International Journal of Scientific Research in Chemistry (IJSRCH) | Online ISSN: 2456-8457
© 2018 IJSRCH | Volume 3 | Issue 4

The reflection of feeding the mulberry leaves treated with water solution of seed powder of Syzigium cumini (L) into Profiles of Protein (Total) in the fifth instar larvae of silk worm, Bombyx mori (L) Race - bivoltine, crossbreed: [(CSR6 x CSR26)] x [CSR2 x CSR27)].

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ABSTRACT

The seed of the fruit is used in various alternative healing systems like Ayurveda , Unani and Chinese medicine. Wine and vinegar are also made from the fruit. It has a high source in vitamin A and vitamin C. The present Attempt is concerned with the analysis of effect of Syzigium cumini (L) seed powder treatment on the levels of protein in the silkworm, Bombyx mori (L). Aqueous solutions of seed powder of Syzigium cumini (L) of different strengths (10 ppm; 20 ppm; 40 ppm & 50 ppm) was utilized to treat the leaves of mulberry. Treated mulberry leaves were fed to the fifth instar larvae of bivoltine, crossbreed race of silkworm, Bombyx mori(L) [(CSR6 x CSR26)] x [CSR2 x CSR27)]. The feeding the larvae with treated leaves was carried for first four days. The larvae fed with untreated & water treated leaves were also maintained. Total protein bioassay was carried out on fifth day through the use of silk glands, fat bodies and haemolymph. The total protein content of salivary glands of untreated group of larvae was found measured 22.817 units. Syzigium treatment was found resulted into increase in the salivary gland proteins (total) from 21 to 104 percent. Fat body total protein was increased from 34 to 90 percents through the Syzigium treatment. The haemolymph total proteins were found increased from 22 to 68 percent through the Syzigium treatment. Treating the leaves of mulberry with Syzizium powder may have had effect on the increase in the levels of amino acids. It may be followed by accelerated rate of synthesis of protein in the body of fifth instar larvae of silkworm, Bombyx mori (L).

Keywords : Bombyx Mori (L), Total Proteins, Syzigium Cumuni (L).

I. INTRODUCTION

The tree was introduced to Florida in 1911 by the USDA, and is also now commonly grown in Suriname, Guyana and Trinidad and Tobago. In Brazil, where it was introduced from India during Portuguese colonization, it has dispersed spontaneously in the wild in some places, as its fruits are eagerly sought by various native birds such as thrushes, tanagers and the great kiskadee. This species is considered an invasive in Hawaii. There is close interlinking between the life of insect herbivores and metabolites in plants. The metamorphosis in insects is said to be in the orchestrate progression. The insect metamorphosis is closely interlinked with plant metabolites. According to Bowers, et al (1966) the chemical constituents of plants (Roots; Stems; Leaves and Fruits) could have been the factors of growth & metamorphosis for

insects. The plant eating insects are able to avoid poor quality food. That is to say, the insects are able to select food from variety available for them. The larvae of silkworm, Bombyx mori (L) are monophagous. They are feeding exclusively on the leaves of mulberry Morus alba (L). For the purpose of getting qualitative silk cocoons, it is essential to fortify either the quality of food (mulberry leaves) appetite of larval instars of silkworm, Bombyx mori (L). According to Murugan and George (1992), the factors responsible for influencing the growth, development & subsequent physiology of body of silkworm larvae include: quality of nutrition, that is to say the biochemical status of nutrients in the food (Leaves of mulberry, Morus alba L); quantity of hormones (hormonal level) in the body & the conditions of climate (environmental conditions). Each and every element in body of larva is primarily derived from it's source of food material. The leaves of mulberry, Morus alba (L) are exclusive source of nutrients for the life of larval instars of silkworm, Bombyx mori (L). The leaves of mulberry, Morus alba (L) are containing the nutrients and many stimulants for the life of larval instars of silkworm, Bombyx mori (L) (Ito, 1960,1961; Nayar & Fraenkel, 1962; Ito, et al, 1964; Ito & Hyashiya, 1965). The quality of the nutrition (leaves of mulberry, Morus alba L.) serves a lot to accelerate the growth, metamorphosis in larval instars of silkworm, Bombyx mori (L). The entire credit of life of silkworm, Bombyx mori (L) goes to the nutrients in the leaves of mulberry, Morus alba (L). Therefore, the leaves of mulberry, Morus alba (L) forms the physiological foundation for sericulture. The leaves of mulberry are the mulberry, Morus alba (L). The leaves of mulberry, Morus alba (L) biochemically constituted with proteins, lipids, carbohydrates (Murali, 1992) & minerals (Subramanyam Reddy, 1992). The biochemical profile of the leaves of mulberry, Morus alba (L.) exert influence on the corresponding diversity of larval mid-gut enzymes capable of hydrolyzing the biocompounds in the body of larval instars of silkworm, Bombyx mori (L). The proteins; lipids; carbohydrates (glycogen) are stored in the body tissues of larval instars of silkworm, Bombyx mori (L) especially, the fat bodies.

