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Susceptibility of Laboratory-Reared *Anopheles stephensi* (Diptera: Culicidae) and Field-Collected *Culex quinquefasciatus* Larvae to *Bacillus thuringiensis* serovar. *israelensis* and *Bacillus sphaericus* in Lahore, Pakistan

Nusrat Jahan and Naila Hussain
Department of Zoology, G.C. University, Katchery Road, Lahore 54000, Pakistan

Abstract.- Susceptibility of late 3rd instars laboratory-reared *Anopheles stephensi* and field-collected *Culex quinquefasciatus* in Lahore, Pakistan to a technical powder of *Bacillus thuringiensis* serovar. *israelensis* (*Bti*) and a technical powder of *Bacillus sphaericus* (*Bsph*) were studied in the laboratory. At 24 h post-treatment the LC_{50} and LC_{95} values for *An. stephensi* amounted to 0.041 and 0.11 ppm, respectively. At 48 h post-treatment, these values were reduced to 0.025 ppm (LC_{50}) and 0.083 ppm (LC_{95}). In comparison, *Cx. quinquefasciatus* larvae at 24 h post-treatment required 0.048 ppm *Bti* to achieve LC_{50} level and 0.128 ppm to achieve LC_{95} level; the 48 h LC_{50} for this species was exceptionally low (0.002 ppm). In general, both species exhibited somewhat similar levels of larval susceptibility to the technical powder of *Bti*. The LC_{50} and LC_{95} values of *Bsph* against *An. stephensi* at 24 as well as 48 h post-treatment were rather high, exceeding 1 ppm at the LC_{95} level. However, *Cx. quinquefasciatus* larvae were highly susceptible to *Bsph* with LC_{50} and LC_{95} values of 0.043 and 0.12 ppm, respectively at 24 h, and at 48 h posttreatment, 0.008 ppm (LC_{50}) and 0.11 ppm (LC_{95}).

Key Words: Malaria vector, *Bacillus sphaericus*, *Culex quinquefasciatus*, laboratory bioassays.

INTRODUCTION

In Pakistan, malaria is common in rural and urban areas and the upsurge of the disease in the country is associated with many factors including the development of resistance to malaria drugs, such as chloroquine and sulfadoxine-pyremethamine, prolonged warmer climate suitable for malaria transmission (Bouma *et al.,* 1996), a chronic decline in vector control activities, massive increase in human population growth, extensive irrigation network and poor sanitary conditions (Country Report, 2003).

In the city of Lahore (Punjab Province), mosquitoes of three genera, *Culex, Anopheles,* and *Aedes* predominate. Large populations of these mosquitoes occur almost year round. Among these, *Anopheles* spp. not only cause a severe biting nuisance primarily in summer months but are also a serious threat to public health due to their potential for malaria transmission. Thus far, more than 22 species of *Anopheles* have been reported from Pakistan (Country Report, 2003). Among these, *An. culicifacies* is a confirmed primary vector of malaria in rural areas (Mahmood *et al.,* 1984); whereas vectorial capacity of *An. stephensi* has been confirmed only in the laboratory (Pervez and Shah, 1989). *An. stephensi* has been generally considered to be a malaria vector of urban areas (Rehman and Mutalib, 1967); however, recent evidence from rural areas of Punjab, suggests that it may be an important vector in rural areas as well. Rowland *et al.* (2000) reported that in rural areas *An. stephensi* was five times more prevalent than *An. culicifacies* and malaria cases peaked when *An. culicifacies* had disappeared.

Culex quinquefasciatus, is a major vector of lymphatic filariasis throughout the tropics, has been incriminated in the transmission of West Nile virus in Pakistan and India (Peiris and Amerasinghe, 1994).

Larval and adult mosquitoes have primarily been controlled with a variety of chemical pesticides. The emergence of resistance in insect populations to chemical pesticides has led to increased interest in biological control agents, including some naturally occurring entomopathogenic bacteria, such as *Bacillus*

* Corresponding author: dr.nusratjahan@gcu.edu.pk
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thuringiensis serovar. *israelensis* (*Bti*) and *Bacillus sphaericus* (*Bsph*). These two biological insecticides, due to their environmental safety and specificity to nematoceran Diptera (especially mosquitoes), have become mosquito control agents of choice almost throughout the world (Becker, 1998; Fillinger *et al.,* 2003).

To date, studies concerning *Bti*, *Bsph*, or other biological control agents against mosquitoes in Pakistan including vectors of malaria are rather limited (Rathor *et al.,* 1985). The present study evaluates larvicidal effects of one technical powder each of *Bti* and *Bsph* against laboratory–reared *An. stephensi* and field collected *Cx. quinquefasciatus*.

