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Acute Toxicity of Selected Insecticides and Their Safety to Honey Bee (*Apis mellifera L.*) Workers Under Laboratory Conditions

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Abstract

Objectives: The honey bee, *Apis mellifera L.*, is widely used for the production of honey, wax, pollen, propolis, royal jelly and venom and crop pollination. Since honey bees can be exposed to insecticides in sprayed flowering crops, therefore, this study aimed to assess the acute toxicity and safety index of five commonly used insecticides to honey bee workers in laboratory.

Methods: Bees were exposed to the insecticides: Imidacloprid, Thiamethoxam, Esfenvalerate, Indoxacarb and Chlorantraniliprole by two methods of exposure: topical application and feeding techniques. LD_{50} and LC_{50} values for each insecticide to honey bees were determined after 24 and 48 h from treatment.

Results: The LD_{50} values in µg per bee were 0.0018 (indoxacarb), 0.019 (esfenvalerate), 0.024 (thiamethoxam), 0.029 (imidacloprid) and 107.12 (chlorantraniliprole). The LC_{50} values (mg L^{-1}), for each insecticide, were as follows: indoxacarb, 0.091; esfenvalerate, 0.014; thiamethoxam, 0.009; imidacloprid, 0.003 and chlorantraniliprole, 0.026, after 24 h from exposure. In general, the neonicotinoid insecticides were the most toxic to bees by feeding technique, and indoxacarb, esfenvalerate were the most toxic by contact method while chlorantraniliprole had slightly or non- toxic effect by the two methods. Thus, all of the tested insecticides are harmful to the honey bees except chlorantraniliprole.

Keywords: Insecticides; *Apis mellifera L.*; Acute toxicity; Safety index; LD_{50}; LC_{50}

Introduction

The importance of bees lies not only in the production of honey, wax, pollen, propolis, royal jelly and venom, but also in the role they have in the pollination of entomophilous crops. The honey bee is credited with approximately 85% of the pollinating activity that ultimately enhances the production and productivity of the crop [1]. Unfortunately, honeybee populations are in decline since the 1990s, possibly due to a combination of pests, diseases, poor diet, colony collapse disorder and the increasing use of different pesticides [2,3]. Pesticides are often considered an easy, quick and inexpensive solution for managing weeds and insect pests in agriculture and in urban landscapes. Pesticide contamination poses considerable risks to the surroundings and non-target organisms [4].

Neonicotinoid insecticides are successfully applied to control pests in a variety of agricultural crops; however, they may not only affect pest insects but also non-target organisms such as pollinators [5]. They are neurotoxicants and therefore have been of particular concern for sub lethal effects in honeybees. This class of insecticides was considered a major milestone for integrated pest- and resistance-management programs. The neonicotinoid insecticides include imidacloprid, acetamiprid, clothianidin, thiamethoxam, thiacloprid, dinotefuran, nithiazine, and nitenpyram, which are marketed under a variety of trade names [6].

Pyrethroids act on the nervous system as a primary target organ and exert their neurotoxic effects primarily by altering the conductance of sodium channel, leading to hyperexcitation [7]. Honey bees often thought to be extremely susceptible to insecticides in general, exhibit considerable variation in tolerance to pyrethroid insecticides [8].

Indoxacarb belongs to the oxadiazine chemical family and is being registered for the control of lepidopterous pests in the larval stages. Insecticidal activity occurs via blockage of the sodium channels in the insect nervous system and the mode of entry is via the stomach and contact routes [9].

Chlorantraniliprole (Coragen®) is a new anthranilicdiamide insecticide, efficacious for the control of lepidopteran pests and some species of Coleoptera, Diptera and Hemiptera that attack fruit and vegetables in both open field and glasshouse situations. While the compound has been shown to be highly effective against pests, it has also been shown to be highly selective for beneficial parasitoid, predator and pollinator species, a trait that has been reported on a number of occasions [10,11]. Therefore, this study aimed to assess the acute toxicity of five commonly insecticides, used in control of agricultural pests, to the honey bee workers under the laboratory conditions.

