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# Haemocyte dynamics in silkworm *Bombyx mori* genotypes infected with fungal pathogen *Beauveria bassiana*

*M. Sheeba Praveena*
*sheeba.praveena.a@gmail.com*
*Sri Padmavati Mahila Visvavidyalayam, Tirupati, Andhra Pradesh*

*G. Savithri*
*ganta.savithri@gmail.com*
*Sri Padmavati Mahila Visvavidyalayam, Tirupati, Andhra Pradesh*

## ABSTRACT

***The silkworm, Bombyx mori L. is a delicate and sensitive lepidopteron insect, which has been domesticated for silk production since time immemorial. Due to continuous domestication, silkworm becomes susceptible to various diseases. During the course of infection, the cellular defense mechanism in silkworm is mediated by different types of hemocytes. In the present study, popular bivoltine and multivoltine breeds were selected to carry out an experiment to understand the day to day changes in total hemocyte count (THC) in silkworm larvae inoculated with Fungal Pathogen Beauveria Bassiana (Balsamo) Vuillemin. During the course of progressive infection of Fungal Pathogen Beauveria bassiana, significant enhancement of total haemocyte count (THC) was observed till the third day in Pure Mysore x CSR2; later gradual reduction of the haemocyte population was noticed and in (CSR2 X CSR27) X (CSR6 X CSR 26) (Bivoltine Double hybrid) course of progressive infection of Fungal Pathogen Beauveria bassiana, significant enhancement of total haemocyte count (THC) was observed till the fourth day and later gradual reduction of the haemocyte population was noticed.***

**Keywords:** *Lepidopteron, Beauveria Bassiana, Haemocyte, Breeds.*

## 1. INTRODUCTION

Silkworm (*Bombyx mori*) is one of the important economic insects spinning a cocoon. But the silkworm always suffers from diseases in the sericulture production, especially white muscardine caused by infection with entomopathogenic fungi. *Beauveria bassiana* was the first discovered fungus causing the devastating white muscardine disease to the silkworms and are always a troublesome pathogenic fungus in sericulture (Arnold, J. W *et al* 1979, Jone *et al* 1979, Nittono *et al* 1960). *B. bassiana* works by direct penetrating into the insect cuticle and colonize *in vivo* (Balavenkatasubbaiah M, *et al* 2001). Since in insects the haemolymph is considered as a medium for maintaining constancy in the internal environment, it may be presumed that the haemolymph may show some changes in physical properties and chemical composition when an insect is infected with fungal, which may lead to high mortality of the particular insect species. Haemocytes are a complex of several types of cells that circulate within the haemolymph, but are sometimes attached loosely to other tissues or are enmeshed within them. In insects, several types of haemocytes are observed in the haemolymph (Arnold, 1979; Jones, 1979). Nittono (1960) classified the blood cells in the silkworm, *Bombyx mori* L. into six types *viz*., Prohaemocytes, Plasmatocytes, Granulocytes, Spherulocytes, imaginal spherulocytes (observed only at the adult stage, but occasionally in pupa on the day before emergence) and Oenocytes. Balavenkatasubbaiah *et al*., 2001 and Balavenkatasubbaih and Nataraju, (2005) reported five types of hemocytes *viz*., Prohaemocytes, Plasmatocytes, Granulocytes, Spherulocytes and Oenocytes in the silkworm.

Muscardine caused by *Beauveria bassiana* is the most contagious disease, leads to 30-40 per cent cocoon crop loss in total loss due to diseases (Nataraju *et al* 2005). The degree of variations in the number of haemocytes can be used as an index for diagnosis of the disease. In view of the significance of haemocytes in defence mechanism the present study was aimed to enumerate total and differential haemocyte count during the development of fungal pathogen *Beauveria bassiana* in 5th instar silkworm larvae in different breeds of silkworm

## 2. MATERIALS AND METHODS

Silkworm hybrid of Pure Mysore x CSR2 and (CSR2 X CSR27) X (CSR6 X CSR 26) (Bivoltine Double hybrid) was selected for the study. The silkworm larvae were brushed and reared in the laboratory under optimum conditions as suggested by 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 $10^6$ spores/ ml @ 50 ml/100 worms for 45 sec). The larvae treated with double distilled water were used as a control. After 24 hours of induction of pathogen, haemolymph was collected every day by clipping third pair of abdominal legs and drained into prechilled eppendoff tubes. The haemolymph was used for the enumeration of total and differential haemocyte count by using phase contrast microscope. Total haemocyte count was enumerated in the haemolymph of treated and control batches following the method described by Tauber and yeager (1935) using haemocytometer. The total haemocyte count (THC) per mm3 of haemolymph was calculated using the formula suggested by Jones (1962).

