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EFFECT OF PHYTOHORMONES ON THE PHYSIOLOGICAL AND PHYTOCHEMICAL TRAITS IN THE SEEDS OF *CEIBA PENTANDRA*

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ABSTRACT

The main aim of our present study was to test the efficiency of seeds germination of *Ceiba pentandra* plant under various circumstances, such as to algal extract of *Oscillatoria willei, Chlorella vulgaris* and synthetic hormones like kinetin and IBA (Indole butyric acid) on growth parameters of *Ceiba pentandra* seeds. The *Oscillatoria willei* had shown very excellent results at 60mg/l00ml extract when compared to the *Chlorella* species and other synthetic hormones. The present study also focus for the assessment of primary and secondary metabolities in seedlings grown in different aqueous concentration of natural algal extracts and synthetic hormones. In the present investigation, observed that the primary and secondary metabolities are rich in *Oscillatoria* at 60mg/l00ml extract concentration when compared with natural algal extract of *Chlorella* and synthetic hormones Kinetin and IBA. The present investigation, suggested the extract of *Oscillatoria* is playing the important role as the growth hormone or growth promoter and as fertilizer in *Ceiba pentandra* seeds. The further investigation is needed to study the in detail the effect of *Oscillatoria* extract on *Ceiba pentandra* seeds.

KEYWORDS: *Ceiba pentandra*, Algal extracts, Phytohormones, Synthetic hormones, Seeds germination, Phytochemical traits.

INTRODUCTION

Ceiba pentandra is a fastest growing and emergent tropical forest tree species that can grow up to 60m height belongs to the family Bombaceae native to Mexico, Central America, Caribbean region and middle east. It is commonly called as **Kapok** tree or the English name, cotton tree/silk cotton tree, is derived from the floss and is a universal trade

name. Various part of the plant are essential, used for various illness as laxative, as foliage for goat, trunk for domestic plank and wood pulp for paper (Emmanuel *et al* 2011). The bark contains a blackish mucilaginous gum which swells in water and resembles trasgacanth; it is astringent and is used in India and Malaya for bowel-complaint and West Africa for diarrhoea. It is also used for skin medicine on skin-infection, used for tooth-troubles in Senegal. The fruit is collected for the valuable kapok floss and used for stuffing pillows, mattresses and cushions. Due to its water repellent and buoyant, making it ideal for life jackets, lifeboats and other naval safety apparatus. It is an excellent material for insulating iceboxes, refrigerators, cold-storage plants, offices, theatres and aeroplanes. It is a good sound absorber and is widely used for acoustic insulation; it is indispensable in hospitals, since mattresses can be dry sterilized without losing original quality (Rex Immanuel and Ganapathy, 2007). Because of its wide range of uses, it has been cultivated in the boundaries of farmlands and social forestry plantations in India.

Kapok trees *C. pentandra* are cultivated as well as found naturally in the evergreen forests of South India. They bloom at night and flowers emit a strong odour that attracts nocturnal pollinators like bats (Thiruchenthil Nathan 2005). The extensive literature survey revealed that *Ceiba pentandra* is important medicinal plant with diverse pharmacological spectrum. The plant shows the presence of many chemical constituents which are responsible for varied pharmacological and medicinal property. The evaluation needs to be carried out on *Ceiba pentandra* in order to uses and formulation of the plant in their practical clinical applications, which can be used for the welfare of the mankind (Elumalai and Nikhitha, 2012). However, the potential of this tree species to grow and survive in the semi arid region of Hyderabad Karnataka region is not well known. Therefore, the present investigation was carried out to understand the effect of Phytohormones in the germination aspects of *Ceiba pentandra* seeds mainly to enhance the plantation and propagation of the of *Ceiba pentandra* plant.

