Chapter 3

MATERIALS AND METHODS

3.1 Location of Study

All experiments in the present study was conducted in the nursery of Dr. T.C Joseph Memorial Botanical Garden, of the Department of Botany, Union Christian College, Alwaye with geographic position +10° 7' 30.65", +76° 20' 3.32" that comes under Ernakulam district of Kerala State, India (Fig.3.1.1 a,b). Ernakulam district has wet monsoon type of climate. The location of experimental study and the nursery experiences heavy rainfall during southwest monsoon season followed by northeast monsoon season. During the other months the rainfall is considerably less. March, April and May months are the hottest. December to February months are the coldest.
The annual rainfall ranges from 3233 to 3456mm at different places of Ernakulam district. The district received an average 3359.2mm (based on 1901-99 data) of rainfall annually. Rainfall during Southwest monsoon season contributes nearly 67.4% of total rainfall of the year, followed by the northeast monsoon, which contributes nearly 16.6%, and the balance of 16% is received during the month of January to May as summer/pre-monsoon showers.

The mean monthly maximum temperatures, ranges from 28.1 to 31.4°C and the minimum ranges from 23.2 to 26°C. The maximum temperature occurs during March and April months and the minimum temperature occurs during December and January months. The humidity ranges from 68 to 89% during morning hours and 64 to 87% during evening hours. The maximum humidity is observed during May to October months.

The wind speed ranges from 6.7 to 10.9 km/hr with mean speed of 9.1 km/hr. The wind speed is high during the period from March to September. The PET (potential evapo-transpiration) ranges from 94.5 to 159.2mm. The maximum PET occurs during March and minimum occurs during June.

On the basis of morphological features and physico-chemical properties, the soils of Ernakulam district are classified as Lateritic, Hydromorphic saline, Brown hydromorphic, Reverine alluvium and Coastal alluvium. The soil of the study area is Lateritic that are well-drained, low in organic matter and plant nutrients. The major crops grown are coconut, tapioca, rubber, areca nut, pepper, cashew and spices (Shyam, 2007).
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3.2 Basic information on the study species

Up-to-date nomenclature of all the nine species was worked out in accordance with the International Code of Botanical Nomenclature and a few synonyms are included in the nomenclature part by which the species are known in National (Hooker, 1871-96), Regional (Gamble, 1915-1935) and State (Rama Rao, 1914; Bourdillon, 1902) Floras. Local names used for the species were gathered from literature and also during field and herbarium studies. A brief description of each species was prepared based on fresh collections and by literature scrutiny, giving details of habitat, leaves, inflorescence, flowers, fruits and seeds. Data on flowering and fruit ripening periods were also gathered based on field and herbarium data. The natural distribution pattern of each of the species in the forests of Kerala was gathered from field, herbarium studies and literature, along with their natural stands in the forests of Kerala (Sasidharan, 2007, 2010; Barndis, 1971). Wood characteristics such as density, grain, and texture were mostly gathered from Nazma, et al. (1981). Phytochemical characters for each species were also gathered (Chandrasena, 1935).
3.3. Seed collection

Seeds were collected from selected mature trees from homesteads or natural stands. Seed collected before ripening or maturity may either give poor germination or may not germinate at all, therefore knowledge about fruit ripening and seed dispersal is a prerequisite. First hand information on phenological data were gathered by literature scrutiny and then by herbarium and through field exploration. A thin variation in judging the maturity of seeds may result in failure of nursery; usually seeds that ripen very early or very late gives poor germination per cent and hence seed collection was conducted during the exact season when seeds are widely available in an area.

