



COMPARATIVE TOXICITY AND SELECTIVITY OF THE NEONICOTINOID INSECTICIDES ACETAMIPRID AND IMIDACLOPRID AGAINST THE COTTON WHITE FLY *Bemisia tabaci*, THE COTTON LEAF WORM *Spodoptera littoralis* AND THE HONEY BEE *Apis mellifera*

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ABSTRACT

Bioassays were conducted to study comparative and selective toxicity of commercial formulations of the neonicotinoid insecticides acetamiprid (Mospilan 20% SP) and imidacloprid (Imaxi 35% SC) against *Bemisia tabaci* (Genn.) and *Spodoptera littoralis* (Boisd.) using leaf dipping technique as well as *Apis mellifera* L. by orally mixing with food media. Based on the obtained LC₅₀ values, acetamiprid was more toxic to the tested adult and nymphal stages of *B. tabaci* than imidacloprid. The adult stage was more susceptible than nymph. Acetamiprid showed higher toxicity to eggs, the 2nd and 4th instar larvae of *S. littoralis* than imidacloprid. Moreover, eggs were more susceptible to the two tested insecticides than the larval instars, and the 4th instar larvae were the least susceptible. Acetamiprid applied orally to honey bee workers at a field rate (50 µg a.i./ml) caused 3.33 % mortality with no symptoms of toxicity 24 hr. post treatment. The field rate of imidacloprid (265 µg a.i./ml) gave 90% mortality and obvious symptoms of poisoning were recorded 10 minutes after exposure. The LC₅₀ value for imidacloprid was 59.83 µg a.i./ml. The results revealed that the cyano-substituted compound (acetamiprid) was more toxic to the tested insect pests than the nitro-substituted compound (imidacloprid) and the later was highly toxic to honey bee. So, the efficiency and selectivity of neonicotinoid insecticides are related to the chemical group of the compound as well as the species and developmental stage of the insect.

Key words: Neonicotinoid insecticides, comparative toxicity, *Spodoptera littoralis*, *Bemisia tabaci*, *Apis mellifera*.

INTRODUCTION

During the last five decades intensive use of organophosphates, carbamates and synthetic pyrethroids resulted in high level of economic insect resistance. High level of resistance resulted in disturbance of the equilibrium of the environmental system beside the increase of pest control costs. This has led to search for and development of new compounds such as neonicotinoids (Kodandaram *et al.*, 2010).

The neonicotinoid insecticides, which include imidacloprid, clothianidin, thiamethoxam and

thiacloprid, are among the most important chemicals in crop protection (Elbert *et al.*, 2008) and they are widely used in seed dressings (Sur and Stork, 2003). Neonicotinoids are neurotoxins that act as agonists of insect nicotinic acetylcholine receptors and are lethal through disruption of the insect nervous system (Matsuda *et al.*, 2001). Imidacloprid was the first member of this family and was effective against many insects showing resistance to carbamates, organophosphates and pyrethroids (Cox, 2001). Acetamiprid belongs to second generation of the neonicotinoids and has a broad-spectrum insecticide effective against several groups of insects including lepidopterans,

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coleopterans, hemipterans and thysanopterans. The insecticide has an ingestion and stomach action and has systemic action (Takahashi *et al.*, 1998; Yamada *et al.*, 1999). The use of neonicotinoid insecticides has grown considerably since their introduction in 1990s (Thany, 2010)

Selective toxicity involving low hazard for mammals and high potency to pests is an essential requirement for safe and effective insecticides, a principal type of selective toxicity involves bioactivation in insects and detoxification in mammals (Casida and Quistad, 1998 and 2004). The excellent selective toxicity of the neonicotinoids is conferred in large part by differential sensitivity for insect versus mammalian nAChRs. However, this observation is based on only the parent insecticide. The selectivity profile of neonicotinoids is not shared with desnitro or descyano metabolites and derivatives which exhibit high toxicity to mice and high affinity and/or agonist potency to mammalian nAChRs equal to or greater than that of nicotine (Tomizawa and Casida, 2003).

