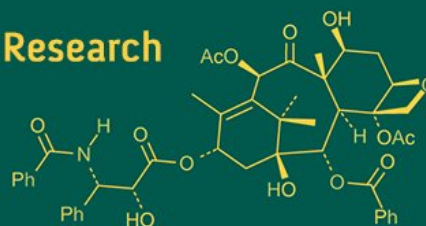


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Effects of gibberellic acid and chitosan on seed germination and Morpho-physiological parameters of sandalwood (*Santalum Album L.*)

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Abstract

Sandalwood (*Santalum album L.*) is an indigenous Indian forestry species of immense economic and cultural value. However, commercial propagation is severely hindered by inherent physical dormancy primarily driven by impermeability of seed coat to water, which restricts germination. To address this constraint, a study was conducted during the year 2025-26 at Research Farm of Agroforestry, All India Coordinated Research Project (AICRP) on Agroforestry and Agricultural Botany section, College of Agriculture, Nagpur to evaluate the effect of pre-sowing seed treatments using chitosan and gibberellic acid. The experiment was laid out in Randomized Block Design with three replications in the greenhouse under shade. The seeds were subjected to eight different soaking treatments, namely Control, Water soaking, GA₃ @ 100 ppm, GA₃ @ 500 ppm, GA₃ @ 1000 ppm, Chitosan @ 100 ppm, Chitosan @ 500 ppm and Chitosan @ 1000 ppm. The data revealed that seeds treated with GA₃ @ 1000 ppm recorded significantly higher germination percentage (43.33%) and morphological and physiological parameters such as plant height, number of leaves, collar diameter, root and shoot lengths, fresh and dry weights of both shoots and roots, leaf area per plant and leaf area index followed by GA₃ @ 500 ppm and Chitosan @ 1000 ppm as compared to other treatments at all observational stages. Thus, it can be concluded that GA₃ @ 1000 ppm proved to be the best treatment to break seed dormancy and significantly enhanced germination to ensure superior quality seedlings and supply of quality planting materials for promoting the cultivation of sandalwood.

Keywords: Sandalwood, seed dormancy, germination, gibberellic acid, chitosan, morpho-physiological parameters

Introduction

Indian Sandalwood (*Santalum album L.*) stands as a highly coveted species within Indian forestry, renowned globally for its premium commercial timber, valuable heartwood and aromatic essential oil. The species demonstrates a broad geographic range occurring naturally across latitudes from 30°N to 40°S. Within India, indigenous populations are primarily concentrated in Kerala and Andhra Pradesh; however, fragmented populations have been documented across a diverse array of states including Bihar, Gujarat, Haryana, Maharashtra, Madhya Pradesh, Odisha, Punjab, Rajasthan, Uttar Pradesh, and West Bengal, with more recent sightings even reported in states of Himachal Pradesh and Assam.

As a moderately sized evergreen, Sandalwood typically reaches a height of 12 to 15 meters with a girth ranging from 1 to 2.4 meters, characterized by both erect and slenderly drooping branches (Sen Sarma, 1982) [14]. This adaptable species flourishes at elevation ranging from sea level up to altitudes of 1,800 meters. It particularly favors cool climates with extended dry periods and moderate annual rainfall of 600 to 1,600 millimeters (Troup, 1921) [18]. Indian Sandalwood exhibits an early reproductive cycle, often initiating flowering between two to three years of age, though specific flowering and fruiting seasons can vary. The tree produces succulent, globose to elongated drupe fruits that ripens to deep purple black. Each fruit encases a spherical seed enclosed within a moderately hard brown endocarp, though the fruit's overall shape can range from globose and ovate to elongated with tapering ends. Propagation predominantly relies on seeds which are yielded either once or twice annually, typically between June and September or November and February. Sandalwood seeds

demonstrate a remarkably prolonged period of seed dormancy (Doran *et al.*, 2005) [6]. Consequently, the germination process is protracted, requiring anywhere from 4 to 12 weeks to complete (Srinivasan *et al.*, 1992; Srimathi *et al.*, 1995) [16, 17]. This intense dormancy is multifaceted; it is primarily driven by a seed coat that is impermeable to both water and oxygen, alongside other factors such as mechanically resistant seed coats and rudimentary or physiologically immature embryos (Das and Tah, 2013) [5]. Seed dormancy is the most limiting factor for seed germination and thus it causes prolongation in seed germination.

