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Effect of seed encapsulation on germination and seedling quality of *Santalum Album* L.

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Abstract
Seed encapsulation technique has a significant impact on germination and seedling quality in forestry crops. *Santalum album* is one of the tropical trees of commercial value that is being raised in large scale for raising plantations in both forested and non-forest areas. It is imperative to overcome the bottlenecks in sandal seed such as seed dormancy, prolonged germination period and poor plant establishment. Encapsulation of GA_3 pretreated sandal seeds using different concentrations of sodium alginate such as 2, 4, 6 and 8% was tried and found that both 6 and 8% concentrations of Sodium alginate encapsulation helped improve germination capacity significantly to 60 and 55% respectively compared to control (38.2%). The Seed vigour index (SV) for the 6 and 8% Sodium alginate concentrations were 853 and 732 while the Sturdiness Quotient (SQ) were observed to be 5.72 and 4.92. However the Chlorophyll Stability Index (CSI) did not vary significantly among the treatments. It was evident that encapsulating pretreated Sandal seeds helps quality planting stock production.

Keywords: Sandal, encapsulation, sodium alginate, germination, seedling vigour index

Introduction
Sandal (*Santalum album* L.) a member of the family *Santalaceae*, is one of the most economically important tree species occupying a prominent position in Indian forestry. Sandalwood is one of the highly priced aromatic wood employed in essential oil extraction for perfumery, soap making, carving and medicinal purposes apart from religious and cultural utility (Subasinghe, 2013) [22]. For more than 5000 years, India has been the traditional leader of sandalwood oil production for perfumery and pharmaceuticals. According to a The Hindu news report, 52 tonnes of Sandalwood was sold at a remarkable price of 40 crore rupees in an auction held during 2015 at Marayoor by Kerala Forest Department. Arun Kumar *et al*. (2016) [4] reported that the cost of Indian Sandalwood oil that is extracted from the heartwood fetches US$5000 per kilogram in the international market as per the Tropical Forestry Services in Western Australia. Current sandalwood and sandal oil prices in India are indicated at Rs. 12.00 lakh/ton and Rs. 22,000/kilogram respectively. The price of Indian Sandalwood of good heartwood class at present is over Rs.6500 per kilogram and that of oil is over Rs.1,23,000 per kg, while the price at the international market is about 15 to 20 % higher than the domestic market (Gowda, 2011) [8].

Sandal is housed in the deciduous forests of South India. In India, the principal sandal tracts are most parts of Karnataka and adjoining districts of Maharashtra, Tamil Nadu and Andhra Pradesh It grows in a wide range of soils but is most common in sandy soils associated with laterites or rocky red soil zones. Over 70 years ago, nearly 90% of the natural sandalwood populations occurred in the southern part of Karnataka and northern part of Tamil Nadu. Owing to extensive changes in the land use patterns, habitat destruction and indiscriminate exploitation of the sandal resources for generations, the natural stock of the species has been rapidly dwindling in the country (Arun Kumar *et al.*, 2012) [3]. As of 2014, India has 20,725 ha of sandalwood plantation, but it could be a decade or more before the country can harvest any of it as the sandalwood plants are still very young. In the current scenario sandal is in the IUCN RED list, enlisted in the vulnerable category and hence regeneration of sandal is given crucial attention.

Excessive harvesting without replenishment of this invaluable resource has substantially reduced the turnover of sandalwood industry, resulting in global shortage and soaring of market prices. Sandalwood populations are now sparse and devoid of larger girth classes and