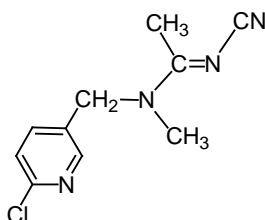
This work aimed to study comparative and selective toxicity of commercial formulations of two neonicotinoid insecticides *i.e.* acetamiprid (Mospilan 20% SP) and imidacloprid (Imaxi 35% SC) against different developmental stages of *Bemisia tabaci* and *Spodoptera littoralis* as insect pests as well as *Apis mellifera* as a beneficial insect.

MATERIALS AND METHODS

Test Insecticides

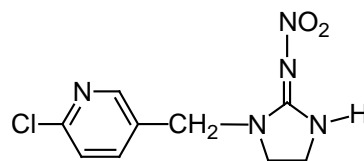
Acetamiprid (Mospilan 20% SP)

Chemical name (IUPAC): (E)-N¹-[(6-chloro-3-pyridyl) methyl]-N²-cyano-N¹-methylacetamide.



Imidacloprid (Imaxi 35% SC)

Chemical name (IUPAC): (E)-1-(6-chloro-3-pyridylmethyl) -N- nitroimidazolidin-2-ylideneamine.



Test Insects

The cotton whitefly, *Bemisia tabaci* (Genn.) (Hemiptera: Aleyrodidae)

For rearing, a stock culture of *B. tabaci* was established from infested tomato fields at the Tenth of Ramadan area, Sharkia Governorate. Tomato leaves bearing nymphs and pupae were brought to the laboratory and were placed with castor bean plants in pots in a wooden cages (60 cm height and 40 cm diameter) covered by fine mesh nylon clothes. Whitefly adults that had emerged from the tomato leaves had been maintained on the castor bean plants for oviposition. The plants were kept under controlled conditions 25±2°C and 70±5 RH for hatching of eggs and development of the nymphs without any exposure to pesticides (Mann *et al.*, 2012 with modifications).

The Egyptian cotton leafworm, *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae)

A laboratory susceptible colony of the Egyptian cotton leaf worm, *S. littoralis* was used in this study. The culture was initiated from freshly collected egg masses supplied by the Division of Cotton Pests, Branch of Plant Protection Research Institute at Zagazig, Sharkia Governorate and has been reared for 5 years without any exposure to pesticides under controlled conditions according to El-Defrawy *et al.* (1964). The egg masses were kept in glass jars covered with muslin and fastened with rubber bands under laboratory conditions of 25±2 °C and 70±5 RH till hatching. The newly hatched larvae were transferred into 2 kg capacity rearing jars where enough fresh castor bean leaves bottomed with sheets of towel paper to absorb excess humidity. Fresh castor bean leaves, *Ricinus communis* were provided to the larvae daily. The accumulated feces and debris were cleaned out daily. After pupation, pupae were collected and placed in clean jars until adult emergence. Newly emerged moths were sexed and kept in mating jars (5 males and 5 females for each jar), saturated 15% sugar solution cotton wool pieces were placed and

changed daily. Jars were supplied with fresh leaves of tafla, *Nerium oleander* as an oviposition site. Egg masses were collected daily and transferred into the rearing jars.

Honey bees, *Apis mellifera* L. (Hymenoptera: Apidae)

Honey bee workers needed for laboratory tests, were collected from the peripheral combs of the colony at the apiary of Plant Protection Research Institute, Zagazig, Sharkia Governorate.

Bioassay Methods

Bioassay methods were conducted with commercial formulations of acetamiprid (Mospilan 20% SP) and imidacloprid (Imaxi 35% SC).

Toxicity against *B. tabaci* using leaf dipping technique

Serial dilutions of each test insecticide (μg a.i./ ml) that gave 20-80% mortality for nymphs/ were freshly prepared in distilled water. Nymphal-dip bioassay protocol was carried out according to Cahill *et al.* (1996) and Nauen *et al.* (2005) with slight modifications. Adults of *B. tabaci* were collected from the rearing colony with a hand aspirator and were confined in a wooden cage (60 cm height and 40 cm diameter) covered by fine mesh nylon clothes on a castor bean plant for oviposition as mentioned before. After 24 hours, adults were removed and the plants were kept under controlled conditions 25 ± 2 °C and 70 ± 5 RH for hatching of eggs and development of the nymphs. When the fourth instar nymphs were predominant (14-16 days old), third instar nymphs were removed from infested leaves with a camel's hair brush and the fourth instar nymphs were counted and subjected to bioassay. The infested leaves were dipped for 10 seconds in the tested insecticide solution. There were 5 replicates for each concentration. Only distilled water was used for the untreated control. Mortality was recorded after 24 hours post treatment.

