Impact of seed priming techniques on germination and seedling performance in sandal (*Santalum album L.*)

By

CHITRA P (2017-17-005)

THESIS

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DECLARATION

I, hereby declare that this thesis entitled **"Impact of seed priming techniques on germination and seedling performance in sandal (*Santalum album L.*)"** is a bonafide record of research done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

Vellanikkara

Date: 19-09-2019

CHITRA P

(2017-17-005)

CERTIFICATE

Certified that this thesis entitled **"Impact of seed priming techniques on germination and seedling performance in sandal (*Santalum album L.*)"** is a record of research work done independently by **Ms. Chitra P (2017-17-005)** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

Vellanikkara,
Date: 19-09-2019

Dr. Jijeesh C M
(Major Advisor, Advisory Committee)
Assistant Professor
Department of Silviculture and Agroforestry
College of Forestry
Vellanikkara.

CERTIFICATE

We, the undersigned members of advisory committee of **Ms. Chitra, P. (2017-17-005)** a candidate for the degree of **Master of Science in Forestry** with major in Silviculture and Agroforestry, agree that this thesis entitled **"Impact of seed priming techniques on germination and seedling performance in sandal (*Santalum album*, L.)"** may be submitted by her in partial fulfillment of the requirement for the degree.

Dr. Jijeesh C. M.
(Chairman, Advisory Committee)
Assistant Professor
Department of Silviculture and Agroforestry
College of Forestry
Vellanikkara.

Dr. T. K. Kunhamu
(Member, Advisory Committee)
Professor and Head
Department of Silviculture and Agroforestry
College of Forestry
Vellanikkara.

Dr. V. Jamaludheen
(Member, Advisory Committee)
Professor
Department of Silviculture and Agroforestry
College of Forestry
Vellanikkara.

Dr. A. V. Santhoshkumar
(Member, Advisory Committee)
Professor
Department of Forest Biology and Tree Improvement
College of Forestry
Vellanikkara.

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INTRODUCTION

Santalum album, also known as the East Indian Sandalwood is a partial root parasitic tree native to South India. It is a globally renounced timber species valued for its fragrant heartwood and essential oil. Sandal is a small to medium sized evergreen tree growing to a height of 12 to 15 meters and a girth of 2 to 2.4 meters (Parthasaradhi and Rai, 1989) with elevated branching as well as narrow drooping. Preferably growing in the lowland tropical forests and woodlands, the species is found to occur naturally in India, China, Indonesia and Philippines and introduced to other parts of the world. The tree burgeons well from the sea level up to 1800 m altitude. Although the species thrives in a wide array of soil and climate, its growth is greatly retarded in highly alkaline, waterlogged or very cold places. The species has attracted the global market due to its multitudinous usefulness and is one of the most expensive timber in the world.

The demand for the sandalwood across the globe is outstripping its supply. Moreover, the natural population of sandal is dwindling over the past three decades. The high price of the sandal wood in the market has led to the illegal felling of the trees in their natural habitat. The illegal trading of the commodity is pervading. Added to these pressures, the trees in India face heavy threat from the spike disease. All these factors have led to the categorization of 'Vulnerable' according to the IUCN red list 2010 after its production has dwindled by 80 per cent in the last decade. An estimated number of 100,000 trees above in Marayoor from a report by Varghese (1976) have been recently corrected as approximately 60,000 trees in the protected area. Also in India, the economically viable sandal trees are reported to be commercially extinct due to illegal harvesting and over exploitation. Hence, the need of the hour is to regenerate and conserve the species in its natural habitat as well as plantations.

One among the major constraints in raising sandal is the poor germination rate of the seeds and slow rate of establishment of the seedlings in the field. The hard seed coat makes the seed difficult to germinate. The artificial propagation methods have not been successful in sandal, hitherto. Therefore, new strategies to enhance the speedy germination and uniform growth of the seedlings of sandal has to be achieved to raise good quality planting stock. The fruits of sandal are succulent drupes with a diameter of 0.3 to 0.5 inch. The fruits obtain a purplish black colour at maturity and contains a single seed with brown endocarp which is moderately hard. The seeds are spherical in shape having a diameter range of 0.5 to 1 cm and the weight of an individual seed

varies from 0.1 to 0.2 g. Nagaveni and Ananthapadmanabha (1986) grouped the seeds as small, medium and big based on the seed size and weight, and found that 82 to 87% of a seed lot of sandal is constituted by medium sized seeds (0.1 to 0.2 g weight and 7 to 8 mm size). It is said that the weight of the seed is inversely proportional to the germination rate whereas the seedling vigour is directly proportional to the seed weight. The germination in sandal is sporadic and completes within a period of 4 to 12 weeks (Srimathi *et al.*, 1995). Different pre – treatments like soaking in cowdung, acid scarification, hot water soaking and gibberellic acid have been practiced in sandal till date and currently, gibberellic acid 500 ppm is the best pretreatment for sandal seeds. A pretreatment of 0.5% gibberellic acid has resulted in 35 to 45% germination in sandal seeds (Sudhir *et al.*, 2013) whereas the seeds of sandal recorded a cumulative germination percentage of 74 per cent in 115 days (Sutheesh *et al.*, 2016).

Seed priming is a controlled seed hydration treatment in which the metabolic activity is enhanced, but suspended before radicle protrusion. It inducts a particular physiological state in plants by the treatment of natural and/or synthetic compounds to the seeds prior to germination. During priming, the seeds are soaked in water (hydropriming) or PEG (osmopriming) or salt ($CaCl_2$, $CaSO_4$ or NaCl, etc.) or any other chemical prior to germination. The beneficial effects of seed priming include faster emergence, better establishment and lower incidence of re-sowing, more vigorous plants, better drought tolerance, earlier flowering, earlier harvest and higher yield. Biochemical studies of primed seeds indicated that protein synthesis was increased by osmotic conditioning which can be due to the removal of certain inhibiting factors such as abscisic acid or to the production of promoting factors. The mobilization of the reserved materials stored in the seeds may explain the increase in germination and vigour induced by osmotic conditioning.

Rapid germination and emergence is an important factor of establishment of sandal seedlings. Seed priming is reported to be one of the major development to induce rapid germination and emergence of seeds and to increase seed tolerance to adverse environmental conditions. (Harris *et al.*, 1999). Seed priming has also proved to be successful in reducing the germination time and uniform seedling growth of few important forest tree species. Prolonged nursery period is an important constraint to be overcome for raising quality planting stock of sandal.

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Hence, the present investigation entitled "Impact of seed priming techniques on the germination and seedling attributes of sandal (*Santalum album* L.)" was carried out with the following objective keeping the above aspects in view.

- To evaluate the effect of different seed priming techniques *viz,* biopriming, chemical priming, hydropriming and osmopriming on the germination and seedling growth of *Santalum album* L.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Sandal (*Santalum album*) is one among the esteemed and high – priced timber in the world. The species has attained great importance in the global market due to its fragrant heartwood and the essential oil. In India *Santalum album* is mainly distributed in the Deccan plateau and total extend of its distribution is around 9600 km^2 of which 8200 km^2 is in the states of Karnataka and Tamil Nadu (Srinivasan *et al.,* 1992). In Kerala, it occurs sporadically in the deciduous forest up to 900 m elevation; fairly common at Marayoor. Estimates indicate that the global demand for sandalwood is 5000-6000 tons $year^{-1}$ and that of oil is 100 tons $year^{-1}$ (Joshi and Arun Kumar, 2007). This demand is expected to increase in the coming years and in India, substantial decline in sandalwood production has occurred 1500 tons $year^{-1}$ in 1997-98 to 500 tons $year^{-1}$ in 2007 and 100 tons $year^{-1}$ in 2011-12. Sandal heartwood prices have increased from Rs. 365 ton^{-1} in 1900 to Rs. 6.5 lakhs ton^{-1} in 1999 -2000 and to Rs. 37 lakhs ton^{-1} in 2007 (Joshi and Arun Kumar, 2007). Considerable decline in the natural populations of sandal due to illicit felling and over exploitation has resulted in the categorization of the species as 'Vulnerable' to extinction in the IUCN red list (IUCN 2010). Hence the regeneration of sandal is of great challenge and a concern to the forest department. The poor rate of germination of sandal seeds associated with prolonged period of germination are the major constrains in the regeneration of sandal.

2.1 Seed Germination in *Santalum album*

Sandal fruit is a drupe, globose, 1.25 cm diameter, purplish-black, with hard-ribbed endocarp. Seeds are globose (Luna, 1996) and 0.2 to 1.2 cm in diameter (Srimathi *et al.*, 1995) and 6000 to 7055 seeds weight one kg (Sengupta, 1937; Kumar and Bhanja, 1992). Seed emptiness in this species is low. Nagaveni and Ananthapadmanabha (1986) had graded the seeds of sandal as small, medium and big on the basis of seed size and weight. More than 82 – 87% of the sandal seed lot falls in to medium category, weighing 0.1 to 0.2 g and an average size of 7 to 8 mm. Generally, the seeds of sandal when dispersed by birds in the natural habitat require four to eight weeks to start germination (Venkatesh, 1995). Srimathi *et al.* (1995) stated that sandal seeds exhibit sporadic germination and complete germination within a period of 4 to 12 weeks.

The dormancy of the seeds is a major constraint in the regeneration of sandal. However, exact mechanism behind the dormancy is not known. Baskin and Baskin (1988) concluded that

the sandal seeds possess physiological dormancy or morphophysiological dormancy i.e. seeds contain a minute embryo that should elongate inside the seed before, during or after the loss of physiological dormancy. They inferred that on the basis of the facts that seeds required (1) warm stratification to break dormancy (Srimathi and Rao, 1969) and (2) a prolonged germination period (Beniwal and Singh, 1989). Embryos inside *S. album* seeds are very minute (Rangaswamy and Rao, 1963), but whether they grow before radicle emergence is unknown. Additional information supports that the seeds of Santalum species are dormant at maturity and have a physiological component to their dormancy: germination increases with (1) gibberellic acid (GA3) (Nagaveni and Srimathi, 1980; Ananthapadmanabha *et al.*, 1988; Hirano, 1990; Loveys and Jusaitis, 1994; Cromer and Woodall, 2007; Nikam and Barmukh, 2009; Gamage *et al.*, 2010) and (2) with removal of the fruit wall since the embryo has a low growth potential (Sahai and Shivanna, 1985; Loveys and Jusaitis, 1994; Woodall, 2004; Cromer and Woodall, 2007). Clarke and Doran (2012) speculated that the seeds of this species might have an exogenous kind of dormancy. Das and Tah (2013) stated that the enforced dormancy of sandal seed is likely due to the presence of chemical inhibitors in the seed coat which are impervious to water and gases. However, Prasetyaningtyas (2007) reported that the fruit pulp apparently contains inhibitors but the extracted clean seed has no known dormancy. Dileepa *et al.* 2015 had confirmed the morphophysiological dormancy in sandalwood and suggest that the level is non-deep simple.

Among the different pretreatments like soaking in cowdung solution, acid scarification, hot water soaking and soaking in gibberellic acid that have been practiced in sandal, soaking in gibberellic acid 0.05% is the best pretreatment for sandal seeds. A pretreatment of 0.05% gibberellic acid has resulted in 35 to 45% germination in sandal seeds (Sudhir *et al.*, 2013). The reason for the superiority of GA_3 treatment might be due to several GA signaling factors that are known to induce the expression of genes encoding enzymes that mobilize food reserves, including starches, proteins and lipids, stored in the endosperm during seed germination (Peng and Harberd, 2002). Nagaveni *et al.* (1989) tried different pre-treatment like soaking in water (control), 1% $ZnCl_2$, 0.5% NaOH, 100 ppm IBA, 5% cytozyme, 0.5% thiourea, 100 ppm IAA, 0.5% HCl or a methanolic extract of fresh sandal leaves for 4 h, soaking in 1% H_2O_2 for 2 h; and soaking in 10 ppm kinetin for 3 h. They reported that all treatments increased speed of germination over that of the control, reducing the time for first germinants to appear from 60 to 15-45 days. Srimathi and Rao (1969) reported early and quick germination in 15 days by breaking the false seed coat,

indicating the presence of inhibitory chemicals in the seed coat. Ananthapadmanabha *et al.* (1988) reported that treating with dilute NaOH or dilute HCl or GA_3 can remove the dormancy chemicals from the seed. Pretreatment of seeds with GA_3 500 mg l^{-1} for 16 h resulted in 60% germination under field conditions (Nagaveni *et al.*, 1989). Sutheesh *et al.* (2016) proved that treatment with GA_3 is the best pretreatment in sandal, however, the organic pretreatments like soaking in cow dung slurry and cow urine also produced good germination. The sulphuric acid and boiling water treatments reduced the germination of the seeds below the control. Sudheesh *et al.* (2016) stated that poor rate of germination associated with long germination period of 140 – 150 days is the major constraint in the regeneration of sandal.

2.2 Problems in Regeneration of *Santalum album*

Natural regeneration of sandal occurs by means of seeds. Seeds are usually dispersed by birds and normally take 4 to 8 weeks to germinate (Venkatesan, 1995). Seeds have a post drop dormancy period up to two months due to their impermeable outer covering and retain their viability for 6 to 12 months. Germination is hastened by soaking seeds in 0.05 per cent gibberellic acid. Soaked seeds are sown in germination trays filled with vermiculite or with sieved sand and soil in 1:2 ratio. The germination media in trays must be treated with nematicide and fungicide, periodically as a prophylactic measure.

Artificial regeneration in sandal was achieved by dibbling seeds in pits, sowing on mounds and trenching around mother trees for wounding the roots for inducing root sucker production. Planting nursery raised, vegetatively multiplied and tissue culture raised seedlings are also carried out (Rai and Kulkarni, 1986). Vegetative propagation is achieved through stem cuttings, grafting, air layering or through root suckers; but rooting of stem cuttings has been achieved only in 15- 20 per cent of cuttings (Rao and Srimathi, 1976; Uniyal *et al.*, 1985; Balasundaran, 1998; Sanjaya *et al.*, 1998). Micropropagation through axillary shoot proliferation, somatic embryogenesis and adventitious shoot induction has also been reported (Bapat *et al*, 1990; Bapat and Rao, 1999; Gairola *et al.*, 2007). A growing sandal tree under natural conditions can put up an increment of 1 kg of heartwood $year^{-1}$ and a girth of one cm $year^{-1}$ (Venkatesan, 1980; Rai, 1990).

There is drastic decline in the natural sandal population due to factors like recurring annual fires in the natural habitats, excessive grazing, illicit felling and spreading of spike disease etc. which are further accelerated by the man made activities (Venkatesh and Srimathi, 1981). The decreasing

rate of population can also be attributed to the poor germination rate of seeds, over-exploitation and failure of natural as well as artificial regeneration (Jeeva *et al.*, 1998). The freshly collected seeds of sandal exhibit after-ripening for a period of 60 days after which the seeds attain physiological maturity (Sreenivasan *et al.*, 1992). The sandal at nursery stage is susceptible to diseases and pests. The major pathogens infecting the seedlings causing severe economic loss in nurseries were *Rhizoctonia*, *Phytophthora*, *Pythium* and *Fusarium oxysporum* (Rathore, 2007). The poor regeneration in sandal can also be attributed to the low seed setting and lack of seedling vigour. Exploitation of the good quality trees at a faster rate furthered by illicit felling has led to a great decline in population which furthered the fragmentation of the population leaving behind inferior trees (Balasundaram, 2010). The cumulative seedling mortality rate of sandal in Nachivayal reserve forest in Marayoor was reported to be 94.5% at 2 years after the emergence of the seedlings sown in mounds (Balasundaram, 2010).

2.3 Seed Priming

Seed priming is one of the key technologies to achieve seed enhancement through rapid germination of seeds and optimizing the seedling establishment in the field. It is a controlled seed hydration treatment in which the metabolic activity is enhanced, but suspended before radicle protrusion. It inducts a particular physiological state in plants by the treatment of natural and/or synthetic compounds to the seeds prior to germination. During priming, the seeds are soaked in water (hydropriming) or PEG (osmopriming) or salt ($CaCl_2$, $CaSO_4$ or NaCl etc.) or any other chemical prior to germination. The efficiency of priming is dependent on many factors and is strongly depends on the plant species and the method of priming. Physical and chemical factors such as osmotic and water potential, priming agent, duration, temperature, presence or absence of light, aeration, and seed condition also influence priming success and determine germination rate and time, seedling vigor, and further plant development (Hussain *et al.* 2006, Varier *et al* 2010). Priming may augment the events happening at the beginning of the germination, but the whole process is interrupted at a given state, which is the same for all concerned seeds. It also induces structural and ultrastructural modifications that could facilitate subsequent water uptake and attenuate initial differences between the seeds in terms of imbibition, which results in a more uniform germination. On withdrawal of priming conditions, seed germination is usually faster and more uniform. The beneficial effects of seed priming include faster emergence, better

establishment, lower incidence of re-sowing, more vigorous plants, better drought tolerance, earlier flowering, earlier harvest and higher yield. Several chemicals were employed to bring about priming in various crops. In addition, plants can acquire resistance to abiotic stress after treatment with several natural or synthetic compounds such as Butenolide, Selenium, $CuSO_4$, $ZnSO_4$, KH_2PO_4, ethanol, Putrescine, Paclobutrazol, Choline, and Chitosan (Demir, *et al,.* 2012).

The increased germination rate and uniformity achieved through priming can be attributed to metabolic repair during imbibition (Burgass and Powell, 1984) or may be due to the buildup of germination-enhancing metabolites (Basra *et al*., 2005). Nonetheless, the actual cellular events happening during seed priming greatly remains uncertain, earlier studies in priming related the reduction in leakage of metabolites (Styer and Cantliffe, 1983), increase in: RNA and protein synthesis (Fu *et al*., 1988), the expression of β-tubulin (De Castro *et al*., 1995) and nuclear DNA synthesis in the radicle cells of seeds (Saracco *et al*., 1995; Liu *et al*., 1997), faster embryo growth (Dahal *et al*., 1990), nuclear replication (Lanteri *et al*., 1993; 1996), and minimal chromosomal damage (Sivritepe and Dourado,1995) as promoting effectors of seed priming.

The increase in germination by priming may be associated with a change in plant hormone biosynthesis and signaling. Priming has increased gibberellins (GA)/ abscisic acid (ABA) ratio (El-Araby *et al*. 2006), and this may lead to direct effect on a priming impact in gene expression pattern (Schwember and Bradford, 2010). A more uniform endogenous GA concentration may help to synchronize endosperm weakening, embryo cell elongation, and reserve mobilization (Sung et al. 2008). Ethylene also straightly influences speed of and percentage of germination. Priming has been reported to initiate repair and reactivation of pre-existing mitochondria and to initiate the biogenesis of new ones (Sun *et al*., 2011). It may thus afford a higher level of energy over a short time to sustain final germination (Nascimento, 2013).

Seed priming, commonly used to synchronize individual seed germination (Taylor and Harman, 1990) is reported to foster a distinct physiological state in plants through treatment of seeds before germination by natural and synthetic compounds. In addition, the plants neutralize the adverse effects of abiotic stress by seed priming (Ashraf and Foolad, 2005; Patade *et al*., 2009).

The seed germination benefits from the seed priming mainly due to activation of enzymes associated with endosperm utilization (Habib *et al.*, 2010), mobilization of storage proteins and changes in hormonal balance (Iqbal and Ashraf, 2013). Moreover, seed priming is identified to synthesize proteins that play crucial role during germination in several plant species (Gallardo *et al.*, 2001). Furthermore, the rapid and uniform germination through seed priming can also be attributed to stimulation of antioxidant activities (Chiu *et al.*, 2002; Afzal *et al.*, 2012).

Re-drying of the seeds, following seed priming treatment, to their original moisture content is an inevitable step which will otherwise do harm to the primed seeds (Thomas *et al.*, 2000) thereby affecting the seed quality (Parera and Cantliffe, 1992). Inappropriate re-drying of the seeds may cause reduction in the lag time of imbibition affecting seed germination (Heydecker and Coolbear, 1977; Brocklehurst and Dearman, 1983). Re-drying must be attained slowly to maintain the advantages obtained during priming.

2.3.1 Hydropriming

The seed priming technique in which seeds are soaked in water to initiate the pre-germination activities suspending radicle emergence is called as hydropriming. According to Kaya *et al* (2006), the duration of hydropriming is determined by controlling the seed imbibition and the hydrated seeds are dried after a particular period of hydration under shade conditions (Mc Donald, 2000). After soaking, seeds are re-dried to their original weight with forced air under shade (Bennett and Waters, 1987). It is necessary to dry the seeds after soaking as storing of improperly dried seeds will do more harm than good (Thomas *et al.* 2000). Hydropriming is the simplest among the seed priming, techniques which depends on seed soaking in pure water and re-drying to original moisture content prior to sowing. As no additional chemical substances are used as a priming agent, this method is a low-cost and environmentally friendly technique.

The protoplasm of seeds subjected hydropriming have a lower viscosity and exhibit higher permeability to water and nutrients and also hold water against dehydrating forces (Thomas *et al.*, 2000). The advantage of hydropriming is the enhancement of physiological and biochemical events taking place in seeds even when the germination is suspended by low osmotic potential and negligible matric potential of the imbibing medium (Basra *et al.*, 2003).

Increase in the seedling growth correlated with higher water uptake by primed seeds is the predominant feature in the case of hydropriming (Yagmur and Kaydan 2008).

Fujikura et al. (1993) reported hydropriming as a simple and inexpensive method of seed priming and according to Abebe and Modi (2009), it is a very important seed treatment technique for rapid germination and uniform seedling establishment in various grain crops. A study in barley by Jaudi and Sharifzadeh (2006) identified that hydropriming can improve rate of germination, length of coleoptile and root, dry mass accumulation and seedling vigor index. Abebe and Modi (2009) suggested hydropriming as a successful seed treatment technique for rapid germination and uniform seedling establishment in various grain crops. It was found to be the most successful method for improving seed germination in onion (Caseiro *et al.*, 2004). Filho and Kikuti (2008) suggested that although priming can increase rate of germination and speed of seedling emergence it had no effect on the yield of cauliflower.

Hydropriming in chickpea has resulted in three to four fold increases in root and shoot height in comparison with seedlings obtained from non-primed seeds in drought condition (Kaur *et al.*, 2002) which may be attributed to the faster emergence of roots and shoots and the enhanced root and shoot height which results in vigorous plants, better drought tolerance under adverse conditions (Amzallag *et al.*, 1990; Cayuela *et al.*, 1996; Lee-suskoon *et al.*, 1998). In wheat, hydropriming brought about significant improvement in germination and early growth. In wheat, hydropriming was found to be effective in improving the seeding vigour (Jafar *et al.*, 2012). Sung and Chiu (1995) proposed that emergence force and seedling growth were improved by hydropriming of watermelon seeds.

Daniel *et al.* (1984) hydroprimed lettuce seed in water at 15°C in the dark for different durations and revealed that priming for 20 h in distilled water enhanced the germination up to 86 per cent in lettuce seeds. Moradi and Younesi (2009) reported that both hydropriming and osmopriming improved the percentage and mean emergence time of sorghum seeds at sub-optimal temperature of 15°C. Seed treatment for 12 and 24 h had a positive statistically significant effect on percentage and speed of emergence. Nevertheless priming for 36 h failed to improve emergence percentage and mean emergence time.

Amooaghaie (2011) reported that seedlings from hydroprimed seeds performed higher growth with respect to root and shoot height compared to seedlings from non – primed seeds.