Sandalwood has emerged as a highly adaptable botanical asset, demonstrating significant utility across the agricultural, industrial, and medical sectors. The species acts as a rich biological reservoir of phytochemicals that are increasingly sought after by the modern cosmetics and pharmaceutical sectors. As natural reserves deplete amidst surging global demand, the economic value of sandalwood has escalated dramatically. This lucrative market environment has catalyzed a unified push toward commercial cultivation, drawing collaborative interest from farmers, policymakers and industry executives. To successfully sustain these widespread commercial initiatives, it is imperative to optimize propagation techniques. Specifically, there is a critical need to evaluate various chemical pre-sowing treatments to maximize seed germination rates and ensure the production of high vigour seedlings.

Materials and Methods

The experiment was carried out in the year 2025-26 at Research Farm of Agroforestry, All India Coordinated Research Project (AICRP) on Agroforestry and Agricultural Botany section, College of Agriculture, Nagpur. The field experiment was conducted using Randomized Block Design (RBD) with three replications and eight treatments. The matured uniform fruits of sandalwood were collected from the mother plants situated at AICRP on Agroforestry, Nagpur. To extract the seeds, the collected fruits were initially soaked in water and rubbed against a hard surface to macerate and remove the pulp. Following the extraction, seeds were dried in the shade. Prior to sowing, the seeds were subjected to a 24-hour soaking period in the given eight treatments consisting of T₁ - Control, T₂ - Water soaking, T₃ - GA₃ @ 100 ppm, T₄ - GA₃ @ 500 ppm, T₅ - GA₃ @ 1000 ppm, T₆ - Chitosan @ 100 ppm, T₇ - Chitosan @ 500 ppm, T₈ - Chitosan @ 1000 ppm.

To mitigate the risk of fungal attack, all seeds were treated with fungicide. For each treatment, 50 seeds were sown by dibbling method in polythene bags of size 6 x 8 inches filled with a mixture of FYM, soil and cocopeat in 1:2:1. Red Durlanta (*Durlanta erecta* L.) was co-planted as a primary host plant. The polythene bags were maintained in shaded greenhouse environment.

Observations were recorded on five random plants at 50, 100 and 150 days after sowing (DAS) in each treatment. The observations such as germination percentage, plant height, number of leaves, collar diameter, shoot length, root length, fresh weight of root, fresh weight of shoot, dry weight of root, dry weight of shoot and leaf area per plant, leaf area index (LAI), crop growth rate, net assimilation rate were recorded. The plant height was measured from the base to the tip of the observational plant, measured in centimeter.

The number of leaves was counted on a visual basis. The collar diameter was measured with the help of digital vernier caliper from freshly collected seedlings and recorded in millimeters. The length of the shoot was measured from the collar region to the apex region while the root length was measured from the collar region of root to the root tip and expressed in centimeters. The fresh and dry weight of both root and shoot was recorded and expressed in grams. Leaf area per plant was measured using leaf area meter and expressed in cm². Leaf area index was obtained by measuring a leaf area sample plant and dividing it by the average area occupied by the plant. The crop growth rate was calculated by using the formula suggested by Watson (1958) [20], and expressed as mg cm⁻² day⁻¹. The net assimilation rate was calculated by using the formula suggested by Radford (1967) [13], and expressed as mg cm⁻² day⁻¹. The germination percentage was calculated as:

Germination

(%) = Number of seeds germinated / Total number of seeds sown × 100

All the experimental data were subjected to analysis of variance to determine the significance of differences, following the methodology suggested by Panse and Sukhatme (1954) [10].

Results and discussion

Effect of gibberellic acid and chitosan on seed germination of sandalwood

The effect of the pre-treatment of gibberellic acid and chitosan has been represented in Table 1. Significant variation was observed across the chemical treatments, characterized by a progressive temporal increase in the germination percentage from 50 to 150 DAS.

