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Pyrethroid resistance in adult mosquitoes, *Aedes aegypti* from Jaipur city, Rajasthan

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Abstract
In the present study, resistance status was evaluated in adult mosquitoes of *Aedes aegypti* against two synthetic pyrethroids from urban area of Jaipur, Rajasthan during July, 2015 to January, 2017. Mortality percentage for field and susceptible mosquitoes stain was determined against cypermethrin and permethrin by regression analysis using log probit method by using CDC bottle bioassay. Susceptibility of *A. aegypti* was assessed more to cypermethrin (LC_{50}= 0.0003 and LC_{90}=0.0002) as compared to permethrin (LC_{50}=0.02 and LC_{90}=0.01) on the basis of insecticidal efficacy. Some extent of resistance have been occurred in dengue vectors against pyrethroids due to regular use. Resistance status was expressed as resistance ratio (RR), against permethrin it was observed to be $RRLC_{50}$= 2 and $RRLC_{90}$=2.5 in field collected population, whereas, regarding cypermethrin resistance level was observed at $RRLC_{50}$= 1.5 and $RRLC_{90}$= 3.16 as compared to laboratory susceptible population.

Keywords: *Aedes aegypti*, resistance status, susceptibility, cypermethrin, permethrin

1. Introduction
Aedes aegypti (Diptera- Culicidae) is an important vector transmitting a variety of diseases in humans and domestic animals. It is a primary vector of dengue, yellow fever, chickengunya in India [23, 29]. In Rajasthan state many outbreaks of dengue and chickungunya have been reported in the past [13, 7, 8, 3]. Nearly one-third cases of dengue have been reported in Jaipur till October 29, 2013, the disease affected more than 869 persons in Jaipur [17]. The poor drainage system, prolonged storage of water for domestic and other purposes created the conditions conducive to breeding of *Aedes aegypti* mosquitoes significantly affects pronounced presence of vector. It is highly adapted to the environmental conditions that surround the Jaipur area with the ability occurrence in intense number with the human population [10]. Seasonal and area distribution of the transovarial transmission of dengue virus *Aedes aegypti*, *A. albopictus* and *A. vittatus* has been made in desert and non-desert districts of Rajasthan, India from 2006 to 2007 [3]. Currently, pyrethroid insecticides are the most widely used insecticides for controlling mosquitoes worldwide [2]. This is only class of insecticides recommended by World Health Organization [28] for treating mosquito nets in India. For long period of time indiscriminate and consistently use of it caused emergence of pyrethroid resistance in *Aedes* is a serious threat to control dengue and chickengunya epidemics. To our knowledge there have been no work done on the mechanism of insecticide resistance in Jaipur City, Rajasthan. Therefore, the present study was undertaken to evaluate pyrethroid resistance in *Aedes* mosquitoes collected from Jaipur, if any.

2. Materials and Methods
2.1 Larva collection
Egg masses, larvae and pupae of *A. aegypti* were collected from containers located in and around houses in area of Jaipur. Survey of immature stages were done by plastic tub, plastic dipper and dropper, scoop net method [28] made up of fine mesh net mounted on an iron handle (25 cm in diameter) at two hrs of sun rising and before two hrs of sunset. Collected eggs, larvae and pupae were immediately carried to laboratory for rearing and further analysis at Department of Zoology, Entomology Laboratory, University of Rajasthan, Jaipur. Adult mosquitoes were kept in rearing jars for propagation of generation at 25±3 °C temperature and 50-85% R.H.

2.2 Adult collection
Adults of Aedes mosquitoes were collected using hand picking, net and aspirator before sunset from the field area. Laboratory adult strain were collected from running mosquitoes culture. Both field and laboratory strains were transferred in CDC bottles to analyze for bioassay study against synthetic pyrethroids i.e. permerthrin and cypermethrin.

2.3 Insecticides
Permerthrin (Technical grade 96.2% purity) and Cypermethrin 25% EC formulation purchased from Chemet Chemicals Pvt, Ltd. Bharuch, Gujrat, India

2.4 Adult Bioassay and Resistance Ratio (RR)
Adults insecticide bioassay was conducted by using CDC bottle bioassay on both susceptible or laboratory reared strain and field collected strain of adult *A. aegypti*. According to the standard method of WHO [27] the adults of mosquitoes were subjected to bioassay with different concentrations of synthetic pyrethroids viz permerthrin and cypermethrin to get LC_{50} and LC_{90} at each strain. Insecticides of different concentrations were prepared from stock concentration i.e. 0.0001 to 1 ppm. Twenty adult mosquitoes were transferred to each CDC bottle after employing the surface film of insecticide doses. A minimum of five replicates were kept for each concentration along with respective control for each strain. Adult mortality was determined after one hour of exposure to insecticides.

