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Residual Toxicity of 8 Different Insecticides on Honey Bee (*Apis mellifera* Hymenoptera: Apidae)

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Abstract: The study examined the acute toxicity of eight agricultural insecticides such as Karate 5 EC (Lamda-cyhalathrine), Deltanete 400 EC (Furathiocarb), Sevin 85 WP (Carbaryl), Sevin XLR plus (Carbaryl), Marshal 25 EC (Carbosulfan), Oncol EC 200 (Benfurocarb), Mesurol WP 50 (Methiocarb) and Neem Azal T/S (Azadirachtin) with 3 different doses including recommended doses (r.d), ½ r.d and ¼ r.d. The mortality ratios were counted 1, 8, 16 and 24 h after each application. Research results indicated that Marshall, Oncol, Deltanate, Mesurol, Karate, Sevin XLR and Sevin WP 85 had the harmful effect on bee while Azadirachtin was environmentally friendly for controlling hazelnut pests. Research results also revealed that Sevin XLR contained the fastest insecticide toxic effects on bees. In the research conducted areas, toxic insecticides use should be avoided and Azadirachtin use should be encouraged to Hazelnut growers.

Key words: Insecticides, toxicity, *Apis mellifera*, Aazadirachtin

INTRODUCTION

Up to now, meeting the needs of human is one of the main problems for mankind. Population pressure led to rising of demand for agricultural products. For this reason, many societies have aimed to counterbalance the human needs and food production. This made the farmers use much more agrochemicals in production activities. Farmers, especially in developed and developing countries have been using excessive agrochemicals in order to increase crop yields. Pretty (1995) suggested that overuse and inappropriate use of agrochemicals have led to environmental problems such as contamination of water, loss of genetic diversity and deterioration of soil quality. Recently, there has been much debate on the indiscriminate use of insecticides that result in environmental pollution and toxicity risk to non-target organisms. Insecticides can contaminate soil, water, turf and vegetation. In addition to killing insects, insecticides can be toxic to a most of other organisms including birds, fish, beneficial insects, bees and non-target organisms (Pimentel, 1995; Liong *et al*., 1998; Kolankaya *et al*., 2001; Incerti *et al*., 2003; Frederick, 2005; Akca *et al*., 2005). Some insecticides no longer effective and are also banned in many countries. Many previous researches therefore have concentrated on the toxicity of insecticides on different non-target organisms (Qadri *et al*., 1994; Venkateswara, 2004). Some of the previous studies especially focused on the effects of insecticide on bees (Liong *et al*., 1998; Bendahou *et al*., 1999; Venkateswara *et al*., 2001; Fletcher and Barnett, 2003; Marletto *et al*., 2003).

In Turkey, agriculture is still a relatively important sector. The agriculture sector contributed 12.9% of gross domestic product and accounted for 32.9% of total employment (Anonymous, 2007). Both forcing the economic factors surrounding the farmers and willingness of Turkish farmers to increase crop yield has caused to intensive use of chemical inputs. Intensive use of fertilizers and pesticides have led to deterioration of land (Tanrivermis, 1999; Kizilkaya *et al*., 2004; Cemek and Kizilkaya, 2006), contamination of ground water (Balkaya *et al*., 1996) and residue accumulation on some crop (Kumbur *et al*., 1996). Using levels of carbamates insecticide, carbamates herbicides, amides, rodenticides and triazine has increased by 61, 90, 100, 50 and 64%, respectively in Turkey during 1994-2001 (Anonymous, 2008). Nowadays, the toxicity of insecticide to non-target

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organisms has become a current issue in Turkey. One of the non-target organisms is bee to which insecticide can be toxic. There is not much information available on toxic effect of pesticides on non-target organisms in Turkey (Kolankaya *et al*., 2001).

Turkey is the primary hazelnut producer of the world. Hazelnut covers almost 584.000 ha of land and provide solid income for >400 thousands families Turkey (Tuncer *et al*., 2001). Since, hazelnut yield highly depends on both diseases and insects, management of insect pests are the major concern of many hazelnut growers in Turkey. There have been almost 150 insect pest species in the hazelnut area of Turkey (Isik *et al*., 1987). For combating the economic damage of insects, Turkish farmers have traditionally used insecticides against hazelnut insect pests.

Carbamotes, organophosphates and recently synthetic pyrethroids are the most comon groups of insecticides being used in the country. Regarding the number of applications, there has been an excessive usage of insecticides. In some hazelnut orchards, the numbers of applications ascend to 4 applications. Based on previous studies, it is apparent that spraying more than twice is unnecessary for controlling target pests (Tuncer and Ecevit, 1997).

Bee keeping is common in most hazelnut orchards in Turkey. Since, insecticide uses affect the bee keeping, recently, there has been relatively much more concern on using insecticides and their effects on beekeeping. However, there has been a limited study about the toxic effects of insecticides on bee. Therefore, the objective of the study is to explore the adverse effects of insecticides used for hazelnut production on bee in the Black Sea region of Turkey.

