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Evaluation of Resistance Against *Bacillus thuringiensis israelensis* WDG in Dengue Vector from Lahore, Pakistan

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Abstract.- In the present study, resistance against biological insecticide i.e. *Bacillus thuringiensis israelensis* (*Bti*) WDG (water dispersible granules) was evaluated in *Aedes aegypti* larvae. Early 4th instars larvae were collected from the slum area of Misri Shah, Lahore, province Punjab, Pakistan during the period of May 2009 to January 2010. Larval bioassays were carried out with early 4th instars susceptible (laboratory-reared) colony to find out diagnostic dose. A range of known concentrations (100, 40, 20, 10, 5, 2.5 and 1.25 ppm) of *Bti* WDG was used for fixed time period (60 min). A diagnostic dose of 10ppm was found post 30 min. exposure whereas, the same concentration cause hundred percent mortality of field collected larvae in 60 min. exposure. The resistance level was expressed as resistance ratio (RR) of lethal time for 50% death determined in field collected and susceptible strain. The results indicated that the field collected larvae were 10 times more resistant than susceptible population with respect to dose, while RR LT_{50} - RR LT_{90} ranged 1.97-2.22 against *Bti* (WDG) in *Aedes aegypti* larvae.

Key words: *Aedes aegypti*, resistance, *Bacillus thuringiensis israelensis* WDG, Pakistan.

INTRODUCTION

Mosquitoes belonging to genus *Aedes*, are medically important, regarding transmission of many viral diseases (yellow fever, encephalitis and dengue fever) to humans. *Aedes aegypti* and *Aedes. albopictus* are both suspected vectors of dengue in Lahore, Pakistan (Jahan *et al*., 2011). Dengue was reported annually since 2006 from Pakistan. Recently in 2011, dengue emerged as an epidemic all over the country especially in province Punjab where ≥20,000 positive cases were found along with 207 deaths in the urban city of Lahore only. There is no proper drug or vaccine, for the control of dengue fever (DF) and dengue hemorrhagic fever (DHF). Primarily, control of DF/DHF depends on controlling the mosquito vectors (Spiegel *et al.*, 2005). Mosquito control includes biological and chemical control.

Biological control is the control by living enemies such as use of predatory bugs, copepods, nematodes, fungi, fishes, bacterial compounds such as *Bacillus thuringiensis* var. *israelensis* (*Bti*) and *Bacillus sphaericus* (*Bsph*). However, the exclusive utilization of microbial control might not be significant in mosquito control management programmes in different climatic conditions (Medina *et al.*, 2003).

Insects including mosquitoes become resistant due to extensive use of various insecticides worldwide (Chandre *et al.*, 1999). Resistance to *Bti* is due to reduced binding of these agents to the epithelial lining of the lumen in the insect gut (Escriche *et al.*, 1995) or by the action of gut proteases that enhanced the process of digestion of insecticides. Resistance against *Bti* was documented in the field populations of *Ae. aegypti* and *Ae. vexans* (Goldman *et al*., 1986; Becker and Ludwig, 1993).

A moderate level of resistance (2.82-fold) to *Bti* was also reported in field collected larvae of *Culex pipiens* as a result of 20 generations of laboratory selection (Saleh *et al.*, 2003). Resistance ratio (RR) in *Cx. quinquefasciatus* colony was found 13-fold in 22 generations against *Bacillus thuringiensis* subspecies *jegathesan*. However, RR dropped to 2.3-fold and remained low in 26-40 generations (Wirth *et al.*, 2004). Furthermore, several studies revealed that *Bsph* strains 2362, 1593M, and C341 have showed the significant level of resistance in field populations of the *Cx. pipiens* and *Cx. quinquefasciatus* larvae in India, France, Brazil, China, and Thailand (Rao *et al.*, 1995; Yuan *et al.*, 2000; Mulla *et al.*, 2003).

To date, no study was reported on the susceptibility status of dengue vectors against different biological or chemical control agents from

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Pakistan. Since the disease cases re-emerged annually in urban areas of Pakistan, it is a dire need to study the susceptibility status of dengue vectors against different control agents for effective control strategy in future. The main objective of the current study was to evaluate the resistance / susceptibility status of early 4th instars *Ae. aegypti* larvae against microbial larvicide (*Bti* WDG) in selected locality of Lahore, Pakistan.

