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Effects of Fenitrothion and Deltamethrin Insecticides on Certain Biochemical Parameters of *Culex pipiens* larvae

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ABSTRACT

The efficiency of two insecticides, fenitrothion (Organophosphorus) and deltamethrin (Pyrethroid) against the 3rd instar larvae of *Culex pipiens* were evaluated under the laboratory conditions. LC50 values for fenitrothion and deltamethrin were 0.089 and 0.0016 ppm, respectively. Also, the effect of the tested insecticides on some biochemical parameters was carried out through evaluation the effect of sublethal concentration of the tested compounds on the activity of acetylcholinesterase, aspartate aminotransferase (GOT), alanine aminotransferase (GPT) and protein content after treatment the 3rd larval instar for 24 hr. The results indicated that deltamethrin and fenitrothion reduced in the activity of acetylcholinesterase reached to 69.98, 68.0, 73.43 and 65.99% compared to the untreated check with moribund and alive larvae, respectively. As for the transaminases GOT, fenitrothion showed great inhibition on GOT of moribund larvae compared with negligible reduction than the untreated value in alive larvae, showing 97.34 and 16.15% reduction, respectively. On the contrary, deltamethrin caused stimulatory effects on GOT activity in both moribund and alive larvae, reaching 41.15 and 30.21% increase than the normal value, respectively. As for total protein, data clearly showed that both the tested insecticides caused slight alteration in such parameter

Keywords: fenitrothion, deltamethrin, Culex pipiens, ChE, GOT, GPT ,Transaminase, total protein.

INTRODUCTION

Mosquito's uptil now are playing an important role in the transmission of several diseases especially in the tropical countries. In Egypt, different mosquito species are involved in the transmission of malaria, filarial and rifit valley fever. *Culex pipens* is the main vector of filariasis and RVF in Egypt.

Most *Culex* species are resting outdoors; expect *Cx. pipens* which is a domestic mosquito, with over 50% resting on non-sprayable surfaces in the house, such as mosquito nets, clothing hangings and furnatures. For this reason indoor residual house spraying is of limited use, particularly when the insecticide involved does not have volatile or fumigant properties. Furthermore, *Cx. pipens* adults have developed high levels of resistance to organochlorine and organophosphate insecticides in many areas. Therefore, larviciding has become the principal method of chemical control for most species of *Culex*, especially in urban and semi-urban areas (Gratz, (1991), WHO, (1995)

Environmental sanitation should be an integral part of effective *Cx. pipines* control. Before implementing chemical control, breeding sites should be mosquito-proofed or destroyed wherever possible. This may involve unblocking drains water flow, draining areas of flooded land, filling in small collections of polluted water and repairing chiped or cracked concrete lids of septic tanks. In the special situation posted by pit latrines which have a free water surface (i.e not covered with scum) cesspits and septic tanks, all of which may produce vast numbers of *Cx. pipens*, a layer of expanded polystyrene beads, one cm deep can applied to prevent breeding. However care should be taken on investigate the likelihood of beads being scattered about the environment as a result of treated sites flooding or being emptied (Grant, *et. al.*1984).

The present investigation aims to determine the effects of two insecticides; fenitrothion (organophosphorus compound) and deltamethrin (pyrethroid compound) on some biochemical parametres includes, the activity of three key enzymes; acetylcholinesterase, aspartate aminotransferase (GOT), alanine aminotransferase (GPT) and protein content in the immature stage, 3th instar larvae of *Culex pipens* under laboratory conditions.

MATERIALS AND METHODS

1- Rearing and Maintenance of Larvae

Larvae of *Culex pipens* were collected from El-Qualyobeia Governorate from a locality sites which was not previously treated or rarely exposed to insecticides application. They were transferred to the breeding bawls half filled with water and reared in the laboratory under constant conditions of 28-30ºC and 70-80% R.H. Tetramine food substance (Protein 4810, Fats 27% and Fiber 33%) was sprinkled twice a day on water surface. Pupae were pipette, placed in groups into plastic containers half-filled clean water and transferred to emerging adult cages (33 x 33 x 33cm) with wire screen roof an sides. Emerged adults were provided 18% sucrose solution and pigeon blood. Suitable containers for egg-laying were placed into the cages 36-48 hrs after providing the blood meal.