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Table 1: Acute contact toxicity of 5 pesticides against honey bee workers after 24 and 48 h from treatment under laboratory conditions.

Insecticide	After 24 h from treatment			After 48 h from treatment		
	LD_{50}[a] (µg /bee) (95% CL)	Toxicity Index	Slope ± SE	LD_{50}[a](µg /bee) (95% CL)	Toxicity Index	Slope ± SE
Indoxacarb	0.0018 (0.0025 - 0.0013)	100	1.38 ± 0.145	0.0012 (0.0015 - 0.0008)	100	1.45 ± 0.163
Esfenvalerate	0. 019 (0.023 - 0.016)	9.47	1.54 ± 0.144	0.015 (0.017 - 0.012)	8	1.14 ± 0.15
Thiamethoxam	0.024 (0.031- 0.019)	7.5	1.35 ± 0.143	0.020 (0.022 - 0.017)	6	1.56 ± 0.165
Imidacloprid	0.029 (0.033 - 0.024)	6.2	1.68 ± 0.147	0.026 (0.028 - 0.023)	4.6	1.69 ± 0.168
Chlorantraniliprole	107.12 (111.23 - 103.44)	0.00102	0.39 ± 0.068	95.65 (98.25 - 88.75)	0.00125	0.45 ± 0.069

Materials and Methods

Honey bee

Honey bee foraging workers were collected between 9.00 AM and 12.00 noon from healthy hives maintained in the apiary of El- Sabahia Research Station, Agriculture Research Center, Alexandria, Egypt. The bees shaken from the frames into wooden cage (with two sides screen wire and a hole in the top of the cage) and then transported to the laboratory of Eco-toxicology in the department of plant protection, Faculty of Agriculture, Damanhour University, Egypt.

Insecticides

Imidacloprid (Best 25% WP), Thiamethoxam (Actara 25% WG), Esfenvalerate (Fenirate-S 5% EC), Indoxacarb (Avaunt 15% EC) and Chlorantraniliprole (Coragen (20% SC) purchased from Agrochem. Co., Egypt, was used in this study.

Toxicity bioassays

For Topical application method: To evaluate direct contact toxicity, five concentrations of formulated insecticides (a.i.) were prepared using acetone as solvent to obtain mortality in the range of 20-80 %. These concentrations were (0.1, 0.05, 0.025, 0.012, 0.0062) for imidacloprid; (0.05, 0.025, 0.0125, 0.005, 0.0025) for thiamethoxam; (0.5, 0.25, 0.12, 0.06, 0.03) for esfenvalerate, (2.5, 1.25, 0.62, 0.31, 0.15) for chlorantraniliprole and (0.75, 0.37, 0.18, 0.09, 0.04) for indoxacarb. One treatment with acetone only served as the untreated control. There were three replicates with 20 bees each. The honey bee workers were anaesthetized with carbon dioxide (CO_2). An Arnold hand micro applicator apparatus H-66 Arnold, [12] was used to apply the determined dosage of each diluted insecticide on the ventral mesothorax of honey bee workers. They treated with two microliter-drop of insecticide concentration. Control honey bee received 2µl of acetone only. Treated bees were kept after application in the mentioned cages and fed with honey [13].

