EGYPTIAN ACADEMIC JOURNAL OF

BIOLOGICAL SCIENCES

TOXICOLOGY & PEST CONTROL

F

ISSN
2090-0791

WWW.EAJBS.EG.NET

Vol. 12 No. 2 (2020)

Egypt. Acad. J. Biolog. Sci., 12(2): 189-201 (2020)

Egyptian Academic Journal of Biological Sciences
F. Toxicology & Pest Control
ISSN: 2090 - 0791
http://eajbsf.journals.ekb.eg/

Evolution of Resistance to Chlorpyrifos and lambda-cyhalothrin Insecticides against *Culex Pipiens* Populations

Gharib, A. M.; Mahmoud, M. M. El-Hassawy and Fouad, A. F. Ali
Plant Protection Department, Faculty of Agriculture, Al-Azhar University, Cairo, Egypt
E.mail: **amohiy31@yahoo.com**

ARTICLE INFO
Article History
Received:7/7/2020
Accepted:19/10/2020
Keywords:
Culex pipiens, Chemical insecticide, Insecticides resistance, Enzymes.

ABSTRACT

The susceptibility of *Culex pipiens* larvae collected from Al-Asher of Ramadan, Sharkia Governorate, to chlorpyrifos and lambda-cyhalothrin insecticides was investigated for 20 successive generations. For multiple generations, the instar larvae of field parent strain were exposed to LC_{30} of the previous generation to that insecticide. Total protein and lipids content as well as activities of detoxifying enzymes (i.e. acetylcholinesterase, non-specific esterases and glutathione-S-transferase) were determined in each generation. Bioassay tests showed that larval *Cx. pipiens* developed 144.31 and 761.85-fold resistance to chlorpyrifos and lambda-cyhalothrin, respectively, after 20 successive generations of selected pressure. Total protein content declined while total lipids increased gradually with proceeded the generations. In general, the activities of detoxifying enzymes increased gradually with raising generation numbers which indicate that the increased resistance is likely to be associated with the increased activity of target and metabolic enzyme systems.

INTRODUCTION

Mosquitoes (Diptera: Culicidae) spread in different climatic environments to reach every area where humans live and transmit to them many diseases. Mosquitoes are considered to be the essential vector of many pathogens and parasites such as viruses, protozoans, bacteria, and nematodes, which cause dangerous diseases, such as, malaria, yellow fever, dengue, chikungunya fever, Zika fever, and filariasis. *Culex*, *Aedes*, and *Anopheles* are considered the responsible vectors of these diseases (Jang *et al*., 2002 and Barbosa *et al*., 2011). In Egypt, the common house mosquito is *Culex pipiens*, which causes infections and disability in persons (Kady *et al*., 2008). It is the main vector of Bancroftian filariasis (Yadav, 2012). Filariasis is a widespread disease in many regions of Egypt with an incidence ranging from 4 to 6% in Sharkia Governorate (Rashed, 1981).

Vector control is a very important part of the global strategy for the management of mosquito associated diseases, and insecticide application is the most important component in this effort (Liu, 2015). Chemical insecticides are used repeatedly in mosquito control programs in Egypt leading to increase insect resistance accompanied by dangerous effects on human, nontarget organisms, and the environment (Barbosa *et al*., 2011; Mahyoub *et al*., 2016 and Merdan and Ghareeb, 2016).

The development of insecticide resistance in mosquitoes occurs mainly due to two

major mechanisms i.e., target-site insensitivity and metabolic detoxification (Hemingway *et al.*, 2004). However, understanding the nature of resistance helps to create efficient strategies for mosquito control (Zaim and Guillet, 2002). The major cause of resistance mechanism in mosquitoes is the detoxification and degradation of insecticides by overproduction of various metabolic enzymes (Viswan *et al.*, 2018).

The aim of this study was to test the susceptibility of *Cx. pipiens* populations, collected from Al-Asher of Ramadan, Sharkia Governorate, to the most commonly used insecticides for mosquito control (chlorpyrifos and lambda-cyhalothrin) and to assess the relative activities of detoxification and target enzymes in association with resistance.

MATERIALS AND METHODS

Toxicological Studies:

1- Test Mosquitoes Strains:

A susceptible reference strain of *Cx. pipiens* was obtained from the Research Institute of Medical Entomology (Ministry of Health Populations, Giza, Egypt). The reference strain was not exposed to any control agents since it was colonized in the insectary.

Cx. pipiens larvae were collected from 10th of Ramadan El-Sharkia Governorate and reared to adults in the laboratories of Plant Protection Department, Faculty of Agriculture, Al-Azher University, Cairo under controlled conditions of 26 ± 2C°, 70± 5% relative humidity and 14:10 L.D photoperiod to G_{20} to ensure the homogeneity of the colony.

2- Insecticides Used:

Lambda-cyhalothrin insecticide (10% EC) was purchased from Dotra chemicals Co. While **C**hlorpyrifos insecticide (40% EC) was purchased from Kafr El Zayat Co.

These insecticides were recommended by the World Health Organization's Pesticide Evaluation Scheme (WHOPES) for use against mosquito (WHO, 2006).

Larval Bioassays:

Resistance Development in *Cx. pipiens* to Chemical:

Bioassays according to the WHO protocol were undertaken (WHO, 2005). The 4th instar larvae of the field parents strain were exposed every generation to LC_{30} of the previous generation to that insecticide for 20 successive generations. The LC_{50} value for each generation was determined. Based on the LC_{50} values of the selected strains compared with that of the susceptible strain, the resistance ratios were estimated as follows:

Lethal concentrations for 50% and 90% mortality levels, with 95% confidence limit (CL) and line parameters of log dose-probit response lines (Ld-p Lines) were determined using a probit analysis computer program (Karaagac, 2012). The rates of development of resistance were studied through the pattern and slope of the mortality regression lines.

$$\text{Resistance Ratio (RR)} = \frac{LC_{50} \text{ of the selected strain}}{LC_{50} \text{ of the susceptable strain}}$$

Biochemical Assay:

1- Preparation of Samples for Biochemical Studies:

Samples were collected from the 4th instar larvae of susceptible strain, parent strain, and a selected strain of each generation after selection with the tested insecticides. Batches of 50 early 4th instar larvae were homogenized in glass homogenizer at 4 ^{O}C in 1 ml of 0.1 M ice-cold phosphate buffer pH 8.0 was prepared from the stock solutions of NaH_2PO_4 and Na_2HPO_4. The homogenate was centrifuged at 10,000 r.p.m. at 4^{O}C for 30 min., solid debris and cellular matrix were discarded. The supernatant was collected and stored at -20^{o}C. The supernatant fraction was used for determining the total protein, lipids, and activities of α and β-esterases, glutathione S-transferase (GST), and acetylcholinesterase (AChE).

2- Determination of Total Protein Content:

Total protein was determined using a diagnostic kit produced by Diamond Company according to the method described by (Young, 2001).

3- Determination of Total Lipids Content:

Total lipids were determined using a diagnostic kit produced by Diamond Company according to the method described by (Zollner and Kirsch, 1962).

