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ORAL TOXICITY AND REPELLENT EFFECT OF SOME INSECTICIDES AGAINST HONEYBEE WORKERS

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ABSTRACT

These experiments were carried out to evaluate toxic and repellent impact of 6 insecticides i.e. Jojoba oil, Dimilin, Protecto, Biovar, Biorinza and Dursban against honeybee workers at Zagazig center, Sharkia Governorate on blooming Egyptian clover fields during the two successive seasons of 2008/2009. Results showed that all the tested compounds comparing to Dursban are safe to forging honeybee workers when fed orally with serial of concentrations started with field rate, 1/2 field rate till 1/4 field rate after 24, 48 and 72 hours of application. Dursban recorded 100% mortality after 48 and 72 hours with field rate while rest of tested compounds did not exceed 17.5% mortality with Jojoba oil after 72 hours at field rate. In case of repellent effect, Dursban recorded the highest repellent impact in initial, after one and two days for both studied seasons, Jojoba extract recorded highest repellent effects against honeybee workers in initial time in both seasons recorded 47.36 and 39.70%, respectively.

Keywords*:* Honeybees, Apis mellifera, Bioinsecticides, Toxicity, Repellent.

INTRODUCTION

The wide and misuse of the insecticidal or chemical compounds resulted in great damage caused to beneficial insects, including honeybees and beekeeping allover the world. (Abbas *et al*., 1996). The exposure of honeybees to pesticides is an ever-changing problem for bee and beekeeper because each year new pesticides as well as new formulations of the established ones appear in the marketplaces been devastating to honeybees. Therefore, the poisoning of bees by insecticides is a major problem affecting the efficiency of a bee, not only in the production of honey and other products, but also in crop pollination (Yousif-Khalil *et al*., 1987; Yousif-Khalil and Shalaby, 1992).

Honeybee pollination increases the productivity of field and horticultural crops without displacing other necessary farm commodities and without environmental pollution. Honeybee improve the pollination especially cotton flowers which are adapted to bee visitation. Moreover, cross-pollination can prevent the expression of less desirable recessive genes, promote plant vigor and improve fruit and seed set.However, the insecticidal application especially to flowering plants hindered the role of honeybees as insect pollinators. Such problems drove the scientists' attention to find out another safer and less expensive agents to control insect pests, the successful use of plant extracts, bioinsecticides and insect growth regulators (IGRs) may be also considered as new approaches in pest control.

The present work aims to throw some light on the possibility of exposing honeybee workers to some chemical agents i. e. bioinsecticides, plant extracts and IGRs and therefore, the side effects of using such toxicants against honeybees.

MATERIALS AND METHODS

Tested compounds:

1- **Jojoba oil**: (plant oil formulated as E. C) produced by Egyptian natural oil Co. used at the rate of 500ml/feddan.

2- **Dimilin**®: (diflubenzuron).

A commercial water dispersible powder containing 25% diflubenzuron, clorophenyl)-3-(2.6-diflubenzoyl) urea was available for testing. The suspension at the rate of. 0.5kg formulation/Fadden. Produced by Dow Agro science.

3**. Bioinsecticides**:

Protecto®: ***Bacillus thuringiensis*** var. ***Kurstaki*** 32.000 IU/mg). The recommended rate is 300gm / 100L water.

Biovar®: an atomopathegenic fungi 32000 viable spore/mg) containing the fungus ***Beauveria bassiana*** applied at rate of 200g/100 L water.

Biorinza®: ***Metarhizium anisopliae*** sore Biorinza 10% W.P (32 x 10^6 spores/ml) Rate 200 g / 100 L water. All Biocides produced by Insect Pathogen Unite Plant Protection Research Institute, Agricultural Research Center Dokki, Giza, Egypt.

4. Dursban®:

(Chlorpyrifos) 48% EC. 0.0 – diethyl 0- (3.5.6 – tri chloro -2- pyrinyl. phosfothioate. Used the rate of 1 L/feddan. Produced by Dow Agro science.

