EGYPTIAN ACADEMIC JOURNAL OF
BIOLOGICAL SCIENCES
ENTOMOLOGY

A

ISSN
1687-8809

WWW.EAJBS.EG.NET

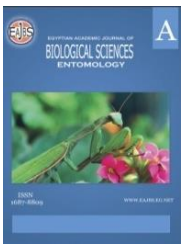
Vol. 16 No. 1 (2023)

Citation: *Egypt. Acad. J. Biolog. Sci. (A. Entomology) Vol. 16(1) pp.113-131 (2023)*
DOI: 10.21608/EAJBSA.2023.289170

Egypt. Acad. J. Biolog. Sci., 16(1):113-131(2023)

Egyptian Academic Journal of Biological Sciences
A. Entomology

ISSN 1687- 8809
http://eajbsa.journals.ekb.eg/



Toxicological and Biochemical Changes of Some insecticides on *Culex pipiens* L. from Egypt

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ARTICLE INFO

Article History
Received:13/1/2023
Accepted:27/2/2023
Available:3/3/2023

Keywords: Biochemical changes, Toxicological changes, *Culex pipiens*, Larvae pesticides.

ABSTRACT

The domestic mosquito *Culex pipiens* (*Diptera: Culicidae*) is one of the most important public health pests spread in Egypt, so we studied biochemical and toxicological changes to traditional and bio-pesticides on the third instar larvae of the domestic mosquito. Three organic pesticides (chlorpyrifos, lambada cyhalothrin, imidaclopride) and two biopesticides (emamectin benzoate, spinosad) at different concentrations treated on larvae and calculating the value of $LC_{50\ after}$ treatment and the rate of resistance were studied. The results showed that the South strain of domestic mosquito larvae were high resistance against chlorpyrifos ethyl, it was found that the most efficient tested pesticide was spinosad, where it was in the South strain 55,227-fold, and in the East strain, The relative potency of imidacloprid was 12.17-fold and in the West strain, imidacloprid was the first category, followed by spinosad. Activity acid phosphatase, alkaline phosphatase, acetylcholinesterase and glutathione -s- transferase in larvae, were determined the highest activity in South strain indicated that this strain had the highest resistance.

INTRODUCTION

Mosquitoes (*Diptera: Culicidae*) spread in different climatic environments to reach every area where humans live and transmit to them many diseases. Mosquitoes are the essential vector of many pathogens and parasites such as viruses, protozoans, bacteria, and nematodes, which cause dangerous diseases, as malaria, yellow fever, dengue, chikungunya fever, Zika fever, and filariasis. *Culex*, *Aedes*, and *Anopheles* mosquitoes are considered the responsible vectors of these diseases (Jang *et al*., 2002 and Barbosa *et al*., 2011).

In Egypt, the common mosquito species is *Culex pipiens*, which causes infections and disability in persons (Kady *et al*., 2008).

Vector control is a very important part of the global strategy for insecticide application is the most important component of this effort (Liu, 2015).

Also, WHO (2013) reported that there were about 219 million cases of malaria in 2010 with an estimated 660000 deaths. Most deaths occur among children living in Africa where a child dies every minute from malaria. Mosquitoes also transmit animal diseases like the fowl pox of poultry, myxomatosis of rabbits, rift valley fever of sheep, encephalitis of horses and

birds (Muga *et al*., 2015).

In this study, mosquitoes in the Culex pipiens complex were collected at various sites throughout California and tested for esterase, GST, and kdr activities. The esterase, GST, and kdr activities were compared to the corresponding activities found in a pyrethroid-Susceptible laboratory strain Culex quinquefasciatus (CQ1). A correlation was found between elevated esterase activities and kdr assay indicating that further investigation should be done to Fig out the potential role of enzyme detoxification and kdr assay in conceding resistance to pyrethroids class**,** (Ahmed, et *al, 2012).*

Effect of fenoxycarb, dinotefuran, imidacloprid, phenthoate and thiocyclam insecticides on the greenhouses population of the tomato leaf miner, Tuta absoluta, was evaluated. Data declared that the five tested insecticides had high toxic on 3rd instar larvae. Imidacloprid was the most effective toxicant against larvae and moths, so it had a very low resistance coefficient. And also, produced a higher induction effect of AChE enzyme activity than the other three insecticides) Radwan EMM and Taha HS. 2012).

MATERIALS AND METHODS

All experiments of the present work were conducted during the period of 2020-2022 in the Toxicology Environmental Research Unit, Agriculture Faculty, Ain Shams University.

Strains of *Culex Pipiens:*

The field strains larvae mosquito was collected from Cairo Governorate from (Hadayek El-Kobba) named North strain, (Helwan) named South strain, (Al-Marj) named - East strain and (Azbakeya) named West strain. the susceptible strains of *Culex. pipiens* was obtained from the Research Institute of Medical Entomology, Ministry of Health, Dokki, Giza, Egypt.

Insecticides:

Three organic pesticides (chlorpyrifos, lambada cyhalothrin, imidaclopride) and two biopesticides (emamectin benzoate, spinosad) at different concentrations

Rearing of *Culex pipiens* populations:

The third instar larvae of the domestic mosquito.was bred under laboratory conditions of the Toxicology Environmental Research Unit, Agriculture Faculty, Ain Shams University, under controlled conditions 26 ± 2°C, 70 ± 5% relative humidity and 14: 10 L.D photoperiod. Larvae of *Culex. pipiens* were transferred to white enameled and shallow trays about 30 cm in diameter containing 2-3 liters of dechlorinated water.These trays were always covered with a mesh screen to prevent oviposition by escaped adult mosquitoes.

The larvae were fed daily on bread until pupation and water replaced every two days. The pupae were transferred from the trays to plastic cups containing dechlorinated tap water and were maintained in cages with netting cover wood frames (30×30×30 cm) until adults emerged. (Mohsen and Mehdi 1989).

Female mosquitoes were fed three days after their emergence on the blood of a pigeon, female plucked its feathers from the chest and abdomen area female tied its wings and tied its legs and I sat on top of the breeding cage all night. He also put a small bowl of water inside the cage.Then the egg boats were transferred by a small brush to containers containing the food of the larvae, and the emergence of full-grown and careful not to rot. The water was replaced every three days, and this method was repeated until the appearance of the third generation of full-grown adults.

Then samples were taken from the third instar larvae.

The breeding of the insect and the numbers of its farm was carried out under conditions temperature of 21 ± 2° C, a humidity of 55 ± 5%, (Sharrook *et.al*.,1991).

Experiments on Larval Stage:

Bioassay insecticides were performed by dipping technique according to WHO (2017). The concentations of the tested (0.01 ppm, 0.1 ppm, 1 ppm and 10 ppm) was dissolved in 100 ml distilled water in the cup to obtain the desired concentration, Larvae were exposed to a wide range of insecticide concentrations, 20 larvae were transformed to each cup and each pesticide's concentration had four replicates Three organic pesticides (chlorpyrifos, lambada cyhalothrin, imidaclopride) and two biopesticides (emamectin benzoate, spinosad) at different concentrations applied mosquito larvae. then this range was narrower to only 4 concentrations that yield 10-95% mortality. For insecticides, 3rd larval instar was used, and the control test was in the same condition without insecticides.

