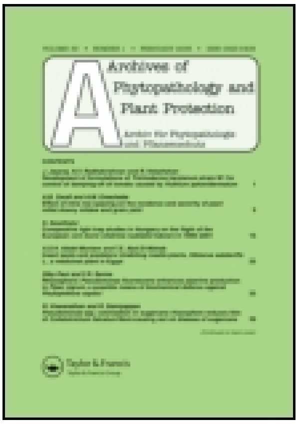

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Toxicity and persistence of selected neonicotinoid insecticides on cowpea aphid, Aphis craccivora Koch (Homoptera: Aphididae)

Aly A. Abd-Ella[a]
[a] Plant Protection Department, Faculty of Agriculture, Assiut University, 71526, Assiut, Egypt.
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Toxicity and persistence of selected neonicotinoid insecticides on cowpea aphid, *Aphis craccivora* Koch (Homoptera: Aphididae)

Aly A. Abd-Ella*

Plant Protection Department, Faculty of Agriculture, Assiut University, 71526 Assiut, Egypt

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Field strain of cowpea aphid, *Aphis craccivora* Koch, was treated by selected neonicotinoid insecticides to evaluate their toxicity and persistence against this pest in Assiut governorate, Egypt. Under faba bean field conditions, acetamiprid, imidacloprid, thiamethoxam and dinotefuran registered significantly high percent reduction of the pest at one, seven, fifteen and 21 post treatment. The residual effects of these insecticides showed that the LT_{50} for acetamiprid, imidacloprid, thiamethoxam and dinotefuran were 5.8, 6.2, 6.95 and 4.2 days, respectively. The application of these insecticides on the cowpea aphid under field conditions induced yield increases. The toxicity of these neonicotinide insecticides were tested against field strain of cowpea aphids using leaf-dib bioassay under field and laboratory conditions. The toxicity index showed that thiamethoxam, acetamiprid and imidacloprid have the highest aphicidal activity, with LC_{50s} 0.60, 0.71 and 1.16 mg/L, respectively, while dinotefuran was the least toxic one with LC_{50} 23.41 mg/L. Results of this study indicated that neonicotinoid insecticides were highly effective against cowpea aphid under field and laboratory conditions.

Keywords: pest control; residual effect; leaf-dipping method; bioassay; aphid

Introduction

The cowpea aphid, *Aphis craccivora* Koch, is one of the most serious pests of legumes, such as faba bean, cowpea and pea in Egypt (El-Ghareeb et al. 2002). It causes direct damage by feeding, which may induce plant deformation and indirect damage caused either by honeydew or by transmission of viruses (Schepers 1988). It has been reported to cause considerable loss in yield in different parts of the country (Bishara et al. 1984). Controlling aphid in crops is very important to increase the quality and quantity of the products. Chemical control is the major effective method that is used by farmers. In recent years, selective insecticides (e.g. neonicotinoids) were introduced into the market instead of traditional insecticides because insect pests became resistant to the most conventional insecticides and are increasingly replacing the organophosphates and methylcarbamates (Tomizawa et al. 2007). The favourable selectivity of the neonicotinoids occurs largely at the target level, which is the agonist binding site of the nicotinic acetylcholine receptor (Nauen et al. 2001; Tomizawa et al. 2007). Neonicotinoids, exemplified by the major imidacloprid, thiamethoxam, acetamiprid and dinotefuran (Figure 1), are the most important new class of insecticides of the past three decades. It is effective against homopteran pests, such as aphids (Elbert et al. 1991).

*Email: alyrageh@yahoo.com; aly.abdella@agr.au.edu.eg

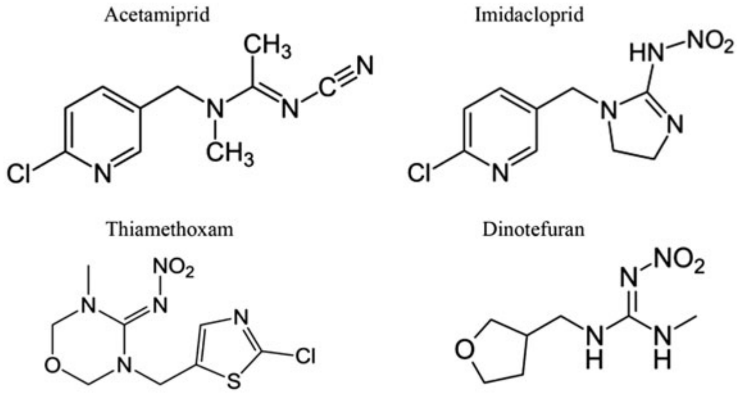

Figure 1. Structure of acetamiprid, imidacloprid, thiamethoxam and dinotefuran used against cowpea aphid, *A. craccivora*, under field and laboratory conditions.

