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EFFECT OF DIFFERENT ENVIRONMENTAL CONDITIONS ON GERMINATION OF TWO MAJOR SPECIES OF LITSEA (LAURACAE) OF UTTARAKHAND HIMALAYA

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Abstract: Present investigation covers monitoring on seed germination (under the influence of different growth regulators) on two important tree species of the region, viz- Litsea elongata, and Litsea monopetala. This analysis was considered necessary since the beneficial effect of pre-soaking treatment of seeds with growth regulators and other substances have been reported in the literature repeatedly. It is now generally agreed that such a treatment leads to an increase in tissue hydration, respiratory activity, redistribution of nutrient reserves, and enhancement of the seedling growth, thus it is bound to help us in knowing the causes of the dormancy of seeds in forest tree species on the one hand and seems to be the potential approach to keep pace with new concepts and developments to meet the increasing demands of the country especially through the release of new seedling and the samplings growth in the green houses and laboratory conditions on the other. The critical survey of the literature reveals that the quality of the seeds of forest species in terms of their variability, germination and overall capacity for raising healthy seedlings would play a very important role like those of the agricultural crops, it is a general realization, however, that research with respect to the morphological aspects, of seeds the influence of different growth regulators on germination of these taxa has been carried out in a limited and sporadic manner only. As such and particularly in view of the changed emphasis on forest ecosystem, it is earnestly felt that this work should help us to draw right conclusion leading to proper orientation of the forestation programme in future.

Keywords: Growth regulators; *Litsea elongata*; *Litsea monopetala*; Seed germination; Tissue hydration. Postal Address: Sita Kuni, 195. Nagendra Saklani Marg. Tea Estate, Near Bangali Kothi, Banjarawala, Dehradun-248001, Uttarakhand, India

INTRODUCTION

The forests of the Uttarakhand Himalayas cover nearly 70% of the total land. Profoundly influence the economy of this reason by providing timber, fruits, resins, and the other Minor products keeping in mind the everincreasing demands of the common populace and due to the ever growing realization that the continuous deforestation of the trees, point towards a constant danger is loaming around the

corner, particularly for the protection of forests and forest areas. As such attempts are being geared up to enhance the tree population and production of timber on a large scale. The recent trust on the forestation programmes have provided an impetus to study various facets of seed biology because the foresters and planners are keen to adapt both mono and mixed culture of trees. As such, their interest in data on seed germination, seedling ontogeny tree growth as well as the selection of plasters hardly needs any

emphasis. Lauraceae, a large pan-tropical family mainly composed of large trees or shrubs, consists of approximately 55 genera and over 4000 species worldwide, with much of the known diversity the described from neotropics (Meissner, 1864). Trees of the laurel family predominate in the world's laurel forests, which occur in a few humid subtropical and mild temperate regions of the northern and southern hemispheres. Populations of many species are dramatically declining worldwide, but the causal mechanism remains debated among different human-related threats. Coping with uncertainty is critical to several issues about the conservation and future of biodiversity, but remains challenging due to difficulties associated with the experimental manipulation and/or isolation of the effects of such threats under field conditions. Human activity is considered as one of the major cause of genetic erosion and extinction of species on temperate Himalaya through over exploitation, habitat destruction and degradation and exotic species introduction.

The maintenance of sufficient genetic diversity, both within and among populations, is one of the main goals in conservation planning as genetic diversity provides the template for adaptation and evolution of populations and/or species. The family is largely characterized by trimerous flowers, bi- or tetrasporangiate anthers with apical valvate dehiscence, and a unicarpellate gynoecium containing a single anatropous, apically attached ovule (Cronquist, 1981; Heo *et al.*, 1998).