mature trees have been nearly vandalized. At this juncture, the policy of the Government of Tamil Nadu to abolish their monopoly on sandalwood has generated interest in public and private sectors to raise sandalwood plantations. The Tamil Nadu Forest has also undertaken efforts to aid regeneration of Sandal in the forest areas in addition to encouraging sandal cultivation outside forest areas inorder to revive the dwindling sandal populations, enhance stocking in the natural forest tracts and sustainable availability of sandal wood. However the complex, ecological factors that play a role in the establishment and growth of sandal is a challenge. Sandal, though the seeds are slightly larger, inherent problems of dormancy and need for host plant for nursery establishment renders seedling raising a cumbersome process. Besides, at nursery stage damping off of juvenile planting stock is a serious problem that needs to be overcome. Seed treatment is a very important tool to improve seed germination and vigour. It has also helped to increase the yield of many different crops by providing protection from pre and post emergent insects and diseases and ensures better establishment of seedlings across varied environmental conditions. Artificial seed production by encapsulating meristematic tissues in alginate beads has been practised since years, however the problems encountered in this method are many (Redenbaugh, 1990) [17]. True seed replacing meristematic tissue inside the hydrogels have been found to result in satisfactory seed beads (Pazderu and Koudela, 2013) [16]. Hence, in this study, the effect of seed encapsulation technique on the germination capacity, seedling quality and chlorophyll stability index in *Santalum album* has been evaluated with scope for minimising seed loss and for improving production of quality sandal seedlings.

Materials and Methods

Fruit collection and processing

Mature dark purple fruits of *Santalum album* (Sandal) were collected from 22 trees in Forest campus, Coimbatore and bulked into one seedlot. The fruits were soaked in water for about an hour and scrubbed off the pulp thoroughly by manual sand abrasion to extract the seeds. Washed and dried the seeds under shade (30±1 °C; RH 65 ± 2%) for two days.

Seed Moisture Content

The seeds were tested for moisture content on fresh weight basis by oven dry method (ISTA, 1999) [9]. About 5g of seed samples were taken in triplicates and determined the exact wet weights. The seeds were dried in a hot air oven at 103°C for 17 hours. The sample seeds were then cooled in desiccators and the dry weight was found. The moisture content was calculated using the following formula,

$$\text{Moisture content (\%)} = \frac{\text{(Initial weight - Dry weight) g}}{\text{Initial weight (g)}} \text{ x } 100$$

Seed pretreatment

Sandal seedlot was soaked in GA_3 500 ppm for 24 hours. The GA_3 treated seeds were divided into two sublots- (1) for control (2) for encapsulation.

Seed encapsulation

For seed encapsulation, 2, 4, 6 and 8% (w/v) of sodium alginate solution was prepared with distilled water which was used as the complexing agent. Calcium chloride solution (2% w/v) was used as gel matrix. Both the gel matrix and complexing agent were sterilized by autoclaving at 15 lb pressure at 121˚c for 15 minutes. Encapsulation was accomplished by mixing the seed into the sodium alginate solution for 2 minutes. Seed along with alginate solutions were pipetted out using a broadly cut tip of the micropipette and dropped individually into calcium chloride solution to form the encapsulation. The seeds were allowed to stand in the gel matrix for at least 30 min to polymerize the beads. The bead containing the entrapped seeds were retrieved from calcium chloride solution and washed 2-3 times with sterilized distilled water. The encapsulated seeds for each percentage of alginate-gel matrix combinations were tested for germination

Germination Test

Germination test was conducted as per ISTA rules (1999) [9]. Seed germination percentage was determined by sowing 25 seeds in 4 replications in sand beds with regular watering once a day. The germination test was completed on the 30th day of the test beginning from appearance of first sprout.

Morphological studies

The root length, shoot length, total plant height and collar diameter were measured at the end of the germination period. Shoot length & Root length was taken by measuring scale and expressed in centimeters. Collar thickness was measured using Mitutoyo Digimatic Vernier Caliper and expressed in millimeters.

Seed Vigour Index

Seed Vigour Index is calculated by determining the product of germination percentage and seedling length of the same seed lot. The seed lot showing the higher seed vigour index is considered to be more vigorous (Abdul-Baki and Anderson, 1973) [1].

Sturdiness Quotient

Based on the ratio between seedling height and collar diameter the Sturdiness Quotient of the seedling was derived.

$$\text{Sturdiness Quotient} = \frac{\text{Height of the seedling in cm}}{\text{Collar Diameter in mm}}$$

Chlorophyll Estimation

The Chlorophyll A and Chlorophyll B and total Chlorophyll (Arnon, 1949; Witham *et al*., 1971) [2, 25], in leaves were measured.