Nauen et al. (1996) found that imidacloprid was more active than primicarb by 23 fold against *Myzus persicae* using dipping bioassay technique, but Abdu-Allah (2012) indicated that primicarb was more toxic than imidacloprid by 17.83 fold against *A. craccivora*. Jarande and Dethe (1994) added that imidacloprid was effective in reducing the incidence of aphids, whiteflies and jassids on brinjal (auberggine) and in increasing seedlings' total leaf chlorophyll over those of untreated plants. Under field and laboratory conditions, Wang et al. (1995) tested the efficacy and toxicity of the new insecticide imidacloprid on cotton aphid *Aphis gossypii*. In the field, cotton aphids were controlled with imidacloprid 37.5 gm/ha; after 5 days, control was above 95% and after 7–10 days, control still above 90%. Babu and Santharam (2001a, 2001b) evaluated the bio-efficacy of imidacloprid 200 SL (at 100, 150, 200 and 250 ml/ha) with dimethoate and methyl-o-demeton at 500 ml/ha as standards on ground nut. Imidacloprid at 100 ml/ha effectively controlled aphids and was superior to the standard chemicals. They estimated the acute toxicity of imidacloprid against *A. craccivora*. The test revealed that the median lethal concentration was 0.327 ppm. Abdu-Allah (2012) stated that imidacloprid showed less toxicity against *A. craccivora* and *Brevicoryne brassicae* (LC_{50} 2.04 mg/L and LC_{50} of 2.14 mg/L, respectively) after 48 h using leaf-dip bioassay technique.

Thiamethoxam is a new neonicotinoid insecticide belonging to thianicotinyl compounds and is the first example of the second generation of neonicotinoid insecticides. Senn et al. (1998) indicated that dose rates of thiamethoxam between 10 and 200 gm a.i./ha were sufficient for controlling target insect pests, such as aphids, rice hoppers, rice bugs, mealy bugs and some lepidopterous species, under laboratory and field trials. Mohamed and Mohamady (2010) evaluated the efficacy of thiamethoxam against field and laboratory strains of *A. craccivora*. They stated that thiamethoxam (neonicotinoid) was the most effective one, followed by diafenthiuron (thiourea compounds), carbosulfan (carbamate) and esfenvalerate (pyrethroid) in the all tested governments, except Sharkia; diafenthiuron was the least effective one (LC_{50} 232.80 ppm), while carbosulfan was the least effective one at Gharbia Governorate (LC_{50} 109.7 ppm).

Lacombe (1999) stated that acetamiprid, the new neurotoxic insecticide belonging to the chloronicotinyl family, is well adapted for orchard protection, with its rapid shock action and persistence (longer than 3 weeks), good control with acetamiprid against the main aphids present in pome fruits, stone fruits and citrus (in particular *Dysaphis plantaginea, Aphis pomi*, and *Myzus persicae*); it is regularly obtained, equal to or better than standard products. Many authors tested the toxicity of acetamiprid on different aphid species under laboratory and field conditions (Chinnabbai et al. 1999; Franco 1999; Chalam et al. 2003; Foster et al. 2003; Hohn et al. 2003; Liu et al. 2011; Shi et al. 2011). The results indicated that acetamiprid was highly effective for the control of different aphids. Dinotefuran is a recently developed neonicotinoid insecticide that is highly effective against a broad range of insect pests and has low mammalian toxicity (Mori et al. 2001; Nault et al. 2004; Wilde et al. 2004; Wang et al. 2005).The present study aimed to elucidate the effect of different neonicotinoid insecticides (acetamiprid, imidacloprid, thiamethoxam and dinotefuran) on *A. craccivora* under field and laboratory conditions.