There is variation in the food consumption in phytophagous insects. This may be for varied biochemical processes, ultimately for successful adaptations (Slansky, 1982). It has been suggested that, there is a functional difference between the activity of digestion by the digestive fluid in mid gut & tissue of mid gut. It has been reported by Horie, et al (1963) that, molecular proteins are hydrolyzed into peptides by digestive fluid content & into aminoacids with peptidases in the mid gut tissue. Likewise, the polysaccharides, are digested in the insect gut lumen by digestive fluid & disaccharides and/or trisaccharides get hydrolysed into their constituent monasaccharide sugars mainly in the gut tissue (Horie, 1967). Yamafugi and Yonezawa (1935) reported the analogy of insect lipase, the lipid digesting enzyme of the insect mid gut with pancreatic lipase of vertebrates. The attempts towards production of the qualitative silk through the improvement in the efficiency of consumption & utilization of food by larval instars of silkworm, Bombyx mori (L) include: improvement in the quality of mulberry leaves & supplementation of nutrient biocompounds like soya protein; potassium iodide, copper sulphate, other mineral salts, herbal products (or drugs) like digoxin (Vitthalrao & Kulkarni, 2011) kho-go (Desai, et al, 2011) and stevia inulin (Shubhangi Pawar, et al, 2017). Quality of mulberry leaves get reflected into the quality of the cocoons spun by fifth instar larvae of silkworm, Bombyx mori (L). There are reports on Use of soya protein; potassium iodide, copper sulphate, mineral salts, herbal products for improvement of the quality of leaves of mulberry, Morus alba. Herbal products are well known for the acceleration of metabolism in the body of larval instars of silkworm, Bombyx mori (L).

The Syzygium cumini (L) is a large evergreen tree, belong to family myrtacae. It is a medicinal plant. Various parts of this plant are used in controlling the

diabetes like diseases. The fruits and the seeds of Syzigium are used in folk medicine. The seeds of syzigium are excellent source of glycosides. The flavonol glycosides have been isolated from the roots of this plant. In one of the earlier studies in author's laboratory, the glycosides are reported for the fortification of digestion in fifth instar larvae of silkworm, Bombyx mori (L). The seed powder of Syzigium cumini (L) is reported for contents of glycoside (5, 7-dihydroxy-6, 2 dimethoxyisoflavone-7-O-alpha-L-rhamnoside). The aim present attempt is to screen correct dosage for treating the mulberry leaves with seed powder of Syzigium cumini (L) and appropriate time of feeding treated mulberry leaves to the fifth instar larvae of silkworm, Bombyx mori (L).

MATERIAL & METHODS

The work was divided into the steps like: Silkworm Rearing; Syzigium solution Preparation; Grouping the Fifth Instar Larvae; Treating the mulberry leaves and feeding the larvae; Protein Bioassay and Statistical analysis.

