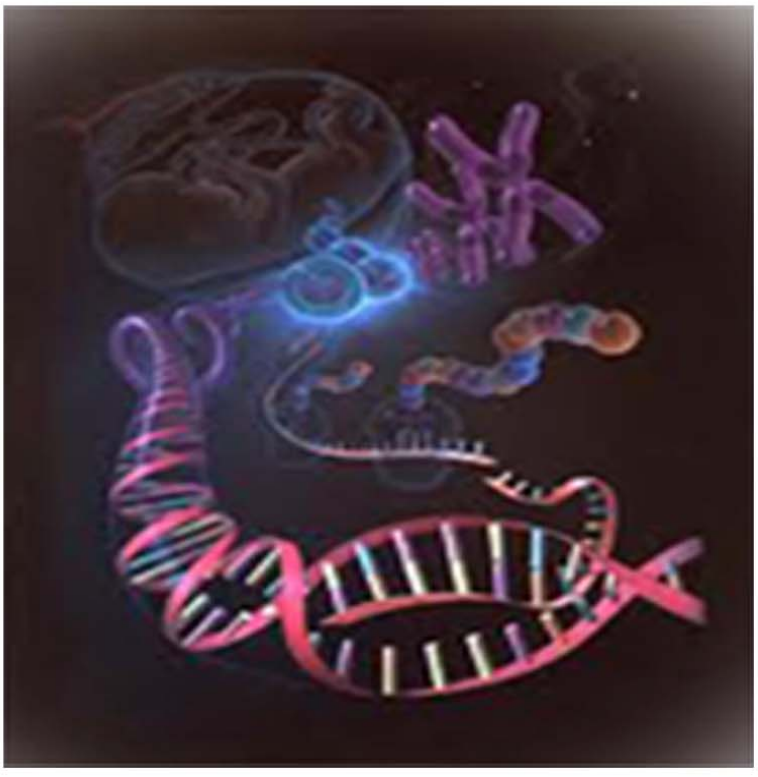
IJLBPR

ISSN 2250 - 3137
Vol1. 3, No. 1, January 2014

International Journal of

Life Sciences Biotechnology and Pharma Research



International Journal of Life Sciences Biotechnology and Pharma Research
Hyderabad, INDIA

www.ijlbpr.com

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International Journal of Life Sciences Biotechnology and Pharma Research

ISSN 2250-3137 www.ijlbpr.com
Vol. 3, No. 1, January 2014

Research Paper

DAY TO DAY ANALYSIS OF AMYLASE AND TREHALASE ACTIVITY IN THE HAEMOLYMPH OF SILKWORM *BOMBYX MORI* L. INFECTED WITH FUNGAL PATHOGEN *BEAUVERIA BASSIANA* (BALS.) VUILL.

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Increased trend of amylase activity was recorded in the haemolymph of healthy larvae up to the 5th day (0.475 mg/ml to 0.667 mg/ml) of the instar and then the enzyme activity was declined on the 6th day (0.626 mg/ml). In experimental larvae the enzyme activity was elevated up to 3rd day (0.462 mg/ml to 0.523 mg/ml) and then the activity of the enzyme was declined significantly (0.490 mg/ml to 0.467 mg/ml) during the rest of the instar. Whereas the activity of trehalase was increased in haemolymph of 5th instar silkworm from the 1st day to 6th day (1.49 mg/ml to 2.97 mg/ml). In comparison to control the trehalase activity shows a negligible increase up to 3rd day (1.5 mg/ml to 1.76 mg/ml) of the inoculated 5th instar larvae then the enzyme activity was declined significantly during the rest of the instar (1.64 mg/ml to 1.44 mg/ml).