Li *et al.* (2011) concluded from his laboratory experiment on effect of hydropriming on the seeds of pyrethrum that hydropriming has significantly reduced the mean germination time and increased the germination percentage. According to Srivastava *et al.* (2010), hydropriming was the most suitable priming technique in mustard. Shah *et al.* (2012) also suggested that hydropriming as an effective seed priming method for enhancing the seedling vigour and nutrient uptake in green gram. According to Umair *et al.* (2012), hydropriming significantly increased the seed yield of green gram and also enhanced the antioxidant enzyme activities.

2.3.2 Biopriming

Seed biopriming is one of the most suitable methods for the application and subsequent establishment of bacterial antagonists in the spermospheres and rhizospheres (El Zain, 2006). Biopriming has potential advantages over seed coating (Muller and Berg, 2008) due to the fact that biopriming helps in establishing the bacteria in the seed which attributes to the better stability and shelf life of the seeds. Biopriming with *Pseudomonas fluorescens* in tomato seeds reported to exhibit higher germination, field emergence and reduction in Fusarium wilt in tomato seedlings (Asha *et al.*, 2011).

Biopriming involves treating the seeds with a biocontrol agent such as *Pseudomonas aureofaciens* Kluyver AB254 and hydrating for 20 h under warm conditions (23° C) in moist vermiculite or on moist germination blotters in a self - sealing plastic bag and the seeds were removed from the above condition before radicle emergence (Callan *et al.*, 1990). Biopriming with PGPR improves seed germination percentage of *Cicera rietinum* L plants under saline conditions and also increased the shoot height, root length and dry matter (Mishra *et al.*, 2010). In wheat, seed biopriming with different salinity tolerant isolates of *Trichoderma* were successful in improving germination percentage and reducing the reduction in percentage of germination during salinity stress (Rawat *et al.*, 2011). Biopriming of sunflower seeds with *Pseudomonas fluorescens* UTPf76 and UTPf86 improved the ability of seeds to invigorate and seedlings to grow uniformly (Moeinzadeh *et al.*, 2010).

Karthika and Vanangamudi (2013) reported that biopriming with Azospirillum 20% for 12 h in hybrid maize showed significant increase in germination, root length, shoot height, total dry matter production and vigour index when compared to priming by other biocontrol agents and non – primed seeds. A study in pea seed by Negi *et al.* (2008) reported that priming by

Pseudomonas fluorescens reported to show marked increase in shoot and root dry weight and were effective in enhancing plant growth activities and yield performances in the field. Biopriming with *Azospirillum brasilense* and *Pseudomonas striata* (20%) greatly increased seed germination, root and shoot height, dry matter accumulation, vigour index and lower disease occurrence in hybrid seed maize than hydropriming and control.

Biopriming using co-flocs, which consists *A. brasilense* MTCC125 and *P. fluorescens* MTCC-4828 reported striking increase in the germination (85.5%) and vigour index (1970.1) in rice (Joe and Sivakumar, 2011). Nayaka *et al.* (2009) revealed from a study in maize that biopriming with pure culture and the formulations of *P. fluorescens* increased the seed germination, seedling vigour, plant height and yield as well as a dwindling rate of incidence of *F. verticilloides* greater extend when compared to non – primed seeds. Raj *et. al.* (2003) concluded that biopriming with *P. fluorescens* isolate in pearl millets increased the growth of seedlings and induced the resistance against downy mildew. The time required for flowering was also advanced by 5 days with 22% increase in grain yield and 20 to 75% resistant to downy mildew.

Kalaivani (2010) revealed that biopriming with 20% Azospirillum for 12 h maize seeds imparted greater germination (95%) over non-primed seed (70%) and hydroprimed seeds (85%). The study also indicated that biopriming the seeds with phosphobacteria at 20% concentration for 12h could also impart a higher germination of 95 per cent. Zorita and Canigia (2009) bioprimed the wheat seeds with liquid formulation of *A. brasilense* and proclaimed that the crop showed higher vigorous vegetative growth with greater shoot and root dry matter accumulation. Priming also increased the number of harvested grains and grain yield.

Biopriming of pearl millet seeds with *Pseudomonas fluorescens* triggered the plant growth inducing resistance against downy mildew disease caused by the fungus *Sclerospora graminicola* (Raj et *al*., 2003). El-Mougy and Abdel-Kader (2008) reported that biopriming with any one of the strains of *T. viride*, *T. harzianum*, *B. subtilis* and *P. fluorescens* revealed a remarkable reduction in disease incidence than non – primed seeds. The reduction in disease incidence was greatly observed in pre – emergence stage that the post – emergence stage.

A study in safflower to understand the effect of biopriming with Pseudomonas strain in combination with nitrogen application at 180 kg ha^{-1} reported that maximum grain yield (1940.4 kg ha^{-1}) was obtained when compared to the treatment combinations and control (Sharifi, 2012).

Study by Mariselvam (2012) revealed that when bhendi seeds were bioprimed with *P. fluorescens* at 60% for 12 h + foliar spray of *P. fluorescens* at 2g lit^{-1} of water on 30^{th} and 45^{th} DAS, plant growth and development was maximum and induced a steady increase in chlorophyll content (17-20%), seed yield (49%) and quality of post primed seed.

2.3.3 Osmopriming

Osmoconditioning or osmopriming is a seed priming technique in which seeds are soaked in osmotic solutions. It is the soaking of seeds in aerated, low-water-potential solutions. Osmopriming exposes seeds to a low external water potential to restrict the rate and extent of imbibition. The process of osmopriming is analogous to a prolonged early imbibition of seeds that sets in motion a gradual progression of various pre-germinative metabolic activities. Thus, it is helpful to use osmopriming as a model to study the transition of seeds from a dry and physiologically quiescent to a hydrated and physiologically active state (Chen and Arora, 2011). Usually water potential of priming agent varies from −1.0 down to −2.0 MPa.

Osmopriming is a long established seed priming technique to improve seed quality (Bray *et al.,* 1995), seed viability and vigour (Senaratna and McKersie, 1983; Bruggink *et al.,* 1991; Bailly *et al.,* 1998; Ouyang *et al.,* 2002; Wenfan *et al.,* 2010). Polyethylene glycol (PEG) is more commonly used as water potential lowering agent due to its nontoxic nature and large molecular size, which lowers water potential without penetrating into the seeds during soaking (Thomas *et al.*, 2000). Osmopriming using PEG 6000 is reported to reduce the leakage of solute during germination (Kmetz-Gonzalez and Struve, 2000; Chen and Sung, 2001; Ashraf and Foolad, 2005). The reduced rate of hydration during germination by PEG priming is reported to allow sufficient time for metabolic repair (Toole *et al*., 1957; Villiers and Edgecumbe, 1975). An increase in the concentration of PEG is in proportionate with a decrease in electrical conductivity of the germinating seed. This trend which occurs due to reduction of water uptake was observed in radish and eggplant (Rudrapal and Nakamura, 1988), corn (Sung and Chang, 1993) soybean (Senaratna and Mc Kersie, 1983) and Onion (Choudhury and Basu, 1988).

Dearman *et al*. (1986) reported that on osmopriming with PEG (342 g kg^{-1} water and dried back to 9% moisture content) in onion, the seeds exhibited better storability over a period of 18 months at 10^0 C with no reduction in the viability and germination rate. A similar study by

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Dearman *et al.* (1987) concluded that fresh seeds of carrot and leek primed in PEG (273 and 342 g/kg of water, respectively), showed storability of the seeds at 10° C for 12 months with little or no loss viability under all priming durations in leek (10, 14 and 17 days) and for the 10th and 14th days priming duration in carrot. On the contrary considerable loss in viability of carrot seeds were observed in the priming duration of 17 days after 12 months storage.

Osmopriming with PEG for 48 hours was reported to be an effective technology to enhance germination and seedling vigour in peanut seeds (Fu *et al.*, 1988). Karansingh and Kakralya, (2000) concluded that osmopriming in green gram with PEG for 48 hours resulted in increased seed germination, vigour, storability and field performance. Bino *et al.*, (1992) suggested that a priming period of 14 days in PEG 6000 solution has significantly increased the rate of uniform germination in tomato seeds. Nagarajan (2003), from his study on Asiatic carrot concluded that when osmoprimed with PEG 6000 (-0.5 Mpa and -1.0 Mpa) for 3 days had shown an increase in germination by 38%, vigour index by 47% and dry matter accumulation by 36% in one month old seedlings. Osmopriming with PEG 6000 (-1.0 Mpa) for 7 days increased the seedling dry weight from 10.68 to 11.77 mg in sunflower (Kaur, 1992), 267 to 429 mg in capsicum, 256 to 400 mg in tomato and 200 to 331 mg in onion (Jagdish, 1993). Gayathri (2001) studied that there was a noted increase in the seedling vigour index of tomato seeds from 1280 to 1540 when primed with PEG 6000 (-1.0 Mpa) for 3 days. Pallavi (2004) has indicated an increase in seedling vigour index of cauliflower seedlings on the basis on seedling dry weight when primed with PEG 6000 (-1.0 Mpa) for 3 days.

2.3.4 Chemical Priming

Various chemicals were employed in the seed priming technique from time to time. The common priming agents in seed priming include KNO_3, KCl, K_3PO_4, KH_2PO_4, $MgSO_4$, $CaCl_2$, mannitol, etc. (Farooq *et al.*, 2005). Seed priming with KNO_3 increased germination percentage germination index, root length, shoot height and seedling fresh weight of tomato plants (Nawaz *et al.*, 2011). In rice, seed priming with KCl and $CaCl_2$, reduced the emergence time and increased the energy and index of seedling emergence. Seedlings from primed seeds had greater length, number of roots and enhanced fresh and dry biomass compared to control. Seed priming had altered the pattern of nitrogen and calcium homeostasis both seeds and seedlings, which in turn enhanced the α-amylase activity and reducing sugars content (Farooq *et al.*, 2006). Seed priming

with copper sulphate and zinc sulphate appreciably increased the final caryopses germination and increased seed emergence by 43% and 29%, respectively in maize (Foti *et al.*, 2008).

Seed priming with KH_2PO_4 had revealed to have good potential to enhance germination, emergence and plant growth of wheat (Das and Choudhury, 1996; Ghana and Schillinger, 2003; Korkmaz and Pill, 2003). In *Triticale*, seed priming with KH_2PO_4 increased germination percentage and seedling growth (Yagmur and Kaydan, 2008).

The studies on the oil seed Sesame (*Sesamum indicum* L.) had indicated that soaking seeds with $MnSO_4$ 0.5% for 12 h and followed by humid invigoration of 9 h was effective in increasing germination (22.7%), radical protrusion (54%), shoot height (36.4%), root length (78%), dry matter production (63.5%) and vigour index (92%) over the control (Vijayalakshmi and Sundaralingam, 2018).

2.4 Effect of seed priming on the biochemical parameters of seed

Priming is associated with the change in physiological and biochemical parameters of the seeds. Mir (2013) reported a steady increase in the total sugar, shoot and root length and seedling dry weight when hybrid maize seeds were subjected to hydropriming. Whereas, Sharanappa (2013) reported that PEG priming (-1.0 Mpa) increased the total sugar and total protein in the seed by 2.71% and 2.58%, respectively. There was a decrease in the seed leachate conductivity. The studies on PEG priming (-1.0 Mpa and -1.5 Mpa) in two varieties of wheat (Sowmya, 2007) indicated an increase in the total sugar, total protein and lipid content with priming. The growth attributes of the resultant seedlings exhibited a positive correlation with the total sugar and total protein content of the seeds. Biopriming of garden pea seeds using *Trichoderma viride* and *Pseudomonas fluorescens* had resulted in an increase in the germination percentage, plant height, plant dry weight as well as an increase in the chlorophyll content (62.1%), total sugar (31.7%) and total protein (17.2%) (Manjubala, 2015). The study also revealed that biopriming with *Trichoderma viride* and *Pseudomonas fluorescens* at 40% for 4 h resulted in 42 per cent reduction in seed rotting and enhanced the seed quality and seedling performances in the field.

The storability studies on maize seeds by biopriming with *P. fluorescens* reported that biopriming with *P. fluorescens* 80% for 12 h resulted in an increase in germination (11%), root (65%) and shoot (88%) length, dry weight accumulation (38%) and vigour index (83%) over non-

primed seeds after a storage period of six months (Kalaivani, 2010). Kavitha (2011) concluded that at sixth month of storage, rice seeds bioprimed with *P. fluorescens* 60% for 12 h brought about significant increment in germination (11%), root and shoot height (14%), dry weight accumulation (19%) and vigour index (12%) over non-primed seed. Mariselvam (2012) proclaimed that biopriming in bhendi seeds with *P. fluorescens* @ 60% for 12 h resulted in an 18 per cent increment in germination in cloth bag whereas the rate of germination declined to a 15 per cent increment in case of polyethene bag over non-primed seed when subjected to 10 months storage period under ambient conditions. In spite of the increased germination percentage, the amount of total free amino acids and reducing sugars recorded a lower value over the non-primed seeds.

2.5 Application of seed priming in forest tree species

Perusal of literature indicated that only limited studies are available on seed priming of forest tree species and most of the studies are confined to various pines (Haridi, 1985; Hallgren *et.al.*, 1989; Bourgeois *et al.*, 1991; Su-juan *et al.*, 2012) and those included mainly the priming techniques like hydropriming and osmopriming. Adebisi *et al.*, (2011) tried hydropriming in the seeds of *Cordia millenni* and which was successful in increasing the germination percentage and seedling vigour.

Osmopriming with 20% PEG increased the germination percentage, mean germination time, seedling vigor index and seedling height in *Gmelina arborea* (Adebisi *et al.*, 2013). Venudevan and Srimathi (2013) studied the positive influence of hydropriming in *Aegle marmelos* and concluded that highest germination and seedling growth attributes were achieved by hydro priming of seeds for 6 hours. Osmopriming in *Azadirachta indica* seeds had induced hydrogen peroxide during germination that could be the signaling molecule for germination improvement (Pandey and Pati, 2016). Chemical priming was found to reduce the mean germination time and improve germination index, seedling diameter, seedling length and dry weight in *Aegle marmelos* (Singh, 2017).

Rodriguez *et al.*, (2015) studied the effect of both hydropriming and biopriming on seed germination and seedling vigor of two Mexican fir tree species, *Abies hickelii* and *Abies religiosa*. They suggested biopriming in combination with hydropriming has become a viable treatment for increasing seed germination rate and seedling vigor. Results of the study indicated that treating *A.*

hickelii and *A. religiosa* with both hydropriming and biopriming with certain strains of Plant Growth-Promoting Rhizobacteria (PGPR) could improve germination rates up to 91% for *A. hickelii* and up to 68% for *A. religiosa*. Moreover, priming methods did not show negative impact on the growth of *A. religiosa* and actually improved growth in *A. hickelii*.

In *Casuarina equisetifolia* germination increased from 46% in the control to 65% after soaking in 1.5% KNO3 for 36 hours. Both higher and lower concentration, and shorter duration of soaking showed a lower germination in that experiment (Maideen *et al.*, 1990).

In the study on *Ziziphus mauritiana*, KNO3 was less effective than GA_3, thiourea and BA in all germination parameters except root length (Murthy and Reddy, 1989). Among several compounds studied, thiourea proved the most effective germination stimulant for *Ziziphus mauritiana*. A 24 h soaking in a 1% solution enhanced total germination percentage from 41% (control) to 78% at 30°C, which was considered the optimal germination temperature. In addition it alleviated the deleterious effects of sub-optimal temperatures, both in terms of total germination and vigour.

Osmopriming was effective in imparting higher germination percentage and speed of germination, higher seedling growth and uniformity and lower electrical conductivity of seeds. Osmotic priming with aerated solutions of polyethylene glycol improved both final germination and rapidity of germination in loblolly and short-leaf pines (Hallgren, 1989). Hydropriming and chemical priming showed to improve the highest physiological potential and increased germination performance in *Guazuma ulmifolia* (Tay and Novembere, 2010).

MATERIALS AND METHODS

MATERIALS AND METHODS

The investigations on the impact of seed priming techniques on germination and seedling performance in sandal (*Santalum album*, L.) carried out at the College of Forestry Kerala Agricultural University, Thrissur from March 2018 to April 2019. The materials used and methodology followed in the present study are presented in this chapter.

Study area

Geographically, the area is located at 40 meters above mean sea level at 10° 32'N latitude and 76° 26'E longitude. The area experiences a warm and humid climate with distinct rainy season. The soil of the study area is oxisol. The predominant parent material is metamorphic gneiss. The soils and subsoils are porous and extremely well drained.

3.1 MATERIALS

The seeds of *Santalum album* obtained from the Marayoor provenance were selected for the present study. Marayoor is considered as the best sandal provenance in India. The Kerala Forest Department is maintaining about 8,500 acres of natural sandalwood in the Nachivayal Reserve Forest I and II of Marayoor sandal division. The Marayoor sandal Division is situated between 77^0 5' to 77^0 15' East longitude and 10^0 10' to 10^0 20' North latitude and is situated the Devikulam Taluk of Idukki District. The area has gneissic metamorphic rocks from the Archaean shield. The predominant rock type is biotitic gneiss and it is also associated with hornblende biotitic gneiss in certain areas. The soil is sandy loam in texture. The soil reaction varies from slightly alkaline to strongly acidic depending on the vegetation type. Climate in Marayoor is comparatively drier. It is cool from December to February and hot during March to May. The Marayoor Sandal Division area being on the leeward side of the Ghats has low rainfall of about 1000 to 1500 mm. Precipitation from the South-West monsoon is comparatively low and is only as much as what the North East monsoon bestows on these regions. The rainy season, though lasts from June to the end of November, is frequently interrupted by spells of hot weather.

The seeds were obtained from the Vana Samrakshana Samithi, Marayoor Forest Department, Kerala during the collection season February to March. The seeds were globose to spherical with an average weight of 0.1 g having an average diameter of 0.74 cm. The collected seeds were cleaned and thoroughly mixed to improve the homogeneity.

3.1.1 Seed characters

The following characters were determined from the seeds seed samples. This was replicated ten times. The weight of thousand seeds were determined using an electronic balance and expressed in grams. From the samples, 50 seeds were randomly selected to determine the individual seed weight, and diameter. The seed colour was determined visually.

3.2 METHODS

Four priming methods viz., hydropriming, biopriming, chemical priming and osmo priming were adopted in the present study. Biopriming agent selected for the study was *Pseudomonas fluorescens*, the osmopriming agent was PEG 6000 and that of chemical priming was $MnSO_4$. Although KNO_3 was the chemical priming agent selected initially, as the preliminary trials did not give the results it was avoided from the study. The steps after obtaining seeds to subjecting them to different priming techniques are given in the following sections.

3.2.1 Seed Sampling

The sandal seeds received from Marayoor provenance (seeds procured from selected superior trees in Nachivayal Reserve Forest) were thoroughly mixed to improve the homogeneity of the samples. The entire seed lot was spread on the floor and mixed by scooping the seeds from side to side and from top to bottom. After thorough mixing, the seeds were evenly spread out on a smooth surface and the whole lot was divided into four equal parts. Scooping was continued on all four parts also. The process was repeated four times. In order to conduct the various priming experiments seeds were randomly selected from the seed lot.

3.2.2 Surface Sterilization of the Seeds

The seeds were surface sterilized using 1% Mercuric chloride. The seeds were immersed in Mercuric chloride solution for 3 minutes and were thoroughly washed using distilled water to remove the traces of the solution.

3.2.3 Seed Priming Methods

The sandal seeds were subjected to four seed priming methods viz., hydropriming, biopriming, chemical priming and osmopriming. The untreated seeds were kept as control.

3.2.3.1 Hydropriming

In order to hydroprime, the seeds were soaked in double distilled water for the duration 3, 6, 9 and 12 days. To start the hydropriming, 400 seeds weighing 39.3 g to 42.2 g were taken and the volume of the sample was estimated using a measuring cylinder. The seeds were then transferred to a glass bottle and were soaked in distilled water in the ratio of 1: 2.

3.2.3.2 Biopriming

The priming agent for biopriming selected in the present study was *Pseudomonas fluorescens*. The biopriming agent *P. fluorescens* was obtained from Department of Microbiology, College of Horticulture, Kerala Agricultural University. Suspension culture of *Pseudomonas fluorescens* at 10^8 c.f.u. ml^{-1} were obtained. For the study 20 g of the suspension culture of *P. fluorescens* @ 10^8 c.f.u. ml^{-1} is said to produce 100% concentration for 50 sandal seeds. Hence, the ratio of suspension culture to the number of seeds to be primed were taken in the ration 2:5. The biopriming of seeds were carried out at four different concentrations of the suspension culture viz., 25, 50, 75 and 100% for 2, 4, 6 and 8 days. During the treatment distilled water was added to make up the volume of priming solution sufficient to suspend the seeds within it. The glass bottle with seeds and suspension culture was covered with aluminum foil. The seeds were stirred at regular interval to prevent the suspension culture from hardening.

3.2.3.3 Osmopriming

Osmopriming was achieved using Polyethylene Glycol 6000 (PEG 6000) (Nice). Osmotic solutions of PEG 6000 were made at 5%, 10%, 15% and 20% concentrations in distilled water and a volume double the volume of seeds were added. Priming was completed at different durations viz., 3, 6, 9 and 12 days for each level of concentration. The lid of the glass bottles were then covered with aluminium foil.

3.2.3.4 Chemical Priming

Chemical priming was initially carried out using KNO_3 at different levels viz., 0.5 M, 1 M, 2 M, and 3 M at 3, 6, 9 and 12 days. The absence of germination in KNO_3 primed sandal seeds forced to change the priming chemical as $MnSO_4$. The seeds were primed at 0.4 M, 0.6 M, 0.8 M and 1 M concentrations of $MnSO_4$ for 3, 6, 9 and 12 days. $MnSO_4$ (Nice) solutions of different

molarity were made in distilled water to a volume double the volume of seeds. The $MnSO_4$ salt was thoroughly dissolved in water to obtain a homogenous solution and seeds were soaked in it. The glass bottles with seeds and priming solution was covered with an aluminum foil.

The Table 1 shows the various priming treatments adopted for the present study. The Plate 1 narrates the different stages during seed priming.

