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Effect of direct spray of insecticides on mortality of honeybee, *Apis mellifera* L. (Hymenoptera: Apidae) on mustard crop (*Brassica napus*)

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Abstract

The effect of direct spray of insecticides on mortality of honeybee, *Apis mellifera* L. (Hymenoptera: Apidae) on mustard crop (*Brassica napus*) cultivar DGS-1 were studied at Jammu (India). Under field conditions the application of methyl-demeton resulted in 100 per cent bee mortality within one hour of spraying followed by imidacloprid (76.5, 100 per cent), acetamiprid (55.0, 62.5 per cent), diamethoate (47.5, 59.00 per cent) and thiamethoxam (42.5, 51.5 per cent) during 2014-15 and 2015-16 respectively. However, the per cent mortality of honey bee was not observed in treatment of control. Toxicity order of insecticides were methyl-demeton > imidacloprid > acetamiprid > diamethoate > thiamethoxam. After 3 hours of spraying the mortality was 100 per cent of methyl-demeton and imidacloprid followed by acetamiprid (77.5, 80.0 per cent), diamethoate (70.0, 79.0 per cent) and thiamethoxam (50.0, 56.5 per cent) whereas no mortality was observed in control treatment. Similar trend was obtained in all the treatments but with increased per cent bee mortality after 7 hours of spraying. After 12 and 24 hours of spraying the mortality was 100 per cent for all the treatments except thiamethoxam, which recorded (99.0, 97.5 per cent) during 2014-15 and 2015-16 respectively. It is thus evident that methyl-demeton followed by imidacloprid, acetamiprid, and diamethoate were more toxic to the honey bees forager and thiamethoxam having insecticidal properties was found to be less toxic of honey bees forager. Statistical analysis of the data revealed that effects of different insecticides at different hours of treatment on mortality of bees were highly significant.

Keywords: *Apis mellifera* L., *Brassica napus*, Insecticides, Direct mortality, Pollination

Introduction

Honeybees provide pollination services to several cultivated and wild species, thereby, maintaining biological diversity (Sharma & Abrol 2005, Frankie *et al*. 2009) [20, 10]. 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) [9]. 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) [8]. 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) [1], 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) [11].

Pollinator-plant interactions are complex phenomena, influenced by many overlapping effects (Stark *et al*. 1995) [23]. 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) [24, 7]. Conservation of honeybees for crop pollination is vital to agricultural production (Kremen *et al*. 2002) [13]. In India, 90% of the pollination of crops grown across 50 million hectares is done by bees (Singh *et al*. 1989) [22]. Although poorly studied, a harmonious compromise between pest management and honeybee pollination of crops in India is clearly important.

The exotic honeybee, *Apis mellifera* L., has fully acclimatized to the various agroclimatic and geographical conditions of Jammu and Kashmir State. mustard (*Brassica napus*) 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) [17]. This requires the application of insecticides to combat the pests (Sihag 1988) [21], directly or indirectly affecting the foraging activity of honeybees and ultimately crop yield. The aim of present studies is not only the determination of the safest insecticides to the honey bees but also the evaluation of the dose of most commonly used insecticides which can control the insect pests and less harming to honey bees.

Materials and Methods

The experiment was conducted during 2014-15 and 2015-16 at the University Research Farm, Sher-e-Kashmir University of Agricultural Sciences & Technology, Jammu. The effect of direct spray of insecticides was evaluated for mustard (*Brassica napus*) cultivar (DGS-1). The crop was sown on plots (5 × 5 m) with planting geometry 45 × 20 cm. Six treatments (control, methyl-demeton, imidacloprid, acetamiprid, diamethoate and thiamethoxam) with four replications each were laid out in a randomized block design. In order to evaluate the effect of direct spray, 80 foragers of *A. mellifera* in 20 each replications 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, 3, 5, 7, 12 and 24 hr after spraying. The honey bees *Apis mellifera* L. that did not move or respond were considered dead (Obeng-Ofori *et al*., 1998). Dead bees counts were made and mean population was calculated according to Baba-Tierto Niber (1994) [5] using the formula: Mortality (%) =

$$\frac{\text{No. of dead insects} \times 100}{\text{Total No. of insects}}$$

The values, after square root transformation were subjected to ANOVA (Analysis of Variance) (Panse and Sukhatme, 1954). One way analysis of variance was applied to find out the differences between treatments imidacloprid (T_1), thiamethoxam (T_2), acetamiprid (T_3), diamethoate (T_4), methyldemeton (T_5) and water control (T_6) on honey bee *Apis mellifera* L.

