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 27^0N 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 is 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.

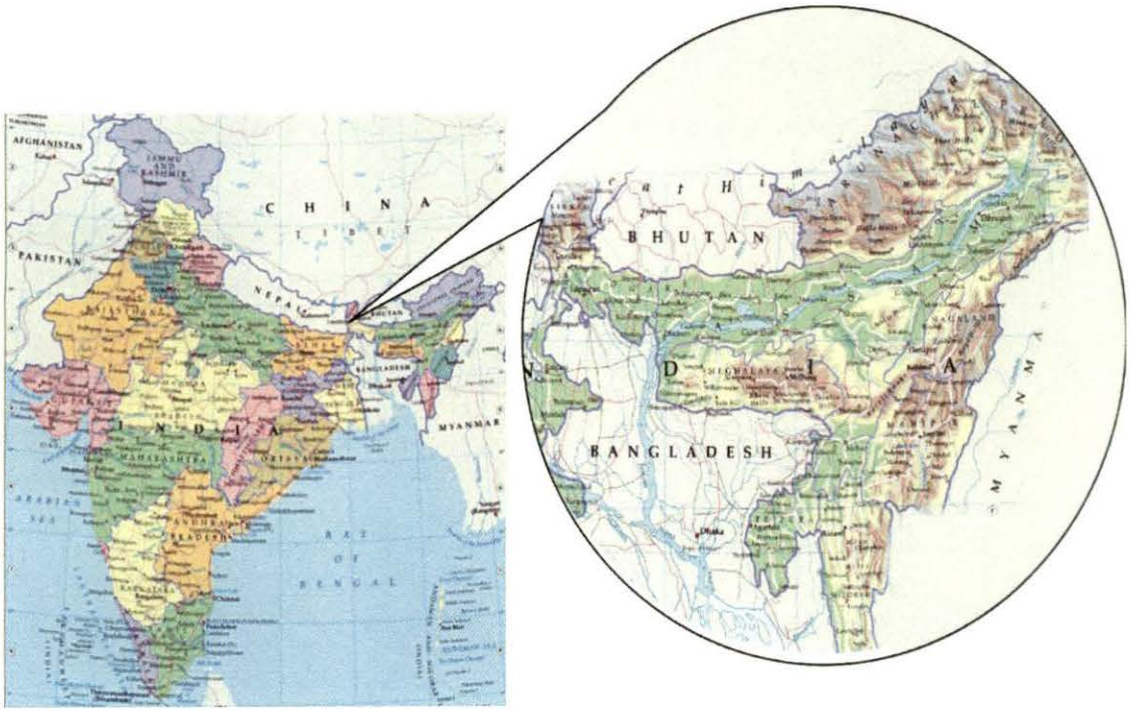

Plate 1 showing different regions of Eastern and North Eastern India where muga culture is in practice

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. On the whole the area has a humid and warm climate excerpt having a short spell in winter, December – February.

3.3 Experimental Site :

Rearing :

The experimental rearings of muga silkworm were conducted at Khagrabari Research Extension Centre (Cooch Behar, West Bengal), Regional Muga Research Station (Boko, Assam), Central Silk Board, India and at Khagrabari State Sericulture Farm (Cooch Behar, West Bengal), Govt. of West Bengal as well as in the adopted farmers field at Nalongibari, Dangarhat of Cooch Behar – I Block and Hatidura, Atialiguri of Cooch Behar – II Block.

Experimental rearings of muga silkworm were conducted at the instructional plantation of Acharya B. N. Seal College, Cooch Behar, West Bengal.

All rearings were conducted in natural out door condition.

Grainage:

Grainage operation and other studies were conducted at Muga Research Laboratory (MRL), 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 (Pearse *et al.*, 1987). 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 :

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.

3.7 Seasonal influence of grainage parameters of muga silkworm :

Muga silkworm rearing were conducted during all the seasons under consideration, namely February – March, April – May, May – June, July – August, August – September, October – November, November – December, December – February and January – March and after harvesting cocoons subsequent grainage operations were done in the laboratory.

Grainage parameters like potential fecundity (PF), realized fecundity (RF), fecundity up to 3 days (3DRF), egg retention within the female body (ER), hatching number (HN) and hatching percentage (HP) were observed in all the seasons. Data

obtained from each grainage were recorded and analyzed statistically by a suitable method.

