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SUSCEPTIBILITY OF KARACHI MOSQUITOES TO TWO VARIETIES OF *BACILLUS THURINGIENSIS* BERLINER

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Laboratory bioassay of two strains of bacteria *Bacillus thuringiensis* var. *israelensis* (Bti) and *Bacillus thuringiensis* var. (Btk) were conducted against three larval age group of *A. aegypti* and *C. tarsalis*. Both bacterial varieties were found to be effective in all age groups of mosquitoes. In comparison *Bti* proved to more effective than *Btk*. In 24 hr exposure the LC_{50} of *Bti* and *Btk* were 0.06 ppm to 0.14 ppm and 0.15 ppm to 0.26 ppm for *A.aegypti and* 0.15 ppm to 0.2 ppm and 0.27 ppm to 0.55 ppm for *C.tarsalis* respectively. Younger larvae of both species of mosquitoes were more susceptible than older one.

Key words: Susceptible, Mosquitoes, *B. thuringiensis*

Introduction

During the last decade, strains of *Bacillus thuringiensis* Berliner have been developed into microbial pesticides for use against mosquitoes. Hall *et. al*. [1] reported several strains of *B. thuringiensis* with high levels of activity in bioassays against 5 species of *Aedes* and *Culex*. Later Goldberg and Margalit [2] isolated a new strain as *Bt.* var. *israelensis* from the milieu of a mosquito breeding pool in a drying river bed in Israel. These investigators observed that it rapidly kills larvae of mosquitoes. They also demonstrated that pathogenic activity was due to an endotoxin which has a property of heat stability and they designated it as serotype H-14. The bioassays of this new strain of *B. thuringiensis* have been conducted against several species of mosquitoes [3-5] and also black flies [6,7].

The objective of the study reported here was to determine the effect of age of mosquito larvae on their susceptibility to two varieties of bacterial *Bt.* var. *isrealensis* and *Bt.* var. *kurstaki*. To accomplish this their efficacy was assayed in laboratory conditions against two species of mosquitoes found in Karachi area.

Materials and Methods

Mosquito rearing: Both species of mosquitoes were reared under constant conditions of 28° ± 2° and 65% ± 5% RH for adults and 28° for larvae with 12 hours photoperiod. Adults were fed on white rats 3 times weekly and maintained between blood meals on 10% sucrose solution. The eggs were collected on moist paper towelling. Larvae were maintained in rearing tubs (30 cm x 15cm 1000 larvae per tub in 2.5 liters distilled water) and were fed on 1:1 mixture of liver powder, egg albumin and yeast. In order to ensure that the larvae tested on a given day were all in same age, special egg hatching procedures were employed. The eggs were soaked in distilled water for 30 min. under vacuum in a common laboratory desicator. The egg paper was then removed to prevent further hatch.

Bioassay procedure. Laboratory reared mosquitoes were bioassyed in 250 ml beaker in total volume of 100 ml of distilled water. Larvae of desired age were selected at random, placed in groups of 20 and 1 ml of food slurry was added to each beaker followed by the appropriate dilution of the *Bti* and *Btk*, formulated in 10 ml distilled water. The containers were then kept at the temperature 28° ± 2° and 70% RH until mortality was recorded at 24 hr post-treatment. Thus the larvae were exposed continuously to biopesticide for the period of 24 hr. Three age groups were tested for both species of mosquitoes at 2nd, 3rd and 4th instar larvae. Five groups of untreated controls (100 larvae) were carried out during each test.

The bacterial pesticides *Bti.* and *Btk.* were supplied by Abbott laboratory Chicago, USA. One gram of powder (biopesticide) was weighed and 100 ml distilled water was mixed in a waring blender and operated for one minute. Further dilutions were prepared from this stock solution which was continuously agitated. Fresh aqueous solutions were prepared weekly and refrigerated after mixing [8].

During screening tests, 8 or 9 doses were tested. Experiments were conducted so that at least 5 doses would give mortalities between 10% to 90%. Each dose was replicated 4 times in every test. The assay for a given age group was then repeated on three separate occasion. Thus a minimum of 240 larvae were tested at any given age group and dose. The results were expressed as LC_{50} values. The mortality percentages were corrected by Abbott's formula [9]. The LC_{50}'s were calculated from the tests carried out with *Bti* and compared with *Btk* in order to assess relative activity [10].

Results and Discussion

Table 1 and the graphs (Fig. 1-3) shows susceptibility of the 2nd, 3rd and 4th instar larvae of *A. aegyti* and *C. tarsalis* to *Bti* and *Btk*.

