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RESEARCH ARTICLE

Plant growth promoting rhizobacterium (PGPR): An effective consortium for sandal (*Santalum album* L.) seed germination

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Abstract

Plant growth promoting rhizobacteria (PGPR) is a consortium of beneficial microorganisms having broad spectrum of mechanisms promoting plant growth. Sandal (*Santalum album* L.) seeds, in general have poor and staggered germination which is a major constraint in nursery management. Seed biopriming with beneficial microbes is one of the cheapest and eco-friendly seed enhancement techniques resulting in rapid, uniform, high crop establishment and yield. Fresh mature sandal seeds were procured from the Nachivayal Reserve Forest, Marayur Sandal Division, Kerala, India. The trial was conducted in two factorial Completely Randomized Design (CRD) with different concentrations of PGPR II (25, 50, 75 and 100 %) and different duration of the treatment (1, 2, 3, 4, 6 and 8 days) as factors. The effect of post-priming storage of one day and one week was also analyzed. The trial results in positive responses with respect to the germination percentage and seedling establishment. The highest germination was obtained for the seeds bioprimed at 50 % for 3 days (76.6 %) for post-priming storage of one week and highest seedling growth was obtained for the seeds bioprimed at 100 % for post-priming storage of one day and at 50 % for 3 days for post-priming storage one week. Both germination and seedling characters are its peak at biopriming with PGPR II at 50 % for 3 days subjected to post-priming storage of one week and hence recommended as the best treatment.

Keywords: biopriming; germination; plant growth promoting rhizobacteria; post-priming storage; sandal

Introduction

Priming is the controlled hydration technique in which the seed metabolic activity is enhanced but suspended prior to radicle protrusion (1). During priming, a specific physiological state is induced in plants using natural and/or synthetic compounds to the seeds before germination (2). The various priming agents used includes water (hydropriming) or Polyethylene glycol (PEG) (osmopriming) or salt ($CaCl_2$, $CaSO_4$ or NaCl etc.) or another chemical or living bacterial inoculums (biopriming) prior to germination (3, 4). Seed priming with living bacterial inoculum is known as biopriming (5), which involves the application of plant growth promoting rhizobacterium (PGPR) resulting in enhanced germination, plant growth and disease resistance (6). In addition, it ameliorates a wide variety of biotic, abiotic and physiological stresses to seeds and seedlings. Biopriming agents include useful microorganisms like fungi and bacteria e.g. *Trichoderma, Pseudomonas, Bacillus, Rhizobia* etc. Biopriming also offers an opportunity to replace the use of chemicals to control pests and diseases. During biopriming, the bacteria enter/adhere the seeds and acclimatize under familiar conditions. PGPR can produce Indole-3-acetic acid (IAA) like, compounds (7), which improves plant growth via improved seed emergence period, plant growth and crop yield. Seed priming has additionally proven to be successful in lowering the germination time and uniform seedling germination of few vital tree species (8, 9).

Sandalwood (*Santalum album* L.) is a slow growing, evergreen tree species known for its heavy, fine grained, aromatic wood and essential oil which fetches higher price in the international market. *Santalum album* is one of the most important industrial and medicinal plants in the world and has a long record of use in conventional medical systems. The demand of sandalwood is escalating in global market. The annual demand for sandalwood for instance, is projected to be 5000 to 6000 MT and that for oil to be 100 to 120 MT (10). The declining natural sandal populations due to over-exploitation, recurring annual fires, spike disease, illegal felling and smuggling have resulted in the International Union for Conservation of Nature (IUCN) red list categorising this species as 'Vulnerable' (11). Sandal is distributed throughout India, with over 90 % of its stock in Karnataka and Tamil Nadu which account for approximately 9600 km^2 (12, 13).

The fruit is a drupe and the hard seed coat makes it hard to germinate the seed. In addition, the morpho-physiological dormancy confirmed in the sandal seeds makes germination difficult (14). Therefore, new techniques to improve the germination and uniform growth of the seedlings of sandal must be achieved for the production of high-quality planting stock. The germination in sandal is poor and scattered and sometimes may take a year to complete. Prolonged nursery period is a major

hurdle in raising high-quality planting stock of sandal. Considering the high demand and diminishing supply of sandal trees, there is great potential for raising it, not only in forest lands but also in private lands such as home gardens and other agroforestry systems. Many pre-sowing treatments are tried to invigorate sandal seeds with varying degrees of success of which the Gibberellic Acid (GA_3) treatment is reported to be the best. One previous study conducted at College of Forestry with different concentration of priming agents like water, Manganese sulfate ($MnSO_4$), PEG 6000 and the PGPR *Pseudomonas fluorescens* Migula for various duration indicated that higher duration of treatment and post-priming storage for one day was the best in terms of germination and seedling attributes; and the biopriming treatment was superior to other priming methods. The study also indicated that the post priming storage of seeds for one month failed to germinate (15).

PGPR consortium can be beneficial to growth and stimulate the growth directly or indirectly by various means like nitrogen fixation and phosphate solubilisation. PGPRs also play a pivotal role in crop production by indirect means which includes siderophore production and *in situ* antagonism of soil-borne root pathogens (16–19). PGPR are also involved in phytohormone production and bioremediation (20, 21). PGPR Mix II of Kerala Agriculture University (KAU) is a consortium of highly compatible rhizobacteria having broad spectrum of antagonistic properties. With this backdrop, the current study is focussed to assess the impact of biopriming with PGPR Mix II of KAU (consortium of *Bacillus subtilis* (Ehrenberg) Cohn and *P. fluorescens*) and post-priming storage of seeds on the germination and seedling attributes of *S. album.*

Materials and Methods

The present trial was conducted at the College of Forestry, KAU, Thrissur, from May 2019 to June 2020. Sandal seeds were procured from the Nachivayal Reserve Forest I and II of Marayur sandal Division, Kerala, India (77^0 5' to 77^0 15' E longitude and 10^0 10' to 10^0 20' N latitude) during October to November 2019 and brought to the laboratory. The seed lot was mixed 4 times and quarter divided to improve homogeneity (22).

Biopriming with plant growth promoting rhizobacteria (PGPR) II

The suspension cultures of the inoculant (PGPR mix II) were obtained from the Department of Microbiology College of Agriculture, Vellayani, KAU. Treating 20 g of the powder formulations of the PGPR II at 10^8 CFU $mL^{-1}$for 50 seeds produces a concentration of 100 %. The experiment was conducted in a two factorial Completely Randomized Design (CRD). 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.

