Original Research Article

Susceptibility of the dengue vector *Aedes aegypti* to various insecticides in Lahore, Pakistan

ABSTRACT

Objective

Present studies were carried out to examine the susceptibility of larvae and adults of *Aedes aegypti* (*Ae. aegypti*) from laboratory and field populations of Lahore city against four major groups of commonly used insecticides; Imidacloprid 5% SC, phenyl-pyrazoles* (fipronil 2.5% EC), pyrethroids (Deltamethrin 2.5% SC) and organophosphates (DDVP 50% EC) world health organization standard procedures were observed.

Methods

For laboratory strain, adults and larvae were collected from the Insectary of the National Institute of Malaria Research and Training (NIMRT), Lahore, Pakistan. Insecticides of four major groups. Larval bioassays were conducted according to the World Health Organization procedure with minor modifications. Larval mortality was finalized after 48 hours for Deltamethrine, Imidacloprid, DDVP, and 72 hours for Fipronil.

Results

Larval and adult toxicity results were different for wild and laboratory strains exposed to different groups of insecticides. Regression analysis showed non-significant trends in mortality. Imidacloprid proved to be the most toxic to wild larval strains of *Ae.aegypti* collected from different localities of Lahore while Fipronil was also active for wild larval samples.

Conclusion

Deltamethrin showed the least activity against both adults and larval strains. The susceptibility of the field strains was lower than laboratory strains; the ratio varies from insecticide to insecticide.

Keywords: *Aedes. aegypti,* neonicotenoids, phenylpyrazoles, pyrethroids, organophosphates

Introduction

Dengue fever is a disease widespread throughout the world, there is no safe affordable, and effective vaccine available. For the purpose to control the disease vector control is an effective method (Kuno 1997, Mohanty and Prakash 2008). However, Dengue Fever is less widespread in the temperate than in tropic countries. The life cycle of pathogens is delayed by the cold weather and due to the shorter life span mosquitoes usually die before pathogens become infective (Busvine 1980) Climate is mostly favorable for the breeding of mosquitoes in Pakistan (Schafer and Lundstrom

2001) *Ae.aegypti* (Linnaeus) and *Ae. albopictus* (Skuse) are the important vectors of dengue fever in tropical and subtropical regions of the world (Lee and Yap1999). Major diseases like dengue fever and dengue hemorrhagic fever (DHF) are spread by *Ae.aegypti*. Neurotoxic insecticides are either neuro inhibitory or neuro excitatory. The behavioral effects of neuro excitation are tremors, hyperactivity, and rigid paralysis, in the case of neuro inhibition, lower immobility and flaccid paralysis occur. The mortality is caused by energy depletion and neuromuscular fatigue (Michael 2003). In Pakistan, a major strategy to prevent and control diseases like dengue fever is the use of insecticides. During the dengue outbreak in 2011, insecticides used were reportedly not effective. Although some previous studies indicated that there is a high level of resistance to some commonly used insecticides, the level of resistance was not studied for the *Aedes* population in Lahore. According to the World Health Organization (WHO) standards and control program, there is a continuous need to monitor insecticide resistance. The present study was conducted to determine the activity of different groups of insecticides against laboratory and field strains of larvae and adults of *Ae.aegypti.*

Material and Methods

Mosquito collection sites

For wild strain, larvae were collected from those sites in Lahore where insecticides were used at high frequency and quantity. For laboratory strain, adults and larvae were collected from the Insectary of the National Institute of Malaria Research and Training (NIMRT), Lahore, Pakistan.

Mosquitoes

For wild strain of *Ae. aegypti*, first star larvae were collected from the field using plastic trays and were kept under laboratory conditions of controlled temperature (25掳C 卤2) and relative humidity respectively (70% 卤5) with a constant photoperiod (light: dark, 14h:10h). These larvae were fed on a mixture of biscuits, dried yeast and milk (1:1:1). After 3-5 days these wild larvae hatched into adults, and were used for adult bioassays, for, larvae bioassays laboratory strains were used. Adults took insectary from larvae collected in 2009 from indoor and outdoor populations of mosquitoes in Lahore, Pakistan.

