Original Research Article

~~Bio toxicity~~ Bio-toxicity of Different Insecticides in *Culex quinquefasciatus* Larvae in Lahore Punjab, Pakistan

ABSTRACT

Purpose: Mosquitoes are medically important vector and transmit several viral diseases which cause devastating effect on human. New classes of insecticides, such as neo-necotinoids (imidacloprid) and phenylepyrazoles (fipronil) which were registered. Although these group of insecticides are used but comparative study of insecticides has not yet been taken in Pakistan. Therefore, this study was done to determine bio toxicity of different insecticides in *culex quinquefasciatus* larvae in lahore, punjab, pakistan

Methods: In the present study, bio toxicity of four insecticides from four major groups: neo-nicotenoids (imidacloprid 5% SC) phenyl-pyrazoles (fipronil 2.5% EC), pyrethroids (deltamethrin 2.5% SC) and organophosphates (DDVP 50% EC) were tested against *Culex quinquefasciatus (Cx. quinquefasciatus)* Samples were collected from different localities to determine the susceptibility of both species against tested insecticides.

Results: The findings of the study displayed Larval toxicity results were different for Kot Lakhpat and Lahore College for Women University Lahore for each insecticide. Kot Lakhpat samples were considered as reference samples because these were exposed to insecticides at a very low extent as compared to LCW samples. Regression analysis of variance showed significant positive trend in mortality. Fipronil was proved to be most toxic against *Cx. Quinquefasciatus* having LC_{50} = 0.003 µl/ml and 0.006 µl/ml n KotLakhpat and L samples respectively.

Conclusion: Deltamethrin showed least efficacy against both localities representing its high tolerance and resistance against pyrethroids. Lahore College for Women University samples were more resistant than Kot Lakhpat as resistant ratio varies from 1.8-2.30 for insecticides to insecticides in *Cx. Quinquefasciatus* respectively.

Key words: *Cx. quinquefasciatus*-insecticides – Punjab-neo-nicotenoids -phenyl-pyrazoles - organophosphates -LC_{50}

INTRODUCTION

Mosquitoes are medically important vector and transmit several viral diseases which cause devastating effect on human[1]. High population explosion of *Cx quinquefasciatus* in Lahore district has become a severe biting nuisance primarily in summer months[2]. *Cx. Quinquefasciatus* is vector of *Bancraftian filariasis* in human and domestic animals. Filariasis is common disease in tropical area as there are 45 million cases of lymphatic filariasis are reported in south East Asia[3]. Outbreaks of filariasis need to use of larvicides for sustainable mosquitoes control in semi-arid zone of Asia[4]. Conventional methods for mosquito control have relied on the application of different insecticides[5]. Active ingredients in insecticide products and repellent used for mosquito control are made up of synthetic pyrethroids, organophosphate, carbamate, or organochlorines. Use of larvicides to control immature mosquito's population is considered less controversial than adulticides for vector control programs[6].Unfortunately resistance has developed by many mosquito species to all these major groups of insecticides[7-8]. Resistance within class or cross resistance may be developed in many species. Resistance can be passed from immature stages to adult if expose to insecticides with same mode of actions. In addition many mosquitos' species vary in developing resistance to different adulticides and larvicides[6].*Cx. quinquefasciatus*became resistant to many insecticides groups such as pyrethroid, organophosphate and carbamate in Saudi Arabia, Northern Thailand and America[9-10]. In view of the recently increased development of resistant, four groups of insecticides were used in bioassay against *Cx. quinquefasciatus*in which pyrethroids (deltamethrin) and organophosphate (DDVP) are conventionally used in Pakistan. New classes of insecticides, such as neo-necotinoids (imidacloprid) and phenylepyrazoles (fipronil) which were registered last decade and not commonly used for mosquitoes control in Pakistan. Although these group of insecticides are used but comparative study of insecticides has not yet been taken in Pakistan. An attempt was done to determine the comparative efficacy of different groups of insecticides against *Cx. quinquefasciatus*larvae.

