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Efficacy of *Bacillus thuringiensis* var. Kurstaki in the control of two mosquito species (*Anopheles stephensi* and *Culex quinquefasciatus*)

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Abstract: *Bioinsecticide Bacillus thuringiensis var. kurstaki (Btk) was used for controlling the mosquito species (Anopheles stephensi and Culex quinquefasciatus) which gave a significant ($p<0.05$) mortality in both species. The higher concentration of Btk was highly effective compared to the control ones. The controlling effect was dose and time dependent. Among the studied mosquitoes the C. quinquefasciatus (LC_{50} 0.154%) was more susceptible than A. stephensi (LC_{50} 0.372%) towards the bioinsecticide Btk.*

Key words: *Bacillus thuringiensis var. kurstaki (Btk), Mosquitoes, Anopheles stephensi, Culex quinquefasciatus*
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Introduction

Mosquitoes, the dipteran insects acts as pests and vectors of many dreaded human diseases such as malaria, filaria, dengue/ dengue hemorrhagic fever (DHF) *etc*. Among the anophelines, *A. stephensi* is an important vector of malaria while among the culicines, *C. quinquefasciatus* and *Aedes aegypti* are the vectors of filaria and dengue/DHF respectively. Development of resistance to various types of insecticides such as organochlorides, organophosphates and carbamates (Singh and Bansal, 2001; Bansal and Singh, 2002, 2007; Shanmugasundaram *et al.,* 2008) poses serious threat to the conventional control measures for vectors. Such insecticides pollute water bodies, air and land. There is every likelihood of development of pesticide resistant strains of mosquitoes due to over dependence on chemical toxicants. In recent years, several pathogenic strains of the *Bacillus thuringiensis* (*Bt*) and *B. sphaericus* were reported to posses a high level of biological activity against mosquito larvae in laboratory and field studies (Davidson *et al.,* 1981; Barjac and Sutherland, 1990). Highly mosquitocidal strains of *B. sphaericus* produce a protein binary toxin which is toxic to mosquito larvae. Many western country researchers have reported biological activity of *Bt* to control the mosquito larvae both in field and laboratory as a safer insecticidal alternative (Charles *et al.,* 1996; Nielsen-Le-Roux *et al.,* 1997; Wirth and Georghiou, 1997; Berber *et al.,* 2004). Although this bacterium is highly toxic to mosquito larvae, but in India very few reports are available on this aspect (Balaraman and Hoti, 1987; Vasuki *et al.,* 1995). The present study was therefore undertaken to evaluate the efficacy of *Btk* for the control of mosquito vectors in laboratory.

Materials and Methods

Fully fed female *A. stephensi* and *C. quinquefasciatus* were collected early in the morning from sheep and cattle sheds in Erode town with the help of mosquito collecting jar. Cages (32x22x21cm) were used for rearing of mosquitoes, provided with cotton pads soaked in 10% glucose solution. Enamel tray (15x15x6 cm) filled with 500 ml water was kept in the cages for laying eggs. The eggs were maintained in the laboratory at 25±1°C and 70% relative humidity with a photoperiod of 12 hr. The newly hatched larvae were transferred into plastic rearing trays (21x15x9 cm) and fed on 40% brewers yeast and used for the trials. Pupae were separated from the trays and maintained separately. The *B. thuringiensis* var. kurstaki (*Btk*) used in this study were purchased from Tamil Nadu Agricultural Department at Thindal in Erode, TN, India. Growth and sporulation conditions were followed as described by Stewart *et al.* (1981).

Preparation of sample: 4.42×10^{10} spores in 2 milliliter of bacterial samples 2.2×10^{10} spores per milliliter from cultures incubated for 48hr were transferred into sterile Eppendorf tubes and kept at -70°C until use. Experimental concentrations (2, 4, 6, 8 and 10%) were prepared from the stock culture of *Btk* as per method described by Berber *et al.* (2004) for bioassay tests. Control tests were also conducted by adding only tap water.

Bioassays: The tests were carried out in the laboratory at a temperature of 28±1°C and 70% RH. The tests were repeated four to five times to investigate variation and average was taken for deriving inferences.

To each of the beaker containing different test concentrations, 25 healthy, 4th instar larvae were released. Percent mortalities were calculated 8 hr later by counting both dead and moribund larvae as per instructions given by WHO (1981). Larvae were considered moribund if they failed to flex head to siphon when provoked with a glass rod.

