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Effect of insecticides on foraging behaviour and pollination role of *Apis mellifera* L. (Hymenoptera: Apidae) on toria (*Brassica campestris* var. *toria*) crop

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Abstract

The effects of insecticide application on foraging and pollination by honeybees in toria (*Brassica campestris* var. *toria*) cultivar RSPT-1 were studied at Jammu (India).Under field conditions the application of betacylfluthrin, betacyfluthrin + imidacloprid and carbaryl resulted in 100% bee mortality within one hour of spraying. After 48 hours, 100% mortality was recorded in all the treatments except malathion (94%). The post-spraying effects of the insecticides were much higher during the first hour after treatment, but after 48 hr there was 100% mortality in all treatments except imidacloprid (43%). Residual effects after spraying were high for flowers sprayed with imidacloprid (76% mortality), demeton-o-methyl, carbaryl, and ethiprole, moderate for betacyfluthrin (49%), betacyfluthrin + imidacloprid, and profenophos, and low for malathion (12%). The residual effect decreases with time and after 96 hours of spraying, the residual effect was reduced in almost all the insecticides. The number of foraging bees were greatly reduced in all treatments 24 hr after spraying, compared to levels before spraying, recovering considerably after 3 days, and normal after 7 days. Open pollination resulted in 1.80 times more yield compared to caged condition and crop pollinated by bees alone. This study suggests that both protective application of insecticides and use of honeybees for pollination are essential for maximum crop yields.

Introduction

Honeybees provide pollination services to several cultivated and wild species, thereby, maintaining biological diversity (Sharma & Abrol 2005, Frankie *et al*. 2009). Bee poisoning or killing of bees from pesticides continues to be a serious problem for beekeepers. Most bee kill occurs when pesticides are applied or allowed to drift on to flowering crops or weeds. Most (99%) bee kills results from bees picking up the pesticides when foraging (Eckert & Shaw 1960). The hazards of insecticidal application on flowering crops include direct mortality, fumigative effects, repellent effects and toxicity of the residues present on various floral parts and in nectar (Desneux *et al*. 2007). A highly toxic insecticide generally reduces the foragers of a colony within a short period of time, up to one-third to a half within 24-48 hr (Aliouane *et al*. 2009), thus adversely affecting both the production and marketing segments of the honey and beekeeping industry. A prolonged repellent effect may deprive flowers of the pollination benefits of insect visits, while a short repellency will deter the insect pollinators from visiting the treated bloom for a brief period, but thereafter allow them to resume foraging activities (with minimal residual hazards) without compromising the yield potential of the crop (Halm *et al*. 2006).

Pollinator-plant interactions are complex phenomena, influenced by many overlapping effects (Stark *et al*. 1995). The uses of pesticides for pest control on the one hand, and the role of honeybees for crop pollination on the other, have become essential components of modern agriculture. Unfortunately, these two practices are not always compatible, as honeybees are susceptible to many of commonly used pesticides (Sundararaju 2003, Brittain *et al*. 2010). Conservation of honeybees for crop pollination is vital to agricultural production (Kremen *et al*. 2002). In India, 90% of the pollination of crops grown across 50 million hectares is done by bees (Singh *et al*. 1989). Although poorly studied, a harmonious compromise between pest management and honeybee pollination of crops in India is clearly important.

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The exotic honeybee, *Apis mellifera* L., has fully acclimatized to the various agro-climatic and geographical conditions of Jammu and Kashmir State. Toria (*Brassica campestris* var. *toria*) is an oilseed crop that attracts not only a large numbers of insect pollinators, especially honeybees for nectar and pollen, but also other insects that feed on flowers, leaves and fruits, thereby causing serious economic losses (Perveen *et al*. 2000). This requires the application of insecticides to combat the pests (Sihag 1988), directly or indirectly affecting the foraging activity of honeybees and ultimately crop yield.

In view of the above, the present investigation was planned with the aim of determining the impact of insecticides on the pollination activities of honeybees and seed production in *B. campestris* var. *toria*.