4- Determination of α- and β-Esterases Activities:

The activities of α- and β-esterases were determined according to the method described by **(Van Asperen, 1962)** using α-naphthyl acetate and β-naphthyl acetate as substrates. Alpha- and beta-esterases activities were determined using the extinction coefficient of (**Grant *et al.*, 1989)**. The specific esterase activity was determined by dividing esterase activity by total protein (mg/ml) in each sample to get nMole/min/mg protein.

5- Determination of Acetylcholinesterase Activity:

Acetylcholinesterase (AChE) activity was measured using acetylthiocholine iodide (ATCh) as a substrate according to (Ellman *et al*., 1961).

The change in absorbance was measured against blank at wavelength 412 *nm* using Jenway 6105 spectrophotometer for at least 10 minutes. Following enzyme assay. Specific AChE activity was determined in μMole/min/mg protein

6- Determination of the Glutathione S-transferase Activity:

Glutathione s-transferase activity was measured according to the procedure of (Grant *et al*., 1989) which is based upon catalysing the reaction of 1-chloro-2,4-dinitrobenzene (CDNB) with reduced glutathione (GSH) through the thiol group to form S-(2,4-dinitrophenyl) glutathione which absorbs light at 340 *nm*.Following enzyme assay. Specific GST activity was calculated as nMole/min/mg protein using the extinction coefficient for CDNB at 340 nm (9.6 mM/ml).

Statistical Analysis:

LC_{50} and LC_{90} values were estimated using log-probit software program LdpLine® model "Ehab soft" (Bakr, 2000). The number of dead larvae was counted 24 hours post-exposure. Percent mortality was calculated for each test. Mortality data from bioassays were corrected by natural control mortality using Abbott's formula (1925).

Data for biochemical analysis were performed to one-way analysis of variance (ANOVA) by using Costat program (1988) and significant differences among the means values were determined according to (Duncan, 1955) multiple range test at probability levels of $P = 0.05$.

RESULTS AND DISCUSSION

When field strain was exposed to chlorpyrifos continually in each generation, its resistance ratio increased to reach 144.31 folds in generation 20 (Table 1). The same pattern was observed with mosquitos exposed to lambda-cyhalothrin to reach 761.85 folds resistance in generation 20 (Table 2). The same results were obtained by Merdan and Ghareeb (2016) and Tageldin *et al*. (2018) who reported that continuous exposure to insecticides leading to increase mosquito resistance. Moreover, El- Sheikh (2011) found that larval *Cx. pipiens* collected from Diarb Negm location, Sharkia Governorate developed 99- and 1900-folds resistance to Malathion and lambda-cyhalothrin, respectively. The high level of resistance to lambda-cyhalothrin than chlorpyrifos may be due to frequent exposure in nature to pyrethroids either directly for mosquito control or through drift (El- Sheikh, 2011). In contrast, Nikookar *et al*. (2019) found that the resistance level of field *Cx. pipiens* collected from Iran were lower to pyrethroids compared to organophosphate insecticides.

Tables (1&2) show that the slope of Ld-p Lines increased with proceeding the selection, which indicates increasing homogenisty of individuals.

Table 1: Rate of development of resistance of field strain to chlorpyrifos in 4th instar larvae of the *Cx. pipiens* during selection for 20 successive generations.

Generations	LC_{30} (ppm)	LC_{50} (ppm)	LC_{90} (ppm)	Slope± S.E.**	R.R.*** (fold)
Susceptible Strain	0.0067 (0.0025-0.009)	0.0131 (0.0065- 0.0185)	0.0682 (0.0491- 0.1434)	1.7932 ± 0.1512	——
P-strain*	0.0288 (0.0231-0.0339)	0.0568 (0.0494-0.0657)	0.2998 (0.2187-0.4745)	1.7739 ± 0.1811	4.385
G_1	0.0483 (0.0333-0.0617)	0.0968 (0.0793-0.1127)	0.5276 (0.3998-0.81)	1.7398± 0.2086	7.462
G_2	0.0658 (0.0483-0.081)	0.1339 (0.1146-0.1542)	0.7604 (0.542-1.3033)	1.6994± 0.2095	10.308
G_3	0.1099 (0.0872-0.1281)	0.1824 (0.1624-0.2017)	0.6285 (0.4965-0.9147)	2.3850± 0.2954	14.000
G_4	0.1418 (0.1211-0.1588)	0.2243 (0.2046-0.2473)	0.6885 (0.5458-0.9866)	2.6317±0.3048	17.231
G_5	0.155 (0.1134-0.1834)	0.2776 (0.2474-0.3162)	1.1539 (0.7793-2.5534)	2.0714±0.3655	21.385
G_6	0.2077 (0.1496-0.2429)	0.3701 (0.3303-0.4422)	1.5181 (0.9512-4.6041)	2.0907±0.4396	28.462
G_7	0.2877 (0.2365-0.3284)	0.5043 (0.4509-0.5791)	1.9872 (1.4096-3.5848)	2.1518± 0.2992	38.769
G_8	0.4564 (0.3822-0.5084)	0.6985 (0.6426-0.775)	1.9765 (1.4965-3.319)	2.8373± 0.4475	53.769
G_9	0.6351 (0.564-0.696)	0.9412 (0.869-1.0265)	2.4622 (2.0349-3.2594)	3.0688±0.3184	72.385
G_{10}	0.749 (0.6539-0.8239)	1.0922 (1.0125-1.1823)	2.7461 (2.2644-3.7191)	3.2006±0.3823	84.000
G_{11}	0.8153 (0.6878-0.9086)	1.1637 (1.0738-1.2484)	2.7767 (2.3274-3.7195)	3..3934±0.4500	89.538
G_{12}	0.8624 (0.7423-0.9514)	1.2194 (1.1334-1.3067)	2.8431 (2.3829-3.7971)	3.4860±0.4518	93.769
G_{13}	0.9135 (0.7962-1.0009)	1.2953 (1.2079-1.3938)	3.0414 (2.5157-4.163)	3.4572±0.4533	99.615
G_{14}	0.9762 (0.87-1.058)	1.3615 (1.2745-1.467)	3.0691 (2.552-4.1417)	3.6305±0.4592	104.692
G_{15}	1.0159 (0.8624-1.1224)	1.422 (1.3237-1.5204)	3.235 (2.687-4.4807)	3.5901±0.5212	109.385
G_{16}	1.0596 (0.9149-1.1614)	1.4732 (1.3778-1.5765)	3.2964 (2.7362-4.5573)	3.6641±0.5230	113.308
G_{17}	1.1378 (1.0027-1.2349)	1.5807 (1.4817-1.7063)	3.53 (2.8908-5.0065)	3.6731±0.5269	121.615
G_{18}	1.1185 (0.8988-1.2571)	1.5934 (1.4703-1.7081)	3.7838 (3.0489-5.8015)	3.4120±0.5875	122.538
G_{19}	1.2788 (1.1158-1.3891)	1.7447 (1.6413-1.8697)	3.7282 (3.0757-5.296)	3.8863±0.5955	134.231
G_{20}	1.376 (1.2285-1.4796)	1.8756 (1.7621-2.04)	3.9985 (3.2486-5.8572)	3.8984±0.6017	144.308

* Field parental strain before chlorpyrifos selection.
**S. E., Standard Error
***R.R., Resistance ratio = LC_{50} for chlorpyrifos -resistant strain / LC_{50} for susceptible strain

Table 2: Rate of development of resistance of field strain to lambda-cyhalothrin in 4th instar larvae of the *Cx. pipiens* during selection for 20 successive generations.

