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Effect of *Beauveriabassiana*infection and subsequent dusting of plant powder on protein content of midgut (MG), silkgland (SG), Fat bodies (FB) tissue and haemolymph (HL) on 3rd day of fifth instar larvae of PM and CSR2 *B. mori* L.

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ABSTRACT

In the present investigation, the silkworm *Bombyx mori* L. larvae inoculated with *Beauveria bassiana*and subsequent dusting of plant powder was studied. The protein content from midgut, silkgland, fatbody and hemolymph was observed on 3rd day of fifth instar larvae from control and experimental groups. Due to inoculation of *B. bassiana*reduction in protein content was observed. Significant increased or similar protein content was observed in inoculated and subsequent dusting of plant powder.The secondary metabolites in plant powder fight against the fungal disease observed no change in biochemical mioteis but due to the plant products increased the level of biochemical than the inoculated, which is useful for larvae to complete their normal life cycle.

Key Words:*Bombyx mori, Beauveria bassiana,*Protein content

INTRODUCTION

Principal classes of organic compounds forms the carbohydrates, proteins and lipids. In biochemical processes proteins, carbohydrates and lipids plays important role during growth and development of insects (Ito and Horie, 1959; Wyatt, 1961 and 1967). Protein plays an important role in the formation of structures in organisms. It is derivative of high molecular weight polypeptides. Proteins are also used for energy purpose like carbohydrates and fats. The tissue protein is used as last source of energy. It is used only when carbohydrates or fats are not available. Proteins occurred in all parts of cells, they are most abundant biological molecules. Proteins occur in variety of forms like small peptides to huge polypeptides with millions of molecular weight. Proteins perform variety of functions in various organisms and different cells in the same organism. Proteins make antibodies, proteins hormones and contractile elements of the cell. In insects proteins are present abundantly in fat bodies and the haemolymph protein synthesis in insect embryogenesis was studied by Chen (1971).

During larval development in insect fatbody responsible for the synthesis of various major haemolymph proteins and serves at the same times as the place of storage of these components (Shigematsu, 1958; Chipendale and Kelbey, 1969; Locke and Collins, 1967; Kulkarni and Mehrotra, 1970; Price, 1973, Thomson *et al*., 1975; Wyatt, 1978; Woodring *et al*., 1976; Bhawane and Bhanot, 1989). Any stress on the animal causes the metabolic adjustments in its tissues through modifications or modulations of proteins (Bano*et al*., 1981; Assen and Hanke, 1983). The total protein content of structural and soluble proteins involved in the architecture and metabolism of a cell. The role of glucose in the metabolism of silkworm has been revised by Chitra and Sridhara (1973).

Therefore, in the present study infection of *B. bassiana*subsequent dusting of plant powder on changes in biochemical mioteis like protein content was studied on 3rddayof fifth instar larvae of PM and CSR2 race.

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MATERIAL AND METHODS

Animal:

For present study the disease free layings of multiviltine pure mysore i.e. (PM X PM) and bivoltine CSR2 i.e. (CSR2 X CSR2) breeds of *Bombyx mori* were used. The cocoons were brought from Sericulture Grainage Center, Sankeshwar road, Gadhinglaj, District-Kolhapur, Maharashtra, India. Silkworms are reared in departmental rearing house of department of zoology, Shivaji University, Kolhapur, Maharashtra, India and hatched larvae were fed with mulberry M5, S36 and V1 varieties cultivated in Departmental mulberry garden. The silkworm rearing was done by following standard protocol suggested by Krishnaswami (1978, 1979).

Fungus:

The fungus culture of *Beauveria bassiana* was made available from Microbial Type Culture Collection (M.T.C.C), institute of Microbial Technology, Chandigarh, India. Culture was maintained as per the procedure of Govindan *et al.*,(1998).

Dusting of plant powder:

Dusting of plant powder to *B. bassiana* because the fungal spores inoculated to silkworm through skin by deeping the individual larvae in solution containing the fungal spores. Therefore used the plant powder for dusting prepared the effective formulations by using lime. Each plant powder prepared the three concentration 2.5%, 5% and 10% and observed the reduction in mortality and used for further experimentation.