For each bioassay, Larval mortality was recorded after 48 hours after exposure.Corrected mortality was calculated by Abbott's formula (Abbott, 1925). Probit regression lines were estimated to the LC_{50} (lethal concentration) and slope values by probit analysis program according to (Finney, 1971). The LC_{50} values were expressed as ppm. The same procedures were applied with both susceptible and field strains.

This ratio was gradually increased due to the selection pressure by tested insecticides. 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 slope of the mortality lines.

Biochemical Studies:

Enzymes activities were conducted in 3rd larvae.

Preparation of Samples for Biochemical Studies:

Samples were collected from the 3rd instar larvae of field strains and susceptible strain. Batches of 100 early from 3rd 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 containing 0.1 % Triton X-100, 0.1 M (6 gm) solution of (NaH_2PO_4) per 500 ml distilled water and 0.1 M (7.1 gm) solution of (Na_2HPO_4) per 500 ml distilled water.

Buffer solutions of 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. The supernatant was collected and stored at 20^{o}C. The supernatant fraction was used for determining glutathione S-transferase (GST), acetylcholinesterase (AChE) and activities of alkaline and Acid phosphatase

Determination of Glutathione S-transferases Activity:

Glutathione S-transferase activity was measured according to the procedure of (Grant *et al*., 1989), which is based on 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.

The Procedure:

For assay, 2 ml GST substrate buffer and 400 µl CDNB solution were each transferred to a cuvette using a pipet. 50 µl of larvae homogenate was added and then 50 µl of GSH solution was added. The cuvette was equilibrated at room temperature for 15 min and the change in absorbance was measured at 340 nm by Jenway 6105 spectrophotometer for 10 minutes against a blank prepared from substrate buffer, CDNB solution and GSH solution.Following enzyme assay. Specific GST activity was calculated as nMole/min/mg protein

Determination of Acetylcholinesterases (AChE) Activity:

Acetylcholinesterase (AChE) activity was measured using acetylthiocholine iodide (ATCh) as a substrate according to Ellman *et al*. (1961). Thiocholine, the product of the hydrolysis of the substrate, reacts with 5,5-dithio bis (2-nitrobenzoic acid) (DTNB) to

produce a yellow anion 5-thio-2-nitrobenzoic acid which absorbs light at 412 nm.

The Procedure:

1- Buffer: Phosphate buffer 0.1 M, pH 8.0 was prepared as described previously.

2- Substrate solution: Acetylthiocholine iodide (ATCh) 0.075 M (21.67 mg/ml distilled water). This solution was used successfully for 10-15 days if kept refrigerated.

3- Reagent: Dithiobisnitrobenzoic acid (DTNB) 0.01 M of the 5,5-dithio bis (2-niotrobenzoic acid), (39.6 mg) were dissolved in 10 ml phosphate buffer 0.1 M (pH 7.0) and 15 mg of sodium bicarbonate were added. The reagent was made up of a buffer of pH 7.0 which was more stable of pH 8.0.

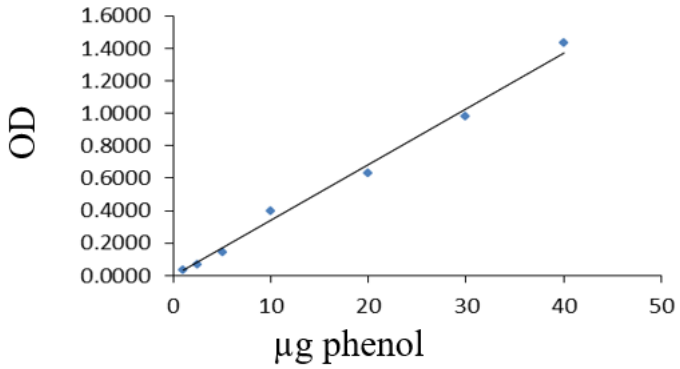
For kinetic microassay, 20 µl of the substrate solution was added to 3.0 ml phosphate buffer pH 8.0, 100 µl DTNB (reagent) and 50 µl from 4th instar larvae homogenate. The blank for such a run consisted of buffer, substrate, and DTNB solutions. The change in absorbance was measured against a blank at 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

Determination Of Acid and Alkaline Phosphatases Activities:

Acid phosphatase (ACP) and alkaline phosphatase (AlP) activities were determined according to the method described by Powell and Smith (1954). The phenol released by enzymatic hydrolysis of di-sodium phenyl-phosphate reacts with 4-amino-antipyrine, and by the addition of potassium ferricyanide, the characteristic brown color is produced.

Preparation of Phenol Standard Curve:

A stock of phenol was prepared by dissolving 1 g pure crystalline phenol in 1 liter 0.1 N HCl. 10 ml of the stock solution (containing 10 mg) was diluted to 100 ml with distilled water. Aliquots of the diluted phenol equal to 1, 2.5, 5, 10, 20, 30, and 40 µg phenol were pipetted into test tubes and the volume was completed to 1 ml with distilled water. 1.1 ml of buffer was added followed by 0.8 ml of NaOH, 1.2 ml of $NaHCOH_3$, 1 ml of 4-amino antipyrine, and 1 ml of potassium ferricyanide. Each tube was mixed well after each addition and the developed color was measured at 510 nm. The standard curve was plotted by O.D. (Optical Density) against phenol concentration.



Reaction Mixture and An Assay of Phosphatase Activities:

The reaction mixture consists of 1 ml carbonate buffer (pH 10.4) for Alk-P, 01 ml of citric buffer (pH 4.9) for Ac-P, 01 ml of 0.010 Molar di-sodium phenyl phosphate (substrate), and 0.10 ml pupal tissues homogenate. The mixture was mixed gently and incubated for exactly 30 minutes at 37°C. At the end of the incubation period, 0.80 ml of 0.5 Normal NaOH was added to stop the reaction. Then 1.2 ml of 0.5 Normal $NaHCO_3$ was added, followed by 1 ml of 4-amino-antipyrine solution and 1 ml of K- ferri-cyanide. The produced color was measured at once by a BECKMAN spectrophotometer (DU 7400) at 510 nm. The enzymatic activity is expressed as µg phenol released/min/g body weight.

Statistical Analysis:

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 the (Duncan, 1955) multiple range test at probability levels of P = 0.05.

RESULTS AND DISCUSSION

Toxicity insecticides for *Culex Pipiens* Larvae:

The percentage of death and the corrected percentage of death were calculated, and the results were obtained after 48 hours by observing the dead larvae from the treatment with pesticides, drawing toxicity lines, calculating the value of Lc_{50}, Lc_{90} and calculating the resistance rate by comparing with the susceptible strain.

Levels of resistance of the field populations of the insects under investigation were calculated as follows:

$$\text{Resistance Ratio (RR)} = \frac{\text{LC}_{50} \text{ of the selected field strain}}{\text{LC}_{50} \text{ of the susceptible strain}}$$

The following criteria proposed by (Mazzarri and Georghiou, 1995) were adopted to classify the resistance level of populations: low (RR<5), moderate (5<RR<10), and high (RR>10).