TABLE 1. SUSCEPTIBILITY OF LARVAE OF *A. A EGYPTI, C.TARSALIS* TO TWO SPECIES OF *BACILLUS THURINGIENSIS*.

Larval stage (instar)	LC_{50} ppm						LC_{90} ppm					
	B.T. Israelensis		B.T. Kurstaki		Relative Activity		B.T. Israelensis		B.T. Kurstaki		Relative Activity	
	Aedes aegypti	*Culex tarsalis*	*Aedes aegypti*	*Culex tarsalis*	*Aedes aegypti*	*Culex tarsalis*	*Aedes aegypti*	*Culex tarsalis*	*Aedes aegypti*	*Culex tarsalis*	*Aedes aegypti*	*Culex tarsalis*
2nd. instar	0.06	0.15	0.15	0.27	2.5	1.8	0.12	0.28	0.3	0.58	2.5	2.87
3rd. instar	0.1	0.17	0.2	0.5	2.0	2.94	0.25	0.37	0.54	0.9	2.16	2.43
4th. instar	0.14	0.2	0.26	0.55	1.857	2.75	0.25	0.35	0.38	1.1	1.52	3.14

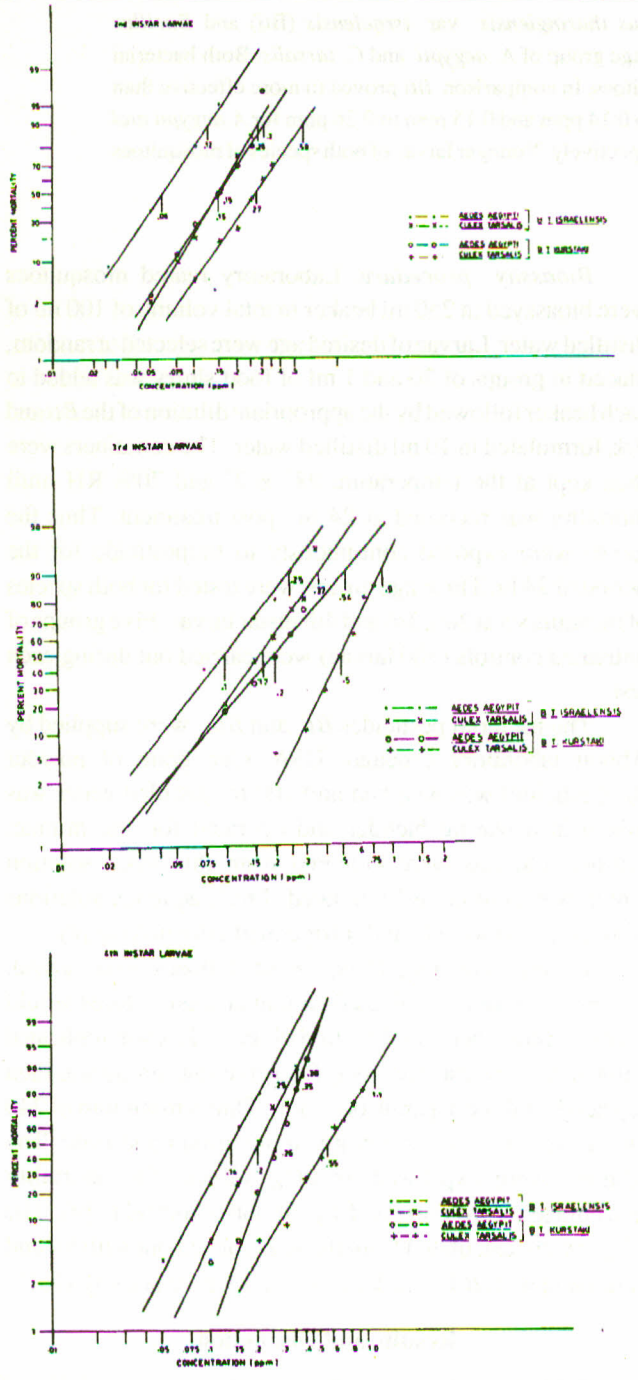

Fig. 1-3. Dosage mortality lines indicating relative susceptibility of *A. aegypti* and *C. tarsalis* larvae to *Bti.* and *Btk.*

Treatment with BTI . The LC_{50} value for 2nd, 3rd, 4th instar larvae of *A. aegypti* was 0.06 ppm, 0.1 ppm and 0.14 ppm respectively, after 24 hr. In case of *C. tarsalis*, the LC_{50} value for 2nd, 3rd and 4th instar larvae was 0.15 ppm, 0.17 ppm and 0.2 ppm respectively, after 24 hrs. exposure with *Bti.* It was also quite apparent from the figures, and the Table 1 that *A.aegypti* were more susceptible than *C. tarsalis.*

