# EFFECT OF DIFFERENT NUTRITIONAL CONDITIONS OF SILKWORM *BOMBYX MORI* (LEPIDOPTERA: BOMBYCIDAE) ON ITS SUSCEPTIBILITY TO *BEAUVERIA BASSIANA* (HYPHOMYCETES: MONILIALES)

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ABSTRACT: Effect of nutritional conditions of silkworm *Bombyx mori* on the susceptibility to *Beauveria bassiana* was studied and discussed in this paper. There was significant difference in the susceptibility of silkworm to *B. bassiana* when the nutritional conditions of silkworm were changed. Feeding with V1 variety mulberry leaves increased the susceptibility of silkworm to the fungus. Feeding silkworm with tender leaves also increased the susceptibility of silkworm to *B. bassiana*. The $LT_{50}$ value was 6.18 days when tender leaves were provided and it was 6.34, 6.62 and 6.59 days with medium, matured and normal leaves, respectively. Feeding with tree mulberry leaves resulted in less mortality when compared to the others.

KEY WORDS: *Beauveria bassiana*, *Bombyx mori*, nutritional condition, susceptibility.

Muscardines caused by fungal pathogens are the most virulent and contagious diseases which occurs throughout the sericultural areas of the world (Steinhaus, 1949). White muscardine caused by *Beauveria bassiana* (Bals.) Vuill. is one of the most common silkworm diseases which are predominant during rainy and winter seasons in India. The loss due to white muscardine varies from 5 to 50 % in different countries (Jayaramaiah & Kuberappa, 1987). Selvakumar et al. (2002) reported that incidence of muscardine was high during rainy (17.96 %) and winter (17.36 %) seasons. A variety of factors may determine or influence the susceptibility of a host to infection by *B. bassiana*. These include the fungal strain, the host's physiological state, nutrition, defense mechanisms and a number of other diverse factors such as the environment.

A variety of factors may determine or influence the susceptibility of a host to infection by *Beauveria bassiana*. These include the fungal strain, population density of host, the host's physiological state, age nutrition, genetics, exposure to injuries, defense mechanisms and a number of other diverse factors such as the environment. The susceptibility of silkworm to most of the pathogens is controlled by poly genes. Due to this, this character is prone to modification by external factors such as food, temperature and chemicals.

The susceptibility of silkworm to most of the pathogens is controlled by poly genes. Due to this, this character is prone to modification by external factors such as food, temperature and chemicals. There is some evidence indicating that the food quality influences the susceptibility of silkworm to viral infections. Silkworm reared on the artificial diet with mulberry leaf powder was more resistant to infection than the one without mulberry leaf powder (Matsubara & Hayashiya, 1969). They also indicated that silkworms reared on the artificial diet containing mulberry leaf powder from the spring season were more tolerant to infection than that containing leaf powder of autumn. Silkworms fed with artificial diet containing low protein; sucrose and high cellulose tend to have increased susceptibility to viral infections (Watanabe & Imanishi, 1980). The diet with low protein lowered the protease level in the gut juice of silkworm resulting in low anti-viral activity (Watanabe et al., 1989).

Mulberry (*Morus* spp.) leaves constitute the only food source to the silkworm *Bombyx mori*. There are different varieties of mulberry cultivated in India for rearing mulberry silkworm. The quality of mulberry leaf varies according to the variety, season, age of the plant and height of the plant. When poor quality leaves are provided to silkworm they become undernourished and such silkworms will be more susceptible to the pathogens. Nutritional effect on the disease development and cocoon traits in silkworm was studied by Mareppa et al. (1999) with respect to BmNPV and Bhaskar et al. (1999) with respect to BmCPV and found significant influence. Selvakumar et al. (2005) also studied on the influence of environmental and nutritional factors on the development of flacherie disease in silkworm.

In the present paper influence of mulberry leaf quality on the susceptibility of silkworm to the fungal pathogen *B. bassiana* is discussed.

## MATERIALS AND METHODS

Influence of mulberry leaf quality on the susceptibility of silkworm to *B. bassiana,* was studied in three experiments. Larvae of a popular bivoltine silkworm hybrid (CSR2 × CSR4) were used for the experiments. *B. bassiana* was cultured in Petri plates using Sabouraud's dextrose agar. Conidia were harvested by brushing the surface of three week-old culture into a 500 ml glass beaker containing 50 ml sterile distilled water using a sterile camel hair brush. A drop of tween-20 was added to the beaker containing distilled water and conidia to keep the conidia dispersed. The conidial suspension was prepared by mixing the solution using a magnetic stirrer for 5 minutes and its concentration was determined based on counts made with an improved Neubauer haemocytometer. The required concentration of *B. bassiana* inoculum ($1 \times 10^5$ conidia/ml) was prepared by suitably diluting the stock inoculum with sterilised distilled water.