Fig No: 1 Habit of *Ceiba pentandra*

Fig No: 2 Fruit of *Ceiba pentandra*

MATERIALS AND METHODS

The Present study was carried out in the Plant Biotechnology Laboratory, Department of Botany, Gulbarga University, Kalaburagi (Lattitude and Longitude of 17.3104^0 N and 76.8730^0 E respectively) Karnataka, India. The fruits of *Ceiba pentandra* plant were collected to obtain the seeds in May 2014 from the Gulbarga University campus. The collected and dried seeds of *Ceiba pentandra* were first sterilesed with mercuric chloride solution and later with distilled water. The different varying concentrations of 10mg, 20mg, 40mg, 60mg, 80mg and 100mg of natural algal extracts of *Oscillatoria*, *Chlorella* and synthetic hormones such as Kinetin and IBA were weighed and dissolved in 100ml of water separately, later sterilised seeds were placed over the blotting paper in the petriplates and were wetted with solutions 10mg/100ml, 20mg/100ml, 40mg/100ml, 60mg/100ml, 80mg/100ml, 100mg/100ml concentrations of *Oscillatoria*, *Chlorella* , Kinetin and IBA, totally six petriplates with ten number of seeds in each petriplate was taken and seventh petriplate with same number of seeds but without phytohormones and algal extracts wetted with only water every time serve as a control. Growth of the seeds in response to algal extracts and phytohormones was calibrated by considering percentage of seeds germination on third day, sixth day and ninth day in comparison with control.

The germinated seeds in different concentrations of algal extracts and synthetic hormones were collected separately on tenth day and were shade dried for further phytochemical estimations.

1. Estimation of total Carbohydrate (Anthrone`s method)

Reagents

2.5N Hydrochloric acid,

Anthrone reagent: M200mg of Anthrone was dissolved in 100ml of ice cooled 95% concentrated H_2SO_4.

Standard Glucose Solution: (Stock) Dissolve 100mg in 100ml distilled water.

Working Standard: 10ml of stock diluted to 100ml with distilled water and few drops of toluene was added and stored in refrigerator.

100mg of the sample was hydrolyzed in boiling water bath for 3h with 5ml of 2N hydrochloric acid and cooled to room temperature. The contents were neutralized with solid

sodium carbonate until the effervescences ceases. The volume was made to 100ml by adding distilled water and centrifuged. The supernatant was collected and used for quantitative estimation of carbohydrates. 0.5ml and 1ml of extract taken separately and the volume was made up to 1ml by adding distilled water, 4ml of Anthrone reagent was added to the same and kept the mixture in boiling water bath for 8 minutes. The content was cooled and read the absorbance at 630nm. Standard graph was plotted using glucose and the amount of carbohydrates present in sample was calculated. The standard graph drawn by plotting concentration of the standard on the X-axis versus absorbance on the Y-axis. From the graph the amount of carbohydrates present in the sample tube was calculated.

$$\text{Carbohydrate in mg/100gm} = \frac{\text{Graphical value}}{\text{Wt. of the plant material}} \times \frac{\text{volume of total extract}}{\text{volume taken for reading}}$$

2. Estimation of proteins (Lowry *et al*., 1951)

Reagents

2% $NaNO_3$ in 0.1 N NaOH -Reagent-A

0.5% ($CuSO_4$) 1% potassium sodium tartarate - Reagent-B

Alkaline Copper Solution: Mix 50 ml of Reagent A + 1ml of Reagent B prior to use (Reagent C).

Follin-Ciocalteau Reagent: Reflux gently for 10 hours a mixture consisting 100g sodium tungstate ($Na_2WoO_42H_2O$), 25g of sodium molybdate ($Na_2MoO_42H_2O$), 700ml of water, 50ml of 85% phosphoric acid 1.5 liter flask. Add 150g Lithium sulphate, 50 ml water and few drops of Bromine water. Boil the mixture for 15 minutes without condenser to remove excess bromine cool the mixture and dilute to tint. Determine the acid concentration of the reagent by titration with 1N NaOH to a phenolphthalein end point.

Protein Solution

Accurately 50mg of Bovine Serum Albumin (fraction) dissolved in distilled water and the volume was made to 50ml by adding distilled water in a standard flask.

