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EVALUATION OF RESISTANCE AGAINST DELTAMETHRIN AND CYPERMETHRIN IN DENGUE VECTOR FROM LAHORE, PAKISTAN

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ABSTRACT

The resistance/susceptible status of *Aedes aegypti* (field strain) from slum area of Misri Shah Lahore, Pakistan was determined during May 2009 to January 2011. Two insecticides i.e. deltamethrin 1.5% EC and cypermethrin 10% EC which were sprayed in various localities of Lahore from the last ten years were used to evaluate resistance/susceptible status of field collected adult females of *Aedes aegypti* by CDC bottle bioassays. The resistance level was expressed as resistance ratio (RR) of lethal time at 50% death determined using field collected and susceptible strains. Current study indicated RR LT_{50} 1.95 and 1.47 against deltamethrin and cypermethrin in adult females of *Aedes aegypti* respectively. In addition, the tested population was less resistant against cypermethrin as compared to deltamethrin. In comparison of percent mortalities, there was no significant difference between the two insecticides against adult females.

Key words: *Aedes aegypti*, resistance, deltamethrin, cypermethrin, Pakistan.

INTRODUCTION

Aedes aegypti is the primary vector of dengue (Gubler, 1998) while another species *Aedes albopictus* (Asian tiger mosquito) acts as a secondary vector, both are involved in the spread of dengue fever in Asia (CDC, 2001).

Dengue fever affects 50 million people annually, and approximately two fifth population of the world expected to be at risk of dengue (WHO, 1997; 2008). More than 100 countries including South East Asia, South and Central America, Mexico and Africa have dengue as an epidemic. It occurs predominantly in many areas of Southeast Asia. According to World Health Organization, (WHO, 2008) about 2.5% deaths occur per year mostly among children due to dengue epidemics all over the world.

Suspected vectors of dengue in Lahore, Pakistan are *Ae. aegypti* and *Ae. albopictus* (Jahan *et al*., 2011). In Pakistan, Dengue Hemorrhagic Fever (DHF) was first reported from Karachi in 1994 (Chan *et al.*, 1994) and in 2006, 5,522 cases were reported from all over the country by Health Directorate of Pakistan. Recently, a severe dengue epidemic occurred in 2011 with > 20000 positive cases and > 200 deaths in urban city Lahore, province Punjab. The dengue situation in Pakistan is alarming. To control the disease, vector control is the only option for having no vaccine and proper antiviral drug.

Mosquitoes are being primarily controlled by a large number of chemical insecticides. Chemical control has become the most important method in mosquito control since the introduction of organic insecticides in 1940s (Yap *et al*., 2003). The chemical insecticides are classified as i) organochlorines, ii) organophospates, iii) carbamates and iv) pyrethroids (WHO, 1997). The annual use of insecticides against dengue vectors from 2000-2009 was 394 tones of organophosphates and 154 tones of pyrethroids (WHO, 2011).

Insects including mosquitoes become resistant due to extensive use of various chemical pesticides worldwide (Chandre *et al.*, 1999). A wide-range of organophosphate (malathion) resistance has been reported in the malaria vectors such as *An. culicifacies, An. stephensi*, *An. albimanus*, *An. arabiensis and An. sacharovi.* Resistance levels to different pyrethroids have also been documented in *An. gambiae* of Kenya (Vulule *et al*., 1994) and East and West Africa (Chandre *et al*., 1999; Vulule *et al*., 1999), *An. funestus* of South Africa (Hargreaves *et al*., 2000; Nikou *et al*., 2003), *An. arabiensis* in Tanzania (Kulkarni *et al*., 2006) and Mozambique (Casimiro *et al*., 2006), *An. stephensi* of India and Dubai strains (Hodjati and Curtis, 1997) and *An. albimanus* of Guatemala (Brogdon and Barber, 1990).

