



## Selection of cypermethrin resistance in mosquito *Culex pipiens* larvae in Assiut, Egypt

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### Abstract

To induce cypermethrin resistance in *Culex pipiens* larvae, about 20000 4<sup>th</sup> instar larvae of *Cx. pipiens* were collected from Assiut field and exposed to cypermethrin selection pressure for fifteen generations using dipping method. This study revealed that the resistance ratio (RR<sub>50</sub>) of the parent strain (G<sub>0</sub>) was 14.49-fold as compared with susceptible strain. This RR<sub>50</sub> value increased gradually from each generation to the next one as a result of selection pressure with cypermethrin. The regression line for cypermethrin against the parent field strain showed slope value 2.38 which indicate a moderate level of heterogeneity of parent strain to cypermethrin. The first five generations during the course of cypermethrin selection showed that resistance ratio (RR<sub>50</sub>) was increased gradually from one generation to the next (The RR<sub>50</sub> increased from 14.49-fold in G<sub>0</sub> to 135.40-fold resistance in G<sub>5</sub>). By the 6<sup>th</sup> generation, the rate of resistance development to cypermethrin was fairly rapid where; the RR<sub>50</sub> to cypermethrin increased from 135.40-fold in G<sub>6</sub> to 272.00-fold resistance in G<sub>11</sub>. With the beginning of the 12<sup>th</sup> generation, the development of resistance to cypermethrin was increased very slowly during the last four generations from 279.92-fold in G<sub>12</sub> to 295.52-fold resistance in G<sub>15</sub> (plateau phase). Finally, the slope values were volatile until the eleventh generation (2.38 – 3.63) and then relatively proven in the last four generations (4.55 – 4.52). This is evidence of increasing homogeneity of selected strain in the last five generations.

**Keywords:** insecticides, cypermethrin, resistance, *Culex pipiens*, mosquito, selection.

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## 1. Introduction

The major mosquito vectors span the *Culex*, *Aedes*, and *Anopheles* genera. *Culex sp.* is the major vector of filariasis and Japanese encephalitis, *Aedes* of dengue and dengue hemorrhagic fever, and *Anopheles* of malaria. *Culex quinquefasciatus* is a member of the *Cx. pipiens* complex *Linnaeus* and one of the main subspecies found in Africa (Drummond *et al.*, 1951; Subra, 1981). *Culex quinquefasciatus* (Diptera: Culicidae) is widely distributed in tropical and subtropical areas and is the most important vector of filarial parasite *Wuchereria bancrofti*, although *Anopheles gambiae s.l.* and *An. funestus* also play a role in selected areas (Guillemaud *et al.*, 1998; Maxwell *et al.*, 1990). Insecticides play a pivotal role in controlling major vectors of diseases such as mosquitoes. For the last 70 years, pesticides have been widely used to control insects. The wide use of organic insecticides to control medically and agriculturally important pest species has been a powerful agent of selection in natural populations of many insect species which have developed various degrees of resistance (Georghiou and Lagunes-Tejeda, 1991). The resistance problems continued with the switch to deferent groups of insecticides such as organophosphates, carbamates and pyrethroids. Focal spraying limits the insecticides of choice largely to pyrethroids due to the speed of control required to protect the occupant of the safety margin needed for insecticides used in such close contact with people. The development of pyrethroid resistance in

*An. gambiae* is particularly important given the recent emphasis by the WHO and other organizations on the use of pyrethroid-impregnated bednets for malaria control. Current strategies for malaria vector control rely heavily on chemical means for both the indoor residual spraying (IRS) of insecticide and the use of insecticide-treated nets (ITNs) (World Health Organization (WHO), 2013). Use non-pyrethroid insecticides if Long-lasting Insecticidal Nets (LLIN) and Indoor Residual Spraying (IRS) are to be deployed together in the same geographical location in order to reduce selection pressure for pyrethroid resistance (WHO, 2014). The dynamics of pesticide resistance genes in natural populations is dependent on the classical evolutionary factors: mutation, migration, drift and/or selection. For the mosquito *Cx. pipiens*, it has been shown that mutation is a limiting factor (Raymond and Marquine, 1994). Resistance genes are obviously advantageous in treated areas, so that their frequencies increase with selection. Until recently, the evolution of resistance could only be studied by bioassays, which measure the overall result of resistance genes present in a population. Unfortunately, the extensive use of insecticides has led to the selection of insecticide resistance in malaria vectors. To date, only a limited number of insecticide chemical classes are available for mosquito control. Only four classes of insecticide (Carbamates, organophosphates, organochlorines and pyrethroids) are available for IRS, whereas the use of LLINs depends exclusively on pyrethroids. The two major causes of insecticide resistance

