CHAPTER- 3

MATERIALS & METHODS

MATERIALS AND METHODS

3.1 Materials

Leaf samples of different types (tender, medium and mature) were collected from castor and tapioca plants in four seasons from various places of Kokrajhar, BTAD, Assam. The leaves were properly cleaned and then dried in hot air oven at 90°C for about two hours. The dried leaves were ground in an electric grinder to make it powder. The powdered leaf sample were kept separately in polythene bags which were subsequently used for analysis (For moisture analysis, fresh leaves were used immediately after collection).

3.2 Methods

3.2.1 Biochemical analysis of leaves of host plants

The biochemical compositions were analyzed using the standard methodologies for each constituent. The average of four seasons were taken. The data generated were statistically analyzed. The standard procedures for extraction and estimation of the chemical constituents are described below.

3.2.1.1 Determination of Total protein content:

The total soluble protein content was estimated by using method of Lowry *et al.* (1951).

Reagents:

a) 2% Na_2CO_3 in 0.1 N NaOH

b) 0.5% $CuSO_4.5H_2O$ in 1% sodium citrate or sodium potassium tartrate

c) Alkaline copper solution: 1 ml of reagent ‘b’ mixed with 50 ml of reagent ‘a’.

d) 1N Folin-Ciocalteau reagent (Commercial reagent) diluted with water to give a solution 1 N in acid.

Extraction:

The residues left after 80% acetone extraction was hydrolyzed in 5ml of 1 N NaOH for overnight and centrifuged at 5000 rpm for 20 min. Supernatant was kept aside and residue was again extracted with 5 ml of 1 N NaOH for 1 hour and then centrifuged. Both the supernatants were pooled and made the volume 10 ml.

Procedure:

A 0.5 ml aliquot was taken in a test tube and mixed with 5 ml of reagent (c) solution and allowed to stand for 10 min. Thereafter, 0.5 ml of reagent (d) was added with instant mixing.

After 30 min, absorbance was recorded at 570 nm through spectrophotometer (Model SL177) against reagent blank. Standard curve was prepared with a graded concentration of bovine-serum albumin.

3.2.1.2 Determination of lipid and free amino acid content

Sadasivam and Manickam (2005) method was used to determine free amino acid and lipid.

Sample Processing

Weight of the sample was taken before loading in the Soxhlet apparatus and solvent was heated so that the steam passes through the plant material vaporizing the volatile compounds. The vapour flowed through a coil where they condensed back to liquid which was then collected in the receiving vessel. The dried and ground plant part was extracted with ethanol by Soxhlet extraction. It was concentrated to dryness under reduced pressure and controlled temperature (40-50°C) using rotary evaporator.

Sample Preparation

The extract received as a result of Soxhlet extraction was further condensed using a rotary evaporator. The sample was extracted using ethanol and was further used for analysis of lipids and amino acid contents.