Biochemical analysis:

Homogenate preparation:

The biochemical study was done on 3rd day of fifth instar. The larvae dissected out in cold ringer solution. The tissue were weighted on the electronic balance and prepared the homogenate of midgut tissue for total protein estimation was prepared in 2N NaoH. For haemolymph biochemical, study the haemolymph was collected by pricking the prolegs of silkworm larvae in small vials precoated with phenylthiourea to prevent the melonization. Then the samples of haemolymph centrifuged immediately at 3000 rpm for 15 minutes for total protein estimation samples were prepared in 2N NaoH.

Estimation total Protein by Lowry's method:

The protein concentration in the tissue and haemolymph from all the experimental groups were estimated by Lowry *et al.*, (1951) method.

RESULT

Protein:

i. Midgut:

In control group of CSR2, midgut tissue showed the high protein content (8.21mg/gm) than the midgut of PM (7.28mg/gm). In the *B. bassiana*inoculated protein content of midgut decreased by 70.60% in PM but in the CSR2 race no change was observed.

Dusting of *C. longa* after innocualtion of fungus *B. bassiana*observed no significant change in protein content in midgut of CSR2, but decreased percentage was noticed by 27.06% in PM. Dusting of *A. mexicana*plant powder showed the 14.28% decreased protein in midgut in PM and in the CSR2 37.63% decrease was observed in protein content. The dusting of *C. multiflorum*resulted in increase of 96.20% and 37.62% in midgut proteins of PM and CSR2 race respectively.

The above observation revealed that inoculation of fungus in PM race responsible for the reduction of protein from midgut tissue at significant level but in CSR2 race results were

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more or less similar to its control group. The results of dusting of antimicrobial plant powder used in the present study after inoculation of fungus midgut tissue of silkworm races under study showed the opposite results in their two races. In PM race except *C. multiflorum*three is reduction of protein from midgut tissue but in CSR2 race there is in general increase of protein of midgut over 37% but the application of *C. longa* did not affect the protein content in CSR2 race.

ii. Silkgland:

In control groups, PM silkgland tissue showed the high protein content i.e. 15.93mg/gm than in the CSR2 i.e. 11.37mg/gm. In the *B. bassiana*inoculated protein content in silkgland was decreased by 43.06% in PM race but in CSR2 race the silkgland protein was increased by 42.56%. The dusting of *C. longa* after the inoculation of fungus *B. bassiana*the silkgland protein was decreased by 45.13% in PM race and increased percentage was noticed in CSR2 by 4.66%. The dusting of *A. mexicana*plant powder the silkgland protein was decreased by 24.48% in PM and increased in CSR2 by 20.49%. The dusting of *C.multiflorum*plant powder also noticed similar results i.e. decreased silkgland protein in PM by 21.46% and increased the silkgland protein in CSR2 by 11.43% observed on 3rd day of the experiment. The above results showed the *B. bassiana*inoculation causes the decreased protein in silkgland in PM and increased silkgland protein the in CSR2. After the dusting of plant powder the PM race showed the decreased silkgland protein in all the groups. The maximum decrease was observed in PM race of *C. longa* treated group. In CSR2 race the silkgland protein was increased in all groups as compared to control. The maximum silkgland protein observed in *A. mexicana*treated group.

iii. Fat body:

In control group of both races the high protein content in fat body tissue was observed in PM race (12.26mg/gm) than in the CSR2 (11.60mg/gm). The *B. bassiana*inoculation causes the decreased fatbody protein by 89.32% in PM race and in the CSR2 increased the fat body protein by 9.31%. After the inoculation of *B. bassiana,* larvae subsequently treated with the plant powder, the dusting of *C. longa* showed the decreased percentage of fat body protein by 42.95% in PM race. While the percent decrease was observed in the CSR2 by 8.18%. The dusting of *A. mexicana*powder showed the decreased fat body protein by 33.0% in PM race while in the CSR2 the decrease was observed by 17.52%. The dusting of *C. multiflorum*plant powder the decreased was observed in PM by 33.25% while in CSR2 race the fat body protein was increased by 22.5% on 3rd day of experiment.

