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Laboratory evaluation of comparative toxicity of *Bacillus thuringiensis* subspecies to larvae of *Plutella xylostella* and *Bombyx mori*

C. GAILCE LEO JUSTIN, R.J. RABINDRA, S. JAYARAJ AND M. RANGARAJAN *
Centre for Plant Protection Studies, Tamil Nadu Agricultural University, Coimbatore 641 003.

ABSTRACT

Laboratory bioassay tests on the comparative efficacy of eight *Bacillus thuringiensis* (*B.t.*) subspecies against third instar larvae of *Plutella xylostella* L. as well as its safety to second instar larvae of *Bombyx mori* L. revealed that *B.t.* subsp. *kurstaki* (Bactospeine) was the most toxic to larvae of *P. xylostella* followed by *B.t.* subsp. *thuringiensis* and *B.t.* subsp. *kurstaki* (lab culture). The *B.t.t.* and *B.t.k.* (Bactospeine) were found to be less toxic to the larvae of *Bombyx mori* than the other species.

KEY WORDS: *Bacillus thuringiensis* subspecies, toxicity, *Plutella xylostella*, *Bombyx mori*

INTRODUCTION

The pathogenicity of *Bacillus thuringiensis* Berliner to any species of the host may vary greatly according to the variety of this pathogen employed (Angus and Norris, 1968; Yamvrias and Angus, 1970). It has been shown in a number of studies (Ridet, 1966; Burgerjon and Biache, 1967; Angus and Norris, 1968) that the ranking of the various known varieties of *B.t.* on the basis of the amount of whole culture necessary to evoke paralyses and/or death varies with the insect species tested. Hence it was of interest to identify a subspecies of *B.t.* that would be most toxic to the local strain of *Plutella xylostella* L. and less toxic to the silk worm *Bombyx mori* L.

MATERIALS AND METHODS

Lyophilized cultures of *B.t.* subspecies (*aizawai, darmstadiensis, entomocidus, galleriae, kurstaki, tolworthi*) available in the Department of Agricultural Microbiology, Tamil Nadu Agricultural University as well as those (*Sotto & thuringiensis*) received from the Division of Entomology, Indian Agricultural Research Institute, New Delhi were revived on nutrient agar and the pure clones were transferred on agar slopes. Similarly, *B.t.* subsp. *kurstaki* was also grown from Bactospeine, a commercial preparation. The slope cultures of all the subspecies were allowed to sporulate and then harvested on the same day in 10 ml aliquots of sterile distilled water aseptically. These suspensions were then mixed well and 10 ml of each inoculum was added to 200 ml aliquots of sterile nutrient broth in 250 ml capacity conical flasks. These conical flasks were incubated undisturbed as static cultures at room temperature. Complete sporulation was obtained on the fifth day. Employing differential staining technique, the presence of crystals in the culture was confirmed (Narayanan, 1985). The spore-crystal mixtures thus obtained were standardized based on haemocytometer count of spores (Fast, 1974) and all the cultures were adjusted to carry 10^7 spores/ml. Before using these suspensions in the bioassays, Teepol was added as a surfactant at 0.1% and mixed thoroughly.

Cauliflower leaves cut into circular discs of 6.0 cm diameter were dipped in the *B.t.* suspension containing 0.1% Teepol for 30 sec and the excess fluid drained off. After the leaf discs treated with suspensions had dried under room temperature, they were placed inside plastic containers (10 x 15 cm) with a moistened filter paper placed at the bottom of the containers to prevent the drying up of leaf discs. Third instar larvae of *P. xylostella* starved for 6 h were released on the treated leaves @ 10 / leaf disc. The treatments were replicated three times. Mortality counts were recorded at 6 h intervals for 48 h. Suitable controls were maintained.

For *B. mori*, the same method of treatment as described for *P. xylostella* was adopted except that the spore strength tested was 10^5 spores/ml and the leaves used were that of mulberry. The larvae were allowed to feed on the treated leaves for 24 h and subsequently on untreated mulberry leaf bits. Second instar larvae of *B.mori* were used for bioassay.

RESULTS AND DISCUSSION

The results of laboratory bioassays on the relative efficacy of eight different subspecies of *B.t.* including Bactospeine, the commercial preparation against *P. xylostella* indicated that all the subspecies at a dose of 10^7 spores/ml were equally effective causing 100 per cent mortality of third instar larvae of *P. xylostella* in 48 h. However, the LT_{50s} of different subspecies

* Department of Microbiology,
Tamil Nadu Agricultural University, Coimbatore 641 003.

varied. It was found that the *B.t.* subspecies, *kurstaki* cultured from the commercial product - Bactospeine recorded the lowest LT_{50} followed by *B.t.* subsp. *thuringiensis* and *kurstaki* (grown from lyophilized pure culture) (Table 1). There were not much differences in the LT_{50s} of the other *B.t.* subspecies *viz.*, *aizawai, darmstadiensis, entomocidus, galleriae, sotto* and *tolworthi.*