3.3.1. Method of Fruits and Seed Collection

The method of fruit and seed collection largely depends on the type of fruits and their seed dispersal mechanism and hence different mechanisms were adopted, whenever required in different study species. Even though there are several methods for collection, in the present experimental study the method that is suitable for a specific plant was performed. Fallen seeds and fruits were collected from the floor under mother trees in plant species such as Cassia fistula, Mimusops elangi, Pongamia pinnata, Pterocarpus marsupium, Saraca asoca and Terminalia arjuna. To ensure that the fruits and seeds collected are of the same year, the floor of the selected mother trees were swept and polythene sheets were spread. Fruits, which were not fully ripened, and insect attacked at fruiting stage were discarded by hand picking at the time of procurement from the field itself. This can reduce the entry of seed born pest in storage to some extent that may cause greater loss of seeds and affects the whole storage process. In Cassia fistula and Mimusops elangi, some plants produces deformed as well as heavily infested fruits, and hence such trees were not selected for fruit and seed collection. In Cassia fistula, Pongamia pinnata,
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Pterocarpus marsupium and Terminalia arjuna, fruits can be procured more economically by loping the terminal branchlets. In Bombax ceiba, lopping of terminal branchlets is not practical as they have delicate branchlets besides prickles on their entire stem, therefore a pole with a hook with attached bag was used. The same method of collection was also useful in Saraca asoca too.

3.3.2 Quantity of Seeds to be collected

Knowledge of approximate number of fruits and seeds that weighs per kilogram, average weight of fruits and seeds, are valuable to determine the quantity of fruits to be collected from the field as seeds of most of the plantation tree species are seasonal. The above-mentioned characters are qualitative and vary widely within the plants, between plants as well as with different localities of collection. The number of fruits that has to be collected is largely based on number of seedlings required for planting and the number of seedlings produced in a trial depends on germination percentage, pretreatments used, viability loss due to storage and post harvest loss. In the present study as there was several aspects of propagation involved, four times the actual number of plants required were collected.

The fruits collected from the field are packed in gunny bags and the loose seeds from the field are packed separately in paper covers or polythene bags and then taken to the lab for processing and plantation trial experiments.

3.3.3. Seed Extraction and Processing

The method of seed extraction from fruits, differ from species to species and it is based on the delicacy of seeds and type of fruits each plant has. The fruits of Bombax ceiba were spread under sun for natural splitting of the capsular fruit, after which the fruits are longitudinally split and seeds were
pushed out by using a pointed needle as seeds are found concealed inside the silk cotton fibres.

Fruits of *Cassia fistula* are pods with an outer dry pericarp and an inner pulpy material along with transverse partitions for each seeds. Fruits are externally hit using a mallet and then are able to split open longitudinally. The exposed seeds were then dislodged by hit and the few others that are left over were detached using a needle. Seeds thus obtained are washed to remove the gelatinous material completely and then spread under shade for drying.

Seeds of *Pongamia pinnata* and *Pterocarpus marsupium* were extracted only on the day of sowing as their exposed seeds were damaged by pest and fungal infection. The wood hard pericarp was given a hit at the tip of fruit, which leads to formation of a longitudinal split through which seeds can be dislodged. *Pterocarpus marsupium* fruits has hard pericarp with lateral wings all around, hence after cutting the wings using a scissors a small cut is made at the tip at 45° along the sides using a secateur without harming the seed and then it is expelled out through the opening using a needle (Fig 3.3.1 a).

![Pongamia pinnata](image1.png) ![Mimusops elangi](image2.png)

**Fig 3.3.1 Seed extracion**
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*Mimusops elengi* have fleshy pericarp and hence it was depulped in water immediately after collection. Fruits were soaked in bucket full of water for three days until it becomes soft. Changing water in the bucket enhances peeling of skin. Gentle crushing and rubbing the fruits together by hand helped in the separation of seeds. The soft pulp floats on water surface and the freed seeds sink to the bottom were collected by decantation. After decanting process seeds were again washed in running water to remove traces of pulp if any and then spread in shade for sun drying (Fig 3.3.1b).