MATERIALS AND METHODS

Study area:In this study two different localities KLP, Lahore College for Women University Lahore (LCWU) were selected for the collection of mosquitoes larvae to find out the degree of susceptibility against insecticide in different field populations *Cx. quinquefasciatus.* Larvicides susceptibility test were carried out against *Cx. quinquefasciatus* from September 2010 and august 2011during the high prevalence period of respective vectors.

Stock solution preparation: Four technical grade insecticides, insecticides were used as larvicides: neo-nicotenoids (imidacloprid 5% SC) phenyl-pyrazoles (fipronil 2.5% EC), pyrethroids (deltamethrin

2.5% SC) and organophosphates (DDVP 50% EC) taken from Ali Akbar group of industries. Stock solutions of four insecticides were prepared in distilled water. Subsequent concentrations of stock solutions for the larvae bioassays were prepared by using formula:

$$= \frac{\text{Required concentration in µl/ml} \times \text{required volume in ml}}{\text{Concentration \% (gm/l)} \times 10}$$

$$= \text{---------} \ \text{µl/ml}$$

Larval susceptibility bioassay: Larval susceptibility bioassay was conducted according to World Health Organization procedure[11]. For the larval bioassay test, fourth instars larvae were introduced in 250 ml of insecticides concentration. Bioassay was carried out in plastic cups. Five different concentrations for each insecticide ranging from 0.001 to 0.5µl/mlwere used to determine sub lethal concentration. Bioassays were done in three replicates for each concentration in order to get valid results. Controls were carried into distilled water. Larval mortality was recorded after 48 hours for deltamethrin, imidacloprid, DDVP and 72 hours for fipronil. In susceptibility bioassay procedure moribund larvae was considered as dead larvae. The temperature was maintained at 25± 2°C and 70 ± 5% relative humidity.

Statistics: Data taken from bioassays were expressed in mean ± S.E.M by using Minitab statistical software (Version 13.20). LC_{50} with their 95% confidence intervals was estimated by using EPA probit analysis program (version 1.5). Results were statistically significant when $P \leq 0.05$. Duncan's multiple range tests was applied to compare the concentrations of insecticides with significant difference at the 5 % level using New Costat.

RESULTS AND DISCUSSION

Mean mortality and lethal concentrations of insecticides revealed that fipronil, GABA gated chloride channels non-competitive antagonist showed outstanding performance against*Cx. quinquefasciatus*in both localities. Imidacloprid represented next effective insecticides after Fipronil(Table 1 and 2). Table 3 showed that the comparative toxicities of all tested insecticides in terms of LC_{50} against*Cx. quinquefasciatus*in both localities. In KLP, Cx. *quinquefasciatus,* LC_{50} values recorded against imidacloprid, fipronil, deltamethrin and DDVP were 0.011, 0.003, 0.054 and 0.026 µl/ml after treatment. LC_{50} values of imidacloprid, fipronil, deltamethrin and DDVP were 0. 0.024, 0.006, 0.124 and 0.049 µl/ml was recorded in LCW samples. *Cx. quinquefasciatus* represented the resistant ratio was high in deltamethrin (2.30) and low in DDVP (1.88). While imidacloprid and fipronil showed intermediate resistant ratio as both localities were never sparyed by these insecticides. Analysis of variance indicated that significant positive trends in mortality were observed between different concentrations of insecticides in KLP and LCW samples. Analysis of variance indicated that significant positive trends in mortality(d.f = 4; $P < 0.05$) were observed between different concentrations of insecticides in KLP and LCW samples.

