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Comparative Risks of Several Insecticides towards Honeybee Workers

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Authors' contributions

This work was carried out in collaboration between both authors. Author MMA conceived, designed the research and wrote the manuscript. Both authors conducted the experiments, analyzed the data, reviewed, read and approved the manuscript.

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ABSTRACT

The risk level of several insecticides of various chemical classes was estimated for honeybee workers, *Apis mellifera* L. (Hymenoptera: Apidae). Lethal time calculation was used to risk assessment for honeybees. Bioassay tests were conducted with six insecticides [dinotefuran (neonicotinoid), methomyl (carbamate), profenofos (organophosphate), azadirachtin (botanical-bioinsecticide), spinosad (bioinsecticide - an extract of the fermentation broth of soil actinomycete) and chlorfluazuron (IGR)] on honeybee workers by the insecticide / food mixture technique, at seven concentrations as ratios of recommended field rate [F (ug a. i. mL^{-1})], for 15 days. Results revealed that dinotefuran was significantly the most toxic to bees, which gave the shortest median lethal times (LT_{50s}), 4.4, 4.9, 5.8, 6.4 and 10.3 days at concentrations of $1F\times10^{-2}$, $5F\times10^{-3}$, $1F\times10^{-3}$, $5F\times10^{-4}$ and $1F\times10^{-4}$, respectively. Moreover, it gave 100% bee mortality after one day exposure time, at two higher concentrations, ($1F\times10^{-1}$) and ($5F\times10^{-2}$). The toxicity order of the tested insecticides for honey bees (Based on LT_{95s}) varied by the reducing in their concentrations, whereas it was: dinotefuran > methomyl > profenofos > azadirachtin > chlorfluazuron > spinosad, at the higher concentrations and this became azadirachtin > dinotefuran > profenofos > chlorfluazuron > methomyl > spinosad at the lowest concentrations. It was concluded that the

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interaction among insecticide concentration, exposure time and its chemical class plays a great role in the risk level on honeybee workers. Spinosad and chlorfluazuron were significantly less toxic in comparison to the other insecticides tested and they can be safely applied to crops.

Keywords: Dinotefuran; methomyl; profenofos; azadirachtin; chlorfluazuron; spinosad; bioassay tests; Apis mellifera; lethal time.

1. INTRODUCTION

Honey bees are significantly important to the environment, conserving biodiversity by providing essential pollination for a wide range of crops and wild plants. And for the important ecological and economic value of honey bees, there is a need to maintain healthy bee insects, not just locally or nationally, but globally. Pesticides have been targeted as a major factor, causing not only direct losses, but also reductions in honey and wax production and pollination benefits. The role played by honey bees in increasing the crop yield is 10-20 times greater than their values of honey production [1,2]. The increase in pesticides application for agriculture has exposed honey bees to a continual array of chemicals, including insecticides, fungicides, herbicides and insect growth regulators. As a result, residues of many pesticides and metabolites have been found in honey, beeswax and pollen, as well as adult and pupal bees [3,4,5,6]. A number of these compounds have also been shown to have sub-lethal effects on bees, causing delayed development, shortened adult longevity and immune system impairment [7,8]. Insecticides caused a serious threat to bees because bees are insects and, therefore, are susceptible to any poison that was designed to kill insect pests. Consequently, strict toxicity studying was and still is required before such chemicals can be registered for applying to crop protection [9,10, 11]. Neonicotinoids exhibited a significantly higher toxicity compared to all the other chemical classes [12,13,14,15,16,17,18,19,20,21]. In addition, several botanical insecticides, which are often touted as safe and environmentally friendly, might generate acute toxicity and sub-lethal effects on honey bees [22,23,24]. Many studies have well demonstrated that the time of exposure may strongly impact on mortality of honey bees exposed to sub-lethal doses [25,26,27]. Frequently, bees expose to pesticides and ingest their residues from contaminated pollen and nectar of crop plants and weeds [28]. Sub-lethal doses can also lead to mortality of 20 or 30% of honey bees [11]. Generally, sub-lethal doses create toxic effects that do not kill the honey bees but still affect their health [29,30]. The classic principle of toxicology was “the concentration makes the toxicant,” and its modern version is “the concentration and the time of exposure make the toxicant.” These two factors, concentration and time help us understand the severity effects that pesticides may have on honey bees and their risk [11]. The purpose of this study is to compare the risk levels of various insecticides which belong different chemical classes, dinotefuran (neonicotinoid), methomyl (carbamate), profenofos (organophosphate), azadirachtin (botanical-bioinsecticide), spinosad (bioinsecticide - an extract of the fermentation broth of soil actinomycete) and chlorfluazuron (IGR) on honeybee workers.