Pods of *Saraca asoca* were first spread over a polythene sheet under sun till the pods turn brown and split open. Seeds were then dislodged from the fruits by shaking. No processing or separations of seeds are applicable for commercial seedling production in *Terminalia arjuna* as their pericarp is woody and seeds are delicate. Seeds are easily damaged by slight hit or by forceful breaking of pericarp.

3.4. Seed Storage

Fruits or seeds after conditioning were packed in gunny bags, cloth bags, polythene bags or plastic bottles for storage. Conditioned seeds were used for storage in *Bombax ceiba, Cassia fistula, Mimusops elengi* and *Saraca asoca*, whereas seeds as well as fruits were used for storage in *Pongamia pinnata* and *Pterocarpus marsupium* (Fig 3.4.1 a, b). Fruits as such were stored after conditioning in *Terminalia arjuna*. Seeds and fruits were stored in closed shelves in dry condition for all the study species. In order to prevent the loss of moisture, seeds of recalcitrants such as *Bombax ceiba, Pongamia pinnata, Pterocarpus marsupium* and *Saraca asoca* were mixed with sieved sand and kept in earthen pots.
3.5. Seed Pest and Disease

For seed pathological studies, seed samples were collected during the seeding year 2007 to 2009. The pooled samples, soon after collection and extraction, were labeled and brought to the laboratory in cloth bags. The seeds were sun dried in shade to reduce the moisture content to about 10-15% and stored separately in cloth bags at room temperature between 25-28°C. The standard blotter test recommended for seed testing was employed (ISTA, 1996). A random sample of 400 seeds was used for data collection of each species. Wet sterilized blotters of 9 or 11 cm size were used. The plates were incubated at 25°C under 12 hours of alternating cycles of light and darkness for 7 days and were examined on the 8th day with the help of a compound microscope for microbial growth. The relative percent incidence (RPI) of each microorganism was calculated using the following formula.

\[
\text{R.P.I} = \frac{\text{Number of seeds with organism}}{\text{Total number of seeds tested}}
\]

For gathering data on seed pests, observations were made on fruits and seeds; random samples were taken from the seed lot and the number of affected seeds recorded and percent infestation calculated. Infested seeds were kept in the laboratory for observation (Fig 3.5.1 a-f).
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a) Seed rot in *Pongamia pinnata* caused by fungi

b) Damaged seeds of *Cassia fistula* by *Aspergillus*

c) Infested seeds of *Bombax ceiba*

d) Infested seeds of *Saraca asoca*

e) Infested fruits of *Saraca asoca*

f) Infested fruits of *Cassia fistula*

**Fig 3.5.1** Some pests and disease infected seeds and fruits
Based on the nature and extent of damage by various pests, they were categorized as follows; infestation above 50 per cent was rated as heavy, 25-50 per cent as moderate and up to 25 per cent as mild.

3.6. Nursery Tools and Implements

**Hoe:** It is commonly known as *mummitee* in south India, which was used for digging up loam soil for potting purpose and also for turning the soil and coir pith efficiently.

**Pickaxe:** It has one end pointed and the other end flat. It is a heavy implement and the centre hole has a wooden handle. It was very useful in loosening surface soil and also for digging (Fig 3.6.1e).

**Hosepipe:** for convenient watering purpose in seedbeds, poly potted seedlings and root-trainers; hosepipe made of rubber was used, which was connected to the garden tap with the help of a rubber connector.

**Knife:** A three-in-one knife was used which was useful for activities such as pruning, collecting twigs for rooting of cutting procedure.

**Labels:** Even though labels of different types are available for labeling the plants depending on their permanent and temporary usage, labels made of aluminum sheet foils or thick paper labels
coated with wax were used for nursery seedlings, root-trainers, mist chambers, seed and fruit samples.

**Secateur:** This was used for collecting twigs for rooting of cutting technique and also for pruning the seedlings after cutting collection. Secateurs with blade made from first-grade carbon steel, which are anticorrosive, very sharp, long lasting and easy to handle were used (Fig 3.6.1b).

