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Research Article

Toxic and Biochemical Effects of Certain Biocides and Chlorpyrifos-Ethyl on *Culex pipiens* (L.) Mosquito Larvae under Laboratory Conditions

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Abstract:

Culex pipiens (L.) (Diptera: Culicidae) is regarded as a significant pathogen vector. Using the standard procedures advised by the WHO organizations, the toxicity of three insecticides (two biocides and chlorpyri-fos-ethyl) was assessed in this study against field and laboratory of third-instar *C. pipiens* larvae at varying concentrations after 24, 48, and 72 hours.Additionally, three insecticides were identified using a biochemical assay, total protein, invertase, protease, glutathione-S-transferase (GST), α, β esterases (ESts), and acetylcholin-esterase (AChE) activity for both field and lab strains. The findings demonstrated that the field strains taken from Arab El-Raml, Al Menofia Governorate, were less susceptible to the tested pesticides than the laboratory strain, and that the percentage of death increased gradually as the insecticide concentration increased. When comparing the field strain to the laboratory strain, the biochemical impacts revealed that all pesticides raised the activity levels of both acetyl cholinesterase (AChE), glutathione S-transferase (GST), and α, β esterases (ESts), indicating that the field strain had the highest resistance. On the other hand, following pesticide treatment, total protein, inverses, and protease levels dropped. Detoxifying enzyme activity often increased gradually as generation numbers grew, suggesting that elevated resistance is probably linked to elevated activity of target and metabolic enzyme systems.

1. Introduction

Mosquitoes represent a major public health threat, transmitting serious human diseases, Mosquitoborne diseases are common in over 100 countries worldwide, affecting over 700,000,000 people annually. (Ghosh et al., 2012). Mosquitoes are an important vector for a number of diseases that impact both humans and animals, inclouding Japanese encephalitis, Dengue, Chikungunya, Filariasis, Yellow Fever, and Schistosomiasis (James, 1992). Additionally, mosquitoes can trigger both local (skin allergies) and systemic (angioedema) allergic reactions (Gubler, 1998).

Culex molestus is a competent vector for the Usutu virus, whereas Belgian *C. pipiens* is a competent vec-tor for the West Nile virus. (Soto et al., 2023). The rise of *C. pipiens* tolerance to various insecticides has made management more challenging, giving transmitted diseases a growing chance (Zayed et al., 2006). Evaluated the biological insecticides *B. thuringiensis* var. israelensis (Bti) to address the concerns of environmen-tal safety and vector resistance to chemical larvicides. The biological agent has proven to be extremely safe for the environment and effective at controlling mos-quito species in a range of breeding habitats. (Mulla et al., 1984; Karch et al., 1992).

Spinosad is a neurotoxin that binds to nicotinic acetylcholine and gamma-amino butyric acid receptors. Under a variety of environmental circumstances, ex-tracts and formulations based on spinosad have been tested against larvae of several species belonging to the genera *Aedes*, *Anopheles*, *Culex*, and *Psorophora*. Spi-nosad has a good chance of preventing mosquito larvae (Romi et al., 2006).

Additionally, discovered that Bti was one of the most efficient weapons for eradicating *C. pipiens* larvae. Emamectin benzoate, azadirachtin, diflubenzuron, and then Beauveria bassiana are listed in that order. Better control, cost savings, and amount savings can all be achieved by using various binary mixes of these tried-and-true control approaches(Zahran et al., 2013).

The aim of this work was to study toxic and bio-chemical effects of spinosad, (Bti) and Chlorpyrifos ethyl aganist 3rd instar *C. pipiens* larvae in both field and laboratory individuals strains under laboratory conditions.

2. Materials and Methods

2.1. Insecticides used:-

The Trading formulation of organophosphorus Larveno Plus 40% EC (Chlorpyrifos ethyl) and Biolave 1.2 % WG (*Bacillus thuringiensis* var *israelensis*) both provided by Kafr El-zayat Pesticides and Chemicals Company, Kafr El-zayat, Egypt, and Natular 20% EC (spinosad) was obtained from Lotus Agriculture Development Company, Egypt.

2.2 Instruments and chemicals:

Bovine albumin standard was obtained from Stan-bio Laboratory (Texas, USA). Commasie brilliant blue G-250 was purchased from Sigma (Sigma Chemical Co.). p-Nitroanisole (97% purity) was obtained from Ubichem Ltd. (Ham Pshire). The nicotinamide ademine dinucleotide phosphate (NADPH) was purchased from BDH Chemicals Ltd. (Poole, England). Other used chemicals were obtained at high quality from El-Gomhouria Drug Company, Egypt.