Keywords: Bombyx mori, Beauveria bassiana, Haemolymph, Amylase and Trehalase

INTRODUCTION

Silkworm *Bombyx mori* is completely domesticated tiny insect with economic significance. Due to centuries of this domestication, it has lost much of its natural resistance and shows neither morphological nor behavioral adaptations to escape predators, parasites, pests and pathogens. Diseases in silkworm *Bombyx mori* are fairly common in occurrence and are serious in inflicting losses. Among the many constraints that influence the success of cocoon production, the menace of disease is the prime one. The major diseases affecting mulberry silkworm are flacherie, grasserie, muscardine and pebrine. The white muscardine is common during winter and rainy seasons and this disease is prevalent in all sericultural countries. White muscardine is caused by fungi *Beauveria bassiana.* The fungal pathogen is an aggressive parasite of different insect host species. Not only does it have a wide host range but also insects are attacked from larval to adult stages. This versatility helps the fungus to persist in the sericulture ecosystem by

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harboring on the mulberry and silkworm pests. Haemolymph is a dynamic fluid tissue with a close metabolic relationship with other tissues and organs. Enzymatic changes in infected larvae would envisage the metabolic stress that the insect experience during the development of pathogen.

In most insects, carbohydrates reserves are present as glycogen and trehalose which can be readily converted into glucose for the support of all life processes. Enzymes provide the energy needed for metabolic reactions essential to immune health. Enzymes have been shown to stimulate the body's natural defenses while breaking down offending pathogenic immune complexes. This helps relieve stress on the body and strengthen the immune system, which can speed the healing process. Changes in enzymatic action along with metabolic modulations during the progress of pathogen play an important role in understanding the interaction between the host and pathogen as a part of a survival strategy. In view of this, the present study has been carried out to understand the dynamics of amylase and trehalase activity during the progress of fungal pathogen in silkworm *Bombyx mori*.

MATERIALS AND METHODS

PM x CSR_2 was selected for the study. Silkworms were reared under optimum conditions (Dandin *et al.,* 2003). On 1st day of the fifth instar, the larvae were inoculated by dipping in sublethal concentration of fungal condia spore suspension (2.15 x 10^6 conidia spores/ ml @ 50 ml/100 worms for 45 s) and larvae treated with double distilled water were used as control. After 24 h of the inoculation of fungal pathogen *Beauveria bassiana*, haemolymph was collected everyday into pre-chilled centrifuge tubes with a pinch of phenyl thiourea by clipping third pair of abdominal legs of silkworm larvae and the haemolymph was taken for the enzymatic studies. Amylase activity was analyzed by the method Noetling and Bernfeld (1948) and Baker (1991) using the 3, 5 dinitro salicylic acid (DNS) reagent as modified by Ishaaya and Swirsiki (1976) and trehalase activity was analyzed by the method of Ishaaya and Swirsiki (1976) in the haemolymph of both infected and healthy silkworm larvae. The experimental data recorded and the data was subjected to statistical analysis by following T-test.

RESULTS AND DISCUSSION

Amylase

The activity levels of amylase enzyme in the haemolymph of silkworm *Bombyx mori* inoculated with fungal pathogen *Beauveria bassiana* during 5th instar are presented in Table 1 and Graph 1. Increased trend of amylase activity was recorded in the haemolymph of healthy larvae up to the 5th day (0.475 mg/ml to 0.667 mg/ml) of the instar and then the enzyme activity was declined on the 6th day (0.626 mg/ml), whereas in experimental larvae the enzyme activity was elevated up to 3rd day (0.462 mg/ml to 0.523 mg/ml) and then the activity of the enzyme was declined significantly (0.490 mg/ml to 0.467 mg/ml) during the rest of the instar. Compared to control, the enzyme activity was declined significantly in the fungal infected larvae.

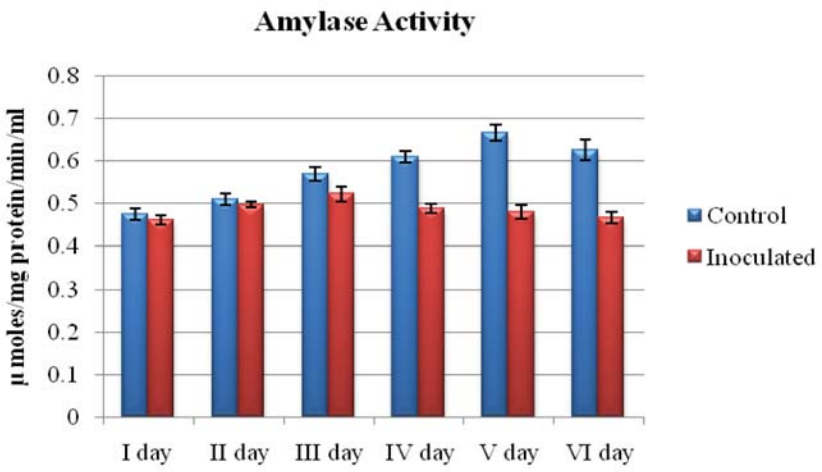
Amylase is one of the key enzymes involved in digestion and carbohydrate metabolism in insects (Daone *et al.,* 1975; Buonocore *et al.,* 1976; Horie and Watanabe, 1980). Amylase refers to a group of enzymes whose catalytic function

