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6.1. The muga silkmoth, *Antheraea assama* Ww (Lepidoptera: Saturniidae) is an exclusively Indian species endemic to the North-eastern part of India., specially in the Brahmaputra valley of Assam and Meghalaya. The species is fundamentally wild in nature. In late eighties of the twentieth century trial for rearing this species in the plains of Coochbehar District has been initiated. Since ninties of the same century its cultivation has been commercialized in this non-traditional area. But the success is not at par with that in its homelands. Research work on this species in the non-traditional area has started recently.

The present endeavour is a preliminary attempt to study the rearing success, biochemical profile of haemolymph of the larvae of *A. assama*, their major diseases and the resistance pattern of the larvae against bacteriosis. The biochemical profiles assessed from larval haemolymph included total protein, lipid and carbohydrate, and amino acids and polypeptides.

6.2. The rearing success was assessed during four prime cropping seasons of 2004 – 2006, such as Chotua (Feb-Mar), Jethua (Apr-May), Bhodia (Aug-Spt) and Katia (Oct-Nov). Of these, Chotua and Bhodia are the seed crops while Jethua and Katia constitute the commercial crops. The ERR value was the best during the Katia season. During this season, the moderate atmospheric temperature and relative humidity ranges appeared to be congenial for muga culture in non-traditional area of Coochbehar.

6.3. A survey of nature and intensity of disease revealed higher frequencies of bacteriosis and virosis and negligible existence of pebrine. The extent of larval death due to bacteriosis ranged from about 8-24 per cent at different seasons with highest value during Bhodia and lowest death during the Katia season. The virosis ranged between about 10 and 21 per cent, the highest, again, during Bhodia and the lowest during Katia season. Higher temperature and high relative humidity during Bhodia promoted both the fatal diseases.

6.4. The symptoms of the diseases were more or less same as in case of *Bombyx mori* for respective diseases. In case of bacteriosis the worms lose appetite and become sluggish. With the progress of disease, worms become flaccid and turn dark brown. The pulsation of dorsal vessel may become rapid and for sometime the larvae wriggle as if in pain, and then vomit brown fluid. Excreta turn soft and sticky. During moulting the exuvium cannot be shed properly. In toxicosis sudden cessation of feeding, lifting of heads, spasm and tremor, paralysis and distress are the symptoms. These are followed by sudden collapse and death. In chronic toxicosis ingestion is reduced, faecal pellet become irregular-shaped and occasional vomiting occurs. After a few days clasping power of the leg is lost, body become flaccid and subsequently death occurs.

6.5. The susceptibility of the larvae to bacteriosis was variably higher when these were reared on the principal host plant som (*Persea bombycina* Kost) than the larvae reared on another principal host plant, soalu (*Litsaea polyantha* Juss.).

6.6. Result of morphological, staining and biochemical characterization indicate the existence of the genera *Pseudomonas*, *Klebsiella*, *Citrobactor*, *Proteus*, *Providentia* and *Bacillus* in the bacteriosis affected muga silkworm in Coochbehar district of West Bengal.

A few gram positive and gram-negative cocci has been isolated from the muga silkworms suffering from bacteriosis needs further investigation for their identification at the specific level. Further investigation is required to study the pathogenecity of the isolated bacteria during different seasons and also to explore possible preventive/control measures.

6.7. The fifth instar larvae had higher quantities of protein, lipid and carbohydrate contents in their haemolymph, precisely more than three times those of the fourth instar larvae. This happened due to much higher quantity of food consumption by the fifth stage larvae and consequent accumulation of resources of higher quantity. Because this stage is the crucial period regarding energy storage for metamorphosis from larva to pupa and for formation of cocoon as well as for gamete formation especially the heavily yolk-laden oocytes.

