3. METHODOLOGY

3.1. The general features of the study sites

The studies were undertaken in three village clusters, viz., Hosapet (herein after called Cluster 1), Beechagondanahalli (herein after called Cluster 2), and Chowdadenahalli (herein after called Cluster 3) of Kolar District, Karnataka State (Figure 1). The name given to the cluster is based on the village from where maximum number of farmers has been identified for the study. The district has a major mulberry growing area under irrigated condition. Of the total area under mulberry cultivation of the Karnataka State (88,903 ha) as reported during 2002-03, an area of 24,655 ha (27.7%) was in Kolar District alone (Anonymous, 2003 b). The mulberry cultivation was traditionally practised in all the 11 taluks of the district and more intensively in Kolar, Chintamani, and Siddlaghatta. The Cluster 1 identified for the study is in Jangamakote Hobli, Siddlagatta Taluk and Clusters 2 and 3 are in the Vemgal Hobli, Kolar Taluk.

Of the 45 farmers (15 from each cluster) identified for the study, 30 (10 from each cluster) with 0.2 to 0.4 ha improved mulberry variety V₁ were selected for the INM treatment (Plate 1) based on their willingness to adopt the integrated practices and also based on the suitability of their holdings. The remaining 15 farmers (5 from each cluster) with similar extent of improved mulberry variety (Plate 2) were allowed to continue their conventional practices (control). The number of farmers selected for the INM practice and control could not be kept equal due to heterogeneity in the mulberry plantation and socio-economic status of the identified farmers. Hence, due care was taken to adopt the farmers who predominantly had V₁ variety with wider spacing and comparable socio-economic status. This also minimizes the error while
analyzing the impact of INM at the end of the study period. Study on INM was also undertaken simultaneously at the Regional Sericultural Research Station (RSRS), Kodathi, Bangalore Urban District under CSRTI, Mysore (herein after called RSRS Farm), in 0.40 ha V1 mulberry garden of which 50 per cent was considered for INM treatment and the remaining 50 per cent as control with recommended practices (Plate 3).

3.2. Agro-meteorological conditions

The selected village clusters fall under Eastern dry zone of Karnataka State, situated at an altitude of 900 m above mean sea level (MSL) representing similar agro-climatic conditions. The latitude of the area is 13-14° North and longitude 78° East. The mean maximum and minimum temperature recorded in the area was 39°C and 19°C, respectively. The average annual rainfall recorded was 730 mm with a mean relative humidity of 75%. The meteorological data mentioned were recorded for the years 2003, 2004 and 2005 at the identified village clusters as well as at RSRS Farm.

3.3. Grouping of socio-economic factors

Before imposition of the INM treatments, the selected farmers (INM and control) were personally interviewed to collect the benchmark information on sericultural practices adopted besides their personal and socio-economic details using a pre-tested schedule (Annexure). Based on the data collected on independent variables, the selected participants were classified as suggested by Singhvi et al. (1994) and Jagadish (1994).
3.3.1. Personal profiles

i) Age : (actual number of years completed by an individual at the time of interview)

Young < 30 years
Middle 30 - 50 years
Old > 50 years

ii) Education level :

No education No education/ineducated
Low education Primary or Middle school education
High education High school and above

iii) Land holding :

Actual holdings (to have a common denominator 0.80 ha dry land was made equal to 0.40 ha wet land as per Karnataka Land Reforms Act 38 of 1966).

Marginal farmers < 1 ha
Small farmers 1 - 2 ha
Big farmers > 2 ha

iv) Family size :

Small family < 5 members
Medium 5 - 10 members
Big > 10 members

v) Annual agricultural income (in Rs) :

Low Below 65,000
Medium Above 65,000 & below 1, 24,000
High Above 1, 24,000

3.3.2. Socio-economical characteristics: To study the influence on technology adoption, the following socio-economical characteristics viz., social participation, mass media and extension participation, extension contact, and cosmopolitan nature of the participants were considered and the participants were given the scores as regular (2), occasional (1) and never (0) and further classified as indicated below.
i) **Social participation:** While recording the scores for the social participation, the farmers’ membership or their involvement in the farmers’ co-operative society, milk co-operative society, sericultural co-operative society, self-help group (SHGs) and village/taluk/zilla panchayats were considered and classified as:

<table>
<thead>
<tr>
<th>Level</th>
<th>Classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>Score 1 or below</td>
</tr>
<tr>
<td>Medium</td>
<td>Score 1-2</td>
</tr>
<tr>
<td>High</td>
<td>Score &gt; 2</td>
</tr>
</tbody>
</table>

ii) **Mass media participation:** The score for farmers’ mass media participation was recorded based on the participation in the radio / TV programmes, and ability of reading newspapers and sericultural magazines, etc. and classified as:

<table>
<thead>
<tr>
<th>Level</th>
<th>Classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>Up to 50% of the eligible score</td>
</tr>
<tr>
<td>High</td>
<td>Above 50% of the eligible score</td>
</tr>
</tbody>
</table>

iii) **Extension participation and extension contact:** The participation of the farmers in the extension programmes, *viz.*, group discussion, demonstration, film shows, exhibition, and field days, resham krishi melas, etc. was considered while scoring for extension contact the individual contact with the state/central or lead farmers was considered and classified as:

<table>
<thead>
<tr>
<th>Level</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>0 - 30%</td>
</tr>
<tr>
<td>Medium</td>
<td>30 - 60%</td>
</tr>
<tr>
<td>High</td>
<td>Above 60%</td>
</tr>
</tbody>
</table>

iv) **Cosmopolitan nature:** The farmers’ frequency of visit to the hobli, taluk and district headquarters was considered and classified as:

<table>
<thead>
<tr>
<th>Level</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>up to 5</td>
</tr>
<tr>
<td>High</td>
<td>above 5</td>
</tr>
</tbody>
</table>
3.4. **Sericultural practices**

The important mulberry cultivation and silkworm rearing practices recommended in the *Handbook of sericulture technologies* (Dandin *et al.*, 2003) were selected and degree of adoption level was recorded by three-point scoring pattern as advocated by Gopala (1991). The percentages of adoption level were calculated based on the level of adoption by the number of selected participants as against the total number of participants adopted for study. The participants were classified into three groups as low adoption level (0-39 per cent), moderate adoption level (40-59 per cent), and high adoption level (60 per cent and above). The data were tabulated and subjected to chi square analysis.

