Egypt. Acad. J. biolog. Sci., 2 (1): 39- 43 (2010) F. Toxicology &Pest control
Email: egyptianacademic@yahoo.com ISSN: 2090 - 0791
Received: 25/4/2010 www.eajbs.eg.net

# Biochemical and Toxicological studies on different field strains of cowpea aphid, *Aphis craccivora* (Koch)

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## ABSTRACT

Field strains of *copea Aphis craccivora* selected (in October 2008) from different Governorates (Behira, Gharbia, Sharkia and Faiyoum) were treated by different groups of insecticides, to evaluate their efficacy against this pest and to study some biochemical characters of these strains. The novel compound 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 ($LC_{50}$ 109.7ppm.) Differences in protein content, protein patterns, esterase activity, alkaline and acidic phosphatase were detected and differed from population to another.

## INTRODUCTION

The sap sucking insects like aphids constitute one of the major and important economic pests of several different plants in Egypt (Hassanein *et al*., 1971). Several species of *Aphis sp.* damage a broad range of vegetables like beans, cotton, tobacco, tomato and ornamental plants (Greathead, 1986). Foliar applied insecticides are recommended when insect populations reach damaging levels but this is favorable to the development of insect resistance to many groups of insecticides (Georghiou, 1990). Aphids are difficult to be controlled because of their mobility, tremendous reproductive ability and resistance to many synthetic pesticides (Van Lenteren, 1990), however outbreaks have occurred in association with insecticidal use for controlling other pests (Slosser *et al*., 1989). In general the novel compounds including neonicotinoids, the newest major class of insecticides have the potency and systemic action of crop protection against piercing sucking pests. They have low toxicity to mammals, birds and fishes (Foster *et al*., 2003 insecticide resistance in agricultural pests is undesirable from crop production point of view, some insecticides present the opportunity to investigate physiological mechanisms that confer resistance beside minimum danger to humans and natural enemies (Ishaaya., 1990). Current work was to evaluate the toxicity of used insecticides against *A. craccivora* as one of the main pests, and to study some biochemical characters of these strains.

## MATERIAL AND METHODS

**Insect Strains:**

**1-Laboratory susceptible strain:** Susceptible strain of cow pea aphid, *A. craccivora* (Koch) has been reared in laboratory for 30 generations under standard conditions at 22± 2 $C^0$ and 70 ±5 R.H. and a photoperiod of 12:12 (Light :Dark) hrs.

**2- Field strains:** Field strains of the aphid; *A. craccivora* were collected from faba bean fields of Behira, Gharbia, Sharkia, and Faiyoum Governorates. Aphids were collected in first October 2008. Field populations were tested directly for comparative studies of toxicological activity of tested materials and for biochemical studies.

**Bioassay:**

The leaf-dip bioassay was used corresponded to that described by Moores *et al.* (1996) with slight modifications. Faba bean leaves were dipped in the aqueous solution of any of the tested insecticides for about 10 seconds and allowed to dry on paper towel. They were then placed upside down on an agar bed in

small petri-dish (60 mm diameter).Ten apterous adults of *A. craccivora* were placed on the treated leaf surface, while leaves dipped in water serve as controls. Three replicates batches of aphids (i.e, 30 insects) were used per each insecticide concentration, and certain concentrations (4-7) being used for each insecticide. Petri-dishes containing aphids were kept in rearing chamber temperature until mortality was recorded.

**Insecticides used:**

**Diafenthiuron** (Polo 50% SC), Thiourea insecticides Chemical name: 1-*tert*-butyl-3-(2,6-di-isopropyl-4-phenoxyphenyl).

**Carbosulfan** (Marshal 25 % WP), Carbamate insecticide.

Chemical name: 2,3 – dihydro - 2,2 - dimethylbenzofuran-7-yl (dibutylaminothio) methyl carbamate.

**Esfenvalerate (**Fast 20%…….) Pyrethroid insecticide. Chemical name: [*S*-(*R**,*R**)]-cyano(3-phenoxyphenyl)methyl4-chloro-2-(1-ethylethyl)benzeneacetate.

**Thiamethoxam: (**Actara 25 % WP) Neonicotinoid insecticide.

Chemical name: 3-(2-chloro-1,3-thiazol-5-ylmethyl) -5- methy l-1, 3,5 -oxadiazinan-4-ylidene (nitro) amine.

**Data analysis:**

$LC_{50}$, slope values and fiducial limits were estimated by using a software package ″LD-P line″, copyright of Dr. Ihab M.Bakr, Plant Protection Research Institute.