Materials and methods

Insecticides

Four neonicotinoid insecticides were tested (trade name, formulation type and percentage of active ingredient and application ratio): acetamiprid (Mospilan 20% SP, 25 g/100 L), imidacloprid (Admir 20% SC, 50 cm^3/100 L), thiamethoxam (Actara 25% WP, 50 g/100 L) and dinotefuran (Ochin 20% SG, 50 g/100 L).

Insect field strain

Field strains of cowpea aphids, *A. craccivora*, were collected from faba bean, *Vicia faba*, fields of Assiut University Experimental Farm during 2011/2012 and 2012/2013 seasons.

Field trials, sampling methods, experimental design and pest inspection

The field tests were conducted on faba bean field at Assiut University Experimental Farm, Assiut, Egypt, during 2011/2012 and 2012/2013 seasons. The faba bean seedlings were cultivated on 15 September. Normal agricultural practices were applied. The experimental area was divided into plots, 3.5 m long and 3 m wide (1/400 feddan). Tested insecticides were distributed in a randomised complete block design and each insecticide replicated three times. The knapsack sprayer with one nozzle covering 200RCBDL per feddan (1 feddan = 0.42 hectar) was used for application. The aphid populations were counted in 10 plants for each replicate before and after treatment at periods of 1, 7, 15 and 21 days after treatment (DAT). The percentages of infestation reduction were calculated according to Henderson and Tilton's equation (1955).

$$\text{Reduction } \% = \left(1 - \frac{N \text{ in Co before treatment} \times n \text{ in } T \text{ after treatment}}{N \text{ in Co after treatment} \times n \text{ in } T \text{ before treatment}}\right) \times 100$$

where n = insect population, T = treatment and Co = control.

Residual efficacy of foliar applications of neonicotinoid insecticides

The objective of this study was to examine the residual activity of acetamiprid, imidacloprid, thimathoxam and dinotefuran against cowpea aphids, *A. craccivora*, on faba bean. Faba bean leaves were collected daily from one to 21 days after spraying each insecticide and control plots. Faba bean leaves were transferred to Petri dishes (9 cm diameter). In each tested insecticide and control, separate batches of at least 20 apterous adults, approximately of same size, were transferred to a Petri dish and placed on the treated surface of the collected leaves. Three replicates (i.e. 60 insects) were used per insecticide. Mortality was recorded after 24 and 48 h using a binocular microscope. Percentage of mortality was corrected by Abbott's formula (Abbott 1925). The median lethal exposure time (LT_{50} = time required to kill 50% tested aphids) of each insecticide was determined by a computerised probit analysis program and expressed in day.

Laboratory bioassay

The leaf dip-bioassay technique (O'Brien et al. 1992)have evaluated these insecticides for their efficacy in the field for controlling *A. craccivora* and the results are reported separately. Reported here are the results of laboratory tests to determine the concentration of these insecticides that is required to kill 50% (LC_{50}) and 90% (LC_{90}) of adults with a modification in the toxicity tests. Five to six concentrations of aqueous solution were used. Twenty apterous adults, approximately of same size, were dipped for 10 s per concentration triple time. The treated aphids were allowed to dry at room temperature for about half an hour. Control batches of aphids were similarly dipped in a solution of distilled water. After the treated batches of aphids had dried, they were individually transferred to Petri dishes (9 cm diameter) and held for 24 h at 22 + 2 °C, 60 + 5 RH% and photoperiod 12:12 (L:D). Aphid mortality was recorded 24 h after treatment with a binocular microscope. An aphid was considered dead if it was incapable of coordinated forward movement. The toxicity experiment of each compound was repeated twice and the results were corrected by Abbott's formula (Abbott 1925). Median lethal concentrations (LC_{50}) and slope values of neonicotinoid insecticides were determined by probit regression analysis program and expressed in ppm.

Statistical analysis

Data were analysed using one-way ANOVA and presented as mean ± SEM (standard error mean). Means were separated by Duncan's multiple range test (DMRT). Figures and statistical analysis were done using Graph Pad Prism 5^{TM} software (San Diego, CA).