In Pakistan, dichlorodiphenyltrichloroethane (DDT) was the most important insecticide used for malaria control in early 1969s. In 1979, resistance to DDT was detected in *An. annularis, An. culicifacies*, *An. subpictus* and *An. stephensi* resulting in an increase in malaria transmission rates. Therefore, benzene hexachloride (BHC) was introduced in 1972. Then in 1976, organophosphates (malathion and sumithion) were also introduced. DDT was banned due to resistance development in malaria vector in 1979, but BHC use was continued to 1983. From 1984-1996, the main insecticide used was malathion for the control of malaria vector. Later on, resistance was reported to malathion in *An. stephensi* (Rathor *et al*., 1986) and pyrethroids (deltamethrin) were used for indoor residual spraying from late 1996-2003 (Country Report, 2003).

Previously no report was available on the insecticide susceptibility/resistance status of *Ae. aegypti* from Lahore, Pakistan. Moreover, the dengue cases have been reported annually in urban areas of Pakistan from the last few years. To control any vector, it is important to study its susceptibility status against specific insecticide. Therefore, it is necessary to develop different strategies to overcome insecticide resistance against dengue vectors from different localities of Lahore, Pakistan. The main goal of the current study was to evaluate the resistance in *Ae. aegypti* adults against most commonly used chemical insecticides (deltamethrin and cypermethrin) in selected municipality of Lahore, Pakistan.

MATERIALS AND METHODS

Mosquitoes: Immature *Aedes* larvae were collected from artificial containers such as discarded jars, tyres, plastic tubs found in Misri Shah (slum area) (31° 35' 14" N, 74° 19' 50" E) in North of Lahore. All the collections were conducted between 17:00-18:30 pm from May 2009-January 2010. The susceptible strain of *Ae. aegypti* was maintained in GCU insectory since 2006 and used as a reference strain.

Identification of species: *Ae. aegypti* larvae were identified on the basis of morphological characteristics using identification key (Huang, 1977) and reared in the laboratory at standard conditions.

Insecticides used for CDC (Centers for Disease Control and Prevention) bottle bioassays: The two chemical insecticides (pyrethroids) used were deltamethrin 1.5% EC (emulsifiable concentrate) and cypermethrin 10% EC marketed by Kanzo Urban Pest, Lahore (Pakistan).

Experimental Protocol: Insecticides susceptibility of field collected *Ae. aegypti* mosquitoes was determined by (CDC) bottle bioassays. The bottles were prepared as described by Brogdon and McAllister (1998). Tests were performed with 5 different concentrations of deltamethrin 1.5% EC (20, 5, 2.5, 1.25, 0.625 µg/ml) and cypermethrin 10% EC (10, 5, 2.5, 1.25, 0.625 µg/ml) in technical grade acetone Merck®, respectively. Each concentration was replicated three times and three untreated bottles were used as control that consisted of only acetone. The name and the concentration (µg/bottle) of insecticides on the bottle and lid were recorded. One ml of different concentrations of each insecticide was poured in the treated and the same volume of acetone in the untreated/control bottles. Each bottle was caped immediately to avoid premature evaporation. After having dose in each bottle, the inside was coated thoroughly by rotating the bottle in all directions including circular and linear movements. Lid was removed from each bottle and finally rolled on the table until the solvent has evaporated. The bottles were left open without their lids overnight for complete dryness.

For each insecticide, 20 non-bloods fed female mosquitoes, four-five days old were used in each bottle for determining the diagnostic dose in the susceptible adult females. After exposure, mosquitoes were maintained at 25±3°C and 70-80% relative humidity. The number of cumulative mortality was recorded after every 10 minutes. The resistance status was determined by the diagnostic dose of both insecticides used for the field collected adults *Aedes* mosquitoes. The diagnostic dose was used for susceptible and field collected populations of *Ae. aegypti* to evaluate the resistance status.

