Methodology

The present dissertation was designed to assess the influences of plantation forest on phyto-diversity of the study area and the main theme behind the selection of methodology is to compare different types of plantation with adjacent natural vegetation that was predominant in entire Terai-Duars belt and considered to be the standard land form. The comparison emphasized on the vegetation structures and phyto-sociological attributes along with a number of other aspects. Accordingly, a wide array of methodologies has been followed to carry out such diverse type of task and they are detailed below with proper citation.

5.1. SELECTION OF PLANTATION AND NATURAL TRACTS OF VEGETATION

The study area includes two Forest Divisions- Darjeeling and Jalpaiguri division and three administrative districts - Darjeeling, Jalpaiguri and Alipurduar. These two forest divisions were visited consulting with the respective DFOs and the forest ranges having plantation of different ages and types as well as the natural vegetations adjacent to that, were selected for the study. Preference was given to those areas having plantations and natural vegetation under same environmental and ecological conditions. Three such sampling sites were spotted in different parts of Terai-Duars Belt.

Site I: North Rajabhatkahwa (NRVK) area in Buxa Tiger Reserve, where along with Natural vegetation a plenty of Teak plantation, Jarul plantation and Jarul-Benteak plantation were found.

Site II: Sursuti Beat in Lataguri Reserve Forests where both the natural vegetation and plantations (Teak plantation, Sal-Chilauni plantation and mixed plantations) are available.

Site III: Noth Sevoke area in Mahananda Wlidlife Sanctuary with good quality natural vegetation along with Teak and Jarul plantation.

5.2. VEGETATION STRUCTURE

Three-tire Nested Quadrate (20m x 20m for trees or canopy; 5m x 5m for shrubs or under-storey and 1m x 1m for ground cover) was adopted for sampling vegetation (Misra, 1968; Shimwell, 1971; Tripathi & Misra, 1971; Phillips, 1959; Malhotra, 1973; Das & Lahiri, 1997 and Kadir, 2001).

5.2.1. Identification of specimens

Identification of the specimens was done in the field as far as possible with their local names while unidentified voucher specimens were collected. But as the study sites were located in and around the reserved area and the Dept. of forest did not allowed to collect the plant material from reserved or other category of protected forest. Jain & Rao (1977) was followed for the preparation of Harbarium.

5.2.2. Phytosociological analysis

For both the different types of plantations and the natural vegetations, the number of trees, shrubs and herbs were recorded to find their Frequency, Density, Abundance, Relative Frequency, Relative Density and Relative Abundance and finally to compute Important Value Index following Misra (1966), Das & Lahiri (1997) and Rai (2006). Random sampling plot survey has been done in consecutive three seasons during Pre-Monsoon, Post-Monsoon and in the winter.

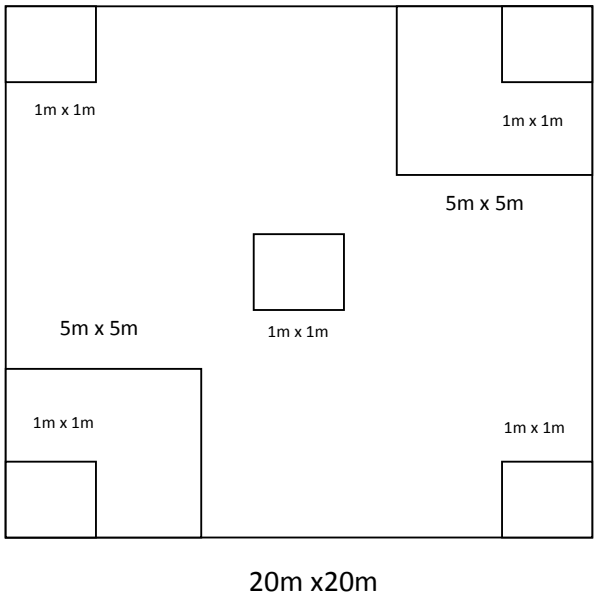

Figure 5.1. Layout of nested quadrate

5.2.2.1. Frequency

Frequency is defined as the degree of dispersion of an individual species in a community and indicates the chance of its occurrence in a particular habitat. Relative frequency (RF) is the percentage of frequency of a particular species to the total frequency of all other species in the same habitat. They are calculated as follows.

$$\text{Frequency (F)} = \frac{\text{Number of quadrates in which the species occurs}}{\text{Number of quadrates studied}}$$

$$\text{Relative Frequency (RF)} = \frac{\text{Frequency value for a species}}{\text{Total of Frequency value for all the species}} \times 100$$

5.2.2.2. Density

Density of a species is its abundance in unit area of a particular habitat and expresses its numerical strength in a community. It is good indicator of rarity or dominance of a species and of standing biomass and productivity of the habitat (Ambashant *et al.* 1995; Rai, 2006).