Generations	LC_{30} (ppm)	LC_{50} (ppm)	LC_{90} (ppm)	Slope± S.E.**	R.R.*** (fold)
Susceptible Strain	0.0056 (0.0027-0.0068)	0.0092 (0.0056-0.0137)	0.0312 (0.0304-0.0847)	2.4191± 0.1642	——
P-strain*	0.0118 (0.0095-0.0136)	0.0193 (0.0173- 0.0213)	0.0649 (0.0512- 0.0944)	2.4347± 0.2968	2.065
G_1	0.0143 (0.012- 0.0161)	0.0235 (0.0212- 0.0262)	0.0788 (0.0601- 0.1218)	2.4345± 0.3024	2.500
G_2	0.0191 (0.0141- 0.0224)	0.0317 (0.0286- 0.0352)	0.1088 (0.0775- 0.2207)	2.3923± 0.4389	3.478
G_3	0.034 (0.0252- 0.0397)	0.0506 (0.0426- 0.065)	0.1339 (0.1144- 0.2914)	3.0343± 0.3231	5.543
G_4	0.044 (0.0318- 0.0533)	0.0755 (0.0652- 0.0842)	0.2819 (0.2159- 0.4506)	2.2397± 0.3297	8.152
G_5	0.0887 (0.0575- 0.107)	0.1522 (0.1352- 0.1704)	0.5694 (0.3813- 1.5234)	2.2369± 0.4875	16.522
G_6	0.2166 (0.1706- 0.2468)	0.3546 (0.322- 0.4033)	1.1823 (0.8295- 2.4589)	2.4503± 0.4426	38.587
G_7	0.4318 (0.3589- 0.4831)	0.6495 (0.5987- 0.7084)	1.7608 (1.3788- 2.7371)	2.9588± 0.4463	70.543
G_8	1.1508 (1.0067- 1.2526)	1.6331 (1.5236- 1.7849)	3.8417 (3.0589- 5.8178)	3.4497±0.5258	177.50
G_9	1.785 (1.268- 2.0195)	2.5494 (2.3718- 2.7916)	6.0918 (4.4915- 15.0283)	3.3877± 0.8636	277.07
G_{10}	1.8036 (1.2344- 2.0948)	2.8574 (2.6102- 3.2243)	8.7977 (5.9962- 25.1023)	2.6241±0.6281	310.54
G_{11}	2.2633 (1.8351- 2.5031)	3.2075 (2.9977- 3.5123)	7.52 (5.7595- 13.8787)	3.4633± 0.7053	348.59
G_{12}	2.8242 (2.4505- 3.0847)	4.0285 (3.7566- 4.3969)	9.5968 (7.625- 14.6336)	3.3996±0.5239	437.94
G_{13}	2.9565 (2.4411- 3.2838)	4.1949 (3.9052- 4.5125)	9.8644 (7.8882- 15.3166)	3.4512±0.5884	455.98
G_{14}	3.5254 (2.9195-3.8853)	4.9848 (4.6601- 5.4356)	11.6228 (9.0332- 19.9762)	3.4858± 0.6599	531.30
G_{15}	3.4748 (2.1395-3.947)	4.8876 (4.5615- 5.4454)	11.2516 (8.0773-42.9508)	3.5392± 1.0947	541.85
G_{16}	3.6621 (2.9431- 4.078)	5.0149 (4.6659- 5.336)	10.8128 (8.7953- 16.8422)	3.8409± 0.7289	545.11
G_{17}	3.0391 (1.3578- 3.7657)	5.0733 (4.4291- 5.6798)	17.7488 (11.1244- 111.045)	2.3564±0.7181	551.41
G_{18}	4.4532 (3.7375- 4.8529)	6.012 (5.6717- 6.4943)	12.5196 (9.9843- 20.8374)	4.0230± 0.8019	653.48
G_{19}	5.1089 (4.4126- 5.4964)	6.75 (6.3875- 7.3537)	13.3338 (10.687- 22.1074)	4.3348± 0.8768	733.70
G_{20}	5.3044 (4.3949- 5.7691)	7.0088 (6.6395- 7.5421)	13.8478 (11.1023- 23.8006)	4.3336± 0.9412	761.85

* Field parental strain befor lambda-cyhalothrin selection.

****S**. E., Standard Error

***R.R., Resistance ratio = LC_{50} for lambda-cyhalothrin resistant strain / LC_{50} for susceptible strain

Figures 1&2 show the content of total protein (mg/ml) and total lipids (mg/ml) in 4th instar larvae of susceptible, parent field, and resistant strains. The content of total protein which normally contains both soluble and insoluble fractions declined gradually with raising the generation number (i.e. increase the resistance pattern, Fig.1). The reduction in total protein has been subjected to wide speculations by many investigators. Ramdev and Rao (1986) reported that haemolymph volume changes under insecticide stress resulting in an alteration in protein concentration. Wilkins *et al*. (1998) found high intracellular protease activities in the Malathion resistant strain of *Musca domestica* which responsible for protein digestion. El- Barky *et al*. (2008) postulated that the total protein in 4th instar

larvae of *S. littoralis* was significantly decreased when treated with the insecticide due to inhibition of DNA and RNA synthesis.

On the other hand, lipid content increased gradually with raising generation number (Fig 2). This piece of result is in accordance with that obtained by Kalra (1970) who found that larval of *Culex pipiens fatigans* containing a high amount of lipids were able to resist better the toxic effect of DDT. Canavoso *et al*. (2001) documented that the quantity of lipids available for the energy reserve seems to be the result of a balance between the catch of food and the request for reserve by processes such as reproduction, maintenance, and growth, and this balance is disturbed by any toxicant.

The result shown in Fig 1&2 are in agreement with those obtained by Bouaziz *et al*. (2011) and Shaurub and El- Aziz (2015).

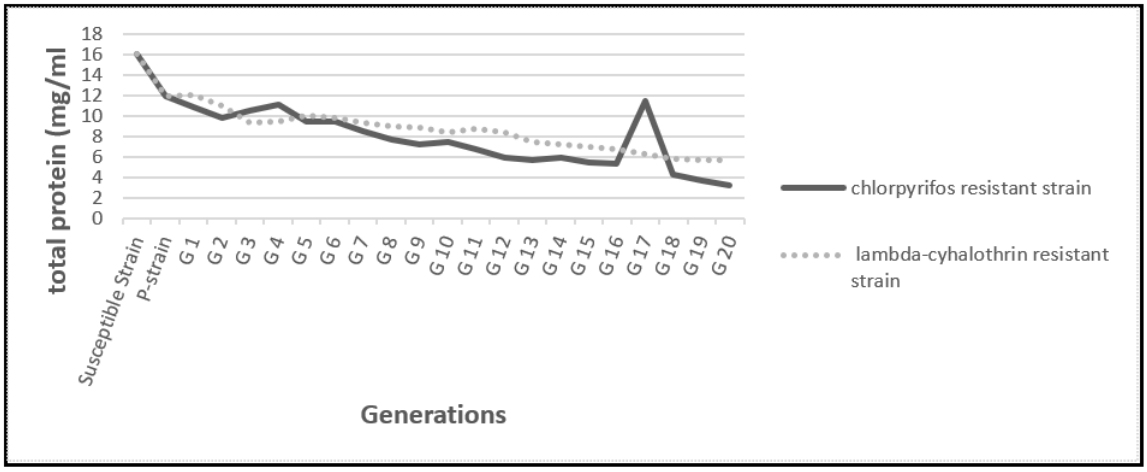

Fig. 1: The total protein (mg/ml) in 4th instar larvae of susceptible, field parent and insecticide resistant strains during 20 generations of selection pressure**.**

