**Current Science International**
ISSN 2077-4435

Volume : 04 | Issue : 04 | Oct.-Dec.| 2015
Pages: 646-657

# Spinosad as an Alternative Larvicide for Mosquito *Culex pipiens* Control

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## ABSTRACT

Insecticide resistance has been known to be prevalent in several insect species including mosquito. It has become a major problem in vector control programs due to pesticide resistance through detoxification enzymes. The present study aimed to compare between the larvicidal action of some organophosphorus insecticides (fenitrothion, malathion and temephos) commonly used in larval control programmes and one bio- insecticide (spinosad). Five populations of *Culex pipiens* mosquito larvae were tested, one was laboratory (reference) population and the other four were collected from El-Fayoum, Giza, El-Sharkia and Kafr El-Shakh Governorates. The highest larvicidal effect was recorded in spinosad treatment followed by temephos, fenitrothion then malathion. Enzyme – based metabolic mechanisms of spinosad were investigated based on the biochemical assay principle against the laboratory population of *c. 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. The use of spinosad as an alternative larvicide was discussed.

***Key words:*** Insect, mosquito, control programs, pesticide, enzymes.

## Introduction

Vector borne diseases are among the major causes of illness and death in tropical and subtropical countries and worldwide more than one million people die due to vector borne diseases every year. Mosquitoes are one of the important vectors transmitting a variety of diseases for human and domesticated animals. In Egypt, the mosquito *Culex pipiens* is the main vector of filariasis and some arboviruses such as Rift Valley Fever and West Nile Fever viruses (Southgate 1979; Hanafi *et al*., 2011). *C. pipiens,* therefore, is the main target in control programs against these diseases. *C. pipiens* larvae breed in different kinds of water bodies such as wet pit latrines, septic tanks, cesspits, cesspools, drains and canals containing stagnant water polluted with organic waste. They also breed in polluted water associated with home industries, for example coconut husk pits. Other breeding sites are pools and unused wells used for dumping garbage (Zayed 2015 and Walaa *et al*., in press). Mosquito larvae control of this species has been achieved mainly by the use of organophosphorus (OP) insecticides, insect growth regulators and bacterial larvicides (WHO 2006).

Larval control is most practicable where vector borne diseases in low transmission level and their vector breeding sites are limited in number and relatively permanent (WHO 2005). Few chemical insecticides are safe enough for use in water that is used for drinking and bathing. The most commonly used is the organophosphorus insecticide temephose, which is effective and safe in drinking water at a concentration of 1 part per million (WHO 2005 & 2006). In Egypt, current programmes are largely dependent on organophosphorus insecticides, which are the main WHO-recommended synthetic insecticides for larval control.

However, long term and intensive use of insecticides often lead to emergence of resistance. Insecticide resistance is one of the major obstacles in the control of medical and agricultural arthropod pests (Georghiou & Taylor, 1986).

The appearance of such problems has been accompanied by growing interest to use new safe bio-insecticide with a new mode of action specially when dealing with water (Salgado, 1997 and Salgado, 1998). Spinosad is 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.

This study has been able to establish the fact that spinosad as a larviciding tool will be effective in Egypt. Spinosad has number of advantages over OP insecticides. Beside its higher insecticidal action against mosquito larvae, it is biodegradable with no significant effect on non- target creatures and minimal risk to human health (Garza- Robledo *et al.,* 2011; Kemabonta and Nwankwo, 2013). It also showed a persistence insecticide action

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for weeks (Prabhu *et al.,* 2011) and this naturally derived insecticide has been reported to have no adverse effects on predatory insects such as ladybirds, lacewing, big- eyed bugs or minute pirate bugs (Kirst *et al.,* 1992). 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).

Today, 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 (Soderlund *et al*., 1989, WHO 2012). To fully develop this strategy, a thorough knowledge of the mechanism of insecticide resistance is essential.

Enzyme assay has been commonly used due to its rapid, simple and sensitive method for identification of mechanisms underlying the insecticide resistance in mosquito population even at low frequencies (Lee, 1990 and Brogdon, 1989). The present study aimed to evaluate the insecticidal effect of spinosad as alternatives for the traditional organophosphorus insecticides that used for larval control of *C. pipiens* as well as the effect of this bio- insecticide on the biochemical contents of *C. pipiens* was studied.

## Materials and Methods

**Toxicological studies:**

*Reference laboratory strain of mosquitoes:*

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

*Field mosquito strains:*

Larvae of *C. pipiens* were collected from four governorates in Egypt as shown in fig (1) (El-Fayoum, Giza, El-Sharkia and Kafr El-Shakh Governorates). Mosquitoes were reared basically as those described by Chapman and Barr, 1969. They hold in the insectary in which temperature was maintained at 25±3 C$^{o}$ and humidity between 70- 80%.

