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Monitoring the Effect of Insecticide Selection on *Culex pipiens* (Diptera: Culicidae) Larval Susceptibility to Malathion and Lambda-Cyhalothrin

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ABSTRACT
The susceptibility of *Culex pipiens* collected from different localities of the Sharkia Governorate, Egypt, to malathion and lambda-cyhalothrin was investigated for 11 successive generations. Larval *Cx. pipiens* developed 57 and 305-fold resistance to malathion and lambda-cyhalothrin, respectively after 11 successive generations of selection pressure. The susceptibility of unselected generations, due to non-exposure to both insecticides for 11 generations, was increased to 1.1-fold in F_{11} for malathion and to 1.5-fold in F_{11} for lambda-cyhalothrin. Acetylcholinestrase (AChE) activity increased gradually in both selected and unselected generations until the 11th generation. The activity of AChE in generations (F_1-F_7) selected with malathion was significantly lower than that of the lambda-cyhalothrin selected and unselected generations. General esterase activity increased 3.7 and 3.0-fold (malathion) and 4.2 and 3.6-fold (lambda-cyhalothrin) compared with the susceptible strain (LS-CP), when either α-napthyl acetate (NA) or β-NA were used as general substrates in F_{11} selected generations, respectively. A significant increase in glutathione-S-transferase (GST) activity was noticed in the F_{11} generation recording 6400 and 8800 μmoL min^{-1} mg^{-1} protein for malathion-and lambda-cyhalothrin-selected generations, respectively. The ratios recorded in the 11th generations were 3.6 and 4.9 fold as compared with LS-CP for malathion and lambda-cyhalothrin selected generations, respectively. Our results indicate that the *Cx. pipiens* mosquito strain from Egypt can increase resistance to malathion and lambda-cyhalothrin if these insecticides are continuously or rotationally used to control this species. Increased resistance is likely to be associated with increased activity of target and metabolic enzyme systems.

Key words: *Culex pipiens*, malathion, lambda-cyhalothrin, AChE, esterases, GST

INTRODUCTION
Culex pipiens Linnaeus (Diptera: Culicidae) is an important vector of several human pathogens such as West Nile virus, Rift Valley Fever virus and Bancroftian filariasis (Claire and Callaghan, 1999). *Cx. pipiens* is found in tropical areas (Bourguet *et al*., 1998) and different species have been reported in parts of Africa, Russia, Australia, North and South America (Azari-Hamidian, 2007). *Cx. pipiens* is both a nuisance and a disease vector (Dehghan *et al*., 2011), affecting more than 700 million people annually (Taubes, 2000). In Egypt, *Cx. pipiens* is one of the most common mosquito species in urban and rural areas and causes a human health risk (Zahran and Abdelgaleil, 2011).

The control of mosquitoes depends primarily on continued applications of different insecticide classes (Rozendaal, 1997), which rotational use may result in continued satisfactory control against field populations of house mosquitoes (Shin *et al*., 2012). Although they are effective, their continuous and repeated use has resulted in the widespread development of resistance (Perumalsamy *et al*., 2010). As insects become resistant, more insecticides are used which can cause human and environmental health problems. Widespread insecticide resistance to commonly used and less expensive insecticides has been a major obstacle in implementing cost-effective and safe integrated mosquito management.

Insecticide resistance in mosquitoes is essentially achieved through two mechanisms: target insensitivity and increased detoxification (Nauen, 2007). The former is associated with target modifications that lower their affinity for the considered insecticide. Increased detoxification results from an increased activity of detoxifying enzymes such as esterases and GSTs and the specific carboxylesterase which was reported for malathion resistance in *Drosophila melanogaster* by Ashour *et al*. (1987a).

The effect of resistance on the chemical control of mosquitoes is very difficult to determine due to a large number of associated factors that may impact on successful control in the field. Understanding the relationship between insecticide resistance and metabolic resistance mechanisms is important in order to address the knowledge gap between control strategies and developing resistance (Rajatileka *et al*., 2011). The aim of this study was to test the susceptibility of *Cx. pipiens* populations, collected from different localities at Sharkia Governorate in Egypt, to the most commonly used insecticides for mosquito control (malathion and lambda-cyhalothrin) and to assess the relative activities of detoxification and target enzymes in association with resistance.