Table 1. Seed priming treatments adopted in the study

Method	Treatment code	Treatment substrate	Duration in (days)	Concentration
Hydropriming	T1	Distilled water	3	-
	T2	Distilled water	6	-
	T3	Distilled water	9	-
	T4	Distilled water	12	-
Biopriming	T5	*Pseudomonas fluorescence*	2	25%
	T6	*P. fluorescence*	2	50%
	T7	*P. fluorescence*	2	75%
	T8	*P. fluorescence*	2	100%
	T9	*P. fluorescence*	4	25%
	T10	*P. fluorescence*	4	50%
	T11	*P. fluorescence*	4	75%
	T12	*P. fluorescence*	4	100%
	T13	*P. fluorescence*	6	25%
	T14	*P. fluorescence*	6	50%
	T15	*P. fluorescence*	6	75%
	T16	*P. fluorescence*	6	100%
	T17	*P. fluorescence*	8	25%
	T18	*P. fluorescence*	8	50%
	T19	*P. fluorescence*	8	75%
	T20	*P. fluorescence*	8	100%
Osmopriming	T21	Poly Ethylene Glycol (PEG 6000)	3	5%
	T22	Poly Ethylene Glycol (PEG 6000)	3	10%
	T23	Poly Ethylene Glycol (PEG 6000)	3	15%
	T24	Poly Ethylene Glycol (PEG 6000)	3	20%
	T25	Poly Ethylene Glycol (PEG 6000)	6	5%
	T26	Poly Ethylene Glycol (PEG 6000)	6	10%
	T27	Poly Ethylene Glycol (PEG 6000)	6	15%
	T28	Poly Ethylene Glycol (PEG 6000)	6	20%
	T29	Poly Ethylene Glycol (PEG 6000)	9	5%
	T30	Poly Ethylene Glycol (PEG 6000)	9	10%
	T31	Poly Ethylene Glycol (PEG 6000)	9	15%
	T32	Poly Ethylene Glycol (PEG 6000)	9	20%
	T33	Poly Ethylene Glycol (PEG 6000)	12	5%

	T34	Poly Ethylene Glycol (PEG 6000)	12	10%
	T35	Poly Ethylene Glycol (PEG 6000)	12	15%
	T36	Poly Ethylene Glycol (PEG 6000)	12	20%
Chemical Priming	T37	$MnSO_4$	3	0.4 M
	T38	$MnSO_4$	3	0.6 M
	T39	$MnSO_4$	3	0.8 M
	T40	$MnSO_4$	3	1.0 M
	T41	$MnSO_4$	6	0.4 M
	T42	$MnSO_4$	6	0.6 M
	T43	$MnSO_4$	6	0.8 M
	T44	$MnSO_4$	6	1.0 M
	T45	$MnSO_4$	9	0.4 M
	T46	$MnSO_4$	9	0.6 M
	T47	$MnSO_4$	9	0.8 M
	T48	$MnSO_4$	9	1.0 M
	T49	$MnSO_4$	12	0.4 M
	T50	$MnSO_4$	12	0.6 M
	T51	$MnSO_4$	12	0.8 M
	T52	$MnSO_4$	12	1.0 M
Non-primed (Control)	T53	-	-	-

3.2.4 Post Priming Process

The primed seeds taken out of the priming agents were thoroughly washed with distilled water thrice and were placed on a piece of Whatman filter paper, allowing dehydration under shade at 25° C till the seeds retrieved the original moisture level as that of pre-priming stage.

3.2.5 Seed Storage

The re-dried seeds were transferred to paper bags and stored for 1day and 1 month in glass containers with tight lid under ambient conditions.

3.2.6 Seed Pre – Treatment

The primed sandal seeds were pretreated with 500 ppm GA_3 (Merck SRL) for overnight prior to sowing.

3.2.7 Sowing

Seeds were sown in plastic germination trays filled with sand medium. The treated seeds were sown at a depth of one cm below at a spacing of 5 cm x 5 cm. They were sown in five rows

Plate 1. Stages of priming in sandal seeds

1a. Selection of seed samples for priming treatments

1b. Recording the initial seed weight

1c. Surface sterilization with 1% Mercuric chloride

1d. Seeds soaked in different priming media

1e. Redrying the seeds to the original moisture content

and ten columns. There were 106 treatment combinations and each treatment had three replications of 50 seeds each. It was watered regularly and uniformly with a rose can until germination was completed.

3.2.8 Germination Study

The sown seeds were daily observed to record the germination. The first germination was recorded 8 days after sowing (Plate 2).

Daily germination counts were recorded for a period of 60 days from the start of germination by which germination was complete. Number of seedlings emerging on each day was recorded. From these observations, germination percentage, Peak Value of germination (PV), mean daily germination (MDG) and Germination Value were calculated. The germination percentage was calculated using the formula:

$$\text{Germination percentage (\%)} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds sown}} \times 100$$

Speed of germination was calculated using the formula:

$$\text{Speed of Germination} = \frac{n_1}{d_1} + \frac{n_2}{d_2} + \frac{n_3}{d_3} + \cdots + \frac{n_n}{d_n}$$

Where n is the number of seeds germinated on n^{th} day and d is the corresponding number of days. The germination value (GV) was calculated using the following formula suggested by Czabator (1962):

$$GV = MDG \times PV,$$

Where, GV = Germination Value,

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 germination test, divided by the number of days from sowing to the end of the test. Peak Value of germination actually denotes the speed of germination, which is the maximum mean daily germination, recorded at any time during the period of test.

Plate 2. Stages of seed germination in sandal

2a. Breaking of the seed endosperm

2b. Radicle protrusion

2c. Emergence of the plumule

2d. The cotyledons detach from the seedling

2e. Fully emerged seedlings of sandal

3.2.9 Seedling growth and biomass production

In order to find the effect of priming on seedling parameters, 12 uniform seedlings having 4-6 leaves belonging to each priming treatments were be transplanted to polythene bags containing the medium soil, sand and cow dung in the ratio 3:1:1 for six months. The growth attributes, biomass production and physiological observations were made at 90^{th} and 180^{th} days after transplanting (DAT). The various seedling parameters studied are described below (Plate 3).

3.2.9.1 Seedling height

The height of the sample plants were counted from the ground level to the tip of the main shoot using a meter scale 90^{th} and 180^{th} days after transplanting (DAT) and the mean plant height was recorded (cm).

3.2.9.2 Collar girth

The collar girth of 4 uniform seedlings from each treatment was measured using a Vernier caliper at 90^{th} and 180^{th} DAT and was expressed as the mean girth in mm.

3.2.9.3 Leaf number and Leaf area

Leaves from each seedling were detached to record the total leaf area. The total leaf area of three sample seedlings were recorded using systronics Leaf area Meter 21 I and the average leaf area (cm^2) was determined at 90^{th} and 180^{th} DAT; the leaves of the three sample plants were counted and the mean was determined.

3.2.9.4 Root length and Number of Roots

The length of 4 uniform seedlings from each treatment was measured using a meter scale and was expressed as mean length (cm) at 90^{th} and 180^{th} DAT. The number of roots from the taproot was individually counted for each seedling and recorded.

3.2.9.5 Fresh weight

The fresh weight of leaf, stem and root were separately recorded for each seedling using an electronic balance. The fresh weight of the seedling was calculated by adding the fresh weight of leaf, stem and root at 90 and 180 DAT. The total fresh weight was expressed in grams.

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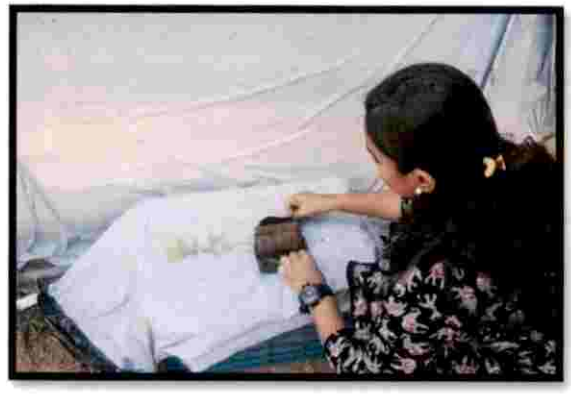
Plate 3. Morphometric observations on sandal seedlings

3a. Sandal seedlings belonging to different priming treatments

3b. Measuring the collar girth of seedling

3c. Laying the seedling horizontally for opening the poly bag



3d. Removal of the potting mixture without damaging the seedlings

3e. Obtaining the seedling for destructive sampling

3f. Recording of the biometric observations

3.2.9.6 Dry weight

Each biomass component of the seedling separately dried in a brown paper bag in a hot air oven maintained at 70^0C for 48 h to estimate the dry weight.

3.3 Physiological Parameters

3.3.1 Chlorophyll content

The chlorophyll content of the seedling was estimated using chlorophyll meter (SPAD – 502, Minolta) from three selected leaves (leaves from the top and middle portion of the seedlings) in the seedling. The chlorophyll content of the leaf is expressed as mg g^{-1}.

3.4 Growth analysis indices

3.4.1 Vigour Indices

The vigour index I (VI I) and vigour index II (VI II) of the seedlings was calculated using the formula given by Abul-Baki and Anderson, 1973:

$$\text{VI I} = \text{GP} \times (\text{SL} + \text{RL})$$

$$\text{VI II} = \frac{\text{GP} \times \text{TDW}}{100}$$

Where, VI I= Vigour index I, VI I= Vigour index II, GP = Germination percentage, SL = Shoot height and RL = Root length and TDW= Total dry weight

3.4.2 Root: Shoot Ratio

The root: shoot ratio of the seedlings were worked out at 90^{th} and 180^{th} DAT using the following formula (Hunt, 1990):

$$\text{Root: Shoot Biomass Ratio} = \frac{\text{Root dry weight (g)}}{\text{Shoot dry weight (g)}}$$

3.4.3 Leaf Area Ratio

According to Radford (1967) Leaf Area Ratio is an expression of the amount of leaf area formed per unit of biomass. It is expressed as $cm^2\ g^{-1}$ of plant dry weight.

$$\text{Leaf Area Ratio} = \frac{\text{Leaf Area per Plant}}{\text{Plant Dry Weight}}$$

3.4.4 Leaf Weight Ratio

Leaf weight ratio is an expression of the leaf dry weight to the whole plant dry weight. It was suggested by Kvet *et al.*, (1971).

$$\textit{Leaf Weight Ratio} = \frac{\text{Leaf Dry Weight}}{\text{Plant Dry Weight}}$$

3.4.5 Specific Leaf Area

Specific leaf area was determined by dividing total leaf area by leaf dry weight per plant and the average value was expressed in $cm^2\ g^{-1}$ (Hunt, 1990).

$$\text{Specific Leaf Area} = \frac{\text{Total leaf area per plant}}{\text{Total leaf dry weight per plant}}$$

3.4.6 Specific Leaf Weight

Specific leaf weight was estimated as the ratio of leaf dry weight per plant to the total leaf area per plant and is expressed as $g\ cm^{-2}$ (Pearce *et al.*, 1967).

$$\text{Specific Leaf Weight} = \frac{\text{Leaf dry weight per plant}}{\text{Total leaf area per plant}}$$

3.4.7 Absolute Growth Rate

Absolute growth rate is the height increment attained by a plane within a definite time interval and is generally denoted as $cm\ day^{-1}$ (Hunt, 1990).

$$\text{Absolute growth rate} = \frac{h_2 - h_1}{t_2 - t_1}$$

Where, h_1 is the height of seedling at t_1 and h_2 is the height of seedling at t_2

3.4.8 Relative Growth Rate

Relative growth rate (RGR) is a measure of increase in dry matter per unit biomass per unit time ($g\ g^{-1}\ day^{-1}$) was calculated from the formula proposed by Williams (1946):

$$\text{Relative growth rate} = \frac{\log_e W_2 - \log_e W_1}{t_2 - t_1}$$

Where, W_1= dry weight estimate at time t_1

W_2 = dry weight estimate at time t_2

3.4.9 Net Assimilation Rate

Net assimilation rate (NAR) is an index of the productive efficiency of plant calculated in relation to the total leaf area. NAR was calculated from the equation given by Williams (1946):

$$\text{Net assimilation rate} = \frac{W_2 - W_1}{t_2 - t_1} \times \frac{\log_e L_2 - \log_e L_1}{L_2 - L_1}$$

where, W_2 = dry weight at time t_2

W_1= dry weight at time t_1

L_2 = leaf area at time t_2

L_1= leaf area at time t_1 and it was expressed in (g cm^{-2}) day^{-1}

3.5 Electrical conductivity of seed leachates

The electrical conductivity of the seed leachates was determined from the readings of conductivity meter and the conductivity was expressed as dS cm^{-1}. In order to measure the electrical conductivity 50 normal and undamaged seeds were taken for each priming treatment and was primed in a 100 ml beaker replicated thrice at a constant temperature. Care was taken to immerse the seeds completely in the priming solution and the beakers were covered with aluminum foil. When each treatment is completed, the leachate is filtered to a conical flask. 5 ml of the leachate is then transferred to a 25 ml standard flask and the final volume was made to 25 ml by adding distilled water. Thereafter, the electrical conductivity of the seed leachates was measured using a direct reading conductivity meter (CDC 40101).

3.6 Biochemical Analysis

The methodology for determination of biochemical constituents like total carbohydrate, protein and crude fat content is given in the following sections.

3.6.1 Total carbohydrate

The total carbohydrate of the control and primed seeds was estimated using the method suggested by Yemm and Wills (1954) and is expressed in mg g^{-1}.

Reagents:

a. Ethanol 80% (w/v)
b. Anthrone (0.2% in conc. H_2SO_4)
c. Standard Glucose.

Procedure:

Seed sample weighing 250 mg was homogenized in 10 ml of warm 80% ethanol and was centrifuged at 3000 rpm for 20 minutes. The supernatant was collected to a test tube and was evaporated to dryness on a water bath and was cooled to room temperature. Later, 1ml of distilled water was pipetted to each sample, shaking thoroughly and followed by the addition of 4 ml of Anthrone reagent. The mixture was again heated on a boiling water bath for 10 minutes and the test tubes were cooled under running water. The absorbance was immediately measured at 620 nm against reagent blank. A standard curve was drawn using graded concentrations of glucose and the amount of total carbohydrate was calculated using the following formula.

$$\text{Total carbohydrate} = \frac{\text{X mg} \times \text{V} \times 1000}{\text{Amount of aliquot pipetted} \times \text{W}}$$

Where, X= the amount of carbohydrate obtained from the standard curve

V= Volume of the sample taken

W= Weight of the seed sample taken

3.6.2 Total Protein

The total protein was estimated by the Lowry *et al* method (1951) and is expressed in mg g^{-1}.

Reagents:

a. Phosphate buffer (0.01 M, pH 7.6): A mixture of 16 ml of 0.2 M monobasic sodium phosphate and 84 ml of 0.2 M dibasic sodium phosphate was made up to 200 ml using DDW.
b. Reagent A: 2% Na_2CO_3 in 0.1 N NaOH in DDW.
c. Reagent B: 0.5% $CuSO_4.5H_2O$ in 1% sodium potassium tartarate in DDW.
d. Reagent C: Reagent A and reagent B mixed in the ratio of 50:1 just before use.
e. Reagent D: Folin- ciocalteau phenol and distilled water mixed to the ratio 1:2 prior to use.

Procedure:

Seed sample weighing 0.5 mg was homogenized using 10 ml of 0.01 M phosphate buffer and was centrifuged at 3000 rpm for 20 minutes. The supernatant was collected and made up to 25 ml using double distilled water in a volumetric flask. One ml of this solution is pipetted to a test tube. Also one ml of distilled water and 5 ml of reagent C was added and kept for 10 minutes undisturbed. 0.5 ml of reagent D is added and vortexed. The intensity of blue colour developed was read at 660 nm after 30 minutes in a spectrophotometer (Plate 4). The total protein content in the sample was estimated using the following formula.

$$\text{Total Protein} = \frac{\text{X mg} \times \text{V} \times 1000}{\text{Amount of aliquot pipetted} \times \text{W}}$$

Where, X= the amount of protein obtained from the standard curve

V= Volume of the sample taken

W= Weight of the seed sample taken

3.6.3 Crude Fat

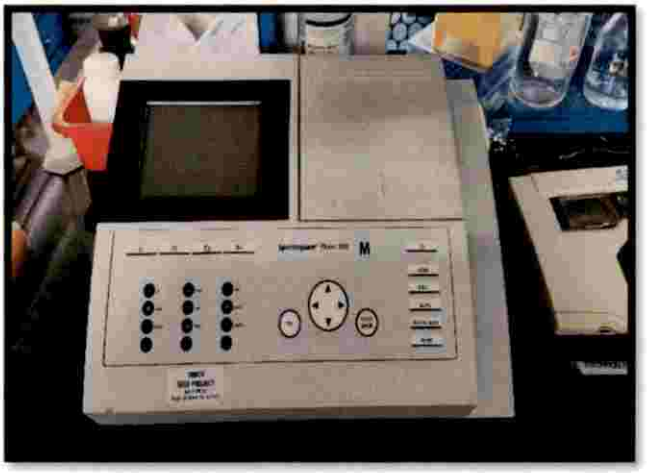
The crude fat content was estimated by Soxhlet method of extraction (Kennedy, 1949) and is expressed in %.

Reagent

1. Petroleum ether (60 – 80^0 C)

Plate 4. Instruments used to conduct biochemical analysis

4a. Centrifuge



4b. Spectrophotometer

4c. Soxhlet Apparatus

4d. SPAD 502 Plus Chlorophyll Meter

Procedure

Twenty gram of primed seed samples of each treatment with 3 replications were weighed and ground to a homogenous mixture using pestle and mortar. In order to facilitate fast and optimum extraction of the crude fat, the ground sample was packed between 2 cm thick cotton layers when transferred to the thimble. The pre dried extraction flask was connected beneath the thimble and was placed in the mantle. Further, to reduce the extraction time, 150 ml of the solvent was slowly poured in to the ground sample in thimble from top, until the complete solvent with extract reached the extraction flask. The thimble was then connected to the condenser. The temperature was adjusted to 70^0 C and the extraction was carried out for 2.5 h by the time which the colour of solvent became transparent. Once the extraction was completed, the thimble and solvent flask were detached from the apparatus and the content of the flask was emptied to a dry pre – weighed beaker. The excess solvent present in the extract was removed by keeping the beaker on a hot water bath until a constant weight was obtained. The beaker was then cooled and the final weight was recorded (Plate 4). The amount of crude fat was expressed in percentage. The crude fat in the given sample was calculated using the following formula:

Plate 5. Crude fat obtained from the seeds of sandal

$$\text{Crude fat (\%)} = \frac{W_2 - W_1}{\text{Weight of the sample}} \times 100$$

where, W_2 = Final weight of beaker with crude fat

W_1 = Initial weight of the beaker

3.7 Statistical Analysis

The statistical analysis was conducted using the IBM SPSS 25 software. One-way analysis of variance to understand the variation among treatments within same priming method, the significance of the study and the correlation between seed quality and seedling performance. Z-test was conducted to compare the growth of seedlings at 90^{th} and 180^{th} DAT in MS Excel. Pearson's correlation was also conducted to study the correlation between biochemical constituents of the seeds and the seedling growth.

Principal Component Analysis, a dimension reduction technique was conducted for the 12 seedling growth attributes of sandal. The principal component are extracted based on the Eigen value criteria (Eigen value >1). From the study, two components were extracted. The first component accounts for the 99 percentage of the variance and the second component accounts for what is left over. Hierarchical cluster analysis was carried out for the 39 treatments (including control) based on the Euclidean Distance.

RESULTS

RESULTS

The results of the study on the "Impact of seed priming techniques on the germination and seedling performances in sandal (*Santalum album*, L.)", are presented in this following sections. The chapter deals with the results of germination and seedling growth of sandal with respect to the seeds stored for one day. The seeds stored for one month failed to germinate and hence subsequent seedling growth was absent.

4.1 Seed characteristics of sandal

The seed characteristics such as dimensions, colour, individual seed weight and 1000 seed weight of the *Santalum album* procured from Marayoor Sandal Division, Kerala, used for the present investigation were examined. The sandal seeds were with brown hard seed coat and the average size ranged from of 0.7 to 0.8 cm. The seed diameter varied from 0.5 to 1 cm. The individual seed weight ranged from 0.15 to 0.27 g where the seed kernel weighed an average of 0.01 to 0.02 g. The 1000 seeds weighed 250 g.

4.2 Effect of various seed priming techniques on germination attributes of sandal

The effect of duration and concentration of various priming agents on the germination attributes of the sandal seeds compared with non-primed seeds are presented in the following sections.

4.2.1 Hydropriming

The variation in the germination attributes of the sandal seeds stored for one days after priming after hydropriming compared to control are presented in Table 2. Perusal of data reveals that hydropriming could not improve the germination percentage in sandal when compared to control or non-primed seeds. The highest germination (45.33 %) was obtained when the seeds were subjected to hydropriming for 3 days and the minimum was obtained in hydropriming 9 days (10.67%) under one day storage. However, the MDG, Peak Value and Germination value were higher compared to control indicating a faster and uniform germination.

The speed of germination of seeds hydroprimed for 3 days were approximately same as that of the non – primed seeds.

The MDG and PV of the seeds hydroprimed for three days were almost double compared to control seeds. The differences in germination among the treatments are highly significant ($F = 14.931$, $p = 0.001$). There was a decrease in the germination attributes of the sandal seeds with the increase in the priming duration. The germination percentage of the seeds subjected to priming 3 days and control were on par as well as the germination percentage of seeds subjected to hydropriming 9 days and 12 days were on par. Hence, the hydropriming of the seeds beyond 6 days is not beneficial.

4.2.2 Biopriming with *Pseudomonas fluorescens*

Biopriming with *Pseudomonas fluorescens* influenced the germination parameters of the sandal seeds (Table 3). The germination percentage seeds subjected to biopriming with different concentration and duration of biopriming varied significantly ($F = 5.08$, $p = 0.01$).

Results indicated that the germination percentage was highest (88%) in seeds subjected to biopriming with *P. fluorescens* at 100% for 8 days. Other biopriming concentration and duration also enhanced the germination percentage of the sandal seeds. The highest germination observed in this priming was on par with other treatments like seeds bioprimed at 25%, 50% and 75% for 4 days and 8 days; seeds bioprimed at 50%, 75% and 100% for 6 days and 25% and 50% for 2 days. The least germination rate was obtained from seeds bioprimed at 75% for 2 days, which even was significantly higher than the germination percentage of non-primed seeds (46.67%). Hence, the biopriming method have significant influence in the germination parameters.

The highest mean daily germination, PV, germination value and speed of germination was observed in seeds subjected to biopriming at 100% for 8 days. Although the mean daily germination was minimum in seeds bioprimed at 75% for 2 days, it had no significant differences with seeds bioprimed at 50% and 75% for 2 days as well as with seeds bioprimed at 25%, 50%, 75% and 100% for 6 days. The peak value of germination was highest in seeds bioprimed at 100% for 8 days followed by biopriming 50% for 8 days. The germination value and speed of germination were highest in seeds subjected to biopriming at100% for 8 days (18.54 and 12.19, respectively) whereas these parameters were the lowest in control (0.73 and 2.79, respectively). Although comparatively higher germination was observed in the seeds bioprimed at 25% and 75% (85.33%) for .8 days, the two treatments had a remarkably lower germination value (6.54 and 6.34) and germination speed (9.48 and 8.78).

Table 2: Effect of hydropriming on the germination attributes of sandal seeds

Hydropriming (days)	Days Taken to Initiate Germination	Days Taken to Complete Germination	Germination Percentage	Mean Daily Germination	Peak Value	Germination Value	Speed of germination
3	17	48	45.33 ± 2.00^{a}	1.53	1.57	0.85	2.36
6	18	57	30.00 ± 6.35^{b}	0.52	0.57	0.30	1.50
9	22	37	10.67 ± 0.66^{c}	0.28	0.28	0.08	0.54
12	13	59	11.33 ± 5.03^{c}	0.20	0.20	0.04	0.40
0 (Control)	13	56	46.00 ± 5.69^{a}	0.83	0.86	0.73	2.39
Values within the same column with similar superscripts are homogenous.							

