

Trichoderma viride based seed biopriming strategies for quality seedling production in East Indian Sandalwood

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Abstract

Seed biopriming is one of the cheapest, eco-friendly, and proven seed enhancement techniques to obtain better germination and seedling performance in many crops. Sandal (*Santalum album* L) seeds, in general, have poor and staggered germination which leads to difficulty in nursery management. In this study, the impact of plant growth-promoting fungi *Trichoderma viride* on the germination and seedling growth of sandal was studied. Fresh mature sandal seeds were procured from the Nachivayal Reserve Forest, Marayur Sandal Division, Kerala, India. The experiment was conducted in two factorial CRD with concentrations of *T. viride* (25, 50, 75 and 100%) and duration of treatment (1, 2, 3, 4, 6 and 8 days) as factors. The effect of post priming storage (periods one day and one week) was also studied. The application of *T. viride* improved the germination and seedlings performance of *S. album*. The highest germination was obtained on biopriming at 100% concentration for 3 days (82.72%) for one week, followed by 100% concentration for 1 day (73.3%) for storage of one day. Post priming storage studies indicated that the storage of bioprimed seeds (100% for 3 days) for one week recorded better seedling growth. Hence, we recommend the biopriming of sandal seeds with *T. viride* at 100% for 3 days stored for one week for the quality seedling production of sandal in the nursery.

1. Introduction

Sandal (*Santalum album* L) is one of the most esteemed and high priced precious timber of the world because of its fragrant essential oil and heartwood. The annual demand for sandalwood is estimated to be 5,000 to 6,000 MT and that of the oil to the tune of 100 to 120 MT (Bhaskar *et al.* 2010). Over-exploitation and illicit felling and has resulted in a severe decline in the natural populations of this species followed by the categorization of it as 'Vulnerable' in the IUCN red list (Arunkumar *et al.* 2019). Despite the high demand for sandalwood the supply is diminishing as the natural population is rapidly dwindling in India due to the undesirable changes in land use patterns, habitat destruction and indiscriminate exploitation of the sandal resources for generations. Furthermore, the area under sandalwood plantation in India is reported to be 20,725 ha and the majority of plantations occur in the state of Karnataka, Tamil Nadu and Kerala (Mishra *et al.* 2018). To cope up with the declining share of sandal in international markets and to conserve it in natural habitat, most of the sandal growing states had relaxed the rules and regulations for the cultivation of sandal and there is a need for large-scale domestication of this species. Moreover, the benefit-cost analysis of sandal both as monocrop and in combination with the crops has been reported to be a lucrative option for farmers (Viswanath *et al.* 2009; Divakara *et al.* 2018). The poor rate of germination and prolonged germination period are the major constraints in the regeneration of sandal. This results in considerable difficulties in the mass production of sandal for meeting the requirements of the forest department, farmers and agroforestry practitioners.

Seed priming is one of the key technologies to achieve seed enhancement through the rapid germination of seeds and optimisation of the seedling establishment in the field. It is a controlled seed treatment during which the metabolic activity is enhanced, but suspended before radicle protrusion. Biopriming is one of the foremost suitable techniques that use beneficial microorganisms to protect against pathogens and enhance plant growth. It is considered as a safer, cheaper and simple method for biological control (El-Mougy and Abdel-Kader, 2008). *Trichoderma* species are free-living fungi that are present in soil and associated with the roots of many plant species. It is a biocontrol agent used to suppress and control soil-borne plant pathogenic fungi and also shows promising plant growth-promoting potential hence an important component of bio-fertilizers (Druzhinina *et al.* 2011; Sharma *et al.* 2012; Martínez-Medina *et al.* 2014). There are reports that storage of primed seeds is an issue and in some cases which report that priming is detrimental to the storage of the seeds. However, the storing of primed seeds has been shown to improve the viability, germination and seedling performance. With this background, the present study was formulated to assess the impact of biopriming with *Trichoderma viride* and post priming storage of seeds on the germination and seedling attributes of *Santalum album*.

2. Materials and method

The experiment was conducted at the College of Forestry, Kerala Agricultural University (KAU), South India, from May 2019 to June 2020.

2.1. Plant material

Fresh sandal seeds were harvested from October to November 2019 from the Marayur provenance (Nachivayal Reserve Forest I and II) of Kerala, India (77° 5' to 77° 15' E longitude and 10° 10' to 10° 20' N latitude) and bought to the Seed Technology laboratory. The extraction procedure was overnight soaking of fruits in water and then manual depulping. The extracted seeds were dried in shade for 48 h, cleaned and mixed for homogeneity. Examination of physical characteristics indicated that the sandal seeds were with a brown hard

seed coat and the seed diameter ranged from 0.5 to 1.0 cm. Seed moisture content ranged from 10–15%. The individual seed weight ranged from 0.15 to 0.27 g whereas the seed kernel weighed an average of 0.01 to 0.02 g and the 1000 seeds weighed 250 g.

2.2. Priming procedure

The powder formulations of *T. viride* (Kerala Agricultural University isolate) of 10^8 CFU ml⁻¹ were procured from the Department of Microbiology, College of Agriculture, Vellanikkara, KAU. To produce a 100% concentration of *T. viride*, 50 sandal seeds were treated with 20 g of the powder formulations of the *T. viride* @ 10^8 CFU ml⁻¹. Hence, the ratio of powder formulations to the number of seeds to be primed was 2:5. Four concentrations (25, 50, 75 and 100%) and six durations (1, 2, 3, 4, 6 and 8 days) of the priming agent constituted the treatments of the study. Sandal seeds with bioinoculant were kept in glass bottles, covered with aluminium foil and agitated at regular intervals to prevent the solidifying of priming agent at ambient temperature (29–30°C). On completion of priming the seeds were thoroughly washed with distilled water thrice and dried with Whatman No. 1 filter paper in shade at room temperature till the seeds achieved the initial moisture levels. The re-dried seeds were transferred to brown paper bags, kept in glass containers and stored at ambient conditions for post priming storage. The seeds were pre-treated with 500 mg l⁻¹ (w/v) of GA₃ (Merck) 12 hours before sowing. The seeds were spread for germination in plastic germination trays of dimensions 30x30x5 cm filled with sand medium. Each treatment had three replications, with each replication consisting of 50 seeds. The germination trays were irrigated uniformly twice a day until the completion of germination. Seeds germinating daily (Daily seed germination) were recorded for each treatment and the percentage of germination was calculated. The germination value (GV) was calculated as suggested by Czabator (1962):

$$GV = MDG \times PV$$

Where, MDG = Final Mean Daily Germination, PV = Peak value of Mean Daily Germination.

The MDG is calculated as the cumulative percent of full seed germination at the end of the germination test, divided by the number of days from sowing to the end of the test. Peak Value of germination denotes the speed of germination, which is the maximum mean daily germination, recorded at any time during the period of the test.

2.3. Seed chemical analysis

To find the chemical changes associated with priming, the seeds after priming were immediately subjected to chemical analysis in five replications. The electrical conductivity of the seed leachates, which is an absolute indication of seed membrane stability, was determined using the conductivity meter (CDC 40101). The total carbohydrate was estimated by the Anthrone method (Yemm and Wills, 1954) and protein content was estimated by the method suggested by Lowry et al. (1951). The crude fat content of the primed seeds was estimated by Soxhlet extraction (Kennedy, 1949).

2.4. Seedling growth estimation

To find the impact of priming on the seedling parameters, 12 uniform seedlings having 4–6 leaves belonging to each priming treatment were transplanted during November 2020 to polythene bags containing the medium soil, sand and Farm Yard Manure in the ratio 3:1:1 and kept in the nursery for six months. The seedling dimensions and dry biomass were recorded at 180 days after transplanting (DAT). The height, collar diameter, number of leaves, root length and number of roots per taproot were recorded. The dry weight of the biomass components was determined by drying to constant weight in a hot air oven maintained at 70°C. The vigour index of the seedlings was estimated as suggested by Abdul-Baki and Anderson (1973), the root: shoot ratio of the seedlings as suggested by Hunt (2012) and seedling quality index as by the method of Dickson et al. (1960).

The statistical analysis was conducted using R package – Agricolae taking the concentration and duration as the factors and Tukey's HSD (Honestly Significant Difference) for the post-hoc test under R environment (Mendiburu, 2015).

