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Effect of insecticides on foraging behaviour of honeybee *(Apis mellifera* L.) on mustard (*Brassica napus*)

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Abstract
The present studies on effect of insecticides on foraging behaviour of honey bees (*Apis mellifera* L.) on mustard (*Brassica napus*) were conducted. Five insecticides methyl-demeton >acetamiprid >imidacloprid >diamethoate >thiamethoxam were sprayed on the mustard field during blooming and checked the pollinator visiting rates and compared with no-treatment control. Honeybee visitation rate was lower in insecticide treated plots with different degree. The repellent effect of *Apis mellifera* L. was observed after 24 hrs of spraying followed by methyl-demeton (79.4, 85.6, 86.6, 85.6 per cent), acetamiprid (75.5, 79.5, 80.6, 81.0 per cent), imidacloprid (73.7, 79.6, 75.0, 71.4 per cent), diamethoate (70.3, 73.4, 76.3, 77.1 per cent) and thiamethoxam (66.1, 70.7, 67.0, 64.5 per cent) during 2014-15 and 2015-16 respectively. However, the repellent effect of honey bees was not observed in treatment of control. The order of repellency due to different insecticides was methyl-demeton provide average values of (79.4, 85.6, 86.6, 85.6 per cent) >acetamiprid (75.5, 79.5, 80.6, 81.0 per cent) >imidacloprid (73.7, 79.6, 75.0, 71.4 per cent) >diamethoate (70.3, 73.4, 76.3, 77.1 per cent) >thiamethoxam (66.1, 70.7, 67.0, 64.5 per cent). Only after 7 days after treatment, honeybee visitation rates were recovered relative to the control. It is thus evident that methyl-demeton followed by acetamiprid, imidacloprid, and diamethoate were more repellent effect of the honey bee forager and thiamethoxam having insecticidal properties was found to be less repellency of honey bee forager.

Keywords: *Apis mellifera* L., Pesticide, Repellency tendency, Pollination behaviour

Introduction
Among the oilseeds, rapeseed and mustard (*Brassica* species) occupies an important position and India ranks third both in area and production at global level [1]. Flowering *Brassicas* are not only visited by a large number of insect pollinators especially honey bees for nectar and pollen but they also attract insect pests such as mustard aphid which feeds upon flowers and developing seeds causing serious economic losses. Consequently, the insecticides are to be applied for crop protection which severely harms to the pollinating species. But the impacts of these insecticides on the foraging activity of wild pollinators, on the contaminated crops have not received much attention. Recently declines of various pollinators have been reported worldwide [2, 3]. The European honey bee (*Apis mellifera* L.) is relied upon extensively worldwide for pollinating approximately 75% of crop species in agricultural and horticultural cropping systems at a value of $170–$200 billion per year globally [4–6]. When foraging for pollen and nectar in flowering plants, honey bees can be exposed to a diverse array of pesticides, including: insecticides, fungicides, and herbicides [7–11], that can cause direct or indirect toxic effects to honey bees [12]. 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 [13]. Although many environmental and anthropogenic factors remain under investigation for their role in annual honey bee colony losses, pesticide is a major factor among those [14]. Effect of insecticides as repellent to honey bees has already been documented by some workers [15-19]. 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 [20], thus adversely affecting both the production and marketing segments of the honey and beekeeping industry. Thus, the present study was undertaken with a view to the adverse effect of some insecticides on foraging activity of bees.

Materials and Methods

The present experiment was conducted during *rabi* 2014-15 and 2015-16 at the Entomological Research Farm (600 m^2), Sher-e-Kashmir University of Agricultural Sciences & Technology, Jammu, India. Evaluate the effect of insecticides on foraging behaviour of honeybee *(Apis mellifera* L.) on mustard (*Brassica napus*). The insecticides viz., (control, methyl-demeton, imidacloprid, acetamiprid, diamethoate and thiamethoxam) were sprayed with the help of knapsack sprayer on the field plots of 5 m x 5 m size of mustard var. 'DGS-1' during its peak flowering period in randomized block design with six treatments and four replications. The spray was made between 10.00-12.00 hr during the maximum activity period of honey bees. Insecticide solutions were prepared with the recommended concentrations and applied with the field rates. Initial observation on the number of foraging of bees were recorded by visual count were made 24 hr before spraying, and then after 24, 48, 72 hr and one week after spraying of insecticidal application in $1m^2$ area for one minutes in treated and control plots between 10.00-12.00 hours. The population of bees recorded on the basis of visual counts as adopted by [21].