increasing metabolic detoxification and decreasing target site sensitivity (Hemingway and Ranson, 2000). In metabolic detoxification, the insecticide is prevented from reaching its site of action by detoxification enzymes. Decreased target site sensitivity, by contrast, reduces the rate at which the insecticide binds to its target site. The first case of pyrethroid resistance was recorded in West Africa more precisely in Côte d'Ivoire (Elissa *et al.*, 1993). Many other cases have been described in Benin (Chandre *et al.*, 1999), in Burkina Faso (Chandre *et al.*, 1999) and in Mali (Fanello *et al.*, 2003). In West Africa, *A. gambiae* resistance to the four major classes of insecticides available for public health has been reported (Chandre *et al.*, 1999; Elissa *et al.*, 1993; Fanello *et al.*, 2003). However, the success of control methods is threatened by resistance to the main insecticides such as pyrethroids in malaria vectors.

## 2. Materials and Methods

### 2.1 Insecticide

The toxicant used in the present experiment was the insecticide Cypermethrin; Ripcord (10% E.C) (Shell International Chemical CO.).

### 2.2 Insect strains

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

#### 2.2.1 Susceptible strain (S-strain)

The susceptible strain used in the present

study was brought from the Institute of Veterinary and Medical Insects in Cairo, Egypt which reared in the laboratory for 7 years away from any insecticidal pressure.

#### 2.2.2 Parent field strain (F-strain)

The field strain was wild larvae of *Cx. pipiens* which was collected in season of abundance from Faculty of Agriculture Farm, Al-Azhar University, Assiut, Egypt.

### 2.3 Collecting and rearing techniques

Two strains transferred to the laboratory of Plant Protection Department, Faculty of Agriculture, Assiut University, Assiut, Egypt and reared under laboratory conditions according to the method recommended by WHO (1981).

### 2.4 Toxicity studies

#### 2.4.1 Laboratory induction of Cypermethrin resistant strain (CYP-R-strain) of *Cx. pipiens* larvae

A part of the field parent strain was selected with technical cypermethrin according to the method recommended by WHO (1981). The fourth instar larvae of the parent strain (G<sub>0</sub>) and successive generations was used in this trial. The LC<sub>50</sub> values for the same generation were used as the selective pressure of Cypermethrin for each successive generation. The selection pressure was

applied using the dipping method. About 3000 to 4000 4<sup>th</sup> instar larvae were selected for each generation and larvae survived after treatment were reared under standardized laboratory conditions. Emerged adults constituted the parents of succeeding generation. Selection was carried out for every generation throughout successive fifteen generations. LC<sub>50</sub> value of Cypermethrin in each generation was determined as mentioned before. The resistance ratio for the Cypermethrin in each generation at LC<sub>50</sub> (RR<sub>50</sub>) was calculated as:

$RR_{50} = LC_{50} \text{ of the tested generation} / LC_{50} \text{ of S-strain}$   
(Robertson *et al.*, 2007).

### 3. Results and Discussion

To induce cypermethrin resistance in *Cx. pipiens* larvae, about 20000 4<sup>th</sup> instar larvae of *Cx. pipiens* were collected from the field and exposed to cypermethrin selection pressure for fifteen generations using dipping method. LC<sub>50</sub> values (as µg/L), confidence limits of LC<sub>50</sub> and slope values were obtained for each generation. Each generation was selected by cypermethrin at a concentration equivalent to the LC<sub>50</sub> value of this generation. Resistance ratio (RR<sub>50</sub>) was calculated as mentioned above. All obtained results are recorded in Table (1) and Figure (1). Results in Table (1) revealed that the resistance ratio (RR<sub>50</sub>) of the parent strain (G<sub>0</sub>) was 14.4-fold. This RR<sub>50</sub> value increased gradually from each generation to the next one as a result