Haemocytes in five 1 mm2 × Dilution × Depth factor of chamber

No. of squares counted

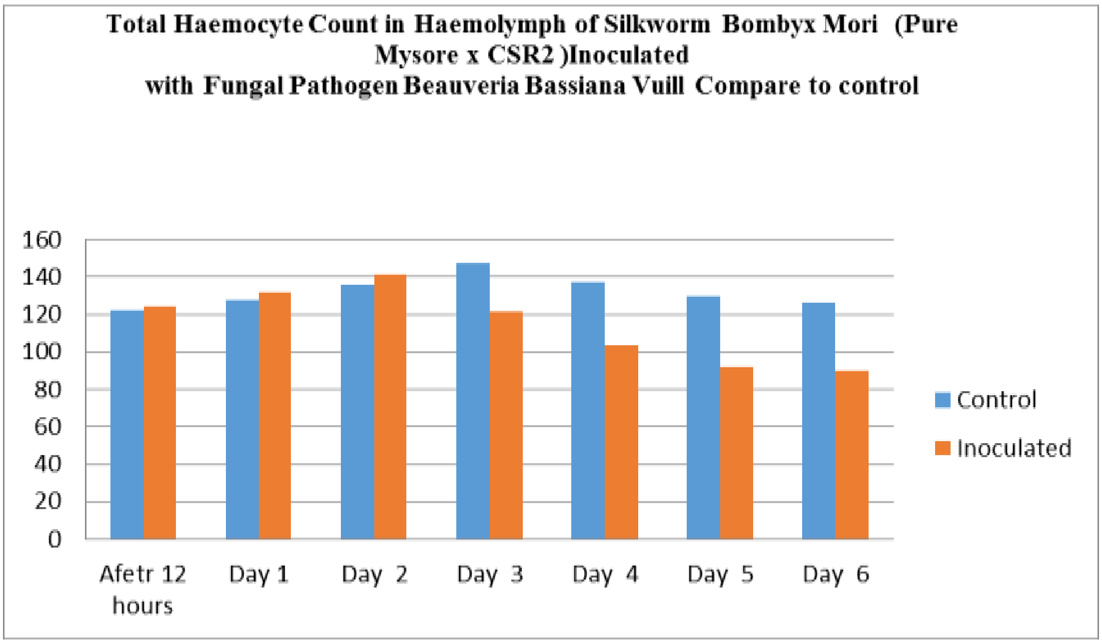
Differential haemocyte count (DHC) was estimated by counting different haemocytes from a haemocyte population of 200, based on the morphological features as described by Nittono (1960). Statistical analysis was performed by the following t-test

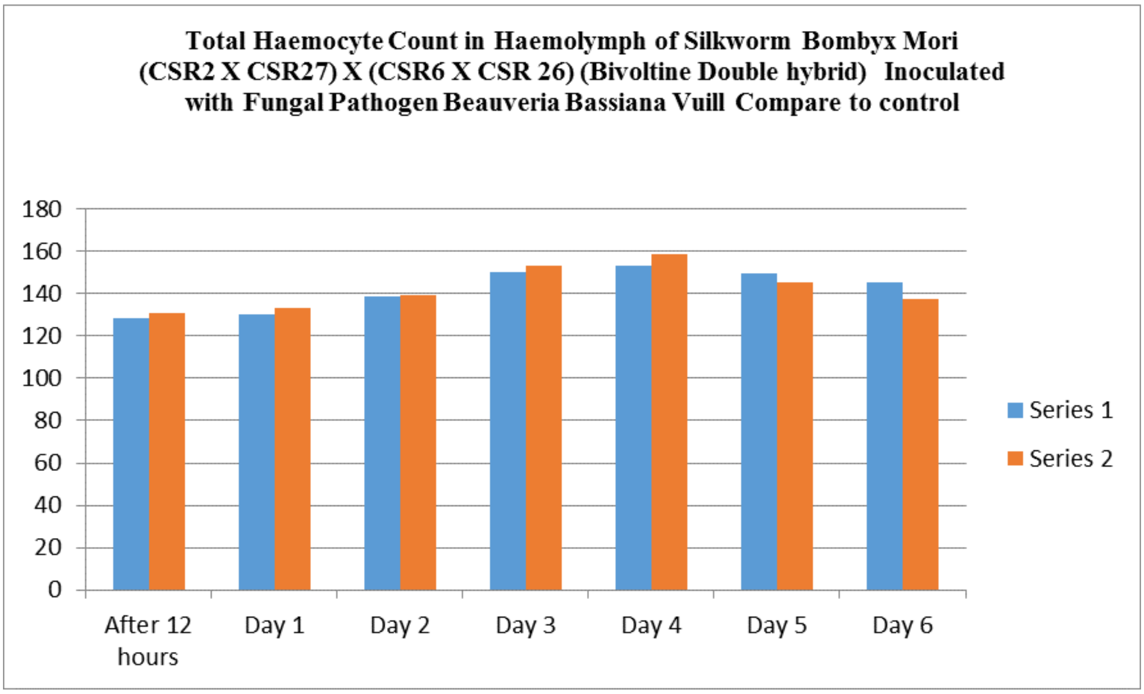
**Haemolymph sample loading**

**Table 1: Total Haemocyte Count in Haemolymph of Silkworm *Bombyx Mori* Inoculated with Fungal Pathogen *Beauveria Bassiana* Vuill Compare Control with the cross breed and double hybrid**