**Biochemical assay:**

Adults of *A. craccivora* from laboratory strain and field populations were collected for investigation. The whole insects were homogenized in 0.9% Sodium Chloride solution by using hand glass homogenizer on ice jacket. The homogenate was centrifuged at 4000 rpm for 15 min. at $4C^0$ and was stored at -20 $C^0$. The supernatant obtained was used for determination of total protein according to Bradford (1976).

**1) Determination of total enzymes activity:** Total esterase was determined according to the method of Van Asperen (1962). Acid and Alkaline phosphatise were determined according to the method described by Powel and Smith (1954).

**2) Fractionation of total protein by using denaturing SDS.PAGE:** In a solution of SDS and 2-mercaptoethanol, proteins dissociated into subunits with rod like shape, in which the diameter of the rods is thought to be constant, while the long axis varies according to the molecular weight. The value can be determined by comparing the electrophoretic mobility of unknown protein with the mobility of known standard protein marker, (Laemmli, 1970).

## RESULTS AND DISSCUSION

**Toxicity of different compounds aginst aphids:-**

The obtained data in table (1) clearly indicated a great effect of the novel compound thiamethoxam on all of the tested aphids field strains. $LC_{50}$ was 0.14, 0.64, 0.36, 0.44 and 0.75 ppm for susceptible, Behira, Gharbia, Sharkia and Faiyoum respectively.

Table (1): Toxicity of the used insecticides against adults of cowpea aphid, *A. craccivora* field populations and susceptible strain in October 2008.

| Governorates / Insecticides | susceptible strain | | Behira | | Gharbia | | Sharkia | | Faiyoum | |
|---|---|---|---|---|---|---|---|---|---|---|
| | $LC_{50}$ | Slpoe±SE* | $LC_{50}$ | Slpoe±SE* | $LC_{50}$ | Slpoe±SE* | $LC_{50}$ | Slpoe±SE* | $LC_{50}$ | Slpoe±SE* |
| **Thiamethoxam** | 0.14 | 1.29±0.34 | 0.64 | 0.76±0.32 | 0.36 | 0.91±0.44 | 0.44 | 1.34±0.43 | 0.75 | 1.66±0.21 |
| **Diafenthiuron** | 1.50 | 1.37±0.76 | 23.6 | 1.05±0.59 | 18.3 | 0.99±0.30 | 232.8 | 0.82±0.23 | 27.93 | 1.06±0.32 |
| **Carbosulfan** | 2.55 | 1.03±0.82 | 18.8 | 1.21±0.13 | 109.7 | 1.03±0.21 | 78.04 | 0.96±0.14 | 46.07 | 1.03±0.20 |
| **Esfenvalerate** | 7.62 | 1.11±0.33 | 33.9 | 0.86±0.21 | 77.61 | 0.87±0.26 | 142.9 | 1.05±0.12 | 132.7 | 1.61±0.27 |

Diafenthiuron which belongs to novel insecticides; thiourea compounds recorded the least effect on the Sharkia population with the greatest $LC_{50}$, 232.80 ppm among all insecticides used while it recorded 18.30 ppm in Gharbia followed by 23.60 and 27.93 ppm in Behira and Faiyoum respectively, it may be due to the high rate of IGI in Sharkia Governorate. Carbosulfan (carbamate) insecticides had moderate efficiency in all tested Governorates except Gharbia ($LC_{50}$, 109.70 ppm), while it was recorded 18.80, 78.04 and 46.07 ppm in Behira, Sharkia and Faiyoum populations respectively. On the other hand the pyrethroid compound, esfenvalerate was the least effective one on the susceptible strain and also on the Behira and Faiyoum populations.

The present data proved that, the neonicotinoid insecticides thiamethoxam was the most effective one. These results agree with Afzal *et al.* (2002); Ulaganathan and Gupta (2004); Dewar *et al.*(2004) and Mainfisch *et al.* (2001) who demonstrated that from the Novel compound, thiamethoxam exhibits, exceptional systemic characteristics and

provides excellent control of a broad range of commercially important pests, such as aphids, jassids, whitefly and thrips.

**Biochemical characters of different aphid field strains:-**

The protein content of susceptible strain and each strain from four tested Governorates was studies.

The obtained results (Table 2) revealed that field strains had higher total protein concentration than the susceptible laboratory strain.