Table 1: Day to Day Changes in Amylase Activity (µ moles/mg protein/min/ml) in the Haemolymph of Silkworm *Bombyx mori* L. Inoculated with Fungal Pathogen *Beauveria bassiana* (Bals.) Vuill with Reference to Control During 5th Instar

Treatments	Days of 5th instar					
	I	II	III	IV	V	VI
Control	0.475±0.013	0.510±0.013	0.569±0.016	0.610±0.013	0.667±0.019	0.626±0.025
Inoculated	0.462±0.010 NS	0.498±0.006 NS	0.523±0.018***	0.490±0.011****	0.481±0.017****	0.467±0.014****

Note: Mean±Standard Deviation; NS = Not Significant; ***P≤0.01; ****P≤0.001.

Graph 1: Histogram Showing the day to day Changes in Amylase Activity (µ moles/mg protein/min/ml) in the Haemolymph of Silkworm *Bombyx mori* L. Inoculated with Fungal Pathogen *Beauveria bassiana* (Bals.) Vuill with Reference to Control During 5th Instar



is to hydrolyze sugar and starch. Chatterjee *et al* (1989) reported the presence of two different forms of amylase activity in digestive fluid and haemolymph. Abraham *et al.* (1992) noticed that amylase activity of digestive fluid was 40 fold higher than that of haemolymph. The presence of this enzyme is in abundance during larval development in both diapausing and nondiapausing strains imply that this enzyme has some important physiological role. The function of haemolymph amylase is not fully understood although Wyatt (1967) suggested its possible involvement in the degradation of fat body glycogen.

The investigation revealed the decreased amylase activity in the haemolymph of infected silkworm with reference to the control. It was directly related to low intake of food as a consequence of fungal infection. Christopher and Mathavan (1985) suggested that the rational food consumption by a lepidopteran larvae was correlated directly with the activity of amylase. The larva receiving 100% food found to have the highest amylase activity, which declined as the percentage of food offered was reduced. In contrast to this, Gururaj *et al.* (1999b) found that the activity of amylase increased significantly in the haemolymph from 48 h to 144 h of infection with BmNPV.

Trehalase

Table 2 and Graph 2 show the results on trehalase activity. In control the activity of trehalase showed a trend of increase in haemolymph of 5th instar silkworm from the 1st day to 6th day (1.49 mg/ml to 2.97 mg/ml). In comparison to control the trehalase activity shows a negligible increase up to 3rd day (1.5 mg/ml to 1.76 mg/ml) of the inoculated 5th instar larvae then the enzyme activity was declined significantly during the rest of the instar (1.64 mg/ml to 1.44 mg/ml).

Trehalase plays an important role in energy supply to an insect (Wyatt, 1967) and midgut trehalase serves as an indicator of energy reserves resulting from the availability of carbohydrate nutrients. Trehalase is the one of the most important carbohydrolases in insects occurring in the gut, flight muscles, fat bodies, labial glands, haemolymph and also in the silk glands of silkworm. It causes the breakdown of trehalose into glucose for internal supply for chitin synthesis, muscular activity during flight, cocoon formation and other metabolic process. The enzyme catalyzes the hydrolysis of trehalose into two glucose molecules.