A chilled glass Teflon tissue homogenizer (ST–2) Mechanic-Preczyina, Poland) was used in homogenizing insects were homogenized for biochemical analysis.

A deep freezer at –20º C was used to keep the insect homogenates supernatants till using for biochemical assays. Double beam Ultraviolet/Visible (UV-Vis) Spectrophotometer (Spectronic 1201, Milton Roy Co., USA) was used to measure absorbance of colored substances or metabolic compounds.

2.3 Toxicological studies

2.3.1 Treated Insect

Field larvae of *C. pipiens*, Culicidae, order Diptera were obtained from Arab El-Raml, Al Menofia, Egypt. Larvae were collected and transferred in a plastic whirl – pack bags (Nasco) half-filled with water from the breeding place and brought to the laboratory of Research Institute of Medical Entomology ADD, El Agouza, Giza, Egypt. They were put in 2000 ml tank for conducting the experiments.

In the laboratory, the third (3^{rd}) instar larvae were picked up and identified using the suggested key of (Harbach, 1988) for the Egyptian Culicidae mosquitoes. In plastic pans filled with dechlorinated tap water, lar-vae were raised. They were fed Tetra-Min dry fish food flakes, which were scattered throughout the water's sur-face. The larvae were kept in a dish with tap water and fed a mixture of fish feed while being raised under conventional conditions, which included a temperature of (25±2) ^{O} C and 75 ± 5% humidity, as well as a 12/12 light/dark cycle. The Research Institute of Medical En-tomology provided the insecticide-susceptible strain of *C. pipiens* that was utilized in the research.

2.3.2 Larvicidal Bioassay:

Larvicidal activity of *B. thuringiensisisraelensis*, spinosad and chloropyrifos-ethyl on *C. pipiens* larvae was assessed by using the standard method according to (WHO, 1981). In brief twenty-five larvae of 3^{rd}. instar were taken and treated with different concentration of insecticides (5×10^{-4}, 1×10^{-3}, 5×10^{-3}, 2×10^{-2} and 2×10^{-1}mg/ L) for chlorpyrifos-ethyl, (1×10^{-8}, 5×10^{-8}, 1×10^{-7}, 5×10^{-7}, 1×10^{-6} and 1×10^{-5} mg/ L) for spinosad and five different concentrations (1×10^{-6}, 5×10^{-6}, 1×10^{-5}, 5×10^{-5}, and 1×10^{-4} mg/ L) for *B. thuringiensisisraelensis.* Similarly, the untreated larvae were used as control. For each concentration, three replicates were maintained at the same time. Mortality counts were carried out after 24,48 hr. and 72hr. of treatment. Mortality percentages were calculated and corrected according to (Abbott, 1925). Values of LC_{50} confidence limits and slop functions were calculated and ascertained using probit analysis according to (Finney, 1971).

$$\% \text{ Mortality} = \frac{\%M \text{ Treatment} - \%M \text{ Control}}{100 - \%\ M \text{ Control}} \times 100$$

2.4. Biochemical Studies

2.4.1 Sample preparations

The homogenates of the insect were made in accordance with (Amin, 1998). Using a chilled glass Tef-lon tissue (ST–2) homogenizer, the treated insect larvae with the LC_{50} value of each toxicant were collected and homogenized in distilled water at a ratio of 50 mg insect in /1.0 ml of water. The homogenates were collected and centrifuged in a chilled centrifuge at 8000 rpm for 15 minutes at 2º C. The separated pellet (precipitate) was discarded and the floated supernatant was collected and used for the carried in Vivo biochemical (enzymatic) studies.

The harvested supernatant can be used freshly or stored at less than 0^0 C for a week without a significant loss of the enzyme activity.

2.5 Enzymatic activity Measurements

2.5.1 Determination of acetylcholine esterase (AChE) activity

Acetylcholinesterase (AChE) activity was measured according to the method described by (Simpson et al., 1964) using acetylcholine bromide (AChBr) as a substrate.

2.5.2 Determination of esterases activity

Alph esterases (α-) and beta esterases (β-) enzymes were determined according to (Van Asperen, I962) using α-naphthyl acetate or β-naphthyl acetate as substrates in the same array.

