5. DISCUSSION

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5.1. Climatic factors and their impact on ERR

Among the four seasons, the best ERR has been obtained from the Katia crop, that represents the ideal time for the commercial crop harvesting. An approximate climatic condition having T_{max} of 27 – 29 ^{0}C, T_{min} of 19 – 22 ^{0}C, RH_{max} of 83 – 86% and RH_{min} of 74 – 78% during this season appear to be conducive for getting the best ERR in Coochbehar. A temperature range higher than this coupled with higher range of RH is the worst condition when ERR becomes the least as in case of Bhodia season. Conversely, the higher T_{max} and T_{min} during Bhodia season, lower RH_{max} and RH_{min} during the Jethua crop and a lower T_{max} and T_{min} during the Chotua season are responsible for poor ERR. The least ERR value of only 28.43% has been obtained from the Chotua crop. In spite of luxuriant growth of food plant, the Bhodia season yields a poorer ERR due to the combined effect of higher ranges of temperature and relative humidity, a condition that render the fourth and more particularly the fifth instar larvae susceptible to two fatal diseases, virosis and bacteriosis.

Among the four diseases considered in the four crop seasons, the occurrence of pebrine and mycosis is very negligible, more particularly the incidence of mycosis is almost nil in the new area of study. But the incidence of both bacteriosis and virosis is quite frequent. The decending order of the incidence of both bacteriosis and mycosis during the four seasons has been Bhodia > Jethua > Chotua > Katia.

5.2. Impact of two major host plants on the larval and cocoon characters

It has been recorded that the larvae reared on soalu plants gain more larval weight, about 14 per cent higher weight than the larvae reared on som plant. This result corroborated with the earlier findings of researchers of CSB (CSB annual report 1985-'86). Soalu leaves contain higher moisture and starch contents (Dutta *et al.*, 1997). As a result the larval green weight has increased more. Furthermore higher quantity of fat is accumulated by the larvae for producing greater amount of yolk for a relatively higher number of egg when fed with soalu leaves (CSB Annual Report 1985-'86).

Since the water content of the body has no significant differences for the consumption of two different host plants, it appears that the larvae have well regulated genetic mechanism of the maintenance of water balance.

As a consequence of higher body weight, the cocoon weight also higher in case of rearing on soalu plants than on som plants. Similarly, higher shell weight has also been obtained due to feeding on soalu leaves. But the silk ratio has always been higher when fed with som leaves. This implies that the larvae of *A. assama* have a greater efficiency of conversion and assimilation of som leave nutrients to silk. Such a result corroborates earlier observations by several authors in both traditional and non-traditional geographical areas (CSB Annual Report 1985-'86; Ray, 2001).

5.3. Incidence and extent of major diseases of the larvae during the four prime crop seasons in the farmers' field and their impact on ERR

The present study reveals that every year 11.30 to 14.94 per cent larvae of *A. assama* die out of bacteriosis in the non-traditional area of Coochbehar district. The incidence has been the highest during Bhodia rearing (August – September). But during Katia rearing (October – November) there has been a low incidence of bacterial flacherie (Table 5 and Fig. 1). The highest incidence of bacteriosis during Bhodia season is promoted by ambient temperature and relative humidity both of which were very high and the host plant leaves contain high moisture content. Again, the congenial temperature and humidity ranges during October – November (Katia) crop have yielded best ERR value by way of reducing the bacterial flacherie as well as virosis.

Cocoon crop loss due to silkworm diseases is 30 – 40 per cent in India (Janakiraman, 1961), over 30 per cent in developing countries of South East Asia and 10 per cent in advanced countries like Italy, Japan (Nanavathy, 1965) and China (Kellog, 1928). Among the various diseases, bacteriosis is of serious concern (Krishnaswami, 1978; Vanitha Rani *et al.*, 1994). Chakravorty and Dutta (2008) has reported that the bacterial flacherie of muga silkworm was recently occurred in severe form in all the growing tract of not only in Coochbehar but also in Assam causing a 100 per cent crop loss.