MATERIALS AND METHODS

Test mosquitoes: A mixed population of *Cx. pipiens* was established from larvae collected from five field localities in Sharkia Governorate, Egypt, during July and August of 2010. The collected *Cx. pipiens* larvae identified in the Research Institute of Medical Entomology, Mosquito Department, Egyptian Ministry of Health, which the source of a laboratory susceptible (SS) strains (LS-CP strain) of the same species that maintained in the laboratory for 15 years without any insecticidal exposure. The collection localities were Al-Asher of Ramadan (PR), Diarb Negm (PD), Faquos (PF), El-Salhia (PS) and El-Zagazig (PZ) (Fig. 1). The collected larvae were transferred to an insect rearing room and reared in plastic trays (30×20×10 cm) containing 3000 mL tap water and 1.0 g of sterilized rodent diet. The first generation (F1) was obtained from rearing 2500 fourth instar larvae (500 larvae/locality) in one tray. Pupae were subsequently transferred to adult cages for adult emergence. Adult mosquitoes were maintained on a 10% sucrose solution and were allowed to blood feed on a domestic pigeon for oviposition under an approved animal use protocol. All stages were maintained at 27±2°C, 65-75% Relative Humidity (RH) and 14:10 h light:dark cycle (WHO, 1975).

Insecticides and reagents: Malathion (Malathion, 57% EC) and lambda-cyhalothrin (Lambda, 5% EC) were used in this study. Malathion and lambda-cyhalothrin formulations were obtained from a local manufacturer, Kafr El-Zayat for Pesticides and Chemicals Company, Kafr El-Zayat City, Gharbia Governorate, Egypt. α-Naphthyl acetate (α-NA), β-naphthyl acetate (β-NA), α-naphthol and β-naphthol were obtained from Alfa Aesar Co (Karlsruhe, Germany). 5,5-dithiobis nitrobenzoic acid (DTNB), acetylcholine iodide substrate (ATChI), 1-chloro-2,4-dinitrobezene

