

Influence of Systemic Fungicide on the Hematology of Silkworm *Bombyx mori* L. Infected with *Beauveria bassiana*

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Systemic fungicides viz., Bavistin and Beyleton are reported to have curative effect against the infection of *Beauveria bassiana* in silkworm *Bombyx mori* L. To understand the influence of the systemic fungicides on the disease suppression/development, hematological studies were carried out. There was an increase in the percent total hemocyte count, granulocyte and plasmatocyte in silkworm treated with the systemic fungicides. It possibly indicates the influence of systemic fungicide on the hemocyte mediated defense system leading to the higher resistance and suppression of disease development.

Key words: Systemic fungicide, Hemocytes, Hemogram, Total hemocyte count (THC), Differential hemocyte count (DHC), Granulocyte, Plasmatocyte

Introduction

Certain fungicides such as Carbandazim and Traizole have been reported to have curative effect against muscardine disease, caused by the infection by fungal pathogen *Beauveria bassiana* in silkworm *Bombyx mori* L. (Zhou *et al.*, 1990; Sreedharan *et al.*, 1991; Virendra Kumar *et al.*, 1997). Two systemic fungicides, Bavistin (1 – 2% Bavistin, a carbandazim fungicide 50% WP, Rallis India Ltd., India) and Bayleton (0.05 – 0.1% Bayleton 25% WP, a Triazole compound, Rallis India Ltd., India) have been reported to control muscardine disease in silkworm, *Bombyx mori* L. (Virendra Kumar *et al.*, 1997). In order to understand the influence of these systemic fun-

gicides on the host physiology in defense against the muscardine disease development, hematological studies were carried out as hemocytes play an important role in defense against infections in insects.

Materials and Methods

The hemocyte response of silkworm by the infection of *B. bassiana* and the role of systemic fungicide were evaluated using healthy 4th instar silkworms of NB18 breed. The response was quantified based on the total hemocyte count and differential hemocyte count. The total hemocyte count (THC) is the number of cells in specified volume of hemolymph. The differential hemocyte count (DHC) provides the relative proportion of different types of hemocytes in the blood. The THC and the DHC per mm³ of hemolymph were estimated at 72 and 144 hrs after percutaneous inoculation with conidia of *B. bassiana* and *per os* feeding of the systemic fungicides. The THC and DHC were estimated using Neubauer hemocytometer counter. The prohemocytes, plasmatocytes, granulocytes, oenocytes and spherulocytes were identified based on their morphological characters under phase contrast microscope.

The two systemic fungicides, Bavistin - (1 – 2% Bavistin, a carbandazim fungicide 50% WP, Rallis India Ltd., India) and Beyleton - (0.05 – 0.1% Bayleton 25% WP, a Triazole compound, Rallis India Ltd., India) were fed to silkworm through mulberry continuously for two days to 4th instars silkworm inoculated topically with conidia of *B. bassiana* (4×10^6 conidia/ml). There were two treatment concentrations viz., Bavistin 1.00 (BV1) and 2.00% (BV2) and Beyleton 0.05 (BL1) & 0.1% (BL2) tested for each systemic fungicide. There was an inoculated control and normal control with larva neither pathogen inoculation nor treatment with fungicide. The treatments were given immediately after 3rd moult. Each treatment had ten rep-

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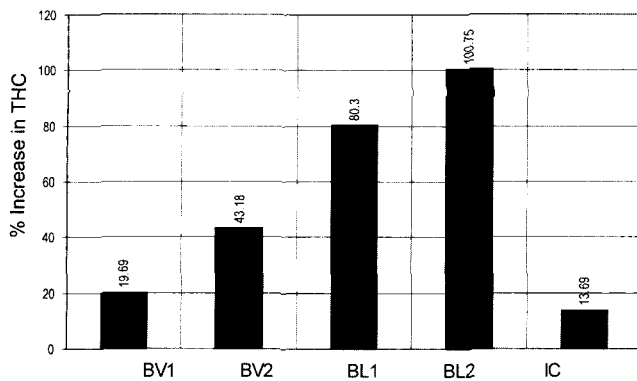


Fig. 1. Percent increase/decrease in total hemocyte count in systemic fungicide and inoculated control over normal control (72 hrs). BV1: Bavistin 1%; BV2: Bavistin 2%; BL1: Beyleton 0.05%; BL2: Beyleton 0.1% and IC: Inoculated control.

lications and each replication was represented by one larva. The hemolymph was collected directly from each larva on to the hemocytometer and estimated the THC and DHC per mm^3 of hemolymph. Observations were also recorded with regards to disease development in treated and control batches.