The recent energetic push on afforestation policy has provided a grand impulse to study certain aspects of seed biology, particularly those of the tree species; this is because the foresters and forest planners are keen to adapt the mono culture as well as mixed-culture plantations. Thus the state Forest Departments are making consumptive efforts to meet the growing requirements for wood through raising large-scale plantation of species of industrial consequence in addition to the important timbers, large quantity of fuel-wood for domestic purpose

is also needed and as such, fast growing tree are required to be cultivated. Simultaneous the production of a high percentage of viable seed germination at a speedy rate is very important in propagating such tree. The study of certain aspects of floral biology including morphology of style and stigma process of seed development and their structure, germination and establishment of the seedlings provide a useful background for understanding the Germination development. Percentage expressed as the percentage of pure seeds which produce normal seedling. This is very important, especially for the tree species, since it involves considerable expense to raise this plantation in the forest on a large scale. It has been observed that generally 25 to 30 days are required for seed germination. The seeds of several tree species germinate well at constant temperature whereas others require diurnal temperature fluctuations. The germination can be recognized by emergence from the seed for seed populations, it becomes possible to mark grades of germination ability (germinability) or capacity, which is simply the maximum percentage of which germinate under favorable seeds conditions. The main aim of the laboratory germination test is to estimate the maximum number of seed which can germinate in optimum condition. The objective of seed testing for most purposes may be narrowed down, the provisions of factual information of the field plant value of a seed lot by means of purity and germination tests. Dormancy, germination and some early events in seedling development are thought in many cases to be regulated by various hormones. Two questions might therefore be asked; what hormones are present in the seeds, do these hormones arise during development and are any of the events of the seed maturation, influenced or controlled by endogenous hormones. To answer the above questions we need only recall the seed were the first discovered higher sources of auxins, gibberellins and cytokinins, since these times it has become firmly established that both developing and mature seeds of very many

species are rich in these three hormones. Indeed it is not unlikely that seeds contain these substances to some extent or another, especially during their development. In the present work the seed germination (under the influence of different growth regulators) have been studied out on two important tree species of *Litsea* from Uttarakhand region, viz- *Litsea* elongata and *Litsea* monopetala.

This analysis was considered necessary since the beneficial effect of pre-soaking treatment of seeds with growth regulators and other substances have been reported in the literature repeatedly. It is now generally agreed that such a treatment leads to an increase in tissue hydration, respiratory activity, redistribution of nutrient reserves, and enhancement of the seedling growth, thus it is bound to help us in knowing the causes of the dormancy of seeds in forest tree species on the one hand and seems to be the potential approach to keep pace with new concepts and developments to meet the increasing demands of the country especially through the release of new seedling and the samplings growth in the green houses and laboratory conditions on the other. The critical survey of the literature reveals that the quality of the seeds of forest species in terms of their variability, germination and overall capacity for raising healthy seedlings would play a very important role like those of the agricultural crops, it is a general realization, however, that research with respect to the morphological aspects, of seeds the influence of different growth regulators on germination of these taxa has been carried out in a limited and sporadic manner only. As such and particularly in view of the changed emphasis on forest ecosystem, it is earnestly felt that this work should helps us to draw right conclusion leading to proper orientation of the forestation programme in future. Optimum germination on filter papers requires sufficient quantity of water too little creates external and internal impediments and too much can restrict oxygen diffusion in to the seed. Seeds with cracked of scarified seed coats and those with

seed coats completely removed leak more solutes than those with intact seed coats. Dormancy germination and some early events in the seedling development are thought in many cases to be regulated by various hormones. Turning attention to seed Physiology and seedling growth substances control several important processes in plants such as their germination root and shoot elongation as well as flowering and fruiting. The study of viability and germination of the seeds is essential because they impart success to tree development. Besides this, the germination pattern and seedling morphology are also to be understood and analyzed since these aspects have an important bearing on the formation of healthy and juvenile plants.

EXPERIMENTAL

The present studies have been carried out in the seeds of following two tree species of family Lauraceae.

- a) Listea elongate,
- b) Litsea monopetala.

Collection of the seed materials: Litsea elongata (Nees) Hook, is monoecious autotrophic tree bearing essential oils in which leaves are well developed and nearly always evergreen, usually alternate, gland-dotted, aromatic and simple. The seeds and flowers were collected from the mature trees growing in the Kund forest range of (Agastyamuni) Rudraprayag District, during March 2013. The seeds of Litsea monopetala were collected from the mature trees growing in Deewalikhal forest area of Chamoli District during the month of Jan.-Feb. 2013. These immature seeds were fixed in different in fixatives for anatomical studies and mature seed dried in open air for 25 days and stored at room temperature till the experimentation.