Adult treatment was carried out according to Hameed *et al.* (2010). Castor bean leaf discs of diameter 5 cm were cut and dipped in the test solution for 20 seconds. Leaf discs were then air dried on towel tissue papers and placed in petri-dishes. Adults (mixed sex population) were immobilized by cooling (after 2 hr. of

starvation). Thirty to forty adults were placed in petri-dish with treated leaves and covered. There were five replicates for each concentration. For the untreated control, only distilled water was used. The mortality was recorded after 24 hours.

Toxicity against *S. littoralis* using leaf dipping technique

A range of each test insecticide concentrations (μg a.i. / ml) that gave 10 – 90% mortality for eggs, 2nd or 4th instar larvae were freshly prepared in distilled water.

Egg treatment

Newly laid egg-masses of uniform age (0-1 day old) were obtained after starting oviposition in the rearing colony. The hairs covering the egg mass were carefully removed with a fine brush. The eggs in the upper layers of the egg-masses were removed gently under the binocular (using a small piece of paper). Eggs in the base layer were examined carefully under binocular microscope and any defected eggs were removed in order to count the number of eggs in the remaining lower layer, which was divided into patches, each contained about 100 eggs. The egg patches (0-1 day old) were immersed for 5 seconds in each concentration. The treated egg-masses were allowed to dry and then placed in 9 cm petri-dishes and held at 25 ± 2 °C for hatching. Glass jars provided with water-treated tafla leaves were prepared to provide 0-1 day old egg-masses used as untreated control. Three replicates were used for each concentration. Daily inspection of the untreated eggs was done for 2 days after treatment to check hatching. Once all eggs in the untreated control experiment had hatched out, the eggs in treatments were observed under binocular and the rate of hatching was recorded. Eggs normally hatched 3-5 days after oviposition. Consequently eggs that had not hatched after 8 days were counted as dead. Unhatchability percentages were recorded for each tested concentration. Unhatched percentages of eggs representing eggs mortality.

Larval treatment

Castor bean leaves were dipped for 30 seconds in each concentration then left to dry for one hour and offered to starved larvae. The 2nd

and the 4th instar larvae of the tested strain were confined with treated leaves in glass jars covered with muslin for 24 hr. Leaves of the untreated control were dipped in water. Five replicates were done for each concentration, and each replicate contained 20 larvae. Mortality percentages were recorded after 24 hr., post treatment.

Toxicity to honey bees, *A. mellifera*

Oral Toxicity of a field rate of acetamiprid (50 µg a.i./ml water) and imidacloprid (265 µg a.i. /ml water) against honey bee workers was studied. A range of imidacloprid concentrations in µg a.i./ml water that gave 10-90% mortality were used to establish toxicity regression line for the insecticide. For each tested compound at each concentration and for the controls, 30 honey bees (three cages) were used. Bees were placed in feeding cages of 9×12×20 cm under room conditions (26±3 °C) and (65±5 % RH). The oral toxicity of the tested compounds against honey bee workers was evaluated by mixing with food media on 1:1 (W:V) sugar syrup, containing the tested compound was introduced in piece of wax comb (4×4 cm.) in each cage according to the methods of Szczepanski and Gromiszowa (1979) and Khedr (2002). The bees were starved for 2 hours before exposure. The control workers were fed on sugar syrup only. Symptoms of poisoning on honey bees were recorded and the dead bees were counted after 24 hr.