Statistical analysis

Honeybee visitation rates were calculated as percentage relative to the pre-treatment counts. The percentage data were statistically among insecticides along the time series by ANOVA after square root transformation [22]. When significant, means were separated by DMRT (Duncan's Multiple Range Test) by using SAS 9.1 (Statistical Analysis Software) programme.

Results & Discussion

2014-15 Studies

The data presented in (Table 1) showed that after 24 hrs of spraying the repellent effect of *Apis mellifera* L. was observed significantly higher in treatment with methyl-demeton 79.4 per cent followed by acetamiprid 75.5 per cent, imidacloprid 73.7 per cent and diamethoate 70.3 per cent. The lowest repellent effect of bees was recorded in thiamethoxam 66.1 per cent. However, the repellent effect of honey bees was not observed in treatment of control. The order of repellency due to different insecticides was methyl-demeton >acetamiprid >imidacloprid >diamethoate >thiamethoxam. Similar trend was obtained in all the treatments but with decrease per cent in repellent effect of bees was observed after 72 hrs of spraying. All the treatments showed population density built up to pre-treatment level, and some increase in number of bee visit was observed after 7 days of sprayingwhereas no repellent effect was observed in control treatment which showed some increased in number of bees.

Table 1: Effect of insecticidal treatments on *Apis mellifera* L. visiting flowering of mustard during 2014-15 (25% of bloom)

Treatment	Concentration g or ml/L	24 hrs before spray	Mean bee visitors at different intervals after spray			
			24 hrs	48 hrs	72 hrs	7 days
Imidacloprid 17.8 SL	0.3	28.5 (5.4)	7.5 (2.9)	10.8 (3.4)	13.8 (3.8)	25.8 (5.2)
Thiamethoxam 25 WG	0.3	30.3 (5.6)	10.3 (3.4)	13.8 (3.8)	17.3 (4.3)	28 (5.4)
Acetamiprid 20SP	0.3	27.5 (5.3)	6.8 (2.8)	8.5 (3.1)	11.5 (3.5)	24.3 (5.0)
Diamethoate 30 EC	2.5	29.5 (5.5)	8.8 (3.1)	12.0 (3.6)	14.5 (3.9)	28 (5.4)
Methyldemeton 35 EC	1.5	26.8 (5.3)	5.5 (2.5)	8.0 (3.0)	10.3 (3.3)	21.5 (4.7)
Water (Check) CD (p=0.05)	-	26.8 (5.3) NS	29.0 (5.5) 0.41	31.8 (5.7) 0.46	34.3 (5.9) 0.44	36.8 (6.1)0.45

Values in parenthesis are √n+1 transformed values.

Effect of second spray (at fall bloom stage)

The data presented in (Table 2) showed that after 24 hrs of spraying the repellent effect of *Apis mellifera* L. was observed significantly higher in treatment with methyl-demeton 85.6 per cent followed by imidacloprid 79.6 per cent, acetamiprid 79.5 per cent and diamethoate 73.4 per cent. The lowest repellent effect of bees was recorded in thiamethoxam 70.7 per cent. However, the repellent effect of honey bees was not observed in treatment of control. The order of repellency due to different insecticides was methyl-demeton >imidacloprid >acetamiprid >diamethoate >thiamethoxam. After 48 hrs of spraying the repellent effect of bees was observed significantly higher in treatment with methyl-demeton 79.7 per cent followed by acetamiprid 71.8 per cent, imidacloprid 69.9 per cent and diamethoate 62.1 per cent. The lowest repellent effect of bees was recorded in thiamethoxam 60.3 per cent. Whereas no repellent effect was observed in control treatment the order of repellency due to different insecticides was methyl-demeton >acetamiprid >imidacloprid >diamethoate >thiamethoxam. Similar trend was obtained in all the treatments but with decrease per cent in repellent effect of bees was observed after 72 hrs of spraying. All the treatments showed population density built up to pre-treatment level, and some increase in number of bee visit was observed after 7 days of spraying. However, the repellent effect of honey bees was not observed in treatment of control which showed some increased in number of bees.