The data revealed that treatment T₅ (GA₃ @ 1000 ppm) recorded the significantly highest germination percentage of 32.00%, followed closely by treatment T₈ (Chitosan @ 1000 ppm) at 27.33% and T₄ (GA₃ @ 500 ppm) at 25.33% at 50 DAS. T₈ was found to be at par with T₅. As the seedlings progressed to 100 DAS, treatment T₅ maintained strict physiological dominance, peaking at 42.67%. Treatment T₄ reached 37.33% which was at par with T₅, narrowly outperforming T₈, which recorded 36.67%. By the final observational stage at 150 DAS, the maximum germination percentage was sustained by treatment T₅ at 43.33% again followed by T₄ at 38.67% and T₈ at 37.33%. T₄ maintained the statistical parity with T₅.

The progressive improvement observed following gibberellic acid applications demonstrates its biochemical capacity to antagonize abscisic acid (ABA) signaling. This effectively shifts the hormonal balance from a state of quiescence to active metabolic growth (Shu *et al.*, 2016 [15], Vishal & Kumar, 2018) [19]. As reported by Berry *et al.* (2022) [4], seeds treated with high concentrations of GA₃ (1000 ppm) recorded a maximum germination capacity in sandalwood, proving its efficacy in overcoming non-deep morphophysiological dormancy. Polaiah *et al.* (2020) [11], also mentioned comparable results where the highest germination percentage was observed in seed treated with GA₃ @ 1000 ppm for *Santalum album* L.

Chitosan pretreatment also demonstrated substantial efficacy in breaking dormancy. Ali *et al.* (2025) [2] revealed that chitosan pretreatment significantly enhanced the germination. The robust performance of Chitosan is

corroborated by Liu *et al.* (2022) [8]. and Malerba & Cerana (2016) [9], who highlighted chitosan's dual-action mechanism as a biopolymer that promotes controlled hydration while upregulating metabolic mobilization in recalcitrant woody seeds.

Effect of gibberellic acid and chitosan on morphological parameters of sandalwood

The effect of different concentrations of chitosan on the morphological characters such as plant height, number of leaves, collar diameter, shoot length, root length, fresh and dry weight of both root and shoot was assessed and depicted in Tables 1, 2 and 3.

The pre-treatment using both gibberellic acid and chitosan has significantly enhanced the plant height, number of leaves and collar diameter of seedlings. The data recorded at 50, 100, and 150 DAS revealed a consistent upward trajectory. At 50 DAS, T₅ (GA₃ @ 1000 ppm) recorded the highest plant height at 15.43 cm, number of leaves at 10.67 and collar diameter measuring 2.46 mm at 50 DAS. This was followed by T₄ (GA₃ @ 500 ppm) and T₈ (Chitosan @ 1000 ppm). The plant heights further expanded to 17.00 cm, with number of leaves increasing to 12.67 and collar diameter to 3.13 mm at 100 DAS. This was narrowly followed by T₄ and T₈ while T₄ was found to be statistically at par with T₅. At the final stage of 150 DAS, T₅ achieved an impressive peak height of 18.40 cm, with highest number of leaves at 19.33 and collar diameter at 3.18 mm followed by T₄ and T₈. T₄ maintained statistical parity with T₅. Similar observations were made by Polaiah *et al.* (2020) [11], on Sandalwood regarding plant height, number of leaves and collar diameter. Also, results from chitosan treatments regarding plant height and number of leaves are in parallel with conclusions revealed by Qahraman (2026) [12]. Additionally, the structural benefits of chitosan treatments on collar diameter align with the extensive morphological studies conducted by Aleksandrowicz-Trzcińska *et al.* (2015) [1], on Scots Pine (*Pinus sylvestris* L.).