Data Analysis

The mortality percentages of each compound were corrected using Abbott's formula (Abbott, 1925). The toxicity regression lines were drawn according to Finney (1972) and the LC₅₀, LC₉₀ and slope values were calculated using log-probit software program Ldp Line model "Ehabsoft" (Bakr, 2000). Toxicity index (T.I) at the LC₅₀ level was determined using Sun's equation (Sun, 1950) as follows:

$$\text{Toxicity index} = \frac{\text{LC}_{50} \text{ of the highest efficient treatment}}{\text{LC}_{50} \text{ of the other compound}} \times 100$$

RESULTS AND DISCUSSION

Toxicity of Acetamiprid and Imidacloprid Against Nymphs and Adults of *B. tabaci* Using Leaf Dipping Technique

The results of bioassays of acetamiprid and imidacloprid against the fourth nymphal instar nymphs and adults of *B. tabaci* are shown in Table 1 and Fig. 1. The obtained data reveal that acetamiprid was more toxic to the tested adult and nymph stages than imidacloprid. The LC₅₀ values of acetamiprid and imidacloprid were 30.60 and 151.29 µg a.i./ml, respectively (for nymph) and 12.26 and 73.40 µg a.i./ ml (for adult). The corresponding LC₉₀ values were 81.90 and 3271.02 µg a.i. /ml (for nymph) and 133.89 and 492.63 µg a.i. /ml (for adult). The low value of the slope of the toxicity regression line of imidacloprid as well as the high value of its LC₉₀ may due to heterogeneity of nymph population used in that experiment. Several investigators reported that acetamiprid was highly effective in controlling all stages of *B. tabaci* and was more effective than imidacloprid in reducing whitefly population (Horowitz *et al.*, 1998; Takahashi *et al.*, 1998; Naranjo and Akey, 2005).

Toxicity of Acetamiprid and Imidacloprid Against Eggs And Larvae of *S. littoralis*

Acetamiprid and imidacloprid toxicity to eggs, the 2nd and the 4th instar larvae of *S. littoralis* using leaf dipping technique are shown in Table 1 and Fig. 2. Regarding eggs, the LC₅₀ values of acetamiprid and imidacloprid were 460.20 and 553.12 µg a.i./ml, respectively. The corresponding LC₉₀ values were 2677.84 and 2027.08 µg a.i. /ml. As for the larval instar, the LC₅₀ values for acetamiprid were 990.79 and 1972.61 µg a.i. /ml (2nd and 4th instar) whereas they were 1551.07 and 2706.78 µg a.i./ml for imidacloprid, respectively. The corresponding LC₉₀ were 2470.33 and 6491.45 µg a.i./ml (acetamiprid); and 2967.08 and 13944.37 µg a.i./ml (imidacloprid). The slopes of the toxicity regression lines for eggs, the 2nd and the 4th instar larvae were 1.68, 3.23 and 2.48, respectively for acetamiprid, whereas they were 2.27, 4.55 and 1.80, respectively for imidacloprid.

Table 1. Comparative toxicity of acetamiprid and imidacloprid to *Bemisia tabaci*, *Spodoptera littoralis* and *Apis mellifera* at the LC₅₀ and LC₉₀ levels

Treatments	LC ₅₀		LC ₉₀		Slope (±SE)	Toxicity index (LC ₅₀)		
	(µg a.i./ ml)	*CL	(µg a.i./ml)	CL				
<i>B. tabaci</i>	Acetamiprid	Adults	12.26	9.23-16.66	133.89	65.83 - 606.75	1.23 ± (0.24)	100
		Nymphs	30.60	27.34-33.53	81.90	68.81 - 107.22	2.10 ± (0.36)	40.07
	Imidacloprid	Adults	73.40	57.63 - 92.07	492.63	320.60 - 997.78	1.55 ± (0.21)	16.70
		Nymphs	151.29	115.18 - 205.50	3271.02	1479.05 - 14079.96	0.96 ± (0.15)	8.10
<i>S. littoralis</i>	Acetamiprid	Eggs	460.20	389.18-542.57	2677.84	1957.85 -4166.22	1.68 ± (0.16)	2.66
		2 nd instar larvae	990.79	897.94 -1093.60	2470.33	2048.82 -2974.06	3.23± (0.33)	1.24
		4 th instar larvae	1972.61	1733.16 - 2254.55	6491.45	5173.95 - 8875.10	2.48 ± (0.23)	0.62
	Imidacloprid	Eggs	553.12	378.04 - 826.32	2027.08	1558.96 - 4950.70	2.27 ±(0.18)	2.22
		2 nd instar larvae	1551.07	1417.32 - 1681.26	2967.08	2628.10 - 3525.71	4.55 ± (0.49)	0.79
		4 th instar larvae	2706.78	2314.29 - 3175.40	13944.37	10465.22-20597.25	1.80 ± (0.16)	0.45
<i>A. mellifera</i>	Imidacloprid	Workers	59.83	49.58 - 70.09	257.30	200.87 - 368.13	2.02± (0.22)	20.49

