Annals of Agric. Sci., Moshtohor **ISSN 1110-0419**
Vol. 59(2) (2021), 577 – 584 **https://assjm.journals.ekb.eg**

Efficacy of Chlorpyrifos-ethyle and *Bacillus thuranginsis israelinsis* against *Culexpipiens* (L.)

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Abstract

Culex pipiens (L.) (Diptera: Culicidae) is the most important medical insect in many parts of the world. Biological and natural chemicals have many advantages over the traditional ones in case of mosquito control. The efficacy of two insecticides belonging to different groups chlorpyrifos-ethyle (oranophosphate) and *Bacillus thuringiensis israelensis*) (bio-insecticides) were evaluated against the field and laboratory individuals of late 3rd instar larvae of *Culex pipiens* at different concentrations and three periods of exposure under laboratory conditions. Results obtained showed that the laboratory strain showed higher susceptibility to the tested insecticides than the mosquito populations collected from Abo-Rawash City, Giza Governorate and the mortality percentage was increased gradually with increasing the insecticide concentrations and the mortality percentage showed significant differences between concentrations and control. Also, The activity of acetyl cholinesterase (AChE), the glutathione *S*- transferase (GST), Total proteins and the activities of both Aspartate aminotransferase AST(GOT) and Alanine aminotransferase ALT(GPT) were determined were determined after 72 hrs of insecticidal exposure.

Key words: *Culex pipiens*;Toxcity; Chlorpyrifos; biochemistry mechanisms .

Introduction

Man has suffered from the activities of mosquitoes since time immemorial and it is ranked as man's most important insect pest. The genera of mosquito have been incriminated as the main vectors: *Culex*, *Aedes*, and *Anopheles*, which transmit several infectious diseases to human; for example, filariasis, Japanese encephalitis, dengue and yellow fever viruses, and malaria **(Barbosa *et al*. 2011 and Jang *et al.* 2002)**

In Egypt, the widespread house mosquito *Culex pipien molests* (Forsk) has been recorded in all governorates without any exception **(Kady *et al*. 2008)** causing a health problem and nuisance to humans **(Zahran *et al.* 2011**).

Mosquito control represents an important strategy for prevention of diseases transmission and epidemic outbreaks. The efficacy of the most used insecticides belonging to different groups (organophosphate, carbamate, synthetic pyrethroid and insect growth regulator) was tested by **(Emtithal & Abd El-Baset, 2012)** against four different field populations of *Cx. Pipiens*. For many decades, the scientists have been engaged in searching the effective and efficient of the mosquito control program based on chemicals. Chlorpyrifos-methyl (Reldan®), an OP used in agriculture to control stored product pests, may be more suitable, being classified by WHO as unlikely to present acute hazard in normal use, whereas carbosulfan and the majority of pyrethroids recommended for treatment of mosquito nets are classified by WHO as Class II, i.e. moderately hazardous **(WHO, 2002)**. The primary mechanism of toxicity of organophosphorus pesticides, such as chlorpyrifos, is cholinesterase inhibition (AChE). Inhibition of the enzyme acetylcholinesterase (AChE) results in an accumulation of acetylcholine (AChE) at choline receptors, resulting in continuous nerve stimulation **(Giesy *et al.* 1999)**.

The resistance to conventional insecticides is the major problem in mosquito control program. The traditional insecticides are environmentally nonsustainable and harmful the natural enemies, consequently may due to disturbance in the natural balance and most mosquito species are becoming physiologically resistant **(Karunamoorthi and Sabesan, 2013). Daaboub, *et al*., (2017**) studied the susceptibility of larval stage of mosquito *Cx. pipiens* against organophosphate chlorpyrifos and carbamate propoxur insecticides in Southern Tunisia. All samples were resistant to chlorpyrifos (RR>1, $p<0.05$) and the tolerance to this insecticide was varied between 1.8 and 1318. The appearance of such problems has been accompanied by growing interest to use new safe bioinsecticide with a new mode of action specially when dealing with water (**Salgado, 1997 and Salgado, 1998).** The commercial products whose active principles are based on *Bacillus thuringiensis israelensis* were tested against *Cx. pipiens* to determine their effectiveness against field and laboratory strains of its 3rd larval instar **Lopes, *et al*., (2010).**