Data analysis: The results were analyzed using Probit-analysis Program of Raymond (1985) to determine LT_{50} and LT_{90}. Resistance ratio (RR) was calculated by dividing the lethal time of the field strain by the lethal time of the susceptible strain. Percentage of mortalities of the field collected population against two insecticides was compared for significance by one-way analysis of variance ANOVA, Tukey's mean of separation procedure and Student's t-distribution test at 95% confidence interval of the difference (SSPS version 16.0; SSPS Inc., Chicago, IL).

RESULTS AND DISCUSSION

Insecticide resistance has been developed due to the continuous use of chemical insecticides. The understanding of resistance in mosquitoes towards different insecticides plays an important role in mosquito control program. In the present study, the diagnostic dose for deltamethrin (1.5% EC) in the adult females (susceptible population) was 2.5 µg/ml post 30 minutes exposure (Fig. 1). Field collected mosquitoes were found resistant using diagnostic dose (2.5µg/ml) since 100% mortality occurred at 50 minutes exposure as compared to 30 minutes for the same mortality in susceptible (laboratory reared) strain against deltamethrin (Fig. 3). Moreover, the percentage mortalities of field collected mosquitoes were found 30% more resistant as compared to susceptible strain against deltamethrin at 30 minutes exposure (Table I).

The diagnostic dose against cypermethrin was 5 µg/ml required for hundred percent mortality of *Ae. aegypti* adult females at 30 minutes exposure (Fig. 2). Field collected females indicated 100% mortality against diagnostic dose (5 µg/ml) of cypermethrin at 40 minutes exposure as compared to 30 minutes for the same mortality in susceptible population (Fig. 4). Regarding percent mortalities, 60% mortality occurred in field collected females at 30 minutes post exposure as compared to 100% mortality in susceptible population for the same exposure time. Field collected population was found 40% more resistant as compared to susceptible female mosquitoes (Table I). In general, a comparison between the two insecticides indicated that cypermethrin was less effective with 5 µg/ml diagnostic dose and 60% mortality as compared to deltamethrin with 2.5 µg/ml diagnostic dose and 70% mortality at 30 minutes post exposure. Moreover, there was no significant difference ($p>0.05$) with respect to resistance status between two insecticides in field collected population from Misri Shah, Lahore ($P=0.79$) (Table I). Evaluation of resistance status also indicated that resistance ratio at LT_{50} was 1.95 and 1.47 against deltamethrin and cypermethrin respectively (Table II).

These results indicated that though both insecticides used in current study belong to the same pyrethroid group, field collected population was found more resistant against deltamethrin as compared to cypermethrin. In addition, one possible reason of this difference could be due to the difference in active ingredients of these insecticides.

The diagnostic dose 10 µg/ml for *Ae. aegypti* and *An. darlingi* was reported by Zamora Perea *et al*. (2009) against deltamethrin 2.5% WP. The difference in diagnostic dose in our findings could be due to the difference in active ingredients and different regional conditions. Moreover, lower active ingredient i.e. 1.5% EC of deltamethrin indicated lower diagnostic dose (2.5 µg/ml) as compared to above mentioned study. In general, many authors documented that technical grade of any insecticide is less active for causing mortality in the field conditions as compared to the same formulation with low active ingredients against *Aedes*/*Anopheles* mosquitoes. Therefore, with the same insecticide depending upon the active ingredients, diagnostic dose could be different.