2.5.3 Determination of Glutathione S-transferase (GST) activity

The conjugation of reduced glutathione (GSH) with 1-chloro-2,4-dinitrobenzene (CDNB) is catalyzed by glutathione S-transferase (GST) via glutathione's -SH group. As per (Habig et al., 1974), approach, the conjugate S-(2,4-dinitro-phenyl) -L- glutathione could be identified.

2.5.4 Determination of invertase activity

The method demonstrated by (Amin 1998). and re-fined and adjusted by (Ishaaya and Swirski, 1976). was utilized to assess the digesting enzymes of insects.

2.5.5 Determination of proteolytic enzymes activity

Proteolytic enzymes activity was measured as

described by (Tatchellet al., 1972) and (lee and Takabashi 1966) with some modifications by measuring the increase in the concentration of the released free amino acids, which split from the substrate protein (albumin) during incubation at 30 ^{O}C for an hour.

2.5.6 Determination of total proteins

Total proteins were calculated using the protocol outlined in (Bradford I976). To make the protein reagent, 100 mg of Coomassie Brilliant blue G-250 were dissolved in 50 milliliters of 95% ethanol.

2.6 Statistical analysis:

All experiments were applied in 3 replicates of the insect homogenates, and the biochemical determinations outcomes were combined from three separate determinations. Costat statistical software was used to do a one-way analysis of variance (ANOVA) on the data. (Cohort Software, Berkeley). At $P < 0.05$ significance, means were compared using the Duncan's multiple range test.

3. Results and Discussion

In many regions of the world, *C. pipiens* is the most significant insect for medical purposes. The effec-tiveness of the investigated substances Chlorpyri-fos-ethyl, spinosad, and *Bacillus thuranginsis* israelin-sis against field and laboratory specimens of *C. pipiens* larvae in their third instar at varying doses was assessed. The following outcomes were noted and discussed:

3.1 Toxic effects:

3.1.1 Effect of Chloropyrifos-ethyl

Larvicidal effects of Chloropyrifos-ethyl against the 3rd instar larvae of *C. pipiens* for the field and laboratory strains following various exposure periods, as summarized in Table (1). The collected results showed that the field strain of Chlorpyrifos-ethyl was less efficient than the laboratory strain against the third instar larvae

After 24 hours of treatment, the field strain of *C. pipiens* third instar larvae showed a high percentage of larval death of 86%, while the laboratory strain showed a high percentage of larval mortality of 2×10-1 mg/L, or 96%. Additionally, Table (1) displayed the LC_{50} and slope value of Chlorpyrifos-ethyl against susceptible field and laboratory strains of *C. pipiens* larvae. Based on LC_{50} results, it was evident that Chlorpyrifos-ethyl was the most hazardous, with an LC50 of 6.5 ×10-3 mg/L in the laboratory strain and 1.1 ×10-2 mg/L in the field strain after 24 hour exposure period.

Also, Table (1) results show that the slope values for the field and laboratory strains were 1.22 ± 0.04l and 1.31 ± 0.015, respectively, indicating homogeneity between the two sets of strains. The outcomes corroborate those of (Abd El-Samie and Abd El-Baset 2012) study, which found that the laboratory colony was more susceptible to Chloropyrifos than field populations of *C. pipiens* that were gathered from the Assiut Governorate and Shakira.

Table 1. Larvicidal activity of Chlorpyrifos-Ethyl against the 3rd instar larvae of *C. pipiens* (Laboratory and field strains) after 24 hrs.

Strain	**Laboratory strain**	**Field strain**
Concentrations (mg/ L)	**Mortality (%) hrs**	**Mortality (%) hrs**
	24h± SE	**24h± SE**
5×10^{-4}	12.0± 4.00	5.3± 2.30
1×10^{-3}	18.7± 2.31	13.3± 2.31
5×10^{-3}	44.0± 4.00	36.0± 4.00
2×10^{-2}	80.0± 4.00	74.7± 6.11
2×10^{-1}	96.0± 8.00	86.7± 4.62
Control	0	0
LC_{50} (mg/ L)	6.5×10^{-3}	1.1×10^{-2}
% C.L	$(3.8–11.0)\times10^{-3}$	$(0.82–1.6)\times10^{-2}$
Value of Slope	1.31±0.015	1.22±0.04