3. Results

Biopriming with varying concentrations and durations and post priming storage of the seeds significantly influenced germination in sandal (Fig. 1 and Table 1). The results are presented for the post priming storage of sandal seeds for one day (S₁) and one week (S₂). For S₁, the highest germination was obtained on biopriming at 100% for 1 day (73.30%) and the lowest was on biopriming at 25% for 8 days (2.61%). Except in 100% concentration, the increasing concentration of bioinoculant increased the germination but the increasing duration of priming decreased the germination. The imbibition (time to initiate germination) and germination periods corresponding to

the highest germination were 18 and 71 days respectively. The imbibition period varied from 17–24 days, and the germination period, time to complete germination varied from 24–71 days. The MDG, PV and GV were highest for biopriming at 75% for 1 day (1.86, 3.72 and 6.93, respectively). For S_2 (Fig. 1), biopriming with *T. viride* at 100% for 3 days recorded the highest germination (82.72%) and the lowest was on biopriming at 100% for 6 days (1.22%). Treatment corresponding to the highest germination had an imbibition period of 17 days and a germination period of 40 days. The biopriming at higher durations decreased the germination for all the treatments. The imbibition period for the entire treatment ranged from 15–21 days and the germination period from 24–56 days. The MDG, PV and GV were highest for biopriming at 50% for 4 days (3.21, 6.41 and 20.56, respectively). Analysis of variance revealed a significant difference in germination percentage due to biopriming concentration and duration. Post priming storage of seeds increased the per cent germination and decreased the germination period in sandal seeds.

Table 1
Effect of biopriming with *Trichoderma viride* on the germination attributes of sandal seeds subjected to post priming storage of one day and one week

<i>Trichoderma viride</i> (10 ⁸ CFU ml ⁻¹)		and one week									
		Imbibition period		Germination period		Mean Daily Germination		Peak Value		Germination Value	
		Post priming storage									
Concentration	Duration (days)	S ₁	S ₂	S ₁	S ₂	S ₁	S ₂	S ₁	S ₂	S ₁	S ₂
25%	1	21	17	32	33	1.66	2.12	3.31	4.24	5.49	9.00
	2	16	16	34	34	0.74	2.44	1.47	4.88	1.08	11.92
	3	24	19	28	24	0.32	0.38	0.64	0.75	0.21	0.28
	4	19	16	28	42	0.68	0.67	1.36	1.33	0.92	0.89
	6	19	21	24	48	0.63	0.58	1.25	1.17	0.78	0.68
	8	21	16	30	33	0.13	1.33	0.03	2.67	0.00	3.56
50%	1	24	15	67	34	0.54	1.88	2.48	3.76	1.33	7.09
	2	17	20	35	48	1.09	1.13	2.17	2.25	2.36	2.53
	3	21	18	50	48	0.58	1.27	1.16	2.54	0.67	3.23
	4	21	15	29	34	0.59	3.21	1.17	6.41	0.69	20.56
	6	21	16	32	56	0.50	0.98	1.00	1.96	0.50	1.93
	8	19	21	66	52	0.21	0.29	1.00	0.58	0.21	0.17
75%	1	19	15	29	30	1.86	1.57	3.72	3.13	6.93	4.91
	2	20	21	70	33	0.20	0.39	1.00	0.79	0.20	0.31
	3	16	15	35	25	0.49	0.96	0.97	1.92	0.47	1.84
	4	24	15	32	29	0.38	1.21	0.75	2.41	0.28	2.91
	6	21	17	28	41	0.61	0.90	1.21	1.80	0.74	1.63
	8	16	16	41	30	0.56	1.47	1.12	2.93	0.63	4.30
100%	1	18	18	71	56	1.55	1.25	3.10	2.50	4.80	3.13
	2	17	15	37	35	0.78	1.20	1.57	2.40	1.23	2.88
	3	19	17	34	40	1.15	3.10	2.29	6.20	2.63	19.22
	4	19	15	37	55	0.68	1.31	1.35	2.62	0.91	3.43
	6	20	16	35	56	1.06	1.34	2.11	2.68	2.24	3.59
	8	23	18	39	53	1.26	0.25	2.51	0.49	3.16	0.12
S ₁ Post priming storage for one day, S ₂ Post priming storage for one week											

Biopriming concentration and time caused significant variations in electrical conductivity and biochemical content of seeds ($p = 0.01$). The electrical conductivity of seed leachates could be reduced most of the biopriming treatments and the highest EC was recorded for the seeds bioprimed at 75% for 6 days (1.302 dS cm^{-1}) followed by 25% for 6 (0.830 dS cm^{-1}) and 8 days (0.757 dS cm^{-1}) and, the lowest was in biopriming for 75% for 1 day (0.217 dS cm^{-1}) (Table 2). The carbohydrate concentration of the seeds after priming ranged from 0.110 mg g^{-1} (25% for 3 days) to 0.722 mg g^{-1} (75% for 2 days). The highest protein content was observed on biopriming at 100% for one day (0.059 mg g^{-1}) and the lowest was at 25% for 1 day (0.020 mg g^{-1}). The highest crude fat content was observed on biopriming at 100% for 6 days (76.6%) and the lowest was at 75% for 1 day (34.0%).

Table 2

Effect of biopriming with *Trichoderma viride* on electrical conductivity and biochemical composition of the sandal seeds

<i>Trichoderma viride</i> (10 ⁸ CFU ml ⁻¹)	Duration (days)	Electrical conductivity (dS/cm)	Total carbohydrate (mg/g)	Total protein (mg/g)	Crude Fat (%)
25%	1	0.522 ^{fg}	0.313 ⁱ	0.020 ^a	53.0 ^{cdef}
	2	0.624 ⁱ	0.365 ⁱ	0.057 ⁱ	55.6 ^{defgh}
	3	0.396 ^d	0.722 ⁱ	0.047 ^{hi}	46.0 ^{bc}
	4	0.577 ^{ghi}	0.289 ^{ghi}	0.064 ⁱ	43.4 ^b
	6	0.830 ⁱ	0.147 ^{bc}	0.049 ^{hi}	67.6 ⁱ
	8	0.757 ⁱ	0.259 ^{ef}	0.042 ^{fgh}	47.0 ^{bcde}
50%	1	0.460 ^e	0.213 ^d	0.039 ^{efg}	66.6 ⁱ
	2	0.330 ^{bc}	0.285 ^{fgh}	0.049 ⁱ	65.0 ⁱ
	3	0.591 ^{hi}	0.284 ^{fgh}	0.036 ^{def}	66.6 ⁱ
	4	0.411 ^{de}	0.447 ⁱ	0.044 ^{ghi}	53.2 ^{cdefg}
	6	0.672 ⁱ	0.138 ^b	0.027 ^b	49.0 ^{bcde}
	8	0.628 ⁱ	0.160 ^{bc}	0.031 ^{bcd}	66.2 ⁱ
75%	1	0.217 ^a	0.281 ^{efg}	0.038 ^{efg}	34.0 ^a
	2	0.524 ^{fg}	0.110 ^a	0.059 ⁱ	59.2 ^{fghi}
	3	0.537 ^{gh}	0.175 ^c	0.044 ^{ghi}	48.8 ^{bcde}
	4	0.320 ^b	0.358 ⁱ	0.037 ^{def}	63.4 ^{hi}
	6	1.302 ^{if}	0.338 ⁱ	0.026 ^{ab}	58.6 ^{fghi}
	8	0.388 ^{cd}	0.379 ^{ide}	0.051 ⁱ	60.8 ^{fghi}
100%	1	0.440 ^{de}	0.304 ^{hi}	0.059 ⁱ	62.0 ^{ghi}
	2	0.667 ⁱ	0.392 ⁱ	0.039 ^{efg}	60.8 ^{fghi}
	3	0.731 ⁱ	0.268 ^{efg}	0.031 ^{bcd}	62.0 ^{ghi}
	4	0.628 ⁱ	0.248 ^e	0.035 ^{cde}	57.0 ^{efghi}
	6	0.595 ^{hi}	0.439 ⁱ	0.047 ^h	76.6 ⁱ
	8	0.469 ^{ef}	0.213 ^d	0.029 ^{bc}	68.0 ⁱ
SEM		0.011	0.005	0.001	8.08
Main effects					
Concentration					
25%		0.618 ^D	0.357 ^D	0.0465 ^D	52.2 ^A

Note: Values within the same column with similar superscripts are homogenous

SEM: Standard Error of mean

<i>Trichoderma viride</i> (10 ⁸ CFU ml ⁻¹)	Duration (days)	Electrical conductivity (dS/cm)	Total carbohydrate (mg/g)	Total protein (mg/g)	Crude Fat (%)
50%		0.515 ^A	0.254 ^A	0.0377 ^A	61.2 ^B
75%		0.548 ^B	0.270 ^B	0.0425 ^C	54.2 ^A
100%		0.588 ^C	0.311 ^C	0.0400 ^B	64.4 ^C
SEM		0.0044	0.002	0.0005	6.66
Duration					
1		0.410 ^A	0.275 ^B	0.0390 ^A	54 ^A
2		0.536 ^C	0.288 ^C	0.0510 ^C	60.2 ^B
3		0.564 ^D	0.372 ^E	0.0395 ^A	55.8 ^A
4		0.484 ^B	0.335 ^D	0.0450 ^B	54.2 ^A
6		0.850 ^E	0.265 ^B	0.0372 ^A	63 ^B
8		0.560 ^D	0.252 ^A	0.0382 ^A	60.4 ^B
SEM		0.00534	0.002	0.0006	4.04
Note: Values within the same column with similar superscripts are homogenous					
SEM: Standard Error of mean					

