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Laboratory evaluation of toxicity of *Bacillus thuringiensis* subsp. *kurstaki* to larvae of mulberry silkworm, *Bombyx mori* L.

P.D. KAMALA JAYANTHI* AND K. PADMAVATHAMMA

Department of Entomology, S.V. Agricultural College, Tirupathi-517502, A.P. (India)

ABSTRACT : The toxicity of *Bacillus thuringiensis* subsp. *kurstaki* to different larval instars of mulberry silkworm, *Bombyx mori* L. was evaluated. The per cent larval mortality in all the instars of *B. mori* was higher at higher concentrations of *B.t.* but decreased with the decrease in *B.t.* concentrations. Higher mortality percentages were observed in the early instars as compared to later instars. The mortality rate decreased with the increase in age of the larvae. The LC_{50} was 1.40×10^1, 4.20×10^2, 1.0×10^3, 2.0×10^5 and 6.30×10^6 spores ml^{-1} for the first, second, third, fourth and fifth instar larvae, respectively. Longer incubation period was observed at lower concentrations of *B.t.* Higher concentrations of *B.t.* were associated with reduced pupation, higher pupal mortality, higher malformed adult emergence and lower emergence of normal adults in all the instars.

The raising of the domesticated silkworm, *Bombyx mori* L. is a popular agroforestry option in India. Sericulture can be practiced even in the most modest circumstances to provide a higher-value silk fibre with a ready world market, as also other useful by-products, such as high-protein animal feed and industrial oils. Recent trend of using insect pathogens particularly *Bacillus thuringiensis* Berliner (*B.t.*), one of the entomobacterial pathogens, for the control of almost all lepidopterous crop pests (Narayanan and Gopalakrishnan, 1988) is encouraging but fraught with risk to silkworm. This consideration necessitated to investigate the effect of *B.t.* on silkworm, *Bombyx mori* L., to obviate the threat to sericulture industry.

MATERIALS AND METHODS

'Dipel', a wettable powder formulation of *B.t.* subsp *kurstaki,* obtained from Lupin Laboratories Limited, Bombay, was multiplied by feeding bacteria contaminated groundnut leaves to the third and fourth instar larvae of *Spodoptera litura* (Fabricius). The bacteria were isolated from the diseased *S. litura* larvae and pure culture was prepared from it as described by Kiraly *et al.* (1974). Ten ml of distilled water was added to each agar slant and growth was harvested. The bacterial suspension so obtained was used as stock suspension.

For the development and maintenance of the mass culture of mulberry silkworm, *B. mori,* in the laboratory, the eggs were collected from SDRSI (Society for Development of Rural Sericulture Industry) Grainage, Tirupathi. The rearing of silkworm was followed in the

* Present address : CPD - Entomology, International Crops Research Institute for Semi-Arid Tropics (ICRISAT), Asia Region, Patancheru, Hyderabad-502324, A.P. (India).

same way as the commercial rearings are undertaken. The usual procedure of sterilization, hygenic conditions, brushing and chawki rearing of the larvae were followed as per Krishnaswami *et al.* (1988). The rearings were operated at an average room temperature of 27 ± 2°C and relative humidity of 70 to 80 per cent.

All the larval instars of uniform size from the laboratory culture were used for this study. Nine concentrations of *B.t., viz.*, 1×10^9, 1×10^8, 1×10^7, 1×10^6, 1×10^5, 1×10^4, 1×10^3, 1×10^2 and 1×10^1 spores ml^{-1} suspensions were used for infecting the larvae of each instar. Fifty larvae of all the instars starved for 6 hours were placed individually in glass vials (7.5 cm × 2.5 cm). Each of the nine concentrations of *B.t.* suspensions were smeared on leaves separately. The suspensions had 0.1 per cent teepol which served as wetting agent. The treated leaves were air-dried in shade and fed to the larvae for 24 hours. Fresh uncontaminated mulberry leaves were provided daily to *B. mori* larvae as suggested by Krishnaswami *et al.* (1988). Another set of 50 larvae of each instar were fed with mulberry leaves treated with sterile distilled water which served as control. Each treatment was replicated thrice. These experiments were conducted at 27±2°C with a relative humidity of 75 to 80 per cent. Daily observations were made on larval mortality, incubation period, rate of pupation, pupal mortality and emergence of malformed and normal adults. Microscopic examination was conducted in doubtful cases by preparing smears from dead larvae. The mortality rates of the larvae inoculated with different concentrations of *B.t.* (spores ml^{-1}) were analysed by probit anlaysis to determine the LC_{50}, lethal concentrations for 50 per cent larval mortality (Finney, 1964).