The above results showed that the *B. bassiana*inoculation responsible for reduction in fat body protein in PM. The dusting of plant powder in both the races showed the decreased fat body protein except the *C. multiflorum*treated group of CSR2 race.

iv. Haemolymph:

The high protein in haemolymph was observed in PM i.e 1.8mg/ml than the CSR2 race i.e. 1.45mg/ml in their control groups. The *B. bassiana*inoculation showed the decreased protein content in haemolymph of both the races. The percent decreased was noticed in PM by 45% and in the CSR2 by 65.5% protein in haemolymph. The dusting of plant powder after the inoculation of *B. bassiana,* the dustingof *C. longa* plant powder showed the decreased protein in haemolymph by 7.22% in PM and by 27.58% in the CSR2. The dusting of *A. mexicana*powder increased haemolymph protein observed in PM race by 16.66% while in CSR2 race no change was observed as compared to its control group. The dusting of *C. multiflorum*plant powder showed the decreased haemolymph protein in both the races, the decreased percentage was observed

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maximum in CSR2 race i.e. 28.27% while in PM the protein was decreased by 5.55%.
From the above results, it became cleared that the inoculation of *B. bassiana*causes the reduction in haemolymph protein content. The maximum reduction was observed in CSR2 race than PM. The dusting of *C. longa* and *C. multiflorum*plant powder showed decreased the protein in haemolymph in both the races. The *A. mexicana*dusting plant powder showed the increased protein in haemolymph in PM race as compared to control. But the application of *A. mexicana*plant powder did not affect the haemolymph protein content in CSR2 race.

DISCUSSION

The Muscardine disease caused by *B. bassiana*affects on the normal physiology of silkworm *B. mori* L. The protein content is decreased due to the infection in silkworm. The results obtained in *B. bassiana*infected dusting of plant powder, the protein content was estimated on 3rd day of 5th instar larvae. On 3rd day of experiment in control group, the maximum midgut tissue protein was observed in CSR2 race than the PM race. After the *B. bassiana*inoculation, theprotein content was decreased in PM race but the increased protein observed in CSR2 race as compared to their control groups.The pathogens are reported to induce the several biochemical and physiological alteration in insect tissue (Bergold, 1963; Benz, 1963; Martignoni, 1964).The *B. bassiana*inoculation responsible for the reduction in silkgland protein. In fatbody tissue the protein content was decreased in all the groups in *B. bassiana*inoculated and subsequent oral treatment of plant extracts in both races. In insects the most important place for protein syntheisis is fat body which is also sensitive to the inoculation of BmNPV in silkworm as reported by Etebari*et al.,* (2007a). After the plant extract treatment the maximum fatbody protein was observed in *C. multiflorum*treated group in both races. In haemolymph the protein content was decreased in *B. bassiana* inoculated and plant extracts treated group in both races on the 3rd day. The Sarma*et al.,* (1994) observed that significant decrease of total protein content in the haemolymph of BmNPV infected silkworm larvae. For some times the protein level was increased in pathogen inoculated larval tissue because due to the bursting of cell membrane after the cell death and released the cellular protein, other factors have also been outlined as repoted by Matindoost (2006). The initial decrease of protein, due to the infection of *B. bassiana* may be the interference of *B. bassiana*with the process of protein synthesis of host or may be the structural destruction of tissue. Some workers (Boctor, 1978; Cheung *et al.,* 1978; Salama *et al.,* 1983) reported the similar biochemical chages in the haemolymph of insect treated with *B. thuriengensis.*

In present experiment dusting of plant powder treated larval groups showed decreased mortality rate as compared with inoculated group. The treatment of *Curcuma longa* containing terpenoid, curcumin, *Argemone mexicana* seeds contains alkaloids *C. multiflorum*contains the steroids, saponins, alkaloids, tannins *B. spectabilis* leaves contains the tannins, phenols and pinitol could fight against the fungal disease observed no change in biochemical mioteis but due to the plant products increased the level of biochemical than the inoculated, which is useful for larvae to complete their normal life cycle.

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