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DAY TO DAY CHANGES IN PROTEIN LEVELS OF *SILKWORM BOMBYX MORI* L. INFECTED WITH BEAUVERIA BASSIANA (BALS) VUILL

D. Thirupathamma* and G. Savithri

Department of Sericulture, S.P. Mahila Visvavidyalayam, Tirupati -517502.

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***Author for Correspondence**
Thirupathamma Dasari
Department of Sericulture, S.P. Mahila Visvavidyalayam,
Tirupati -517502

ABSTRACT

Day-to-day variations in protein levels were examined in three tissues viz., integument, midgut and silkgland of 5th instar silkworm *Bombyx mori* during the progress of fungal pathogen *Beauveria bassiana*. Inoculation of fungal pathogen *Beauveria bassiana* resulted gradual reduction of protein content in the three tissues selected for the study.

KEYWORDS: *Bombyx mori*, *Beauveria bassiana*, Protein content, Integument, Midgut, Silkgland.

INTRODUCTION

The success of sericulture industry, primarily depends on the successful harvest of cocoon crops. Perhaps the major problem for sericulture in a tropical country like India is the high incidence of diseases. The major diseases affecting mulberry silkworm are pebrine (protozoan disease), flacherie (bacterial disease), grasserie (viral disease) and muscardine (fungal disease). ***Beauveria bassiana*** (Bals.) Vuill. is one of the most destructive fungal pathogen of silkworm ***Bombyx mori*** causing white muscardine disease, which is common in all sericulture zones of the world. It is known to attack more than 150 insect species (Bell 1974). Pathogens were reported to induce several biochemical and physiological alterations in insect tissues (Bergold (1963) and Martignoni (1964).

Proteins are the known biomolecules which regulate and integrate several physiological and metabolic processes in the body through hormones, enzymes and nucleoproteins. These biomolecules plays a vital role in the synthesis of microsomal detoxifying enzymes that helps to detoxify the toxicants when entering into the animals. 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 or modulations of proteins (Bano *et al* 1981; Assem and Hanke, 1983). The total protein content consists of structural and soluble proteins involved in the architectures and metabolism of a cell. In view of the significance of the protein molecules in various metabolic activities, day to day changes in the protein content in various tissues of *Beauveria bassiana* infected 5th instar silkworm *Bombyx mori* were examined.

MATERIALS AND METHODS

For the present study PM × CSR2 silkworm strain was selected. Silkworms are reared in the laboratory under optimum conditions as suggested by Dandin *et al* (2003). Immediately after fourth moult the heatlhy larvae were selected from the rearing stock and grouped into two sets. Each group consists of 4 replications with 100 larvae for each group. One set of larvae was treated with fungal spore suspension with sub lethal concentration (3.25 x 10^6 spores/ ml @ 50 ml/100 worms) and another set of larvae were treated with double distilled water and used as control. Every day, silkworms from both the sets were randomly selected from 1st day to 7th day of 5th instar silkworms and dissected in physiological saline solution and collected the three tissues viz., integument, midgut and silkgland for protein analysis. Protein content was determined by the method of Lowry *et al* (1951).

RESULTS AND DISCUSSION

Day to Day changes in total protein content were examined in three tisses viz., integument (Table-1 and Graph-1), midgut (Table-2 and Graph-2) and silkgland (Table-3 and Graph-3) of 5th instar silkworm *Bombyx mori* during the progress of fungal pathogen. In the integument decreased trend of protein levels were noticed in the experimental larvae from 1st day to 7th day (45.1 to 30.14 mg/g wet wt of tissue) of 5th instar silkworm, whereas in control the protein levels were increased up to third day (46 to 48.15 mg/g wet wt of tissue) then a gradual reduction of protein content was recorded (41 to 31.28 mg/g wet wt of tissue). In the midgut no significant variation was recorded in the initial stages of fungal infection, i.e., 1st and 2nd day (42 and 45 mg/g wet wt of tissue), then significant reduction of protein content was noticed till the end of the instar (40.16 to 35.14 mg/g wet wt of tissue) compared to control. Highly significant protein levels were noticed on 6th day of inoculated silkworms. In the silkgland of experimental larvae, no significant reduction of the protein content was recorded up to 3rd day (37 to 40.14 mg/g wet wt of tissue) and then the significant reduction of protein content was noticed (39.8 to 26.14 mg/g wet wt of tissue) during the rest of the instar with reference to control (48.76 to 38.9 mg/g wet wt of tissue). Increased protein content was recorded in the silkgland of healthy larvae up to the 5th day (38.14 to 48.96 mg/g wet wt of tissue) of the instar and then the protein content was declined on the 6th and 7th day of the instar (49.49 and 38.9 mg/g wet wt of tissue).

