Comparative Toxicity of Some Conventional Insecticides Against *Culex Pipiens* L. Mosquito Larvae from Different Districts in Egypt

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ABSTRACT: The susceptibility of *Culex pipiens* larvae to some insecticides commonly used in mosquito control programs [cypermethrin (Sparkill®), deltamethrin (Embrator®), temephos (Temepest®) and spinosad (Tracer®)] was investigated, also variations in esterases and glutathione S- transferases (GST) activities among three field populations (Abou homoss, Nadi El Said, Montaza) were measured and compared with a laboratory susceptible strain (S). The highest level of resistance against the tested insecticides was found in Abouhomoss strain (27.3, 22.2 and 24.8- fold) and the lowest level of resistance was recorded in Montaza strain (12.75, 15.17 and 8.17-fold) towards cypermethrin, deltamethrin and temephos, respectively. On the other hand, all strains recorded no resistance against spinosad (Tracer®). All field strains revealed significantly higher levels of GST and esterases activities compared with the laboratory susceptible strain. The results of the present study suggest that esterases and glutathione S- transferases enzymes have major role in *Culex pipiens* resistance to the evaluated insecticides.

Keywords: *Culex pipiens*, estrases, glutathione S-transferases, cypermethrin, deltamethrin, temephos, spinosad.

INTRODUCTION

Mosquitoes are the most important arthropod disease vectors, transmitting nine dreadful human diseases in over 100 countries, causing mortality of nearly two million people every year (Knudsen and Slooff, 1992; Klempner *et al.*, 2007), therefore, the mosquito control continues to be an important strategy in preventing the mosquito-borne diseases (Nauen , 2007; Billingsley *et al* ., 2008; Midega *et al*., 2010). Mosquito control relies mainly on the chemical control using organophosphate, carbamate and pyrethroid insecticides. The extensive and indiscriminate applications of synthetic chemical insecticides lead to widespread development of resistance by mosquitoes and unwarranted toxic or lethal effects on non-target organisms (Roberts and Andre, 1994; Nauen , 2007) .

Insecticide resistance is a complex evolutionary phenomenon, which can potentially cause large problems in the control of agricultural insect pests and disease vectors, and it is an increasing problem for mosquito control in different parts of the world (Canyon and Hii, 1999; Katyal *et al*., 2001; Saleh *et al*., 2003; Nazny *et al*., 2005; Tawatsin *et al*., 2007). It is necessary, from time to time, to monitor the susceptibility status of local mosquito vectors to the insecticides used in the control programs. Documentation of insecticide resistance will identify insecticides that are no longer effective and is a critical first step towards developing resistance management programs (Panlawat *et al*., 2005).

The more efficient detoxification mechanisms, also known as metabolic resistance mainly occurs due to an increase in the expression or activity of three major enzyme families: esterases (EST), glutathione-S-transferases and the cytochrome P450 superfamily of enzymes (Li *et al*., 2007; Braga and Valle 2007; Russell *et al.*, 2011).

Measuring the activity of these enzymes in natural populations is an important step in monitoring insecticide resistance mechanisms worldwide and should be conducted together with the surveillance of control efficacy to prevent significant changes in susceptibility to the insecticides being used (Coleman and Hemingway, 2007; Polson *et al.*, 2011). Metabolic-based resistance mechanisms are important in conferring insecticide resistance. Detoxifying enzymes, primarily esterases, glutathione-S- transferases and monooxygenases , may be qualitatively or quantitatively changed to confer resistance (Cui, *et al*., 2007) . Glutathione-S-transferase enzymes (GST) play an important role in detoxification of xenobiotic compounds including insecticides. GSTs can produce resistance to a range of insecticides by conjugating reduced glutathion (GSH) to the insecticide or by its primary toxic metabolic products (Hemingway, 2000; Enayati *et al*., 2005). There is limited information on susceptibility levels of mosquito vectors to insecticides in Alexandria and Bouherra. So the objective was to determine the current susceptibility status of *Culex pipiens* larvae, the primary vector of filariasis, to some insecticides commonly used in mosquito control programs. Moreover, activities of esterases and glutathione S- transferases (GST) activities among three field populations (Abou homoss, Nadi El Said, Montaza) with a laboratory susceptible strain to investigate the role of these enzymes in *C. pipiens* resistance to the commonly used insecticides.