In the first experiment, the effect of feeding mulberry leaves from three different mulberry varieties was studied. Mulberry varieties V1, S36 and K2 were used for the study. Newly ecdysed fourth instar larvae (out of third moult) were counted (100 each) and topically inoculated by dipping them in *B. bassiana* inoculum suspension of $1 \times 10^5$ conidia/ml concentration. The inoculated larvae were then reared separately by providing mulberry leaves of the three varieties in plastic rearing trays (90 cm × 60 cm). High relative humidity (95±5 % RH) was provided by keeping wet foam pads in the rearing trays and a temperature of 25±1°C was maintained in the rearing room. Three replications were maintained for each feed schedule. Muscardine incidence was recorded for ten days post inoculation. $LT_{50}$ and $LT_{90}$ values were calculated for each feed schedule by probit analysis (Finney, 1971).

Similar experiments were conducted with four different maturities of leaves (tender, medium, matured and normal) and also with mulberry leaves from different sources (tree leaves, bush leaves and shade leaves). Each treatment had 3 replications and 100 larvae were kept per replication. The mortality due to muscardine was recorded every day post inoculation on daily basis and $LT_{50}$ and $LT_{90}$ was calculated for each treatment following probit analysis (Finney, 1971).

## RESULTS

Influence of feeding three different mulberry varieties on the susceptibility of silkworm to *B. bassiana* is presented in Table 1. It is observed that mortality due to white muscardine was comparatively less up to 8 DPI (71.67%) when the larvae were fed with K2 variety mulberry leaves. The feeding of V1 variety resulted in maximum mortality (100%) followed by S36 (89%) by 8 DPI. However, by 9 DPI, feeding on different varieties of mulberry resulted in 100 % mortality due to muscardine.

Results of feeding leaves of different maturity *viz.*, tender, medium and matured leaf vis-à-vis normal leaves on the susceptibility of silkworm to *B. bassiana* are presented in Table 2. Mortality started appearing on 5 DPI and 100% mortality was recorded by 8 or 9$^{th}$ day in the last three treatments while it was on 7 PDI when fed with tender leaves. The $LT_{50}$ value was 6.18 days when tender leaves were provided. It was 6.34, 6.62 and 6.59 with medium, matured and normal leaves, respectively. The $LT_{90}$ value varied between 8.07 and 8.65 days in the different batches.

Observations made on feeding mulberry leaves from different sources *i.e.,* tree, shade grown plants and mulberry grown in the form of bush on the susceptibility of silkworm to *B. bassiana* are presented in Table 3. It is observed that leaves from tree mulberry resulted in less mortality by 8 DPI 9 74.07%) than from the others. However by 9 DPI, 100% mortality due to muscardine was recorded, irrespective of quality of mulberry fed. The $LT_{50}$ value with tree leaf was 6.88 days and with shade and bush leaves it was 6.42 and 6.55 days, respectively.

## DISCUSSION

Stressed hosts are more susceptible to entomopathogens than non stressed hosts (Steinhaus, 1958; Vago, 1963). Stresses brought about by malnutrition, metabolic imbalances, physical and other factors may result in infection by potential pathogens or by the activation of chronic to acute infection. Certain stresses may enhance the chronic infection or activate latent (occult viral infections).

In the present study there was significantly reduced mortality up to 8 DPI when the larvae were fed with K2 variety mulberry leaves. The V1 variety feeding resulted in maximum mortality followed by S36 after 8 days of inoculation. However, in all the varieties 100% mortality was observed by 9 days post inoculation. Here, the difference in the humidity conditions in these varieties might have influenced the difference in mortality. It is well known that the V1 variety is superior in nutritional point of view to silkworm (Sarkar et al., 2000). But its leaf moisture content was high and also it retains leaf moisture for longer periods. This may enhance the bed humidity facilitating fast growth of the fungus. Declining nutrient and water content in the mature foliage of perennial plants was reported to reduce the growth rates of lepidopteran larvae compared with those of closely related species feeding on younger leaves or the foliage of herbaceous plants (Krischik & Denno, 1983). It has also been reported that that high protein concentrations in an insect's diet can counter balance the toxic effect of secondary metabolites, such as alkaloids (Costa & Gaugler, 1989).

The effect on feeding leaves of different maturity leaves *viz.*, tender, medium and matured leaves revealed that mortality was very fast with tender leaves when compared to other quality leaves. The $LT_{50}$ value was low when tender leaves were provided, which was followed by medium, matured and normal leaves respectively. Usually the tender leaves which contain high moisture are provided to young age worms (80-85 %). Medium leaves contain 65 to 70 % leaf moisture and mature leaves contain 55 to 60 % moisture. The tender leaves with high moisture content when fed to fifth instar larvae may grow faster (Rahamathulla et al., 2004) but it will increase the bed humidity to higher levels. This predisposes to *B. bassiana* infection at a greater rate. The concentration of secondary metabolites in plants is said to be higher in young leaves than in older leaves, but older leaves contain fewer nutrients (*i.e.* nitrogen and water) (Fenny, 1992).