The shoot growth parameters measured in terms of height, collar diameter and number of leaves (Table 3) varied significantly due to biopriming concentration and duration ($p = 0.01$) at both storage periods. For S_1 , the seedlings bioprimered at 25% for 4 days (25.2 cm) recorded the highest height and biopriming at 25% for 3 days recorded the lowest value (10.4 cm). The collar diameter was highest on biopriming at 50% for 3 days (16.67 mm) followed by 100% for 3 days (11.00 mm) and the lowest was at 100% for 3 days (3.33 mm). The seedlings bioprimered at 25% for 4 days (41.9) followed by 75% for 4 days (39) recorded the largest number of leaves and the lowest was in biopriming at 100% for 6 days (18.6). During S_2 height of the seedlings was maximum (24.5 cm) in biopriming at 100% for 3 days and minimum (13.4 cm) for 50% for 6 days and 75% for 2 days. The highest collar diameter was recorded on biopriming at 50% for 2 days (3.34 g) and the lowest was at 75% for 6 days (1.62 mm) and a maximum number of leaves were recorded on biopriming at 100% for 1 day (23.7) and the minimum was at 50% for 1 day and 75% for 2 days (15.7). Root growth and total seedling length in sandal seedlings were significantly affected by biopriming concentration and duration (Table 4) for S_1 and S_2 . S_1 resulted in variation of seedling root length from 18.2 cm (25% for 2 days) to 4.87 cm (100% for 6 days). The highest total seedling length was observed in seedlings bioprimered at 25% for 4 days (41.9 cm) and the lowest was at 100% for 6 days (18.6 cm). For S_2 , a maximum root length of 27.53 cm was observed in biopriming at 50% for 8 days and the lowest was at 50% for 4 days (9.17 cm) and the highest number of roots were recorded in biopriming at 50% for 3 days, 75% for 1 day and 100% for 3 days (7.67) and the lowest was at 75% for 8 days (4.33). The highest seedling length was observed at 25% for 1 day (40 cm) and the lowest was at 75% for 2 days (22.8 cm).

Table 3
Effect of biopriming with *Trichoderma viride* on shoot growth parameters of the sandal seedling for the post priming storage of one day and one week

<i>Trichoderma viride</i> (10 ⁸ CFU ml ⁻¹)		Shoot Height (cm)		Collar Girth (mm)		Number of Leaves	
		Post priming storage					
Concentration	Duration (days)	S ₁	S ₂	S ₁	S ₂	S ₁	S ₂
25%	1	19.5 ⁱ	20.7 ^{bcd}	3.67 ^a	2.01 ^{bcdef}	37.6 ⁱ	20.0 ^{ab}
	2	15.7 ^{efg}	14.0 ^a	5.00 ^{abc}	2.90 ^{cdefg}	33.9 ⁱ	22.3 ^b
	3	10.4 ^a	-	3.67 ^a	-	23.3 ^{bcde}	-
	4	25.2 ⁱ	19.4 ^{bcd}	9.67 ^{bc}	1.73 ^{bcd}	41.9 ⁱ	16.3 ^a
	6	20.6 ⁱ	-	6.33 ^{abc}	-	37.8 ⁱ	-
	8	-	16.1 ^b	-	2.19 ^{bcdefg}	-	23.0 ^b
50%	1	18.9 ^{hi}	18.4 ^{bcd}	6.33 ^{abc}	3.01 ^{efg}	34.0 ⁱ	15.7 ^a
	2	18.8 ^{hi}	15.4 ^{ab}	6.00 ^{abc}	3.34 ^g	36.8 ⁱ	20.7 ^b
	3	13.4 ^{cde}	14.3 ^a	7.67 ^{bc}	1.78 ^{bcde}	22.3 ^{bcd}	20.3 ^{ab}
	4	13.3 ^{cde}	16.6 ^{bc}	8.67 ^{bc}	2.60 ^{bcdefg}	18.9 ^a	19.0 ^a
	6	12.1 ^{bc}	13.4 ^a	4.33 ^{abc}	2.93 ^{defg}	20.4 ^b	19.0 ^a
	8	16.7 ^{ghia}	13.8 ^a	7.67 ^{bc}	2.99 ^{defg}	25.9 ^{defg}	22.0 ^b
75%	1	15.0 ^{efg}	18.0 ^{bcd}	3.67 ^{ab}	1.82 ^{bcdef}	21.8 ^{bcd}	17.3 ^a
	2	15.6 ^{efg}	13.4 ^a	3.33 ^{ab}	1.66 ^{bc}	24.4 ^{cdef}	15.7 ^a
	3	-	-	-	-	-	-
	4	21.8 ⁱ	18.1 ^{bcd}	11.00 ^{cd}	1.65 ^b	39.3 ⁱ	19.7 ^a
	6	16.4 ^{fgh}	18.7 ^{bcd}	5.00 ^{abc}	1.62 ^a	31.4 ^h	21.3 ^b
	8	15.8 ^{efg}	20.4 ^{bcd}	5.00 ^{abc}	1.76 ^{bcde}	23.6 ^{cdef}	19.3 ^a
100%	1	16.3 ^{fgh}	16.8 ^{bc}	3.33 ^a	3.06 ^{fg}	28 ^{efgh}	23.7 ^b
	2	12.3 ^{bcd}	21.9 ^{cd}	6.33 ^{abc}	3.02 ^{efg}	30.8 ^{hi}	19.7 ^a
	3	16.9 ^{gh}	24.5 ^d	16.67 ^d	2.53 ^{bcdefg}	34.0 ⁱ	23.0 ^b
	4	16.9 ^{gh}	19.2 ^{bcd}	3.33 ^a	1.81 ^{bcdef}	30.5 ^{ghi}	21.7 ^b
	6	13.7 ^{cdef}	-	3.67 ^a	-	18.6 ^a	-
	8	12.7 ^{bcd}	-	5.00 ^{abc}	-	28.2 ^{fgh}	-
SEM		0.517	0.517	1.28	1.28	0.871	0.871
Main effects							
Concentration							
Note: Values within the same column with similar superscripts are homogenous							
SEM: Standard Error of mean, S ₁ Post priming storage for one day, S ₂ Post priming storage for one week							

<i>Trichoderma viride</i> (10 ⁸ CFU ml ⁻¹)	Shoot Height (cm)		Collar Girth (mm)		Number of Leaves	
	Post priming storage					
25%						

Table 4
Effect of biopriming with *Trichoderma viride* on root growth parameters of the sandal seedling for the post priming storage of one day and one week

