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Pre-sowing seed treatment of carrageenan oligomers promotes seed germination of *Eucalyptus citriodora* Hook

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Abstract
Seed germination is a vital event in the life cycle of a plant which determines its efficiency of survival. Irradiated carrageenan (ICR) is considered as gene regulator acting as signal molecules of the plant. *Eucalyptus* is one of the important essential oil-bearing plants and its oil is extremely valuable in medical and cosmetics applications. The study was aimed to find out the efficacy of best soaking concentration of ICR on the germination of *Eucalyptus*. The seeds were treated with aqueous solution of different concentrations *viz*. un-irradiated carrageenan (UCR) of 30 mg L^{-1} and four aqueous concentrations of ICR (irradiated at 250 KGy) *viz*. 30, 60, 90 and 120 mg L^{-1}. Double distilled water (DDW) used as the control. The effect of ICR was found significant for the germination. Out of five tested concentrations of ICR, 60 mg L^{-1} proved optimum for the seed germination of *Eucalyptus*.

Keywords: *Eucalyptus citriodora* hook, irradiated carrageenan, seed germination

Introduction

Germination is an important stage in the life cycle of each plant, which controls the population dynamics with major sensible implications (Ashraf and Foolad 2005; Ali *et al*., 2010; Ali *et al*., 2014, 2021) [3, 4]. Various plant growth promoters like gibberellins and indole acetic acids and other chemical agents like KNO_3 are advocated to intercept dormancy and enhance germination. *Eucalyptus citriodora* Hook., preferred for high oil content, provides the potential for fast establishing high oil-yielding plantations. High seed germination rate counts for successful propagation of a plant. Therefore, one of the important aspects of crop cultivation is the potential seed germination. Factually, viability of commercially available *Eucalyptus* seeds is extremely variable; even the germination percentage of seeds from reliable sources in India is usually around 30-50%. It may be as low as 5-10%, if seeds were procured from local source (Weiss, 2002) [28]. Poor emergence of *Eucalyptus* species and delayed full emergence are serious limitations, not only in achieving economical seed usage however conjointly in avoiding the extra production prices of 'pricking in'.

Carrageenan is a mixture of water-soluble, linear, sulfated galactans, gel-forming, viscosifying polysaccharides that are obtained commercially by extraction from different species of red seaweeds (Rhodophyceae). Recently, degradation by radiation processing of the polysaccharides has gained much attention due to its technological effectiveness in producing oligomers of small molecular mass (Naeem *et al*., 2020, Naeem *et al*., 2021a, b, c) [19, 20, 21]. The purpose of this research was to determine if the ICR could be exploited for improving seed germination efficiency and seedling performance of *Eucalyptus citriodora*. To overcome this situation, the author hypothesized whether the seed germination in Eucalyptus could be enhanced by using aqueous solutions of pre-soaking of seeds of irradiated carrageenan.

Materials and Methods

Carrageenan material was purchased from Sigma-Aldrich, USA. The material was sealed in an exceedingly glass ampule with atmospheric air. It had been unbroken in water for swelling over night at room temperature to obtain 4% aqueous solution. Then, the carrageenan solution was stirred for many hours till the contents were fully dissolved. The samples of carrageenan were irradiated using Co-60 Gamma Chamber, GC-5000 at the BARC, Mumbai, India, at the dose rate of 2.4 kGy/h. The samples were exposed at a total dose of 250 kGy. Various aqueous concentrations of irradiated carrageenan (ICR), *viz*. ICR 30, 60, 90 and 120 mg L^{-1}, at the side of deionized water (control) and un-irradiated carrageenan (UCR; 30 mg L^{-1}), were finally prepared using double distilled water for spray treatment and utilized for this study.