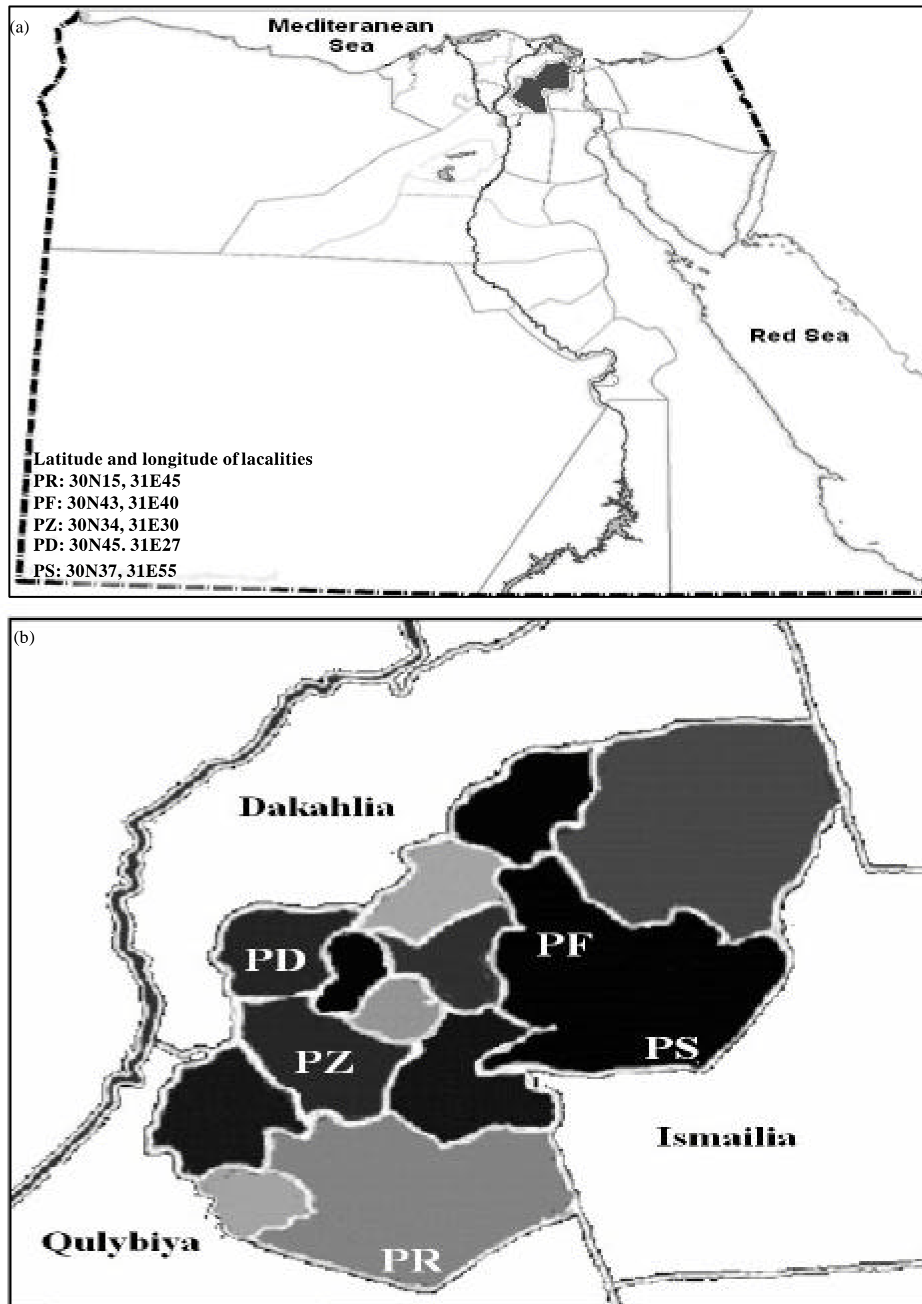

Fig. 1(a-b): Localities of the collected *Culex pipiens* populations, (a) Whole Egypt map indicate Sharkia governorate in shadow and (b) Collecting localities and their latitude and longitude in Sharkia governorate which PR: Al-Asher of Ramadan, PD: Diarb Negm, PF: Faquos, PS: Salhia and PZ: El-Zagazig

(CDNB), reduced glutathione, fast blue B salt and Bovine Serum Albumin (BSA) were from Sigma-Aldrich (St. Louis, MO, USA). All commercial reagents and other chemicals used in this study were of analytical quality with the highest purity available and purchased from commercial suppliers.

Selection pressure and bioassay: Fourth instar larvae of the first generation (F_1), which considered the parent generation, were divided into three groups of approximately 3000 larvae each. Two of the three groups were subjected separately to selection with either malathion or lambda-cyhalothrin for 11 successive generations at concentrations that caused 50-60% mortality every generation (selected strain). The third group was reared in parallel under the same conditions for the same number of generations but without insecticidal contamination so as to be used as an unselected strain. After 24 h of insecticide-exposure, the surviving larvae of each group (selected strain) were transferred separately into clean plastic trays containing fresh tap water until pupation. The resulted pupae were transferred into adult cages until adult emergence and reared using the same procedure described above.

Larval bioassays were performed on the selected and unselected generations as well as LS-CP strain. Batches of 25 fourth instar larvae were exposed to a range of insecticide concentrations prepared in 100 mL of tap water in 4 OZ plastic cups (Bio-Serv, Frenchtown, NJ). For each tested insecticide, serial concentrations were prepared to give mortalities between 10 and 90%. At least five concentrations ranging 0.01-1.0 mg mL^{-1} (malathion) and 0.00001-0.01 mg mL^{-1} (lambda-cyhalothrin) were used for each assay with three replicates for each concentration. After 24 h of treatment at 27±2°C, the larval mortality was recorded. Three similar batches of 25 fourth instar larvae each were introduced to clean plastic cups containing tap water only to be used as a control. For each generation, the toxicity regression lines of the three populations (malathion and lambda-cyhalothrin selected strains and the unselected strain) were established and the LC_{50} and slope values were determined. *Cx. pipiens* larval susceptibility to the tested insecticides in each generation of the selected strain was expressed as a resistance ratio (RR) obtained by dividing the LC_{50} values of the selected or unselected generations by the LC_{50} values of LS-CP.