<i>Trichoderma viride</i>		Root Length (cm)		Number of Lateral Roots		Total Seedling Length (cm)	
(10 ⁸ CFU ml ⁻¹)		Post priming storage					
Concentration	Duration (days)	S ₁	S ₂	S ₁	S ₂	S ₁	S ₂
25%	1	18.10 ^{hi}	19.27 ^d	3.67 ^{ab}	7.33 ^b	37.6 ⁱ	40.0 ^{de}
	2	18.20 ⁱ	10.63 ^{bc}	5.00 ^{abc}	6.67 ^b	33.9 ⁱ	24.6 ^b
	3	12.97 ^{efg}	-	3.67 ^{ab}	-	23.3 ^{bcde}	-
	4	16.73 ^{ghi}	9.33 ^b	9.67 ^{bc}	5.00 ^{ab}	41.9 ⁱ	28.7 ^{bcd}
	6	17.23 ^{hi}	-	6.33 ^{abc}	-	37.8 ⁱ	-
	8	-	14.2 ^{bcd}	-	5.33 ^{ab}	-	30.3 ^{bcde}
50%	1	15.13 ^{fghi}	10.37 ^{ab}	6.33 ^{abc}	5.67 ^{ab}	34.0 ⁱ	28.8 ^{bcd}
	2	17.97 ^{hi}	12.53 ^{bcd}	6.00 ^{abc}	4.67 ^{ab}	36.8 ⁱ	27.7 ^{bc}
	3	8.9 ^{bcde}	13.00 ^{bcd}	7.67 ^{bc}	7.67 ^b	22.3 ^{bcd}	27.3 ^{bc}
	4	5.57 ^{ab}	9.17 ^b	8.67 ^{bc}	7.33 ^b	18.9 ^a	25.8 ^b
	6	8.37 ^{bcd}	11.33 ^{bc}	4.33 ^{abc}	7.00 ^b	20.4 ^{ab}	24.7 ^b
	8	9.2 ^{cde}	27.53 ^e	7.67 ^{bc}	5.00 ^{ab}	25.9 ^{defg}	41.4 ^e
75%	1	6.80 ^{bc}	10.8 ^{bc}	3.67 ^{ab}	7.67 ^b	21.8 ^{bc}	28.8 ^{bcd}
	2	8.83 ^{bcde}	9.40 ^b	3.33 ^a	5.00 ^{ab}	24.4 ^{cdef}	22.8 ^a
	3	-	-	-	-	-	-
	4	17.43 ^{hi}	9.43 ^b	11.00 ^{cd}	6.00 ^{ab}	39.3 ⁱ	27.5 ^{bc}
	6	15 ^{fghi}	10.07 ^a	5.00 ^{abc}	7.00 ^b	31.4 ^{hi}	28.8 ^{bcd}
	8	7.83 ^{bcd}	10.67 ^{bc}	5.00 ^{abc}	4.33 ^a	23.6 ^{cdef}	31.1 ^{bcde}
100%	1	11.7 ^{def}	9.40 ^b	3.33 ^a	5.67 ^{ab}	28 ^{efgh}	26.2 ^b
	2	18.5 ⁱ	16.53 ^{cd}	6.33 ^{abc}	4.67 ^{ab}	30.8 ^{hi}	38.5 ^{cde}
	3	17.03 ^{ghi}	15.83 ^{bcd}	16.67 ^d	7.67 ^b	34.0 ⁱ	40.4 ^{de}
	4	13.93 ^{fgh}	12.60 ^{bcd}	3.33 ^a	6.00 ^{ab}	30.5 ^{ghi}	31.8 ^{bcde}
	6	4.87 ^a	-	3.67 ^{ab}	-	18.6 ^a	-
	8	15.5 ^{fghi}	-	5.00 ^{abc}	-	28.2 ^{fgh}	-
SEM		0.777	0.777	1.28	1.28	0.871	0.871
Main effects							
Concentration							
Note: Values within the same column with similar superscripts are homogenous							
SEM: Standard Error of mean, S ₁ Post priming storage for one day, S ₂ Post priming storage for one week							

<i>Trichoderma viride</i> (10 ⁸ CFU ml ⁻¹)	Root Length (cm)		Number of Lateral Roots		Total Seedling Length (cm)	
	Post priming storage					
25%						
	13.87 ^C	8.91 ^A	4.72 ^A	4.06 ^A	29.1 ^C	20.6 ^A
50%	10.86 ^B	13.99 ^B	6.78 ^B	6.22 ^B	26.4 ^B	29.3 ^B
75%	9.32 ^A	8.39 ^A	4.67 ^A	5.00 ^{AB}	23.4 ^A	23.2 ^A
100%	13.59 ^C	9.06 ^A	6.39 ^{AB}	4.00 ^A	28.4 ^C	22.8 ^A
SEM	0.317	0.504	0.523	0.491	0.356	0.907
Duration						
1	12.93 ^{BC}	12.46 ^{BC}	4.25 ^A	6.58 ^C	30.4 ^C	30.9 ^C
2	15.88 ^D	12.28 ^{BC}	5.17 ^{AB}	5.25 ^{ABC}	31.5 ^{CD}	28.4 ^{BC}
3	9.72 ^A	7.21 ^A	7.00 ^{BC}	3.83 ^{AB}	19.9 ^A	16.9 ^A
4	13.42 ^C	10.13 ^B	8.17 ^C	6.08 ^{BC}	32.6 ^D	28.5 ^{BC}
6	11.37 ^B	5.35 ^A	4.83 ^{AB}	3.50 ^A	27.1 ^B	13.4 ^A
8	8.13 ^A	13.10 ^C	4.42 ^{AB}	3.67 ^{AB}	19.4 ^A	25.7 ^B
SEM	0.388	0.618	0.641	0.601	0.436	1.11
Values within the same column with similar superscripts are homogenous						
Note: Values within the same column with similar superscripts are homogenous						
SEM: Standard Error of mean, S ₁ Post priming storage for one day, S ₂ Post priming storage for one week						

Among seedling biomass obtained for S₁, the highest total biomass (Table 5) was recorded on priming at 100% concentration for 1 day and 25% for 2 days (2.6.0 and 0.960 g, respectively) and the lowest value was at 100% for 6 days (0.31 g). The dry weight biomass of the components viz. leaf and shoot were the highest on bioprimer at 100% for 1 day (1.682 and 0.391 g respectively) and that of the root was highest on priming at 25% 4 days (0.441 g). The lowest leaf dry weight was observed on priming at 100% for 6 days (0.120 g), shoot weight at 50% for 1 day (0.067 g) and root weight at 75% for 4 days 0.047 g). The highest total biomass production obtained for S₂ was 0.960 g (25% for 2 days and 100% for 1 day) and the lowest was 0.18 g (50% for 8 days). When the component biomass was examined, the highest leaf dry weight was on bioprimer at 50% for 2 days (0.617 g), shoot weight was at 25% for 8 days (0.153 g) and root weight was at 50% for 3 days (0.370 g) and the lowest values of leaf, shoot and root dry weight were recorded at 100% for 1 day (0.093 g), 100% for 3 days (0.053 g) and 50% for 8 days (0.50 g), respectively. Analysis of variance revealed significant differences in total dry weight and weight of leaf shoot and root at 1% level.

Table 5

Effect of biopriming with *Trichoderma viride* on biomass production of the sandal seedling for the post priming storage of one day and one week

<i>Trichoderma viride</i>		Dry weight (g)							
(10 ⁸ CFU ml ⁻¹)		Leaf		Shoot		Root		Total	
		Post priming storage							
Concentration	Duration (days)	S ₁	S ₂	S ₁	S ₂	S ₁	S ₂	S ₁	S ₂
25%	1	0.260 ^{cdef}	0.263 ^{abcd}	0.178 ^{abc}	0.110 ^{ab}	0.137 ^{abc}	0.104 ^{abc}	0.593 ^{cde}	0.840 ^{ef}
	2	0.162 ^{bcd}	0.51 ^{cde}	0.118 ^{ab}	0.100 ^{ab}	0.053 ^a	0.140 ^{abc}	0.320 ^b	0.960 ^f
	3	0.242 ^{bcde}	-	0.150 ^{ab}	-	0.110 ^{abc}	-	0.484 ^{bcd}	-
	4	0.558 ^g	0.143 ^{ab}	0.147 ^{ab}	0.103 ^{ab}	0.441 ^d	0.093 ^{abc}	1.280 ⁱ	0.643 ^{de}
	6	0.329 ^{ef}	0.000 ^a	0.213 ^{ab}	0.000 ^a	0.097 ^{abc}	0.000 ^a	0.553 ^{cde}	0.37 ^{bc}
	8	-	0.193 ^{abc}	-	0.153 ^b	-	0.070 ^{abc}	-	0.897 ^{ef}
50%	1	0.249 ^{bcdef}	0.223 ^{abc}	0.067 ^{ab}	0.140 ^{ab}	0.070 ^{ab}	0.127 ^{abc}	0.382 ^{bc}	0.91 ^f
	2	0.551 ^g	0.617 ^e	0.171 ^{abc}	0.143 ^{ab}	0.107 ^{abc}	0.120 ^{abc}	0.853 ^{ghi}	0.00 ^a
	3	0.313 ^{def}	0.460 ^{cde}	0.120 ^{ab}	0.130 ^{ab}	0.187 ^{abcd}	0.370 ^d	0.656 ^{ef}	0.477 ^{cd}
	4	0.398 ^f	0.380 ^{bcde}	0.291 ^{bc}	0.130 ^{ab}	0.093 ^{abc}	0.160 ^{bc}	0.799 ^{fgh}	0.740 ^{ef}
	6	0.220 ^{bcde}	0.300 ^{bcd}	0.080 ^{ab}	0.090 ^{ab}	0.167 ^{abc}	0.178 ^{bc}	0.440 ^{bc}	0.83 ^{ef}
	8	0.602 ^g	0.217 ^{abc}	0.222 ^{abc}	0.103 ^{ab}	0.187 ^{abcd}	0.050 ^{ab}	1.071 ⁱ	0.18 ^{ab}
75%	1	0.656 ^g	0.540 ^{de}	0.147 ^{ab}	0.120 ^{ab}	0.1600 ^{abc}	0.207 ^c	1.004 ^{ghi}	0.490 ^{cd}
	2	0.140 ^{abc}	0.410 ^{cde}	0.264 ^{bc}	0.090 ^{ab}	0.0767 ^{ab}	0.193 ^{bc}	0.484 ^{bcd}	0.30 ^{bc}
	3	-	-	-	-	-	-	-	-
	4	0.173 ^{bcd}	0.253 ^{abcd}	0.133 ^{ab}	0.103 ^{ab}	0.047 ^a	0.120 ^{abc}	0.340 ^b	0.477 ^{bcde}
	6	0.898 ^h	0.457 ^{cde}	0.198 ^{abc}	0.067 ^{ab}	0.277 ^{bcd}	0.187 ^{bc}	1.153 ⁱ	0.740 ^{cdef}
	8	0.328 ^{ef}	0.400 ^{cde}	0.173 ^{abc}	0.060 ^{ab}	0.100 ^{abc}	0.097 ^{abc}	0.624 ^{def}	0.840 ^{def}
100%	1	1.682 ^{ia}	0.093 ^a	0.391 ^c	0.063 ^{ab}	0.400 ^{cd}	0.053 ^{ab}	2.602 ⁱ	0.960 ^f
	2	0.364 ^{ef}	0.287 ^{abcd}	0.187 ^{abc}	0.097 ^{ab}	0.0733 ^{ab}	0.107 ^{abc}	0.631 ^{def}	0.670 ^{ef}
	3	0.344 ^{ef}	0.117 ^{ab}	0.211 ^{abc}	0.053 ^{ab}	0.100 ^{abc}	0.127 ^{abc}	0.660 ^{ef}	0.643 ^{ef}
	4	0.329 ^{ef}	0.300 ^{abcd}	0.133 ^{ab}	0.0800 ^{ab}	0.1967 ^{abc}	0.170 ^{bc}	0.691 ^{efg}	0.37 ^{bc}
	6	0.120 ^{ab}	-	0.116 ^{ab}	-	0.080 ^{ab}	-	0.307 ^b	-
	8	0.264 ^{cdef}	-	0.096 ^{ab}	-	0.077 ^{ab}	-	0.458 ^{bc}	-
SEM		0.068	0.068	0.046	0.046	0.039	0.039	0.049	0.082
Main effects									
Note: Values within the same column with similar superscripts are homogenous									
SEM: Standard Error of mean, S ₁ Post priming storage for one day, S ₂ Post priming storage for one week									