After priming, the seeds with the bioinoculant were kept in glass bottles wrapped with aluminium foil to prevent contamination and stirred at regular intervals to prevent hardening of the priming agent. After priming operations, the seeds were washed thrice with sterile distilled water and dried with Whatman No.1 filter paper in shade at 25 °C till the seeds achieved the initial moisture content before priming. The seeds after drying are stored in brown paper bags, kept in glass containers and stored at ambient conditions for one day and one week. Prior to sowing the seeds are pre-treated with 500 mg L^{-1} (w/v) of GA_3 (Merck) overnight. The seeds were line sown in plastic germination trays of size 30 × 30 × 5 cm with sand as germination medium. There were three replications for each treatment and 50 seeds constituted one replication (1, 23, 24).

The germination trays were irrigated uniformly twice a day with a rose can until the germination was completed. Daily germination counts were recorded and the germination percentage was calculated. The germination value (GV) was calculated with the following formula (25):

$$GV = MDG \times PV \quad \text{(Eqn. 1)}$$

Where, MDG = Final mean daily germination, PV = Highest value of mean daily germination.

The mean daily germination 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.

Seed chemical analysis

For biochemical analysis, the seeds after priming were immediately subjected to chemical analysis in 5 replications. The electrical conductivity of the seed leachates, a clear augury of seed membrane stability, was determined using the conductivity meter (CDC 40101). The total carbohydrate was estimated by Anthrone method and was expressed in mg g^{-1} (26) and protein content were estimated by the standard method and was expressed in mg g^{-1} (27). The crude fat content of the primed seeds was estimated by Soxhlet extraction and expressed in percentage (%) (28).

Seedling growth estimation

To find the impact of biopriming on the seedling growth parameters, 12 uniform seedlings of 4–6 leaf stage were transplanted to polythene bags containing soil, sand and Farm Yard Manure (FYM) as potting medium in the ratio 3:1:1 and kept in the nursery for six months. The seedling growth and biomass production 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 per the standard method (29).

$$VI = GP \times (SL + RL) \quad \text{(Eqn. 2)}$$

Where, VI = Vigour index, GP = Germination percentage, SL = Shoot height and RL = Root length.

The root: shoot ratio of the seedlings was worked out using the formula:

$$\text{Root: Shoot ratio} = \frac{\text{Root dryweight (g)}}{\text{Shoot dryweight (g)}} \quad \text{(Eqn. 3)}$$

The seedling quality index was calculated as:

$$\text{Seedling quality index} = \frac{\text{Total seedling weight (g)}}{\frac{\text{Height (cm)}}{\text{Diameter (mm)}} + \frac{\text{Shoot dryweight (g)}}{\text{Root dryweight (g)}}} \quad \text{(Eqn. 4)}$$

The statistical analysis was conducted using Agricolae taking the concentration and duration as the factors and Tukey's Honestly Significant Difference (HSD) for the post-hoc test under R environment (30). The results are presented separately for the post -priming storage of sandal seeds for one day and one week.

Results and Discussion

Seed germination

Concentration and duration of PGPR II at two post-priming storage periods significantly influenced the germination percentage of sandal (Fig. 1) and other germination attributes also varied (Table 1). For seeds stored for one day after priming, the highest germination (29.36 %) occurred in the treatment with PGPR II at 75 % for 6 days, while the lowest (1.22 %) was recorded at 50 % for 3 days. The corresponding imbibition and germination periods were 18 and 35 days, respectively. The highest vigour indices MDG (4.55), PV (3.41) and GV (15.56) were obtained with 25 % for 2 days, whereas the lowest values occurred at 50 % for 1 day.

For seeds stored for one week, PGPR II at 50 % for 3 days resulted in the highest germination (76.60 %), while no germination was observed at 100 % for 3 days and 25 % for 1 day. The corresponding imbibition and germination periods were 18 and 35 days. Maximum MDG (6.76), PV (6.57) and GV (44.41) were recorded at 25 % for 3 days and the minimum at 100 % for 3 days and 25 % for 1 day. The concentration × duration interaction had a highly significant effect on germination percentage for both one-day ($F = 65.667$; $p < 0.01$) and one-week storage periods ($F = 1909.205$; $p < 0.01$).

Post-priming storage seeds for one week resulted in a higher germination which was 2.5 times more compared to the highest germination for seeds sown immediately after priming. Perusal of data also indicate that the imbibition period, time to initiate germination was spread over 16–21 days and germination period, time to complete germination was 21–47 days for different treatment combinations and these periods were 16 and 35 days in treatment with highest germination. All non-germinated seeds were subjected to a cutting test at the end of the germination trial

Table 1. Effect of biopriming with PGPR II on the germination attributes of sandal seeds subjected to post priming storage of one day and one week

PGPR II		Imbibition period		Germination period		Mean daily germination		Peak value		Germination value	
		Post priming storage for									
Concentration	Duration (days)	1 day	1 week	1 day	1 week	1 day	1 week	1 day	1 week	1 day	1 week
25 %	1	21	-	35	-	1.52	-	1.83	-	2.79	-
	2	21	18	24	42	4.56	2.05	3.42	2.32	15.56	4.76
	3	20	19	28	42	2.00	6.76	0.19	6.57	0.38	24.45
	4	21	23	28	34	2.27	2.00	2.27	0.52	5.14	1.04
	6	19	16	27	37	2.32	4.20	2.51	2.00	5.82	8.40
	8	22	32	26	35	1.35	0.82	1.50	1.00	2.03	0.82
50 %	1	21	15	35	41	1.00	1.17	0.25	1.33	0.25	1.56
	2	19	15	28	25	2.50	2.00	0.38	0.34	0.96	0.69
	3	21	18	21	35	1.86	2.26	1.86	2.46	3.45	5.56
	4	14	16	28	35	2.13	4.67	1.21	4.12	2.58	19.22
	6	20	16	25	34	2.43	1.78	0.72	1.33	1.76	2.37
	8	20	18	28	46	2.00	0.75	1.08	0.52	2.15	0.39
75 %	1	15	20	30	23	0.89	1.91	1.00	2.10	0.89	4.01
	2	19	19	28	34	1.43	1.19	1.00	1.51	1.43	1.80
	3	21	18	38	25	2.80	0.94	1.17	1.00	3.27	0.94
	4	18	15	26	48	2.36	1.29	1.63	0.72	3.84	0.93
	6	18	16	35	21	2.22	0.41	1.57	0.53	3.49	0.22
	8	23	15	47	24	1.58	0.53	1.19	0.72	1.88	0.38
100 %	1	19	16	28	45	1.50	0.82	0.86	0.53	1.29	0.43
	2	18	17	28	48	2.56	2.70	1.64	2.16	4.20	5.83
	3	16	-	36	-	1.50	-	0.86	-	1.29	-
	4	19	15	26	23	2.00	0.94	1.23	1.29	2.46	1.21
	6	13	15	32	47	3.44	2.06	2.21	2.67	7.63	5.51
	8	21	15	28	34	1.50	1.37	0.86	1.53	1.29	2.09