MATERIALS AND METHODS

Bacillus formulations

A technical powder of *Bti* (VectoBac® containing 5000 International Toxic Units (ITU)/mg of *Bti*) and a technical powder of *Bsph* (VectoLex® 1,380 ITU/mg of *Bsph* (Valent Biosciences Corp; Illinois, USA) were used in the experiments.

Mosquito larvae

An. stephensi (a strain maintained since 1979 at the Malaria Research Center, Lahore) were reared under standardized conditions at 27±3°C, 80±3% RH and a photoperiod of 16:8 (L:D) h. Larvae for the bioassays were reared in batches of 300 each, in 1200 ml deionised water in stainless steel trays (35x30x5 cm). Each batch was fed with two drops of 10% sugar and a yeast suspension of 0.02% daily for first instars and thereafter with finely ground fish food that is available in local market. Measured amounts of larval food (with respect to larval age) were applied to the surface of water until the larvae developed to late 3rd instars (6-9 days post-hatching). *Cx. quinquefasciatus* (3rd instar) were obtained from periodic field collections made from stagnant water pools at Jinnah Gardens, Lahore.

Bioassay procedure

For mosquito bioassays, 25 late 3rd instars of each species were placed in disposable cups (7.8 cm diameter) containing 200 ml distilled water. From a 100 ppm stock suspension of each biocide, serial dilutions (10, 1, 0.1, 0.01, 0.001, and 0.0001ppm) in deionized water were made using a magnetic stirrer. Three replicates of each concentration with three untreated cups serving as control were used in each evaluation to determine the range of larval mortality. Larval mortality in each treated cup was scored at 24 and 48 h post-treatment with *Bti* or *Bsph*; any mortality in corresponding control cups was also checked at these times. Mortality in treatments was corrected for any control mortality in each evaluation. and percentage reduction in each group was calculated using the following formula;

$$\text{Percentage reduction (\% RD)} = NC - NT / NC \times 100$$

Where NC = No. of larvae in control and NT = No. of larvae in treatment

Data analysis

Bioassays data from all replicates of each mosquito species for each biocide were individually pooled and analyzed using computer software SPSS 10 for Probit – regression analysis to estimate the dosage response of exposed larvae (Finney, 1971); LC_{50}, LC_{95}, at 95% confidence limits of each lethal level and slope values were determined in each group.

RESULTS

Susceptibility data of laboratory-reared 3rd instar *An. stephensi* and field-collected *Cx. quinquefasciatus* to the technical powder of *Bti* are shown in Table I. At 24 h post-treatment the LC_{50} and LC_{95} values for *An. stephensi* amounted to 0.041 and 0.11 ppm, respectively. At 48 h post-treatment, these values were reduced to 0.025 ppm (LC_{50}) and 0.083 ppm (LC_{95}). In comparison, *Cx. quinquefasciatus* larvae at 24 h post-treatment required 0.048 ppm *Bti* to achieve LC_{50} level and 0.128 ppm to achieve LC_{95} level; the 48 h LC_{50} for this species was exceptionally low (0.002 ppm). In general, both species exhibited somewhat similar levels of larval susceptibility to the technical powder of *Bti*. Table II summarizes laboratory susceptibility data of *An. stephensi and Cx. quinquefasciatus* to *Bsph*. The LC_{50} and LC_{95} values of *Bsph* against *An. stephensi* at 24 as well as 48 h post-treatment were rather high, exceeding 1 ppm at

the LC_{95} level. However, *Cx. quinquefasciatus* larvae were highly susceptible to this biocide with LC_{50} and LC_{95} values of 0.043 and 0.12 ppm, respectively. At 48 h posttreatment, these values declined to 0.008 ppm (LC_{50}) and 0.11 ppm (LC_{95}).

Table I.- Susceptibility of 3rd instar laboratory-reared *An. stephensi*[1] and field-collected *Cx. quinquefasciatus*[2] mosquitoes to a technical powder of *B. thuringiensis* serovar. *israelensis* (VectoBac®, containing 5,000 International Toxic Units/mg).

Lethal concentration (ppm)					
Time (h)	LC_{50}	95% CL	LC_{95}	95% CL	Slope
An. stephensi					
24	0.041	0.008-0.18	0.11	0.06-0.59	5.15
48	0.025	0.004-0.078	0.083	0.042-0.46	3.28
Cx. quinquefasciatus					
24	0.048	0.021-0.115	0.128	0.081-0.332	5.78
48	0.002	0.001-0.018	0.11	0.068-0.29	2.91

[1]Laboratory strain maintained since 1970 at Lahore, Pakistan.
[2]Field-collected from Lahore, Pakistan.