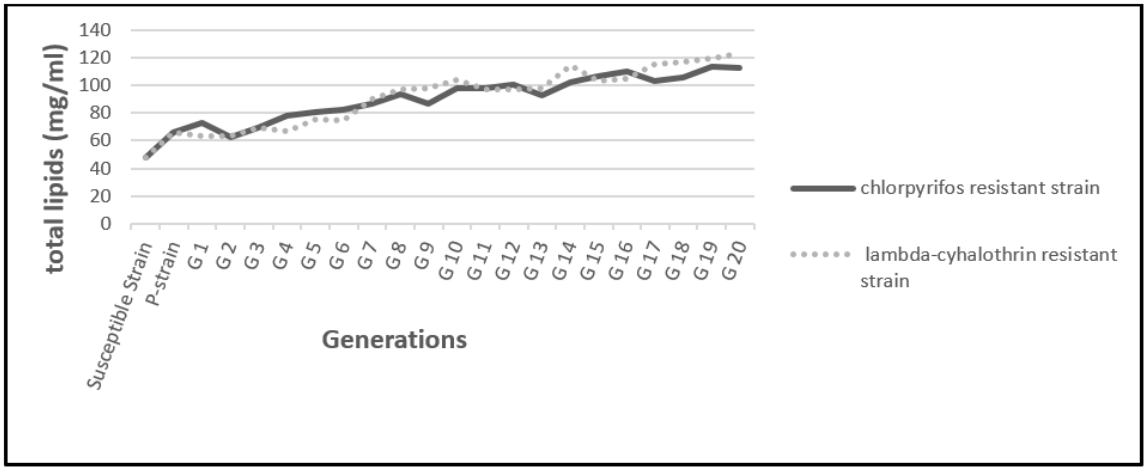

Fig. 2: The total lipids (mg/ml) in 4th instar larvae of susceptible, field parent and insecticide resistant strains during 20 generations of selection pressure.

The elevated level of studied enzymes in different insect species and mosquitoes have been known to enhance the resistance to insecticides. The enzymes involved in insecticides detoxification may be qualitatively and /or quantitatively changed to give resistance.

Hemingway *et al*. (2004) reported that the development of insecticide resistance in mosquitoes occurs mainly due to two major mechanisms i.e., target-site insensitivity and metabolic detoxification. The former inhibits the binding of the insecticides in the target

site and the latter results in increased or modified activities of some detoxifying enzymes. The authors mentioned that pyrethroids resistance is target- site insensitivity of the voltage-gated sodium channel. Mutation in the voltage-gated sodium channel gene termed as knockdown resistance (Kd_r) mutation leads to the development of resistance to synthetic pyrethroids. The Kd_r mutation has been reported in association with resistance to lambda-cyhalothrin from *Cx. pipiens* in Morocco region (Alout *et al*., 2016).

Figure (3) shows that GST activity increased progressively as mosquito generations proceeded. GSTs are a major family of detoxification enzymes. They catalyze the conjugation of the tripeptide glutathione to electrophilic centers of lipophilic compounds, thereby increasing their solubility and aiding excretion from the cell (Brown, 1986). Thus, GSTs play a vital role in protecting tissues against oxidative damage and oxidative stress. The GSTs in insects are primarily of interest because of their role in insecticides resistance. They are involved in the *O*-dealkylation or *O*-dearylation of organophosphorous insecticides (Hayes and Wolf, 1988) and as a secondary mechanism in the detoxification of organophosphate metabolites (Hemingway *et al*., 1991). Although GSTs have not been implicated directly in pyrethroids resistance, there are reports of elevated GSTs in pyrethroids resistance (Grant and Matsumura, 1988 and Wu *et al*., 2004). For example, Brooke *et al*. (2001) found that GSTs play a minor role as a detoxifying enzyme in pyrethroids resistant to *An. funestus*.

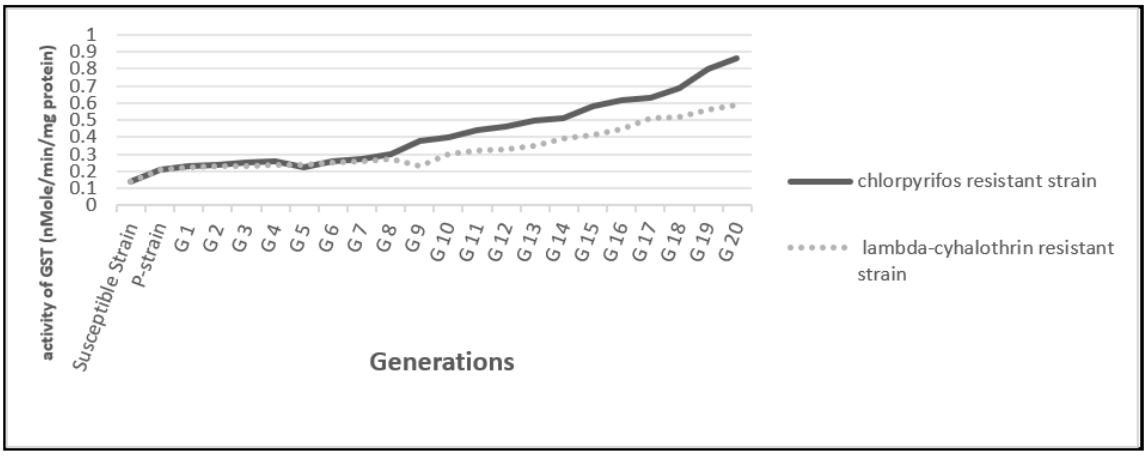

Fig.3: Specific activity of Glutathione S-transferase (GST) in 4th instar larvae of susceptible, field parent, and insecticide-resistant strains during 20 generations of selection pressure.

Figure (4) shows that AChE activity increased with raising generation number. Measuring AChE activity in susceptible and resistant strains is an important factor in measuring resistance level (Yang *et al*., 2011). AChE is a key enzyme in the nervous system, hydrolyzing acetylcholine neurotransmitter and terminating neural impulses, and are the target for both organophosphates and carbamate insecticides. Alterations in AChE in resistant insects result in a decreased sensitivity to inhibition by insecticides. El- Sheikh (2011) found a positive correlation between AChE activity and the level of insecticide resistance in *Cx. pipiens*. Liu (2015) reported that high insensitivity of AChE can be mainly due to mutations in the ace- I gene. Karunaratne *et al*. (2018) reported that increase AChE activity in resistant insects results in decreasing its sensitivity to be inhibited by the insecticides.