For feeding treatment: To evaluate oral toxicity, five concentrations of formulated insecticides (a.i.) were diluted with water, to obtain the appropriate five concentrations: ($1.8x10^{-3}$, $9x10^{-4}$, $4.5x10^{-4}$, $2.2x10^{-4}$, $1.1x10^{-4}$) for imidacloprid; ($5x10^{-4}$, $2x10^{-4}$, $1 x10^{-4}$, $5x10^{-5}$, $2x10^{-5}$) for thiamethoxam, ($3x10^{-3}$, $1x10^{-3}$, $7.5x10^{-4}$, $3x10^{-4}$, $1x10^{-4}$) for esfenvalerate, ($1x10^{-4}$, $5x10^{-5}$, $2.5x10^{-5}$, $1x10^{-5}$, $5x10^{-6}$) for chlorantraniliprole and ($6x10^{-4}$, $3x10^{-4}$, $1x10^{-4}$, $7x10^{-5}$, $3x10^{-5}$) for indoxacarb. One treatment with water only served as control. There were three replicates with 20 bees each. The concentrations were dissolved in sucrose solution 20% (W/V). Bees were deprived of food for 2 h before treatment. One part of concentrated insecticide dilution was mixed with 19 parts of sugar solution 20% (W/V). The resulted dilution was put in glass tubes (20 cm^3) in the top side of cages for feeding of bee workers. A mixture of one part of water and 19 parts of sugar solution 20% (W/V) was used for check reason [14]. After 24 h number of dead bees in each cage was recorded in both contact and oral treatments.

The safety index of different insecticides was calculated by the formula of Hameed:

$S.I. = LC_{50}/NRC$

Where S.I. is the safety index, LC_{50} is the median lethal concentration of insecticide (%) and NRC the normal recommended concentration of each formulated insecticide.

Results and Discussion

Laboratory experiments were carried out to determine the LD_{50} values, after 24 and 48 h from treatment of honey bee workers with five insecticides and their confidence limits as presented in Table 1. Based on LD_{50} values, after 24 and 48 h from treatment, indoxacarb was the most toxic compound followed by esfenvalerate, thiamethoxam, imidacloprid and chlorantraniliprole, respectively. For bees as non-target insects, the US EPA (2018) classified pesticides based on LD_{50} values into three categories as non-toxic (>11µg/bee), moderately toxic (2-10.9µg/bee) and highly toxic, (<2µg/bee).Thus, all of the tested insecticides are considered highly toxic to honey bees except chlorantraniliprole .Therefore, indoxacarb, esfenvalerate, thiamethoxam, imidaclopridare classified as highly toxic to honey bees, where chlorantraniliprole was considred non- toxic (non-harmful) to bees. Based on toxicity index (T.I.) for tested insecticides, indoxacarb was the most toxic insecticide followed by esfenvalerate, thiamethoxam, imidacloprid and chlorantraniliprole, respectively.

Results in Table 2 show the LC_{50} values of tested insecticides and safety indices for *Apismellifira* L. According to the LC_{50} and T.I. values, the tested insecticides can be arranged in ascending order from the most to the least toxic as follows: imidacloprid, thiamethoxam, esfenvalerate, chlorantraniliprole and indoxacarb, respectively.

The safety index of each insecticide was calculated on the basis of its recommended spray concentration against its LC_{50} value Table 2. The safety index values were as follows: 0.0016, 0.0180, 0.0093, 0.0217 and 0.2390, for imidacloprid, thiamethoxam, esfenvalerate, chlorantraniliprole and indoxacarb, respectively. Thus, according to the safety index, imidacloprid, esfenvalerate and thiamethoxam are the least safe insecticides to honey bees, while chlorantraniliprole and indoxacxb are the safest insecticides to honey bees.

Table 2: Acute feeding toxicity of 5 pesticides against honey bee workers after 24 and 48 h from treatment under laboratory conditions.

Pesticide	After 24 h from treatment			After 48 h from treatment		
	LC_{50}[a] (mg/L) (95% CL)	Toxicity Index	Safety index	LC_{50}[a](mg/L) (95% CL)	Toxicity Index	Safety Index
Imidacloprid	0.003 (0.008 - 0.001)	100.0	0.0016	0.0006 (0.0009 - 0.0004)	100.0	0.0003
Thiamethoxam	0.009 (0.012 - 0.004)	33.33	0.0180	0.0009 (0.001 - 0.0005)	66.66	0.0018
Esfenvalerate	0.014 (0.018 - 0.012)	21.42	0.0093	0.011 (0.09 - 0.013)	5.45	0.0071
Chlorantraniliprole	0.026 (0.029 - 0.023)	11.53	0.0217	0.022 (0.025 - 0.017)	2.72	0.018
Indoxacarb	0.091 (0.096 - 0.087)	3.40	0.2390	0.086 (0.084 - 0.089)	0.88	0.229

[a]Lethal dose (or concentration) causing 50% mortality after 24 and 48 h with 95% confidence limits.
[b]Slope value ± standard error of the mean for the dose - (-concentration) mortality regression line.