RESULTS AND DISCUSSION

The data on the incubation period of *B.t.*, larval mortality, rate of pupation, pupal mortality and extent of emergence of adults due to bacterial inoculation affecting all the instars of *B. mori* are presented in Tables 1-3. All the larval instars of silkworm were susceptible to *B.t.* subsp. *kurstaki.* The per cent larval mortality decreased with the increase in age and decrease in concentration. Incubation period was longer at lower concentrations with the increased pupation rates. The per cent pupal mortality increased with the increase in bacterial concentrations (Tables 1-3). Similar reports were documented by Al-Azawi (1964), who observed the susceptibility of silkworm larvae to *B.t.* subsp. *kurstaki.* He found that the per cent mortality decreased with the increase in age of the larvae and increased with the increase in bacterial concentrations. Also, Byakod (1974) observed the susceptibility of all the instars of *B.mori* to *Bactospeine* at various concentrations. Later, Manchev (1980) showed higher mortality percentages in early instars when compared with later instars in *B.t.* treated larvae of *B.mori.* In the present study, the incubation period was less and larval mortality was higher at higher concentrations of bacteria in all the instars. Also, it is evident that higher concentrations of bacteria were associated with reduced pupation, higher pupal mortality, higher malformed adult emergence and lower emergence of normal adults in all the instars of *B. mori* (Tables 1-3).

On the basis of LC_{50} values (Table 4) and the regression lines for all the larval

Table 1. Effect of *B.t.* on first and second instar larvae of *B. mori*

*B.t.**	Incubation period				Larval mortality (%)		Rate of pupation (%)		Pupal mortality (%)		Malformed adults (%)		Normal adults (%)	
	I		II		I	II	I	II	I	II	I	II	I	II
	Range	Mean	Range	Mean										
1×10^9	1-3	2.0	1-3	2.0	92.00	90.00	8.00	10.00	100.00	100.00	0.00	0.00	0.00	0.00
1×10^8	1-3	2.0	1-3	2.0	91.33	85.00	8.67	15.00	100.00	100.00	0.00	0.00	0.00	0.00
1×10^7	1-3	2.0	1-4	2.5	86.33	81.67	13.67	18.33	100.00	100.00	0.00	0.00	0.00	0.00
1×10^6	1-4	2.5	1-6	3.5	83.67	78.67	16.33	21.33	100.00	98.00	0.00	2.00	0.00	0.00
1×10^5	2-4	3.0	2-6	4.0	78.00	75.67	22.00	24.33	100.00	95.25	0.00	1.95	0.00	2.80
1×10^4	2-4	3.0	2-7	4.5	74.67	73.33	25.33	26.67	98.75	89.07	1.25	1.95	0.00	8.98
1×10^3	2-5	3.5	2-7	4.5	59.67	69.33	40.33	30.67	92.44	82.42	1.17	1.42	6.39	16.16
1×10^2	2-6	4.0	2-8	5.0	42.00	49.67	58.00	50.33	86.25	74.15	0.99	1.13	12.76	24.72
1×10	2-6	4.0	3-8	5.5	36.00	37.00	64.00	63.00	72.36	70.00	0.56	1.01	27.08	28.99
Control	0.00	0.00	0.00	0.00	0.00	0.00	100.00	100.00	0.00	0.00	0.00	0.00	100.00	100.00

* Concentration in spores^{-ml}.

Table 2. Effect of *B.t.* on third and fourth instar larvae of *B.mori*

*B.t.**	Incubation period				Larval mortality (%)		Rate of pupution (%)		Pupal mortality (%)		Malformed adults (%)		Normal adults (%)	
	III		IV		III	IV	III	IV	III	IV	III	IV	III	IV
	Range	Mean	Range	Mean										
1×10^9	2-3	2.5	1-3	2.0	83.00	90.00	17.00	10.00	100.00	100.00	0.00	0.00	0.00	0.00
1×10^8	2-4	3.0	1-3	2.0	80.33	85.00	19.67	15.00	96.45	100.00	3.21	0.00	0.34	0.00
1×10^7	2-5	3.5	1-4	2.5	76.00	81.67	24.00	18.33	89.75	100.00	2.79	0.00	7.46	0.00
1×10^6	3-5	4.0	1-6	3.5	72.67	78.67	27.33	21.33	88.12	98.00	2.25	2.00	9.63	0.00
1×10^5	3-5	4.0	2-6	4.0	66.00	75.67	34.00	24.33	81.24	95.25	2.00	1.95	16.76	2.80
1×10^4	3-6	4.5	2-7	4.5	51.67	73.33	48.33	26.67	79.99	89.07	1.85	1.95	18.16	8.98
1×10^3	4-6	5.0	2-7	4.5	50.00	69.33	50.00	30.67	75.33	82.42	1.12	1.42	23.55	16.16
1×10^2	5-7	6.0	2-8	5.0	42.67	49.67	57.33	50.33	72.07	74.15	1.10	1.13	26.83	24.72
1×10	5-7	6.0	3-8	5.5	37.33	37.00	62.67	63.00	68.99	0.00	0.95	1.01	30.06	28.99
Control	0.00	0.00	0.00	0.00	0.00	0.00	100.00	100.00	0.00	0.00	0.00	0.00	100.00	100.00

* Concentration in spores^{-ml}.