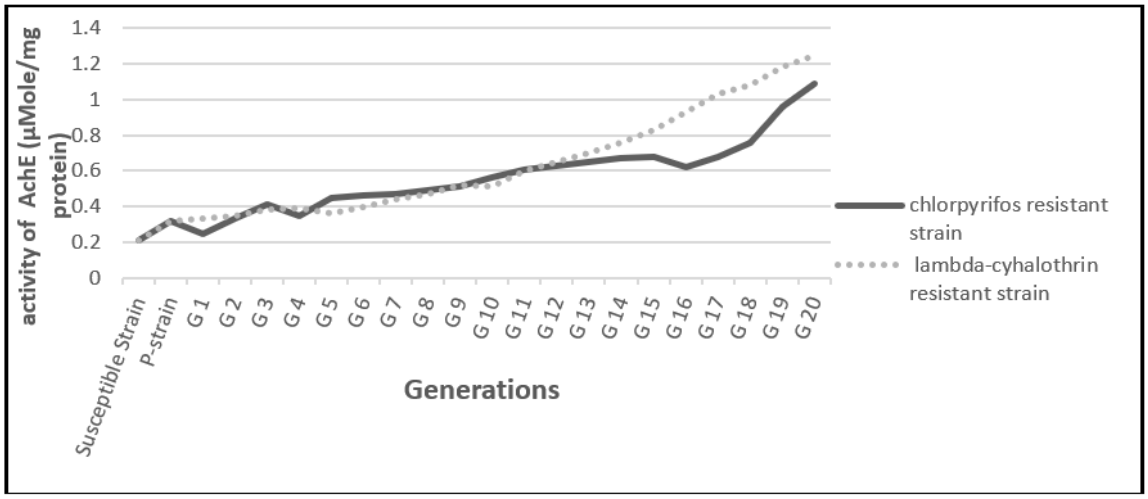

Fig. 4: Specific activity of acetylcholinesterase (AChE) in 4th instar larvae of susceptible, field parent and insecticide-resistant strains during 20 generations of selection pressure.

The two common esterases (α and β) are involved separatory or in combination in *Cx. pipiens* resistance. These esterases act by rapidly binding and slowly turning over the insecticide (Hemingway and Karunaratne, 1998). Previous investigators showed an association between elevated levels of esterases activities and organophosphate resistance in mosquito larvae (Hemingway and Ranson (2000), Zayed *et al*. (2006), Vézilier *et al*. (2013) and Liu, 2015).

Results of Table (3) and Figures (5&6) show a significant increase in activity of α and β esterases in selected strain than susceptible strain which agreed with that obtained by (Macoris *et al*., 2003) who observed a higher level of α and β – esterases activity in the field populations of *Ae. aegypti* which is accompanied by higher resistance ratios. The present results proved that these enzymes are good biomarkers in detecting resistance to organophosphates.

The result of Table (3) indicated that the ratio of activity to α esterases between selected and susceptible strains was 7.13& 4.63 in the case of chlorpyrifos and lambda-cyhalothrin, respectively. The same trend was observed with β - esterases. This indicates that: - (1) regardless of the examined insecticide, α- esterases have higher activity for detoxification than β esterases. The same result was obtained by Nikookar *et al*. (2019). (2) Both esterases are more efficient in detoxifying organophosphate than pyrethroids insecticides. Gordon and Ottea (2012) reported that there was no correlation between elevated esterase level and pyrethroids resistance in *Cx. quinquefasciatus*

The elevated activities of detoxifying enzymes obtained from the present study (Fig 3, 4, 5 and 6) are in accordance with that obtained by Akiner and Eksi (2015).

The findings obtained from this research provide valuable information on the involvement of metabolic mechanisms in insecticide resistance in the field population of *Cx. pipiens* collected from 10th of Ramadan, Sharkia Governorate. This information is pivotal in implementing a successful control program for this important vector.

Table 3: Specific activity of Alpha and Beta-esterases in 4th instar larvae of the parent - (p) strain, chlorpyrifos (R*) and lambda-cyhalothrin (R**) resistant strains of *Culex pipiens* during 20 generations of selection pressure.