2. MATERIALS AND METHODS

2.1 Insecticides

Dinotefuran: (RS)-1-methyl-2-nitro-3-(tetrahydro -3-furylmethyl) guanidine.

Methomyl: methyl (1E)-N-(methylcarbamoyloxy) ethanimidothioate.

Profenofos: O-4-bromo-2-chlorophenyl O-ethyl S-propyl phosphorothioate.

Azadirachtin: dimethyl (3S,3aR,4S,5S,5aR,5a1 R,7aS,8R,10S,10aS)-8- acetoxy- 3,3a,4,5, 5a,5a1,7a,8,9,10-decahydro-3,5- dihydroxy-4-{(1S,3S,7S,8R,9S,11R)-7-hydroxy-9-methyl-2,4,10-trioxatetracyclo [6.3.1.03,7.09,11] dodec-5-en-11- yl}- 4- methyl-10[(E)-2-methylbut-2-enoyloxy]-1H,7Hnaphtho[1,8a,8-bc:4,4a-c’]difuran-3,7a- dicarboxylate.

Spinosad: (a mixture of 50-95% of spinosyn A and 50-5% spinosyn D) Spinosyn A: (2R, 3aS, 5aR, 5bS, 9S, 13S, 14R, 16aS, 16bR) - 2- (6-deoxy-2,3,4-tri-O-methyl- α- Lmannop-yranosyloxy) – 13 - (4- dimethylamino - 2, 3, 4, 6-tetradeoxy- β-Derythrop-yranosyloxy)-9-ethyl-2, 3, 3a, 5a, 5b, 6, 7, 9, 10, 11, 12, 13, 14, 15, 16a, 16bhexadecahydro- 14- methyl -1H-8 oxacyclododeca [b] as-indacene-7,15-dione. Spinosyn D: (2S, 3aR, 5aS, 5bS, 9S, 13S, 14R, 16aS, 16bR) - 2- (6-deoxy-2, 3, 4- tri- O- methyl-α-Lmannop-yranosyloxy) -13- (4-dimethylamino -

2, 3, 4, 6- tetradeoxy- β-Derythrop-yranosyloxy)-9-ethyl-2, 3, 3a, 5a, 5b, 6, 7, 9, 10, 11, 12, 13, 14, 15, 16a, 16b hexadecahydro-4,14-dimethyl-1H-8-oxacyclododeca [b] as-indacene-7,15-dione.

Chlorfluazuron: 1-[3,5-dichloro-4-(3-chloro-5-trifluoromethyl-2-pyridyloxy) phenyl] -3-(2, 6-difluorobenzoyl) urea.

The trade name, formulation, producing company, insecticide class and recommended field rate of the tested insecticides were presented in Table 1.

2.2 Honeybee Workers

Apis mellifera L. workers of one day age were obtained from hives maintained in an apiary at the experimental farm of the Faculty of Agriculture, Benha University, Egypt. They were then placed in the laboratory refrigerator at 4^{o}C for approximately 10 min to slow bee movement. Then they were transferred to wooden three-hole Benton cages, with 5 bees per cage.

2.3 Bioassay

Bees were deprived of food for 4 h prior to insecticide exposure. Bee workers were fed on candy [(4 powdered sugar: 1 honey) - 40 g /cage] which contained 1 mL of insecticide water solution (stock solutions) to give the required concentration level (in case of control treatment only 1 mL of water was added). The experimental cages of each insecticide were divided into 7 concentrations, 1F×10^{-1}, 5F×10^{-2}, 1F×10^{-2}, 5F×10^{-3}, 1F×10^{-3}, 5F×10^{-4} and 1F×10^{-4}, where F was the recommended field rate of the applied insecticides. Each concentration and an untreated control consisted of five repetitions. Bee cages were held in an incubator (24 h darkness; 32±2ºC; 70% RH) [31]. Mortality was recorded after 1, 2, 3, 5, 7, 10 and 15 days of the experiment.

2.4 Statistical Analysis

A probit computer program was used to determine the lethal times for the insecticides [32,33]. A significant difference between LT_{50} values (the time required for 50% of the insects to die following exposure to a level concentration of the test insecticide) was based on overlap of 95% confidence intervals [34].

