

Characterization of Insecticide Cross-resistance in a Fenitrothion-resistant Strain of *Culex pipiens* Larvae

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Received on: 28/1/2016

Accepted for publication on: 15/2/2016

Abstract

Insecticide resistant strains of mosquitoes is considered as a main problem in controlling malaria and dengue diseases. In the present study, a fenitrothion resistant strain (FEN-R-strain) of *Culex pipiens* larvae in Assiut University, Assiut Egypt, proved to have a high level of cross-resistance against the organophosphate insecticides. Cross-resistance to malathion ($RR_{50} = 634.86$ -fold) was higher than that of fenitrothion ($RR_{50} = 426.70$ -fold), whereas the cross-resistance to diazinon ($RR_{50} = 97.86$ -fold) was lower than that of fenitrothion. RR_{50} on the other hand, the resistant strain had low level of cross-resistance toward chlorpyrifos (RR_{50} were 31.8-fold) as compared with the other tested organophosphates. The results revealed that larvae of FEN-R-strain displayed slightly high cross-resistance to the pyrethroid insecticide deltamethrin ($RR_{50} = 22.32$ -fold), moderate cross-resistance to cypermethrin ($RR_{50} = 16.90$ -fold) and permethrin ($RR_{50} = 12.64$ -fold) and less cross-resistance level was to fenvalerate ($RR_{50} = 1.67$ -fold). Larvae of FEN-R-strain displayed high cross-resistance to the carbamate insecticide propoxure ($RR_{50} = 10.18$ -fold), low cross-resistance or tolerance to carbaryl ($RR_{50} = 4.13$ -fold) and very low cross-resistance or tolerance to methomyl ($RR_{50} = 1.96$ -fold). Data also indicated that larvae of the FEN-R-strain of *Culex pipiens* displayed slight cross-resistance to the bioinsecticide spinosad ($RR_{50} = 250.53$ -fold) and low cross-resistance or tolerance to avermectin ($RR_{50} = 8.52$ -fold). Larvae of FEN-R-strain showed moderate cross-resistance or tolerance to the insect growth inhibitor insecticide pyriproxyfen ($RR_{50} = 10.30$ -fold).

The variability in cross-resistance values in larvae of FEN-R-strain of *Cx. pipiens* to different conventional insecticides seem to indicate the absence of single mechanism controlling resistance among and within the insecticides classes (organophosphate, pyrethroid, carbamate, bioinsecticide and IGR insecticides). This pattern suggests multiple mechanisms of resistance, i.e. metabolic resistance and insensitive target site.

Keywords: Insecticide resistant, Cross-resistance, Mosquito, *Culex pipiens*, Fenitrothion.

Introduction

The common and widely distributed mosquitoes across Egypt, *Culex pipiens* (Linnaeus) has been incriminated as the main vector of bancroftian filariasis (Southgate, 1979), and Japanese encephalitis. Over 120

million people from 83 countries are physically disabled by lymphatic filariasis caused by *Culex* mosquitoes (WHO, 1995). For the last 50 years, pesticides have been widely used to control mosquitoes. In 1989, among the 504 arthropod species that had

become resistant to one or more insecticide families, 114 were mosquito species, the most important vector of human diseases (Georghiou and Lagunes-Tejeda 1991). A limited resistance mechanisms, including modification of target site, or changes in rate of metabolism involving esterases, glutathione S-transferases or monooxygenases operate in all insects. The development of resistance has been apparent since the 1950's, but the scale of the problem has been poorly documented. Few new public health insecticides have been developed for controlling of disease vectors for the past three decades without good stewardship. These insecticides will cease to be effective for vector control. This may have a dramatic effect in disease endemic countries, as few affordable alternative insecticides can rapidly be made available for vector control. The ability to optimally measure and manage insecticide resistance in field populations of insects is crucial to the long term sustainability of insecticide-based disease control campaigns by (Coleman and Hemingway, 2007). The objective of this study have investigate resistance pattern of a fenitrothion resistant strain (FEN-R-strain) of *Cx. pipiens* larvae.

