MATERIALS AND METHODS

LOCATION: The experimental site of Research Extension Centre Farm is situated above 627 mASL and its lies 24.98º N Latitude and 92.83º E Longitude at Dima Hasao (North Cachar Hills) district of Assam.

Location Map of Umrangso, District. Dima Hasao, Assam

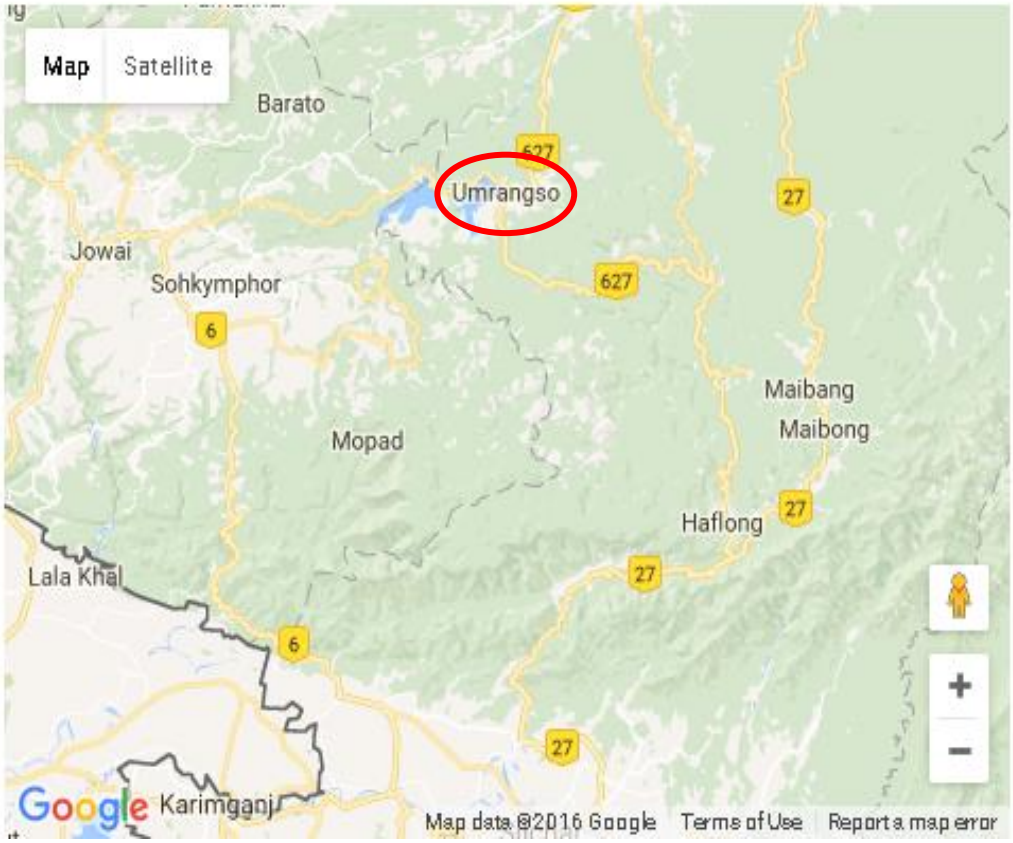

Figure 1: Map of Dima Hasao (North Cachar Hills)
Source: Satellite Map

MATERIALS AND METHODS

3(a).1 Materials

Quercus serrata 300 number of plants each of the same age (20 years), height and girth were taken at Research Extension Centre. farm of Umrangso, Dima Hasao district, Assam for Spring and Autumn season leave yield during the year 2013 and 2014 respectively.

3(a).2 Methods

3(a).2.1 Studies of leaf yield of *Q.serrata*

Pruning and pollarding of *Q. serrata* plants were done during the first week of December followed by application of Farm Yard Manure and Chemical Fertilizer for spring season and light pruning, removal of 1/3 biomass/ clipping off of leave during the second week of August for Autumn season followed by application of Farm Yard Manure and Chemical Fertilizer to rejuvenate the plant for maximum flush of new leaves.