2- Insecticides Used

Two insecticides were used in this study; i.e. fenitrothione 50Ec (Sumithion®) ([(O, O- dimethyl O-(3-methyl - 4- nitrophenyl) phosphorothioate)], and deltamethrin 2.5 % Ec (Decis®) ([(*S*)-α-cyano-3-phenoxybenzyl). The studied insecticides were obtained from (Sumitomo chemicals company, Cairo, Egypt).

3- Laboratory Bioassay Procedure of tested insecticides

The selected insecticides were evaluated against the 3rd instar larvae using the standard bioassay technique (WHO, 1981) performed in single use cups containing 100 ml aqueous media. Fenitrothion and deltamethrin were tested at concentrations ranged from 0.2 to 0.00001 ppm in distilled water. Each bioassay was conducted in triplicate, 20 starved larvae in each replicate. After 24 h of exposure, both of the dead and alive larvae were counted, the mortality percentages were calculated and expressed in form of log/probit regression lines and the LC50 values were calculated according to the method of Finney (1971), using the computer program Sigma Plot for Windows, Version 2.0, which calculated the corrected mortality percentage based on the Abbott's formula (Abbott, 1925).

4- Biochemical studies:

C. pipiens larvae were collected after 24hr of treatment and homogenized in distilled water. Homogenates were centrifuged at 5000 rpm for 15 min. The supernantant was placed in tubes for analysis as mentioned by (Assar2012).

5- Determination of Some Biochemical Parameters

Acetylcholinesterase activity: The activity of acetylcholinesterase was determined in the total larval homogenate accoding to the method described by Ellman *et al*., (1961).

Transaminas activity: The activities of both aspartate aminotransferase (GOT), alanine aminotransferase (GPT) were determined in the larval homogenate according to the method of Reitamn and Frankle (1957).

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).

6- Statistical Analysis: Statistical Analysis was performed for all the estimated parameters by Student *t*-test using Sigma Plot program Version 2.0 software.

RESULTS AND DISCUSSION

1- Efficiency of tested insecticides against 3rd instar mosquito larvae

Data concerning the insecticidal activites of the two tested compounds, the organophosphate fenitrothion and the synthetic pyrethroid deltamethrin, against 3rd instar larvae of *Culex pipen*s in laboratory are illustrated graphically as toxicity regression lines in (Figures 1) and the calculated values tabulated in Table (1). The obtained data clearly indicated that deltamethrin was more effective against the 3rd instar larvae of *Culex pipiens* than fenitrothion. The calculated LC50 value of deltamethrin was 0.0016 ppm while this value was 0.089 ppm. Thus it could be concluded that deltamethrin was more toxic as larvicide than fenitrothion at lab-evaluation by about 55 times or fold.

Also, the main criteria of the toxicity lines reveales that the slope values of both lines were 2.31 and 1.68. This means that the response of individual of tested strain toward deltamethrin was more homogeneous than towared fenitrothion, that the Ld-p line of this compound was more steeper than that the other compound.

In this connection, many investigators reported that field populations of *Culex pipiens* larvae were varied in their responses to the tested insecticides. The degree of susceptibility of mosquito's population was differed from one location to another for each the tested insecticides. The chemical structure and the back history of usage insecticides in each location affecting the toxicity of insecticides against investigated population of *Culex pipiens*.

In this respect, Kawakami (1989) reported that high resistance to fenitrothion and fenthion in *Culex pipiens*, larvae may reflect the history of application of these compopounds to the breeding site. Also Grant *et.al* (1984) stated that, field collected population of *Culex pipiens* from Sutter and Yuba counties,California proved more susceptible to chloropyrifos than malathion, they attributed this finding to the more wide spread use of malathion for filed control.

In addition, Selim (1998) found that, the field population larvae of Shobra EL-Khema collected from Faculty of Agruculture revealed more susceptible to all of pyriproxyfen, deltamethrin, cyphenothrin, malathion, fenitrothion and diazinon than both of Shalakan, Fac. and EL-Suff population of *Culex pipiens* .Also, the same auther reported that deltamethrin proved to be the most effective insecticides than other tested compounds against the population of Shobra EL-Keima.