3.5. **Cropping pattern**

For better understanding of the economic status of the selected farmers, the annual agricultural income was calculated based on the crops grown, mean crop yield, and the prevailing market prices for the previous three years (2000, 2001, and 2002).

3.6. **Crop determinants**

The participating respondents were asked to rank the cropping scheme determinants, *viz.*, prices, cost of cultivation, adaptation to local climate and soil, experience in cultivation, etc. to record the participants’ preference to the technologies.

3.7. **Application of FYM and chemical fertilizers in mulberry**

To understand the soil fertility status, information on the various inputs applied to mulberry gardens was collected by personal interview using the pre-tested schedule and visit to the farm. The data were recorded on the quantity and schedule of use of FYM and chemical fertilizer.
3.8. Soil fertility status

In order to fulfill the main objective of the study i.e., monitoring the soil fertility status, the initial fertility level of all the 45 selected farmers’ mulberry gardens as well as the mulberry plot at RSRS Farm was assessed by studying the physical, chemical and biological properties.

3.8.1. Soil sampling: Soil sampling was carried out by following a standard method (Jackson, 1973). For this, samples were collected by digging pits between rows of mulberry at three different depths i.e., from 0 to 15 cm (surface), 15 to 30 cm (middle) and 30 to 60 cm (lower) and scraping the walls from all sides at each depth (Plate 4). The process was repeated at 4-5 locations for each garden and the samples were thoroughly mixed on a newspaper. After dividing the sample into four equal parts, the two opposite parts were rejected and the other two parts were mixed again. By repeating the process, 250 g of soil samples were finally retained for each depth. The top soils (0 to 22 cm) were considered for analyzing the physical properties and 15 to 30 cm depth soil was assessed for soil microflora. The soils were analysed for pH and EC for all the three depths. They were also analysed for OC, P, and K and micronutrients like Bo, Cu, Fe, Mn and Zn by taking composite sample from 0 to 30 cm depth.

3.8.2. Physical properties of the soil

In order to study the dynamics of the nutrients in the soil, knowledge of the physical properties of soil is important. Under the present study, the soil bulk density (g/cc), total porosity (%), maximum water holding capacity (%), and volume expansion (%) were recorded for the INM and control plots by ‘Keens cup’ method as described by Gupta (1999).
3.8.3. Chemical properties of the soil

i) **Determination of soil pH:** Soil pH is an indication of the soil reaction (hydrogen ion concentration) in terms of acidity, neutral, or alkalinity. The pH of the soils was tested following standard method (Jackson, 1973). For this, 10 g oven dried soil from each sample was taken in 250 ml conical flask and 25 ml distilled water was added to make a soil solution of 1:2.5 ratio. Solutions in the flasks were then shaken for 30 minutes continuously on a rotary shaking machine for homogenization. The pH of the soil solution was then measured using an Elico® digital pH meter calibrated with standard buffer solution.

ii) **Determination of electrical conductivity:** The electrical conductivity is an indicative of the salt concentration of the soil solution and is an important parameter influencing the absorbability or toxicity levels of salts. The EC value of more than 1.0 m mohs /cm is inhibitory to the plant growth. For determining EC, the solutions as prepared for pH (1:2.5) were filtered through Whatman No.1 filter paper. The suspension was then analysed for EC using a pre-calibrated Elico® digital electrical conductivity meter (Jackson, 1973).

iii) **Estimation of organic carbon:** The organic carbon content of the soil samples was estimated by the modified Walkley and Black (1934) rapid titration method. In this method, the organic matter in the soil gets oxidized by potassium dichromate and concentrated sulphuric acid utilizing the heat of diluted H₂SO₄. The excess potassium dichromate not reduced by the organic matter of the soil is then determined by back titration with standard ferrous sulphate solution. For this, 0.5 g of soil from each sample was taken in a dry 250 ml conical flask and 10 ml of 1 N potassium dichromate (K₂Cr₂O₇) was added followed by 20 ml of concentrated H₂SO₄ rapidly. The flasks were then kept without disturbance for 30 min and 200 ml distilled
water was added in each flask followed by addition of 10 ml of orthophosphoric acid and 1 ml of diphenyl amine indicator. The contents were titrated with 1 N ferrous sulphate solution till the colour changes from blue-violet to green. Simultaneously, a blank was also run without soil and estimated the OC.

iv) **Estimation of available phosphorus:** Available phosphorus was estimated by the method of Olsen *et al.* (1954). In this method, the available phosphate reacts with ammonium molybdate in an acid medium to form molybdo-phosphoric acid. The molybdo-phosphoric acid then turns to a blue coloured complex through reduction reaction with stannous chloride (SnCl₂). Optical density (O D) was then read at 660 nm wavelength using an Elico® spectrophotometer. A standard curve was also prepared using standard phosphate solution. For estimation, 1 g soil was taken from each sample into a 250 ml conical flask. To each flask, 25 ml sodium bicarbonate (NaHCO₃ of pH 8.5) and a pinch of carbon black were added and kept shaking for 30 min and filtered through Whatman No.1 filter paper. Similarly a blank was prepared without soil. Five ml of clean filtrate was taken from each flask to a 25 ml volumetric flask and 5 ml ammonium molybdate solution was added to each followed by the addition of 10 ml distilled water and one ml stannous chloride solution. Finally, the solution was made up to 25 ml by adding distilled water and was kept undisturbed for 30 min for colour development and was read at 660 nm wavelength in an Elico® spectrophotometer. Phosphorus availability was determined (kg/ha) by interpolating the readings into a standard curve.