Insecticide resistance become a major problem in vector control programs due to pesticide resistance through detoxification enzymes. Enzyme – based metabolic mechanisms of spinosad were investigated based on the biochemical assay principle against the laboratory population of *cx. pipiens*. The obtained

results showed that there were no significant difference in the activity of alfa esterase, acetylcholinesterase and invertase enzymes after the larvae treatment with spinosad ,while there were significant differences in protease and beta esterase activities after 6, 24 and 48 hour post treatment, respectively .**(Moselhy, *et al*.,2015).** On the other hand, , **Gharib, *et al*., (2020)** Investigated the susceptibility of *Cx. pipiens* larvae collected from Al-Asher of Ramadan, Sharkia Governorate, to chlorpyrifos and lambda-cyhalothrin insecticides 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.

The present study was carried out to investigate effect of (chlorpyrifos-ethyl) and bio-insecticide *Bacillus thuringiensis israelensis* on the larval mortality and their impacts on some biochemical parameters of *Cx. pipiens* larvae .

Materials and Methods

1. Toxicological studies:

1.1: Rearing of lab strain of *Culex pipiens* The egg rafts of common house mosquito, *Culex pipiens* were obtained from the Research and Training Center on Vectors of Diseases (RTC), Faculty of Science, Ain Shams University. The mosquitoes were reared for at least ten generations in insectary rooms, under controlled laboratory conditions at temperature 27±2 ºC, and relative humidity RH 70±80%, for photoperiods14:10 (light: dark) hours. Egg rafts were placed in white enamel dishes 35-40 cm in diameter and 10cm in depth filled with 1500 ml of distilled water. Newly hatched larvae were fed on fish food (Tetra-/Min, Germany) as a diet sprinkled twice daily over the water surface of the breeding pans **(Kasap and Demirhan, 1992)**.Distilled water in each dish was stirred daily and changed every two days to avoid scum formation on the water surface or on the walls and bottoms of pans. Small air pump was used to aerate the breeding water gently every day for about 5 minutes. Pupae were collected routinely and separated in plastic containers filled with distilled water then introduced into screened wooden cages until emergence. Adults were reared in (24 x 24 x 24 cm) wooden cages and provided daily with cotton pads soaked in 10% sucrose solution for a period of four days. After this period the females were allowed to take a blood meal from a pigeon host. To obtain best blood feeding, sucrose was removed 24 hours prior to blood meal. Oviposition containers filled with distilled water were placed in adult cages 48 hours after the females had been provided with blood meals. Deposited egg rafts were collected routinely and placed in white enamel dishes. When mosquito larvae developed to the 2nd instar, they were poured into clean pans (25x 30x15cm) containing 3 liters of tap water left for 24 hours and observed daily **(Gerberg,1970 and Kasap and Demirhan, 1992)**. Early third larval instars were used for toxicological studies.

1.2 Field mosquito strains

Larvae of *Cx. pipiens* were collected from (Abo-Rawash City, Giza Governorate). Mosquitoes were reared basically as those described by **Chapman** and **Barr, 1969**. They hold in the insectary in which temperature was maintained at 27±2 C^o and humidity between 70- 80%.

1.3 Insecticides used:-

1- The commercial formulation of organophosphorus (dursban 48% EC) (**Chlorpyrifos-Ethyl)** (C9H11Cl3NO3PS): dimethoxy-sulfanylidene-(3,5,6-trichloropyridin-2-yl)oxy-λ5-phosphane, was supplied by Dow Agrosciences 2- 2- The Bio-inseticide (Diple 2x 6.4%WP) **(*Bacillus thuringiensis var israelensis)*** which was by Valent Bioscience.

