Chapter - III

MATERIALS AND METHODS

3.1 Geographical location :

Cooch Behar district of West Bengal is under Terai region and is situated in the north-eastern part of West Bengal, adjacent to Kokrajhar and Dhubri district of Assam. Terai zone is situated between $25^0$57' N and 27^0 N latitude and $88^0$25' E and $89^0$54' E longitude. This northern region of West Bengal is situated along the foot of Karseong and Kalimpong hills and Bhutan hills in the north, Bihar border on the west and Assam border on the east. It includes Siliguri Sub-Division of Darjeeling district and entire district of Jalpaiguri and Cooch Behar and Islampur Sub-Division of North Dinajpur District. The total geographical area of the zone is 12025 sq Km., which is 13.5% of the state area. Rural population comprises about 90% of the population of the zone. Cooch Behar district of West Bengal lies between $26^0$57'40" N and $26^0$32'20" N latitude and $88^0$47'44" E and $89^0$54'35" E longitude. The altitude of the district is 43m above MSL.

3.2 Climate :

The climate of the zone is sub tropical and humid in nature. Average annual rainfall about 80% is received from south-western monsoon during the rainy months of June to September. The range of minimum temperature of the area is 11.19 – 30.24^{0}C while the maximum is 20.54 - 34.24^{0}C. The relative humidity of the area at 8.30 am is 58-89% respectively in March – July. The relative humidity in the afternoon at 5.30 pm is 48 – 81% respectively in March – November. The maximum temperature never rises above 35^{0}C and the high temperature is recorded only for a few days. Autumn and spring are very pleasant and the minimum and maximum temperatures ranges from 19 ^{0}C to 27^{0}C and 17^{0}C to 25^{0}C during these two seasons. Winter is moderate and the minimum temperature dose not fall below 10^{0}C.

3.3 Experimental site :

3.3.1 Outdoor rearing :

Experimental rearing of muga silkworm was conducted at the instructional plantation and adopted farmer's field of Acharya B.N. Seal College, Cooch Behar, West Bengal. All rearings were conducted in natural outdoor condition.

3.3.2 Indoor rearing :

Indoor rearing and other studies were conducted at Muga Research Laboratory, P.G. Department of Zoology, Acharya B.N. Seal college, Cooch Behar, West Bengal.

3.4 Insect :

Among most successful animal groups in terms of species, insects are predominant one an estimate of total number of leaving species ranges from 10 – 30 million. Muga silkworm *Antheraea assama* Westwood is a highly heterogeneous unique and semi domesticated multi voltine strain of Saturniidae family of Lepidopteran insect endemic to Assam, adjacent foot hill of Meghalaya, Nagaland, Arunachal Pradesh and Mizoram. However, it grows under semi-domesticated conditions in North Eastern States of India but it has immense possibility of expansion in area and increasing productivity under befitting agro-ecological situation of Terai zone of West Bengal.

As it is wild in nature, so rearing was done in the field. At the time of rearing 4-6 big trees together, cover with a mosquito net (rearing net) to kept them away from the natural enemies like birds, snakes, wasps, britle, lizard etc.

3.5 Food plants or host plants :

Muga silkworm is polyphagous in nature. It feeds on various plants, viz. *Persea bombycina* Kosterm (*Machilus bombycina* King), *Litsea monopetala* Pers, *Litsea polyantha* Juss, *L. citrata*, *L. Salicifolia*, *Magnolia sphenocarpa* and *Zizyphus jujuba*. In present investigation, larvae were reared on the two principal host plants namely *Persea bombycina* King (som) and *Litsea polyantha* Juss. (soalu).

3.6 Rearing of silkworm :

3.6.1 Outdoor :

During different seasons in a year, rearing was done at different field alternately. Immediate after completion of rearing the pruning of tree, liming of soil, organic manuring, bleaching powder spray etc. were done for the purpose of increasing new leaves, changing of soil

pH, growth and free from larvae eating hunter ants, beetles etc. respectively. During dry season March – April proper irrigation was maintained to avoid the water scarcity. Rearing were carried out during different seasons namely summer, rainy, winter, autumn and spring utilizing seven period viz. February – March, March – April, April – May, June – July, August – September, October – November and December – February.