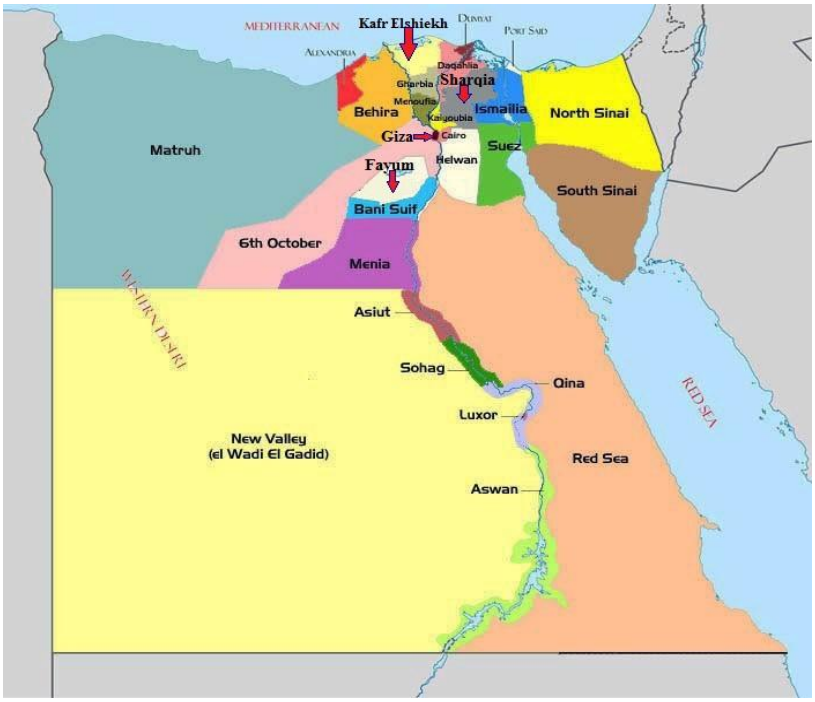


**Fig. 1:** Location of mosquito collection sites from some governorates in Egypt.

*Larvicides:*

Three organophosphorus insecticides were used that, recommended by the World Health Organization's Pesticide Evaluation Scheme (WHOPES) for use against mosquito larvae (WHO 2006); fenitrothion, malathion and temephos. The three organophosphorus insecticides and their methods of application were obtained from the

WHO susceptibility test Kit. The tested concentrations were made by dilution in ethanol on a volume/ volume bases using the procedure pre-described by the World Health Organization expert committee on insecticides (197I). Also spinosad (natural compound) was tested as a new larvaicide against mosquito larvae in the form of technical ingredient 94%. Spinosad was dissolved in distilled water and test was conducted according to the procedure prescribed by Dow company®. Each insecticide was prepared at concentrations that shown in table (1).

**Table 1:** Name, classification, mode of action and concentrations of the tested insecticides.

| Insecticide | Family | Mode of action | Concentration (p.p.m.) |
|---|---|---|---|
| Fenitrothion | Organophosphate | Acetyl cholinesterase inhibitor | 1E-05 [a], 5E-05, 1E-04, 5E-04, 1E-03, 5E-03, 0.025, 0.125. |
| Malathion | Organophosphate | Acetyl cholinesterase inhibitor | 5E-05, 1E-04, 5E-04, 1E-03, 5E-03, 0.025, 0.125. 0.625, 3.125. |
| Temephos | organophosphate | Acetyl cholinesterase inhibitor | 1E-05, 5E-05, 1E-04, 5E-04, 1E-03, 5E-03, 0.025, 0.125. |
| Spinosad | Bio- insecticides | Nicotinic Acetylcholine receptor | 5E-10, 1E-10, 5E-09, 1E-09, 5E-08, 1E-08, 5E-07, 1E-07, 5E-06, 1E-06, 5E-05. |

*a = 1E-05= 0.00001*

*Larval bioassays:*

Larval bioassay was performed according to the WHO standard method (WHO 1975). For each insecticide five to eleven concentrations were tested to give mortality between20% to 100% and to determine 50% and 90% lethal concentrations ($LC_{50}$and $LC_{90}$). For bioassay, four replicates for each concentration were tested against the 3rd larval instar for organophosphorus insecticides and the 2nd larval instar for spinosad. Test beakers of 500 ml capacity, each containing 249 ml tap water were prepared. In each, 1 ml of each concentration was infiltrated under the water surface with a pipette. A group of 25 larvae was introduced in each beaker after 30 min of preparing the insecticide solution. The test was run at the same temperature as that at which the larvae were reared. Larvae were left for 24 h and mortality was then recorded. Incase of spinosad mortality was recorded after 24h and 48h. Moribund larvae were considered dead. Larvae pupating during exposure period were excluded from calculation. Tests in which pupation exceeded 10% were repeated. The control tests were set up by adding 1 ml of ethanol into water and mortality never exceeded 4%. Curve mortalities of different insecticides were drown using several dilutions and the lethal concentrations were calculated using IBM, SPSS software.