Table 2: Effect of insecticidal treatments on *Apis mellifera* L. visiting flowering of mustard during 2014 -15 (50% of bloom)

Treatment	Concentration g or ml/L	24hrs before spray	Mean bee visitors at different intervals (days) after spray			
			24 hrs	48 hrs	72 hrs	7 days
Imidacloprid 17.8 SL	0.3	28.3 (5.4)	5.8 (2.6)	8.5 (3.1)	12.5 (3.7)	25.3 (5.1)
Thiamethoxam 25 WG	0.3	29.0 (5.5)	8.5 (3.1)	11.5 (3.5)	14.8 (4.0)	28.0 (5.4)
Acetamiprid 20SP	0.3	29.8 (5.5)	6.0 (2.6)	8.3 (3.0)	10.8 (3.4)	24.8 (5.1)
Diamethoate 30 EC	2.5	31.0 (5.7)	8.3 (3.0)	11.8 (3.6)	14.5 (3.9)	30.0 (5.6)
Methyldemeton 35 EC	1.5	29.5 (5.5)	4.3 (2.3)	6.0 (2.6)	8.0 (3.0)	25.0 (5.1)
Water (Check) CD (p=0.05)	-	31.5 (5.7) NS	33.5 (5.9) 0.29	35.5 (6.0) 0.33	37.8 (6.2) 0.26	39.3 (6.3) 0.30

Valuesin parenthesis are √n+1 transformed values.

2015-16 Studies

The data presented in (Table 3) showed that after 24 hrs of spraying the repellent effect of *Apis mellifera* L. was observed significantly higher in treatment with methyl-demeton 86.6 per cent followed by acetamiprid 80.6 per cent, diamethoate 76.3 per cent and imidacloprid 75.0 per cent. The lowest repellent effect of bees was recorded in thiamethoxam 67.0 per cent. However, the repellent effect of honey bees was not observed in treatment of control. The order of repellency due to different insecticides was methyl-demeton >acetamiprid >diamethoate >imidacloprid >thiamethoxam. Similar trend was obtained in all the treatments but with decrease per cent in repellent effect of bees was observed after 72 hrs of spraying. All the treatments showed population density built up to pre-treatment level, and some increase in number of bee visit was observed after 7 days of spraying. Whereas no repellent effect was observed in control treatment which showed some increased in number of bees.

Table 3: Effect of insecticidal treatments on *Apis mellifera* L. visiting flowering of mustard during 2015-16 (25% of bloom)

Treatment	Concentration g or ml/L	24 hrs before spray	Mean bee visitors at different intervals (days) after spray			
			24 hrs	48 hrs	72 hrs	7 days
Imidacloprid 17.8 SL	0.3	28.0 (5.4)	7.0 (2.8)	10.0 (3.3)	13.0 (3.7)	21.5 (4.7)
Thiamethoxam 25 WG	0.3	28.8 (5.5)	9.5 (3.2)	12.5 (3.7)	16.3 (4.2)	26.0 (5.2)
Acetamiprid 20SP	0.3	25.8 (5.2)	5.0 (2.4)	7.8 (3.0)	9.5 (3.2)	21.8 (4.8)
Diamethoate 30 EC	2.5	28.5 (5.4)	6.8 (2.8)	9.5 (3.2)	12.8 (3.7)	27.0 (5.3)
Methyldemeton 35 EC	1.5	28.0 (5.4)	3.8 (2.2)	5.5 (2.5)	8.0 (3.0)	20.8 (4.7)
Water (Check) CD (p=0.05)	-	26.5 (5.2) NS	28.3 (5.4) 0.45	29.3 (5.5) 0.42	33.3 (5.9) 0.34	35.5 (6.0) 0.50

Values in parenthesis are √n+1 transformed values.