**Sieve:** Small and medium sized sieve made of nylon mesh was used to sieve sand and soil. Sieve made of wire mesh was used for depulpping of fruits in *Mimusops*

**Sprayers:** Small type hand sprayers with half litre and one litre capacity were used for spraying pesticides and fungicides in the garden and in mist chambers. Pneumatic type sprayers were used for spraying growth boosters and watering in mist chambers (Fig 3.6.1 a, d).

**Trowel:** It is made of steel with plastic handles. It was used for filling potting mixture in polythene bags and also in root-trainers (Fig 3.6.1c).

**Watering can:** Plastic can of five litre capacity was used which had a connecting pipe starting from the base at an angle 45°. At the end of outlet pipe a fine shower of water was available as it was fitted with brass rose with fine holes.
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Fig 3.6.1 Some tools used in Nursery work

a) Hand sprayer  
b) Secateur  
c) Trowel

d) Pneumatic sprayer  
e) Pickaxe
3.7. Nursery Methods

The period of propagation is usually a very narrow segment of a plant's life, that in woody perennial species ranging from few months to one or two years. To enhance the quality of propagules, propagators manipulate the environment of propagules by managing micro climatic conditions, edaphic and biotic factors. Therefore site selection for nursery construction is an important decision. The selection of site and further preparations were done before six months before the first sowing.

3.7.1. Nursery Preparation

Nursery of T.C. Joseph memorial Botanical Garden was utilized for this purpose with alterations made according to the need of experiments such as area for mist chamber, racks for maintaining germination trays and root-trainers. For continuous and adequate water supply two water tanks (both with 500 litre capacity) were kept 7 m, higher to the level of nursery; in addition to the source of water supply from a pond in the nearby area and also the water from rain harvest project. The site of nursery was a location with deep loamy soil, which is the right type of soil required for potting purpose. The additional requirement of soil was met by digging up the organic top soil in a newly cleared tree dominated area within the college campus and was transported to the nursery using trolleys. Fencing to the garden within which the nursery was located was furnished and maintained throughout the study period so as to avoid entry of grazing animals and humans intervention. Design inside the plant nursery was made in such a way that it contains path for workers, soil storage shelter, working area, compost making area, store for keeping nursery implements.
3.7.2. Preparation of Seed Sowing Media

Preparation of nursery beds:

Raised standard nursery bed of the size 1.2 m x 8 m with 0.3 m height was prepared. To protect the raised soil, bricks were used as bed frame; otherwise the raised soil along with seeds or seedlings may wash off during rainy season. A trench was dug to a depth of 0.3 m from which stones, roots of other plants were dug out and removed from the nursery area. After the preparation of seedbeds, they were raked to make sure that it has no stones or lumps in it and pressed with wooden board to ensure that the surface stays perfectly leveled. A top dressing of sand was used so that topsoil does not cakes when watered and to avoid splash during rains (3.7.1c).

Preparation of Germination Trays with Polyurethane sheets:

Germination trays of the size 25 x 37 cm were used inside which polyurethane sheets of 2 cm thickness was spread. The sheets were dipped in water and then treated with Carbendazim solution (1 gm per litre) to avoid fungal infection. Seeds were then sown on the polyurethane sheets. A sample of 24 seeds was used in each experiment.

Preparation of Polythene Bags with Potting Mixture:

Potting mixture was prepared in the ratio 1 soil: 1 sand: 1 farmyard manure and was used to fill the polythene bags. In the present study, all the control plants during germination, growth of seedlings and also for rooting of cutting technique was provided with the same medium. The germinated seedlings in nursery beds were transplanted to polythene bags with potting mixture(Fig 3.7.1a and d).
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**Fig 3.7.1 Materials for preparing potting medium**

**Preparation of Polythene Bags with Coir pith:**

The pressed and sterilized coir pith cakes was dipped in water for 12 hours; then mixed with loamy soil and sand in the ratio 1:1:1. After filling the
medium in polythene bags, they were treated with Carbendazim solution (1 gm per litre) as a precaution against fungal infection. This was done 24 hours prior to the direct dibbling of seeds into it for germination. In addition to this the growth performance of seedlings and rooted cuttings up to three months were also utilized (Fig 3.7.1e).