Materials & Methods

The experiment was conducted during 2009-10 and 2010-11 at the University Research Farm, Sher-e-Kashmir University of Agricultural Sciences & Technology, Jammu. It aimed to explore the effects of insecticide application on *Apis mellifera* and the subsequent effect on pollination, measured as seed number and seed weight. We also aimed to determine whether insecticide application influenced honeybee visitation rates to flowers, and the impact of bees on pollination. It was anticipated that, after insecticide application, there would be a reduction in flower visitation by honeybees and that this would lead to a reduction in pollination of the crop plants.

The effect of insecticides was evaluated for toria (*Brassica campestris* var. *toria*) cultivar (RSPT-1). The crop was sown on plots (5 × 4 m) with planting geometry 75 × 30 cm. Nine treatments (control, betacyfluthrin, carbaryl, betacyfluthrin + imidacloprid, profenophos, demeton-o-methyl, ethiprole, malathion, imidacloprid) with three replications each were laid out in a randomized block design. The recommended concentrations of the insecticides were sprayed on the marked plots, while the control was sprayed with water.

In order to evaluate the effect of direct spray, 60 foragers of *A. mellifera* in three replications of 20 each were confined in nylon netting cages (0.5 m cube). The cages were placed in each plot before spraying so that there was direct spraying onto the bees. After spraying, the cages were removed and placed in the laboratory. The bees were presented with a 50% sugar solution in each cage. Mortality was recorded 1, 4, 8, 12, 24 and 48 hr after spraying.

To study the effect of fumigation and drift on honeybee mortality, three cages of nylon nettings with confined bees as described above were hung in the crop after spraying. The bees were provided with 50 per cent sugar solution in each cage. Mortality counts were made after 1,4,8,12,24 and 48 hr.

Bees in cages were provided with treated flowers collected from the sprayed plots 24, 46, 72 and 96 hours after spraying in order to assess the toxicity of insecticide residues.

The numbers of honey bees visiting treated and untreated plots of *B. campestris* var. *toria* were used to study the effect of insecticides on the foraging activity of bees. The experiment was a completely randomized block design with nine insecticidal treatments and one untreated control. Spraying was done with a Knapsack sprayer at the full-bloom stage. Initial observations were made 24 hr before spraying, and then after 24, 48, 72 hr and one week after spraying. Bee counts were made on 5 marked plots (1x1 m).

The contribution of honeybee pollinators to toria pollination versus self pollination and *A. mellifera* pollination was estimated by excluding them from a set of plants. In both sampling rounds, the toria plants with insect-proof nets (1x1.5x2m) placed over them, were put in field sites, while other plants were left for open pollination. The role of bee pollination was assessed by placing a nucleus hive (four bee frames) inside the cages with the plants.

Seed yield was compared to detect treatment differences. Crop yield variables such as siliqua per plant, seeds per siliqua, 1000 seed weight (g), yield per hectare (kg ha^{-1}) were recorded. The data were analyzed by Anova following Sokal & Rholf (1981), using the Critical Difference between mean values assessed at p = 0.05.

Results

When applied as a direct spray,all the insecticides had significant impacts on mortality of honeybees, with slightly different time courses (Table 1). The order of cumulative acute direct toxicity was: betacyfluthrin > carbaryl > betacyfluthrin + imidacloprid > ethiprole > demeton-o- methyl > profenophos > imidacloprid > malathion.