MATERIALS AND METHODS

Insect strains: A *Culex pipiens* L. (Diptra: Culicidea) colony maintained in the laboratory of Medical and Veterinary Insects, Department of Economic Entomology, for more than 10 years was used as susceptible strain (S). The field strains were collected from different ponds from Abou Homouss (El-Bouherra governorate), Montaza and Nadi El Said districts (Alexandria governorate). Larvae were cultured in the laboratory for one generation. Mosquitoes were reared at 27±1 °C, 70±5% RH, and a photo regime of 14: 10 hr (light: dark). adults were provided with a 10 % sucrose solution as food source. A pigeon was introduced twice a week to the adults for blood feeding. Larvae were reared in dechlorinated water under the same temperature and light conditions and were fed daily with baby fish food.

Insecticides used: Cypermethrin (Sparkill® 25% EC) was provided by Anchor Co. Egypt, deltamethrin (Embrator®) 2.5% EC was supplied by KZ CO. Egypt, temephos (Temepest®) 50% EC was obtained from Kalyanyi industries, India and spinosad (Tracer®) 24% SC was provided by Dow Agrosciences CO.

Larvicidal bioassay: The larval susceptibility test was conducted according to WHO guidelines (WHO, 1975 and 1981) using early fourth instar larvae. Sufficient numbers of larvae were kept in the same breeding water till the test was carried out. Series of each tested insecticide concentrations were prepared in addition to control were replicated four times. Lots of 25 larvae

were used for all the experiments that have been conducted at 27± 1 °C and 75± 5 RH. Mortality counts were carried out after 24hr of treatment. Mortality percentages were calculated and corrected according to Abbott (1925). Values of LC_{50}, confidence limits and slop functions were calculated and ascertained using probit analysis according to Finney (1971).

Biochemical analysis:

Sample preparation: Batches of 200 mg early fourth instar larvae, from each mosquito strain, were homogenized in 2ml of 0.1 M phosphate buffer pH 7.4 using a glass homogenizer immersed in ice cubes. The homogenates were centrifuged for 30 min at 10.000 xg at 4°C using Cryofuge 20-3, Heraeus Christ Centrifuge. The supernatant was used as crude enzyme extract for enzymes assay.

Protein measurements: The protein concentration of enzyme extract was determined by the method of Bradford (1976). Absorbance at 595 nm was carried out. Each sample was replicated three times and the protein concentration extrapolated from a standard curve using bovine serum albumin.

Glutathione S- transferases assay: GST activity was measured according to the method of Asaoka and Takahashi (1983). Results were presented as a specific activity ±SD.

Esterases assay: Esterase activity was measured according to He (2003) using α- naphthyl acetate as a substrate. Production of α- naphthol was monitored with a spectrophotometer at 320 nm. All assays were done in triplicate at 37°C. The reaction mixture (1 ml) contained 50 mM sodium phosphate (PH 7.0) , 10 mM $MgCl_2$, 50 mM substrate and 100 µl crude esterase homogenate. The enzyme blank reference cuvette was used without the protein as a control.

Statistical analysis: Data was subjected to analysis of variance (ANOVA) (CoStat Statistical Software, 1990). The standard deviation (SD) of four replications was calculated. Means were compared with each other using Student- Newman Keuls (SNK) test (LSD at $P < 0.05$).