The effect of feeding mulberry leaves from different sources *i.e.* tree leaves, shade grown leaves and bush leaves, on the muscardine disease development indicated that the tree leaves induced less mortality when compared to the others. The $LT_{50}$ value with tree leaf was more. The plants growing under shade produce thin leaves with low dry matter content due to low photosynthetic activity (Nagarjunaiah, 1976). As reported by Balakrishna et al. (1999) shading creates a stress environment for mulberry and all the parameters were decreased when it was grown in shade.

Nutrition is a very important factor regulating the susceptibility of insects to entomopathogens and inadequate nutrition often leads to its increased susceptibility. Conversely, diet can also decrease the susceptibility of insect pests to entomopathogensc Hyphomycetes. Ekesi et al. (2000) reported that thrips (Megalurothrips *sjostedti* as less susceptible to *Metarhizium anisopliae* on certain cow- pea cultivars because of plant derived fungistatic compounds.

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Table 1. Influence of feeding leaves of different mulberry varieties on the susceptibility of silkworm to *B. bassiana*.

| Mulberry variety | Cumulative mortality due to white muscardine in days after inoculation (%) | | | | | | $LT_{50}$ (Days) | $LT_{90}$ (Days) |
|---|---|---|---|---|---|---|---|---|
| | 5 | 6 | 7 | 8 | 9 | 10 | | |
| V1 | 5.67 ±1.155 | 30.33 ±4.041 | 55.33 ±3.512 | 100.00 ±0.00 | 100.00 ±0.00 | 100.00 ±0.00 | 6.55 | 8.58 |
| S36 | 4.67 ±1.528 | 29.33 ±2.517 | 50.67 ±2.082 | 89.00 ±3.00 | 100.00 ±0.00 | 100.00 ±0.00 | 6.67 | 8.79 |
| K2 | 2.67 ±0.577 | 22.00 ±3.606 | 39.33 ±1.528 | 71.67 ±3.512 | 100.00 ±0.00 | 100.00 ±0.00 | 6.94 | 9.11 |
| SE± | 0.94 | 2.82 | 2.06 | 2.14 | 0.00 | 0.00 | | |
| CD at 5% | 2.31 | 6.89 | 5.03 | 5.33 | NS | NS | | |

Table 2. Influence of feeding leaves of different maturity on the susceptibility of silkworm to *B. bassiana*.

| Leaf maturity | Cumulative mortality due to white muscardine in days after inoculation (%) | | | | | | $LT_{50}$ (Days) | $LT_{90}$ (Days) |
|---|---|---|---|---|---|---|---|---|
| | 5 | 6 | 7 | 8 | 9 | 10 | | |
| Tender | 12.00 ±2.00 | 42.00 ±3.00 | 100.00 ±0.00 | 100.00 ±0.00 | 100.00 ±0.00 | 100.00 ±0.00 | 6.18 | 8.07 |
| Medium | 7.67 ±1.53 | 34.00 ±2.00 | 84.67 ±2.52 | 100.00 ±0.00 | 100.00 ±0.00 | 100.00 ±0.00 | 6.34 | 8.29 |
| Mature | 4.67 ±0.58 | 23.67 ±2.52 | 52.00 ±3.00 | 100.00 ±0.00 | 100.00 ±0.00 | 100.00 ±0.00 | 6.62 | 8.61 |
| Normal | 5.00 ±1.00 | 29.00 ±1.00 | 57.67 ±3.06 | 95.00 ±3.00 | 100.00 ±0.00 | 100.00 ±0.00 | 6.59 | 8.65 |
| SE± | 1.13 | 1.84 | 2.03 | 1.23 | 0.00 | 0.00 | | |
| CD at 5% | 2.61 | 4.25 | 4.68 | 2.82 | NS | NS | | |

Table 3. Influence of feeding mulberry leaves from different sources on the susceptibility of silkworm to *B. bassiana*.

| Mulberry type | Cumulative mortality due to white muscardine in days after inoculation (%) | | | | | | $LT_{50}$ (Days) | $LT_{90}$ (Days) |
|---|---|---|---|---|---|---|---|---|
| | 5 | 6 | 7 | 8 | 9 | 10 | | |
| Tree | 3.67 ±0.58 | 24.33 ±3.22 | 42.00 ±3.00 | 74.67 ±3.06 | 100.00 ±0.00 | 100.00 ±0.00 | 6.88 | 9.06 |
| Shade grown | 6.00 ±2.00 | 35.00 ±3.00 | 72.33 ±3.06 | 100.00 ±0.00 | 100.00 ±0.00 | 100.00 ±0.00 | 6.42 | 8.42 |
| Bush | 5.00 ±1.00 | 32.33 ±3.06 | 57.33 ±2.52 | 97.33 ±2.52 | 100.00 ±0.00 | 100.00 ±0.00 | 6.55 | 8.61 |
| SE± | 1.09 | 2.52 | 2.34 | 1.87 | 0.00 | 0.00 | | |
| CD at 5% | 2.66 | 6.18 | 5.73 | 4.57 | NS | NS | | |