(A). Silkworm Rearing:

The egg cards or disease free layings (DFL) of biivoltne, crossbreed race: [(CSR6 x CSR26)] x [CSR2 x CSR27)] of silkworm, Bombyx mori (L) were procured through the sericulture unit of Agriculture Development Trust, Malegaon. Black boxing was followed for incubation. The early age larvae (First and Second instared larvae) (Chawki) and late age larvae (Third; Fourth and Fifth instared larvae) were reared in the laboratory of "Dr. APIS" through the methods prescribed by Krishnaswami, et al (1978) & explained in earlier attempts by Khyade (2004); Vitthalrao & Kulkarni (2011); Desai, et al, (2011) Shubhangi Pawar, et al (2017); Ramprakash Verma, et al (2018); Pranita Rajendra Vare, et al (2018); Manisha Mahendra Nalwade, et al (2018); Seema K. Dongare, et al (2018) and the others. The larvae were fed with fresh and appropriate quality leaves of mulberry, Morus alba (L) procured from sericulture unit at Malegaon Sheti Farm of Agricultural Development Trust Baramati, Shardanagar, (Malegaon Khurd). The schedule of feeding prescribed by Sharad G. Jagtap (2014) was followed for both early age larvae (First and Second instared larvae) (Chawki) and late age larvae (Third; Fourth and Fifth instared larvae). The fifth instared larvae were preferred for the analysis of effect of treating the mulberry leaves with aqueous solution of seeds of Syzigium cumini (L) and them for total protein contents.

(B). Syzigium solution Preparation: The ripen fruits of Syzigium cumini (L) were collected from Malegaon Sheti Farm of Agricultural Development Trust Baramati, Shardanagar, (Malegaon Khurd). They were identified and confirmed for species through the Botanical Survey of India, Pune. Seeds were separated and allowed for shade drying. It was followed by preparation of seed powder through the use of domestic mixture. Known quantity of this powder was kept for maceration in distilled water for twenty four hours. Macerated content was allowed for filtration through muslin cloth. Volume of filtrate and weight of residue were accounted for knowing the strength of seed powder in the solution. The filtrate was further utilized for preparation of aqueous solution known strength. Four different concentrations of solution were prepared, which include: 10 ppm; 20 ppm; 40 ppm and 50 ppm.

(C). Grouping the Fifth Instar Larvae: Soon after the fourth moult, the the fifth instared larvae were divided into six groups, each with hundred individuals. The groups include: Untreated Control; Water treated Control and four treated groups. The four treated groups include: 10 ppm; 20 ppm; 40 ppm and 50 ppm. 400 ml of aqueous solution of seed powder was used to treat 100 grams of fresh mulberry leaves. The treatment was carried out for half an hour before feeding. The treated mulberry leaves were drained off completely & then fed to the fifth instar larvae of silkworm, Bombyx mori(L) in respective groups. Feeding treated mulberry was carried out for the first four days of fifth instars.

(D).Treating the mulberry leaves and feeding the larvae:

Mulberry leaf treatment was carried half an hour before each feeding. 2000 ml of aqueous solution of seed powder of each strength was used to treat 500 grams of fresh mulberry leaves for feeding the group of hundred larvae for each time. Fresh leaves of mulberry, Morus alba (L) were weighed. The known volume of solution of each strength was taken in separate glass jar. Known quantity of mulberry leaves was kept immersed separately in aqueous solution of each strength. The treatment was carried out for half an hour before feeding. The treated mulberry leaves were drained off completely & then fed to the fifth instar larvae of silkworm, Bombyx mori (L) in respective groups. Four feedings were followed (5.00 a.m.; 11.00 a.m. ; 5.00 p.m. ; 11.00 p.m.). Five hundred grams leaves of mulberry, Morus alba (L) were used for feeding the group of hundred larvae for each time. The feeding treated mulberry was carried out for the first four days of fifth instars. The larvae fed with untreated mulberry leaves and water treated mulberry leaves were also maintained.

(E). Bioassay of Total Proteins from the Salivary Glands; Fat bodies and Haemolymph:

The bioassay of total proteins from salivary glands; fat bodies and haemolymph was carried out on fifth day of fifth instar. Twenty larvae from each group were selected randomly. Ten larvae were utilized for total protein estimation from silk glands. And remaining ten larvae were used for haemolymph total protein estimation. The chloroform soaked cotton pads were used for the provision of anaesthetia to the fifth instar larvae of silkworm, Bombyx mori (L). Weight of individual larva was recorded. Individual larva was dissected open from dorsal side. Both the silk glands from individual larva were separated. The larval dissection was carried in chilled saline (0.9 percent sodium chloride solution). The fifth instar larvae are dissected for silk glands and fat bodies in chilled saline (0.9 percent NaCl). Similarly, the fat bodies from each dissected larva were separated. The tissues were blotted separately and weighed accurately on electronic balanace. Both the tissues were washed separately in ice cold saline. There after, each tissue was blotted; blotted & weighed accurately on electronic balance. Each tissue was then processed for fragmentation followed by homogenization in chilled in chilled distilled water. Clean & sterilized morter & pestle were used for tissue homogenization. Each tissue assay sample was processed for keeping at 37oC for twenty four hours in the solution of sodium hydroxide of normal (1.0 N) strength.