Most of the researchers are of the openion that incidence of bacteriosis is associated with high environmental temperature with high relative humidity (Sengupta, 1988; Chisti *et al.* 1991). According to Lu Yup-lian and Fu-an (1995), bacterial diseases are common in silkworms and tend to occur in the hot and humid summer and autumn rearing seasons. A group of researchers in Central Muga and Eri Research & Training Institute, Lahdoigarh, Jorhat, Assam has recorded the highest mortality rate of muga silkworm due to bacteriosis in Lower Assam area during late summer (CSB Annual Report 2001-'02). The environmental contiguity of Coochbehar to that endemic area of Assam is already known. Thus, the highest incidence and crop loss due to bacteriosis in this non-traditional area corroborates the findings in endemic or traditional area. Thus, the late summer, i.e. the wet part of the summer has a relevance to the incidence of both bacteriosis and virosis in muga larvae at higher proportion in the district of Coochbehar. Dutta *et al.* (2007) has reported that in Assam the bacterial disease of muga silkworm is though common throughout the year, but is severe in summer and rainy seasons in almost all the Muga growing tracts. They have also reported that there is a large outbreak of the disease in several areas of Assam, Meghalaya and in West Bengal causing complete loss of the crop. Peak occurrence of the disease is found at the late larval ages between middle of fourth instar and fifth instar of silkworm. The present observations corroborate the earlier findings of various researchers from all over the world in the field of sericulture. According to Veeraiah *et al.* (2006), an excessive temperature and humidity coupled with varied fluctuations in climatic conditions of tropical countries along with poor hygienic condition contributes to low productivity and crop failure due to the diseases. An easily available labour system with less mechanized small-farm concept of tropical countries added further in this juncture.

Virosis is the disease prevalent in the area of study next to bacteriosis. During the three years' observation, pebrine and mycosis appear to be very insignificant and demands no attention in muga rearing.

5.4. Impact of the two principal food plants on the incidence of bacteriosis

The impact of the two principal host plants on the incidence of bacteriosis has been recorded as the percentage of larval loss due to the disease. The results indicate that susceptibility to bacteriosis is variably higher in case of som as food plant, as is evident from the Pearson correlation values. The differences are significant in all the seasons except during the Jethua season. The higher incidence of bacteriosis during Bhodia season is associated with high temperature and higher relative humidity in the atmosphere, the condition prevents profuse transpiratory water loss from larval body and induces bacterial multiplication.

5.5. Detection and characterization of the bacterial pathogens causing bacteriosis (bacterial flacherie)

For identification of bacteria infected worms the clinical symptoms as described by Thangavalu *et al.* (1988), Chishti *et al.* (1991), Govindan and Devaiah (1995a) and Sanakal *et al.* (1996) have been followed. The diseased worms have lost appetite and become sluggish. Initially there is no colour change, but with the progress of disease, worms become flaccid (soft) 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, spasms and tremors, paralysis and distress are the symptoms, followed by sudden collapse and death. In chronic toxicosis, ingestion is reduced, faeces become irregular-shaped and occasional vomiting occurs. After a few days, loss of clasping power of the legs, flaccidity and subsequent death has been observed. These clinical symptoms of bacteriosis also confirm the similar characteristics noticed by Choudhary *et al.* (2002), Das *et al.* (2006), Chakravorty, *et al.* (2007) and Dutta *et al.* (2007). Furthermore, identification of the disease on the basis of clinical features has confirmed by the isolation of bacteria population from the diseased larvae.

The infected larvae collected from the five different places (Plate II) of Coochbehar district were studied separately for the identification of pathogens. The bacteria recovered and isolated from the diseased worms belong to the group cocci and bacilli (Plate VII). On the basis of biochemical characterization (Plate VIII) they have been classified into six groups (Table 8). Some of them are gram positive, rod shaped and a few of them spore forming (aerobic spore bearer). A large number of bacteria have been gram positive and gram negative Cocci. A few are Cocco-bacilli. Both capsulated and non-capsulated bacteria have been found. Most of the bacteria are motile though a few non-motile bacteria have also been recovered from the cultures. Large number of gram-negative bacteria recovered from the culture are lactose fermenting, few belong to NLF (non-lactose fermenting) variety. Bacteria found in group I and group III are facultative aerobics (Table 8).