3.6.2 Indoor rearing :

The indoor rearing was carried out following the methodology of Thangavelu and Sahu (1983) within the rearing cell. The branches of the food plant, som and soalu were kept immerged in water contained in earthen pots and these were kept in a stand in three tiers and the whole setup was covered with polythene cover except the ground surface (Plate 1 and 2). The rearing was conducted inside the room. The polythene cover was knitted on all sides except on the front and the front sheet was used like door curtain enabling rearing operation and these also facilitated maintaining the desired level of crop loss in outdoor rearing,. Indoor rearing was done in seven different season namely February – March, March – April, April – May, June – July, August – September, October – November and December – February. In each seasons, eggs after hatching were reared following the recommended schedule until the attainment of cocoon, adult moth and egg.

3.7 Diet :

For outdoor and indoor rearing, the larvae were fed with the leaves of som (*Machilus bombycina*) and soalu (*Litsea polyantha*). Trees were used after two months of pruning. For consumption besides pure som and pure soalu, different combinations were used. Combination be depict in table 3.

Table 3 : Combination of feed of muga silkworm

T_1	1st instar on som and rest on soalu
T_2	1st and 2nd instar on som and rest on soalu
T_3	1st to 3rd instar on som and rest on soalu
T_4	1st to 4th instar on som and rest on soalu
T_5	1st instar on soalu and rest on som
T_6	1st and 2nd instar on soalu and rest on som
T_7	1st to 3rd instar on soalu and rest on som
T_8	1st to 4th instar on soalu and rest on som

Plate 1 : Rearing cell for muga silkworm, the rearing stand completely closed on all sides with polythene cover.

Plate 2 : Polythene cover of the front portion partly removed for feeding and other operations.

3.8 Rearing performances :

Twenty-five disease free laying (eggs laid by a single disease free female) were reared in mass from hatching still spinning. The cocoons were harvested on sixth day from the onset of spinning, as this time gap is more than sufficient for the transformation of larvae into pupae. After the emergence of moth and coupling them, after laying eggs were collected upto 6th days for counting fecundity.

From the view point of economic importance, the following parameters and their method of evaluation were considered :

1. Larval duration calculated from the date of hatching to the date of spinning (in days)
2. Weight of single larva (gm)
3. Effective rate of rearing by percentage which was calculated as :

$$\text{ERR \%} = \frac{\text{Number of cocoons harvested}}{\text{Number of larvae reared}} \times 100$$

$$\text{ERR Number} = \frac{\text{Number of cocoons harvested}}{\text{Number of larvae reared}} \times 10000$$

4. Single cocoon weight (gm)
5. Single shell weight (gm)
6. Shell ratio (SR%) which was determined as :

$$\text{SR\%} = \frac{\text{Single shell weight (gm)}}{\text{Single cocoon weight (gm)}} \times 100$$

7. Absolute silk content (Kg) which was determined by :

   ERR number x Single shell weight (gm)

8. Fecundity = Number of egg lay by single gravid female moth

All the parameters were recorded on fresh weight basis. Twenty larvae as well as cocoons (10 males and 10 females) were taken for each of the three replications for assessment of quality of cocoon. For fecundity twenty gravid females were taken for each of the three replications. All

values of rearing results comprising rearing performance as well as quality of cocoons were subjected to suitable statistical analysis.

3.9. Consumption and utilization of food by muga silkworm larvae :

In order to determine the consumption and utilization of som and soalu leaves a colony of muga silkworm was raised in the laboratory and was maintained from brushing till spinning in indoor. Three replication with fifty larvae per replication were maintained for the study of nutritional efficiencies. Aliquot was kept for dry weight determination.

An additional larval batch was also maintained as above for determining dry weight values. The healthy larvae were counted daily in each replication and unequal, weak, unhealthy if any, were replaced by healthy ones of the same age from the reserve stock. The left over leaf (LOL) and excreta were collected carefully and separated daily at 9 AM. The excreta and leaf were dried at 60^0C to a constant weight. The experiment was conducted five consecutive years (2003-2008) considering five seasons per year February – March, April – May, June – July, August – September and October – November.