Percentage of density of a species to that of all the species in same the habitat/ community is termed as Relative density (RD).

$$\text{Density (D)} = \frac{\text{Total number of individuals of a species in all the quadrates}}{\text{Number of quadrates studied}}$$

$$\text{Relative Density (RD)} = \frac{\text{Density value for a species}}{\text{Total of Density value for all the species}} \times 100$$

5.2.2.3. Abundance

Abundance indicates the commonality of a species in a habitat under study. Relative abundance is also the percentage of abundance of a species to that of all the species occurring in that habitat and the formulae for calculating them are as follows.

$$\text{Abundance (A)} = \frac{\text{Total number of individuals of a species}}{\text{Number of quadrate in which the species occurs}}$$

$$\text{Relative abundance (RA)} = \frac{\text{Abundance value for a species}}{\text{Total of abundance value for all the species}} \times 100$$

5.2.2.4. Importance Value Index (IVI)

Important Value Index is valuable statistical measures for the analysis of phyto-sociology and plant community and it provides an overall idea of a species and its importance in the plant community. It is derived by summing up Relative Frequency, Relative Density and Relative Abundance.

Importance Value Index (IVI) = RA + RD+ RF

5.2.3. Biodiversity Indices

As Diversity indices are important surrogates for measuring biodiversity (Sarkar and Margules, 2002) different diversity indices were studied to comprehend the diversity of plant communities, in both the plantation and natural vegetation, in time and space. Different biologists have formulated a numbers of diversity biodiversity indices of which following three were selected for diversity study in case of present work.

5.2.3.1. Species Diversity Index [Shannon-Weiner Index (1963)]

Shannon-Weiner Index (1963) is one of the widely used indices for measuring species diversity, which is the expression of community structure and indicates the complexity of a habitat, of an ecosystem and incorporates both the species richness and evenness components. Higher value of this index i.e. higher diversity is encountered in case of low dominance showing large number of species and large evenness components.

Shannon-Weiner Index (1963)

$$H' = - \Sigma [(ni/N)\ln(ni/N)]$$

Where, 'H" is index value

'ni' number of individuals of a species

'N' total number of species in the habitat type

5.2.3.2. Species Richness [Menhinick's Index (1964)]

Species richness is nothing but another mode of expression of the diversity and is defined as the number of species present in a sample or habitat per unit area and thus based on the total number of species and total number of individuals in a sample or habitat. Menhinick's index (1964) was chosen for the understanding of species richness. This index emphasizes the rare species unlike Shannon-Weiner Index (1963).

Menhinick's Index (1964)

$$D = S/\sqrt{N}$$

Where, 'D' is the index value

'S' total number of species

'N' total number of individuals of all species.

5.2.3.3. Similarity Index [Sorensen's Index (1968)]

Similarity index is much more important in case of present study as it help to compare different habitat type and their stability for migration and evolution. Here Sorensen's Index (1968) of similarity was followed to compare plantations and natural vegetation.

Sorensen's Index (1968)

$$S = 2C/(a+b)$$

Where, 'S' is the index value

'C' number of species common to both sites

'a' number of species in site A

'b' number of species in site B

5.2.3.4. Concentration of dominance [Simpson's index (1949)]

To measure concentration of dominance Simpson Index (1949) was used.

Simpson's Index (1949)

$$\lambda=\Sigma pi^2$$

where, ' λ' is the index value

'pi' is the proportional abundance of the i^{th} species

Pi= ni/N

'ni' number of individuals of a species

'N' total number of species in the habitat type

5.3. ESTIMATION OF ABOVEGROUND HERBACEOUS BIOMASS

Biomass is an important parameter to understand the functional aspect of an ecosystem (Cornet, 1981) and it also helps to understand the physical and chemical attributes of the soil. For the present study, estimation of biomass was a crucial aspect for comparing the primary productivity of natural vegetation with that of planted forest. Only above ground herbaceous biomass was estimated for the present work following "harvest and estimate" method (Tadmor *et al.* 1975; Scurlock *et al.* 1999, 2002) based on a single harvest at the peak of live biomass.

Above ground herbaceous biomass was collected or harvested by clipping at ground level from 25cm×25cm area of each upper right and lower left quadrate of 1m×1m during the post monsoon vegetation survey when the productivity reaches at

peak. Harvested biomass were placed in perforated paper bags and dried to constant mass at 80°C using hot air oven and weighed (Garnier *et al*. 2007 and Das *et al.* 2008). The experimental data were processed using MS Office Excel 2007.

5.4. RECOGNITION OF RARE, ENDEMIC AND THREATENED ELEMENTS

Eastern Himalaya is renowned for its endemic flora as well as the other category of threatened elements (Das, 1986; Bhujel, 1996; Das, 2002). The study area is located just at the foot of the Darjeeling Himalaya which is in important part of Eastern Himalaya from the view point of phyto diversity as well as its overall biodiversity. Keeping this in mind and it was assumed that there may be some threatened category of floral elements, the area was screened for Rare, Endemic and Threatened [RET] plants. RET elements were recognized with the help of Red Data Book for Indian Flora (Nayar & Sastry, 1987, 1988, 1990; Ahmedulla & Nayar, 1987; Bhujel, 1996; Ahmedulla, 2000; Bhujel & Das, 2002; Rai, 2006), Flora of India (Botanical Survey India) and following the IUCN guidelines (IUCN, 2014) for determination of different classes of threatened plants.