Calculated the amount of chlorophyll present in the extract mg chlorophyll per g tissue using the following equations:

mg chlorophyll A/g tissue = 12.7(A663) - 2.69(A645)*V/ 1000*W

mg chlorophyll B/g tissue = 22.9(A645) – 4.68(A663)*V/1000*W

mg total chlorophyll tissue = 20.2(A645) + 8.02(A663)*V/1000*W

Where

A = absorbance at specific wave lengths
V= final volume of chlorophyll extract in 80% acetone
W= fresh weight of tissue extracted.

Chlorophyll Stability Index (CSI)

CSI was estimated by following procedure of Koleyoreas (1958) [11] and expressed in percentage

$$CSI = \frac{\text{Total chlorophyll content (treated)}}{\text{Total chlorophyll content (control)}} \times 100$$

Statistical analysis

The experiments were carried out in Completely Randomized Design. Four replications were maintained for each treatment. One way ANOVA was used to test the effect of treatments on various plant parameters employing statistical software GENSTAT 5. Means that exhibited significant differences were compared by Duncan's Multiple Range test (DMRT) (α = 0.05) (Panse and Sukhatme, 1995) [15]. The percentage values of germination was subjected to arc sine transformation before subjecting to ANOVA.

Results and Discussion

The initial moisture content of Sandal seeds was found to be 6.483%. Seed encapsulation showed significant influence on germination capacity of sandal seeds. The germination of non-encapsulated control seeds was recorded as 38.2%. Encapsulated seeds of Sandal showed improved germination capacity compared to control seed. All 4 concentrations of Sodium alginate were equally good in encapsulating sandal seeds. Highest germination of 60% was recorded by 6% Sodium alginate and closely followed by 4% Sodium alginate (58.7%). Collar diameter of seedlings increased to significant levels on encapsulation for both 6% and 8% alginate concentrations recording 2.51mm and 2.72 mm respectively (Table 1 and Figures 1, 2 and 3).

Seed Vigour Index (SVI) was enhanced significantly in all four concentrations of Sodium alginate encapsulation, with 6% Sodium alginate concentration recording SVI of 853 compared to control (533). Seed vigour, is a comprehensive feature that comprises several parameters which determine the seed quality and uniformity in field germination under different environmental conditions (Finch-Savage and Bassel, 2016) [7]. Vigour testing is a more sensitive measure of seed physiological quality (Marcos-Filho, 2015) [13] and in the present study influence of encapsulation on physiology of sandal seed has been evidenced through high SVI. This may be due to sufficient moisture availability inside the alginate capsule for a longer period which is essential to trigger physiological changes inside the dormant sandal seed when compared to non encapsulated control seeds.

The Sturdiness Quotient (SQ) for different treatment ranged between 4.92 (8% Sodium alginate) and 9.43 (2% Sodium alginate). However, control seeds were on par with 2% Sodium alginate with SQ of 8.80. Sodium alginate concentrations of 4% and 6% ranked next to 8% Sodium alginate for SQ. The lower the SQ the higher the survival capacity of seedling under harsh conditions (Jaenicke, 1999) [10]. Edralin and Mercado (2010) [6] states that a sturdiness quotient above 6 imply that height of the seedlings is relatively greater than collar diameter which reflects spindly, weak and undesirable nature of seedlings. Black spruce seedlings with sturdiness quotients > 6 had recorded severe casuality when exposed to wind, drought, and frost (Roller, 1977) [18]. In this study 8% Sodium alginate was found favourable for enhancing SQ of Sandal seedlings. This could be due to the fact that apart from moisture availability to encapsulated seeds, the thick coating over the seed has rendered protection from any insect damage until the germination period. As a result sturdy seedlings could develop by efficiently utilising the available nutrients stored in the sandal seed. Budiman *et al.* (2015) [5] in his studies on *Anthocephalus cadamba* suggested that sturdiness quotient must strongly parallel the diameter for predicting field performance. In this study, biochemical parameters such as Chlorophyll A, B and total Chlorophyll and Chlorophyll Stability Index (CSI) did not vary significantly for encapsulated seeds on comparison with control (Table 2). The CSI is a measure which indicates how well chlorophyll performs under stress conditions. Higher CSI helps to withstand stress which leads to increased photosynthetic rate and more biomass production. However, it was found that seed encapsulation did not influence the CSI in sandal.