v) **Estimation of available potassium:** Potassium was extracted from the soil with the help of saturated ammonium acetate solution and the potash content was estimated using Elico® flame photometer as described by Tandon (1993). For this, 1 g of dry soil was taken from each sample in a 250 ml conical flask and 20 ml of neutral
ammonium acetate solution was added and kept for shaking up to 30 min on a rotary shaker and filtered through Whatman No.2 filter paper immediately. Potassium concentration in the extract was then determined with the help of a flame photometer. Potassium in the soil was determined (kg/ha) by interpolating the readings into a standard curve.

vi) **Estimation of available soil micronutrients:** The method advocated by Lindsay and Norvell (1978) was followed for determining the available soil zinc, iron, manganese and copper. The method consists of using DTPA (diethylene triamine penta acetic acid) extractant for simultaneous extraction of the micronutrients. The content of these were determined in a GBC® atomic absorption spectrophotometer (AAS). For this, 10 g of soil from each sample was taken in a 150 ml conical flask and 20 ml of DTPA extractant was poured and the contents were shaken on a horizontal shaker for 2 h. The solutions were filtered through Whatman No. 40 filter paper. The clear extract of the samples was then fed to the instrument having an appropriate hallow cathode lamp and the readings were noted and the available micronutrient were calculated.

For determination of available boron, hot water extraction method developed by Berger and Truog (1939) was followed. Ten g powdered soil with 20 ml distilled water was refluxed in a 100 ml conical flask for 5 min. After cooling, 2 to 3 drops of calcium chloride solution was added. The solution was later filtered in 50 ml plastic beakers using Whatman filter paper No. 42. One ml of filtered solution was then taken in porcelain basins and 4 ml of curcumin oxalic acid reagent was added to each. The solutions were evaporated on a water bath at 55 ±3°C. Further, the residue was baked for 10-15 min to ensure complete drying. After cooling the porcelain basins, 25 ml of ethyl alcohol was added to each. The content was shaken with a glass rod and
filtered. The colour was read in an Elico® Spectrophotometer using green filter at 540 
m\text{\textmu}m \text{ } wavelength. Simultaneously, standard curve was developed with 0.25, 0.5, 1.00, 
and 1.5 ppm concentrations of standard boron solution by plotting boron 
concentration on X axis and spectrophotometer reading on Y axis to calculate the 
boron content of the soil.

3.9. Irrigation water analysis

The main source of irrigation water in the study area was bore wells drilled up 
to a depth of 300 to 400 m. The underground water contained high concentrations of 
dissolved salts that perhaps influenced the various properties of the soil. Thus, in 
order to find out the quality of irrigation water and its influence on soil properties, the 
samples were chemically analysed for the presence of various salts in combination of 
carbonates, bicarbonates, chlorides and sulphates of sodium, potassium, calcium, and 
magnesium. The pH, EC, sodium adsorption ratio (SAR), and residual sodium 
carbonate (RSC) of water samples were also determined to assess the hazardous 
nature of the irrigation water as per the methods described by Gupta (1999).

i) Sampling: The irrigation water samples were collected from the bore wells 
during summer before the onset of monsoon. Initially, water from bore wells was 
pumped out for 30 min and then about one litre of water was collected in a clean 
bottle, labeled and transported to the laboratory for analysis within 24 h.

ii) Determination of pH: Twenty five ml of water sample was taken in 50 ml 
glass beakers and pH was read by using an Elico® digital pH meter. Before 
measurement of pH, the instrument was calibrated with standard buffers at pH 4.0, 
7.0, and 9.2.
iii) **Determination of EC:** Twentyfive ml of water sample was taken in 50 ml glass beakers and EC was determined by using an Elico® electrical conductivity bridge after calibrating the instrument with standard buffers.

iv) **Determination of sodium:** The sodium concentration in water samples was determined in meq/litre by flamephotometric method (Jackson, 1973). The standard curves were prepared with the known concentrations of sodium solution and the readings were plotted on X axis. After atomizing the water samples, the readings were plotted on Y axis. From the standard curve, the concentration of sodium in meq / litre was determined.

v) **Determination of calcium and magnesium:** Twentyfive ml of water sample was taken in a conical flask. Five ml of NH₃ buffer solution was added to adjust pH to 10 followed by 3-5 drops of erichrome black indicator (EDT). The solution was titrated against the Versants solution of 0.2 N till the wine red colour changed to blue and the readings were recorded for calculating the available calcium and magnesium.

vi) **Determination of carbonates and bicarbonates:** Twentyfive ml of water was taken in a porcelain container and 1-2 drops of phenolphthalein indicator was added. The solution was titrated against standard sulphuric acid of 0.05 N till the pink colour disappeared and the reading was recorded. Then, 1-2 drops of methyl orange indicator was added to the solution and titration was continued till the yellow colour changed to orange when the readings were recorded and carbonate and bicarbonate contents estimated.

vii) **Determination of chlorides:** The chlorides of the water sample were determined by titrating the sample against standard silver nitrate solution of 0.05 N till the brick red precipitate appeared (potassium chromite was used as an indicator).
viii) **Sodium Adsorption Ratio (SAR):** The SAR is an index of the sodicity of water. The sodium ions in the irrigation water react with soil clay and increase the exchangeable sodium percentage of soil, converting it into alkaline if the proportion of sodium to calcium and magnesium is high.

ix) **Residual Sodium Carbonate (RSC):** The bicarbonate effect of irrigation water is expressed in terms of RSC. The bicarbonate and carbonate ions present in the irrigation water may precipitate as calcium and magnesium carbonates as the soil solution get concentrated and increase the relative proportion of sodium to other cations. RSC was calculated from the analysed data for carbonates and bicarbonates, calcium, and magnesium.