Results of morphological, staining and biochemical characterization indicate the existence of the genera *Pseudomonas, Klebsiella, Citrobactor, Proteus, Providentia* and *Bacillus* in the bacteriosis afflicted muga silkworm in Coochbehar district of West Bengal. Chitra *et al.* (1975) have also identified three species of *Proteus,* three species of *Pseudomonas*, two species of *Bacillus* involved in bacterial diseases in *B. mori.* Enomoto *et al.* (1987) have reported that *Pseudomonas, Proteus, Bacillus* and many other bacteria cause septicemia in *B. mori.*

A. assama also suffers in Assam, the original residence, frequently from bacteriosis (Senapati *et al.*, 2001 and Senapati *et al.*, 2002) but the authors could not describe any bacteria responsible for the disease. Choudhury *et al.* (2002) made preliminary report on the existence of the bacterial strain AC-3 associated with bacteriosis in Assam. Dutta *et al.*, 2007 mentioned that the bacteria like *Pseudomonas, Bacillus* etc. are involved in bacteriosis disease of muga silkworm in Assam.

A number of similar pathogenic bacteria have been recorded in the present investigation. A few gram positive and gram-negative cocci has been isolated from the muga silkworms suffering from bacteriosis needs further investigation for their identification up to specific level. Further investigation is required to study the pathogenecity of bacteria isolated in different seasons and also the exploration of possible preventive/control measures. After such studies the extension of muga silkworm rearing in this non-traditional area would be feasible for commercial viability.

5.6. Seasonal variations of haemolymph total protein, lipid and carbohydrate contents of fourth and fifth instar larvae and consequent cocoon weight, shell weight and silk ratio after rearing on two major host plants

Compared with the fourth instar, the fifth instar larvae consumes several times higher quantity of food. Obviously, higher amount of nutrients are accumulated as storage resource in the late fifth stage larval body. Consequently, more than four times higher protein content and more than three times higher lipid and carbohydrate contents have been accumulated in the haemolymph of the late fifth stage larvae than in the fourth stage. A higher quantum of resource accumulation towards the end of larval stage is crucial to a lepidopteran species in which the adults are non-feeding, short-lived and emerge out from the cocoon as sexually matured individuals. At the cost of accumulated resources plus the resources available from hydrolysis of most of the larval tissues, the development of imaginal structures and synthesis of huge quantity of silk production is possible.

It has been found that the protein and carbohydrate contents in the haemolymph of the larvae fed with soalu leaves are higher though their reflection on shell weight and silk ratio are not correspondingly higher. Hence, soalu is not superior food for getting higher silk output. This is also corroborative from earlier results (CSB Annual Report 1985-'86). On the other hand, larvae fed on som leaves though contain lower protein and carbohydrate contents in their haemolymph, the cocoon traits and silk ratio are superior. Higher silk ratio obtained from the larvae fed with som has also been reported in Assam (CSB Annual Report 1985-'86).

Pooled analytical data (Table 9) reveal that significantly higher protein content occurs in the haemolymph of female than its corresponding male stage and the total haemolymph lipid content has been found significantly higher in male than in its female counterpart. For obvious reason females have comparatively higher protein content than male, since they require more reserve materials for egg production, which confirm the earlier findings of Tojo *et al.* (1980), Kar *et al.* (1994) and Das and Deb, 2005. Haemolymph total protein has a strong positive relation with the advancement of larval stage from fourth to the fifth stage. Larval weight has increased progressively due to higher consumption of food which is indicated by higher level of protein concentration in the fifth instar stage. Level of total protein recorded highest during Bhodia crop. Change of haemolymph total protein content in different seasons might be associated with the feeding behaviour as well as the quality of leaf available to the larvae. The differential nutritive value of the leaves caused by seasonal variation may be responsible for variation in the quantity of haemolymph protein level. Higher photoperiod during summer season seems to induce higher protein bio-synthesis (Tazima, 1978). This may be the reason for a higher protein level in Bhodia season. Som leaves have higher protein content than in the soalu leaves (Dutta *et* al., 1997). Yet the larvae reared on som leaves contain lower level of haemolymph protein content. This is an unique observation that warrants further investigation for understanding the causes. The soalu leaves having higher moisture content may trigger quantitatively higher intake of food (Anantharaman *et.al.*, 1993), may be one reason.