*Resistance ratio (RR) calculation:*

Levels of resistance of the field populations of the insects under investigation were calculated as follows:
Resistance ratio (RR) = LC50 of filed population/LC50 of laboratory colony

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) or high (RR > 10).

**Biochemical assay:**

The concentration of spinosad that cause 50% ($LC_{50}$) after 48h was used against second instar larvae to measure total protein, Acetylcholinesterase, α and β esterase, protease and invertase.

*Preparation of insects for analysis:*

The survived 2nd instar of larvae *C. pipiens* were collected before insecticide application (0 hour) and after 6h, 24h and 48h after exposure to spinosad and also for control weighted and mechanically homogenized in distilled water (50 mgL1 ml) by a chilled glass telfon homogenizer. Homogenates were centrifuged at 8000 r.p.m. for 15 min at 5°c in a cooling centrifuge. The deposits were discarded and the supernatant were kept in a deep freezer till use.

*Determination of total protein content:*

Total protein quantification of mosquito homogenates was performed using Bradford reagent with bovine serum albumin as the standard protein (Bradford, 1976) to normalize enzyme activity levels by protein content.

*Determination of Acetylcholinesterase (AChE):*

Acetylcholinesterase (AChE) activity was measured according to simpson *et.al.*, (1964), using acetylcholine bromide (AChBr) as substrate, the produced color was measured calorimetrically at 515 nm.

*Determination of alpha and beta esterases (α and β-esterases) activity:*

Alpha and beta esterases (α and β-esterases) were determined according to Van Asperen (I962) using α-naphthyl acetate or β-naphthyl acetate as substrates, respectively. The developed color was read at 600 or 555 nm for α- and β-naphthol produced from hydrolysis of the substrate, respectively. The enzyme activity was expressed as mole naphthol released/ individual/ min.

*Determination of Proteolytic activity:*

Proteolytic activity measured as described by Tatchell *et al*., (1972), with some modifications, by measuring the increase in free amino acids split from substrate protein (albumin). The developed color was read at 570 nm. Zero adjustment was against reagent blank containing everything and 100 µl distilled water of the supernatant. D, L alanin was used as the standard and the amino acids were expresses as µg alanine / min / g.b.wt.

*Determination of invertase activity:*

Invertase enzyme was determined according to Amin (1998) using sucrose as substrates glucose used as a standard. Appropriate dilutions of enzyme supernatant used to obtain a linear production of glucose equivalents. Generally, for each test, invertase activity determined from triplicate analyses of three groups of seedlings. The enzyme activity expressed as µg glucose released /min/gm fresh weight.

*Statistical analysis:*

Mortality data and values of biochemical assay were calculated by using Microsoft Excel (Microsoft Corporation, 2007). Mortality data were re-modified, statistical analysis and graphing employed IBM, SPSS V.18, for Windows. Lethal concentration statistical values were calculated and utilized to characterize the susceptibility of mosquito species to insecticides (Robertson and Preisler 1992).

## Results

The response of the five *C. pipiens* populations (one lab and four fields ones) to the different insecticides using the WHO bioassay method and the baseline susceptibility were shown in table 2 and figures 2, 3, 4,5 and 6.

**Toxicological studies:**

Table (2) shows the $LC_{50}$ and $LC_{90}$ of the four tested insecticides and resistance ratio (RR) of *C. pipiens* larvae obtained from (El-Fayoum, Giza, El-Sharkia and Kafr El-Shakh Governorates) in comparison with laboratory colony.

The results revealed that the bio- insecticide, spinosad was the most effecting tested insecticide, that it had the lowest $LC_{50}$ and $LC_{90}$ values against the laboratory colony and the four field populations; El-Fayoum, Giza, El-Sharkia and Kafr El-Shakhafter.

The $LC_{50}$ and $LC_{90}$ values of spinosad against laboratory colony after 24 hour was (1.15E-06 and 0.0005ppm, respectively) and after 48 hour (9.35E-09 and 2.13E-06 ppm, respectively). Among the tested organophosphate insecticides, fenitrothion and temephose were effective against laboratory colony that they recorded $LC_{50}$ values (1.51E-03, 1.2E-3 ppm, respectively) and $LC_{90}$ values (0.26, 0.05ppm, respectively), while malathion recorded high $LC_{50}$ and $LC_{90}$ values against reference one (5.58E-03, 2.44ppm, respectively.

Mosquitoes that collected from El-Fayoum were more susceptible to all used insecticides than the other populations that spinosad after 24h, 48h, fenitrothion, temephos and malathion exhibited $LC_{50}$ values against them (1.75E-09, 1.98E-10, 6.07E-05, 8.18E-05 and 3.43E-04ppm, respectively) and $LC_{90}$ values(1.3E-06, 3.02E-07, 0.0072, 0.02 and 0.203ppm, respectively).