Working Standard

10ml of stock solution was diluted to 50ml with distilled water in a standard flask, this solution contain 200µg proteins.

500mg of the powdered material was ground in pestle and mortar with a small quantity of buffer (5-10ml) and centrifuged. The supernatant was used for the quantitative estimation of proteins, 0.1ml of test solution mixed with 0.9ml of distilled water to make final volume 1ml. To this 5ml of alkaline copper reagent was added and incubated for 10 minutes at room temperature 0.5ml of Foline ciocalteu reagent (FCR) was added to the mixture thoroughly and kept in dark for 30 minutes. The absorbance of blue coloured solution was measured at 660nm. A standard graph was plotted and used to calculate the amount of protein in the sample.

$$\text{Proteins in mg/100gm} = \frac{\text{Graphical value}}{\text{Wt. of the plant material}} \times \frac{\text{volume of total extract}}{\text{volume taken for reading}} \times 100$$

3. Estimation of Lipids

Principle: Oil from a known quantity of the seeds is extracted with petroleum ether. It is then distilled off completely, dried, the oil weighed and the % of oil is calculated.

Materials

Petroleum ether (40^{o}c-160^{o}c)

Whatman No 1 filter paper

Absorbant cotton

Soxhlet apparatus

Procedure

Fold a piece of filter paper in such a way to hold the powdered seed material. Wrap around a second filter paper which is left open at the top like a thimble. A piece of cotton wool is placed at the top to evenly distribute the solvent as it drops on the sample during extraction. Place the sample packet in the tubes of the Soxhlet extraction apparatus. Extract with petroleum ether for 6 hrs without interruption by gentle heating. Allow to cool and dismantle the extraction flask. Evaporate the Pet.ether on a steam or water bath until no odour of ether remains. Cool at room temperature. Carefully the dirt or moisture outside the flask was removed and weighed the flask. Repeat heating until constant weight is recorded.

$$\text{Lipid/Oil in ground sample \%} = \frac{\text{Weight of oil(g)}}{\text{Weight of sample(g)}} \times 100$$

RESULTS

In this experiment an effort was made to see the comparative efficiency between the two natural algal extracts and two synthetic hormones. In which it was found that the seeds treated with 60mg of *Oscillatoria* extracts shows the good germination of 60% on 3rd day, 6th day as well as on 9th day of treatment equally similar and highest results were observed. The lowest germinations were observed in the seeds treated with both the synthetic hormones particularly IBA in which no growth was observed in any concentration from 20mg-100mg but only germination was observed in the seeds treated with 10mg of IBA, 60% on 3rd day, 40% on 6th day and 40% on 9th day. Later, the seeds of above performed activity treated with various extracts with different concentrations were subjected for the estimations of primary metabolites.

The maximum concentration of carbohydrates was observed in the seeds treated with the phytohormones Kinetin at concentration of 80mg/100ml was 26mg/100g, second highest concentration of carbohydrates was at concentration 60mg/100ml of *Oscillatoria* was 18mg/100g.

The maximum concentration of proteins was observed in the seeds treated with the natural extract *Chlorella* at concentration of 40mg/100ml was 6.4mg/100g, second highest concentration of proteins was in control *i.e.*, 06mg/100g.

The maximum concentration of Lipids was observed in the control *i.e.*, 0.57g/100g and second highest concentration of lipids was observed in the seeds treated with the phytohormone *Oscillatoria* at concentration 60mg/100ml was 0.32g/100g.

The results obtained from this experiment shows the algal natural extracts can be the best alternative to synthetic hormones. The algal members belonging to the class cynophyceae and chlorophyceae can be the good biofertilizers as well as good growth hormones, further research has to be carried out in this direction.