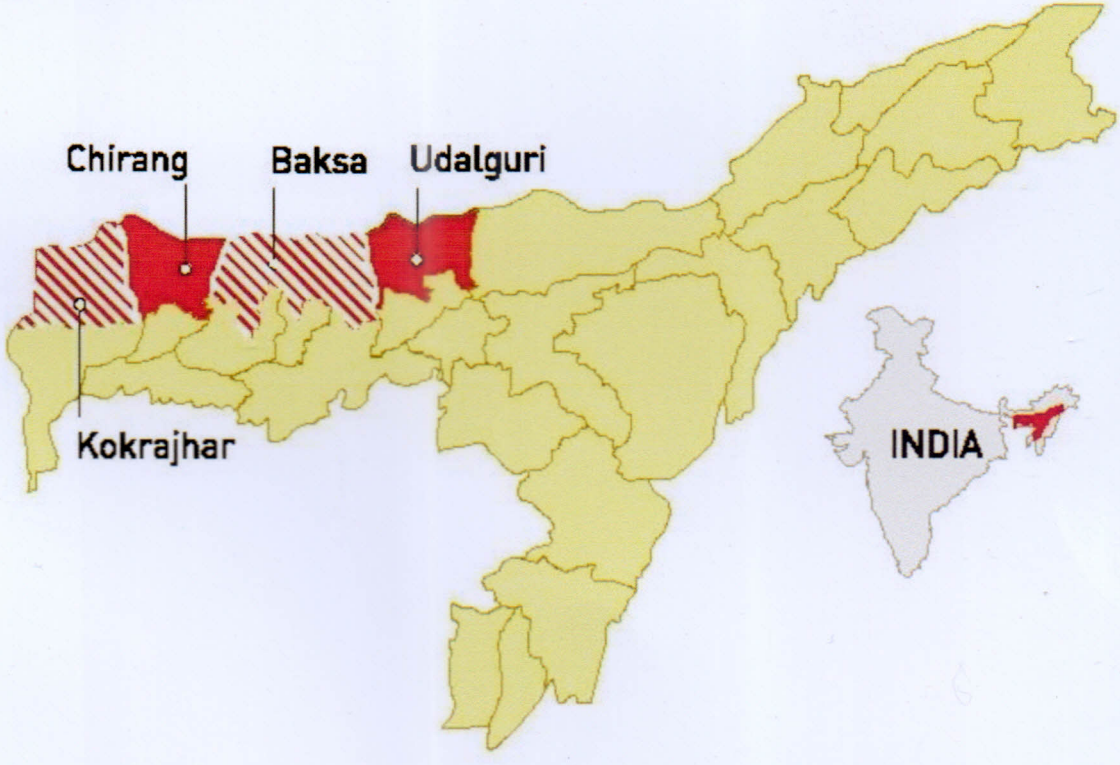

Plate: 1
Map of Assam showing Kokrajhar District in BTAD
(Source: www.mapofindia.com)

3.2.1.3 Determination of crude fibre content

The crude fibre content was determined by the method of A.O.A.C. (1970). 4 g of moisture and fat free sample was digested with 200 ml of 1.25 % sulphuric acid (H_2SO_4) for 30 min. The acid solution was decanted and the material was washed with hot water to remove the acid. The acid free residue was treated with 200 ml of 1.25 % sodium hydroxide (W/V) solution for 30 min. After decantation of top layer, solid material was filtered through previously weighed filter paper. The residue was made free from alkali by repeated washing with hot water and then washed with alcohol and finally with ether. The material was then dried in an oven at 100°C for five hours and weighed (W_e). The material was transferred to a crucible, heated in a muffle furnace (Make: INSIF) at 60°C for three hours, cooled and weighed again (W_a). The difference in weight (W_e-W_a) represented the weight of crude fibre.

$$Crude\ fibre\ content\ (\%) = \frac{(W_e - W_a)}{Weight\ of\ leaf\ sample} \times 100$$

3.2.1.4 Determination of moisture content

The moisture content was determined by the method of A.O.A.C. (1970). For this, fresh leaf samples (10 g) were accurately weighed in aluminium moisture box and dried in an oven at 100°C with air circulation until a constant weight was obtained. The moisture content was expressed in percentage on fresh weight basis using the following relationship.

$$Moisture\ content\ (\%) = \frac{Initial\ weight - Final\ weight}{Weight\ of\ the\ leaf\ sample} \times 100$$

3.2.1.5 Determination of total soluble sugar content

Total soluble sugars were determined by the Anthron methods (Sadasivam and Manickam, 1996) through adding 3 ml Anthron reagent to 0.1 ml filtrate, then heated for 10 min in a boiling water bath, cool rapidly and the developed green colour was read at 630 nm by spectrophotometer.

3.2.1.6 Determination of carbohydrate content

The carbohydrate content in the leaves were estimated by Anthrone method (Sadasivam and Manickam, 2005). In this method, carbohydrates were first hydrolysed into simple sugars using dilute hydrochloric acid. In hot acidic medium, glucose was dehydrated to hydroxymethyl furfural. This compound formed with anthrone a green colored product with an absorption maximum at 630 nm.

50 mg of the leaf sample was taken into a boiling tube and hydrolysed it by keeping in boiling water bath for 1 hour with 2.5ml of 2.5 N HCl and cooled to room temperature. It was neutralized with solid sodium carbonate until the effervescence ceases. The volume was made up to 50ml and centrifuged it. The supernatant was collected and 0.5 and 1ml aliquots were taken for analysis. The standards were prepared by taking 0, 0.2, 0.4, 0.6, 0.8 and 1ml of the working standard (0 served as blank). The volume was made up to 1ml in all the tubes including the sample tubes by adding distilled water. Then 2ml of anthrone reagent was added, heated for ten minutes in a boiling water bath. It was cooled rapidly and absorbance was taken at 630nm.