3. RESULTS AND DISCUSSION

The results of Table 2 show, at the highest concentration tested (1F×10^{-1}), dinotefuran (neonicotinoid) and methomyl (carbamate) were the most toxic to honeybee workers that gave 100% mortality after one day exposure time, followed by profenofos (organophosphate) which had the least lethal times, LT_{15}, LT_{50} and LT_{95} of 1.0, 1.9 and 4.9 days, respectively, whereas spinosad (bioinsecticide) gave the longest lethal time (LT_{95}), 23.7 days. Lethal times (LT_{95s}) of both azadirachtin (botanical-bioinsecticide) and chlorfluazuron (IGR) were longer than profenofos and at same time, shorter than spinosad. At concentration of (5F×10^{-2}), dinotefuran is still the most toxic and gave 100% of bee mortality after one day exposure time. Methomyl, profenofos

Table 1. The trade name, formulation, producing company, insecticide class and recommended field rate of the tested insecticides

Insecticide	Producing company	Insecticide class	Recommended field rate (F) ug (a.i) mL^{-1}
Dinotefuran (Oshin 20% SG)	Sumitomo Chemical Co., Japan.	Neonicotinoid	250.0
Methomyl (Lannate 90% WSP)	E. I. du Pont de Nemours, USA.	Carbamate	1350.0
Profenofos (Selecron 72% EC)	Syngenta chemical Co. AG, Switzerland.	Organophosphate	1353.6
Azadirachtin (Achook 0.15% EC)	Bahar Agrochem. and Foods Pvt. Ltd., India.	Bioinsecticide (botanical)	562.5×10^{-2}
Spinosad (Tracer 24% SC)	Dow AgroSciences Co., India.	Bioinsecticide (an extract of the fermentation broth of soil actinomycete bacterium, *Saccharopolyspora spinosa*)	60.0
Chlorfluazuron (Topron 5% EC)	Agrochem. Co., Egypt.	Benzoyl phenyl urea (IGR)	100.0

Table 2. The lethal times of tested insecticides to the honeybee workers at 7 concentrations as ratios of the field recommendation rate