In present study, the diagnostic dose 5 µg/ml against cypermethrin 10% EC, was observed for susceptible population of *Ae. aegypti* while da-Cuncha *et al*. (2005) reported 8 µg/ml post 30 minutes exposure with the different formulation of same insecticide cypermethrin 250 EC using Rockefeller (susceptible) strain of *Ae. aegypti.* There is no previous report documented on the insecticide resistance in *Ae. aegypti* by any method (CDC bottle bioassays/WHO paper testing) from Lahore, Pakistan. Previously, a limited work has been reported on resistance in *Anopheles* and *Culex* mosquitoes in Pakistan. Resistance against DDT, dieldrin, malathion, fenitrothion, and propoxur in *An. culicifacies and An. stephensi* mosquitoes has been confirmed from 10 districts of province Punjab. The field population of *An. culicifacies* was resistant (mortality less than 80%) in all 50 tests conducted with DDT and dieldrin, whereas, resistance against the same insecticides was also observed in 7 districts of the province Punjab against *An. stephensi* (Rathor *et al*., 1980, 1983). The same authors also reported resistance against malathion in both malaria vectors of Pakistan from Lahore (Rathor *et al*., 1986). Tahir *et al*. (2009) also documented resistance in males and females *Cx. quinquefasciatus* to deltamethrin from five different localities (Jaman, Mohlanwal, Noopur Bhatta A1, Yohanaabad-2 and GCU Lahore) of Lahore, province Punjab. The authors further reported that all populations were susceptible to 5% deltamethrin with 100% mortality post 24 hrs exposure.

A high level of resistance to DDT was also observed by Fonseca-Gonzalez *et al*. (2006) in *Ae. aegypti* population by using WHO and CDC bioassays. The same authors found 5-35% mortality of *Ae. aegypti* from four different populations of Colombia. Moreover, it was also observed that DDT resistant populations were not found resistant against pyrethroids. In our results, the mortality rate of field collected *Ae. aegypti* against deltamethrin (1.5% EC) and cypermethrin was 70% and 60% post 30 minutes exposure whereas, Fonseca-Gonzalez *et al*. (2009) reported 73% mortality to deltamethrin (99.9%) in *An. nuneztovari* post 30 minutes exposure by CDC bottle bioassays in Columbia.

Table 1. A comparison of percent mortalities of susceptible and field collected populations of *Aedes aegypti* adult females against deltamethrin and cypermethrin

Time (minutes)	Deltamethrin 1.5% EC		Cypermethrin 10% EC	
	Susceptible %mortality	Resistant % mortality	Susceptible %mortality	Resistant %mortality
0	0	0	0	0
10	40	0	0	0
20	80	50	80	20
30	100	70	100	60
40	100	90	100	100
50	100	100	100	100

One way ANOVA at 95% CL between two insecticides on field collected populations, $P>0.05$: Not significant.

Table 2. Evaluation of resistance/susceptible status as resistance ratio (RR) of *Aedes aegypti* adults from Lahore (Misri Shah), Pakistan

Insecticide	Mosquito strains	LT_{50} (minutes) 95%CL	LT_{90}(minutes) 95%CL	Resistance ratio RR_{LT50}	Resistance ratio RR_{LT90}
Deltamethrin 1.5 %EC (2.5 µg/ml)	S	12.380 (7.038-15.574)	22.394 (18.730-31.360)	1.95	1.65
	R	24.193 (20.443-27.591)	37.109 (32.954-44.213)		
Cypermethrin 10% EC (5 µg/ml)	S	18.208	20.961	1.47	1.68
	R	26.877 (24.060-29.786)	35.399 (32.040-41.710)		

S: Susceptible strain, **R**: Resistant strain, **CL**: Confidence limit, **RR** $_{LT50}$: Resistance ratio is the ratio of LT50 between the field collected and susceptible strains of *Aedes aegypti* larvae.

In conclusion, the field collected populations of *Ae. aegypti* was resistant against the two insecticides used in this study as compared with susceptible (laboratory reared) strain. Moreover, *Aedes* females were more resistant to deltamethrin as compared to cypermethrin which is probably due to the frequent spray of the deltamethrin from the last few years in Lahore. The monitoring of insecticide susceptibility/resistance status in mosquitoes can reduce the rising problems of resistance in mosquito species. The current study of insecticide resistance status in dengue vector indicated that regular testing, recording and analysis of vector for susceptibility tests against different insecticides in different localities for effective vector control is needed in Pakistan.