5.5. DOCUMENTING NON TIMBER FOREST PRODUCES

To collect data on Non Timber Forest Produces (NTFPs) of the Terai-Duars belt from both the Natural and plantation forest and to compare these two types of vegetations in respect of Non timber forest produces, a combination of methods were used. Maximum amount of data was collected during the vegetation study as a number of knowledgeable and local people on behalf of forest department accompanied the author then. Mainly the methodology followed by Jain, 1981, 1987, 1991; Rai *et al.* 1998; Rai & Bhujel, 1999; Rai, 2002; Jain & Mudgal, 1991; Sarkar, 2011, 2014; Santra & Roy, 2002 and Das, 2005 were followed for this purpose. During the survey, enquiry was made with the local people about the NTFPs which they collect. Few local markets in or around the study area were also visited to gain an overview of NTFP used for different purpose and the materials and voucher specimens were collected, processed and identified following the methodology described previously.

5.6. RECORDING OF ETHNOBOTANICAL KNOWLEDGE AND PLANTS

For recording ethnobotanical or Traditional knowledge and related plants and plant materials from the plantation and natural vegetation and the adjoining areas of Terai-Duars belt, the methodology suggested and adopted by Schutles (1962), Jain (1981, 1987, 1991), Rai *et al.* (1998), Rai and Bhujel (1999) and Rai (2002), Sarkar (2011) were followed with some modification in some cases. Sometimes information was collected by various technique like open interview and discussion with local informants. When questionnaire is used to collect data, the same was prepared by following Jain & Mudgal (1991) and Tag (2007).

The field work was carried out over 3 years from 2008 to 2010 in different forest of Terai-Duars belt of west Bengal and the fringes. Most of the information was collected during the phyto-sociological study and from the knowledgeable forest workers belonging to the tribal community, from local people found nearby, the cowboys, firewood collectors, fodder collectors, *Oajhas* or rural medicine men at the time of their collection of plant materials from the forest. In some cases, where found nearby, village markets were visited in search of those plant materials collected from the forest and are sold. In some cases the elder and the knowledgeable person were also visited and consulted to collect the ethno-botanical knowledge of the area.

5.7. SURVEY AND DOCUMENTATION OF MEDICINALLY IMPORTANT PLANTS

Terai-Duars region is a rich source of important and rare medicinal plant (Das *et al.* 2010a) that corresponds to the rich and wide phyto-diversity it harbours. In the present study medicinally important plants, their uses and distribution in both the plantation areas and natural vegetation were documented. Most of the data were collected during the study of vegetation and phyto-sociology. Available literature on medicinal plants were also consulted for identification and documentation and the methodology suggested and followed by Jain (1991), Rai *et al* (1998), Rai and Bhujel (1999, 2002, 2007a), Santra & Roy (2002) and were adopted.

5.8. CHARACTERIZATION OF SOIL

Different workers had reported the modification of soil characters by plantation (Ehrenfeld, 2003; Thapa *et al.* 2011). That's why the soil parameters like- soil texture, moisture content, soil organic carbon, nitrogen and available potash and phosphorus were included in the present study. Soil samples were collected as per method prescribed by Misra *et al.* (2009) from natural and plantation forests from two layers (0 – 15cm and 15 – 30cm depth).Various physico-chemical properties of the soil were analyzed using standard methods (Piper, 1966; Jackson, 1958): Soil P^H using digital P^H meter 335 (Systronic), moisture by gravimetric method, soil texture by Bouyoucos hydrometric method (Allen *et al.* 1974), organic carbon (Walkley & Black, 1934; Walkley, 1947), N_2 content by Kjeldal method (Allen *et al.* 1974), Available sulphur by colorimetric method (Anderson & Ingram, 1993; Ensminger, 1954) and phosphorus by Bray's method-I (Bray & Kurtz, 1945). The soil samples were analysed in Department of Tea Science, University of North Bengal.

5.9. IMPACT OF AGGRESSIVE WEEDS

Impact of aggressive weeds on local flora was assessed following a combination of methodology suggested by Misra (1968), Acharyya (1998), Rai (2004) and Ghosh (2006). Both the invaded and non invaded area under same environmental conditions were surveyed to collect the phyto-sociological data, relative density,

relative abundance and relative frequency and IVI of the weeds in invaded areas were calculated and the processed data was analysed for their impacts on plant society of the study area.

5.10. ASSESSMENT OF ALLELOPATHIC EFFECTS

To assess the allelopathic effects of different plantation species (Teak, Sal, Jarul and Chilauni) native plants, few of them were selected which were encountered during the phyto-sociological survey. Selection of plants which were supposed to have allelopathic effects was based on their stenches of planting i.e. which were used to raise plantation over large areas whereas the species on which allelopathic effects were assessed were selected on the basic of their occurrences, medicinal or other importance, their conservational status, availability of seeds etc. The methodology suggested and used by Putnam and Duke (1978); Kadir (2001); Datta & Ghosh (1987) and Ghosh (2006) were followed.

5.10.1. Collection of seeds

Mature seeds of the test plants [*Andrographis paniculata* (Burm. f.) Wall *ex* Nees, *Plumbago zeylanica* L., *Ocimum gratissimum* L., *Senna occidentalis* (L.) Link, *Oxalis corniculata* L. were collected from study area and Garden of medicinal plants, University of North Bengal and stored at 4°C in brown envelops for the assessment.