Materials and Methods

1. Insecticides

The toxicants used in the present experiments belonged to organophosphates, the synthetic pyrethroids, carbamates, bioinsecticides and IGR compounds. Organophosphorus compounds were: Chlorpyrifos; Dursban (48% E.C) (Dow Chemical CO.), Diazinon; Diazin (5% G) (K.Z. CO.), Fenitrothion

; Sumithion (50% E.C) (Sumitomo Chemical CO.) and Malathion; Agrothion (57% E.C) (Agro Chemical CO.). Carbamate compounds were: Carbaryl; Sevin (1% Dust) (K.Z. CO.), Methomyl; Lannate (90% W.S.P) (Dobon di numorz CO.USA) and Propoxur Technical grade. (Sumitomo Chemical CO.).

Pyrethroid compounds were: Cypermethrin; Ripcord (10% E.C) (Shell international Chemical CO.), Deltamethrin; K-othrin (2.5% W.p) (K.Z. CO.), Fenvalerate; Sumicidin (20% E.C) (Sumitomo Chemical CO.) and Permethrin, Technical grade. (Sumitomo Chemical CO.), Bioinsecticide compounds were: Abamectin; Vertemic (1.8% E.C) (Syngenta. CO.) and Spinosad; Spinotor (24% S.C) (Dow Agro Chemical CO.) and IGR compound was: Pyriproxyfen; Admiral (10% E.C) (Sumitomo Chemical CO.).

2- Chemicals Used as surfactants

TritonX100 (100% purity, BDH Chem, Ltd. Poole England)

3- Insect strains

Two strains of *Cx. pipiens* were used in this study as follows:

A- Susceptible strain (S-strain)

The susceptible strain used in the present study was brought from the Institute of Veterinary and Medical Insects in Cairo, which reared in the lab for 5 years away from any insecticidal pressure.

B-Resistant strain fenitrothion (FEN-R-strain)

This strain was obtained by selecting a part of the parent field strain (which was collected in season of abundance, from Faculty of Agriculture Farm, Assiut, University, Assiut Go-

vernorate.) (~10000 4th instar larvae) with the OP insecticide fenitrothion. Selection pressure was carried out by dipping the 4th instar larvae for fifteen successive generations at the LC₅₀ level of each generation.

4- Collecting and rearing techniques

All strains transferred to laboratory of Plant Protection Department, Fac. Agric., Assiut University and reared under laboratory conditions of 25±2 C° and 60±5% R.H. throughout the study. Mosquito (S-strain, fenitrothion resistant strain) rearing was maintained in enamel trays. Transformed pupae were collected from the aforementioned trays by means of a wide mouth glass dropper, then pipette into Petri dishes that were placed in the adult cages (30cm dimensions). The emerging males were fed on 10% sucrose solution and females were fed on pigeon breast blood meat, respectively. Suitable containers for egg-laying were provided to the cages 48 hours after adult emergence and female feeding for egg-laying. Receptacles containing egg rafts were daily collected from the cages, then the newly hatched larvae were then transferred to the breeding trays, each containing 2 inch high tap water and provided with 500 larvae of the same age. After twenty four hours, the hatched larvae were fed on fresh yeast and protein which was evenly sprinkled on the water surface twice daily. The left over yeast, that was not ingested, was carefully removed with a medicinal dropper. Mass-rearing colony of all stages was maintained at 25±2 C° and 60±5% R.H. Temperature and relative humidity readings were daily

measured using a thermograph and hydrograph, respectively.

5- Bioassay tests

A- Toxicity studies

Sensitivity of investigated strains (S-strain and FEN-R-strains) of *Cx. pipiens* to the tested insecticides was checked in accordance with procedure described by the WHO (1981). The 4th instar larvae were used in the course of the present study. To the beakers, which were used for testing the insecticides a volume of 225 ml of tap water was added. Then 0.5 ml of different concentrations, in acetone, of each insecticide plus Triton X₁₀₀ was separately added to those beakers. The final concentration of Triton X₁₀₀ was 10 ppb. The serial concentrations of each insecticide tested were sufficient to cover almost a complete range of mortality. Three replicates per each concentration were used and each replicate contain 20 4th instar larvae. Mortality was recorded 24 h after treatment. Control was made with 0.5 ml acetone plus Triton X₁₀₀. Temperature was maintained at 27±1 C° during bioassay. The mortality percentages were corrected using Abbot's formula (1925). Concentration-mortality regression lines were analyzed using a computer program modified from the method of Finney (1971) to estimate the LC₅₀, the confidence limits and slopes of LCp lines.