| | Total Haemocyte Count (THC) | | | |
|---|---|---|---|---|
| V Instar | Control Pure Mysore x CSR2 | Inoculated Pure Mysore x CSR2 | Control (CSR2 X CSR27) X (CSR6 X CSR 26) (Bivoltine Double hybrid) | Inoculated (CSR2 X CSR27) X (CSR6 X CSR 26) (Bivoltine Double hybrid) |
| 12 hours after inculation | 122.75±0.12 | 124±0.16 NS | 128.11±3.45 | 131.01±2.138** |
| Day 1 | 127.5±0.59 | 131.5±1.13 *** | 130.13 ± 2.90 | 133.14±0.98*** |
| Day 2 | 135.5±1.08 | 141.36±2.18*** | 138.36±6.12 | 139.17±1.32 NS |
| Day 3 | 147.13±0.96 | 121.18±1.87**** | 150±33.4 | 153.18±0.13*** |
| Day 4 | 136.98±5.78 | 103.78±4.23**** | 153.21±0.34 | 158.34±1.34 * |
| Day 5 | 129.56±1.34 | 91.95±1.30**** | 149.24±1.98 | 145.12±0.34**** |
| Day 6 | 126.12±2.13 | 89.76±2.13**** | 144.67±0.89 | 137.67±2.15**** |

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






## 3. RESULTS AND DISCUSSIONS

Present investigation tried to analyze cellular immunopotency in Bombyx mori larvae during the development of fungal pathogen i.e. Beauveria bassiana by enumerating the population density of total haemocytes. The haemotological investigations envisaged, no significant change in total haemocyte count in control on the first day of in both the breeds of the 5 the instar silkworm larvae. First day onwards elevation of total haemocyte count was noticed in both the breeds the trend continued up to 3 rd day of the instar in case crossbreed and till 4 the day of the instar in Bivoltine Double hybrid. Then decline of haemocyte count was observed in both breeds i.e., on the 4$^{th}$ day in cross breed (control: 136.98±5.78, Inoculated:103.78±4.23) and 5$^{th}$ day in Bivoltine Double hybrid (control: 149.24±1.98, Inoculated :145.12±0.34). Beauveria bassianna inoculated cross breed silkworms showed a decrease in total haemocyte count (THC) when compared to control and the reduction of THC (4.12) in Bivoltine Double hybrid is lower than the cross breed, which indicates a higher degree of defense mechanism compared to cross breed. Significantly higher total haemocyte count was recorded in double hybrid (CSR2 XCSR27) X (CSR6 X CSR 26) (Bivoltine Double hybrid) than cross breed (Pure Mysore x CSR2) in both the control groups and the same trend noticed in inoculated silkworms. Haemocytes play an essential role in defending insects against invading parasites and pathogens. The effective physiological mechanisms of phagocytosis, encapsulation, and other related defense mechanisms were primarily due to the availability of circulatory immune cells i.e., haemocytes. Initial elevation of the number of haemocytes up to 3 rd and 4 th day in the cross breed and bivoltine double hybrid respectively indicates the primary role of haemocytes to fight against the fungal pathogen Beauveria bassianna. Ananda kumar and Ann Sandhya Michael (2011) reported a significant increase of plasmatocytes and granulocytes in Bombyx mori infected with Bacillus thuringiensis. Gradual reduction of haemocytes was recorded from 3 rd and 4 th day of Beauveria bassianna infection in Cross breed and bivoltine double hybrids, as haemocytes reported to be involved in cell mediated defense mechanism. Butt et al 1988; Butt and humber 1989; Vey and Gotz 1986 reported that, Once the entomophagus fungi have penetrated in the host integument and gained access to nutrient rich haemocoel, they are confronted with host humoral and cellular defenses. Nappi (1981) and Eslin and Prevost (1998) reported the increase of the haemocyte number in the haemolymph of insects as a response to parasitism which indicates the activation of host defence mechanism. Crossely (1968) observed an increased number of phagocytic blood cells during muscle autolysis and regeneration in the larvae of Calliphora. Rizki, (1957) reported the increase in the proportion of plasmocytes and morphological modification at the end of larval development in Drosophilla. The findings infer that haemocytes appear in the haemolymph in greater numbers at certain stress conditions and at certain physiological state.

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