The present finding about indoxacarb is in agreement with the report of EPA (2000) which reported that indoxacarb and its R-enantiomer is "practically non-toxic" by dietary intake and "highly toxic" by contact for honey bee. Previous work by [15] reported that indoxacarb was the most toxic compound by direct contact to honey bee *Apismellifera*, compared with the pyrethroidcypermethrin and the neonicotinoid imidacloprid, The toxicity of indoxacarb as observed in the present investigation is not in agreement with Zhu et al., [16] who found that the LD_{50} value was 1.80µg/bee and LC_{50} value of 1140mg/L, for formulated indoxacarb to *Apismellifera*. Also, the obtained LC_{50} value for indoxacarb is differ from that reported by Rui-xian et al., [17], which was 3.54mg/L. Steen and Dinter [18], reported that the application of indoxacarb in apple orchards caused no effects on honey bee mortality. Atkins [19] found that the toxicity of pyrethroids to honey bees ranges from relatively low (e.g., LD_{50} = 8,780ng per bee for fluvalinate, to highly toxic (e.g., LD_{50} = 83ng per bee for lambda-cyhalothrin, to extremely toxic (e.g., LD_{50} = 29ng per bee for cyfluthrin. Esfenvalerate is a type II pyrethroid used in orchards for protection against beetles and lepidopterans [20]. There are several reports for topical LD_{50} of esfenvalerate for *Apismellifera*, among them: 0.01µg/bee [21]; 0.03µg/bee [22]; 0.02µg/bee [22], all these findings are within the range of the present results. Kadala et al., [20] compared the cellular efficiency of some pyrethroids on *Apismellifera* and Bombusterrestris, to explore their differential effects on bee species. For this purpose, esfenvalerate was chosen because it is currently used in agriculture (including in the EU) so that Apis and Bombus have a similar risk to be exposed to it. It possesses a phenoxyphenyl radical just like 85% of pyrethroids authorized in Europe (this radical is involved in the molecular interaction with sodium channels) and it has a unique chlorobenzyle radical (a property it shares with no other pyrethroid, except the tau-fluvalinate molecule). In addition, it is a type II pyrethroid since it has a cyanide radical (-CN), and it induces specific type II toxicological symptoms in insects. They reported that its toxicity for bees is quite high (LD_{50} equals 0.06µg/bee), which is in the range of our results. Also, our results show that esfenvalerate is more toxic than thiamethoxam and the latter is more toxic than imidacloprid, when applied topically on the thorax, by contrast to those of Poquet et al., [24] who arranged these three insecticides according to their toxicity to honey bee as follow: thiamethoxam>imidacloprid>esfenvalerate.

Are classified as highly toxic to honey bees, where chlorantraniliprole was considered non-toxic (non-harmful) to bees. Based on toxicity index (T.I.) for tested insecticides, indoxacarb was the most toxic insecticide followed by esfenvalerate, thiamethoxam, imidacloprid and chlorantraniliprole, respectively.

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Different neonicotinoid insecticides have slightly different chemical structures, some of which are more toxic to bees. The "nitroguanidine" neonicotinoids (including imidacloprid, dinotefuran, clothianidin, and thiamethoxam) are a subgroup of neonicotinoids that contain a nitro functional group ($-NO_2$) instead of a cyano functional group (-C≡N) in their molecular structure. This slight difference in their molecular structure affects how these two subgroups of neonicotinoids bind to an insect's receptor site. The nitro-group neonicotinoids are much more toxic to bees than the cyano-group neonics, which include acetamiprid and thiacloprid [5], because the presence of this functional group (nitro group) grants to the pesticide great affinity with the nicotinic acetyicholine receptor and, therefore, its high toxicity [25].