Insecticide	Concentration		Lethal times and their 95% confidence limits (Days)		
	Field recommendation rate (F)	ug (a.i.) mL^{-1}	LT_{15}	LT_{50}	LT_{95}
Dinotefuran	1F×10^{-1}	25.0	NC$_1$	NC$_1$	NC$_1$
Methomyl		135.0	NC$_1$	NC$_1$	NC$_1$
Profenofos		1353.6×10^{-1}	1.0(0.9-1.2)^{a}	1.9(1.7-2.1)^{a}	4.9(4.3-5.8)^{a}
Azadirachtin		562.5×10^{-3}	2.3(1.9-2.6)^{b}	3.7(3.4-4.1)^{b}	8.2(7.4-9.6)^{b}
Spinosad		6.0	2.0(1.8-3.0)^{b}	5.2(3.6-7.6)^{bc}	23.7(13.6-37.4)^{c}
Chlorfluazuron		10.0	4.7(4.2-5.0)^{c}	6.3(6.0-6.6)^{c}	10.2(9.4-11.5)^{b}
Dinotefuran	5F×10^{-2}	12.5	NC$_1$	NC$_1$	NC$_1$
Methomyl		67.5	1.2(0.1-2.3)^{a}	2.5(0.6-3.5)^{a}	7.3(6.2-11.2)^{a}
Profenofos		676.8×10^{-1}	2.3(1.9-2.7)^{ab}	3.8(3.5-4.1)^{a}	8.2(7.2-10.0)^{a}
Azadirachtin		2812.5×10^{-4}	4.2(3.7-4.6)^{c}	5.6(5.3-5.9)^{b}	8.8(7.9-10.5)^{a}
Spinosad		3.0	3.0(2.5-3.6)^{b}	10.1(6.1-14.7)^{c}	67.0(53.4-75.9)^{c}
Chlorfluazuron		5.0	4.7(4.0-5.1)^{c}	7.1(6.7-7.6)^{c}	14.0(12.2-17.6)^{b}
Dinotefuran	1F×10^{-2}	2.5	3.2(2.4-3.8)^{a}	4.4(3.8-4.8)^{a}	7.2(6.6-8.4)^{a}
Methomyl		13.5	3.8(2.4-4.9)^{abc}	7.5(6.3-8.3)^{b}	21.4(17.2-32.2)^{c}
Profenofos		1353.6×10^{-2}	4.2(3.4-4.9)^{abc}	7.6(7.1-8.1)^{b}	12.2(11.2-13.9)^{b}
Azadirachtin		562.5×10^{-5}	4.5(3.9-4.8)^{bc}	5.9(5.6-6.2)^{c}	9.3(8.4-11.3)^{ab}
Spinosad		0.6	3.5(1.9-4.6)^{ab}	15.3(11.0-25.0)^{d}	160.6(116.5-239.4)^{d}
Chlorfluazuron		1.0	5.6(4.7-6.3)^{c}	10(9.0-11.8)^{d}	24.8(18.3-44.0)^{c}
Dinotefuran	5F×10^{-3}	12.5×10^{-1}	3.4(2.5-4.0)^{a}	4.9(4.3-5.2)^{a}	8.4(7.5-10.6)^{a}
Methomyl		67.5×10^{-1}	4.8(3.9-5.6)^{abc}	10.5(9.5-11.9)^{d}	36.2(27.0-58.6)^{d}
Profenofos		676.8×10^{-2}	4.8(4.2-5.4)^{b}	8.0(7.1-8.9)^{c}	17.3(15.3-20.5)^{bc}
Azadirachtin		2812.5×10^{-5}	4.8(4.2-5.1)^{b}	6.5(6.1-6.9)^{b}	10.0(9.3-12.9)^{a}
Spinosad		0.3	4.6(2.5-6.0)^{bc}	19.6(14.5-39.6)^{e}	193.9(173.0-243.9)^{e}
Chlorfluazuron		0.5	6.3(5.6-6.8)^{c}	9.8(9.0-11.0)^{d}	19.7(16.0-28.3)^{cd}
Dinotefuran	1F×10^{-3}	2.5×10^{-1}	4.1(3.5-4.5)^{a}	5.8(5.4-6.3)^{a}	10.4(9.3-12.1)^{a}
Methomyl		13.5×10^{-1}	5.5(3.9-6.7)^{ab}	14.3(12.2-18.3)^{c}	64.8(39.9-88.5)^{c}
Profenofos		1353.6×10^{-3}	5.4(4.6-6.0)^{bc}	8.4(7.8-9.1)^{b}	22.5(18.9-28.7)^{b}
Azadirachtin		562.5×10^{-5}	5.1(4.7-5.5)^{b}	6.7(6.3-7.2)^{a}	10.4(9.5-11.7)^{a}
Spinosad		0.6×10^{-1}	NC$_2$	NC$_2$	NC$_2$
Chlorfluazuron		0.1	7.0(6.0-7.8)^{c}	13.3(11.1-20.0)^{c}	37.4(23.5-62.0)^{c}
Dinotefuran	5F×10^{-4}	12.5×10^{-2}	4.3(3.8-4.8)^{a}	6.4(5.9-6.9)^{a}	11.7(10.6-13.5)^{a}
Methomyl		67.5×10^{-2}	5.8(3.8-7.1)^{abc}	17.4(14.1-26.5)^{d}	101.4(82.0-128.5)^{c}
Profenofos		676.8×10^{-3}	5.7(4.9-6.2)^{b}	9.2(8.5-10.0)^{b}	25.4(21.1-32.6)^{b}
Azadirachtin		2812.5×10^{-6}	7.1(6.7-7.4)^{c}	8.4(8.1-8.7)^{b}	10.9(10.4-11.8)^{a}
Spinosad		0.3×10^{-1}	NC$_2$	NC$_2$	NC$_2$
Chlorfluazuron		0.5×10^{-1}	7.7(7.1-8.3)^{c}	11.6(10.4-13.8)^{c}	21.8(17.1-34.7)^{b}
Dinotefuran	1F×10^{-4}	2.5×10^{-2}	4.7(3.8-5.3)^{a}	10.3(8.2-17.5)^{ab}	36.4(20.2-57.0)^{b}
Methomyl		13.5×10^{-2}	6.2(4.4-7.9)^{ab}	32.6(20.7-57.8)^{c}	255.7(210.6-313.0)^{c}
Profenofos		1353.6×10^{-4}	5.9(4.0-7.4)^{ab}	16.1(13.2-22.4)^{bc}	78.1(44.6-92.6)^{b}
Azadirachtin		562.5×10^{-6}	7.1(6.7-7.4)^{b}	8.6(8.3-8.9)^{a}	11.6(10.9-12.7)^{a}
Spinosad		0.6×10^{-2}	NC$_2$	NC$_2$	NC$_2$
Chlorfluazuron		0.1×10^{-1}	10.1(8.8-13.5)^{c}	17.9(13.4-45.1)^{bc}	44.4(24.4-66.6)^{b}