Bioassay of total proteins from silk glands and fat bodies was carried in triplicate (for assay sample three test tubes were taken). 1 ml assay sample was transferred to each test tube. Addition of 5.0 ml Lower‘s —C solution was made in each of the test tube mixed well and kept for 15 min to allow the formation of copper protein complex. A blank was also prepared simultaneously. After 15 min, 0.5 ml Folin‘s phenol regent was added to each tube and mixed well. Then they were allowed to develop colour for 30 min at room temperature. After it, the optical density was recorded at 660 nm on spectrophotometer. The results were replicated three times. The protein concentration of assay sample was calculated by referring the optical density obtained for sample and by using standard graph and expressed in the unit as µg proteins per mg tissue.

Through pricking the prologs with sterilized needle, the haemolymph from fifth instar larvae was collected in separate small vials precoated with phenyl thiourea (phenyl thiourea prevent melanization of content). Volume of haemolymph was measured. Each vial was weighed accurately. Weight of empty vial was subtracted to get the weight of haemolymph (mg/ml). It was stored at – 200 C and used for bioassay of total proteins. For estimation of total proteins, in a clean centrifuge tube 0.1 ml of haemolymph was taken, to this 1ml of 10% TCA solution was added and centrifuged for 10 min at 3000 rpm. The content was mixed with 2ml of 0.1 N NaOH and

processed for estimation of proteins by Lowry's method as described earlier.

(F). Statistical analysis:

Consistency in the results is qualitative parameter in research studies. Therefore, the whole experimentation in the present study was repeated for thrice. The data of all the three attempts was collected and subjected for statistical analysis. The statistical parameters for analysis considered in the study include mean, standard deviation, percent change & significance through student t – test introduced by William Sealy Gosset (a chemist working for the Guinness brewery in Dublin, Ireland. "Student" was his pen name) (https://en.wikipedia.org/wiki/Student%27s_t-test) and explained by Norman & Baily (1955).

Table 1. Contents of Total Protein in Silk Glands, Fat Bodies and Haemolymph in the fifth instar larvae of silkworm, *Bombyx mori* (L) (Race: Bivoltine Cross Breed [(CSR6 x CSR26) x CSR2 x CSR27)] fed with the leaves of mulberry, *Morus alba* (L) (M-5: variety) treated with aqueous solution of seed powder of *Syzigium cumuni* (L).

Group → Tissue	Untreated Control	10 ppm	20 ppm	40 ppm	50 ppm
Silk Glands	22.817 (±0.445) 00.000	27.608 (±0.649) 20.997	31.432 (±0.751) 37.756	32.923 (±2.756) 44.291	46.614 (±5.631) 104.29
Fat Bodies	14.951 (±0.783) 00.000	20.157 (±1.018) 34.821	25.571 (±1.651) 71.032	26.905 (±1.203) 79..954	28.518 (±3.427) 90.743
Haemolymph	13.563 (±0.684) 00.000	16.666 (±0.855) 22.878	17.373 (±1.547) 28.091	18.043 (±1.617) 33.031	22.786 (±3.445) 68.001

- Each figure is the mean & three replications.
- Figure in parenthesis with ± sign is the standard deviation.
- Figure below parenthesis is percent change.

* : P<0.05

** : P<0.01

*** : P<0.001

The results on the contents of total protein in the fifth instar larvae of bivoltine, crossbreed, silk worm, Bombyx mori (L) fed with mulberry Morus alba (L) (M-5: variety) leaves treated with water solution of seed powder of Syzigium cumini (L) are summarized in table 1 and Figure- 1.

The total protein contents of silk glands; fat bodies and haemolymph of the fifth instar larvae of bivoltine race [(CSR6 x CSR26) x CSR2 x CSR27)] silkworm, Bombyx mori (L) recipients of untreated leaves of mulberry, Morus alba (L) (M-5: variety) (untreated control group) in present attempt were found measured 22.817 (±0.445); 14.951 (±0.783) and 13.563 (±0.684) units respectively.