Considerable variation in $LC_{50}$ and $LC_{90}$ values of the tested insecticides, spinosad after 24h, 48h, temephos, fenitrothion and malathion against Giza population $LC_{50}$ values (5.38E-06, 1.05E-07, 4.5E-03, 8.8E-03 and 7.46E-02 ppm, respectively) and $LC_{90}$ values (0.0123, 2.24E-04, 0.86, 1.96 and 76.73ppm, respectively).

Larvae of the mosquitoes that collected from El-Sharkia recorded $LC_{50}$ values (5.33E-06, 8.57E-08, 1.3E-03, 4.43E-3 and 9.86E-02 ppm after 24h and 48h treatment with spinosad, 24h from temephos, fenitrothion and malathion treatment, respectively, and $LC_{90}$ values (0.043, 2.33E-04, 0.98, 12.39 and 27.48ppm, respectively).

Kafr El-Shakh mosquitoes were most susceptible to spinosad followed by temephos than to the other used organophosphorus insecticides fenitrothion and malathion $LC_{50}$ and $LC_{90}$ values of spinosad after 24h (2.88E-06, 0.00191ppm, respectively) and48h (4.75E-08, 2.7E-05ppm, respectively while the $LC_{50}$ and $LC_{90}$ values of temephos (4.8E-03, 0.49ppm, respectively and (9.29E-03, 3.214ppm, respectively for fenitrothion, and (5.19E-02, 18.41ppm, respectively for malathion.

According to the larval bioassay results, all tested population showed low resistance ratio to spinosad after 24h [El- Fayoum (1.5E-03), Kafr El-Shakh (2.5), El-Sharkia (4.63) and Giza (4.67)]. All populations also showed low ratios of resistance to temephos [El- Fayoum (0.04), El-Sharkia (1.08), Giza (3.75) and Kafr El-Shakh (4)]. Also fenitrothion showed low ratios of resistance in populations of [El- Fayoum (0.04), El-Sharkia (2.93)] and moderate resistance in [Giza (5.83), Kafr El-Shakh (6.16)]. Malathion had low ratios of resistance in populations of [El- Fayoum (0.06), and Kafr El-Shakh (0.06)], while Giza and El-Sharkia mosquito recorded significant increase in resistance ratio to the toxicity of malathion that they recorded 13.37 and 17.67 folds, respectively.

**Table 2:** Toxicity of the four tested insecticides and their resistance ratio in the larvae of *Culex pipiens* collected from different Egyptian governorates in comparison with laboratory colony.

| Insecticides | Population | Slope value | $LC_{50}$ (ppm) | $LC_{90}$ (ppm) | (RR) $LC_{50}$ |
|---|---|---|---|---|---|
| Spinosad after 24 h. | Laboratory colony | 0.490 | 1.15E-06 (4.23E-07-5.41E-06) | 0.0005 (5.3E-05- 0.03) | - |
| | El- Fayoum | 0.445 | 1.75E-09 (6.92E-10-3.81E-09) | 1.3E-06 (4.1E-07-7.8E-06) | 1.5E-03 |
| | Giza | 0.382 | 5.38E-06 (2.27E-06-1.82E-05) | 0.0123 (0.00156-0.2692) | 4.67 |
| | El Sharkia | 0.328 | 5.33E-06(2.01E-06-2.213E-05) | 0.043(0.0036-2.10378) | 4.63 |
| | Kafr ElShakh | 0.454 | 2.88E-06(1.5E-06-6.99E-06) | 0.00191(0.00041-0.01707) | 2.5 |
| after 48 h | Laboratory colony | 0.543 | 9.35E-09 (6.1E-09-1.4E-08 | 2.13E-06 (1.11E-06-4.9E-06) | - |
| | El- Fayoum | 0.402 | 1.98E-10(5.45E-11-5.E-10) | 3.02E-07 (1.02E-07-1.5E-06) | 0.02 |
| | Giza | 0.385 | 1.05E-07(6.27E-08-1.86E-07) | 2.24E-04 (5.7E-05 -0.002) | 11.23 |
| | El Sharkia | 0.373 | 8.57E-08(5.06E-08-1.52E-07) | 2.33E-04 (5.7E-05-0.002) | 9.16 |
| | Kafr ElShakh | 0.466 | 4.75E-08(3.1E-08-7.4E-08) | 2.7E-05 (1.04E-05-9.42E-05) | 5.08 |
| Fenitrothion. after 24 h. | Laboratory colony | 0.575 | 1.51E-03(9.5E-04-2.32E-03) | 0.26 (0.1102-0.8492) | - |
| | El- Fayoum | 0.618 | 6.07E-05(1.26E-05-1.65E-04) | 0.0072 (0.00212-0.0745) | 0.04 |
| | Giza | 0.546 | 8.8E-03 (5.6E-03-1.49E-02) | 1.96 (0.6442-9.9312) | 5.83 |
| | El Sharkia | 0.372 | 4.43E-3 (2.33E-03-8.93E-03) | 12.39 (1.9276-299.2265) | 2.93 |
| | Kafr ElShakh | 0.505 | 9.29E-03(5.68E-03 -.017) | 3.214 (0.91622-20.9894) | 6.15 |
| Malathion. after 24 h. | Laboratory colony | 0.485 | 5.58E-03 (3.2E-03-9.16E-03) | 2.44 (1.05- 7.57) | - |
| | El- Fayoum | 0.462 | 3.43E-04 (5.7E-05 1.13E-03) | 0.203 (4.34E-02 -3.61) | 0.06 |
| | Giza | 0.425 | 7.46E-02 (0.0437-0.135) | 76.73 (21.04 - 478.63) | 13.37 |
| | El Sharkia | 0.524 | 9.86E-02 (6.3E-02-0.163) | 27.48(10.30-101.86) | 17.67 |
| | Kafr ElShakh | 0.503 | 5.19E-02 (3.3E-02-8.5E-02) | 18.41(6.95-68.08) | 0.06 |
| Temephos. after 24 h. | Laboratory colony | 0.781 | 1.2E-3 (0.8E-03-1.7E-03) | 0.05 (0.03- 0.11) | - |
| | El- Fayoum | 0.555 | 8.18E-05 (4.8E-05-1.3E-4) | 0.02 (0.01 -0.04) | 0.04 |
| | Giza | 0.562 | 4.5E-03 (2.9E-03-7.2E-03) | 0.86 (0.32- 3.52) | 3.75 |
| | El Sharkia | 0.446 | 1.3E-03 (7.1E-04-2.3E-03) | 0.98 (0.29- 6.71) | 1.08 |
| | Kafr ElShakh | 0.639 | 4.8E-03 (3.3E-03-7.4E-03) | 0.49 (0.22-1.53) | 4 |