The data in Table (1) and Fig. (1) showed the toxicity of chlorpyrifos on field and Susceptible strains after 48 hours of treatment, the south strain showed the highest resistance against chlorpyrifos in the north strain, The East strain and the west strain, The R.R.were 38,13,6 and 4 according to the Susceptible strain respectively. The West strain was less resistant compared to the other strains.

Table 1: Toxicity and rate of resistance for chlorpyrifos on field strains and susceptible strain in 3rd instar larvae of *Culex pipiens*

Strains		LC_{50} (ppm)	LC_{90} (ppm)	Slope ± S.E.	R.R
Field Strains	North Strain	0.075	1.115	1.091 ± 0.138	13
	South Strain	0.243	4.376	1.021 ± 0.128	42
	East Strain	0.036	5.567	0.587 ± 0.128	6
	West Strain	0.025	2.329	0.651 ± 0.133	4
Susceptible Strain		0.0058	0.254	0.782 ±0.152	1

S. E., Standard Error

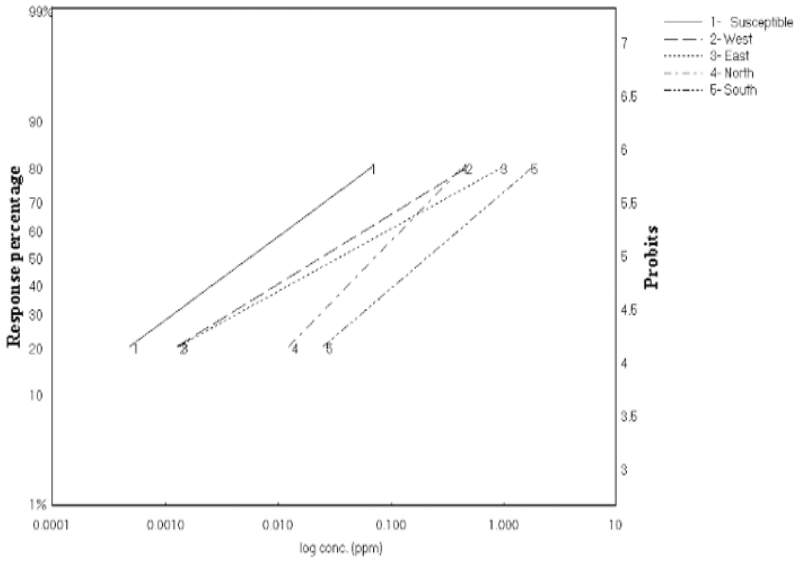


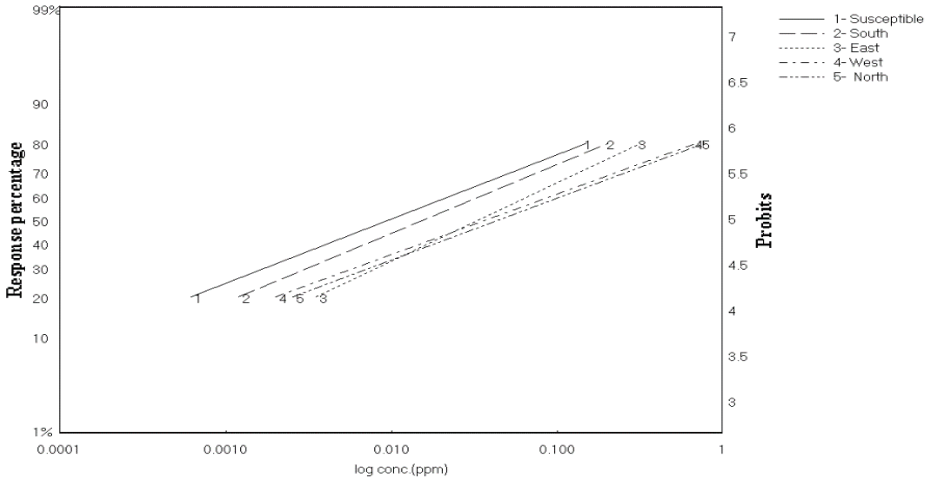
Fig. 1: Toxicity lines of chlorpyrifos on field strains and susceptible strain in 3rd instar

The data in Table (2) and Fig. (2) showed the toxicity of lambda-cyhalothrin on field and Susceptible strains, The North strain showed the highest resistance against lambda-cyhalothrin, in West strain, The East strain, and The South strain The R.R. were 4.592,3.878,3.469and 1.633 according to Susceptible strain, respectively. The South strain was less resistant compared to the other strains.

Table 2: Toxicity and rate of resistance for lmbada cyhalothrin on field strains and susceptible strain in 3rd instar larvae of *Culex pipiens.*

Strains		LC_{50} (ppm)	LC_{90} (ppm)	Slope ± S.E.	R.R
Field Strains	North Strain	0.045	3.500	0.677 ± 0.071	4.592
	South Strain	0.016	0.795	0.753 ± 0.073	1.633
	East Strain	0.034	1.047	0.860 ± 0.077	3.469
	West Strain	0.038	3.268	0.662 ± 0.071	3.878
Susceptible Strain		0.0098	0.639	0.706 ± 0.072	1

S. E., Standard Error



Fig, 2: Toxicity lines of lambada-cyhalothrin on field strains and susceptible strain in 3rd instar larvae of *Culex pipiens*

The data in Table (3) and Fig. (3) showed the toxicity of imidacloprid on field and Susceptible strains, The South strain showed the highest resistance against Imidacloprid, followed by The East strain, The Wests strain and The North strain The R.R. were 345,34,18 and 17 according to Susceptible strain respectively. The North strain was less resistant compared to the other strains.

Table 3: Toxicity and rate of resistance for imidacloprid on field strains and susceptible strain in 3rd instar larvae of *Culex pipiens*

Strains		LC_{50} (ppm)	LC_{90} (ppm)	Slope ± S.E.	R.R
Field Strains	North Strain	0.0046	0.361	0.676 ± 0.093	23
	South Strain	0.061	1.306	0.962 ± 0.095	305
	East Strain	0.0055	0.103	1.012 ± 0.129	27.5
	West Strain	0.0029	0.746	0.531 ± 0.095	14.5
Susceptible Strain		0.0002	1.133	0.331 ± 0.103	1

S. E., Standard Error

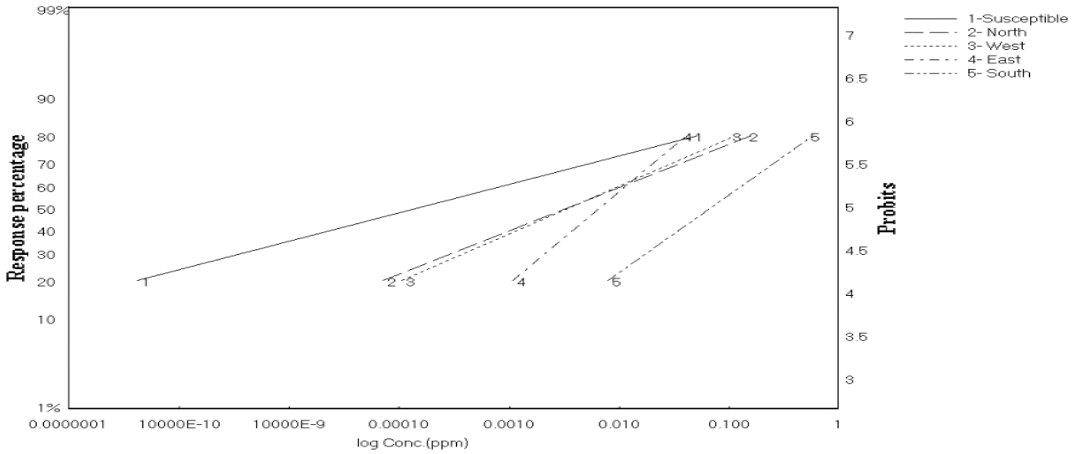


Fig. 3: Toxicity lines of imidacloprid on field strains and susceptible strain in 3rd instar larvae of *Culex pipiens*.