It has been noted that the mortality rate of the larva due to bacteriosis is higher when those were fed on som compared to diseased larvae reared on soalu and it has been observed in all the seasons. Though the result has no significant statistical correlation with the host plant, but it required an extensive study to find the actual cause of the differences in mortality. During 1986-'87, Central Silk Board, Government of India first tried to evaluate the feasibility of muga silkworm culture in Pakhihaga village of Coochbehar district of West Bengal considering soalu as the host plant (CSB Annual Report 1986-'87). Soalu is a natural plant in the terai region and som has been planted later for the rearing of muga silkworm. Som gives better silk ratio in both Coochbehar and Assam. This might be due to better nutrient constituent of the leaves as evaluated by Dutta *et al.* (1997). The authors have noted that crude fat, total nitrogen, protein, soluble sugar, phosphorus and calcium contents are higher in som than in soalu leaves. Though the silk ratio is less, but in Coochbehar district, disease incidence (in per cent mortality) is also less when soalu has been considered for muga silkworm rearing. It requires a thorough exploration for the actual cause. However, soalu leaves contain significantly higher quantity of starch, potassium and moisture than in the som leaves (Dutta *et al.*, 1997). It may so happen that some micronutrient deficiency in the planted som leaves in this region be the cause. A round the year comparative analytical study of som and soalu leave contents of Coochbehar and Assam may suggest better understanding of the differences in the constituents in the food leaves, particularly in som.

According to Belgaum *et al.* (1991), in the normal larvae of *B. mori*, the quantity of haemolymph protein content remains relatively constant from the first up to the fourth instar, thereafter increasing nearly two fold on day four of the fifth instar and attains the highest level on the day nine. Haemolymph protein level generally increases during each instar but decline at the time of moulting. The levels are low in early instars but increase considerably in the subsequent instars in lepidoptera (Wyatt and Pan, 1978). Such results corroborate the present findings irrespective of the host plants.

Males of *A. assama* have more lipid content than their female counterparts. Similar results have been obtained in other insects by several workers (Chinya and Ray, 1976; Gupta and Pathak, 1984; Quader *et al.*, 1988 and Saha *et al.*, 1994). Saha and Khan (1996) have reported for *B. mori* that high lipid content in adult male is required for active participation in mating. The lipid might be used for storage activity in the tissue for future structural development and functional growth (Blum, 1985). Part of lipid might be converted to maintain the titre of protein and amino acid (Bora and Handique, 1999) as high titre of these components are required in lepidopterans for imaginal tissue formation (Chapman, 1982). Stored fats are very important to *A. assama* because during the pupal period these stores are used to support metamorphosis and the energy demands of adult imposed by reproduction and flight (Ziegler, 1991).

Larval body water content has no relation to the host plant. But there have been significant differences among the sexes when the larvae are fed with soalu plant. Males always have low water content than their female counterpart. It has been observed in both the batches of larvae when fed with som or soalu. Shapiro (1979) is of the openion that the body water content of insect, *Galleria mellonella* (L) is affected by fluctuation in the environmental temperature, which in turn directly influences the blood volume (i.e. the size of the insect). Therefore a thorough study of body weight along with water content in different seasons and of the larvae fed with different host plant leaves is required to find out whether any relationship is there between the larval body water content and silk ratio in the muga silkworm.

Larval weight was significantly higher when reared on soalu than on som plant. Cocoon weight was significantly higher in case of larvae reared on soalu leaves. Shell weights though have non-significant differences, was higher in case of som as the host plant. Silk ratio has been higher in the cocoons obtained from som leaves as diet. It can be inferred from the observation that the larvae fed with som plant gives better silk ratio. Similar results were also obtained by Thangavelu, Chakraborty and Bora of Central Silk Board (CSB Annual Report 1985-'86). Variation of larval weight, cocoon weight, shell weight and shell ratio per cent in different seasons of muga silk moth in Coochbehar district have also been recorded by Ray *et al.* (2005). Our recorded result has clear conformity to the earlier findings.