Quality seedling is important because in afforestation programmes seedlings cannot receive the sufficient care which is being extended to horticultural trees. After planting, the seedlings need to survive without extra nourishment or irrigation. Studies on different tree species have shown that productivity and field survival are related to the seedling quality (Valli, 1996). In this study it has been envinced that seed encapsulation using Sodium alginate has beneficial effect on sandal germination, SVI and SQ. Similar studies have been reported where sodium alginate concentrations at various ranges have been employed for different systems (Sakamoto *et al*., 1995; Vij *et al*., 2001; Mondal *et al*., 2002) [19, 24, 14]. Despite the availability of various artificial and natural polymers for encapsulation, the most widely used is sodium alginate due to its simple gelling ability, cost-effectiveness and non-toxic nature. Adding up of bulking agents that possess nutrient values to the alginate is also in the formulation of alginate beads (Lewis *et al.,* 1996; Shaban and El-Komy, 2000) [12, 21]. Sarrocco (2001) [20] has studied that alginate matrix can entrap nutrients without any adverse effect on seedling emergence.

Table 1: Effect of seed encapsulation of *Santalum album* on seedling morphological parameters

Treatments	Germination %	Arc sine germination (%)	Root length (cm)	Shoot Length (cm)	Seedling height (cm)	Collar diameter (mm)	Seedling Vigour Index (SVI)	Sturdiness Quotient (SQ)
2% sodium alginate	53.7 [a]	47.17 [a]	4.82	8.58	13.4	1.423 [c]	717 [a]	9.43[a]
4% sodium alginate	58.7 [a]	50.08 [a]	4.97	8.45	14.73	2.288 [b]	683 [a]	6.47[b]
6% sodium alginate	60 [a]	50.80 [a]	5.17	9.03	14.2	2.510 [a]	853 [a]	5.72[b]
8% sodium alginate	55 [a]	47.95 [a]	4.7	8.70	13.4	2.72 [a]	732 [a]	4.92[c]
Control	38.2 [b]	38.17 [b]	5.5	8.38	13.88	1.588 [c]	533 [b]	8.80[a]
Grand Mean	53.1	46.83	5.03	8.63	13.92	2.105	704	7.07
S.e.d.	4.74	2.772	0.358	0.528	0.958	0.1411	136.9	0.510
L.s.d.	10.1	5.909	0.763	1.126	2.043	0.3008	291.8	1.088
	S	S	NS	NS	NS	S	S	S