of selection pressure with cypermethrin. The RR<sub>50</sub> values were 14.49, 28.65, 47.83, 56.16, 73.29, 135.40, 192.96, 214.64, 228.87, 234.90, 243.68, 272.00, 279.92, 283.25, 291.57 and 295.52 for G<sub>0</sub>, G<sub>1</sub>, G<sub>2</sub>, G<sub>3</sub>, G<sub>4</sub>, G<sub>5</sub>, G<sub>6</sub>, G<sub>7</sub>, G<sub>8</sub>, G<sub>9</sub>, G<sub>10</sub>, G<sub>11</sub>, G<sub>12</sub>, G<sub>13</sub>, G<sub>14</sub> and G<sub>15</sub>, respectively. Results in Table 1 showed that the slope values for the corresponding generations were 2.38, 3.17, 3.29, 3.07, 4.44, 3.33, 3.03, 3.23, 3.29, 3.63, 3.63, 3.63, 4.55, 4.67, 4.76 and 4.52, respectively. From the above-mentioned results, it could be concluded that: First, the field parent strain in the present study exhibited low level of resistance to cypermethrin (14.49). This may be due to a large portion of the field population avoid exposure to insecticides, providing susceptible individuals for repopulation of cypermethrin resistant *Cx. pipiens* mosquitoes to become 295.52-fold resistance after selection with cypermethrin for 15 generations. Also, the regression line for cypermethrin against the parent field strain showed slope value 2.38 which indicates a moderate level of heterogeneity of the parent strain to cypermethrin. Second, the results in Table 1 and Figure 1 revealed that in the first five generations during the course of cypermethrin selection, the resistance level (RR<sub>50</sub>) was increased gradually from one generation to the next (The RR<sub>50</sub> increased from 14.49-fold in G<sub>0</sub> to 135.40-fold resistance in G<sub>5</sub>). By the 6<sup>th</sup> generation, the rate of resistance development to cypermethrin

was fairly rapid where the RR<sub>50</sub> to cypermethrin increased from 135.40-fold in G<sub>6</sub> to 272.00-fold resistance in G<sub>11</sub>. With the beginning of the 12<sup>th</sup> generation, the development of resistance to cypermethrin was increased very slowly during the last four generations from 279.92-fold in G<sub>12</sub> to 295.52-fold

resistance in G<sub>15</sub> (plateau phase). Finally, the slope values were volatile until the eleventh generations (2.38 – 3.63) and then relatively proven in the last four generations (4.55 – 4.52). This is evidence of increasing homogeneity of the selected strain in the last five generations.

Table (1): Toxicity of Cypermethrin to the 4<sup>th</sup> instar larvae of *Culex pipiens* in relatively successive selected generations for detecting resistance level.

Generation	LC <sub>50</sub> ug/l	95% confidence limits		Slope ± SE <sup>a</sup>	R R <sub>50</sub> <sup>b</sup>
		Lower	Upper		
P <sup>c</sup>	1.492	1.006	1.885	2.38±0.47	14.49
1	2.951	2.305	3.511	3.17±0.82	28.65
2	4.927	4.192	5.864	3.29±0.84	47.83
3	5.784	5.020	6.686	3.07±0.60	56.16
4	7.549	6.872	8.464	4.44±0.76	73.29
5	13.946	11.828	16.303	3.33±0.58	135.40
6	19.875	16.613	23.602	3.03±0.55	192.96
7	22.108	17.778	26.295	3.23±0.76	214.64
8	23.574	19.418	27.454	3.29±0.60	228.87
9	24.195	20.445	27.827	3.63±0.62	234.90
10	25.099	18.932	29.400	3.63±0.85	243.68
11	28.016	24.135	32.385	3.63±0.61	272.00
12	28.832	25.539	32.514	4.55±0.67	279.92
13	29.175	25.921	32.828	4.67±0.68	283.25
14	30.032	25.964	33.614	4.76±0.75	291.57
15	30.439	26.165	34.520	4.52±0.88	295.52

<sup>a</sup> = SE: standard error. <sup>b</sup> = RR<sub>50</sub>: resistance ratio = LC<sub>50</sub> of the selected generation / LC<sub>50</sub> of the Susceptible strain. <sup>c</sup> = P: Parent field strain.

From the results in Table (1), cypermethrin resistant strain of *Cx. pipiens* from the field parent strain was obtained. The difference in RR<sub>50</sub> values among the tested insecticides against *Cx. pipiens* larvae in the present study may be due to several factors. According to Kuhr and Dorrough (1976) it is suggested that the speed with which resistance

develops in an insect population depends on many factors but of major importance is the intensity of selection, the frequency of resistant genotypes in the population, the number of genes involved in conferring resistance, whether the gene expression is dominant or recessive, history of exposure to other toxicant and counteracting effect of natural selection.

Georghiou and Taylor (1986) reported that resistance does not evolve at the same rate for all organisms that come under selection pressure.