The data in Table (4) and Fig (4) showed the toxicity of emamectin benzoate on field and Susceptible strains, The East strain showed the highest resistance against imidacloprid, The North strain, The South strain and The West strain The R.R. were 2.387, 1.484, 1.323 and 1.161 according to Suscptible respectively. The West strain was less resistant compared to the other strains.

Table 4: Toxicity and rate of resistance for emamactin benzoate on field strains and susceptible strain in 3rd instar larvae of *Culex pipiens*.

Strains		LC_{50} (ppm)	LC_{90} (ppm)	Slope ± S.E.	R.R
Field Strains	North Strain	0.046	0.376	1.407 ± 0.115	1.484
	South Strain	0.041	2.088	0.752 ± 0.09	1.323
	East Strain	0.074	2.382	0.851 ± 0.092	2.387
	West Strain	0.036	0.998	0.892 ± 0.094	1.161
Susceptible Strain		0.031	0.557	1.018 ± 0.103	1

S. E., Standard Error

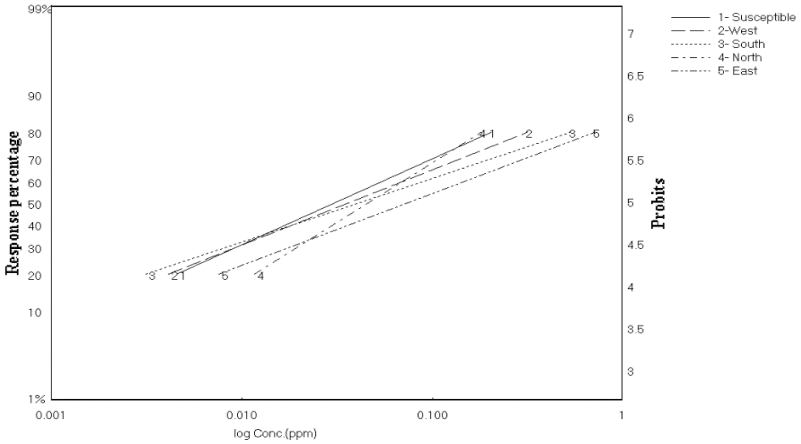


Fig (4): Toxicity lines of emamactin benzoate on field strains and susceptible strain in 3rd instar larvae of *Culex pipiens* .

The data in Table (5) and Fig (5) showed the toxicity of spinosad on field and susceptible strains t, The East strain showed the highest resistance against spinosad, in The South strain, The North strain, The West strain, The R.R.78,44,34 and 3 according to Suscptible respectively. The West strain was less resistant compared to the other strains.

Table 5: Toxicity and rate of resistance for spinosad on field Strains and susceptible strain in 3rd instar larvae of *Culex pipiens*.

Strains		LC_{50} (ppm)	LC_{90} (ppm)	Slope ± S.E.	R.R
Field Strains	North Strain	0.0034	0.061	1.019 ± 0.108	34
	South Strain	0.0044	0.152	0.832 ± 0.101	44
	East Strain	0.0078	0.612	0.676 ± 0.098	78
	West Strain	0.0003	0.041	0.6± 0.087	3
Susceptible Strain		0.0001	0.03	0.522±0.088	1

S. E., Standard Error

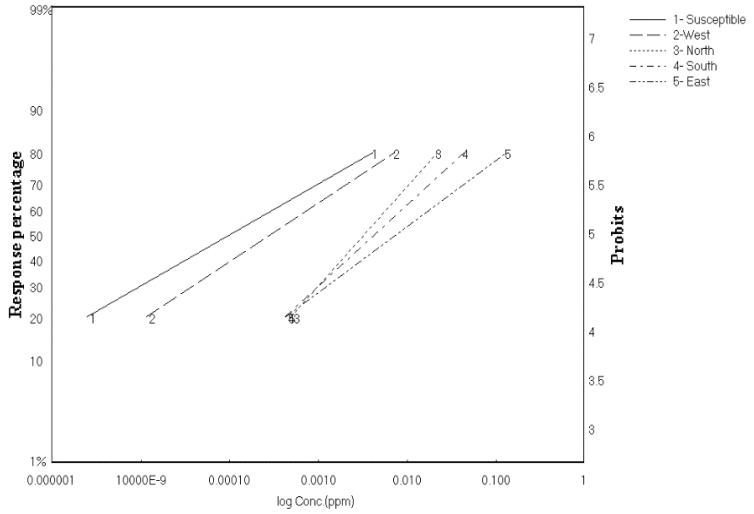


Fig. 5: Toxicity lines of spinosad on field strains and susceptible in 3rd instar larvae of *Culex pipiens*

Toxicity Index and Relative Potency:

The data in Table (7) and Fig (6) showed that the most effective pesticides on The

North strain were spinosad, imidacloprid, lambda-cyhalothrin, emamectin benzoate, and chlorpyrifos resulting in 100%, 73.39%,7.55 %, 7.39% and 4.53% respectively Imidacloprid had the highest value of 22.06-fold.

Table 7: Effect of chlorpyrifos, lambda-cyhalothrin, imidacloprid, emamectin benzoate and spinosad on North strain in 3rd instar larvae of *Culex pipiens*.

Insecticides	LC_{50} (ppm)	LC_{90} (ppm)	Slope ± S.E.	Toxicity Index %*	Relative Potency **
Chloropyrifos	0.075	1.115	1.09 ± 0.138	4.53	1
lambda cyhalothrin	0.045	3.5	0.677 ± 0.071	7.55	1.66
Imidacloprid	0.0046	0.361	0.676 ± 0.093	73.91	16.30
Emamactin benzoat	0.046	0.376	1.407 ± 0.115	7.39	1.63
Spinosad	0.0034	0.061	1.019 ± 0.108	100	22.06

$$*\text{Toxicity Index} = \frac{\text{The value of } Lc_{50} \text{for the most efficient pesticides}}{\text{The value of } Lc_{50} \text{ for the other pesticide}} \text{ X } 100$$

by Sun (1950)

$$**\text{Relative Potency} = \frac{\text{The value of } Lc_{50} \text{for the less efficient pesticides}}{\text{The value of } Lc_{50} \text{ for the other pesticide}}$$