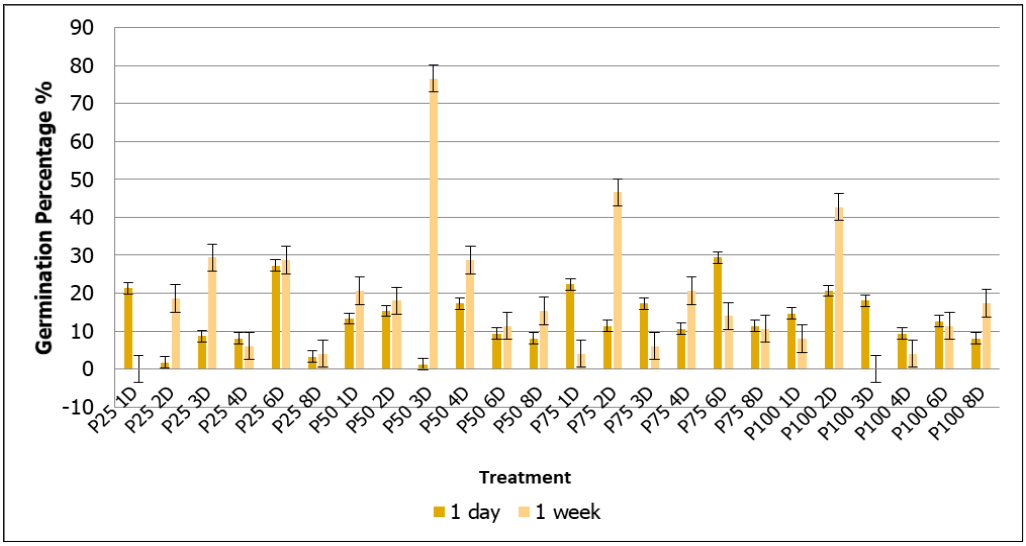

Fig. 1. Comparison of germination percentage of sandal seedlings bioprimed with PGPR mix II subjected to post priming storage of 1 day and 1 week.

and embryo conditions suggested that the seeds were dead.

Seed biochemical composition

Most biopriming treatments reduced the electrical conductivity (EC) of seed leachates, indicating improved membrane stability. EC ranged from a maximum of 0.962 dS cm^{-1} at 25 % for 2 days to a minimum of 0.247 dS cm^{-1} at 100 % for 1 day (Table 2). Carbohydrate content varied from 0.370 mg g^{-1} (100 % for 1 day) to 0.127 mg g^{-1} (75 % for 4 days). The highest protein content (0.077 mg g^{-1}) was recorded at 25 % for 4 days, while the lowest (0.030 mg g^{-1}) occurred at 75 % for 3 days. Crude fat content was highest (74 %) at 25 % for 2 days and lowest (44.6 %) at 75 % for 6 days. Analysis of variance showed that the interaction between concentration and duration significantly influenced EC (F = 116.519; $p < 0.01$), carbohydrate (F = 310.594; $p < 0.01$), protein (F = 102.167; $p < 0.01$) and fat content (F = 25.153; $p < 0.01$).

Seedling growth and biomass production

The variation in the germination and biochemical parameters of the sandal seeds due to priming showed significant variation in growth and biomass production also. For the post-priming storage of one day, the seedlings bioprimed at 100 % concentration for 2 days (27.2 cm) recorded the highest height and biopriming at 75 % for 2 days recorded the lowest value (11.7 cm). The collar diameter was highest on biopriming at 100 % for 6 days (3.18 mm) and the lowest was at 50 % for 6 days (1.85 mm). The seedlings bioprimed at 75 % for 4 days (24) recorded the largest number of leaves (Table 3) and the lowest was on biopriming at 50 % for 6 days (14.3). Post-priming storage of seeds for one day resulted in the seedlings with a maximum root length of 16.97 cm (50 % for 4 days) and a minimum length of 4.23 cm (50 % for 8 days) and the highest root number was 10.67 (100 % for 3 days) and the lowest was 2.33 (75 % for 2 days). Post-priming storage of seeds for one week resulted in seedlings with the largest height of 25.5 cm (100 % for 6 days) and the lowest height of 6.1 cm (100 % for 3 days), the highest collar diameter was recorded at 50 % for 3 days (2.25 mm) and the lowest was at 25 % for 6 days (1.46 mm) and the largest number of leaves were recorded in biopriming at 100 % for 2 days (22) and the lowest was at 50 % for 1 day (10.31). Maximum root length of 14.2 cm was observed in biopriming at 50 % for 3 days and the lowest was at 50 % for 1 day (1.67 cm) and the highest number of roots were recorded in biopriming at 50 % for 3 days (11.33) and the lowest was at 100 % for 4 days and 50 % for 2 days (4.00). The statistical analysis revealed that the interaction effect of concentration and duration was significant for shoot height (F = 27.06, $p < 0.01$), collar diameter (F = 41.76, $p < 0.01$) leaf number (F = 49.14, $p < 0.01$), root length (F = 60.503, $p < 0.01$) and number of roots (F = 60.898, $p < 0.01$) for one day storage. The interaction effect was also significant for shoot height (F = 30.197, $p < 0.01$), collar diameter (F = 31.041, $p < 0.01$) leaf number (F = 27.324), root length (F = 60.503, $p < 0.01$) and number of roots (F = 8.741, $p < 0.01$) for one week storage.