Table 3. Effect of *B.t.* on fifth instar larvae of *B. mori*

*B.t.**	Incubation period		Larval mortality (%)	Rate of pupation (%)	Pupal mortality (%)	Malformed adults (%)	Normal adults (%)
	Range	Mean					
1×10^9	3-5	4.0	56.67	43.33	82.12	9.71	8.71
1×10^8	4-6	5.0	52.00	48.00	77.07	8.23	14.70
1×10^7	5-8	6.5	48.00	52.00	72.12	8.00	19.88
1×10^6	5-8	6.5	41.67	58.33	68.99	7.52	23.49
1×10^5	8-10	9.0	33.33	66.67	65.19	7.12	34.69
1×10^4	10-11	10.5	30.67	69.33	50.17	6.56	43.27
1×10^3	11-12	11.5	27.67	72.33	50.00	5.00	45.00
1×10^2	11-13	12.0	16.00	84.00	45.00	4.75	50.25
1×10	11-13	12.0	12.33	87.66	42.22	3.25	54.53
Control	0.00	0.00	0.00	100.00	0.00	0.00	100.00

*Concentration in spores^{-ml}.

stadia depicted in Fig. 1, it is evident that the fifth stadium larvae required higher spores ml^{-1} (6.30×10^6) than the other larval instars. Likewise, gradual increase in LC_{50} values as the larval stage increased was reported by Deshmukh and Mathai (1991) in the case of *S.litura* to Dipel SL.

Obviously, *B.t.* subsp. *kurstaki* was toxic to *B.mori.* Hence, it may be judiciously used in pest control programmes in silk producing areas. Studies have to be carried out in evolving mutants of *kurstaki* which are non-sporulating and equally potent on lepidopterous pests. There are several strains and serotypes under *B.t.* other than *B.t.* subsp.*kurstaki* which

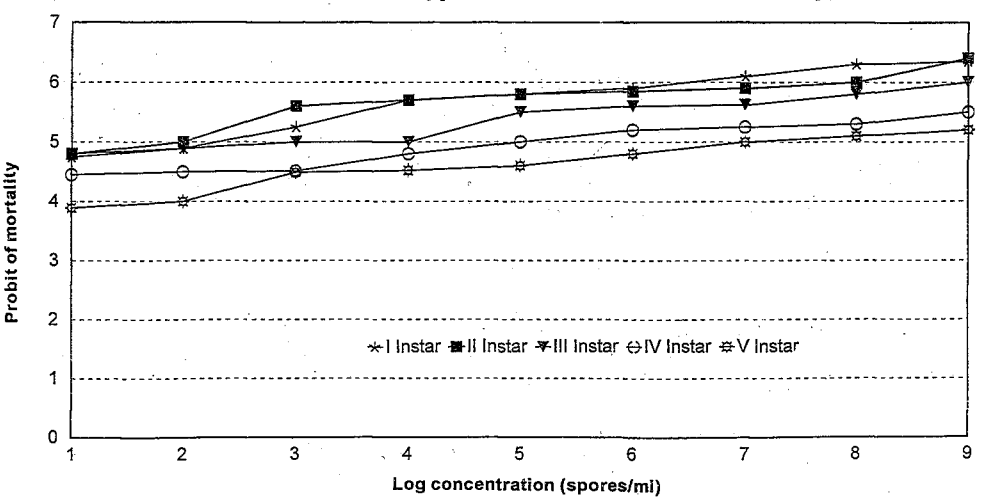

Fig. 1. Concentration - mortality regression lines for the first, second, third, fourth and fifth instar larvae of *Bombyx mori* fed with serial dilutions of *B.t.*

Table 4. LC_{50} values for different instars of *B. mori*

Larval instar	Heterogeneity χ^2 (n-2)	Regression equation	LC_{50} (spores ml^{-1})	Fiducial limits
I	11.919	y=0.2357x + 4.4871	1.40×10^1	1.4×10^2 1.0×10^1
II	12.379	y=0.1829x + 4.7024	4.20×10^2	2.67×10^2 8.39×10^1
III	2.642	y=0.1718x + 4.4840	1.0×10^3	3.1×10^3 3.2×10^2
IV	1.573	y=0.1522x + 4.1933	2.0×10^5	5.7×10^5 6.9×10^4
V	1.189	y=0.1598x + 3.6243	6.3×10^6	2.1×10^7 1.8×10^6

Fiducial limits were calculated by using equivalent deviate (p=0.05) 1.96

could also be tested for their potential pathogenicity. Maramarosch (1986) reported that a product of *B.t.* subsp. *aizawai* serotype 7 was highly toxic to common cutworm, *Agrotis ipsilon* (Hfn.) but harmless to *B.mori.* This was developed recently by genetic engineering and is being used as a microbial insecticide in Japan.

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