3.10. **Estimation of soil microflora**

The bacteria, fungi, and actinomycetes were estimated by determining the number of colony forming unit/s (CFU/s or cfu/s) per gram of soil on yearly basis in order to monitor the build-up of microbial population in the rhizosphere of both INM treated and control fields. For conducting the experiment, the following steps were taken. Before collection of the soil samples, the tools like shovel, secateur, kolugudali, etc., were sterilized with 5 per cent formaline solution and oven dried at 50°C to avoid contamination. The glasswares were sterilized in a hot air oven at 250 °C for one h.

i) **Collection of soil samples in rhizosphere habitat:** By randomly selecting 25 plants from each farmer’s field, the soil samples from close proximity of root zones at 15-30 cm depth were collected in sterilized polythene bags. These plants were labeled by tagging for collection of the soil samples for 2nd and subsequent years. For preparation of composite samples, all the 25 soil samples collected from each farmer’s garden were separately mixed together.
ii) Isolation of microbes from rhizosphere habitat: The microflora present in the soil was isolated by dilution plate technique (Waksman, 1922). The composite soil sample of each replication was air dried, powdered and sieved through a 2 mm mesh and the fine soil stored in sterilized polythene bags. Ten g of the soil was suspended in 90 ml of sterilized distilled water in 250 ml Erlenmeyer flasks and shaken thoroughly to have a 1:10 dilution. The flasks were kept undisturbed for 20-30 min for the soil particles to settle. One milli litre of 1:10 solution was then transferred to a sterilized test tube containing 9 ml sterilized distilled water. The process was repeated several times to obtain the final dilution of 1:10,000.

iii) Preparation of microbial culture media and pouring of media in plates: The different culture media, viz., potato dextrose agar, nutrient agar, and actinomycetes agar were prepared for the isolation of fungi, bacteria, and actinomycetes, respectively. The required amounts of ingredients for the respective medium were weighed in an electronic balance and taken in sterilized conical flasks. The ingredients were mixed thoroughly with required quantity of distilled water. The mouth of each flask containing ingredients was covered with a sterilized cotton plug and a layer of aluminium foil to avoid the contamination. The flasks containing different media were then sterilized at 20 lb/sq inch (at 120°C approx.) using a vertical autoclave and cooled down at room temperature.

Five milli litre of final dilution of each rhizosphere sample was mixed with 100 ml of respective lukewarm medium and poured into five petri plates at the rate of 20 ml/plate separately. The inoculated petri plates were then inverted and incubated at 28 ±2°C for the growth of fungi and actinomycetes for seven days in a BOD incubator, while for bacteria the plates were incubated at 30°C for 2-3 days.
iv) **Observation of microbial population:** After incubation, the growth of fungi, bacteria, and actinomycetes was recorded and the population in terms of CFUs/g soil was calculated.

v) **Macroscopic examination of microflora:** First, all the colonies developed on the agar surface in different petri plates were observed macroscopically. The solid puffy growth on medium indicated the presence of fungi while watery growth showed the presence of bacteria. However, the actinomycetes appeared on medium like a shiny colony in the form of pustule (pearl-like) in different colours (white, gray, milky, *etc.*).

vi) **Microscopic examination of microflora:** Bacteria were first stained with gram staining method as described by Buchanan and Gibbs (1975). For this, smears of bacterial cells from 3 to 4 day-old colonies were prepared on microscopic slides by spreading the bacterial cells using a drop of sterilized water and air-dried. The bacterial cells were then flooded with ammonium oxalate crystal violet solution (0.8 g of ammonium oxalate dissolved in 80 ml of distilled water and mixed with 2 g of crystal violet having 90 per cent dye content dissolved in 20 ml of 95 per cent ethyl alcohol) for a minute. The slides were washed in running tap water for 2 sec and immersed in iodine solution (1 g iodine + 2 g potassium iodide dissolved in 300 ml distilled water) for a minute and again washed in running tap water till the excess stain was removed. The slides were then flooded with 95 per cent ethanol for 30 sec to decolourize and counter stained with safranin solution for 30 sec. Finally, the slides were washed in running tap water and blot dried to examine microscopically. All the slides were examined under a high-resolution phase contrast Leica® binocular microscope to identify the bacteria on the basis of gram reaction as gram -ve and gram +ve. The bacteria, which retained the colour of crystal violet as purple, were
designated as gram +ve, while those, that didn’t retain the colour of crystal violet but retained the colour of safranin (red/pink) were designated as gram -ve.

The fungi were identified on the basis of their morphological characters, which consisted of vegetative and reproductive structures including the shape, size, colour, and arrangements/attachment of spores on the sporophores or the fruiting bodies. These structures were examined under the microscope directly after preparation of mounted slides. For this, a drop of cotton blue-lacto phenol stain was taken on a clean glass slide and a small piece of fungal hypha along with the fruiting bodies taken with the help of a pointed needle from the fungal colonies developed on agar medium in different plates. Later, the fungal hyphae were spread as a thin layer on the lactophenol and covered with a cover slip for observing under microscope. All the morphological characters were pooled and compared with those of all recognized groups of fungi which have already been published in monographs/books/journals (Booth, 1971; Domosch et al., 1980; Ellis, 1971; Ellis and Ellis, 1985; Gillman, 1956; Nelson et al., 1983; Rotem, 1994).

