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INSECTICIDE SELECTION, RESISTANCE AND ESTERASE KINETICS IN THREE CLONES OF THE GREEN PEACH APHID, *Myzus persicae* (SULZER)

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Abstract: One susceptible and two resistant clones of the green peach aphid, *Myzus persicae* (Sulzer) were selected by 10 ppm of fenitrothion for six generations. The three tested aphid clones were: A susceptible clone with normal chromosomal karyotype and red colour (S); a resistant clone with a normal karyotype and red colour (RDR); and a resistant clone with A1,3-autosomal translocation and green colour (GNR). Each clone was started with parthenogenic female. Values of LD_{50} and slope for two carbamates (carbaryl and pirimicarb) and two organophosphates (fenitrothion and tetrachlorvinphos) were determined topically for the three tested clones before selection. Data revealed that pirimicarb was the most toxic compound against the susceptible clone but tetrachlorvinphos was the least active one against all tested clones. Fenitrothion was the most toxic compound against each of RDR and GNR. The two resistant clones showed resistance ratio (RR) toward all tested compounds compared to the susceptible clone. Values of RR for RDR and GNR clones, respectively, were 30 and 30.8 fold against pirimicarb; 11.77 and 7.27 fold for tetrachlorvinphos; 5.24 and 4.82 fold against carbaryl; and 2.88 and 3.00 fold for fenitrothion. Carboxylesterase kinetic parameters were determined for each clone and each generation using 1-naphthylacetate as a substrate. Values of enzyme kinetics revealed that selection pressure for several generations resulted in some qualitative modification of the enzyme rather than quantitative change. Changing of the carboxylesterase properties in *M. Persicae* (Sulzer) as affected by selection with 10 ppm of fenitrothion for only six generations may suggest to stop using anticholinesterase compounds in controlling aphids.

Key words: Insecticide selection, esterase kinetics, resistance, *Myzus persicae*

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Introduction

The green peach aphid, *Myzus Persicae* (Sulzer) is one of the most important aphid species world wide. Intensive use of insecticides to control aphids have led to the selection of high level resistant individuals. The mode of resistance in aphids seem to be more than one mechanism. Knowledge of the mechanisms of resistance in aphid species is essential for management strategies. Multiple mechanisms of resistance in aphid species has been reported by several investigators (Gubran *et al.*, 1992; El-Ghareeb, 1993; Moores *et al.*, 1994 and Ezzel-Din, 1997). This problem require management to prevent or delay or reverse the development of resistance. The present study was carried out to investigate the effect of fenitrothion selection pressure on carboxylesterases from three clones of those used in the previous work of Ezz Eldin (1997).

Materials and Methods

I- Chemicals:

I.1. Insecticides:

Four insecticidal chemicals representing two groups were used in the present study.

These insecticides are:

I. 1-1. Carbamates:

- Carbaryl 99% (union carbide): 1-naphthyl N-methylcarbamate.

-Pirimicarb 95% (ICI):2-dimethyl amino–5,5-dimethylpyrimidin-4-yl-dimethylcarbamate.

I. 1-2. Organophosphates:

- Fenitrothion E.C. 50% (Sumitomo): 0,0-dimethyl O-(3-methyl-4-nitrophenyl) phosphorothioate.

- Tetrachlorvinphos 99% (Shell): Z-2-chloro-1-(2,4,5-trichlorophenyl)-vinyl 0.0-dimethyl phosphate.

Chemicals Used for General Carboxylesterase Assay:

Fast blue B salt (48) was purchased from T.C.I.; mono and dibasic potassium phosphate, 1-naphthyl acetate (1-NA), 4-aminoantipyrin and potassium ferricyanide from Wako Pure Chem Ind. LTD.

II. Rearing Insects and Selection:

Three clones of the green peach aphid, *Myzus Persicae* (Sulz.), were tested. a susceptible clone with a normal chromosomosal karyotype and red color (S); a resistant clone with a normal karyotype and red color (RDR) and a resistant clone with $A_{1,3}$ – autosomal translocation and green color (GNR). The Karyotype of each clone was previously identified by Yanagihara (1986). Each clone was started with a parthenogenic female received from Prof. Hajimu Takada, Kyoto University. All clones were maintained on cabbage leaves in the laboratory at 25 ± 1° C and

photoperiod of 16 : 8 (L:D) in plastic cages covered with muslin cloth. Apterous adult females of approximately similar size were used in all experiments.