Results

Efficacy of foliar applications of neonicotinoid insecticides on cowpea aphid

Data analyses for these experiments have been constructed in tables and figures to facilitate comparison between different neonicotinoid insecticides used against the cowpea aphid, *A. craccivora*, under field and laboratory conditions. Data represented in Table 1 and Figure 2 showed that all treatments caused a significant reduction in aphid population after one, seven, 15 and 21 DAT compared to control. Acetamiprid, imidacloprid and thimathoxam showed a high efficiency against cowpea aphid under field conditions compared to dinotefuran. There was no significant difference between acetamiprid, imidacloprid and thiamethoxam on percentage of reduction of cowpea aphid population at one, seven, 15 and 21 DAT. Acetamiprid, imidacloprid, thiamethoxam and dinotefuran registered a highest significant reduction percent of aphids at one, seven, 15 and 21

Table 1. Efficacy of selected neonicotinoid insecticides against the cowpea aphid, *A. craccivora*, population according to Henderson–Tilton on faba bean plants under field conditions.

Insecticides	Dose	% Reduction of cowpea aphid population ± SE			
		1 DAT	7 DAT	15 DAT	21 DAT
Acetamiprid 20% SP	25 g/100 L	99.50 ± 1.2a	92.54 ± 1.3a	85.24 ± 3.2a	78.48 ± 2.2a
Imidacloprid 20% SC	50 cm^3/100 L	95.88 ± 2.2a	90.75 ± 1.8a	86.94 ± 1.2a	75.55 ± 1.1a
Thiamethoxam 25% WP	50 g/100 L	99.05 ± 1.5a	95.64 ± 1.2a	87.65 ± 2.5a	75.45 ± 4.5a
Dinotefuran 20% SG	50 g/100 L	82.06 ± 1.1b	75.73 ± 1.2b	66.33 ± 4.1b	44.77 ± 3.1b

Notes: SE: stander error, DAT: day after treatment.
Means followed by the same letter(s) within the same column are insignificantly different ($p = 0.05$) according to DMRT.