T₅ (GA₃ @ 1000 ppm) produced a superior shoot length of 12.78 cm and root length of 8.45 cm of seedling at 50 DAS followed by T₄ (GA₃ @ 500 ppm) and T₈ (Chitosan @ 1000 ppm) both functioning at par with T₅. As the seedlings progress to 100 DAS, T₅ displayed an impressive surge, reaching 14.57 cm shoot length and 9.43 cm root length, followed by T₄ and T₈. At 150 DAS, the highest shoot and root length was found in treatment T₅ gaining 14.79 cm and 9.58 cm respectively. This was followed by T₄ and T₈ both functioning at par with T₅. Polaiah *et al.* (2020) [11], revealed that GA₃ priming significantly maximize shoot and root length in sandalwood. Similar results regarding efficacy of chitosan on shoot and root length were reported by Zulkadir (2022) [21], on *Zea mays* L.

Regarding the fresh and dry weights of shoots and roots, treatment T₅ (GA₃ @ 1000 ppm) consistently exhibited the maximum biomass accumulation across all observational stages. At 50 DAS, T₅ recorded the significantly highest fresh shoot and root weights of 0.25 g and 0.10 g, respectively, as well as the highest dry shoot and root weights of 0.06 g and 0.06 g, respectively. This superior performance was closely followed by treatments T₄ (GA₃ @ 500 ppm) and T₈ (Chitosan @ 1000 ppm). The growth trend continued at 100 DAS, with T₅ surging to maximum fresh

weights of 0.47 g (shoot) and 0.13 g (root), along with peak dry weights of 0.11 g (shoot) and 0.07 g (root), which were the highest compared to the control. By 150 DAS, T₅ maintained its physiological dominance, achieving peak fresh weights of 0.50 g (shoot) and 0.21 g (root) and maximum dry weights of 0.17 g (shoot) and 0.09 g (root). At this final stage, T₄ demonstrated a superior root fresh weight compared to T₈, while both T₈ and T₄ identically achieved dry root weights of 0.05 g, 0.06 g, and 0.07 g at the 50, 100, and 150 DAS stages, respectively. These findings align closely with Polaiah *et al.* (2020) [11], who reported that GA₃ seed priming significantly maximizes the fresh and dry weights of shoots and roots in sandalwood. Similarly, Zulkadir (2022) [21], demonstrated that chitosan acts effectively to significantly increase both fresh and dry biomass in *Zea mays* L.

Effect of gibberellic acid and chitosan on physiological parameters of sandalwood

The effect of the pre-treatment of gibberellic acid and chitosan has been represented in Tables 3 and 4. Significant variation was observed across the chemical treatments, characterized by a progressive temporal increase from 50 to 150 DAS.

T₅ (GA₃ @ 1000 ppm) consistently maintained a statistically superior leaf area and leaf area index achieving 5.49 cm² leaf area with 0.28 LAI at 50 DAS as compared to T₁ (Control). This was followed by T₈ (Chitosan @ 1000 ppm) and T₄ (GA₃ @ 500 ppm) both functioning statistically at par with the superior treatment T₅. T₅ maintained its physiological dominance at 100 DAS by securing highest leaf area of 8.92 cm² and LAI of 0.45. This was followed by T₄ and T₈. At 150 DAS, T₅ achieved 9.69 cm² leaf area and 0.49 LAI which is highest among all treatments. Arun *et al.* (2020) [3], reported similar observations in cowpea (*Vigna unguiculata* L. Walp) related to leaf area whereas seed priming with chitosan similarly enhanced the leaf architecture as reported by Godase *et al.* (2023) [7].

Evaluated over distinct physiological intervals of 50-100 DAS and 100-150 DAS, T₅ (GA₃ @ 1000 ppm) maintained absolute dominance. Between 50 and 100 DAS, T₅ recorded 0.057 mg cm⁻² day⁻¹ and 0.159 mg cm⁻² day⁻¹ of CGR and NAR respectively, which is significantly higher as compared to control. This was followed by T₈ (Chitosan @ 1000 ppm) which achieved a CGR of 0.041 mg cm⁻² day⁻¹ and NAR of 0.143 mg cm⁻² day⁻¹ across the respective intervals, narrowly outperforming T₄ (GA₃ @ 500 ppm) which achieved 0.035 mg cm⁻² day⁻¹ CGR and 0.134 mg cm⁻² day⁻¹ NAR at respective intervals. The treatment T₅ maintained a physiological dominance, escalating CGR to 0.085 mg cm⁻² day⁻¹ and NAR to 0.178 mg cm⁻² day⁻¹ in the final 100-150 DAS period as compared to control. T₄ (GA₃ @ 500 ppm) recorded highly competitive rates of 0.163 mg cm⁻² day⁻¹ NAR and CGR of 0.052 mg cm⁻² day⁻¹, slightly followed by T₈ (Chitosan @ 1000 ppm) achieved 0.157 mg cm⁻² day⁻¹ NAR and 0.056 mg cm⁻² day⁻¹ CGR in the respective intervals. Arun *et al.* (2020) [3], reported similar results regarding crop growth rate in cowpea (*Vigna unguiculata* L. Walp). Godase *et al.* (2023) [7], reported similar results on chitosan regarding crop growth rate in lablab bean (*Lablab purpureus* L. Sweet).