The developed actinomycetes in the plates were identified on the basis of colony characters like growth pattern on agar media as suggested by Shirling and Gottlieb (1966). Later, the sporophore characters were noted by direct microscopic examination after the preparation of slide. Based on the morphology of the sporophores (rectus, flexibles, retinaculum apertum, spira, etc.), as detailed by Pridham et al. (1958), the actinomycetes genera were confirmed. The identified microbes (fungi, bacteria, and actinomycetes) from the present study were also compared with Mulberry Pathology Culture Collection Bank at CSRTI, Mysore, for confirmation of their identity.
vii) **Categorization and frequency of isolated microflora:** After isolation and identification of microflora from rhizosphere habitats, they were categorized based on their behaviour into three groups *viz.*, beneficial, harmful, and saprophytic. The increase (+) or decrease (−) of the beneficial/harmful/saprophytic microflora in treated soil over the control (farmers’ practices/CSRTI recommendations at RSRS Farm) was calculated.

The percentages of abundance and frequency of various forms of microflora were calculated by applying the appropriate formulae. Based on the frequency of occurrence (%), the individual forms of microflora were also expressed as most dominant, frequent, occasional, and rare (Das *et al.*, 1998).

**3.11. Experimental details:** There were two treatments, one comprising INM concept and the other absolute control consisting of farmer’s conventional practice. Under the INM, importance was given to prepare FYM at farmers’ level using scientific methods of aerobic as well as anaerobic composting techniques. Attempts were also made to recycle the farm residue through vermicomposting technology. Further, efforts were directed to build-up soil organic matter following *in situ* incorporation of green/green leaf manures. The incorporation of various biofertilizers along with FYM/compost was also made as an integrated practice. The methods followed for green manuring, biofertilizer application, and composting and vermicomposting are as follows:

3.11.1. **Green manuring:** The study was conducted using three green manure legume crops, *viz.*, sunhemp (*Crotalaria juncea*), daincha (*Sesbania aculeata*), and cowpea (*Vigna sinensis*) to find out the suitable one under the local agro-climatic conditions and agronomical practices employed in the region. The trials at RSRS Farm were conducted in 3 blocks of 5 replications each in 0.40 ha in one-year old mulberry
(RFS-175) garden having a spacing of (150+90) × 60 cm (paired row system) (Plate 5 a) and at farmers’ gardens with a plot size of 2 cents each in five replications. Two sowing periods (5 and 10 days after pruning of mulberry) (Plate 5 b and c) and seed quantities at 20, 25, 30, 37.5, and 50 kg /ha/crop were tested (Plate 6, 7, and 8). After pruning (before sowing), the land was ploughed thoroughly. The seeds were soaked for 4 to 5 h in water and surface dried in shade. Approximately one kg of arrow root/ragi floor/gum paste was prepared for bacterization of 10 kg green manure seeds. Before sowing, the legume seeds were coated with a culture of the crop and region specific Rhizobium (at the rate of 20 g / kg of green manure seeds) isolated from the rhizosphere of local mulberry gardens and cultured at GKVK, University of Agricultural Sciences, Bangalore. The inoculant was mixed and stirred with the paste and seeds were mixed in it for bacterial coating. The seeds were then evenly spread on a plastic sheet under the shade for drying and pelleting (Plate 9 a). The inoculated seeds were broadcast immediately between mulberry rows and were covered by a layer of soil with the help of a garden rake or by light surface ploughing (Plate 9 b). When the green manure plants attained the stage of flowering (about 50%), when the plants developed nodulated roots, they were incorporated in soil up to a depth of 15 cm using a tractor plough/power tiller/bullock plough (Plate 10, 11, and 12).

The data were recorded at 50% initiation of flowering stage or 45th day after sowing. The number of green manure plants/sq m at 5 randomly selected spots for each replication was counted in order to record the total number of plants per hectare/crop. The survival percentage was recorded for each green manure crop. The fresh weight and dry weight (by oven drying till the constant weight was obtained) of green manure plants (10 plants per replication) from the same sample was recorded in order to calculate the moisture percentage of green manure crops. Similarly, the weed
flora monocots and dicots by number and biomass (monocots and dicots) were also recorded. Weed suppression percentage was calculated by comparing the number and biomass of weeds in the treatment and control plots. In each replication, 10 plants were selected at random and the average plant height from ground level to the tip of the terminal shoot and also the length of the primary root were measured using a meter scale and recorded in cm (Plate 13). The mean values for number of nodules/plant, were counted in 10 plants at random for each replication and classified as small (up to 1 mm dia), medium (1-2 mm dia), and big nodules (>2 mm dia). The fresh and dry weight of nodules (after oven drying) was also recorded. The nutrient contents of the nodules/green manure crops/weeds were analyzed as described in subsequent paragraphs in the chapter. The nitrogen, phosphorus, potash, and crude protein of the green manure crops incorporated in soil were estimated based on the yield of above the ground biomass and percentage of nutrient content of green manure crop. Glyricidia (Glyricidia maculata) and karanj (Pongamia pinnata) were used as green leaf manures. The foliage of these crops was incorporated by making the trenches between rows of mulberry, 1’ away from mulberry row and at 1’ breadth and 1’ depth.

3.11.2. Use of Azotobacter / Azospirillum biofertilizer: Research conducted at CSRTI, Mysore and Karnataka State Sericultural Research and Development Institute (KSSRDI), Bangalore with Azotobacter / Azospirillum under field conditions revealed that mulberry responded well under low fertilizer nitrogen level. To find out the effect of these free-living nitrogen-fixing bacteria under Eastern dry region (Kolar District.), studies were undertaken using Azotobacter chroococum and Azospirillum sp. developed at CSRTI, Mysore, at the rate of 20 kg/ha/yr. The experiment was initiated with five treatments and three replications in one holding in each village.
Treatment-1: *Azotobacter* + 75% chemical nitrogen

Treatment-2: *Azotobacter* + 50% chemical nitrogen

Treatment-3: *Azospirillum* + 75% chemical nitrogen

Treatment-4: *Azospirillum* + 50% chemical nitrogen

Treatment-5 (Control): Full dose of chemical nitrogen (350 kg/ha/yr)

The inoculation was done by mixing *Azotobacter/Azospirillum* biofertilizer with 200 kg of FYM and applied 2-3 days after pruning. Biofertilizer was applied between mulberry rows near rhizosphere by making small furrows and then covered with soil. Three crops’ leaf analysis was undertaken to find out the suitability of the biofertilizer for the local soil conditions. *Azotobacter* population after inoculation was assessed in both the INM and control plots employing serial dilution plate technique as described with Waksman No. 77 medium.