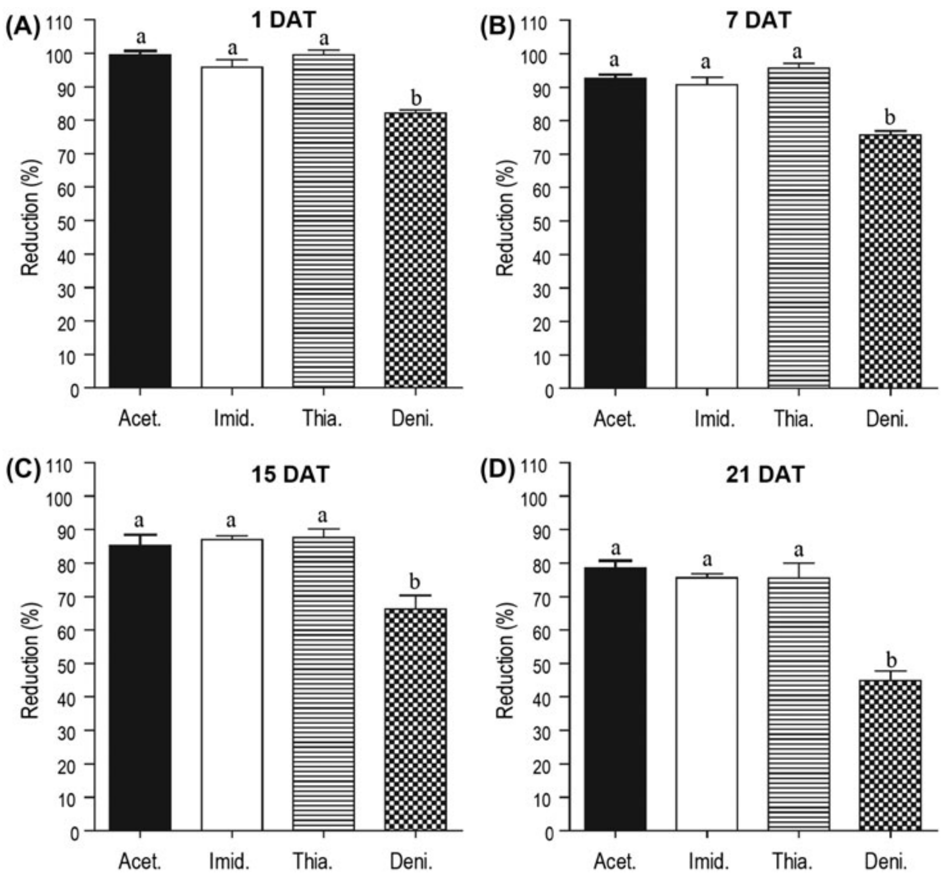

Figure 2. Efficacy of selected neonicotinoid insecticides on the cowpea aphid, *A. craccivora*, population under field condition, 1 DAT: one day after treatment (A), 7 DAT: seven days after treatment (B), 15 DAT: 15 days after treatment (C) and 21 DAT: 21 days after treatment (D). [Acet.: Acetamiprid, Imid.: Imidacloprid, Thia.: Thiamethoxam, Dino.: Dinotefuran]. Reduction % ± SE according to Henderson and Tilton's equation, 1955. Columns headed by the same letter (s) within the same figure are insignificantly different ($p = 0.05$) according to DMRT.

DAT, with an average of 99.50, 92.54, 85.24 and 78.48%; 95.88, 90.75, 86.94 and 75.55%; 99.05, 95.64, 87.65 and 75.45% and 82.06, 75.73, 66.33 and 44.77%, respectively.

Effect of foliar applications of neonicotinoid insecticides on yield of faba bean

In the present study, the application of acetamiprid (25 gm/100 L), imidacloprid (50 cm^3/100 L), thiamethoxam (50 gm/100 L) and dinotefuran (50 gm/100 L) against the cowpea aphid, *A. craccivora*, under field conditions increased the yield production per feddan (e.g. 830, 740, 810 and 650 kg/feddan, respectively) and, subsequently, yield percentage (e.g. 118.36, 94.74, 113.15 and 71.19% respectively) Table 2 and Figure 3. The application of neonicotinoid insecticides under field conditions to suppress the cowpea aphid, *A. craccivora*, population increased the yield production of faba bean by about 70–120%.

Residual efficacy (persistent) of neonicotinoid insecticides

The residual effect of neonicotinoid insecticides on faba bean leaves against the cowpea aphid, *A. craccivora*, was estimated. A batch of the aphids was allowed to feed on the field-treated leaves after each treatment and the mortality was recorded after 24 and 48 h. The median lethal exposure time (LT_{50}) of dinotefuran was shorter than acetamiprid, imidacloprid and thiamethoxam. The LT_{50} of dinotefuran at 50 gm/100 L after 24 and 48 h was 4.2 and 4.8 days, respectively (Figure 4(D)). Higher LT_{50} values of thiamethoxam at 50 gm/100 L were observed after 24 and 48 h, 6.95 and 7.85 days, respectively (Figure 4(C)). The LT_{50} of acetamiprid at 25 gm/100 L after 24 and 48 h was 5.8 and 6.8 days, respectively (Figure 4(A)). The LT_{50} of imidacloprid at 50 cm^3/100 L after 24 and 48 h was 6.2 and 7.4 days, respectively (Figure 4(B)).

Toxicity to the cowpea aphid

Comparison between the LC_{50s} of the tested neonicotinoid insecticides for the cowpea aphid, *A. craccivora*, under laboratory conditions (Table 3) showed that the most toxic insecticide by ppm (unit weight of active ingredient) was thiamethoxam (0.60),

Table 2. Estimated average yield per feddan in kilo of faba bean and percentage of increase yield in control and after treatment of neonicotinoid insecticides against cowpea aphid, *A. craccivora*.

Insecticides	Dose	Average yield/feddan (kg) ± SE	Yield increase (%) ± SE
Control	0	380 ± 43.59d	–
Acetamiprid 20% SP	25 g/100 L	830 ± 47.26a	118.42 ± 12.39a
Imidacloprid 20% SC	50 cm^3/100 L	740 ± 32.15b	94.74 ± 8.46b
Thiamethoxam 25% WP	50 g/100 L	810 ± 20.00a	113.15 ± 5.26a
Dinotefuran 20% SG	50 g/100 L	650 ± 15.28c	71.05 ± 3.98c

Notes: Feddan = 0.42 hectare, yield increase (%) = [average yield (kg) in treatment – average yield (kg) in control/average yield (kg) in control] × 100.
Means followed by the same letter(s) within the same column are insignificantly different ($p = 0.05$) according to DMRT.