Potential fecundity (PF) = Potentiality to egg production by a gravid female moth.
Realized fecundity (RF) = No. of egg laying by a gravid female moth.
Fecundity up to 3 days (3DRF) = No. of egg laying by a gravid female moth upto 3 day during laying period.
Egg retention (ER) = After successful laying egg retention within the female body.
Hatching number (HN) = No. of hatched larva from a single laying.
Hatching percentage (HP) = Percentage hatched larva of respective realized fecundity.

During grainage after moth emergence, natural coupling was allowed after decoupling females laid eggs on *Kharika*. Intrinsic factor influencing seed production were recorded as pupal period, emergence period, mating period, oviposition period and incubation period.

Pupal period = Period between spinning of larva (cocooning) and emergence of adult (days).
Emergence period = Time gap between head appearance and emergence of hole body (minute).
Mating period = Time span (duration) of coupling (hrs.).
Oviposition period = Duration of laying (days).
Incubation period = Time from laying of eggs to hatching of larva (days).

Day-wise cocoons were harvested from the main seed crop and pre seed crop rearings after moth emergence were allowed to couple naturally. From the onset of egg laying day wise collection of eggs were maintained and also kept the collected eggs separately in the hatching box to evaluate the day wise hatching percentage.

All the treatments were replicated thrice taking average layings of 25 coupling as one replication.

All the intrinsic and grainage parameters were studied separately nourished on both som and soalu plant during all the nine periods.

3.8 Manipulation of abiotic factors :

3.8.1 Manipulation of temperature :

To find out the temperature suitable for seed production, five (5) set of temperature ranges were taken under consideration namely T_1 (15^{0}C), T_2 (20^{0}C), T_3 (25^{0}C), T_4 (30^{0}C), T_5 (35^{0}C) during the grainage operations cocoons obtained from seed crop rearing of March – April and August – September. The hatching number (HN) and hatching percentage (HP) were studied.

3.8.2 Manipulation of relative humidity (RH) :

To determine the RH suitable for seed production, four (4) humidity range were selected namely R_1 (65%), R_2 (75%), R_3 (85%), R_4 (above 90%) and hatching number and hatching percentage were recorded.

3.8.3 Manipulation of temperature and RH :

To study the combined effect of temperature and humidity all the 5 temperature regimes and 4 humidity regimes were combined i.e. under each temperature range 4 humidity range were selected. The total experiment was conducted in Environmental Test Chamber regulating the desired temperature and humidity. Parameters like incubation period, hatching duration and average hatching percentage during both the seasons were studied.

3.8.4 Manipulation of temperature, humidity and photoperiod :

After screening of the effect of temperature and relative humidity were observed with five (5) photoperiod regimes namely 6 L, 9 L, 12 L, 15 L and 18 L condition in Environmental Test Chamber.

3.9 Synchronization and Mating behaviour :

3.9.1 Synchronization :

3.9.1.1 Synchronization in normal condition :

After harvesting of cocoons were allowed to emerge, male and female moths emerged upto 4 days were allowed to couple in all possible combinations and 16 combinations were taken under consideration as depicted in table 7.

Table 7 Different mating combinations in normal condition of muga silkworm

Treatment	Coupling Combinations
T_1 :	1st day emerged male x 1st day emerged female
T_2 :	1st day emerged male x 2nd day emerged female
T_3 :	1st day emerged male x 3rd day emerged female
T_4 :	1st day emerged male x 4th day emerged female
T_5 :	2nd day emerged male x 1st day emerged female
T_6 :	2nd day emerged male x 2nd day emerged female
T_7 :	2nd day emerged male x 3rd day emerged female
T_8 :	2nd day emerged male x 4th day emerged female
T_9 :	3rd day emerged male x 1st day emerged female
T_{10} :	3rd day emerged male x 2nd day emerged female
T_{11} :	3rd day emerged male x 3rd day emerged female
T_{12} :	3rd day emerged male x 4th day emerged female
T_{13} :	4th day emerged male x 1st day emerged female
T_{14} :	4th day emerged male x 2nd day emerged female
T_{15} :	4th day emerged male x 3rd day emerged female
T_{16} :	4th day emerged male x 4th day emerged female .