Results

Observations at 72 hrs post-inoculation (Fig. 1), in silk-worm indicated that the inoculated control group developed the symptoms of the disease *viz.*, formation of oily speck, death with flaccidity, gradual change into hardened body condition and finally characteristic mummification with larva becoming hard dry white stick having white powdery covering. In treatments with different concentrations of systemic fungicide, the larvae did not develop symptoms of the disease. There was percent increase in THC in all inoculated control as well as all systemic fungicide treatments. The percent increase in THC in treatment BL2 was maximum with increase in percentage of cells over normal control being 100.75%. It was followed by BL1 (80.30%). In the inoculated control, the increase was 13.63%. In case of BV1, the maximum % increase in THC over normal control was 19.69% and BV2 was 43.15%.

At 144 hrs (Fig. 2), the disease symptoms were not seen in the treated batches. The % increase in THC was only in treatment BL2 (57.22%) and BV1 (1.73%). It was negative in all other treatments with the reduction ranging from -10.98% (BL1) to -50.86% (Inoc. Control).

The result with regards to the differential count at 72 hrs post inoculation (Fig. 3), indicated significant % increase in granulocyte in number over the normal control. The

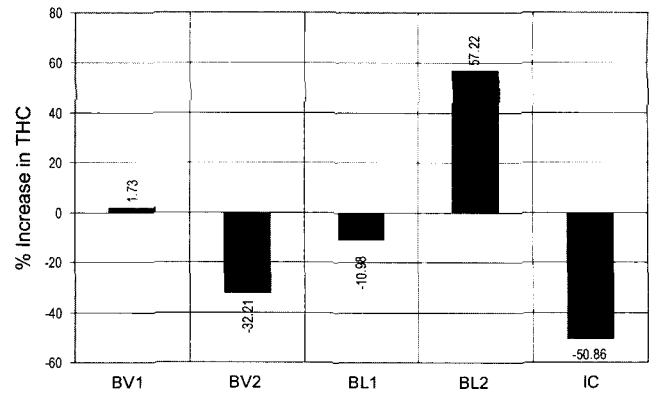


Fig. 2. Percent increase/decrease of total hemocyte count in systemic fungicide and inoculated control over normal control (144 hrs). BV1: Bavistin 1%; BV2: Bavistin 2%; BL1: Beyleton 0.05%; BL2: Beyleton 0.1% and IC: Inoculated control.

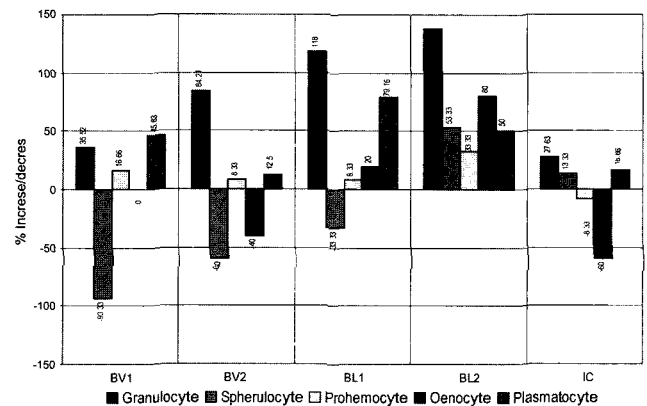


Fig. 3. Percent increase/decrease in different hemocyte count in systemic fungicide treated larvae and inoculated control over normal control (72 hrs). BV1: Bavistin 1%; BV2: Bavistin 2%; BL1: Beyleton 0.05%; BL2: Beyleton 0.1% and IC: Inoculated control.

increase was 138% (BL2), 118% (BL1), 84.21% (BV2) and 35.22% (BV1). The increase was by 27.63% in inoculated control. Similar trend of increase in plasmatocyte count was recorded where the percent increase was 50% (BL2), 79.16% (BL1), 12.50% (BV2) and 45.83% (BV1). The prohemocytes also showed marginal increase in count in all treatment with maximum in BL2 (33.33%). However the count was lower in inoculated control. The count with regards to spherulocyte and oenocytes showed trend of decrease with the exception in treatment BL2 where there was increase by 53.33% and 80%, respectively.