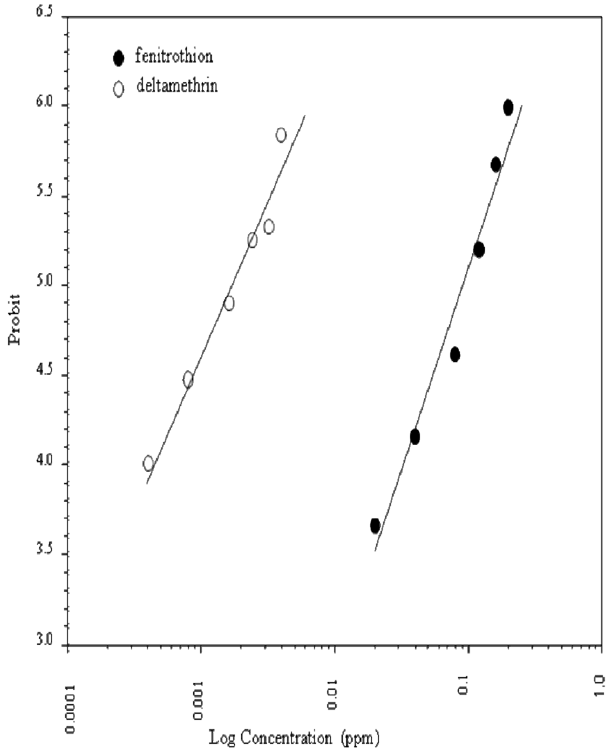

Figure 1. Toxicity regression lines of fentrothion and deltamethrin against 3rd instar larvae of *Culex pipiens* after 24 hr of treatment.

Table 1. Values of LC_{50}, slope and regressions values of the two tested insecticides, fenitrothione and deltamethrin against 3rd instar lave of *Culex pipiens*.

Tested Insecticide	LC_{50} (ppm)	Slope	r (regression)
Fenitrothion	0.089	2.31	0.98
Deltamethrin	0.0016	1.68	0.97

2- Effect of tested insecticides on Biochemical Parameters

Data concerning the effects of sublethal concentrations (LC50) of the organophosphate fenitrothion and the synthetic pyrethroid deltamethrin on the activity of the three vital enzymes AchE, GPT and GOT as well as total protein content of the alive and dead 3rd instar larvae of the mosquito *Culex Pipiens* under laboratory conditions and after 24hrs exposure in water are tabulated in Tables (2&3). Examination of the obtained results indicated the role of insecticides treatment on the activity and content of the studied biochemical aspects. Insecticide type and status of larvae after exposure (alive or moribund) showed noticeable influences in this respect. Different trends of responses were noticed between the studied enzymes and total protein content.

In case of acetylcholinesterase enzyme (AchE), data in Tables(2&3) indicate the inhibitoy effect of deltamethrin pyrethroid onAChE enzyme activity even it is not consider as the specific site of action for this insecticide .The percent reduction in AChE activity than the normal value reached 69.98 and 68.01%with moribund and alive larvae, respectively. The organophosphorus insecticides showed considerable inhibition in AChE activity in both moribund and alive larvae,i.e. 73.43 and 65.99% reduction than the untreated check. Such finding is in agreement with that obtained by many researchers,i.e. Marchi and Addis(1990), Nance *et.al*.(1990), Bonning et.al.(1991), Xu et.al.(1994), Bourguet et.al.(1996), Mouches et.al.(1990).

As for transaminase enzymes, data in the same tables indicate that exposure of mosquito larvae to the LC50's of fenitrothion or deltamethrin resulted in slight inhibition in GPT of moribund larvae than the normal values, the reduction percent in GPT enzymes of moribund larvae reached 37.81 and 19.40% with the mentioned insecticides, respectively. The contrary was recorded with the alive larvae, where insecticide treatments resulted in GPT simulation, showing 91.54 and 47.26% increase than normal level. With fenitrothion and deltamethrin, respectively. Such finding is quite strange because it is contradicting with the well known mode of action of insecticides on vital enzymes in mammals and insects. (Sergieva and Gracheva, 1992).