3.11.3. Use of phosphate solubilizing bacteria (*Bacillus megaterium* var. *phosphaticum*): The phosphate solubilising bacterial biofertilizer (Seriphos developed at KSSRDI, Bangalore) was used to supplement the requirement of chemical phosphatic fertilizer. The application was made at the rate of 25 kg/ha in 5 equal split doses by mixing with 200 kg of FYM and incorporated into the soil near the root zone. The biofertilizer was used along with the *Azotobacter* biofertilizer (Plate 14).

3.11.4. VAM inoculation:

i) **Stock culture preparation:** Nursery beds of 3 x 1 m size were prepared by proper digging and weeding and 15 kg of sand was mixed for better aeration. The prepared beds were surface sterilized by burning dry weeds on the surface to eliminate native VAM flora and pathogens. After cooling the beds, ash from the burnt-out materials was mixed with the beds thoroughly by digging. Finally, 15 cm
deep furrows were made from one end to the other in each bed at a distance of 20 cm from each other. Soil-based VAM inocula as starter culture (mixed culture of *Glomus mosseae* and *G. fasciculatum*) obtained from CSRTI, Mysore was applied in each furrow at the rate of 7.0 kg per bed containing 15 to 20 spores/g dry soil. About 150-200 good quality maize seeds per nursery bed were sown on the VAM culture applied to beds in the furrows and the furrows were closed. In the beginning, water was sprinkled on the nursery bed once every 3 to 4 days for proper germination of the seeds. Then, regular flow irrigation was followed once in 7 days (Plate 15). The maize plants were allowed to grow for 90 days and the maize roots and soil were tested for VAM colonization and VAM spore load, respectively (Plate 16). Once the required spore load (10 to 15 spores/g dry soil) and root colonization (60 to 70%) were ensured, the maize plants were cut 15 cm above the ground level. The roots of these plants were cut in to small bits and mixed with the soil from at a depth of 15 cm (Plate 17). This was used as soil-based VAM inoculum for further treatment. No fertilizer was used during the culture preparation.

ii) **Inoculation of established mulberry garden:** The established mulberry garden/s in RSRS Farm and farmers’ holdings were inoculated with soil-based mixed culture of VAM at the rate of 1000 kg/ha by maize intercropping method developed by Katiyar *et al.* (2000). For this, 10 cm deep furrows were first opened up adjacent to the plant roots from one end to another between the mulberry rows by country plough. Soil-based VAM inoculum having 10 to 15 spores/g dry soil was introduced as a thin layer by placing in furrows. Maize seeds at a distance of 60 cm from each other were sown on the thin layer of inoculum. The furrows were later covered with soil and regular irrigation was followed. The maize seeds were allowed to germinate and the plants were grown for 3 months (Plate 18). During this period, the colonization of
VAM in maize roots was ascertained from time to time. Similarly, the roots of mulberry plants were also examined for VAM colonization. Once the required percentage of root colonization in mulberry as well as maize plants were ascertained, the plants were cut above the ground level leaving the roots of maize behind in the field (Plate 19). Finally, the whole plot was ploughed to mix the maize roots in the soil for further cross inoculation of mulberry roots by the active VAM propagules.

**iii) Assessment of VAM spore load:** The number of VAM spores in the rhizosphere of mulberry soil was assessed following wet sieving and decanting technique (Gerdmann and Nicolson, 1963). For this, a known quantity of soil (1 kg) was first collected and mixed in a substantial volume of water in a bucket and mixed thoroughly by hand till all the lighter organic fractions, including spores and roots, float on the water surface. The heavy soil particles and other fractions were sedimented at the bottom. The floating lighter materials were decanted through a series of sieves of decreasing pore diameter. The soil was repeatedly washed and decanted to remove all the lighter fractions. Finally, each fraction from the different sieves was resuspended in water and collected on filter paper for microscopic observations of VAM spores and their counts. This gave an account on the total number of VAM spores present in a kg of soil. The number of spores per gram of soil was calculated from the total number of spores.

**iv) Assessment of VAM root colonization:** VAM root colonization in the rhizosphere of mulberry was assessed following the technique described by Phillips and Hayman (1970). For this, fine roots were collected from mulberry and washed with running tap water to remove dirt and debris. The roots were cut into 1 cm bits and treated with 40 ml of 2.5 per cent KOH solution in a test tube and autoclaved at 120°C for 3 min. KOH was removed by washing several times with running water and
treated with alkaline H₂O₂ (3 ml of 20 per cent NH₄OH in 30 ml of 3 per cent H₂O₂) for 40 to 45 min. This treatment removed the pigments, rendering the root bits to look white for better observation of VAM. After KOH or H₂O₂ treatment, the roots were treated with 1 per cent hydrochloric acid equal to root mass and kept over night. Roots were then stained in acidic glycerol solution (500 ml glycerol + 450 ml H₂O + 50 ml 1 per cent HCl) containing 0.05 per cent tryphan blue. Tryphan blue solution was then poured out from the test tube and the roots were finally destained in acidic glycerol at room temperature. The stained root bits were then examined under a Leica® binocular research microscope under 10 × 40 magnifications for the presence of mycelia, vesicles, and arbuscles. Percentage of colonization by VAM was then calculated.