Source of variation	Degrees of freedom (DF)	Sum of square	Mean sum of square	F- ratio
Regression Error	t-1 n-t	TrSS SSE	TrMS= $\frac{TSS}{t-1}$ EMS= $\frac{ESS}{n-t}$	$\frac{TrMS}{EMS}$
Total	n-1	TSS		

Critical value of F for comparing with calculated F value was done by finding out the CD as per detail given here under:

CD= tX SE (d) Where S.E= $\sqrt{EMS\left(\frac{1}{ri}+\frac{1}{rj}\right)}$ and t is the critical tvalue for error degrees of freedom at 5% level.

Results and Discussion

2014-15 Studies

The data presented in Table 2, 3 and Figure 1 showed that 1 hour after spraying the mortality of *Apis mellifera* L. was observed significantly higher in treatment with methyl-demeton 100 per cent followed by imidacloprid 76.5 per cent, acetamiprid 55.0 per cent and diamethoate 47.5 per cent. The lowest mortality was recorded in thiamethoxam 42.5 per cent. However, the per cent mortality of honey bee was not observed in treatment of control. Toxicity order of insecticides were methyl-demeton > imidacloprid > acetamiprid > diamethoate > thiamethoxam. After 3 hours of spraying the mortality was 100 per cent of methyl-demeton and imidacloprid followed by acetamiprid 77.5 per cent, diamethoate 70.0 per cent and thiamethoxam 50.0 per cent whereas no mortality was observed in control treatment. Similar trend was obtained in all the treatments but with increased per cent bee mortality after 7 hours of spraying. After 12 and 24 hours of spraying the mortality was 100 per cent for all the treatments except thiamethoxam, which recorded 99.0 per cent. It is thus evident that methyl-demeton followed by imidacloprid, acetamiprid, and diamethoate were more toxic to the honey bee forager and thiamethoxam having insecticidal properties was found to be less toxic of honey bee forager. Statistical analysis of the data revealed that effects of different insecticides at different hours of treatment on mortality of bees were highly significant.

Table 1: Effect of direct spray of insecticides on mortality of *Apis mellifera* L. on different hours during (2014-15)

Treatment	Concentration g or ml/L	1 h	3 h	5 h	7 h	12 h	24 h
Imidacloprid 17.8 SL	0.3	15.3 (4.0)	20.0 (4.6)	20.0 (4.6)	20.0 (4.6)	20.0 (4.6)	20.0 (4.6)
Thiamethoxam 25 WG	0.3	8.5 (3.1)	10.0 (3.3)	14.0 (3.9)	16.8 (4.2)	19.8 (4.6)	20.0 (4.6)
Acetamiprid 20SP	0.3	11.0 (3.5)	15.5 (4.1)	18.5 (4.4)	20.0 (4.6)	20.0 (4.6)	20.0 (4.6)
Diamethoate 30 EC	2.5	9.5 (3.2)	14.0 (3.9)	17.0 (4.2)	18.8 (4.4)	20.0 (4.6)	20.0 (4.6)
Methyldemeton 35 EC	1.5	20.0 (4.6)	20.0 (4.6)	20.0 (4.6)	20.0 (4.6)	20.0 (4.6)	20.0 (4.6)
Water (Check)	-	0.0 (1.0)	0.0 (1.0)	0.0 (1.0)	0.0 (1.0)	0.0 (1.0)	0.0 (1.0)
CD (p=0.05)		0.13	0.10	0.10	0.09	-	-

Figures in parenthesis are √n+1 transformed values

Table 2: Mean reduction in number of *Apis mellifera* L. in various treatment over control mortality due to insecticides on different hours during (2014-15)

