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Determination of Lethal Concentration of Some Insecticides to Honey Bee *Apis mellifera* (Apidae, Hymenoptera) with Laboratory Biossays

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Abstract: The study, efficacy of three insecticides including Carbaryl, Carbosulfan and Methiocarb were evaluated in laboratory bioassays and lethal concentrations were determined against honeybee adults. The highest toxicity based on LC_{50} values was observed in carbosulfan (1.3 mg AI L^{-1}). The LC_{50}'s (mg AI L^{-1}) for methiocarb and carbaryl in topical application method were 65.9 and 71.7, respectively.

Key words: *Apis mellifera*, honeybee, lethal concentration, carbary, carbosulfan, methiocarb

INTRODUCTION

Beekeeping is one of the important agricultural activities in Turkey where approximately 74,000 tons of honey is being produced annually. Karadeniz region is one of the high honey production regions of Turkey with an annual honey production capacity of 20,247 ton, comprising 27.4% of the country's total production (Turkstat, 2007). Meanwhile, in this region of Turkey, there is a great production of hazelnut, which also is considered one of the highly valuable incomes for the country. Turkey produces 75.2% (570,000 ton) of total World hazelnut production from 640,000 ha of orchards. Blacsea region ranked first in terms of hazelnut production area with 459,800 ha (Anonymous, 2007; FAO, 2007).

The most important cause of yield decrease in hazelnut production is the hazelnut pests. If required pest managements and control tactics are not applied yield decreases can reach up to 30%. Consecutively, producers in this region apply chemicals twice a year in order to control of these pests. The number of insecticide applications can, sometimes, be up to 4 times a year (Tuncer and Ecevit, 1997; Tuncer *et al*., 2001). Among some of common chemicals used in these hazelnut orchards are Carbaryl, Carbusulfan and Methiocarb.

Because, both beekeeping and hazelnut production are very important agricultural activities in the region, we tried to determine the toxicities of these chemicals for honeybees.

MATERIALS AND METHODS

The experiment was designed as an (3×4×3) factorial treatment arrangement in a randomized complete block design with four replications. Factors and the levels for each factors consisted of 3 chemicals: Sevin 85 WP (Carbaryl), Marshal 25 EC (Carbosulfan) and Mesurol WP 50 (Methiocarb) and 3 counting duration: 3, 24 and 48 h after each application (Table 1).

Laboratory conditions were maintained at 25±2°C, 70±5% R.H and 14:10 h light: dark. Worker honeybees, which were younger than 20 days old, were used in the study.

Bioassays of adult bees were conducted by topical application of insecticide diluted in acetone (0.5 μL) to the ventral abdomen of each bee. Following the treatment, the bees were held in disposable plastic boxes (10×20×7 cm) having filter paper on bottom. Polyethylene sheets containing small holes were used together with rubber to cover open side of boxes. Ten adult honeybees were put into each box for each treatment. Test concentrations were prepared with acetone. In control boxes only distilled water was used.

The mortality was counted 3, 24 and 48 h after each application. Experimental mortality was corrected for control mortality (<10%), in which bees were treated with acetone only.

The mortality data was corrected by Abbott's Formula (Abbott, 1925). Statistical analysis was performed by SAS (1998). Analysis of Variance (ANOVA) was performed to compare the means of tested insecticides. Once, it was determined that differences existed among the means, pair wise multiple comparisons were made using both Duncan (1995) Multiple Range Test and the LSD test. The * indicate significant differences at $p<0.01$. Additionally, all data were analyzed and LC_{10}'s, LC50's and LC90's were generated with R Program (Ritz and Streibig, 2005).

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Table 1: Insecticides used in the experiments

Compounds	Trade name	A	Concentrations used in bioassay (mg ai L^{-1})
Carbaryl 85%	Sevin 85 WP	150 g	250,200,150,100,75,50,25,10,5
Carbosulfan 250 g L^{-1}	Marshall 25 EC	125 mL	4,3,2,1,0.5
Methiocarb 50%	Mesurol WP 50	100 g	100,75,50,25,10,7.5, 5, 2.5,1

A: Recommended application rate (g da^{-1}-mL da^{-1})

Table 2: Mortality rate (%) of the bees associated with time after application and comparison of recommended application rates of bioassayed insecticides with laboratory efficiency results