Biochemical assays

Preparation of mosquito extracts: For total protein, acetylcholinesterase, general esterases and GST determinations, samples of larval homogenate ware prepared by homogenizing 20 fourth instar larvae of each tested generation as well as the LS-CP strain. Larvae were homogenized in 250 μL of 0.1 M ice-cold sodium phosphate buffer, pH 7.4, containing 0.02% Triton X-100 using a plastic mini pestles in 1.5 mL centrifuge tubes. Debris was pelleted at 10,000 rpm for 15 min at 4°C. The supernatant was then separated in clean 0.5 mL eppendorf tubes and stored at -20°C until used within 15 days.

Acetylcholinesterase: AChE activity in whole larval homogenate was estimated according to the procedure described by Ellman *et al.* (1961). Twenty larvae/batch were homogenized as mentioned above and the supernatant was decanted, kept on ice and used as the crude enzyme preparation. In 10 mL glass test tubes, 10 μL of the crude enzyme was added to 1.5 mL of phosphate buffer, pH 7.2, containing 0.39 μM of 5,5-dithiobis nitrobenzoic acid (DTNB). The reaction was initiated with the addition of 50 μL of acetylcholine iodide substrate (ATChI) (final conc. in 1560 μL = 7.8 μM). The 300 μL of the previous mixture was transferred into ELISA plate's wells in triplicates. Absorbance was recorded initially after 5 min at 450 nm in 96-well microplate using Microplate Autoreader, EL311S (Bio-TEK Instrument, Highland Park, Winooski, VT) (Ashour *et al.*, 1987b). Reading was repeated after exactly 30, 60 and 90 sec. The mean absorbance change per 30 sec (ΔA/30 sec) was determined. Blank contains the same components except the substrate was used as control. Rates were converted to nmol min^{-1} mg^{-1} using the extinction coefficients of 9.25 mM^{-1} 300 μL^{-1} for 2-nitro-5-mercaptobenzoate (Grant *et al.*, 1989).

Esterases: Colorimetric esterases (EST) activity assays were determined using the general substrates of α and β-NA as described by Gomori (1953) with modifications. Measurements were performed in 96-well microplates using microplate autoreader. For each reaction mixtures, 480 μL phosphate buffer (0.1 M, pH, 7.4) with 0.02% Triton X-100, 20 μL of protein solution, 500 μL of α or β-NA substrate solutions (final concentration in 1500 μL total volume = 2.5 mM) and 500 μL of Fast Blue B salt solution (consists of 2 parts of 1% Fast Blue B salt and 5 parts of 5% SDS) were mixed in 10 mL glass tubes and incubated at 30°C for 5 min. A thousand five hundred μL total volume of the mixture was measured in 5 replicates (300 μL $well^{-1}$) for each sample. The Optical Density (OD) was measured at 450 nm during the first 5 min of the reaction and rates were converted to nmol min^{-1} mg^{-1} using the extinction coefficients of 9.25 mM^{-1} 300 μL^{-1} for 1-naphthol (Grant *et al.*, 1989). Activities were corrected for non-enzymatic hydrolysis using reactions without protein as controls.

Glutathione-S-transferase: GST activity assays were done by the modified method of Grant and Matsumura (1988). Twenty five μL of larval homogenate prepared as previously mentioned, 75 μL of chloro-2, 4-dinitrobenzen (CDNB) and 75 μL of reduced glutathione (fin. conc. 5 mM) were mixed with 750 μL of phosphate buffer, pH 7.4. Reactions were allowed to take place for 5 min at 37°C and then terminated by adding 75 μL of trichloroacetic acid to make final assay volume of 1000 μL $test^{-1}$ tube. Three replicates were used for each measurement and activities were corrected for non-enzymatic hydrolysis using reactions without protein as controls. The conjugation of glutathione to CDNB is accompanied by an increase in absorbance at 340 nm. The rate of increase was directly proportional to the GST activity in sample. These measurements were done using spectrophotometer (Spectronic 20, Bausch and Lomb, USA) by measuring absorbance at 340 nm at 30°C after 15 min from the addition of glutathione. Rates were converted to nmol min^{-1} mg^{-1} using the extinction coefficients of 8.5 O.D. mM^{-1} 1000 μL^{-1} for CDNB (Grant *et al.*, 1989).