5.10.2. Preparation of extract

For preparation of extracts fresh leaves of the plantation species were collected in airtight zip pouch, brought to the laboratory and washed thoroughly. Then 100 g of fresh leaves were crushed in 250 ml of distilled water using Sandoz mixer grinder machine, filtered through muslin cloth and then Whatman No.1 filter paper and the final volume was adjusted to 1000 ml and used as mother or stock solution (100 %). Then different solution of desired concentrations 25 %, 50 %, 75 %, were prepared by proper dilution with distilled water from the stock solution (Hoque *et al*. 2003).

5.10.3. Germination tests

Before germination tests, healthy seeds of test plants were soaked in 0.1% $MgCl_2$ solution for 3 minutes for surface sterilization and washed with 1% $AgNO_3$ solution to remove adhering $MgCl_2$ and then washed several times with distilled water. Then 20 healthy seeds placed in a sterile 15cm glass petriplates lined with single layer of Whatman filter paper which was moistened sufficiently by adding 15ml of the test solutions. This was set in three replicates along with a control in which the filter paper was moistened with 15ml of distilled water. The petriplates were kept under constant temperature (room temperature) for germination which is indicated by the emergence of radical. After the germination started it was left for 14 days more for

recording different parameters like number of seeds germinated, length of roots and shoots, number of lateral roots etc.

Then the data collected from the experiment were processed and analysed statistically using MS Excel 2007. Different formulae which were used to calculate percentage of viability, germination percentage, percentage of inhibition of germination, percentage of inhibition of shoot length and root length, shoot and root vigour index etc. are as mentioned below.

Germination Percentage:

The percentage of germination was calculated using the formula which was followed by Lama (2004), Ghosh (2006) and Acharyya (1998).

$$\text{Germination percentage} = \frac{\text{Number of seeds germinated}}{\text{Number of seeds sown}} \times 100$$

Percentage of inhibition or stimulation:

Saxena *et al* (1995) was followed to calculate the percentage of inhibition or stimulation.

$$\text{Percentage of inhibition or stimulation} = \frac{\text{Germination \% in desired solution} - \text{Germination \% in Control solution}}{\text{Germination \% in control solution}} \times 100$$

Percentage of viability:

To determine the percentage of viability and the percentage of non-viability of seeds the formula suggested by Acharyya (1998) and Lama (2004) were adopted.

$$\text{Percentage of viability} = \frac{\text{Number of viable seeds in desired solution}}{\text{Number of viable seeds in control solution}} \times 100$$

Percentage of inhibition or stimulation of root length, shoot length and seedling length:

Percentage of inhibition or stimulation of root, shoot and seedling length were calculated by applying the following formula (Acharyya, 1998).

$$\text{Inhibition or stimulation of root length (\%)} = \frac{\text{Root length in desired solution} - \text{Root length in Control}}{\text{Length of root in Control solution}} \times 100$$

$$\text{Inhibition or stimulation of shoot length (\%)} = \frac{\text{Length of shoot in desired solution} - \text{Length of shoot in Control solution}}{\text{Length of shoot in Control solution}} \times 100$$

$$\text{Inhibition or stimulation of seedling length (\%)} = \frac{\text{Length in desired solution} - \text{Length in Control}}{\text{Length of seedling in Control solution}} \times 100$$

Shoot vigour index, root vigour index and seedling vigour index:

Shoot vigour index, root vigour index and seedling vigour index were determined adopting the formula suggested by Thind and Malik (1988) and followed by Acharyya (1998), Lama (2004) and Ghosh (2006).

Shoot vigour index = Percentage of germination × shoot length

Root vigour index = Percentage of germination × root length

Seedling vigour index = Percentage of germination × seedling length

Shoot-root Ratio:

For determination of shoot root ratio Bajpai *et al.* (1995) was followed

$$\text{Shoot: Root} = \frac{\text{Length of shoot}}{\text{Length of root}}$$

Fresh weight of seedlings in control solution and different extract concentration was measured to determine the inhibition or stimulation of fresh weight of seedlings.