III- Experimental:

III-1- Bioassay:

All bioassay tests were carried out in the laboratory under controlled temperature of 25 ± 1°C. Concentrations of the tested insecticides were dissolved in acetone and aphid were treated topically with 0.2 µl/ individual on the dorsal side using electrically operated ISCO microapplicator model 131 fitted with a 1 ml Agla all glass syringe having a curved blunt-ended 20-gauge hypodermic needle. Each tested concentration was replicated at least three times, each of twenty individuals per replicate. Control aphids were treated with acetone alone. After topical application, aphids were kept in plastic cups with pieces of cabbage leaves, and held at 25 ±1° C. for 24 hours. An aphid was considered dead if it was incapable of a coordinated forward movement. The LD_{50} values in ng of toxicant per aphid were determined by a computerized probit analysis program.

III. 2. Carboxylesterase assay:

III. 2-a. Esterase preparation:

One hundred apterous adults of approximately similar size from each clone were homogenized in 2 ml of 0.05 M potassium phosphate buffer (PH 7.2) by glass homogenizer on ice bath. The homogenate was centrifuged at 10.000 xg for 10 min. The supernatant was adjusted to 20 ml by the same buffer.The diluted homogenate was used in carboxylesterase assay and for determining its protein content.

III. 2-b. Carboxylesterase activity:

To determine the kinetic properties, 1.8 ml of the potassium phosphate buffer (0.05 M; PH 7.2) was placed in a test tube, followed by 1 ml of enzyme solution. One hundred µl of 1-naphthyl acetate (1-NA) dissolved in acetone was added as a substrate. The reaction was incubated for 20 min. at 30 °C. At the end of the incubation time, 100 µl from each of 4-aminoantipyrine (0.4%) and potassium ferricyanide (0.6%) in distilled water were added. Absorbance at 500 nm, was measured 5 min after the addition of potassium ferricyanide using sequoia – turner model 340 spectrophotometer. A control tube containing everything except the enzyme solution was used to correct for nonenzymatic spontaneous hydrolysis of the substrate. An extinction coefficient of 0.0136 was used to calculate the concentrations of the 1-naphthol that was released form the reaction of aphid carboxylesterase with 1-NA. the kinetic parameters of esterase activity toward 1-NA was evaluated by applying double reciprocal treatments (Lineweaver

and Burk, 1934). The enzymatic half- life (t0.5), as a measure of the time required for a given enzyme concentration to reduce any substrate concentration to one – half of its initial level was calculated from a combined relationship of V_{max} and K_m (Main and Braid, 1962).

The present study was carried out based on the results of the previous work of Ezz Eldin (1997)

Results and Discussion

Table 1 shows the topical LD_{50} values and slopes of LDp lines of two carbamates (Carbaryl and pirimicarb) and two organophosphates (fenitrothion and tetrachlorvinphos) against the susceptible (S) and two resistant (RDR and GNR) clones of the green peach aphid, *Myzus persicae* (Sulzer). Pirimicarb was the most toxic compound against the susceptible clone, followed by fenitrothion, carbaryl and tetrachlorvinphos. The LD_{50} values for the previous compounds were 2.27, 16.33, 495 and 593 ng/aphid, respectively. Both of resistant clones showed different ranking order. Fenitrothion was the most toxic compound against each of RDR and GNR, followed by pirimicarb, carbaryl and tetrachlorvinphos. Values of LD_{50} for RDR clone were 47, 68, 2592 and 6977 ng/aphid, respectively. While LD_{50} values for GNR clone were 49, 70, 2387 and 4313 ng/aphid for the same corresponding compounds. Quantitatively, primicarb was more toxic than fenitrothion, carbaryl and tetrchlorvinphos by 7.19, 218 and 261 fold, respectively, in S clone. While fenitrothion was more toxic than pirimicarb, carbaryl and tetrachlorvinphos by 1.44, 55.15 and 148.45 fold, respectively, in RDR clone and by 1.43, 48.71 and 88.02 fold in GNR clone for the same corresponding insecticides.

Table(1): LD_{50} (ng/aphid), slope and resistance ratio values of two carbamates (Carbaryl and pirimicarb) and two organophosphates (Fenitrothion and tetrachlorvinphos) against susceptible (S) and two resistant (RDR and GNR) clones of *M. persicae*.

Compound	S		RDR			GNR		
	LD_{50}	Slope	LD50	Slope	RR	LD_{50}	Slope	RR
Carbaryl	495	2.21	2592	3.41	5.24	2387	2.58	4.82
Pirimicarb	2.27	1.84	68	4.98	30	70	5.11	30.82
Fenitrothion	16.33	3.15	47	5.23	2.88	49	4.96	3.00
Tetrachlorvinphos	593	2.58	6977	2.17	11.77	4313	2.85	7.27

The fact that fenitrothion was the most active compound against resistant clones and not the susceptible one suggests that higher activity of mixed function oxidases (MFO) in resistant clones may be

the reason.. Since fenitrothion is an organophos-phorus compound with phosphor-rothioate moiety, higher MFO activity can enhance the activation of fenitrothion to its phosphate analoge (fenitroxon). The later compound is known as other phosphate to be more potent anticholinesterase than its parent compound (Attia *et al.,* 1979; and Ezz Eldin, 1997).