RESULTS AND DISCUSSION

Toxicity of the tested insecticides against four strains of *C. pipiens*:

Toxic effect of the selected insecticides against *Culex pipiens* 4^{th} instar larvae of the laboratory and field strains, collected from different locations was evaluated. The probit analysis of the obtained data illustrated the insecticidal activity of the selected insecticides as LC_{50} values (Table 1). Data showed that the 4^{th} instar larvae of the field strains demonstrated varied levels of resistance to the tested insecticides. It is clear that Abou homoss strain has the highest levels of resistance against the tested insecticides with resistance ratios of 27.3 , 22.2 and 24.8 – fold towards the evaluated insecticides cypermethrin (Sparkill®), deltamethrin (Embrator®) and temephos (Temepest®) , respectively. This high level of resistance may be due to the intensive use of insecticides in surrounding agricultural areas. Regarding Nadi El Said strain,

the resistance ratios were 15.6, 17.6 and 10.7 – fold towards cypermethrin (Sparkill®), deltamethrin (Embrator®), and temephos (Temepest®), respectively. Montaza strain recorded the lowest resistance ratios against the tested insecticides (12.75, 15.17 and 8.17-fold towards cypermethrin, deltamethrin and Temephos, respectively). The present results clearly suggest the differential resistance ratio of the three *Culex pipiens* field strains to cypermethrin, deltamethrin and temephos when they were compared with the lab. strain. The strategy for the control of vector population with the restricted group of insecticides is very crucial and facing challenge nowadays. Rotational use of different groups of insecticide rather than the use of different members of same group of insecticides is more effective to reduce and deal with the resistance problem. Carbamates and organophosphates must be used in rotation in order to maintain the pyrethroids susceptibility (Nauen, 2007). . Resistance against 5% deltamethrin was reported in *Culex quinquefasciatus* from Lahore, Pakistan (Tahir *et al.*, 2009). On the other hand, all strains recorded no resistance against spinosad (Tracer®), therefore, Tracer® can be used as a good alternative for mosquito control. This result agreed to a large extent with the findings of Darriet *et al*. (2005) who found that spinosad was significantly more effective against *An. gambiae* than against the other two mosquito species (*Cx. quinquefasciatus* and *Ae. aegypti)*, and was more effective against *Cx. quinquefasciatus* than against *Ae. aegypti*. No significant difference was noted between the susceptible and resistant strains of each mosquito species. Currently, mosquito control depends on chemical or biological insecticides that cause as small toxic effect as possible against man and the environment. In this regard, spinosad proved to be a valid alternative for eliminating the larvae of many culicid species because it is a mixture of two natural compounds produced during the fermentation of spinosad and it has LC_{50} of 5000mg/kg for rats (Tomlin, 2000). This larvicide, which has been noted for its successful use for control of *Ae. aegypti* and *An. albimanus* larvae in Mexico (Bond *et al.* 2004), merits detailed evaluation with other mosquito species, especially because the absence of cross-resistance with common insecticides (pyrethroids, carbamates, and organophosphates) which makes spinosad a potential candidate for disease vector control, particularly in areas which where mosquitoes are resistant to insecticides. Perez *et al.* (2007) concluded that spinosad was as effective as temephos granules in eliminating the immature stages of *Aedes* spp.The present results also agreed with Jones (2012) who found that the susceptibility of *Culex quinquefasciatus* to spinosad did not differ between the laboratory reference strain (Sebring-S) and field collected mosquitoes.

Table (1): Median lethal concentrations of some evaluated insecticides against three field strains of *Culex pipiens* compared to the laboratory strain.

insecticide	Mosquitostrain	LC_{50} (mg/l)	Lower limit (mg/l)	Upper limit (mg/l)	RR*	Slope ± S.E
	Lab	0.0016	0.0007	0.0039	1.0	1.35 ± 0.109
Cypermethrin	Abou homoss	0.04368	0.06	0.090	27.3	1.5 ± 0.125
(Sparkill®) 25%EC	Nadi El Said	0.0249	0.0171	0.081	15.56	1.35 ± 0.112
	montaza	0.0204	0.0192	0.029	12.75	1.41 ± 0.113
	Lab	0.0006	0.0005	0.0007	1	1.45 ± 0.150
Deltamethrin	Abou homoss	0.0133	0.0155	0.024	22.16	1.5 ± 0.162
(Embrator®)2.5%EC	Nadi El Said	0.0106	0.007	0.024	17.6	1.41 ± 0.152
	montaza	0.0091	0.01	0.0153	15.1	1.48 ± 0.158
	Lab	0.0006	0.0004	0.0009	1	1.63 ± 0.131
Temephos	Abou homoss	0.0149	0.0199	0.027	24.83	1.78 ± 0.147
(Temepest®)50%EC	Nadi El Said	0.0064	0.0053	0.0117	10.66	1.66 ± 0.136
	montaza	0.0049	0.0049	0.006	8.16	1.67 ± 0.135
	Lab	0.14	0.103	0.277	1	2.04 ± 0.190
Spinosad	Abou homoss	0.714	0.678	1.3005	5.1	2.9 ± 0.250
(Tracer®) 24% SC	Nadi El Said	0.336	0.2505	0.5808	2.4	2.68 ± 0.238
	montaza	0.49	0.385	0.875	3.5	2.28 ± 0.217