* CL = Confidence limits

The obtained results (Table 1) show that acetamiprid was more toxic to eggs, the 2nd and the 4th instar larvae of *S. littoralis* than imidacloprid. Moreover, eggs were more susceptible than the tested larval instars. The two tested insecticides were more toxic to the 2nd larval instar as compared with the 4th instar. These results are in agreement with several previous studies on toxicity of acetamiprid and/or imidacloprid to 4th instar larvae of

insecticide-susceptible strains of *S. littoralis* and *Heliothis virescens* (Lagadic *et al.*, 1993); 7 days old larvae of *Spodoptera litura* (Ramanagouda and Srivastava, 2009); eggs of the codling moth *Cydia pomonella* (L.) and oriental fruit moth *Grapholita molesta* (Busck) (Brunner *et al.*, 2005; Magalhaes and Walgenbach, 2011); eggs, the 2nd and the 4th larval instars of diamondback moth *Plutella xylostella* (Yamada *et al.*, 1999).

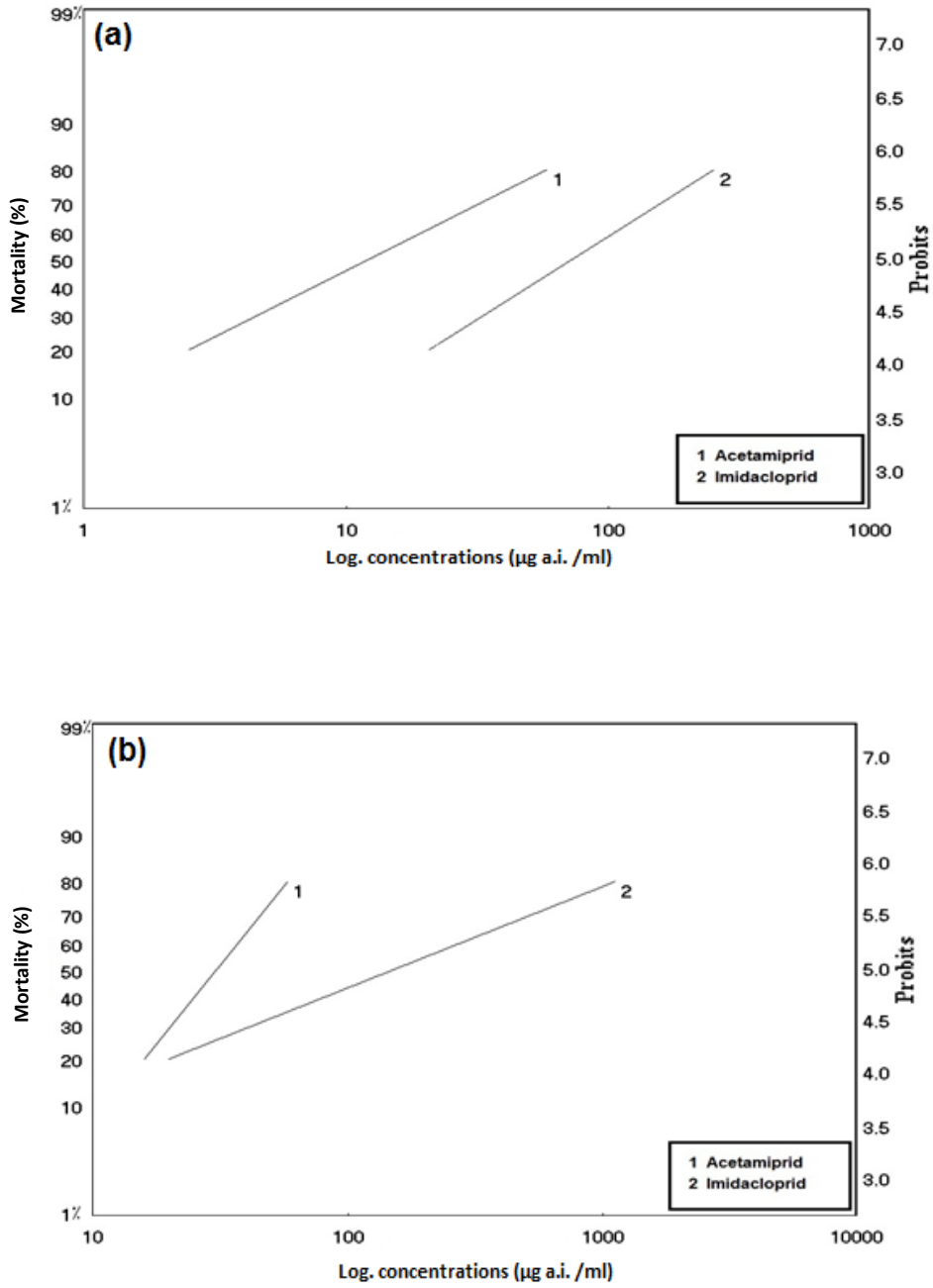


Fig. 1. Toxicity regression lines of (1) acetamiprid and (2) imidacloprid tested by leaf dipping technique against *Bemisia tabaci* : (a) adults and (b) nymphs