Tested honeybee workers:

Honeybee workers needed for laboratory tests were collected from the peripheral combs of the colony. To avoid the genetic variations as possible, tasted workers were collected from one colony headed by open mated Carniolan queen from the Educational Apiary of Plant Protection Research Institute (Sharkia Branch).

2. Effect of the tested compounds on honeybee workers:

2.1. Oral toxicity:

The oral toxicity of the tested compounds against honeybee workers was evaluated by feeding on 1:1 (W: V) sugar syrup, polluted with the tested compounds according to the methods of Szepanaski, and Gromiszowa (1979).

The control workers were fed on unpolluted sugar syrup. Three concentrations i.e. the (f.r), 1/2(f.r) and 1/4 (f.r) were tested for each compound. Three replicates of 40 workers each were used. After application, bees exposed to the same concentration were placed together in small feeding cage of 9x12x20Cm and fed on unpolluted syrup under room conditions (26±3ºC) and (65±5% R.H).

Dead bees were counted after 24, 48 and72 hours. Mortality counts were corrected according to Abbott's Formula (1925).

2.2. Repellent activity:

The repellent effect of the tested compounds on forager workers visiting treated fields was studied according to Yousif-Khalil (1987) during flowering period of Egyptian clover (*Triflalium alexandrinum*) in 2008 and 2009 seasons at Zagazig center, Sharkia Governorate. The experimental field was selected at a distance of 500m. from a private fixed apiary located northern the field. The field was divided into plots of 15x4m each. Four plots were considered for each compound, as well as control plot. An area of 15x4m. was left untreated between each two treatments as belt. The tested compounds were applied at the recommended rates at 8 a.m, the bee foragers visiting one m^2 were counted on the plots treated with the tested toxicants as well as on control plots by counter's square. Counting was performed once after two hours from spraying at the first day (initial repellency). Thereafter, the counts were made 3 times daily in the next two days, i.e. at 12 noon, 2 p.m and 4 p.m (Yousif–Khalil 1987 and Yousif-Khalil and Shalaby, 1992). Percent of repellency was calculated and recorded using the following equation:

$$\text{Repellency\%} = \frac{\text{No. of bees/m}^2 \text{ in untreated plots} - \text{No. of bees / m}^2 \text{ treated}}{\text{No. of bees / m}^2 \text{ in untreated plots}} \times 100$$

RESULTS AND DESUSSION

1. Studies of tested compounds on honeybee under laboratory conditions:

Oral toxicity:

The toxicity of the tested compounds by ingestion to honey bee workers was investigated. The tested materials were offered in (1:1) sugar syrup in four successive concentrations i.e. the recommended field rate (f.r) as well as (1/2 f.r) and (1/4 f.r), Control workers were offered (1:1) sugar only. The corrected mortality percentages after 24, 48, and 72 hours were calculated and are presented in Table (1) obtained results could be explained as follow:

In tested bioinsecticides; Biovar, Biorinza and Protecto obtained results indicated that the tested bioinsecticides recorded very slight toxicity to exposed honeybee workers orally. For instance, the mortality percentages in bees fed on sugar syrup polluted with the recommended field rate of Biovar, Biorinza, Protect and Jojoba oil ranged between 2.5-10%, 0.0 – 7.5, 2.5-15% and 5.0-17.5 after 24, 48 and 72hr of bees exposure to Biovar, Biorinza, Protecto and Jojoba oil, respectively. The corresponding percent of mortality in honey bee workers fed on syrup polluted with 1/2 and 1/4 recommended field rate recorded 0.0% for the tow concentration of Biovar, Biorinza, Protecto and Jojoba oil after 24hr. Generally, it could be concluded all that the tested compounds are safe to forging honey bee workers. Similar results were also reported by Vandenberg (1990) who mentioned that Protecto and *Beauveria bassiana* are considered safe for honey bees. Also Alves *et al*.,

(1990) found that *Metarhizium anisopliae* and *Beauveria bassiana* caused 9.5 and 26.2% mortality when sprayed on to bees. Dimetry *et al.,* (2005) found that Neem-Azal have insignificant adverse effect of the worker bees compared with to control colonies and also Neetu-Singh and Karantak (2005) found that *B.t.* safety to honey bee, *Apis ceranss*. Brighenti *et al.* (2007) found that *B.t.* K. was safe to honey bee and also Ellis and Hayes (2009) found that *Bacillus thuringiensis* Berliner, harmful to bees.

