Evaluation of resistance against deltamethrin in *Aedes* mosquitoes from Lahore, Pakistan.

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**ABSTRACT**

In the current study, resistance status of *Aedes aegypti* was evaluated against pyrethroid insecticide. The resistance in field collected population against Deltamethrin 2.5% EC was compared with susceptible (laboratory reared) population. Field population was collected from two localities of Lahore i.e. Government College University and Government Islamia College for Women Cooper Road, Lahore during July-September, 2010. CDC Bottle Bioassays were carried out on adult female mosquitoes (susceptible) in order to determine the diagnostic dose. A range of concentrations (10, 5, 2.5 and 1.25 µg/ml) of deltamethrin was used for 60 minutes exposure. Diagnostic dose 1.25 µg/ml was found in post 30 minutes exposure. The same concentration caused 100% mortality of field collected populations from GCU and Govt. Islamia College for Women in 30 and 40 minutes exposure respectively. Resistance level was expressed as resistance ratio (RR) of lethal time for 50% death determined in field collected and susceptible strain. Results of bioassays indicated that *Ae. aegypti* field collected population from Government Islamia College for Women Cooper Road, Lahore was resistant to Deltamethrin at RR$_{LT50}$=1.3 and RR$_{LT95}$=1.37 as compared to laboratory reared susceptible population. However, field population of *Ae. aegypti* from Government College University, Lahore was found susceptible at RR$_{LT50}$=1.03 and RR$_{LT95}$=1.0.

**Key words:** *Aedes aegypti*, resistance, Deltamethrin, CDC Bioassay.

**INTRODUCTION**

Many pathogenic diseases spread through different species of mosquitoes due to their hematophagus nature. Among these diseases Dengue fever (DF) and its complications Dengue hemorrhagic fever (DHF) and Dengue shock syndrome (DSS) have posed serious threats since last few decades. The vector of Dengue belongs to genus “*Aedes*” (Subfamily: Culicinae). Two important species *Aedes aegypti* and *Aedes albopictus*, are involved in the spread of DF/ DHF in North and South America, Africa, Europe (Gratz, 1967) and South East Asian Region (SEAR) (Tewari *et al.*, 2004) including Pakistan.

*Aedes aegypti* is a black colored mosquito and has silvery white scales in the form of lyre shaped pattern on the thorax region. These mosquitoes primarily breed in artificial containers like earthen jars, plastic and metal drums, used tyres, potted plants and main-hole covers etc (Chareonviriyaphap *et al.*, 2003). Dengue is caused by “Flaviviruses” having four distinct serotypes “DEN-1, DEN-2, DEN-3 and DEN-4” (Westaway & Blok, 1997). WHO (2008) has reported dengue as re-emerging disease in SEAR and considered as it more fatal vector borne disease spreading rapidly all over the world.

There is no vaccine or proper treatment for dengue, therefore, vector control is the only option to control the disease. Chemical insecticides are
primarily being used to control mosquito vectors. There are four classes of insecticides used for the control of mosquitoes; (1) organochlorine, (2) organophosphate, (3) carbamates and (4) pyrethroids. Pyrethroids have been successful in eradication of mosquitoes in last few decades (Nauen, 2007). Globally vector control measures are facing challenges due to the development of resistance in vector population against wide variety of insecticides (Chandre et al., 1999). Resistance in insects against commonly used insecticides was reported by many authors (Brengues et al., 2003; Liu et al., 2006; Nauen, 2007). It has become necessary to develop effective resistance management strategies due to increase in vector resistance and highly evolved resistance mechanisms (McCaffery & Nauen, 2006). Surveillance to the susceptibility status of vector is a necessary step to evaluate resistance in nature against particular insecticide (Brogdon & McAllister, 1998a).

The knowledge of resistance status of Dengue vector may provide an effective data for the development of integrated vector control strategies. The main objectives of the present study were; to evaluate the resistance status of Aedes mosquitoes against Deltamethrin commonly used insecticide in Lahore, Pakistan and to find the level of resistance in two selected localities which will help in proper management of vector population control particular in areas of Lahore.

MATERIALS AND METHODS

Mosquitoes Rearing and Maintenance

Aedes aegypti were reared according to standardized conditions in the laboratory and maintained in GCU insectary since last 05 years. In the present study this colony was used as susceptible strain for the evaluation of resistance.