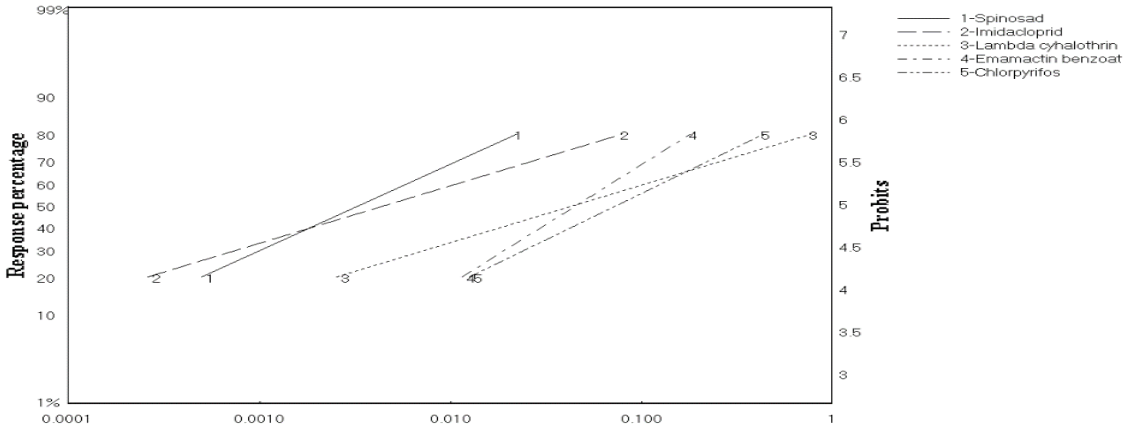


Fig. 6: Toxicity lines of chlorpyrifos, lambda-cyhalothrin imidacloprid, emamectin benzoate and spinosad on North strain in 3rd instar larvae of *Culex pipiens*.

The data in Table (8) and Fig (7) showed that the most effective pesticides on The South strain were spinosad, lambda-cyhalothrin, emamectin benzoate, imidacloprid and chlorpyrifos resulting in 100%,27.5%,10.73%,6.38 % and 1.81 % respectively, spinosad had the highest value of 55.227 fold.

Table 8: Effect of chloropyrifos, lambda-cyhalothrin, imidacloprid, emamactin benzoate and spinosad on South strain in 3rd instar larvae of *Culex pipiens*

Insecticides	LC_{50} (ppm)	LC_{90} (ppm)	Slope ± S.E.	Toxicity Index %*	Relative Potency**
Chloropyrifos	0.243	4.376	1.021 ± 0.128	1.811	1
lambda cyhalothrin	0.016	0.795	0.753 ± 0.073	27.5	15.188
Imidacloprid	0.061	1.306	0.962 ± 0.095	7.213	3.983
Emamactin benzoat	0.041	2.088	0.752 ± 0.09	10.73	5.927
Spinosad	0.0044	0.152	0.832 ± 0.101	100	55.227

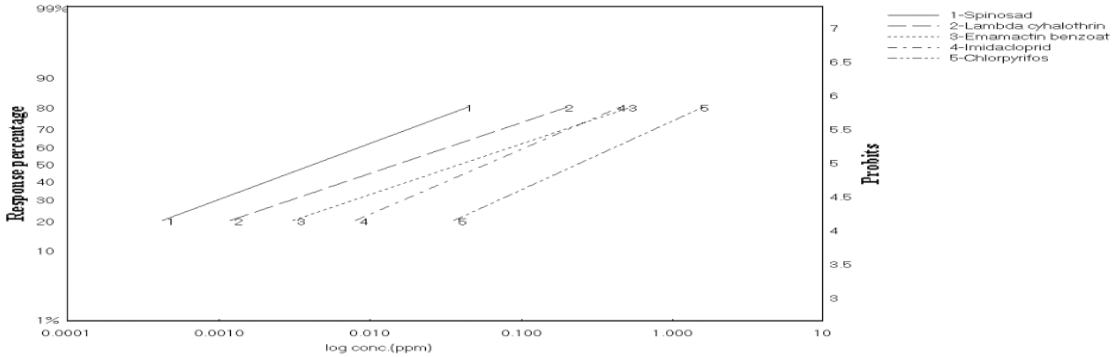


Fig. 9: Toxicity lines of chlorpyrifos, lambda-cyhalothrin, imidacloprid, emamactin benzoate and spinosad on South strain in 3rd instar larvae of *Culex pipiens*.

The data in Table (9) and Fig (10) showed that the most effective pesticides on The East strain were imidacloprid, spinosad, lambda-cyhalothrin, chlorpyrifos and emamactin benzoate resulting in 100%,87.17%,20%,18.89% and 9.189% Imidacloprid had the highest value of 10.88 fold.

Table 9: Effect of chloropyrifos, lambda-cyhalothrin, imidacloprid, emamactin benzoate and spinosad on East strain in 3rd instar larvae of *Culex pipiens*

Insecticides	LC_{50} (ppm)	LC_{90} (ppm)	Slope ± S.E.	Toxicity Index %*	Relative Potency**
Chloropyrifos	0.036	5.567	0.587 ± 0.128	15.278	2.06
lambda cyhalothrin	0.034	1.047	0.86 ± 0.077	16.176	2.18
Imidacloprid	0.0055	0.103	1.012± 0.129	100	13.45
Emamactin benzoat	0.074	2.382	0.851 ± 0.092	7.432	1
Spinosad	0.0078	0.612	0.676 ± 0.098	70.513	9.49

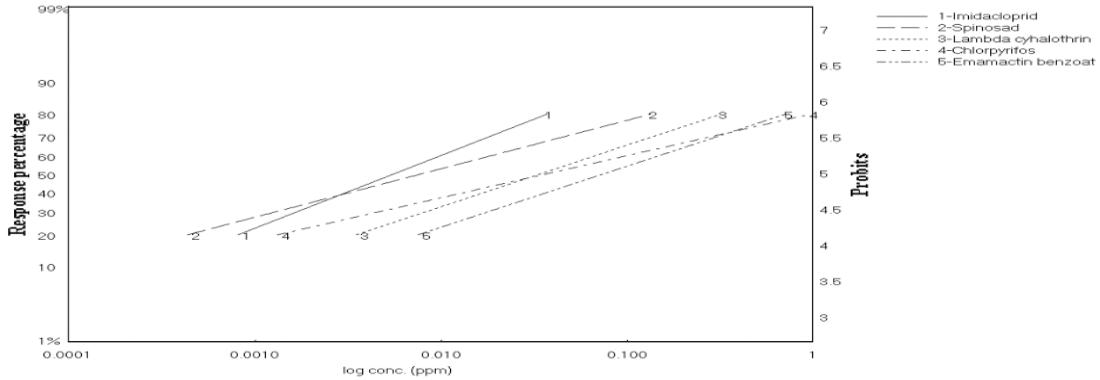


Fig. 10: Toxicity lines of chloropyrifos, lambda-cyhalothrin, imidacloprid,emamactin benzoate and spinosad on East strain in 3rd instar larvae of *Culex pipiens* after 48 hours of treatment.

The data in Table (10) and Fig. (11**)** showed that the most effective pesticides on The West strain were spinosad, imidacloprid, chlorpyrifos, emamactin benzoate and lambda-cyhalothrin resulting in 100%,8.33%,1.2%,0.83% and 0.78% respectively, spinosad had the highest value of 126.67-fold.