*RR=Resistance Ratio

Activity of glutathione S-transferase (GST) and esterases in the tested strains of *Culex pipiens* :

GST and esterases activities in the susceptible laboratory strain and three field strains are shown in Fig. (1). Activities of GST and esterases were found to be significantly higher in all field strains compared to the activities of the susceptible one. The GST activities in the field strains (Abou homoss , Nadi El Said and montaza strains) were 2.57, 2.00 and 1.65 - fold , respectively, of that of the laboratory strain Abou homoss strain recorded the highest esterases activity (2.26- fold) compared with that of the laboratory strain, while Nadi El Said and montaza strains esterases activities were 2.11 and 1.83-fold of the laboratory strain esterases activities. There was a significant correlation between all enzyme activity levels and insecticide resistance phenotype by populations. The most probable reason for this, as explained by Ahmed and Wilkins (2002), when an insecticide enters an organism, before reaching its target site, it could meet with different enzyme and protein obstacles and as a result of interactions with these enzymes the insecticide is degraded. The latter results agreed to a large extent with the findings of Bisset *et al.* (2011) who recorded high levels of resistance in all tested strains of the mosquito *Aedes aegypti* and also they found that resistance ratios were highly correlated with esterase activity (P = 0.00001). On the contrary, the present finding of the correlation between the GST activities and the resistance ratio was disagreed with Bisset *et al.* (2011) who reported that neither GST nor monooxygenases were associated with the increase in *Aedes aegypti* resistance to temephos.

The increased detoxification is a common mechanism of resistance to pesticides (Openoorth, 1985). In *Culex pipiens*, such a mechanism is often involved in resistance to organophosphates . However, the low levels of

organophosphate and pyrethroid resistance could be conferred by either the elevated esterase or monooxygenase enzymes (Penilla *et al*., 1998).

Several earlier workers stated that pyrethroids do not serve as substrates for GST (Reidy *et al*., 1990; Grant and Matsumura , 1989) . So, other enzymes systems have been proposed as being responsible for conferring metabolic detoxification of pyrethroids. However , induction of GST activity has been reported not only after exposure to organophophates and organochlorides but also against pyrethroid (Yu and Nguyen , 1996; Kostarpoulos *et al*., 2001). Reidy *et al*. (1990) and Grant and Matsumura (1989) reported that there were correlation between the elevated levels of GST and resistance to pyrethroids for *Tribolium castaneum* and *Aedes aegypti*, respectively. Therefore, the significantly higher level of GST activity might play a role in pyrethroid resistance in *Culex pipiens* along with esterase activity.