Field Collection

The status of resistance was evaluated in the field collected Aedes aegypti against Deltamethrin (2.5% EC) in two different localities (Govt. College University (GCU) and Govt. Islamia College for Women (GICW), Cooper Road) of Lahore from July –September, 2010. Mosquito larvae were collected weekly from July 2010 to 2nd week of September 2010. Larvae were identified on the basis of short and thick siphon along with the black hooks at the thorax region. Moreover, Aedes aegypti had biforked comb rows on the last abdominal segment as compared to needle like comb rows of Aedes albopictus (Rueda, 2004).

Insecticide used for CDC Bottle Bioassays

Deltamethrin 2.5% EC (emulsifiable concentrate) was kindly provided by Health Directorate Cooper Road Lahore and was used to evaluate the susceptible and resistance status in laboratory reared and field collected adults Aedes aegypti females. Deltamethrin contained 2.5 gm active ingredient by weight in 100 ml of water by volume (25000 µg/ ml). Evaluation of resistance was carried out using CDC bottle bioassays (Brogdon & McAllister, 1999b). The purpose of CDC bioassay was to measure the rate of mortality of a population at a given dose of insecticide (usually the diagnostic dose). Preliminary tests were performed to determine the diagnostic dose (Benedict, 2007).
Experimental protocol

Preliminary test for diagnostic dose

A diagnostic dose is an amount of insecticide needed to kill 100% susceptible population within specific time (30 min-1 hr). A range of insecticide concentrations (10, 5, 2.5, 1.25 μg/ml) was used to find the diagnostic dose for the susceptible population (laboratory reared *Ae. aegypti*). Each concentration was dissolved in 1 ml technical grade acetone with 3 replicates in 250 ml reagent bottles. Each concentration was properly labeled on the bottle and lid. Three untreated bottles containing 1 ml of acetone only acted as control.

Performance of Bottle Bioassays

Bottles were prepared the day before used for testing. Each concentration (1ml) was transferred with the help of sterile disposable syringe to the relevant labeled bottle. Even coating was done on all surfaces with circular movement and upright / linear position. When the liquid disappeared lids were taken off and bottles were left on an undisturbed clean surface over night in dark. Bottles were lined up without their lids. A group of 20 non-blood fed female mosquitoes (laboratory reared) were first introduced into the control bottle and then into the experimental ones. After the determination of diagnostic dose, the experiment was performed in the same manner with wild collected mosquitoes.

Data analysis

Data collected from bottle bioassay was analyzed by using SPSS computer software for Probit-regression analysis. In each group LT$_{50}$ (lethal time for 50% death) and LT$_{95}$ (lethal time 95% death) was calculated. Resistance ratio (RR) was calculated by dividing lethal time for wild strain (field population) with the lethal time for laboratory reared susceptible strain.

RESULTS AND DISCUSSION

The results indicated that 100% mortality occurred with deltamethrin at concentration of 1.25 μg/ml (diagnostic dose) in the laboratory reared susceptible *Ae. aegypti* adult females post 30 minutes exposure (Fig. 1).

The *Aedes* population collected from Government Islamia College for Women Cooper Road, Lahore showed low levels of resistance with respect to percent mortalities in the specific time post exposure, since 100% mortality required 40 minutes as compared to 30 minutes in laboratory reared susceptible population. In contrast population collected from GCU was found susceptible since 100% mortality occurred in 30 minutes post exposure comparable with laboratory reared susceptible population (Table.1; Fig. 2). However, Lethal times for 50% and 95% deaths i.e. LT$_{50}$-LT$_{95}$ were 13.137 and 25.114 minutes respectively with deltamethrin 2.5 % EC in laboratory reared (susceptible) adult females. For populations from GCU and Government Islamia College For Women Cooper Road Lahore, the LT$_{50}$-LT$_{95}$ were 13.599 and 25.219, 17.329 and 34.56 respectively (Table. 2).

Synthetic Insecticides are used worldwide to control the vector population. Regular use of insecticides has led to the development of high rate of resistance
in insect vectors (Nauen, 2007). Thus the development of resistance has become a critical issue in all groups of insect vectors (Hemingway & Ranson, 2000). Resistance / susceptible status play an important role in any vector control program and the knowledge of this status helps to control any vector population in a particular locality.