The results of the study shows a series of variations in total protein content in the three tissues viz., integument, midgut and silkgland of 5th instar larvae infected with fungal pathogen *Beauveria bassiana*. As a whole decreased trend of protein content was noticed in the three tissues of silkworm inoculated with *Beauveria bassiana* compared to control. The reduction of the protein levels in the different tissues of experimental larvae may be due to the interference of fungal pathogen with the structural and physiological functions of the infected larvae in which it leads to functional impairment of midgut and decreased intake of food, absorption and assimilation into body fluid in turn to different tissues. Another possible reason for the reduction of protein content in different tissues may be due to the invasion of fungal hyphae into different tissues of infected silkworm and draw the nourishment and selective utilization of specific protein fraction by *Beauveria bassiana* for the growth and development of the fungal pathogen. It may also be due to inhibition of protein synthesis owing to non-availability of energy for protein synthesis due to starvation. Decreased levels of protein in inoculated larvae may also be attributed to the drastic degradation of structural proteins.

Changes in total protein metabolism during infection have been reported by several authors. Kodaira (1961) opined that the aberrations may be inducted in *Beauveria bassiana* infected silkworm due to coagulation of protein substance with the increased moisture requirement of fungal pathogen *Beauveria bassiana*. Degtyareva (1967) reported a marked disruption of protein metabolism accompanied by a decrease in total proteins in the larvae of *Leptinotarsa decemlineata* sprayed with a mixture of spore suspension of *Beauveria bassiana* and DDT.

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 consequence of two possibilities, firstly the reduction in protein content is caused by the formation of polyhedral in the midgut and secondly it is caused by starvation due to a dysfunction of the midgut. Smith and Grula (1981) reported that *Beauveria bassiana* can readily utilize many proteins, amino acids and carbohydrates for germination and growth of fungal spore. Cheung and Grula (1982) reported that the physical presence of the fungus in the alimentary tract tissue and /or toxin liberation in the immediate area most likely leads to a malfunction of the digestive tract and subsequent starvation. The ultimate cause of death, however, is not starvation alone since uninfected larvae survive without food for 48 h and beyond. The same authors recorded a 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.

Sam Devadas (1991) reported a significant reduction in the haemolymph and gut protein contents inoculated with *Serratia marcescens,* which was interpreted as due to fast multiplication of bacteria. Rajasekhar and Pathak (1994) observed the significant reduction of total protein concentration in the silkglands of flacherie infected larvae, the decrease was suggested to be due to a decrease in the level of protein and aminoacids in the haemolymph of diseased worms which affects the formation of silk protein in the glands. Govindan *et al* (1998) noticed the reduction in protein concentration in infected individuals and suggested that it may be due to starvation as a consequence of fungal infection and selective utilization of specific protein fraction by *Beauveria bassiana.* According to Srikanth *et al* (1988) the food intake is lower in parasitised larvae than in unparasitised. Perhaps this is one of the reasons for less protein content in the haemolymph.

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. Rajitha *et al* (2013) observed reduction of protein content in the advanced stage of *Beauveria bassiana* infection, this may be attributed to the consequences of changes in the metabolism of proteins and amino acids of haemolymph by the developing pathogen and cessation of feeding by the host organism.