3.11.5. **FYM and compost preparation**: FYM preparation in the three village clusters was unscientific where cattle dung, wastes, etc. were dumped in a corner of their land without proper care. Mostly, half of such wastes only were converted into some sort of manure. To overcome this, various residues including silkworm litter, left-over leaves, weeds, etc. were dumped in convenient sized pits located in slightly elevated places to avoid water stagnation and protected by thatched shed or compost pits for anaerobic composting. The composting materials were inoculated with *Trichoderma viride* fungus for enhancement of decomposition. Later, the pits were treated with cow dung slurry as starter culture and regularly sprinkled with water to maintain the optimum moisture for microbial activity. The materials were turned two to three times to hasten the process of decomposition. At the end of decomposition, the composted samples were collected, nutrient content tested, and microbial population was assessed to know the quality of compost (Plate 20).

Vermicomposting was taken up with selected farmers as well as at RSRS Farm by releasing the earthworms (*Eudrilus eugeniae, Eisenia fetida*, and *Perionyx*...
excavatus) following chamber method (Plate 21). The generated compost samples were tested for their nutrient status including microflora before harvest. The compost/FYM/vermicompost produced were regularly applied to the adopted mulberry gardens.


3.12.1 Estimation of mulberry production: Mulberry yield parameters were recorded at RSRS Farm by measuring the longest shoot length, total shoot length (cm) in a plant as well as by counting the number of shoots/plant and leaves/plant. The weight of leaves/plant and weight of stem/plant were recorded, finally the leaf yield/ha was estimated.

3.12.2. Assessment of mulberry leaf quality: The leaf quality was assessed by chemical analysis and also by bioassay (silkworm rearing) conducted following the standard procedures as described below. The leaves were collected at 60 - 65 days after pruning in all the selected mulberry gardens as well as at RSRS Farm. The 4th to 10th leaves of the plants from the apex were selected for sampling and from each mulberry garden 75 to 100 leaves were collected as a representative composite sample. After the sample leaf collection, primary data were recorded in a format as given below:

- Sample number:
- Name of the farmer:
- Acreage of the land:
- Spacing followed:
- Number of plants in the field:
- Number of leaf harvests per year:
➢ Age of the garden:

➢ Total leaf production (kg/ha/yr):

3.12.3. Leaf moisture content: Fresh weight of the leaves was recorded on 60th day after pruning and the leaf yield (excluding shoot)/net plot was calculated. The leaves were washed and kept in triplicate samples under shade for air drying for 2 to 3 days followed by oven drying for 24 - 48 h at 55 - 60°C. The dry weight of the samples was recorded to calculate the leaf moisture percentage as per the following formula:

\[
\text{Leaf moisture \%} = \frac{(\text{Fresh weight} - \text{Dry weight}) \times 100}{\text{Fresh weight}}
\]

The collected leaves were washed with 0.2 per cent detergent and then cleaned in tap water followed by distilled water. After proper drying of leaf samples in hot air oven, the dry samples were ground after removing the petioles and sieved to get uniform powder. These leaf samples were preserved in desiccators to avoid absorption of atmospheric moisture and processed for analysis of different biochemical constituents.

3.12.4. Estimation of nitrogen: The total process of nitrogen estimation was done through three distinct steps, i.e., digestion of the leaf samples, distillation, and titration as per the Kjeldhal method suggested by Tandon (1993).

i) Digestion of the leaf samples: A quantity of 0.1 g of finely powdered mulberry leaf sample was taken in a digestion tube followed by adding 10 ml of concentrated H₂SO₄. A pinch of catalyst (K₂SO₄ and CuSO₄ in 5:1 ratio) was added to a few ml of 30 per cent H₂O₂ to speed up the digestion. All the digestion tubes were kept in a digestion unit and heated up to 300°C for 1.5 h, when the solution became colourless.

ii) Distillation: All the digestion tubes were kept sequentially in Tecator kjeltec system distillation unit, which automatically added 10 ml of 40 per cent NaOH, and
the contents were distilled with alkali. Twentyfive ml of 4 per cent boric acid solutions was taken separately in conical flasks followed by adding two drops of mixed indicator (bromocrysal green 5 g + methyl red 0.1 g in 100 ml absolute alcohol) which became purple red in colour. Then the solutions were kept in the distilling system for allowing the distillation vapour (ammonia) to get trapped in the boric acid solution. After completion of distillation, the solution became bluish green in colour.

**iii) Titration:** The conical flasks were taken out from the Kjeltec system and the contents were titrated with 0.1 N H₂SO₄ until the colour became purple red. A blank was also made with the distilled water, which was also titrated to calculate the total nitrogen percentage.

\[
\text{Total N (\%)} = \frac{14 \times (\text{sample titration value-blank value}) \times \text{normality of the acid} \times 100}{\text{Sample weight (g)} \times 1000}
\]

**3.12.5. Di-acid digestion of samples for estimation of potassium and phosphorus:**

As per the method of Tandon (1993), 0.5 g of leaf sample dry powder was taken in a 50 ml conical flask and 10 ml of di-acid (mixture of HNO₃ and HClO₄ in 9:4 ratio) was added to the sample. It was kept for digestion on a hot plate at 120 - 150°C for about 45 min when the colour disappeared. After completion of digestion, all the samples were cooled and diluted to 25 ml with double distilled water and filtered through Whatman No. 1 filter paper. The potassium percentage in leaf samples was estimated using a flame photometer (Elco make) as described below:

**i) Standard stock solution:** 1.9069 g of analytical grade KCl was dissolved in deionized water and the volume made up to one litre to get 1000 ppm potash. By following the serial dilution the final standard solutions of 0, 5 and 10 ppm were prepared from 100 ppm potash.
ii) **Standard curve**: For the estimation of potassium, a standard curve was prepared by setting the instrument at a concentration of 10 ppm using a standard filter and suitable conversion factor was calculated for finding out potassium in leaf samples.