As for the transaminases GOT, data in the same tables indicate different trend of the enzyme response between the two tested insecticides. The organophosphate fenitrothion showed great inhibition on GOT of moribund laravae compared with negligible reduction than the untreated value in alive larvae, showing 97.34 and 16.15% reduction, respectively. On the contrary, the pyrethroid deltamethrin caused stimulatory effects on GOT activity in both moribund and alive larvae, reaching 41.15 and 30.21% increase than the normal value, respectively.

The obtained resulted didn't give a clear indicator to the effect of the tested compounds on transaminase activity but it could be concluded that the tested insecticides gave an alteration in transaminase activity as mentioned by many other investigators.

However, the obtained results are in agreement with that obtained by other investigators which studied the effect of deltamethrin on the 3rd in star larvae of the cotton leafworm, *spodoptera litterolis* (boised) (El-malla et. al., 1983; Ahmed *et. al*., 1987). In this respect, Assar *et al* (2012).said that the effect of cyromazine on the activity of aspartate aminotransferase (GOT) and alanine aminotransferase (GPT) of 4th instar larvae of *C. pipiens* treated as 2nd instar larvae. The obtained results revealed that cryomazine induced inhibitory effect on the GOT and GPT at 0.1 ppm and induced. A significant stimulatory effect on total GOT and GPT at 1 ppm. The inhibitory effect of some IGR'S on theacitivity of AST and ALT on *C. pipiens* larvae was in accordance with those obtained by Saha *et al.* (1986) using JHA against *Chrysocoris stollii*; Abdel-Hafez *et al.* (1988) using diflubenzuron and riflumuron against *S. littoralis* ; Ahmed *et al.* (1990) using chlorofluazuron against *S. Littoralis* ; Sokar (1995) using hexaflumuron against *S.littoralis* ; Abdel –Aal (2002) using chlorfluazuron ,flufenoxuron and pyriproxyfen against *S. littoralis.* EL-sheikh (2002) using pyriproxyfen against *A. ipsilon* and Assar *et al.*(2010) using consult and match against *M. domestica*. The stimuatory effect induced on the total AST and ALT by cryomazine agree with the results obtained by JHA against *Chrysicoris stollii* (Saha *et al.,* 1986); pyroproxyfen against *P. gossypiella* and *E. insulana* (Anan *et al*., 1993); pyriproxyfen, flufenoxuron and chlorfluazuron against *S. littoralis* (Abdel- Aal, 2002), hexaflumuron against *S. littoralis* (Sokar, 1995); and mimic,Applaud and admiral against *M. domestica* (Assar *et al.*, 2010).

In case of the role of transaminase enzymes in the insect , it was reported that transaminase enzymes were considered as key enzymes in the formation of non essential amino acids, which if formed inside the body not taken from outside in metabolism of nitrogen waste and gluconeogensis (Mordue and Goldworthy, 1973).The same authors stated that the change in transaminase levels have been correlated with anabolism or catabolism of protein. Maintainance of the balanced " amino acid pool " in insects is the result of various biochemical reactions carried out by a group of enzymes called amino- transferases (Meister, 1957).In addition, Glbert (1967) reported that the level of ALT varies with the amount of synthesized protein.The amino transaminase alanine is one of the components of oxidative metabolism of proline, which is utilized during the initial periods of flights; it acts as a catalytic agent in the carbohydrate metabolism. Azmi *et al.* (1998) stated that the ransaminases (ALT and AST) enzymes help in the production of energy and serve as a strategic link between the carbohydrates and protein metabolism and Are known to be altered during var ious physiological and pathological conditions.

However, the obtained data related to the effect of the two tested insecticides on the activity of the two tested transaminase, may be due to the effect on the role of the transamination process which affect in turn the determined activity of such two enzymes.

As for total protein, data in the same table indicated that deltamethrin pyrethroid caused slight inhibitory effect in amount of the vital biochemical aspect of mosquito larvae, showing 34.23 and 6.3% reduction than untreated larvae, respectively. The organophosphate fenitrothion showed different influence on total protein content due to the status of the exposed larvae. A great inhibition was recorded in moribund larvae (77.23%) compared with slight simulation in alive larvae (+ 30% than the untreated). Similar changes of protein content were also reported by other investigators when they tested the effect of two organophosphorus pesticides on the 4th instar larvae of *S. littoralis* (El-Herrawie *et al*., 1985).