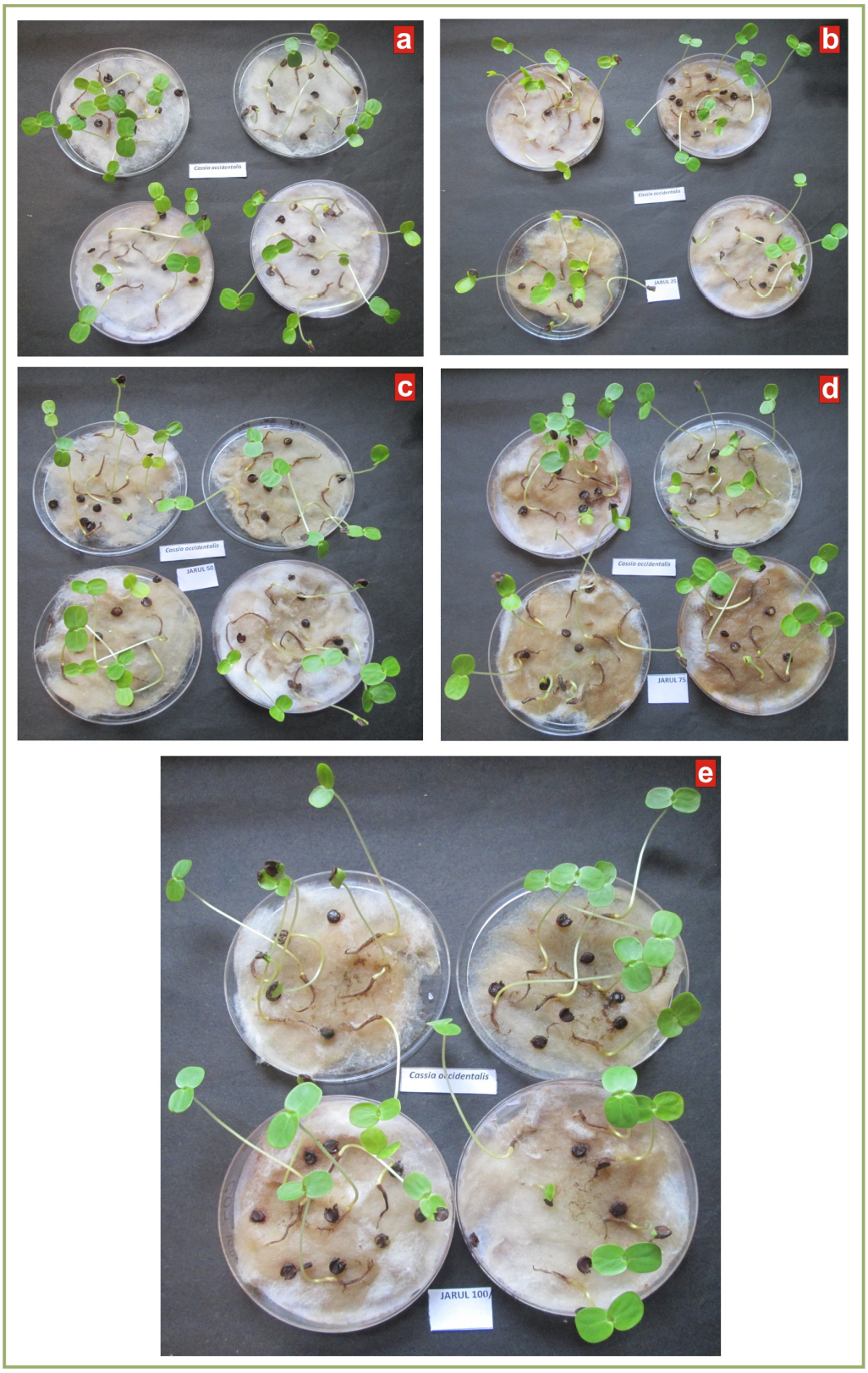

Figure 5.2. Experimental set up for allelopathic effect of Jarul on *S. occidentalis*: **a.** Control; **b.** 25%; **c.** 50%; d. 75%; **e.** 100%