<i>Trichoderma viride</i>	Dry weight (g)							
(10 ⁸ CFU ml ⁻¹)	Leaf	Shoot			Root		Total	
	Post priming storage							
Concentration								
25%	0.259 ^A	0.169 ^A	0.147 ^A	0.0778 ^{AB}	0.131 ^A	0.0739 ^A	0.539 ^A	0.317 ^A
50%	0.389 ^B	0.348 ^B	0.194 ^{AB}	0.1161 ^B	0.135 ^A	0.1667 ^B	0.699 ^B	0.631 ^C
75%	0.366 ^B	0.381 ^B	0.188 ^{AB}	0.0700 ^A	0.110 ^A	0.1339 ^B	0.6010 ^A	0.642 ^B
100%	0.515 ^C	0.125 ^A	0.225 ^B	0.0489 ^A	0.151 ^A	0.0761 ^A	0.891 ^C	0.230 ^A
SEM	0.0277	0.0208	0.0188	0.0108	0.0159	0.0114	0.033	0.0321
Duration								
1	0.712 ^D	0.290 ^{BC}	0.196 ^A	0.1033 ^C	0.1967 ^B	0.1317 ^B	1.146 ^D	0.506 ^C
2	0.304 ^B	0.417 ^D	0.185 ^A	0.0975 ^{BC}	0.0775 ^A	0.1400 ^B	0.572 ^B	0.698 ^C
3	0.225 ^A	0.117 ^A	0.111 ^A	0.0458 ^{AB}	0.0992 ^{AB}	0.1242 ^B	0.4500 ^A	0.314 ^{AB}
4	0.364 ^C	0.290 ^{BC}	0.176 ^A	0.1042 ^C	0.1917 ^B	0.1358 ^B	0.775 ^C	0.475 ^{BC}
6	0.388 ^C	0.166 ^{AB}	0.127 ^A	0.04 ^A	0.1550 ^{AB}	0.09 ^{AB}	0.614 ^B	0.393 ^{AB}
8	0.298 ^B	0.223 ^{BC}	0.123 ^A	0.0792 ^{ABC}	0.0908 ^{AB}	0.0542 ^A	0.538 ^{AB}	0.327 ^A
SEM	0.0339	0.028	0.0233	0.0233	0.0194	0.014	0.0408	0.0393
Note: Values within the same column with similar superscripts are homogenous								
SEM: Standard Error of mean, S ₁ Post priming storage for one day, S ₂ Post priming storage for one week								

When the seedling growth indices were compared, for S₁, the highest root: shoot ratio was observed in the seedlings bioprimered at 25% for 2 days (0.719) and the lowest was at 75% for 4 days (0.172). The vigour and Dickson quality indices were higher on bioprimering at 100% for 1 day (2052 and 0.245 respectively and the lowest was 50% for 8 days (414) and 25% for 2 days (0.031), respectively (Table 6). For S₂, the highest root: shoot ratio was obtained on bioprimering at 75% for 2 days (0.832) and the vigour and quality indices at 100% for 3 days (3332) and 25% for 2 days (0.112) respectively and the lowest root: shoot ratio and vigour and quality indices were obtained on bioprimering at 50% for 2 days (0.147), 50% for 8 days (414) and 100% for 1 day (0.027), respectively.

Table 6
Effect of biopriming with *Trichoderma viride* on seedling quality of the sandal seedling for the post priming storage of one day and one week

<i>Trichoderma viride</i>		Root : Shoot ratio		Vigor index		Dickson quality index (DQI)	
(10 ⁸ CFU ml ⁻¹)		Post priming storage for					
Concentration	Duration (days)	S ₁	S ₂	S ₁	S ₂	S ₁	S ₂
25%	1	0.424 ^{de}	0.39 ^{cde}	1303 ^e	1869 ^f	0.049 ^a	0.046 ^{abc}
	2	0.231 ^{bc}	0.370 ^{cd}	565 ^{de}	1357 ^{de}	0.031 ^a	0.112 ^{ef}
	3	0.407 ^{de}	-	140 ^a	-	0.066 ^{ab}	-
	4	0.719 ^f	0.388 ^{cd}	531 ^{de}	534 ^b	0.148 ^{de}	0.028 ^a
	6	0.251 ^{bcd}	-	379 ^{cd}	-	0.068 ^{abc}	-
	8	-	0.204 ^{ab}	-	886 ^c	-	0.041 ^{abc}
50%	1	0.269 ^{cd}	0.360 ^{bc}	816 ^e	1229 ^{de}	0.046 ^a	0.066 ^{bcd}
	2	0.199 ^{ab}	0.174 ^a	933 ^e	998 ^{cd}	0.066 ^{ab}	0.155 ^f
	3	0.630 ^{ef}	0.638 ^{de}	431 ^{cd}	1109 ^{cd}	0.086 ^{de}	0.106 ^{def}
	4	0.202 ^b	0.339 ^{bc}	214 ^b	1875 ^f	0.079 ^{cd}	0.090 ^{cde}
	6	0.620 ^{ef}	0.593 ^{de}	217 ^b	909 ^c	0.093 ^{de}	0.090 ^{cde}
	8	0.313 ^{de}	0.191 ^a	242 ^{bc}	414 ^b	0.152 ^{de}	0.054 ^{abcd}
75%	1	0.275 ^{cd}	0.332 ^{bc}	786 ^{de}	901 ^c	0.149 ^{de}	0.084 ^{bcd}
	2	0.235 ^{bcd}	0.832 ^f	227 ^b	200 ^a	0.051 ^{ab}	0.052 ^{abcd}
	3	-	-	-	-	-	-
	4	0.172 ^a	0.342 ^{bc}	314 ^{bc}	638 ^b	0.033 ^a	0.040 ^{abc}
	6	0.470 ^{de}	0.370 ^{cd}	355 ^{cd}	714 ^{bc}	0.160 ^e	0.063 ^{bcd}
	8	0.305 ^{de}	0.288 ^{ab}	362 ^{cd}	917 ^c	0.067 ^{ab}	0.036 ^{abc}
100%	1	0.195 ^{ab}	0.438 ^{cde}	2052 ^f	1225 ^{de}	0.245 ^f	0.027 ^a
	2	0.185 ^a	0.300 ^{ab}	597 ^{de}	1077 ^{cd}	0.074 ^{cd}	0.061 ^{bcd}
	3	0.227 ^{bc}	0.765 ^{ef}	883 ^e	3332 ^f	0.069 ^{abc}	0.030 ^{ab}
	4	0.497 ^{de}	0.716 ^{ef}	509 ^{de}	1528 ^{ef}	0.069 ^{abc}	0.037 ^{abc}
	6	0.426 ^{ade}	-	459 ^{cd}	-	0.045 ^a	-
	8	0.286 ^{cde}	-	922 ^e	-	0.056 ^{ab}	-
SEM		0.0806	0.0806	25.7	25.7	0.0148	0.0108
Main effects							
Concentration							
Note: Values within the same column with similar superscripts are homogenous							
SEM: Standard Error of mean, S ₁ Post priming storage for one day, S ₂ Post priming storage for one week							