The seeds procured from the Central Institute of Medicinal and Aromatic Plants (CIMAP), Bangalore, India, were soaked in the Petri dishes. The healthy seeds of uniform size were surface-disinfested in 0.1% $HgCl_2$ for five minutes followed by an intensive removal with sterilized double-distilled water (DDW). This practice was done 5-6 times to ensure that no residual of Hg remained adhering to the seed surface. Each treatment was replicated five times. The pre-sterilized Petri dishes were lined with filter paper, moistened from below with sterilized cotton pads. The seeds were treated with double distilled water served as control and aqueous solution of un-irradiated carrageenan (UCR) at 30 mg L^{-1} concentrations. In addition to that four different aqueous solution of ICR (irradiated at 250 kGy) *viz*. 30, 60, 90 and 120 mg L^{-1} were also used as seeding treatment. Five mL each of DDW, UCR and different ICR solutions were added to each Petri dish on 1st, 3rd, 5th, 7th, 9th, 11th and 13th day of treatment. Petri dishes were transferred to a dark, using a BOD incubator, maintained at temperature of 25±2ºC and 70% RH, and the seeds were allowed to germinate. Germination was recorded every day for consecutively 15 days. The seeds were considered to have germinated when the emergent radical was approximately 2 mm long. Finally, the samplings were done at seedling stages.

Seed viability was calculated by Tetrazolium viability test in keeping with following formula.

Viability (%) = (number of viable seeds / number of total seeds per treatment) ×100

Germination potential of the eucalyptus seeds was calculable in accordance with the International Rules for Seed Testing (ISTA, 1999) [14]. Germination percentages, using five replicates of twenty five seeds, were determined by putting the seed samples in ninety mm Petri dishes on filter papers (Whatman No. 1) moistened with five mL of distilled water. Enclosed pet dishes were placed in incubator at 25±2°C with twelve hours photoperiod by fluorescent light illumination. Germination in terms of radical emergence (at least 2 mm) was assessed every day from the second day till no additional body structure (radical) emergence was noted on two successive days.

Final germination percentage was expressed as GP (%) = (n/N) ×100

Where, n: the final number of germinate seeds, N: the total number of seed per treatment

After five days, germination energy was calculated during the germination tests. This indicates the uniformity of the seed germination and germination rate.

The germination energy was calculated by using the formula by (Wang and Sang, 2008)

GE (%) = (n/N) ×100

Where, n: the number of germinated seeds after the appointed time, N: the total number of seed per treatment

The speed of germination was calculated by using the formula given by (Maguire, 1962)

GS (%) = (n/t) ×100

Where, n: the number of seeds newly germinated at time t, t: the number of days from soaking to the nth count

The moisture content in fresh seed samples was calculated by employing the formula:

Moisture content = [(fresh weight of seed – dry mass of seed) / fresh mass of seed] × 100

Two weeks after presoaking, total length of seedling, root and shoot length, seedling fresh and dry weight and seedling vigour index I and II were recorded. Healthy seedlings were taken at the end of the experiment. Total length of seedling was measured from tip of the primary root to apex of hypocotyls with the help of scale and expressed in millimeters. From the germination test, normal seedlings were selected randomly in each treatment of all the replications on 15th day. The root length was measured from the tip of the primary root to base of hypocotyl and mean root length was expressed in millimeters. The standard seedlings used for shoot length measurement, were also used for the measurement of shoot length. The shoot length was measured from the bottom of the first leaf to the base of the hypocotyl and mean shoot length was expressed in millimeters.

The same seedlings preferred for fresh weight. The weights of seedlings were measured and mean weight was calculated and expressed in milligrams. The same normal seedlings selected for shoot and root length measurement were put in butter paper pocket and kept in an oven maintained at 75 ± 2°C for 24 hours. After drying, the seedlings were kept in desiccators for one hour cooling. The weights of dried seedlings were recorded and mean weight was calculated and expressed in milligrams. Vigour index is an associate degree index combining the seedlings growth with seed germination ability. The seedling vigour index (SVI) I was calculated by adopting the strategy recommended by Abdul-Baki and Anderson (1973) and expressed in number by using the following formula:

Seedling vigour index I = Germination (%) x (shoot + root length)

The seedling vigour index (SVI) II was calculated by using the following formula:

Seedling vigour index II = Germination (%) x seedling dry weight (root + shoot)

Each Petri dish was treated as one replicate and all the treatments were replicated five times. The information was analyzed statistically using SPSS-17 statistical software (SPSS Inc., Chicago, IL, USA). Mean values were statistically compared by Duncan's Multiple Range Test (DMRT) at *p<0.05 % level using different letters.