Treatment	Concentration g or ml/L	1 h	3 h	5 h	7 h	12 h	24 h
Imidacloprid 17.8 SL	0.3	76.5 (61.0)	100 (90.0)	100 (90.0)	100 (90.0)	100 (90.0)	100 (90.0)
Thiamethoxam 25 WG	0.3	42.5 (40.7)	50.0 (45.0)	70.0 (56.8)	84.0 (66.4)	99.0 (89.4)	100 (90.0)
Acetamiprid 20SP	0.3	55.0 (47.9)	77.5 (61.7)	92.5 (74.1)	100 (90.0)	100 (90.0)	100 (90.0)
Diamethoate 30 EC	2.5	47.5 (43.6)	70.0 (56.8)	85.0 (67.2)	94.0 (75.8)	100 (90.0)	100 (90.0)
Methyldemeton 35 EC	1.5	100 (90.0)	100 (90.0)	100 (90.0)	100 (90.0)	100 (90.0)	100 (90.0)
Water (Check)	-	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)

-Figures in parenthesis are angular transformed values

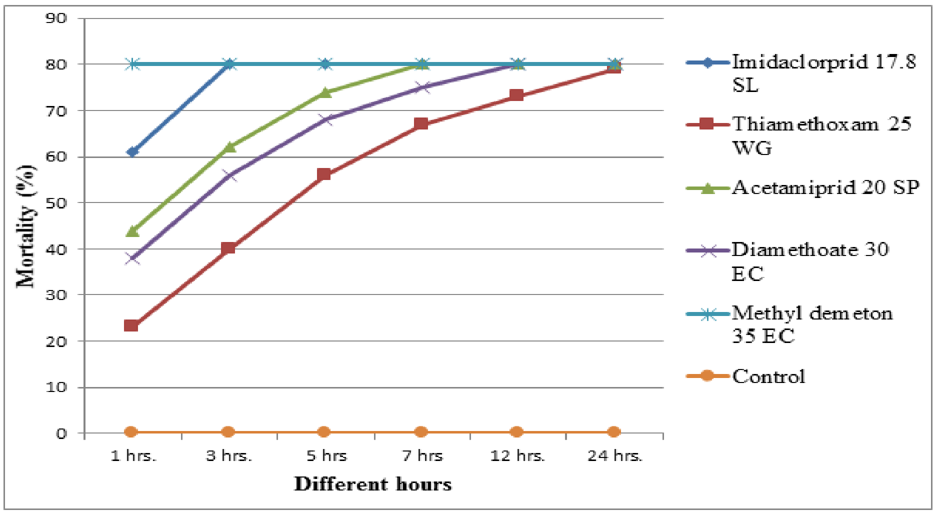

Figure 1: Effect of direct spray of insecticides on mortality of *Apis mellifera* L. during (2014-15)

2015-16 Studies

The data presented in Table 4, 5 and Figure 2 showed that 1 hour after spraying the mortality of *Apis mellifera* L. was observed significantly higher in treatment with methyl-demeton and imidacloprid 100 per cent followed by acetamiprid 62.5 per cent and diamethoate 59.0 per cent. The lowest mortality was recorded in thiamethoxam 51.5 per cent. However, the per cent mortality of honey bee was not observed in treatment of control. Toxicity order of insecticides were methyl-demeton Similar as imidacloprid > acetamiprid > diamethoate > thiamethoxam. After 3 hours of spraying the mortality was 100 per cent of methyl-demeton and imidacloprid followed by acetamiprid 80.0 per cent, diamethoate 79.0 per cent and thiamethoxam 56.5 per cent whereas no mortality was observed in control treatment. Similar trend was obtained in all the treatments but with increased per cent bee mortality after 7 hours of spraying. After 12 and 24 hours of spraying the mortality was 100 per cent for all the treatments except thiamethoxam, which recorded 97.5 per cent. It is thus evident that methyl-demeton Similar as imidacloprid followed by acetamiprid, and diamethoate were more toxic to the honey bee forager and thiamethoxam having insecticidal properties was found to be less toxic of honey bee forager. Statistical analysis of the data revealed that effects of different insecticides at different hours of treatment on mortality of bees were highly significant.