1.4 Larvicidal assay

The bioassay was assessed by using the standard method according to **(WHO, 2005)** with some modification. Batches of 25 3rd instar larvae of *Cx. pipiens* were transferred to five small test cups, by a plastic dropper. Different concentrations of insecticides (00.1, 0.01, 0.001 and 0.0001 µl.L^{-1} for Chloropyrifos Ethyl) and (10, 7.5, 5, 2.5 and 1.25µg.$L^{-1}$for *Bacillus thuringiensis israelensis*) were assayed. (3/replicate) were usually applied for each concentration including the control. The larval mortality was recorded after 24, 48 and 72 hrs post-treatment.

2. Preparation of Samples for Biochemical Assay

The field and laboratory strain of 3rd larval instar of *Cx. pipiens* larvae were collected after 72 hrs from all concentrations of treatment and homogenized in distilled water. Homogenates were centrifuged at 5000 rpm for 15 min. The supernatant was placed in tubes as a source of biochemical assays of enzymes analysis as mentioned by **(Assar2012)**.

Determination of some biochemical parameters

2.1 Acetylcholinesterase activity (AChE)

Acetylcholinesterase activity was evaluated depending on **(Simpson *et al.* 1964)** method. Acetylcholine bromide (AChBr) was used as a substrate.

2.2 Glutathione S-transferase activity (GST)

The activity of glutathione *S*-transferase (GST) was evaluated depending on **(Kao *et al.* 1989)** method CDNB was used as a substrate.

2.3 Transaminas activity: The activities of both Aspartate aminotransferase AST(GOT) and Alanine aminotransferase ALT(GPT) were determined in the larval homogenate according to the method of **Reitamn and Frankle (1957)**.

2.4 Total protein content:

A standard and quantitative assay for determination the total protein content in larval homogenate has been carried out based on the method of **Bradford (1976).**

Measure activity ratio for each enzyme was calculated according to the following equation:

Activity ratio =

$$\frac{\text{Enzyme activity in treated larvae}}{\text{Enzyme activity in control}}$$

3. Statistical Analysis

Mortality and the percentages of enzyme activation were subjected to probit analysis for calculating LC_{50} and LC_{90} **(Finney, 1971**), other parameters statistic used (LDP-line) for the goodness of fit (Chi -square test) **(Duncan, 1955).**

Results Aand Discussion

Culex pipiens (L.) (Diptera: Culicidae) is the most important medical insect in many parts of the world. Biological and natural chemicals have many advantages over the traditional ones in case of mosquito control. Chloropyrifos, and *Bacillus thuranginsis israelinsis* were evaluated for their efficiency against the field and laboratory individuals of late 3rd instar larvae of *Culex pipiens* at different concentrations and three periods of exposure under laboratory conditions. The values of the insecticidal activates of the two tested compounds, the organophosphate chloropyifos-ethyl and the bioinsecticide *Bacillus thuringiensis israelensis*, against 3rd instar larvae of *Culex pipiens* with (laboratory and field strains) are illustrated in Tables (1&2).

1. **Larvicidal activity of chlorpyrifos-ethyl against the 3rd instar larvae of *Culex pipiens (*laboratory and field strains) after different exposure times.**

The obtained data clearly indicate that cholrpyrifos-ethyl was more effective against the 3rd instar larvae of laboratory strain than field strain .The high percentages of larval mortality were recorded with concentration of 0.1 µlL^{-1} (70.66, 96 and 100%), , in case of laboratory ,while the high percentage of larval mortality of field strain of *Culex pipiens* 3rd instar lavae were (70.66, 86.60 and 94.66%), after 24, 48 and 72 hours of application, respectively. Moreover, the obtained results Table (1) show LC_{50}, LC_{90} and slope values of the chlorpyrifos-ethyl against susceptible laboratory strain of *Culex pipiens*. Based on LC_{50} values, it clear that chlorpyrifos-ethyl was the most toxic compound (LC_{50} = 0.01, 0.001 and 0.0001µlL^{1}), in laboratory strain, while this values were increased to (LC_{50} = 0.0165, 0.0035 and 0.0012 µlL^{1} in field strain after 24, 48 and 72hrs. of exposure,respectively.