The percent mortality was corrected by using Abbott's formula [1] when the control mortality was less than 20 percent.

$$\text{Abbott's formula: } \frac{\%\text{ test mortality} - \%\text{ control mortality} \times 100}{100 - \%\text{ control mortality}}$$

Stastical analysis was done by regression analysis using log probit method [11]. Lethal concentrations (LC_{50} and LC_{90}) were calculated with their 95% confidence intervals. The resistance ratio (RR) calculated by dividing the LC_{50} a nd LC_{90} (lethal concentrations) value of each strain by the LC_{50} and LC_{90} value for baseline susceptible strain and considered to be significant at 95% fiducial limits (FL).

3. Results and Discussion
The results revealed that synthetic pyrethroids were very effective at lower concentration against adults of *A. aegypti*. In general, a comparison between two synthetic pyrethroids indicated that cypermethrin was more effective than permethrin. Regarding lethal concentrations, LC_{50}=0.0003, LC_{90}=0.0002 and LC_{50}=0.1, LC_{90} =0.0316, were recorded for field and susceptible mosquitoes strain, respectively, against cypermethrin. Experiments carried out with permetrhrin showed (LC_{50}-0.02, LC_{90}-3.162) and (LC_{50}-0.01, LC_{90}-1.584) for both field and susceptible *Aedes* population (Table-1). Earlier, susceptibility of adults of three mosquito vector species was evaluated with different diagnostic doses of pyrethroids in arid zone of Rajasthan [4, 5, 6]. Similar to present work, at Masore deltamethrin was found to be 12.5 and 47 times more effective than permethrin against *Anopheles stephensi* and *Anopheles culicifacies* [12]. Further, exposure to the discriminating doses of deltamethrin and permethrin induced 100% mortality indicating susceptibility status in *A. aegypti* [18]. In contrast to current results, *Aedes* mosquitoes from three localities of Central African Republic showed mortality rates > 98% against deltamethrin [23].

Moreover, bioassay with permethrin and cypermethrin evaluated some extent of resistance in field population of *A. aegypti* mosquitoes. The resistance ratio (RR) was determined by dividing lethal concentration of field strain by laboratory susceptible strain and observed to be $RRLC_{50}$= 2 and $RRLC_{90}$=2.5 against permethrin whereas $RRLC_{50}$= 1.5 and $RRLC_{90}$= 3.16 against cypermethrin, respectively (Table-1). *A. aegypti* mosquitoes population belonging to urban area of Jaipur was found resistant against permethrin and cypermethrin. Insecticides as synthetic pyrethroids using at regular basis has led to the development of the resistance in insect vectors [14, 22]. Earlier, susceptible level of some anophelilines from Rajasthan was studied and found that all species were resistant to DDT and dieldrin, but in contrast to our results susceptible to permethrin [4, 6]. The results are in conformity with resent studies that have reported an increased deltamethrin resistance in several field populationss of *A. aegypti* and *A. albopictus* in India [9, 21]. Regular use of permethrin in Singapore and Kuala Lumpur for the control of dengue since long period attributed as a possible reason of rising in resistance in *Aedes* mosquitoes [20, 25]. At Lahore resistance level was found to be $RRLT_{50}$=1.3 and $RRLT_{95}$=1.37 as compared to laboratory reared susceptible population of *A. aegypti* against deltamethrin [15]. Further, resistance status was recorded as $RRLT_{50}$=1.95 and $RRLT_{95}$=1.47 against deltamethrin and cypermethrin whereas percent mortalities had no significant differences against two synthetic pyrethroids [16]. *A. aegypti* were also found to be highly resistant to deltamethrin (0-37%) followed by permethrin (2-55%) [19, 27], in other study resistance level in *Culex* and *Anopheles* mosquitoes supported to our present work [26].

Table 1: Evaluation of resistance status in *Aedes aegypti* against cypermethrin and permethrin from Jaipur, Rajasthan

Insecticides	Mosquitoes strain	No. of mosquitoes observed	Lethal concentrations (in ppm)		Resistance Ratio (RR)	
			LC_{50}	LC_{90}	RR at LC_{50}	RR at LC_{90}
Cypermethrin Permethrin	Field	100	0.0003	0.1	1.5	3.16
	Susceptible	100	0.0002	0.0316		
	Field	100	0.02	3.162	2	2.5
	Susceptible	100	0.01	1.584		

4. Conclusion
In the present study field populations were found at beginning to develop resistance against synthetic pyrethroids in *Aedes* mosquitoes. The presence of resistance in the natural population was probably due to the impacts from using of pyrethroids in domestic areas and fogging of insecticides under vector control programe at regular basis around the city. Detection of resistance will help in public health personnel, serving as an early warning of the impending problem of uncertain levels of pest control under field conditions. An appropriate resistance reduction strategy is needed in order to achieve natural insecticidal susceptibility in nature. To provide more confimation to resistance status in *Aedes* mosquitoes biochemical assay and molecular studies for kdr

mutations is recommended.

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