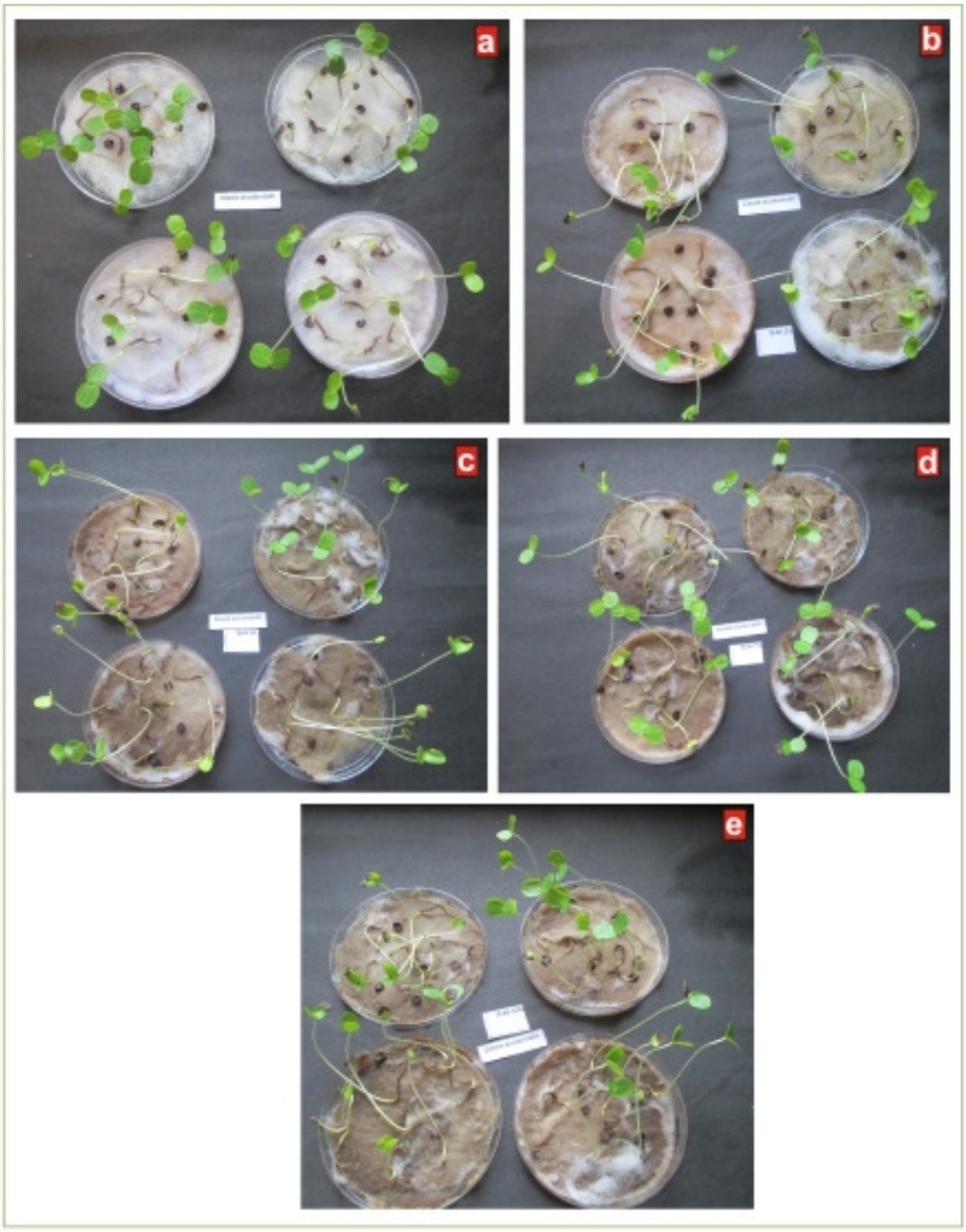

Figure 5.3. Experimental set up for allelopathic effect of Teak on *S. occidentalis*: **a.** Control; **b.** 25%; **c.** 50%; **d.** 75%; **e.** 100%

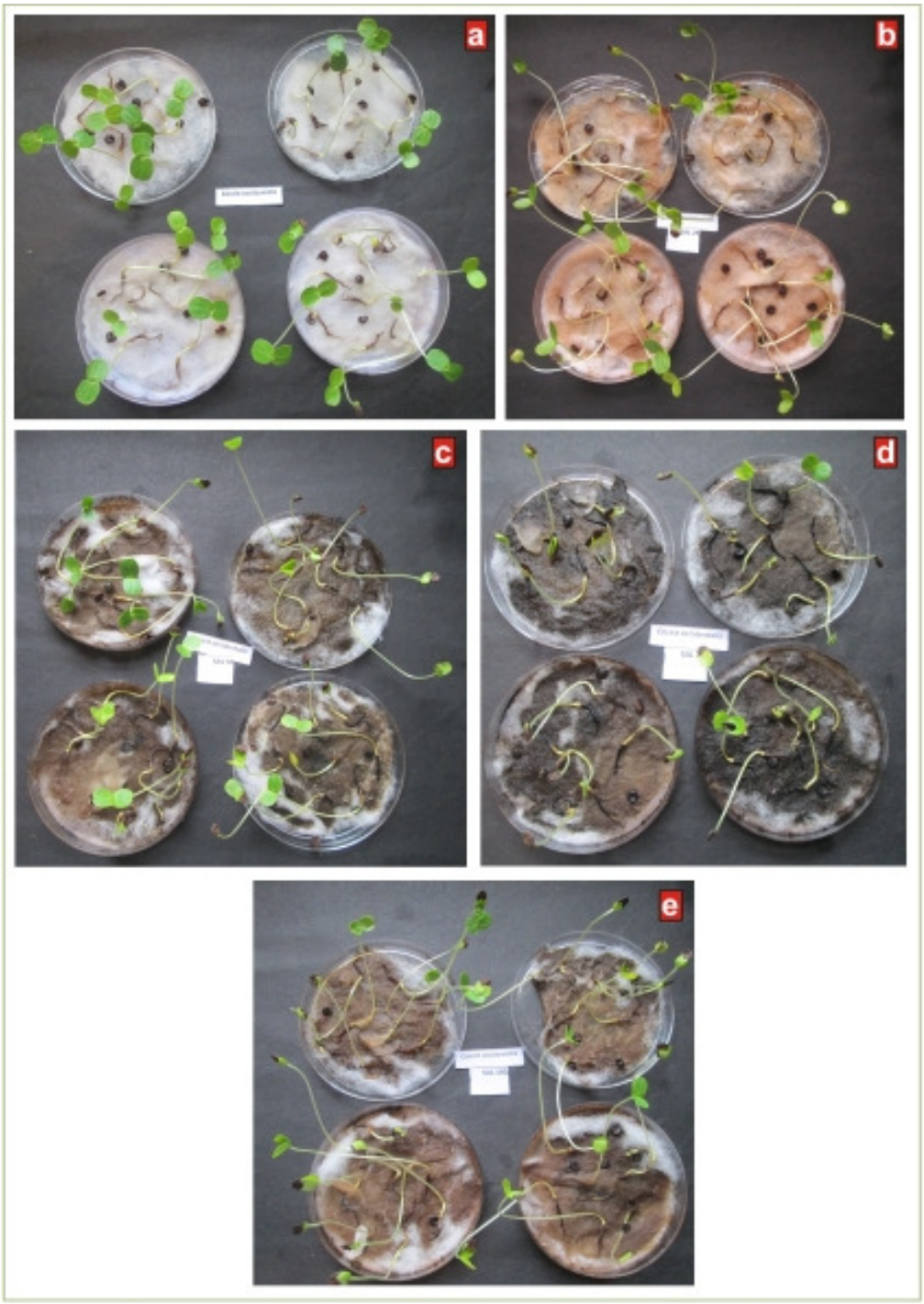

Figure 5.4. Experimental set up for allelopathic effect of Sal on *S. occidentalis*: **a.** Control; **b.** 25%; **c.** 50%; **d.** 75%; **e.** 100%