Insecticides

Insecticides of four major groups: imidacloprid 5% SC, phenylpyrazoles (fipronil 2.5% EC), pyrethroids (deltamethrin 2.5% SC), and organophosphates dichlorvos (DDVP 50% EC) were used during this study. Stock solutions of four insecticides, DDVP, deltamethrin, fipronil, and imidacloprid were prepared in distilled water.

Larval bioassay

Larval bioassays were conducted according to the World Health Organization procedure (WHO, 1981) with minor modifications. For the larval bioassay, fourth instars larvae (batches of 20) were introduced in 100 ml of water in plastic cups containing different concentrations of the insecticides. Into each cup, 0.02g mixture was added to feed larvae. Five different Volumes for each insecticide ranging from 0.001 to 0.5碌l were used to determine lethal concentrations. Bioassays were done in

three replicates for each concentration to get valid results. In the control test, distilled water was used. Plastic cups were inspected after 24h and the numbers of dead larvae were recorded. Larval mortality was finalized after 48 hours for deltamethrin, imidacloprid, DDVP, and 72 hours for fipronil. In bioassay moribund larvae were considered dead.

Adult bioassay

Adult bioassays were conducted according to the World Health Organization procedure (WHO 1981) with some modifications. Five days old adults (batches of 25) were exposed to Whatman # 1 filter paper treated with 0.05% Imidacloprid, 0.01% Fipronil, 0.5% Deltamethrin, and 0.5% Dichlorvos (DDVP) in a glass test tube under the same laboratory conditions as for larvae. In control, no treatment was given to filter paper. After 24 h mortality was observed.

Data analysis

Data from bioassays were expressed in mean 卤 S.E.M by using Minitab statistical software (Version 13.20). LC50 with their 95% confidence intervals was estimated by using the EPA Probit analysis program (version 1.5). Results were statistically significant when P 鈦0.05. Duncan's multiple range tests were applied to compare the concentrations of insecticides with a significant difference at the 5 % level using New Co stat. Log concentration regression lines were drawn for the insecticides used in larval and adult bioassays. The resistance status was determined according to WHO criteria.

Results

From table 1, it is evident that larval mortality for fipronil ranges from 18-99% for laboratory strain of *Ae. aegypti* at 0.005- 0.003磅 and field strain ranges from 15-97% at 0.008-0.048磅 showing its high activity as compared to other insecticides. From all tested insecticides against *Ae. aegypti* larvae, deltamethrin proved to be as its percentage mortalities ranges from 15-85% at 0.2-0.65磅 in the laboratory strain and for field strain it decreases to 10-80% at 0.3-0.7 磅. Bioassays results indicate that the percentage mortality of *Ae.aegypti* exposed to larvicides was lower in the field populations than in the laboratory strain.

As far as LC50 is concerned, deltamethrin shows the highest LC50 values 0.4 and 0.52 磅 for laboratory and field strain depicting its low effectiveness in the control of *Ae.aegypti*.The LC50 values were recorded in the lab and field strain of *Ae. aegypti* after treatment with fipronil were 0.012 and 0.022磅 respectively. While imidacloprid and DDVP showed intermediate activities (table 1).

The diagnostic doses of imidacloprid, fipronil, deltamethrin, and DDVP are 0.05磅, 0.03磅, 0.7磅, and 0.5磅 respectively (table1). Hundred adult females derived from wild larvae after one generation were exposed to each group of insecticides. The lowest percentage mortality rate (75%) was observed in the deltamethrin-treated group, representing the development of resistance in the field population. The highest percentage mortality rate (90%) was observed in the fipronil-treated group. According

to WHO criteria of adult susceptibility test *Ae.aegypti* population showed high tolerance to imidacloprid, fipronil, and DDVP but high resistance to deltamethrin.