The differential count at 144 hrs post inoculation (Fig. 4), indicated trend of increase in granulocyte in number in treatment BL2 (47.89%) and BV1 (12.60%) while in BL1, BV2 and inoculated control the trend was on the decrease -15.12%, -44.53% and -54.62% respectively

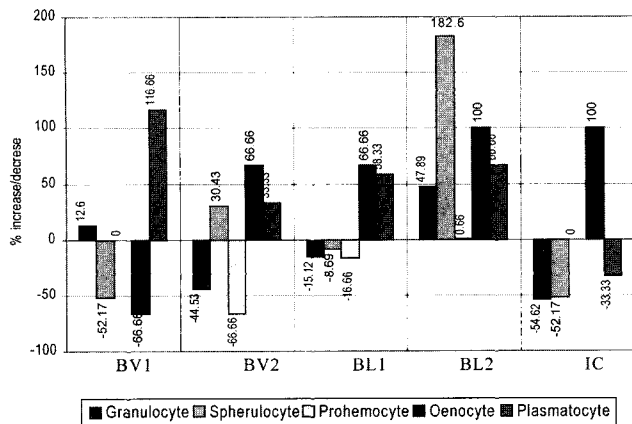


Fig. 4. Percent increase/decrease of different hemocytes in systemic fungicide and inoculated control larvae over normal control (144 hrs). BV1: Bavistin 1%; BV2: Bavistin 2%; BL1: Beyleton 0.05%; BL2: Beyleton 0.1% and IC: Inoculated control.

over the normal control. However the prohemocyte showed the trend of increase. It was 116.66% (BV1), 66.66% (BL2), 58.33% (BL1) and 33.33% (BV1). The spherulocyte (182.60%) and Oenocyte (100%) also has shown significant increase in trend in treatment BL2 and BV2.

Discussion

The two fungicides whose role is under study, on feeding through mulberry for two days to 4th and 5th instars silkworm infected topically with conidia of *B. bassiana* (4×10^6 conidia/ml) resulted in reduction in mortality due to muscardine by over 90% as against 100% mortality in inoculated control. Bavistin at 1% conc. reduced the mortality by 90% in 4th instars and 91% in final instars silkworm while at 2%, the reduction was 92% and 96% respectively. Beyleton at 0.05 and 0.1% concentration, the reduction in mortality was by 82% and 88% during 4th instars and by 88% and 92% during 5th instars, respectively (Virendra Kumar *et al.*, 2002). It is observed from the result of this study that there was a trend of increase in THC and granulocyte as well as spherulocyte count in silkworm fed on systemic fungicides over the normal control especially during the first 72 hrs p. i. The significant increase in the THC and DHC, especially with granulocyte and plasmatocyte count is an indication of effort of insect to eliminate the invader by cellular defense response. In insects, the main hemocyte mediated response is encapsulation (Carton and Nappi, 1997; Ratcliffe, 1993). This cellular immune reaction is influenced by the genetic and physiological parameters of the host

and the parasitoid. The number and type of host hemocytes are two of key factors required for a successful immune reaction (Eslin and Prévost, 1998). The conidia of *B. bassiana* in silkworm enter through the integument and spread infection by budding off hyphal bodies in the hemocoel, which spreads the infection in the host. Phagocytosis of hyphal bodies and live conidia of lowly pathogenic fungi such as *Isaria fumoso-rosea* and *Harziella entomophila* has been reported (Kawakami, 1965). However, the granulocytes failed to phagocytose the live conidia of highly pathogenic fungi (Govindan *et al.*, 1998). It is possible that the virulent pathogens such as *B. bassiana* are able to overcome the phagocytic activity and encapsulation and destroy the phagocytes (Hou and Chang, 1985; Vey and Vago, 1971). It is also observed that many pathogens are phagocytised and digested when the number are below the lethal dose. The phenol oxidase system, which is involved in phagocytic process, melanin production around the wounds and formation of humoral and cellular capsules has been studied in silkworm. Phenol oxidases have been detected with polyclonal antibody in oenocytoids of silkworm (Iwana and Astida, 1986).

It is possible that the systemic fungicides especially Beyleton at 0.1% concentration (BL2) influence the physiological status of the host to enhance the defense response through increased production of hemeocytes which would actively participate to eliminate the invading pathogen. There may also be influence on other defense systems in insects as is indicated in treatments involving Bavistin. In treatment involving Bavistin the suppression of disease development is significantly high but the level of increase in THC and DHC is low as compared to treatment with Beyleton. It is possible that the influence on the hemogram of insect is only an approach in insects. There may be other defense systems in insects influenced by the systemic fungicides to defend against muscardine.

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