The experiment was undertaken in two main seed crop-rearing seasons namely March – April and September-October. Parameters like coupling efficacy, fecundity and hatchability were recorded.

3.9.1.2 Synchronization by short-term cold preserved condition :

Preservation of adult moth after emergence upto 3 days at 10 ± 1^0C in BOD incubator were done and coupled in all possible combinations : 15 with a control batch of fresh

Table 8 Different mating combinations in preserved condition of muga silkworm

Treatment	Coupling Combinations
T_1 :	Fresh male x Fresh female
T_2 :	1Day Preserved Male x Fresh Female
T_3 :	2Day Preserved Male x Fresh Female
T_4 :	3Day Preserved Male x Fresh Female
T_5 :	Fresh Male x 1Day Preserved Female
T_6 :	Fresh Male x 2Day Preserved Female
T_7 :	Fresh Male x 3Day Preserved Female
T_8 :	1 Day Preserved Male x 1 Day Preserved Female
T_9 :	1 Day Preserved Male x 2 Day Preserved Female
T_{10} :	1 Day Preserved Male x 3 Day Preserved Female
T_{11} :	2 Day Preserved Male x 1 Day Preserved Female
T_{12} :	2 Day Preserved Male x 2 Day Preserved Female
T_{13} :	2 Day Preserved Male x 3 Day Preserved Female
T_{14} :	3 Day Preserved Male x 1 Day Preserved Female
T_{15} :	3 Day Preserved Male x 2 Day Preserved Female
T_{16} :	3 Day Preserved Male x 3 Day Preserved Female

male and female during March – April and September-October. Coupling efficacy, fecundity and hatchability were studied as the effect of preservation.

The hatched larva (hatching yield), the ultimate indicator of output of grainage operation was calculated in both the seasons with the formula as expressed below:

Hatching yield = [Coupling efficacy (%-age) x fecundity x hatching percent]

hatched larvae per 100 couplings

3.9.2 Mating behaviour :

3.9.2.1 Mating duration :

To find out the optimum period of coupling for successful fertilization of eggs, male moths were allowed to mate for 11 different durations. For this standardization 11 treatments were selected from 2 hours to 12 hours with one hour interval, egg laying and egg retention were taken as quantity production parameters and hatching number and hatching percentage were taken as quality production.

9.3.2.2 Repeated mating :

After standardization of mating hours, subsequently another experiment was undertaken to determine the mating times : multiple coupling capacity of male moths . In this experiment males were exploited upto 6 times (T_1 to T_6) during September – October and upto 4 (T_1 to T_4) times during March – April with fresh female : unmated and egg laying, retention and hatchability were taken as key parameters.

3.10 Characterizations of seed cocoons :

Female cocoons were available from 4.00 to 8.50 g. Preliminary screening of seed cocoon was based on cocoon weight, length and diameter and their individual as well as combined reflection on fecundity, hatchability and egg vigour were the key parameters Cocoons were grouped into four category viz. light, average, moderate and heavy.

Furthermore, cocoons were grouped under seven weight ranges separately for male and female with a same limit of weight. The groups were extreme low, lower, low, medium, high, higher and extreme high. Distribution patterns of weight range percentage

Table 9 Different female cocoon groups selected for primary screening

Treatment	Weight (g)	Length (cm)	Width (cm)
Light (T_1)	4.50 - 5.49	3.50-4.00	5.52-5.90
Average (T_2)	5.50 – 6.49	4.00-4.50	5.90-6.30
Moderate (T_3)	6.50 - 6.49	4.50-5.00	6.30-6.70
Heavy (T_4)	7.50 and above	5.00-5.50	6.70-7.10

were calculated. After emergence, fresh males and females were utilized for mating. Although 49 combinations alongwith one control batch (randomly selected cocoon) were selected and Parameters like fecundity, hatchability and egg vigour were recorded.