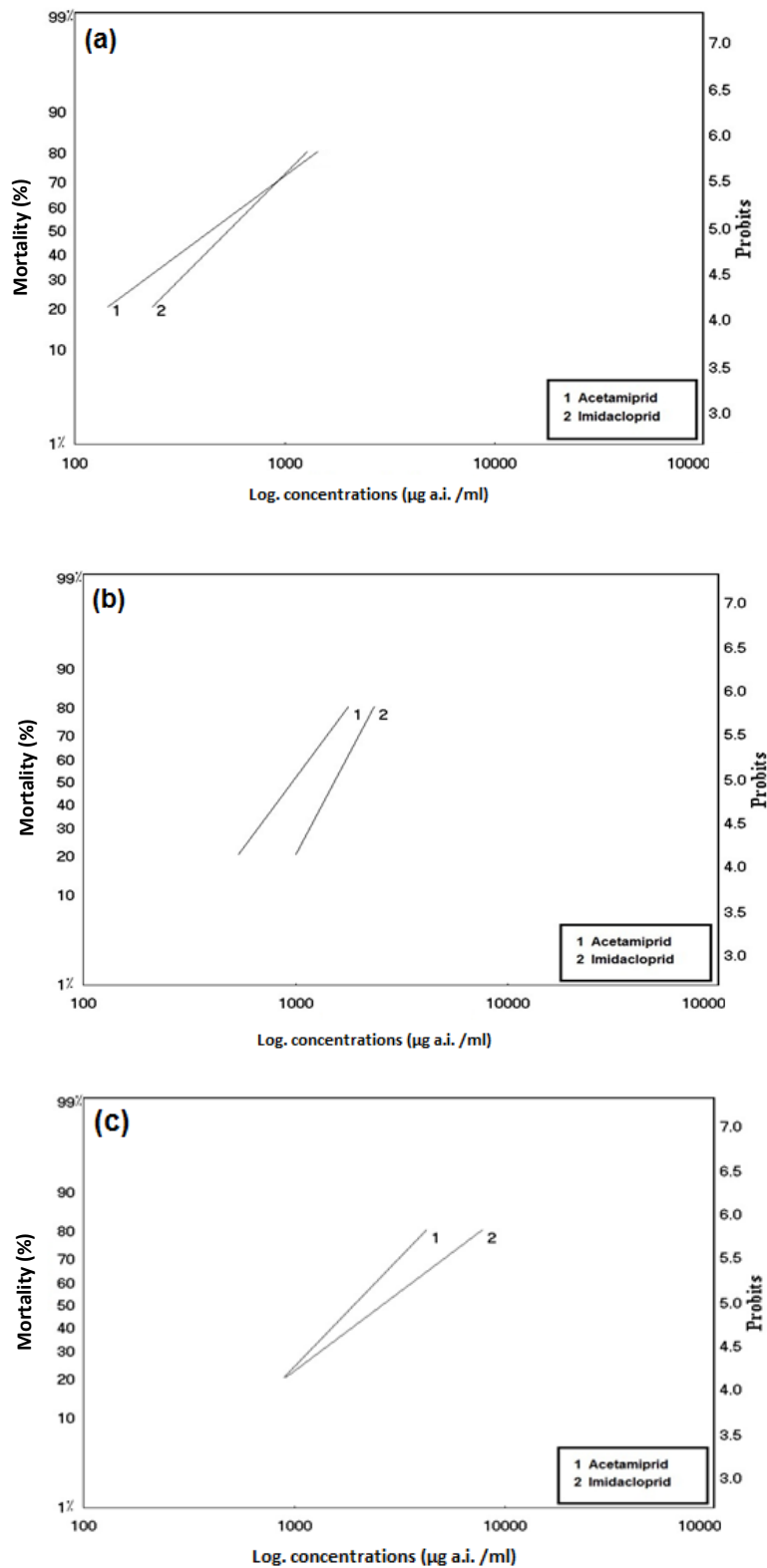


Fig. 2. Toxicity regression lines of (1) acetamiprid and (2) imidacloprid tested by leaf dipping technique against *Spodoptera littoralis*: (a) eggs; (b) the 2nd larval instar and (c) the 4th instar larvae

Toxicity of Acetamiprid and Imidacloprid to Honey Bee, *A. mellifera* L.

Oral Toxicity of a field rate of acetamiprid (50 µg a.i./ml) and imidacloprid (265 µg a.i./ml) against honey bee workers was studied. The results show that the insecticide acetamiprid caused 3.33 % mortality with no symptoms of toxicity 24 hr., post treatment, whereas imidacloprid treatment gave 90% mortality. Moreover, obvious symptoms of poisoning on honey bee, such as shaking, hyperactivity, uncontrolled movements and inability to take up a correct position of the body, consequently the inability to feed were recorded after 10 minutes from exposure.

Data obtained from the toxicity regression line for imidacloprid against honey bee indicate that the LC₅₀ and LC₉₀ values were 59.83 and 257.30 µg a.i. /ml and the slope was 2.02 (Table 1 and Fig. 3). This result is in agreement with Suchail *et al.* (2001) who found that acute intoxication by imidacloprid or its metabolites resulted in the rapid appearance of neurotoxicity symptoms, such as hyperresponsiveness and hyperactivity. Also, Iwasa *et al.* (2004) reported that the nitro-substituted neonicotinoids were the most toxic to the honey bee in laboratory studies

with LD₅₀ values of 18 ng/bee for imidacloprid and cyano-substituted neonicotinoids exhibited a much lower toxicity with LD₅₀ values of 7.1 µg/bee for acetamiprid. Yang *et al.* (2008) reported high toxicity of imidacloprid against non-target organisms like honey bees.

Conclusively, based on the chemical structure of the two tested neonicotinoid insecticides, these results reveal that the cyano-substituted compound (acetamiprid) is more toxic to the tested insect pests (*B. tabaci* and *S. littoralis*) than the nitro-substituted compound (imidacloprid). At the same time, imidacloprid is highly toxic to the non-target beneficial insect (honey bee) as compared to the very low toxicity of acetamiprid. The tested neonicotinoids are more effective against the cotton white fly *B. tabaci* as compared with the cotton leaf worm *S. littoralis*. Moreover, the adults of *B. tabaci* are more sensitive than the nymphs. *S. littoralis*, eggs are more susceptible than the tested larval instars and the 2nd larval instar is more susceptible than the 4th instar. So, the efficiency and selectivity of neonicotinoid insecticides are related to the chemical group of the compound as well as the species and developmental stage of the insect.

