

## Original Research Article

### Studies on Biochemical Changes in *Bombyx mori* L. Races at Different Developmental Stages

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#### ABSTRACT

##### Keywords

Silkworm,  
*Bombyx mori*  
L, Diapause,  
 $\alpha$ -amylase,  
Acid  
phosphatase,  
Alkaline  
phosphatase

The present investigation was an attempt to analyze and compare the changes in  $\alpha$  – amylase, acid phosphatase and alkaline phosphatase in *Bombyx mori* pure races namely pure mysore and NB<sup>4</sup>D<sup>2</sup> in all the four remarkably different developmental stages, the egg, larva, pupa and the moth. The protein content of all the three enzymes was found to increase in both the races of *Bombyx mori* during developmental stages. But the protein content of  $\alpha$  – amylase and alkaline phosphatase were higher in V instar stage whereas, acid phosphates were more in pupa stage. All these Biochemical parameters were higher in female adult moth than in male adult moth. The specific activity of these enzymes showed a similar trend like that of enzyme activity.

## Introduction

The silkworm *Bombyx mori* has been considered as a good laboratory tool has been exploited commercially for the production of silk as well as a bioreactor for the production of recombinant proteins (Wu *et al.*, 2008). This typical insect has a different stages in its life cycle represents the most advanced form of metamorphosis, completes life cycle through serial progression of four distinct stages of development; egg, larva, pupa and adult (moth) and hence termed holometabolous insect. The number of life cycles (generations) per year depends on the silkworm strain and it varies with the

environmental conditions particularly temperature, relative humidity, light and nutrition. Silkworm strains which go through multiple generations (5–6) in a year are polyvoltines or multivoltines. These strains do not undergo egg diapause, which is an adaptation to tropical condition in which there is no severe winter. Under natural conditions, silkworm strains which undergo only one generation in a year are univoltine strains. This is an adaptation to overcome harsh winters in temperate countries (Savithri *et al.*, 2013), and the silkworms which exhibits two generations (during spring-autumn season) in a year are

Bivoltine strains. The qualitative and quantitative characters are entirely different from one another, hence their physiological studies is of great importance in understanding their differences. The multivoltine race Pure Mysore is named for robustness and good survivability and bivoltine NB4D2 is popular for its commercial traits like quality and quantity of silk produced (Rohith *et al.*, 2008), they are the most commonly used pure races for the production of commercial hybrids in India (Rohith and Subramanya, 2010). Research activities concerning different digestive enzymes in the silkworm *Bombyx mori* were initiated after the pioneering works of Matsumura (Matsumura, *et al.*, 1934). Major biomolecules such as proteins, carbohydrates and lipids play an important role in biochemical process underlying growth and development of insects (Ito and Horie, 1959).

Keeping this in view, the present investigation aims at comparative analysis of some of the enzyme activity during different developmental stages in selected multivoltine and bivoltine race of *Bombyx mori*. Protein is necessary for various biological activities during development, metamorphosis and maintenance of various physiological functions in different tissues (Kumar *et al.*, 2011). The  $\alpha$ -amylases ( $\alpha$ -1, 4-glucan-4- glucanohydrolases; EC 3.2.1.1) are the hydrolytic enzymes and are one of the key enzymes involved in digestion and carbohydrate metabolism in insects (Baker, 1989). Alkaline phosphatases (ALP, EC 3.1.3.1) are abundant enzymes mainly involved in removing phosphate groups from organic molecules. In insects, ALPs are involved in several biological processes and respond to stress, pathogenesis, or infection (Eguchi, 1995; Sukhanova *et al.*, 1996; Miao, 2002). ALPase activities in the blood and midgut tissues are strongly related to silk protein synthesis, digestion, and

absorption of phosphorylating substances in silkworm larvae (Wu, 1993)

## Materials and Methods

**Collection of the material** - The multivoltine race, Pure Mysore and Bivoltine race NB4D2 were obtained from the Department of Sericulture, Manasagangothri, Mysuru.

**Preparation of the extract** - 5g samples at each stage of development were homogenised using Potter-Elvehjem homogeniser for 5 minutes at room temperature and the volume was made up to 5 ml with distilled water. The homogenate was centrifuged at 3000 rpm for 10 min at 50C, and the resultant supernatant was used as crude enzyme extract and used for the various biochemical analyses.

**Biochemical studies** - Protein was estimated as described by Lowry *et al.*, (1951) using BSA as standard.  $\alpha$  - Amylase activity was assayed according to the procedure of Bernfeld (1955), alkaline phosphatase and acid phosphatase activity were estimated according to the procedure of Linhardt and Walter (1963).

