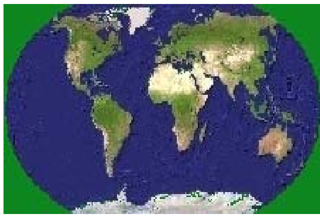

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Biochemical activity in the haemolymph of silkworm, *Bombyx mori* L. during the infection of fungal pathogen, *Beauveria bassiana* (Bals) Vuill

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Abstract
The silkworm larvae fed on mulberry leaves treated with *Beauveria bassiana* on 1stday of Vth instar and its effect on protein, lipid, carbohydrate and trehalose content in the haemolymph of infected silkworm were analysed. The present study indicated that, inoculation of fungal pathogen resulted significant reduction in protein (158.09 ± 4.86 to 143.28 ± 4.76 mg/ ml), lipid (30.53 ± 1.12 to 26.54 ± 0.73 mg / ml) and carbohydrate (3.68 ± 0.14 to ± 6.23 ± 0.78 mg/ ml) content of haemolymph compared to control group. Significant elevation of trehalose (3.14 ± 0.09 to 7.12 ± 0.22 mg/ ml) content was noticed in the inoculated group from 1st to 6th day of the Vth instar silkworm larvae.

Keywords: *Bombyx mori, Beauveria bassiana,* biochemical contents, haemolymph

1. Introduction
Mulberry silkworm, *Bombyx mori* is a poikilotherm, susceptible to several diseases [7,19]. There are no silkworm races at present, which deemed as totally resistant to diseases or pests [16]. Among the fungal diseases, white muscardine is caused by a pathogenic fungus, *Beauveria bassiana* (Balsamo) Vuillemin (1912). *B.bassiana* is an aggressive parasite; the fungal spore germinates and proliferates rapidly in the various tissues ultimately causing death of silkworm. Insect haemolymph, a complex mixture of proteins, lipids, carbohydrates, nucleic acids, hormones and their degradation product, is primarily responsible for supplying nutrients, transferring metabolic wastes to maintain normal growth and development. Haemolymph is the only extracellular fluid of insects with diverse functions and 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 [18]. Pathogens induce several biochemical and physiological alterations in insects [13]. The progress of infection by a pathogen in the host tissue can be monitored by studying the degree of variation in metabolic constituents [20].The present study was designed to find out the biochemical activity of haemolymph in silkworm, *B. mori* during the infection of fungal pathogen, *B.bassiana.*

2. Materials and Methods
2.1. Rearing of *B.mori*
The disease free layings of PM x CSR_2 hybrid silkworm, *B.mori* was procured from the Government Grainage Center, Konam, Nagercoil. The silkworm larvae were reared as per rearing method of Krishnaswami [10].

2.2. Fungus culture
The fungal culture of *B.bassiana* was obtained from the Institute of Microbial Technology (IMTECH), Chandigarh, India. The fungal culture was maintained as per the procedure of Govindan *et al*. [8].

2.3. Treatment of *B. bassiana* to silkworm, *B. mori*
LD_{50} values are important to evaluate the toxicity level and allow the determination of the sub-lethal doses. The present study began with the determination of 96 hrs; LD_{50} of *B. bassiana* to PMxCSR2.The treatment of LD_{50} concentration of *B.bassiana* was given to freshly moulted, two hours starved fifth instar silkworm larvae. The treated mulberry leaves were shade dried before providing to larvae. One group was fed with pathogen treated mulberry leaves (inoculated control).The another group was fed with fresh mulberry leaves applied with

3. Results and discussion

The results on the investigation of biochemical changes in protein, lipid, carbohydrate and trehalose (mg/ml) content in haemolymph of *B. bassiana* infected silkworm larvae are presented in Table 1. Results showed that the larvae fed with *B.bassiana* treated leaves at 1st to 6th day of the fifth instar recorded decrease in protein (158.09 ± 4.86 to 143.28 ± 4.76 mg/ ml), lipid (30.53 ± 1.12 to 26.54 ± 0.73 mg / ml) and carbohydrate (3.68 ± 0.14 to ± 6.23 ± 0.78 mg/ ml) content compared to control group (161.17 ± 4.72 to 205.42 ±5.48 mg/ ml, 30.24 ± 1.11 to 33.57 ± 0.92 mg /ml, 3.44 ± 0.12 to 7.11 ± 0.29 mg /ml respectively). Increased trend of trehalose was noticed in both inoculated (*B.bassiana*) (3.14 ± 0.09 to 7.12 ± 0.22 mg/ml) and control (2.85±0.11 to 6.42 ± 0.14 mg/ml) groups.

The data from the table clearly indicate that the inoculation of fungal pathogen resulted gradual reduction of protein, lipid and carbohydrate contents in the haemolymph of fifth instar silkworm larvae. However, the trehalose content increased in the inoculated group compared to control.

