Egypt. Acad. J. biolog. Sci., 1(1): 65-69 (2009) C. Physiology & Molecular Biology
Email: egyptianacademic@yahoo.com ISSN: 2090-0767
Received: 10/11/2009 www.eajbs.eg.net

Development of resistance in field strain of *Aphis craccivora* to the dinotefuran insecticides from the new class neonicotinoids and its effect on some enzymes content.

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ABSTRACT

Resistance of cowpea aphid colony to the newest insecticide, dinotefuran was tested by selection pressure for 20 generations. Resistance ratio reached 148.8 fold in relation to susceptible strain. The LC_{50} value of the parent (first generation) was 5.39 ppm with slope value 1.06 ±0.53 which mean that this strain was not homogenous to this insecticide. The value of LC_{50} increased slowly with selection pressure, reached 22.24 ppm for the 10^{th} generation. Up to 15^{th} generation with the same selection pressure LC_{50} value increased to reach to 66.19 ppm and reached to 230.71 ppm in case of 20^{th} generation. Resistance ratio values in relation to susceptible strain, increased until the 10^{th} generation to 14.34 fold, and fastly increasing to 42.7 fold and 148.8 fold to 15^{th} and 20^{th} generation respectively.

Selection pressure produced some differences in total protein content and SDS-protein patterns. The analysis of esterase patterns obtained by the native- PAGE and by using α naphthyl acetate as a substrate which revealed a minor was measured.

Keywords: Resistance, *Aphis craccivora,* Dinotefuran, Neonicotinoids, Enzymes

INTRODUCTION

Unfortunately, many of conventional insecticides are harmful to human and beneficial organisms and caused ecological disturbance. This situation led to great demand for safe and more selective insecticides affecting specially harmful pests. One of these new approaches was the development of novel compounds acting selectively on some groups of insects by affecting biochemical sites such as respiration (diafenthiuron), the nicotinic acetylcholine-receptor (Neonicotinoids) or salivary gland of sucking pests (pymetrozine) (Ishaaya and Horowitz.1998).

Neonicotinoids have become one of the most extensively used insecticides for both crop protection and health applications. As with other classes of insecticides, resistance to Neonicotinoids is a significant threat and has been identified in several pest species (Zewen *et al*., 2003).

Also, neonicotinoids compounds effectively circumvent the known carboxylesterase, modified acetylcholinesterase (MACE) and knock-down (KDr) insecticides resistance mechanisms in aphid, Myzus persicae species, (Foster *et al*., 2003). So through this study we tried to elucidate the development of resistance of certain strain of Aphis craccivora to the novel compound nicotinic acetylecholine receptor agonists, dinotefuran which belong to the third generation of the new class: neonicotinoids.

MATERIALS AND METHODS

1- Insecticide used: Dinotefuran (MTI-446 20% SG), nitroguanidine insecticides.

Chemical name:

N-methyl-*N*'-nitro-N''-[(tetrahydro-3-furanyl)methyl]guanidine.

2-Insect Strains:

Laboratory susceptible strain:

The laboratory susceptible strain of cow pea aphid, *A. craccivora* (koch) was originated from a field population, had reared for 30 generation ever since free from exposure to insecticides under laboratory conditions (22±2^{0}C,70±5% relative humidity and photoperiod 12 :12 light : dark), the insects were kept on faba bean seedlings, *Vicia fabae* which grown in plastic pots. The pots with faba bean seedlings were maintained in another place without any exposure to insecticides.

Dinotefuran resistance strain:

This stain was reared described previously on faba bean seedlings and subjected to continuous exposure of the selected dinotefuran (MTI-446 20% SG) under laboratory conditions. The selection pressure with insecticide used was carried out of various *A. craccivora* stages reared on faba bean to LC_{50} of the insecticide by spraying using 1 liter hand spray.

The strain has been remained under continuous selection pressure for about 20 generations. This strain was used for biochemical studies.

3- Bioassay:

The leaf-dip bioassay corresponded to that described by Moores *et al*., 1996 with slight modifications was used. Faba bean leaves were dipped in the aqueous solution of any of the tested insecticide 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 *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 scored mortality.

4 - Biochemical assay:

Esterase patterns of samples of susceptible and dinotefuran- resistant strain were separated by polyacrylamide gel electrophoresis into two groups based on their ability to hydrolyze the following substrate: α-naphthyl acetate.

5- 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.