3.12.6. **Estimation of phosphorous**: The elemental content of phosphorous in the leaf samples was determined by colorimetric method using a spectrophotometer (Systronics). The optical density (DD) (absorbance) readings were taken at 420 nm wavelength of visible spectrum.

i) **Reagents used**:

- Ammonium molybdate - ammonium venadate in HNO₃: A quantity of 22.5 g (NH₄)6Mg7O₂₄.4H₂O was dissolved in 400 ml of distilled water. In addition, 1.25 g of ammonium venadate was also dissolved separately in 300 ml boiling distilled water. The two solutions were allowed to cool at room temperature. After cooling, 250 ml of concentrated HNO₃ was added and diluted to 1 litre.

- Phosphate standard solution: A quantity of 0.2195 g analytical grade KH₂PO₄ was dissolved and diluted to 1 litre. This solution contained 50 mg P/ml (500 ppm).

ii) **Standard curve**: Zero, 1, 2, 3, 4 and 5 ml of phosphate standard solutions were transferred to 50 ml volumetric flask to get 0, 1, 2, 3, 4 and 5 ppm phosphorus, respectively. Ten ml of vanadomolybdate reagent was added to each flask and the volume made up to 25 ml with de-ionized water by proper shaking. The solutions were kept for 30 min for the development of colour. The readings were taken at 420 nm in spectrophotometer (Systronics) using blue filter. A standard graph was prepared by plotting the absorbance values against each phosphorus standard concentration. Phosphorus content in leaf samples was determined following the procedure indicated
above and the readings were taken at 420 nm against the blank reagent. The O D values were plotted on the standard curve and the percentage of phosphorus was recorded.

3.12.7. Estimation of chlorophyll content: The total chlorophyll content of the mulberry leaves was extracted and estimated following the method of Ronen and Galun (1984). One hundred mg of the fresh leaf sample was cut into small pieces and was taken into a clean test tube containing 50 ml of dimethyl sulfoxide (DMSO). The test tubes were covered with aluminium foils and were kept in hot water bath for one hour at 60°C for extraction of chlorophyll pigments. The sample tubes were then taken out and cooled to room temperature. The supernatant green chlorophyll solution was taken into a quartz cuvette and the optical density was measured in a spectrophotometre (Hitachi) at 645 and 663 nm wavelengths of light spectrum against the DMSO blank reagent. Total chlorophyll content was expressed as mg/ g fresh weight.

3.12.8. Estimation of total sugars: Total sugar content of the mulberry leaf samples was analysed and estimated as per the method of Dubois et al. (1956). One hundred mg of oven-dried mulberry leaf powder was homogenized with 10 ml of hot 80 per cent ethyl alcohol using a mortar and pestle. The homogenate was centrifuged at 5000 rpm for 10 min in a Remi® centrifuge. The supernatant sugar extract was taken into a separate test tube and the volume was made up to 50 ml with 80 per cent ethanol. From this, 1 ml of aliquot was taken into a test tube and 1 ml of 5 per cent phenol was added with vigorous shaking. Then, 5 ml of concentrated sulphuric acid was added with the stream of acid being directed against the sample surfaces. The contents were shaken and cooled in water to room temperature. The optical density of the sample possessing golden yellow colour was read at 490 nm using a Spectrophotometer.
against the reagent blank. A standard graph was prepared simultaneously with different concentrations (10, 20, 30, 40, 50 to 100 mg/ml) of D-glucose. The total soluble sugars were estimated and expressed as per cent on dry weight basis.

3.13. Silkworm cocoon yield and economics of INM package: The impact of green manuring, inoculation of biofertilizers, and compost/vermicompost/enriched FYM with reduced doses of fertilizers on the mulberry leaf quality (nutritional status of leaf) was assessed by silkworm rearing (bioassay) following the improved rearing techniques (Dandin et al., 2003). Three silkworm rearings were conducted at RSRS using CSR2 × CSR4 bivoltine hybrid. From the total number of larvae brushed, 300 larvae were retained after fourth moult and reared in each tray in three replications for INM treated and control plot till spinning. The rearing from the day of brushing through spinning was conducted using the leaves harvested from INM and control plots separately. The rearing parameters observed were weight of 10 matured larvae (5th day in V instar) and effective rate of rearing (ERR) by weight and by number (number of cocoons harvested for 300 larvae maintained and converted to number on the basis of 10,000 larvae brushed). For cocoon weight (CW), ten cocoons from each replication were randomly picked and weighed in a sarotorius balance and the average weight recorded in g. The cocoons then were cut open and the pupae were separated and the average shell weight (SW) was recorded in g. Then shell percentage was determined using the following formula:

\[
\text{Shell percentage} = \frac{\text{Average shell weight}}{\text{Average cocoon weight}} \times 100
\]

At farmers’ level, cocoon yield per 100 disease free layings (two boxes of eggs), rate per kg of cocoon and total income from cocoon sales were recorded from the farmers’ pass books and bidding slips (receipts issued at the Govt. Cocoon Market). The total income from cocoon production before and after imposition of
INM package was also worked out. The economics of the INM package was calculated by including the cost of the organic, inorganic, and biofertilizers and compare with that of the recommended dose of fertilizers cost by CSRTI, Mysore.

The data recorded were statistically analyzed by ANOVA. Correlation matrix was determined for each set of selected parameters to obtain the degree of correlation. Paired T-test value for finding out the level of significance between INM treated and control farmer holdings was also calculated for various soil fertility parameters, mulberry leaf yield and quality, cocoon yield and income from cocoon sales both before and after the study period as suggested by Gomez and Gomez (1983).