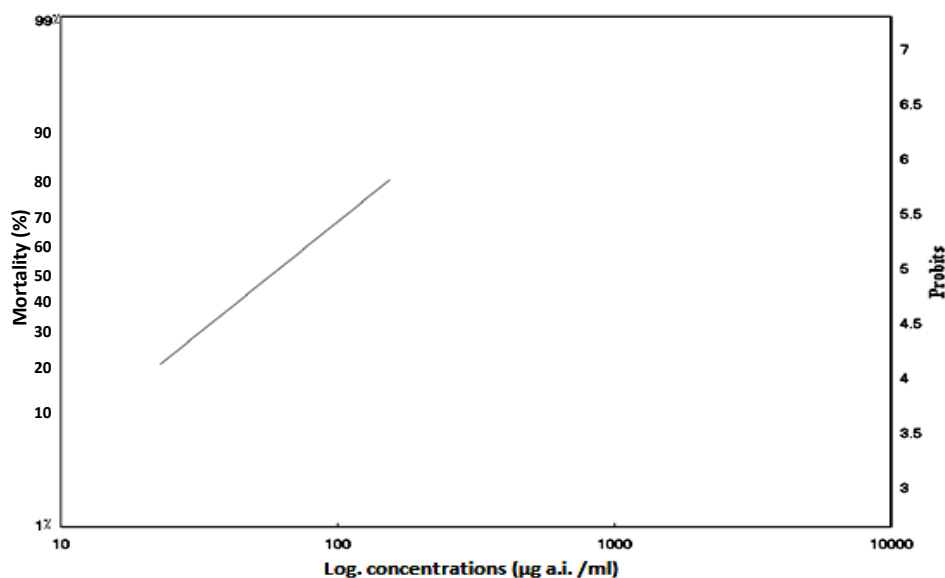


Fig. 3. Toxicity regression line of imidacloprid tested by oral administration against workers of honey bee *Apis mellifera*

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السمية المقارنة والاختيارية لمبيدات النيونيكوتينويدات الحشرية أسيتامبريد وإيميداكلوبريد ضد ذبابة القطن البيضاء، دودة ورق القطن ونحل العسل

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تم دراسة السمية الاختيارية والمقارنة لبعض المستحضرات التجارية للمبيدات الحشرية من جيل النيونيكوتينويدات متمثلة في الأسيتامبريد (موسيلان ٢٠% مسحوق قابل للذوبان) والإيميداكلوبريد (إيماكسي ٣٥% مركز معلق) ضد كل من حشرة الذبابة البيضاء ودودة ورق القطن، حيث معاملة الحشرات بطريقة غمر الأوراق بالإضافة إلى نحل العسل والذي تم معاملته بالخلط مع الغذاء، وبناء على القيم المتحصل عليها للتركيز القاتل لـ ٥٠% من الأفراد المعاملة كان مبيد الأسيتامبريد أكثر سمية للأطوار الكاملة و حوريات الذبابة البيضاء عن الإيميداكلوبريد، وكانت الأطوار الكاملة أكثر حساسية للمبيد من الحوريات، كما أظهر مبيد الأسيتامبريد سمية عالية على البيض والعمر اليرقي الثاني والرابع لدودة ورق القطن مقارنة بالإيميداكلوبريد كما كان البيض أكثر حساسية لكلا المبيدان المختبران مقارنة بالأعمار اليرقية المختبرة في حين كان العمر اليرقي الرابع أقل حساسية للمبيد، وأدت معاملة شغالات نحل العسل بالأسيتامبريد بالفم بالمعدل الحفلي (٥٠ ميكروجرام مادة فعالة/ملييلتر) إلى موت ٣.٣٣% بدون ظهور أعراض تسمم خلال ٢٤ ساعة من المعاملة، في حين أن المعدل الحفلي للإيميداكلوبريد (٢٦٥ ميكروجرام مادة فعالة/ملييلتر) تسبب في موت ٩٠% من الأفراد وصاحب ذلك أعراض تسمم خلال ١٠ دقائق من المعاملة، وكان التركيز القاتل لـ ٥٠% من شغالات النحل المعاملة ٥٩.٨٣ ميكروجرام مادة فعالة/ملييلتر، وأظهرت النتائج وجود مجموعة سيانيد في مركب الأسيتامبريد زادت من سمية المركب للأفات الحشرية المختبرة مقارنة باستبدالها بمجموعة نيترو في مركب الإيميداكلوبريد ذو السمية العالية لنحل العسل، وبناء عليه فإن كفاءة واختيارية المبيدات الحشرية التابعة للنيونيكوتينويدات تكون مرتبطة بالمجموعة الكيميائية الموجودة على المركب بالإضافة إلى نوع وطور الحشرة.

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