<i>Trichoderma viride</i>	Root : Shoot ratio		Vigor index		Dickson quality index (DQI)	
(10 ⁸ CFU ml ⁻¹)	Post priming storage for					
25%						
	0.339 ^{AB}	0.226 ^A	486 ^B	774 ^B	0.0603 ^A	0.0378 ^{AB}
50%	0.372 ^B	0.376 ^B	476 ^B	1089 ^C	0.0870 ^B	0.0935 ^C
75%	0.243 ^A	0.361 ^B	341 ^A	562 ^A	0.0768 ^{AB}	0.0459 ^B
100%	0.303 ^{AB}	0.370 ^B	904 ^C	1194 ^C	0.0931 ^B	0.0258 ^A
SEM	0.0329	0.0333	10.5	38.6	0.00603	0.00442
Duration						
1	0.291 ^{AB}	0.380 ^{BC}	1265 ^C	1306 ^C	0.1222 ^C	0.0559 ^B
2	0.213 ^A	0.419 ^C	607 ^B	908 ^B	0.0556 ^A	0.0951 ^C
3	0.316 ^{AB}	0.351 ^{BC}	389 ^A	1110 ^C	0.0552 ^A	0.0338 ^{AB}
4	0.397 ^B	0.446 ^C	418 ^A	1144 ^C	0.0823 ^{AB}	0.0488 ^{AB}
6	0.442 ^B	0.241 ^{AB}	378 ^A	406 ^A	0.0917 ^{BC}	0.0382 ^{AB}
8	0.226 ^A	0.161 ^A	382 ^A	554 ^A	0.0688 ^{AB}	0.0328 ^A
SEM	0.0403	0.0408	12.9	47.3	0.00739	0.00541
Note: Values within the same column with similar superscripts are homogenous						
SEM: Standard Error of mean, S ₁ Post priming storage for one day, S ₂ Post priming storage for one week						

To find the seedling growth and biomass parameters which strongly influenced by the biopriming, principal component analysis with Varimax Rotation was conducted for S₁ and S₂. Extracted the principal component (PC) were analysed based on the Eigenvalues and percentage of variance. Based on Eigenvalue and total variance explained by different principal components (Table 7) at 180 days after transplanting, for One day after storage (S₁), the first PC exhibited the highest loadings on shoot length, root length, collar diameter, number of leaves, number of roots, leaf area and total seedling length. The first PC could explain 48.38% of the variance in response to the priming treatment. The PC2 had higher loading on biomass parameters such as dry weight leaf, dry weight root, dry weight stem and total dry weight and explained 18.76% of the variance. The results of a principal component factor analysis with varimax rotations for one week of storage (S₂) are shown in Tables 8–9. For S₂, first two PCs explained 78.9% of variation (Table 8). The PC1 had higher loading for all the seedling as well as biomass parameters (Table 10) which indicated that both seedling and biomass attributes contribute to 66.11% variance in response to the priming. The PC2, a much lower proportion of variance (12.77%) in response to the treatment. Hence for S₁, the maximum impact of priming was on the seedling parameters of sandal, and for S₂ priming has an impact on both growth and biomass attributes of sandal seedlings.

Table 7

Total Variance Explained by different principal components at 180 DAT for seedlings obtained from seeds subjected to biopriming with *Trichoderma viride* and post priming storage of one day

Component	Initial Eigen values			Rotation Sums of Squared Loadings		
	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	5.3	48.4	48.4	4.1	37.4	37.4
2	2.1	18.8	67.1	3.3	29.7	67.1
3	0.9	8.1	75.2			

Table 8

Factor loadings of seedling attributes at 180 DAT for seedlings obtained from seeds subjected to biopriming with *Trichoderma viride* and post priming storage of one day

Variables	Principal Component	
	1	2
Shoot length	0.85	0.40
Root length	0.79	0.15
Collar diameter	0.71	0.45
Number of leaves	0.69	0.40
Number of roots	0.71	-0.02
Leaf dry weight	0.03	0.90
Root dry weight	0.15	0.78
Shoot dry weight	0.20	0.52
Total dry weight	0.11	0.97
Leaf area	0.66	-0.11
Total seedling length	0.89	0.29

Table 9

Total Variance Explained by different principal components at 180 DAT for seedlings obtained from seeds subjected to biopriming with *Trichoderma viride* and post priming storage of one week

Component	Initial Eigenvalues			Rotation Sums of Squared Loadings		
	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	7.3	66.1	66.1	5.1	45.9	45.9
2	1.4	12.8	78.9	3.6	33.0	78.9
3	0.5	4.9	83.8			

Table 10
Factor loadings of seedling attributes at 180 DAT for seedlings obtained from seeds subjected to biopriming with *Trichoderma viride* and post priming storage of one week

Variables	Component	
	1	2
Shoot length	0.89	-0.22
Root length	0.83	-0.34
Collar diameter	0.84	-0.23
Number of leaves	0.89	-0.17
Number of roots	0.82	0.11
Leaf dry weight	0.71	0.54
Root dry weight	0.68	0.53
Shoot dry weight	0.75	0.16
Total dry weight	0.82	0.55
Leaf area	0.75	-0.41
Total seedling length	0.93	-0.30

To find the best-performing treatments, cluster analysis was conducted for the seedlings obtained from seeds subjected to post priming storage of one day and one week. Hierarchical clustering with Euclidian distance measure grouped the treatments into homogenous clusters with maximum homogeneity within the cluster. For S_1 , clustering at height 30 (Fig. 1), leads to the formation of two homogenous clusters, one with treatments 6 (25% for 8 days) and 15 (75% for two days) which were with lower seedling traits compared to the second cluster (Fig. 2). The superior cluster at height 20 gave two homogenous clusters one with superior seedling traits which contain treatments, 1, 2, 4, 5, 7 and 8 (25% for one day, 25% for two days, 25% for four days, 25% for six days and 50% for one day, respectively) and the other with minimum value. At the height 10, the superior cluster gave two homogenous clusters, one with maximum value (4) and the rest in the second cluster. Hence, biopriming with *Trichoderma viride* at 25% for four days produces better seedlings. All the treatments at 25% concentration were superior to the rest of the treatments.

Results of the hierarchical clustering for S_2 are displayed on the dendrogram (Fig. 3). Based on the dendrogram, at height 30, two homogenous clusters were obtained, the one with treatments 3, 5, 15, 23 and 24 which were inferior in seedling traits and another one with superior seedling traits including the rest of the treatments. At the height 20, three homogenous clusters were obtained for the superior cluster at the height 10 and the cluster containing the treatments 20 and 21 were superior in seedling traits compared to other clusters. The examination of seedling traits indicated that the treatment 21 i.e., biopriming with *Trichoderma viride* at 100% for 3 days was better compared to 20 (biopriming at 100% for 2 days).

When the seedling growth indices were compared, for S_1 , the highest root: shoot ratio was observed in the seedlings bioprimed at 25% for 2 days (0.719) and the lowest was at 75% for 4 days (0.172). The vigour and Dickson quality indices were higher on biopriming at 100% for 1 day (2052 and 0.245 respectively) and the lowest was 50% for 8 days (414) and 25% for 2 days (0.031), respectively (Table 6). For S_2 , the highest root: shoot ratio was obtained on biopriming at 75% for 2 days (0.832) and the vigour and quality indices at 100% for 3 days (3332) and 25% for 2 days (0.112) respectively and the lowest root: shoot ratio and vigour and quality indices were obtained on biopriming at 50% for 2 days (0.147), 50% for 8 days (414) and 100% for 1 day (0.027), respectively.

