

TOXICITY AND BIOCHEMICAL EFFICACY OF NOVEL PESTICIDES AGAINST *Aphis craccivora* Koch (HEMIPTERA: APHIDIDAE) IN RELATION TO ENZYMES ACTIVITY



Shehawy, A. A.¹ and A. N. Z. Alshehri².

1-Plant Protection Research Institute,(A.R.C.) Giza, Egypt.

2-Biology Dept, Univ. College, Umm Al-Qura Univ, Makkah, Arabia.

ABSTRACT

Resistance development is a key part in pest management; Experimental trials included five conventional insecticides; Mospilan, Imidor (Neonicotinoid), Actellic (Organophosphate), Chess (Azomethine pyridines) and Aphox (Carbamate) to investigate their toxicity against both field and laboratory strains of *Aphis craccivora* Koch. The potency levels of the ongoing insecticides against the *A. craccivora* were 1.29, 1.43, 1.75, 1.97, 2.75 folds in laboratory strain more than that of field strain respectively. Thus, results showed that the field strain was more resistant for all compounds than that of laboratory strain. On the other hand, the results of the biochemical aspects of detoxification enzymes; Mixed Function Oxidase (MFO), Glutathione-S-transferases (GST), α and β -esterases demonstrated that, all tested insecticides induced impact on these detoxifying enzymes in both laboratory and field strain of *A. craccivora*.

INTRODUCTION

The use of pesticides and chemicals are the most important means and methods used to reduce the spread of harmful insects. However, the excessive use of others and thoughtful pesticides has led to a breach the ecosystem as such as pollutants in addition to the phenomenon of resistance. Insecticide resistance has developed within many classes of pesticide, and over 500 species of insects are resistant to one or more insecticides. Insecticide resistance causes economic losses of several billion dollars worldwide each year. Hence, the information associated to susceptibility in some aphid species is essential for an effective pest management programs (Ketoh *et al.*, 2005; Rozman *et al.*, 2007 and Cosimi, *et al.*, 2009). The cowpea aphid, *Aphis craccivora* Koch is the most injurious pest attacking several crops, as it prevents plant normal growth, affects flowers and bud production. This aphid also, transmits serious viral diseases and consequently reducing quantity and quality of the yield Shehawy (2007). Most of the insecticides are subjected to enzymatic reaction after their penetration into the site effect in the tested insect. It had also been clearly demonstrated that several enzymatic systems in resistant insects such as aliphatic esterases, phosphatases, and non-specific esterases play an important role in the detoxification mechanisms of insecticides. A member of the esterase cluster probably plays a role in the detoxification of xenobiotic esters. (Gacar and Tasksn, 2009). P450 enzymes (mixed function oxidase, cytochrome P450 monooxygenase), one of the most important enzyme system involved in insecticide detoxification or activation, are a complex family found in most organisms(Nadia Helmy *et al.* 2010). Cytochrome P450 monooxygenases

(CYPs), glutathione-S-transferases (GSTs) and α and β -esterases (ESTs) are the three major detoxifying enzymes in most organisms, at least one of them is involved in detoxification of insecticides in insects (Bull, 1981). In insects, the diverse functions of P450 enzymes range from synthesis and degradation of ecdysteroids and juvenile hormones to the xenobiotics metabolism (Feyereisen, 2005). Also, Terriere (1984) stated that such increase in enzymatic activities has been shown to protect insects from insecticide poisoning as part of defense mechanism. The aim of this study is to evaluate five recommended insecticides with possible insecticidal activity for their toxic efficacy against both laboratory and field strain of cowpea aphid, *A. craccivora*. Evaluation of the relationship between the efficacy of the tested compounds and some biochemical aspects; i.e. mixed function oxidase, alkaline phosphatase and esterase's activities in aphid species also was the target of this study.