Outstanding performance of fipronil in this study was agreement with Pridgeon*et al.* (2008) who reported relative potency of 19 insecticides against female *Cx. quinquefasciatusAe. aegypti* and *An. quadrimaculatus*. These tested insecticides had different mode of actions. Pesticides were applied (0.5-1µl) topically to 5- to 7 days adult females mosquitoes. In order to investigate the efficacy of each insecticides six concentrations were applied to estimate 0-100% mortality. After treatment these females' mosquitoes were transferred to plastic cups provided with 10% sucrose solution. Mortality was recorded after 24 hours. Among 19 pesticides, fipronil was considered highly effective against all tested species *Cx. quinquefasciatus*with LD_{50} values 3.3 $\times 10^{-7}$ µg/mg. This study also revealed that imidacloprid being relatively new insecticide showed low activity with LD_{50} values 1.2 $\times 10^{-3}$µg/mg against *Cx. Quinquefasciatus[12]*. Therefore it was seemed to be effective insecticide for control of*Cx. quinquefasciatus*.These results were coinciding with study of Liu *et al*. in which imidacloprid was considered as moderately toxic when applied to three strains of *Cx. quinquefasciatus*in United States[13]. However the use of fipronil as larvicides was controversial as it is broad spectrum insecticides and harmful for non-target aquatic organisms[14].

DDVP showed intermediate and deltamethrin showed least efficacy against *Cx. quinquefasciatus*. DDVP showed intermediate and deltamethrin showed least efficacy against *Cx. quinquefasciatus*. The results of this study coincide with study of Tahir*et al*. in which 5% deltamethrin bioassay was done against *Cx. quinquefasciatus*in order to detect resistance level in Punjab. All the individuals of populations were not resistant to 5% of deltamethrin although tolerance level was high in that area where organophosphate might have exposed to insecticides with same mode of action as cross resistance developed[2]. Pyrethroidand OPs cross resistance based upon esterase was studied in many insects' species[15].Kasai *et al*. reported that larvae of *culex* genera showed high resistance as response etofenprox, new insecticides of pyrethroids group used as larvicides in china and Japan[16]. The frequent use of these pyrethroids can lead to development of resistance against all pyrethroids and lessens the effectiveness of spatial repellent.

Cross resistance can be developed in mosquitoes between pyrethroid and non pyrethroids insecticides if both share the same target site mechanisms. This phenomenom was well explained in study of Sathantriphop*et al.* for potency of insecticides (pyretroids, organochlorines, carbamates and organophosphates were recorded for 1 hour against *Cx. quinquefasciatus*. It was revealed that samples of *Cx. quinquefasciatus*females developed resistant against pyrethroidsand organochlorines and susceptible to malathion. As organochlorines and pyrethroids targeted sodium channels so mosquitoes developed cross resistance between these groups of insecticides[17].

As the data represented samples of both localities were least susceptible against pyrethroids. The use of low concentrations of pyrethroids for mosquitoes control well thought-out effective and safe[18-19]. The main problem was development of resistance in vectors which can be managed by monitoring susceptibility status vectors control programs. Resistance developed in mosquitoes to pyrethroids especially deltamethrin and permethrin was resulted due to household use of pyrethroids. Insecticidal products such as liquid, mat, coil and cream formulations have ingredients of pyrethroids. These products play an important role in development of resistance in *Cx. quinquefasciatus*.

The present study revealed that insecticides play an imperative role in vector control. Applications of larvicides are principal methods for control of vector borne diseases[20]. Resistance in mosquitos' population was due to incomplete and infrequent coverage in examining and reporting. The extensive application of pyrethroid and organophosphate for mosquito and agriculture pest control caused indirect contribution for development of resistant species to these classes of insecticides.

Declaration

e. Ethics approval

The University Institute of Public Health Committee and the Research Ethics group of the University of Lahore gave ethical approval.

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Table 1: Mean mortality (X ± S.E.M) of KLP samples of *Cx. quinquefasciatus*in response to different concentrations of four different insecticides during 2010 and 2011