Results

The effect of pre-soaking treatment on the viability of seeds was found statically insignificant (Table 1). The effect of pre-soaking treatment on the percentage of germination was found significant. Treatment T_4 (ICR-60) recorded significantly higher germination (13.7 %) compared to control. The effect of pre-soaking treatments was statistically significant on the germination energy. Treatment T_4 (ICR-60) recorded significantly higher germination energy (13.3 %) compared to control (Table 1). The speed of germination as influenced by pre-soaking treatments was found statistically insignificant (Table 2). The effect of pre-soaking treatments on the moisture content was found significant. Pre-soaking of seeds with treatment T_4 (ICR-60) recorded 8.4 % higher moisture content compared to control. The effect of pre-soaking treatments of ICR was significant on the seedling fresh weight. Treatment T_4 (ICR-60) gave 44.4 % higher seedling fresh weight compared to control (Table 2).

Table 1: Effect of pre-soaking seed treatment of different concentrations of aqueous solution of irradiated carrageenan on seed viability percent and germination percent and germination energy of *Eucalyptus citriodora* Hook. Means within a column followed by the same letter (s) are not significantly different ($p \leq 0.05$). The data shown are means of five replicates ± SE.

Treatments	Viability (%)	Germination (%)	Germination energy (%)
T 1 (Control: DDW)	94.00 ± 1.06^a	69.50 ± 0.76^c	64.00 ± 1.05^b
T 2 (UCR-30 mg L^{-1})	94.00 ± 1.09^a	70.20 ± 0.83^c	65.50 ± 1.08^{ab}
T 3 (ICR-30 mg L^{-1})	95.00 ± 1.00^a	74.40 ± 0.79^b	67.80 ± 1.04^{ab}
T 4 (ICR-60 mg L^{-1})	96.00 ± 1.21^a	79.00 ± 0.87^a	72.50 ± 1.12^a

T 5 (ICR-90 mg L^{-1})	95.00 ± 1.10^{a}	78.10 ± 0.87^{a}	71.30 ± 1.06^{ab}
T 6 (ICR-120 mg L^{-})	95.00 ± 1.11^{a}	76.80 ± 0.88^{ab}	70.10 ± 1.03^{ab}

UCR= Unirradiated carrageenan ICR=Irradiated carrageenan

Table 2: Effect of pre-soaking seed treatment of different concentrations of aqueous solution of irradiated carrageenan on speed of germination, moisture content and seedling fresh weight of *Eucalyptus citriodora* Hook. Means within a column followed by the same letter (s) are not significantly different ($p \leq 0.05$). The data shown are means of five replicates ± SE.

Treatments	Germination speed (%)	Moisture content (%)	Seedling fresh weight (mg)
T 1 (Control: DDW)	3.20 ± 0.20^{a}	14.85 ± 0.29^{c}	45.10 ± 0.73^{e}
T 2 (UCR-30 mg L^{-1})	3.22 ± 0.16^{a}	14.98 ± 0.31^{bc}	50.40 ± 0.88^{d}
T 3 (ICR-30 mg L^{-1})	3.27 ± 0.15^{a}	15.45 ± 0.23^{abc}	55.16 ± 0.80^{c}
T 4 (ICR-60 mg L^{-1})	3.33 ± 0.17^{a}	16.10 ± 0.23^{a}	65.10 ± 0.86^{a}
T 5 (ICR-90 mg L^{-1})	3.31 ± 0.16^{a}	15.80 ± 0.22^{ab}	62.00 ± 0.98^{b}
T 6 (ICR-120 mg L^{-1})	3.29 ± 0.17^{a}	15.70 ± 0.30^{abc}	59.90 ± 0.83^{b}

UCR= Unirradiated carrageenan ICR=Irradiated carrageenan

Table 3: Effect of pre-soaking seed treatment of different concentrations of aqueous solution of irradiated carrageenan on seedling dry weight, total seedling length, length of shoot and root of *Eucalyptus citriodora* Hook. Means within a column followed by the same letter (s) are not significantly different ($p \leq 0.05$). The data shown are means of five replicates ± SE.