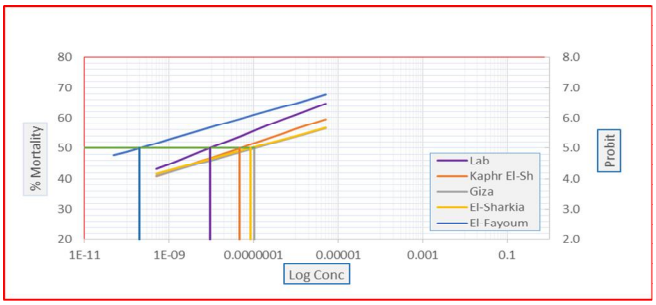


**Fig. 2:** Probit mortality curve of *Culex pipiens* larvae collected from different Governorates after spinosad treatment.

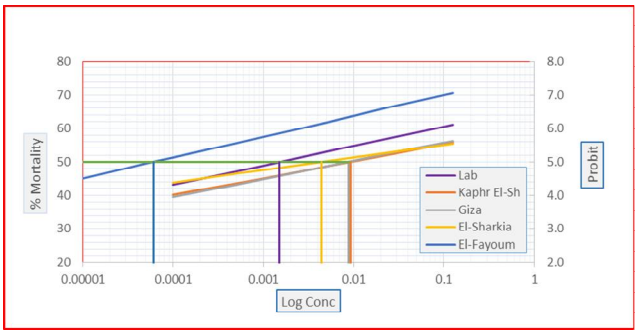


**Fig. 3:** Probit mortality curve of *Culex pipiens* larvae collected from different Governorates after fenitrothion treatment.

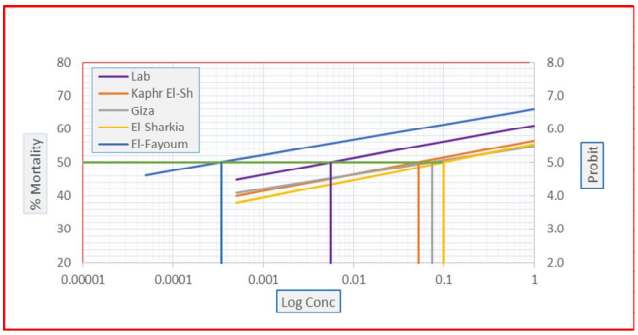


**Fig. 4:** Probit mortality curve of *Culex pipiens* larvae collected from different Governorates after malathion treatment.

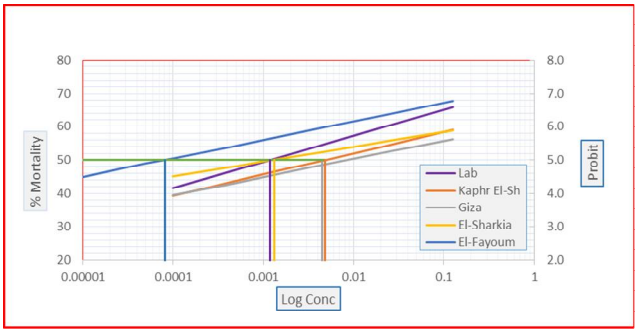


**Fig. 5:** Probit mortality curve of *Culex pipiens* larvae collected from different Governorates after temephos treatment.