RESULTS AND DISCCUSION

Dinotefuran resistant strain of *A. craccivora:*

The cow pea aphid, A. craccivora were exposed to selection pressure up ward 20 generations. The LC_{50} values were obtained from toxicity regressions lines of the tested generations. The resistance ratio was used as an indication to the increasing of tolerance or resistance due to the selection pressure by the tested insecticide. The slope values of toxicity lines were taken as an indication of the degree of homogeneity of the tested population to insecticide. The obtained data are summarized in table (1).

These data showed that, LC_{50} of the parent (first generation) was 5.39 ppm with slope value 1.06 ± 0.53 which mean that this strain wasn't homogenous to this insecticide.

The value of LC_{50} increased slowly with selection pressure, reached to 22.24 ppm for the 10th generation and the slope value also increased to 2.94 ±0.52 which mean that this strain become to be homogenous. Up from 15th generation with selection pressure the data showed high resistance increasing, and the value become 230.71 ppm, and the slope values started to decrease, which mean that the strain started to become non- homogenous.

Table 1: Development of resistance in the cow pea aphid, *A. craccivora* exposed to laboratory selection pressure with dinotefuran insecticide.

Generation	LC_{50}(mg Litre^{-1})	Slope± SE	Resistance ratio(fold)
Susceptible strain	1.55(1.22-2.30)	2.78±0.64	-
Dinotefuran-selected strain:			
Parent	5.39(2.45-9.55)	1.06±0.53	3.47
5th generation	10.27(7.34-16.42)	1.66±0.35	6.62
10th generation	22.24(18.70-27.07)	2.94±0.25	14.34
15th generation	66.19(48.02-90.71)	1.90±0.42	42.70
20th generation	230.7(158.53-384.98)	1.30±0.31	148.80

Resistance ratio values in relation to susceptible strain, increased until the 10th generation to 14.34 fold, and fastly increasing to 42.7 fold and 148.8 fold to 15th and 20th generation respectively.

Results obtained indicated that, resistance was grown slowly until the 10th generation. This finding is completely agreement with Wang *et al*., (2002) who selected for a low level of resistance to imidacloprid, approximately 8 fold, in a line of cotton aphid, *A. gossypii*. Also, testing of field collected strains of tobacco whitefly, *B. tabaci* showed a slow but steady increase in resistance level to imidacloprid (Elbert *et al.*, 2001), also agree with Prabhaker *et al.*, (1997) who mentioned that high levels resistance to imidacloprid for silver – leaf whitefly, *B. argentifolii* was obtained after exposing whiteflies to imidacloprid over 32 generation, and reached resistance level of 82 fold compared with unselected strain.

Esterase patterns with α- naphthyl acetate:

Esterase analysis with native (PAGE) revealed 7 major bands with a high capability of hydrolyzing α- naphthyl acetate table (2) and Fig. (1). Comparison between susceptible and dinotefuran- resistant strain indicated that esterase patterns of susceptible consisted of five bands no.1,2,3,5and 7,with Rm values 0.068, 0.193, 0.310, 0.539 and 0.719, while dinotefuran – resistant strain had more one additional band no. 4 with Rm value 0.432. Comparing the susceptible strain with the field strain revealed two additional bands no. 4 and 6 and the disappearance of band no. 2.

Scanning densitometric of α-naphthyl acetate esterase patterns (table 2, fig 2) revealed that, the band no.3 represent the highest band concentration (27.02) in dinotefuran –resistant strain, while in susceptible stain the same band represent 22.73. The concentration of appearance band no. 4 in dinotefuran- resistant strain was 19.56%, in contrast to susceptible strain the band no. 4 was absent.

Table 2: Rm values of esterase bands in susceptible and dinotefuran- resistant strain of *A. craccivora* (Koch) with α-naphthyl acetate as substrate.

Band No.	Rm value	Susceptible strain	Resistance strain
1	0.014	+	+
2	0.130	+	+
3	0.311	+	+
4	0.448	-	+
5	0.509	+	+
6	0.636	+	+

These results agree with Choi *et al*., (2001) who stated that, esterase activity was higher in imidacloprid- resistant strain than susceptible by 1.4 times. Also, O'Brien *et al*., (1992) found that, carboxylesterase activity was higher significantly in resistant aphid, *A. gossypii* strains to chlorpyrifos.

Generally, treatment the strain of A. craccivora by sub lethal doses from LC_{50} resulted in slight highly of LC_{50} from the first to the 10^{th} generation while after 15 generations, resulted in high values of LC_{50}, where insecticide can be used for short period and stop its use for a while before applied again.