		Mean per cent mortality after (hr)						
Treatment	**Concentration**(%)	**1**	**2**	**4**	**8**	**12**	**24**	**48**
Betacyfluthrin	0.007	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Imidacloprid	0.0025	15.0	34.2	55.8	79.2	100.0	100.0	100.0
Betacyfluthrin +Imidacloprid	0.004	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Carbaryl	0.10	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Demeton-o- methyl	0.03	15.0	45.0	72.5	90.8	100.0	100.0	100.0
Ethiprole	0.07	24.2	50.8	70.8	95.8	96.7	100.0	100.0
Malathion	0.07	3.3	5.0	20.0	40.0	61.7	79.2	94.2
Profenphos	0.07	10.8	31.7	53.3	80.8	98.3	100.0	100.0
Control	-	0.0	0.0	0.0	0.0	0.8	2.5	4.2
CD		**4.3**	**2.8**	**2.5**	**2.1**	**1.2**	**1.3**	**1.1**

Table 1: Effect of direct spray of insecticides on mortality of honeybee, *Apis mellifera* in toria seed crop. CD is the Critical Difference at p = 0.05

Treatment	**Concentration (%)**	**Mean per cent mortality after (hr)**					
		1	**4**	**8**	**12**	**24**	**48**
Betacyfluthrin	0.007	44.2	99.2	100.0	100.0	100.0	100.0
Imidacloprid	0.0025	4.2	9.2	15.8	28.3	32.5	43.3
Betacyfluthrin + Imidacloprid	0.004	39.8	88.3	100.0	100.0	100.0	100.0
Carbaryl	0.10	47.5	94.8	100.0	100.0	100.0	100.0
Demeton-o-methyl	0.03	12.5	34.2	65.0	81.7	95.8	100.0
Ethiprole	0.07	22.5	41.7	63.3	84.2	95.8	100.0
Malathion	0.07	42.5	66.7	78.3	89.2	100.0	100.0
Profenophos	0.07	15.8	34.2	61.7	82.5	100.0	100.0
Control	---	0.0	0.0	0.0	0.0	2.5	5.0
CD		**4.4**	**2.8**	**1.7**	**1.3**	**1.2**	**1.1**

Table 2: Post-spraying effects of insecticides on mortality in the honeybee, *A. mellifera*, in *toria* seed crops. CD is the Critical Difference at p = 0.05

The insecticides varied considerably in their post-spraying effects (Table 2). The percentage mortality after one hour of fumigation due to different insecticides was in the order: carbaryl > betacyfluthrin >malathion> betacyfluthrin + imidacloprid > ethiprole > profenophos > demeton-o-methyl > imidacloprid. Mortality increased with time, and within 12 hr 100% mortality was recorded for betacyfluthrin, carbaryl, and betacyfluthrin +

imidacloprid. Imidacloprid was safest of all the insecticides at only 28% mortality after 12 hr. After 48 hr, 100% bee mortality was recorded in all treatments except imidacloprid (Table 2).

The insecticides retained residual effects on sprayed crops for variable lengths of time (Table 3). Imidacloprid caused the highest mortality, with high values also for demeton-o-methyl, carbaryl and ethiprole. Malathion was the safest of all the insecticide residues. Mortality of honeybees feeding on sprayed flowers decreased with time for all insecticides, but some persisted even 72 hr after spraying. There were pronounced reductions in the residual effects 96 hr after spraying, with no treatment different from the control.

Treatment	**Concentration (%)**	**Mean per cent mortality after (hr)**			
		24	**48**	**72**	**96**
Betacyfluthrin	0.007	49.2	29.2	7.5	0.8
Imidacloprid	0.0025	75.8	35.8	6.7	0.0
Betacyfluthrin + Imidacloprid	0.004	48.3	40.8	6.7	1.7
Carbaryl	0.10	35.8	30.8	0.8	0.0
Demeton-o- methyl	0.03	69.2	31.7	7.5	1.7
Ethiprole	0.07	68.3	33.3	9.2	3.3
Malathion	0.07	11.7	4.2	0.8	0.0
Profenophos	0.07	36.4	24.2	6.7	0.0
Control	-	0.0	0.0	6.7	0.0
CD		**5.0**	**6.2**	**2.3**	**6.5**

Table 3: Effects of insecticide residues on mortality of honeybee foragers . CD is the Critical Difference at $p = 0.05$

There was a significant reduction in the number of honeybees visiting sprayed plants relative to the control, and relative to before spraying (Table 4). Numbers were recovering 72 hr after spraying, but were still significantly less than the normal even 3 days after spraying. After 7 days numbers were back to normal more or less at par with the control treatment. The order of repellency due to different insecticides was: betacyfluthrin + imidacloprid > carbaryl > demeton-o-methyl > imidacloprid > profenophos > betacyfluthrin > ethiprole >malathion. Insecticide applications delayed foraging, and behaviours such as agitation, aggressiveness and self-cleaning were observed to increase.