**Statistical analysis** - All the experiments were done in triplicate and data thus obtained was reported as mean  $\pm$  standard deviation (SD).

## Results and Discussion

**Estimation of protein** - The protein content in multivoltine and bivoltine races of *Bombyx mori* was estimated at different developmental stages are presented in table 1. The protein content in multivoltine race was found to be increased by 33,45,25,91,124,62,43 and 143% in I, II III, IV and V instars (larval stages), pupa and adult male and female moth respectively

when compared with egg protein content. Whereas the protein content in bivoltine race was found to be higher by 20, 28, 130, 203, 207, 85, 130 and 392% in I, II III, IV and V instars, pupa, adult male and female moth respectively when compared with egg protein content. The highest protein content was found to be in V instar larva and adult female moth in both the races. The lowest protein content was observed in II and I instar in multivoltine and bivoltine races respectively. The protein content was significantly lower in adult male moth in both the races when compared with adult female moth of *Bombyx mori*. The egg protein content was not significantly different between the races, however high protein content was observed in bivoltine race of *Bombyx mori* from III instar's larval stage. Nearly 90% higher protein content was observed in adult female moth of bivoltine race of *Bombyx mori*.

**Assay of  $\alpha$ - amylase** - The activity and specific activity of  $\alpha$ - amylase in Multivoltine and bivoltine races of *Bombyx mori* was estimated at different developmental stages are given in figure 1 and figure 2. The activities were higher by 1.33, 3.67, 9.33, 10.33, 12.67, 5.33, 6.33 and 7.67 folds in I, II III, IV and V instars larva, pupa, and adult male and female moth respectively when compared with the activity in egg. The activity was steadily increasing up to V instar's larval stage. The decreased activity was observed in pupa, adult male and female moth when compared with V instar's larval stage. Adult female moth showed higher activity. The specific activity also showed a similar trend. However the maximum specific activity was observed in V instar's larval stage. The adult male moth showed higher specific activity than in adult female moth. When comparison is drawn between the two races, both activities and specific activities were higher in multivoltine race of *Bombyx mori*

up to pupa stage. In both the races, specific activities were higher in adult male moth.

**Assay of alkaline phosphatase** - The activity and specific activity of alkaline phosphatase in multivoltine and bivoltine races of *Bombyx mori* at different stages of development were given in figure 3 and 4.

**Multivoltine race** - In multivoltines race the activity were increased by 1.38, 0.76, 3.90, 3.80, 0.90, 0.09, 0.52 and 0.72 folds in I, II III, IV and V instars larva, pupa and adult male and female moth respectively when compared with the activity in egg. The maximum activity was found in III instar larva whereas minimum activity was observed in adult male moth. The specific activity also showed a similar trend, but lower specific activity was observed in pupa stage of development. The adult male moth showed maximum specific activity than adult female moth.

**Bivoltine race** - In bivoltine race of *Bombyx mori* the activity was increased by 2.01, 1.16, 2.75, 0.37, 3.30, 0.55, 0.82 and 0.92 folds in I, II III, IV and V instars, pupa and adult male and female moth respectively when compared with the activity in egg. The maximum activity was found in V instar and least activity was observed in IV instar of larval stage. No significant differences were observed in the activities of adult female and adult male moth. Similar trend was observed for the specific activity. However Maximum specific activity was observed in III instar stage and adult male moth than adult female moth. Increased activity was observed in adult male moth and adult female moth of multivoltine race when compared with the bivoltine race.

**Assay of acid phosphates** - The activity and specific activity of acid phosphatase in multivoltine and bivoltine races of *Bombyx mori* at different stages of development were given in figure 5 and 6.

**Multivoltine race** - In multivoltine race the activity were increased by 1.35, 1.67, 2.65, 1.35, 2.99, 11.62, 12.61 and 13.28 folds in I, II III, IV and V instars, pupa and adult male and female moth respectively when compared with the activity in egg. The maximum activity was found in pupa and adult moth. The specific activity also showed a similar trend, however the specific activity in adult male moth showed higher activity than in adult female moth.

**Bivoltine race** - The activities were increased in bivoltine race by 1.5, 2.0, 2.5,

4.0, 2.24, 11.2, 10.2 and 11.8 folds in I, II III, IV and V instars, pupa and adult male and female moth respectively when compared with the activity in egg. Though the activity was highest in adult female moth, no significant differences were observed between pupa, adult male and adult female moth. Highest specific activity was observed in pupa stage. When comparison is drawn between the two races, no significant differences in the activities were observed except in IV instar larva where bivoltine race showed higher activity.