4. Discussion

Recently, biopriming has gained importance as a pre-sowing treatment to enhance the rate and uniformity of seed germination and seedling quality. The unique achievements due to priming are manifested as increased germination rate, uniform germination, and increased total germination percentage (Basra et al. 2005). In addition to disease control, certain strains of *Trichoderma* are found to be effective as bio-stimulants of growth and development in many crops (Harman et al. 2004; Bhardwaj et al. 2014). The most crucial

element of a plant's life cycle is seed germination. and the increased and uniform germination determines the growth and vigour of the seedlings. Results of the study indicated that sandal seed germination was affected by the concentration and duration of the biopriming agent *T. viride*. Post priming storage of seeds was found to influence the germination characteristics of the sandal seeds. The studies on storage of post primed seeds are limited and a few studies indicated the detrimental effect of priming on viability (Schwember and Bradford 2005; Hill et al. 2007) while others reported positive influence on viability (Dearman et al. 2008; Venkatasubramanian and Umarani 2010; Abnavi and Ghobadi 2012). Post priming storage of the seeds for one day (S_1) resulted in the highest germination on biopriming at 100% for 1 day (73.30%) which was followed by that at 75% (36%) and 25% (34.61%) for one day. The biopriming at a higher concentration for lower priming duration resulted in better germination in the sandal seeds for S_1 and most of the treatment combinations could produce less than 20% germination only. Meanwhile, for S_2 biopriming with *T. viride* at 100% for 3 days recorded the highest germination (82.72%) followed by priming at the concentration 50% for 4 days (72.6%) and 25% for 2 days (55.4%). The biopriming at higher durations was detrimental to seed germination especially for 6 and 8 days and most of the treatment combinations could produce above 25% germination. There was an increase of 112% in the highest germination obtained S_2 compared to S_1 the period to complete germination was reduced from 71 days (S_1) to 40 days for S_2 in sandal seeds. Sandal normally takes 4–12 weeks to complete germination (Srimathi et al. 1995; Suthesh et al. 2016). Hence, the present study's achievement of a shorter germination period (about six weeks) was a significant achievement. Increased germination rate and uniformity can be ascribed to the metabolic repair during imbibition (Bray et al. 1989), enhancement of germination promoting metabolites (Basra et al. 2005) and osmotic adjustments (Bradford, 1986). In addition, the synthesis of growth hormones, enhancement of nutrient availability, and provision of biological control attributable to fructification of antibiotics or siderophores (Deepa et al. 2010) which is reported to enhance germination. Moreover, *T. viride* produces IAA which is the major auxin in plants that regulates cell division, enhances photosynthetic activities, and activated the translocation of carbohydrates thereby enhancing root initiation, flowering, fruit setting and ripening etc. (McDonald, 1999). Both exogenous and endogenous IAA inhibits seed germination in an ABA-dependent manner, indicating that auxin acts synergistically to inhibit seed germination (Liu et al. 2013) which may be the basic mechanism behind biopriming, on pre-treating with GA_3 which acts antagonistically to ABA (Weiss and Ori, 2007) breaks dormancy and led to the germination of the seeds.

The electrical conductivity of seed leachates was measured immediately after priming. The loss of cell membrane integrity and increasing leakage are thought to represent the beginning of the ageing process (McDonald, 1999). The higher EC of the seed leachates is associated with seed deterioration and loss of vigour (Perry and Harrison, 1977) but lower values indicate the precise integrity of cellular membranes during priming which prevents the leakage from the cells (Copeland and Mc Donald, 2001). Chitra (2019) reported that the electrical conductivity of sandal seeds without any priming (control) was 1.11 dS cm^{-1} and most of the biopriming treatments with *T. viride* recorded the lowest values compared to this. Only the treatment 75% at 6 days (1.32 dS cm^{-1}) recorded the highest value than control. Correlation analysis indicated that the EC of the seed leachates was negatively correlated with the germination at S_1 ($r = -0.405$, $p = 0.05$). The bulk electrical conductivity (EC) test of seed soak water was significantly and negatively correlated with total seed germination (radicle emergence) of seed lots in *Cyanus segetum*, *Prunella vulgaris*, *Valeriana officinalis* and *Centaurea nigra* (Marin et al. 2018). The lower EC of the seed leachates on biopriming in this study implies that seed leakage is reduced, resulting in improved sandal seed membrane integrity. This is due to a cell repair mechanism triggered by enzyme activity. In comparison to hydropriming and control, Rinku et al. (2017) found that biopriming resulted in a low EC. This further indicates improved membrane integrity of the cells resulting in the better utilization of the mobilized seed reserves and hence enhanced germination and fastened the subsequent seedling growth.

Pierson's bivariate correlation was fitted among the germination at S_1 and biochemical parameters. Seed germination was significantly and positively correlated with carbohydrate ($r = 0.703$, $p = 0.01$) and protein ($r = 0.647$, $p = 0.01$). During germination, storage reserve materials are quickly degraded and used as energy sources for the radicle protrusion and subsequent seedling development. According to Zhao et al. (2018), soluble sugar and soluble protein contents at different germination stages were positively and significantly correlated with germination rate, but those reserves in dry seeds were not significantly correlated. During germination, starch is hydrolyzed to sugars, while protein is degraded by proteases and transformed to polypeptide and amino acids by proteases (Palmiano and Juliano, 1972; Levin, 1974). These hydrolysis products may be sufficient to supply the energy requirements of seeds during germination (Bewley and Black 1994). The function of carbohydrates is to serve as a carbon source for the seeds during the germinative process as they display features that confer advantages to germination, such as being fast-use reserves for energy production (Buckeridge et al. 2004). No correlation was obtained between germination and the fat concentration of seeds. Similar to our study, lipid concentration in seeds *Dalbergia nigra* remained constant or increased slightly during germination (Ataíde et al. 2013). Sharifi (2012) also reported that the biopriming increased the biochemical components of the seeds like carbohydrate, protein, and oil content in safflower.

The seedling growth and biomass production considerably varied due to biopriming and the hierarchical cluster analysis indicates that for S₁, *T. viride* at 25% for 4 days produces the better seedlings and all the treatments at 25% concentration were superior to the rest of the treatments but the germination ranged from 3 to 35% in these treatments and highest was on biopriming at 100% for 1 day (73%) and vigour index and quality indices of the seedlings were also higher in this treatment. For S₂, biopriming with *T. viride* at 100% for 3 days was the best treatment which corresponds to the highest germination, quality index and vigour index. Hence, this treatment can be suggested to improve the germination and seedling growth of the sandal. *Trichoderma* spp. is a plant growth-promoting fungi (PGPF) with the ability to produce siderophore, phosphate-solubilising enzymes, and phytohormones (Doni et al. 2013). Many microorganisms like *Trichoderma* spp. produces organic acids and phosphatase that solubilise the unavailable phosphate to its available form that can be easily absorbed by plants. It also helps to increase the nitrogen use efficiency in plants (Rakshit et al. 2015). *Trichoderma* induces IAA production and so it helps root growth and seedling quality in different plants (Morales and Stall, 2004; Graval *et al.* 2006). Hence, the *Trichoderma* based priming strategies are useful in quality seedling production.

Declarations

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1. Ethical Approval:

Not using any human and/or animal.

2. Competing Interests:

The authors declare no competing interests.

3. Authors' contributors:

Anjali KS carried out the experiment, collected the data, and wrote the manuscript with the help of Jijeesh CM and Arshad A. Krishnamoorthi S, Santhoshkumar AV, Jamaludheen V and Fasilkhan Nageri worked out almost all of the technical details, like analysis tools, research methodologies, and appropriate statistical tools, and performed the statistical calculations for all the experiments. All authors contributed critical feedback and helped shape the research, analysis, and manuscript.

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5. Availability of data and materials

All data are analysed is available to the manuscript not published elsewhere.