Treatment	**Concentration (%)**	**before spray**	**Visitor numbers N days after spraying (% change from before spraying)**			
			1	**2**	**3**	**7**
Betacyfluthrin	0.007	9.8	-38.9	-30.4	-5.0	+3.3
Imidacloprid	0.0025	10.0	-58.5	-34.5	-1.3	+8.5
Betacyfluthrin +Imidacloprid	0.004	12.8	-37.9	-29.2	-28.7	+2.6
Carbaryl	0.10	10.2	-62.3	-46.0	-23.0	+1.6
Demeton-o-methyl	0.03	13.7	-68.0	-38.4	-8.9	0.0
Ethiprole	0.07	10.3	-17.0	-10.3	-1.2	+6.8
Malathion	0.07	10.0	-15.4	-10.1	+11.8	+24.7
Profenophos	0.07	10.3	-46.8	-30.7	-25.9	+1.6
Control	-	10.0	+5.8	+19.0	+7.0	+2.0
CD		**1.1**	**6.4**	**5.3**	**7.3**	**4.7**

Table 4: Comparative effect of some insecticides on bee visitation on treated bloom of toria. CD is the Critical Difference at $p = 0.05$

Quantitative yield parameters showed significant differences under the different modes of pollination in the toria and cauliflower seed crop (Table 5). The numbers of siliquae per plant under open and honeybee-pollination were twice those formed under closed caged conditions. The siliqua filling was almost doubled in plants open to pollinators. Seed weight in general was lower under caged conditions. More healthy and sound seeds were obtained in open pollination than under caged conditions. Plants having free access to insect pollinators yielded 1.80 times more seed than those without access.

Yield attributes	**honeybees**	**open**	**closed**
siliquae per plant	258.3 ± 0.3	276.3 ± 0.1	122.7 ± 0.3
seed per siliqua	22.1 ± 0.1	29.5 ± 0.2	15.7 ± 0.2
pod length (cm)	6.23 ± 0.01	7.37 ± 0.18	5.26 ± 0.01
1000 seed weight (g)	5.65 ± 0.01	5.97 ± 0.04	2.15 ± 0.01
seed yield (kg ha^{-1} ÷ 100)	12.77 ± 0.02	13.73 ± 0.01	7.50 ± 0.02

Table 5: Seed yield and yield parameters in toria as influenced by varied mode of pollination.

Discussion

Organophosphates and carbamates are known to be highly toxic to honeybee workers when sprayed in cotton fields (Arzone & Patetta 1987, Brar *et al.* 1992). Although carbaryl, oxy demeton methyl and imidacloprid are highly toxic, they can be applied in the late evening with minimum hazard (Sihag 1991), but Suhail *et al.* (2001) found that application of diafenthion and malathion on cucumber during flowering resulted in 35% and 67% mortality of honeybees 48 hr after application. Scott-Dupree *et al.* (2009) reported that foliar applications of neonicotinoid insecticides, deltamethrin or spinosad affected bee foraging.

Several investigators have studied the fumigation effect of insecticides on honeybees (Eckert & Shaw 1960, Johansen & Mayer 1990, Anonymous 1991). Johansen & Mayer (1990) found that carbaryl and fluvalinate had no fumigation effects on bees one hour after spraying, but malathion and monocrotophos were equally hazardous from 1 to 12 hr after spraying. Cumulative mortality of all the insecticides they studied (oxydemeton methyl, formothion, dimethoate, malathion and phosphamidon) was much less than found here, ranging between 7% to 18% and not significantly different from the control.