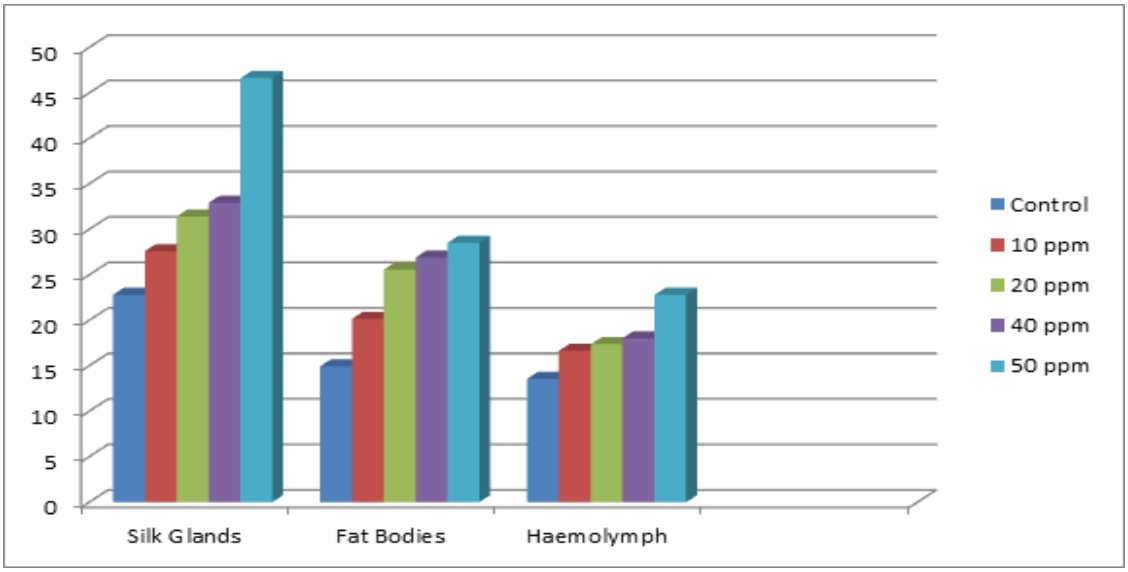
The quantitative estimation of total protein of silk glands; fat bodies and haemolymph of the fifth instar

larvae of bivoltine race [(CSR6 x CSR26) x CSR2 x CSR27)] silkworm, Bombyx mori (L) recipients of leaves of mulberry, Morus alba (L) (M-5: variety), treated with aqueous solution of seed powder of Syzigium cumini (L), with 10 ppm strength in present attempt was found measured 27.608 (±0.649); 20.157 (±1.018) and 16.666 (±0.855) units respectively. In comparison with the control group, there was 20.997; 34.821 and 22.878 percent increase in total proteins respectively in of silk glands; fat bodies and haemolymph of the fifth instar larvae of bivoltine race [(CSR6 x CSR26) x CSR2 x CSR27)] silkworm, Bombyx mori (L) through treating leaves of mulberry, Morus alba (L) (M-5: variety) with 10 ppm aqueous solution of seed powder of Syzigium cumini (L).

The total proteins of silk glands; fat bodies and haemolymph of the fifth instar larvae of bivoltine race [(CSR6 x CSR26) x CSR2 x CSR27)] silkworm, Bombyx mori (L) recipients of leaves of mulberry, Morus alba (L) (M-5: variety), treated with aqueous solution of seed powder of Syzigium cumini (L), with 20 ppm strength in present attempt were found measured 31.432 (±0.751); 25.571 (±1.651) and 17.373 (±1.547) units respectively. In comparison with the control group, there was 37.756; 71.032 and 28.091 percent increase in total proteins respectively in of silk glands; fat bodies and haemolymph of the fifth instar larvae of bivoltine race [(CSR6 x CSR26) x CSR2 x CSR27)] silkworm, Bombyx mori (L) through treating leaves of mulberry, Morus alba (L) (M-5: variety) with 20 ppm aqueous solution of seed powder of Syzigium cumini (L).