Table 3: Effect of biopriming on the germination attributes of sandal seeds

Biopriming		Days Taken to Initiate Germination	Days Taken to Complete Germination	Germination Percentage	Mean Daily Germination	Peak Value	Germination Value	Speed of germination
Duration (days)	Concentration (%)							
2	25	10	31	81.33 ± 7.85^{ab}	2.63	2.73	7.21	7.10
	50	10	52	73.33 ± 6.65^{bc}	1.44	1.73	2.50	5.11
	75	10	54	66.67 ± 1.76^{c}	1.24	1.32	1.64	4.29
	100	9	42	70.67 ± 4.37^{bc}	1.65	1.97	3.33	5.91
4	25	7	36	82.67 ± 3.47^{ab}	2.34	2.99	7.03	9.17
	50	7	32	82.67 ± 1.15^{ab}	2.57	2.87	7.41	8.58
	75	8	37	83.33 ± 4.66^{ab}	2.12	2.73	5.81	8.22
	100	8	29	82.67 ± 1.76^{c}	2.12	2.89	6.15	8.24
6	25	8	50	$78.00 \pm 4.37b^{bc}$	1.54	2.33	3.61	6.67
	50	9	51	76.67 ± 1.15^{bc}	1.47	2.07	3.05	6.11
	75	7	48	78.00 ± 3.71^{b}	1.59	2.10	3.36	6.85
	100	7	41	78.67 ± 1.33^{b}	1.97	2.56	5.06	7.47
8	25	7	40	85.33 ± 10.72^{a}	2.19	2.96	6.54	9.48
	50	7	21	86.67 ± 5.29^{a}	3.45	3.91	13.53	11.26
	75	7	38	85.33 ± 2.40^{a}	2.25	2.80	6.34	8.78
	100	7	21	88.00 ± 2.90^{a}	4.27	4.33	18.54	12.19
Control		13	56	46.00 ± 5.69^{d}	0.83	0.86	0.73	2.39
Values within the same column with similar superscripts are homogenous.								

4.2.3 Osmopriming with Polyethylene Glycol 6000

The results of experiment on effect of osmopriming on the germination attributes of sandal seeds are presented in Table 4. Highly significant variation in the germination percentage of the sandal seeds ($F = 125.16$, $p = 0.01$) were observed due to duration and concentration of PEG soaking. The germination attributes of the seeds decreased with increasing concentration and duration of osmopriming.

Although the highest germination percentage was obtained in seeds primed with PEG at 5% (78%), there was no statistically significant variation in germination attributes till sixth day of soaking. With regard to Mean Daily Germination, the highest value was recorded for the seeds subjected to PEG priming at 15% concentration for 3 days and lowest value (0.18) was recorded for those treated for 12 days at 10 and 20% PEG. The higher germination value and speed of germination were also observed on osmopriming at 5% concentration for 3 days. The seeds subjected to different treatments under osmopriming with PEG 6000 for 9 days and 12 days showed significantly poor germination attributes when compared to control.

4.2.4 Chemical priming with $MnSO_4$

Table 5 depicts the effect of chemical priming ($MnSO_4$) on germination percentage, Mean Daily Germination, Peak Value, Germination Value and Speed of Germination in sandal seeds. The germination parameters of the seeds decreased with increasing concentration and duration of priming. The germination percentage of the sandal seeds showed highly significant variation due to treatments ($F = 209.82$, $p=0.01$). The highest germination was observed in the seeds subjected chemical priming for 3 days at 0.8 M concentration (88%) followed by those soaked at 0.4, 0.6 and 1.0 M concentrations for the same duration. The rest of the treatments could not initiate germination above the control and hence recorded lower germination attributes compared to control.

Among the 16 treatments belonging to chemical priming, only seeds exposed to priming with $MnSO_4$ at 0.4 M, 0.6 M, 0.8 M and 1 M for 3 days were found to have the highest germination percentage, MDG, PV, GV, and speed of germination. The highest MDG (3.35), PV (3.46) and PV (11.60) was observed in the seeds soaked in $MnSO_4$ 0.4 M solution for 3 days. Meanwhile,

Table 4. Effect of osmopriming on the germination attributes of sandal seeds

Osmopriming		Days Taken to Initiate Germination	Days Taken to Complete Germination	Germination Percentage	Mean Daily Germination	Peak Value	Germination Value	Speed of germination
Duration (days)	Concentration (%)							
3	5	11	44	78.00 ± 4.16[a]	1.77	2.05	3.64	5.76
	10	11	53	76.00 ± 2.40[a]	1.40	1.97	2.79	5.76
	15	11	60	78.00 ± 3.05[a]	1.30	1.71	2.37	4.98
	20	12	49	78.00 ± 2.00[a]	1.59	1.89	3.02	5.43
6	5	11	44	72.00 ± 4.80[a]	1.66	1.83	3.06	5.09
	10	13	49	70.67 ± 0.66[a]	1.44	1.77	2.55	4.67
	15	13	46	73.33 ± 1.33[a]	1.59	1.84	2.95	4.63
	20	15	54	71.33 ± 1.76[a]	1.32	1.64	2.18	4.30
9	5	28	60	22.67 ± 1.33[c]	0.35	0.38	0.14	0.87
	10	37	63	10.00 ± 2.30[de]	0.15	0.15	0.03	0.32
	15	38	52	3.33 ± 2.40[e]	0.06	0.04	0.00	0.12
	20	20	47	7.33 ± 1.76d[e]	0.15	0.15	0.02	0.32
12	5	40	62	14.67 ± 1.76[cd]	0.23	0.23	0.06	0.45
	10	43	62	13.33 ± 2.40[de]	0.18	0.18	0.03	0.34
	15	42	61	12.67 ± 1.76[d]	0.20	0.20	0.04	0.38
	20	47	62	11.33 ± 3.52[de]	0.18	0.17	0.03	0.32
Control		13	56	46.00 ± 5.69[b]	0.83	0.83	0.73	2.39
Values within the same column with similar superscripts are homogenous.								

Table.5: Effect of chemical priming on the germination attributes of sandal seeds

$MnSO_4$ Priming		Days Taken to Initiate Germination	Days Taken to Complete Germination	Germination Percentage	Mean Daily Germination	Peak Value	Germination Value	Speed of germination
Duration (days)	Concentration (M)							
3	0.4	9	26	86.67 ± 2.90^{a}	3.35	3.46	11.60	8.41
	0.6	9	40	86.67 ± 4.00^{a}	2.21	3.00	6.63	8.22
	0.8	9	42	88.00 ± 2.30^{a}	2.10	3.18	6.70	9.10
	1.0	9	37	86.00 ± 3.05^{a}	2.33	3.17	7.43	8.14
6	0.4	22	41	12.00 ± 3.05^{c}	0.29	0.32	0.10	0.64
	0.6	36	40	4.00 ± 1.33^{c}	0.06	0.05	0.00	0.08
	0.8	30	35	4.00 ± 2.3^{d}	0.11	0.10	0.01	0.11
	1.0	33	39	2.67 ± 1.15^{d}	0.10	0.08	0.01	0.19
9	0.4	22	44	16.00 ± 2.30^{d}	0.36	0.36	0.13	0.73
	0.6	36	42	2.00 ± 1.15^{c}	0.04	0.04	0.00	0.08
	0.8	44	49	1.33 ± 2.30^{d}	0.02	0.02	0.00	0.04
	1.0	49	58	1.33 ± 2.00^{d}	0.02	0.02	0.00	0.04
12	0.4	52	62	2.67 ± 0.66^{d}	0.04	0.04	0.00	0.07
	0.6	60	60	1.33 ± 1.33^{d}	0.02	0.02	0.00	0.03
	0.8	59	62	4.00 ± 1.76^{d}	0.06	0.06	0.00	0.10
	1.0	61	61	1.33 ± 0.66^{d}	0.02	0.02	0.00	0.03
Control		13	56	46.00 ± 5.69^{b}	0.83	0.86	0.73	2.39
Values within the same column with similar superscripts are homogenous.								

the speed of germination was the highest (9.1) in seeds primed in 0.8 M $MnSO_4$ for 3 days. The control seeds showed better germination parameters than seeds soaked for a longer duration above three days.

4.3 Effect of seed priming techniques on the electrical conductivity of seed leachates

The changes in electrical conductivity of seed leachates primed at different duration and concentration of priming agents are given in the following section.

4.3.1 Effect of hydropriming on the electrical conductivity of seeds leachates

The results of the effect of hydropriming on the electrical conductivity of seed leachates are presented in Table 6. The results revealed that there was a highly significant variation (F=10.12, p=0.01) of the electrical conductivity between the hydropriming treatments. The electrical conductivity was increasing with the increase in priming duration, indicating that hydropriming for longer durations are not suitable for improving the seed quality of sandal.

Table 6. Effect of hydropriming on the electrical conductivity of sandal seed leachates

Hydropriming (Days)	Electrical Conductivity ($dScm^{-1}$)
3	1.11 ± 0^{a}
6	1.53 ± 0^{b}
9	1.61 ± 0^{b}
12	1.96 ± 0^{c}
Values within the same column with similar superscripts are homogenous.	

4.3.2. Effect of biopriming on the electrical conductivity of seeds leachates

The results of the effect of duration and concentration of *Pseudomonas fluorescens* on the electrical conductivity of sandal seed leachates are presented in Table 7, which indicated that there was a highly significant variation among the treatments (F=25.24, p=0.01). The variation in the electrical conductivity of bioprimed seeds were found to be independent of biopriming treatments, whereas the electrical conductivity of the seeds were critically reduced when compared to the hydroprimed seeds indicating that biopriming is a better method to improve the seed quality of sandal.

Table 7. Effect of biopriming on the electrical conductivity of sandal seed leachates

Biopriming		Electrical Conductivity ($dScm^{-1}$)
Duration (Days)	Concentration (%)	
2	25	0.03 ± 0^{a}
	50	0.04 ± 0^{b}
	75	0.04 ± 0^{b}
	100	0.04 ± 0^{b}
4	25	0.04 ± 0^{b}
	50	0.04 ± 0^{b}
	75	0.03 ± 0^{a}
	100	0.03 ± 0^{a}
6	25	0.06 ± 0^{d}
	50	0.06 ± 0^{d}
	75	0.05 ± 0^{c}
	100	0.04 ± 0^{b}
8	25	0.05 ± 0^{c}
	50	0.04 ± 0^{b}
	75	0.04 ± 0^{b}
	100	0.03 ± 0^{a}
Values within the same column with similar superscripts are homogenous.		

4.3.3. Effect of osmopriming on the electrical conductivity of seeds leachates

The results of the effect of osmopriming on the electrical conductivity of sandal seed leachates are presented in Table 8, which indicated that there was a significant variation (F=33.36, p=0.04) among the treatments. However, the variation in electrical conductivity due to osmopriming treatments found to follow a different pattern from hydropriming as well as biopriming. The electrical conductivity of osmoprimed seeds was found inversely related to the concentration of Polyethylene Glycol 6000. Moreover, the values were found to be increasing with increase in priming duration. Hence, osmopriming at 20% of PEG 6000 for 3 days was the best treatment to improve seed quality in sandal.

4.3.4. Effect of chemical priming on the electrical conductivity of seeds leachates

The results of the effect of chemical priming on the electrical conductivity of sandal seed leachates are presented in Table 9, which indicated that there was a significant variation (F=26.43, p=0.03)

Table 8. Effect of osmopriming on the electrical conductivity of sandal seed leachates

Osmopriming		Electrical Conductivity ($dScm^{-1}$)
Duration (Days)	Concentration (%)	
3	5	1.02 ± 0^{ab}
	10	0.75 ± 0^{ab}
	15	0.62 ± 0^{a}
	20	0.46 ± 0^{a}
6	5	1.22 ± 0^{bc}
	10	1.00 ± 0^{bc}
	15	0.63 ± 0^{a}
	20	0.83 ± 0^{ab}
9	5	1.32 ± 0^{c}
	10	1.08 ± 0^{bc}
	15	0.93 ± 0^{bc}
	20	1.07 ± 0^{bc}
12	5	1.69 ± 0^{c}
	10	1.29 ± 0^{bc}
	15	1.16 ± 0^{bc}
	20	0.99 ± 0^{bc}
Values within the same column with similar superscripts are homogenous.		

Table 9. Effect of chemical priming on the electrical conductivity of sandal seed leachates

Chemical Priming		Electrical Conductivity ($dScm^{-1}$)
Duration (Days)	Concentration (M)	
3	0.4	0.65 ± 0^{cd}
	0.6	0.81 ± 0^{cd}
	0.8	0.55 ± 0^{bc}
	1	0.61 ± 0^{c}
6	0.4	0.58 ± 0^{b}
	0.6	0.88 ± 0^{d}
	0.8	0.71 ± 0^{cd}
	1	0.66 ± 0^{bc}
9	0.4	0.72 ± 0^{cd}
	0.6	0.98 ± 0^{d}
	0.8	0.59 ± 0^{b}
	1	0.90 ± 0^{a}
12	0.4	0.13 ± 0^{a}
	0.6	0.16 ± 0^{a}
	0.8	0.22 ± 0^{ab}
	1	0.14 ± 0^{a}
Values within the same column with similar superscripts are homogenous.		

among the treatments. Unlike the other priming methods, the variation in electrical conductivity due to chemical priming did not follow a definite trend. The electrical conductivity of the chemical primed seeds were least in the seeds primed for longer duration.

4.4 The effect of seed priming techniques on the biochemical composition of the sandal seeds

The effect of the duration and concentrations of the different priming agents on the seed reserve materials *viz*, total carbohydrate, protein and crude fat are given below.

4.4.1. The Effect of hydropriming on the biochemical content of the sandal seeds

The results of the effect of hydropriming on the biochemical character of the sandal seeds such as total carbohydrate, protein and crude fat are presented in Fig. 1-3. While the total carbohydrate ($F=414.88$, $p=0.01$) and crude fat ($F=972.91$, $p=0.01$) exhibited a highly significant difference among the treatments, the difference in the total protein ($F=6.74$, $p=0.03$) content among treatments was significant at 5% level only. Total carbohydrate content in the non-primed seeds were superior to the primed seeds. Besides the lower values of carbohydrate content shown by the hydroprimed seeds, it can be concluded that the carbohydrate content was inversely proportional to the priming duration.

On the contrary, the total protein content showed an increase with the increase in the priming duration. The total protein content of the non-primed seeds were found to be on par with that of the seeds hydroprimed for 6 days and 9 days. Meanwhile, the crude fat content was observed to be the highest in non-primed seeds. No significant increase in the crude fat was obtained with hydropriming in sandal seeds.

4.4.2. The Effect of biopriming on the biochemical composition of the sandal seeds

The results of the effect of biopriming on the total carbohydrate ($F=7.82$, $p=0.01$), protein ($F=5.46$, $p=0.01$) and crude fat content ($F=81.83$, $p=0.01$) are presented in Fig. 4-6. All the three components were observed to exhibit very high significant variation among the biopriming treatments. The results revealed that the biopriming of the sandal seeds had significantly increased the total carbohydrate content of the seeds when compared to control while the total carbohydrate content decreased with increasing priming duration.

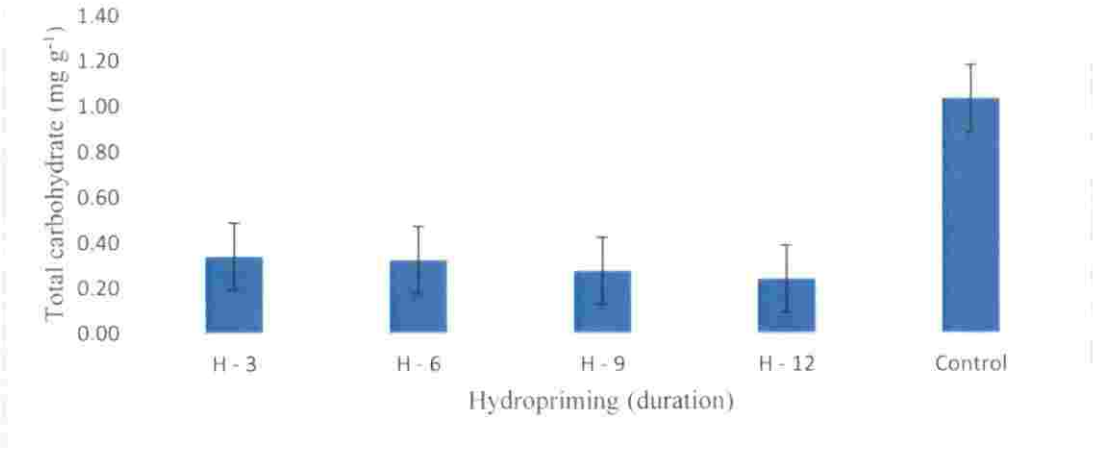


Figure 1. Effect of hydropriming on the total carbohydrate content of sandal seeds

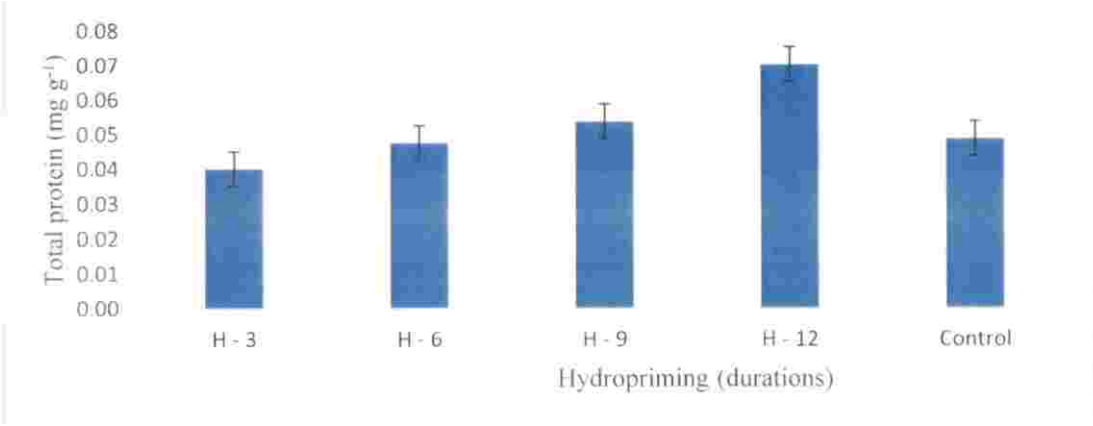


Figure 2. Effect of hydropriming on the total protein content of sandal seeds

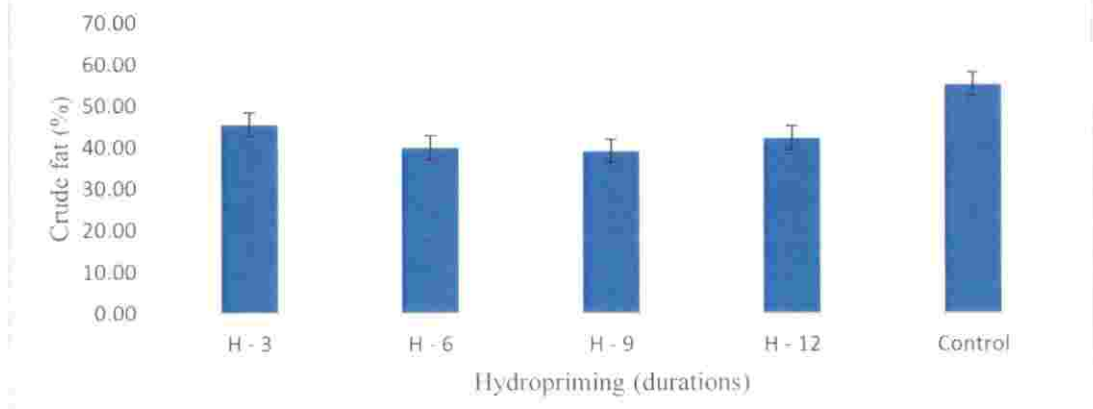


Figure 3. Effect of hydropriming on the crude fat content of sandal seeds

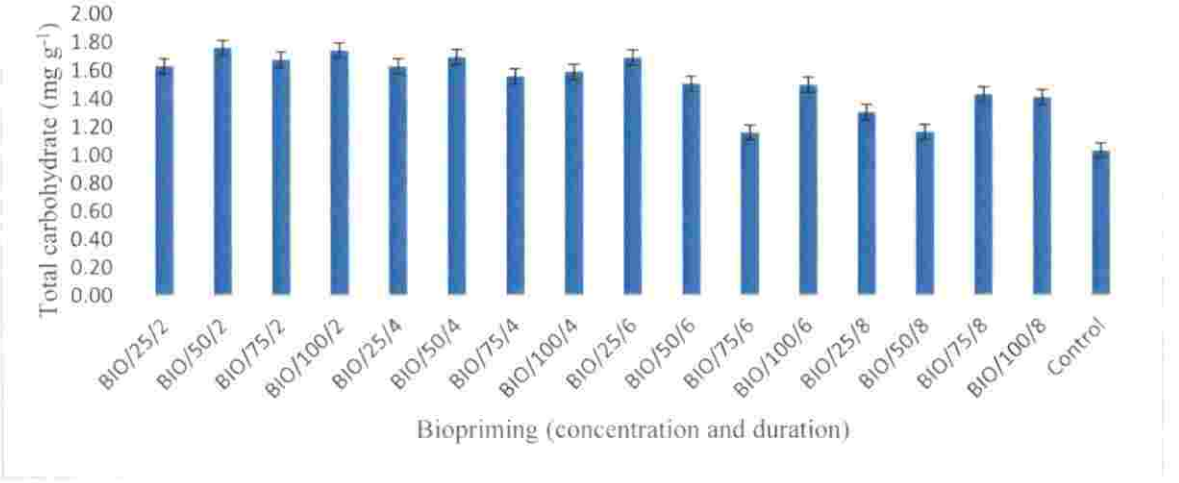


Figure 4. Effect of biopriming on the total carbohydrate content of sandal seeds

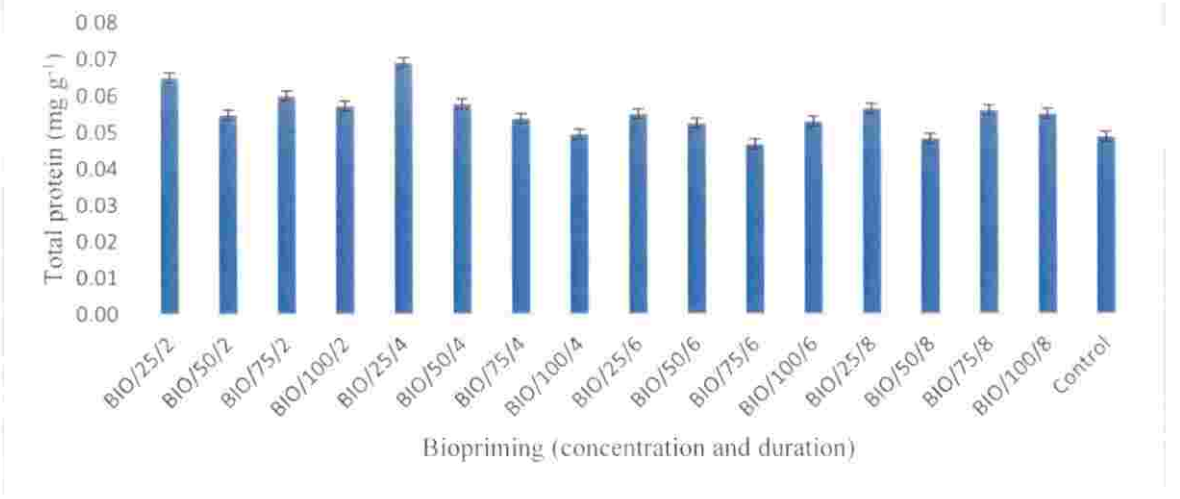


Figure 5. Effect of biopriming on the total protein content of sandal seeds

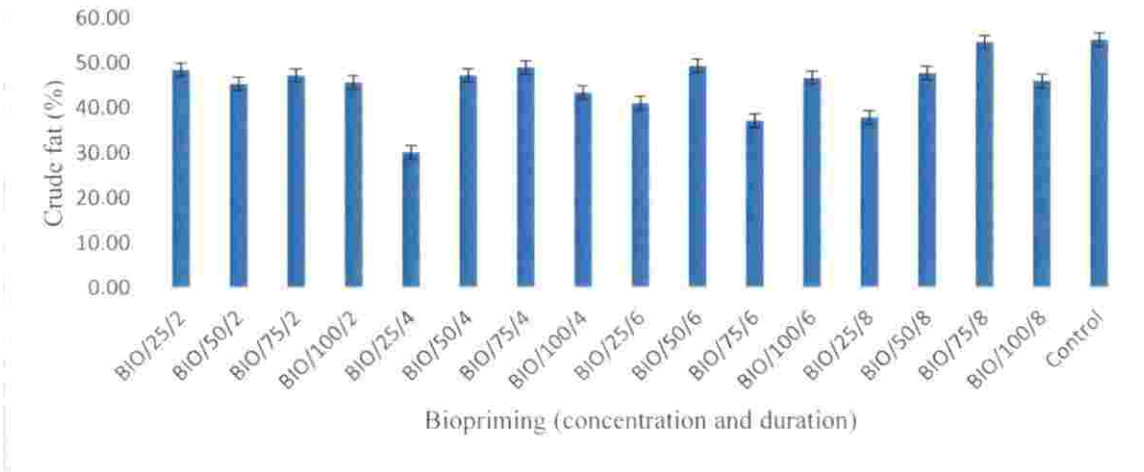


Figure 6. Effect of biopriming on the crude fat content of sandal seeds

The seeds bioprimed at 50% for 2 days recorded the highest total carbohydrate content (1.76 mg g^{-1}), whereas it recorded the least protein content (0.05 mg g^{-1}).