The LC_{50} was 0.12 ppm, 0.25ppm and 0.25 ppm for 2nd, 3rd and 4th instar larvae of *A.aegypt,* for *C.tarsalis* the values were 0.28 ppm, 0.37 ppm and 0.35 ppm respectively. These values showed the change in the pattern. In case of LC_{50} the younger larvae were more susceptible rather than the older ones but the LC_{90} values showed no significant difference in the toxicity with 3rd and 4th instar larvae.

Treatment with BTK. The LC_{50} against 2nd, 3rd and 4th instar larvae of *A.aegypti* were 0.15 ppm, 0.2 ppm and 0.26 ppm respectively. Simultaneously, the LC_{50} values against 2nd, 3rd and 4th instar larvae of *C.tarsalis* were 0.27 ppm, 0.5 ppm and 0.55 ppm respectively. These results showed that *Bti* was more active than *Btk.* Thus, relative activity was calculated as 2.5 ppm, 2.0 ppm, 1.85 ppm for 2nd, 3rd and 4th instar larvae of *A.aegypti* and as 1.8 ppm, 2.94 ppm and 2.75 ppm for *C. tarsalis* respectively.

The LC_{90} values of *Btk* against 2nd, 3rd and 4th instar larvae of *A. aegypti* were 0.3 ppm, 0.54 ppm and 0.38 ppm respectively. For *C. tarsalis* the values were 0.58 ppm, 0.9 ppm and 1.1 ppm respectively. The data of LC_{90} values for 3rd and 4th instar larvae of *A. aegypti* and *C. tarsalis* with the treatment of *Btk* followed the same pattern as with *Bti.* The LC_{90} values calculated for *Btk* were also higher as *Bti.* The relative toxicity figures for 2nd, 3rd and 4th instar larvae of *A. aegypti* were 2.5 ppm, 2.16 ppm and 1.52 ppm; for *C.tarsalis* were 2.87 ppm, 2.43 ppm and 3.14 ppm respectively to the age groups.

The data indicated that these two varieties of *B. thuringiensis* were highly pathogenic to both species of mosquitoes. The excellent efficacy of these pathogens were very encouraging. As compare to *Btk* the variety *Bti* has proved to be more toxic against early stage of larvae than late. The values of LC_{50} in 24 hr exposure were enough to inactivate the larvae of *A.aegypti* and *C.tarsalis* respectively.

In variety *Btk*, few larvae died immediately and rest of them died slowly, a bit late. But in case of *Bti,* larval death was immediate and this was probably due to a quick-acting toxin.

It was determined that the high mortality causing factor was toxins in the spore of bacilli. The variability of the activity of strains or varieties of *B. thuringiensis* against insects depends on the serotype of the toxins, e.g. *Bti* possesses serotype H-14 and *Btk* contains serotype 3a, b [11]. In both *Bti* and *Btk* strains, serologically identical crystals K-1 were present and mosquitocidal activity is correlated with this crystal type [12].

Microscopic examinations revealed that septicemia was common place, indicating that actual invasion of the host body take place at some stage in the infection process with subsequent multiplication of bacterial cell within the tissue and haemolymph.

These investigations indicated that the variety Bti, as compared to *Btk.* was very effective in the laboratory against larvae of *A. aegypti* and *C. tarsalis*. This proves that it has a great potential for field control of species Aedes and Culex and other mosquitoes. However, differences in the rapidity and type of activity betwen *Bti* and *Btk* may make it desirable to combine the two to obtain more effective field control of mosquitoes. Further research on field trials should be carried out to evaluate the efficacy of these bacteria for the control of mosquitoes under natural conditions. As reported by Goldberg and Margelit [2], Garcia and Desrochers [5], anticipated field rates are estimated at between 1/4 and 2 kg/ha. This is equivalent of Ca 0.4 to 2.9 billion LC_{50} units/ha and 0.2 to 1.2 billion IU/ha based on 600 IU/mg bioassayed against *A. aegypti.* According to Ignoffo *et. al* · [13] that the kind of water, pond sediment and sunlight-UV, and settling in water might reduce available *Bti*. Thus research on these facts may be needed to increase field effectiveness. Our laboratory evidences indicate that these pathogens are promising controlling agents for mosquitoes and future efforts should be made to improve activity, stability, formulation and application procedure for open field.

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