

**AMINO ACID PROFILES IN THE HAEMOLYMPH OF SILKWORM *BOMBYX MORI* L.
INFECTED WITH FUNGAL PATHOGEN *BEAUVERIA BASSIANA* (BALS.) VUILL.**

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ABSTRACT: Examined the day to day changes of amino acid profiles in the haemolymph of 5th instar silkworm *Bombyx mori* L. during the development of fungal pathogen *Beauveria bassiana* (Bals.) Vuill. For the qualitative determination of amino acids Thin Layer Chromatography (TLC) was performed. The qualitative changes were observed in amino acid at different lengths on 1st and 6th day after inoculation of *Beauveria bassiana*. More number of amino acid spots were noticed on 1st and 6th day of inoculated larvae compared to control.

Key words : *Bombyx mori*, *Beauveria bassiana*, Haemolymph, Amino acids, TLC

INTRODUCTION

Textile industry is a very important component of India's economy and silk forms a small, yet important part of it because it is a high value fiber. Silk is the most elegant textile in the world with unparalleled grandeur, natural sheen and inherent affinity for dyes, high absorbance, light weight, soft touch and high durability and is known as the queen of textile in the world ever. Sericulture being an agro-based enterprise plays a predominant role in shaping the economic destiny of rural people. It holds promise as an employment generating industry, especially in rural and semi-urban areas. Sericulture means cultivation of silkworms, which finally produces silk. Diseases in silkworm *Bombyx mori* are fairly common in occurrence and are serious in inflicting losses. Diseases in the silkworm *Bombyx mori* can be grouped under four major categories, namely the microsporidian disease, viral diseases, bacterial diseases and fungal diseases. These diseases are popularly known as pebrine, grasserie, flacherie and muscardine respectively. A disease outbreak is a major reason for not achieving the expected cocoon yield parameters. *Beauveria bassiana* is an aggressive parasite, the fungal spore germinates and proliferates rapidly in the various tissues ultimately causing death of silkworm. The progress of infection by a pathogen in the host tissue can be monitored by studying the degree of variation in metabolic constituents. Amino acids are the essential components of all living cells. They are the building blocks of proteins. Several functions have been ascribed to the FAA in insect haemolymph, such as Osmoregulation (Beadle and Shaw 1950), Protein synthesis (Buck 1953), Cocoon spinning (Wyatt 1961; Fukuda and Matuda 1953) etc. Many insects including the silkworm are known to contain usually large amount of free amino acids (Ramsay 1958; Florkin 1960; Levenhook 1962; Chen 1966; Venkata Rami Reddy 1984; Parenti *et al* 1985; Sinha *et al* 1990, 1991; Siva Prasad and Murali Mohan 1990). These reports indicate that the amino acids are essential for growth and survival of the silkworm. In view of the importance of amino acids in the metabolism, the present study was carried out to understand the qualitative changes in amino acids in inoculated as well as in control silkworm.

MATERIALS AND METHODS

PM x CSR₂ was selected for the study. Silkworms were reared in the laboratory under optimum conditions (Dandin *et al* 2003). On the 1st day of the fifth instar, the larvae were inoculated by dipping in sublethal concentration of the fungal conidia spore suspension (2.15 x 10⁶ conidia spores/ ml @ 50 ml/100 worms for 45 sec) and larvae treated with double distilled water were used as control. After 24 hours of the inoculation of fungal pathogen *Beauveria bassiana*, haemolymph was collected every day 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 study. Qualitative analysis of amino acids was carried out by using Thin Layer Chromatography (TLC).

RESULTS AND DISCUSSION

Amino acids play an important role in maintaining the proper osmotic balance in haemolymph of arthropods (Sutcliffe 1963). Their nature and arrangement influence the physical, chemical and biochemical properties of the proteins. The amino acids and their derivatives participate in intracellular functions such as nerve transmission, regulation of cell growth, biosynthesis of various compounds like hormones, purines, pyrimidines, porphyrins and some vitamins (Rodwell 1993). The physiological state of the cell can be understood by means of amino acid quality as the best diagnostic tool (Adibi 1980). The changes in total amino acid content also affect the protein balance in the silkworm because amino acids are the intermediates for protein synthesis. The effect of disease on the total and individual amino compounds varies depending on the host and pathogen interactions.

Thin Layer Chromatography (TLC) analysis showed the presence of different kinds of amino acids at different lengths. In the present study no significant changes were seen in qualitative amino acid composition in the haemolymph of silkworm infected with fungal pathogen *Beauveria bassiana* compared to control. Five amino acid spots were observed on the 1st day of inoculated silkworm larvae whereas in healthy silkworm larvae three amino acid spots were recorded. On 2nd and 3rd day four amino acid spots and on 4th and 5th day three amino acid spots were observed in both experimental and control. On the 6th day four amino acid spots were observed in experimental larvae and in control three spots were noticed. More number of amino acid spots were noticed on 1st and 6th day of inoculated larvae compared to control. It could be due to elevation of protein synthesis in inoculated larvae to meet the energy demand against fungal pathogen in the initial stage of infection. Enhancement of amino acid spots at the later stage of infection may be due to the degradation of structural proteins.