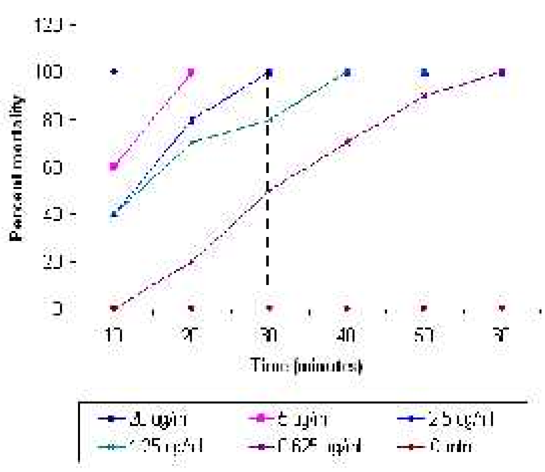

Figure. 1. A range of concentrations (deltamethrin) used for finding diagnostic/saturation dose in susceptible *Aedes aegypti* adult females by CDC bottle bioassays.

2.5 µg/ml= Diagnostic dose (100% mortality) at 30 minutes exposure.

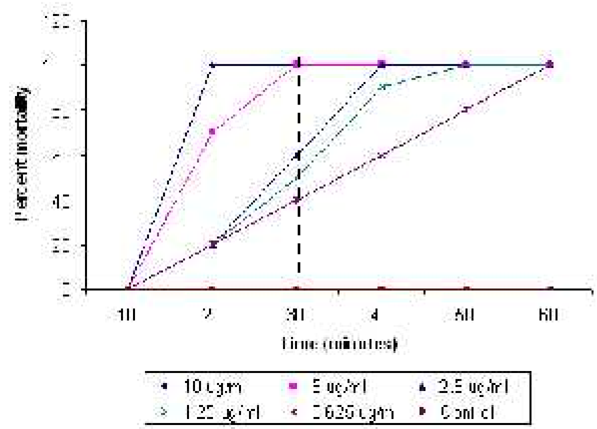

Figure. 2. A range of concentrations (cypermethrin) used for finding diagnostic/saturation dose in susceptible *Aedes aegypti* adult females by CDC bottle bioassays.

5 µg/ml= Diagnostic dose (100% mortality) at 30 minutes exposure.

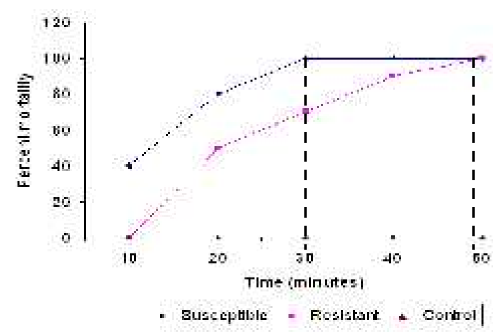

Figure. 3. A comparison of susceptible and field collected populations of *Aedes aegypti* adult females against diagnostic dose (2.5 µg/ml) of deltamethrin.

Resistant population: Hundred percent mortality at 50 minutes exposure.

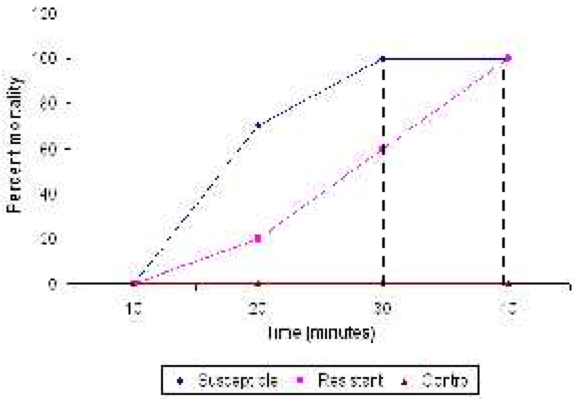

Figure. 4. A comparison of susceptible and field collected populations of *Aedes aegypti* adult females against diagnostic dose (5 µg/ml) of cypermethrin.

Resistant population: Hundred percent mortality at 40 minutes exposure.

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