Seed weight: A total of 1000 clean seeds of each species were weighted and from this the number of seeds/Kg. was determined by employing the following formula:

No. /Kg. =
$$\frac{1000 * 1000}{\text{Weight of Thousand seeds}}$$

Moisture content: Three seed samples weighing 10 g. each in both the species were taken for calculating the moisture content of the air dried seeds. Subsequently the samples contained in glass and dried in thermostatically controlled oven for 17 h at 105°+ 2°C. Later these were cooled to room temperature and weighed again kept in oven till the weight of the samples became constant for finding out the average dry weight, the moisture content of the seeds was calculated using the following formula. (ISTA, 2008).

Moisture content = $\frac{\text{Fresh weight-oven dry weight *100}}{\text{Fresh weight}}$

Seed Germination: Three growth regulators *viz*. GA₃, IAA, NAA had been selected for the present studies. Since GA₃ and IAA have been repeatedly reported in the literature to stimulate seed germination and NAA (an ethylene derivative) has been shown to be highly effective in bringing about morphogenetic changes at various stages of plant growth. Aqueous solutions of above three growth regulators in 4 concentrations such as 10 ppm, 50 ppm, 100 ppm, 200ppm, and a control set treated with distilled water are selected for present studies. The seeds were pre-soaked for 48 hours at room temperature for 25°c in aqueous solution of different concentrations of GA3, IAA and NAA separately. Before pre-soaking, the seeds obtained from mature fruit by putting certain pressure and kept in sunlight for 15 days, but both the taxa the micropylar regions of seeds were scarred by means of a sharp- edged blade to facilitate imbibitions. As separate control set with seeds soaked in distilled water for the same duration was also arranged for comparison. Seeds were thoroughly washed with distilled water pre- soaking in different concentrations. After soaking in specified solution the seeds were kept for germination tests in Petri plates lined with Whatman's filter paper No1, and moist in different concentrations of solutions of GA3 IAA and NAA in a cabinet type thermostat constant temperature at 25°C for each set 40 seeds of Litsea elongata and 50 seeds of Litsea monopetala with three replication were tested.

The germination was scored under each treatment on each alternate day up to 25 days from the day of sowing. As a rule, the germination observations have been recorded and continued up to one month till there was no more germination.

Seedling Growth: Measurement of the hypocotyls length have been recorded one each alternate day up to the final day of the experimentation. Expansions of cotyledons have also been recorded by measuring the length of the cotyledonary leaves during the experiment for three *Litsea species*.

Statistical Analysis: All the data collected for different experiments during the study were compiled and processed for statistical treatment. The data were analyzed for the mean and standard error. Analysis of Variance (ANOVA) was used to test the significance of difference between treatment means.

RESULTS AND DISCUSSION

Influence of different environmental conditions of Growth substances on various parameters of Seed Germination

Under laboratory conditions, the seed of *Litsea* initiated germination 15-20 days after sowing in growth substances as well as in distilled water (as a control set). The germination value and germination percentages were calculated for different concentrations of the growth substances also in order to compare the vigour and speed of germination. The number of seeds per kilogram and moisture content of the study material of *L. elongata* and *L. monopetala* were recorded as 4132 and 6849 seeds/kg and moisture recorded 38.8% and 31.6% respectively.