5.7. Day-wise change of haemolymph biochemical profiles of the fifth instar larva reared on single or different combinations of principal host plants and their impact on cocoon characters

Likewise in *B. mori* the study reveals that haemolymph protein content increases rapidly in fifth instar larvae. Rapid increase of protein content in fifth instar healthy larvae is directly related to the increased metabolic activity at that stage particularly for enormous growth of silkgland (Sinha *et. al.*, 1992; and Bashamohideen and Ameen, 1998). Increase of larval body weight with the higher consumption of food lead to higher level of protein synthesis and concentration in the fifth instar stage for subsequent pupal programming. According to Shigematsu (1958), high rate of increase of the level of haemolymph protein in the fifth instar larva is the result of active synthesis of protein by the fat body and their release into the haemolymph. Sowri and Sarangi (2002) demonstrated in *B. mori* that in day wise change of haemolymph protein, the value increased significantly on daily basis and reaching its peak on the last day before the larva ripened for cocooning. Our results on muga silkworm corroborate such findings and also in *A. assama* studied earlier by Handique *et al.* (2000). Haemolymph lipid content also increases rapidly in fifth instar with the progress of day. Carbohydrate represents a major part of the total caloric intake of insects and serve as a temporary store of glucose. The day wise study of fifth instar also reveals that, the carbohydrate profile also undergoes similar incremental change with two pauses in day 3 and day 6. In general all the haemolymph biochemical parameters undergo rapid increase from third day of fifth instar onwards. According to Das *et al.* (2004) after the third day onwards of fifth instar larvae the food is utilized for the purpose of silk gland development. In case of *Bombyx mori* silk glands enlarges rapidly during, the fifth larval life, particularly during the later part of life of this instar. This is possible because of a metabolic shift in this instar from lipogenesis at the early phase for reproductive allocation of food resources to glycogenesis at the late phase for silk protein allocation (Inagaki and Yamashita, 1986). In case of *A. assama* presumably similar compartmentalization of food allocation happens for the two prime functions. And for that purpose a high level of building materials must be available in the haemolymph. Because of this reason haemolymph biochemical parameters attain higher quantity from the third day onwards. Lipid and carbohydrate contents attain their peak on the eighth day and the protein on the last day i.e. on ninth day. Increased accumulation of carbohydrate in the haemolymph is associated with the increased energy requirement of the larvae during fifth instar (Sowri and Sarangi, 2002). Levels of protein and sugar in the fifth instar depend on the feeding activity of the *B. mori* worm (Gururaj, *et al.*, 1999). In *A. assama* there has not been significant differences on the day wise increment of haemolymph biochemical parameters particularly for the change of host plant after the third instar stage.

There is no significant statistical correlation of the larval characters (larval weight, water content) or cocoon characters (cocoon weight, shell weight, silk ratio) for the change of host plant after the third instar stage. But silk ratio (in per cent) is always comparatively of higher value, obtained from the larva reared on soalu plant up to third stage and then transferred to som plant (for last two instars).

5.8. Studies on free amino acids in haemolymph of fourth and fifth instars healthy and diseased worms reared on two principal host plants

Muga silk contains 17 amino acid species. These are Gly, Ala, Val, Leu, Ilu, Ser, Thr, Asp, Glu, Phe, Tyr, Lys, His, Arg, Pro, Trp and Cys. All these amino acids are available in the leaves of both som and soalu except tryptophan (Sinha and Sinha, 1991). Both the plants have 20 amino acids, but the total pool is much higher in soalu leaves (Sinha and Sinha, 1991). But som leaves contain higher protein content (Dutta *et al.*, 1997). Therefore, it is imperative that amino acid pool available from food sources has no strict relevance to the quantum of silk synthesis, the larvae that consume soalu leaves ultimately show low silk ratio in the resultant cocoons. Similarly higher protein content of som leaves has very little linkage to the final performance of the larvae in terms of egg and silk production. Therefore, some of the amino acids are synthesized in the larvae for silk and vitellin and vitellogenin synthesis.