The nitro- and cyanoguanidine groups of neonicotinoids display similar binding affinity for honey bee nAChR [26, 27]. The relative tolerance of bees toward the cyanoguanidines is likely due to rapid cytochrome $P4_{50}$ detoxification because the toxicity of both acetamiprid and thiacloprid can be increased from 2_{50}- to 1,100-fold in the presence of a $P4_{50}$ inhibitor [28].

Thiamethoxam, was highly toxic insecticide to *A. mellifera* by oral administration as by spraying [29,30]. Costa et al., [31] found that the two neonicotinoids, thiamethoxam and acetamiprid were highly toxic to honey bee, *A. mellifera*, even those did not show the same speed of mortality. In our study, the toxicity of thiamethoxam is in agreement with those of European Food Safety Authority [32], since the acute oral LD_{50} and the acute contact LD_{50} of thiamethoxam for *Apismellifera* were: 0.005µg/bee and 0.024µg /bee, respectively, and Iwasa et al., [28] who found that the LD_{50} value for thiamethoxam equals 0.03µg/bee after 24 h. On the other hand, the obtained mortality of thiamethoxam is not in agreement with those of Laurino et al., [33], who found that LD_{50} values after 24 and 48 h were: 5.200ng/bee and 3.313ng/bee respectively. Their LC_{50}values after 24 and 48 h were: 0.134ng/bee and 0.126ng/bee, respectively.

Since imidacloprid has been in use for much more time than other neonicotinoids, its toxicity on honey bees has been extensively investigated and the several available LD_{50} determinations were critically collated and discussed by Doucet-Personeni et al., [34]. Imidacloprid was highly toxic to the honeybees as well as wild bees, as reported by Singh, [35] and acts as an agonist to nicotinic Acetylcholine Receptors (nAChRs) present in high density in insect nervous tissue [36]. The oral LD_{50}s, however, showed large variability over the different studies with neonicotinoids [37,33]. A similar high toxicity of imidacloprid and thiamethoxam was also found for the bumble bee Bombusterrestris [38]. The lower toxicity of the cyano-group neonicotinoids can be attributed to their fast biotransformation [39,40,41] and the existence of different nAChR subtypes [42]. For contact exposure Iwasa et al., [28] ranked the neonicotinoid insecticides based on their 24-h LD_{50} as follows: for the nitro-group: imidacloprid (18ng bee-1) > clothianidin (22ng bee-1) > thiamethoxam (30ng bee-1) > dinotefuran (75ng bee-1) > nitenpyram (138ng bee-1); and for the cyano-group: acetamiprid (7µgbee-1) > thiacloprid (15µgbee-1). Metabolites of neonicotinoids were shown to contribute to the toxicity [26,43,44,45]. So far, most studies were conducted on metabolites of imidacloprid: those with a nitroguanidine-group (oleofin-, hydroxy-, and dihydroxy-imidacloprid) were more toxic (oral LD_{50}) compared to the urea-metabolite and 6-chloronicotinic acid [26]. Also, the metabolite of thiamethoxam, was highly toxic for bees [43]. A metabolic study by Suchail et al., [40] quantifying imidacloprid and its metabolites 5 hydroxyimidacloprid and olefin in honeybee concluded that imidacloprid was responsible for immediate neurotoxicity symptoms, whereas its metabolites must have been responsible for mortality, since it occurred post-ingestion at which time no imidacloprid was detected. The mortality data for imidacloprid obtained in this investigation are diverged from previous works as such: LC_{50} - 48 h: between 41 and 81 ng/bee, LD_{50} - 48 h: between 49-104ng/bee [26]; LC_{50}- 48 h 4-41ng/bee [26]. The obtained contact LD_{50} value for imidacloprid is similar to this obtained by Bovi et al., [46] which was 0.030µg/bee. Also our acute LD_{50} of imidaclopridis within the range reported by Iwasa et al., [28] and Suchail et al., [44] who found that theLD_{50}s of nitro-substituted compound, imidacloprid, were 0.018µg/bee and LD_{50} = 0.06µg/bee after 48 h, respectively.