Table 1. Larvicidal activity of chlorpyrifos-ethyl against the 3rd instar larvae of *Culex pipiens* (laboratory and field strains)after different exposure times.

Strain	**Laboratory strain**			**Field strain**		
Concentration	**Mortality (%) / hrs.**			**Mortality (%) / hrs.**		
***µlL^{-1}**	**24hr.**	**48hr.**	**72hr.**	**24hr.**	**48hr.**	**72hr.**
0.1	70.66	96.00	100	70.66	86.60	94.66
0.01	48.00	78.66	98.60	41.30	56.00	65.30
0.001	29.33	41.30	64.00	22.66	33.30	41.30
0.0001	21.33	24.00	50.66	9.30	20.00	30.66
Control	0	0	0	0	0	0
LC_{50}	0.01	0.001	0.0001	0.0165	0.0035	0.0012
95%F.l*	(0.004 - 0.012)	(0.001-0.002)		(0.009 - 0.031)	(0.002-0.006)	
LC_{90}	6.25	0.05	0.01	1.96	0.36	0.12
95%F.l*	(1.21 - 105.83)	(0.03 - 0.01)		(0.62 - 10.78)	(0.14 - 1.34)	
Slope±SE**	0.45 ± 0.06	0.79 ± 0.06	0.78± 0.09	0.62 ± 0.07	0.63 ± 0.07	0.64± 0.06

* Fiducially Limits *µlL^{-1} = Micro Liter /Liter.

**Slope of the concentration-inhibition regression line± standard error.

The susceptible laboratory strain exhibited relatively high slope values (as expected) to tested compound. Slope values of laboratory strain ranged from about 0.45 ± 0.06 to 0.78 ± 0.09 indicating that the laboratory strain is homogenous. While the slope values of field strain ranged from about 0.62 ± 0.07 to 0.64 ± 0.06). The slope values in field or in lab strain proved that the homogeneity between field and laboratory individuals. These results agree with those obtained by **Emtithal & Thanaa (2012)** who indicated that the laboratory colony showed higher susceptibility to chlorpyrifos than the field populations of *Cx. pipiens* populations collected from Sharkia and Assiut Governorates. Also, **Daaboub, *et al.*, (2017)** studied the susceptibility of larval stage of mosquito *Cx. pipiens* against organophosphate chlorpyrifos and carbamate propoxur insecticides in Southern Tunisia. They found that samples were resistant to chlorpyrifos (RR>1, p<0.05) and the tolerance to this insecticide was varied between 1.8 and 1318.

2. Larvicidal activity of *Bacillus thuringiensis var* i*sraelensis* **against the 3rd instar larvae of *Culex pipiens* (laboratory and field strains) after different exposure** times**.**

Results in Table (2) show the mortality percentages of bio pesticides *Bacillus thuringiensis var* i*sraelensis (Bti)* on the 3rd instar larvae of *Culex pipiens* mosquito as bio-indicator, with a serial number of concentration (10, 7.5, 5, 2.5and 1.25µgL-1) corresponding to laboratory and field strains after different exposure times and the lethal dose concentration LC_{50} and LC_{90} of *(Bti)* on *Culex pipiens* larvae. Also, from these date it is clear that the exposure time has an important effect on the values of LC_{50} in this study. In most cases, the LC_{50} values had synergistic interactions with time; thus, it increased after 72h of exposure when compared to 24 h of exposure (Table 2). Very high concentrations of the *(Bti)* gave high mortality in laboratory and field strain of *Culex pipiens* larvae after24,48and 72 hours of exposure. Where as the high percentages of larval mortality were recorded after 24, 48 and 72 hours of application (69.3, 94.6 and 100%) for lab and field strain were (61.3, 80, and 98.6%) , respectively with increasing the time of insecticide exposure for *Culex pipiens* larvae.