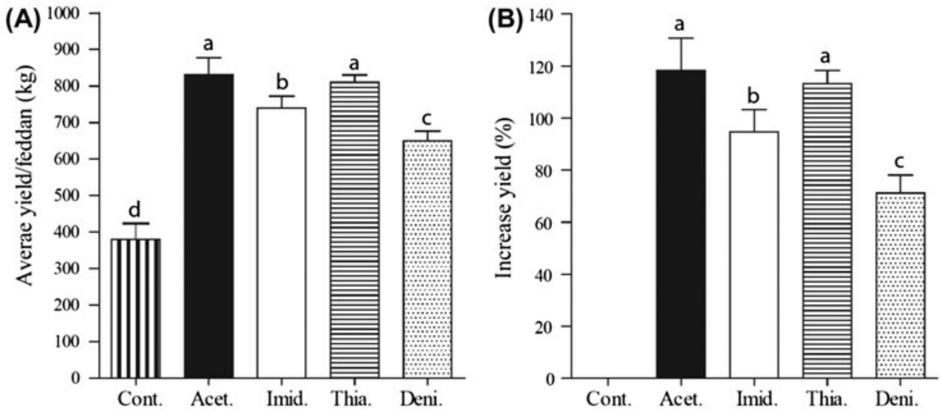

Figure 3. Average yield per feddan in kilo of faba bean (A) and percentage of yield increase (B) in control and after treatment of neonicotinoid insecticides against cowpea aphid, *A. craccivora*. [Acet.: Acetamiprid, Imid.: Imidacloprid, Thia.: Thiamethoxam, Dino.: Dinotefuran]. Columns headed by the same letter(s) within the same figure are insignificantly different ($p = 0.05$) according to DMRT.

followed by acetamiprid (0.71), imidacloprid (1.16) and dinotefuran (23.41). The LC_{50s} for thiamethoxam, acetamiprid and imidacloprid are not significantly different (95% FL overlap), while the LC_{50s} for these insecticides are significantly different from dinotefuran. Y value for each line estimated by probit regression was equal to zero when LC_{50} (x) was converted to log base 10. The slopes of the regression line of the tested insecticides (Table 3) in ascending order are imidacloprid (0.96), thiamethoxam (1.13), dinotefuran (1.20) and acetamiprid (1.34). Comparison between the LC_{90s} of the tested neonicotionid insecticides for cowpea aphid (Table 3) showed that the lowest effective one is acetamiprid (6.46), followed by thiamethoxam (7.89), imidacloprid (24.90) and dinotefuran (213.70). The LC_{90s} for thimathoxame and imidacloprid are not significantly different and thiamethoxam and acetamiprid took the same trend (95% FL overlap), while the LC_{90s} for the dinotefuran are significantly different from each other. Based on the toxicity index, thiamethoxam was more toxic than acetamiprid, imidacloprid and dinotefuran by 1.18, 1.93 and 39.06 fold, respectively. Also, acetamiprid was more toxic than imidacloprid and dinotefuran by 1.63 and 33.01 fold, respectively. In addition, imidacloprid was more toxic than dinotefuran by 20.52 fold. These results indicated that thiamethoxam, imidacloprid and acetamiprid were the most toxic neonicotinoid insecticides against cowpea aphid, followed by dinotefuran.

Discussion

The cowpea aphid, *A. craccivora*, has recently emerged as an important pest of field faba bean in Egypt (El-Ghareeb et al. 2002) and cause considerable loss in yield in different parts of the country (Bishara et al. 1984). Foliar application of neonicotinoid insecticides might be a good tool in the integrated pest management programme. Therefore, several controlled assays were performed in order to determine the effects of selected neonicotinoid insecticides acetamiprid, imidacloprid, thiamethoxam and dinotefuran against cowpea aphid and its mortality, to reduce aphid population and to estimate the increase in the yield of baba bean by the application of these insecticides.