3.2.1.7 Determination of total phenol content

The total phenol content in the leaf samples was estimated by the Folin-Ciocalteau method described by Mallick and Singh (1980). 0.5 g of leaf sample was weighed and extractions were done with 10 ml 80% ethanol and grind it with mortar. Centrifuged the homogenates 10,000 rpm for 20 mins and saved the supernatant. Re-extracted the residue with 3 times the volume of 80% ethanol. The supernatant was pooled and evaporated to dryness which was then re-dissolved with 50 ml of water. 3 ml of the extract was taken in test tubes and volume was equalized to 3 ml with water. 0.5 ml of

Folin-Ciocalteau reagent was added. After 3 min, 2 ml of 20% sodium carbonate solution was added to each tube and then kept in boiling water bath for exactly 2 min. The tubes were then cooled and the intensity of color was measured at 650nm in a PC based UV-Vis spectrophotometer (Make: Systronics, Model: 2202). The total phenol content was estimated from a standard curve prepared with known concentration of catechol and was expressed as mg phenols per 100g leaf on fresh weight basis.

3.2.1.8 Determination of tannin content

The tannin content in the leaf samples was estimated by the Folin-Denis method described by Schanderl (1970). 0.5 g of powdered leaf sample was weighed and taken in a 250 ml conical flask and 75 ml distilled water was added to it. The flask was gently heated and boiled for 30 min. The solution was then centrifuged in a centrifuge (Research Centrifuge, Make: Remi, Model: R-24) at 2000 rpm for 20 min and the supernatant was collected in 100 ml volumetric flask and volume made up to 100 ml. Then, 1ml of the sample extract was transferred to a 100 ml volumetric flask containing 75 ml water to which 5 ml of Folin-Denis reagent, 10ml of sodium carbonate solution and diluted to 100 ml with water. It was then shaken well and absorbance was read at 700nm in a PC based UV-Vis spectrophotometer (Make: Systronics, Model: 2202) after 30 min. The tannin content was estimated from a standard curve prepared with known concentration of tannic acid and was expressed as percentage on dry weight basis.

3.2.2 Rearing performance of eri silkworms on different host plants during different seasons

Rearing of eri silkworm on castor and tapioca leaves was conducted in Kokrajhar District following standard methodology (Sarmah *et al.*, 2013). Rearing was carried out maintaining one dfls (300 worms) per treatment per replication and there were three replications per treatment. A life cycle of eri silkworm is shown in Plate: 2, which includes various stages of the silkworm i.e., egg, larva, pupa and adult (moth). It is worth mentioning that larval stage is the only feeding stage.

3.2.2.1 Rearing techniques

Depending upon the nutritional requirements and micro-climatic conditions to be maintained during rearing, eri silkworm rearing was divided into two main phases, *viz.*, (i) young age silkworm rearing (1st and 2nd instar larva) and (ii) late age silkworm rearing (3rd to 5th instar larva).

i) Young age silkworm rearing

Young age worms reared under good rearing conditions providing them with tender and good quality tender leaves as well as water soaked foam pad and paraffin paper were put on the rearing tray.

ii) Late age silkworm rearing

The late age worms consumed around 90% leaves supplied during the entire larval period. The mature and semi-matured leaves depending upon the type of food plants. The feeding of dried, yellow and diseased leaves were avoided.

The tray rearing technique was adopted in the present study. During late stage, the larvae were provided with entire shoot and three cross bar sticks made up of bamboo were provided for better aeration and maintenance of hygiene which was found to very much useful to reduce larval mortality in late stage.

iii) Feeding and its frequency

After collection, the leaves were washed in water and preserved in the leaf preservation chamber covering with wet gunny cloth/bag all around. The castor leaves provided without petiole in tray rearing. 4-5 feedings was given per day at regular intervals during the young age rearing. In late age rearing, increased feeding frequency to 5 feedings per day. In night times, excess leaves were provided to fulfill the required consumption throughout the night.