Also, results presented in Table (2) show that LC_{50} value, of *(Bti)* were (LC_{50} = 6.137, 3.783and 2.856µgL^{-1}), in laboratory strain, while this values were increased to (LC_{50} = 6.831, 5.092 and 4.142 µgL^{-1}) in field strain after 24,48 and 72hrs. of exposure , respectively. The slope values of susceptible laboratory strain exhibited relatively high slope values to *(Bti)* laboratory strain, ranged from about 1.6004 ± 0.193 to 2.589 ± 0.211) indicating that the laboratory strain is homogenous. While the slope values of field strain ranged from about to 2.097 ± 0.219 to 3.391 ± 0.252). The slope values in field or in laboratory strain proved that the heterogeneity between field and laboratory individuals; *Bacillus thuringiensis israelensis* has been used in large scale due to its specificity for Culicidae. Our results show a toxic effect of *Bti* against the 3rd instar larvae of *Cx. pipiens.* Thus, laboratory and field strains show that *Bti* has a reduction in the abundance of mosquito larvae after *Bti* application in different sites.The *Bti* which specifically affects the Culicidae, contains spores and parasporal crystals of the serotype of *Bti* H -14, that must be ingested by the larvae of the mosquito to cause mortality. After ingestion, the parasporal crystals are solubilized in the larval alkaline midgut, followed by proteolytic activation of proteins into soluble crystals. The toxin binds to a receptor cells of the midgut wall to form pores in cell, leading to larval death **Gill *et al.* (1992) ; Bauer *et al.*(1995) and Mansouri *et al.* (2013).**

3. Biochemical activity of chlorpyrifos-ethyl and *Bacillus thuringiensis israelensis* **against the 3rd instar larvae of *Culex pipiens* (laboratory and field strains) after 72hrs. exposure time**

Acetylcholinesterase (AChE), Glutathione S-transferase activity (GST), Total protein (TSP) content and the activities of both Aspartate Aminotransferase AST(GOT) and Alanine Aminotransferase ALT(GPT) were determined in 3rd instar larvae of *Cx. pipiens* which survived after 72 hrs. from exposed to different concentrations of the two tested insecticides. Results presented in Table 3 reveal that the highest values (GST) of chlorpyrifos were 2.27 and 5.00 with activity ratio about 1.62 and 1.56 in the 3rd instar larvae of *Cx. Pipiens* for both laboratory and field strains respectively,and significantly increased compared with control, . While, GST showed insignificant different for *(Bti)* compared with control. Also the obtained results clear that AChE activities, after chlorpyrifos and *(Bti)* treatments in comparison with untreated control, were generally inhibited (**Table 3)**. Where as the acetylcholinesterase values were 3618.6, 1368.0 and 1488.67 with activity ratio about 2.43 and 0.49 in 3rd instar larvae of *Cx. pipiens* of labouratry strain, exposed to chlorpyrifos, and *(Bti)* ,respectively .on the other hand Chlorpyrifos exhibited significantly the AChE activity compared to *(Bti)* about 2.43 and 0.92 in field strain, respectively.

Table 2. Larvicidal activity of *Bacillus thuringiensis israelensis* against the 3rd instar larvae of *Culex pipiens* (laboratory and field strains) after different exposure times.

Strain	**Laboratory strain**			**Field strain**		
Conc.	**Mortality (%)**			**Mortality (%)**		
***µgL^{-1}**	**24hr.**	**24hr.**	**24hr.**	**24hr.**	**48hr.**	**72hr.**
10	69.30	94.60	100	61.30	80	98.60
7.5	56.00	74.60	86.66	56	66.60	78.60
5	36.00	39.00	62.22	40	46.60	50.60
2.5	24.00	28.00	34.66	16	17.30	20.00
1.25	17.33	21.30	25.33	6.60	9.30	8.00
Control	0	0	0	0	0	0
LC 50 95%F.l*	6.137 (5.17-7.54)	3.783	2.856 (1.49-4.19)	6.831 (5.948 - 8.062)	5.092 (4.55-5.72)	4.142 (2.539 - 6.134)
LC90 95%F.l*	38.793 (25.04-77.61)	14.823	8.925 (7.56- 25.75)	27.904 (20.34-44.46)	16.431 (13.33-21.84)	9.888 (8.640-24.318)
Slope±SE**	(1.60 ± 0.193)	(2.16±0.198)	(2.59 ± 0.21)	(2.097± 0.219)	(2.52 ± 0.22)	(3.39 ± 0.252)

* Fiducially Limits *µgL^{-1} = Micro gram /Liter.