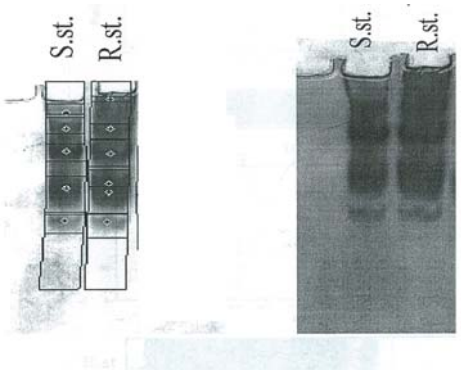

Fig.1: Polyacrylamide gel electrophoresis isozyme patterns in two tested strains of cowpea aphid with α-naphthyl acetate.
S. st: susceptible strain R. st:resistant strain

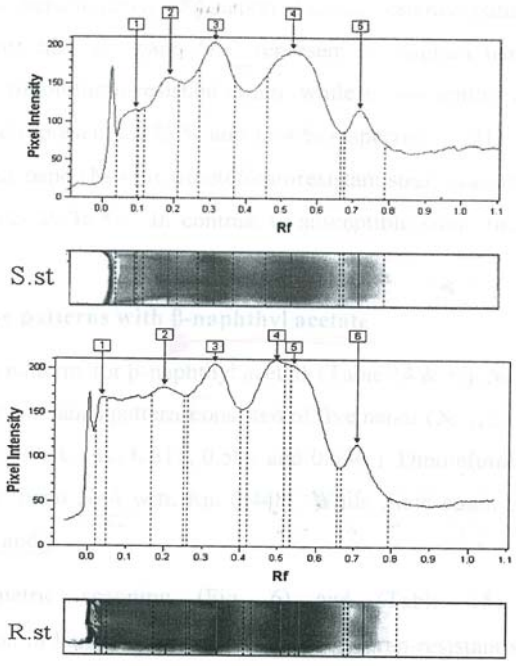

Fig. 2: Desitometric scanning of esterase patterns illustrated in Fig.(1).
S. st: susceptible strain R. st: resistant strain

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ARABIC SUMMARY

تطور صفة المقاومة فى آفة من الفول الحقلية لآحد المبيدات الحديثة (Dinotefuran) من عائلة (Neonicotinoids) وتأثيرها على بعض محتوياتها الأنزيمية.

السيد سليمان مقبل- عزة اسماعيل محمد

المعمل المركزى للمبيدات- مركز البحوث الزراعية

استهدفت الدراسة تطور صفة المقاومة لأحد المبيدات الحديثة dinotefuran معمليا. وكذلك دراسة بعض الفروق البيوكيميائية بين السلالة المقاومة و السلالة الحساسة و تشمل انزيمات الاستيريزمن حيث النشاط و التفريد الكهربى.

وقد تم اختبار مقاومة آفة المن للمبيدات الحديثة مثل مبيد dinotefuran تحت ضغط انتخابى من الرش المتتابع لمدة ٢٠ جيلا متتاليا باستخدام الجرعة تحت المميتة LC_{50} . وكانت قيمة التركيز القاتل لجيل الآباء هى 5.39 جزء فى المليون وباستمرار الضغط النتخابى زادت قيمة هذا التركيز حتي وصلت الي 22.24 فى الجيل العاشر بقيمة 14.34 ضعف. ومع استمرار الضغط الانتخابى فقد بدأت زيادة كبيرة فى قيمة التركيز النصفى القاتل و بالتالى فى المقاومة . و كانت قيمةالتركيز النصفى القاتل فى الجيل الخامس عشر 66.19 بقيمة 43.70 ضعف و فى الجيل العشرين كانت قيمة التركيز النصفى القاتل 230.71 جزء فى المليون و بقيمة 148.80 ضعف.

وكذلك تم استخدام ألفا نفثيل أسيتات كوسط تأثيرى لانزيمات الاستريزات وقد أوضحت النتائج التعرف علي ٧ حزم انزيمية و امتازت السلالة المقاومة بوجود ٦ حزم انزيمية مقابل ٥ حزم للسلالة الحساسة وكانت نسبة التشابه بين استيريزات السلالة المقاومة و الحساسة 0.909 و قد تميزت السلالة المقاومة بوجود الحزمة الانزيمية رقم٤ .