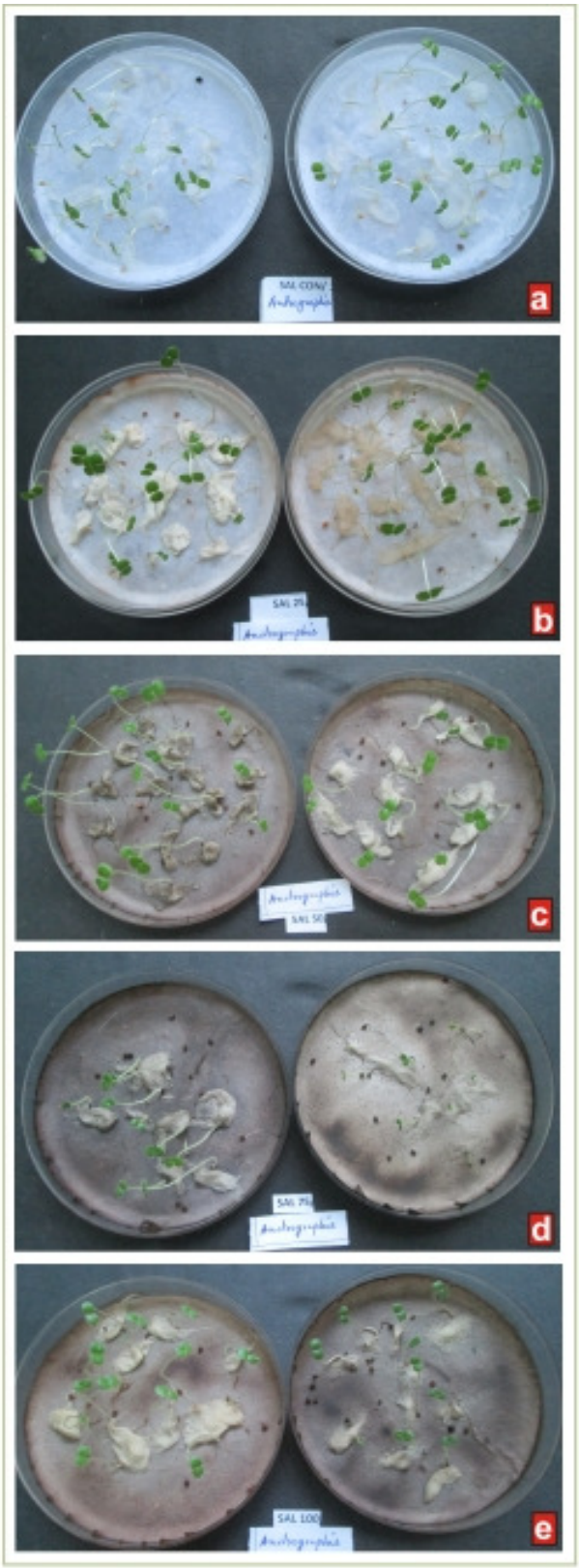

Figure 5.5. Experimental set up for allelopathic effect of Sal on *A. paniculata*: **a.** Control; **b.** 25%; **c.** 50%; **d.** 75%; e. 100%