The amino acids obtained by Sharma *et al.* (1995) and in this study are almost the same except the amine form of glutamic acid and aspartic acid which have been obtained by Sharma *et* al. (1995) and proline and cysteine in the present observation. Furthermore, Sharma *et al.* (1995) did not obtain glutamic acid, proline and cysteine in the fourth stage and proline and cysteine in the fifth stage larvae. There is no earlier record of proline and cysteine in the haemolymph of *A. assama*. Proline is very essential for the proper growth of the larvae (Inokuchi, 1969; Bose *et al.*, 1989). Sulphur containing amino acid cysteine is important in intermediary metabolism and serves as a source of sulphhydryl group for the synthesis of coenzymes and hormones (Gilmour, 1961). In *A. assama* it has been recorded that the nonessential amino acids dominate quantitatively over the essential amino acids like in other arthropods (Claybrook, 1983; Sharma *et al.,* 1995). According to Dhavalikar (1962a, b) there are seventeen amino acids present in the muga silk. Sixteen amino acids are common with the present observation but it has not been possible to detect tryptophan. This leaves a scope to assess whether tryptophan is present in the leaves of both som and soalu plants as obtained by Sinha and Sinha (1991). However, tryptophan 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 (Tazima, 1978). The larvae fed with soalu leaves show a higher quantity of total free amino acids than the larvae fed with som leaves. The higher quantity may be due to the presence of a higher amount of amino acids in the soalu leaves (Sinha and Sinha, 1991). The unidentified peaks found in all the samples might be the derivatives of some amino acids. This aspect, too requires a thorough study for their identification and significance.

Incident of disease is more frequent during the fifth instar stage. Hence in this instar the diseased worms had 3.0 times less amino acid content in their haemolymph than in their healthy counterparts. A lower content of amino acids in the diseased worms might be due to an interference of the pathogen in the larval physiology (Sinha *et al.,* 1988b), utilization of free amino acids for their own requirement or due to utilization of free amono acids in antibacterial protein synthesis. Chitra *et al.* (1974) and Kodama and Nakasuji (1968) have reported a fall in the gut pH, due to bacterial infections. This may also impair amino acid absorption in gut. The total quantity of haemolymph free amino acids of diseased larvae is so low. The acidic amino acids, aspartic acid and glutamic acid have been drastically reduced in the diseased worms, which are reasonably be responsible for the retarded growth of the diseased worms (Arai, 1977; Ito and Arai, 1966). Both aspartate and glutamate play active role in amino acid nutrition as donors of amino groups in the transamination reactions (Bheemeswar and Sreenivasaya, 1952). The basic amino acids, arginine, lysine and histidine are involved in the regulation of haemolymph osmotic pressure (Florkin and Jeuniaux, 1974), which were obtained in a very low quantity in the diseased worms than in their healthy counterparts. Glycine plays an important role in detoxication mechanism (Friedler and Smith, 1954; Shyamala, 1964). This investigation records a higher concentration (1.33 – 2.36 times) of glycine in diseased worms when compared with that of healthy larvae. This warrants further investigation to find out the actual role of glycine in diseased worms whether this is utilized for the synthesis of anti-bacterial glycine-rich peptides / polypeptides effective against Gram-negative bacteria.

5.9. Studies on the different types of protein and peptides by HPLC and electrophoresis

HPLC: The protein / peptide peaks appeared at 17-18 min. and at 32-33 minutes in HPLC study are the major constituents of haemolymph protein content of all the samples. The peaks appeared at around 5 min and 18 minutes are the most polar regarding solubility, those appeared around 44 min and 50 minutes are the least polar (even may be non-polar), and the major peak appeared at 32-33 min may be considered comparatively neutral protein constituents of the haemolymph. One of the major peaks of healthy fifth instar appeared around 44 min sharply goes down in all worms suffering from different stages of bacteriosis which requires further investigation to find out its role. One extra protein / peptide peak (in number) appeared in worms at early stage of bacteriosis and at bacterial toxicosis stage (though the appearance time is different), but not at late bacteriosis stage. The causal relation of such decline needs further investigation to explain its role and immunological significance, if any, in diseased worms. The appearance and disappearance of some of the peaks of diseased worms compared to those of healthy ones also need further investigation.