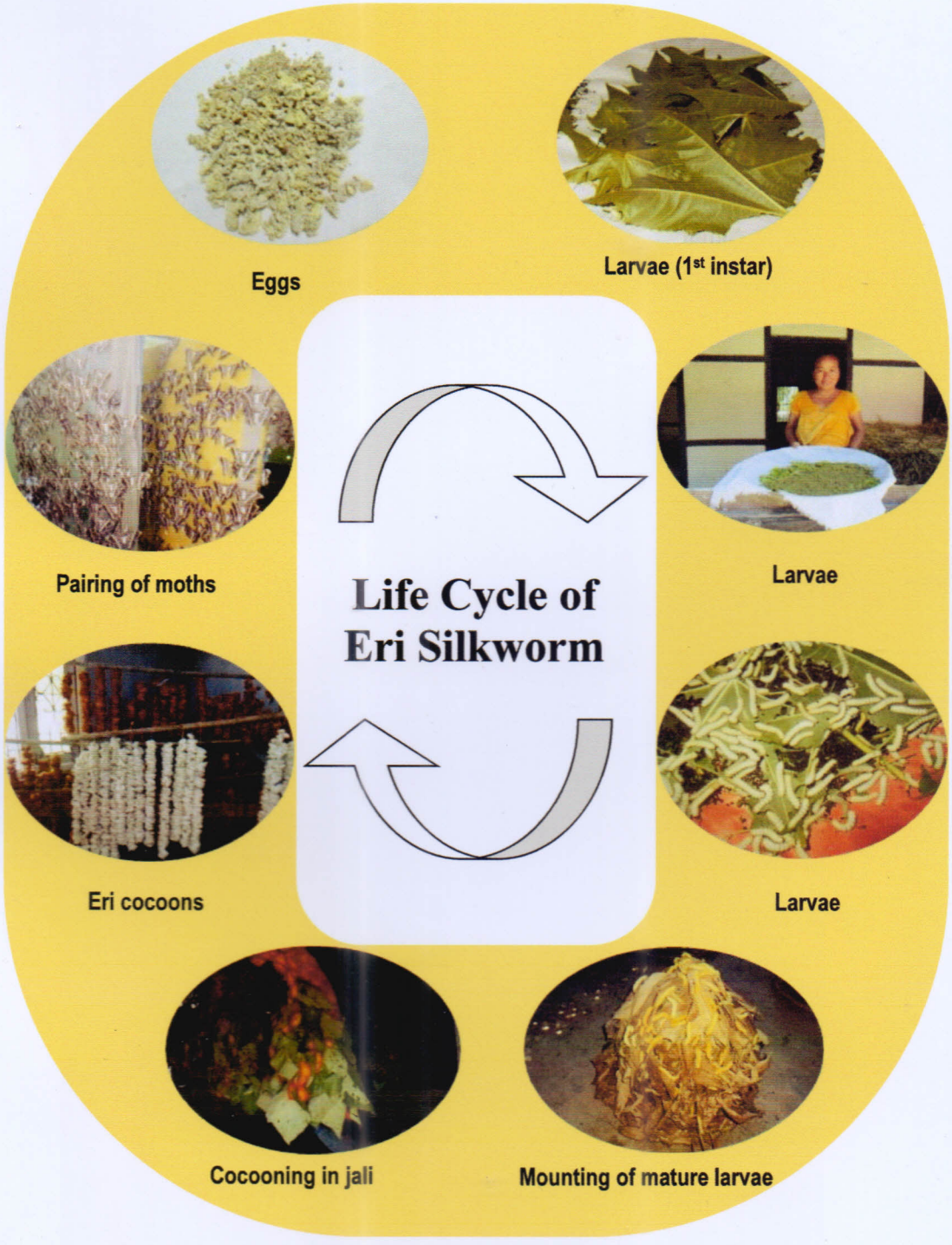

Plate: 2
Life Cycle of eri silkworm

iv) Bed cleaning

Only one cleaning was given during 1st stage, in 2nd stage, two times bed cleaning given, one after first moult and another before second moult. Three bed cleanings resorted to during 3rd and 4th stages, first after second moult, second in the middle and the third before 3rd moult, similarly in between 3rd and 4th stages. The daily bed cleaning was done in 5th stage, preferably in the morning after one or two feedings.

v) Matured worm collection and mounting

Collapsible plastic mountages were used for spinning of the cocoons.

vi) Harvesting of cocoons

Cocoons were harvested after 5-6 days of spinning in summer and 8-9 days in winter.