Treatments	Seedling dry weight (mg)	Total seedling length (mm)	Shoot length (mm)	Root length (mm)
T 1 (Control: DDW)	09.00 ± 0.33^{d}	20.70 ± 1.37^{d}	18.58 ± 0.61^{e}	2.12 ±0.25^{b}
T 2 (UCR-30 mg L^{-1})	10.08 ± 0.32^{cd}	23.27 ± 1.38^{cd}	20.92 ± 0.53^{d}	2.35 ± 0.27^{ab}
T 3 (ICR-30 mg L^{-1})	11.03 ± 0.31^{bc}	25.94 ± 1.25^{bc}	23.34 ± 0.57^{c}	2.60 ± 0.22^{ab}
T 4 (ICR-60 mg L^{-1})	12.84 ± 0.36^{a}	32.60 ± 1.25^{a}	29.60 ± 0.60^{a}	3.00 ± 0.20^{a}
T 5 (ICR-90 mg L^{-1})	12.40 ± 0.37^{b}	31.10 ± 1.36^{a}	28.23 ±0.56^{ab}	2.87 ± 0.27^{ab}
T 6 (ICR-120 mg L^{-1})	11.98 ± 0.35^{ab}	29.33 ± 1.22^{ab}	26.59 ± 0.62^{b}	2.74 ± 0.20^{ab}

UCR= Unirradiated carrageenan ICR=Irradiated carrageenan

Like seedling fresh weight, dry weight was also affected by pre-soaking treatments significantly. Pre-soaking of seeds with T_4 (ICR-60) gave 42.7 % higher seedling dry weight compared to control (Table 3). The pre-soaking treatments also affected significantly the total length of seedling. Treatment T_4 (ICR-60) recorded significantly 57.5% higher length as compared with control. The effect of pre-soaking treatments on shoot length was statistically significant. Pre-soaking of seeds with T_4 (ICR-60) recorded 59.3 % higher shoot length compared to control (Table 3). The root length of *Eucalyptus citriodora* seedlings was also affected significantly by the pre-soaking treatments. Pre-soaking of seeds with T_4 (ICR-60) gave 41.5 % higher root length compared to control (Table 3). The effect of pre-soaking of seeds on the seedling vigour index I was significant. Treatments T_4 (ICR-60) recorded 79.0 % higher vigour index I as compared to control (Fig. 1). Like vigour index I, vigour index II was also influenced by pre-soaking treatments significantly. Pre-soaking of seeds with T_4 (ICR-60) gave 62.2 % vigour index II compared with control (Fig. 2).