Chemical insecticides such as DDT (dichlorodiphenyltrichloroethylene) and Malathion were used to control mosquitoes in the last three decades in Pakistan (Country Report, 2003). These were replaced by Deltamethrin, Fenthion, Temephos etc. due to resistance reported against DDT and Malathion in Anopheline and Culex species in Punjab, Pakistan (Rathore et al., 1980); Malcolm & Boddington, 1989). Resistance against 5% Deltamethrin was also reported in Culex quinquefasciatus from Lahore, Pakistan (Tahir et al., 2009).

In our laboratory, diagnostic dose against deltamethrin 1.5 % EC was found 2.5 µg / ml (unpublished observation) more than current study (1.25 µg/ml) against deltamethrin 2.5 % EC post 30 minutes exposure. It appeared that more active ingredients in a formulation required lesser amount to kill 100 % populations of Aedes aegypti as compared to the formulation with fewer active ingredients.

The strategy for the control of vector population with the restricted group of insecticides is very crucial and facing challenge now a days. Rotational use of different groups of insecticide rather than the use of different members of same group of insecticides is more effective to reduce and deal with the resistance problem. Carbamates and organophosphates must be used in rotation in order to maintain the pyrethroids susceptibility (Nauen, 2007). An effective long term resistance management strategy is needed in order to sustain insecticides susceptibility in nature and this is a target to be achieved in Pakistan to control vector borne diseases.

Table 1: Percent mortalities in susceptible (laboratory reared) and field collected populations against diagnostic dose (1.25 µg/ml) of Deltamethrin 2.5% EC

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>*Susceptible population (laboratory reared)</th>
<th>**Field population GCU</th>
<th>**Field population (coop road)</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>45 % mortality</td>
<td>40 % mortality</td>
<td>35 % mortality</td>
<td>0 %</td>
</tr>
<tr>
<td>20</td>
<td>75 % mortality</td>
<td>75 % mortality</td>
<td>60 % mortality</td>
<td>0 %</td>
</tr>
<tr>
<td>30</td>
<td>100 % mortality</td>
<td>100 % mortality</td>
<td>80 % mortality</td>
<td>0 %</td>
</tr>
<tr>
<td>40</td>
<td>100 % mortality</td>
<td>100 % mortality</td>
<td>100 % mortality</td>
<td>0 %</td>
</tr>
<tr>
<td>50</td>
<td>100 % mortality</td>
<td>100 % mortality</td>
<td>100 % mortality</td>
<td>0 %</td>
</tr>
<tr>
<td>60</td>
<td>100 % mortality</td>
<td>100 % mortality</td>
<td>100 % mortality</td>
<td>0 %</td>
</tr>
</tbody>
</table>
*Hundred percent mortality in susceptible population in thirty minutes, ** hundred % mortality in field collected populations (GCU & Cooper road) in thirty and forty minutes respectively

Table 2: Probit regression analysis for LT<sub>50</sub> and LT<sub>95</sub> of susceptible (laboratory reared) and field collected populations

<table>
<thead>
<tr>
<th>Lethal time for 50% death</th>
<th>Susceptible population (laboratory reared)</th>
<th>Field collected population (GCU, Lahore)</th>
<th>Field population (Cooper Road, Lahore)</th>
<th>Resistance ratio of field collected population (GCU)</th>
<th>Resistance ratio of field collected population (Cooper Road, Lahore)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LT&lt;sub&gt;50&lt;/sub&gt;</td>
<td>13.137</td>
<td>13.599</td>
<td>17.329</td>
<td>1.03</td>
<td>1.31</td>
</tr>
<tr>
<td>LT&lt;sub&gt;95&lt;/sub&gt;</td>
<td>25.114</td>
<td>25.219</td>
<td>34.566</td>
<td>1.004</td>
<td>1.37</td>
</tr>
</tbody>
</table>

**Fig., 1:** Evaluation of diagnostic dose against different concentrations of Deltamethrin 2.5% EC as percent mortalities in susceptible (laboratory reared) Ae. aegypti female

*Diagnostic dose (1.25 µg/ml) has given 100% mortality post 30 minutes exposure
Fig. 2: A comparison of susceptible (laboratory reared) and field collected populations (GCU and Government Islamia Cooper Road College, Lahore) as percent mortalities against diagnostic dose (1.25 µg/ml) of Deltamethrin 2.5% EC

REFERENCES