Effect of GA₃ on hypocotyl development of *Litsea elongata*

The length of the hypocotyl was measured from the collar up to the attachment of the hypocotyl and cotyledonary leaves. As the germination gets initiated, radicle and hypocotyl growth were noticed highly influenced by the three growth regulators. The hypocotyl development of *Litsea elongata* on 15, 23, 27, 31, 35, 39, and 43 days

except 19 days were found highly significant with respect of different GA₃ concentrations. The recorded data on 19 days was found null variance under different GA₃ concentrations. The highly significant variance was developed from the treatments source, showing the influence of treatments on hypocotyl development under different concentrations of GA₃. Amongst different concentrations of GA₃, 200ppm (1.04) concentration showed most superiority (Figure 1) over the other concentrations followed by 100 ppm (0.97) while the minimum hypocotyl development was recorded under control conditions (0.80). The treatment effectiveness of 50 ppm and 100 ppm was recorded close consistent, showing the basic indifference of the concentrations.

Table-1. Morphological Characters of Flower, Ovule Seed and fruits of *Litsea*.

Ovule Ocea and naits of Ensea.			
S.No.	Parameters	Litsea	Litsea
	studied	elongata	monopetala
1.	Shape	Oval	Rounded to
			slight oval
2.	Colour	Light-	Dark-brown
		brown	
3.	Ornamentation	Smooth,	Smoth, dull,
		shining,	micropyler
		micropyler	end is flat
		end is	
		pointed	
4.	Fruit colour	Black	Black
5.	Fruit shape	Ovoid	Ovoid
6.	Fruit length	12-14	5-8mm
	(mm)		
7.	Length (mm)	13.0	5-10mm
8.	Width (mm)	8.0	6.5mm
10.	Moisture contents in seed	31.6%	42.5%

Effect of IAA on hypocotyl development of Litsea elongata

The highly significant variance was developed from the treatments source, showing the influence of treatments on hypocotyl development under different concentrations of

IAA. Amongst different concentrations of IAA, 100 ppm (1.06) concentration showed most superiority (Figure 2) over the other concentrations followed by 200 ppm (1.05) while the minimum hypocotyl development was recorded under control conditions (0.80).

Effect of NAA on hypocotyl development of Litsea elongata

Amongst different concentrations of GA₃, the maximum hypocotyl development was recorded under 200 ppm (1.12) concentration treatments which showed most superiority (Figure 3) over the other concentrations followed by 100 ppm (1.10) while the minimum hypocotyl development (0.80) was recorded under control conditions.

Effect of GA₃ on hypocotyl development of Litsea monopetala

Amongst different concentrations of GA₃, the maximum hypocotyl development was recorded under 50 ppm (2.01) concentration treatments which showed most superiority (Figure 4) over the other concentrations followed by 100 ppm (2.00) while the minimum hypocotyl development (0.95) was recorded under control conditions.

Effect of IAA on hypocotyl development of Litsea monopetala

Amongst different concentrations of IAA, the maximum hypocotyl development was recorded under 50 ppm (1.08) concentration treatments which showed most superiority (Figure 5) over the other concentrations followed by 200 ppm (1.06) while the minimum hypocotyl development (0.95) was recorded under control conditions.

Effect of NAA on hypocotyl development of Litsea monopetala

Amongst different concentrations of NAA, the maximum hypocotyl development was recorded under 50 ppm (2.01) concentration treatments which showed most superiority (Figure 6) over the other concentrations followed by 100 ppm (2.00) while the minimum hypocotyl development (0.95) was recorded under control conditions.

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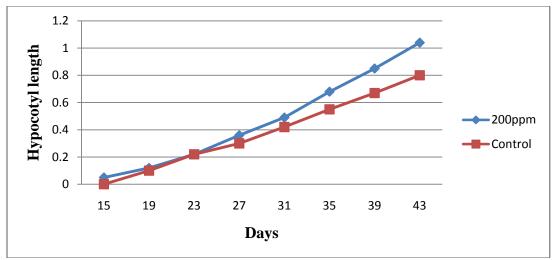


Figure 1. Effect of GA₃ (200 ppm) concentration on the hypocotyl development with respect to control in *Litsea elongata*