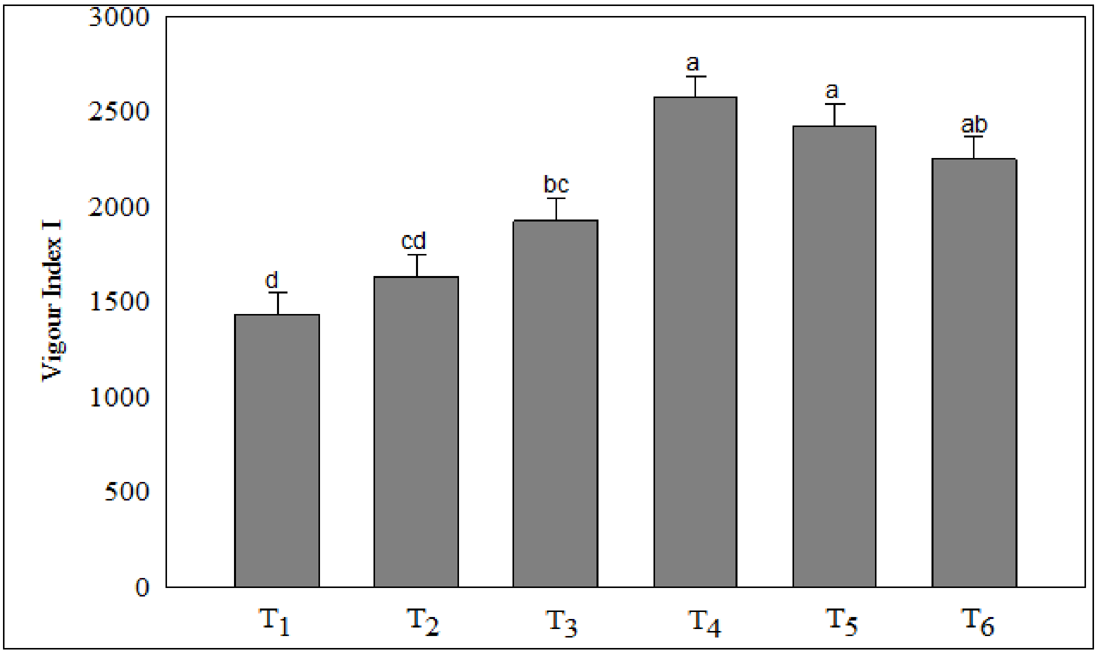

Fig 1: Effect of pre-soaking seed treatment of different concentrations of aqueous solution of irradiated carrageenan (ICR) on vigour index I of *Eucalyptus citriodora* Hook. Bars showing the same letter (s) are not significantly different at $p \leq 0.05$ as determined by Duncan's multiple range test. Error bars (┬) show SE.

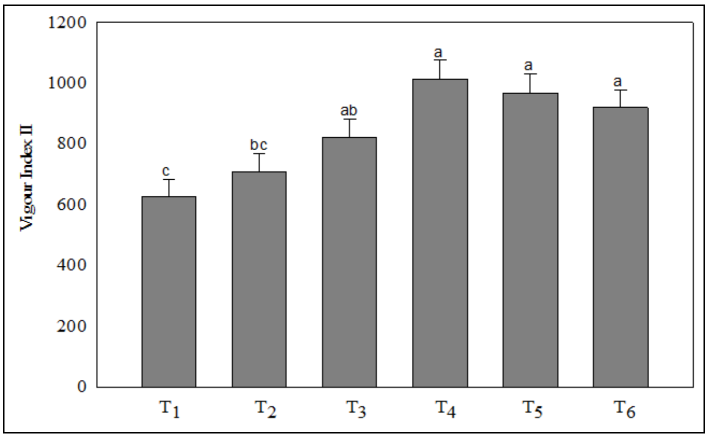

Fig 2: Effect of pre-soaking seed treatment of different concentrations of aqueous solution of irradiated carrageenan (ICR) on vigour index II of *Eucalyptus citriodora* Hook. Bars showing the same letter (s) are not significantly different at $p \leq 0.05$ as determined by Duncan's multiple range test. Error bars (┬) show SE.

Discussion

Seed germination establishes the survival of most plant species. A rise in germination and increased seedling vigor obviously resulted in the establishment of good and healthy crop with increased productivity. Factually, a uniform seed germination and the speed of root and shoot development ensure increased yield per unit area as well as quality-seed production (Black *et al*., 2000; 2006) [5, 6]. Increase in seed quality attributes may be due to enlarged embryos, superior rate of metabolic activity and respiration and optimal utilization and mobilization of metabolites to growing points (Taiz and Zeiger, 2006) [25]. Bio-regulators, when used in appropriate concentration, either as pre-soaking or pre-sowing treatment or as foliar spray treatment, influence the plant architecture in a typical manner. Pre-soaking seed treatments, i.e. the aqueous solution of different concentrations (30-120 mg L^{-1}) of ICR stimulated significantly all the germination parameters seed germination parameters including viability percentage, germination percentage, germination energy, germination speed, moisture content, seedling fresh and dry weights, length of seedling, shoot and root length of seedling, and seedling vigor index (I and II).

Application of the irradiated natural polysaccharides stimulated the plant biological activity remarkably in terms of promotion of seedling growth. All the oligosaccharides were almost uniformly effective in stimulating the seedling growth (Guan *et al*., 2009; Ali *et al*., 2014; 2021; Naeem *et al*., 2021c) [1, 10, 21]. It is fascinating to note that the seeds pre-soaked with solutions of ICR, compared to those pre-soaked in water, showed improved seedling growth, indicating phytohormones-like action of the irradiated polysaccharides. This variation in treated and untreated seeds might be due to alterations in physiology of embryos and synthesis of specific germination enzymes that hastened the overall developmental processes after pre-soaking treatment with irradiated polysaccharide. The applied treatment not only accelerated the seed germination rate and time but also improved the seedling vigor as indicated by higher shoot and root length and increased seedling fresh and dry weights. Enhanced seedling fresh and dry weights might be due to increased cell division within the apical meristem of seedling roots, which might have caused the increase in plant growth.