MATERIALS AND METHODS

The present study was carried out at the Department of Sucking and Piercing Insects, Plant Protection Research Institute (PPRI), Agricultural Research Center (ARC), Egypt and Biology Department, University College, Umm Al-Qura University, Makkah, Saudi Arabia.

Rearing Technique:

Two strains of the cowpea aphid one of them was collected from Faba bean field: Elbagor, Monufia (Governorate) under ($20.7 \pm 1^\circ\text{C}$ and 53 ± 7 R. H.) and the second strain was a laboratory one, which was reared under constant conditions (21°C and 65 ± 5 R. H.) in Piercing and Sucking Insects Department, PPRI, ARC, Egypt.

Insecticide used:

Group	Common name	Trade name	Iupac name
Neonicotinoid	Acetamiprid	Mospilan	(E)-N-[(6-chloro-3-pyridinyl)methyl]-N-cyano-N-methylethanimidamide
	Imidacloprid	Imidor	1-(6-chloro-3-pyridylmethyl)-N-nitroimidazolidin-2-ylideneamine
Organophosphate	Pirimiphose-methyl	Actellic	O-[2-(diethylamino)-6-methyl-4-yl]O,O-dimethyl phosphorothioate
Pymetrozine	Azomethine pyridines	Chess	(E)-4,5-dihydro-6-methyl-4-[(3-pyridylmethylene)amino]-1,2,4-triazin-3(2H)-one
Carbamate	Pirimicarb	Aphox	[2-dimethylamino-5,6-dimethylpyrimidin-4-yl]N,N-dimethyl carbamate

Methods of application:

A serial stock concentration of insecticides were prepared as follows Acetamiprid (140, 70, 52, 35, 17.5 and 8.75 ppm), Imidacloprid (252, 262.5,

196.9, 131.25, 65.6 and 32.8 ppm), Pirimiphos-methyl (3750, 1875, 1406.25, 937.5, 468.75 and 234.375 ppm), Pymetrozine (202.4, 151.01, 113.4, 75.6, 37.8 and 18.9 ppm) and Pirimicarb (500, 250, 187.5, 125.5, 93.75 and 46.88 ppm) Which equivalent to 2 \times recommended field concentration, 3/4, 1/2, 1/4, 1/8 and 1/16 of the recommended field concentration for each insecticide. Apterous adult aphids homogeneous in age and size comprised ten (10) replicates (10 adults / replicate) for each concentration. A control (untreated check) test was run parallel with the serial concentrations using water only. For each concentration, young whole leaves (2–3 cm) of plants were dipped in the pesticide formulation for 10 seconds and then left for air-dried. The treated leaf was placed in Petri dish and 10 adults were introduced and kept under laboratory conditions ($25\pm1^{\circ}\text{C}$ and $65\pm5\%$ R. H.). After 24 hours of exposure, the mortality counts were recorded. The insect was considered alive if it was able to move at least one leg or antennae during probing with a camel's hair brush. If no movement or only very slight twitching was observed the aphid was considered dead (Harlow and Lampert, 1990). All mortality data were corrected for natural mortality using Abbott's formula (Abbott, 1925). The data were analyzed according to (Finney, 1971) to estimate LC₅₀, LC₉₀ and slope values were estimated. Sun, (1950) described the toxicity index as a mean for comparing the degree of the insecticides toxicity.

Determination of enzymes activity:

Effect of five insecticides on the activities of four detoxification enzymes [mixed function oxidase (MFO), alpha esterases (α -esterases), beta esterases (β -esterases), and glutathione-S-transferases (GSTs)] were evaluated. *A. craccivora* were treated topically with LC₅₀ of tested insecticide for 24hrs, then, preserved in refrigerator until analysis, after that, the specimen homogenized in distilled water using a Teflon homogenizer surrounded with a jacket of crushed ice for 3 minutes. The homogenates were centrifuged at 8000 rpm. for 15 minutes at 5°C, and the supernatants were used directly to determine the activity mixed function oxidase MFO, alkaline phosphates, α and β -esterases. P-nitroanisole o-demethylation was assayed to determine MFO activity according to the method of Hansen and Hodgson (1971) with slight modification. α -esterases and β -esterases were determined according to Van Asperen (1962) using α -naphthyl acetate or β -naphthyl acetate as substrates. GST catalyzes the conjugation of reduced glutathione (GSH) with 1-chloro 2,4dinitrobenzene (CDNB) via the -SH group of glutathione. The conjugate, S-(2, 4-dinitrophenyl)-L-glutathione could be detected as described by the method of Habig *et al.* (1974).

RESULTS AND DISCUSSION

1. Comparison on the basis of LC values, toxicity index and potency level values of insecticides on field and laboratory strains of *A. craccivora*.

Data obtained in Table (1) cleared that the LC₅₀ values of different concentrations of insecticides namely Acetamiprid, Imidacloprid, Pirimiphos-

methyl, Pymetrozine and Pirimicarb against the laboratory strain of *A. craccivora* were 30.8, 133.8, 297.5, 64.8 and 96.1 ppm respectively. Whereas, LC₅₀ values were 39.845, 195.1, 521.1, 127.7 and 264.7 ppm respectively in case of field strain.

The resistant ratio in the field strain of *A. craccivora* compared with the laboratory strain, the tested insecticide; Acetamiprid, Imidacloprid, Pirimiphose-methyl, Pymetrozine and Pirimicarb at LC₅₀ and LC₉₀ were 1.29&1.43, 1.46&1.93, 1.75&1.22, 1.97&1.68 and 2.78&3.46; respectively. According to Sun (1950) the toxicity index method is used to determine the efficiency or degree of toxicity of different insecticides by comparing them with a standard compound as follows:

$$\text{Sun's toxicity index} = \frac{\text{LC}_{50} \text{ or } \text{LC}_{90} \text{ of standard material}}{\text{LC}_{50} \text{ or } \text{LC}_{90} \text{ of standard material}} \times 100$$

The potency levels expressed as number of folds were obtained by dividing the LC₅₀ or LC₉₀ for the least effective materials by corresponding figure for each material. The obtained data showed general similarity of the trend of both the toxicity index and potency levels at the tow mentioned levels of toxicity against the field and laboratory strain except Pirimicarb at LC₉₀ in case of LC₉₀ against the laboratory strain. For simplicity, results would be compared at LC₅₀ values.

On the ground of the toxicity index at LC₅₀ level, the insecticide; Imidacloprid, Pirimiphose-methyl, Pymetrozine and Pirimicarb were 20.41, 7.63, 31.2 and 15.03 as toxic as the toxicity of Acetamiprid against the Field colony strain of *A. craccivora* and 23.0, 10.35, 47.75 and 33.08 as toxic as the toxicity of Acetamiprid against the laboratory strain of *A. craccivora* (Table 2). Concerning the potency levels compared with Pirimiphose-methyl, the Acetamiprid, Imidacloprid, Pymetrozine and Pirimicarb were 13.0, 2.67, 4.08 and 1.96 times as toxic as the toxicity of Pirimiphose-methyl against the field colony, 9.66, 2.22, 4.59 and 3.09 folds as toxic as toxicity of Pirimiphose-methyl against the laboratory stain of *A. craccivora*.

The obtained results are in agreement with those obtained by Abd El-wahab (2009) who suggested that the neonicotinoid proved that it has highly and fastly lethal effects against some aphids. Horowitz *et al.*, (1998) who carried out comparative bioassays of two chloronicotinyl insecticides namely; Acetamiprid and Imidacloprid, against the whitefly *Bemisia tabaci* (Gennadius).