Table 10: Effect of chloropyrifos, lambda-cyhalothrin, imidacloprid, emamactin benzoate and spinosad on West strain in 3rd instar larvae of *Culex pipiens*

Insecticides	LC_{50} (ppm)	LC_{90} (ppm)	Slope ± S.E.	Toxicity Index %*	Relative Potency**
Chloropyrifos	0.025	2.329	0.651 ± 0.133	1.2	1.52
lambda cyhalothrin	0.038	3.268	0.662 ± 0.071	0.789	1
Imidacloprid	0.0029	0.746	0.531 ± 0.095	10.345	13.103
Emamactin benzoat	0.036	0.998	0.892 ± 0.094	0.833	1.06
Spinosad	0.0003	0.041	0.6 ± 0.087	100	126.67

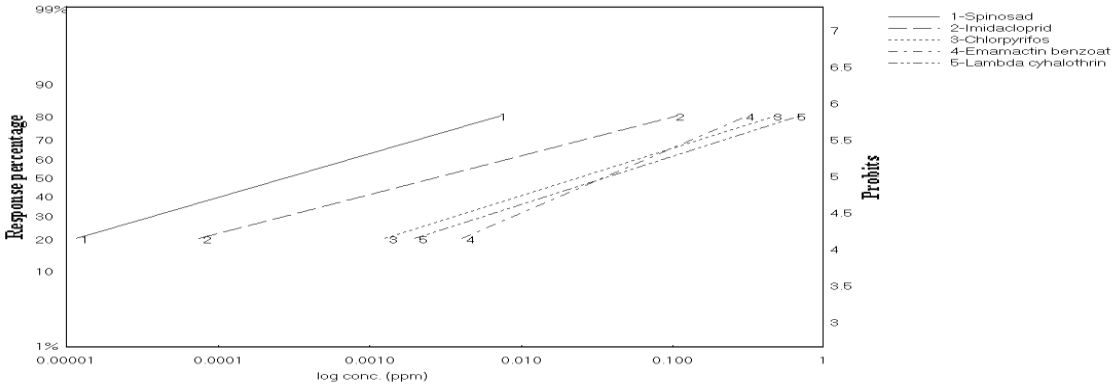


Fig.11: Toxicity lines of chloropyrifos, lambda-cyhalothrin, imidacloprid, emamactin benzoate and spinosad on West strain in 3rd instar larvae of *Culex pipiens*.

These obtained results are in harmony with Thompson *et al.*, (2000) Spinosad was a new insecticide that shows promise as a mosquito control agent. Watson (2000) suggested that Spinosad is a neurotoxin with a novel mode of action involving the nicotinic acetylcholine receptors and GABA receptors. It kills the target insects through activation of the acetylcholine nervous system by nicotinic receptors which results in continuous activation of motor neurons and leads to insect death due to exhaustion (Salgad, 1998 and Thompson *et al.*, 2000). Liu *et al.* (2004) reported that susceptible strains were as susceptible to imidacloprid as to permethrin in larval assays.

Christos *et al*., (2008) Spinosad was a secondary metabolite of the aerobic fermentation of the naturally occurring soil actinomycete Saccharopolyspora spinose which produces a mix of compounds known as spinosyns A and D. Christos *et al*., (2008) Structurally, Spinosad can be described as a macrocyclic lactone containing a unique tetracyclic ring to which two different sugars are attached. Garza- Robledo *et al.*, 2011; Kemabonta and Nwankwo, (2013) established the fact that Spinosad as a larviciding tool will be effective in Egypt. Spinosad has advantages over OP insecticides. Besides its higher insecticidal action against mosquito larvae, it is biodegradable with no significant effect on non-target creatures and minimal risk to human health. El-Sheikh (2011) found that larval *Cx. pipiens* collected from Diarb Negm location, Sharkia Governorate developed 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. It was surprising that Spinosad showed the highest residual activity.Marcombe *et al*., (2011) suggested that chemical may be a promising alternative to chemical insecticides.Abd El-Samie and Abd El-Baset (2012) found resistance (resistant strain) in *Culex. pipiens* to chlorpyrifos toxicity. After 15 generations of selection pressure using chlorpyrifos against the third instar larvae of *Culex. pipiens*, resistance increased by 24.56-fold in the resistant strain as compared with the

control. Soderlund *et al.,* (1989); WHO (2012) verified that resistance management in the context of integrated vector management has evolved as the favored approach to prevent, delay or reduce the impact of insecticide resistance.Hossam El-Din *et al*. (2013) showed lethal concentration LC50 of the formulation of Emamectin benzoate against C. pipiens was found to be 1.24, 0.10 and 0.07 ppm after 24, 48 and 72 hr of treatment. Mohamed and Reda (2014) concluded that Imidacloprid (20% SL) is the most potent Imidacloprid whereas Acetamprid (20% SL) is the most toxic Acetamprid. El-Sayed and Nahla (2015) concluded that lambda-cyhalothrin and lufenuron induced significant metabolic alterations in Cx. pipiens late third-instar larvae by acting on different secondary targets. Mohammed *et al*. (2016) recorded that, inappropriate continuous use of the same larvicide might be responsible for resistance development and stability, also, Cx. pipiens resistance might be inherited by incomplete dominant factors in malathion selection and recessive factors. Nermeen *et al.* (2018) showed that the biochemical assays imidacloprid significantly (P <0.01) decreased the activity levels of both AChE and ATPase enzymes in reducing mosquito-transmitted diseases. imidacloprid, can be used as a successful control method. Tasneem *et al.* (2018) Based on the LT50 values, emamectin benzoate and abamectin demonstrated high efficiency against Culex pipiens (42.60 and 43.61 hours). Matowo *et al.* (2019) reported that *the Culex. pipiens* complex the greatest biting nuisance inside people's and showed resistance to most public health insecticides possible. Resistance varied at a fine geographical scale, between adjacent wards, and seasons, which warrants some modifications to current insecticide resistance monitoring strategies. Nikookar *et al*. (2019) found that the resistance level of field *Cx. pipiens* collected from Iran was lower to pyrethroids compared to organophosphate insecticides. Yahya *et al.* (2019) found that The expression of the four quantified detoxification genes differs significantly in third-larval instars exposed to chlorpyrifos and/or imidacloprid compared with controls. Gravid females also fail to lay eggs in water to which either of the insecticides or the binary mixture is added, although they do lay eggs in cups containing water only. Chronic exposure to sublethal concentrations of chlorpyrifos or imidacloprid has significant adverse effects on development and thus the reproductive fitness of C. pipiens and, accordingly, could be used in the population control of these mosquitoes. Doaa *et al.* (2020) showed promising larvicidal activity against *Culex. Pipiens* in the larvicidal assay, acetamiprid and thiamethoxam, with LC_{50} values of 0.0093 and 0.0305 ppm after 24 hrs, 0.0078 and 0.0206 ppm and 0.0065 and 0.0137 ppm after 72 hrs of insecticidal exposure.Wang *et al.* (2020) found larval density of Culex. tritaeniorhynchus correlated positively with water depth (r = 0.927 p = 0.003). used Spearman correlation analysis to evaluate the relationship between larval density and the physicochemical characteristics of the breeding habitat. Ahmed (2021) showed that spinosad has the highest larvicidal toxicity followed by pyrethroids and then OP insecticides. Shaimaa *et al.* (2022) concluded that chlorpyrifos has a different toxicological effect on the tested mosquito larvae

Role of Biomarker in Resistance Larvae of *Culex pipience:*

The biomarkers of mosquito larvae taken from different regions north, south, east and west of Cairo were studied to estimate the enzymes responsible for the occurrence of resistance: Glutathione S-transferase (GST) enzyme, acetylcholinesterase (ACHE) enzyme, alkaline phosphatase (AlP) enzyme and acid phosphatase (ACP) enzyme in microchemical analyzes component of the Plant Protection Research Institute.

