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Comparative toxicity of certain insecticides against laboratory and field populations of *Aphis craccivora* Kock and *Rhopalosiphum padi* (L.) (Homoptera:Aphididae)

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ABSTRACT

The responsibility of field population of *Aphis craccivora* Kock and *Rhopalosiphum padi* (L.) was investigated toward certain insecticides. The role of carboxylesterase activity as a metabolic mechanism was also studied. Based on the comparisons of the LC_{50} of field and laboratory populations for each species, the resistance factors indicated that the field population of *A. craccivora* was less susceptible by 5.77, 1.91, 1.54 and 1.03-fold resistance for malathion, chlorpyrifos, methomyl and cypermethrin insecticides, respectively, while field population of *R. padi* was less susceptible than the laboratory population by 5.14, 1.26, 9.21 and 1.58-fold resistance for the same corresponding insecticides, respectively. Carboxylesterase activity by using 1-naphthyl acetate (1-NA) as a substrate in susceptible and field population for each species revealed that based on the enzymatic half life ($t_{0.5}$) values, the carboxylesterase activity in field population of *A. craccivora* and *R. padi* were higher than carboxylesterase of the corresponding

susceptible populations by 1.7 and 2.25-fold, respectively. These results suggested that carboxylesterase activity plays a role in the insensitivity of the field populations of the two tested aphid species.

INTRODUCTION

Extensive use of insecticides has led to widespread development of resistance in many insect species and this represents one of the major threats to the future success of chemical pest control (Devonshire, 1989). In the past 50 years more than 500 arthropod species have become resistant to the toxicological action of insecticides (Brattsten, 1989) and this included more than 20 resistant aphid species (Devonshire, 1989). The mechanism of resistance is complex and sometimes varies in different biotypes of the same species, even for the same insecticides.

There are at least two toxicological significant mechanisms of insecticides resistance. These are target site resistance and metabolic resistance (Oppenoorth, 1984). A great deal of information is available on three major metabolic detoxification enzyme systems which influence the toxicity and resistance to insecticides; i.e., microsomal polysubstrate monoxygenases, glutathion transferases and hydrolases (Oppenoorth, 1984). In Upper Egypt, the black legume aphid *Aphis craccivora* Kock is one of the most important insect pests attacking the bean crop. The birdcherry aphid *Rhopalosiphum padi* (L.) is also one of the most important insect pests infesting the wheat crop during the spring season. The economic infestation of the *A. craccivora* and *R. padi* can be controlled with foliage sprays of various insecticides, but the control of these aphids in the field was become more difficult in recent years.

In the present study, we reported the incidence of insecticide resistance in field population of *A. craccivora* and *R. padi* against certain insecticides. The role of the carboxylesterase activity as a metabolic mechanism was also demonstrated.

MATERIALS AND METHODS

I-Chemicals:

1-Insecticides: Four insecticides were used in the present study. These insecticides were, two organophosphates, malathion (EC, 90% Amer. Cyan. Co.) and chlorpyrifos (EC 48%, Dow Chem. Co.), one carbamate insecticide methomyl (S.P. 90% DuPont Co.) and one pyrethroid cypermethrin (Technical grade, 93% Purity, Shell Chem. Co.).

2-Chemicals used for enzyme assay: Anhydrous mono- and dibasic potassium phosphate, Triton X-100, 1-naphthyl acetate (1-NA), 1-naphthol, 4-amino antipyrin, and potassium ferricyanide were obtained from Sigma Chemical Co.

II-Insects: Two different aphid species were used in this study, Black legume aphid *Aphis craccivora* Kock and Birdcherry aphid *Rhopalosiphum padi* (L.). A laboratory susceptible clone and field population of each species were used in all experiments.

1-Laboratory susceptible clones: Susceptible clone of *A. craccivora* was started with parthenogenic female collected from bean plant in Assiut University Experimental farm and maintained in the laboratory on lablab leaves for five years. Susceptible clone of *R. padi* was also started with parthenogenic female reared under the laboratory condition on wheat plant in a small pots 15 cm diameter. Both of the two species clones were maintained at 25±1°C and a photoperiod of 16:8 (light:dark).