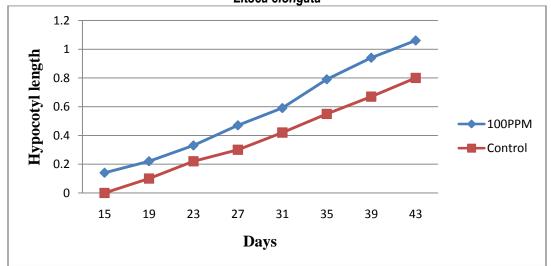


Figure 2. Effect of IAA (100 ppm) concentration on the hypocotyl development with respect to control in *Litsea elongata*

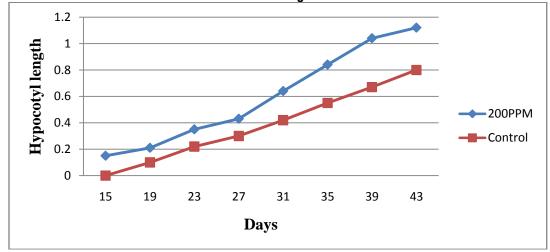


Figure 3. Effect of NAA (200ppm) concentration on the hypocotyl development with respect to control in *Litsea elongata*

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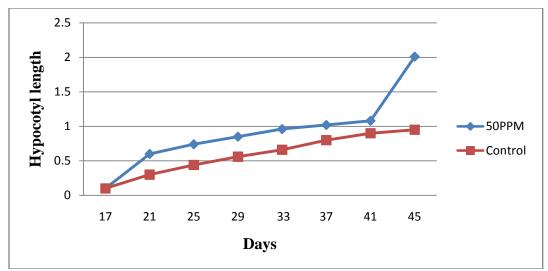


Figure 4. Effect of GA₃ (50ppm) concentration on the hypocotyl development with respect to control in *Litsea monopetala*

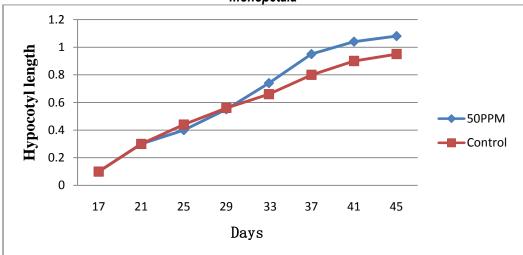


Figure 5. Effect of IAA (50ppm) concentration on the hypocotyl development with respect to control in *Litsea monopetala*

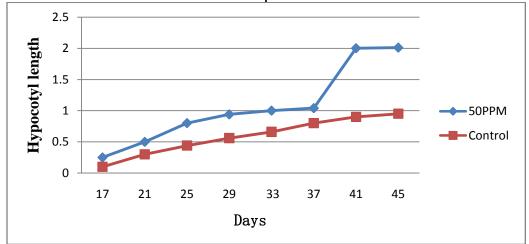


Figure 6. Effect of NAA (50 ppm) concentration on the hypocotyl development with respect to control in Litsea monopetala

Highly significant variation was revealed with respect of different GA3, IAA and NAA concentrations. The maximum hypocotyl development was recorded under 50 ppm (2.01) of GA₃ concentrations followed by 100 ppm (2.00), whereas under IAA and NAA treatment of Litsea monopetala, the maximum hypocotyl development was recorded to 50 ppm. The seed phase is the most important stage in the life cycle of higher plants as regards survival; dormancy and germination are natural mechanisms to ensure this. The seed is often well equipped to survive extended periods of unfavourable conditions, and the embryo is protected by one or several layers of other tissues. These include endosperm, perisperm, seedcoats, and fruit tissues, which protect the embryo from physical damage and nourish it (in the case of the endosperm); all contribute to spreading the seeds after abscission as these surrounding layers play an important part in the regulation of dormancy and germination. For many tree species native to the northern hemisphere, with seed maturing and dispersing in late fall to early spring, dormancy is an acquired trait to carry them over the winter conditions ready for germination next spring. Pandey et al. (2007) revealed that the people of Uttarakhand State use L. elongata and L. monopetala in ethnoveterinary practices such as stomach disorders and bone fractures. Plant bioregulators has been used extensively for initiation of rooting in cuttings of different ornamental plants. The synthetic root promoting chemicals that have been found most reliable in stimulating adventitious root production in cuttings are indole-3-butyric acid (IBA), indole-3-acetic acid (IAA) and naphthalene acetic acid (NAA). Indole-3-butric acid has been used more widely because it is non toxic to plants over a wide concentration range (Singh, 2002).