**Slope of the concentration-inhibition regression line± standard error.

Table 3. Biochemical activity of chlorpyrifos-ethyl and *Bacillus thuringiensis israelensis* against the 3rd instar larvae of *Culex pipiens (*laboratory and field strains) after 72hrs. exposure time**.**

Treatment After 72 hrs.		**GST (umol sub conjugated/min. /mg protein) Mean ± SD**		**AChE (µg AchBr/min. /mg protein) Mean ± SD**		**Total protein (Mg/g.b.wt.) Mean ± SD**		**AST (GOT)** (unit/ml) **Mean ± SD**		**(ALT) GPT)** (unit/ml) **Mean ± SD**	
Strain		**Lab.**	**Field**	**Lab.**	**Field**	**Lab.**	**Field**	**Lab.**	**Field**	**Lab.**	**Field**
Chloropyrifos-ethyl	**Mean**	2.27 ± 0.15$^{a}_{B}$	5.00±0.50 ^{aA}	3618.67 ±58.41^{aA}	2460.00 ±55.76$^{a}_{B}$	17.50 ±0.47$^{b}_{B}$	27.00 ±1.53$^{c}_{A}$	66.67 ±1.20$^{b}_{B}$	110.00 ±7.64$^{c}_{A}$	12.20 ±0.64$^{b}_{A}$	14.50 ±1.32$^{b}_{A}$
	Activity ratio	**1.62**	**1.56**	**2.43**	**3.12**	**0.76**	**0.75**	**0.21**	**0.28**	**0.49**	**0.36**
Bacillus thuringiensis	**Mean**	1.43 ± 0.04$^{b}_{B}$	3.30±0.26 ^{bA}	1368.00±34.77 ^{bcA}	725.00 ±36.56 ^{bB}	21.20 ±1.20$^{a}_{B}$	31.00 ±1.15$^{b}_{A}$	292.33 ±3.38$^{a}_{A}$	310.00 ±8.74$^{b}_{A}$	23.97 ±1.18$^{a}_{B}$	37.00 ±1.00$^{a}_{A}$
	Activity ratio	**1.02**	**1.03**	**0.92**	**0.92**	**0.92**	**0.86**	**0.92**	**0.78**	**0.96**	**0.93**
Control	**Mean**	1.40 ± 0.03$^{b}_{B}$	3.20±0.25 ^{bA}	1488.67 ±17.13^{bA}	789.00 ±11.79 ^{bB}	22.97 ±0.45$^{a}_{B}$	36.00 ±2.00$^{a}_{A}$	319.33 ±1.20$^{a}_{B}$	398.00 ±2.65$^{a}_{A}$	25.00 ±0.58$^{a}_{B}$	40.00 ±1.15$^{a}_{A}$
LSD at 0.05	**Treatment (T)**	0.48		705.67		3.14		43.13		9.56	
	Strain (S)	0.34		498.98		2.22		30.49		6.76	
	T*S	0.69		997.96		4.45		60.99		13.52	
F-value	**Treatment**	17.09		950.50		12.0		1594.25		126.51	
	Strain	157.53		343.85		135.69		256.01		110.40	

a, b & c: There is no significant difference (P>0.05) between any two means, within the same column have the same superscript letter.

A, B & C: There is no significant difference (P>0.05) between any two means for the same attribute, within the same row have the same superscript letter.

