



## EFFECT OF *BACILLUS THURINGIENSIS* ON ACID PHOSPHATASE ACTIVITY IN *SPODOPTERA LITURA* (FAB.) DURING DEVELOPMENT

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### ABSTRACT

In present investigation the acid phosphatase activity was measured in the fat body of *Spodoptera litura* during the development in the last instar larvae and pupae. The larvae were fed with castor leaves treated with sublethal concentration of *Bacillus thuringiensis kurstaki*. The control and treated larvae showed decline in the enzyme activity with a minimum on the last day. But there was increase in activity throughout the pupal stage. The specific activity values were also lower than the control. The activity of acid phosphatase, a marker enzyme of the lysosomal system, in the larval fat body of *Spodoptera litura* decreases at the same time as the cessation of feeding. The increased acid phosphatase activity detected in the pupal period is due to the high levels of ecdysteroids present during this period.

**KEY WORDS:** Acid phosphatase, *Spodoptera litura*, Biolep, p-nitrophenol.

### INTRODUCTION

Fat body is a vital multi-functional tissue found in the visceral cavity of insect life stages (Telfer and Kuntel, 1991 Arresse *et al.*, 2010). During metamorphosis, the larval fat body being major site of biosynthetic activity undergoes a chronologically ordered sequence of alterations and is completely remodeled by the time adult emerges (Wang and Haunerland, 1991). Acid phosphatase which is considered as a marker of lysosomal activity has been implicated in the histolysis and reorganization of tissues in vertebrates and invertebrates. In holometabolous insects where there is a complete metamorphosis in their life cycle, the larval tissues are completely reorganized during the pupal stage and the adult tissues are formed anew (histogenesis) (Thompson, 1975). During feeding stages, large amount of proteins, lipids and glycogen are stored in large granules. These reserve materials are meant to be used up during the formation of adult organs during the pupal period in the case of holometabolous insects. Therefore, insect tissues are ideal for the study of lysosomal hydrolytic enzymes (Bhawane and Bhanot, 1994). Detoxification enzyme, acid phosphatase (ACP) in insects is generally demonstrated as the enzymatic defense against foreign compounds and play significant roles in maintaining their normal physiological functions (Li and Liu, 2007). Phosphatases are proficient of transphosphorylation in addition to hydrolysis. Phosphatases thus play an important role in the metabolism of carbohydrates, phospholipid and nucleotides. Acid phosphatase is vital in biological processes that need high level of energy, such as development, growth, gamete maturation and histolysis (Ray *et al.*, 1984). The tobacco leaf eating caterpillar,

*Spodoptera litura* Fabricius is a polyphagous pest that feeds on more than 150 different host plants and widely distributed throughout tropical and subtropical regions (Gong *et al.*, 2014). It has triggered devastating destruction to many vital field crops and vegetables, such as cotton, soybeans and cabbage (Gandhi *et al.*, 2016). The activity of acid phosphatase, a marker enzyme of the lysosomal system, in the larval fat body of *Spodoptera litura* increases at the same time as the cessation of feeding (Prasad Rao, 1990). The present study was undertaken to determine the effect of *Bacillus thuringiensis kurstaki* viz. Biolep on the lysosomal enzyme acid phosphatase in the fat body of last instar larvae of *Spodoptera litura*.

### MATERIAL AND METHODS

*Spodoptera litura* culture was maintained in the laboratory and fed with castor leaves. The sixth instar larvae were separated after they molted. The larvae and pupae were used for the experiments. The experimental larvae were fed with castor leaves treated with sublethal concentration of Biolep. Control larvae were fed with castor leaves. Fat body from the insects were removed, rinsed in saline and weighed before homogenization. Fat body was homogenized in ice cold citrate buffer, pH 5.0. Homogenate was centrifuged at 5000xg. The supernatant was used as enzyme sample. Acid phosphatase was estimated by the method described by DeLoach and Mayer, (1979) using p-nitrophenyl phosphate (PNP) as the substrate. Protein estimation was carried by the method of Lowry *et al.* (1951).

**TABLE 1:** Acid Phosphatase activity in the fat body of Biolep treated final instar larvae of *Spodoptera litura*.

Age of last instar larvae	mmol pNP released/hr/g fat body		mmol pNP released/hr/mg Protein	
	Control	Treated	Control	Treated
2 days	67.28±3.40	60.12±2.01	0.76±0.01	0.63±0.04
4 days	56.58±2.20	41.16±3.29	0.60±0.05	0.51±0.03
6 days	49.97±1.67	35.56±1.57	0.54±0.06	0.48±0.02
Prepupa	94.86±1.64	53.01±2.58	1.60±0.05	0.73±0.04
Pupa	156.00±2.48	66.21±2.66	1.95±0.02	0.86±0.02

Each value is a mean of 3 samples ± SD

## RESULTS AND DISCUSSION

Acid phosphatase activity showed variation in the larva and pupa of *Spodoptera litura*. (Table1). The control last instar larvae showed a decrease from 67.28 mmol to 49.97mmol p- nitrophenol released/ hr/g/fat body. The activity then increased during the prepupal stage. There was further increase throughout the pupal stage. Specific activity also showed same pattern. In the larvae treated with Biolep, fat body acid phosphatase activity varied from 60.12 mmol to 35.56 mmol p-nitrophenol released/ hr/g/ fat body. The prepupa and pupal values were 53.01 mmol and 66.21mmol p-nitrophenol released/hr/g/fat body. The specific activity values also declined in the larval period but increased in the pupal stage. The treated larvae and pupae showed similar pattern. The non-feeding pre-pupal and pupal stage fat body of *Spodoptera litura* show increase in the acid phosphatase activity.

The activity of acid phosphatase, a marker enzyme of the lysosomal system, in the larval fat body of *Spodoptera litura* increases at the same time as the cessation of feeding (Prasad Rao, 1990). There was reduction in the acid phosphatase activities in the tobacco cutworm *Spodoptera litura* (Fab.) after treatment with nucleopolyhedrosis (NPV) (Senthil-Nathan *et al.*, 2005). The lower activities in acid phosphatases were induced in larvae of *Heliothis armigera* after exposure to LC<sub>50</sub> of the product Bactospine -a *Bt* commercial formulation. (Abdeen and Moawad, 1986).

The fat body undergoes a gradual but significant alteration which is morphological as well as histological during postembryonic development of *S. litura*. The larval fat body appears fairly synthetic which releases proteins and other macromolecules synthesized by it into the haemolymph, thereafter it gradually changes into a dense structure which is primarily a storage tissue. These findings corroborate well with earlier reports of lepidopteran as well as dipteran insects (Levenbook, 1985). In *S. litura* protein content of the fat body gradually increases during postembryonic development from early instar larval stage to pre-pupal stage (Kirankumar *et al.*, 1997). The morphological alteration in fat body structure and its compaction during the larval-pupal transformation seen in *Spodoptera litura* is associated with the massive sequestration and accumulation of hexamerins in protein granules most likely act as a amino acid resources for metamorphosis (Chauhan *et al.*, 2017). Thus, acid phosphatase activity appears to increase during the pupal stage in *Spodoptera litura*.

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