With regard to the dry biomass accumulation (Table 4), for the seedlings obtained after post-priming storage of one day, the highest total biomass recorded on priming at 75 % concentration for 1 day (1.600 g) and the lowest values was at 100 % for 8 days (0.344 g). The dry weight biomass of the components viz. leaf and shoot were the highest on biopriming at 75 % for 1 day (0.860 g) and 50 % for 8 days (0.4311 g) respectively and that of root was highest on priming at 75 % for 1 day (0.663 g). The lowest leaf dry weight was observed on priming at 50 % for 4 days (0.197 g), shoot weight at 50 % for 4 days (0.0933 g) and root weight at 100 % for 8 days (0.0933 g). The highest total biomass production obtained after post-priming storage of one week was 1.41 g (75 % for 3 days) and the lowest was 0.21 g (50 % for 1 day). When the component biomass were examined, the highest leaf dry weight was on biopriming at 50 % for 6 days, 75 % for 2 days and 100 % for 2 days (0.3 g), shoot weight was at 25 % for 2 days (0.191 g) and root weight was at 100 % for 2 days (0.196 g) and the lowest values of leaf, shoot and root dry weight were recorded at 50 % for 1 day (0.11 g), 50 % for 1 day (0.036 g) and 50 % for 1 day (0.063 g), respectively. The interaction of concentration and duration of bio-inoculant was also significant for in dry weight of stem (F = 2.778, $p < 0.01$), leaf (F = 38.459, $p < 0.01$) and root (F = 10.858, $p < 0.01$) and total dry weight (F = 44.801, $p < 0.01$) for one day storage. The interaction was also significant for dry weight of stem (F = 2.314, $p < 0.05$), leaf (F = 10.387, $p < 0.01$) and root (F = 29.689, $p < 0.01$) and total dry weight (F = 10.177, $p < 0.01$) for one week storage.

In order to find the best performing treatments, cluster analysis was conducted for the seedlings obtained from seeds subjected to post priming storage and respective dendrograms were made for one day storage (Fig. 2) and one week storage (Fig. 3). Hierarchical clustering with Euclidian distance measure grouped the treatments into homogenous clusters with maximum homogeneity within the cluster. For the seedlings obtained after one day storage, at a distance of 40, two distinct clusters were obtained, the one with minimum value i.e. treatments PGPR II, 50 % 3 days, P25 % 2 days and P25 % 8 days were eliminated. At distance of 20, two distinct clusters are obtained of which superior cluster is treatment P100 % 3 days which is superior among all other treatments. For one week storage, at a distance of 40 % two distinct clusters are obtained, the one with minimum value i.e. treatments PGPR II 100 % concentration for 4 days, 75 % for 8 days, P 75 % for 6 days, P50 % for 8 days, 25 % for 1 day and 25 % for 8 days was eliminated in the next level. At the distance 20, two distinct clusters were obtained; the cluster with superior treatment was containing the treatments PGPR II 50 % 3 days, 50 % 6 days and 100 % 6 days. At the distance 10, three clusters are obtained, the one with maximum value is cluster with treatment PGPR II 50 % for 3 day and 50 % 6 days and of which the one with superior seedling performance was the biopriming with PGPR II at 50 % for 3 days. Hierarchical cluster analysis enabled the identification of biologically meaningful groups of priming treatments based on overall seedling performance, allowing the elimination of inferior treatments and the selection of superior ones. This multivariate approach provided an objective basis for identifying the best-performing biopriming combinations-particularly PGPR II at 50 % for 3 days-across different post-priming storage periods.

The growth analysis indices of the seedlings also varied due to concentration and duration of bioinoculant and post- priming storage. For the post-priming storage of one day, the highest root: shoot ratio was observed in the seedlings bioprimed at 50 % concentration for 4 days (0.763) and the lowest was at 75 % for 6 days (0.176). The vigour index was maximum at 75 % for 1 day (859) and minimum at 50 % for 8 days (143) and the Dickson quality index was the highest on biopriming at 75 % for 3 days (0.173) and the lowest at 50 % for 4 days (0.04) (Table 5). Seedlings obtained after the post-priming storage of one week, the highest root: shoot ratio was obtained on biopriming at 100 % for 6 days (0.922) and the vigour and quality indices at 50 % for 3 days (3223) and 50 % for 3 days (0.139) respectively and the lowest root: shoot

Table 2. Effect of biopriming with PGPR II on electrical conductivity and biochemical composition of the sandal seeds

PGPR II	Duration (days)	Electrical conductivity (dS/cm)	Total carbohydrate (mg/g)	Total protein (mg/g)	Crude Fat (%)
25 %	1	0.484^{e}	0.238^{cd}	0.039^{bc}	57.0^{cde}
	2	0.518^{f}	0.257^{de}	0.037^{ab}	75.2^{i}
	3	0.659^{f}	0.278^{ef}	0.069^{i}	73.2^{i}
	4	0.763^{g}	0.344^{i}	0.077^{i}	64.6^{efghi}
	6	0.765^{g}	0.359^{i}	0.062^{i}	72.4^{i}
	8	0.962^{g}	0.338^{i}	0.055^{ghi}	63^{defgh}
50 %	1	0.302^{ab}	0.137^{a}	0.049^{efg}	56.2^{cde}
	2	0.337^{ab}	0.178^{b}	0.061^{i}	64^{efghi}
	3	0.339^{bc}	0.215^{c}	0.051^{efgh}	69.4^{ghi}
	4	0.435^{cd}	0.281^{efg}	0.045^{cde}	51.8^{abc}
	6	0.506^{f}	0.350^{i}	0.047^{def}	54.6^{bcd}
	8	0.597^{f}	0.305^{gh}	0.036^{ab}	45.2^{a}
75 %	1	0.282^{a}	0.339^{i}	0.039^{bc}	58.2^{cdef}
	2	0.372^{bc}	0.316^{hi}	0.042^{bcd}	45.8^{ab}
	3	0.408^{c}	0.305^{fgh}	0.031^{a}	73.4^{i}
	4	0.422^{cd}	0.127^{a}	0.058^{i}	45.8^{ab}
	6	0.470^{e}	0.305^{gh}	0.053^{fghi}	44.6^{a}
	8	0.752^{fg}	0.178^{b}	0.059^{i}	57.4^{cde}
100 %	1	0.247^{a}	0.370^{i}	0.057^{hi}	61.2^{defg}
	2	0.322^{ab}	0.320^{hi}	0.046^{de}	76.2^{i}
	3	0.368^{bc}	0.313^{hi}	0.037^{ab}	60.0^{cdef}
	4	0.368^{bc}	0.239^{cd}	0.061^{i}	70.4^{hi}
	6	0.469^{e}	0.142^{a}	0.059^{i}	66.2^{fghi}
	8	0.559^{f}	0.320^{hi}	0.056^{hi}	59.6^{cdef}
SEM		0.0107	0.00491	0.00115	1.616
Main effects					
Concentration					
25 %		0.692^{D}	0.302^{D}	0.0565^{C}	69.0^{C}
50 %		0.419^{B}	0.244^{A}	0.0482^{A}	58.2^{B}
75 %		0.452^{C}	0.262^{B}	0.0470^{A}	54.2^{A}
100 %		0.378^{A}	0.284^{C}	0.0527^{B}	65.6^{C}
SEM		0.00436	0.002	0.000471	0.66
Duration					
1		0.399^{A}	0.271^{B}	0.0460^{A}	58.2^{A}
2		0.677^{E}	0.268^{A}	0.0465^{A}	65.4^{B}
3		0.479^{C}	0.278^{BC}	0.0470^{A}	69.0^{C}
4		0.512^{D}	0.248^{A}	0.0602^{D}	58.2^{A}
6		0.453^{B}	0.289^{D}	0.0552^{C}	59.4^{A}
8		0.391^{A}	0.285^{CD}	0.0515^{B}	56.2^{A}
SEM		0.00534	0.0245	0.000577	0.808

Values within the same column with similar superscripts are homogenous.