Total protein: Protein concentrations were determined according to the method of Bradford (1976) by incubating 10 μL of larval homogenate with 290 μL of Bio-Rad protein assay solution for 10 min. Absorption was then measured at 570 nm and bovine serum albumin was used as the standard.

Statistical analysis: Mortality data were subjected to probit regression analysis using a Probit polo pc plus software v 3.1 (LeOra Software Inc., Cary, NC) which automatically corrected for control mortality according to the method of Finney (1971) and the lethal concentrations which gave 50% (LC_{50}) mortalities were calculated. Data of acetylcholinesterase, esterases and GST in selected and unselected generations were subjected to SPSS 10.0 for Windows software package for statistical analyses. One-way analysis of variance (ANOVA) was performed and variant among groups (selected, unselected and susceptible strains) were determined by means of the Duncan test (Duncan, 1955).

RESULTS

Development of resistance: As a result of continuous exposure to malathion or lambda-cyhalothrin for 11 successive generations, the resistance ratio to malathion when compared with LS-CP strain (Table 1) was increased from 24.7 in F1 to 57.2 fold in F_{11} based on LC_{50} values (0.296 mg L^{-1} in F_1 to 0.687 mg L^{-1} in F_{11}). The slope values of malathion-selected generations fluctuated around the value of the F_1 generation (2.22) suggesting that the field strain didn't alter

Table 1: Resistance development of *Cx. pipiens* either continuously selected for resistance to malathion or unselected for 11 successive generations[a], LC_{50} = Lethal concentration inducing 50% mortality, LS-CP refers to an insecticide susceptible *Cx. pipiens* laboratory strain

Generations	n[b]	LC_{50}, mg L^{-1} (95% CL)[c]	Slope (SE)	X^2	RR[d]
LS-CP	450	0.012 (0.001-0.106)	0.90 (0.18)	6.89	1.0
Selected generations					
F1	375	0.296 (0.234-0.366)	2.22 (0.40)	1.17	24.7
F3	450	0.388 (0.334-0.441)	3.67 (0.61)	2.51	32.3
F5	450	0.419 (0.344-0.500)	2.94 (0.52)	3.76	34.9
F7	375	0.510 (0.450-0.569)	3.57 (0.46)	1.20	42.5
F9	450	0.606 (0.556-0.655)	2.19 (0.59)	2.69	50.5
F11	375	0.687 (0.624-0.776)	2.15 (0.40)	0.51	57.2
Unselected generations					
F1	375	0.254 (0.205-0.310)	2.59 (0.40)	2.95	21.2
F3	450	0.054 (0.032-0.088)	1.01 (0.14)	6.22	4.5
F5	450	0.021 (0.010-0.038)	0.90 (0.11)	4.61	1.8
F7	375	0.017 (0.010-0.028)	0.95 (0.12)	4.40	1.4
F9	450	0.013 (0.005-0.021)	0.83 (0.10)	2.46	1.1
F11	375	0.013 (0.006-0.023)	0.81 (0.11)	1.22	1.1

[a]Data are shown from 3 replicates of bioassay, [b]Total number of larvae tested population^{-1}, [c]CL means confidence limit, [d]Resistance ratio (RR) = LC_{50} of the selected or unselectedgenerations/LC_{50} of the susceptible strain

Table 2: Resistance development of *Cx. pipiens* either continuously selected for resistance to lambda-cyhalothrin or unselected for 11 successive generations[a], LC_{50} = Lethal concentration inducing 50% mortality, LS-CP refers to an insecticide susceptible *Cx. pipiens* laboratory strain