B- Cross-resistance studies

The cross-resistance value for each tested insecticide was calculated as:

LC₅₀ value of resistant strain (FEN-R-strain)/ LC₅₀ value of susceptible strain (S-strain).

Results and Discussion

To investigate the cross-resistance spectrum in FEN-R-strain (426.7-fold resistance to fenitrothion) of *Cx. pipiens* larvae, the toxicity of thirteen insecticides including organophosphates (chlorpyrifos, diazinon and malathion), pyrethroids (cypermethrin, deltamethrin, fenvalerate and permethrin), carbamates (carbaryl, methomyl and propoxur) bioinsecticides (avermectin and spinosad) and the insect growth inhibitor pyriproxyfen were tested against 4th instar larvae of FEN-R-strain and S-strain. Resistance ratios (RR50) were calculated by dividing the LC₅₀ of the FEN-R-strain by the LC₅₀ value of the S-strain for the same insecticide. The data presented in Table 1 and Figures 1 and 2. Results indicate that the FEN-R-strain of *Cx. pipiens* larvae exhibited different levels of

cross-resistance to the tested organophosphates insecticides, the cross-resistance could be ranked in descending order of resistance ratio (RR50) as follow: malathion (634.86-fold), diazinon (97.86-fold) and chlorpyrifos (31.8-fold). The cross-resistance to the tested pyrethroid insecticides could be ranked in descending order: deltamethrin (22.32-fold), cypermethrin (16.0-fold), permethrin (12.64-fold) and fenvalerate (1.67-fold). Carbamate insecticides could be ranked as follow: propoxur (10.18-fold), carbaryl (4.13-fold) and methomyl (1.96-fold). Bioinsecticides could be ranked as follow: spinosad (250.53-fold) and avermectin (8.52-fold). The cross-resistance to the tested insect growth inhibitor insecticide pyriproxyfen was 10.30-fold.

Table 1. Toxicity and cross resistance of certain insecticides against the 4th instar larvae of the fenitrothion selected strain (FEN-R-strain) and the susceptible strain (S-strain) of *Cx. pipiens*.

S	Insecticides	S-strain	FEN-R-strain				
		LC ₅₀ µg/L	LC ₅₀ µg/L	C. Limits 95%		Slope ± S.E	RR ^(a)
				Lower	Upper		
Organophosphorus							
1	Fenitrothion	0.720	307.227	229.012	381.436	3.70±0.85	426.71
2	Chlorpyrifos	0.601	19.222	12.836	25.280	1.91±0.35	31.98
3	Diazinon	0.427	41.789	30.259	52.719	2.10±0.45	97.86
4	Malathion	0.116	73.644	60.237	91.375	2.35±0.38	634.86
Pyrethroids							
5	Cypermethrin	0.103	1.741	1.374	2.279	2.19±0.33	16.90
6	Deltamethrin	0.266	5.938	5.434	6.498	5.94±1.10	22.32
7	Fenvalerate	0.334	0.558	0.510	0.605	6.31±1.11	1.67
8	Permethrin	0.367	4.640	4.171	5.113	5.29±0.92	12.64
Carbamates							
9	Carbaryl	0.380	1.571	1.306	1.974	2.77±0.45	4.13
10	Methomyl	5.869	11.516	9.556	13.912	2.93±0.46	1.96
11	Propoxur	1.730	17.611	13.080	21.563	2.60±0.47	10.18
Bioinsecticides							
12	Avermectin	0.257	2.190	1.747	2.625	2.79±0.48	8.52
13	Spinosad	0.017	4.259	3.849	4.630	6.29±0.97	250.53
Insect growth inhibitor							
14	Pyriproxyfen	0.033	0.340	0.289	0.389	3.70±0.69	10.30

(a) RR: Cross resistance = LC₅₀ of the tested insecticide in FEN-R strain /LC₅₀ of the same insecticide against S-strain.

