



International Journal of Emerging Technologies in Computational and Applied Sciences (IJETCAS)

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DYNAMICS OF PROTEINS AND FREE AMINO ACIDS IN SILKWORM *BOMBYX MORI* L INFECTED WITH *BEAUVERIA BASSIANA* (BALS) VUILL

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Abstract: Examined the total protein and free amino acid dynamics occurring in the haemolymph of *Beauveria bassiana* infected silkworm *Bombyx mori* compared to control. Protein content was elevated significantly in the initial stage of inoculation i.e from 1st to 3rd day of inoculation (168.7 to 200.8 mg/ml), from 4th day to 6th day the biomolecules shown decreased trend (189 to 152.58 mg/ml) . Significant reduction of free amino acid (41 to 26.6 mg/ml) was noticed in the silkworm larvae inoculated with *Beauveria bassiana*.

I. Introduction

Beauveria bassiana (Bals) Vuill is one of the most destructive fungal pathogen of silkworm *Bombyx mori* L causing white muscardine disease, which is common in all sericulture zones of the world. With in lepidoptera the muscardine remains confined to the haemocoel attaching and/or destroying and denaturing the haemolymph and fat body cells prior to death (Gardener, 1977). Haemolymph is a dynamic fluid tissue with a close metabolic relationship with other tissues and organs. The progress of a fungal pathogen can monitored by studying the degree of variation in several biochemical constituents in the haemolymph, So far no systematic investigation has been made on comprehensive and comparative studies on protein and amino acid content in the haemolymph during the development of fungal pathogen in 5th instar silkworm *Bombyx mori*. Hence present study was carried out to understand the dynamics of the chief biomolecules during the progress of fungal pathogen *Beauveria bassiana*.

II. Materials and Methods

Silkworm hybrid of PM x CSR₂ was selected for the study. Silkworm larvae were brushed and reared in the laboratory under optimum conditions according to Dandin *et al* (2003). Immediately after fourth moult i.e. on the first day of the fifth instar, the larvae were inoculated, by dipping in fungal spore suspension (2.15 x 10⁶ spores/ ml @ 50 ml/100 worms for 45 sec). The larvae treated with double distilled water were used as control. After 24 hours of the induction of pathogen, day to day biochemical variations occurring in the haemolymph were examined. Haemolymph was collected by clipping third pair of abdominal legs of larvae. The haemolymph was drained into pre-chilled centrifuge tubes with a pinch of thiourea for the biochemical analysis. Total protein content (Lowry *et al* 1951) and amino acids (Moore and Stein 1954) were examined.

III. RESULTS AND DISCUSSION

A. Total proteins:

No significant change was observed in protein content (168.7 mg/ml) on the 1st day of induction of fungal pathogen and then shown a significant elevation of the biomolecule on the 2nd and 3rd day of inoculation (185.05 mg/ml and 200.8 mg/ml) compared to control (176.55 mg/ml and 184.05 mg/ml). Decreased trend of protein content was observed from the 4th day (189 mg/ml) of inoculation. In the control increased trend of total protein content was noticed (169.13 mg/ml to 214.5 mg/ml) with the advancement of age in 5th instar.

The initial enhancement of protein content in inoculated silkworm may be due to elicit humoral as well as cellular response to encounter microbial inoculation. The humoral factors present in the haemolymph are attributed as a lysozymal function to bacterial substances (Powning and Davidson, 1973 and 1976). Gajendra Pal Singh *et al* (2011) reported that the protein levels in haemolymph were increased in infected larvae and he suggested that during infection the synthesis and release of proteins from fat bodies are greatly increased. Kodaira (1961) opined that the aberrations may be induced in the infected silkworm due to coagulation of proteinaceous materials with the increased moisture requirement of *Beauveria bassiana*. Variation in the volume of haemolymph is also known to influence the levels of circulating proteins as per the report of Engelmann and Penny (1966). Degtyareva (1967) also reported a marked disruption of protein metabolism accompanied by some decrease in total proteins, a marked increase in globulin content after three days when

larvae of **Leptinotarsa decemlineata** was sprayed with a mixture of spore suspension of **Beauveria bassiana** and DDT. Watanabe *et al* (1968) observed hypoproteinemia in the haemolymph of silkworms infected with BmNPV. They attributed this to fat body being the centre of haemolymph protein synthesis and the most susceptible tissue to NPV.

In the present study reduction of protein content was recorded in the advanced stage of disease, it may be due to consequences of metabolism of proteins and amino acids of haemolymph by the developing pathogen and cessation of feed by the host organism. Peter *et al* (1982) recorded relatively rapid decline in some proteins, decrease in total amino acids, disappearance of minor sugars and a small increase in glucose after infection with **Beauveria bassiana** and explained that these changes may be due to cessation of feeding and difficulty in processing the food material when larvae were infected. Quantitative and qualitative changes in protein profiles of various tissues of tropical tasar silkworm **Antheraea mylitta** D was studied by Kumar *et al* (2011). They reported decreased protein trend in inoculated larvae and suggested that it may be due to drastic degradation of structural proteins. But interestingly there was no increase in amino acid content it means that the proteins degraded might be utilized by pathogen for rapid development. Kawase and Hayashi (1971) reported that hypoproteinemia occurred in silkworm larvae infected with CPV especially in later stages of infection. They concluded that hypoproteinemia is the consequences of two possibilities, firstly the reduction in protein content is caused by the formation of polyhedral bodies in the midgut, and secondly it is caused by starvation due to a dysfunction of the midgut.