Generations	n[b]	LC_{50} mg L^{-1} (95% CL)[c]	Slope (SE)	X^2	RR[d]
LS-CP	450	0.00002 (0.000019-0.00003)	1.88 (0.18)	2.32	1.0
Selected generations					
F1	375	0.0001 (0.00004-0.00010)	1.72 (0.24)	4.58	5.0
F3	450	0.0001 (0.00008-0.00016)	1.56 (0.26)	7.48	5.0
F5	450	0.0002 (0.0001-0.0003)	1.39 (0.30)	4.29	10.0
F7	375	0.0008 (0.0005-0.0014)	1.03 (0.19)	5.76	40.0
F9	450	0.0016 (0.0010-0.0026)	1.00 (0.14)	2.22	80.0
F11	450	0.0061 (0.0004-0.0010)	1.17 (0.21)	3.44	305.0
Unselected generations					
F1	375	0.00010 (0.00009-0.00018)	1.72 (0.20)	2.81	5.0
F3	450	0.00008 (0.00006-0.00011)	1.40 (0.18)	5.43	4.0
F5	450	0.00007 (0.00005-0.00010)	1.36 (0.17)	5.57	3.5
F7	375	0.00007 (0.00005-0.00009)	1.21 (0.16)	4.34	3.5
F9	450	0.00005 (0.00003-0.00006)	1.08 (0.16)	5.32	2.5
F11	450	0.00003 (0.00002-0.00006)	0.90 (0.15)	6.71	1.5

[a]Data are shown from 3 replicates of bioassay, [b]Total number of larvae tested population^{-1}, [c]CL means confidence limit, [d]Resistance ratio (RR) = LC_{50} of the selected or unselected generations/LC_{50} of the susceptible strain

its homogeneity as it may be already homogenous from initial field collection for response to malathion. When unselected generations were tested against malathion (Table 1), the susceptibility increased as LC_{50} values decreased from 0.254 mg L^{-1} in F_1 to 0.013 mg L^{-1} in F_{11}. There is a dramatic drop was noticed in malathion sensitivity of the unselected generation from F_1 to F_3. Both slope and resistance ratio of unselected generations decreased as the rearing in the laboratory was continued (Table 1), suggesting an increase of susceptibility and heterogeneity.

Table 2 shows *Cx. pipiens* susceptibility to lambda-cyhalothrin through 11 successive generations of selection as well as without any insecticidal selection. The resistance ratio increased

from 5 (F_1) to 305 (F_{11}) fold, respectively in the selected generations. The slope of the log concentration-probit line decreased in F_{11} compared with F_1 indicating an increase of heterogeneous individuals in the population. The unselected generations showed a decrease in resistance to lambda-cyhalothrin, with the resistance ratio dropping from 5 in F_1 to 1.5 fold in F_{11} (Table 2). In the same way of unselected generations to malathion, the slope of the unselected to lambda-cyhalothrin was decreased indicating an increase of heterogeneity.

Biochemical analysis: AChE activity (Fig. 2) was markedly decreased in the F_1, F_3, F_5 and F_7 malathion-selected generations compared with lambda-cyhalothrin selected or unselected generations. The normal activity was recovered in the malathion-selected F_9 and F_{11} generations in which no significant differences in activity were observed compared with unselected generations. AChE activity in lambda-cyhalothrin-selected generations was always markedly high compared with malathion-selected or unselected generations.

The EST activity was measured in the unselected, selected generations as well as in the laboratory LS-CP (Table 3). The activity ratio was calculated compared with that in LS-CP. General esterase activity was significantly increased in generations selected with both insecticides. The highest activity was scored in F_{11} following continuous exposure to malathion and lambda-cyhalothrin. Continuous exposure to lambda-cyhalothrin showed markedly high EST activity compared with the malathion selected F_{11} generation. General esterase activity was not significantly altered through 11 successive generations without exposure to insecticides. The