Also data of Dimilin indicate that percent of mortality of honeybee workers demonstrated orally at the rate of field application induced mortality percentage of 5.00%, 7.5% and 12.5% for Dimilin after 24, 48 and 72hr, respectively.

On the other hand, Dursban caused sharp mortality percentage of honey bee workers that demonstrated orally at the rate of field application induced percent of mortalities 95.0%, 100% and 100% after 24, 48 and 72 hour, respectively.

Table (1): Toxicity date of the tested insecticides applied orally to F1 Carniolan honeybee workers under laboratory conditions.

Compound	Recomm-ended rate/fed.	1 f.r*		1/2 f.r		1/4 f.r	
		No.	Mor.%	No.	Mor.%	No.	Mor.%
After 24 hour							
Biovar	2 gm/L	39.0	2.5	40.0	0.0	40.0	0.0
Biorinza	2 gm/L	40.0	0.0	40.0	0.0	40.0	0.0
Protecto	3 gm/L	39.0	2.5	40.0	0.0	40.0	0.0
Jojoba	2.5 gm/L	38.0	5.0	40.0	0.0	40.0	0.0
Dimilin	2.5 gm/L	38.0	5.0	39.0	2.5	40.0	0.0
Dursban	1m/L	2.0	95.0	7.0	82.5	10.0	75.0
Control	-	40.0	-	40.0	-	40.0	-
After 48 hour							
Biovar	2 gm/L	37.0	7.5	40.0	0.0	40.0	0.0
Biorinza	2 gm/L	38.0	5.0	40.0	0.0	40.0	0.0
Protecto	3 gm/L	37.0	7.5	39.0	2.5	40.0	0.0
Jojoba	2.5 gm/L	35.0	12.5	37.0	7.5	40.0	0.0
Dimilin	2.5 gm/L	37.0	7.5	38.0	5.0	40.0	0.0
Dursban	1m/L	0.0	100.0	3.0	92.5	7.0	82.5
Control		40.0	-	40.0	-	40.0	-
After 72 hour							
Biovar	2 gm/L	36.0	10.0	38.0	5.0	40.0	0.0
Biorinza	2 gm/L	37.0	7.5	39.0	2.5	40.0	0.0
Protecto	3 gm/L	34.0	15.0	37.0	7.5	39.0	2.5
Jojoba	2.5 gm/L	33.0	17.5	36.0	10.0	38.0	5.0
Dimilin	2.5 gm/L	35.0	12.5	37.0	7.5	39.0	2.5
Dursban	1m/L	0.0	100.0	0.0	100.0	0.0	100.0
Control	-	40.0	-	40.0	-	40.0	-

***f.r= Recommend rate**

The respective percent mortalities at 1/2 the recommended field rate recorded 82.5%, 92.5% and 100% after 24, 48 and 72 hour doses of 1/4 the recommended field rate recorded 75%, 82.5 and 100% after 24, 48 and 72 hour. Similar results were also reported by Salman and Abd-El-Raof (1983) who mentioned the Dursban was more toxic to honey bees than to

Spodoptera littoralis and also Kalita and Kahman (1995) found that chlorpyrifos was the most toxic to *Apis mellifera* followed by oxydemethon methyl, neem oil in laboratory studies and also Neetu-Singh and Karantak (2005) recorded that no insecticide was safe to honey bee. Generally Dursban is very toxic to honey bees.

2. Repellent activity:

These experiments were carried out at Zagazig center, Sharkia Governorate on blooming Egyptian clover fields during the two successive seasons of 2008/2009.