Table 2: Total protein, total enzymes, alkaline and acid phosphatase content from different Governorates and susceptible strains.

| Samples (All body) | Total protein mg/ml | Total Esterase mM/mg protein | Acid phosphatase U/L | Alkaline phosphatase U/L |
|---|---|---|---|---|
| susceptible strain | 15 | 1.25 | 37.4 | 238.9 |
| Faiyoum | 45 | 2.77 | 144.5 | 198 |
| Gharbia | 39 | 1.61 | 102.1 | 173 |
| Sharkia | 25 | 1.88 | 94.4 | 163.4 |
| Behira | 17 | 1.36 | 87.9 | 189.6 |

Faiyoum strain showed the highest one with respect to total protein content with 45mg/ mg protein while the strain which had the lowest protein content, was attained by Behira strain 17mg/ mg protein followed by susceptible strain 15mg/ mg protein. Also the total esterase content of Faiyoum strain was reached to 2.77mM/mg protein followed by Sharkia, Gharbia, and Behira strains (1.88, 1.61 and 1.36 mM/mg protein, respectively). The esterase content of the susceptible strain was 1.25 mM/mg protein.

Acid and Alkaline phosphatases activities were illustrated in table (2). Data showed that acid phosphatase activity in the four field strains increased sharply more than the susceptible strain (37.4 U/ mg protein). It was 144.5, 102.1, 94.4, and 87.9 U/ mg protein for Faiyoum, Gharbia ,Sharkia and Behira, respectively, while alkaline phosphatase activity decreased in case of all tested populations than susceptible strain which recorded 238.90 U/ mg protein while other populations recorded 198, 173, 163.4, and 189.6 U/ mg protein for Faiyoum, Gharbia ,Sharkia and Behira, respectively.

The lower level of Alkaline phosphatase of aphid field strains than susceptible strain in the present work agree with Abd-Hafez (1978) and Farag (1978) who reported that organophosphorus resistant laboratory strain of *S. littoralis* have much lower Alk-phos. activity than the susceptible strain. The presence of lower level of Alk-pase content in field populations than the susceptible strains might indicate that this lower level of content Alkaline phosphatase accompanies resistance rather cause it,i.e resistance gene(s) might be associated with gene (s) responsible for this low content (Amin, 1992). With respect to Acid phosphatase activity, our data agreed with Farag (1981), who stated that cypermethrin and curacron resistant laboratory strains of *S. littoralis*, had higher level of acid phosphatase activity as compared to the susceptible strain. El-Gendy *et al*. (1985), stated that the higher acid phosphatase activity in laboratory strain of the larvae of *S. littoralis* might explain why that strain was more tolerant than other strains for the tested organophosphorus compounds.

Table (3) and Fig. (1) cleared that two protein bands were appeared in field populations and also in susceptible strain (band number 27 and 29) whereas band no. 18 appeared only in sus. strain with Mw 75.78 KD. Also one band occurred only in both susceptible strain and Sharkia population (band no. 1 with Mw. 138.39), while it did not appear in the others.

Table 3: Changes in protein patterns of A. craccivora from four Governorates and susceptible strain during October of the year 2008.

| Band No. | Rm value | MW. kd | Behira | Faiyoum | Sharkia | Gharbia | Sus. |
|---|---|---|---|---|---|---|---|
| 1 | 0.051 | 138.39 | | | • | | • |
| 2 | 0.062 | 131.37 | | | | | |
| 3 | 0.118 | 131.03 | | | | | |
| 4 | 0.125 | 126.55 | | | | | |
| 5 | 0.126 | 119.80 | | | | | |
| 6 | 0.129 | 117.84 | | • | | | |
| 7 | 0.135 | 113.52 | | | | | |
| 8 | 0.136 | 107.48 | | | | | |
| 9 | 0.165 | 103.17 | | | | | |
| 10 | 0.166 | 96.61 | | • | | | |
| 11 | 0.171 | 91.23 | | | | • | |
| 12 | 0.176 | 89.91 | | | • | | |
| 13 | 0.256 | 87.31 | | | • | | |
| 14 | 0.296 | 85.25 | | • | • | | • |
| 15 | 0.303 | 81.32 | | | • | | |
| 16 | 0.332 | 78.31 | • | • | | | |
| 17 | 0.372 | 77.50 | | | | • | |
| 18 | 0.392 | 75.78 | | | | | • |
| 19 | 0.406 | 73.52 | • | | | • | |
| 20 | 0.443 | 70.13 | | • | | | |
| 21 | 0.493 | 68.81 | | • | | | |
| 22 | 0.552 | 66.30 | | | | • | |
| 23 | 0.601 | 63.91 | • | | • | | |
| 24 | 0.653 | 60.30 | | • | • | | |
| 25 | 0.702 | 58.50 | | | | • | |
| 26 | 0.758 | 56.10 | | | | | |
| 27 | 0.793 | 49.30 | • | • | • | • | • |
| 28 | 0.817 | 36.20 | | • | • | | |
| 29 | 0.858 | 35.21 | • | • | • | • | • |
| 30 | 0.908 | 34.20 | • | | | • | |
| 31 | 0.920 | 33.25 | | • | | • | |
| 32 | 0.950 | 30.23 | | | | | |
| 33 | 0.970 | 28.13 | | • | | | |
| 34 | 0.990 | 26.20 | | | | | |
| 35 | 0.999 | 20.20 | | | | | |