The increase in seedling vigor-index and seedling dry-weight was presumably due to improved germination rate and root and shoot lengths of the seedlings. These results are in conformity with those reported by Yonemoto *et al*., (1993) who argued that oligo-alginate had superior effect on shoot elongation of, tobacco grains, and rice. Similarly, Tomoda *et al*., (1994) [26] observed that the germination and root growth rates of barley were positively affected by a supplement of oligo-alginate. Wheat seeds soaked with solution of chitosan polymer (10-20 kDa) or of its oligomers (2-4 kDa) at a concentration of 50 mg L^{-1} each for 3 to 18 hours, significantly improved the stem and root lengths of seedlings as recorded six days as reported by Krivtsov *et al*. (1996) [16]. Similarly, promotive effect of chitosan on the germination of soybean seeds was reported by Lee *et al*., (1999). Further, Iwasaki and Mastubara (2000) also reported that unsaturated alginate oligosaccharides mixture promoted the root growth of lettuce seedlings. The results are also in agreement with those of Hien *et al*., (2000) [11], who observed that dry matter of peanut shoot was significantly increased due to application of 100 mg L^{-1} of degraded alginate. Similarly, Yue *et al*., (2001) [30] reported that treated maize seeds with chitosan solution resulted in a positive effect on endogenous hormone contents in germinating seeds and seedling leaves; it promoted the seedling growth.

A similar chitosan-mediated promotive effect has been reported by Chandrkrachang (2002) [7] on the rate of seed germination regarding cucumber (*Cucumis sativus* L.), chili (*Capsicum annuum* L.), pumpkin (*Cucurbita maxima* L.) and cabbage (*Brassica oleracea* L.). In the same way, Sui *et al*., (2002) [24] found that the seeds coated with small molecular weight oligomers of chitosan showed positive effects on seed germination index, growth of seedlings and root length with regard to rapeseed (*Brassica chinensis*), c.v. Aikangqin. Likewise, application of 493 kDa chitosan oligomers improved vegetative growth of soybean sprouts (No *et al*., 2003) [23]. The present results are also in agreement with those of Hu *et al*., (2004) [12]. They tested the effect of various concentrations of alginate-derived oligosaccharides (ADO) were tested on the maize seed germination. They found that all treatment markedly enhanced the growth promotive ability in roots and shoot. They also suggested that ADO probably improved germination via promotion of the amylase activity and acceleration of the metabolic activities of the seeds.

According to, Wisniewska-wrona *et al*., (2007) [29] all the tested chitosan forms stimulated the germination parameters of radish seeds; the chitosan oligomers applied at 0.01% proved most advantageous, which increased the seedling length and the germination percentage considerably as compared to the control. Similar observation was reported by Cho *et al*. (2008) [8], who found that application of oligo-chitosan (476 kDa) increased the total weight, length and thickness of sunflower hypocotyls as compared with the control. These findings lend support from the results obtained by Idrees *et al*. (2012) [13], who observed that pre-soaking the seeds in an aqueous solution of irradiated sodium alginate (ISA) at 80 mg L^{-1} significant improved various seed germination parameters, *viz*. germination percentage, seed viability and relative water content of seed of *Foeniculum vulgare* Mill. (fennel).

El-Mohdy (2013) [9] reported that radiation-induced oligomers of sodium alginate (low molecular weights), improved the seed germination percentage and the seedling emergence rate in case of faba bean (*Vicia faba*) due to pre-soaking seed treatment with oligo-alginate solution, as compared with the control. He concluded that the seedling emergence rate increased with decreasing the molecular weight of alginate oligomers. Similarly, the results of Chao *et al*., (2013) and Ali *et al*., (2021) [2] indicated that the seeds of pepper (*Capsicum annuum* L.) and *Eucalyptus citriodora* Hook., respectively, when soaked in different aqueous solutions of irradiated chitosan enhanced the germination rate.

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this manuscript.

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