The biochemical parameters such as, proteins, carbohydrates, lipids, nucleic acids *etc*., vary significantly during the life cycle of all living organisms. Proteins are the essential constituent of living cells and play a crucial role in all biological processes. Nagata and Yashitake [17] explained the quantitative variation in these biomolecules in the body of insects depends upon the nutritional status of the food and their utilization during growth and metamorphosis. Kodaira [9] suggested that the aberrations of protein content in the infected silkworm may be due to coagulation of protein substance with the increased moisture requirement of fungal pathogen, *B. bassiana*. Degtyareva [4] 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 *B. bassiana* and DDT. The reason for the decrease in protein content may be either by activated proteolysis or impaired protein synthesis in the tissues during infection.

The present investigation was correlated with Rajitha *et al.* [21] who observed the reduction of protein content in the advanced stage of *B.bassiana* infection in *B.mori* and 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. Thirupathamma and Savithri [24] examined the protein levels in three tissues viz., integument, midgut and silkgland of fifth instar silkworm, *B.mori* infected with *B.bassiana.* They reported that during the progression of fungal pathogen, *B.bassiana,* resulted gradual reduction of protein content in the three tissues.

Lipids always represent the major component of the fat body and the main source of metabolic fuel in insect. Significant reduction of lipid content was observed in the present study during the course of fungal pathogen. It might be due to the utilization of lipids in metabolic activity of host to combat against the infection of the pathogen. Increased lipase activity in infected haemolymph may also be the reason for decreased content of lipid in the haemolymph [5, 15, 3, 20]. Mallikarjuna *et al.* [12] noticed the reduction of lipid content in the haemolymph of *B. bassiana* inoculated larvae and suggested that the lipids are used as a source of energy required for the growth and development of fungus. In contrast to the present study, increased level of lipid content was recorded by Aboul-ela *et al.* [1], who noticed double folded elevation of lipid level in the haemolymph of *Plodia interpunctella* larvae after treatment with *Bacillus thuringiensis.*

Carbohydrates are non-reducing disaccharide, the principal haemolymph sugar, is maintained at a steady state in insects through homeostatic regulation at all stages of the life cycle [26]**.** In the inoculated *B.mori*, the carbohydrate content reduced when compared to control. The present work was in agreement with Rajitha *et al.* [21], who reported that inoculation of fungal pathogen resulted gradual reduction of protein, lipid and carbohydrate contents in the haemolymph during the progress of *B. bassiana*. It may be due to the utilization of carbohydrates by the pathogen and low food intake.

The major source of trehalose in the haemolymph appears to be from the breakdown of glycogen in the fat body [23, 14]. Unni *et al*. [25] explained that the higher concentration of trehalose in the haemolymph may be due to the release of trehalose as a result of histolysis of various tissues or release from the fat body. The present work was supported by Rajitha *et al*. [21]. They reported that the significant elevation of trehalose content in infected haemolymph might be due to the conversion of glycogen in to trehalose and its subsequent release in to the haemolymph. Another reason may be the efficiency of infected larvae to utilize the available haemolymph trehalose for deriving energy to put forth growth.

Table 1: Biochemical changes in proteins, lipids, carbohydrates and trehalose (mg/ml) contents in the haemolymph of *B.bassiana* infected silkworm larvae.

Biochemical activity	Treatments	I day	II day	III day	IV day	V day	VI day
Protein	Control	161.17±4.72	169.33±5.16	174.08±3.06	183.18±3.48	197.05±1.23	205.42±5.48
	Inoculated	158.09 ±4.86 (1.91)	172.07±3.45 (1.62)	192.28±4.32 (10.40)	178.35±2.63 (-2.64)	160.53±4.87 (18.53)	143.28±4.76 (30.25)
Lipid	Control	30.24±1.11	31.57±1.23	32.09±1.09	32.24±1.34	33.19±1.08	33.57±0.92
	Inoculated	30.53±1.12 (0.96)	30.76±0.45 (-2.57)	29.03±1.19 (-9.54)	28.33±1.10 (-12.13)	27.14±1.21 (-18.23)	26.54±0.73 (-20.94)
Carbohydrate	Control	3.44 ± 0.12	3.93± 0.32	4.20 ± 0.27	5.66 ± 0.42	6.90 ± 0.18	7.11± 0.29
	Inoculated	3.68 ± 0.14 (6.98)	4.06± 0.23 (3.31)	4.38 ± 0.17 (4.28)	5.97 ± 0.54 (5.48)	6.54 ± 0.33 (-5.22)	6.23 ± 0.78 (-12.37)
Trehalose	Control	2.85 ± 0.11	3.12± 0.12	4.85± 0.33	5.02 ± 0.11	5.94 ± 0.21	6.42 ± 0.14
	Inoculated	3.14 ± 0.09 (10.18)	3.78 ± 0.17 (21.15)	5.27 ± 0.42 (8.66)	5.88 ± 0.19 (17.13)	6.35 ± 0.10 (6.90)	7.12 ± 0.22 (10.90)

Note: Values in parentheses indicate the percentage change over the normal control

5. Conclusion

The present study indicates the reduction of biochemical molecules such as, protein, lipid and carbohydrates and the elevation of trehalose content in the haemolymph of *B.mori* infected with *B.bassiana.*

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