In earlier studies Arzone and Patetta (1987) [4] who found that most of the organophosphates and carbamates were highly toxic to honeybees as compared to endosulfan. The present findings are also in conformity with Brar *et al.* (1992) [6] who found that carbaryl was highly toxic to honeybee, *A. mellifera* when sprayed in cotton fields. In another study, Mayer *et al.* (1994) [14] found that carbaryl, oxydemeton methyl, imidacloprid were highly toxic to honeybee, *A. mellifera* and can be applied in the late evening with minimum hazard to honeybees. Wightman and Whitford (1982) [27] also found that demeton-s-methyl and endosulfan are not entirely safe for honeybee (*A. mellifera*) and thus are not suitable for seed crops during the flowering period.

Arzone (1986) [3] demonstrated that the endosulfan, carbaryl and demeton-o-methyl were more toxic to bees and can be dangerous to bees and other pollination if allowed to come in contact not only with the flowering crop but also with honeydew, propolis or water likely to be imbibed or collected by the insects. Thakur and Kashyap (1989) [25] found out that demeton-s-methyl was highly toxic to honeybee, *A. mellifera* followed by dimethoate. Similar results have also been reported by Hameed *et al.* (1973) [12]. Rana and Goyal, (1991) [18] found out that both methyl demeton (0.025%) and dimethoate (0.03%) when sprayed on Chinese sarson (*Brassica chinensis*) were significantly toxic to honeybees as they reduced the colony strength in terms of brood area and the number of bee frames and killed the foragers. Similarly Anon (1991) [2] found out that fluvalinate and endosulfan was less toxic as compared to malathion, phosphamidon and dimethoate to honeybee, *A. c. indica* foraging on rapeseed crop. Thakur *et al.* (1985) [26] reported endosulfan least toxic to *A. mellifera* when sprayed on mustard field followed by permethrin, cypermethrin, sumicidin, decamethrin and fenpropathrin. Suhail *et al.* (2001) [19] found that application of Polo (diafenthion) and endosulfan on cucumber during flowering period resulted in 34.64 per cent and 66.64 per cent mortality of honeybees, respectively at 48 hours after insecticide application.

Table 4: Effect of direct spray of insecticides on mortality of *Apis mellifera* L. on different hours during (2015-16)

Treatment	Concentration g or ml/L	1 h	3 h	5 h	7 h	12 h	24 h
Imidacloprid 17.8 SL	0.3	20.0 (4.6)	20.0 (4.6)	20.0 (4.6)	20.0 (4.6)	20.0 (4.6)	20.0 (4.6)
Thiamethoxam 25 WG	0.3	10.3 (3.4)	11.3 (3.5)	15.0 (4.0)	16.5 (4.1)	19.5 (4.5)	20 (4.6)
Acetamiprid 20SP	0.3	12.5 (3.7)	16.0 (4.1)	20.0 (4.6)	20.0 (4.6)	20.0 (4.6))	20.0 (4.6)
Diamethoate 30 EC	2.5l	11.8 (3.6)	15.8 (4.1)	17.8 (4.3)	20.0 (4.6)	20.0 (4.6)	20.0 (4.6)
Methyldemeton 35 EC	1.5l	20.0 (4.6)	20.0 (4.6)	20.0 (4.6)	20.0 (4.6)	20.0 (4.6)	20.0 (4.6)
Water (Check)	-	0.0 (1.0)	0.0 (1.0)	0.0 (1.0)	0.0 (1.0)	0.0 (1.0)	0.0 (1.0)
CD (p=0.05)		0.10	0.10	0.09	0.04	0.04	-

Figures in parenthesis are √n+1 transformed values

Table 5: Mean reduction in number of *Apis mellifera* in various treatment over control mortalitydue to insecticides on different hours during (2015-16)