Table 3. Effect of biopriming with PGPR II on shoot and root growth parameters of the sandal seedling for the post priming storage of one day and one week

PGPR 11		Shoot height (cm)		Collar diameter (mm)		Number of leaves		Root length (cm)		Number of lateral roots	
		Post priming storage for									
Concentration	duration (days)	1 day	1 week	1 day	1 week	1 day	1 week	1 day	1 week	1 day	1 week
25 %	1	21.80^{e}	-	1.98^{a}	-	21.30^{de}	-	9.27b^{c}	-	3.67^{a}	-
	2	-	10.50^{a}	-	2.11^{c}	-	16.70^{ab}	-	11.33^{d}	-	5.00^{ab}
	3	19.00^{d}	15.30^{b}	2.58^{bc}	1.98^{bc}	20.70^{de}	20.30^{bc}	15.83^{cd}	9.77^{c}	6.33^{b}	6.67^{bc}
	4	12.40^{a}	19.70^{bc}	2.75^{cd}	2.57^{cd}	18.00^{bc}	16.30^{a}	7.90^{b}	9.53^{c}	4.67^{ab}	9.33^{c}
	6	12.00^{a}	21.00^{c}	2.61^{c}	1.46^{a}	23.70^{de}	18.00^{bc}	9.10^{b}	8.2^{bc}	5.00^{ab}	6.33^{bc}
	8	-	-	-	-	-	-	-	-	-	-
50 %	1	16.00^{c}	11.50^{a}	2.91^{d}	1.59^{a}	22.30^{de}	14.30^{a}	17.63^{d}	5.73^{a}	4.67^{ab}	5.33^{ab}
	2	14.90^{b}	11.30^{a}	2.68^{cd}	1.98^{bc}	19.00^{bc}	16.70^{ab}	14.63^{cd}	5.17^{a}	8.00^{bc}	4.00^{a}
	3	-	22.90^{cd}	-	3.44^{e}	-	19.30^{bc}	-	19.10^{e}	-	12.33^{d}
	4	22.00^{e}	22.10^{cd}	1.98^{a}	1.90^{bc}	15.00^{ab}	20.70^{bc}	16.97^{d}	4.93^{a}	8.00^{bc}	5.33^{abc}
	6	17.30^{cd}	22.80^{cd}	1.85^{a}	2.41^{cd}	14.30^{a}	17.30^{bc}	9.63^{bc}	12.97^{d}	5.67^{ab}	11.33^{cd}
	8	13.60^{ab}	-	3.11^{d}	-	19.30^{bc}	-	4.23^{a}	-	3.67^{a}	-
75 %	1	22.30^{e}	12.20^{a}	2.21^{b}	1.76^{b}	14.30^{a}	19.00^{bc}	15.40^{cd}	6.63^{ab}	9.33^{bc}	5.00^{ab}
	2	11.70^{a}	19.40^{bc}	2.77^{cd}	2.99^{d}	19.30^{bcd}	21.00^{bc}	10.03^{bc}	8.80^{bc}	3.67^{a}	6.33^{bc}
	3	14.70^{b}	20.00^{bc}	2.24^{b}	3.08^{d}	21.70^{de}	24.70^{c}	12.77^{c}	9.70^{c}	9.33^{bc}	5.67^{bc}
	4	21.40^{e}	20.00^{c}	2.21^{b}	1.82^{b}	24.00^{e}	16.70^{ab}	16.47^{d}	10.53^{cd}	4.97^{ab}	9.67^{cd}
	6	17.20^{cd}	-	3.11^{d}	-	21.70^{de}	-	11.03^{bc}	-	4.77^{ab}	-
	8	18.20^{cd}	-	2.19^{b}	-	20.30^{d}	-	8.67^{b}	-	5.00^{ab}	-
100 %	1	16.30^{c}	16.10^{b}	2.67^{c}	1.76^{b}	20.00^{d}	16.70^{ab}	15.50^{cd}	9.43^{c}	5.00^{ab}	8.67^{c}
	2	27.20^{f}	16.70^{b}	2.62^{c}	2.87^{d}	23.70^{de}	22.00^{bc}	13.43^{c}	11.03^{d}	3.67^{a}	5.67^{bc}
	3	23.10^{e}	6.10^{a}	2.81^{cd}	2.04^{c}	20.70^{de}	17.70^{bc}	24.03^{e}	8.43^{bc}	12.67^{d}	5.00^{ab}
	4	15.00^{bc}	-	2.41^{b}	-	22.30^{de}	-	12.47^{c}	-	7.33^{bc}	-
	6	18.50^{d}	25.50^{d}	3.18^{d}	1.81^{b}	20.30^{d}	15.70^{a}	4.30^{a}	17.20^{e}	6.00^{ab}	4.00^{a}
	8	11.30^{a}	19.30^{bc}	2.16^{b}	2.31^{cd}	21.30^{de}	16.30^{a}	6.97^{b}	12.50^{d}	3.67^{a}	8.00^{c}
SEM		1.37	1.6	0.151	0.176	1.1	1.49	0.717	1.02	1.18	1.34