Haemolymph proteins serve as a storehouse for amino acids (Pequeux *et al* 1979) and host mobilization or parasitic degradation of these proteins could lead to elevated levels of some Free Amino Acids (FAA) in the plasma. Stentiford (1999) reported that the concentrations of several FAAs, notably serine, were reduced in early infections of *Nephrops norvegicus* by *Hematodinium*, while at later stages several FAAs were increased in concentration. The decrease in the concentration of some amino acids in the early stage infection is consistent with the parasite utilisation of these compounds as the haemolymph is colonised. The increased total FAA concentration that occurred in later stages of infection may be due to widespread disruption of the major organs and tissues and liberation of intracellular compounds by cellular damage due to infection of *Nephrops norvegicus* by *Hematodinium* (Field and Appleton 1995). However, the elevated total FAA concentration is not due to an increase in all components of FAAs, but rather to elevated levels of a few FAAs, which suggests either selective release or selective utilisation amino acids. Rutherford *et al* (1977) explained the variable effects in amino acids encountered in nematode infections reflect the dynamic relationship between host and parasite.

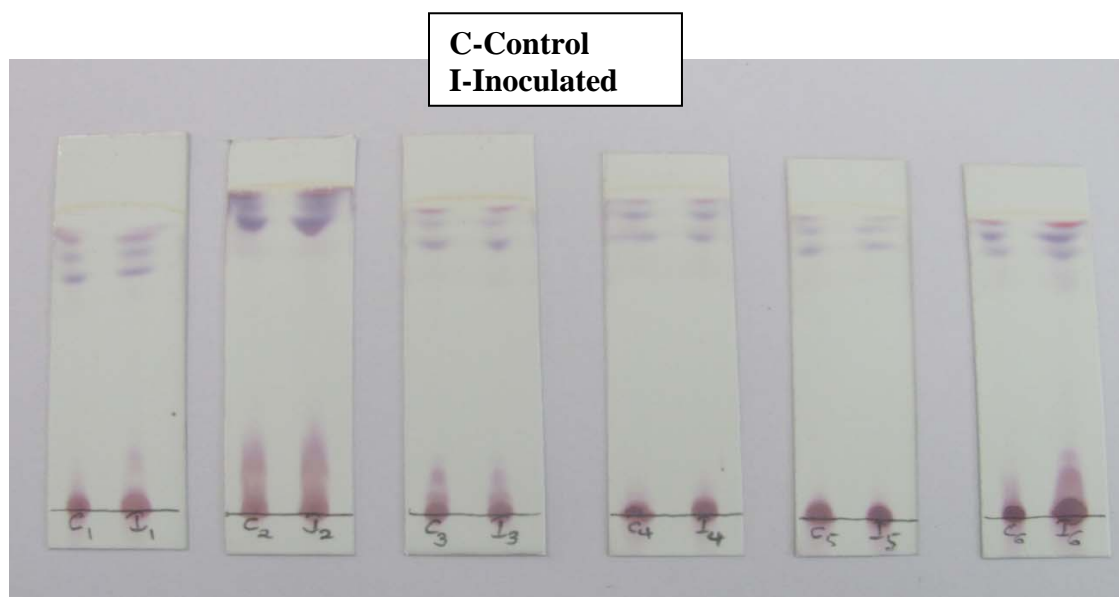


Fig-1: Day to day changes in amino acid profiles of the haemolymph in 5th instar silkworm *Bombyx mori* L. during the development of fungal pathogen *Beauveria bassiana*.

In contrast to the study Sinha *et al* (1990) noticed seventeen amino acids are common to both healthy and pebrine-infected *Antheraea mylitta* larvae and reported that the healthy larvae contain glutamine in addition to the seventeen amino acids. Sinha *et al* (1991) noticed the presence of twelve amino acids, namely aspartic acid, serine, glycine, lysine, arginine, methionine, threonine, histidine, α -alanine, proline, valine and leucine/isoleucine in both healthy and pebrine-infected *Antheraea mylitta* moths. The healthy moths showed two more amino acids viz., asparagines and tyrosine.

Amino acids are the basis of all life processes, as they are absolutely essential for every metabolic process. They perform many important functions such as: building cells, protecting the body from viruses or bacteria, repairing damaged tissue and carrying oxygen throughout the body. In insects, haemolymph proteins have a diversity of functions like metabolic, endocrine, reproductive and immunological. Haemolymph serves as the transport milieu for the exchange of essential materials between cells, tissues and organs (Mullins 1985; Karpells *et al* 1990; Lovallo *et al* 2000). It is evident from the various reports that amino acids are essential for growth and survival of the silkworm and act as indicator to assess the health conditions of any organism. In the present investigations variations in the amino acid levels of *Bombyx mori* larvae infected with *Beauveria bassiana* indicates the aberrations in the protein metabolism of host cells.

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