Reviewing the aforementioned results, it could be concluded the important role of insecticide type and larvae status on the response of the vivo enzyme systems of mosquito larvae. No clear trend was noticed with cholinesterase as well as transminase enzymes activities in relation to insecticide treatment and larval conditions. Fluctuated influences were also recorded with total protein content of insecticide treated larvae.

Table 2. Effect of exposure of 3rd instar larvae of *C. pipiens* to fenitrothion and deltamethrin on the relative enzymes activities and total protein content

		(Amount of enzymes and total protein content) in treated and untreated larvae.			
		Fenitrothion		Deltamethrin	
Biochemical aspects	Untreated check	moribund	alive	moribund	alive
ChE (U/L)	3908±0.111	1.038±73.44	1329±0.275	1173±0.371	1250±0.101
GPT(unit/ml)	0.201±0.121	0.125±0.191	0.385±0.095	0.162±0.111	0.296±0.172
GoT(unit/ml)	0.192±0.121	0.051±0.181	0.161±0.211	0.271±0.311	0.250±0.111
Total protein (g/dL)	1.335±0.101	0.304±0.161	1.740±0.185	0.878±0.081	1.250±0.121

Table 3. Relative changes (-, +) in the amounts of enzymes of treated mosquito larvae compared with normal levels

	Activity of enzymes (%) comparing with untreated larvae.			
	Fenitrothion		Deltamethrin	
Biochemical aspects	moribund	alive	moribund	alive
ChE (U/L)	(-73.43)	(-65.99)	(-69.98)	(-68.01)
GPT (unit/ml)	(-37.81)	(+91.54)	(-19.40)	(+47.26)
GoT (unit/ml)	(-97.34)	(-16.15)	(+41.15)	(+30.21)
Total protein(g/dL)	(-77.23)	(+30.34)	(-34.23)	(-6.37)

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تأثير مبيدى الفينتروثيون و الدلتاميثرين على بعض المقاييس البيوكيميائية ليرقات الكيولكس بيبينز

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يهدف هذا البحث الى تقييم كفاءة مبيدين ، هما الفينيتروثيون والدلتاميثرين ضد يرقات العمر الثالث لبعوض كيولكس بيبينز وتم التقييم تحت الظروف المعملية . وكانت قيم التركيز المميت النصفى (LC50) للفينيتروثيون و دلتاميثرين 0.089 و 0.0016 جزء في المليون، على التوالي. كما تم اختبار تأثير المبيدات الحشرية على بعض المقاييس الكيميائية الحيوية من خلال تقييم تأثير التركيز المميت النصفى للمركبات المختبرة على نشاط انزيم أستيل كولينستريز، انزيمى أمينوترانسفيريز (ALT&AST)، والمحتوى البروتين الكلى بعد معاملة يرقات العمر الثالث لمدة 24 ساعة. أشارت النتائج إلى أن دلتاميثرين و فينيتروثيون قد سجلا انخفاض معنوى واضح في نشاط أستيل كولينستريز وصل الى 69.98 و 68.0 و 73.43 و 65.99٪ بالمقارنة على التوالي بالمقارنة بالغير معامل . أما بالنسبة لانزيم الترانس امينيز ALT ، أظهر فينيتروثيون تثبيط كبير على نشاط انزيم ALT لكل اليرقات المريضة بالمقارنة بانخفاض ضئيل عن اليرقات غير المعاملة (اليرقات الحية)، والتي بلغت نسبة الخفض فيها 97.34 و 16.15٪ ، على التوالي. و على العكس من ذلك، تسبب دلتاميثرين فى احداث تاثيرات تنشيطية لانزيم ALT في كل من اليرقات المعاملة والغير معاملة بلغت 41.15 و 30.21٪ زيادة عن القيمة الطبيعية ، على التوالي. أما بالنسبة للبروتين الكلي، فقد أظهرت النتائج بوضوح أن كلا من المبيدين المختبرين قد سجلا تغييرا غير معنويا فى هذا المعيار 0