Main effects										
Concentration										
25 %	10.9^{A}	11.1^{A}	1.65^{A}	1.35^{A}	13.0^{A}	11.9^{A}	7.02^{A}	6.47^{AB}	3.28^{A}	4.56^{A}
50 %	14.0^{B}	15.1^{B}	2.09^{B}	1.89^{C}	15.3^{A}	14.7^{B}	10.52^{B}	7.98^{B}	5.00^{AB}	6.39^{A}
75 %	17.6^{C}	11.9^{A}	2.46^{C}	1.61^{AB}	20.2^{B}	13.6^{AB}	12.39^{C}	5.94^{A}	5.44^{B}	5.22^{A}
100 %	18.5^{C}	15.6^{B}	2.64^{C}	1.8^{BC}	21.4^{B}	14.7^{B}	12.78^{C}	9.77^{C}	5.89^{B}	4.44^{A}
SEM	0.56	0.652	0.0616	0.0718	0.449	0.608	0.359	0.415	0.484	0.549
Duration										
1	19.1^{C}	9.93^{B}	2.44^{BC}	1.27^{B}	19.5^{B}	12.5^{B}	14.45^{C}	5.45^{B}	4.75^{AB}	4.75^{AB}
2	13.4^{AB}	14.46^{C}	2.02^{A}	2.48^{C}	15.5^{A}	19.08^{C}	9.53^{B}	9.08^{C}	3.83^{A}	5.25^{B}
3	14.2^{B}	18.56^{D}	1.91^{A}	2.63^{C}	15.8^{A}	20.5^{C}	13.16^{C}	11.75^{D}	7.08^{B}	7.42^{B}
4	17.7^{C}	15.43^{CD}	2.34^{B}	1.57^{B}	19.8^{B}	13.42^{B}	13.45^{C}	6.25^{B}	5.42^{AB}	6.08^{B}
6	16.2^{BC}	17.33^{CD}	2.69^{C}	1.42^{B}	20.0^{B}	12.75^{B}	8.52^{B}	9.59^{C}	5.25^{AB}	5.42^{B}
8	10.8^{A}	4.82^{A}	1.86^{A}	0.57^{A}	15.7^{A}	4.08^{A}	4.97^{A}	3.12^{A}	3.08^{A}	2.00^{A}
SEM	0.685	0.798	0.0754	0.0879	0.55	0.745	0.359	0.508	0.592	0.672

Values within the same column with similar superscripts are homogenous.

Table 4. Effect of biopriming with PGPR II on biomass production of the sandal seedling for the post priming storage of one day and one week

PGPR II		Dry weight (g)							
		Leaf		Shoot		Root		Total	
		Post priming storage for							
Concentration	duration (days)	1 day	1 week	1 day	1 week	1 day	1 week	1 day	1 week
25 %	1	0.489^{c}	-	0.323^{c}	-	0.180^{d}	-	0.882^{ab}	-
	2	-	0.196^{a}	-	0.216^{ab}	-	0.120^{cd}	-	0.490^{ab}
	3	0.424^{bc}	0.160^{a}	0.193^{b}	0.126^{ab}	0.186^{de}	0.110^{c}	0.804^{ab}	0.400^{a}
	4	0.442^{c}	0.230^{a}	0.197^{b}	0.233^{ab}	0.300^{e}	0.076^{ab}	1.036^{cd}	0.540^{c}
	6	0.791^{d}	0.190^{a}	0.133^{a}	0.090^{ab}	0.183^{de}	0.200^{de}	1.133^{d}	0.490^{ab}
	8	-	-	-	-	-	-	-	-
50 %	1	0.293^{b}	0.110^{a}	0.204^{b}	0.036^{ab}	0.120^{b}	0.063^{a}	0.656^{a}	0.210^{a}
	2	0.364^{b}	0.260^{ab}	0.109^{a}	0.100^{ab}	0.177^{cd}	0.160^{d}	0.649^{a}	0.520^{ab}
	3	-	0.980^{d}	-	0.163^{ab}	-	0.213^{de}	-	1.260^{d}
	4	0.407^{bc}	0.190^{a}	0.093^{a}	0.056^{ab}	0.207^{de}	0.096^{c}	0.920^{c}	0.350^{a}
	6	0.593^{cd}	0.300^{ab}	0.158^{a}	0.096^{ab}	0.140^{c}	0.166^{d}	0.918^{c}	0.580^{cd}
	8	0.384^{b}	-	0.431^{c}	-	0.173^{cd}	-	1.018^{cd}	-
75 %	1	0.860^{e}	0.550^{cd}	0.220^{b}	0.350^{b}	0.663^{f}	0.186^{d}	1.600^{e}	1.150^{d}
	2	0.576^{cd}	0.270^{ab}	0.320^{c}	0.113^{ab}	0.147^{cd}	0.230^{e}	1.071^{d}	0.620^{bcd}
	3	0.853^{de}	0.220^{a}	0.216^{b}	0.0933^{ab}	0.447^{e}	0.090^{c}	1.582^{e}	1.410^{d}
	4	0.533^{cd}	0.300^{ab}	0.227^{bc}	0.126^{ab}	0.350^{cde}	0.110^{bc}	1.196^{de}	0.570^{c}
	6	0.524^{cd}	-	0.220^{bc}	-	0.083^{a}	-	0.818^{ab}	-
	8	0.573^{cd}	-	0.196^{b}	-	0.240^{e}	-	1.189^{d}	-
100 %	1	0.604^{d}	0.180^{a}	0.116^{a}	0.160^{ab}	0.217^{de}	0.096^{c}	0.971^{c}	0.440^{a}
	2	0.533^{cd}	0.300^{bc}	0.113^{a}	0.156^{ab}	0.287^{e}	0.196^{de}	0.993^{cd}	0.650^{cd}
	3	0.560^{cd}	0.230^{a}	0.200^{b}	0.160^{ab}	0.354^{e}	0.126^{cd}	1.222^{e}	0.420^{a}
	4	0.542^{cd}	-	0.138^{a}	-	0.120^{ab}	-	0.820^{ab}	-
	6	0.600^{d}	0.250^{ab}	0.169^{a}	0.200^{ab}	0.373^{e}	0.070^{a}	1.216^{e}	0.520^{ab}
	8	0.162^{a}	0.400^{cd}	0.071^{a}	0.083^{ab}	0.093^{ab}	0.120^{cd}	0.344^{a}	0.600^{cd}
SEM		0.0535	0.0608	0.0472	0.0632	0.03918	0.0231	0.0759	0.106
Main effects									
Concentration									
25 %		0.358^{A}	0.13^{A}	0.104^{A}	0.111^{A}	0.136^{A}	0.084^{A}	0.643^{A}	0.322^{A}
50 %		0.340^{A}	0.26^{B}	0.166^{AB}	0.075^{A}	0.140^{A}	0.117^{A}	0.693^{A}	0.452^{A}
75 %		0.653^{C}	0.24^{B}	0.230^{B}	0.11^{A}	0.269^{C}	0.103^{A}	1.243^{C}	0.458^{A}
100 %		0.500^{B}	0.23^{B}	0.134^{A}	0.11^{A}	0.220^{B}	0.102^{A}	0.928^{B}	0.439^{A}
SEM		0.0134	0.0248	0.0193	0.0258	0.016	0.00944	0.0242	0.0433
Duration									
1		0.562^{D}	0.2^{ABC}	0.177^{A}	0.136^{A}	0.295^{C}	0.086^{BC}	1.027^{C}	0.45^{B}
2		0.368^{B}	0.25^{BC}	0.136^{A}	0.146^{A}	0.1425^{AB}	0.176^{D}	0.678^{A}	0.57^{B}
3		0.459^{C}	0.33^{C}	0.137^{A}	0.150^{A}	0.2475^{B}	0.135^{CD}	0.902^{B}	0.57^{B}
4		0.481^{C}	0.18^{AB}	0.163^{A}	0.104^{A}	0.262^{C}	0.072^{AB}	0.993^{BC}	0.365^{AB}
6		0.627^{D}	0.19^{AB}	0.166^{A}	0.096^{A}	0.171^{AB}	0.109^{BC}	1.021^{BC}	0.397^{B}
8		0.280^{A}	0.1^{A}	0.174^{A}	0.021^{A}	0.138^{A}	0.03^{A}	0.638^{A}	0.151^{A}
SEM		0.0164	0.0304	0.024	0.0316	0.0196	0.0116	0.0379	0.053