S-Significant at 5% level of significance; NS-Non significant

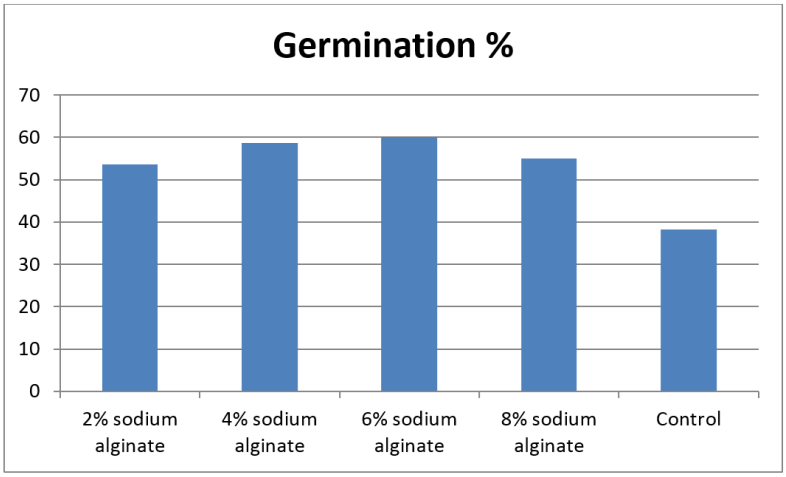

Fig 1: Effect of seed encapsulation on germination of *Santalum album*

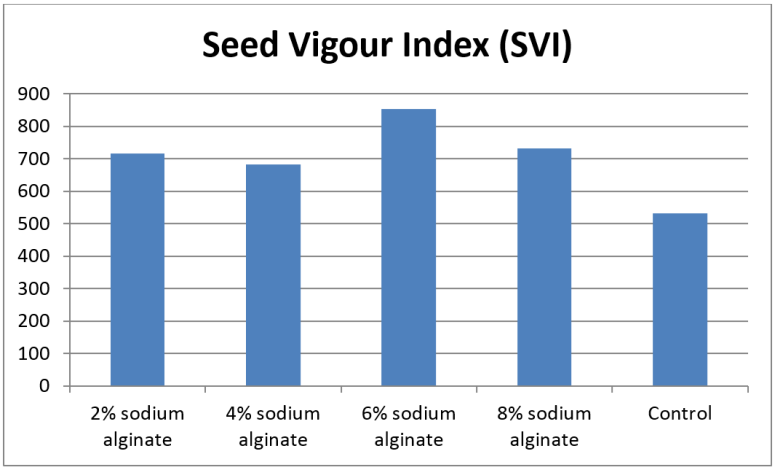

Fig 2: Effect of seed encapsulation on Seed Vigour Index of *Santalum album*

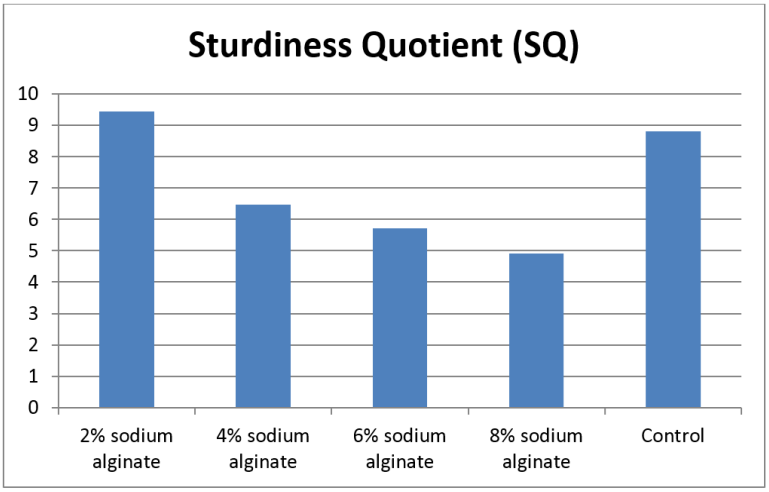

Fig 3: Effect of seed encapsulation on Sturdiness Quotient of *Santalum album* seedlings

Table 2: Effect of seed encapsulation of *Santalum album* on Chlorophyll content

Treatments	Chlorophyll A mg/g tissue	Chlorophyll B mg/g tissue	Total Chlorophyll mg/g tissue	CSI
2% sodium alginate	0.2325	0.2133	0.446	94.3
4% sodium alginate	0.2398	0.2135	0.453	95.8
6% sodium alginate	0.2808	0.2485	0.529	111.9
8% sodium alginate	0.2615	0.2148	0.476	100.7
Control	0.2348	0.2023	0.437	92.3
Grand Mean	0.2499	0.2185	0.468	99.0
S.Ed.	0.02548	0.02395	0.0475	10.04
L.Sd.	0.05431	0.05105	0.1012	21.41
	NS	NS	NS	NS

S-Significant at 5% level of significance; NS-Non significant

Plate 1: Encapsulated seed and germinated seedlings of Sandal

Plate 2: Morphological studies on Sandal seedlings

Conclusion

From the above study, it is suggested to deploy either 6% and 8% sodium alginate concentration for production of encapsulated seeds of *Santalum album*. Nursery production of quality seedlings of Sandal can be increased through seed encapsulation technique. This would also enable afforestation programs which involve sandal regeneration in natural pockets and stressed sites. Successful utilization of encapsulation of sandal seeds can be extended to propagules with suitable amendments which may be of value in breeding programs and allows the propagation of elite genotypes.

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