Initial repellency:

Jojoba and Bio insecticide data presented in Tables (2&3) indicate that the initial repellency against honey bee foragers visiting treated blooming Egyptian clover fields recorded 47.4%, 36.84%, 38.16% and 30.26% during 2008; 39.7%, 31.75, 33.3% and 28.57% during 2009, respectively. Biorinza caused the least repellency in both. However, Jojoba oil extract was the most potent in the first and second seasons.

On the other hand, Dursban induced the highest initial repellency in both seasons, recording 82.9% and 74.61%, respectively while Dimilin caused the least effect, recording 36.8% and 36.5% repellency in the two seasons, respectively.

Residual repellency:

Obtained results indicate that the percentage of forager bees repellency on the first day after spray with Jojoba extract, Protecto, Biovar and Biorinza were 22.4%, 29.27%, 33.7% and 23.9% in the first seasons and 23.86%, 21.59%, 22.73% and 48.75% in the second one, respectively.

Repellency percentages on the second day were 20.28%, 23.78%, 25.175% and 16.1% in the first season; 22.13%, 18.85%, 18.00% and 14.75% in the second one, respectively.

Biovar induced the highest significant repellency on the first and second day in the first season while in the second season Jojoba oil induced the highest significant repellency on the second day in the first and second season on the other hand; the differences the four compounds extract were mostly significant in the first season.

As shown in Tables (2&3) the percentages of forager bees repellency on the first day spray with Dimilin and Dursban were 31.59% and 59.1 in the first season; 33.17% and 71.5% in the second one, respectively. Repellency percentages on the second day were 23.37 and 49.1% in the first season; 25.175% and 61.54% in the second one for Dimilin and Dursban, respectively. Dursban induced the highest significant repellency on the first and second day in both season However, Dimilin induced the least significant repellency. This phenomenon could be attributed to weather factors that were higher in the season especially in the first day.

Nauman *et al.,* (1994) who tested the repellency of neem seed extract to *Apis mellifera* using a feeding dish choice bioassay and under field condition on blooming canola (rape). Silva *et al*., (2003) found that plant extracts (Ocimum, Ofalsa, Melissa, Oronye and Eucalyputs leaves) did not in crease honey bees frequency on quintal and ferontana**.**

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Kanga *et al.*, (2003) found that *Metarhizium anisopliae* was harmless to the honey bees (adult bees) on contrary Carreck *et al*., (2007) found that honey bees effectively transported the an etomopathegenic *Metarhizium anisopliae* to the flower is causing infection and mortality to adults. Ellis and Hayes (2009) found that *B.t*. is not harmful to Bees.

Kandasamy (1987) found that Dimilin has low toxicity to honey bees and predators and is therefore useful in integrated pest management programmer. Mansour *et al.,* (1984) assessed repellent activity of some insecticides including O. P. that demonstrated high initial repellent and medium residual effect. Al-Ansary and El-Zogby (1992) recorded residual toxicity of Chlorpyrifos until 6 days. Kanga *et al*., (2003) found that chlorpyrifos demonstrated residual toxicity until the 6^{th} day after treatment It is concluded that the insecticides are highly toxicity to *Apis mellifera*. Seema *et al*., (2007) found that chlorpyrifos repelled honey bees through contact action at 0.2% concentration.

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السمية المعدية و الفعل الطارد لبعض المبيدات الحشرية على شغالات نحل العسل