Treatment	Concentration g or ml/L	1 h	3 h	5 h	7 h	12 h	24 h
Imidacloprid 17.8 SL	0.3	100 (90.0)	100 (90.0)	100 (90.0)	100 (90.0)	100 (90.0)	100 (90.0)
Thiamethoxam 25 WG	0.3	51.5 (36.3)	56.5 (48.7)	75.0 (60.0)	82.5 (65.3)	97.5 (80.9)	100 (90.0)
Acetamiprid 20SP	0.3	62.5 (52.2)	80.0 (63.4)	100 (90.0)	100 (90.0)	100 (90.0)	100 (90.0)
Diamethoate 30 EC	2.5	59.0 (50.2)	79.0 (62.7)	89.0 (70.6)	100 (90.0)	100 (90.0)	100 (90.0)
Methyldemeton 35 EC	1.5	100 (90.0)	100 (90.0)	100 (90.0)	100 (90.0)	100 (90.0)	100 (90.0)
Water (Check)	-	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)

Figures in parenthesis are angular transformed values

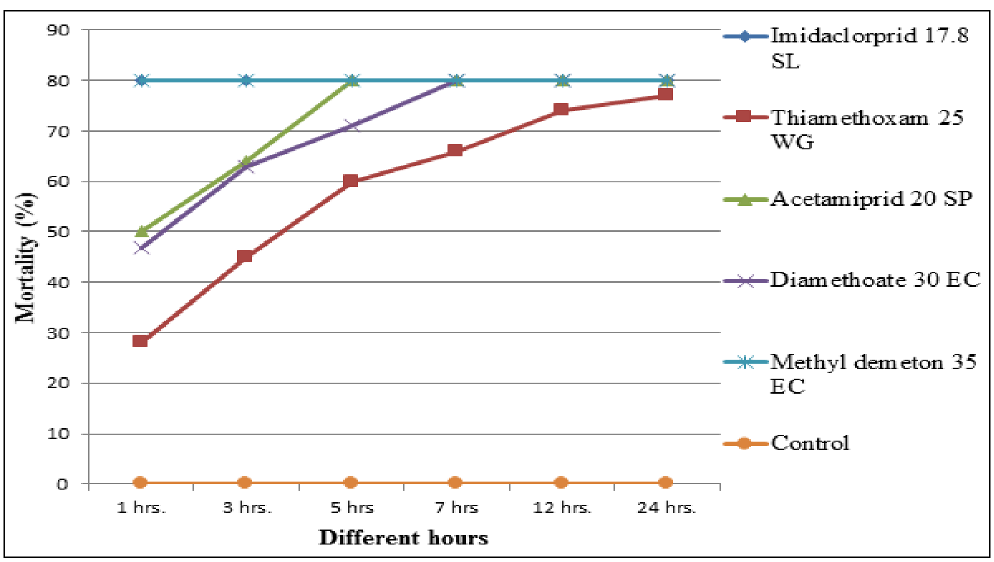

Fig 2: Effect of direct spray of insecticides on mortality of *A. mellifera* during (2015-16)

Conclusion

Under field conditions the application of methyl-demeton resulted in 100 per cent bee mortality within one hour of spraying followed by imidacloprid (76.5, 100 per cent), acetamiprid (55.0, 62.5 per cent), diamethoate (47.5, 59.00 per cent) and thiamethoxam (42.5, 51.5 per cent). However, the per cent mortality of honey bee was not observed in treatment of control. Toxicity order of insecticides were methyl-demeton > imidacloprid > acetamiprid > diamethoate > thiamethoxam. After 3 hours of spraying the mortality was 100 per cent of methyl-demeton and imidacloprid followed by acetamiprid (77.5, 80.0 per cent), diamethoate (70.0, 79.0 per cent) and thiamethoxam (50.0, 56.5 per cent) whereas no mortality was observed in control treatment. Similar trend was obtained in all the treatments but with increased per cent bee mortality after 7 hours of spraying. After 12 and 24 hours of spraying the mortality was 100 per cent for all the treatments except thiamethoxam, which recorded (99.0, 97.5 per cent) during 2014-15 and 2015-16 respectively. It is thus evident that methyl-demeton followed by imidacloprid, acetamiprid, and diamethoate were more toxic to the honey bees forager and thiamethoxam having insecticidal properties was found to be less toxic of honey bees forager. Statistical analysis of the data revealed that effects of different insecticides at different hours of treatment on mortality of bees were highly significant.

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