Discussion

The present study was performed to determine the susceptibility of *Ae.aegypti* to different groups of insecticides. In this study, it was observed that laboratory strain was more susceptible than field strain. From the laboratory results of mortality after exposure to different groups of insecticides, fipronil was the most active followed by imidacloprid. The LC50 values of imidacloprid and fipronil were markedly low. New classes of insecticides, such as imidacloprid and fipronil were registered in the last decade and are not commonly used for mosquito control in Pakistan, therefore *Ae.aegypti* were more susceptible to these insecticides.

From our results, the outstanding performance of fipronil was in agreement with Pridgeon et al. (2008) who reported relative potency of 19 insecticides against female *Ae.aegypti* (L). The insecticides used for bioassay against *Ae. aegypti* larvae and adults have different modes of action for killing. . To investigate the efficacy of each insecticide, six concentrations were applied to estimate 0-100% mortality. These females鈥 mosquitoes were transferred to plastic cups provided with 10% sucrose solution. Mortality was recorded after 24 hours. Among 19 insecticides used fipronil was considered highly effective against all tested *Ae. aegypti*... Similarly, Sandra (2010) also conducted a study in which toxic baits were prepared by using active ingredients of five insecticidal groups (macrocyclic lactones, neonicotenoids, phenylpyroles, pyrethroids, and pyrroles) in sucrose solution. These toxic baits were used against *Anopheles quadrimaculatus, Ae.taeniorhynchus,* and *Culex quinquefasciatus*. In *Ae. taeniorhynchus*, imidacloprid was considered to be the most toxic insecticide in the present study.

Previous studies and the present study reveals that insecticides used previously for control of dengue vectors caused the development of resistance. *Ae.aegypti* was highly resistant to the pyrethroid group of insecticides. Cross-resistance can be developed in mosquitoes between pyrethroid and non-pyrethroid insecticides. This phenomenon was well explained in the study of Sathantriphop et al. (2006). In that study, the potency of insecticides (pyrethroids, organochlorines, carbamates, and organophosphates) was recorded for 1 hour against *Cx. quinquefasciatus* and *Ae. aegypti females*. When *Ae. aegypti* females were tested against insecticides, it was found that resistant to permethrin, and highly tolerant to deltamethrin but high susceptibility was observed to organophosphate (fenitrothion).

Our study emphasized the susceptibility of *Ae. aegypti* to commonly used (DDVP and Deltametnrin) insecticides and newly introduced fipronil and imidacloprid in Lahore city. Our results reveal an increase in the level of tolerance to operational usage of larvicides and adulticides after dengue outbreaks in Lahore. The main problem was the development of resistance in vectors which can be managed by monitoring susceptibility status in vector control programs. Resistance developed in mosquitoes to these insecticides especially deltamethrin (pyrethroids) resulted from household use. Insecticidal products such as liquid, mat, coil, and cream formulations have ingredients

of pyrethroids. These products play an important role in the development of resistance in *Ae. aegypti*. Frequent use of insecticides with the same modes of action can accelerate the development of resistance.

The use of low concentrations of pyrethroids and organophosphates for mosquito control was effective and safe (Chareonviriyaphap et al. 1999, Somboon et al. 2003). Thus insecticides play an imperative role in vector control. The application of larvicides and adulticides are principal methods for the control of vector-borne diseases (Reiter and Gubler 1997). Resistance in the mosquitos population was due to incomplete and infrequent coverage in examining and reporting.

Conclusion

The extensive application of pyrethroids for mosquito and agriculture pest control caused an indirect contribution to the development of resistance to these classes of insecticides. Therefore the present study declares the need for mosquito control professionals to search for strategies to prevent or delay the development of insecticide resistance in Lahore city, Pakistan.

Conflict of Interest

The authors did not declare a conflict of interest.