It was found that the protein and carbohydrate contents of haemolymph of larva fed with soalu leaves were higher along with larval weight, cocoon weight and shell weight as well. Larvae fed with som leaves contained higher lipid content in haemolymph, and silk ratio per cent was also higher. Pooled analytical data revealed significantly higher protein content in the haemolymph of female than its corresponding male and total haemolymph lipid was found significantly

higher in male than in the females. Larval body water content had no relation to the host plant. But there were significant differences among the instars. The lowest water content was recorded in the fifth instar and highest in the fourth instar.

6.8. Day wise study recorded a rapid increase of haemolymph protein, lipid and carbohydrate contents in fifth instar larva. Lipid and carbohydrate content attained their peak on eighth day and the protein on last day i.e. on ninth day. A comparative study was undertaken to assess the protein, lipid and carbohydrate profiles in the haemolymph of the larvae reared exclusively on som, exclusively on soalu, up to 3rd instar on som then on soalu and finally, up to 3rd instar on soalu and then on som plant. There were no significant differences on the day wise increment of haemolymph biochemical parameters for the change of host plant at third instar stage.

There was no statistical correlation of the larval character (larval weight, water content) or cocoon characters (cocoon weight, shell weight, shell ratio) for the change of host plant at third instar stage. It should be noted that the silk ratio per cent is higher in the larva which were reared on soalu plant up to third instar then transferred to the som plant. Therefore this combination of the host plant might be recommended for commercial rearing.

6.9. In *A. assama* it was recorded that the nonessential amino acids dominated the essential amino acids like in other arthropods. The study recorded proline and cysteine in *A. assama*. There is no earlier record of

haemolymph free proline and cysteine in *A. assama*. But proline is very essential for the proper growth of the larvae and the sulphur containing amino acid, cysteine is important for intermediary metabolism and serves as a source of sulpho-hydral (-SH) group for the synthesis of coenzymes and hormones. In this study tryptophan could not be detected from the samples, though it is a component of muga silk filament. *A. assama* may have the ability to convert indole acetic acid to tryptophan likewise in *B. mori* larvae. The larvae fed with soalu leaves showed a higher quantity of amino acids than the larvae fed on som leaves.

Aspartic acid and glutamic acid were drastically reduced in the diseased worms which were directly involved in proper growth process. Arginine, lysine and histidine are involved in the regulation of haemolymph osmotic pressure, which were obtained in a very low quantity in the diseased worms.

A higher concentration of glycine occurred in the diseased worms. This increase needs further investigation as this amino acid play crucial role in detoxification mechanism.

6.10. Altogether 10-15 protein / peptide peaks were obtained from the haemolymph samples of fourth and fifth instar healthy and fifth instar diseased muga silkworm by HPLC assay. The protein / peptide peaks appeared at 17-18 min. and at 32-33 minutes are the major constituents of haemolymph protein. One of the major peak of healthy fifth instar appeared around 44 min sharply goes down in all worms suffering from bacteriosis which requires further investigation to find out its role. One extra protein / peptide peak appeared (in number) in worms at early

bacteriosis and at bacterial toxicosis stage (though the appearance time is different), but not in late bacteriosis needs further investigation to explain its role and immunological significance, if any, in diseased worms.

The SDS-PAGE assay of the haemolymph samples revealed that all the healthy worms had irrespective of their sexes, five major peptides of 108-112, 32-33, 26-28, 18-19, and 17 kDa. The major bands having 56, 26 and 23 kDa molecular weight are female specific and 42 and 24 kDa peptides are male specific. The major peptides having molecular weight of 32-33 and 17 kDa were present in all samples and these can be considered as essential peptides of muga silkworm having general physiological significance. A peptide band having molecular weight at the range of 23-24 kDa recorded from our study needs further investigation as it is very similar to the 23 kDa antibacterial protein molecule recorded by earlier researchers from pupal stage.

Thus, present preliminary observation on the incidence of diseases and haemolymph biochemical parameters of healthy and bacteriosis affected *A. assama* gives a future direction of research and the preliminary findings *at* molecular level will help researchers to find out immunological linkage of the new peptides / amino acids, if there be any.