The two resistant clones showed resistance ratios (RR) toward all tested compounds compared to the susceptible clone (Table 1). The highest RR values were 30 and 30.8 for pirimicarb; followed by 11.77 and 7.27 against tetrachlorvinphos; 5.24 and 4.82 for carbaryl; and 2.88 and 3.00 for fenitrothion, for RDR and GNR clone, respectively. However, RR values were less than 10 fold for all of the tested compounds except pirimicarb which exhibited around 30 fold for each of resistant clones. The high resistance ratio in the two resistant clones toward pirimicarb out of all tested anticholinesterase insecticides suggests that more than one mechanism might be involved in resistance. Multiple mechanisms of insecticide resistance have been reported in several aphid species by some other investigators (Yun – qin *et al.,* 1987; Hamilton *et al.,* 1981 and Kerns and Gaylor, 1992). Special mechanism of resistance against pirimicarb was also reported (Kwon *et al.,* 2009)

High slope values in Table 1 indicated high homogeneity (as expected in parthenogenic insects) among the tested clones. Slope values ranged from 1.84 to 5.23. Interestingly, very high slope values were found in RDR and GNR clones toward pirimicarb (4.98 and 5.11, respectively) compared with less slope value (1.84) for S clone toward the same compound. This result confirms the possibility of some variability within individuals.

Carboxylesterase Kinetics:

Based on Michaelis – Menten assumptions, carboxylesterase kinetics were applied to 1-naphthyl acetate hydrolyzing enzymes in the three tested clones of *M. persicae* (Sulzer). Values of K_m, V_{max} and $t_{0.5}$ are listed in Table 2. Kinetic parameters of generation number 1 in Table 2 (unselected aphids) were obtained from the same clones which used to obtain the toxicity data presented in Table 1. Little differences were observed in V_{max} values among the three clones before selection pressure. V_{max} values of RDR and GNR clones in the first generation (before selection) were higher than that of S clone by 1.38 and 1.33 fold, respectively. While in the last selected generation, the corresponding values were 1.32 and 1.12 fold, respectively.

Regarding enzyme Michaelis – Menten dissociation constant (K_m), big change in K_m values of the three clones at certain generation of selection was detected. The big change in K_m value in S, RDR and GNR clones were at the 5^{th}, 3^{rd} and 6^{th} generations, respectively

Table(2): Michaelis-Menten kinetic parameters and t0.5 values for the hydrolysis of 1-naphthyl acetate (1-NA) by carboxylesterases from a susceptible (S) and two resistant (RDR) clones of the green peach aphid. Km is the dissociation constant, Vmax is the maximum velocity and t0.5 was calculated from 0.693 Km/Vmax with the units of time x enzyme

Clone	S							RDR							GNR						
Generation	1	2	3	4	5	6	7	1	2	3	4	5	6	7	1	2	3	4	5	6	7
K_m	$1.67X10^{-4}$	$1.67X10^{-4}$	$1.75X10^{-4}$	$1.603X10^{-4}$	$6.67X10^{-5}$	$6.5X10^{-5}$	$3.33X10^{-5}$	$1.56X10^{-4}$	$1.4X10^{-4}$	$2.7X10^{-5}$	$2.27X10^{-5}$	$2.17X10^{-5}$	$2.72X10^{-5}$	$1.43X10^{-5}$	$1.56X10^{-4}$	$1.4X10^{-4}$	$1.15X10^{-4}$	$1.065X10^{-4}$	$8.13X10^{-5}$	$4.55X10^{-5}$	$4.49X10^{-5}$
V_{max} µmol/min/mg protein	0.06	.061	0.069	0.065	0.077	0.074	0.076	0.083	0.10	0.118	0.110	0.111	0.127	0.10	0.08	0.10	0.077	0.081	0.074	0.071	0.085
$t_{0.5}$	1.928	1.903	1.758	1.71	0.6003	0.61	0.304	1.303	0.9702	0.1586	0.143	0.1355	0.148	0.0991	1.17	0.9702	1.035	0.911	0.761	0.4441	0.366