**Table.1** Protein content in *Bombyx mori* at different developmental stages

Silkworm Races	Protein content (mg/g)								
	Egg	I-instar	II-instar	III-instar	IV-instar	V-instar	Pupa	Adult male moth	Adult female moth
Multivoltine (Pure mysore)	7.00 ± 0.80	9.34 ± 0.72	10.17 ± 0.82	8.74 ± 0.75	13.41 ± 1.25	15.67 ± 1.28	11.31 ± 0.97	10.01 ± 0.87	17.01 ± 1.81
Bivoltine (NB <sup>4</sup> D <sup>2</sup> )	6.50 ± 0.52	7.80 ± 0.61	8.30 ± 0.59	15.00 ± 0.97	19.70 ± 1.27	20.00 ± 1.86	12.00 ± 1.06	15.00 ± 1.36	32.00 ± 2.72

The values are average of 3 experiments

**Figure.1**  $\alpha$ -amylase activity and specific activity in multivoltine race of *Bombyx mori*.

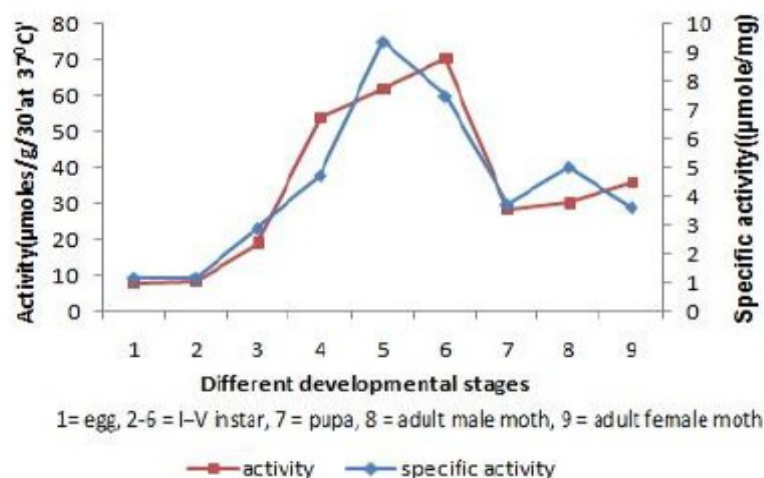


Figure.2  $\alpha$  Amylase activity and specific activity in bivoltine race of *Bombyx mori*

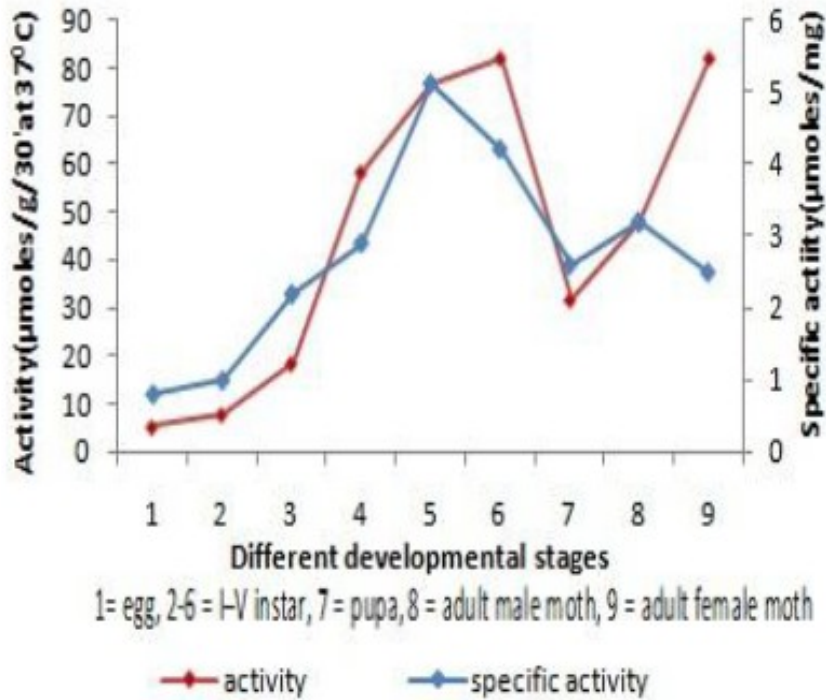


Figure.3 Alkaline phosphatase activity and specific activity in multivoltine race of *Bombyx mori*