Cheung and Gula (1982) reported that the biochemical changes in haemolymph components of corn earworm, **Heliothis zea** Boddie larvae infected with the fungus were due to general starvation rather than due to specific activities of the fungus. Ambika (1990) reported that the depletion in haemolymph protein and amino acids in the larva by the developing pathogen of **Beauveria bassiana** dilutes the haemolymph. The reduction in haemolymph proteins was interpreted as due to starvation as a consequence of fungal infection, selective utilization of specific protein fraction by the pathogen and its interference with normal host protein metabolism as the pathogen attacks and destroys the fat body tissue and the alteration of osmotic relations of haemolymph with other body tissues. Sam Devadas (1991) reported a significant reduction in haemolymph and gut protein content after inoculation of **Serratia marcescens**, which was interpreted as due to fast multiplication of the bacteria. Similar observations were made by stapiro and Ignoffo (1971), Sinha *et al* (1991), Sarma *et al* (1994) and Rajasekhar and Pathak (1994). Govindan *et al* (1998) suggested that the reduction in protein content in infected individuals may be due to starvation as a consequence of infection and also selective utilization of specific protein fraction by **Beauveria bassiana** and blockage of the haemocoel with negative hyphae to isolate proteinaceous materials. The pathogen presumably interferes with the physiology and metabolic activities of the infected larvae in which it leads to functional impairment of midgut and decrease in intake of food, which could lead to a decrease in the protein content (Savithri *et al*, 2006). Ramesh Babu *et al* (2009) suggested that the survival ability of an animal to stress majorly depends on its protein synthetic potential any stress on an animal invokes compensatory metabolic adjustments in its tissues through modifications of proteins. Nath *et al* (1997), Etebari and Matindoost (2004 a, b) and Etebari *et al* (2007) suggested that silkworm compensates the deficiency of energy caused by the stress with intensive breakdown of protein to amino acids and entering them to TCA cycle as a keto-acid.

B. Free Amino Acids:

Decreased trend of free amino acids was observed in both inoculated (41 mg/ml to 19.95) and control (44.6 mg/ml to 24.3 mg/ml) from 1st day to 5th day of inoculation in 5th instar. And it was noticed significant enhancement of amino acid content on the 6th day of both the batches i.e. inoculated (26.6 mg/ml) and control (30.53 mg/ml). Significant reduction of FAA was noticed in the silkworm larvae inoculated with **Beauveria bassiana** compared to control.

In the present study increased levels of protein with decreased free amino acid levels indicated faster mobilization of free amino acids to meet the energy requirements through the TCA cycle through transamination. These free amino acids may be utilized in synthesis of structural proteins to meet the defense mechanism in response to fungal inoculation. Sam Devadas (1991) reported decreased protein content in haemolymph of **Serratia marcescens** infected larvae without any change in free amino acid levels, it could be due to the fast multiplication of pathogen and utilization of amino acids by the pathogen.

On the 6th day of inoculation there was increase in free amino acid levels in haemolymph of both control and inoculated larvae. It may be due to supply of free amino acids to the silk gland through haemolymph for protein synthesis in the formation of silk. Changes in the amino acid pool in different tissues and the consequent supply to the silk gland through the circulating medium of haemolymph was observed by Noguchi *et al* (1974). Nataraju *et al* (2005) reported enhanced trend of the amino acid content in the haemolymph of infected animal at the end of the 5th instar and attributed this may be due to dehydration. Rajasekhar *et al* (1992) opined that the

infected larvae may experience diarrhoea and vomiting which leads to dehydration during host – pathogen interaction.