Generations	Specific activity of total Alpha-esterases (nMole/min/mg protein)				Specific activity of total Beta-esterase (nMole/min/mg protein)			
	R* - strain	R/P*	R** - strain	R/P*	R* - strain	R/P*	R** - strain	R/P*
Susceptible Strain	$1.10\times10^{-4}\pm2.42\times10^{-5}$ f	1.00	$1.10\times10^{-4}\pm2.42\times10^{-5}$ k	1.00	$1.02\times10^{-4}\pm7.86\times10^{-6}$ e	1.00	$1.02\times10^{-4}\pm7.86\times10^{-6}$ g	1.00
P-strain	$1.60\times10^{-4}\pm2.50\times10^{-5}$ ef	1.45	$1.60\times10^{-4}\pm2.50\times10^{-5}$ jk	1.45	$1.05\times10^{-4}\pm1.62\times10^{-5}$ e	1.05	$1.05\times10^{-4}\pm1.62\times10^{-5}$ fg	1.05
G 1	$1.72\times10^{-4}\pm2.78\times10^{-5}$ ef	1.56	$1.64\times10^{-4}\pm1.11\times10^{-5}$ ijk	1.50	$1.42\times10^{-4}\pm1.49\times10^{-5}$ de	1.42	$1.07\times10^{-4}\pm1.22\times10^{-5}$ fg	1.07
G 2	$2.10\times10^{-4}\pm2.90\times10^{-5}$ ef	1.91	$1.94\times10^{-4}\pm2.88\times10^{-5}$ hijk	1.76	$1.63\times10^{-4}\pm2.19\times10^{-5}$ cde	1.63	$1.04\times10^{-4}\pm1.97\times10^{-5}$ g	1.04
G 3	$2.25\times10^{-4}\pm2.36\times10^{-5}$ def	2.04	$1.39\times10^{-4}\pm1.32\times10^{-5}$ jk	1.26	$1.84\times10^{-4}\pm1.96\times10^{-5}$ bcde	1.84	$1.15\times10^{-4}\pm2.25\times10^{-5}$ efg	1.15
G 4	$2.53\times10^{-4}\pm3.49\times10^{-5}$ cdef	2.30	$2.06\times10^{-4}\pm3.10\times10^{-5}$ hijk	1.87	$1.97\times10^{-4}\pm1.45\times10^{-5}$ bcde	1.97	$1.18\times10^{-4}\pm1.25\times10^{-5}$ efg	1.18
G 5	$2.63\times10^{-4}\pm2.92\times10^{-5}$ cdef	2.39	$2.13\times10^{-4}\pm8.84\times10^{-6}$ ghijk	1.93	$2.23\times10^{-4}\pm3.04\times10^{-5}$ abcde	2.23	$1.27\times10^{-4}\pm5.66\times10^{-6}$ efg	1.27
G 6	$2.85\times10^{-4}\pm5.40\times10^{-5}$ cdef	2.59	$2.35\times10^{-4}\pm4.11\times10^{-5}$ fghijk	2.13	$2.34\times10^{-4}\pm3.28\times10^{-5}$ abcde	2.34	$1.32\times10^{-4}\pm1.62\times10^{-5}$ efg	1.32
G 7	$3.08\times10^{-4}\pm4.26\times10^{-5}$ cdef	2.80	$2.51\times10^{-4}\pm2.16\times10^{-5}$ fghijk	2.28	$2.63\times10^{-4}\pm4.12\times10^{-5}$ abcde	2.63	$1.38\times10^{-4}\pm1.14\times10^{-5}$ efg	1.38
G 8	$3.30\times10^{-4}\pm4.54\times10^{-5}$ bcdef	3.00	$2.64\times10^{-4}\pm1.69\times10^{-5}$ efghij	2.40	$2.53\times10^{-4}\pm2.64\times10^{-5}$ abcde	2.53	$1.42\times10^{-4}\pm3.84\times10^{-6}$ efg	1.42
G 9	$3.97\times10^{-4}\pm6.18\times10^{-5}$ abcdef	3.60	$2.79\times10^{-4}\pm1.73\times10^{-5}$ defghij	2.54	$2.90\times10^{-4}\pm1.07\times10^{-5}$ abcde	2.90	$1.58\times10^{-4}\pm3.05\times10^{-5}$ defg	1.58
G 10	$3.95\times10^{-4}\pm7.29\times10^{-5}$ abcdef	3.59	$2.70\times10^{-4}\pm4.07\times10^{-6}$ defghij	2.46	$3.09\times10^{-4}\pm4.40\times10^{-5}$ abcde	3.09	$1.67\times10^{-4}\pm3.36\times10^{-5}$ cdefg	1.67
G 11	$4.22\times10^{-4}\pm1.23\times10^{-4}$ abcdef	3.84	$2.97\times10^{-4}\pm2.79\times10^{-5}$ defghij	2.70	$3.41\times10^{-4}\pm6.83\times10^{-5}$ abcde	3.41	$1.76\times10^{-4}\pm3.02\times10^{-5}$ cdefg	1.76
G 12	$4.56\times10^{-4}\pm8.99\times10^{-5}$ abcdef	4.14	$3.20\times10^{-4}\pm3.59\times10^{-5}$ cdefgh	2.91	$3.64\times10^{-4}\pm6.92\times10^{-5}$ abcd	3.64	$1.58\times10^{-4}\pm2.44\times10^{-5}$ defg	1.58
G 13	$4.96\times10^{-4}\pm6.14\times10^{-5}$ abcdef	4.51	$3.44\times10^{-4}\pm2.42\times10^{-5}$ bcdefg	3.13	$3.79\times10^{-4}\pm4.28\times10^{-5}$ abcd	3.79	$1.95\times10^{-4}\pm1.14\times10^{-5}$ bcdefg	1.95
G 14	$5.43\times10^{-4}\pm1.38\times10^{-4}$ abcde	4.94	$3.63\times10^{-4}\pm1.02\times10^{-4}$ abcdef	3.30	$3.89\times10^{-4}\pm2.43\times10^{-5}$ abcd	3.89	$2.19\times10^{-4}\pm5.65\times10^{-5}$ abcdef	2.19
G 15	$5.82\times10^{-4}\pm1.59\times10^{-4}$ abcde	5.29	$3.85\times10^{-4}\pm6.68\times10^{-5}$ abcdef	3.50	$4.03\times10^{-4}\pm1.11\times10^{-4}$ abc	4.03	$2.28\times10^{-4}\pm4.02\times10^{-5}$ abcde	2.28
G 16	$6.38\times10^{-4}\pm1.81\times10^{-4}$ abcd	5.80	$4.18\times10^{-4}\pm7.57\times10^{-5}$ abcde	3.80	$4.21\times10^{-4}\pm1.26\times10^{-4}$ ab	4.21	$2.57\times10^{-4}\pm5.09\times10^{-5}$ abcde	2.57
G 17	$6.61\times10^{-4}\pm3.19\times10^{-4}$ abc	6.01	$4.11\times10^{-4}\pm2.43\times10^{-5}$ abcde	3.73	$4.09\times10^{-4}\pm1.95\times10^{-4}$ abc	4.09	$2.66\times10^{-4}\pm6.40\times10^{-5}$ abcd	2.66
G 18	$7.36\times10^{-4}\pm2.49\times10^{-4}$ ab	6.69	$4.56\times10^{-4}\pm6.63\times10^{-5}$ abc	4.15	$4.31\times10^{-4}\pm1.22\times10^{-4}$ ab	4.31	$2.72\times10^{-4}\pm6.48\times10^{-5}$ abc	2.72
G 19	$7.56\times10^{-4}\pm1.11\times10^{-4}$ a	6.87	$4.79\times10^{-4}\pm7.69\times10^{-5}$ ab	4.35	$4.53\times10^{-4}\pm1.50\times10^{-4}$ a	4.53	$3.02\times10^{-4}\pm3.51\times10^{-5}$ ab	3.02
G 20	$7.84\times10^{-4}\pm2.06\times10^{-4}$ a	7.13	$5.09\times10^{-4}\pm8.08\times10^{-5}$ a	4.63	$4.66\times10^{-4}\pm3.32\times10^{-5}$ a	4.66	$3.08\times10^{-4}\pm2.41\times10^{-5}$ a	3.08

* R/P, Ratios of Alpha or Beta-esterases activity between selected strain and susceptible strain.
-Each value represents the mean of three replicates.
-Means in the same column followed by the same letter are not significantly different at the 5% level of probability (Duncan's test).

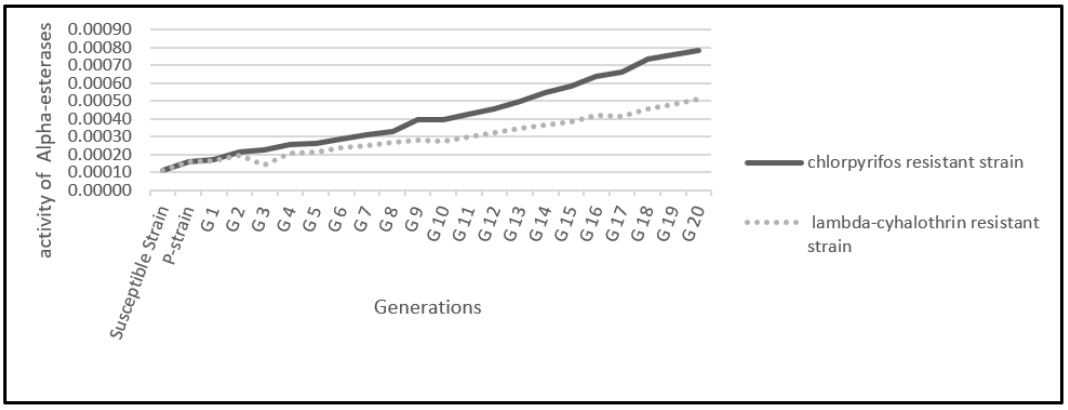

Fig. 5: Specific activity of Alpha-esterases (nMole/min/mg protein) in 4th instar larvae of susceptible, field parent, and insecticide-resistant strains during 20 generations of selection pressure

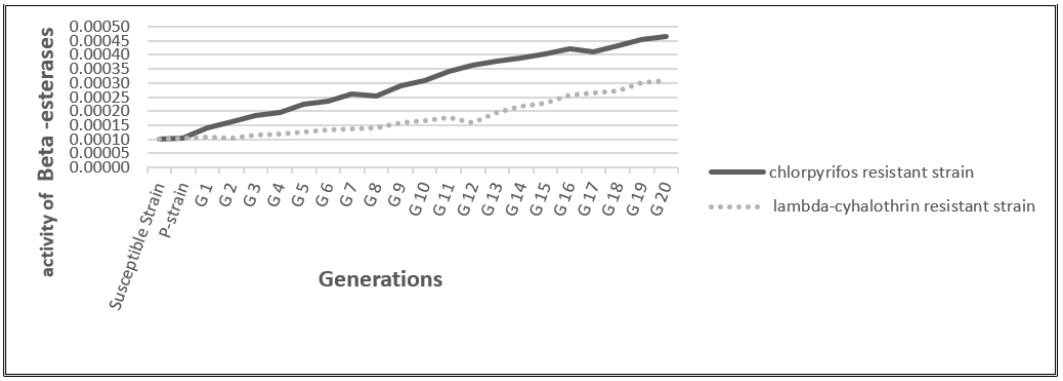

Fig. 6: Specific activity of Beta-esterases (nMole/min/mg protein) in 4th instar larvae of susceptible, field parent and insecticide-resistant strains during 20 generations of selection pressure.