SDS-PAGE: The assay for haemolymph protein / peptides was done in both fourth and fifth instar healthy larvae, in case of fifth in both the sexes. But the assay in bacteriosis-afflicted larvae was done in only the female fifth instar at different stages of the disease. The SDS-PAGE assay of the haemolymph samples reveals that the healthy fourth stage worms have six major peptides of 112, 58, 33, 28, 19, and 17 kDa. There are sexual dimorphisms of the number of peptides in fifth stage, female have 15 and males have 14 peptide bands. The major bands appeared in fifth instar healthy female larvae are of 112, 56, 32, 26, 23, 18 and 17 kDa. Males also have such major bands of 108, 42, 33, 28, 24, 18 and 17 kDa. Out of these, the bands of 56 and 42 kDa may be considered as female and male specific respectively. The remaining peptide / proteins of both the sexes, having minor differences in molecular weight, apparently seem to be of similar nature. The major peptides having molecular weight of 32-33 and around 17 kDa have been present in all the bacteriosis afflicted worms as well as in the healthy worms. Therefore, these can be considered as essential peptides of muga silkworm having general physiological significance. Bacteriosis afflicted worms have expressed peptides of 116 and 119 kDa, which are heavier than the peptide found in the healthy worms. The numbers of protein / peptide bands recorded by Dipali Devi (1993) in fourth (16 in Jarua season and 19 in other rearing seasons) and in the fifth (19 in Jarua and 21 in other seasons) instars do not conform to the present observation. The differences warrant further study regarding changes in the number of peptide bands in haemolymph particularly with respect to seasonal environmental impact, if any. The reduced band width / peak volume of the protein / peptides of the haemolymph of the diseased worms might be due to hydrolysis of larval protein / peptide by the protease released by the infected bacteria (Choudhury, *et al.* 2005). The difference in band patterns in the larvae of this non-traditional area warrants a thorough scrutiny. 23 kDa protein recorded by the other researchers (Choudhury, *et al.*,. 2004; Sharma, *et al.*, 2005 a, b; Unni, *et al.*, 2006) is more or less similar to 23-24 kDa peptides obtained in this investigation. Unni, *et al.* (2005) have isolated and purified a 23 kDa protein from disease induced pupae of *A. assama* and tested its anti-bacterial activity against *P. aruginosa* AC-3 and on *Pseudomonas* DAS-01. Thus this preliminary observation on haemolymph proteins of *A. assama* in newly explored geographical area gives a future direction of research and to find out immunological linkage of the new peptides, if there be any.

Brey and Hultmark (1998) have compiled the immune mechanism at both cellular and molecular level in various insects. They have classified the anti microbial antibody type of molecules found in various insects i.e. bacteriolysins, hemolin, cercopins, diptericins, sarcotoxin II, Melittin, andropin, hymenopticin etc. in addition to lysozyme like molecules. A group of researchers of Seribiotech Research Laboratory, Bangalore, National Institute of Immunology, New Delhi and Central Sericultural Research and Training Institute, Mysore, have purified and characterized an 11.5 kDa antibacterial protein from vaccinated *B. mori* (Abraham *et al.*, 1995). Unni, *et al.* (2005) isolate a 23 kDa antibacterial protein from the pupal stage of muga silkworm which is very much similar to 23-24 kDa protein observed in the present investigation from the fifth instar stage of *A. assama*. Kockum *et al.* (1984) isolated induced bacteriostatic polypeptide with molecular mass higher than 20 kDa from *Hyalophora cecropia*. From the molecular weights it appears in this study that in diseased worms a protein in the molecular mass range of 45 – 47 kDa occurs in all the three stages of bacteriosis but absent in healthy one. This may be the earliest expression against the bacterial attack in general aspect. Another protein of 20 kDa has been expressed in the larvae suffering from bacteriosis at late stage and is continued to the toxicosis stage. This one may be the next step of expression against the pathogens. Finally, one 66 kDa protein has been found at the toxicosis stage. All these proteins require purification and characterization in order to confirm whether these are truly antibacterial proteins. An antibacterial zone inhibition test is also required to ascertain their functional significance.

Thus this observation on the incidence of diseases and haemolymph biochemical parameters of healthy and bacteriosis afflicted *A. assama* gives a future direction of research and to explore the immunological linkage of the new peptides and higher abundance of haemolymph free glycine, if there be any.