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أجريت تجارب هذه الدراسة لتقدير الفعل السام و الطارد لستة مبيدات حشرية هى: زيت الجوجوبا، ديميلين، برتكتو، بيوفار، بيورنزا و دورسبان على شغالات نحل العسل فى حقول البرسيم المزهرة بالزقازيق بمحافظة الشرقية فى موسمى ٢٠٠٨و ٢٠٠٩ . أظهرت النتائج ان كل المركبات المختبرة مقارنة بالدورسبان آمنة للشغالات السارحة بعدما غذيت بسلسلة من التركيزات بدءاً بالتركيز الحقلى و حتى ربع التركيز الحقلى بعد ٢٤ ، ٤٨ و ٧٢ ساعة من المعاملة و قد سجل مركب دورسبان ١٠٠٪ موت بعد ٤٨ و ٧٢ ساعة بينما باقى المركبات لم تتعد نسبة الإبادة ١٧.٥٪ لمركب زيت الجوجوبا بعد ٧٢ ساعة من المعاملة. بالنسبة للفعل الطارد سجل مركب دورسبان أعلى نسبة طرد فى الأثر الفورى و بعد يوم و يومين من المعاملة فى كلا الموسمين، بالنسبة للمركبات الأخرى سجل مركب زيت الجوجوبا أعلى نسب طرد فورى لشغالات نحل العسل فى كلا الموسمين مسجلا ٤٧.٣٦ و ٣٠.٧٠٪ على الترتيب.

قام بتحكيم البحث

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Table (2): Repellent effects of the tested insecticides against honeybee workers (expressed as the average number of forager's m^2) at 2 hours intervals on sprayed clover fields during flowering period of 2008.

Days of application		Initial		1 day					2 days				
Treatment		No.	% R.	12 noon	2 p.m	4 p.m	Total	% R.	12 noon	2 p.m	4 p.m	Total	% R.
Control		19	0	1.8.5	17.25	15.75	51.25	0	9.25	11.5	15	35.75	0
Plant extract (Jojoba)		10	**47.36%**	14.75	15	10	39.75	**22.440**	7.5	10	11	28.5	**20.28%**
Bioinsecticides	Protecto	12	**36.84%**	12.25	12	12	36.25	**29.276**	8	9	10.25	27.25	**23.78%**
	Biovar	11.75	**38.1%**	10.75	12.25	11	34	**33.7%**	7.25	9.25	10.25	26.75	**25.17%**
	Biorinza	13.25	**30.26%**	12.25	14.5	12.25	39	**23.9%**	9.75	10.5	9.75	30	**16.15%**
IGR	Dimilin	12	**36.840**	11.75	11.75	10.75	34.25	**33.17**	7	8.5	11.25	26.75	**25.17**
O.P	Dursban	3.25	**82.89%**	6.75	3.25	4.25	14.25	**71.50**	4.75	4.25	4.75	13.75	**61.54%**
Mean ºC	-	32	-	-	-	33	-	-	-	-	34	-	-
R. H%	-	46%	-	-	-	41%	-	-	-	-	38%	-	-

Table (3): Repellent effects of the activity of the tested insecticides against honey bee workers (expressed as the average number of foragers m^2) at 2 hour intervals on sprayed clover fields during flowering period of 2009.

Days of application		Initial		1 day					2 days				
Treatment		No.	% R.	12 noon	2 p.m	4 p.m	Total	% R.	12 noon	2 p.m	4 p.m	Total	% R.
Control		15.75	**-**	18	17.25	8.75	44	**-**	12.5	10.5	7.5	30.5	**-**
Plant extract (Jojoba)		9.5	**39.70**	14.5	11.25	7.75	33.5	**23.86**	9.25	8.75	5.75	23.75	**22.13**
Bioinsecticides	Protecto	10.75	**31.75**	16.75	13.5	6.25	34.5	**21.59**	10.5	7.5	6.75	24.75	**18.85**
	Biovar	10.5	**33.30**	14.25	12	7.75	34	**22.73**	11	8.5	5.5	25	**18**
	Biorinza	11.25	**28.57**	15.75	12.25	7.75	35.75	**18.75**	10	9.25	6.75	26	**14.75**
IGR	Dimilin	10	**36.5**	13.75	13	7.75	34.5	**21.59**	8.75	8.5	6	23.25	**23.37**
O.P	Dursban	4.00	**74.61**	9	5.5	3.5	1.8	**59.1**	6.25	4.75	2.75	13.75	**49.10**
Mean °C	-	26.9	**-**	-	-	31	-	**-**	-	-	32	-	**-**
R.H%	-	53%	**-**	-	-	50%	-	**-**	-	-	53%	-	**-**