Treating leaves of mulberry, Morus alba (L) (M-5: variety), with aqueous solution of seed powder of Syzigium cumini (L), with 40 ppm strength and feeding them to the fifth instar larvae of bivoltine race [(CSR6 x CSR26) x CSR2 x CSR27)] silkworm, Bombyx mori (L) for continuous four days was found reflected into quantity of total proteins in silk glands; fat bodies and haemolymph, which measured 32.923 (±2.756); 26.905 (±1.203) and 18.043 (±1.617) respectively. In comparison with the control group, there was 44.291; 79.954 and 33.031 percent increase in total proteins respectively in of silk glands; fat bodies and haemolymph of the fifth instar larvae of bivoltine race [(CSR6 x CSR26) x CSR2 x CSR27)] silkworm, Bombyx mori (L) through treating leaves of

Figure 1. Contents of proteins in Silk Glands; Fat Bodies and Haemolymph in the fifth instar larvae of silkworm, Bombyx mori (L) (Race: Bivoltine Cross Breed [(CSR6 x CSR26) x CSR2 x CSR27)] fed with the leaves of mulberry, Morus alba (L) (M-5: variety) treated with aqueous solution of seed powder of Syzigium cumuni (L).



mulberry, Morus alba (L) (M-5: variety) with 40 ppm aqueous solution of seed powder of Syzigium cumini (L).

The quantitative estimation of total protein of silk glands; fat bodies and haemolymph of the fifth instar larvae of bivoltine race [(CSR6 x CSR26) x CSR2 x CSR27)] silkworm, Bombyx mori (L) recipients of leaves of mulberry, Morus alba (L) (M-5: variety), treated with aqueous solution of seed powder of Syzigium cumini (L), with 50 ppm strength in present attempt was found measured 46.614 (±5.631); 28.518 (±3.427) and 22.786 (±3.445) respectively. In comparison with the control group, there was 104.29; 90.743 and 68.001 percent increase in total proteins respectively in of silk glands; fat bodies and haemolymph of the fifth instar larvae of bivoltine race [(CSR6 x CSR26) x CSR2 x CSR27)] silkworm, Bombyx mori (L) through treating leaves of mulberry, Morus alba (L) (M-5: variety) with 50 ppm aqueous solution of seed powder of Syzigium cumini (L).

Change in the strength of aqueous solution of seed powder of Syzigium cumini (L) from 10 ppm to 50 ppm for treating the leaves of mulberry, Morus alba (L) (M-5: variety) and feeding the fifth instar larvae of bivoltine race [(CSR6 x CSR26) x CSR2 x CSR27)] silkworm, Bombyx mori (L) was observed to exert considerable change amount of the total protein of silk glands; fat bodies and haemolymp. Significant increase in the levels of total proteins of silk glands; fat bodies and haemolymph of the fifth instar larvae of silkworm, Bombyx mori (L) fed with mulberry leaves treated with various concentrations of aqueous solution of seeds of Syzigium, herbal formulation may be explained away as due to enhanced break down of contents of mulberry leaves. The contents of seeds of Syzigium cumini (L) may improve appetite & digestion. Some of the herbal powders contain insect juvenoids (like eugenol) which are known to increase the capability of consumption & utilization of food by insects like silkworm. Insect juvenoid activity of the contents of seed powder of Syzigium cumini (L) should be analyzed. According to Sen (1988), there is enhanced synthesis of poly (A) RNA in phytophagous insects through exogenous compounds. It may be possible for contents of seed powder of Syzigium cumini (L) to accelerate the rate of synthesis of total proteins in the tissues like silk glands; fat bodies and haemolymph in fifth instar larvae of silkworm Bombyx mori (L). According to Applebaum (1985), continuous feeding in insects get reflect into improvement in the rate of metabolism through the enhanced activities of the enzymes. Individual dosage of maceratives of seed powder of Syzigium cumini (L) responsible for improved contents of total proteins of silk glands; fat bodies and haemolymph should screened out. This may help to improve the technology of rearing the larval instars of silkworm, Bombyx mori (L) for commercial silk fiber.

II. ACKNOWLEDGEMENT

Academic inspiration and help received from International Science Community Association (ISCA) and Agriculture Development Trust (ADT), Shardanagar (Baramati) deserve appreciations.

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