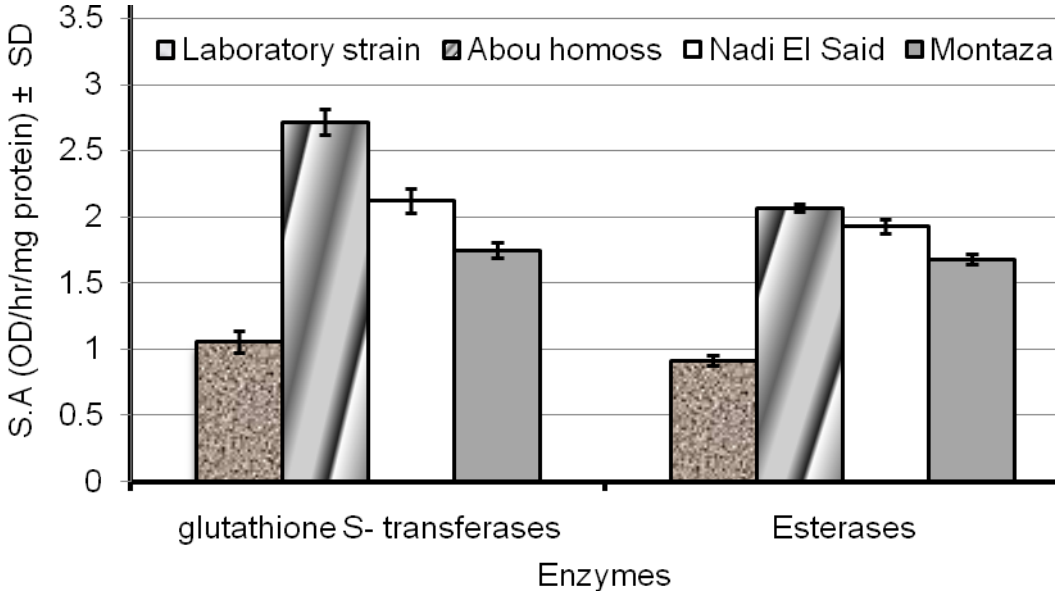

Figure 1: Activity of gluatthione S- transferases and esterases in the field and laboratory strains of *Culex pipiens* .

The results of the present study showed that the field populations have highest levels of esterases and glutathione S-transferases activities and resistance to the tested insecticides. These results suggest that esterases and glutathione S-transferases enzymes have major role in metabolic resistance to the used insecticide.

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الملخص العربي

مقارنة سمية بعض المبيدات الحشرية التقليدية ضد يرقات بعوض الكيولكس بيبينز من مناطق مختلفة في مصر

حسام الدين مجدى زهران

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تم دراسة حساسية العمر اليرقي الرابع لبعوض الكيولكس بيبينز لأربعة من المبيدات المعتاد إستخدامها ضمن برامج مكافحة البعوض و هي سيبرمثرين (سباركل®)، دلتامثرين (إمبراطور®)، تيميفوس (تيمبست®) و الإسبينوساد (تريسر®). كذلك تم دراسة الإختلاف فى النشاط النوعى لإنزيمات الإستيريز و الجلوتاثيون – إس ترانسيفيريز فى ثلاثة سلالات حقلية [أبو حُمص (محافظة البحيرة) ونادى الصيد والمنتزه (محافظة الأسكندرية)] ومقارنتها بالسلالة المعملية الحساسة وذلك لمعرفة الدور الذى تلعبه هذه الإنزيمات فى تطور ظهور المقاومة فى البعوض تجاه تلك المبيدات المختبرة. وقد وجد أن أعلى مستوى مقاومة للمبيدات المختبرة كان فى سلالة أبو حمص بمقدار 27.3 ، 22.2 ، 24.8 ضعف. أقل مستوى مقاومة كان فى سلالة المنتزه بمقدار 12.75 ، 15.17 ، 8.17 ضعف تجاه المبيدات المختبرة سيبرمثرين (سباركل®)، دلتامثرين (إمبراطور®)، تيميفوس (تيمبست®) على الترتيب. على الجانب الآخر، وجد أن جميع السلالات المختبرة لم تسجل أي مقاومة تجاه المبيد سبينوساد (التريسر®). هذا وقد أظهرت جميع السلالات الحقلية المختبرة زيادة معنوية فى النشاط النوعى لإنزيمات الإستيريز و الجلوتاثيون – إس ترانسيفيريز مقارنة بالسلالة المعملية الحساسة .

وقد اتضح من خلال نتائج الدراسة الحالية أن لإنزيمات الإستيريز و الجلوتاثيون – إس ترانسيفيريز لها دور أساسي فى تطور ظهور المقاومة فى بعوض الكيولكس بيبينز تجاه المبيدات المختبرة .