The length of the hypocotyl was measured from the collar up to the attachment of the hypocotyl and cotyledonary leaves. As the germination gets initiated, hypocotyl growths

were noticed highly influenced by the three growth regulators. The hypocotyl development of Litsea elongata was observed significant influence by the different GA₃ concentrations. Since, GA₃ have been repeatedly reported in the literatures to stimulate morphogenetic changes at various stages of plant growth. From the analysis of the result it can be known that the significant influence was developed from the different concentrations of GA3 treatments. When the affectivity of different concentrations of GA3 was considered 200 ppm (1.04) concentration showed most superiority over the other concentrations while the minimum hypocotyl development was found under control conditions (0.80). The treatment effectiveness of 50 ppm and 100 ppm was recorded close consistent. showing the basic indifference of the concentrations. The hypocotyl development was found significant influence with the treatment of arowth hormone IAA under different concentrations. The highly significant influence was observed from the treatments of varied concentrations of IAA. Amongst the of IAA, 100ppm concentrations (1.06)concentration showed most superiority over the other concentrations. The influence of NAA on hypocotyl development of Litsea elongata was found insignificant. The maximum hypocotyl development was shown under 200 ppm (1.12) concentration treatments which showed the consistency of influence with the GA₃ treatment. When all the three growth regulators were examined, NAA can be considered as the most influential to the hypocotyl development of Litsea elongata.

The hypocotyl development of *Litsea* monopetala was found highly influenced by GA₃ treatment. As far as the effect of concentrations of GA₃ was discussed, 50 ppm of GA₃ which found the most effective (2.01) can be consider the most appropriate concentration for the treatment of *Litsea* monopetala seeds to obtain the maximum hypocotyl development. The treatment with IAA hormone to the seeds of

Litsea monopetala had been observed a significant influence on hypocotyl development. Amongst different concentrations of IAA, the most suitable concentration; 50 ppm (1.08) can be used to enhance the hypocotyl development in Litsea monopetala. The effect of NAA to the hypocotyl development of Litsea monopetala was being observed highly influence. 50 ppm (2.01) concentration showed most effective when different concentrations were examined, which the value increased more than double from the control value (0.95). The rate of influence by hormones GA3 and NAA was found consistent which concludes that both the treatments can be used to enhance the hypocotyl development of Litsea monopetala. The findings support the report of Chauhan et al. (2009) who studied the influence of various concentrations of growth regulators i.e. GA3 and IAA in Black gram and Horse gram.

CONCLUSION

Three growth regulators viz. GA₃, IAA, NAA has been selected for the present studies. Since GA₃ and IAA have been repeatedly reported in the literature to stimulate seed germination and NAA (an ethylene derivative) has been shown to highly effective in bringing morphogenetic changes at various stages of plant growth. The hypocotyl development of Litsea elongata was found highly significant with respect of different GA3, IAA and NAA concentrations. Amongst different concentrations of GA₃, 200 ppm (1.04) concentration showed most superiority over the other concentrations followed by 100 ppm (0.97). Under IAA treatment, 100 ppm (1.06) concentration was found most appropriate followed by 200 ppm (1.05) while under NAA treatment the maximum hypocotyl development was recorded under 200 ppm (1.12) concentration followed by 100 ppm (1.10). Highly significant variation was revealed with respect of different GA3, IAA and NAA concentrations. The maximum hypocotyl development was recorded under 50 ppm (2.01) of GA₃ concentrations followed by 100 ppm (2.00), whereas under IAA and NAA treatment of *Litsea monopetala*, the maximum hypocotyl development was recorded to 50 ppm. In the last but not least it can be concluded that the protocols must be developed so as to rescue them from extinction. Further studies must be conducted on its silviculture in order to fast track the need to have more and better quality of seedlings.

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