Values within the same column with similar superscripts are homogenous.

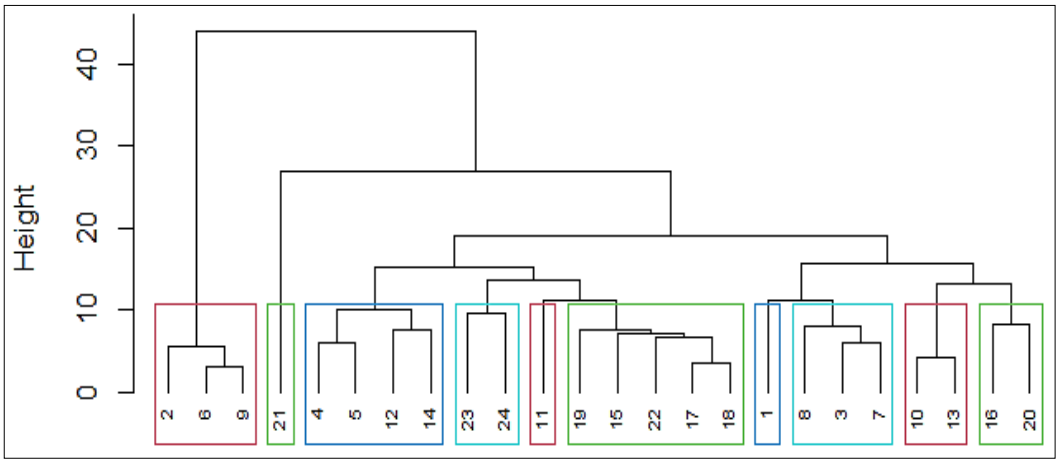

Fig. 2. Cluster dendrogram for 1 day post priming storage treatment comparison.

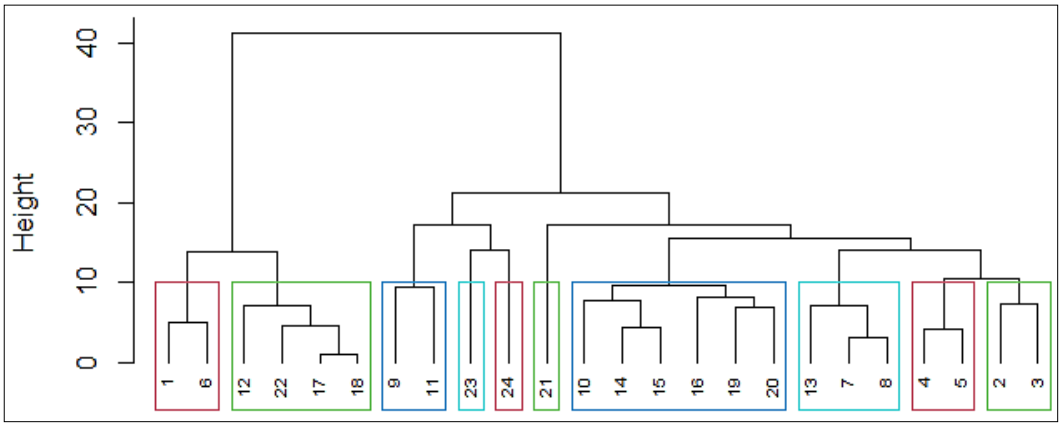

Fig. 3. Cluster dendrogram for 1 week post priming storage treatment comparison.

ratio and vigour and quality indices were obtained on biopriming at 75 % for 4 days (0.054), 75% for 1 day (31.5) and 100 % for 6 days (0.024), respectively. Analysis of variance indicated that the interaction effect of concentration and duration of PGPR II was significant for root: shoot ratio ($F = 7.647$, $p < 0.01$) and vigour index ($F = 62.652$, $p < 0.01$) for one day storage. The interaction was also significant at 180 days for root: shoot ratio ($F = 6.280$, $p < 0.01$) and vigour index ($F = 68.481$, $p < 0.01$).

Sandal is a high-value forest tree, but poor and variable germination-often taking over 48 weeks–is a major constraint in its seed propagation (31, 32). Seed biopriming with PGPR initiates metabolic processes that enhance germination rate and uniformity. In this study, PGPR Mix II (containing *P. fluorescens* and *B. subtilis*) significantly influenced sandal seed germination across concentrations, priming durations and post-priming storage periods. The best result–76.6 % germination–was achieved by treating seeds with 50 % PGPR Mix II for 3 days followed by one-week storage. This treatment also shortened the germination period to 35 days, improving uniformity for nursery planting. Similar benefits of biopriming have been reported in other species (33). The positive effects may be linked to phytohormone production such as Indole-3-acetic acid (IAA) and gibberellins, osmotic adjustments, metabolic repair and modulation of Abscisic acid (ABA)/(GA_3) balance (34–36).