Our results show that residual toxicity was high up to 24 hr after spraying, with significant differences among insecticides in the time course of fading but effects after 48 hr had decreased and were considerably reduced after 96 hr. Palmer-Jones *et al.* (1959) found that endosulfan and endrin could be used without many bee losses in the field. Our findings agree with Atkins & Anderson (1967), who found that most organophosphates were highly toxic to bees, but some (eg profenophos) declined rapidly and had almost disappeared 5 days after application. Thakur & Kashyap (1989) also found that demeton-s-methyl was considerably toxic to honeybees, with 100% kill after 48 hr exposure to treated flowers. Thus it seems that the insecticide was slowly translocated to the nectar (Bai & Reddy 1977). Deposits of demeton-s-methyl were relatively more persistent as compared to other insecticides. Vaidya *et al.* (1996) also observed that demeton-s-methyl persisted for 21 days though phosphamidon lasted only for 9 days. Singh (1969) and Kapil & Lamba (1974) found that malathion was less toxic than others against the workers of *Apis cerana indica*, *A. dorsata* and *A. florea* after spraying on mustard for aphid control.

The use of insecticides on flowering crops may adversely affect the foraging behavior of honeybees. A prolonged repellant effect will deprive the flowers of the pollinating benefits of insect visits, while short repellency will deter only briefly and thereafter allow them to resume their foraging activity (with minimum residual hazards) without compromising with the yield potential of the crops (Delaplane & Mayer 2000). After application of insecticides, foraging was delayed and behaviours such as agitation, aggressiveness and increases in self-cleaning were observed (cf. Vaidya *et al.* 1996). Such repellent effects of insecticides on honeybee foraging has been reported several times (Pike *et al*. 1982, Shires *et al.* 1984). Sharma (1993) found that morning application of insecticides (malathion and oxydemeton methyl) had some repellency effect on insect visitors including honeybees, which spent significantly less time on treated flowers than controls. The mode of action is not fully understood, but visual, olfactory, gustatory and chemical cues (Ramirez *et al*. 2005) are involved. Yang *et al*. (2008) reported that the honeybee foraging behaviour can be affected by imidacloprid concentrations as low as 50 g l^{-1}, the abnormal behaviour influencing orientation to the hive or to the feeding site (Decourtye *et al.* 2004). Observations in regions of extensive *Brassica* culture report a decrease in honey production and crop pollination, behavioural dysfunction, forager disappearances and great mortality during flowering, and also after winter when all *Brassica* pollen has been consumed. In areas where *Brassica* is treated with insecticides, all ages of honeybees are at risk of poisoning and also of bringing back contaminated food. The observed effects might relate to acute, chronic or sub lethal intoxication, all inducing the honeybee death (Desneux *et al.* 2007).

We found that toria crops benefit greatly from cross-pollination, as others have done (Westcott & Nelson 2001), and even just from flower probing ousting flower pests (Sihag 1988). Seed weight in general was lower under caged conditions than under open pollination, and more and better quality seeds were obtained from open pollination. The combined effect was that open pollination yielded 1.80 times more seed than plants having no access to insect pollinators. Sabir *et al*. (2000) found that honeybees maximized seed yield, 1000-grain seed yield and germination percentage in *Brassica campestris*. Sabbahi *et al*. (2005) found a significant improvement in the seed yield when honeybees were present. Munawar *et al.* (2009) reported significant increases in a range of plant parameters when caged with bees as compared to plants without bees.

Thus the yield obtained by having honeybee pollinators and using insecticides together is not just the simple addition of increases obtained under either in isolation: the effect is synergetic (Brittain & Potts 2011). Special care of the management of insect pollinators at the flowering stage of the crop should be made. Most of the tested insecticides were highly toxic to honeybees, causing considerable losses in the apiaries and resulting in reduced toria pollination. The integration of bee behaviour with insecticidal sprays will minimize bee losses and provide safety to honeybees for boosting honey production and pollination. Alternate ecofriendly strategies for pest management may be used. If necessary at all, only pesticides known to be safe for honeybees and other pollinating insects must be used.

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