**Preparation of Root-trainers with Potting Mixture:**

Loamy soil, sand and farmyard manure were mixed in 1:1:1 ratio and were used to fill the root-trainers. The filled in root-trainers were used in germination, growth of seedlings and for rooting of cutting technique studies (Fig 3.7.1b).

**Preparation of Root-trainers with Coir pith:**

The wetted coir pith was mixed with loamy soil and sand in the ratio 1:1:1 and was treated with Carbendazim solution (1 gm per litre). After 24 hours seeds were directly dibbled for germination studies, or treated cuttings were stuck for rooting process.

**3.7.3. Method of Seed Sowing:**

In raised standard nursery bed, seeds of *Bombax ceiba* and *Pterocarpus marsupium* were sown in broadcast method, after they were mixed with sieved sand and that of *Cassia fistula, Mimusops elangi, Pongamia pinnata, Saraca asoca* and *Terminalia arjuna* were sown in drilled lines which were taken 20 cm apart. Seeds were directly sown on soaked polyurethane sheets in germination trays. In all other medium such as polythene bags with potting mixture, Polythene bags with coir pith, root-trainers with potting mixture and root-trainers with coir pith, seeds were directly dibbled in the medium. The sample size for sowing was restricted to twenty-four in all the six of sowing medium.
In the present study six sowing media were used to record the germination performance of fresh untreated seeds, fresh pretreated seeds and stored seeds at 30 days interval up to 3 months in recalcitrant seeds such as *Bombax ceiba*, *Pongamia pinnata*, *Pterocarpus marsupium* and *Saraca asoca*. However germination performance up to one year was gathered for *Cassia fistula*, *Mimusops elangi*, and *Terminalia arjuna*.

3.7.4. Pretreatments:

Even though most of the seeds germinate without pre-treatments, it is of importance in many tree species during commercial production of seedlings when seeds have dormancy due to various reasons. Pre-treatments such as dipping in cold water for 20 hours, dipping in hot water, treating in sulphuric acid, removal of seed coat using knife and application of gibberellic acid (100ppm) was used in all the study species in order to study the effect of pre-treatments and their germination performance in different sowing media (Fig 3.7.2).
3.7.5. Maintenance of Nursery Seedlings

Seedlings either generated directly in polythene bags and root-trainers or transplanted were well arranged inside the nursery and maintained for generating data on growth of seedlings. Plants were given watering twice daily and sprayed with 1% carbendazim solution, (Trade name-Bavistin, BASF, India) which is a growth stimulant as well as a systemic fungicide (Fig 3.7.1f).

3.8. Method in Vegetative Propagation

3.8.1. Auxin treatment and design

A non-auxin control and three Indole-3-butyric acid (IBA) concentrations were designed in this experiment. The four treatments obtained were the non-auxin control, 300ppm, 500ppm and 1000ppm IBA (Fig 3.8.1).

![Indole-3-butyric acid](image)

**Fig 3.8.1** Indole-3-butyric acid

Each auxin solutions were prepared by dissolving the appropriate amount (that is 300mg in 1 litre for 300ppm, 500mg in 1 litre for 500ppm and 1000mg in 1 litre for 1000ppm) of IBA in 50 ml of 70% alcohol and then using de-ionized water to bring the solution to 1000 ml. The prepared auxin solutions were stored at 4°C in opaque bottles and were used within two days.
A randomized complete block design was employed. There were three applications with twenty-four cuttings/treatment

