Chapter - 3

3. MATERIAS AND METHODS

The plants selected for the experimentation were DAHLIA, CHRYSANTHEMUM, ROSE, MUSSAENDA and AZALEA (RHODODENDRON). Potted plants were collected from the nursery of Shillong.

3.1 Plant Description

The plant materials selected for experimentation are *Dahlia*, *Chrysanthemum*, rose, *Mussaenda* and azalea (Rhododendron).

3.1.1 Dahlia (*Dahlia pinnata)*

The autumn flower *Dahlia* is a most important ornamental plant. It belongs to the family Asteraceae. It is an annual erect herb which bears a number of large coloured flower head. The flower is actually an inflorescence containing a large number of compactly arranged flowers. Now it is commercially cultivated in every place as outdoor-ornamental plants. It is one of the versatile flowers with wide range of type size and colour flowers. Now-a-days Dahlia is gaining importance for it is being widely use in cosmetic industry.

3.1.2 Chrysanthemum (*Chrysanthemum moriflolium Ramat)*

A native of China and Japan, it is one of the most popular potted flowering plants and cut flower crops in the world. It is commercially cultivated as an indoor and outdoor plant in most of the European countries, USA and East Asian countries.

It is one of the versatile flowers with wide range of type, size and colour of flowers. It is valued for its attractive flowers, which are used for garlands, and long stemmed bouquets. Further it possesses an excellent transport quality and yield satisfactory under the climatic conditions of our region. The *chrysanthemum* is amongst the earliest flowers known to have been cultivated by man and grown on a commercial scale in the states of Maharastra, Karnataka, Tamilnadu, Andhra Pradesh and some parts of Northern India (Kulkarni 1986).

3.1.3 Rose (*Rosa indica, R. alba)*

Rose is one of the earliest flowers known to man. The rose represents ultimate beauty and perfection. Roses are handsome flowering shrubs popular with gardeners in almost all parts of the world . It belongs to the family Rosaceae. Cultivated roses grow in any reasonable soil, and some kinds bloom continuously through the growing season. As a cutflower Rose is best amongst the flowers.

Not only for aesthitic purposes , but also for commercial purposes it is grown all over the world. Rose produces many important cosmetic items like talc, perfume, cream, and edible essence.

3.1.4 Mussaenda (*Mussaenda Philipiica*)

It belongs to the family Rubiaceae. It is generally cultivated as a garden ornamental plant. *Mussaenda* are branchy shrubs and bear a small flowers in association with a modification of sepal resembling a large colour petaloid flower. *Mussaenda* is commercially ideal for use as cut flowers. The plant can be successfully grown in

pots as well as in garden. Due to its attractive coloured flowers and branchy shrubs *Mussaenda* has an important role in decoration purposes and now it is raised as garden plant for commercial cultivation throughout the world.

3.1.5 Azalea (*Rhododendron conlea)*

Although all **Azaleas** and **Rhododendrons** are classed as Rhododendron by plant taxonomists, the name "Azalea" is commonly used for native deciduous species and some evergreen oriental types. it belogns to the family Ericaceae.

Azalea are branchy shrubs, usually 3-5 ft. high. In spring the flowers are showy, throughout the summer and fall the leaves add a pleasing, deep green colour to the garden. The spectacular spring flowers of azalea make them among the most popular garden shrubs.

3.2 Chemical used

	Chemicals	**Sources**
1.	Indole-3-butyric acid(IBA)	Sigma Chemical Co., USA
2.	α-Napthalene acctic acid (NAA)	Sigma Chemical Co., USA
3.	Gibberellins	
	(a) GA_3 (Gibberellic acid)	Sigma Chemical Co., USA
	(b) $GA_{(4+7)}$	Sigma Chemical Co., USA
4.	(2-chloroethyl) phosphonic acid	Sisco Research Laboratories Pvt.Ltd.
(5)	Cutting aids :	Libigos Agro-Chem. Cal.

(a) Rootex (Chemical formulation of IBA)

(b) Rootone (Chemical formulation of IBA and NAA)

6.	Alcohol	Bengal Chemicals and Pharmaceutical Ltd.

3. 3 Glasswares

Conical flasks, Beakers, Measuring cylinders, volumetric flasks, Glass-rods etc. were of Borosil brand and slides and cover slides were of Blue stur brand.

Other :

(1)	Electronic balance	(SICO, India)
(2)	Microscope	(Nikkon, Japan)
(3)	Hot air oven	(SICO, India)
(4)	Autoclave	(SICO, India)

3. 4 Preparation of chemicals

3.4.1 Indole butyric acid

1 gm of IBA was accurately weight out. The powder compound was first poured into a 1000 ml volumetric flask, then added a little amount of alcohol. After dissolving, the volume of the solution was adjusted to 1000 ml by adding 50% alcohol. This gave 1000 μg/ml stock solution from which lower concentrations (100, 250, 500 1000, 1500 μg/ml) were prepared with 50% alcohol.