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Figures

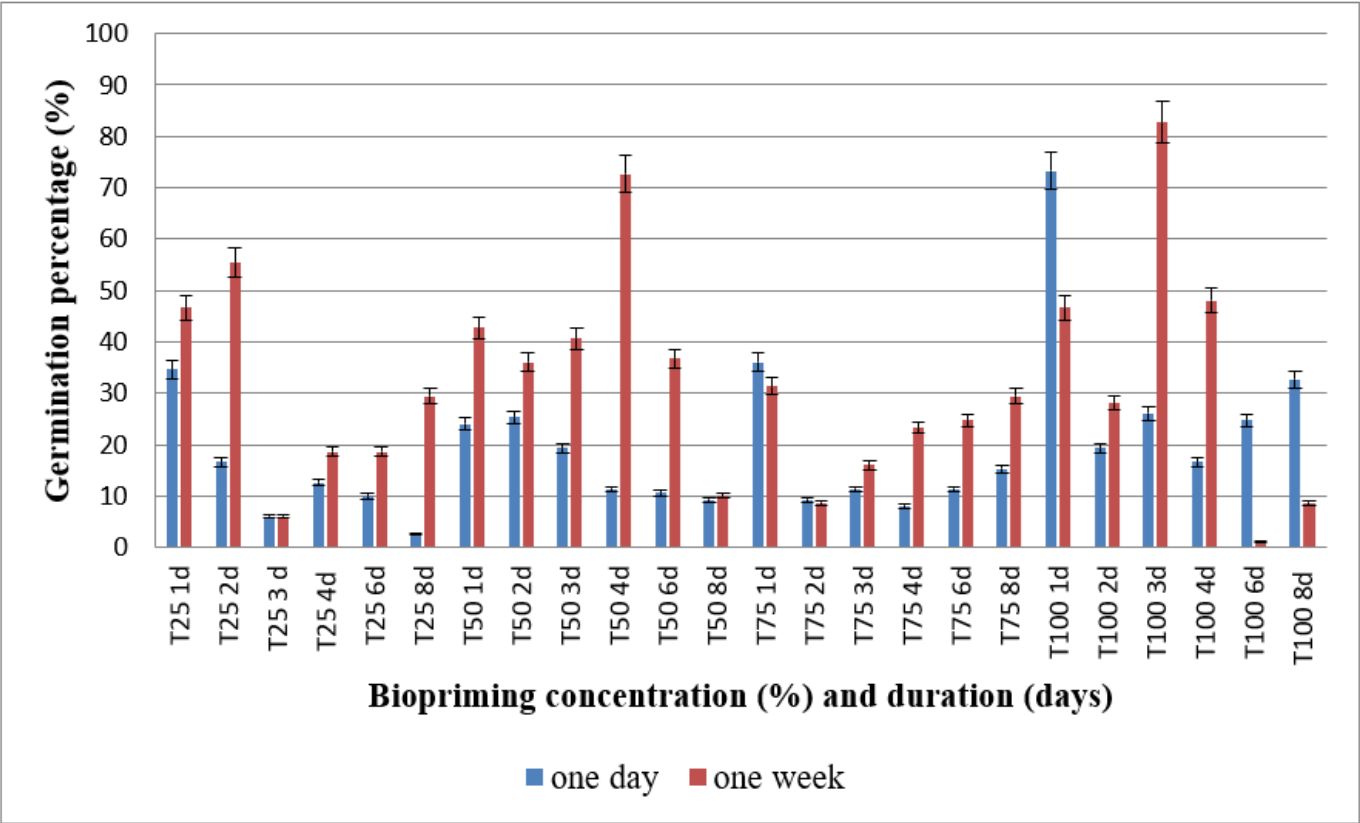


Figure 1

The germination percentage of sandal seeds as affected by the concentration and duration of *Trichoderma viride* for the post priming storage of one day and one week

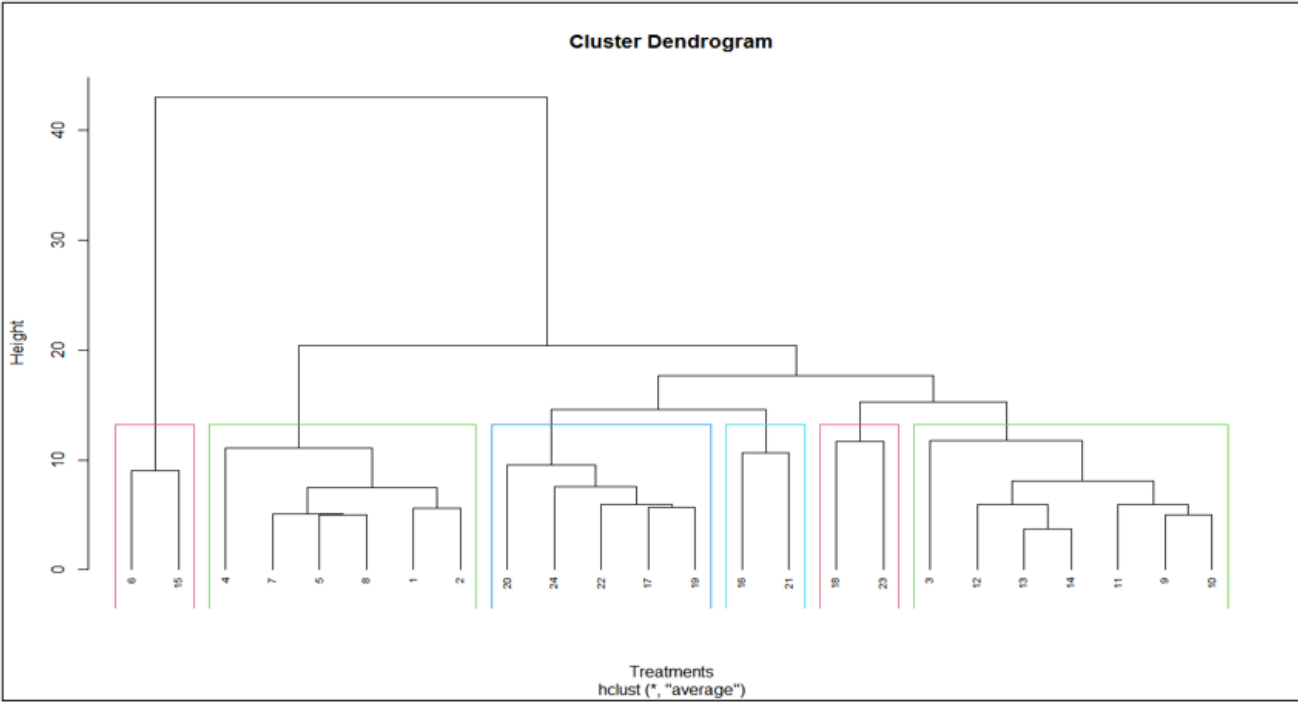


Figure 2

Dendrogram tree processed by R software for the growth and biomass attributes of sandal seedling obtained from the seeds subjected to post priming storage of one day

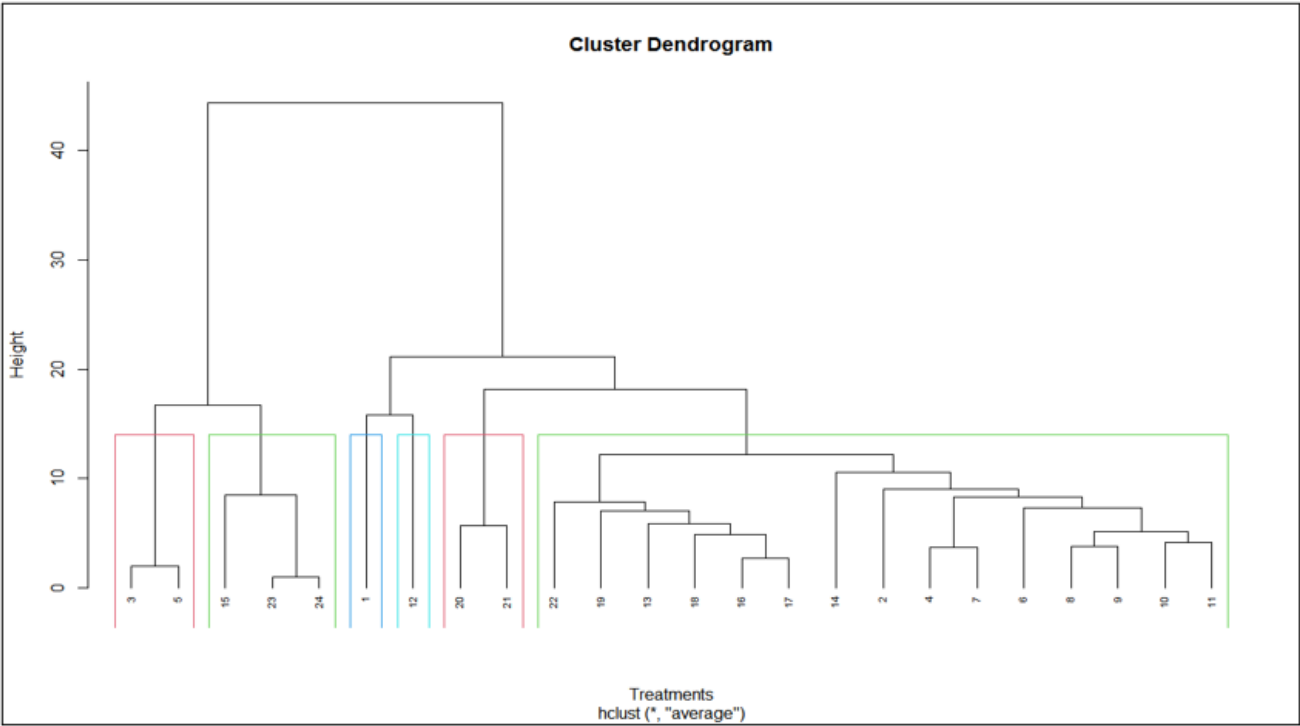


Figure 3

Dendrogram tree processed by R software for the growth and biomass attributes of sandal seedling obtained from the seeds subjected to post priming storage of one week