3.8.2. Collection of Vegetative cuttings and their Handling

Shoot tip with 2-4 nodes were collected from healthy one-year-old seedlings using a clean pruning knife. The bottom cutting was made immediately below the bottom node. In order to reduce the rate of water loss, the bottom leaf of stem cuttings was removed and the remaining leaflets on each petiole or rachis was reduced to one or two and the later were also trimmed by about two thirds. Soon after collection, the cuttings were placed in 2% carbendazim solution to prevent fungal infection and later washed with running tap water. The basal 1 cm part of each cutting was then dried using blotting paper and dipped into the testing solution of IBA for 8-10 seconds. The treated cuttings were then vertically struck into the premade holes in root trainers or plastic cups of 100 cc. The stock plants were managed regularly by pruning, application of carbendazim solution and watering. These plants in root trainers served as stock plants for collection of cuttings (Fig 3.8.2 a and b).

3.8.3. Preparation of rooting media

For the present study three types of potting media were utilized which include Root-trainers with potting mixture, root-trainers with coir pith and plastic cups with coir pith along with root-trainers with potting mixture as control. Samples of twenty-four numbers were maintained for each set of experiment.

Preparation of Root-trainers with Potting Mixture:

Loamy soil, sand and farmyard manure were mixed in 1:1:1 ratio and were used to fill the root-trainers. The cuttings after treatment with auxin was vertically struck into the premade holes in root-trainers filled in root-trainers were growth of seedlings and for rooting of with potting mixture were used as control.
a. Juvenile stem cuttings in Carbendazim solution
b. Sizing of stem cuttings
c. Stem cutting treated with IBA
d. Treated stem cutting planted in root trainers with coir pith
e. Treated cuttings planted in plastic cups with coir pith

**Fig 3.8.2 a-e Stages in Rooting of Cuttings using IBA**
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Preparation of Root-trainers with Coir pith:

Coir pith cakes wetted with water was mixed with loamy soil and sand in the ratio 1:1:1 and was treated with Carbendazim solution (1 gm per litre), 24 hours before the treated stem cuttings were struck into it for rooting process. The root-trainers used were block type, (4 x 6 blocks) each with 100cc (Fig 3.8.2 c, d, e and f).

Disposable Plastic Cups with Coir pith

Well-sterilized coir pith cake was powdered and then mixed with the loamy soil and sand in the ratio 1:1:1 and then filled in plastic cups of 100cc.
They were treated with Carbendazim solution (1 gm per litre), 24 hours before the treated stem cuttings were struck into it for rooting process (Fig 3.8.2 h and i).

### 3.8.4. Design of Mist Chamber and Green house Management.

A glass chamber of the size 1.2m x 0.6x 0.6m was devised in order to enable rooting in hormone (IBA) treated cuttings. The glass chamber was fixed in an iron frame and provided with air holes at a height of 2.5cm from the base. Disposable trays with water were maintained inside the chamber; with perforations 2.5cm above the surface of trays were made. A hygrometer and a thermometer were fixed inside the chamber in order to check the internal moisture content and temperature of the chamber. The root-trainers and plastic cups with hormone treated stem cuttings were placed in trays inside glass chamber for rooting process (Fig 3.8.2 g).

Rooting induction was conducted in glass chambers, which were maintained in shade net to avoid excessive heat. The interior of glass chambers were maintained at 22-35°C and with 72 –95% relative humidity during the rooting period, which were frequently checked by the readings in hygrometer and thermometer that was set inside the glass chamber. The cuttings were provided with intermittent mist to keep the foliage fresh between misting.

In order to control and prevent fungal infections, 2% carbendazim solution was sprayed once in a week during the rooting periods.

### 3.8.5. Transplantation of Rooted Cuttings

After a period of 40 to 60 days, the treated cuttings kept for rooting was taken and examined for rooting percentage and transplanted into different potting media for further growth. The rooted cuttings were transferred into prepared potting media such as polythene bags with potting mixture, polythene bags with coir pith and root-trainers with coir pith (Fig 3.8.2i).
3.8.6. Maintenance of Seedlings and Rooted Cuttings in the Nursery

Rooted cuttings after transplantation were maintained in plant nursery which, were provided with frequent watering and application of 2% carbendazim spray in order to avoid fungal infection. Growth data up to three months were gathered for statistical study.