Table No 1 Effect of different concentrations of algal extracts and phytohormones on Percentage of seeds germination of *Ceiba pentandra*

	Oscillatoria			***Chlorella***			**Kinetin**			**IBA**		
Concentrations	3rd day	6th day	9th day	3rd day	6th day	9th day	3rd day	6th day	9th day	3rd day	6th day	9th day
10mg	-	-	-	-	-	-	-	-	-	60%	40%	40%

20mg	-	20%	20%	-	-	-	20%	20%	20%	-	-	-
40mg	-	-	-	20%	60%	60%	40%	20%	20%	-	-	-
60mg	60%	60%	60%	-	-	20%	-	-	-	-	-	-
80mg	-	-	-	-	-	-	-	20%	20%	-	-	-
100mg	-	-	-	-	-	-	-	-	-	-	-	-

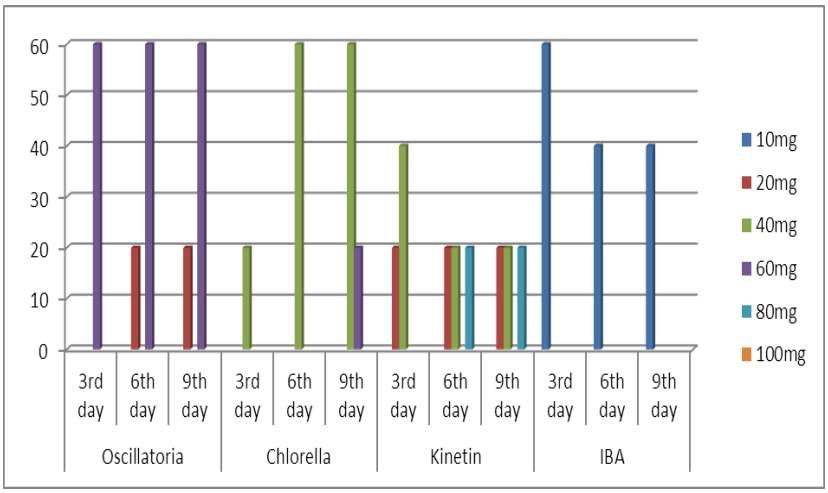

Fig: 3 Effect of different concentrations of algal extracts and synthetic phytohormones on seeds germination of *Ceiba pentandra.*

Table No: 2 Estimations of Primary metabolites in germinated seeds at various concentrations

Concentration of phytohormones/100ml of water	*Oscillatoria*	*Chlorella*	Kinetin	IBA
10mg/100ml	N.G	N.G	N.G	0.6mg/100gm
20mg/100ml	0.5mg/100gm	N.G	0.8mg/100gm	N.G
40mg/100ml	N.G	6.4mg/100gm	N.G	N.G
60mg/100ml	2.3mg/100gm	1.8mg/100gm	N.G	N.G
80mg/100ml	N.G	N.G	1.08mg/100gm	N.G

Control – 0.4mg/100g

N.G – No Germination

Table No: 3 Estimations of Carbohydrates

Concentration of phytohormones/100ml of water	*Oscillatoria*	*Chlorella*	Kinetin	IBA
10mg/100ml	N.G	N.G	N.G	N.G
20mg/100ml	12mg/100gm	N.G	0.10mg/100gm	N.G
40mg/100ml	N.G	18mg/100gm	N.G	N.G
60mg/100ml	18mg/100gm	6mg/100gm	N.G	N.G
80mg/100ml	N.G	N.G	26mg/100gm	N.G

Control – 6mg/100g

N.G – No Germination

Table No: 4 Estimations of Lipids

Concentration of phytohormones/100ml of water	*Oscillatoria*	*Chlorella*	Kinetin	IBA
10mg/100ml	N.G	N.G	N.G	N.G
20mg/100ml	0.11gm/100gm	N.G	0.12gm/100gm	N.G
40mg/100ml	N.G	0.19gm/100gm	N.G	N.G
60mg/100ml	0.32gm/100gm	0.28gm/100gm	N.G	N.G
80mg/100ml	N.G	N.G	0.27gm/100gm	N.G

Control – 0.57/100g

N.G – No Germination

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