Changes in free amino acid content have been reported by several authors. Barlow (1962) observed that there were changes in the ratios between various amino acids as well as a decrease in the amount of polypeptides when the grubs were infected with the milky disease. Variations in the amino acid levels of **Bombyx mori** larvae when infected with NPV and CPV were recorded earlier by Kawase (1966) and it was suggested that polyhedrosis stimulates the infected cell to viral and polyhedral proteins and induces aberrations in the protein metabolism of host cells. Jolly *et al* (1972) and Sinha *et al* (1988) have reported the changes in FAA in the haemolymph of healthy larvae and pupae of tasar silkworm **Antheraea mylitta**. The levels of FAA present in the haemolymph of pebrine-infected larvae and pupae were less than in healthy larvae and pupae which was attributed to their utilization by the pathogen (*Nosema* Sp.). Agarwal *et al* (1974) reported lower levels of amino acids in the haemolymph of pebrine-infected larvae of **Antheraea mylitta** compared to those in the healthy ones. Again, it was suggested that the lower level of proteins and amino acids in the haemolymph of pebrine infected larvae compared to healthy ones may be due to their utilization by the pathogen (*Nosema* sp.). Considerable reduction of amino acid content and hypoproteinemia was recorded by Adolkar (1990) in silkworm larvae infected with both NPV and **Nosema bombycis** and this was attributed to direct consequence of viral lesions or protozoan infection in the fat body of infected larvae. Decreased amino acid content in the haemolymph of host larvae after parasitisation with **Exorista sorbillans** have also been reported by Venkatarami Reddy *et al* (1992). Decrease in the free amino acid content after parasitization can be explained in two ways. The first one is that the parasite absorbs certain amino acids selectively across the cuticle from the haemolymph, resulting inadequate supply of free amino acids. Another possible reason is decreased de novo synthesis of amino acids and/or stepped up carbohydrate biosynthesis.

In contrast, Aboul-ela *et al* (1991) reported no quantitative differences of amino acids were detected in the haemolymph of normal larvae and after their treatment with **Bacillus thuringiensis**. Narayanan (1969) found that the quantity of amino acids present in diseased caterpillar was higher than that present in healthy caterpillar. Elevation in the amino acid level of haemolymph in infested larvae may be due to the hydrolytic breakdown of haemolymph proteins. Similar reports have been made by Drilhon *et al* (1951) and Ramakrishnan and Pant (1971) in case of **Bombyx mori** and **Earias fabia** respectively.

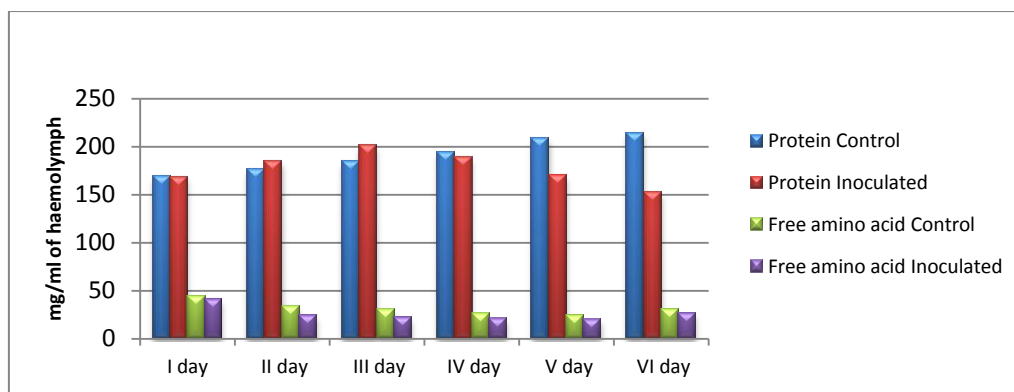
Proteins and amino acids are the essential organic constituents of all living cells. These macromolecules are concerned with the regulation of all biochemical events in the organism (Harper *et al*, 1993). Proteins play an important role in the haemolymph of insects not only in specific transport functions, but also in their enzyme action. Several functions have been ascribed to FAA in insect haemolymph, such as Osmoregulation (Beadle and Shaw 1950); Protein synthesis (Buck 1953); Cocoon spinning (Wyatt 1961; Fukuda and Matuda 1953) etc. The high concentration of these molecules is an indication of great metabolic activity of the tissue. Further investigations on these macromolecules may help to formulate a rational approach for the control of diseases and intensive research on these lines are stressed.

Day to day changes in Protein and Free amino acid (mg/ml) content in haemolymph of silkworm **Bombyx mori** L. during the development of fungal pathogen **Beauveria bassiana** (Bals) Vuill

Proteins						
V instar	I day	II day	III day	IV day	V day	VI day
Control	169.13±12.61	176.55±5.14	184.05±3.42	193.85±3.86	208.35±2.18	214.5±6.94
Inoculated	168.7±5.89 NS	185.05±4.87 *	200.8±4.73 ****	189±3.02 NS	169.8±5.15 ****	152.58±5.88 ****
Free amino acids						
Control	44.6±4.45	33.8±4.73	29.9±1.71	25.7±3.12	24.3±2.29	30.53±1.75
Inoculated	41±2.98 NS	24.4±3.36 ***	22.2±2.74 ***	20.75±2.70 *	19.95±4.51 NS	26.6±1.48 **

* = $P \leq 0.05$, ** = $P \leq 0.02$, *** = $P \leq 0.01$, **** = $P \leq 0.001$,

Figure 1 Histogram showing day to day changes in Protein and Free amino acid (mg/ml) content in haemolymph of silkworm **Bombyx mori L. during the development of fungal pathogen **Beauveria bassiana** (Bals) Vuill**



Acknowledgements:

The Authors are thankful to University Grants Commission, New Delhi for their financial support to carry out the part of the Research work.

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