95% C.L., 95% Confidence limit

3.1.2 Effect of Spinosad

Spinosad's larvicidal activity against *C. pipiens* larvae in their 3rd instar for both field and laboratory strains following varying exposure durations . Following varying exposure times (24, 48, and 72 hours), the results in Table (2) display the mortality percentages of the Spinosad on third-instar larvae of *C. pipiens* with a serial number of concentrations corresponding to labor-atory and field strains. The data in Table (2) of this investigation demonstrated that, after 24, 48, and 72 hours of exposure, a very high concentration of spinosad caused a high death rate in both the laboratory and field strains of *C. pipiens*. However, both the laboratory strain and field strain, the highest percentages of larval death were seen after 24, 48, and 72 hours after admin-istration (26.7%, 61.3%, and 90.7%), respectively. Af-ter 72 hours, the rates were 13.3%, 38.7%, and 77.3%. The results collected was unambiguous in indicating that Spinosad was more efficient than the field strain against the laboratory strain's third instar larvae.

Also, results in Table (2) showed that LC_{50} values of Spinosad were (2.3×10-4, 2.04×10-4 and . 2.3×10-7 mg/L) in the laboratory strain, while these values were increased to LC_{50} values (3.8×10-3 , 3.0×10-5 and 3.6×10-7 mg/L) in field strain after 24, 48 and 72 hrs. of exposure respectively. The collected data obviously indicated that Spinosad was less effective against the 3rd instar larvae of field strain than the laboratory strain.

The acquired results align with the findings of (Liu et al., 2005) who found that in larval experiments, imidacloprid is just as sensitive as permethrin. Also,

(Hossam El-Din et al., 2013) showed lethal concentration LC_{50} of the formulation of Emamectin benzoate against *Culex pipiens* was found to be 1, 0.10 and 0.07 mg/l after 24, 48 and 72 hrs. of treatment. (Bahgat et al. 2007) revealed that spinosad was evaluated against a few *C. species* in the El-Ismailia Governorate, both in the lab and in the field. The results showed that the liquid formula of spinosad the LC_{50} value for *C. pipiens* was 0.002 ppm after a 24-hour period. On *C. pipiens* larvae in their fourth instar, this increased tox-icity was noted. (Moselhy et al., 2015) revealed that Spinosad was the most effecting tested insecticide on *C. pipiens* larvae. (El Sayed et al., 2020) demonstrated that the effectiveness of spinosad was tested on *C. pipiens* larvae at various concentrations, resulting in mortality that increased with concentrations and time, reaching 100% at1000 µl/10 m l after 48 hours

Table 2. Larvicidal activity of Spinosad against the 3rd instar larvae of *C. pipiens* (laboratory and field strains) after (24, 48, 72) hr.

Strain	**Laboratory strain**			**Field strain**		
Concentrations	**Mortality (%) hrs**			**Mortality (%) hrs**		
(mg/L)	**24h ± SE**	**48h ± SE**	**72h ± SE**	**24h ± SE**	**48h ± SE**	**72h ± SE**
1×10^{-8}	4.0±0.0	18.7±2.31	28.0±0.0	0.0±0.0	9.33±2.31	28.0±4.0
5×10^{-8}	8.0±4.0	22.7±2.31	32.0±4.0	2.7±2.31	12.0±0.0	30.7±6.11
1×10^{-7}	13.3±2.30	29.3±6.11	45.3±4.62	4.0±0.0	21.3±2.31	42.7±6.11
5×10^{-7}	18.7±4.62	40.0±4.0	58.7±2.30	5.3±4.62	26.7±6.11	53.3±2.31
1×10^{-6}	21.3±4.61	52.0±4.0	74.6±4.62	8.0±0.0	37.3±8.33	66.7±2.30
1×10^{-5}	26.7±6.11	61.3±4.62	90.7±2.30	13.3±2.31	38.7±4.62	77.3±4.62
LC_{50} (mg/ L)	2.3×10^{-4}	2.04×10^{-6}	2.3×10^{-7}	3.8×10^{-3}	3.0×10^{-5}	3.6×10^{-7}
C.L	$(0.15–36)\times10^{-4}$	$(0.9–4.8)\times10^{-6}$	$(1.5–3.5)\times10^{-7}$	1.7×10-5 –0.94	$(0.5- 18)\times10^{-5}$	$(2.0-6.4)\times10^{-7}$
Value of slope	0.37±0.006	0.46±0.005	0.74±0.007	0.44±0.02	0.40±0.006	0.52±0.005