2-Field populations: Aphid population of *A. craccivora* was collected from bean plants, while *R. padi* was collect from weed plants beside the greenhouse in Assiut University Experimental Farm in March 2000. Apterous adults of approximately the same size were used for all experiments. Fresh samples from the same locality were collected and aphids were transferred alive to the laboratory on the plant leaves in plastic bags immediately before each experiment.

Leaf-Dip Bioassay: A leaf-dip bioassay was carried out to compare the toxicity of the four tested compounds to the laboratory and field populations. Five to seven concentrations of formulated insecticides in aqueous solutions contained 500 ppm triton X-100 as a surfactant were prepared. At each concentration of the insecticide, at least 50 aphids (in replicates of 3) were dipped for 10 seconds in the insecticide solution and allowed at room temperature for about 30 mins. Distilled water contains the surfactant served as control. After the treated batches of aphids had dried, the aphids were transferred to petri-dishes and held at 25±1°C; 60±5% RH and photoperiod 16:8 (L:D). Mortality was assessed 24 hrs. after treatment by examining the aphids using a binocular microscope. An aphid was considered dead if it was incapable of coordinated forward movement. Percent mortality was corrected by Abbott's formula (Abbott, 1925) and values of LC_{50} and slope were determined by a computerized probit analysis programme. The toxicity evaluation for each insecticide tested in each clone or each aphid population was replicated at least 3 times.

Enzyme preparation: Thirty apterous adults of approximately the same size from each tested population were homogenized in 2 ml of potassium phosphate buffer (0.05 M, pH 7.2) using a glass homogenizer. The homogenate was centrifuged at 10,000

r.p.m. for 10 mins. The supernatant was adjusted to 10 ml by the same buffer and kept at 0°C until used.

Carboxylesterase activity: Carboxylesterase activity in homogenates of the laboratory and field population of the two aphid species was tested colourimetrically according to the method of Bracha and Bonard (1966) with slight modifications. The kinetic properties and enzyme activity of each aphid population toward 1-natphthyl acetate (1-NA) as a substrate were determined colourimetrically using Sequoia-Turner model 340 spectro-photometer. In the colourimeter tube, 1.8 ml of the buffer described above was placed. One ml of enzyme solution (representing 3 aphids) was added. Then 0.1 ml of substrate solution (prepared in the same buffer) was added. The reaction was incubated for 20 mins at 30°C. At the end of the incubation period, 100 µl of 4-amino antipyrin (0.4% in distilled water) followed by 100 µl of potassium ferricyanide (0.6% in distilled water) was added. Absorbance at 500 nm was recorded exactly 5 min. after the addition of potassium ferricyanide. Different final concentrations of the substrate ranged between 0.01 and 0.1 mM were used. A complete assay mixture for each substrate concentration but without enzyme solution served as a control. The absorbance data were converted from a calibration curve prepared using several concentrations of 1-napthol under the same experimental conditions.

The kinetic properties of carboxylesterase activity from each aphid species toward 1-napthyl acetate as substrate, i.e. V_{max}, the enzyme maximal velocity and K_m, enzyme Michaelies-Menten dissociation constant, were calculated using the double-reciprocal plots of Lineweaver and Burk (1934). The enzymatic half-life ($t_{0.5}$) describes the time required for a given enzyme concentration to reduce any substrate concentration to one-half

of its initial level. Enzymatic half-life values were computed using the procedure of Main and Braid (1962).

RESULTS AND DISCUSSION

1-Aphicidal activity: Table 1 shows the toxicity and slope values of two organophosphates, one pyrethroid and one carbamate insecticides against the susceptible and field populations of each of the two aphid species, the black legume aphid *Aphis craccivora* Kock and the oat birdcherry aphid *Rhopalosiphum padi* (L.). For the susceptible populations of *A. craccivora* and *R. padi* the LC_{50} values indicated that the descending order of the toxicity for the tested insecticides was the same in the two populations where the cypermethrin was the most toxic insecticide followed by chlorpyrifors, malathion and methomyl. The difference in toxicity between the most toxic (cypermethrin) and the least toxic compound (methomyl) in the susceptible population of *A. raccivora* and *R. padi* was 10.83 and 9.87-fold, respectively.