MATERIALS AND METHODS

The experiment was designed as a 8×4×3 factorial treatment arrangement in a randomized complete block with 4 replications. Factors and the levels for each factors consisted of 8 chemicals: Karate 5 EC (Lamda-cyhalathrine), Deltanete 400 EC (Furathiocarb), Sevin 85 WP (Carbaryl), Sevin XLR Plus (Carbaryl), Marshal 25 EC (Carbosulfan), Oncol EC 200 (Benfurocarb), Mesurol WP 50 (Methiocarb) and Neem Azal T/S (Azadirachtin) Karate 5 EC (Lamda-cyhalathrine), Deltanete 400 EC (Furathiocarb), Sevin 85 WP (Carbaryl), Sevin XLR plus (Carbaryl), Marshal 25 EC (Carbosulfan), Oncol Ec 200 (Benfurocarb), Mesurol WP 50 (Methiocarb) and Neem Azal T/S (Azadirachtin), 4 caunting duration: 1, 8, 16 and 24 h after each application and 3 different doses: recommended doses (r.d), 1/2 and 1/4 r.d (Table 1).

The doses were preparared as mg ai L^{-1} water. Laboratory conditions were maintained at 25±2°C, 70±5% R.H and 14:10 h light:dark. Residual films method was used to determine toxicity of insecticides (Busvine, 1971). Worker honeybees, which were younger than 20 days old, were used in the study.

Honey bees were kept in plastic boxes (10×20×7 cm) having filter paper on bottom. Before bees transferred to the boxes, 1 mL insecticide solution in different doses of each insecticide were applied on filter paper and then allowed to dry for 1 h.

Polyethylene sheets containing small holes were used together with rubber to cover open side of boxes. Ten adult honey bees were released in to each box per treatment. Test concentrations were prepared with distilled water. In control boxes only distilled water was used.

The mortality was counted 1, 8, 16 and 24 h after each application. The mortality data was corrected by Abbott (1925) Formula. Additionally, all data were analyzed using SSPS 11.0 (statistical package for social science) statistical software. Analysis of Variance (ANOVA) was performed to compare the means of tested insecticides. The differences between individual means were tested using the $LSD_{\alpha=0.01}$ test. The * and ** indicate significant differences at $p<0.05$ and $p<0.01$, respectively.

Table 1: Insecticides used in the experiments

Compound	Trade name	Group	Recommended application rate g/da-mL/da	Concentrations used in bioassay (mg ai L^{-1})
Azadrachtin 1%	Neem Azal T/S	Botanical	1	100, 50, 25
Benfurocarb 200 g L^{-1}	Oncol EC 200	Carbamate	150 mL	300, 150, 75
Carbaryl 85%	Sevin 85 WP	Carbamate	150 g	1275, 637.5, 318.7
Carbaryl 480 g L^{-1}	Sevin XLR Plus	Carbamate	200 mL	960, 480, 240
Carbosulfan 250 g L^{-1}	Marshall 25 EC	Carbamate	125 mL	312.5, 156.2, 78.1
Furathiocarb 400 g L^{-1}	Deltanet 400 EC	Carbamate	100 mL	400, 200, 100
L-cyhalothrin 50 g L^{-1}	Karate 5 EC	Prethroid	50 mL	25, 12.5, 6.2
Methiocarb 50%	Mesurol WP 50	Carbamate	100 g	500, 250, 125

RESULTS AND DISCUSSION

Research results revealed that all tested insecticides had the toxic effects on honey bees with the exception of Neem Azal T/S. The results of variance analysis were given in Table 2. There were significant differences among insecticides at different application doses and the different times in terms of mortality percentage of bees ($p<0.01$). The mortality ratio of bee increased associated with time while the percentage of mortality decreased with the dose reduction ($p<0.01$). There were also significant interaction among time, dose and the type of insecticides used ($p<0.01$).

Based on these results, 6 carbamates and one registered pyrethroid compounds significantly decreased the number of living bee. Marshall, Oncol and Deltanate sustained a harmful effect on honey bees at all application time and doses with the exception of the 8 h after application with the dose of r.d ¼. Similarly, Mesurol had the maximum harmful effect on bees at recommended doses and ½ with 16 and 24 h after applications. It was followed by and Karate, Sevin XLR and Sevin WP 85 (Table 3). This finding confirmed the results of the some previous studies (Bendahou *et al*., 1999; Kolankaya *et al*., 2001; Fletcher and Barnett, 2003; Incerti *et al*., 2003; Porrini *et al*., 2003; Chauzat and Faucan, 2007) that they suggested that Carbamates, Organophosphate and pyrethroid chemicals had the toxicity effect to bees.

The other finding of the study was that 1 h after the application; there was no adverse effect of tested insecticides with the exception of Sevin XLR on the number of living bee at all doses while the number of living bee decreased at the other time applications. Interestingly, Sevin XLR was the fastest insecticide in terms of toxic effect on bee.