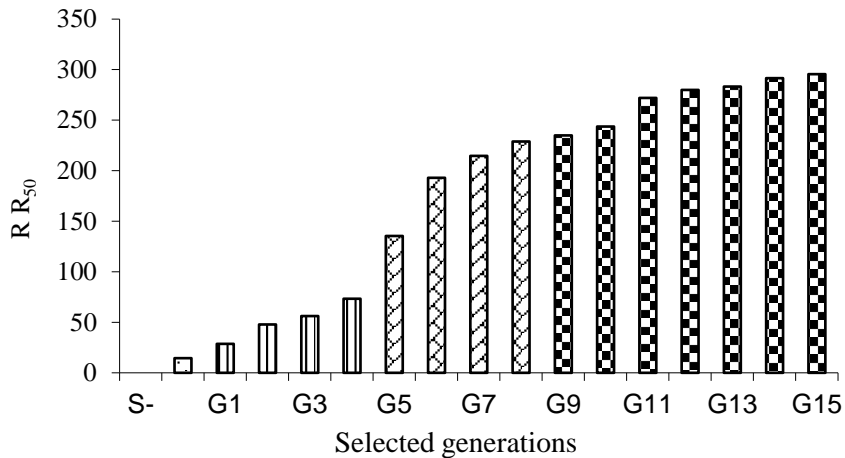


Figure (1): Gradual increase of cypermethrin resistance in *Cx. pipiens* larvae treated with cypermethrin at the LC<sub>50</sub> values for 15 successive generations. The R R<sub>50</sub> was calculated with reference to susceptible strain.

Even within one species, resistance may develop more rapidly in one population than in another. There are many factors that can influence the rate of resistance to insecticide in the field population. These factors are grouped into three categories depending on whether they concern the genetics of resistance, the biology/ecology of the pest, or the operational control used. Genetic factors can be divided to sub factors: frequency of R alleles, number of R alleles, dominance of R alleles, penetrance, expressivity and interaction of R alleles, past selection by other chemicals, and extent of integration of R genome with fitness factors. Biological/ecological factors contain also many sub factors such as generation turnover, offspring per

generation, monogamy, polygamy and parthenogenesis, isolation, mobility, migration, monophagy or polyphagy and fortuitous, survival and refugia. The last operational factors contain sub factor, such as chemical nature of pesticide, relationship to earlier used chemicals, persistence of residue and formation, application threshold, selection threshold, life stage(s) selected, mode of application, space-limited selection and alternating selection. Most factors in the first two categories cannot be controlled and the importance of some not even is determined until resistance has already developed. Nonetheless, some factors that influence the evolution of resistance are under man's control, especially those related to the timing and dose of

insecticide application. The problem is to identify them and determine how their manipulation under existing genetic and biological/ecological constraints may retard the evolution of resistance. It is abundantly clear that merely switching to new insecticides, when the current one is no longer effective cannot continue because of insecticide cross-resistance (Georghiou and Taylor, 1986; Kuhr and Dorough, 1976). Integrated pest management, which will always involve the use of pesticides, is now regarded essential. Recognizing and manipulating factors that influence insecticide resistance and retard resistance development should be an integral part in any resistance management strategy. The practice of using insecticide rotations as a strategy to avoid the development of resistance requires the deployment of insecticide classes over time to reduce selection pressure for resistance to any single class. The conditions required for rotations to delay selection for resistance are that resistance alleles are deleterious in the absence of insecticide selection and that residues of the selecting insecticide have decayed to a point at which they no longer select (Denholm and Rowland, 1992). In the present study,  $RR_{50}$  value became 295.52 in  $G_{15}$  as compared with 14.49 in  $G_0$  indicated that cypermethrin resistance have developed rapidly as affected with selection pressure. From these results, one or more of the following mechanisms may be involved in cypermethrin resistance: knock down

resistance target site mutations (kdr), metabolic resistance mechanisms and/or cuticle alteration. These results were in agreement with the results of some other investigators (Brook *et al.*, 2001; Martinez *et al.*, 1998; Ranson *et al.*, 2000). In Assiut region, Egypt, agricultural area is close to the field where mosquito collected for the experiment. May be using insecticides in agricultural practices play a role in selecting for resistance mechanisms in mosquitos (Antonio *et al.*, 2008; Antonio *et al.*, 2011; Diabate *et al.*, 2002; Yadouleton *et al.*, 2009). In Tanzania, A gambiae population was highly resistant to pyrethroides in the intensive use of agricultural chemicals including insecticides (Matowo *et al.*, 2010).

#### 4. Conclusions

Cypermethrin resistance level in this study from  $G_0$  to  $G_{15}$  representing 295.52-fold resistance suggested that more than one resistance mechanism may be involved in *Cx. pipiens* mosquitos. According to the present results, its highly recommend good management of using agricultural chemicals to avoid selecting kdr mutations and/or metabolic resistance mechanisms. This will help reducing mosquito control failures and improving resistance management strategies.

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