3.2.2.2 Observation on the rearing, grainage and post-cocoon parameter

The following indicators of rearing, grainage and post-cocoon parameters were recorded to assess the best treatment.

i) Fecundity

To record the fecundity, pairs of freshly emerged moth were placed on khorika and kept hanging on a wire. The moths were decoupled after three hours and the females were allowed to lay eggs on the khorika. After three days, the eggs were removed from the khorika and counted to record the fecundity or the number of eggs laid by the female.

ii) Hatching per cent

Hatching percentage was calculated by the formula-

$$Hatchability\ (\%) = \frac{Number\ of\ eggs\ hatched}{Total\ number\ of\ eggs\ laid\ by\ a\ female} \times 100$$

iii) Larval duration

It is estimated as duration from date of hatching followed by brushing of larva till spinning (maturation) and expressed in days.

iv) Mature larval weight

The weight of fully grown larva of 5th instar prior spinning is considered as mature larval weight and expressed in gram (g) which was measured with electronic balance.

v) Effective rate of rearing (ERR)

It indicates the effectives of rearing in terms of total cocoon harvest over total larvae brushed.

$$Effective\ rate\ of\ rearing\ (\%) = \frac{Number\ of\ cocoon\ harvested}{Number\ of\ larvae\ brushed} \times 100$$

vi) Single cocoon weight

The weight of the individual cocoon at the time of harvesting which is otherwise cumulative weight of eri cocoon shell and pupa and expressed in gram (g). It is one of the most important economic parameter.

vii) Single shell weight

The empty shell weight of individual cocoon which is estimated after removing the pupa from eri cocoon and expressed in gram (g). It is the most important economic character for assessment of productivity.

viii) Shell ratio (%)

It is the ratio between single shell weight and single cocoon weight and expressed in percentage.

$$Shell\ ratio\ (\%) = \frac{Single\ shell\ weight\ (g)}{Single\ cocoon\ weight\ (g)} \times 100$$

ix) Count of yarn

The spinned (manual) silk yarns from the cocoons were analysed for the count and the tensile properties at Regional Silk Technological Research Station (RSTRS), Central Silk Board, Muga Farm, Khanapara, Guwahati.

The count of a yarn is defined as the number of hanks to a pound, each hank containing 840 yards of the yarn and is expressed as "s count". The principle is that when the count of a yarn increases, the fineness of it also increases. The count and denier are related by the equation (Shenai, 1980).

$$Count = \frac{5315}{Denier}$$

A lea was made on a warp reel tester of 80 rounds (120 yards, each round is of 1.5 yards). The lea was then weighed and count was determined. The denier of the yarn was then calculated with the above mentioned formula (Shenai, 1980).

x) Tensile properties of yarn

Tensile properties of the silk yarns were measured using Instron 4301 (3343) universal tensile tester at atmospheric temperature of 25±2°C and 65±2% RH. The gauge length and strain rate were 5 cm and 1 cm/min respectively. The silk yarns were pretreated in an environment of ideal temperature and humidity (25±2°C and 65±2% RH respectively) for 24 hours. 10 specimens were tested to obtain average values of tenacity, percentage of strain, toughness and Young's modulus.

3.2.2.3 Statistical analysis

Data were analyzed statistically for test of significance using Fisher's method of "Analysis of variance" as outlined by Sunderaraj *et al.* (1972). The level of significance of 'F' test was tested at 5 per cent. The interpretation of the data was done using critical difference (CD) values calculated at $P < 0.05$.

3.2.3 Role of eri culture in the socio-economic development of the Bodos in Kokrajhar District

- Data on Socio-economy from eri culture in Kokrajhar District were obtained by a questionnaire (enclosed).
- Primary data on status was collected conducting a Survey on eri farmers, eri cocoon traders, District HQ, cocoon bank in Udalguri and Eri Spun Silk Mill, Kokrajhar.
- Secondary data on status of eri culture in Kokrajhar District, Assam was collected from Directorate of Sericulture, BTAD, Assam and the Regional Office, Central Silk Board, Guwahati.
- Appropriate statistical model was ascertained prior collecting and conducting the secondary and primary data.
- The primary and secondary data thus obtained were compiled, statistically analyzed and inferences drawn for establishing sector-wise status of eri culture in Kokrajhar District, Assam.
- The meteorological data of Kokrajhar District was collected on monthly basis for the year 2012 to 2015. The data comprised of maximum and minimum temperature (°C), maximum and minimum humidity (%), rainfall (mm) and no. of rainy days.
- Economics of eri culture/100dfls was calculated. It included quantitative production from eri silkworm rearing, viz. cocoon shell, pupa, tapioca tubers, castor seeds etc.
- Socio-economic status was ascertained by evaluating employment generated, percentage growth of ancillary industries viz. manufacturing of reeling/spinning/weaving industries, etc.