Table - 1: The toxicological status and log probit analysis of the mortality of *Bacillus thuringiensis* var. *kurstaki* to the larvae of two species

Mosquito species	Concentrations (%)	No. of mortality during the end of 8 hr	Percent experimental mortality	Percent corrected mortality	Regression equation	Chi-square	LC_{50} (%)	LC_{90} (%)
Anopheles	2	5	42	36.75	5.506+2.588(3.3010-3.595)			
stephensi	4	8	67	64.01	5.506+2.588(3.6021-3.595)			
	6	10	83	81.46	5.506+2.588(3.7782-3.595)	0.1301	0.3719	1.9943
	8	11	92	91.28	5.506+2.588(3.9031-3.595)			
	10	12	100	100	-	-		
Culex	2	7	58	54.20	5.718+1.861(3.3010-3.599)			
quinquefasciatus	4	9	75	72.74	5.718+1.861(3.6021-3.599)			
	6	10	83	81.46	5.718+1.861(3.7782-3.599)	0.1369	0.1541	3.5019
	8	11	92	91.28	5.718+1.861(3.9031-3.599)			
	10	12	100	100	-	-		

Statistical analysis: The results of larvicidal activity related to different bacterial concentrations was determined at the 95% confidence limit ($p<0.05$). The LC_{50} and LC_{90} values were computed using log probit regression analysis (Finney, 1971) and Chi-square distribution for the expected mortalities (Fisher and Yates, 1963).

Results and Discussion

The results of comparative efficacy of *Btk* against two species of mosquitoes (*A. stephensi* and *C. quinquefasciatus*) and the larvicidal activities of five test concentrations of *Btk* after 8 hr are given in Table 1. In the present study, the author observed some behavioural changes in the *Btk* treated mosquitoes like generally ceased feeding, reduced activity, extreme sluggishness, paralysis after ingestion and finally death. The observed behavioural changes were also observed by Chilcott *et al.* (1990) who have reported that *Bacillus thuringiensis* (*Bti*) treated mosquito larvae generally cease feeding within 1 hr, show reduced activity by 2 hr, extreme sluggishness by 4 hr and general paralysis by 6 hr after ingestion. From the Table 1 it is very clear that all the treated concentrations of *Btk* are very effective even at very low concentration against both mosquito species. The larvae of *C. quinquefasciatus* proved more susceptible than *A. stephensi* (Table 1). Comparatively no significant difference was observed in the larvicidal activities of *Btk* between the mosquito species but their larvae development was significantly controlled as compared to control group. Burke *et al.* (1983) reported that *B. sphaericus* 1543 lost its spore viability after 4 hr of UV exposure while its larvicidal activity remained unchanged. LC_{50} values of *Btk* at 10% concentrations were 0.3719; 0.1541% and LC_{90} were 1.9943; 3.5019% for *A. stephensi* and *C. quinquefasciatus* respectively (Table 1) which indicates that at higher concentration of *Btk* proved more effective in knocking down the 4th instar larvae. Complete susceptibility of many mosquito species towards *Bt* have also been observed by several authors (Laird, 1995; Chilcott and Wigley, 1993; Bansal and Singh, 2006).

Lethal toxicity data indicated that *C. quinquefasciatus* was more susceptible (LC_{50} : 0.154%) at 10% concentration of *Btk* compared to *A. stephensi* (LC_{50} : 0.372%). The present study was confirmed with the work of Ignoffo *et al.* (1981) who found that *B. thuringiensis israelensis* (*Bti*) was more effective against some species of lepidopterans. The LC_{50} for *Bti* against *Trichoplusia ni, Heliothis zea* and *H. virescens* were 109.6, 19.3 and 27.6 µg ml^{-1}, respectively. Corresponding values for *Btk* were 15.9, 2.0 and 7.8 µg ml^{-1}.

Among the five different concentrations studied in the present investigation, minimum percentage of *Btk* are enough to kill the larvae of *C. quinquefasciatus* than *A. stephensi* (Table 1). It was clearly showed that among the two species studied the *C. quinquefasciatus* larvae are more susceptible with short period (Table 1) than the *A. stephensi.* It is evident from the study of Chen *et al.* (1984) on *Bt* showed that the LC_{50s} for *Culex pipiens pallens*, *Anopheles stephensi* and *Aedes albopictus* species were 0.55, 2.05 and $6.37x10^4$ spores ml^{-1}, respectively.

Ali *et al.* (1981) studied the efficacy of *Bti* formulation (R-153-78) and gave LC_{90} values of 4.56-9.84 ppm against the midges and LC_{50} values of 0.13-0.24 ppm against mosquitoes. This study clearly indicates that the *Bt* is more toxic against mosquitoes than the midges. This study was further supporting the present work.

In the present study, different percentage mortalities were observed in the both mosquito species (*A. stephensi* and *C. quinquefasciatus*) were treated against *Btk* (Table 1) at different concentrations. This was due to their mode of action of *Btk* involves the synergistic interaction of four toxic proteins and the mosquito larvae toxicity is due to the crystal protein formed during sporulation than other known bacterial strains.

The 100% mortalities of mosquitoes in 10% concentration of *Btk* may be due to that *Btk* products contain the spores and parasporal crystals of *Btk* H-3a, 3b serotype which must be ingested by the larval stage of mosquito to cause mortality. Following ingestion, the parasporal crystals are solubilised in the alkaline larval midgut, followed by proteolytic activation of the soluble insecticidal crystal proteins. The toxin binds to a receptor on the midgut cell wall resulting in pore formation in the cell, which leads to death of the larva.

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