For pure som, pure soalu and different combinations the experiments were conducted during two main commercial crop rearing season (April – May and October – November). Later on the experiment with pure som and better combinations were conducted during five different seasons namely February – March, April – May, June – July, August – September and October – November. The amount of dry matter ingested, digested and converted were determined by standard gravimetric methods (Waldbauer, 1968). The indices used in the study were followed after (Waldbauer, 1968) and are given below :

3.9.1 Consumption and growth :

3.9.1.1 Reference ratio (RR) = Food Ingestion / Excretion

3.9.1.2. Consumption index (CI) :

$$(CI) = \frac{F}{TA}$$

Where,

F = Dry weight of food ingested

T = Duration of feeding period (day)

A = Mean dry weight of larvae during feeding period.

3.9.1.3. Growth rate

$$(GR) = \frac{G}{TA}$$

Where,

G = Dry weight gain of larvae during feeding period

T = Duration of feeding period (day)

A = Mean dry weight of larvae during feeding period.

3.9.2 Digestibility and Efficiency of Conversion

3.9.2.1 Digestibility

The approximate digestibility (AD) was calculated as :

$$AD\ \% = \frac{\text{Weight of food ingested - weight of faeces}}{\text{Weight of food ingested}} \times 100$$

3.9.2.2 Conversion of ingested food to larval biomass, cocoon, cocoon shell and egg

The efficiency of conversion of ingested food to the larval biomass (ECI %) which calculated as :

$$ECI\ \% = \frac{\text{Final weight of larva - initial weight of larva}}{\text{Weight of food ingested}} \times 100$$

$$ECI\ \%\ \text{to cocoon} = \frac{\text{Weight of cocoon}}{\text{Weight of food ingested}} \times 100$$

$$\text{ECI\% to shell} = \frac{\text{Weight of shell}}{\text{Weight of food ingested}} \times 100$$

$$\text{ECI\% to egg} = \frac{\text{Weight of total egg laid}}{\text{Weight of food ingested}} \times 100$$

3.9.2.3 Conversion of digested food to larval biomass, cocoon, cocoon shell and egg

The efficiency of conversion of digested food to the larval biomass (ECD %) which calculated as :

$$\text{ECD \%} = \frac{\text{Final weight of larva - initial weight of larva}}{\text{Weight of food digested}} \times 100$$

$$\text{ECD\% to cocoon} = \frac{\text{Weight of cocoon}}{\text{Weight of food digested}} \times 100$$

$$\text{ECD\% to shell} = \frac{\text{Weight of shell}}{\text{Weight of food digested}} \times 100$$

$$\text{ECD\% to egg} = \frac{\text{Weight of total egg laid}}{\text{Weight of food digested}} \times 100$$

3.9.3 Ingesta required to produce

$$\text{One gram cocoon} = \frac{\text{Weight of food ingested}}{\text{Cocoon weight}}$$

$$\text{One gram shell} = \frac{\text{Weight of food ingested}}{\text{Shell weight}}$$

$$\text{One gram egg} = \frac{\text{Weight of food ingested}}{\text{Weight of total egg laid}}$$

3.9.4 Digesta required to produce

$$\text{One gram cocoon} = \frac{\text{Weight of food digested}}{\text{Cocoon weight}}$$

$$\text{One gram shell} = \frac{\text{Weight of food digested}}{\text{Shell weight}}$$

$$\text{One gram egg} = \frac{\text{Weight of food digested}}{\text{Weight of total egg laid}}$$

3.10.1 Cocoon yield (gm) = Single Cocoon Weight x ERR%

3.10.2 Egg yield = Total number of egg laid by a single gravid female x ERR%

3.11 Statistical analysis :

For better interpretation of results experiments were laid out on various design of experiment as and when required. All the experiment were replicated thrice. Indoor and outdoor performances were plotted on Three-Factor Factorial Randomized Block Design (RBD). The data of consumption on som and soalu leaves, eight treatments (T_1 to T_8) on different combinations of leaves during two commercial crop rearing season, were plotted on Two Factor Factorial Randomized Block Design (RBD). Data of consumption on som and better combination during five different seasons were plotted on Three Factor Factorial Randomized Block Design (RBD) and their conversion efficiencies to cocoon, cocoon shell and eggs were plotted on Two Factor Factorial Randomized Block Design (RBD).

The relationship between the environmental factors, economic performance of larvae fed on both som and soalu were correlated. The significant levels were taking under consideration from 0.5 % to 0.01 % level of confidence.