			Times after application (h)				
Chemicals	Values	Doses	3	24	48	A	B
Carbaryl	-	Control	0.0±0.0a	0.0±0.0a	9.3±6.4a	1275	6.6
	-	5	5.0±2.9ab	5.0±2.9ab	10.0±0.0ab	-	-
	-	10	13.8±2.4bc	15.0±2.9bc	20.0±4.1bc	-	-
	-	25	17.0±2.4c	24.5±3.2cd	24.5±3.2cd	-	-
	-	50	33.3±1.9d	34.5±2.6d	34.5±2.6d	-	-
	-	75	55.0±2.0e	55.0±2.0e	55.0±2.0e	-	-
	-	100	70.0±4.1f	80.0±0.0f	80.0±0.0f	-	-
	-	150	85.0±3.2g	91.3±3.5g	91.3±3.5g	-	-
	-	200	100.0±0.0h	100.0±0.0g	100.0±0.0g	-	-
$LSD_{\alpha = 0.01}$	10.164	-	-	-	-	-	-
$LD_{10(Min-Max)}$	26.8 (17.2-36.3)	-	-	-	-	-	-
$LD_{50(Min-Max)}$	71.7 (60.9-82.4)	-	-	-	-	-	-
$LD_{90(Min-Max)}$	191.8(102.7-281.0)	-	-	-	-	-	-
Carbosulfan	-	Control	0.0±0.0a	0.0±0.0a	6.8±4.2a	312.5	60.1
	-	0,5	20.8±4.7b	20.8±4.7b	23.3±3.3b	-	-
	-	1	37.5±0.9c	44.3±2.9c	46.3±3.9c	-	-
	-	2	54.5±5.3d	57.0±6.6d	62.0±3.4d	-	-
	-	3	80.5±1.8e	80.5±1.8e	87.3±5.5e	-	-
	-	4	100.0±0.0f	100.0±0.0f	100.0±0.0f	-	-
$LSD_{\alpha = 0.01}$	12.529	-	-	-	-	-	-
$LD_{10(Min-Max)}$	0.3 (0.1-0.5)	-	-	-	-	-	-
$LD_{50(Min-Max)}$	1.3 (0.8-1.9)	-	-	-	-	-	-
$LD_{90(Min-Max)}$	5.2 (1.2-9.1)	-	-	-	-	-	-
Methiocarb	-	Control	0.0±0.0a	5.0±2.9a	17.5±2.5a	500	5.0
	-	1	2.5±2.5ab	10.0±5.8ab	17.5±10.3a	-	-
	-	2,5	10.0±0.0bcd	15.0±2.9ab	20.0±4.1a	-	-
	-	7,5	9.25±3.7cd	16.25±4.4ab	25.5±5.8a	-	-
	-	10	14.5±3.2cd	14.5±3.2ab	28.5±0.9a	-	-
	-	50	18.8±0.8d	18.8±0.8ab	25.3±3.7a	-	-
	-	75	52.3±3.4e	65.3±2.1c	72.3±3.7b	-	-
	-	100	90.0±4.1f	92.5±2.5d	92.5±2.5c	-	-
$LSD_{\alpha = 0.01}$	14.212	-	-	-	-	-	-
$LD_{10(Min-Max)}$	58.1 (45.1-71.1)	-	-	-	-	-	-
$LD_{50(Min-Max}$	65.9 (58.1-73.7)	-	-	-	-	-	-
$LD_{90(Min-Max)}$	99.2 (61.9-136-4)	-	-	-	-	-	-

A: Recommended application rate (mg AI L^{-1} water), B: Recommended dose/(LC_{90}). Valuse are represented as mean±SD

RESULTS AND DISCUSSION

Three compounds (Carbary, Carbosulfan and Methiocarb) were evaluated in toxicity tests. These compounds are effective against honeybee adults under laboratory condition. Toxicity of these compounds to honeybee adult stage is presented in Table 2. It was observed that mortality rate of honeybee adults increased depending on doses. Honeybee adults were most sensitive to carbosulfan and least sensitive carbary. The LC_{50} values ranged from 1,3 mg AI L^{-1} for carbosulfan, to 71.7 for carbaryl. According to lethal concentration values, carbosulfan was most toxic to honeybee adults with the value of 1.3 mg AI L^{-1}. Methiocarb was the second most effective with 65.9 mh AI/L LC_{50} value. They were followed by carbaryl with 71.7 mg AI L^{-1} of LC_{50}'s.

When, the recommended application rate of preparations and LC_{90} values calculated from laboratory bioassays are compared to each other (recommended application rate/LC_{90} value), the highest ratio (60.1-fold) was obtained from carbosulfan and it was followed by carbaryl with 6.6-fold.

Methiocarb had value of 5.0-fold. This study evaluated lethal concentration of 3 chemicals which are being used in Turkish hazelnut orchards for a long time on bees under laboratory condidations. Under the light of the research findings, Carbosulfan, carbaryl and methiocarb had the harmful effect on bees.

CONCLUSION

Beekeeping is common in most hazelnut orchards in Turkey. Turkish farmers have traditionally used insecticides, such as carbaryl, carbosulfan and methiocarb against hazelnut pests. But, there are few results from field and laboratory studies regarding efficiency of these chemicals to bees. Our findings confirmed the results of 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; Akca *et al*., 2009), which suggested that these chemicals had the toxicity effect to bees.

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