Also, the present results revealed that the topical LD_{50} of imidacloprid was higher than the oral LC_{50}. This finding is in agreement with Suchailet al., [47] who reported that ingested LD_{50} values of imidacloprid about 0.005µg/bee; and after contact application, the LD_{50} values were approximately 0.024µg/bee for *A. m. mellifera*. By contrast, Bovi et al., [46] found that ingested LD_{50} (0.107µg/bee) was higher than contact LD_{50} (0.030µg/bee). This may be attributed to the action of detoxification enzymes that act when bees are exposed to pesticides orally. These detoxification enzymes are present in the digestive system, liver, or Malpighian tubules of honey bees [48]. Costa et al., [49] found that the topical LD_{50} of imidacloprid for *Meliponascutellaris* bee was 2.41ng/bee, 24 h from treatment and the oral LC_{50} value was 2.01ng .a.i./µL for 24 h.

Chlorantraniliprole consider an attractive alternative to neonicotinoids, pyrethroids, and older chemistries, especially for use on plants that may attract bees. Because it had low mammalian and avian toxicity, stability of performance across different conditions, and minimal impact on pollinators, natural enemies, earthworms, and other beneficial invertebrates [50]. Upon ingestion, chlorantraniliprole activates the insect Ryanodine Receptor (RyR) located on the sarcoplasmic reticulum of muscles, the endoplasmic reticulum of neurons or other cell types by a selective binding. [51,52] Such binding causes an uncontrolled release and depletion of internal calcium stores, leading to cessation of feeding and lethargic behavior, with muscle dysfunction and paralysis, and finally death of the insect.

The present results confirm that chlorantraniliprole was found to be safe to bees; it has very low acute bee toxicity which is in agreement with several studies such as: Larson et al., [53], who reported that no adverse effects were seen on bee colonies exposed to residues of a selective ryanodine receptor agonist, chlorantraniliprole. Also, neither bumble bees nor honey bees avoided foraging on flowering clover contaminated with residues of chlorantraniliprole. The compound appears to be non-hazardous to bumble bees even when used on lawns where flowering weeds are present. The low acute mortality caused by chlorantraniliprole was expected, because the former usually requires very high doses to achieve repellence and impair development in Hymenoptera [54]. It exhibits insecticidal activity limited to caterpillars, flies and beetles [51,55] and low toxicity against honeybees and bumblebees at the recommended field label rate [56,53].

The differential ryanodine receptor sensitivity to chlorantraniliprole in bee pollinators is the likely reason for the low acute toxicity of this insecticide to bee species [57,55]. Also, Wade et al., [58] found that the insecticide chlorantraniliprole increased larval mortality when combined with the fungicides propiconazole or iprodione, but not alone; the chlorantraniliprole-propiconazole combination was also found to be highly toxic to adult workers treated topically.

In the present study, by comparison the toxicity of imidacloprid, thiamethoxam and chlorantraniliporole, it is clear that, imidacloprid and thiamethoxam were more toxic than chlorantraniliporole to honey bee. Likewise, Ratnakar et al., [1] reported the same results when they compared with the effects of the three insecticides on honey bee *Apismellifera* using the dry film method. They found that chlorantraniliprole relatively safer while thiamethoxam and imidacloprid proved to be more toxic (harmful) to honey bee. Furthermore, previous work by Dinter et al., [59] revealed that chlorantraniliprole have low intrinsic toxicity to honey bees. They reported that chlorantraniliprole formulations provide excellent tools for Integrated Pest Management (IPM) programmes to conserve pollinating honey bees and bumble bees.

Conclusion

Laboratory results revealed that all of the tested insecticides were harmful to honey bees, *Apis mellifera L*., by using topical application and feeding techniques, except chlorantraniliprole. The range of both LD_{50} and LC_{50} values for tested insecticides suggesting that the insecticide risk to honeybees could be minimized by the choice of the insecticide with lower toxicity to bees in crop pest management as chlorantraniliprole in our case.

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