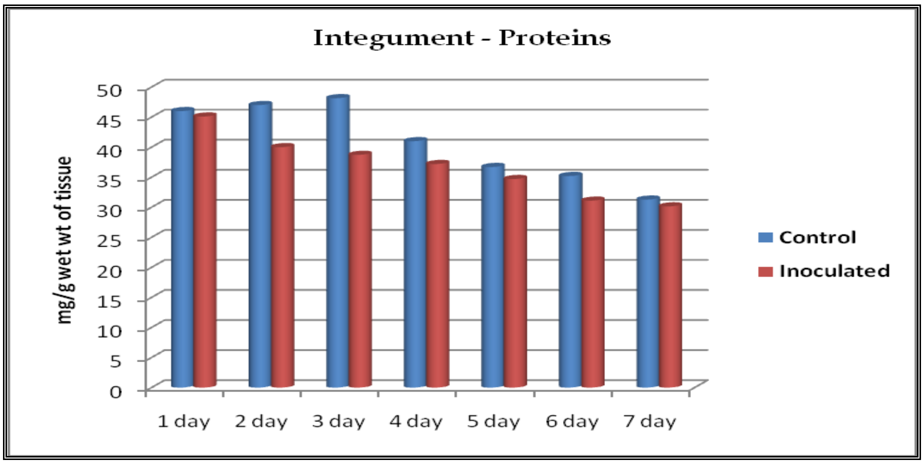
Kumar *et al* (2011) observed quantitative and qualitative changes in protein profiles of various tissues of tropical tasar silkworm *Antheraea mylitta* and reported decreased protein trend in inoculated larvae and suggested that it may be due to drastic degradation of structural proteins. But interestingly there was no increase in amino acid content it means that the proteins degraded might be utilized by pathogen for rapid development.

Elevation of protein levels in the midgut tissue was recorded on the 6th day of inoculated silkworms. The elevation of protein levels may be due to the invasion of vegetative mycelia in the midgut, which also leads to damage of the structural components of the midgut, the release of various antimicrobial substances by the host organism and production of mycotoxins by the fungal pathogen *Beauveria bassiana*, such as beauvericin into the host tissue. It is well supported by Matindoost (2006) and Wago (1995). Matindoost (2006) suggested that the reason for the elevation protein content may be due to the bursting of cell membrane after cell death and release of cellular proteins. Wago (1995) reported that production of antimicrobial substances such as lectin, defensin and attacin with the entry of foreign bodies as part of the defense mechanism may be the reason for the elevation of protein content during viral infection.

Table.1: Day to day variations in protein levels (mg/g wet wt of tissue) in the integument of 5th instar silkworm *Bombyx mori* during the progress of fungal pathogen *Beauveria bassiana* compared to control

Treatments	Days of 5th instar						
	1st day	2nd day	3rd day	4th day	5th day	6th day	7th day
Control	46±1.6	47±1	48.15±2.0	41±0.9	36.7±2.1	35.2±2.1	31.28±1
Inoculated	45.1±1 NS	40±0.5 ****	38.72±1.9 ***	37.2±2.9 ***	34.7±1.1 NS	31.1±2 NS	30.14±2.0 NS

Mean±Standard Deviation; NS = Not Significant; *P<=0.05, **P<=0.02, ***P<=0.01, ****P<=0.001