3.9. Nursery Pests and Diseases

The occurrence of any disease(s), if any, their symptoms and nature of damage caused to seedlings were recorded. The incidence of a disease was estimated either by counting the number of disease patches and approximate area covered by them or percent seedlings affected for a given density of seedlings in sample. The appropriate parts of the diseased seedlings were collected for isolation and identification of the causal organism.

For gathering data on nursery pests, observations were made on seedlings raised in the standard nursery bed, polythene bags and in root trainers. The seedlings were grouped into grids, each with 24 numbers. The number of healthy and affected seedlings within each grid and the nature of damage caused to them were recorded and the pooled average value was recorded as the percent infestation. Observations were made each month. Also, suitable management strategies were standardized based on the pathogen and the intensity of attack. Whenever required, watering and moisture regime of the nursery beds were regulated to avoid flourishing of pathogens in some cases.

3.10. Data Collection and correlation

3.10.1. Data Collection on fruits and seeds

Fruits taken to the lab were first spread on dry concrete slab in the lab for primary data collection. Using an electronic digital weighing instrument, the average number of fruits and seeds and weigh per kilogram was calculated for
each species and also using digital vernier calipers, the average fruit and seed size were obtained.

3.10.2. Germination data and its analysis

Germination percentage of seedlings raised in standard nursery beds, poly pots, germination trays and in root trainers was calculated using the following common formula.

\[
\text{Germination percentage} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds sown}} \times 100
\]

In all germination trials conducted in standard nursery beds, poly pots, germination trays and in root trainers, the starting day for germination is noted. The emergence of cotyledonal leaves is considered as germination in the case of *Cassia fistula, Mimusops elengi* and *Pterocarpus marsupium* where the seeds show epigeal germination, and in *Bombax ceiba, Pongamia pinnata, Saraca asoca* and *Terminalia arjuna* the development of plumule above soil surface was regarded as germination. The germination of last seed in a sample was regarded as completion of germination.

Number of seeds germinated in each day and the day taken for germination were recorded. Cumulative germination percentage was calculated for each treatment at the end of each trial. Germination value was calculated using the following formula as given by Czabator (1962).

\[
\text{G.V} = \text{Final M.D.G} \times \text{P.V}
\]

Where,

- G.V. is the germination value
- Final M.D.G. represents final mean germination, which is calculated at the cumulative percentage of full seed germination at the end of the test divided by number of days from sowing to the end of the test
P.V. is the peak value (the maximum mean daily germination recorded at any time during the test)

Viability percentage was also calculated by using the equation Germination percentage + percentage of sound ungerminated seeds. At the end of the germination period, all remaining ungerminated seeds were cut and examined and the percentage of fresh, viable and possibly viable seeds was recorded as sound ungerminated seeds.

All the germination percentages obtained were subjected to analysis of variance (ANOVA) using transformed and non transformed scales in order to study the comparisons between media, year of study, place of seed collection, effect of pretreatments and effect of storage up to three months in all the study species.

3.10.3. Shoot growth parameters

In the case of seedlings generated in standard nursery beds, and those in germination trays, growth in height was recorded after the establishment of seedlings in poly bags in the nursery. Seeds germinated directly in poly pots and root trainers do not require transplanting and hence growth measures were taken soon after completion of germination. Growth in height of seedlings, collar diameter and cumulative number of leaves produced in each species were recorded and the data obtained were statistically correlated.

While measuring the height of individual seedlings, measure was taken from collar region to terminal bud using a metre scale and expressed in centimeters. The collar diameter was measured using a digital vernier calliper at one-month interval and expressed in centimeter. The total number of leaves produced by individual seedlings was counted at thirty days interval up to three months.
The growth parameters recorded in each case were subjected to ANOVA study and the mean comparison between growing media, vigour of growth in seedlings obtained from fresh and stored seeds were performed.