The total protein content was highest in the seeds primed at 25% for days (0.07 mg/g). In addition, the total protein content was independent of the priming treatments. The crude fat was also found to be independent of the biopriming treatments. The highest percentage of crude fat was obtained from seeds bioprimed at 75% for 6 days (57.01%) and the seeds bioprimed at 75% for 8 days recorded the lowest percentage of crude fat in sandal seeds (48.67%).

4.4.3. The Effect of osmopriming on the biochemical composition of the sandal seeds

The osmopriming treatments imparted highly significant difference in the total carbohydrate ($F=53.18$, $p=0.01$), protein ($F=2.80$, $p=0.01$) and crude fat ($F=149.78$, $p=0.01$) content of the sandal seeds due to different osmopriming treatments. According to the results from Figure 7-9, it is evident that although osmopriming induce an increase in the total carbohydrate content, a remarkable decrease in the total carbohydrate has been observed with increasing duration of the priming. The seeds osmoprimed at 5% for 3 days recorded the highest average value of total carbohydrate (1.90 mg g^{-1}), whereas the non-primed seeds recorded the lowest carbohydrate content (1.03 mg g^{-1}).

The total protein content of the seeds were independent of the osmopriming treatments. The different concentrations of Polyethylene glycol did not affect the total protein content of the

sandal seeds. In spite of the highly significant variation among the treatments, the total protein content of the non-primed seeds were found to be on par with that of the primed seeds. The percentage crude fat content in the seeds also shown results similar to that of the total protein when primed with different concentration of PEG 6000 at 3, 6, 9 and 12 days.

4.4.4. The effect of chemical priming on the biochemical composition of the sandal seeds

The effect of chemical priming on the total carbohydrate ($F=32.99$, $p=0.01$), protein ($F=1.32$, $p=0.87$) and crude fat ($F=81.33$, $p=0.01$) content of the sandal seedlings are presented in Fig. 10-12. The variation in the total carbohydrate content and the percentage crude

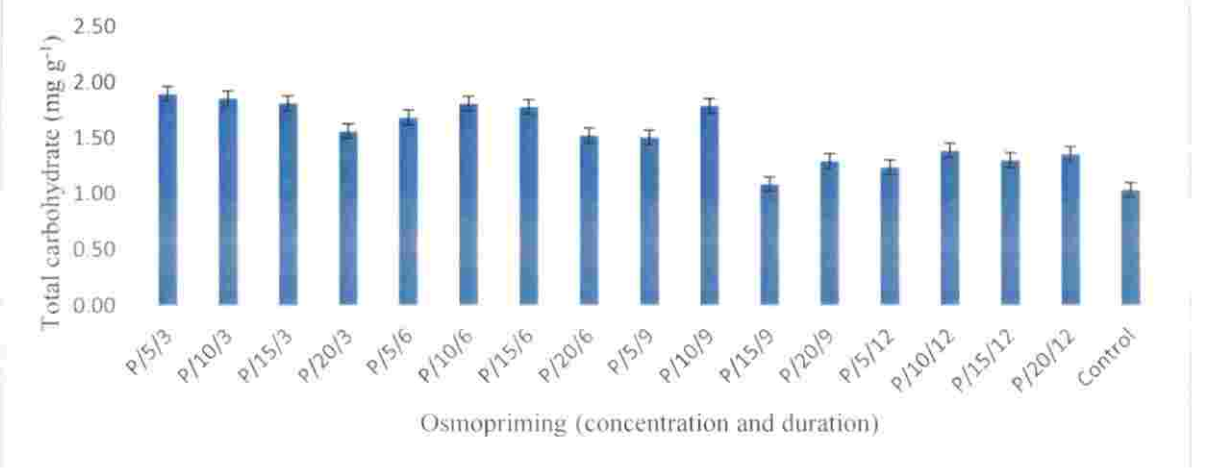


Figure 7. Effect of osmopriming on the total carbohydrate content of sandal seeds

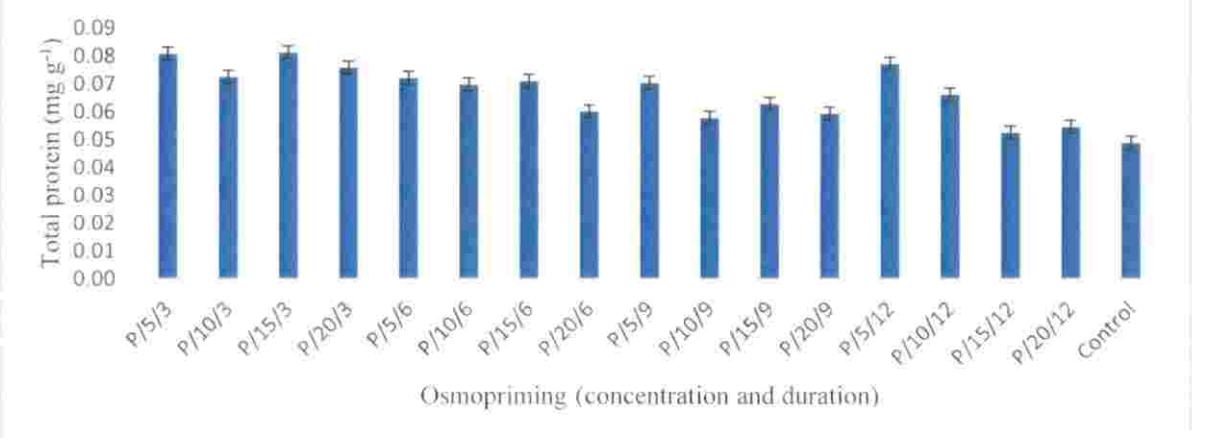


Figure 8. Effect of osmopriming on the total protein content of sandal seeds

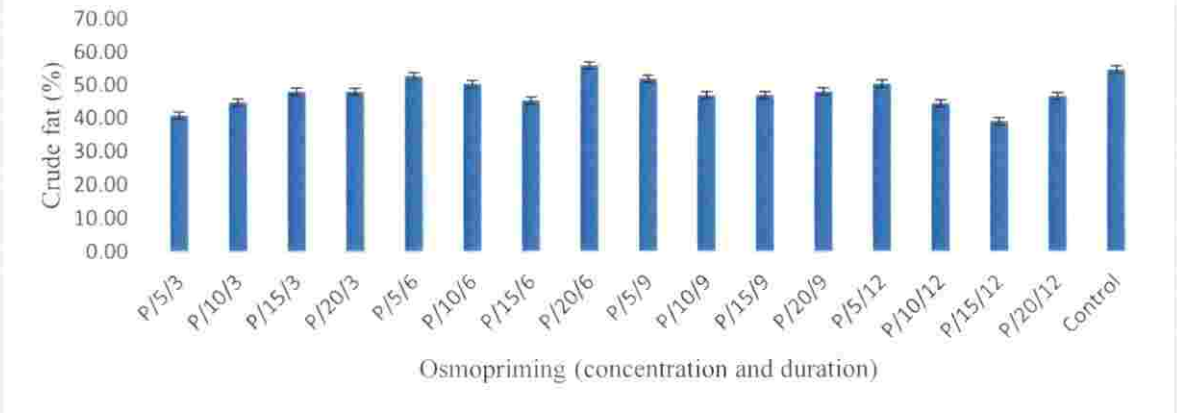


Figure 9. Effect of osmopriming on the crude fat content of sandal seeds

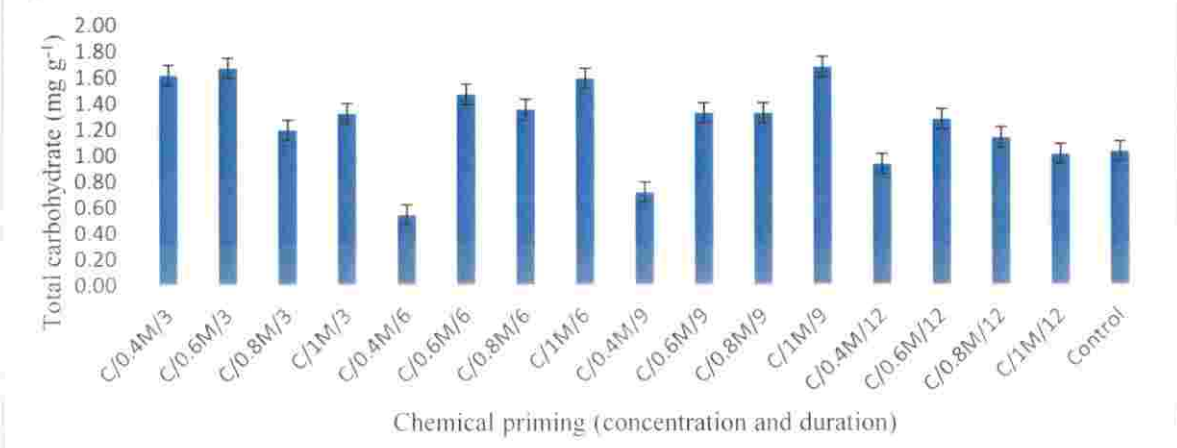


Figure 10. Effect of chemical priming on the total carbohydrate content of sandal seeds

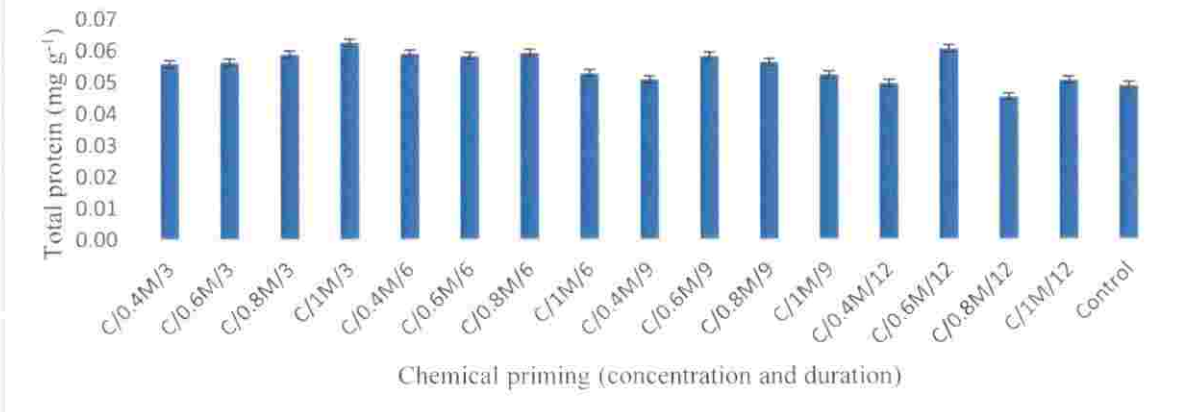


Figure 11. Effect of chemical priming on the total protein content of sandal seeds

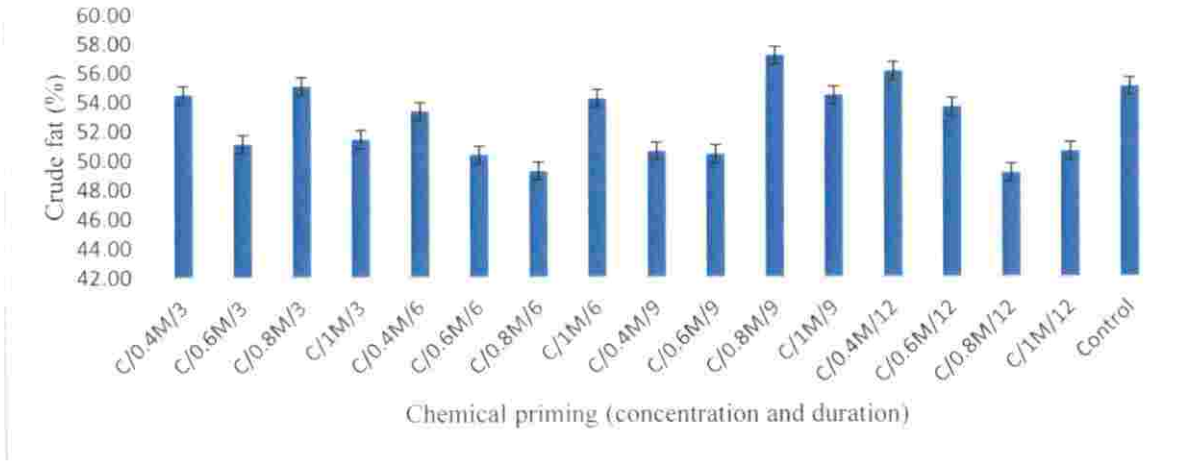


Figure 12. Effect of chemical priming on the crude fat content of sandal seeds

fat in the sandal seeds evinced highly significant variation whereas the total protein did not show any variation.

In accordance with the previous results of osmopriming, the total carbohydrate in the seed was inversely related to the priming duration. Meanwhile, against this assumption, the highest of the total carbohydrate was obtained from seeds primed at 1 M for 9 days (1.68 mg g^{-1}). The total carbohydrate content of the non-primed seeds were found to be higher over the seeds primed with $MnSO_4$ at 0.4 M for 6 days, 9 days and 12 days. The total protein content of the primed seeds and non-primed seeds were found to be on par. The percentage crude fat attained its highest value in seeds primed at 0.8 M for 9 days (57.01%) whereas the lowest value was recorded in seeds primed at 0.8 M for 12 days (48.67%).

4.5 Effect of priming methods on growth, biomass production, growth analysis indices and chlorophyll content of sandal seedlings

Seedling growth measured in terms of height, collar diameter, leaf area, tap root length, number of lateral roots, biomass yield and growth analysis indices and physiological characteristics as affected by the priming techniques are presented in the following sections. The survival rate of the germinated seeds were greatly differing in each priming method. Seedlings from all the treatments of hydropriming and biopriming were available for the study whereas

seedlings from only 6 treatments from osmopriming and 12 treatments from chemical priming were available for conducting the experiments. Seedlings subjected to 5, 10, 15 and 20% PEG 6000 for 3 days and 5 and 10% PEG 6000 in case of osmopriming and seedlings subjected to 0.4, 0.6, 0.8 and 1 M of $MnSO_4$ for 3, 6 and 9 days in case of chemical priming were only available to study the seedling growth attributes of sandal.

4.5.1. Effect of hydropriming on growth attributes and biomass production of sandal seedlings

The effect of hydropriming on the growth attributes and biomass production of sandal seedlings is depicted in the Tables 10 to 13. Analysis of variance revealed significant difference in shoot height of seedlings due to hydropriming at 90 ($F = 41.02$, $p=0.01$) and 180 days after transplanting ($F=230.56$, $p=0.01$). The observations revealed that the highest shoot height at third moth and sixth month were 15.80 cm and 20.85 cm respectively for the seedling obtained after hydropriming for 3 days. While the lowest value of the shoot height at 3rd month was observed in seedlings subjected to hydropriming for 9 days (7.26 cm), the seedlings subjected to hydropriming for 12 days recorded the lowest height during 6th month. The results indicated that hydropriming for 3 days could increase the shoot height whereas an increase in the duration of priming could lead to a decrease in shoot height. Further, the non-primed seeds were found to be superior over seeds hydroprimed for 6, 9 and 12 days in shoot height.

Analogous to the shoot height results, variations in collar girth of the seedlings among the treatments was highly significant at 90 ($F = 4.364$, $p = 0.02$) and 180 DAT ($F=90.26$, $p=0.01$). Although, the seeds hydroprimed for 9 days recorded the highest collar girth value at 90 DAT (2.49 mm), it was having the lowest value at 180 DAT (4.05 mm), whereas the seedlings from the non-primed seeds which recorded the minimal collar girth at 3rd month (1.79 mm) was found to be superior than hydropriming treatments at 6th month (6.33 mm). Collar girth of seedlings obtained after hydropriming for 3 days was 2.10 mm which was on par with that of seeds hydroprimed for 6 days and 9 days (Table 10). At 180 DAT the seedlings hydroprimed for 3, 6 and 9 days were at par in collar girth.

There was significant difference in the number of leaves of sandal seedlings due to hydropriming at 90 ($F = 14.77$, $p = 0.001$) and 180 DAT ($F=20.15$, $p=0.011$). The seedlings obtained from the seeds subjected to hydropriming for 6 days recorded the highest leaf number

(11.33) at 3rd month whereas the seeds subjected to hydropriming for 3 days recorded the highest leaf number at 6th month (20.50). Meanwhile, the value of number of leaves obtained from the seedlings from the seeds subjected to hydropriming for 6 (11.33) and 3 days (10.66) were at par at 3rd month. The number of leaves of the seedlings obtained after hydropriming for 9 (5.33) and 12 (6.00) days were lower compared to control seedlings (8.33) and there was no significant difference among the two treatments at 3rd month. At 180 DAT, the lowest leaf number was recorded in the seedlings germinated from seeds hydroprimed at 9 days (10.75). The number of leaves of seedlings hydroprimed at 9 and 12 days and 3 and 6 days were on par.

At 90 DAT leaf area of the seedlings obtained after hydropriming for different duration ranged from 1.05 cm^2 in hydropriming for 12 days to 7.01 cm^2 for 3 days. The variation in leaf area of the sandal seedlings due to different treatments were found to be highly significant ($F= 15.48$, $p = 0.001$). Similarly, high significant difference in the variations of leaf areas of sandal seedlings due to hydropriming ($F=14.73$, $p=0.001$) was observed at 180 DAT. The leaf area of the seedlings obtained after hydropriming for 3 days was found to be the highest at 90 DAT as well as 180 DAT (8.51cm^2) which in turn was on par with the leaf area of the non-primed seeds (6.95 cm^2). While, the leaf area of the seedling obtained after hydropriming for 9 days (2.02 cm^2) and 12 days (1.95 cm^2) were on par at 90 DAT. The leaf area of seedlings hydroprimed 6, 9 and 12 days were at par at 180 DAT. The hydropriming did not have any influence on the leaf area of the sandal seedlings compared to control.

The effect of hydropriming on the root parameters and total seedling length are presented in Table 11. With regards to the root length, there existed significant variation due to hydropriming at 90 ($F = 5.62$, $p = 0.01$) as well as 180 DAT ($F=80.92$, $p=0.01$). During the third month, the root length was the highest in seeds hydroprimed for 3 days (5.23 cm) and the lowest length was observed in seeds hydroprimed for 9 days (2.2 cm). The root length of the seedling obtained after hydropriming for 3 days was found to be on par with control seedlings, and the root length of the seedlings after hydropriming for 6 days was found to be on par with seeds hydroprimed for 12 days and control. Meanwhile, during 6th month the average root length was the highest in the non-primed seedlings (6.35 cm) and the seedlings hydroprimed at 9 days recorded lowest root length (2.32 cm) and the seedlings primed at 3, 6, 9 and 12 days were on par with respect to root length.

In context of the number of lateral roots of the seedling subjected to hydropriming, there was no significant difference at 90 (F = 3.06, p = 0.71) and 180 DAT (F=1.47, p=0.26). The observations at 90 DAT revealed that the number of lateral roots was higher in non-primed seeds (7) which were on par with those hydroprimed for 3 and 6 days. The number of lateral roots of the seedling obtained after hydropriming for different duration ranged from 3.33 to 4.43. Similarly, the seedlings from non-primed seeds recorded the highest number of lateral roots.

The differences in the total seedling length due to of hydropriming was highly significant at both 90 (F = 41.02, p=0.01) and 180 DAT (F=266.32, p=0.01). The total seedling length ranged from 21.03 to 9.66 cm in different priming durations at 90 DAT. The tallest seedlings (21.03 cm) were produced by the seeds subjected to hydropriming for 3 days and the shortest seedlings were obtained from those hydroprimed for 9 days. The control seedlings recorded the second tallest seedlings (18.26 cm). There was decrease in the total seedling length with the increase in the

Table 10. Effect of hydropriming on the shoot growth attributes of sandal seedlings at 90 and 180 days after transplanting

Hydropriming (Days)	Shoot height (cm)		Collar Girth (mm)		Leaf number		Leaf Area (cm^2)	
	90 DAT	180 DAT	90 DAT	180 DAT	90 DAT	180 DAT	90 DAT	180 DAT
3	15.8 ± 0.91^{a}	20.85 ± 0.26^{a}	2.10 ± 0.13^{abc}	4.14 ± 0.10^{c}	10.66 ± 1.15^{a}	20.50 ± 0.65^{a}	7.01 ± 1.14^{a}	8.51 ± 0.68^{a}
6	12.53 ± 0.75^{b}	16.12 ± 0.36^{c}	2.49 ± 0.38^{a}	4.82 ± 0.12^{b}	11.33 ± 1.52^{a}	18.50 ± 1.55^{a}	4.39 ± 1.56^{b}	5.57 ± 0.63^{b}
9	7.46 ± 1.20^{c}	11.92 ± 0.35^{d}	2.23 ± 0.21^{ab}	4.05 ± 0.08^{c}	5.33 ± 0.57^{c}	10.75 ± 0.85^{b}	2.02 ± 0.04^{c}	4.78 ± 0.23^{b}
12	8.63 ± 0.85^{c}	10.52 ± 0.22^{e}	2.08 ± 0.11^{bc}	4.08 ± 0.02^{c}	6.00 ± 1.00^{c}	12.00 ± 0.71^{b}	1.95 ± 0.19^{c}	4.51 ± 0.24^{b}
0 (Control)	14.16 ± 1.04^{ab}	18.50 ± 0.41^{b}	1.79 ± 0.03^{c}	6.33 ± 0.14^{a}	8.33 ± 1.52^{b}	13.00 ± 1.41^{b}	6.04 ± 1.14^{ab}	8.44 ± 0.58^{a}
Values within the same column with similar superscripts are homogenous.								