MATERIALS AND METHODS

Wild collection of Aedes *larvae as resistant strain*

Immature early 4th instars *Aedes* larvae were collected from artificial containers such as discarded jars, used tires, 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 population of *Ae. aegypti* was maintained in GCU insectory using standard protocol for rearing mosquitoes (Jahan and Hurd, 1997) since 2006 and used as a reference strain.

Identification of species

Field collected *Ae. aegypti* larvae were identified on the basis of morphological characteristics using identification keys (Rueda, 2004).

Test material for larval bioassays

Test strains of microbial larvicide *Bti* WDG (water dispersible granules) VectoBac® by Valent Bioscience Corporation: IL, USA with 3000 ITU (International Toxic Unit/mg) was used to evaluate the diagnostic dose and susceptible/resistance status in the susceptible and field collected early 4th instars larvae of *Ae. aegypti*.

Experimental protocol

Tests were performed with 7 different concentrations of *Bti* (WDG) (100, 40, 20, 10, 5, 2.5, 1.25 ppm) in distilled water. Each concentration was replicated three times and three untreated cups were used as control (containing water) for both susceptible and field collected populations of *Ae. aegypti* larvae. In order to determine a diagnostic dose, 25 early 4th instars larvae were placed in each concentration dissolved above in plastic cups of 200 ml capacity. Mortality in each concentration was counted by separating dead larvae with the help of camel hair brush after every 15 minutes. No food material was added during whole experiment. Moribund larvae were considered as dead. The diagnostic dose (minimum concentration that kills hundred percent of susceptible population in fixed time i.e. 30-60 minutes) was used to evaluate the resistance status in field collected population of *Ae. aegypti* larvae.

Data analysis

The results were analyzed using Probit-regression analysis Raymond (1985) to determine LT_{50} (lethal time in minutes for 50% death) and LT_{90} (lethal time in minutes for 90% death). Resistance ratio (RR) was calculated by dividing the lethal time of the field strain by the lethal time of the susceptible strain.

RESULTS AND DISCUSSION

In the present study, the diagnostic dose of *Bti* WDG for the susceptible early 4th instars of *Ae. aegypti* was 10 ppm post 30 minutes exposure (Fig.1) while the same concentration kills 100% of field collected larvae in 60 minutes (Fig. 2).

Field population of early 4th instars of *Ae. aegypti* was found 10 fold resistant as compared to susceptible *Ae. aegypti* larvae, where 100% mortality occurred against 100 ppm at the same time period i.e. post 30 minutes exposure (Fig. 3). These results indicated that field collected population (Misri Shah, Lahore) of early 4th instars *Ae. aegypti* larvae were highly resistant against *Bti* WDG. The first report of resistance to *Bt* (Dipel) was documented in Indian meal moth (*Plodia interpunctella*). There was 100-fold increase in resistance in a population after 15 generations of laboratory selection with Dipel (McGaughey, 1985) and 250-fold following 36 generations (McGaughey and Beeman, 1988). Goldman *et al.* (1986) observed low level of resistance (2-fold LC_{50}) after 14 generations of selection pressure with *Bti* in the field strain of *Ae. aegypti* larvae. In addition, Saleh *et al* (2003) reported 2.78-fold increase in tolerance to *Bti* in *Cx. pipiens* larvae as a result of 20 generations of

selection pressure. In current study, resistance ratio against *Bti* WDG in early 4th instars *Ae. aegypti* at LT_{90} was 2.22 and LT_{50} was 1.97 (Table I) categorized low level of resistance as compared to the above mentioned authors. However, in the current study, 10X dose required to kill 100% field collected larvae as compared to susceptible (laboratory-reared) population. Becker and Rettich, 1994 reported that higher doses were required to control wild mosquitoes as compared to laboratory conditions.