Agronomical practices: Application of FYM 10 kg / Plant and Urea= 48.0 gm, Single Super Phosphate= 46.5 gm and Murate of potash= 9.3 gm per plant (Nitrogen : Phosphous : Potash =150:50 : 38 and Farm Yard Manure= 7000 kg/hectare) in economical plant spacing of 4´x4´ (6724 no. of plants/hectare).

Leave yield/hectare =Average leaf yield per plant (Kg) x actual no of plant.

100 plants were taken in each treatment (Spring and Autumn crop in 2013 and 2014)

Treatment A : Control
Treatment B : Application of NPK+FYM
Treatment C : Application of FYM

3(b).1 Materials

A total 100 x 3= 300 plants taken from three different treatment *i.e.*. A,B and C which mentioned in 3(a).1 and introduced of *A.proylei* silkworms 100 x10=1000 per treatment of newly hatched silkworm.

3(b).2 Methods

3(b).2.1 Rearing of *Antheraea proyeli* Jolly. during the period 2013 and 2014 in Spring and Autumn crop.

100 x10=1000 (100 silkworms were brushed in one plant) for each treatments as cellular rearing under nylon net in spring and autumn crop in 2013 and 2014.

1. Mature worm weight: Mature silk worm weight was taken just after discharge of the excreta by each male and female larvae.

2. Cocoon weight: Removal of dry leaves after 6th day of spinning by each larva. Ten numbers of silk cocoon male and female each selected randomly for cocoon analysis.

3. Shell weight: Male and female each cocoons considered for taking shell weight after removal of pupa from the cocoon.

4. Shell ratio: Shell ratio of each cocoon was calculated following the formula:

$$\text{Percentage of shell ratio (SR\%)} = \frac{\text{Shell weight}}{\text{Cocoon weight}} \times 100$$

5. Effective rate of rearing: Effective rate of rearing is a performance of assessment indicator for a siik worm rearing. It is calculated as follows:

$$\text{Effective rate of rearing (ERR\%)} = \frac{\text{Total no.of cocoon harvested}}{\text{Total no.of silkworm}} \text{ x } 100$$

6. Cocoon harvested: The total numbers of cocoons spanned for each treatment was counted as cocoon harvested.

7. Larval period: The duration of larva was calculated from the first day of feeding *i.e.*. 1st day of 1st instar till the starting day of spinning for silk cocoon.It is calculated in numbers of days.

3(c).1 Materials

Tender, semimature and mature of *Quecus serrata* leaves to be collected randomly from REC, Umrangso farm. The method of sampling of leaves as described by Kamal and Singh(1970) was followed during the collection of leaves.

3(c).2 Methods

3(c).2.1 Isolation and identification of species from phylloplane of *Q.serrata*. during spring and autumn season in 2013 and 2014.

Serial washing technique of Kamal and Singh (1970),leaf discs were cut out from different categories of leaves with the help of sharp sterilized cork borer. Pieces of different categories of leaves were placed separately in 20ml of sterilized distilled water in 250 ml of erlenmyer flasks and were shaken for 20 minutes at 120 rpm.The extract of the detachable fungal propagules from the leaf surface was determined by plating 1 ml solution from washing to the Petri plates containing PDA media. The cut-out leaf discs upper and lower surface were impringed on the surface of Petri dishes containing PDA media. The Petri dishes were incubated at 28 ± 1^{o}C for 4 days and then the plates are examined for the development of fungal colonies. The experiment was conducted in Spring and Autumn season The isolated fungi were Identified with the help of by Barnatt H.L (1960) "Illustrated genera of Imperfect fungi" and Gilman (1961) "A manual of soil fungi".

3(d).1Materials

Study samples of rhizosphere soil and air samples were collected during different Oak tasar silkworm rearing seasons.

3(d).2Methods

3(d).2.1 Isolation of Fungi from Rhizosphere, Non-rhizophere, Rhizoplane and Air Mycoflora.