The Total protein content activity increased significantly after 72hrs of chlorpyrifos exposure varied between 0.76 and 0.75 in both strains compared with control. While, total protein content activity was 0.92 for laboratory strain of *(Bti)*. The activities of both Aspartate Aminotransferase (AST/GOT) and Alanine Aminotransferase (ALT/GPT) in 3rd larval instar exposure to chlorpyrifos showed insignificant in both tested stains were greatly induced their activity in 3rd larval instar exposed to *(Bti)* than control. GOT, data in (Table 3) indicate different trend of the enzyme response between the two tested insecticides. The organophosphate , chlorpyrifos showed great inhibition on GOT of 3rd instar laravae of *Cx. pipiens* compared with control showing 0.21 and 0.28% reduction, for laboratory and field strains, respectively. On the contrary, the *(Bti)* caused stimulatory effects on GOT activity in both strains, reaching 0.92and 0.78, respectively. The values of GPT showed insignificant differences between the two tested strains were (12.2 and 14.5) while, the values in GPT enzymes of *Cx. pipiens* larvae reached 23.97 and 37 with the *(Bti)* insecticide with activity ratio of 0.96 and 0.93 for laboratory and field strains, respectively.

The obtained results for the two tested insecticides under study showed significant differences between treated and undertreated larvae. The resistance of insects to any insecticide may be a result of a modification in the target site i.e, preventing the inseticide from reaching its action site, or degradation of the insecticide by metabolic enzymes or physiological resistance. **Abdel-Haleem *et al.* 2020).** Also, the obtained results indicate that larvicidal activity of chlorpyrifos was highly toxic against the 3rd larval instar of *Cx. pipiens* than *(Bti)* and these results were confirmed by the biochemical analysis.

The increased detoxification by EST and/or GST was responsible, at least in part, for chlorpyrifos resistance in just one among 5 samples despite several esterases were detected in all resistant samples. So these enzymes were not involved in recorded resistance. Our results are in agreement with previous studies on the role of the EST and the GST in the OPs resistance (**Ben Cheikh *et al.* 1998 ; Liu *et al.* 2005 and Daaboub *et al*., 2017).** Also, the obtained results are agree with(**Gharib, *et al*., 2020)** who investigated the susceptibility of *Cx. pipiens* larvae to chlorpyrifos and lambda-cyhalothrin insecticides 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; Bioassay tests showed that larval *Cx. pipiens* developed 144.31 -fold resistance to chlorpyrifos 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.

The increase in enzyme GST activity after treatment with *Bti*, reflects an established system of detoxification which, is a form of defense of the organisms against the insecticide**.** Similarly, increase of GST activity was found in *Aedes rusticus* after treatment with *Bti* **(Boyer *et al.* 2012).** It was demonstrated that viral infection induces GST increasing in mosquitoes **(Lin *et al.* 2007).**

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فاعلية كلا من الكلوربيروفوس– أثيل و*Bacillus thuringiensis israelensis* على بعوضة الكيولكس

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الملخص

تعتبر حشرة بعوض الكيولكس والتى تتبع عائلة Culicidae ورتبة Diptera من أهم الحشرات الطبية في مناطق كثيرة من العالم وتستخدم الطرق البيولوجية بالإضافة للمبيدات الكيميائية في مكافحتها. تم تقييم سمية كلا من مبيد الكلوروبيروفوس إيثيل (مجموعة المبيدات الفسفورية العضوية) *Bacillus thuringiensis israelensis* (مبيد حيوى) ضد يرقات العمر اليرقى الثالث لبعوضة *Culex pipiens* لسلالة معملية وأخري حقلية مجمعة من مدينة أبو رواش بمحافظة الجيزة,وأيضا دراسة التأثيرات البيوكيميائيه لهذه المركبات علي العمر الثالث ليرقات الكيولكس تحت ظروف المعمل. أظهرت النتائج أن السلالة المعملية حساسيتها عالية مقارنة بالسلالة الحقلية حيث زادت نسبة الموت بزيادة التركيزات وسجلت فروق معنوية بين التركيزات المعاملة والكنترول. كما تم تحديد نشاط أنزيمات أسيتيل كولينستراز (AChE) ، الجلوتاثيون S– ترانسفيراز (GST) ، البروتينات الكلية وأنشطة كل من Aspartate aminotransferase (GOT) AST و (GPT) Alanine aminotransferase ALT) تم تحديدها بعد 72 ساعة من التعرض للمبيدات الحشرية.