Table 1: Effect of gibberellic acid and chitosan on germination percentage, plant height, number of leaves, collar diameter of sandalwood

Treatments	Germination percentage (%)			Plant Height (cm)			No. of leaves			Collar diameter (mm)		
	50 DAS	100 DAS	150 DAS	50 DAS	100 DAS	150 DAS	50 DAS	100 DAS	150 DAS	50 DAS	100 DAS	150 DAS
Control	14.67	23.33	23.33	8.97	9.80	12.13	6.00	6.67	9.00	1.63	2.16	2.23
Water Soaking	18.00	26.00	26.00	10.03	11.67	12.93	7.33	9.33	11.00	1.88	2.34	2.59
GA3 @ 100 ppm	20.67	30.00	30.00	12.13	14.27	14.47	7.67	10.00	13.67	2.01	2.74	2.79
GA3 @ 500 ppm	25.33	37.33	38.67	13.13	15.10	16.50	8.67	10.67	16.67	2.17	2.90	2.92
GA3 @ 1000 ppm	32.00	42.67	43.33	15.43	17.00	18.40	10.67	12.67	19.33	2.46	3.13	3.18
Chitosan @ 100 ppm	17.33	24.67	27.33	10.50	11.93	13.20	6.33	7.67	11.67	1.78	2.45	2.52
Chitosan @ 500 ppm	20.00	29.33	29.33	12.10	13.43	15.67	7.67	9.00	14.00	1.76	2.63	2.63
Chitosan @ 1000 ppm	27.33	36.67	37.33	12.90	14.80	16.80	8.67	10.33	17.00	2.20	2.65	2.73
SE (m) ±	1.77	1.81	1.84	0.73	0.94	0.85	0.50	0.67	0.71	0.11	0.14	0.12
CD @ 5%	5.38	5.48	5.59	2.22	2.84	2.55	1.51	2.04	2.15	0.33	0.43	0.35

Table 2: Effect of gibberellic acid and chitosan on root and shoot length, fresh weight of root and shoot of sandalwood

Treatments	Shoot length (cm)			Root length (cm)			Fresh weight of shoot (g)			Fresh weight of root (g)		
	50 DAS	100 DAS	150 DAS	50 DAS	100 DAS	150 DAS	50 DAS	100 DAS	150 DAS	50 DAS	100 DAS	150 DAS
Control	8.74	9.13	9.43	4.86	4.90	5.05	0.10	0.16	0.20	0.03	0.06	0.06
Water Soaking	9.55	9.68	9.85	5.88	6.13	6.30	0.13	0.19	0.25	0.05	0.06	0.10
GA3 @ 100 ppm	10.93	11.23	11.60	6.05	6.38	8.33	0.14	0.27	0.29	0.07	0.08	0.12
GA3 @ 500 ppm	11.63	13.22	13.64	6.93	7.23	8.88	0.16	0.38	0.39	0.09	0.09	0.14
GA3 @ 1000 ppm	12.78	14.57	14.79	8.45	9.43	9.58	0.25	0.47	0.50	0.10	0.13	0.21
Chitosan @ 100 ppm	9.60	9.77	10.00	5.60	5.85	6.72	0.15	0.19	0.27	0.04	0.08	0.09
Chitosan @ 500 ppm	9.87	10.00	10.75	6.20	6.77	6.92	0.19	0.24	0.29	0.06	0.08	0.11
Chitosan @ 1000 ppm	11.15	11.83	14.20	7.35	8.00	8.13	0.22	0.27	0.33	0.07	0.11	0.17
SE (m) ±	0.57	0.59	0.75	0.40	0.48	0.51	0.009	0.023	0.009	0.005	0.006	0.006
CD @ 5%	1.74	1.78	2.29	1.21	1.45	1.56	0.028	0.069	0.028	0.015	0.019	0.017