Azadirachtin, a botanical pesticide, did not have acute toxicity to bees and the difference between this chemical and control was not statistically different at all application times and doses (Table 3). According to previous studies (Tuncer and Akca, 1996; Aliniazee, 1997; Tuncer and Aliniazee, 1998; Tuncer *et al*., 2007) azadirachtin had a high control affect on some hazelnut pests such as *Hyphantria cunea*, *Parhenolecanium coryli*,

Table 2: The results of variance analysis

Variables	F-value
Insecticides (I)	34.321***
Doses (D)	132.198***
Times (T)	190.491***
Insecticides×doses	6.561***
Insecticides×time	7.739***
Doses×time	23.312***
Insecticides×doses×time	1.834***

***$p<0.001$

Table 3: Mortality rate of the bees associated with time after application

		Mortality (%)			
		Times after application (h)			
Doses	Chemicals	1	8	16	24
Rd	Neem Azal T/S	-	-	3.55±3.55e	3.55±3.55d
	Oncol EC 200	-	70.43±4.43a	83.20±3.97ab	86.33±2.90a
	Sevin 85 WP	-	20.63±2.95c	38.75±4.15d	49.38±6.33c
	Sevin XLR Plus	2.5±2.5	36.13±13.54b	58.28±16.56cd	67.13±14.61b
	Marshal 25 EC	-	70.18±1.31a	72.68±2.77b	84.30±3.97a
	Deltanate 400 EC	-	66.78±7.75a	75.13±2.29b	85.40±4.24a
	Karate 5 EC	-	44.23±8.9b	54.85±6.85c	68.20±6.28b
	Mesurol WP	-	39.43±8.48b	92.20±4.85a	92.20±4.85a
	Control	-	-	-	2.5±2.5d
Rd 1/2	Neem Azal T/S	-	-	2.5±2.5d	2.5±2.5d
	Oncol EC 200	-	51.90±4.94ab	71.53±9.52a	87.33±4.52ab
	Sevin 85 WP	-	16.18±2.92cd	30.48±6.21bc	35.48±3.46c
	Sevin XLR Plus	-	4.58±2.66de	18.38±8.22c	40.00±8.90c
	Marshal 25 EC	-	62.55±1.59a	69.83±4.27a	92.95±4.40a
	Deltanate 400 EC	-	39.63±4.10b	73.30±2.57a	76.73±2.36b
	Karate 5 EC	-	22.50±4.79c	35.00±6.45b	50.00±5.77c
	Mesurol WP	-	40.63±7.24b	83.13±7.80a	90.63±9.38ab
	Control	-	-	-	2.5±2.5d
Rd 1/4	Neem Azal T/S	-	-	-	4.33±2.50d
	Oncol EC 200	-	41.88±4.93b	73.13±4.25a	86.25±5.54ab
	Sevin 85 WP	-	7.78±4.84d	15.60±3.23cd	31.28±8.25c
	Sevin XLR Plus	-	2.78±2.78d	20.28±5.57c	41.03±5.52c
	Marshal 25 EC	-	61.88±5.14a	75.20±6.29a	80.13±3.71ab
	Deltanate 400 EC	-	54.60±6.36ab	71.25±3.37a	76.25±5.66ab
	Karate 5 EC	-	-	15.83±6.55cd	37.20±9.23c
	Mesurol WP	-	25.20±8.54c	53.38±6.57b	68.93±5.92b
	Control	-	-	-	2.5±2.5d

Rd: Recommended doses

Myzocallis coryli, *Archips rosanus* with the exception of except *Curculio nucum* without any adverse effect on bees. This findings did not confirm the results of Naumann and Isman (1996), in which they explored that Azadirachtin could lead to metamorposis disturbances and to the death of bee larvae.

CONCLUSION

This study evaluated acute toxicity of 7 licensed insecticides which are being used in Turkish hazelnut orchards for a long time and one azadirachtin preparations on bees in laboratory condition. Under the light of the research findings, Marshall, Oncol, Deltanate, Mesurol, Karate, Sevin XLR and Sevin WP 85 had the harmful effect on bee while Azadirachtin was environmentally friendly preparation for controlling hazelnut pest. It was clear from upper evidence that azadirachtin deserves serious consideration for inclusion into the hazelnut integrated pest management programs as a more selective approach. For this reason, in the research conducted areas, this toxic insecticides use should be avoided and Azadirachtin use should be encouraged to Hazelnut growers.

Some measures should be put into consideration to practice for reducing the toxic effect of insecticide at the time period of application. Moreover, beehives should be sending away from insecticide application area when spraying toxic insecticides. Future research should focus on the effects of Azadirachtin on other hazelnut pests in order to assess of this botanical pesticide's potential use in organic and environmentally sound hazelnut production. There is also in need of research for finding effective bio-chemicals that haven't any adverse effects on bees for hazelnut pest.

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