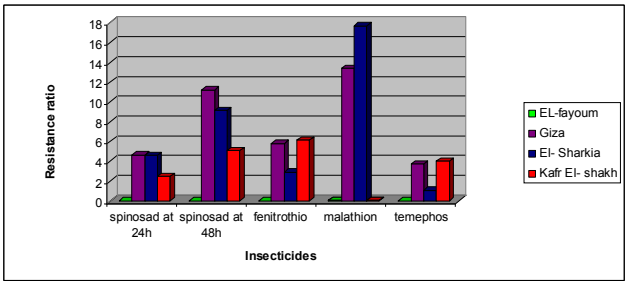


**Fig. 6:** Resistance ratio of field strains of *Culex pipiens* larvae to different insecticides.

**Biochemical assay:**

From the larval bioassay results, the bio- insecticide, spinosad was the most effective larvicide among the tested ones, beside the others organophosphorus compounds are already known their mode of action, so it was only selected to investigate its effect on different biochemical aspects of the laboratory reared mosquitoes that known as strategy for insecticide resistance mechanisms against the traditional insecticides.

The effect of $LC_{50}$ of spinosad on total protein was significantly decreased due to treatment with spinosad compared to the control (fig. 7). With regard to acetylcholineesterase assay all values revealed a significant decrease in AChE activity with time in both treated and control larvae beside that, the activity decreased in post treated larvae if compared with control groups (fig. 8). In non- specific esterases assay, there was no obvious effect for the treatment with spinosad on the α- esterases activity (fig.9), while the exposure to spinosad resulted in elevation the activity of β-esterases if compared with control (fig.10). The results in figure 11 showed that spinosad reduced protease activity compared to control, while exposure to this bio insecticide resulted in varied values of invertase activity, but the values decreased with time in both control and treated groups (fig.12).