Concerning the toxic effect of the four tested insecticides against the field populations of the two aphid species, the results in Table 1 revealed that cypermethrin and chlorpyrifos, respectively, exhibited aphicidal activity higher than the toxic effect of methomyl and malathion. On the other hand, methomyl was the least toxic insecticide against the field population of *R. padi* where malathion was the least toxic one against field population of *A. craccivora.*

Based on the leaf-dip-LC_{50} values, the difference in toxicity between the most and the least toxic insecticides was up to 49.84 and 63.21-fold in field population of *A. craccivora* and

Table (1): LC_{50} (ppm), slope values and resistance factors (RF) for the insecticides tested against susceptible and field population of *A. craccivora* and *R. padi*.

Insecticide	*A. craccivora*		*R. padi*	
	Susceptible	Field	Susceptible	Field
Malathion				
LC_{50}	0.544	3.140	0.326	1.676
Slope	2.57	1.66	2.13	0.88
RF	-	5.77	-	5.14
Chlorpyrifos				
LC_{50}	0.196	0.375	0.534	0.675
Slope	1.55	2.22	1.20	1.18
RF	-	1.91	-	1.26
Methomyl				
LC_{50}	0.602	0.929	0.975	8.976
Slope	1.98	1.58	1.12	1.29
RF	-	1.54	-	9.21
Cypermethrin				
LC_{50}	0.06	0.063	0.090	0.142
Slope	1.83	1.20	1.05	0.95
RF	-	1.03	-	1.58

Resistance factor (RF) = LC_{50} of field population/LC_{50} of susceptible population for the same insecticide and the same species.

R. padi, respectively. These difference in toxicity in each field population was higher than the difference in toxicity in corresponding susceptible population for each tested species. This finding may be due to the presence of amplified esterase genes in the genotypes of each field population.

2-Insecticide resistance in field population: The results in Table 1 shows that comparing with the same susceptible population, the field population of *A. craccivora* was less susceptible than the laboratory population by 5.77, 1.91, 1.54 and 1.03-fold for malathion, chlorpyrifos, methomyl and cypermethrin, respectively. Whereas the field population of *R. padi* was less susceptible than the laboratory one by 5.14, 1.26, 9.21 and 1.58-fold for the same corresponding insecticides, respectively. The above results indicated that generally, the field

the same low level of resistance for all the tested insecticides except methomyl in *R. padi.* The field population of *A. craccivora* had a resistance factor against methomyl (RF = 1.54-fold) whereas the field population of *R. padi* had a considerable higher resistance level for methomyl (RF= 9.21-fold). The great difference in resistance level between the two tested species of aphids against the tested carbamate insecticide may be due to the differences in the back history of insecticides application and/or the differences in genetic diversity in resistance mechanism(s) between the two aphid species.

3 - Resistance mechanism:

Carboxylesterase activity: The kinetic properties of carboxylesterase activity of the four tested populations are shown in Table (2). Based on $t_{0.5}$ values, carboxylesterase of the field population of *A. craccivora* was more active than that of the susceptible one toward 1-naphthylacetate by 1.70-fold. The V_{max} value of the field population was higher than that of the susceptible one by 2-fold. While slight difference in K_m

Table (2): Michaelis-Menten kinetic parameters and $t_{0.5}$ values for the hydrolysis of 1-naphthyl acetate by carboxylesterases from susceptible and field populations of *A. craccivora* and *R. padi.*

Parameter	*A. craccivora*		*R. padi*	
	Susceptible	Field	Susceptible	Field
K_m (M)	4.34×10^{-5}	4.65×10^{-5}	1.54×10^{-4}	6.45×10^{-5}
V_{max} µmol (min/aphid)	0.0003	0.0006	0.0040	0.0038
$t_{0.5}$ (min)	0.090	0.053	0.027	0.012