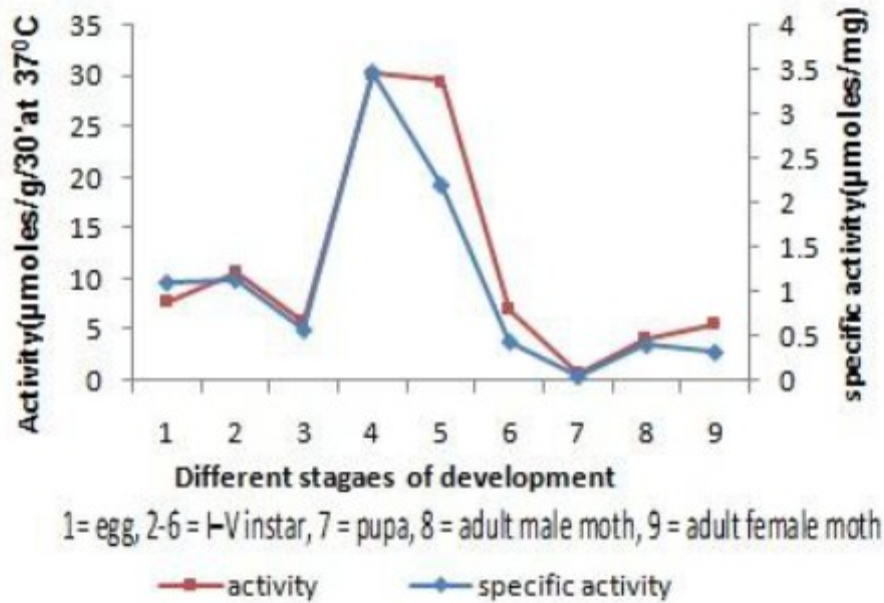


Figure.4 Alkaline phosphatase activity and specific activity in bivoltine race of *Bombyx mori*.

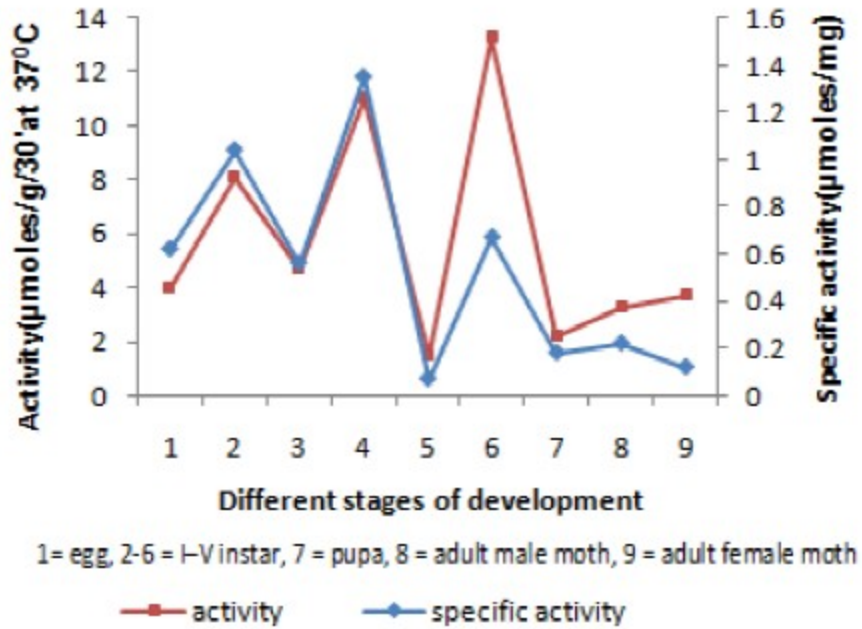
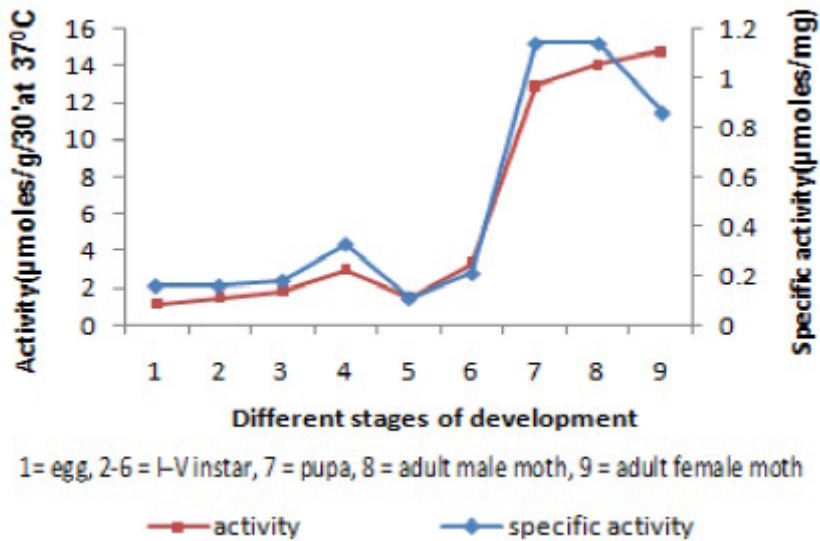
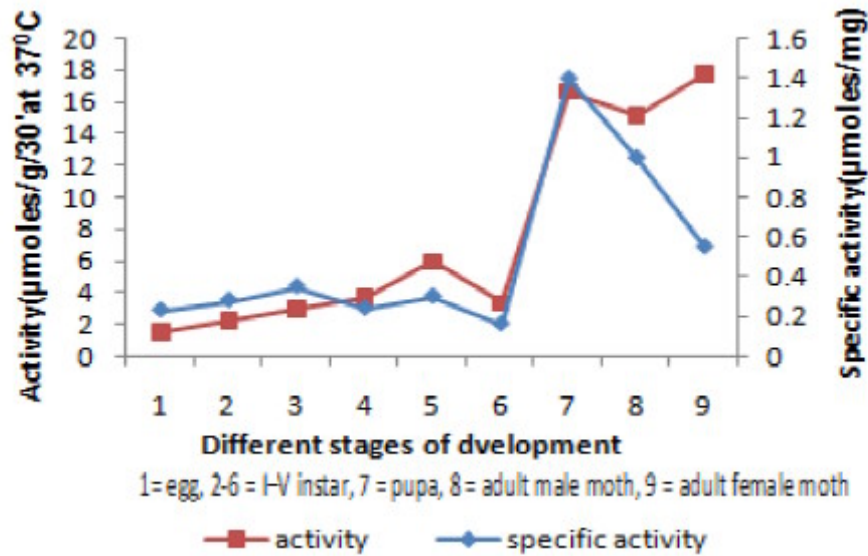