The data in Table (12) and Fig. (36) showed that the North strain had Glutathione S-transferase(GST)enzyme activity was (744.67) µmol and the East strain was (931.33) µmol where there were no significant differences between them, while the West strain was (1004) µmol and the South strain was the most active with (1137.67) µmol which indicates that the resistance rate was It was higher in the South strain among the field strains due to

the increase in Glutathione S-transferase(GST) enzyme activity compared to the Susceptible strain.

The data in Table (12) and Fig. (37) showed that the North strain had Acetylcholine esterase enzyme activity was (235.03) µmol, the East strain was (236.13) µmol and the West strain was (254.17) µmol where there were no significant differences between them, while the South strain was the most active with (360.17) µmol which indicates that the resistance rate was It was higher in the South strain among the field strains due to the increase in Acetylcholine esterase enzyme activity compared to the Susceptible strain.

The data in Table (12)and Fig. (13) showed that the North strain had acid phosphatase (ACP activity was (30.48) µmol, and the West strain was (27.16) µmol where there were no significant differences between them, while the East strain was (39.72) µmol and the South strain was the most active with (48.04) µmol which indicates that the resistance rate was It was higher in the South strain among the field strains due to the increase in Acid phosphatase (ACP) enzyme activity compared to the Susceptible strain.

The data in Table (12) and Fig. (14) showed that The North strain had alkaline phosphatase (AlkP) enzyme activity was (174.68) µmol, the East strain was (163.58) µmol and the West strain was (158.57) µmol where there were no significant differences between them, while the South strain was the most active with (308.85) µmol which indicates that the resistance rate was It was higher in the South strain among the field strains due to the increase in Aalkaline phosphatase (AlkP) enzyme activity compared to the Susceptible strain.

Table 12: Determination of glutathiones-s-transferase, acetylcholine esterase, alkaline phosphatase and acid phosphatase in field strains and susceptible strain larvae of *culex pipiens.*

Strains		Acid phosphatase (µg phosphate/min/ml) ± S.D	alkaline phosphatase (µg phosphate/min/ml) ± S.D	Acetylcholine esterase (ug AchBr /min/gm body weight) ± S.D	Glutathiones-transferase(GST) (u mole sub. Conjugated/ min/mg protein) ± S. D
Field strains	North strain	30.48^{c}± 4.80	174.68^{b} ± 30.52	235.03^{b} ± 15.21	744.67^{c} ± 22.14
	South strain	48.04 ^{a}± 4.24	308.85^{a} ± 30.66	360.17^{a}± 23.65	1137.67^{a} ± 55.99
	East strain	39.72^{b} ± 4.23	163.58^{b} ± 3.13	236.13^{b} ± 26.00	931.33^{c} ± 25.70
	West strain	27.16^{c}± 0.96	158.57^{b} ± 33.08	254.17 ^{b}± 13.67	1004.00 ^{b} ± 63.65
Susceptible strain		20.32^{d}± 2.36	151.11^{b} ± 16.08	196.23^{c} ± 3.08	700.67^{d} ± 11.02
F value		27.08	20.35	34.41	27.08

Means with the same letter within each column were not significantly different from another at the 0.05% level by Duncan's (1955).

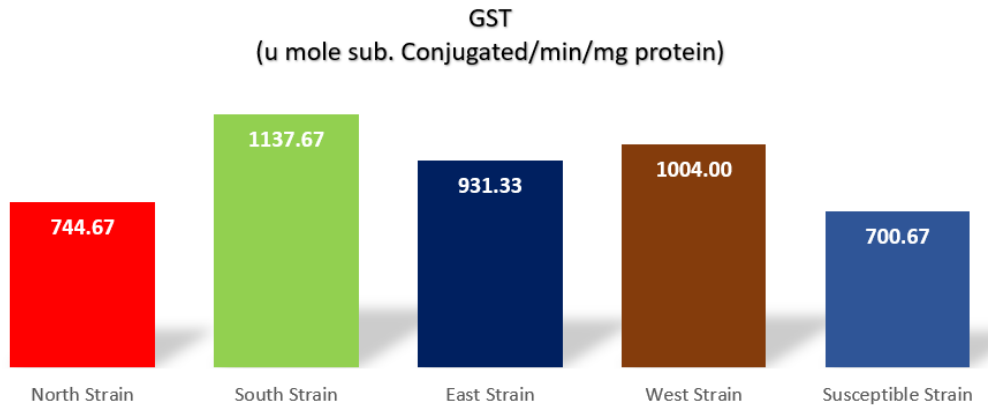


Fig. 12: Mean of GST in larvae of *Culex pipiens* field strains and susceptible strain.

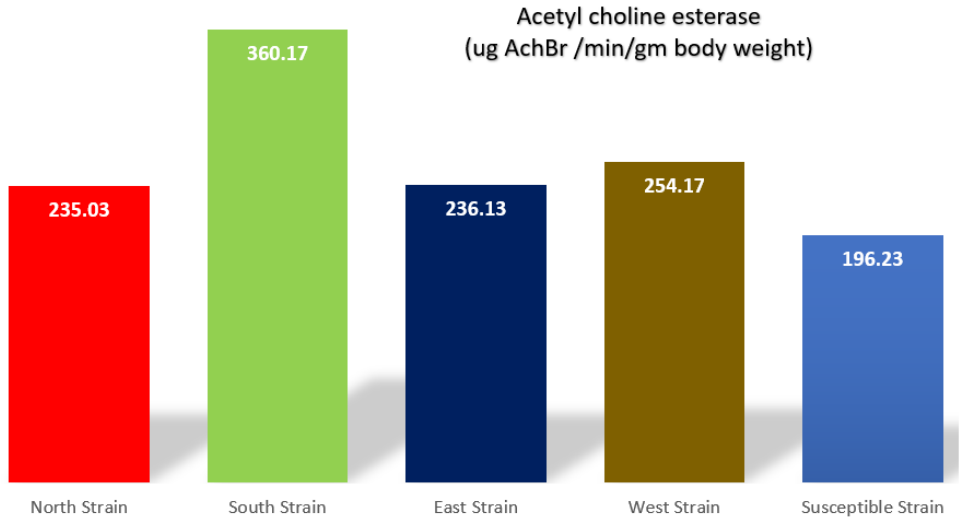


Fig. 13: Mean of Acetylcholine esterase in larvae of *Culex pipiens* field strains and susceptible strains.