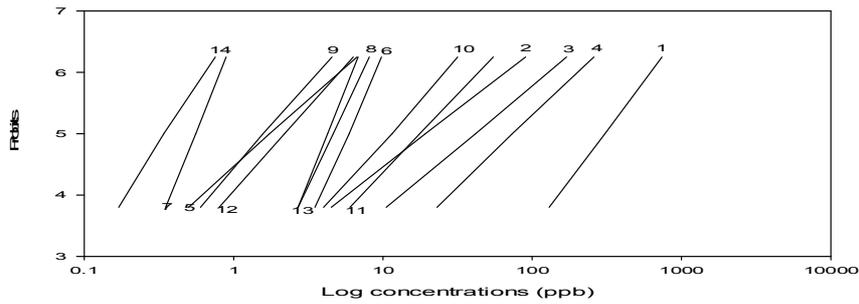


Fig. 1: Relationship between probit equivalent to percentages of mortality and log concentrations ($\mu\text{g/L}$) of (1) fenitrothion, (2) chlorpyrifos, (3) diazinon, (4) malathion, (5) cypermethrin, (6) deltamethrin, (7) fenvalerate, (8) permethrin, (9) carbaryl, (10) methomyl, (11) propoxur, (12) avermectin, (13) spinosad, and (14) pyriproxyfen applied to 4th instar larvae of fenitrothion resistant strain (FEN-R-strain) of *Cx. pipiens*

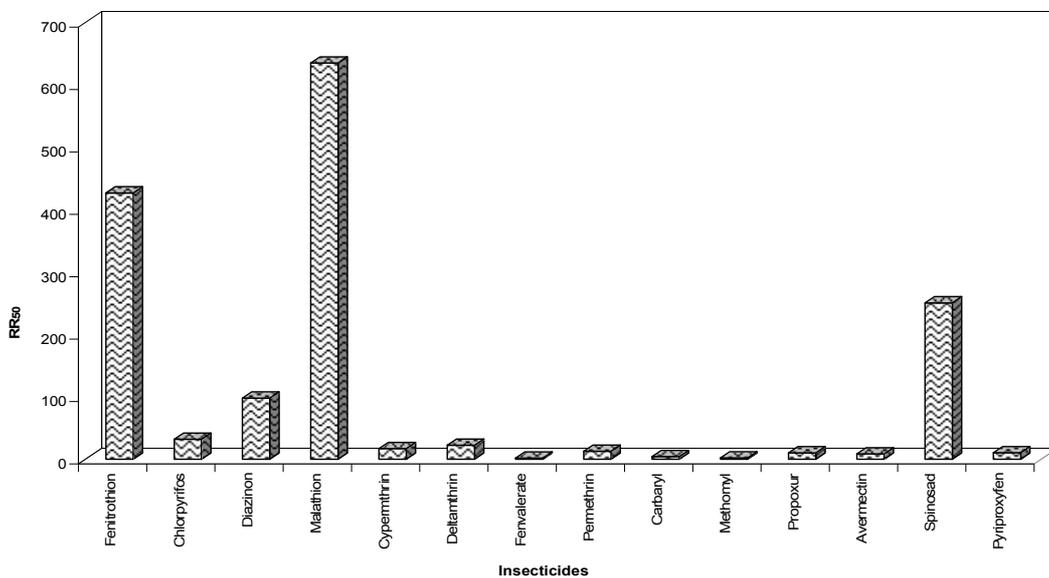


Fig. 2: Resistance ratio (RR_{50}) of certain insecticides in fenitrothion resistant strain (FEN-R-strain) in relation to the susceptible strain (S-strain) of *Cx. pipiens* larvae.

Valles *et al.* (1997), Kerns and Gaylor (1992) and Liu *et al.* (2004) suggested that an insect strain with resistance ratio (RR_{50}) value of <10 is considered tolerant, while that with RR_{50} value of 10-20 is moderately resistant and that with RR_{50} value of >20 is highly resistant. This classification was taken into consideration with the tested insecticides in the present study.

From the above mentioned results, the FEN-R-strain proved to have a very high degree of cross-resistance to all the tested organophosphates but in different levels. Cross-resistance to malathion ($RR_{50} = 634.86$ -fold) was higher than of fenitrothion ($RR_{50} = 426.70$ -fold), whereas the cross-resistance to diazinon ($RR_{50} = 97.86$ -fold) was lower than the RR_{50} of fenitrothion. On the other hand, the resistant strain had an even

lower degree of cross-resistance toward chlorpyrifos (RR_{50} were 31.8-fold) as compared with other tested organophosphates. The high cross-resistance to organophosphates seem to be conform with the hypothesis that cross-resistance was highly observed when the toxicant is chemically similar to the one used in the selection. This is expected as similar insecticidal structure indicates a likely common mechanism(s) of action and detoxification Bracco *et al.* (1999).