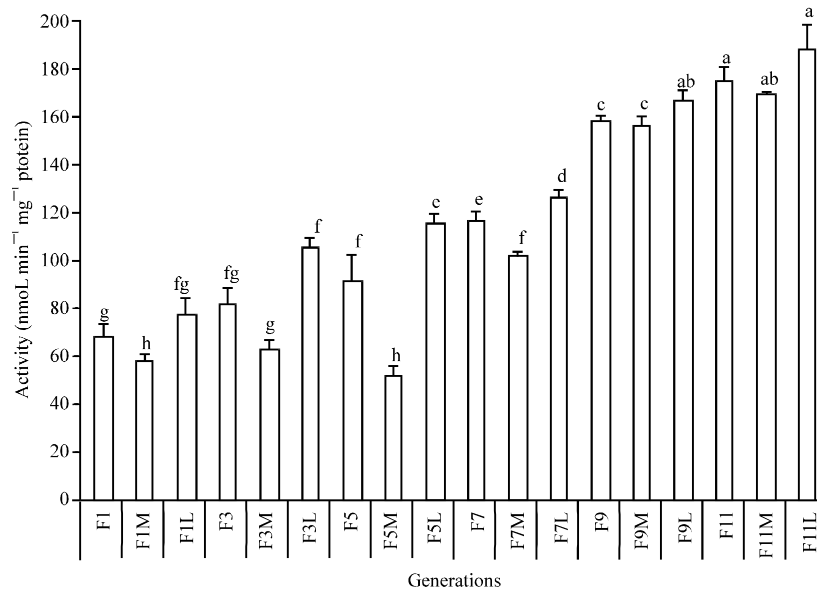

Fig. 2: Acetylcholinesterase activity in malathion or lambda-cyhalothrin selected and unselected *Cx. pipiens* larvae*, F1-F11 represent unselected generations, F1M-F11M represent malathion-selected generations and F1L-F11L represent lambda-cyhalothrin-selected generations, The same letters on bars indicate that there are non-significant differences at p = 0.05 among selected or unselected generations

Table 3: Esterase activity in *Cx. pipiens* malathion or lambda-cyhalothrin selected and unselected larvae*, LS-CP refers to an insecticide susceptible *Cx. pipiens* laboratory strain

	Esterase activity (nmoL min^{-1} mg^{-1} protein)		Ratio**	
Generations	α-NA	β-NA	α-NA	β-NA
LS-CP	8.6±0.5^{e}	7.1±0.3^{d}	1.0	1.0
Malathion-selected				
F1	17.1±3.9^{c}	12.2±3.1^{c}	2.0	1.7
F11	32.0±0.4^{b}	21.2±1.8^{b}	3.7	3.0
Lambda-cyhalothrin-selected				
F1	17.5±3.7^{c}	12.6±3.4^{c}	2.0	1.8
F11	36.3±0.4^{a}	25.9±1.1^{a}	4.2	3.6
Unselected				
F1	12.4±1.5^{d}	7.1±1.2^{d}	1.4	1.0
F11	13.0±2.2^{d}	9.9±0.6^{cd}	1.5	1.4

* Values are shown as Means±SD of three determinations, Activity data are statistically different when numbers followed by different letters within the same column at $p<0.05$, ** Ratio is calculated as mean activity value in selected or unselected generations/mean activity value in the LS-CP

Table 4: Glutathion-S-transferase activity in *Cx. pipiens* malathion or lambda-cyhalothrin selected and unselected larvae*, LS-CP refers to an insecticide susceptible *Cx. pipiens* laboratory strain

Generations	GST activity (µmoL min^{-1} mg^{-1} protein)	Ratio**
LS-CP	1800±600^{d}	1.0
Malathion-selected		
F1	3600±1700^{c}	2.0
F11	6400±300^{b}	3.6
Lambda-cyhalothrin-selected		
F1	4000±1200^{c}	2.2
F11	8800±400^{a}	4.9
Unselected		
F11	1800±400^{d}	1.0
F1	1300±200de	0.7

*Values are shown as Means±SD of three determinations, Activity data are statistically different when numbers followed by different letters within the same column at $p<0.05$, **Ratio is calculated as mean activity value in selected generations/mean activity value in the LS-CP

activity ratios increased 3.7 and 3.0 fold in the malathion selected F_{11} generation compared with 2.0 and 1.7 fold in F_1 generation when α-NA or β-NA were used as general substrates, respectively. For lambda-cyhalothrin selected generations, the activity ratio was increased from 2.0 and 1.8 fold in F_1 to 4.2 and 3.6 fold in F_{11} when α-NA or β-NA were used as general substrates, respectively.