Root sample with adhering soil were dug out carefully from matured plants of Oak and one year seedlings, collect in polyethylene bags and brought to the laboratory .Soil samples were collected from a depth of 0-15 cm from five different spots of the farm. The collected samples were mixed thoroughly and composite

samples obtained from each plot.The samples were then brought to the laboratory immediately in polyethylene bags and stored in refrigerator at 4° C. Five samples were collected in each case of study.

3(d).2.2 For isolation of Fungi from Rhizosphere

Serial dilution plating method (Atlas R M and Parks L C 1997: Mukherjee and Subba Rao 1982) was followed. After removal of superfluous soil, the root system along with the adhering soil (approx 1 gm) was placed in a conical flask containing 10 ml sterile distilled water shaken vigourly. Stock solution 1ml aliquot was transferred with a sterilized pipette to another flask containing 9ml sterilized distilled water and shaken vigorously to obtain a dilution of 10^{-1}, 10^{-2} , $10^{-3}$and 10^{-4} were prepared.

One ml aliquot from dilution of 10^{-3} and 10^{-4} was transferred a septically into sterile Petri-plates with 10^{-12} ml of melted PDA media (15 mltreox). With 0.5 ml 250mg *Streptomycin* to suppress bacterial growth. Five petri plates were provided for each solution. Five replicates of petriplate were maintained for each inoculation. The whole process was carried out in a Laminar Air Flow Chamber. The petridishes were incubated upside down for 5-7 days at 28± 1º C in B.O.D incubator. Fungal population was estimated by counting the number of colonies. Total number of fungi in rhizosphere was calculated on dry weight basis. Pure culture of fungi were maintained on slants of PDA media in culture tubes for identification.

Gilman (1995), Subramanian (1971) and Barnett and Hunter (1972)were consulted for identification of fungi. The following formula was used for determination of relative abundance of a fungal species.

$$\text{Relative abundance (\%)} = \frac{\text{Total no of colonies of individual species}}{\text{Total no of all species}} \times 100$$

3(d).2.3 Isolation of fungi from Rhizoplane:

Roots were washed in running tap water and 1cm root pieces were cut out from tap and lateral roots with sterilized scissor. The roots were then washed in sterile distilled water thrice serially. Root pieces were transfer to PDA media plates supplemented with 0.5 ml streptomycin (0.2 gm/lit). under aseptic condition. The plates were incubated upside down at 28 ± 1º C in a BOD Incubator for 5 days.

3(d)2.4 Isolation of fungi for non Rhizosphere soil.

Soil samples were collected randomly from 5 different spots of Oak plantation of rearing site of Umrangso in 2013 and 2014.One kg soil samples included plant debris were collected with a soil at a depth of 0-15cm from the root region of Oak plants using the conventional sampling method (Johnson and Curl,1972). The samples were placed in clean plastic bags. Five soil sample (four at corner and one at centre) taken from each study site were mixed thoroughly in one composite sample.

1.0gm soil samples were processed for isolation of soil mycoflora. For isolation of soil mycoflora, serial soil dilution plating technique was followed (Johnson and Curl, 1972).Isolation was done within 24hours of collection.1.0 gm soil was transferred to a 250 ml conical flask containing 90ml sterilized distilled water to make a total of 100ml.The suspension was shaken vigorously for 30 minutes to obtain a homogenous solution. Stock solution 1.0ml was pipetted aseptically and dispensed in dilution test tube with 9.0ml sterilized distilled water. Series of soil dilutions of 10^{-1}, 10^{-2} ,$10^{-3}$and 10^{-4} were prepared.1.0ml aliquots from dilution of 10^{-3} and 10^{-4} was transferred aseptically into sterile Petri-plates with 10.0-12.0 ml of melted PDA media suppleted with 0.5ml of 250 mg Streptomycin to s suppress bacterial growth.5 petriplates were provided for each solution. These plates were incubated in BOD incubator for 28 ± 1ºC for 5-7 days. Grown fungi were isolated and identified.

