6.00	6.00	7.5			Nil
06-12-2013	0	7	3.50	3.50	7.5			Nil
07-12-2013	0	1	0.50	0.50	7.5			Nil
08-12-2013	4	3	3.50	3.50	7.5			Nil
09-12-2013	5	1	3.00	3.00	7.5			Nil
10-12-2013	0	4	2.00	2.00	7.5			Nil
11-12-2013	5	1	3.00	3.00	7.5			Nil

A, B= *A. mellifera* colonies, Foraging crop: pomegranate

applying insecticides to crops in bloom (Mader and Adamson, 2012; Hoven *et al*., 2013; May *et al*., 2015).

Conclusion

The coordination between beekeepers and farmers and some preventive and safety measures helps to lower down the probable bee loss. The area where pesticide use is frequent, proper care should be taken to prevent exposure of bee to insecticide and to maintain honey bee hives. Proper precautionary measures (insecticidal sprays were given at late evening hr, minimum and need based use of chemical sprays, use of safer insecticides, closing the bee colony gates for the period of 24 hr and internal feeding with artificial diet before spray)

Table 5. Mortality of honey bees observed in front of colonies at Aalefata (Case III).

Interval in days	A	B	C	SD	Mean	Mor-tality /day	Mean num-ber of frames with bee cluster	Date of spray	Pesticide sprayed (Trade name)	Active ingredient of pesticide sprayed	Diseases observed
23-11-2013 to 25/12/2013	12	18	16	3.05	15.33	5.11	8	24-11-2013	Tata manik	(Acetamiprid 20 % SP)	Nil
26/11/2013 to 1/12/2013	38	22	15	11.79	25.00	4.17	8	28-11-2013	Marshal + Asataf + Saaf	(Carbosulfan 25% EC) + (Acephate 75% SP) + (Carbendazim + Mancozeb75% WP)	Nil
2/12/2013 to 7/12/2013	9	4	2	3.61	5.00	0.71	8		_		Nil
8/12/2013 to 15/12/2013	5	7	4	1.53	5.33	0.76	8		_		Nil
16/12/2013 to 21/12/2013	20	28	37	8.51	28.33	5.67	8	17-12-2013	Chetak + Bavistin	Monocrotophos + Bavistin	Nil
22/12/2013 to 26/12/2013	9	16	3	6.51	9.33	1.56	8		_		Nil
27/12/2013 to 3/01/2014	4	8	2	3.06	4.67	0.67	8		_		Nil
4/01/2014 to 8/01/2014	13	15	5	5.29	11.00	2.20	8	04-01-2014	Nuvan + M-45	(Dichlorvos 76% EC + Mancozeb 75% WP)	Nil
9/01/2014 to 14/01/2014	12	14	8	3.06	11.33	2.27	8		_		Nil
15/01/2014 to 20/01/2014	9	11	3	4.16	7.67	1.28	8		_		Nil
21/01/2014 to 25/01/2014	7	3	2	2.65	4.00	0.67	8		_		Nil
26/01/2014 to 1/02/2014	6	6	2	2.31	4.67	0.67	7.67		_		Nil
2/02/2014 to 7/02/2014	8	13	6	3.61	9.00	1.50	7.67	02-02-2014	Marshal + Asataf + Bavistin	(Carbosulfan 25% EC) + (Acephate 75% SP) + (Carbendazim 50% DF)	Nil
8/02/2014 to 15/02/2014	22	11	18	5.57	17.00	2.43	7.67		_		Nil
16/02/2014 to 20/02/2014	11	14	2	6.25	9.00	1.80	7.67		_		Nil
21/02/2014 to 24/02/2014	15	18	6	6.25	13.00	3.25	7	21-02-2014	Dursban + Nuvan	(Chlorpyriphos 20% EC)+ (Dichlorvos 76% EC)	Nil
24/02/2014 to 28/02/2014	17	23	16	3.79	18.67	3.73	7		_		Nil

A, B and C= Names of *A. mellifera* colonies, Foraging crop: pomegranate , ** 1. The farmer was aware of bee keeping and bee health, 2. The farmer was owner of bee colonies, 3. Colony gates were closed during day after spray (management practices by farmer): to avoid exposure to toxicant, 4. All sprays were made at dusk period (late evening), 5. All observation were recorded during 1000 hr of day, 6. After recording observation the dead bees from trays were discarded, 7. Colony strength: 8 frames (approx. 5000-7000 bees), 8. Normal mortality for a colony with 60000 bees/colony: <100 bees/day

helps to lower down exposure of bees to insecticides.

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