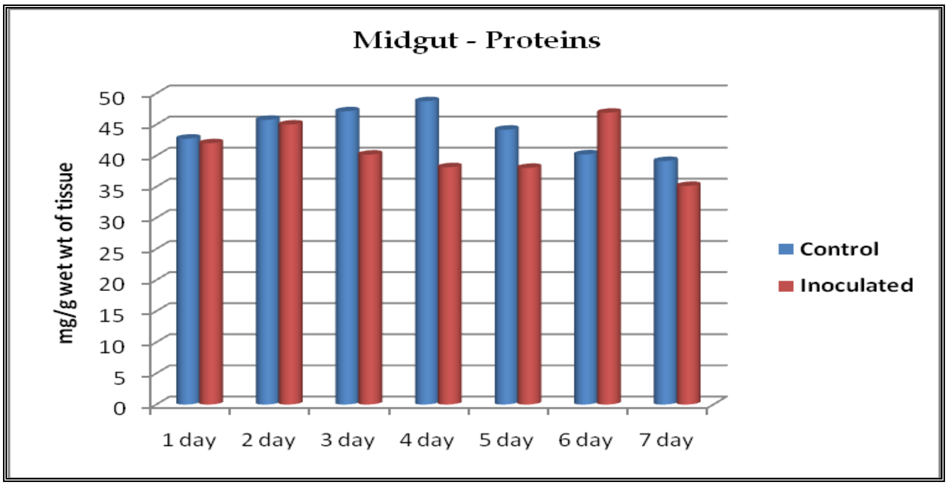


Graph– 1 Histogram showing the day to day variations in protein levels(mg/g wet wt of tissue) in the integument of 5th instar silkworm *Bombyx mori* during the progress of fungal pathogen *Beauveria bassiana* compared to control.

Table. 2: Day to day variations in protein levels (mg/g wet wt of tissue) in the midgut of 5th instar silkworm *Bombyx mori* during the progress of fungal pathogen *Beauveria bassiana* compared to control.

Treatments	Days of 5th instar						
	1st day	2nd day	3rd day	4th day	5th day	6th day	7th day
Control	42.74+2	45.76+1.0	47.14+2.0	48.76+1	44.17+8.0	40.2+1.0	39.14+0.9
Inoculated	42+1 NS	45+6 NS	40.16+2.0 ***	38.14+1.5 ***	38.04+2.0 *	46.9+1 ****	35.14+1.0 NS

Mean±Standard Deviation; NS = Not Significant; *P<=0.05, **P<=0.02, ***P<=0.01, ****P<=0.001

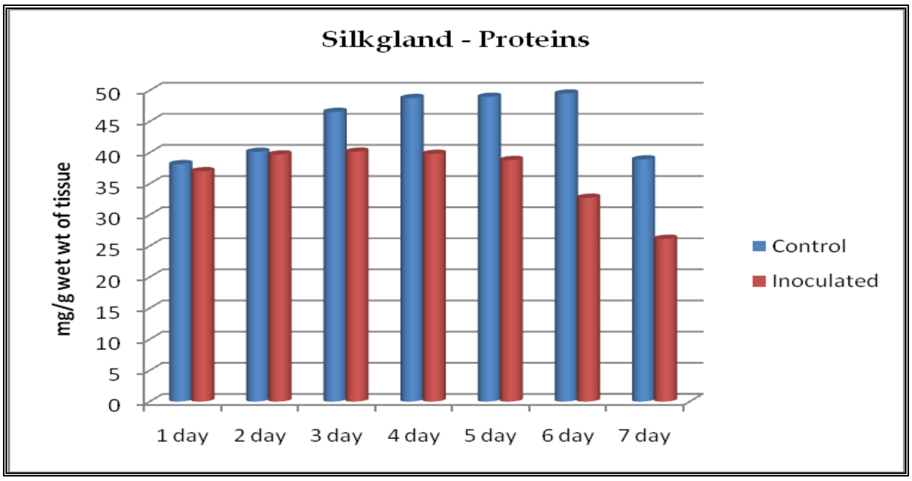


Graph– 2 Histogram showing the day to day variations in protein levels (mg/g wet wt of tissue) in the midgut of 5th instar silkworm *Bombyx mori* during the progress of fungal pathogen *Beauveria bassiana* compared to control

Table- 3 Day to day variations in protein levels (mg/g wet wt of tissue) in the silkgland of 5th instar silkworm *Bombyx mori* during the progress of fungal pathogen *Beauveria bassiana* compared to control

Treatments	Days of 5th instar						
	1st day	2nd day	3rd day	4th day	5th day	6th day	7th day
Control	38.14+2.0	40.12+0.12	46.54+1	48.76+3	48.96+1	49.49+0.4	38.9+1.1
Inoculated	37+4 NS	39.7+1 NS	40.14+2.0 NS	39.8+3 **	38.8+3 **	32.74+2.0 ****	26.14+1.02 ****

Mean±Standard Deviation; NS = Not Significant; *P<=0.05, **P<=0.02, ***P<=0.01, ****P<=0.001



Graph – 3 Histogram showing the day to day variations in protein levels (mg/g wet wt of tissue) in the silkgland of 5th instar silkworm *Bombyx mori* during the progress of fungal pathogen *Beauveria bassiana* compared to control.