3.4.2 α- Naphthalene acetic acid

The same procedure as in the case of IBA was followed for preparation of NAA solutions.

3. 4. 3 GA_3 (Gibberellic acid) solution

1 gm of Gibberellic acid (GA_3) was accurately weighed out. The powdered compound was first poured into a 1000 ml volumetric flask, then added a little amount of distilled water and heated occur a boiling water bath. After dissolving, the volume of the solution was adjusted to 1000 ml by adding distilled water. This gave 1000 µg/ml stock solution from which different concentration (100, 250, 500, 1000 and 1500 µg/ml) were prepared.

3. 4. 4 GA_{4+7}

1gm of GA_{4+7} was accurately weighedout. The powder compound was first poured into a 1000 ml volumetric flask then added a little amount of distilled water and heated over a boiling water bath. After dissolving, the volume of the solution was adjusted to 1000 ml by adding distilled water. This gave 1000 µg/ml stock solution from which different necessary concentration (100, 250, 500, 1000, 1500 µg/ml) were prepared.

3. 4. 5 (2-chloroethyl) Phosphonic acid or Ethrel

1ml of ethrel was taken in a 1000 ml volumetric flask, then added 1000ml of double distilled water. This gave 1000 µg/ml stock solution from which other necessary concentrations (100, 250, 500, 1000, 1500 µg/ml) were prepared.

3. 5 Preparation of Cuttings

Young branches with apical buds were taken as plant cutting for the treatment. The base of the cuttings were first cleaned with distilled water. Sterilized rajor blade was used for making into the cuttings of appropriate length. The excess water was then wiped out, followed by treatment with hormone solution of varying concentration rages.

3.6 Soil, pot and glassware sterilization :

soil were obtained from crop field sieved and throughly washed with tap water followed by distilled water. Sand was sterilized in a autoclave at 15 lb/inch pressure for half an hour. The earthen pots (20x25 cm) were also similarly sterilized.

All the glasswares were washed thoroughly with water to remove foreign materials attached to them. Then again washed with sterile distilled water. Then the glasswares were dried and introduced into hot-air-oven. The temperature of the oven was adjusted to 160 C and allowed to run for a period of one hour (for 3 days). Distilled water was sterilized in an autoclave at a pressure of 15 lb/sq.inch for half an hour. The sterile distilled water was used in preparation of solutions and dilution from stock solutions. Thus every possible care was taken to avoid fungal and bacterial contamination.

3.7 Method of application (Quick dip method)

Concentrated solution of the chemicals were prepared and the basal end of the cuttings (1 cm) were dipped in it for a short time (about 1 min.). Then the cutings

were immediately taken out and alcohol was allowed to evaporate. Then a layer of hormone was retained at the cut end. Cuttings were then planted in pots according to the plan of each experiment.

For the interaction treatment two chemicals like IBA and GA_3, IBA and GA_{4+7}, NAA and GA_3 and NAA and $GA_{(4+7)}$, Ethrel and IBA, Ethrel and NAA were mixed in a beaker. The basal ends of the cuttings were dipped in respective mixtures for a short time (about 1 min.). Then the cuttings were taken out and air dried the end. Cuttings were then planted in pots.

The pots were irrigated regularly. The experiment was continued for 28 day (and where necessary continued to 35 and 42 days). Number of roots per cuttings were recorded as an index of hormonal activity.

The pots were placed in the glass house where the humidity was maintained at 80%. The cuttings were exposed to PAR light 12 hr daily.

3.8 Experimental design

Five set of experiments each with 5 cuttings per pot were maintained for each individual concemtration.

One set of experiment also used for control by treating the cuttings with sterile distilled water.

3.8.1 Combined effects :

Three sets of experiments with five cuttings in each pot were used for each combination.

3.8.2 Preparation of permanent slide

Double stained permanent slides were prepared from the treated zones to examine the origin of adventitious roots in response to the treatments.

3.9 Statistical analysis of the data :

The number of roots produced in response to the treatments were counted at a regular interval of time. Mean per cent rooting of all the replications was calculated out.

The data obtained from the experiments were subjected to the Analysis of variance (ANOVA). The individual factors and their interactions were also analysed by Fisher's method of determination of variance ratios ('F' value) (Panse and Sukhatme 1989)

3.9.1 Critical difference (CD) :

The analysis of varience table gives only a broad indication of performance of the concentration and time as well as their interactions on rooting response. But in order to get the clearer appraisal of the specific *phenomenon* of the different treatment combination as well as the different level of the main factors, the

calculation of the CD was considered necessary. CD was calculated by using the following formula.

$$CD = \sqrt{\frac{\text{Error MSS} \times 2}{n}} \times \text{t value at 5\% or 1\% level for error degrees of freedom}$$

Where, n = total unit/individual unit = The actual number used for calibrating the means.

The calculated CD was utilized in testing the difference between the two mean value as significant or not.