NC$_1$: Not calculated where the mortality of honey bees was 100% at tested days
NC$_2$: Not calculated where the mortality of honey bees was zero at tested days
Different lowercase letters within each column of each concentration indicate significant differences ($p < 0.05$)

and azadirachtin gave the least lethal times (LT_{95s}), 7.3, 8.2 and 8.8 days, respectively. Also, spinosad gave the longest lethal time (LT_{95}), 67.0 days. Although, the lethal time (LT_{95}) of chlorfluazuron was longer than methomyl, profenofos and azadirachtin, it was shorter than spinosad. In general data confirmed that dinotefuran gave the significant shortest lethal times (LT_{50s}), 4.4, 4.9, 5.8 and 6.4 days at the concentrations 1F×10^{-2}, 5F×10^{-3}, 1F×10^{-3} and 5F×10^{-4}, respectively. At the lowest concentration of (1F×10^{-4}), lethal time (LT_{95}) of azadirachtin became significantly the shortest (11.6 days) in comparison to the other insecticides tested. However, toxicity of methomyl and profenofos decreased by the decrease in the concentrations to the lowest one (1F×10^{-4}). Their lethal times (LT_{95s}) increased to 255.7 and 78.1 days in comparison to dinotefuran and azadirachtin insecticides, respectively. On the other hand, the three lowest concentrations of spinosad, $1F\times10^{-3}$, $5F\times10^{-4}$ and $1F\times10^{-4}$, gave no bee mortality, when bees were fed for 15 days on each of them. Moreover, when spinosad was tested at the higher remained concentrations, it gave the significant longest lethal times of LT_{50s} and LT_{95s}.

In general, research results revealed that all tested insecticides had moderate or high risks to honeybee workers. The toxicity order of the tested insecticides significantly varied as follows, dinotefuran > methomyl > profenofos > azadirachtin > chlorfluazuron > spinosad. A risk of neonicotinoids on bees is not only because of their high toxicity but also due to their specific mode of action, result in killing the honey bees if they are exposed to the pesticide residues for a long time, they are more toxic and persist than the majority of organophosphorus, carbamates and pyrethroids [16,18,19,20,21]. The sub-lethal concentrations caused the mortality of honey bees (*A. mellifera* L.). This well explained that the time of exposure may strongly the mortality effect [25,26]. This fact gives evidence of the hazard caused by neonicoitoids to honey bees, since very small concentrations may involve a significant impact on mortality. Previous studies showed the toxicity of the insecticides to honeybee workers of *A. mellifera* was higher with the increase in the exposure period of the insecticides, through the contaminated diets. For example, bee ingested sub-lethal concentrations of imidacloprid for 10 days or 40 days, might cause a high mortality, ranging from 50 to 100%. Moreover, several neonicotinoids show very strong toxicity to bee insects [7,12,13,14,15,17]. On the other hand, the results indicated the toxicity of azadirachtin (the botanical insecticide) to *A. mellifera* may have a strong mortality effects. It can be achieved with an increase in the exposure time. The present results indicated that by decreasing the concentration of the tested insecticides to the lowest concentration, their toxicity order significantly changed as follows, azadirachtin > dinotefuran > profenofos > chlorfluazuron > methomyl > spinosad. Regarding the effect of azadirachtin, negative effects of neem on adult honey bees were observed [35]. They also reported that this insecticide decreased the amount of larvae in colonies. As well as a significant increase in the mortality of adult workers of *A. mellifera* with an increase exposure time of the bees to different concentrations of neem oil was reported [27]. It was also observed that the botanical insecticides had the potential acute toxicity and sub-lethal impacts on honey bees and, herewith, it gives evidence of the importance of evaluating the risks of the side effects of biopesticides. The effects of botanical pesticides were noted, which had been formerly described as "safe" to honey bees [24]. These insecticides led to toxicity to honeybee workers of *A. mellifera*, which indicates that their use should be avoided through the flowering period in crops when the plants are visited by bees. On the other hand, it was found that the exposure of honeybee workers to spinosad treated foliage under laboratory conditions did not result in the increase mortality, indicating that the intrinsic toxicity of spinosad was observed in acute tests of toxicity. It was not seen under conditions of more realistic exposure [36]. Consequently, to protect *A. mellifera* population, it is needed to minimize the bee exposure to highly toxic insecticides, which can be realized through the application of insecticides using the basics of ecological selectivity [37]. The insecticide should be used when the honey bees have lower foraging rates for the crops (i.e., late afternoon) [38]. Another practice that can reduce the effect of the insecticides is the closure of the hive opening and the use of artificial feeding on days when pesticides are applied to prevent the contact of bees with the toxicants [23].

4. CONCLUSION

Under the light of the research findings, the insecticide toxicity to bees was great varied by the interaction among a time exposure, a concentration and an insecticide chemical class. Dinotefuran (neonicotinoid) followed by azadirachtin (botanical-bioinsecticide), profenofos (organophosphate) and methomyl (carbamate) had the harmful effect on bee while spinosad was comparatively less toxic followed by chlorfluazuron (IGR) and can be applied to crops.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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