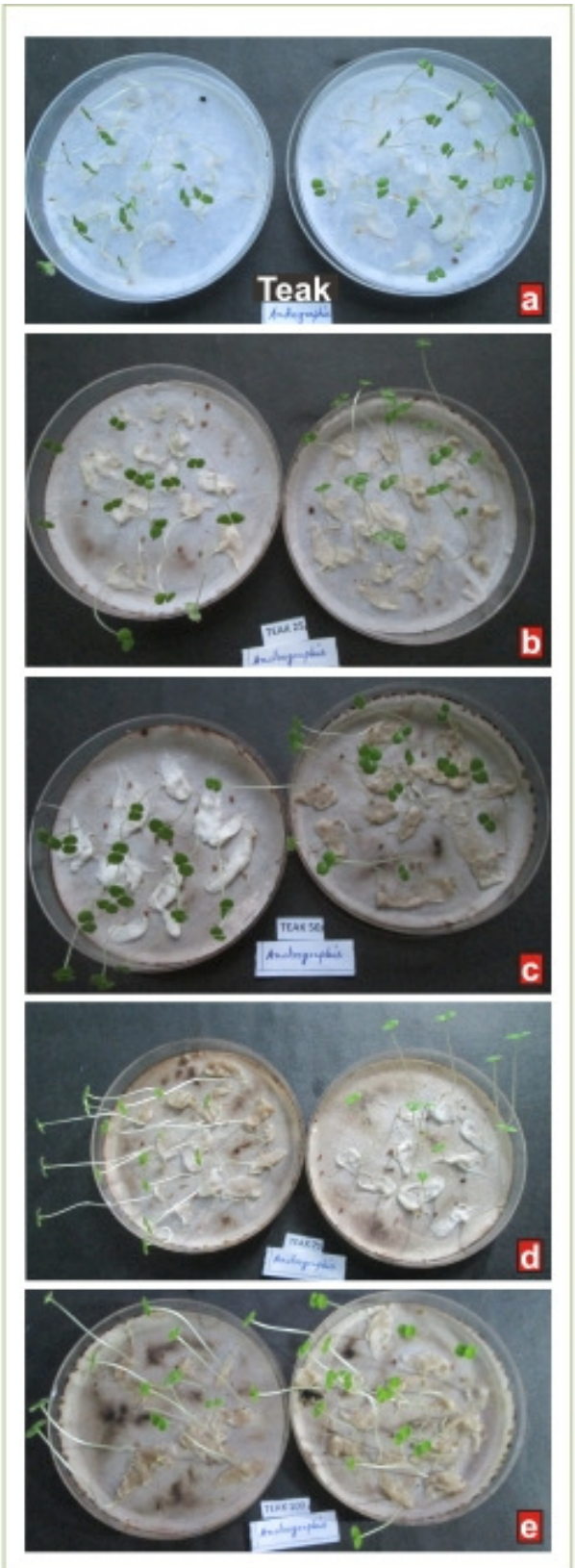

Figure 5.6. Experimental set up for allelopathic effect of Teak on *A. paniculata*: **a.** Control; **b.** 25%; **c.** 50%; **d.** 75%; e. 100%

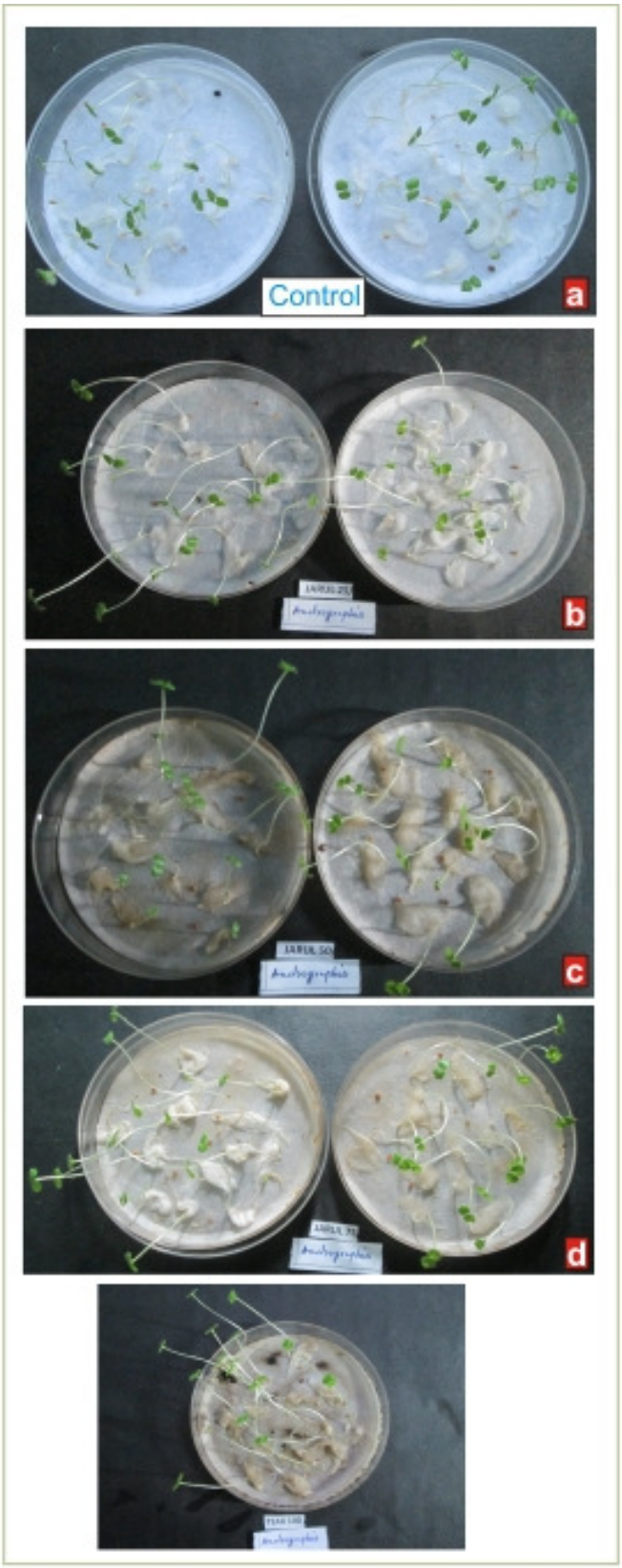

Figure 5.7. Experimental set up for allelopathic effect of Jarul on *A. paniculata*: **a.** Control; **b.** 25%; **c.** 50%; **d.** 75%; **e.** 100%

Figure 5.8: Images of Herbarium specimens: **a.** *Cinnamomum glaucescens*, **b.** *Litsea panamaja*, **c.** *Grewia eriocarpa*, **d.** *Schima wallichii*, **e.** *Aesculus assamica*, **f.** *Aristolochia tagala*, **g.** *Piper boehmerifolia*, **h.** *Bauhinia purpurea*, **i.** *Argyreia roxburghii*