K_m is the dissociation constant, V_{max} is the maximum velocity and $t_{0.5}$ was calculated from 0.693 K_m/V_{max} with units of time.

value (1.07) was found between both populations. These results suggest that the variation in carboxylesterase activity between susceptible and field populations according to $t_{0.5}$ values is dependent on variation in V_{max} than in K_m values. This may lead to suggest that the differences in carboxylesterase activity between the susceptible and field populations of *A. craccivora* toward 1-naphthylacetate are likely to be due to altered amounts of enzymes rather than altered enzymes. This suggestion is in agreement with the finding of other investigations of insecticide resistance in *Aphis gossypii* Glover (El-Ghareeb, 1993) and in *Schizaphis graminum* (Rondani) (El-Ghareeb, 1994).

Interestingly, the $t_{0.5}$ values of *R. padi* (Table 2) revealed that the field population was higher in carboxylesterase activity than the susceptible one by 2.25-fold. A slight difference (<1.1 fold) was found in V_{max} values between the two populations. Values of K_m for the enzymes from the field population to 1-NA was higher (lower affinity) than that of the susceptible one by 2.39-fold. This results suggest presence of altered enzymes in the field population rather than altered amounts of enzymes.

Higher carboxylesterase activity in the enzyme homogenate from the field population of each species compared with that from the susceptible population suggest that carbolxylesterase play a role in the insecticide resistance in both *A. craccivora* and *R. padi.* The possible association between carboxylesterase activity and insecticide resistance in aphid species was also found by several investigators (Devonshire, 1977, Takada and Murakami 1988, Abdel-Aal *et al.*, 1990, Ezz El-Din, 1997 and Mohamed, 1999). In the present study, insecticidal selection pressure in field population may be produces mutant alleles of enzymes which differ from those present in susceptible genotypes. Ibrahim *et al.* (1992) reported that variation in susceptibility of an insect species to insecticides

is an example of the species genetic heterogeneity to the toxic action of these insecticides.

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الملخص العربى

مقارنة سمية بعض المبيدات الحشرية لكل من السلالة الحقلية والمعملية لمّن أفيس كراسيفورا وروبالوسيفم بادى

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دراسة إستجابة المّن أفيس كراسيفورا ، المّن روبالوسيفم بادى لبعض المبيدات الحشرية . كما تم دراسة الدور الذى يلعبه نشاط انزيم الكاربوكسيل إستريز كميكانيكية للمقاومة. وبناء على مقارنة قيم LC_{50} للسلالات الحقلية والسلالات الحساسة لكل من النوعين المختبرين فقد أظهرت السلالة الحقلية للمّن أفيس كراسيفورا درجة تحمل تقدر بـ ٥,٧٧ ، ١,٩١ ، ١,٥٤ ، ١,٠٣ ضعف تجاه مبيدات الملاثيون ، الكلوربيريفوس ، الميثوميل ، السيبرمثرين على الترتيب ، بينما أظهرت السلالة الحقلية لمّن الروبالوسيفم بادى درجات تحمل تقدر بـ ٥,١٤ ، ١,٢٦ ، ٩,٢١ ، ١,٥٨ ضعف تجاه نفس المبيدات المستخدمة على الترتيب .

وباختبار نشاط إنزيم الكاربوكسيل استريز باستخدام ١ نافثيل أسيتات كمادة للتفاعل فى كل من السلالة الحساسة والحقلية لكل نوع فقد دلت قيم $t_{0.5}$ على أن نشاط انزيم الكاربوكسيل استريز فى السلالة الحقلية لأفيس كراسيفورا والروبالوسيفم بادى كان أعلى من نظيره فى السلالة الحساسة لكل نوع بـ ١,٧ ، ٢,٢٥ ضعف على الترتيب . وهذه النتائج تؤدى إلى اقتراح أن نشاط إنزيم الكاربوكسيل استريز يلعب دوراً فى مقاومة السلالات الحقلية لفعل المبيدات لكل من النوعين المختبرين .