Table 10 Wide range of male and female cocoon weight groups selected

Cocoon weight groups	Cocoon weight (g)	
	Male	Female
Extreme low	M_1 : 2.50-2.99	F_1 : 4.50-4.99
Lower	M_2 : 3.00-3.49	F_2 : 5.00-5.49
Low	M_3 : 3.50-3.99	F_3 : 5.50-5.99
Medium	M_4 : 4.00-4.45	F_4 : 6.00-6.49
High	M_5 : 4.50-4.99	F_5 : 6.50-6.99
Higher	M_6 : 5.00-5.49	F_6 : 7.00-7.49
Extreme high	M_7 : 5.50-5.99	F_7 : 7.50-7.99

Where only female cocoons were considered, F_1 to F_7 were treated as T_1–T_7. Weight of cocoons was measured by digital balance with one gram in thousand fraction sensitivity, length, and diameter of cocoons measured by digital slide calipers.

3.11 Short term cold preservation of cocoon, moth and egg :

Before designing preservation schedule, the rearing was conducted during adverse months namely June – July, July – August and August – September and subsequent grainage operations were done. To find out the survivability the parameters like effective rate of rearing : ERR by number which was calculated as ERR number = No. of cocoon harvested per 100 larva reared, pupal period, egg production reflected as fecundity, incubation period and hatchability were recorded.

3.11.1 Preservation of cocoons :

Cocoons were collected before adverse season from May – June commercial rearing and preserved in environmental test chamber at 5^0C, 7^0C and 10^0C for a period of

18 days. Effect of preservation of low temperature on pupal period, moth emergence, coupling efficacy, fecundity and hatchability were studied.

3.11.2 Preservation of adults :

Adults from the cocoon of May – June commercial rearing were taken and preserved in environmental test chamber at 5^0C, 7^0C and 10^0C for a period of 7 days and coupling efficacy, fecundity and hatchability were recorded as grainage performance.

3.11.3 Preservation of eggs :

Eggs were collected for low temperature preservation at 4^0C, 6^0C, 8^0C and 10^0C in BOD incubating 1st : 24 hours , 2nd : 48 hours , 3rd : 72 hours and 4th : 96 hours day laying for 3, 7, 12, 15 and 21 days. Simultaneously a batch of eggs was allowed to hatch in normal condition to measure delay hatching caused by low temperature preservation. Incubation period, days delay and hatchability were recorded.

3.11.4. Continuous preservation of cocoon, moth and egg :

Cocoons were collected from the commercial rearing of May – June and preserved at low temperature. Consequently after emergence of adult from preserved cocoon were preserved and simultaneously eggs laid by preserved adult were taken for preservation. The effect of continuous preservation of cocoon, adult and egg were studied.

3.12 Statistical analysis :

For better interpretation of results the experiment were laid out on various design of experiment as and when required. All the experiment were replicated thrice. Influence of season as well as host plants performance were plotted on two factor factorial Randomized Block Design : RBD . Seasons and host plant were considered as 1st factor and 2nd factor respectively. Manipulation of environmental factor such as different ranges of temperature, relative humidity and photoperiod as well as there different combinations were plotted in to Randomized Block Design : RBD. Synchronization of different day wise preserved male and female moths at normal and controlled temperature condition and coupling them with different combination *i.e.* T_1, T_2, T_3, T_5, T_5, ... T_{16} and the efficacy of mating hour's ranges were laid out in a

Randomized Block Design : RBD. For cocoon characterization different weight groups were considered as a treated and plotted under Randomized Block Design : RBD . On the other hand, influence of different weight groups on grainage performance were laid out in Split Plot Design, where group of male cocoon were consider as main plots and group of female cocoon were consider as sub plots. Preservation of cocoon, moth as well as egg of muga silkworm was carried during different seasons were calculated through Randomized Block Design : RBD . The preservation duration *i.e.* days with temperature ranges were analyzed through two factor factorial combinations were plotted in to Randomized Block Design (RBD), when temperature keeping on first factor where as days were consider as second factor.

The relationship between the environmental factors, intrinsic factors as well as grainage performance of larvae fed on both som and soalu were correlated. Multiple regration of the important parameters were worked out using the formula – $Y = a + b_1x_1 + b_2x_2 + b_3x_3 + b_4x_4 + b_5x_5 + \ldots\ldots\ldots\ldots + b_nx_n$. The co-efficient of determination : $R = r^2$ which is the ratio of predicted and total variance was calculated to represent the variability in dependent variable in percentage due to variation of independent variable. The significant levels were taking under consideration from 0.5 % to 0.01 % level of confidence.