95% C.L., 95% Confidence limit

3.1.3 Effect of Bacillus thuringiensis.

From the gained results, comparing with the mortality percents at all the tested concentrations of Bti on *C. pipiens* larvae after 24, 48 and 72 hrs , it was noticed that the laboratory strain was more affected than the treated field strain of *C. pipiens* larvae population at the highest concentration. The mortality percentage equal 22.6%, 63.4% and 86.7 % for laboratory strain comparing with 17.6%, 54.7% and 75.5% for field strain treated larvae population by Bti after 72 hrs Table(3). However, the LC_{50} values was revealed that the tested (Bti), gave 2.8×10-5, 3.7×10^{-6} and 7.9×10^{-3} mg L. on the treated laboratory strain and 7.9×10^{-3}, 6.1×10^{-5} and . 7.2×10^{-6} g on the field larvae strain after 24, 48 and 72 hours exposure in the same order.

Obviously collected data indicated that Bti was more successful in combating on the third instar larvae of laboratory strain than the field strain.

Table 3. Larvicidal activity of Bti. against the 3rd larvae of *C. pipiens* (laboratory and field. strains) after (24,48,72) hr.

Strain	**Laboratory strain**			**Field strain**		
Concentrations	**Mortality (%) hrs**			**Mortality (%) hrs**		
(mg/ L)	**24h ± SE**	**48h ± SE**	**72h ± SE**	**24h ± SE**	**48h ± SE**	**72h ± SE**
1×10^{-6}	4.6±2.31	18.2±2.31	32.8±2.31	2.8±2.31	16.4±2.31	30.3±2.31
5×10^{-6}	8.2±2.31	31.8±2.31	53.9±4.62	5.8±2.31	27.5±2.31	46.2±4.62
1×10^{-5}	10.7±4.62	38.8±2.31	63.1±2.31	7.8±2.31	33.3±4.62	53.5±2.31
5×10^{-5}	18.4±2.31	56.1±4.62	80.9±4.62	14.0±4.62	48.1±6.11	69.4±4.62
1×10^{-4}	22.6±4.62	63.4±4.62	86.7±6.11	17.6±4.62	54.7±4.62	75.5±6.11
LC_{50} (mg/ L)	3.4×10^{-3}	2.8×10^{-5}	3.7×10^{-6}	7.9×10^{-3}	6.1×10^{-5}	7.2×10^{-6}
C.L	$(0.9–6.5)\times10^{-6}$	$(1.1–7.5)\times10^{-5}$	$(1.5–9.2)\times10^{-6}$	$(0.2–394)\times10^{-4}$	$(0.17-23)\times10^{-5}$	$(2.8–19)\times10^{-6}$
Value of slope	0.49±0.04	0.63±0.03	0.78±0.03	0.49±0.06	0.55±0.03	0.60±0.03

95% C.L., 95% Confidence limit

Bti also plays a significant role in controlling the vector mosquito population as an alternative for synthetic pesticides. For the last two decades, its effectiveness has been reported against *Anopheles*, *Culex*, and *Aedes* (Raymond et al., 2010).

Because Bti is more selective and biodegradable than chemical insecticides, it is seen to be a far superior option. Ever since the Bti was isolated, its paraspores have been very harmful to mosquito larvae. (De Barjac, 1978; Goldbreg and Margalit, 1977).

3.2 Biochemical (Enzymatic) Studies:-

3.2.1 Biochemical activity of Chlorpyrifos-ethyl, spinosad and Bti against 3rd instar larvae of C. pipiens for laboratory and field strains.

C. pipiens in their third instar were treated with Spinosad, Bti, and Chlorpyrifos-ethyl at their LC_{50} val-ues. Acetyl cholinesterase (AChE), glutathione S-transferase (GST), non specific esterases (α, β esterases), total proteins content, invertase, and protease activity were determined in 3rd instar larvae of *C. pipiens* of the three tested insecticides

In both field and laboratory strains of third instar *C. pipiens* larvae, the activity levels of protease, invertase, nonspecific esterases (α, β esterases), glutathione Stransferase (GST), acetyl cholinesterase (AChE), and total proteins were measured in comparison to control. The data in Tables (4a and 4b) demonstrated the impact of Bti, spinosad, and chlorpyrifos-ethyl on a few biochemical components of *C. pipiens* larvae in their 3rd instar. The results demonstrated that after treating the field strain with chlorpyrifos-ethyl, spinosad, and Bti in comparison to the untreated control, there was a rise in the activity of acetyl cholinesterase (AChE), glutathione S-transferase (GST), and nonspecific esterases (α, β esterases). While, total proteins content, invertase, and protease enzymes were decreased in the field strain compared with laboratory strain after chlorpyrifos-ethyl, spinosad and Bti comparison with untreated control .