3(d)2.5 : Isolation of Air mycoflora:

3 petriplates containing sterilized PDA media containing streptomycin (0.2gm/lit)were exposed in the air over the Farm area for 15 minutes against the air

current at two different height(0.75metre and 1.50metre) 3 replication for each height. Petriplates brought laboratory incubated for 5 days

3(e).1Materials

Soil of Oak (*Quercus serrata*) vegetation of Research Extension Centre Farm of Umrangso.

Physico-chemical nature of soil of Oak vegetation in the farm of Research Extension Centre, Umrangso, District Dima Hasao (North- Cachar Hills), Assam.

3(e).2 Methods

3(e).2.1 Determination of Soil pH:

10.0 gm of soil was taken in a beaker and it was mixed with 50ml of distilled water. The soil water mixture was stirred for 20 minutes on a magnetic stirrer. Then pH of soil was measured through an electronic digital pH meter.

3(e).2.2 Estimation of Organic Carbon:

Walkey and Black's (1934) rapid titration method was followed for determination of organic carbon. One gm air-dried and sieved (0.2mm) soil was taken in a dry and clean 500 ml conical flask, 10ml of 1N $K_2Cr_2O_7$ was added to the flask was swirled for a while followed by addition of 20 ml conc. H_2SO_4. The flask was swirled again and allowed to stand for 30 minutes. 10ml of 85% phosphoric was added to it and titrated with 1N $FeSO_4$ solution using diphenylamine as indicator. Same determined as given below.

Organic carbon (%)= $\frac{B-S\times 0.003\times 100}{W}$

Where B=Volume of FeSO4

S=Volume of K2Cr2O7

W=Weight of soil sample(gm)

3(e).2.3 Estimation of Nitrogen:

Nitrogen estimation by Alkaline Potassium permanganate ($KMnO_4$) method (Subbiah and Asija 1956): 20gm of soil was taken in a distillation flask and add 20ml of water and 100ml of 0.32% $KMnO_4$ pipette out 25ml of N/50 H_2SO_4 in a conical flask. And add 2-3 drops of methyl red indicator and dip the end of the delivery tube into it. Pour 100ml of 2.5% NaOH solution into the flask and cork it immediately. Distil the ammonia gas from the distillation flask and collect in H_2SO_4 solution. Continue distillation till the evolution of ammonia ceases completely (test by bringing a moist red litmus paper near the outlet of the condenser, which will turn blue as long as ammonia is being evolved).Titrate the excess of H_2SO_4 against N/50 NaOH and note the volume of NaOH used. The end point is reached when the color changes from pink to yellow.

1. Weight of soil taken=20gm

2. Volume of N/50, H2SO4 taken =25 ml

3. Volume of N/50, NaOH used (titrated value) = X ml

4. Volume of N/50 acid used for NH3 absorption (25-X)ml

(1ml of N/50 H2SO4=0.02 meq. of N 0.28mg N=0.00028 gm N)

Calculation:

a. Percentage of available N = (25-X) x 0.00028 x100/20

b. Available N in the soil (ppm)=(a) x 10,000

c. Available N in the soil (kg/ha)=(b) x 2.24

3(e).2.4 Estimation of Phosphate:

Estimation of available phosphate Bray's method (Bray and Kurtz,1945)

This procedure is primarily meant for soils for soils which are moderately to strong acids (pH around 5.5 or less).This method gives results highly correlated with the crop response to phosphate fertilization.

1.5.0gm soil was taken in 100ml conical flask.

2. Add 50ml of extractant solution to the soil.

3. Shake the contents of the flask for exactly 5 minutes, and filter through Whatman No.42 filter paper.

4. Prepare a blank in which all the reagents are added similarly, except the soil.

3(e).2.5 Determination of Available Potassium in soil (K_2O)

Procedure:

1. 5.0 gm soil in a 150ml of conical flask.

2. Add to it 25 ml N NH_4O Ac solution.

3. Shake the contents of the conical flask on an electric shaker for 5 minutes and filter.

4. Feed the filtrate in the atomizer of the flame photometer, 100 of which has been set with 40 ppm K solution and recorded the reading.

3(f).1 Materials

Leaf samples *Quercus serrata* of different types (tender, semi-mature and mature) were collected from R.E.C. Umrangso farm, in different treatments and in spring and autumn season during the year 2013 and 2014.