SURVEY QUESTIONNAIRE

Eri culture Beneficiaries (Graineurs/Silkworm Rearers/Spinners/Weavers etc.)

Shri Jogesh Deuri, Director, Department of Sericulture, BTC has undertaken his Ph.D. Research works entitled ***"A study on quality aspect of Eri culture for the socio-economic development of the Bodos in Kokrajhar District"*** *under School of Biological Sciences, University of Science and Technology, Meghalaya.. The semi-structured questionnaire has been prepared to identify the specific needs of different stakeholders of the eri culture and to assess their socio-economic status.*

Please fill as per instructions given. Write codes/ values in the box provided at the right hand side)

1.0	**General Information**	
1.1	Name of the District : Kokrajhar	
1.2	Village	
1.3	Name of the Beneficiary: _____ S/o _____W/o_____ Address: City/Town/Village:_____ Tehsil: _____ Pin: _____ Phone/Mobile No.:_____ E-mail, if any _____	
1.4	Age of the respondent (in yrs):	
1.5	Gender (1=Male, 2=Female)	
1.6	Caste (1=SC, 2=ST, 3=Others)	
1.7	Educational Level (1=Illiterate, 2=Primary, 3=Secondary, 4=Graduate and above)	

1.8	Experience in sericulture: 1= 0-5 Yrs: 2=5-10 Yrs: 3=10-15 Yrs: 4= >15 Yrs	
1.9	Type of Sericulture practiced (1=Graineurs (Seed), 2=Commercial silkworm rearing, 3=Spinning (Manual), 4=Automatic spinning machine, 5=Weaving, 6= Dyeing, 7=Fabric Processing, 8=Others (Pls specify)	
1.10	Land holdings (acre)	
1.11	Area under different agricultural activities a) Field crops: b) Horticulture crops: c) Sericulture: d) others	
1.12	No. of family members:	
1.13	Name of the organization/department supported the farmer and type of support	
1.14	Prime Sericulture Sector (1=Mulberry, 2=Tasar, 3=Eri, 4=Muga)	

1.15 Sources of family income

Sl. No.	Source of Income	Approximate Annual Income (Rs. in thousands)				
		2007-08	2008-09	2009-10	2010-11	2011-12
a)	Sericulture a) Rearing (cocoon) b) Grainage c) Spinning d) Weaving Any other					
b)	Income from other activities					
	Total:					

2.0 New Technologies adopted

2.1 Provide details regarding the new Sericulture R&D technologies adopted during last five years?

Year	Name of Technology/ Process/ Product/ Best Sericulture Practice adopted	Implemented by CSB/DOS/ NGO/Others	Whether Beneficial (1= Yes 2= No)	Impact on sericulture yield/ productivity/ Quality or other benefits	Remarks
2007-08					
2008-09					
2009-10					
2010-11					
2011-12					

(Please use additional sheets if required)

2.2 Have you received any extension support/training for using the new technology developed through R&D project?
(1= Yes 2= No)

2.3 If yes, details of training support received

Year	Title /aspect of training	Organization

2.4 Infrastructure available (including community production centre, spinning, weaving, dyeing centre etc.)
i)
ii)
iii)
iv)

3.0 What is the arrangement of marketing?

4.0 Plantation area of host plants available

5.0 The present rearing capacity

6.0 Production cost per 100 dfls

7.0 Income per 100 dfls:
Shell_____ Pupa_____

8.0	Production vis-à-vis value chain 1. Cocoon production ⟶ Pupa and shell 2. Cocoon production ⟶ Pupa and shell ⟶ Yarn 3. Cocoon production ⟶ Pupa and shell ⟶ Yarn ⟶ Fabric	
9.0	Financial supports available	
10.0	The areas on which training/skill development is required	
11.0	What is the infrastructure supports required?	
12.0	Any other aspects you would like to mention for improvement of income and employment?	

Name of the Research Scholar: _____

Signature : _____

Place of Survey : _____ **Date:** _____