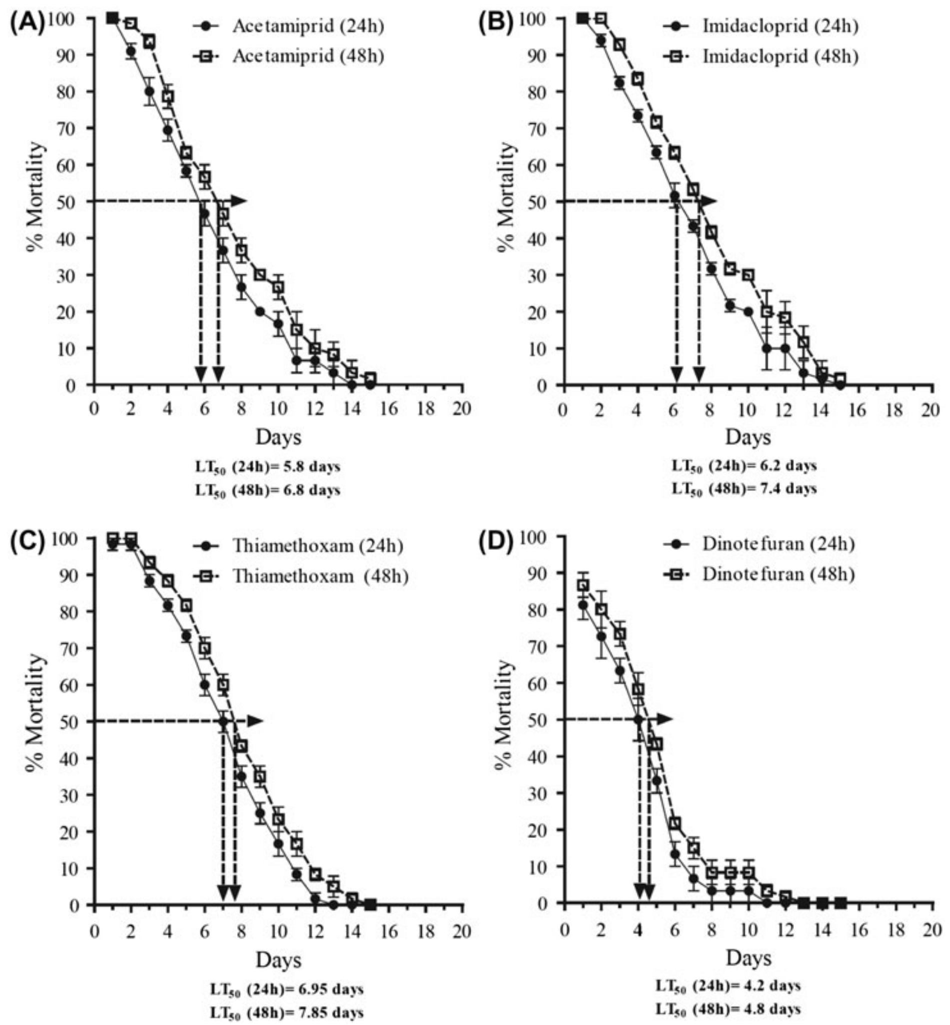

Figure 4. Residual activity of acetamiprid (A), imidacloprid (B), thiamethoxam (C) and dinotefuran (D) on faba bean leaves collected daily after treatments in the field against the cowpea aphid, *A. craccivora*, and the mortality was recorded after 24 and 48 h. (Mortality ± SE, and corrected by Abbott's Formula).

Table 3. Neonicotinoid insecticides toxicity to the adults of field strain of cowpea aphid, *A. craccivora*, after 24 h using leaf-dip bioassay technique.

Insecticides	Slope ± SE	LC_{50} (ppm) (95% FL)	LC_{90} (ppm) (95% FL)	Toxicity index
Acetamiprid	1.34 ± 0.09	0.71 (0.55–0.93) b	6.46 (4.50 – 10.13) c	84.51
Imidacloprid	0.96 ± 0.11	1.16 (0.63–2.18) b	24.90 (10.31–104.56) b	51.72
Thiamethoxam	1.13 ± 0.08	0.60 (0.43–0.77) b	7.89 (5.21–13.28) bc	100
Dinotefuran	1.20 ± 0.10	23.41 (19.12–27.71) a	213.70 (139.27–376.45) a	2.56

Notes: FL: fiducial limits, toxicity index = [(LC_{50} of the most toxic tested compound/LC_{50} of the tested compound) × 100].
LC_{50} and LC_{90} values having different letters are significantly different (95% FL did not overlap).