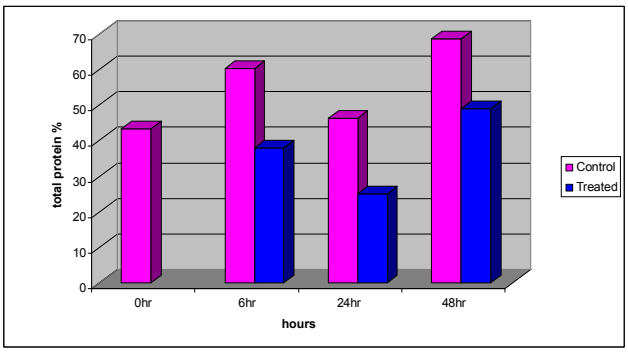


**Fig. 7:** Effect of $LC_{50}$ of spinosad on total protein content of laboratory strain of *culex pipiens* larvae.

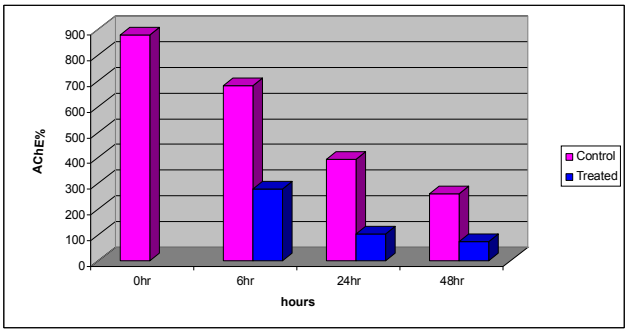


**Fig. 8:** Effect of $LC_{50}$ of spinosad on AChE of laboratory strain of *culex pipiens* larvae.

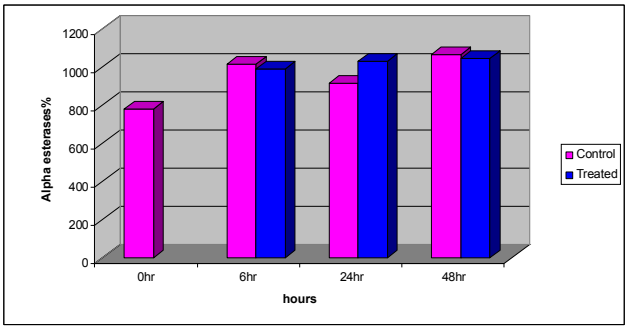


**Fig. 9:** Effect of $LC_{50}$ of spinosad on alpha esterases of laboratory strain of *culex pipiens* larvae.

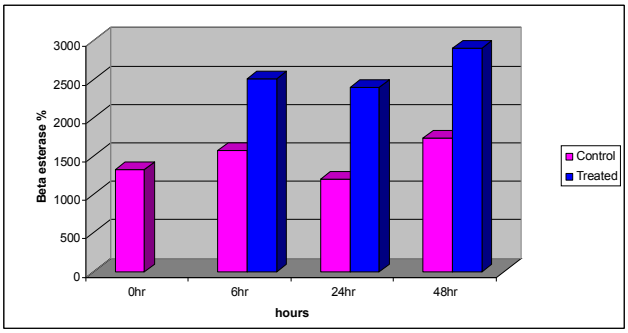


**Fig. 10:** Effect of $LC_{50}$ of spinosad on beta esterases of laboratory strain of *culex pipiens* larvae.

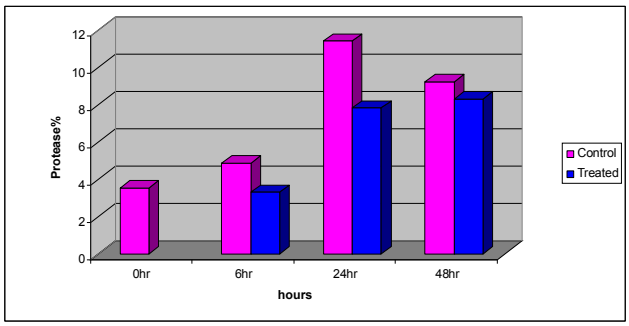


**Fig. 11:** Effect of $LC_{50}$ of spinosad on protease of laboratory strain of *culex pipiens* larvae.

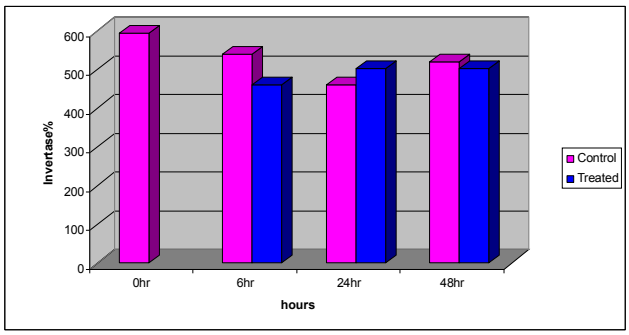


**Fig. 12:** Effect of $LC_{50}$ of spinosad on invertase of laboratory strain of *culex pipiens* larvae.

## Discussion

It is known that larviciding can reduce the number of dangerous adult mosquitoes that transmit disease to human through their bits (Kemabonta and Nwankwo, 2013). Chemical insecticides play a major role in mosquito control. However, the continuous and indiscriminate use of insecticides will lead to the development of physiological resistance in the insect, besides that the release of these chemicals often pollutes the environment and affect non- target organisms. The present study demonstrated larvicidal activity of some traditional insecticides, fenitrothion, malathion and temephos which belong to the same family of insecticides that named organophosphorus insecticides in comparison with one bio- insecticide called spinosad which is a natural product of the fermentation of the bacterium *Saccharopolyspora spinosa*. The toxicity of these compounds were evaluated against the house mosquito, *C. pipiens* that collected from several Egyptian governorates, El-Fayoum, Giza, El-Sharkia and Kafr El-Shakh as well as their effect on laboratory reared colony of this species to determine the insecticides resistance status of *C. pipiens* field populations.

The results clearly indicated that the larvae were more susceptible to spinosad than the other tested insecticides with $LC_{50}$ values at 24h, ranged from 1.75E-09 to 5.38E-06ppm, while, fenitrothion recorded $LC_{50}$ at 24h, ranged from 6.07E-05 to 9.29E-03ppm and temephos from 8.18E-05 to 4.8E-03 ppm. These results agreed with those of (Bahgat *et al.*,2001, Romi *et al.*,2006, Elkady *et al.*,2008, and Shiney Ramya and Ganesh, 2013) in which spinosad was very effective against *C. pipiens* larvae. Also Kemabonta and Nwankwo, 2013 found that spinosad showed better larvicidal activity against *Anopheles* and *Aedes spp.* than temephos. On the other hand malathion recorded the highest values of $LC_{50}$ against all tested populations.

Findings of the present study has been supported by many workers who reported the same results such as results of Nazni *et al.*, 2005 who found that field collected *Culex* from Kuala Lumpur had high level of resistance to malathion, the present findings also in agreement with Bansal and Singh, 2007 who found that resistance of field population of different mosquito species in Rajasthan was high to malathion, while they still sensitive to temephos. Jamal *et al.,* (2011) stated the same results in Kkartoum. Also Akiner and Eksi, 2015 found that *C. pipiens* which were collected from different areas in turkey showed the highest resistance *t*o malathion among different used insecticides.