Rm: Relative mobility. Mw: Molecular weight. Kd: kilo Dalton.

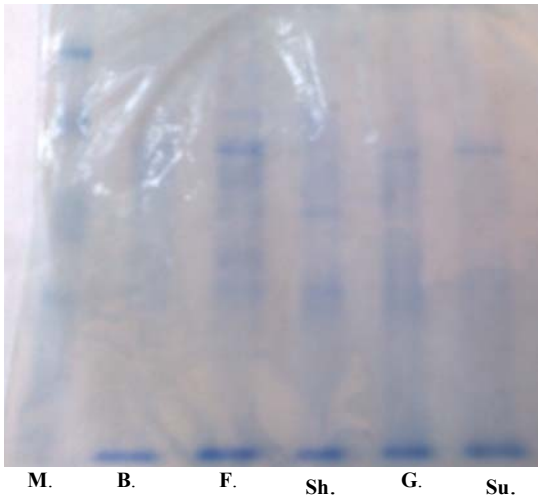


Fig. 1: SDS-polyacrilamide gel electrophoresis of total protein patterns in the different field strains and susceptible strain of *Aphis craccivora*.
M.: Marker, B.: Behira, F.: Faiyoum, Sh.: Sharkia,. G.:Gharbia, Su: susceptible.

Faiyoum and Sharkia populations recorded the highest number of protein bands (11 and 10 bands, respectively), these results agree with the values of $LC_{50}$ where they had the highest $LC_{50}$'samong the other populations and also agree with Hama and Hosoda (1988). They found good relationship between protein content and carboxylesterase activity of *A. gossypii*. Also, Shuai and Wang (2005) stated that, resistance strain of *Myzssyus persicae* developed obvious resistance to alphamethrin, as resistance developed enzyme protein content increased significant correlation between $LD_{50}$'s values and enzymes protein content, as well as esterase activity.

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## ARABIC SUMMARY

### دراسات بيوكيميائية وتوكسيكولوجية علي سلالات حقلية مختلفة لمن الفول

**عزة إسماعيل محمد - عزيزة حسن محمدي**
المعمل المركزي للمبيدات- مركز البحوث الزراعية - الدقي

تم دراسة تأثير أربع مجاميع مختلفة من المبيدات وهي من مجموعات الثيويوريا والكرباماتية و النيوكوتونيدية و البيروثرودية علي بعض السلالات الحقلية والتي تم تجمعها في أكتوبر ٢٠٠٨ من محافظات مختلفة. حيث أستخدمت سلالة محافظة البحيرة ممثلة لغرب الدلتا والغربية ممثلة لوسط الدلتا ومحافظة الشرقية ممثلة لشرق الدلتا والفيوم ممثلة لجنوب الدلتا لتقييم فاعلية هذه المبيدات على هذه الأفة ولدراسة الصفات البيوكيميائية لهذه السلالات.

وقد أعطي مركب thiamethoxam من المبيدات الجديدة أعلى فاعلية يلية مركب diafenthiuron من مركبات الثيويوريا ثم carbosulfan من المبيدات الكرباماتية ثم esfenvalerate من المبيدات البيروثرويدية لكل المحافظات ماعدا محافظة الشرقية حيث أظهر مركب diafenthiuron أقل فاعلية مقارنة بقيم الـ $LC_{50}$ فقد كانت قيمة $LC_{50}$ 232.80 جزء فى المليون بينما أعطي مركب carbosulfan أقل فاعلية في محافظة الغربية حيث كان التركيز النصف مميت ١٠٩.٧ جزء في المليون. وقد وجدت أختلافات في المحتوى الكلي للبروتين وكذلك الحزم البروتينية عند تفريد البروتين بجهاز الألكتروفوريسيس . وقد لوحظ أيضا أختلاف في نشاط انزيم الاستريزيز والفوسفاتيز القاعدي و الحامضي لهذه الأفة من محافظة لأخرى.