3(f)2 : Methods

3(f)2.1 Foliar constituents *Quercus serrata* in spring and autumn season during 2013 and 2014.

Leaves of different types namely tender, semi-mature and mature were collected from plants/trees. For spring crop pruning and pollarding were done during the 1st week of December and for autumn crop light pruning/clipping were done in 2nd week of August. The agronomical practices, application FYM and NPK were done for better quality and quantity of leaves for rearing seasons.

3(f)2.1.1 Determination of moisture content.

Moisture content of the leaves was determined by the method of A.O.A.C (1984).One hundred gram of *Q.serrata* leaves(tender, semi-mature, and mature)were collected separately of three different clean properly with clean cloth to removed dirt and dried separately at 60º C for 24 hours and powdered.

$$\text{Moisture}(\%)=\frac{\text{Fresh weight of leaves} - \text{Dry weight of leaves}}{\text{Fresh weight of leaves}} \times 100$$

3(f)2.1.2 Estimation of Crude Protein:

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

Regents:

i) 2% $NaCO_3$ in 0.1NaOH

ii) 0.5% $CuSO_4.5H_2O$ in1% sodium citrate or sodium potassium tartrates

iii) Alkaline copper solution: 1 ml of reagent 'b' mixed with 50ml of reagent 'a'.

iv) 1N Folin-ciocalteau reagent (Commercial reagent) dilultated with water to give a solution 1N in acid.

Extraction:

The residues left after 80% acetone extraction was hydrolyzed in 5.0ml of 1N NaOH for overnight and centrifuged at 5000rpm for 20 minutes. Supernatant was kept aside and residue was again extracted with 5.0 ml of 1N NaOH for 1 hour and then centrifuged. Both the supernatants were pooled and made the volume 10.0 ml.

Procedure:

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

After 30 minutes absorbance was recorded at 570 mm through spectrophotometer (Model SL.177) against reagent blank. Standard curve was prepared with a graded concentration of bovine-serum albumin.

3(f)2.1.3 Estimation of crude fibre content:

The crude fibre content was determined by the method of A.O.A.C (1984).Four grams of moisture and fat free sample was digested with 200 ml of 1.25 per cent sulphuric acid (H_2SO_4) for 30 minutes. The acid solution was decanted and the material was with hot water to remove the acid. The acid free residue was treated with 200ml of 1.25 per cent sodium hydroxide (W/V) solution for 30 minutes. 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 º C for five hours and weighed (W_e).The was transferred to a crucible, heated in a muffle furnace (Make: INSIF) at 600 ºC for three hours, cooled and weighed again (W_a).The difference in weight (W_e -W_a) represents the weight of crude fibre.

$$\text{Crude fibre content (\%)} = \frac{(We - Wa)}{\text{Weight of leaf sample}} \times 100$$

The crude fibre content was expressed in percentage of moisture and fat free sample on dry weight basis.

↓

2gm sample +2.5 gm ConH_2SO_4=200ml D/W (acidic treatment)

↓

After boiling (30 minutes),the samples are filtered in a muslin cloth and washed it in D/W

↓

Drying samples extract+2.5gmNaOH pelletsP+200m D/W(alkaline treatment)

↓

Again boiling (30 minutes),the samples are filtered in a muslin cloth and washed it inB/W

The extra water is removed by washing alcohol and kept in oven for 24hours at 600^0C

↓

The samples is weighted note down

↓

The acid and alkali treatment, oxidative hydrolytic degradation of the cellulose and degradation of lignin occur. The loss is weight give the fiber content.

3(f).2.1.4 Estimation of Crude fat content:

The crude fat content in the leaf samples was estimated by the A.O.A.C method (1984). Crude fat content was determined by extracting the fat from the sample using a solvent,then determining the weight of the fat recovered. As lipids/fats are relatively non-polar molecules, they were pulled out of a sample using relatively non-polar solvents. With a non-polar solvent, only non-polar molecules in the sample dissolved while polar ones do not.