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REFERENCES

1. Assem, H. and Hanke, W. (1983) The significance of the amino acid during osmotic adjustment in teleost fish. I. Changes in the euryhaline *Sarotherodon mossambicus*, Comp. Biochem. Physiol - 74(A), pp : 531-536.
2. Bano Y, Ali SA and Tariq H (1981) Effect of sub lethal concentration of DDT on muscle constituents of air breathingcat fish, Ani. Sci - 90, pp : 33-37.
3. Cheung PVK and Grula EA (1982) In vivo events associated with entomopathology of Beauveria bassiana for the corn earworm (*Heliothis zea*). J Invertebrate Pathology – 39, pp : 303-313.
4. Dandin B, Jayant Jayaswal and Giridhar (2003) Hand Book of Sericulture Technologies, Central Silk Board, Bangalore.
5. Degtyareva E.N (1967) Protein and carbohydrate metabolism in larvae of the Colarado bettle, *Leptinotarsa decemlineata* say under the influence of the white muscardine fungus, *Beauveria bassiana* (Bals.) Vuill. Zasch Est Kiev – 4, pp : 110-113.
6. Govindan R, Narayanaswamy TK and Devaiah MC (1998) Text book of Principles of Silkworm Pathology, Seri Scientific Publishers, Bangalore.

7. Juliana Bueno Ruiz, Ludimilla Ronqui, Jussara Ricardo de Oliveira and Maria Claudia Colla Ruvolo-Takasusuki(2013) Comparative electrophoretic profile of proteins and esterases in healthy silkworm larvae (*Bombyx mori* Lineu, 1758) and infected with nucleopolyhedrovirus, Acta Scientiarum. Biological Sciences Maringá – 35(1), pp: 77-82.
8. Kawase S and Hayashi Y (1971) As cited by H. Watanabe. In: the cytoplasmic polyhedrosis virus of the silkworm (Ed Aruga H and Tanada Y) University of Tokyo Press, Tokyo Japan. pp: 234.
9. Kodaira Y (1961) Biochemical studies on the muscardine fungi in silkworms, Bombyx mori. In: J. Fac. (Ed.), Text. Sci. Tech. 5. Sinshu. University Sericulture. pp: 1-68.
10. Kumar D, Pandey JP, Jain J, Mishra PK and Prasad BC (2011) Qualitative and quantitative changes in protein profile of various tissue of tropical tasar silkworm, *Antherae mylitta* drury. Int J Zool Res – 7, pp: 147-155.
11. Lakshmi Devi K and K Yellamma (2013) The modulatory role of zinc in the silkworm, *Bombyx mori* (L) Bioscience Discovery, 4(1): 58-68.
12. Lakshmi Velide (2012) Studies on biochemical components of the larval haemolymph, fat body an silkgland of tropical tasar silkworm, *Anthereae mylitta Drury (Daba T.V)*under cold stress condition, European Journal of Experimental Biology - 2 (6) :2238-2242.
13. Lokesh G and Anantha Narayana SR (2011) Changes in the protein profile of silkworm *Bombyx mori* L. (lepidoptera: Bombycidae) in response to the chemical mutagen, I.J.S.N - 2(3), pp: 559- 563.
14. Lowry OH, Rosebrough N F, Farr A L and Randall RJ (1951) Protein measurement with folin phenol reagent. J. Biol. Chem – 193, pp: 267-275.
15. Martignoni ME (1964) Mass production of insect pathogens. In: De Bach P Ed. Biological control of insect pest and weeds. Reinhold, New York. pp: 579-609.
16. Mathavan S and Sudha PM(1989) Effect of *Bacillus thuringiensis israelensis* on the midgut cells of *Bombyx mori* larvae: Histopathological and histochemistry study, J.Invertebr.Pathol - 53, pp: 217-227.
17. Matindoost L (2006) Establishment and characterization of a new cell line from embryonic tissue of *Bombyx mori* and is susceptibilicity to baculovirus (BmNPV), M.Sc thesis on entomology, The University of Guilan, Iran.
18. Rajasekhar C and Pathak JPN (1994) Effects of bacterial flacherie on the total protein concentration of silkgland and haemolymph of *Bombyx mori* L. J Seric – 2, pp: 48-50.