(from 16.03 x 10^{-5} to 6.67 X 10^{-5} M in S, from 14.00 X 10^{-5} to 2.7 X 10^{-5} M in RDR, and from 8.13 X 10^{-5} to 4.55 X 10^{-5} M in GNR). These results indicate that selection pressure by fenitrothion for several generations resulted in changing K_m values for the enzymes from each of susceptible and resistant clones to be lower (higher affinity) than that of the first generation (without selection). Values of $t_{0.5}$ is considered to be a better and practical measure because it takes both K_m and V_{max} into consideration. $T_{0.5}$ values for general carboxylesterases from the three clones (Table 2) indicate that lower $t_{0.5}$ values were detected with the resistant clones. Based on $t_{0.5}$ values, RDR and GNR were 1.48 and 1.65 times more active in hydrolyzing 1-naphthyl acetate than that from S clone, respectively. Selection pressure by fenitrothion for 6 generations resulted in enhancing carboxyl-esterase activity (comparing $t_{0.5}$ in the first generation with that of the 6^{th} selected one) by 6.34, 13.15 and 3.20 fold for S, RDR and GNR clones, respectively. The susceptible clone became very close to the resistant GNR in carboxylesterase activity showing $t_{0.5}$ value of 0.304 (Table 2) after six generations of selection. In summary, these results suggest that using any of organophospohate compounds for several generations in aphids may be lead to high level of resistance. The present study also showed that the resistant clone with normal karyotype (RDR) was able to build up higher level of resistance compared with the translocated karyotype resistant clone (GNR). These results suggest that insecticidal selection pressure may led to chromosomal translocation in RDR clone and / or expression of the resistance gene may be also involved. The time required for the three tested clones to reach high level of biochemical changes is considered very short (less than 6 generations). This biochemical changes were independent of Karyotype of the tested aphid clone. In conclusion, this study highly recommend avoiding using anticholinesterase compounds in controlling the green peach aphid.

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الانتخاب بالمبيدات الحشرية و المقاومة و كينتيكية انزيمات الاستريز فى ثلاثة مستعمرات لمَنْ الخوخ الأخضر ، ميزس بيرسيكا (سولزر)

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تم انتخاب سلالة حساسة ، وسلالتين مقاومتين لمن الخوخ الأخضر ميزس بيرسيكا (سولزر) بتركيز 10 جزء فى المليون من مبيد الفنيتيروثيون وذلك لستة أجيال ، وكانت الثلاثة سلالات للمن هى: سلالة حساسة ذات كاريوتيب عادى ولونها أحمر (S) ، سلالة مقاومة ذات كاريوتيب عادى أيضا ولونها احمر (RDR) ، وسلالة مقاومة بها انتقال كروموسومى $A_{1,3}$ ولونها أخضر (GNR). وقد بدأت تربية كل مستعمرة منهم بأنثى واحدة تتكاثر بكرياً. وقد تم تقدير قيمة LD_{50} ، الميل لاثنين من مركبات الكربامیت (الكرباريل ، البيريميكارب) ، اثنين من المركبات الفوسفورية (الفنيتروثيون ،التتراكلورفينفوس) بطريقة المعاملة السطحية للثلاثة مستعمرات قبل إجراء الانتخاب ، وقد أوضحت النتائج أن مبيد البيريميكارب كان هو الأكثر سمية ضد المستعمرة الحساسة ولكن كان مبيد التتراكلورفينفوس هو الأقل سمية تجاه جميع المستعمرات المختبرة ، وكان مبيد الفنيتروثيون هو الأكثر سمية ضد كل من GNR , RDR. وقد أظهرت المستعمرتين المقاومتين درجة من المقاومة تجاه جميع المركبات المختبرة بمقارنتها بالمستعمرة الحساسة ، وكانت قيم الـ RR 30 ، 30.8 ضعف ضد البريميكارب و 11.77 ، 7.27 ضعف ضد التتراكلورفينفوس و 5.24 ، 4.82 ضعف ضد الكرباريل و 2.88 ، 3.00 ضعف ضد الفنيتروثيون وذلك لمستعمرة ,GNR RDR على التوالى وقد تم تقدير نشاط إنزيمات الكاربوكسيل استريز لكل مستعمرة وكل جيل باستخدام 1– نافثيل أسيتات كمادة للتفاعل وقد أوضحت هذه القيم أن الضغط الانتخابى لبضعة أجيال أسفر عن احتمال لبعض التغيرات النوعية للإنزيم بدلاً من التغيرات الكمية ، وتغير خصائص الكربوكسيل استريز فى الميزس بيرسيكا (سولزر) تأثراً بالانتخاب بـ 10 جزء فى المليون من مبيد الفينيتروثيون لمدى ستة أجيال فقط ربما يؤدى إلى اقتراح التوقف عن استخدام مضادات الكولين استريز فى مكافحة المن.