REFERENCES

Abbott, W. S. (1925). A method for computing the effectiveness of an insecticide. *Journal of Economic Entomol*ogy, 18: 256-267.

Akıner, M. M. and Ekşi, E. (2015). Evaluation of insecticide resistance and biochemical mechanisms of *Culex pipiens* L. in four localities of east and middle mediterranean basin in Turkey. *International Journal of Mosquito Res*earch, 2(3): 39-44.

Alout, H.; Labbé, P.; Berthomieu, A.; Makoundou, P.; Fort, P.; Pasteur, N. and Weill, M. (2016). High chlorpyrifos resistance in *Culex pipiens* mosquitoes: strong synergy between resistance genes. *Heredity*, 116 (2): 224-231.

Bakr, E. (2000). Ld-P Line. [http://www.ehabsoft.com/ldpline/].

Barbosa, S.; Black, IV, W. C. and Hastings, I. (2011). Challenges in estimating insecticide selection pressures from mosquito field data. *PLoS Neglected Tropical Diseases*, 5 (11): 1387-1398.

Bouaziz, A.; Boudjelida, H. and Soltani, N. (2011). Toxicity and perturbation of the metabolite contents by a chitin synthesis inhibitor in the mosquito larvae of *Culiseta longiareolata*. *Annals of biological research*, 2(3): 134-142.

Brooke, B. D.; Kloke, G.; Hunt, R. H.; Koekemoer, L. L.; Tem, E. A.; Taylor, M. E. and Coetzee, M. (2001). Bioassay and biochemical analyses of insecticide resistance in southern African *Anopheles funestus* (Diptera: Culicidae). *Bulletin of entomological research*, 91(4): 265-272.

Brown, A. W. (1986). Insecticide resistance in mosquitoes: a pragmatic review. *Journal of the American Mosquito Control Association*, 2(2): 123-140.

Canavoso, L. E.; Jouni, Z. E.; Karnas, K. J.; Pennington, J. E. and Wells, M. A. (2001). Fat metabolism in insects. *Annual review of nutrition*, 21(1): 23-46.

Costat software. (1988). Microcomputer program analysis, CoHort software, Berkely, CA, USA.

Duncan, D. B. (1955). Multiple range and multiple F tests, *Biometrics*, 11: 1-42.

El-barky, N. M.; Dahi, H.F. and El- Sayed, Y. A. (2008). Toxicological evaluation and biochemical impacts for radient as a new generation of spinosyn on *Spodoptera littoralis* (Boisd.) larvae. *Egyptian Academic Journal of Biological Sciences.*, 1(2): 85-97.

Ellman, G. L.; Courtney, K. D.; Andres, V. and Featherstone, R. M. (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochemical Pharmacol*ogy, 7: 88-95.

El-Sheikh, E. A. (2011). Larval susceptibility of *Culex pipiens* L. Diptera: Culicidae, collected from different localities at Sharkia Governorate to some insecticides. *Zagazig Journal of Agricultural Research*, 38 (3):759-774.

Gordon, J. R. and Ottea, J. (2012). Association of esterases with insecticide resistance in *Culex quinquefasciatus* (Diptera: Culicidae). *Journal of Economic Entomology*, 105(3): 971-978.

Grant, D. F. and Matsumura, F. (1988). Glutathione S-transferase in *Aedes aegypti* larvae: purification and properties. *Insect Biochemistry*, 18(6): 615-622.

Grant, D. F.; Bender, D. M. and Hammock, B. D. (1989). Quantitative kinetic assay for glutathione *S*-transferase and general esterase in individual mosquitoes using EIA reader. *Insecticide Biochemical*, 19: 741-751.

Hayes, J. D. and Wolf, C. R. (1988). Role of glutathione transferase in drug resistance. Glutathione Conjugation: *Mechanisms and Biological Significance*, 350-355.

Hemingway, J. and Karunaratne, S. H. (1998). Mosquito carboxylesterases: a review of the molecular biology and biochemistry of a major insecticide resistance

mechanism. *Medical and Veterinary Entomology*, 12 (1): 1-12.

Hemingway, J.; Hawkes, N.J.; McCarroll, L. and Ranson, H. (2004). The molecular basis of insecticide resistance in mosquitoes. *Insecticide Biochemical. Molecular Biol*ogy, 34: 653-665.

Hemingway, J.; Miyamoto, J. and Herath, P. R. J. (1991). A possible novel link between organophosphorus and DDT insecticide resistance genes in Anopheles: supporting evidence from fenitrothion metabolism studies. *Pesticide Biochemistry and Physiology*, 39 (1): 49-56.

Hemingway, J. and Ranson, H. (2000). Insecticide resistance in insect vectors of human disease. *Annual Review Of Entomology*, 45(1): 371-391.

Jang, Y. S.; Kim, M. K.; Ahn, Y. J. and Lee, H. S. (2002). Larvicidal activity of Brazilian plants against *Aedes aegypti* and *Culex pipiens* pallens (Diptera: Culicidae). *Journal of Applied Biological Chemistry*, 45(3): 131-134.

Kady, G. A. E.; Kamel, N. H.; Mosleh, Y. Y. and Bahght, I. M. (2008). Comparative toxicity of two bio-insecticides (Spinotoram and Vertemic) compared with methomyl against *Culex pipiens* and *Anopheles multicolor*. *World Journal of Agricultural Sciences*,4 (2): 198-205.

Kalra, R. L. (1970). Studies on the mechanism of DDT resistance in *Culex pipiens fatigans*: Lipids of Susceptible and Resistant Strains. *Bulletin of the W. H. O.*, 42 (4), 623-629.

Karaağaç, S. U. (2012). Insecticide resistance. *Insecticides-Advances In Integrated Pest Management*, 469-478.

Karunaratne, S. H. P. P.; De Silva, W. A. P. P.; Weeraratne, T. C. and Surendran, S. N. (2018). Insecticide resistance in mosquitoes: development, mechanisms and monitoring. *Ceylon Journal Of Sciences*, 47: 299-309.

Liu, N. (2015). Insecticide resistance in mosquitoes: impact, mechanisms, and research directions. *Annual Review Of Entomology*, 60: 537-559.

