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Biochemical Alterations in the Haemolymph of *Bacillus thuringiensis* var. *kurstaki* (*B.t.k.*) Infected Larvae of *Spodoptera litura* (Fab)

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The effect of *Bacillus thuringiensis var. kurstaki* (*B.t.k.*) on pathophysiology of *Spodoptera litura* (fab.) larvae was studied at 24, 28 and 72 hour post infection (hpi). The infection resulted in significant alterations in various biochemical parameters of haemolymph, protein, carbohydrate, uric acid and lipid contents were higher in treated larvae as compared to untreated control. The increase was maximum for III, IV and V instar larvae at 72 hpi in case of protein, carbohydrate, lipid and uric acid content.

Key words : *Bacillus thuringiensis var. kurstaki* (*B.t.k.*) *Spodoptera litura*, Biopesticide, Haemolymph.

Introduction :

Bacillus thuringiensis infection induced many pathophysiological and biochemical changes in the haemolymph of insects (Sottani and Habbes, 1995; Salama *et, al* 1999; Tripathi and Singh, 2000). Since, haemolymph of the host insect is reservoir of various biochemical components, thereby the biochemical composition of infected host insect is affected. In the present study an attempt has been made to elucidate the effect of *B. thuringiensis* on alteration in haemolymph composition of the test insect *S. litura* (fab.) which is a serious polyphagous pest of several important crops (Singh and Singh, 1998).

Materials and Methods :

The culture of *S.litura* was maintained on castor *Ricinus communis* leaves at 27 ± 1^{O}C and 70±5 percent Relative Humidity (R.H.). Third, fourth and fifth instar larvae were selected for the present study.

Infection of larvae –

A group of 100 larvae (pre starved for 2h) was fed for a period of 24 hours on castor leaves treated with a suspension of *B.t.k.* having 654.13,

1469.80 and 2445.28 IU/mg for experiments using 3rd, 4th and 5th instar larvae, respectively.

The control group of larvae were fed on untreated and fresh castor leaves. Both groups of larvae were provided fresh untreated castor leaves daily as food. The larvae were sacrified at 24, 48 and 72 hours post infection (hpi) for biochemical estimations.

Sampling of Haemolymph –

Haemolymph was obtained by cutting proleg at its base and collected through micropipette, stored in a vial containing 0.1 ml of extration buffer (0.05 mkcl, 5 mM EDTA, 1 mM PMSF and 1 mM PTV in 1 litre of distilled water) kept in crushed ice. About 50-60 larvae were sacrificed for each experiment. The haemolymph was centrifuged at 3000 rpm for 10 minutes. The plasma so obtained was used for biochemical estimations.

Biochemical Analysis –

Total protein content was estimated by Bradford's method (1976). The carbohydrate content was determined by using the method of Roe (1955). Daily changes in the uric acid content were studied by using colorimetric techniques of Caraway (1955) and lipid content was determined with chloroform - methanol mixture (2:1) by Bligh and Dyer (1959).

Result and Discussion :

A significant hyperproteinemia was observed in infected larvae (Table : 1). Similar results have been observed in *Plodia maculipennis* (Narayan and Jayaraj, 1974). This increase in haemolymph protein of diseased larvae can be attributed to stimulated synthesis of protein producing factors in the insect as the protein requirement is increased for the germination of *B.t.* spores.

Total carbohydrate contents of haemolymph was found significantly higher in diseased larvae than the untreated ones. The results are in corroboration with the finding of Fast and Angus (1965). The increase in carbohydrate content may be due to the germination and maturation of pathogen.

Uric acid content of haemolymph was observed to be relatively higher. The results are in agreement with the results obtained by Sundra Babu and Subramaniam (1973) in *B.t.k.* treated *S. litura*, where uric acid content was significantly increased. It is known that the uric acid is produced principally in the cells of the fat body and released into the haemolymph which is transported to malpighian tubules to be excreted out. Uric acid level progressively decreased in normal larvae, while it increased in infected larvae. This difference reflects modifications in the metabolism of uric acid due to *B.t.* infection.

Total lipid content of haemolymph in III, IV and V instar larvae infected with *B.t.k.* decreased significantly than the untreated control larvae. Similarly Benett and Shotwell (1972) observed 45 percent decrease in infected larvae of *Popilio japonica*. The reason for the lower fat content in infected larvae could be the extended larval period of the treated insects and blocked food ingestion, and the fat reserves might have been utilized for the maintenance during extended larval period.

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Table 1 : Biochemical changes in the haemolymph of *B.t.k.* infected larvae of *Spodoptera litura* (Fab.))

Stage of the larvae (Instar)	Hours post infection (hpi)	Protein (mg/ml)		Carbohydrate (mg/100ml)		Uric Acid (mg/100ml)		Lipid (mg/ml)	
		Control	Treated	Control	Treated	Control	Treated	Control	Treated
III	24	41.96±0.36	43.01±0.41 N.S.	124.56±0.13	128.56±0.5**	10.71±0.07	15.61±0.10**	2.71±0.07	1.44±0.07**
	48	43.01±0.55	50.20±0.10**	125.38±0.32	129.88±0.18**	12.71±0.05	17.73±0.05**	2.32±0.52	1.49±0.22N.S.
	72	52.95±0.46	54.13±0.46 N.S.	130.45±0.19	136.05±0.18**	13.80±0.51	18.43±0.88**	2.89±0.02	1.82±0.03**
IV	24	55.77±0.33	61.21±0.28*	232.35±0.14	234.05±0.74	19.16±1.03	21.76±0.72**	3.27±0.06	2.19±0.02**
	48	56.91±0.91	62.10±0.16*	240.85±0.18	234.05±0.74NS	19.48±0.06	25.60±0.09**	3.41±0.26	2.64±0.39NS
	72	64.28±0.15	70.85±0.53*	244.18±0.89	245.13±0.34**	21.02±1.01	25.97±0.30*	3.87±0.02	2.39±0.07**
V	24	81.27±0.28	87.34±0.49*	275.74±0.17	280.79±0.02**	24.28±0.43	30.72±0.34**	3.93±0.11	2.44±0.01**
	48	82.70±0.37	90.11±0.79*	283.55±0.19	289.39±0.02**	25.75±0.21	31.67±0.10**	4.53±0.10	2.69±0.02*
	72	91.87±0.74	99.02±0.38*	293.64±0.23	304.8±0.18**	27.85±0.22	35.96±0.34**	4.93±0.10	3.11±0.06**

Values given above are Mean±SE

NS – Non Significant

* – Significant at $P < 0.05$

** – Significant at $P < 0.01$