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Table 1: Susceptibility of *Ae .aegypti* larvae (lab and field strains) against various insecticides

Insecticides	Mosquito strains	Effective concentrations mg/l	Larval mortality (%)[a]	Statistical parameters		Resistance Ratio (RR_{50})[b]
				LC_{50}/ µl	Slope	
Imidacloprid 5% SC	Lab strain	0.03-0.13	16-95	0.060	4.00	2.1
	Field strain	0.05-0.20	18-90	0.127	3.72	
Fipronil 2.5% EC	Lab strain	0.005-0.03	18-99	0.012	2.82	1.8
	Field strain	0.008-0.04	15-97	0.022	3.00	
Deltamethrin2.5% SC	Lab strain	0.2-0.65	15-85	0.40	3.66	1.3
	Field strain	0.3-0.70	10-80	0.520	6.06	
DDVP 50% EC	Lab strain	0.05-0.19	17-92	0.104	3.91	1.8
	Field strain	0.07-0.35	19-88	0.187	2.90	

a: Three replicates , 20 larvae each; control mortalities ranged from 0.0% -3.0%

b: RR_{50}: = LC_{50} of field strain / LC_{50} of the lab strain

Table 2: Susceptibility of *Ae .aegypti* adults against various insecticides

Insecticides	Diagnostic dosages/ mg/µl	No. of mosquitoes exposed	Mortality	Susceptibility status
Imidacloprid 5% SC	0.05	100	85	Tolerant
Fipronil 2.5% EC	0.03	100	90	Tolerant
Deltamethrin 2.5% SC	0.7	100	75	Resistant
DDVP s50% EC	0.5	100	80	Tolerant

While susceptibility of *Ae. aegypti* adults against diagnostic dosages of various insecticides,Several mosquitoes exposed and mortality are shown in Figure 1.

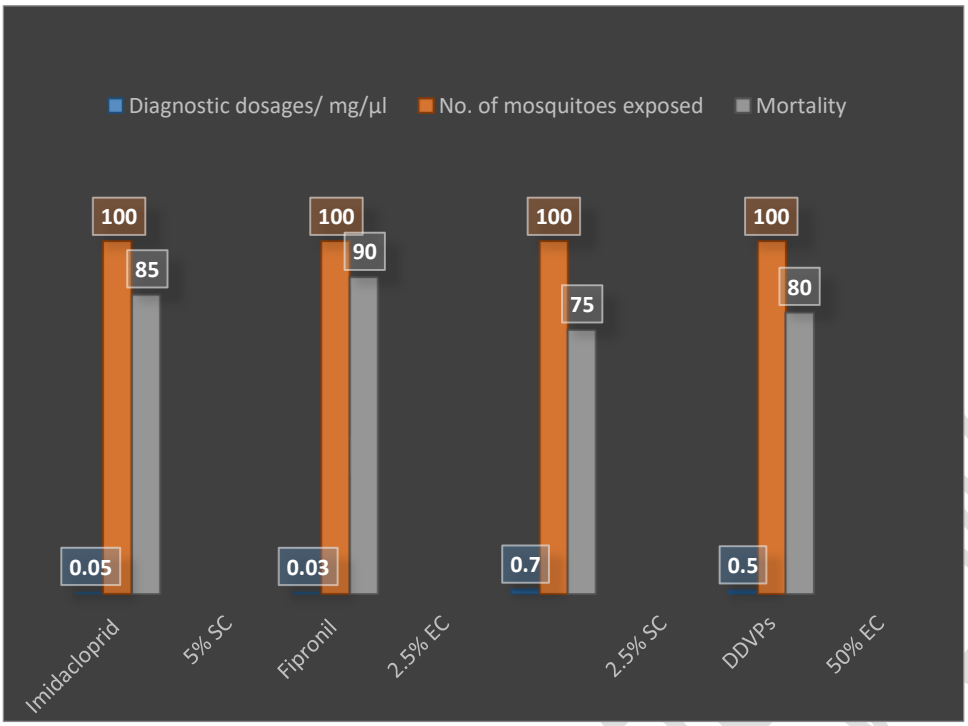

Figure 1: Susceptibility of *Ae. aegypti* adults against diagnostic dosages of various insecticides, number of mosquitoes exposed and mortality