Macoris, M. D. L. G.; Andrighetti, M. T. M.; Takaku, L.; Glasser, C. M.; Garbeloto, V. C. and Bracco, J. E. (2003). Resistance of *Aedes aegypti* from the state of São Paulo, Brazil, to organophosphates insecticides. *Memórias do Instituto Oswaldo Cruz*, 98 (5): 703-708.

Mahyoub, J. A.; Hawas, U.; Al-Ghamdi, K. M.; Aljameeli, M. M.; Shaher, F. M.; Bamakhrama, M. A. and Alkenani, N. A. (2016). The biological effects of some marine extracts against *Aedes aegypti* (L.) mosquito vector of the dengue fever in Jeddah Governorate, Saudi Arabia. *Journal of Pure and Applied Microbial*, 10 (3): 1949-1956.

Merdan Bouthaina, A. and Ghareeb, A. (2016). Detoxifying and serine enzyme activities in developing *culex pipiens* resistance against Carbaryl (Sevin), Malathion and *Bacillus thurengiensis* serovar H-14. *International Journal of Current Res*earch., 8 (7): 34705-34713.

Nikookar, S. H.; Fazeli-Dinan, M.; Ziapour, S. P.; Ghorbani, F.; Salim-Abadi, Y.; Vatandoost, H.; Hanafi, A. A. and Enayati, A. A. (2019). First report of biochemical mechanisms of insecticide resistance in the field population of *Culex pipiens* (Diptera: Culicidae) from sari, mazandaran, north of Iran. *Journal of Arthropod-Borne Diseases*, 13(4): 378-390.

Ramdev, Y. P. and Rao, P. J. (1986). Effects of insecticides on *Achaea janata* Linn. Haemolymph volume, body moisture and cations. *Proceedings of the National Academy of Sciences Section, B*, 52: 232-240.

Rashed, S. S. (1981). Survey of mosquito species in Sharkiya Governorate with special reference to population density of culicids transmitting filarial. *M. Sc. Thesis,*

*Faculty of Sciences Zagazig Univ*ersity, 181.

Shaurub, E. S. H. and El-Aziz, N. M. A. (2015). Biochemical effects of lambda-cyhalothrin and lufenuron on *Culex pipiens* L.(Diptera: Culicidae). *Entomology Journal of Mosquito Research*, 2: 122-126.

Tageldin, R. A.; Zayed, A.; Abd-EI-Samie, E. M.; Mahmoud, H. I. and Zayed, A. B. (2018). Metabolic changes associated with using Lambda-Cyhalothrin insecticide and their effects on resistance development in the mosquito, *Culex pipiens* (Diptera: Culicidae). *Egyptian Academic Journal of Biological Sciences A, Entomology*, 11(1): 43-52.

Van Asperen, K. (1962). A study of housefly esterases by means of a sensitive colorimetric method. *Journal Insecticide Physiol*ogy, 8: 401-416.

Vézilier, J.; Nicot, A.; De Lorgeril, J.; Gandon, S. and Rivero, A. (2013). The impact of insecticide resistance on *Culex pipiens* immunity. *Evolutionary applications*, 6 (3); 497-509.

Viswan, K. A.; Nidhish, G. and Pushpalatha, E. (2018). Biochemical monitoring of detoxifying enzyme levels in field population of mosquitoes: *Culex quinquefasciatus* Say and *Aedes aegypti* (L.). *International Journal of Pharmaceutical and Biological Archives*, 9 (1):36-42.

Wilkins, M. R.; Gasteiger, E.; Sanchez, J. C.; Bairoch, A. and Hochstrasser, D. F. (1998). Two-dimensional gel electrophoresis for proteome projects: The effects of protein hydrophobicity and copy number. *Electrophoresis*, *19* (8-9): 1501-1505.

World Health Organization. (2005). Guidelines for laboratory and field testing of mosquito larvicides (No. WHO/ CDS/ WHOPES/ GCDPP/13). World Health Organization.

World Health Organization. (2006). Guidelines for testing mosquito adulticides for indoor residual spraying and treatment of mosquito nets (No. WHO/CDS/NTD/ WHOPES/GCDPP/3). World Health Organization.

Wu, H. W.; Tian, H. S.; Wu, G. L.; Langdon, G.; Kurtis, J.; Shen, B. and Zhu, C. L. (2004). *Culex pipiens* pallens: identification of genes differentially expressed in deltamethrin-resistant and-susceptible strains. *Pesticide Biochemistry and Physiology*, 79 (3): 75-83.

Yadav, S. (2012). Dose optimization of thermal fogging trials for household insecticide formulation (EC) against *Aedes aegypti* (L.). *Current Biotica*, 6 (1): 71-79.

Yang, J.; Gu, Y.; Huang, X.; Shen, A. and Cheng, C. (2011). Dynamic changes of ICAM-1 expression in peripheral nervous system following sciatic nerve injury. *Neurological Research*, 33(1): 75-83.

Young, D. S. (2001). Effects of disease on clinical lab. Tests, 4th ed AACC.

Zaim, M. and Guillet, P. (2002). Alternative insecticides: an urgent need. *Trends in Parasitology*, 18 (4): 161-163.

Zayed, A. B. B.; Szumlas, D. E.; Hanafi, H. A.; Fryauff, D. J.; Mostafa, A. A.; Allam, K. M. and Brogdon, W. G. (2006). Use of bioassay and microplate assay to detect and measure insecticide resistance in field populations of *Culex pipiens* from filariasis endemic areas of Egypt. *Journal of the American Mosquito Control Association*, 22 (3): 473-482.

Zollner, N. and Kirsch, K. (1962). Colorimetric method for determination of total lipids. *Zeitschrift für die gesamte experimentelle Medizin*,135: 545-550.

ARABIC SUMMARY

تطور مقاومة بعوضة الكيوليكس بيبيينز لمبيدات الكلوربيريفوس واللامدا سيهالوثرين

أحمد محيي غريب محمد، محمود محروس محمود الحصاوى، فؤاد أحمد فهمي على

قسم وقاية النبات - كلية الزراعة - جامعة الأزهر – القاهرة - مصر

تم دراسة حساسية يرقات بعوضة الكيوليكس بيبيينز التي تم جمعها من العاشر من رمضان، محافظة الشرقية، لمبيدات الكلوربيريفوس واللامداسيهالوثرين لمدة 20 جيلًا متتاليًا. حيث تم تعريض العمر اليرقي من السلالة الحقلية في كل جيل لضغط انتخابي من المبيد مقداره LC_{30} من الجيل السابق له. تم تقدير المحتوى الكلي للبروتين والدهون وقياس نشاط إنزيمات AChEs، α-and β-esterases، GSTs في العمر اليرقي لكل جيل. أظهرت نتائج إختبارات التقييم الحيوي، تطور درجة المقاومة لتصل الى 144.31 و761.85 ضعفًا لمبيد الكلوربيريفوس و اللامدا سيهالوثرين على الترتيب، وذلك بعد 20 جيل متتالي من الضغط الانتخابي. كما أظهرت القياسات البيوكيميائية انخفاض في محتوى البروتين وزيادة في محتوي الدهون كذلك زيادة في نشاط الإنزيمات السابقة مع تقدم الأجيال مما يشير إلى أن زيادة درجة المقاومة مرتبطة بزيادة نشاط الإنزيمات الهادمة لهذه المبيدات.