The results are going in line with those reported by Aly A. Abd-ella (2014) who said that The toxicity index showed that Thiamethoxam, Acetamiprid and Imidacloprid have the highest aphicidal activity, with LC_{50s} 0.60, 0.71 and 1.16mg/L, respectively, while dinotefuran was the least toxic one with LC₅₀ 23.41mg/L. Results of this study indicated that neonicotinoid insecticides were highly effective against cowpea aphid under field and laboratory conditions. Imidacloprid achieved Cent percent mortality in resistant population of wheat aphids after treatment Vostrel (1998). The neonicotinoids insecticides were used rapidly worldwide for controlling insects because of their high potency, low mammalian toxicity Mori *et al.* (2002).

2. Enzymatic activity in the field and laboratory strains of *Aphis craccivora*

Another part of the present work was carried out to explore the possible effects of different insecticides on the enzymatic activities i.e., Mixed Function Oxidase (MFO), α and β -esterases and Glutathione-S-transferases (GST) estimated in both field colonies and Laboratory strain of *A. craccivora*.

When comparing between the detoxifying enzymes activity of wild (field) and laboratory strain of *A. craccivora*, it was found that, the significant increasing activity of MFO, α esterases, β -esterases and GSTs in wild strain (field) of *A. craccivora* than that of the laboratory strain before the treatment (Table. 3&4), it means that the field strain was the more resistant strain than that of laboratory one, Similar conclusion achieved by Mona Abd El-aziz and El-Sayed, (2009). The results are in the same line with that of Jean-Baptiste *et al.* (1998) who revealed that the increase in the content of a component of the P450 system results in an increase in resistant insects to the insecticidal action.

In case of field strain, the α -esterases and β -esterases activities were increased significantly by treatment of all insecticides used in this study except α -esterases reduced significantly by Pirimiphose-methyl when compared with those in control, (Table. 3), The insecticide pyridaly exhibited some inducing or reducing the effects on activity of the same enzyme *Schistocerca gregaria* (Teleb *et al.*, 2012). A significant elevation in MFO activities were observed in all treatments compared with the control. The activity of Glutathione-S-transferases (GST) was significantly high in case of treated with Pirimiphose-methyl, Imidacloprid and Pymetrozine (Table. 3). On the other hand, in case of laboratory strain it was found that, A significant elevation in MFO activities in case of treatment with Imidacloprid and Pirimicarb, but it was reduced significantly when treated with Acetamiprid and Pirimiphose-methyl as compared to the control. α -esterases activities were increased significantly by the treatment with Imidacloprid and Pirimicarb, also it decreased significantly when treated with Pymetrozine and Pirimiphose-methyl. β -esterases activities were increased significantly by the treatment with Imidacloprid and reduced after treatment with Pirimicarb, Acetamiprid, Pirimiphose-methyl and Pymetrozine as compared with those of control. The activity of GSTs was significantly high in case of treated with Pirimiphose-methyl and Acetamiprid (Table. 4).

Activity ratio associated to field colony of *A. craccivora* ranged from 0.96 to 2.72 for MFO, 1.55 to 3.14 for α -esterase, 1.33 to 3.59 for β -esterase and 0.96 to 2.86 for GSTs; whereas these values ranged from 0.39 to 1.24 for MFO, 0.55 to 1.15 for α -esterase, 0.53 to 1.96 for β -esterase and 0.79 to 1.69 for GSTs in case of treating the laboratory strain with a forgoing insecticides. The obtained results of detoxification enzymes revealed that were related to their mode of action in field and laboratory strains of *A. craccivora*. Similar conclusion achieved by Ghoneim *et al.* (2014)

Generally, The results showed reduction in α -Est and β -Est activity it may indicated that the tested compounds cannot detoxify by these enzymes. The obtained results of β - Est activity showed reduction in both black and white liquors treatment during all developmental stages, these results explained that this enzyme play no role in the detoxification of tested compounds as stated before by Mona Abd El-aziz and El-Sayed, (2009). Also, these results are in harmony with Terriere (1984). Who stated that these results indicated that this enzyme may play role in detoxifying tested compounds as a self defense to protect them. Esterase – resistant aphids with this mechanism make increased amounts of enzymes called esterases, which break down insecticides before they reach their target sites.