Figure.5 Acid phosphatase activity and specific activity in multivoltine race of *Bombyx mori*



**Figure.6** Acid phosphatase activity and specific activity in bivoltine race of *Bombyx mori*.



The pathway for the biosynthesis of proteins in insects is very much similar to either microbial or mammalian tissues. This view is well supported from a number of reports. The development of ova where there is rapid cell proliferation must involve the synthesis of nucleic acids and proteins. Chen and Levenbook (1966a, b) states; the free amino acids in insects have a distinctly specific effect on protein metabolism. However, it is not certain whether the observed abnormal protein metabolism results from a direct or indirect effect of the mutational process. But the differences observed from the two races of *Bombyx mori* with regard to their free amino acid composition and concentration in the developing ova it fairly supports the view that, the change in protein metabolism must be due to change in their genes. In the present experimental approach, the highest protein content was found to be in V instar larva and adult female moth in both the races. The protein content was significantly lower in adult male moth in both the races when compared with adult female moth. The leaf consumption of V instar larva amounts to up to 75% of the total instars. The high

intake of food by the V instar larva might be to accumulate sufficient energy sources to support its metabolism during non feeding pupal and adult stage.

Ishaaya (1986), while working on protease and amylase activities in the larvae of certain insects have shown that certain protein factors present in the food can stimulate digestive enzymes, probably through a hormonal mechanism. Sree Kumar and Prabhu (1998) clearly demonstrated the possible role of midgut tissue in stimulation of digestive enzyme secretion. In the present investigation, the maximum specific activity was observed in V instar larval stage. This data corresponds to the work of Sree Kumar and Prabhu. The amylase in the digestive juice may have beneficial effect on the survival rate when the larvae are reared on hardened leaves.

The activity of alkaline phosphatase is related to the physiological situation of silkworms and reflects the absorption, digestion and positive transportation of nutrients in the midgut (Eguchi *et al.*, 1990).

In the present investigation, the maximum activity was found in V instar larvae, and least activity was observed in IV instar larvae. This data is in well agreement with the work of Miao (1988).

The silk gland of silkworm is a typical example of an organ, which has developed very rapidly during V instar stage (Umashankar and Subramanya, 2002), which undergoes drastic degeneration during larval-pupal transformation. Materials liberated from degenerating cells will be utilized as a source of energy. Recently studies on lysosomes have been conducted in various kinds of animal cells and it is becoming clear that lysosomes contain a number of hydrolases which play a significant role in autolysis and necrosis. Acid phosphatase is one such lysosomal enzyme. In our experimental approach, maximum activity of acid phosphatase was observed in the pupal stage of development in both the races. This might be due to the larval - pupal metamorphosis.

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