Results of bioassay of *B.t.* subspecies against second instar larvae of *B. mori* showed that all the *B.t.* subspecies produced mortality in the treated larvae which varied from 35.21 per cent in *B.t.* subsp. *kurstaki* (Bactospeine^{R}) to 56.99 per cent in *sotto* (Table 2). Mortality rates in *darmstadiensis, entomocidus, galleriae, kurstaki* (lab culture) and *tolworthi* were on par with that of *sotto*. Considering the LT_{50s} also it was found that *sotto* was the most toxic with the lowest LT_{50} of 32.93 h. The subspecies *thuringiensis* recorded the maximum LT_{50} value of 121.50 h. Eventhough *aizawai* and *kurstaki* (Bactospeine) had lower LT_{50s} when compared to that of *thuringiensis*, their fiducial limits overlapped. Thus, the results of these pathogenicity studies of different *B.t.* subspecies on *P. xylostella* and *B. mori* clearly indicated the difference in insecticidal activity among the various *B.t.* subspecies (Tables 1, 2). The variation in virulence was also observed in terms of speed of kill, eventhough 100 per cent kill of *P. xylostella* was achieved in all the subspecies in 48 h. Chilingaryan *et al.* (1969) in laboratory tests found variation in virulence of strains of *B.t.* subsp. *caucasicus, entomocidus* and *subtoxicus* against larvae of *P. xylostella*. Variation in the toxicity to different lepidoptera had been reported by Angus (1965), Vankova (1964) and Angus and Norris (1968). But Angus (1967) showed that preparations of the purified crystals *i.e.*, the *B.t.* delta endotoxin defined by Heimpel (1967) also differed in toxicity to *B. mori.*

The toxicity of *B.t.* mainly depends upon the delta endotoxin. Atleast 18 varieties representing 12 H-antigen serotypes with different capacity to produce various toxins are known and the delta endotoxins produced by different varieties have different spectra of insecticidal activity. Hence there seems to be a possibility to select and exploit strains with lesser toxicity to silkworm and greater toxicity to other crop pests (Ramakrishnan and Kumar, 1979). In the present studies, the *B.t.k.* (Bactospeine) was found to be more effective against *P. xylostella* but *B.t.t.*, *B.t.k.* (Bactospeine) were less toxic to the silkworm. Hence this subspecies can be exploited for use against *P. xylostella*. Aizawa (1975) isolated a serotype AF-101 with low toxicity to silkworm and high toxicity to other crop pests. There is scope for reducing further the toxicity towards silkworm and increasing the toxicity against *P. xylostella* by genetic engineering. A product of the subspecies *B.t. aizawai*, serotype 7 that is highly toxic to common cut worm, *A. ipsilon*, but harmless to silkworm recently developed by genetic engineering is being used as a microbial insecticide in Japan (Maramorosch, 1986).

TABLE 1. Probit analysis of time-mortality response of third instar larvae of *P. xylostella* to different *B.t.* subspecies at a concentration of 10^7 spores/ml.

B.t. subspecies	No. of larvae used	Chi$^{2@}$	Slope 'b'	LT_{50} (h.)	Fiducial limits 95%
aizawai	30	0.915	5.9175	27.25	24.68 - 29.73
darmstadiensis	30	2.390	7.1546	26.17	23.95 - 28.23
entomocidus	30	0.919	7.3200	27.73	25.55 - 29.86
galleriae	30	3.100	6.2982	29.83	27.37 - 32.51
kurstaki	30	2.760	7.3456	23.15	20.93 - 25.03
sotto	30	0.770	5.9526	26.89	24.36 - 29.34
thuringiensis	30	3.450	6.9426	22.60	20.21 - 24.57
tolworthi	30	1.380	7.0608	26.40	24.17 - 28.50
kurstaki (Bactospeine)	30	1.410	7.7956	21.90	19.71 - 23.70

@ All lines are significantly a good fit ($P < 0.05$)

TABLE 2. Probit analysis of time-mortality response of second instar larvae of *B. mori* to different *B.t.* subspecies at a concentration of 10^8 spores/ml.

B.t. subspecies	No. of larvae used	Chi²@	Slope 'b'	LT_{50} (h.)	Fiducial limits (95%)	Final mortality* (%)
aizawai	30	0.536	2.6322	50.16	40.89 - 75.97	43.07 bc
darmstadiensis	30	0.917	2.4284	39.34	32.96 - 52.79	52.77 a
entomocidus	30	0.434	2.0107	35.22	28.80 - 48.46	52.77 a
galleriae	30	0.491	2.7327	39.12	33.35 - 50.22	52.77 a
kurstaki	30	0.417	1.9946	40.51	32.70 - 61.55	48.84 ab
sotto	30	1.447	2.5672	32.83	28.06 - 40.43	56.99 a
thuringiensis	30	0.346	1.1785	121.50	59.37 -276.22	35.21 c
tolworthi	30	2.393	1.3338	41.59	33.28 - 65.52	54.99 a
kurstaki (Bactospeine)	30	2.438	2.3726	61.09	46.55 -122.10	35.21 c

@ All lines are significantly a good fit ($P<0.05$)

* In a column, means followed by similar letters are not different statistically ($P = 0.05$) by DMRT.

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