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سمية المبيدات الحديثه ضد حشرة من اللوبيا وعلاقتها بالنشاط الانزيمي
أيمن علي شهاوي^١ و عبدالله ناصر زهير الشهري^٢
١-معهد بحوث وقایة النباتات - مركز البحوث الزراعية - الدقى - الجيزة- مصر
٢-قسم الاحياء - الكليه الجامعية - جامعة أم القرى - مكه المكرمه - المملكه العربيه السعوديه

تقييم مستويات المقاومه في الحشرات للمبيدات يعتبر هو مفتاح عمليات المقاومة الناجحة للحشرات. تم في هذه الدراسة اختبار سمية خمس مركبات التي تشمل (الموسيبلان - الایميدور - الاكتيليك - الشيس - الاوفكس) على كل من السلاله الحقليه والسلاله المعمليه لحشرة من اللوبيا فاظهرت النتائج ان المقاومه بالنسبة للسلاله الحقليه مقارنة بالسلاله المعمليه كال التالي ١.٤٧ و ١.٧٥ و ١.٩٧ و ٢.٧٨ و مره على الترتيب. على الصعيد الاخر تم قياس نشاط اربعة انواع من الانزيمات (انزيم MFO وكذلك انزيم جلوتاشيون ترانسفيريز و بيتا ايستيريز و الفا استيريز) في كل من السلالتين نتيجة المعامله بهذه المبيدات. أظهرت النتائج أن كل المبيدات احدثت تغيرات فبنيه في نشاط الانزيمات وكان هناك تباينا في النتائج مما يؤكد أهمية تقدير النشاط الانزيمي في السلالات المعمليه بصفه دوريه للتأكد من فاعلية المبيدات المستخدمه في نطاق مكافحة الافات .

Table (1): Lc values, slope and resistance ratio for five insecticides against field and laboratory strains of cowpea aphid, *aphis craccivora koch.*

Compound	Field strain					Laboratory strain			Resistance Ratio Field/laboratory			
	LC ₅₀	LC ₉₀	Lower limit	Upper limit	Slope	LC ₅₀	LC ₉₀	Lower limit	Upper limit	Slope	LC ₅₀	LC ₉₀
Acetamiprid	39.8	199.2	34.6	45.8	1.8	30.8	138.5	26.8	35.12	1.9	1.29	1.43
Imidacloprid	195.1	1121.6	168.1	229.6	1.6	133.8	582.1	117.3	152.1	2.0	1.46	1.93
Pirimiphose-methyl	521.1	2192.9	443.1	599.0	2.0	297.5	1803.9	218.6	370.4	1.6	1.75	1.22
Pymetrozine	127.7	525.5	96.7	194.4	2.1	64.8	312.8	55.9	75.1	1.8	1.97	1.68
Pirimicarb	264.7	903.4	235.3	304.3	2.4	96.1	260.8	86.2	105.9	2.9	2.78	3.46

Table (2): Toxicity index and relative potency on the basis of lc values for five insecticides against field and laboratory strains of cowpea aphid, *aphis craccivora koch.*

Compound	Field strain					Toxicity index on basis of LC values			Toxicity index on basis of LC values			Laboratory strain	
	LC ₅₀	LC ₉₀	LC ₅₀	LC ₉₀	LC ₅₀	LC ₉₀	LC ₅₀	LC ₉₀	LC ₅₀	LC ₉₀	LC ₅₀	LC ₉₀	
Acetamiprid	100	100	13.0	11.0	100.0	100.0	100.0	100.0	9.66	9.66	13.02	13.02	
Imidacloprid	20.41	17.76	2.67	1.95	23.0	23.79	23.79	23.79	2.22	2.22	3.09	3.09	
Pirimiphose-methyl	7.63	9.10	1.0	1.0	10.35	7.67	10.35	7.67	1.0	1.0	1.0	1.0	
Pymetrozine	31.2	37.9	4.08	4.17	47.75	44.27	47.75	44.27	4.59	4.59	5.76	5.76	

Pirimicarb	15.03	22.0	1.96	2.43	33.08	53.10	3.09	6.91
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Table (3):Detoxification enzymes activities in field strain of *A. craccivora* exposed to five insecticides by leaf dip-technique after 24hr.