19. Rajasekhar R Prasad B Subramanyam Reddy C Bhaskar M Murali K and Govindappa S (1992) Variations in the tissues biochemical composition in the muscardine infected silkworm larvae *Bombyx mori* L. Indian J Comp Anim Physiol -10, pp: 24-27.
20. Rajitha K, Savithri G and Sujathamma P (2013) Dynamics of proteins and free amino acids in silkworm *Bombyx mori* L. infected with *Beauveria bassiana* (bals) vuill International Journal of Emerging Technologies in Computational and Applied Sciences- 4(3), pp: 329-333.
21. Ramesh Babu K, Ramakrishna S, Harish Kumar Reddy Y, Lakshmi G, Naidu NV, Sadak Basha S and Bhaskar M (2009) Metabolic alterations and Molecular mechanism in silkworm larvae during viral infection: A review. African Journal of Biotechnology 8(6), pp: 899-907.
22. Sai Zhang, Yunmin Xu, Qiang Fu, Ling Jia, Zhonghuai Xiang, and Ningjia He (2011) proteomic analysis of larval midgut from the silkworm (*Bombyx mori)* Comparative and Functional Genomics Volume 2011, Article ID 876064, pp: 1-13.
23. Sam Devadas C (1991) Some studies on the pathology of *Serratia marcescens* in silkworm *Bombyx mori* L**.** STS. Dissertation submitted to International Centre for Training and Research in Tropical Sericulture, Mysore.
24. Santosh Kumar Tripathi (2012) Protein level changes under magnetic exposure of larvae in *Bombyx mori*: A multivoltine mulberry silkworm, Academic Journal of Entomology 5 (2), pp: 73-80.
25. Shivankappa B (1991) Studies on few aspects of Physiology and Biochemical genetics in different breeds of silkworm Bombyx mori. L., Ph.D thesis. Bangalore University, Bangalore, India.
26. Smith RJ and Grula EA (1981) Nutritional requirements for conidial germination and hyphal growth of *Beauveria bassiana*, J.Invert.Pathol – 37, pp : 222-230.
27. Srikanth J, Basappa H and Lingappa S (1988) Effect of uzifly parasitization on food consumption and growth of the mulberry silkworm *Bombyx mori* L. Insect Sci Appilc - 9 (3), pp: 373-376.
28. Wago H(1995), Host defense reaction of insects, Jpn.J.Applied Entomol. Zool -39, pp: 1-14.
29. Watanabe H (1970) Enhancement of protein metabolism in the midgut epithelium of the silkworm *Bombyx mori* by infection with a cytoplasmic polyhedrosis virus, J.Invertebr. Pathol - 15, pp: 247-252.

30. Watanabe H (1971) Pathophysiology of cytoplasmic polyhedrosis in the silkworm. In : Cytoplasmic polyhdrosis (Ed. By Aruga H, Tanada Y), Univ. Tokyo press, Tokyo. Pp: 151-167.
31. Watanbe H and Kobayashi M (1969) Effect of virus infection on the protein synthesis in the silkworm *Bombyx mori* L infected with nuclear and cytoplasmic polyhedrosis virus. Jpn. J.Appl. Entomol. Zool - 15, pp: 198-202.