Table 3: Effect of gibberellic acid and chitosan on dry weight of root and shoot, leaf area per plant and leaf area index of sandalwood

Treatments	Dry weight of shoot (g)			Dry weight of root (g)			Leaf area per plant (cm ²)			Leaf area index		
	50 DAS	100 DAS	150 DAS	50 DAS	100 DAS	150 DAS	50 DAS	100 DAS	150 DAS	50 DAS	100 DAS	150 DAS
Control	0.04	0.04	0.06	0.03	0.04	0.05	3.53	3.84	5.06	0.18	0.20	0.26
Water Soaking	0.04	0.05	0.07	0.04	0.05	0.05	3.57	4.03	5.28	0.18	0.21	0.27
GA3 @ 100 ppm	0.04	0.06	0.09	0.04	0.05	0.06	4.51	5.51	6.34	0.23	0.28	0.32
GA3 @ 500 ppm	0.05	0.07	0.11	0.05	0.06	0.07	4.58	5.63	6.95	0.23	0.29	0.35
GA3 @ 1000 ppm	0.06	0.11	0.17	0.06	0.07	0.09	5.49	8.92	9.69	0.28	0.45	0.49
Chitosan @ 100 ppm	0.04	0.06	0.07	0.04	0.05	0.06	4.72	5.35	5.43	0.24	0.27	0.28
Chitosan @ 500 ppm	0.04	0.06	0.09	0.04	0.05	0.07	4.94	5.56	6.46	0.25	0.28	0.33
Chitosan @ 1000 ppm	0.05	0.08	0.13	0.05	0.06	0.07	5.26	5.96	8.12	0.27	0.30	0.41
SE (m) ±	0.002	0.003	0.003	0.002	0.002	0.003	0.193	0.244	0.351	0.010	0.012	0.018
CD @ 5%	0.006	0.009	0.009	0.007	0.006	0.009	0.585	0.741	1.064	0.030	0.038	0.054

Table 4: Effect of gibberellic acid and chitosan on net assimilation rate and crop growth rate of sandalwood

Treatments	Net Assimilation Rate (mg cm ⁻² day ⁻¹)		Crop Growth Rate (mg cm ⁻² day ⁻¹)	
	50-100 DAS	100-150 DAS	50-100 DAS	100-150 DAS
Control	0.082	0.107	0.082	0.107
Water Soaking	0.110	0.113	0.110	0.113
GA3 @ 100 ppm	0.119	0.128	0.119	0.128
GA3 @ 500 ppm	0.134	0.163	0.134	0.163
GA3 @ 1000 ppm	0.159	0.178	0.159	0.178
Chitosan @ 100 ppm	0.108	0.123	0.108	0.123
Chitosan @ 500 ppm	0.121	0.142	0.121	0.142
Chitosan @ 1000 ppm	0.143	0.157	0.143	0.157
SE (m) ±	0.008	0.009	0.008	0.009
CD @ 5%	0.024	0.028	0.024	0.028

Conclusion

The present investigation underscores the profound impact of pre-sowing chemical treatments on the germination, growth, and overall developmental potential of sandalwood seedlings. While the untreated seeds are heavily restricted

by innate dormancy, the exogenous application of gibberellic acid and chitosan effectively overcomes these inherent physiological barriers. Treatment T₅ (GA₃ @ 1000 ppm) significantly delivered superior performance across all evaluated morpho-physiological parameters under nursery

conditions, establishing it as a highly viable protocol for maximizing propagation success in Sandalwood. Furthermore, chitosan maintained robust secondary performance, serving as an effective bio-stimulant with significant potential for improving germination speed and early seedling vigour.

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