DISCUSSION

Previously the only *Bti* evaluation conducted against *An. stephensi* in Pakistan is that of Rathor *et al.* (1985). These authors using an aqueous suspension of *Bti* (ABG-6145) containing 587 ITU *Bti* / mg had reported complete mortality of the larvae at 1 ppm dosage. Our results indicating LC_{95} value of 0.083 ppm at 48 h exposure of *An. stephensi* larvae are comparable to the results of Rathor *et al.* (1985) considering the potency difference of the *Bti* preparation used in the two studies.

In the present study *Bti* TP showed high susceptibility of *Cx. quinquefasciatus* LC_{50}-LC_{95} ranges 0.002-0.11 as compared to *An. stephensi* 0.025-0.083 after 48 hours exposure (Table I). However, LC_{50}-LC_{95} (0.59-1.04) value of *Bsph* (Table II) at 48 h post exposure for *An. stephensi* indicated that *An. stephensi* has lower susceptibility to *Bsph* than *Bti*. Difference in levels of susceptibility of various mosquito species to various test strains of *Bti* and *Bsph* has been reported by many authors (Ali *et al.*, 1984; Majori *et al.*, 1987; Fillinger *et al.*, 2003).

Table II.- Susceptibility of 3rd instar laboratory-reared *An. stephensi*[1] and field-collected *Cx. quinquefasciatus*[2] mosquitoes to a technical powder of *B. sphaericus* (VectoLex®, containing 1,380 International Toxic Units/mg).

Lethal concentration (ppm)					
Time (h)	LC_{50}	95% CL	LC_{95}	95% CL	Slope
An. stephensi					
24	0.73	0.60-0.89	1.24	1.06-1.53	5.8
48	0.59	0.47-0.75	1.04	0.86-1.31	4.06
Cx. quinquefasciatus					
24	0.043	0.030-0.061	0.12	0.08-0.157	4.23
48	0.008	0.007-0.009	0.11	0.073-0.14	3.04

[1]Laboratory strain maintained since 1970 at Lahore, Pakistan.
[2]Field-collected from Lahore, Pakistan.

Laboratory studies of *Mittal et al.* (2001) and Sharma *et al.* (2003) reported LC_{50} for *An. stephensi* larvae 0.14 and 0.221 ppm against *Bti* in Delhi and in foothills of Uttaranchal India respectively. These results are higher than our values mentioned above, suggesting that the present formulation is more toxic for our *An. stephensi* colony.

Majori *et al.* (1987) evaluated Bactimos® WP 3500 ITU/mg and VectoBac® (WP) 2000 ITU/mg against *An. gambiae*. These authors reported LC_{50} 0.081 ppm and 0.110 ppm and LC_{90} 0.231 ppm, 0.375 ppm at 24 h post exposure against the above mentioned two formulations respectively. These values were also higher as compared to our study (0.041-0.11 ppm) for *An. stephensi*. It could be due to the difference in formulation, potency and different species of mosquito. However, LC_{50}- LC_{90}

range 0.04 – 0.107 ppm to Bactimos® for *Culex nigripalpus* (Ali *et al.*, 1984) comparable to our data 0.048– 0.12 ppm for *Cx. quinquefascicatus* at 24 hours post exposure (Table I).

High sensitivity of *An. gambiae* to *Bti* (WDG) was found by Fillinger *et al.* (2003) where LC_{50} - LC_{90} was 0.02 ppm – 0.210 ppm in the laboratory bioassays after 24h exposure. These findings correspond well to our results (0.025-0.083 ppm) for *An. stephensi* 48 hours post exposure. *Culex* and *Anophelese* species are supposed to be more susceptible against *Bsph* Bin toxin than *Aedes* larvae (Charles and Nielson Le - Roux 2000). We confirmed the previous findings that field collected *Culex* species are more susceptible as compared to *Anopheline* after 48 hours exposure to both formulations. However, extensive field tests are needed to investigate the optimum dosage to control *Anoheline* and *Culex* species in Pakistan.

It is evident from this study that *Bti* and *Bsph* are highly toxic larvicides of *An. stephensi* and *Cx. quinquefascicatus* in Lahore Pakistan. These bacteria could be applied in the field with minimum cost effective rates for quick reduction of the larvae. In addition these bacteria are safe to non-targets organisms co-existing with mosquito larvae. Thus these are useful biological control agents for *Anopheline* and *Culex* species of tropical countries.

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