Hidayati *et al.* (2011) stated that the level of resistance to fenitrothion in *Ae. Aegypti* may result from continuous exposure to the selection pressure with malathion which is the same insecticide class. The results that the mosquitoes are more resistant to fenitrothion compared to malathion are similar to the study of Bracco *et al.* (1999). Corena *et al.* (2002) reported that the increase in resistance to temephos over six generations was associated with an increase in resistance to some other organophosphorus insecticides (fenitrothion, fenitrothion and malathion).

Table 1 and Figures 1 and 2 revealed that larvae of the FEN-R-strain displayed relatively high cross-resistance to the pyrethroid insecticide deltamethrin ($RR_{50} = 22.32$ -fold) moderate cross-resistance to cypermethrin ($RR_{50} = 16.90$ -fold) and permethrin ($RR_{50} = 12.64$ -fold) and less cross-resistance was to fenvalerate ($RR_{50} = 1.67$ -fold). As expected, cross-resistance to insecticides structurally unrelated to the selecting OP-insecticide (fenitrothion) was far less than that of other OP-insecticides. This finding supports the use of insecticides structurally unrelated to

overcome the phenomenon of cross-resistance in *Cx. pipiens* larvae.

This result is in agree with Corena *et al.* (2002) who found significant increase in resistance to deltamethrin with temephos selection. A surprisingly relative high increase in resistance was detected to deltamethrin (71.05-fold), low level of resistance was detected with cyfluthrin (5.76-fold) and cypermethrin (1.80-fold).

Table 1 and Figures 1 and 2 revealed that larvae of the FEN-R-strain displayed relative high cross-resistance to the carbamate insecticide propoxure ($RR_{50} = 10.18$ -fold), low cross-resistance or tolerance to carbaryl ($RR_{50} = 4.13$ -fold) and very low tolerance to methomyl ($RR_{50} = 1.96$ -fold). Usually, when a resistant strain is selected with an insecticide, resistance extends to other compounds of the same class of insecticides or to compounds with similar modes of action (Liu *et al.*, 2004).

Larvae of the FEN-R-strain displayed relatively high cross-resistance to the bioinsecticide spinosad ($RR_{50} = 250.53$ -fold) and low cross-resistance or tolerance to avermectin ($RR_{50} = 8.52$ -fold). The level of resistance toward spinosad in FEN-R-strain in the present study was not in agreement with those reported by several investigators. In their review on spinosad, as natural product for larval mosquito control, Hertlein *et al.* (2010) reported that spinosad acts on the postsynaptic nicotinic acetylcholine and GABA receptors of insects and has been demonstrated to possess a unique mode of action not shared by any other known insecticidal class of chemistry

(Salgado and Sparks, 2005). According to Valles *et al.*, 1997, it was revealed that lower susceptibility of 7-fold to abamectin in the field strain is considered tolerant rather than resistant. The present results indicate that the mechanism(s) responsible for high level of resistance against organophosphates confers no cross-resistance to abamectin.

Larvae of the FEN-R-strain displayed relative low tolerance to the insect growth inhibitor insecticide pyriproxyfen ($RR_{50} = 10.30$ -fold). Resistance to the IGR insecticide, pyriproxyfen in larval stage of field populations representing certain *Culex*, *Aedes* and *Anopheles* mosquito species were investigated. The obtained data by Selim (2001) revealed that the LC_{50} value for pyriproxyfen to 4th instar larvae of a field strain of *Cx. pipiens* was 0.0091 $\mu\text{g/ml}$ compared with 0.0009 $\mu\text{g/ml}$ for susceptible strain, indicating that the field strain exhibited 10.11-fold resistance to pyriproxyfen.