Results showed that all treatments caused a significant reduction in the aphid population after 1, 7, 15 and 21 DAT compared to control. Acetamiprid, imidacloprid and thiamethoxam showed a high efficiency against cowpea aphid under field conditions compared to dinotefuran. Similar results were observed by Elbert et al. (1991), who found that imidacloprid is highly effective in controlling aphids, and the trans-laminar transport of this insecticide from the treated upper side of the leaf to the lower surface was excellent. Also, Jarande and Dethe (1994) added that imidacloprid was effective in reducing the population of aphids on brinjal (auberggine) and in increasing the seedlings' total leaf chlorophyll over those of untreated plants. Similar results indicated that neonicotinoid insecticides are highly effective against different aphids and reduced the population of this pest under field conditions (Babu & Sharma 2003; Hohn et al. 2003; Abanowska 2004; Mohamed & Mohamady 2010; Gerami et al. 2011; Abdu-Allah 2012).

The presented data suggests that the application of neonicotinoid insecticides against cowpea aphid that infests faba bean plants under field conditions increased the yield percentage of faba bean by about 70–120%, through its ability to cause high mortality and sever decline in the aphid population. The residual activity of neonicotinoid insecticides in the present study was determined by calculating the LT_{50s} values. Data represented that the LT_{50} for acetamiprid, imidacloprid, thiamethoxam and dinotefuran were 5.8, 6.2, 6.95 and 4.2 days, respectively. Our results are in the same trend of Franco's (1999) results, where he stated that endosulfan plus acetamiprid and acetamiprid alone gave efficient control of *A. gossypii* at high infestation up to 6 days after treatment; endosulfan plus thiamethoxam controlled the pest up to 5 days. Moreover, these results are in accordance with Ayala et al. (1996), who found that imidacloprid, when applied at sowing time and foliage for colonies of *M. persicae* and *A. fabae*, was the most efficient insecticide with persistence of >60 days. He also reported that imidacloprid and aldicarb had similar efficiencies for *A. fabae* control, although there was great variation between fields. The most efficient foliar insecticide for control of *M. persicae* was imidacloprid, with 90% efficiency. Furthermore, in laboratory tests, Kumar and Santharam (1999) stated that the efficacy and persistence of foliar treatment by imidacloprid on cotton at 100 ml/ha resulted in 100% mortality against *A. gossypii* for seven days after treatment. Under field conditions, Abdu-Allah (2012) reported that imidacloprid at 47.6 gm/ha provided similar control to 125 gm/ha of primicarb at 1.5 and 10 days after spraying application against *B. brassicae*. These results indicated that these insecticides can protect the leaves of faba bean for a very long time against the cowpea aphid attack.

In the present study, the toxicity of the neonicotinoid insecticides against cowpea aphid after 24 h was determined using leaf-dip bioassay technique under laboratory conditions. Data showed that the most toxic insecticide by ppm (unit weight of active ingredient) was thiamethoxam (0.60), followed by acetamiprid (0.71), imidacloprid (1.16) and dinotefuran (23.41). In addition, there are no variations in the toxicity of thiamethoxam, acetamiprid and imidacloprid, while the LC_{50s} for these insecticides are significantly different from dinotefuran. Our results are in same line with the results reported by Abdu-Allah (2012), who studied the toxicity of imidacloprid on cowpea aphid, *A. craccivora* and *B. brassicae*, after 48 h using leaf-dip bioassay technique. He stated that imidacloprid was the least toxic compound against *A. craccivora* with LC_{50} 2.04 mg/L and against *B. brassicae* with LC_{50} of 2.14 mg/L.

In conclusion, the battle to control aphids will continue as long as these pests compete with mankind for food resources. Under field conditions, the neonicotionid insecticides still provide a good efficacy against piercing–sucking pests, such as aphids. But, the problem is that these pests can develop resistance very quickly for these insecticides. Therefore, we must use these insecticides in an orderly manner and place them in a programme to control these pests which make them unable to develop a resistance in these insects. Therefore, there is an acute need for determining the susceptibility of aphids to neonicotinoid insecticides and other piercing–sucking pests and the development of resistance to these insecticides as a first step, subsequently; the second step is to study the side effect of these insecticides on the natural enemies under field and laboratory conditions.

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