The Comparison of the tested insecticides on different enzymes Levels between treated and untreated (Control) of *C. pipiens* larvae for field and labora-tory strains, Data showed in the Tables (4a and 4b) AchE, GST, β esterases and α esterases, activity were increased grad-ually with all tested insecticide on *C. pipiens* larvae in field strain compared with laboratory strain while the activity Level of total proton, invertase and protease were decreased in field strain compared with laboratory strain.

The results aligned with the findings of (Denis et al., 1996), which demonstrated that only AChE satisfies the physiological role of neurotransmitter hydrolysis at synapses. Against susceptible and field strain larvae, Spinosad demonstrated the maximum larvicidal effect in the insecticide-resistant strain.

Acetylcholinesterase-modified mosquitoes were demonstrated by (Ana et al., 2011), who also found a notable 20% decrease in energetic resources. (Abdel-Haleem et al., 2020) revealed that Acetamiprid significantly in-creased the activity level of α and β esterases, GST, AChE, and carboxylesterase when compared to Thiamethoxam.

According to research by (Gharib et al., 2020), the activities of detoxifying enzymes progressively in-creased as generation numbers increased, suggesting that the increasing resistance is probably related to the increased activity of target and metabolic enzyme systems.

Jia et al., (2020) suggested the mode of action of Ar-Turmerone may be unrelated to AchE and he add, that all the results show the larvicidal mechanism of Ar-Turmerone is estimated to be stomach poison and the active sites might be the muscle and digestive tissues,.

Kamal and Bulbuli, (2021), mosquitoes exhibited enhanced detoxification throughout the course of a generation, as evidenced by a rise in enzymes linked to metabolic detoxification. (Meta et al., 2022) Increased levels of pesticide resistance have been found in studied insects having cytochrome P450 (CYP), glutathi one Stransferase (GST), and esterase gene super families.

According to research by (Brown, 1986). the GSTs play a vital role in protecting tissues against oxidative damage and oxidative stress and (Brooke et al., 2001) found that GSTs play a minor role as a detoxifying enzyme in pyrethroids resistant to *Anopheles funestus*.

As stated by (Brown, 1986). And according to (Brooke et al., 2001), GSTs are essential for shielding tissues from oxidative stress and damage. However, they also have a little detoxifying enzyme function in pyrethroids that are resistant to *Anopheles funestus.*

Yang et al., (2011), showed Organophosphates and carbamate insecticides target acetylcholine neurotransmitter (AChE), an essential enzyme in the neurological system that hydrolyzes it and stops neural impulses.(El-Sheikh 2011) observed a positive association between AChE activity and the degree of pesticide resistance in *C. pipiens*. Changes in AChE in resistant insects lead to a decreased susceptibility to inhibition by insecticides.

According to (Liu et al., 20015), ace-I gene mutations may be the primary cause of high AChE insensitivity. The research by (Karunaratne et al., 2018), showed that the resistant insects with higher AChE activity had lower susceptibility to pesticide inhibition.

The data in Tables (4a and 4b) indicate a significant increase in Alfa and Beta esterase activity in the selected strain compared to the susceptible strain, which is consistent with the findings of (Macoris et al., 2003) who found higher resistance ratios and higher levels of Alfa and Beta esterase activity in the field populations of *Ae. aegypti*. The current findings demonstrated that these enzymes are useful indicators for identifying organophosphate resistance.

The detoxification of enzymes like esterases (ESTs), mixed function oxidases (MFO), glutathione S-transferases (GST), and acetylcholine-esterases (AChE) is linked to insecticide resistance (Nauen, 2007; Lumjuan et al., 2005).

Similar to how an established detoxification system protects an organism against an insecticide, the detoxi-fication of pesticides in mosquitoes involves three major metabolic detoxification gene families: cytochrome (P450s), esterases, and glutathione S-transferases (GSTs). This is demonstrated by the increase in enzyme GST activity following treatment with chlorpyri-fos-ethyl, spinosad, and Bti. According to (Ranson and Hemingway 2005)., GSTs are soluble dimeric proteins that play a significant role in the metabolism, detoxifi-cation, and excretion of several endogenous and exog-enous substances.