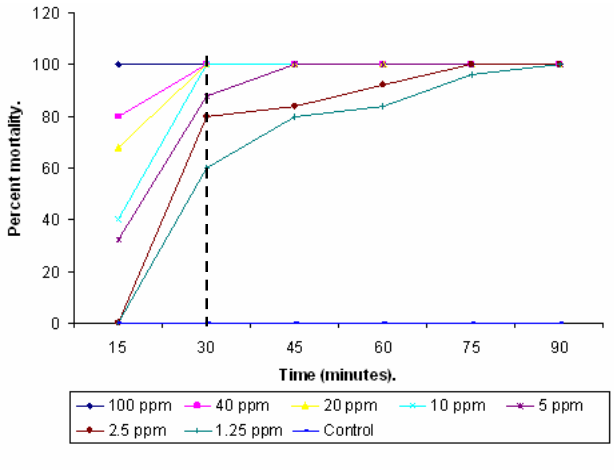

Fig. 1. A range of concentrations (*Bti* WDG) used for finding diagnostic/saturation dose in susceptible *Aedes aegypti* larvae by CDC larval bioassays.
10 ppm = Diagnostic dose (100% mortality) post 30 minutes exposure.

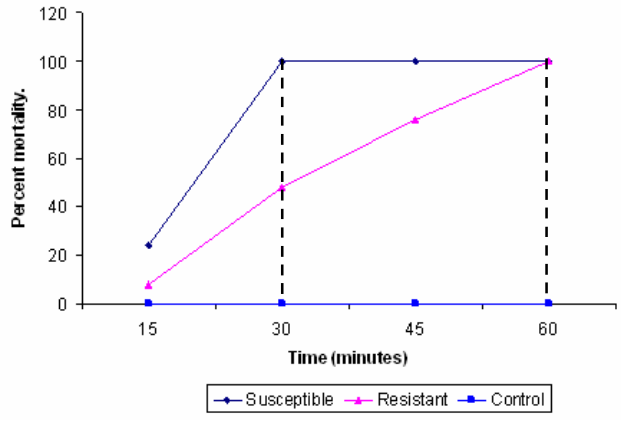

Fig. 2. A comparison of susceptible and field collected populations of *Aedes aegypti* against diagnostic dose (10ppm) of *Bti* WDG.
Susceptible population: Hundred percent mortality post 30 minutes exposure.
Resistant population: Hundred percent mortality post 60 minutes exposure.

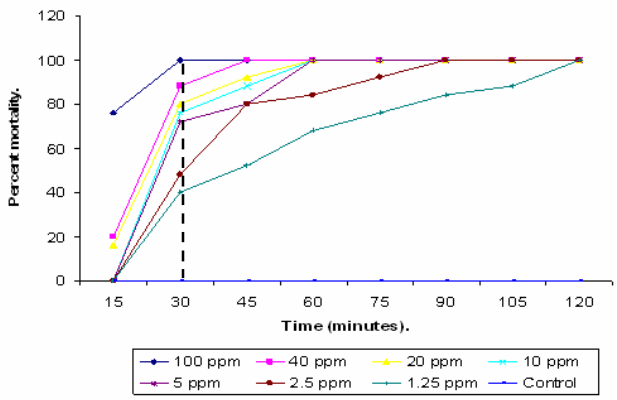

Fig. 3. Evaluation of resistance in field collected *Aedes aegypti* larvae by CDC larval bioassays against various concentrations of *Bti* WDG. 100 ppm = Hundred percent mortality of field collected populations post 30 minutes exposure.

A limited work has been reported against chemical insecticide resistance in *Anopheles* and *Culex* mosquitoes in Pakistan. Resistance against DDT, malathion and dieldrin, in *An. culicifacies* (rural malarial vector in Pakistan) and *An. stephensi* (urban malarial vector in Pakistan) mosquitoes has been confirmed from province Punjab, Pakistan (Rathor *et al.*, 19855).

Although there is no report of the exposure of these larvae against *Bti* WDG in any locality of Lahore, Pakistan. Current study will be useful to evaluate the effectiveness of the *Bti* WDG in future planning for the control of dengue vectors.

In conclusion, insecticide resistance surveillance is essential for resistance management in those areas where selective insecticides are applied for the dengue vector control annually. The monitoring of 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 for effective vector control is needed in Pakistan.

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

Insecticide	Mosquito strain	LT_{50} (min.) 95%CL	LT_{90} (min.) 95%CL	Resistance ratio RR_{LT50}	Resistance ratio RR_{LT90}
Bacillus thuringiensis	S	17.363	21.969	1.97	2.22
Israelensis	R	34.182	48.165		
(WDG) (10 ppm)		(30.213-38.050)	(43.486-55.979)		

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.

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