From the abovementioned results, the FEN-R-strain of *Cx. pipiens* larvae proved to have degree of cross-resistance to all the tested insecticides but in different levels. For example, with respect to the tested OP-insecticides, the FEN-R-strain had high level of cross-resistance toward malathion ($RR_{50} = 634.86$ -fold) whereas RR_{50} value was only 31.98-fold to chlorpyrifos. Among the carbamate insecticides, the FEN-R-strain exhibited high level of cross-resistance against propoxure ($RR_{50} = 10.18$ -fold), whereas RR_{50} value was only 1.96-fold to methomyl. Interestingly, the FEN-R-strain proved to have high level of cross-resistance to

deltamethrin ($RR_{50} = 22.32$ -fold), whereas the RR_{50} values for fenvalerate was less than 1.67-fold. Generally, among the tested anticholinesterase insecticides, cross-resistance levels to OP insecticides were higher than those of carbamate insecticides in the tested FEN-R-strain. However it exhibited high level of cross-resistance toward the tested anticholinesterases compared with the tested pyrethroid insecticides. Moreover the FEN-R-strain exhibited high level of cross-resistance toward the bioinsecticide spinosad ($RR_{50} = 250.53$ -fold), whereas the RR_{50} values for avermectin were less than 8.52-fold.

The variability in cross-resistance values in larvae of the FEN-R-strain of *Cx. pipiens* larvae to different conventional insecticides seem to indicate the absence of a single mechanism controlling resistance among and within the insecticides classes (OPs, pyrethroids, carbamates, bioinsecticides and IGR insecticide). This pattern suggests multiple mechanisms of resistance, i.e. metabolic resistance and insensitive target site. Diverse resistance mechanisms have already been identified in several strains of field collected mosquitoes (Weill *et al.*, 2003 and Liu *et al.*, 2005). Multiple mechanisms acting in concert seem to be common phenomena in insecticide resistance of mosquitoes (Brenques *et al.*, 2003 and Liu *et al.*, 2005). Interactions of these mechanisms obviously results in increasing level of cross-resistance.

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خصائص المقاومة المشتركة للمبيدات علي سلالة مقاومة للفينثروثيون من يرقات بعوض الكيوليكس بيبنز

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الملخص

تعتبر السلالات المقاومة لفعل المبيدات من البعوض مشكلة أساسية في مكافحة أمراض الملاريا والحمى. وفي هذه الدراسة أظهرت سلالة مقاومة لمبيد الفينثروثيون بجامعة أسيوط بمصر مستوى عالي من المقاومة المشتركة تجاه المبيدات الفسفورية المختبرة. وقد أظهرت النتائج أن درجة المقاومة العبورية للمركبات الفوسفورية العضوية المختبرة على السلالة المقاومة للفينثروثيون كانت: كلوروبيروفوس ٣١,٨ ، الديازينون ٩٧,٨٦، الملاثيون ٦٣٤,٨٦. كما أن درجة المقاومة العبورية لمركبات الكارباميت المختبرة على السلالة المقاومة للفينثروثيون كانت: للكارباريل ٤,١٣، الميثوميل ١,٩٦، البروكسر ١٠,١٨. بينما درجة المقاومة العبورية لمركبات البيروثرويد المختبرة على السلالة المقاومة للفينثروثيون كانت: للسيبرمثرين ١٦,٩٠، للدلتا مثرين ٢٢,٣٢، للبيرمثرين ١٢,٦٤، للفينفايرت ١,٦٧. وكانت درجة المقاومة للمركبات الحيوية المختبرة على السلالة المقاومة للفينثروثيون: الأفيريميكتين ٨,٥٢، والاسبينوساد ٢٥٠,٥٣. وقد أظهرت السلالة المقاومة للفينثروثيون درجة مقاومة عبورية ١٠,٣٠ لمركب البيروبروكسفين (IGR) المختبر. ويمكن من النتائج السابقة القول بأن المتحكم في مقاومة سلالة الفينثروثيون للمبيدات التي تشبه الفينثروثيون في التركيب الكيميائي أو طريقة التأثير ميكانيكية واحدة للمقاومة. أما بالنسبة للمبيدات المختلفة في التركيب الكيميائي وطريقة التأثير عن الفينثروثيون فيكون المتحكم في مقاومة السلالة لهذه المبيدات وجود ميكانيكيات أخرى متعددة للمقاومة.