Table 4a. Biochemical activity of Chlorpyrifos-ethyl, spinosad and Bti against 3rd instar larvae of *C. Pipiens* for laboratory strain.

Tested Insecticide		Laboratory strain						
		AChE	**α-esterases**	**β-esterases**	**GST**	**Invertase**	**Protease**	**Total protein**
chloropyrifos ethyl	**T. ±SD**	17.03±1.34	79.97±2.51	32.17±2.47	7.53±0.42	19.60±2.56	1043.33±37.9	35.97±1.85
	C.±SD	13.27±1.02	77.50±2.18	26.80±1.99	7.13±0.49	18.07±2.69	1472±62.80	41.40±2.51
	% increase or decrease than control	28.33	3.19	20.04	5.61	8.47	-29.12	-13.12
Spinosad	**T.±SD**	29.03±2.51	77.33±2.08	29.10±1.54	8.13±0.21	10.57±0.40	1221.67±37.2	29.37±0.93
	C.±SD	23.03±3.06	70.67±3.06	24.13±0.59	6.43±0.25	13.10±0.95	1436.33±72.7	34.63±2.32
	% increase or decrease than control	26.05	9.42	20.60	26.44	-19.31	-14.95	-15.19
B. thuringiensis israelensis	**T.±SD**	27.06±2.51	75.33±3.06	28.40±1.54	6.9±0.26	9.43±0.64	1267.7±47.7	31.23±1.17
	C.±SD	23.03±3.06	70.67±3.06	24.13±0.59	6.43±0.25	13.10±0.95	1436.33±72.7	34.63±2.32
	% increase or decrease than control	19.84	6.59	17.72	15.55	-28.02	-11.74	-9.82

T. = Treatment C. = Control % increase or decrease than control = $\frac{T - C}{C} \times 100$

Table 4b. Biochemical activity of Chlorpyrifos-ethyl, spinosad and Bti against 3rd instar larvae of *C. Pipiens* for and field strain.

Tested Insecticide		Field strain						
		AChE	α-esterases	β-esterases	GST	Invertase	Protease	Total protein
chloropyrifos ethyl	T. ±SD	17.70±1.04	92.73±3.16	27.47±1.86	5.43±0.55	15.03±1.27	766.67±20.8	31.07±2.00
	C.±SD	8.46±0.57	69.67±3.21	24.43±1.16	4.7±0.36	13.37±1.00	1153.67±44.6	52.90±0.66
	% increase or decrease than control	109.22	33.1	12.44	15.53	12.42	-33.55	-41.27
Spinosad	T.±SD	21.33±0.78	97.6±3.04	41.40±2.19	8.57±0.67	10.67±0.76	1133.3±24.8	21.30±0.98
	C.±SD	11.46±1.04	77.03±2.74	39.07±2.49	4.01±0.38	12.53±0.84	1589.3±35.2	48.07±1.90
	% increase or decrease than control	86.13	26.70	5.96	113.72	-14.84	-28.69	-55.69
B. thuringiensis israelensis	T.±SD	18.33±0.18	87.23±3.96	45.10±2.67	5.13±0.32	8.20±0.61	929.0±18.5	36.60±1.90
	C.±SD	11.46±1.04	77.03±2.74	39.07±2.49	4.01±0.38	12.53±0.84	1589.3±35.2	48.07±1.90
	% increase or decrease than control	59.95	13.24	15.43	27.93	-34.56	-41.55	-23.86

T. = Treatment C. = Control % increase or decrease than control = $\frac{T - C}{C} \times 100$

4. Conclusion

We conclude that of the three compounds tested in the laboratory field strains, Chlorpyrifos ethyl was more effective larvicide for labora-tory than field strain on 3rd of *C. pipiens* larvae and increased the activity level of AchE, GST, β esterases and α esterases enzymes. The study showed that role enzymes in resistance on *C. pipiens* larvae, where AchE, GST, β and α esterases were higher in field strain, which indicated a higher rate of resistance. Overall, the activities of detoxifying enzymes increased gradually with raising generation numbers, indicating that the increased resistance is likely to be associated with the increased activity of target and metabolic enzyme systems.

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