Compound	M FO (μ mole substrate oxidized/min/mg protein)	Specific activity of detoxification enzymes			Activity ratio		
		α-esterase (μg α - naphthyl acetate released /min/mg protein)	β-esterase (μg β - naphthyl acetate released /min/mg protein)	GTS (μmole conjugated /min / mg protein)	MFO	α-Est.	β - Est. GSTs
Control	782.0±39.6 ^a	22.66±0.9 ^c	32.66±2.3 ^a	2.53±0.13 ^c	-	-	-
Imidacloprid	2150.0± 104.2 ^a	77.33 ±5.9 ^a	67.0±6.03 ^b	4.23± 0.33 ^b	2.72	3.14	1.73 1.72
Pymetrozine	748.33±48.73 ^a	38.0±3.6 ^b	55.33±2.4 ^b	4.24±0.17 ^b	0.96	1.67	1.43 1.72

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Pirimicarb	1690.0± 95.28 ^b	46.64±4.8 ^b	117.3± 7.43 ^a	2.57±0.93 ^c	2.15	1.89	3.59	1.85
Acetamiprid	1339.0± 45.56 ^b	38.2±3.4 ^b	43.57 ±3.85 ^c	2.42±0.10 ^c	1.76	1.55	1.33	0.96
Pirimiphos-methyl	894.0. ±45.45 ^c	42.47±0.68 ^b	53.33±3.38 ^b	6.8±0.92 ^a	1.13	1.72	1.38	2.86

Table (4): Detoxification enzymes activities in laboratory strain of *A. craccivora* exposed to insecticides by leaf dip-technique 24h.

Compound	Specific activity of detoxification enzymes			Activity ratio				
	MFO (in mole substrate oxidized/min/mg protein)	α-esterase (μg α - naphthyl/ acetate released /min/mg protein)	β-esterase (μg β - naphthyl/ acetate released /min/mg protein)	GTS (umole conjugated /min / mg protein)	MFO	α- Est.	β - Est.	GSTS
Control	722.66±33.2 ^b	15.53±1.5 ^c	28.17±2.9 ^b	1.52±0.11b ^c	-	-	-	-
Imidacloprid	899.33±50.72 ^a	16.0±0.88 ^a	39.0±2.5 ^a	1.44±0.04 ^c	1.24	1.03	1.38	0.95
Pymetrozine	731.66±39.31 ^b	8.57±1.5 ^a	20.17±1.4 ^c	1.20±0.10 ^c	1.01	0.55	1.96	0.79
Pirimicarb	893.66±23.53 ^a	17.8±1.4 ^a	24.0±2.3 ^c	1.24±0.15 ^c	1.23	1.15	0.85	1.20

Acetamiprid	288.33±23.53 ^d	14.17±1.3 ^c	15.1±1.5 ^d	1.82±0.05 ^b	0.39	0.91	0.53	0.82
Pirimiphos-methyl	478.0±28.03 ^c	8.97±0.79 ^d	11.37±0.97 ^d	2.56±0.11 ^a	0.66	0.57	1.89	1.69

Within a column, same letter mean no significant differences at 0.05 level of probability

Within a column, different letters mean significant differences at 0.05 level of probability

Activity ratio = $\frac{\text{Enzyme activity in treated stages}}{\text{Enzyme activity in control}}$