Table 11. Effect of hydropriming on the root growth attributes and total seedling length of sandal seedlings at 90 and 180 days after transplanting

Hydropriming (Days)	Number of Lateral Roots		Root Length (cm)		Total Seedling Length	
	90 DAT	180 DAT	90 DAT	180 DAT	90 DAT	180 DAT
3	5.23 ± 0.20^{a}	2.00 ± 0.41	4.43 ± 1.52^{ab}	3.02 ± 0.35^{b}	21.03 ± 0.85^{a}	23.87 ± 0.35^{a}
6	3.23 ± 1.53^{bc}	2.00 ± 0.41	4.66 ± 1.15^{ab}	2.92 ± 0.10^{b}	15.76 ± 1.61^{c}	19.05 ± 0.40^{b}
9	2.2 ± 0.6^{c}	2.75 ± 0.75	3.33 ± 1.52^{b}	2.32 ± 0.11^{c}	9.66 ± 0.60^{d}	14.25 ± 0.36^{c}
12	3.2 ± 0.26^{bc}	2.00 ± 0.41	3.66 ± 0.57^{b}	2.57 ± 0.05^{bc}	11.83 ± 0.94^{d}	13.1 ± 0.25^{d}
0 (Control)	4.1 ± 0.78^{ab}	3.25 ± 0.25	7.00 ± 2.00^{a}	6.35 ± 0.13^{a}	18.26 ± 1.80^{b}	24.85 ± 0.26^{a}
Values within the same column with similar superscripts are homogenous.						

priming duration. Contrary to the results obtained at 3rd month, the seedling length was the highest in non-primed seeds at 6th month after transplanting (24.85 cm).

The variation in fresh weight of leaf, shoot and root of the sandal seedlings due to hydropriming are presented in Table 12. Analysis of variance revealed a significant difference at five per cent level in the leaf fresh weight of seedlings from seeds subjected to different hydropriming treatments at 90 DAT ($F = 3.09$, $p = 0.03$) and at one per cent significant level at 180 DAT ($F=64.41$, $p=0.01$). At 90 days after transplanting, fresh weight of the leaves was highest in the seedling belonging to the seeds hydroprimed for 3 days (0.29 g) which was on par with that of seeds hydroprimed for 9 days (0.25 g), 12 days (0.27 g) and control (0.27 g). The average leaf weight was found to be highest in seedlings that were not primed (0.85 g), although seedlings hydroprimed at 6 days (0.82 g) were on-par with non-primed seedlings at 180 DAT. The results revealed that the fresh weight of leaves tend to decrease with increase in the priming duration.

Meanwhile, there was no significant difference in the fresh weight of the shoot of the sandal seedlings due to priming duration at 90 ($F = 1.76$, $p = 0.21$) but the shoot weight recorded a highly significant variation at 180 DAT ($F=231.60$, $p=0.001$). The fresh weight of the shoot ranged from 0.22 g to 0.16 g in seedlings subjected to hydropriming at 90 DAT. The shoot weight also showed that non-primed seedlings had the highest shoot weight (0.57 g) with most of the primed seedlings having similar on-par values of shoot weight at 180 DAT. Similarly, there was no significant difference in the root fresh weight of the seedlings due to hydropriming at 90 DAT ($F = 0.008$, $p = 1.00$) whereas the variation was of high significance at 180 DAT ($F=51.24$, $p=0.01$).The seedlings hydroprimed at 6 days as well as the non-primed seeds recorded the highest average root weight (0.40 g), while the seedlings hydroprimed at 3 days and 6 days showed lowest values of average root weight and those were at-par.

The total fresh weight of the seedlings recorded a non-significant variation due to hydropriming treatments at 90 DAT ($F=0.56$, $p=0.44$) whereas the variation was highly significant at 180 DAT ($F=48.04$, $p=0.01$). The total fresh weight of the seedlings subjected to hydropriming treatments as well as control was on par when observed at 90 days after transplanting.

Meanwhile, the observations at 180 DAT recorded the highest seedling fresh weight in seeds of control (1.82 g) which was on par with total fresh weight of seedlings hydroprimed for 6 days (1.52 g). The fresh weight of the seedlings hydroprimed for 3 days and 9 days were on par.

The biomass production (on dry weight basis) of the seedlings obtained after priming treatments varied significantly (Table 13). Results indicated that, there was no significant variation in leaf dry weight of sandal seedling due to hydropriming treatments ($F = 1.57$, $p = 0.25$) at 90 DAT. The leaf biomass of the seedlings ranged from 0.07 to 0.04 g. The highest values were obtained for the seedling obtained after hydropriming for 3 days followed by the control. Significant difference in shoot dry weight due to different hydropriming treatments was obtained ($F = 3.95$, $p = 0.03$) at 180 days after transplanting. The maximum shoot dry weight was recorded in the seedling obtained after hydropriming for 3 days (0.08 g) followed by hydropriming for 6 days (0.06 g). The dry weight of shoot was found be on par for the seedlings belonging to hydropriming for 9, 12 days and control.

The dry weight of the roots also did not show any significant difference ($F = 0.67$, $p = 0.66$) at 90 days after transplanting. The root dry weight of the seedlings ranged from 0.05 g to 0.06 g and the highest values were recorded for the seedling that obtained after hydropriming for 3 days followed by control. There was no significant difference observed in the seedling dry weight of sandal seedlings due to hydropriming ($F=2.54$, $p=0.10$) at 90 days after transplanting. The seedlings from seeds hydroprimed for 3 days possessed maximum dry weight (0.20 g) which was on par with the seedling dry weight of non-primed seeds (0.17 g), where the latter was found to be on par with the dry seedlings of seeds hydroprimed for 6 days, 9 days and 12 days.

Contrary to the results obtained at 90 DAT, the variations in leaf ($F=56.405$, $p=0.01$), shoot ($F=17.90$ $p=0.01$), root ($F=13.02$ $p=0.01$) and plant dry weight ($F=61.66$ $p=0.01$) at 180 DAT were highly significant. It is evident from the values that the non-primed seeds recorded the highest average leaf dry weight (0.26 g), shoot dry weight (0.20 g), root dry weight (0.15 g) and the plant dry weight (0.60 g). The seedlings hydroprimed at 6, 9 and 12 days showed on-par values of shoot and total dry weight. The seedlings hydroprimed at 3 days had on-par values with the non-primed seedlings with respect to shoot dry weight.

Table 12. Effect of hydropriming on the fresh weight of sandal seedlings at 90 and 180 days after transplanting

Hydropriming (Days)	Fresh Weight (g)							
	Leaf		Shoot		Root		Seedling	
	90 DAT	180 DAT	90 DAT	180 DAT	90 DAT	180 DAT	90 DAT	180 DAT
3	0.29 ± 0.00^{a}	0.55 ± 0.02^{b}	0.20 ± 0.02	0.36 ± 0.01^{b}	0.1 ± 0.02	0.26 ± 0.01^{c}	0.59 ± 0.04	1.17 ± 0.04^{b}
6	0.22 ± 0.01^{b}	0.82 ± 0.02^{a}	0.18 ± 0.01	0.30 ± 0.00^{c}	0.1 ± 0.02	0.40 ± 0.01^{a}	0.50 ± 0.03	1.52 ± 0.03^{a}
9	0.25 ± 0.01^{ab}	0.36 ± 0.01^{c}	0.21 ± 0.01	0.28 ± 0.00^{c}	0.1 ± 0.01	0.36 ± 0.01^{b}	0.56 ± 0.02	1.00 ± 0.02^{b}
12	0.27 ± 0.02^{a}	0.35 ± 0.01^{c}	0.22 ± 0.01	0.30 ± 0.01^{c}	0.1 ± 0.01	0.27 ± 0.01^{c}	0.59 ± 0.03	0.92 ± 0.03^{c}
0 (Control)	0.27 ± 0.00^{a}	0.85 ± 0.06^{a}	0.16 ± 0.00	0.57 ± 0.01^{a}	0.09 ± 0.01	0.40 ± 0.01^{a}	0.53 ± 0.02	1.82 ± 0.02^{a}
Values within the same column with similar superscripts are homogenous.								

Table 13. Effect of hydropriming on the dry weight of sandal seedlings at 90 and 180 days after transplanting

Hydropriming (Days)	Dry Weight (g)							
	Leaf		Shoot		Root		Seedling	
	90 DAT	180 DAT	90 DAT	180 DAT	90 DAT	180 DAT	90 DAT	180 DAT
3	0.07 ± 0	0.19 ± 0^{b}	0.08 ± 0^{a}	0.17 ± 0.02^{a}	0.05 ± 0.01	0.15 ± 0.01^{a}	0.20 ± 0.02^{a}	0.51 ± 0.02^{b}
6	0.05 ± 0	0.13 ± 0.01^{c}	0.06 ± 0^{ab}	0.07 ± 0.02^{b}	0.04 ± 0.01	0.08 ± 0.02^{b}	0.15 ± 0.00^{b}	0.28 ± 0.03^{c}
9	0.06 ± 0	0.1 ± 0.00^{d}	0.04 ± 0^{b}	0.09 ± 0.00^{b}	0.04 ± 0.00	0.06 ± 0.02^{bc}	0.15 ± 0.01^{b}	0.25 ± 0.02^{c}
12	0.04 ± 0	0.12 ± 0.01^{cd}	0.05 ± 0.01^{b}	0.08 ± 0.01^{b}	0.04 ± 0.01	0.03 ± 0.01^{c}	0.14 ± 0.02^{b}	0.23 ± 0.01^{c}
0 (Control)	0.06 ± 0.01	0.26 ± 0.01^{a}	0.05 ± 0.00^{b}	0.20 ± 0.01^{a}	0.06 ± 0.00	0.15 ± 0.01^{a}	0.17 ± 0.00^{ab}	0.60 ± 0.01^{a}
Values within the same column with similar superscripts are homogenous.								

4.5.2 Effect of hydropriming on growth analysis indices

The effect of hydropriming on the growth analysis indices like Specific Leaf Area, Specific Leaf weight, Leaf Area Ratio, Leaf Weight Ratio, Root: Shoot Ratio, Vigour Index I and Vigour Index II, Net Assimilation Rate and Relative Growth Rate is given in the Tables 14 to 17.

During the third month after planting, there was significant difference in the specific leaf area due to different hydropriming treatments ($F = 3.45$, $p = 0.05$) whereas the variation was not significant during sixth month ($F = 1.63$, $p = 0.21$). The highest specific leaf area was shown by control seedlings which was on par with those obtained from the seeds hydroprimed for 3 days, 6 days and 9 days at 90 DAT. It can be observed that at 180 DAT, the seedlings hydroprimed at 9 days have the highest specific leaf area value ($48.14 cm^2 g^{-1}$), which is on par with the other hydroprimed seedlings as well as the non-primed seedlings.

The specific leaf weight of the seedlings also recorded the significant differences due to hydropriming treatments at 90 DAT ($F= 3.80$, $p = 0.04$) and a non-significant variation at 180 DAT ($F=0.69$, $p=0.61$). During 3rd month a higher specific leaf weight was recorded in seedlings obtained from the seeds hydroprimed for 12 days ($0.02 gcm^{-2}$). However, the values were on par with seeds hydroprimed for 3 days, 6 days, 9 days and non-primed seeds ($0.01 gcm^{-2}$). The specific leaf weight values of all the hydroprimed seedlings and non-primed seedlings are almost similar and also on-par with each other with seedlings hydroprimed at 12 days and non-primed seedlings recording highest values ($0.03 g cm^{-2}$).

There was significant difference in the leaf area ratio of the seedlings subjected to hydropriming for different duration and at 90 DAT ($F=4.26$, $p=0.02$) and a non-significant variation was observed at 180 DAT ($F=1.52$, $p=0.25$). The highest leaf area ratio was observed in the seedlings obtained from the seeds primed for 3 days (34.31) which was on par with control seedlings (34.67). There was a decrease in the leaf area ratio of the seedlings with increase in priming duration during 3rd month. Meanwhile, during 6th month the leaf area ratio in hydroprimed seedlings and non-primed seedlings having on-par values. Although the highest leaf area ratio value was observed in seedlings hydroprimed at 6 days (20.44).

Leaf weight ratio of the seedlings varied significantly due to hydropriming treatments at 90 (F = 0.35, p = 0.83) as well as 180 DAT (F=4.25, p=0.17).The highest leaf weight ratio was observed in the seedlings obtained from the seeds hydroprimed for 9 days (0.39) followed by control (0.37) seedlings at 90 DAT whereas, the results at 180 DAT indicated that the values of seedlings hydroprimed at 6 days and 12 days were observed to be on-par, with highest values being observed 12 days hydroprimed seedlings (0.52). The seedlings hydroprimed at 3 days recorded the lowest leaf weight ratio (0.36).

The effect of hydropriming on the root: shoot ratio at 90 DAT and 180 DAT are given in Table 15. During 3rd month, the root: shoot ratio of the seedlings showed no significant variation due to hydropriming treatments (F=2.78, p=0.87). The maximum value for the root shoot ratio was obtained from non-primed seeds (1.20) which were found to be on par with seeds hydroprimed for 9 days and 12 days. Although the values of root: shoot ratio recorded non-significant variation, contrary to the results of 3rd month, the values of root: shoot ratio were the highest in the seedlings hydroprimed for 6 days (2.24). The values of all hydroprimed seedlings as well as non-primed seedlings were observed to be on-par (F=1.12, p=0.24) at 6th month.

Vigour index I showed significant variation due to hydropriming treatments (F = 430.31, p=0.01). The vigour index of the non-primed seeds was found to be higher over the hydropriming treatments. The vigour index II (F= 31.39, p=0.01) also showed significant difference in the value due to hydropriming treatments. The maximum value was observed in seeds hydroprimed for 3 days whereas the minimum value was observed in seeds hydroprimed for 9 days and 12 days (Table 16).

The absolute growth rate did not show statistically significant variation among the treatments. Similarly, the relative growth rate also showed similar values in both hydroprimed and non-primed seedlings even though seeds hydroprimed at 3 days and non-primed seedlings had slightly higher but identical values. The values of net assimilation rate also showed that seedlings hydroprimed at 3 days and non-primed seedlings had highest and identical values (0.03) with seeds hydroprimed at 9 days and 2 days recording lowest values (0.01) and being on-par (Table 17).

Table 14. Effect of hydropriming on the growth indices of sandal seedlings at 90 and 180 days after transplanting

Hydropriming (Days)	Specific Leaf Area (cm^2g^{-1})		Specific Leaf Weight (gcm^{-2})		Leaf Area Ratio (cm^2g^{-1})		Leaf Weight Ratio	
	90 DAT	180 DAT	90 DAT	180 DAT	90 DAT	180 DAT	90 DAT	180 DAT
3	96.29 ± 20.95^{a}	45.57 ± 4.02	0.01 ± 0^{b}	0.02 ± 0	34.31 ± 6.57^{a}	16.80 ± 1.94^{a}	0.36 ± 0.02	0.36 ± 0.02
6	80.41 ± 8.01^{ab}	44.35 ± 7.02	0.01 ± 0^{b}	0.02 ± 0	28.83 ± 6.42^{ab}	20.44 ± 3.14^{a}	0.35 ± 0.04	0.46 ± 0.03
9	34.44 ± 3.55^{b}	48.14 ± 3.55	0.02 ± 0^{a}	0.02 ± 0	13.37 ± 0.96^{b}	19.60 ± 2.57^{a}	0.39 ± 0.04	0.40 ± 0.03
12	49.36 ± 10.71^{ab}	38.66 ± 4.87	0.02 ± 0^{ab}	0.03 ± 0	14.86 ± 3.51^{b}	19.77 ± 1.59^{a}	0.31 ± 0.05	0.52 ± 0.04
0 (Control)	101.06 ± 24.67^{a}	33.09 ± 3.15	0.01 ± 0^{b}	0.03 ± 0	34.67 ± 5.40^{a}	14.03 ± 0.97^{a}	0.37 ± 0.07	0.43 ± 0.01
Values within the same column with similar superscripts are homogenous.								

Table 15. The Effect of hydropriming on the root: shoot ratio of the sandal seedlings at 90 and 180 days after transplanting

Hydropriming (Days)	Root : Shoot Ratio	
	90 DAT	180 DAT
3	0.70 ± 0.11	0.92 ± 0.15
6	0.66 ± 0.16	2.24 ± 1.48
9	0.98 ± 0.13	0.76 ± 0.29
12	0.91 ± 0.17	0.35 ± 0.13
0 (Control)	1.20 ± 0.02	0.75 ± 0.03
Values within the same column with similar superscripts are homogenous.		

Table 16. Effect of hydropriming on the vigour indices of the sandal seedlings

Hydropriming (days)	Vigor Index I	Vigor Index II
3	953.51 ± 22.26^{a}	0.09 ± 0.09^{a}
6	473.00 ± 28.00^{b}	0.04 ± 0.04^{b}
9	103.14 ± 3.71^{c}	0.01 ± 0.01^{c}
12	134.07 ± 6.18^{c}	0.01 ± 0.01^{c}
0 (Control)	840.26 ± 47.82^{a}	0.08 ± 0.08^{a}
Values within the same column with similar superscripts are homogenous.		

Table 17. Effect of hydropriming on the growth analysis indices of the sandal seedlings

Hydropriming (days)	Absolute Growth Rate (cm day^{-1})	Relative Growth Rate ($g\ g^{-1}\ day^{-1}$)	Net Assimilation Rate ($g\ cm^{-2}\ day^{-1}$)
3	0.003 ± 0	0.010 ± 0	0.028 ± 0^{ab}
6	0.000 ± 0	0.001 ± 0	0.014 ± 0^{bc}
9	0.001 ± 0	0.001 ± 0	0.011 ± 0^{c}
12	0.002 ± 0	0.002 ± 0	0.020 ± 0^{c}
0 (Control)	0.004 ± 0	0.014 ± 0	0.039 ± 0.00^{a}
Values within the same column with similar superscripts are homogenous.			

4.5.3. Effect of hydropriming on the chlorophyll content of the sandal seedlings.

The results of variation in chlorophyll content at 180 DAT also showed high significant difference in hydroprimed seeds (F=240.25, p=0.01). Contrary to the previous results of leaf area and leaf number, the average chlorophyll content was lowest in seeds hydroprimed at 3 days (16.90 mg g^{-1}) and the highest chlorophyll content was recorded in the non-primed seeds (26.90 mg g^{-1}). The only treatments that were on par were the seeds hydroprimed at 9 days and 12 days (Table 18).

Table 18. Effect of hydropriming on the chlorophyll content of sandal seedlings

Hydropriming (days)	Chlorophyll (mg g^{-1})
3	16.90 ± 0.15^{d}
6	21.23 ± 0.17^{b}
9	18.20 ± 0.20^{c}
12	17.50 ± 0.28^{cd}
0 (Control)	26.90 ± 0.72^{a}
Values within the same column with similar superscripts are homogenous.	

4.5.4. Effect of biopriming on growth attributes and biomass production of sandal seedlings

Impact of biopriming at different duration and concentration on the shoot parameters of sandal seedlings are presented in Table 19. The results indicated that there was no significant difference in heights of seedlings due to biopriming treatments at 90 DAT (F = 1.39, p = 0.20) but highly significant difference was observed at 180 DAT (F=10.15, p=0.01). At 90 days after transplanting, the shoot height of the seedlings varied from 12.83 cm in seeds bioprimed at100%

for 2 days to 18.26 cm in seeds bioprimed at 75% for 8 days. The shoot height of all treatments except seeds bioprimed at 50% for 6 days and seeds bioprimed at 50%, 75% and 100% for 2 days were found to be at par. During 6th month, seeds bioprimed at 100% for 8 days recorded the highest shoot height (24.75 cm) followed by seeds bioprimed at 50% for 8 days (24.03 cm) and the seeds bioprimed at 50% for 2 days recorded the lowest length (16.75 cm). The shoot height of the non-primed seeds were found to be higher than the shoot height of the seeds bioprimed at 50% and 100% for 2 days as well as over the seeds bioprimed at 25% for 4 days whereas the shoot height of non-primed seeds were found to be on par with the shoot height of the seeds bioprimed at 100% for 8 days. In general, most of the biopriming treatments recorded a higher shoot height over the non-primed seeds at 180 days after transplanting.

The variations in collar girth due to different biopriming treatments were found to be highly significant at 90 DAT ($F = 11.20$, $p=0.01$) as well as at 180 DAT ($F=10.15$, $p=0.01$). At 90 DAT, the highest of collar girth was recorded in seedlings produced from seeds bioprimed at 50% for 8 days and was found to be on par with seeds bioprimed at 25% and 75% for 8 days and seeds bioprimed at 50% for 2 days, whereas the minimum collar girth recorded from seeds bioprimed at 25% for 4 days. The lowest collar girth in this treatment was on par with non-primed seeds as well as the seeds bioprimed at 50% and 100% for 4 days and seeds bioprimed at different concentrations for 6 days. Meanwhile, at 180 DAT the highest value of collar girth was again recorded in seeds bioprimed at 100% for 8 days (9.28 mm), further recording highest values of root length (8.35 cm) as well as the total seedling length (33.10 cm). In general, biopriming treatments could impart collar girth values greater than that of the non-primed seeds.

Analysis of variance revealed significant e effect of biopriming treatments on the leaf area at 90 DAT ($F = 2.12$, $p = 0.02$) and 180 DAT ($F=10.15$, $p=0.01$). During the observations recorded at 90 DAT, the highest leaf area was recorded in seeds subjected to biopriming at 100% for 6 days (9.98 cm^2) and the least in seeds bioprimed at 50% for 4 days (1.31 cm^2). All treatments, except biopriming at 100% for 6 days and biopriming at 50% for 4 days, were not statistically different. The variation in leaf area, although highly significant, followed a different trend at 180 DAT. The leaf area of the seedlings due to biopriming treatments with *P. fluorescens* varied from 3.70 cm^2 in seeds subjected to biopriming at 50% for 4 days to a significantly higher value of 13.28 cm^2 in seeds subjected to biopriming at 50% for 8 days where the latter was found to be on par with the

seeds subjected to biopriming at 100% for 6 days and seeds bioprimed at 75% and 100% for 8 days. The seeds subjected bioprimed at all concentrations of *P. fluorescens* for 2 days, seeds bioprimed at 25%, 75% and 100% for 4 days, seeds primed at 25% and 50% for 6 days and seeds bioprimed at 25% for 8 days were found to be on par with that of the non-primed seeds.

The variation in leaf number among treatments was highly significant at 90 (F = 2.84, p=0.01) as well as 180 DAT (F=2.83, p=0.01). At 90 DAT, the maximum number of leaves were produced by seeds bioprimed at 100% for 2 days (14) and the minimum number of leaves were produced by seeds bioprimed at 75% for 6 days (7.33) except which the seeds of all treatments produced greater number of leaves compared to non-primed seeds (8.33). Seeds bioprimed at 50%, 75% and 100% for 2 days, 25% and 75% for 4 days, 25% and 100% for 6 days and 25%, 50%, 75% and 100% for 8 days were statistically not different. The number of leaves per seedlings ranged from 21 numbers in seeds bioprimed at 25% for 6 days to 13 numbers in non-primed seeds at 180 DAT. All the biopriming treatments were performed superior over control with regard to leaf number and the seeds subjected to biopriming treatments at all concentration of *P. fluorescens* for 2, 4 and 8 days and seeds subjected to biopriming for 25%, 50% and 75% for 6 days were on par to each other.