Imidacloprid 5% SC		Fipronil 2.5% EC		Deltamethrin 2.5% SC		DDVP 50% EC	
Concentrations µl/ml	**Mean mortality**	**Concentrations µl/ml**	**Mean mortality**	**Concentrations µl/ml**	**Mean mortality**	**Concentrations µl/ml**	**Mean mortality**
0.01	15 ± 1.15[c]	0.002	10 ±1.00[e]	0.05	16 ± 0.33[c]	0.01	07 ± 1.15[e]
0.02	21 ± 0.58[b]	0.004	15 ± 0.577[d]	0.1	21 ± 1.00[b]	0.03	13 ± 1.15[d]
0.03	29 ± 0.33[a]	0.006	20 ± 0.33[c]	0.2	28 ± 0.33[a]	0.05	20 ± 0.33[c]
0.04	30 ±0.33[a]	0.009	$24 \pm 0.88_{b}$	0.3	30 ± 0.33[a]	0.07	26 ± 1.73 [b]
0.05	30 ±0.00[a]	0.01	30 ± 0.00[a]	0.5	30 ± 0.0[a]	0.1	30 ± 0.33[a]
Control	1.3 ± 0.33	Control	0.00 ± 0.00	Control	0.00 ± 0.00	Control	0.00 ± 0.00

*Values followed by same superscript alphabet in a column are not significantly different at $p = 0.05$ level of significance (Duncan's Multiple Range Test).

Table 2: Mean mortality (X ± S.E.M) of LCW samples of *Cx. quinquefasciatus*in response to different concentrations of four different insecticides during 2010 and 2011

Imidacloprid 5% SC		Fipronil 2.5% EC		Deltamethrin 2.5% SC		DDVP 50% EC	
Concentrations µl/ml	**Mean mortality**	**Concentrations µl/ml**	**Mean mortality**	**Concentrations µl/ml**	**Mean mortality**	**Concentrations µl/ml**	**Mean mortality**
0.01	$1±0.00^{e}$	0.002	$3 ± 0.577^{e}$	0.05	$3 ± 0.33^{d}$	0.01	$00 ±0.0^{e}$
0.02	$11 ± 2.31^{d}$	0.004	$9 ± 1.73^{d}$	0.1	$15 ±1.73^{c}$	0.03	$8 ± 2.31^{d}$
0.03	$19 ± 1.00^{c}$	0.006	$14 ± 1.73^{c}$	0.2	$19 ± 1.53^{c}$	0.05	$14 ± 1.15^{c}$
0.04	$24 ±1.53^{b}$	0.009	$21 ± 0.577_{b}$	0.3	$25 ± 1.73^{b}$	0.07	$22 ± 0.577^{b}$
0.05	$30 ± 0.00^{a}$	0.01	$27 ± 2.08^{a}$	0.5	$30 ± 0.33^{a}$	0.1	$27 ± 1.73^{a}$
Control	1.3 ± 0.33	Control	0.00 ± 0.00	Control	0.00 ± 0.00	Control	0.00 ± 0.00

*Values followed by same superscript alphabet in a column are not significantly different at *p* = 0.05 level of significance (Duncan's Multiple Range Test).

Table 3: Comparative toxicities of different insecticides against samples of both localities of *Cx. quinquefasciatus* during 2010 and 2011

Insecticides	Locality	LC_{50} µl/ml	95% confidence limits		Fit of probit line			Resistance Ratio RR_{50}
			LCL	UCL	Slope ± SE	χ^2 (df)	P	
Imidacloprid 5% SC	KLP LCW	0.011 0.024	0.008 0.021	0.014 0.028	3.69 ± 0.64 5.40 ± 0.96	5.06 (4) 3.39 (4)	0.00 0.00	1 2.18
Fipronil 2.5% EC	KLP LCW	0.003 0.006	0.003 0.005	0.004 0.007	2.57±0.45 3.29 ± 0.52	6.58 (4) 3.48 (4)	0.00 0.00	1 2
Deltamethrin 2.5% SC	KLP LCW	0.054 0.124	0.034 0.100	0.072 0.151	2.75 ± 0.53 2.91±0.403	2.09 (4) 4.62 (4)	0.00 0.00	1 2.30
DDVP 50% EC	KLP LCW	0.026 0.049	0.019 0.040	0.033 0.057	2.41±0.37 3.85±0.73	6.99 (4) 1.46 (4)	0.00 0.00	1 1.88

$RR_{50=}$ LC_{50} of LCW/ LC_{50}ofKL.