Based on $LC_{50}$ values of tested insecticides, a significant difference in resistance ratio was observed in the different populations. All tested populations recorded the lowest resistance ratio at $LC_{50}$ ($RR_{50}$) to spinosad. El Fayoum population of *C. pipiens* exhibits lowest $RR_{50}$ to all tested insecticides, spinosad at 24h and 48h, fenitrothion, temephos and malathion (1.5E-03 and 0.02, 0.04,0.04 and 0.06 folds respectively). On the other

hand Giza governorate recorded low $RR_{50}$ (4.67 folds) to spinosad at 24h, the highest $RR_{50}$ (11.23 folds) was recorded against spinosad at 48h, and temephos exhibited lowest $RR_{50}$ (3.75 folds), while malathion showed higher $RR_{50}$ in Giza and El Sharkia (13.37, 17.67 respectively). These results suggest the presence of organophosphorus resistance in field populations with different degrees.

Several studies were carried out to compare the toxicity of different organophosphorus insecticides and other families of insecticides and evaluated the resistance ratio of different mosquito species to these such as Rong *et al.,* 2012 who evaluated the effects of fenitrothion and other insecticides on *An. gambia* from Benin, Abd El- samie and Abd EL- Baset, 2012 who studied the efficacy of some insecticides on field populations of *C. pipiens* from Egypt, Bhan *et al.,* 2013 who evaluated larvicidal activity of temephos on *An. stephensi* in India, Selvi *et al.,* 2007 and Low *et al.,* 2013 who studied the resistance of different mosquito species to malathion. On other hand many authors studied spinosad as alternative to chemical insecticides such as El- Kady *et al.,* 2008, Prabhu *et al.,* 2011 and Kemabonta and Nwankwo, 2013.

In view of the fact that organophosphorus insecticides resulted in development of resistance in mosquitoes, so several studies were carried to explain the mechanisms of resistance to these insecticides and to recommend another insecticides as well as to compare between their effect on the physiology within the target insects. Qin *et al.,* 2014 summarized the mosquito resistance mechanisms to insecticides in two points; increased metabolic detoxification and reduced target site sensitivity. The organophosphorus insecticide contains ester groups that can be detoxified via hydrolysis of ester bond through the action of same enzyme called detoxification enzymes such as α- and β-esterases (Abd El- samie and Abd EL- Baset, 2012). Evidence of malathion resistance due to elevated α –esterase activity was found which may explain the findings of the present work (Low *et al.,* 2013). Also, Zayed *et al*., 2006 indicated that non-specific esterases may be responsible for resistance in the Egyptian *C. pipiens* larval populations.

To further understand the possible underlying resistance mechanism in the domestic mosquito, *C. pipiens* to spinosad, the present study used biochemical assay detect resistance due to insensitivity of acetylcholineesterase (AChE), protease, invertase, non specific esterase (α- and β-esterases) and total protein, in the laboratory colony reared mosquito larvae.

In the present study, the activity of AChE decreased after exposure to spinosad which agreed with results of El-Kady *et al.,* 2008 who recorded the same effect on *C. pipiens* larvae after spinosad treatment. AChE is enzyme that occurs in the center nervous system and functions by removing acetylcholine from its post synaptic receptors. The result of this action is the hydrolysis of acetylcholine into acetate and prolonged neuron excitation (El-Kady *et al.,* 2008). Inhibition of AChE by bio- insecticides causes a desensitization of acetylcholine receptors and leads to eventual death of the organism.

The present results showed that the activity of β-esterase increased due to exposure to spinosad, while α-esterase activity showed little decrease at 6 and 48 hour and slight increase at 24 hour of exposure, which contradicted with the previous suggestion of Yong *et al.,* 2004 and Gu *et al.,* 2004, and also contradicted with findings of El-Kady *et al.,* 2008, in which spinosad treatment resulted in decrease of the two enzyme activities in the larvae of *C. pipiens*.

The exposure of an organism to a xenobiotic chemical can modify the synthesis of certain metabolites and disturb the functionality of the organism (Rodriguez - Ortega *et al.,* 2003). the total protein in the whole body of *C. pipiens* late 2$^{nd}$ instar larvae in the present study decreased under stress of spinosad, this in agreement with results of Bouaziz *et al.,* 2011; Shaurub and Abd El-Aziz, 2015, in which treatment with insecticides decreased the total main metabolites (protein, carbohydrate and lipid) in mosquito larvae.

Some proteases play roles in insect digestion and development, the exposure to spinosad in the present work resulted in decrease of the protease activity which agreed with suggestion of Rodigue- Ortegua *et al.,* 2003.

The activity of invertase enzyme in the present results varied under stress of time and exposure to spinosad, these results agreed with Shakoori *et al*., 1998 who reported changes in activity levels of invertase in *C. pipiens* larvae treated with both lambda-cyhalothrin and lufenuron. The invertase enzyme belongs to the enzyme group which responsible for digestion of carbohydrates (trehalase, invertase and amylase). Carbohydrates as energy elements play a crucial role in the physiology of the insects; the rate of glycogen in tissues are closely related to several physiological events such as the reproduction, the molt and the flight (Kaufman *et al.* 2008).

According to all the previous results and discussion we can conclude that spinosad, bio- insecticide has high efficacy against the larvae of *C. pipiens* in some Egyptian governorates.

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