The root length (F = 3.49, p = 0.01) and number of lateral roots (F = 5.71, p = 0.01) produced by the seedlings at 90 DAT showed highly significant variation due to biopriming (Table 20). Highest root length was recorded in seeds bioprimed at 50% for 8 days while the shortest root length was recorded by seeds bioprimed at 25% for 4 days. Seeds bioprimed at all concentrations for 2 days and 8 days, seeds bioprimed at 50% for 4 days and seeds bioprimed at 75% for 6 days recorded root lengths higher than non-primed seeds, however the values were at par. The number of lateral roots were found to be higher in non-primed seeds (7.33) and the lowest number of lateral roots were recorded in seeds bioprimed at 100% for 6 days. The root parameters did not follow the similar pattern during 6th month after transplanting. At 180 DAT, the root length recorded highly significant variation (F=10.15, p=0.01) while the number of lateral roots recorded a non-significant variation (F=10.15, p=0.10). The number of lateral roots produced by the biopriming treatments were on par with control.

Table 19. Effect of biopriming on the shoot growth attributes of sandal seedlings at 90 and 180 days after transplanting

Biopriming		Shoot Height (cm)		Collar Girth (mm)		Number of Leaves		Leaf Area (cm^2)	
Duration (Days)	Concentration (%)	90 DAT	180 DAT	90 DAT	180 DAT	90 DAT	180 DAT	90 DAT	180 DAT
2	25	16.33 ± 1.30	21.48 ± 0.19^{cd}	2.58 ± 0.07^{bcd}	7.44 ± 0.06^{d}	10.33 ± 1.20^{bcd}	20.50 ± 0.65^{abc}	7.08 ± 1.44^{ab}	10.36 ± 1.16^{bcd}
	50	13.9 ± 0.25	16.75 ± 0.38^{h}	3.01 ± 0.10^{ab}	7.64 ± 0.13^{d}	11.33 ± 0.66^{abc}	20.75 ± 1.11^{ab}	7.73 ± 1.72^{ab}	10.49 ± 1.09^{bcd}
	75	14.93 ± 0.52	19.65 ± 0.25^{ef}	2.57 ± 0.20^{bcd}	7.53 ± 0.06^{d}	11.66 ± 0.33^{abc}	19.00 ± 0.82^{abcde}	5.52 ± 1.38^{abc}	9.06 ± 0.54^{cdef}
	100	12.83 ± 0.44	16.93 ± 0.15^{h}	2.79 ± 0.15^{abc}	7.48 ± 0.16^{d}	14.00 ± 2.08^{a}	16.50 ± 1.19^{bcdef}	5.56 ± 0.24^{abc}	8.38 ± 0.31^{def}
4	25	15.73 ± 1.53	17.53 ± 0.71^{h}	1.72 ± 0.27^{g}	8.13 ± 0.09^{bc}	9.00 ± 0.57^{cd}	16.00 ± 0.41^{def}	6.41 ± 1.85^{ab}	9.02 ± 0.54^{def}
	50	13.66 ± 0.44	22.85 ± 0.29^{bc}	1.73 ± 0.08^{g}	8.14 ± 0.05^{bc}	9.66 ± 1.20^{bcd}	20.25 ± 0.85^{abcd}	1.31 ± 0.33^{e}	3.70 ± 0.29^{h}
	75	13.16 ± 0.44	21.80 ± 0.18^{c}	2.42 ± 0.13^{cde}	8.29 ± 0.07^{bc}	13.00 ± 1.52^{ab}	17.00 ± 0.71^{abcdef}	6.54 ± 0.93^{ab}	8.22 ± 0.61^{def}
	100	15.70 ± 0.25	19.33 ± 0.22^{fg}	1.91 ± 0.07^{fg}	8.09 ± 0.12^{bc}	13.00 ± 1.52^{ab}	16.50 ± 1.76^{bcdef}	3.87 ± 0.97^{bc}	6.60 ± 0.32^{fg}
6	25	15.76 ± 1.08	20.63 ± 0.15^{de}	2.2 ± 0.13^{defg}	8.15 ± 0.02^{bc}	10.00 ± 0.57^{bcd}	21.00 ± 0.82^{a}	5.78 ± 1.67^{abc}	8.32 ± 0.42^{def}
	50	16.36 ± 0.34	20.50 ± 0.31^{de}	2.03 ± 0.13^{efg}	8.22 ± 0.06^{bc}	10.00 ± 0^{bcd}	16.25 ± 1.11^{cdef}	3.62 ± 0.19^{bc}	7.45 ± 0.24^{efg}
	75	15.06 ± 0.24	22.90±0.09^{b}	2.05 ± 0.15^{efg}	8.36 ± 0.09^{b}	7.00 ± 0.57^{d}	17.50 ± 3.20^{abcde}	3.24 ± 0.91^{bc}	5.78 ± 0.32^{gh}
	100	14.63 ± 0.69	23.00 ± 0.45^{b}	2.07 ± 0.18^{efg}	8.27 ± 0.12^{bc}	11.66 ± 1.66^{abc}	15.25 ± 0.63^{f}	9.98 ± 1.85^{a}	12.32 ± 0.95^{ab}
8	25	15.86 ± 0.54	22.13 ± 0.34^{bc}	3.06 ± 0.09^{a}	8.09 ± 0.12^{bc}	12.00 ± 1.52^{abc}	16.00 ± 0.41^{def}	6.66 ± 1.38^{ab}	9.86 ± 0.68^{cde}
	50	17.56 ± 0.17	24.03 ± 0.17^{a}	3.08 ± 0.09^{a}	8.27 ± 0.12^{bc}	11.66 ± 0.33^{abc}	16.75 ± 1.31^{abcdef}	5.15 ± 1.43^{bc}	13.28 ± 1.44^{a}
	75	18.26 ± 0.46	21.73 ± 0.59^{c}	3.02 ± 0.16^{ab}	7.99 ± 0.06^{c}	11.66 ± 0.33^{abc}	17.25 ± 1.75^{abcdef}	6.97 ± 2.10^{ab}	12.42 ± 1.17^{ab}
	100	17.50 ± 0.47	24.75 ± 0.12^{a}	2.35 ± 0.17^{cdef}	9.28 ± 0.09^{a}	9.33 ± 0.66^{bcd}	17.25 ± 1.49^{abcdef}	7.79 ±1.75^{ab}	11.23 ±0.87^{abc}
	Control	14.16 ± 1.04	18.50 ± 0.20^{g}	1.79 ± 0.03^{g}	6.34 ± 0.14^{e}	8.33 ± 1.52^{cd}	13.00 ± 0.71^{f}	6.04 ± 1.14^{ab}	8.44 ± 0.58^{def}
Values within the same column with similar superscripts are homogenous.									

Table 20. Effect of biopriming on the root growth attributes and seedling length of sandal seedlings at 90 and 180 days after transplanting

Biopriming		Root Length (cm)		Number of Lateral Roots		Total Seedling Length	
Duration (Days)	Concentration (%)	90 DAT	180 DAT	90 DAT	180 DAT	90 DAT	180 DAT
2	25	5.33 ± 0.33^{abcd}	5.28 ± 0.18^{g}	3.33 ± 0.33^{de}	3.50 ± 0.65	19.66 ± 1.36^{abc}	26.75 ± 0.13^{h}
	50	6.33 ± 0.88^{abc}	4.98 ± 0.06^{g}	4.40 ± 0.58^{de}	2.75 ± 0.48	18.30 ± 0.7^{bcd}	21.73 ± 0.34^{k}
	75	5.00 ± 0.57^{abcde}	5.07 ± 0.15^{f}	3.03 ± 0.88^{cde}	2.50 ± 0.29	17.96 ± 1.33^{bcd}	25.35 ± 0.13^{i}
	100	5.66 ± 1.20^{abcd}	5.08 ± 0.39^{g}	2.90 ± 0.66^{d}	1.75 ± 0.75	15.73 ± 1.07^{d}	22.00 ± 0.44^{k}
4	25	2.00 ± 0.00^{f}	5.88 ± 0.09^{f}	4.70 ± 0.98^{abc}	3.50 ± 0.65	20.43 ± 2.00^{ab}	23.40 ± 0.73^{j}
	50	2.33 ± 0.33^{ef}	7.05 ± 0.06^{d}	4.73 ± 0.39^{a}	3.00 ± 0.00	18.40 ± 0.80^{bcd}	29.90 ± 0.24^{cde}
	75	5.33 ± 0.33^{abcd}	7.25 ± 0.13^{d}	4.03 ± 0.32^{e}	2.75 ± 0.48	17.20 ± 0.52^{cde}	29.05 ± 0.27^{e}
	100	3.00 ± 0.57^{def}	7.10 ± 0.09^{d}	3.83 ± 0.44^{bcd}	2.50 ± 0.65	19.53 ± 0.49^{abc}	26.43 ± 0.14^{h}
6	25	3.66 ± 0.33^{cdef}	7.20 ± 0.11^{d}	3.73 ± 0.14^{cde}	3.25 ± 0.48	19.50 ± 1.15^{abc}	27.83 ± 0.13^{f}
	50	4.33 ± 1.45^{bcdef}	7.18 ± 0.08^{d}	3.60 ± 0.73^{cde}	4.00 ± 0.91	19.96 ± 0.88^{abc}	27.68 ± 0.33^{fg}
	75	4.33 ± 0.33^{bcdef}	7.40 ± 0.09^{cd}	2.75 ± 0.43^{cde}	3.25 ± 0.48	17.81 ± 0.30^{bcd}	30.30 ± 0.06^{cd}
	100	5.00 ± 1.52^{abcde}	7.73 ± 0.18^{bc}	2.50 ± 0.06^{cde}	2.00 ± 0.41	17.20 ± 0.75^{cde}	30.73 ± 0.40^{bc}
8	25	6.66 ± 1.20^{ab}	7.20 ± 0.18^{d}	4.20 ± 0.95^{ab}	2.25 ± 0.48	20.06 ± 0.46^{abc}	29.33 ± 0.44^{de}
	50	7.33 ± 0.66^{a}	7.35 ± 0.06^{cd}	3.30 ± 0.36056^{ab}	2.25 ± 0.48	20.86 ± 0.32^{ab}	31.38 ± 0.23^{b}
	75	6.00 ± 0.57^{abc}	8.13 ± 0.09^{ab}	4.30 ± 0.65^{a}	3.25 ± 0.75	22.56 ± 1.04^{a}	29.85 ± 0.59^{cde}
	100	6.33 ± 0.88^{abc}	8.35 ± 0.09^{a}	3.06 ± 0.34^{cde}	3.00 ± 0.82	20.56 ± 0.76^{ab}	33.10 ± 0.18^{a}
	Control	4.10 ± 0.78^{ab}	6.35 ± 0.13^{e}	7.00 ± 2.00^{ab}	3.25 ± 0.25	18.26 ± 1.80^{e}	24.85 ± 0.26^{i}
Values within the same column with similar superscripts are homogenous.							

The total seedling length showed highly significant variation due to priming at 90 (F = 26.79, p=0.01) and 180 DAT (F=10.15, p=0.01). The longest seedlings were obtained from seeds bioprimed at 75% for 6 days (22.56 cm) whereas, the shortest seedling were obtained from seeds bioprimed at 100% for 2 days (15.73 cm). Among the treatments, seeds bioprimed at different concentration for 8 days produced the tallest seedlings at 90 DAT. During 6th month, the seeds subjected to biopriming at 100% for 8 days produced the tallest seedlings (33.10 cm) indicating that the seeds subjected to biopriming for a longer durations produce higher seedling length.

Table 21 depicts the results of the effect of biopriming treatments on the fresh weight of sandal seedlings. Perusal of fresh weight of sandal seedlings at 90 DAT indicated that the weight of the leaves (F = 83.59, p=0.01), shoot (F = 9.29, p=0.01) and root (F = 132.89, p=0.01) exhibited highly significant variation due to biopriming treatments. Among the different biopriming treatments the seeds primed at 25% and 50% for 2 days, 25%, 50%, 75% and 100% for 8 days recorded significantly higher fresh leaf weight over control, whereas the remaining treatments recorded lower leaf weight than non-primed seeds, however the values of the latter were found to be on par with control. The maximum shoot weight was produced by seeds bioprimed at 25% for 2 days (0.27 g) followed by seeds bioprimed at 100% for 8 days (0.26 g) and the minimum fresh weight of shoot was produced by non-primed seeds (0.16 g). The highest root weight was recorded in seeds bioprimed at 25% for 2 days and the lowest root weight was recorded in seeds bioprimed at 25% for 4 days. The observations at 90 DAT indicated that the biopriming treatments for longer duration resulted in higher leaf fresh weight whereas the seeds bioprimed for short durations recorded higher shoot and root weight. During 90 DAT, the total fresh weight of the seedling revealed a highly significant variation (F=10.084, p=0.01) among treatments with highest fresh weight recorded in seedlings bioprimed at 25% for 2 days and the lowest being recorded in seedlings from seeds subjected to biopriming at 25% for 4 days. It can be concluded from the results that biopriming for 2 days significantly increased the fresh weight of the seedlings while the priming duration followed an inverse relationship with fresh weight at longer duration viz., 4 and 6 days which was further found to have impart an increase in the fresh weight of the seedlings.

The results of the effect of biopriming on the fresh weight of shoot (F=35.71, p=0.01) and roots (F=14.38, p=0.01) were significant at 180 DAT while the fresh weight of leaf (F=1.00, p=0.46) and total fresh weight (F=0.99, p=0.48) were not significant. During 6th month, the highest

leaf fresh weight (0.92 g) was recorded in seeds bioprimed at 75% for 6 days which was on par with seeds bioprimed at seeds bioprimed at 25% and 100% for 6 days, seeds bioprimed at 100% for 8 days and the non-primed seeds. The shoot weight of the seedlings recorded the highest value on biopriming at 75% for 6 days while the rest of the biopriming treatments recorded shoot weight lower than that of the control. Meanwhile, the root weight of the sandal seedlings on priming at 75% for 4 days and 25%, 50% and 100% for 8 days were at par in spite of the slightly higher value of root weight recorded by the seeds bioprimed at 100% for 8 days. The total fresh weight of the sandal seedlings at 180 DAT due to biopriming revealed a non-significant variation. It was also found that the biopriming treatments could not impart an increase in the fresh weight of the seedlings compared to control and the values of the each bioprimed seedlings were on par with the non-primed seeds, although the highest value was obtained from the seeds bioprimed at 75% for 6 days (1.89 g).

The results of the effect of biopriming on seedling dry weight presented in Table 22. The leaf ($F=85.67$, $p=0.01$), shoot ($F=41.05$, $p=0.01$), root ($F=13.21$, $p=0.01$) and seedling dry weight ($F=377.09$, $p=0.01$) of sandal showed highly significant variation due to biopriming treatments at 90 DAT. Leaf dry weight ranged from 0.03 g in seeds bioprimed at 75% for 2 days to 0.1 g in seeds bioprimed at 50% for 2 days. A similar trend was observed while recording the shoot dry weight of the seedlings while the root dry weight ranged from 0.03 g in seed bioprimed at 25% and 100% for 4 days to a maximum of 0.08 g in seeds bioprimed at 100% for 8 days. The seedling dry weight was highest (0.24 g) in seeds bioprimed at 50% for 2 days as well as the seeds bioprimed at 100% for 8 days. The seeds bioprimed at 75% for 2 days, 25 and 100% for 4 days recorded seedling dry weight lower than the non-primed seeds but the values were on par.

The effect of biopriming on the dry weight of leaf ($F=42.69$, $p=0.01$), shoot ($F=4.99$, $p=0.01$), root ($F=5.21$, $p=0.01$) and the total dry weight ($F=28.04$, $p=0.01$) were highly significant at 180 DAT. It was evident that seeds bioprimed at 100% for 8 days had produced a higher dry weight compared to the non-primed seeds. Although, each biopriming treatments except seeds bioprimed at all concentration of *P. fluorescens* for 2 days recorded a higher seedling dry weight compared to non-primed seedling, the seeds bioprimed at 5% and 100% for 2 days

Table 21. Effect of biopriming on the fresh weight of sandal seedlings at 90 and 180 days after transplanting

Biopriming		Fresh Weight (g)							
		Leaf		Shoot		Root		Total	
Duration (Days)	Concentration (%)	90 DAT	180 DAT	90 DAT	180 DAT	90 DAT	180 DAT	90 DAT	180 DAT
2	25	0.30 ± 0.03^{a}	0.42 ± 0.01	0.27 ± 0^{a}	0.37 ± 0.01^{fg}	0.27 ± 0.03^{a}	0.28 ± 0.01^{d}	0.84 ± 0.04^{a}	1.06 ± 0.02
	50	0.35 ± 0.03^{a}	0.53 ± 0.01	0.23 ± 0.02^{abc}	0.37 ± 0.01^{fg}	0.19 ± 0.02^{b}	0.30 ± 0.01^{d}	0.77 ± 0.02^{ab}	1.18 ± 0.02
	75	0.24 ± 0.02^{b}	0.45 ± 0.01	0.18 ± 0.02^{cd}	0.38 ± 0.01^{efg}	0.14 ± 0.00^{b}	0.40 ± 0.01^{a}	0.54 ± 0.06^{def}	1.23 ± 0.02
	100	0.29 ± 0.02^{b}	0.41 ± 0.01	0.19 ± 0.02^{cd}	0.37 ± 0.01^{fg}	0.16 ± 0.02^{b}	0.35 ± 0.01^{c}	0.64 ± 0.04^{cde}	1.11 ± 0.01
4	25	0.17 ± 0.03^{b}	0.38 ± 0.01	0.17 ± 0.02^{d}	0.46 ± 0.01^{d}	0.07 ± 0.00^{b}	0.36 ± 0.01^{bc}	0.41 ± 0.04^{g}	1.19 ± 0.01
	50	0.20 ± 0.01^{b}	0.52 ± 0.01	0.17 ± 0.00^{d}	0.51 ± 0.01^{c}	0.10 ± 0.00^{b}	0.39 ± 0.00^{ab}	0.48 ± 0.03^{fg}	1.41 ± 0.01
	75	0.26 ± 0.04^{b}	0.60 ± 0.01	0.22 ± 0.03^{bcd}	0.35 ± 0.01^{g}	0.09 ± 0.02^{b}	0.40 ± 0.01^{a}	$0.57 \pm 0.0.04^{def}$	1.35 ± 0.02
	100	0.25 ± 0.03^{b}	0.63 ± 0.01	0.18 ± 0.00^{cd}	0.40 ± 0.01^{f}	0.11 ± 0.01^{b}	0.36 ± 0.01^{bc}	0.54 ± 0.02^{efg}	1.38 ± 0.02
6	25	0.2 ± 0.04^{b}	0.86 ± 0.01	0.19 ± 0.00^{cd}	0.36 ± 0.01^{fg}	0.11 ± 0.00^{b}	0.35 ± 0.01^{c}	0.50 ± 0.02^{efg}	1.56 ± 0.01
	50	0.2 ± 0.04^{b}	0.70 ± 0.02	0.18 ± 0.00^{cd}	0.34 ± 0.01^{g}	0.09 ± 0.00^{b}	0.37 ± 0.01^{bc}	0.53 ± 0.02^{efg}	1.82 ± 0.01
	75	0.23 ± 0.01^{b}	0.92 ± 0.01	0.18 ± 0.01^{cd}	0.62 ± 0.01^{a}	0.11 ± 0.00^{b}	0.36 ± 0.01^{bc}	0.52 ± 0.04^{efg}	1.89 ± 0.03
	100	0.24 ± 0.02^{b}	0.83 ± 0.02	0.19 ± 0.01^{cd}	0.37 ± 0.01^{fg}	0.10 ± 0.00^{b}	0.36 ± 0.01^{bc}	0.53 ± 0.03^{efg}	1.56 ± 0.03
8	25	0.34 ± 0^{a}	0.67 ± 0.01	0.20 ± 0.00^{cd}	0.36 ± 0.01^{fg}	0.24 ± 0.00^{a}	0.40 ± 0.01^{a}	0.79 ± 0.03^{ab}	1.43 ± 0.03
	50	0.31 ± 0.02^{a}	0.62 ± 0.01	0.22 ± 0.01^{bcd}	0.42 ± 0.04^{de}	0.23 ± 0.01^{a}	0.40 ± 0.04^{a}	0.76 ± 0.04^{abc}	1.44 ± 0.02
	75	0.33 ± 0.02^{a}	0.42 ± 0.01	0.19 ± 0.01^{cd}	0.35 ± 0.01^{g}	0.23 ± 0.02^{a}	0.36 ± 0.01^{bc}	0.76 ± 0.05^{abc}	1.13 ± 0.00
	100	0.28 ± 0.01^{b}	0.88 ± 0.01	0.26 ± 0.00^{ab}	0.46 ± 0.01^{d}	0.12 ± 0.01^{b}	0.42 ± 0.01^{a}	0.67 ± 0.02^{bcd}	1.75 ± 0.01
	Control	0.27 ± 0.00^{a}	0.85 ± 0.06	0.16 ± 0.00^{e}	0.57 ± 0.01^{b}	0.09 ± 0.01^{b}	0.40 ± 0.01^{a}	0.53 ± 0.02^{efg}	1.82 ± 0.01
Values within the same column with similar superscripts are homogenous.									