3.11. Biomass production

Destructive sampling was done at monthly interval for a period of three months in each species raised using different trials to find the oven dried weight of shoot system, root system, root and shoot system together and the shoot/root ratio. The result obtained in each case such as biomass of seedlings obtained from germination of fresh seeds, biomass of seedlings obtained from germination of stored seeds, and the biomass of rooted cuttings in three different potting media for the first three months of growth was analysed statistically by Duncan’s multiple range test.

Representative seedlings from each type were sampled from each treatment at monthly intervals for estimating biomass. The shoot and root portion of the samples were dried separately in hot air oven at a temperature of 68°C – 75°C for about 24 to 48 hours. Dry weight were taken using a precision balance and expressed in grams.

3.12. Statistical Analysis:

For the present study, seeds of *Bombax ceiba, Cassia fistula, Mimusops elangii, Pongamia pinnata, Pterocarpus marsupium, Saraca asoca* and *Terminalia arjuna* collected from two distinct places and germination data were generated on fresh untreated seeds, fresh pretreated seeds and stored seeds up to three months. Seeds were sown in six different media. For statistical study and data correlation 24 seeds were used in each sowing medium.

Germination period as well as number of seeds germinated in the first day of germination and the day in which maximum seeds germinated were
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recorded separately for all the seven species. Germination percent in each trial was also determined. The experiments were repeated in three consecutive years from 2007 to 2009. In addition to this, comparative study on mean germination in different medium of sowing, different location of seed collection and seeds collected in different years was performed.

Growth of seedlings in nursery was recorded from seedlings raised from fresh and stored seeds; also propagules obtained by rooted cuttings were recorded for three months. Forty-eight seedlings and propagules was maintained in nursery in each medium, of which growth data of 24 numbers was recorded. The other 24 was maintained for replacement, whenever there was pest on disease infection or samples taken for biomass study. Growth of seedlings and propagules in three types of media such as polythene bags with potting mixture, polythene bags with coir pith and root-trainers with coir pith was performed and data were generated on a sample of 24 per media. Average height of seedlings for three months (between time intervals of 30 days), average collar diameter of seedlings and average number of leaves at different growth period was recorded. ANOVA table of growth measures such as growth in height, collar diameter and number of leaves were prepared and the level of significance between three different period of growth, medium, year and interaction between year x medium was calculated. Also comparison of growth in mean height in different media was studied.

For rooting of cuttings, 24 numbers of stem cuttings collected from one year old seedlings were used in concentration of IBA, 300ppm, 500ppm and 1000ppm respectively in all the seven study species namely Bombax ceiba, Cassia fistula, Mimusops elangi, Pongamia pinnata, Pterocarpus marsupium, Saraca asoca and Terminalia arjuna. The experiments were conducted in 4 months interval from June 2007 to October 2009, using three rooting medium
such as root-trainers with potting mixture, root-trainers with coir pith and plastic cups with coir pith, of which root-trainers with potting mixture was used as control. Data on effect of different concentration of IBA in callus production, callus and root production and their average number of adventitious roots produced was recorded. Data obtained are represented graphically.

After 8 weeks, the cuttings were evaluated for rooting percentage, mortality percentage, number of roots/cuttings and average root length. The latter two items were assessed only with successful rooting. The data obtained were subjected to factor ANOVA.

Increase in biomass during the first three months of growth in three different growing media was conducted. Biomass study of seedlings rose from fresh and stored seeds and also propagules obtained by rooting of cutting using IBA was conducted with one-month interval up to three months. Two plants with median height was collected from each potting medium and their average fresh weight, dry weight, dry weight of shoot system and dry weight of root system were obtained. Biomass studies were conducted using seedlings and propagules raised during the year 2007 to 2009. The data generated was subjected to Duncan’s multiple range test.