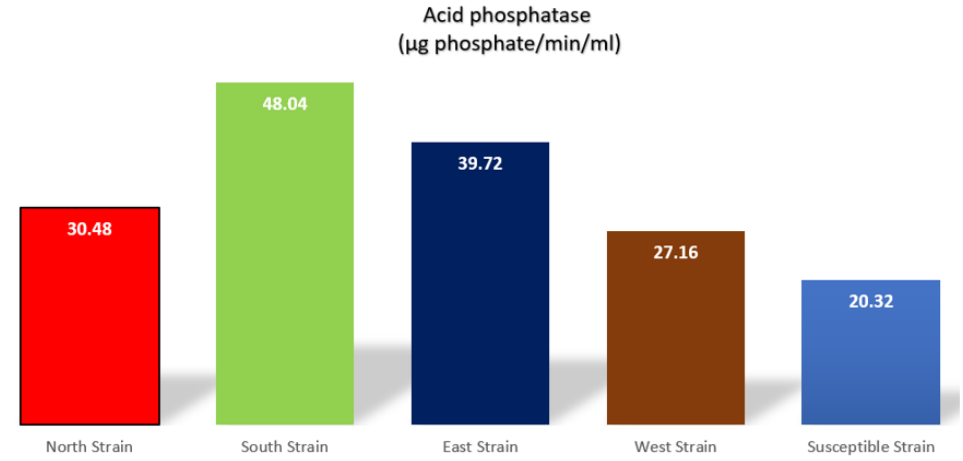


Fig. 14: Mean of Acid phosphatase in larvae of *Culex pipiens* field strains and susceptible strains.

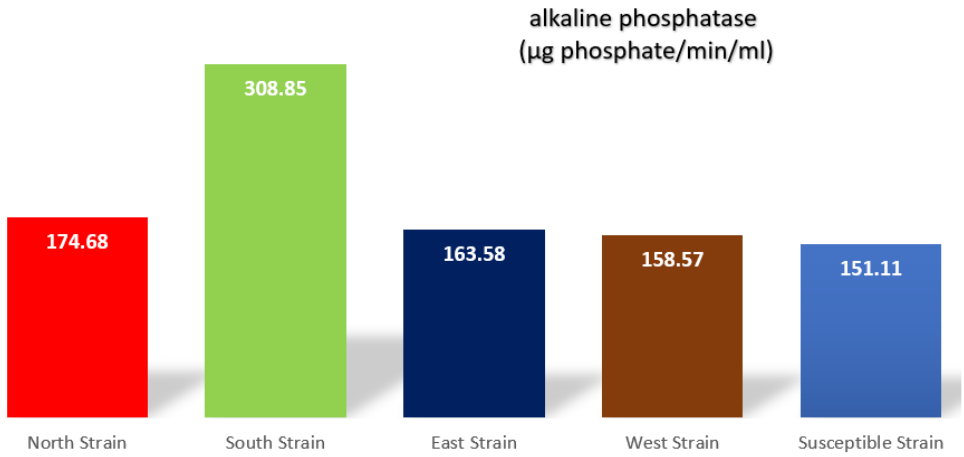


Fig. 15: Mean of Acid phosphatase in larvae of *Culex pipiens* field strains and susceptible strain.

These results were in harmony with Denis *et al.,* (1996) indicated that only AChE fulfills the physiological function of neurotransmitter hydrolysis at synapses, In the insecticide-resistant strain. showed The Spinosad had the highest larvicidal effect against susceptible and field-strain larvae. Ana *et al.,* (2011) showed Acetylcholinesterase-modified mosquitoes, a significant reduction in energetic resources (20% less). Ahmed *et al.,* (2012) found A correlation between elevated esterase activities and kdr assay indicating that further investigation should be done to Fig out the potential role of enzyme detoxification and kdr assay in conceding resistance to pyrethroids class. Isabela *et al*., (2012) Studied that The esterase gene family appeared to be rapidly evolving and each insect species had a unique complement of detoxification genes with only a few orthologues across species. Doaa *et al.,* (2020) showed Acetamiprid a significant increase in the activity level of AChE, GST, carboxylesterase, α, and βesterases than thiamethoxam. Gharib *et al*., (2020) found that activities of detoxifying enzymes increased gradually with raising generation numbers indicated that the increased resistance is likely to be associated with the increased activity of target and metabolic enzyme systems. Jia *et al.,* (2020) suggested All the results that the larvicidal mechanism of ar-turmerone is estimated to be stomach poison and the active sites might be the muscle and digestive tissues, and the mode of action of ar-turmerone may be unrelated to AchE. Kamal and Bulbuli (2021) inferred that mosquito showed increased detoxification in generational time with an increase in enzymes associated with metabolic detoxification. Meta *et al*., (2022) investigated that A strong correlation between increased levels of insecticide resistance has been observed in tested insects with cytochrome P450 (CYP), glutathione-S-transferase (GST), and esterase gene superfamilies.

Conclusion

By studying the toxicity index and the relative potency of different pesticides, it was found that spinosad and imidacloprid were more efficient (22.06 and16.30), respectively after 48 hours in the north strain. In South strain, Spinosad was more efficient (55,227) fold after 48 hours. In East strain, the relative efficacy of Imidacloprid was (13.75) fold after 48 hours. In West strain, was Spinosad more effective, (126.27) fold after 48 hours .The study showed the role of enzymes in resistance on domestic mosquito larvae, where acid phosphatase, base phosphatase, acetylcholinesterase and glutathione S transferase were higher in the South strain, which indicates a higher rate of resistance.

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ARABIC SUMMARY

تغيرات السمية والبيوكيميائية لبعض المبيدات الحشرية على الكيولكس بيبينز
من مصر

عماد سمير كامل منصور [1] , منى حسن حسين هلال [2] , قدرى وشاحى محمود [1] , طه عبد العظيم محمد عبد الرازق [2]

(1) قسم وقاية النبات كلية الزراعة جامعة عين شمس
(2) كلية الدراسات والبحوث البيئية جامعة عين شمس

يعتبر البعوض المنزلى *Culex pipiens* (Diptera: Culicidae) من أهم آفات الصحة العامة المنتشرة في مصر، لذلك قمنا بدراسة التغيرات الكيميائية الحيوية والسمية للمبيدات التقليدية والحيوية على يرقات العمر الثالث للبعوض المنزلي. ثلاثة مبيدات عضوية (كلوربيريفوس ، لامبادا سيهالوثرين ، إيميداكلوبراييد) واثنين مبيد حيوى (إيمامكتين بنزوات ، سبينوساد) بتركيزات مختلفة على يرقات البعوض وحساب قيمةLC50 بعد المعالجة ودراسة معدل المقاومة.

أظهرت النتائج أن سلالة القاهرة الجنوبية من يرقات البعوض المحلية كانت اعلى مقاومة ضد الكلوربيريفوس إيثيل، ووجد أن أكثر المبيدات التي تم اختبارها كفاءة كان سبينوساد ، حيث كانت في السلالة الجنوبية 55227 ضعفًا ، وفي السلالة الشرقية كانت الكفاءة النسبية من الايميداكلوبراييد كان 12.17 ضعفًا وكانت الكفاءة النسبية للايميداكلوبراييد اعلى في السلالة الغربية يليها السبينوساد.

وكان نشاط الفوسفاتيز الحامضي، الفوسفاتيز القلوي، أسيتيل كولينستراز والجلوتاثيون اس ترانسفيراز في اليرقات أعلى في سلالة جنوب القاهرة مما يشير إلى أن هذه السلالة كانت لها مقاومة أكبر ضد المبيدات الحشرية المختبرة.