Effect of second spray (at fall bloom stage)

The data presented in (Table 4) showed that after 24 hrs of spraying the repellent effect of *Apis mellifera* L. was observed significantly higher in treatment with methyl-demeton 85.6 per cent followed by acetamiprid 81.0 per cent, diamethoate 77.1 per cent and imidacloprid 71.4 per cent. The lowest repellent effect of bees was recorded in thiamethoxam 64.5 per cent. However, the repellent effect of honey bees was not observed in treatment of control. The order of repellency due to different insecticides was methyl-demeton >acetamiprid >diamethoate >imidacloprid >thiamethoxam. Similar trend was obtained in all the treatments but with decrease per cent in repellent effect of bees was observed after 72 hrs of spraying. All the treatments showed population density built up to pre-treatment level, and some increase in number of bee visit was observed after 7 days of spraying. Whereas no repellent effect was observed in control treatment which showed some increased in number of bees.

Table 4: Effect of insecticidal treatments on *Apis mellifera* L. visiting flowering of mustard during 2014-15 (50% of bloom)

Treatment	Concentration g or ml/L	24 hrs before spray	Mean bee visitors at different intervals (days) after spray			
			24 hrs	48 hrs	72 hrs	7 days
Imidacloprid 17.8 SL	0.3	29.8 (5.5)	8.5 (3.1)	11.8 (3.6)	14.5 (3.9)	25.8 (5.2)
Thiamethoxam 25 WG	0.3	31.0 (5.7)	11.0 (3.5)	15.0 (4.0)	18.25 (4.4)	29.0 (5.5)
Acetamiprid 20SP	0.3	29.0 (5.5)	5.5 (2.5)	7.5 (2.9)	10.5 (3.4)	26.5 (5.2)
Diamethoate 30 EC	2.5	29.5 (5.5)	6.8 (2.8)	10.3 (3.4)	13.0 (3.7)	28.0 (5.4)
Methyldemeton 35 EC	1.5	29.5 (5.5)	4.3 (2.3)	7.0 (2.8)	11.5 (3.5)	25.0 (5.1)
Water (Check) CD (p=0.05)	-	29.5 (5.5) NS	31.0 (5.7) 0.34	33.3 (5.9) 0.35	35.0 (6.0) 0.31	37.3 (6.2) 0.28

Valuesin parenthesis are √n+1 transformed values.

Although carbaryl, oxy demeton methyl and imidacloprid are highly toxic, they can be applied in the late evening with minimum hazard [23, 24] 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 [25], deposits of demeton-s-methyl were relatively more persistent as compared to other insecticides. [17] also observed that demeton-s-methyl persisted for 21 days though phosphamidon lasted only for 9 days. [26] 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 [27, 28] who observed reduction in the number of honey bee visits up to third day of spraying over the sprayed toria (*Brassica campestris*) crop with imidacloprid. [29] found that dimethoate, phosphamidon, monocrotophos, oxy-demeton methyl and malathion were found to be more toxic than endosulfan to *A. mellifera*. [30] evaluated 8 pesticides against *A. cerana indica* and reported endosulfan as least toxic followed by Lambda cyhalothrin, alpha endosulfan and imidacloprid while betacyflothrin was highly toxic. [31] reported malathion to be most toxic to both *A. mellifera* and *A. cerana*. [32] has also listed endosulfan and metasystox among the insecticides, which could be used with relative safety in the non-foraging periods of bees [33] who observed the sharp decline of the number of bees foraging on "Marostem Kala" (*Brassica oleracea*) almost to zero with the aerial spray of methyl demeton. But they have reported crop to remain toxic up to 5 days to bees. Similarly, repellency effect of oxydemeton-methyl has also been reported by [34], from Virginia involving use of metasystox. But here the repellency effect of this insecticide was found for two weeks on raspberry resulting in reduction of crop yield to a considerable extent because of poor pollination. [35] found that application of endosulfan at flowering stage has little effect on the visitation of beneficial insects (honeybee, *A. mellifera*) and predatory coccinellids. The differences in the present findings and those reported by various workers may be due to

the difference in the concentration of insecticides, crop and agro climatic conditions. The basis of mode of action of repellents is not fully understood [36], but in most of the cases visual, olfactory, and gustatory and possibly common chemical sense [37], of the bees may be involved.

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