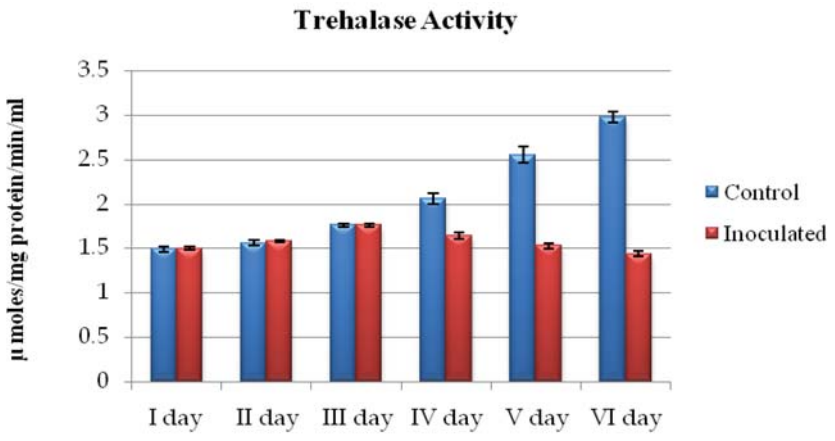
Insignificant elevation of trehalase activity was observed in the initial stage of infection, then the activity of the enzyme was declined in inoculated larvae compared to healthy ones. It appears that the energy demands are stepped up in the host

Table 2: Day to day changes in trehalase activity (µ moles/mg protein/min/ml) in the haemolymph of silkworm *Bombyx mori* L. inoculated with fungal pathogen *Beauveria bassiana* (Bals.) Vuill. with reference to control during 5th instar

Treatments	Days of 5th instar					
	I	II	III	IV	V	VI
Control	1.49±0.03	1.56±0.03	1.76±0.02	2.06±0.06	2.55±0.09	2.97±0.06
Inoculated	1.5±0.02 NS	1.58±0.01 NS	1.76±0.02 NS	1.64±0.04****	1.53±0.03****	1.44±0.03****

Note: Mean±Standard Deviation; NS = Not Significant; ****P≤0.001

Graph 2: Histogram Showing the Day to Day Changes in Trehalase Activity (µ moles/mg protein/min/ml) in the Haemolymph of Silkworm *Bombyx mori* L. Inoculated with Fungal Pathogen *Beauveria bassiana* (Bals.) Vuill with Reference to Control During 5th Instar



in initial stage of infection, when the physiology of the host is altered to combat the disease as a natural response. The decrease in the trehalase activity in the *Beauveria bassiana* inoculated larvae could be attributed to decreased metabolic capabilities of infected larvae. This was also interpreted as due to decreased hydrolysis of trehalose to release glucose molecules under drastic stress conditions and high energy demand (Hasegawa and Yamashita, 1970) as trehalase activity and trehalose levels are inversely related.

In contrast to the present study Sasikala (2007) observed progressively higher trehalase activity in uzi infected 5th instar silkworm larvae. This was attributed to active breakdown of trehalose presumably to meet the energy demands. Higher trehalase activity in the uzi infested tissues over the normal is indicative of higher conversion of glucose during energy needs of both the host and parasite. Yaginuma *et al.* (1990) observed trehalase activity tends to increase during the middle stage of CPV infection in infected midgut. Gururaj *et al.* (1999) noticed no significant change in the haemolymph trehalase activity between BmNPV infected and control larvae till 96 h then enzyme activity was increased in the rest of the instar. He suggested that the increase in the enzyme activity is associated with decreased levels of trehalose.

CONCLUSION

Haemolymph is the only extracellular fluid of insects with diverse functions and a reservoir for the products required for every physiological activity of the insect body, thus changes in the composition of haemolymph, reflect the physiological and biochemical transformations taking place in the insect tissues (Pawar and Ramakrishnan, 1977). As the *Beauveria bassiana* fungus confines to haemolymph till the silkworm larvae approach death, it obviously influences the metabolic profiles, biochemical nature, the volume of haemolymph and the population of haemocytes.

Enzymes are large biological molecules responsible for the thousands of biochemical interconversions that sustain life. Enzymes serve a wide variety of functions, act as catalysts and help in complex reactions occur everywhere in life. Without enzymes, metabolism would neither progress through the same steps, nor be fast enough to serve the needs of the cell. In the absence of enzymes, this occurs so slowly or insignificant. Metabolic pathways within each cell depends on the set of functional enzymes that are present. Enzymes can also reduce free radical damage, inhibit pathogens and potentially provide support for the most challenging health disorders.

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ISSN 2250-3137