Based on mean values, GST activity (Table 4) was increased in the F_{11} generation selected with either malathion or lambda-cyhalothrin. The activity ratios were calculated by comparing them with the activity scored for LS-CP, which increased from 2.0 (F_1) to 3.6 fold (F_{11}) following malathion selection. GST activity increased from 2.2 to 4.9 fold through 11 generations of selection with lambda-cyhalothrin. The activity ratios in the unselected generations, did not alter significantly through 11 generations.

DISCUSSION

Insecticide resistance in members of the *Cx. pipiens* mosquito complex has been documented in many countries such as Tunisia (Daaboub *et al.*, 2008), Cote d'Ivoire and Burkina Faso (Chandre *et al.*, 1998), Saudi Arabia (Amin and Hemingway, 1998), Egypt (El-Sheikh, 2011), USA (McAbee *et al.*, 2004) and China (Li *et al.*, 2002). Our laboratory assays of *Cx. pipiens*, selected and unselected through 11 generations with either malathion or lambda-cyhalothrin, resulted in increased resistance to the tested insecticides. Although the main purpose was not to evaluate the resistance, *Cx. pipiens* developed high resistance levels to lambda-cyhalothrin (305.0-fold) and malathion (57.2-fold) due to continuous exposure to the tested insecticides through 11 generations. The case of developing resistance to lambda-cyhalothrin was recorded in other mosquito species such as *Ae. Aegypti* adults collected from Colombia and many other countries. In the study of Ocampo *et al.* (2011), the susceptibility of larvae and adults of *Ae. Aegypti* from Colombia to malathion, temephos and fenitrothion showed adult resistance to all tested insecticides and larval resistance to temephos and fenitrothion only which explained that temephos resistance are important as it has been the primary chemical for controlling immature stages of *Ae. aegypti* in Colombia. Additionally, temephos pressure on larvae may generate cross-resistance to pyrethroids or in the adult stages to other organophosphates.

In contrast to the insecticide selected strains, the unselected strain of the same population showed increasing susceptibility to malathion and lambda-cyhalothrin through 11 generations. In both cases of developing or reverse resistance to the tested insecticides, *Cx. pipiens* larvae were exhibited resistance to malathion in a rate less than that of lambda-cyhalothrin and reverse to susceptibility faster, suggesting that exposure to malathion in nature may be less than that of lambada-cyhalothrin. Insecticide resistance has been reported in more than 100 mosquito species including *Cx. pipiens* and resistance to one insecticide can induce cross-resistance to another insecticide from the same group or different groups (Hemingway and Ranson, 2000). Regarding this phenomenon, a colony of *Cx. pipiens* Marin in California rapidly developed high levels of resistance following a few generations of selection with permethrin and showed cross-resistance to lambda-cyhalothrin as well as to DDT (McAbee *et al.*, 2004). In Tunisia, *Cx. pipiens* populations from different localities developed high resistance levels to pyrethroid (permethrin and deltamethrin) and organophosphorus (chlorpyrifos) insecticides. The result of these high levels of resistance was stopping use such insecticides in localities of Tunisia where strong resistance was detected (Daaboub *et al.*, 2008).

AChE activity was affected by malathion-exposure. The activity was increased by repeating *Cx. pipiens* larval exposure to malathion until no significant differences were found in the 11th generation among selected and unselected larvae, suggesting that an altered acetylcholinesterase as a target insensitivity may result (Bonning and Hemingway, 1991). In our study, the activities of esterases and GSTs in resistance selected *Cx. pipiens* were determined because of their potential use as biochemical indicators of insecticide resistance (Grant *et al.*, 1989; McAbee *et al.*, 2004).

CONCLUSION

Field collected *Cx. pipiens* larvae showed 24.7 and 5.0 fold resistance to malathion and lambda-cyhalothrin, respectively. Continuous exposure to these insecticides through 11 generations resulted in 57.2 and 305.0 fold increases in resistance to malathion and lambda-cyhalothrin, respectively. Malathion resistance was associated with an altered acetylcholinesterase as well as increased activity of detoxification enzymes (esterases and GST). These data suggest that

Cx. pipiens in Sharkia Governorate, Egypt, may also develop high levels of resistance to these insecticides if exposed to them continuously for several generations. On the other hand, reversions to susceptibility may occur if selection is relaxed by discontinuing the use of these insecticides as part of a resistance management strategy.

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