Biochemical analysis showed that biopriming reduced electrical conductivity of seed leachates compared to controls (15), indicating improved membrane stability and better utilization of seed reserves (37). While protein, carbohydrate and fat contents varied with priming duration and concentration, no strong correlation with germination was found except for a positive association between conductivity and germination in one-week stored seeds. PGPR treatments also enhanced seedling vigour, with 50 % PGPR Mix II for 3 days proving optimal. The growth-promoting effects of *P. fluorescens* and *B. subtilis* are well documented, including phytohormone production, nutrient solubilization, nitrogen fixation, siderophore release and pathogen suppression (38). Overall, the results confirm that short-term post-priming storage combined with appropriate PGPR treatment can substantially improve germination speed, uniformity and seedling quality in sandal.

Conclusion

The present study demonstrates that biopriming with PGPR Mix II is an effective strategy to enhance the germination and seedling performance of *Santalum album* L. Optimised priming schedules particularly the 50 % concentration for 3 days followed by one-week post-priming storage substantially improved germination percentage, reduced germination time and produced vigorous, high-quality seedlings. Bioprimed seeds also exhibited lower electrical conductivity of leachates, indicating improved membrane integrity, along with favourable changes in key biochemical constituents. Given the inherently prolonged and uneven germination of sandal seeds, biopriming offers a simple, cost-effective and eco-friendly seed enhancement technique that can significantly improve nursery efficiency and the production of high-quality planting stock.

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Table 5. Effect of biopriming with PGPR II on seedling quality of the sandal seedling for the post priming storage of one day and one week

PGPR II		Root: Shoot ratio		Vigor index		Dickson quality index (DQI)	
		Post priming storage for					
Concentration	Duration (days)	1 day	1 week	1 day	1 week	1 day	1 week
25 %	1	0.339^{abc}	-	414.5^{d}	-	0.063^{ab}	-
	2	-	0.336^{abc}	-	408^{b}	-	0.072^{c}
	3	0.502^{bc}	0.414^{bc}	199.3^{b}	737^{efgh}	0.071^{ab}	0.045^{bc}
	4	0.511^{bc}	0.197^{ab}	68.8^{a}	175.4^{a}	0.145^{cd}	0.053^{bc}
	6	0.234^{ab}	0.675^{c}	257.7^{bc}	834^{cd}	0.191^{d}	0.035^{ab}
	8	-	-	-	-	-	-
50 %	1	0.370^{abc}	0.468^{bc}	292.7^{cd}	353^{ab}	0.069^{ab}	0.025^{a}
	2	0.573^{bc}	0.460^{bc}	273.8^{bc}	297^{ab}	0.08^{bc}	0.084^{c}
	3	-	0.310^{cbc}	-	3223^{e}	-	0.139^{d}
	4	0.763^{c}	0.390^{abc}	473.1^{d}	777^{c}	0.04^{a}	0.028^{ab}
	6	0.272^{ab}	0.404^{bc}	139.9^{b}	406^{b}	0.058^{a}	0.058^{bc}
	8	0.247^{ab}	-	54.7^{a}	-	0.102^{c}	-
75 %	1	0.774^{c}	0.224^{ab}	593.8^{de}	75.3^{a}	0.151^{cd}	0.126^{cd}
	2	0.346^{abc}	0.597^{c}	110.7^{b}	1316^{d}	0.171^{cd}	0.090^{c}
	3	0.405^{abc}	0.300^{abc}	274.8^{c}	178^{a}	0.173^{cd}	0.056^{bc}
	4	0.531^{bc}	0.054^{a}	279.1^{cd}	630^{bc}	0.086^{bc}	0.045^{bc}
	6	0.176^{ab}	-	483.5^{de}	-	0.079^{ab}	-
	8	0.434^{abc}	-	171.5^{b}	-	0.063^{ab}	-
100 %	1	0.389^{abc}	0.272^{abc}	295.6^{cd}	204^{ab}	0.130^{c}	0.041^{bc}
	2	0.500^{bc}	0.452^{bcd}	597.7^{de}	1182^{d}	0.069^{ab}	0.100^{cd}
	3	0.578^{bc}	0.438^{bcd}	637.8^{e}	375^{ab}	0.094^{bc}	0.053^{bc}
	4	0.232^{ab}	-	148.1^{b}	-	0.096^{bc}	-
	6	0.560^{bc}	0.922^{d}	155.6^{b}	486^{b}	0.146^{cd}	0.024^{a}
	8	0.458^{bc}	0.256^{ab}	52.3^{a}	552^{bc}	0.051^{a}	0.068^{bc}
SEM		0.0807	0.073	29.5	82.3	0.0204	0.0118
Main effects							
Concentration							
25 %		0.264^{a}	0.271^{A}	157^{a}	843^{B}	0.0785^{AB}	0.0342^{A}
50 %		0.3710^{ab}	0.34^{A}	206^{b}	667^{A}	0.0581^{A}	0.0558^{B}
75 %		0.445^{b}	0.229^{A}	319^{c}	467^{A}	0.1204^{C}	0.0530^{B}
100 %		0.453^{b}	0.276^{A}	315^{c}	359^{A}	0.0976^{BC}	0.0479^{AB}
SEM		0.00329	0.0298	12.0	33.6	0.00832	0.0048
Duration							
1		0.468^{bc}	0.244^{BC}	399.2^{c}	158^{A}	0.1031^{B}	0.0481^{B}
2		0.355^{abc}	0.462^{D}	245.6^{b}	801^{C}	0.0801^{AB}	0.0867^{C}
3		0.371^{abc}	0.365^{CD}	278^{b}	1128^{D}	0.0845^{AB}	0.0734^{C}
4		0.509^{c}	0.21^{AB}	242.3^{b}	395^{B}	0.0918^{AB}	0.0318^{AB}
6		0.3110^{ab}	0.323^{BCD}	259.2^{b}	432^{B}	0.1186^{B}	0.0293^{AB}
8		0.285^{a}	0.0649^{A}	69.6^{a}	138^{A}	0.0539^{A}	0.0170^{A}
SEM		0.0403	0.0365	14.7	41.1	0.0102	0.00588

Values within the same column with similar superscripts are homogenous.

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Authors' contributions

AKS, JCM and TKK were primarily responsible for the conceptualization of the research problem and the design of the experimental framework. They led the development of the methodology, coordinated the field and laboratory investigations and handled data collection, software-assisted data processing and statistical analysis. These authors also prepared the initial manuscript draft, including data interpretation, preparation of figures and tables and writing the main sections of the paper. VJ and AVSK actively participated in laboratory work, assisted in the execution of experimental protocols, supported the validation and verification of results and provided essential technical inputs throughout the study. They also contributed to the critical review of the manuscript, offering revisions to improve scientific clarity, coherence and accuracy. Furthermore, they supported the refinement and preparation of the final manuscript for submission. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

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