3(f).2 Methods

Weigh 2-3 gm of the dried food (leaf) sample into extraction thimble.

↓

Place the thimble inside the Soxhlet Apparatus.

↓

Connected a dry pre-weighted solvent flask beneath the apparatus and added required quantity of solvent and connect the condenser.

↓

Adjusted the heating rate to give a condensation rate of 2-3 drops and extract for about 16 hours.

↓

After completing the extraction removed the thimble and reclaim ether using the apparatus.

↓

Complete removal of ether on a boiling bath and dry flask at 105 ºC for 30 minutes. Cooled in a desiccator and weighed.

Calculation: Crude Fat (% of Dry matter) = (weight of fat x/weight of sample) × 100

3(f)2.1.5 Determination of Ash content by A.O.A.C method (1984)

For estimation of total ash %, 1.0gm of leaf sample was dried in a nickel crucible and heated it on a low flame till the organic matter turn to burn.

The crucible were placed in a muffle furnace and heated it 600ºC and stopped when grayish white ash formed. The residue represented the total ash percentage.

3(f)2.1.6 Estimation of Carbohydrate content:

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

50mg of the leaf sample was taken into a boiling tube and hydrolysed it by keeping in boil water bath for 1 hour with 2.5 ml of 2.5 N-HCl and cool to room temperature. Neutralized it with solid sodium carbonate till the effervescence ceases. Made up the volume to 50 ml 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, 0.2, 0.4, 0.6, 0.8 and 1.0ml of the working standard (0 serves as blank)and make up the volume to 1.0ml in all tubes including the sample tubes by adding distilled water. Then 2.0ml of anthrone reagent was added. Heated for ten minutes in a boiling water bath. Cooled rapidly and read the green to dark green color at 630nm.

50 mg sample+2.5ml of 2.5N HCL in to a test tube

↓

Hydrolysed by keeping it in a boiling water bath for 1.0 hour

↓

Neutralized it with Na_2CO_3 until effervescence ceases

↓

Made up the volume to 50 ml and centrifuged

↓

Collected the supernatant and taken aliquots for analysis

↓

Prepared the working standard Zero served as blank

↓

Made up the volume to 1.0 ml in all tubes

↓

Added 2.0ml of Anthrone reagent and heated for 10 minutes

↓

Cooled rapidly and read the absorbance at 630 nm

↓

Carbohydrates hydrolyzed in to sugar using dil HCl. In acidic medium glucose

↓

Dehydrated to hydroxymethyl furfural which formed a green colored Anthrone.

3(g).1.Materials

Oak tasar cocoons (*Antheraea proylei* **Jolly**.) were taken from harvested three different treatments for observed the reeling parameter.

3(g).2 Methods.

Oak tasar cocoons were hot air stifled for 6-7 hrs at 70° C and stored for 2-3 months were used in this study.

Thirty oak tasar cocoons (ten replication of three cocoons each) were wrapped in a coarse cotton cloth and subjected to 30 minutes pressure cooking at 1.05kg/cm pressure.

The cocoons were then soaked in pineapple extract at room temperature (26-31^0C) for 12hrs and cocoons along with the wrapper were taken out from cooking medium and then washed repeatedly with tap water until the associated brown colour and proteinase activity were washed out (Devi *et al.*,2012).

The cocoons were then removed from the wrapper, semidried on blotting papers, deflossed and then subjected to single filament reeling on an eppouvette machine.

Statistical analysis:

All the observed data were analyzed statistically using the technique of analysis of variance. The significant of treatment difference was judged by 'F' test as outlined by Cochran (1977).

The standard error of the differences SED ± was calculated by using following expression.

$$\text{SED} \pm = \sqrt{\text{Error mean square} \times 2} \,/\, \text{pooled number of application}$$

The critical differences (CD) was calculated to test the significance of differences of the treatments. Critical differences (CD) was calculated by using following formula:

$$\text{CD } (5\%) = (\text{SED} \pm) \times \text{'t'}$$

' Where, the =5% tabulated value of the 't' at error degree of freedom.