Table 22. Effect of biopriming on the dry weight of sandal seedlings at 90 and 180 days after transplanting

Biopriming		Dry Weight (g)							
		Leaf		Shoot		Root		Total	
Duration (Days)	Concentration (%)	90 DAT	180 DAT	90 DAT	180 DAT	90 DAT	180 DAT	90 DAT	180 DAT
2	25	0.09 ± 0.03^{a}	0.19 ± 0.01^{l}	0.07 ± 0.01^{a}	0.17 ± 0.01^{def}	0.06 ± 0.01^{b}	0.13 ± 0.01^{g}	0.23 ± 0.02^{a}	0.48 ± 0.01^{i}
	50	0.10 ± 0.01^{a}	0.21 ± 0.01^{kl}	0.07 ± 0.00^{a}	0.17 ± 0.01^{def}	0.06 ± 0.01^{b}	0.13 ± 0.01^{g}	0.24 ± 0.00^{a}	0.51 ± 0.02^{hi}
	75	0.03 ± 0.02^{b}	0.23 ± 0.01^{k}	0.05 ± 0.01^{b}	0.17 ± 0.01^{def}	0.04 ± 0.00^{b}	0.17 ± 0.02^{cdefg}	0.13 ± 0.02^{b}	0.57 ± 0.03^{gh}
	100	0.07 ± 0.01^{b}	0.25 ± 0.02^{jk}	0.06 ± 0.01^{b}	0.19 ± 0.02^{cde}	0.05 ± 0.00^{b}	0.16 ± 0.02^{edf}	0.19 ± 0.02^{b}	0.59 ± 0.04^{fg}
4	25	0.05 ± 0.02^{b}	0.28 ± 0.00^{fgh}	0.05 ± 0.00^{b}	0.19 ± 0.01^{cde}	0.03 ± 0.00^{b}	0.18 ± 0.01^{bcdef}	0.14 ± 0.00^{b}	0.65 ± 0.02^{def}
	50	0.07 ± 0.02^{b}	0.34 ± 0.00^{d}	0.07 ± 0.07^{a}	0.21 ± 0.01^{abc}	0.04 ± 0.00^{b}	0.19 ± 0.01^{bcde}	0.19 ± 0.02^{b}	0.74 ± 0.02^{bc}
	75	0.08 ± 0.00^{a}	0.30 ± 0.01^{efg}	0.06 ± 0.02^{b}	0.18 ± 0.01^{cdef}	0.04 ± 0.01^{b}	0.15 ± 0.01^{defg}	0.19 ± 0.01^{b}	0.63 ± 0.00^{def}
	100	0.05 ± 0.02^{b}	0.32 ± 0.01^{de}	0.07 ± 0.00^{a}	0.19 ± 0.01^{cde}	0.03 ± 0.01^{b}	0.18 ± 0.01^{bcde}	0.16 ± 0.00^{b}	0.69 ± 0.02^{cd}
6	25	0.06 ± 0.00^{b}	0.41 ± 0.01^{b}	0.07 ± 0.00^{a}	0.16 ± 0.01^{ef}	0.04 ± 0.01^{b}	0.19 ± 0.01^{abc}	0.17 ± 0.00^{b}	0.77 ± 0.02^{b}
	50	0.07 ± 0.01^{b}	0.32 ± 0.01^{de}	0.06 ± 0.02^{b}	0.17 ± 0.01^{def}	0.08 ± 0.01^{a}	0.16 ± 0.01^{cdefg}	0.21 ± 0.01^{a}	0.65 ± 0.02^{def}
	75	0.08 ± 0.02^{a}	0.38 ± 0.01^{c}	0.06 ± 0.01^{b}	0.23 ± 0.02^{a}	0.07 ± 0.00^{a}	0.19 ± 0.02^{abcd}	0.22 ± 0.02^{a}	0.80 ± 0.02^{b}
	100	0.08 ± 0.02^{a}	0.30 ± 0.01^{efg}	0.09 ± 0.01^{a}	0.15 ± 0.01^{f}	0.04 ± 0.00^{b}	0.19 ± 0.02^{bcde}	0.22 ± 0.01^{a}	0.63 ± 0.01^{def}
8	25	0.08 ± 0.02^{a}	0.34 ± 0.02^{d}	0.07 ± 0.01^{a}	0.20 ± 0.01^{bcde}	0.07 ± 0.01^{a}	0.14 ± 0.01^{fg}	0.23 ± 0.02^{a}	0.67 ± 0.02^{de}
	50	0.08 ± 0.01^{a}	0.31 ± 0.01^{def}	0.06 ± 0.04^{b}	0.23 ± 0.01^{ab}	0.07 ± 0.00^{a}	0.21 ± 0.01^{ab}	0.22 ± 0.03^{a}	0.75 ± 0.01^{b}
	75	0.09 ± 0.02^{a}	0.27 ± 0.01^{ghi}	0.06 ± 0.00^{b}	0.18 ± 0.01^{cdef}	0.07 ± 0.00^{a}	0.16 ± 0.00^{cdegf}	0.23 ± 0.01^{a}	0.61 ± 0.02^{efg}
	100	0.09 ± 0.01^{a}	0.47 ± 0.01^{a}	0.07 ± 0.07^{a}	0.22 ± 0.00^{ab}	0.08 ± 0.01^{a}	0.23 ± 0.02^{a}	0.24 ± 0.00^{a}	0.92 ± 0.02^{a}
	Control	0.06 ± 0.01^{b}	0.26 ± 0.01^{hij}	0.05 ± 0.00^{b}	0.20 ± 0.01^{abcd}	0.06 ± 0.00^{b}	0.15 ± 0.01^{efg}	0.17 ± 0.00^{b}	0.60 ± 0.02^{fg}
Values within the same column with similar superscripts are homogenous									

seeds bioprimed at 25% and 75%, the seeds primed at 75 % for 8 days were found to be on par with the non-primed seeds. It can be concluded that the biomass production on dry weight basis of the sandal seedlings had improved on subjecting seeds to biopriming for longer durations over control.

4.5.5. Effect of biopriming on growth analysis indices of sandal seedlings

The effect of biopriming on growth analysis indices of sandal seedlings such as specific leaf area, specific leaf weight, leaf area ratio and leaf weight ratio are given in Table 23. At 90 DAT, the specific leaf area recorded the highest value in seeds bioprimed at 75% for 2 days (185.69 cm^2 g^{-1}) followed by biopriming at 25% for 4 days (115.35 cm^2 g^{-1}) and biopriming 100% for 6 days (113.75 cm^2 g^{-1}) and the lowest value was recorded in seeds bioprimed at 50% for 4 days (20.53 cm^2 g^{-1}) indicating that the variation in specific leaf area due to biopriming treatments are highly significant (F = 2.94, p=0.01) during 3rd month after transplanting. The specific leaf area of the non-primed seeds (101.06 cm^2 g^{-1}) was comparatively higher than many of the biopriming treatments.

The specific leaf weight followed a remarkably different trend having its highest value (0.06 g cm^{-2}) in seeds bioprimed at 75% for 2 days as well as seeds bioprimed at 50% for 4 days and the minimum value (0.01 g cm^{-2}) being recorded in all other treatments except biopriming at 100% for 4 days (0.02 g cm^{-2}) and biopriming at 50% (0.02 g cm^{-2}) and 75% (0.03 g cm^{-2}) for 6 days. The variation in specific leaf weight was found to be highly significant (F = 4.84, p=0.01).

The leaf area ratio exhibited a significant variation due to biopriming treatments (F = 2.42, p =0.01) at 90 DAT. The highest of leaf area ratio was recorded in seeds bioprimed at 100% for 6 days (44.37 cm^2 g^{-1}) followed by 25% for 4 days (43.72 cm^2 g-1) and 75% for 2 days (42.56 cm^2 g^{-1}) and the lowest value was recorded in seeds bioprimed at 50% for 4 days (6.75 cm^2 g^{-1}) which was significantly lower than the leaf area ration of the non-primed seeds (34.67 cm^2 g^{-1}). There was no significant variation observed in the leaf weight ratio of the sandal seedlings due to biopriming treatments (F = 0.79, p = 0.68). The values of leaf weight ratio of the sandal seedlings were on par for all the biopriming treatments.

Table 23. Effect of biopriming on the growth indices of sandal seedlings at 90 and 180 days after transplanting

Biopriming		Specific Leaf Area (cm^2g^{-1})		Specific Leaf Weight (gcm^{-2})		Leaf Area Ratio (cm^2g^{-1})		Leaf Weight Ratio	
Duration (Days)	Concentration (%)	90 DAT	180 DAT	90 DAT	180 DAT	90 DAT	180 DAT	90 DAT	180 DAT
2	25	73.36 ± 2.84^{bc}	56.25 ± 8.38^{a}	0.01 ± 0^{c}	0.02 ± 0^{g}	29.68 ± 3.26^{ab}	21.46 ± 2.39^{a}	0.40 ± 0.05^{ab}	0.39 ± 0.03^{f}
	50	77.12 ± 15.60^{bc}	51.59 ± 6.35^{ab}	0.01 ± 0^{c}	0.02 ± 0^{fg}	31.48 ± 6.42^{ab}	20.63 ± 1.93^{a}	0.41 ± 0.02^{ab}	0.41 ± 0.02^{f}
	75	185.69 ± 59.61^{a}	39.84 ± 3.66^{cde}	0.06 ± 0^{a}	0.03 ± 0^{efg}	42.56 ± 11.08^{a}	16.24 ± 1.62^{bcd}	0.25 ± 0.07^{b}	0.41 ± 0.02^{f}
	100	74.30 ± 8.19^{bc}	34.43 ± 3.13^{def}	0.01 ± 0^{c}	0.03 ± 0^{defg}	29.98 ± 3.16^{ab}	14.38 ± 1.36^{cde}	0.40 ± 0.00^{ab}	0.42 ± 0.02^{def}
4	25	115.35 ± 11.34^{b}	32.04 ± 2.39^{defg}	0.01 ± 0^{c}	0.04 ± 0^{def}	43.72 ± 11.42^{a}	14.09 ± 1.22^{cde}	0.36 ± 0.07^{ab}	0.44 ± 0.01^{cdef}
	50	20.53 ± 6.38^{c}	10.88 ± 0.89^{j}	0.06 ± 0^{a}	0.09 ± 0.01^{a}	6.75 ± 1.38^{c}	5.03 ± 0.45^{h}	0.35 ± 0.04^{ab}	0.46 ± 0.01^{bcde}
	75	74.89 ± 8.42^{bc}	27.46 ± 2.33^{fgh}	0.01 ± 0^{c}	0.04 ± 0^{d}	34.17 ± 3.09^{ab}	12.99 ± 0.95^{def}	0.46 ± 0.02^{b}	0.47 ± 0.01^{bcd}
	100	79.73 ± 33.28^{bc}	20.78 ± 1.68^{ghij}	0.02 ± 0^{b}	0.05 ± 0^{c}	23.83 ± 6.25^{bc}	9.69 ± 0.72^{fg}	0.34 ± 0.07^{ab}	0.47 ± 0.00^{bcde}
6	25	96.39 ± 27.86^{b}	20.36 ± 1.31^{hij}	0.01 ± 0^{c}	0.05 ± 0^{c}	33.10 ± 9.95^{ab}	10.91 ± 0.69^{efg}	0.34 ± 0.01^{ab}	0.54 ± 0.02^{a}
	50	50.36 ± 4.57^{c}	23.35 ± 1.02^{fghi}	0.02 ± 0^{b}	0.04 ± 0^{cd}	17.19 ± 1.90^{bc}	11.60 ± 0.60^{ef}	0.35 ± 0.06^{ab}	0.50 ± 0.01^{ab}
	75	39.32 ± 8.85^{c}	15.31 ± 0.65^{ij}	0.03 ± 0.01^{b}	0.07 ± 0^{bn}	14.51 ± 3.02^{bc}	7.27 ± 0.34^{gh}	0.37 ± 0.02^{ab}	0.48 ± 0.01^{bc}
	100	113.75 ± 4.63^{b}	41.81 ± 3.18^{bcd}	0.01 ± 0^{c}	0.03 ± 0^{efg}	44.47 ± 7.61^{a}	19.62 ± 1.71^{ab}	0.39 ± 0.05^{ab}	0.47 ± 0.02^{bcde}
8	25	80.92 ± 12.96^{bc}	28.99 ± 1.26^{defg}	0.01 ± 0^{c}	0.03 ± 0^{defg}	29.00 ± 5.15^{ab}	14.76 ± 1.21^{bcd}	0.36 ± 0.02^{ab}	0.51 ± 0.03^{ab}
	50	57.57 ± 10.28^{c}	42.36 ± 3.83^{bcd}	0.01 ± 0^{c}	0.03 ± 0^{efg}	23.10 ± 4.31^{abc}	17.72 ± 1.72^{abc}	0.40 ± 0.03^{ab}	0.42 ± 0.01^{ef}
	75	71.11 ± 14.82^{bc}	46.55 ± 4.58^{abc}	0.01 ± 0^{c}	0.02 ± 0^{g}	29.24 ± 7.08^{ab}	20.31 ± 1.70^{ab}	0.4 ± 0.02^{ab}	0.44 ± 0.01^{cdef}
	100	86.58 ± 18.93^{bc}	24.04 ± 2.27^{fghi}	0.01 ± 0^{c}	0.04 ± 0^{cd}	31.75 ± 7.54^{ab}	12.33 ± 1.24^{def}	0.36 ± 0.01^{ab}	0.51 ± 0.01^{ab}
	Control	101.06 ± 24.67^{b}	33.10 ± 3.15^{def}	0.01 ± 0^{c}	0.03 ± 0^{defg}	34.67 ± 5.40^{ab}	14.03 ± 0.97^{cde}	0.37 ± 0.07^{ab}	0.43 ± 0.01^{cdef}

Values within the same column with similar superscripts are homogenous

Analysis of variance at 6th month after transplanting, revealed significant effect of biopriming on the specific leaf area (F=12.94, p=0.01), specific leaf weight (F=23.11, p=0.01), leaf area ratio (F=12.22, p=0.01) and leaf weight ratio (F=6.74, p=0.01) of the seedlings. The highest specific leaf area at 180 DAT was recorded in seedlings obtained from seeds bioprimed at 25% for 2 days (56.25 $cm^2 g^{-1}$) and the lowest value was recorded in the seeds bioprimed at 50% for 4 days (10.88 $cm^2 g^{-1}$), a reverse trend was observed with regard to specific leaf weight where the latter showed the highest value (0.09 g cm^{-2}) and the former treatment recorded the lowest value (0.02 g cm^{-2}).

The variation in the leaf area ratio also exhibited the similar pattern as that of the specific leaf area where the seeds bioprimed at 25% for 2 days recorded the highest value (21.46 $cm^2 g^{-1}$) whereas the seeds bioprimed at 50% for 4 days recorded the lowest value (5.27 $cm^2 g^{-1}$). Meanwhile, the leaf weight ratio followed a different pattern with a highest average value recorded by seeds bioprimed at 25% for 6 days (0.54) which recorded significantly lower values of specific leaf area, specific leaf weight and leaf area ratio, and the lowest value was recorded by the seeds bioprimed at 25% for 2 days (0.39).

The variation in root: shoot ratio of the sandal seedlings due to biopriming treatments were non –significant at 90 DAT (F = 1.10, p = 0.38) (Table 24). The maximum value of the ratio was obtained in seeds bioprimed at 50% for 8 days (2.92) and the values of the remaining treatments were found to be on par with the value of non-primed seeds. On contrary, the root: shoot ratio has attained a highly significant variation at 180 DAT (F=4.43, p=0.01) due to different biopriming treatments. The highest average value of the ratio was recorded in seeds bioprimed at 100% for 6 days (1.26) and the biopriming treatments were found to be superior over the non-primed seeds.

The value of vigor index I showed highly significant variation due to biopriming treatments (F = 16.90, p=0.01). In spite of the highest value recorded by seeds bioprimed at 75% for 8 days (1925.61), all the treatments have a significantly higher vigor index over non-primed seeds. The vigor index II also exhibited highly significant variation due to biopriming treatments (F = 7.25, p=0.01). The highest value of vigour index II was recorded in seeds subjected to biopriming at 100% for 8 days (0.21) and the lowest value was recorded in non-primed seeds (0.08) (Table 25).

Table 24. Effect of biopriming on the root: shoot ratio of sandal seedlings at 90 and 180 days after transplanting

Biopriming		Root : Shoot Ratio	
Duration (Days)	Concentration (%)	90 DAT	180 DAT
2	25	0.81 ± 0.19^{a}	0.74 ± 0.07^{f}
	50	0.89 ± 0.20^{a}	0.76 ± 0.06^{def}
	75	0.87 ± 0.23^{a}	0.97 ± 0.05^{bcde}
	100	0.79 ± 0.02^{a}	0.87 ± 0.08^{cdef}
4	25	0.60 ± 0.14^{a}	0.96 ± 0.03^{cde}
	50	0.61 ± 0.05^{a}	0.89 ± 0.02^{cdef}
	75	0.94 ± 0.54^{a}	0.83 ± 0.08^{cdef}
	100	0.45 ± 0.16^{a}	0.98 ± 0.04^{bcd}
6	25	0.56 ± 0.22^{a}	1.19 ± 0.07^{ab}
	50	1.37 ± 0.12^{a}	0.97 ± 0.03^{bcde}
	75	1.18 ± 0.16^{a}	0.86 ± 0.13^{cdef}
	100	0.51 ± 0.08^{a}	1.26 ± 0.16^{a}
8	25	1.14 ± 0.26^{a}	0.70 ± 0.04^{f}
	50	2.92 ± 2.03^{a}	0.94 ± 0.06^{cdef}
	75	1.18 ± 0.18^{a}	0.88 ± 0.05^{cdef}
	100	1.11 ± 0.29^{a}	1.03 ± 0.07^{bc}
	Control	1.21 ± 0.02^{a}	0.75 ± 0.03^{def}
Values within the same column with similar superscripts are homogenous			

The results of the effect of biopriming on the growth indices such as absolute growth rate, relative growth rate and net assimilation rate of sandal seedlings are given in Table 26. The absolute growth rate ($F=5.92$, $p=0.011$), relative growth rate ($F=2.69$, $p=0.001$) and net assimilation rate ($F=2.33$, $p=0.01$) recorded highly significant variation. The values of these indices due to different biopriming treatments were almost similar to each other as well as with control.

Table 25. Effect of biopriming on the vigour indices of sandal seedlings

Biopriming		Vigor Index I	Vigor Index II
Duration (Days)	Concentration (%)		
2	25	$1599.49 \pm 110.95^{bcde}$	0.19 ± 0.02^{ab}
	50	1341.94 ± 51.33^{h}	0.17 ± 0^{abc}
	75	1197.00 ± 88.80^{gh}	0.09 ± 0.01^{ef}
	100	1111.00 ± 75.93^{j}	0.13 ± 0.01^{cde}
4	25	$1689.00 \pm 165.64^{bcde}$	0.11 ± 0^{def}
	50	1521.00 ± 66.82^{cdef}	0.15 ± 0.01^{bcd}
	75	1433.27 ± 44.09^{defg}	0.15 ± 0.01^{bcd}
	100	1614.82 ± 40.59^{bcd}	0.13 ± 0^{cde}
6	25	1521.00 ± 90.40^{cdef}	$0.13 \pm 0.^{cd}$
	50	1530.84 ± 67.61^{cdef}	0.16 ± 0^{abc}
	75	1389.70 ± 24.07^{defg}	0.17 ± 0.01^{abc}
	100	1353.12 ± 59.56^{efg}	0.18 ± 0.01^{abc}
8	25	1712.29 ± 39.51^{abc}	0.19 ± 0.01^{ab}
	50	1808.51 ± 28.45^{ab}	0.19 ± 0.03^{ab}
	75	1925.61 ± 89.40^{a}	0.19 ± 0.01^{ab}
	100	1809.86 ± 69.53^{ab}	0.21 ± 0^{a}
	Control	840.26 ± 47.82^{i}	0.08 ± 0.08^{f}
Values within the same column with similar superscripts are homogenous			

Table 26. Effect of biopriming on the growth analysis indices of sandal seedlings

Biopriming		Absolute Growth Rate (cm day^{-1})	Relative Growth Rate (g g^{-1} day^{-1})	Net Assimilation Rate (g cm^{-2} day^{-1})
Duration (Days)	Concentration (%)			
2	25	0.003 ± 0^{c}	0.01 ± 0^{b}	0.02 ± 0^{c}
	50	0.003 ± 0^{c}	0.01 ± 0^{b}	0.02 ± 0^{c}
	75	0.006 ± 0^{b}	0.01 ± 0.01^{ab}	0.06 ± 0.01^{a}
	100	0.004 ± 0^{bc}	0.01 ± 0^{ab}	0.03 ± 0.01^{bc}
4	25	0.010 ± 0^{a}	0.02 ± 0^{a}	0.04 ± 0.01^{ab}
	50	0.010 ± 0^{a}	0.01 ± 0^{ab}	0.04 ± 0^{bc}
	75	0.003 ± 0^{bc}	0.01 ± 0^{ab}	0.03 ± 0^{bc}
	100	0.010 ± 0^{a}	0.02 ± 0^{ab}	0.04 ± 0^{bc}
6	25	0.010 ± 0^{a}	0.02 ± 0^{a}	0.04 ± 0.01^{bc}
	50	0.010 ± 0^{ab}	0.01 ± 0^{b}	0.03 ± 0^{bc}
	75	0.010 ± 0^{a}	0.01 ± 0^{ab}	0.03 ± 0^{bc}
	100	0.002 ± 0^{c}	0.01 ± 0^{b}	0.03 ± 0^{bc}
8	25	0.003 ± 0^{bc}	0.01 ± 0^{b}	0.03 ± 0^{bc}
	50	0.010 ± 0^{a}	0.01 ± 0^{ab}	0.03 ± 0^{bc}
	75	0.002 ± 0.01^{c}	0.01 ± 0^{b}	0.02 ± 0^{b}
	100	0.010 ± 0^{a}	0.02 ± 0^{b}	0.03 ± 0^{bc}
	Control	0.004 ± 0^{c}	0.01 ± 0^{b}	0.03 ± 0^{bc}
Values within the same column with similar superscripts are homogenous				

4.5.6. Effect of biopriming on the chlorophyll content of the sandal seedlings

With regard to the chlorophyll content in the leaves at 180 DAT, it recorded a highly significant variation ($F=25.01$, $p=0.01$). The seeds subjected to biopriming at 100% for 6 days recorded the highest chlorophyll content (35.73 mg g^{-1}) which was found to be on par with the seeds bioprimed at 25%, 75% and 100% for 2 days, those bioprimed at 75% and 100% for 4 days and seeds bioprimed at 25% for 6 days whereas, the lowest chlorophyll content was recorded by seeds subjected to biopriming at 25% for 8 days (23.30 mg g^{-1}) which was observed to be on par with the other biopriming treatments performed for 8 days duration (Table 27).

Table 27. Effect of biopriming on the chlorophyll content of sandal seedlings

Biopriming		Chlorophyll (mg g^{-1})
Duration (Days)	Concentration (%)	
2	25	32.00 ± 0.30^{bcd}
	50	28.56 ± 0.29^{ef}
	75	33.50 ± 3.75^{abc}
	100	32.90 ± 0.36^{abcd}
4	25	31.30 ± 0.62^{cde}
	50	16.26 ± 0.46^{i}
	75	34.76 ± 0.44^{ab}
	100	35.03 ± 0.26^{ab}
6	25	33.36 ± 0.58^{abc}
	50	30.73 ± 0.62^{cde}
	75	29.90 ± 0.65^{def}
	100	35.73 ± 0.40^{a}
8	25	23.30 ± 0.36^{h}
	50	25.30 ± 0.56^{gh}
	75	25.00 ± 0.76^{gh}
	100	25.33 ± 0.60^{gh}
0	Control	26.90 ± 0.41^{fg}
Values within the same column with similar superscripts are homogenous		

4.5.7. Effect of osmopriming on growth attributes and biomass production of sandal seedlings

The impact of osmopriming on the growth attributes and biomass production of sandal seedlings at are depicted in the Tables 28 to 32.

Table 28 presents the results of osmopriming on the shoot growth attributes of sandal seedlings at 90 DAT and 180 DAT. On 90th day after transplanting, the variation in shoot height of the seedlings were highly significant (F = 10.93, p=0.01) whereas the collar girth variation was non-significant due to PEG priming (F = 1.98, p = 0.14). Among the six PEG priming treatments the PEG priming at 5% for 3 days produced the maximum shoot height whereas the remaining treatments were on par with control. The collar girth of the seedling obtained after osmopriming at different concentrations and durations were higher than that of the non-primed seeds. The collar girth of the seedlings obtained from the seeds subjected to osmopriming were at par. The variations in shoot height (F=230.56, p=0.01) and collar girth (F=25.50, p=0.01) were highly significant in osmoprimed seeds at 180 DAT. At 180 DAT, the highest shoot height were obtained in seeds osmoprimed at 5% for 3 days (20.90 cm), while seedlings osmoprimed at10% for 6 days recorded

the lowest value of shoot height (17.27 cm). The highest collar girth of the seedlings was recorded in seeds subjected to osmopriming at 15% for 3 days which was on par with the seeds osmoprimed at 5% and 10% for 3 days.

The variation in leaf area of the seedlings due to osmopriming was found to be highly significant (F = 5.83, p=0.01) at 90 DAT. The leaf area of the seedlings from seeds subjected to PEG priming at 10% for 3 days (6.98 cm^2) showed the highest value which did not have significant difference from the leaf area of non-primed seeds (6.04 cm^2), whereas the leaf area of seeds subjected to PEG priming at 5% for 3 days (2.30 cm^2) recorded the lowest value and varied significantly when compared to the leaf area of non-primed seeds. The leaf area of the seeds subjected to PEG priming at 15% and 20% for 3 days were also found to be on par with the highest of the leaf area recorded in PEG priming at 10% for 3 days. Similarly, there was high significant difference in the leaf areas of sandal seedlings due to osmopriming at 180 DAT (F=7.07, p=0.01). During the 6^{th} month of observation, the seedlings germinated from the seeds subjected to osmopriming at 10% for 3 days showed the highest value of leaf area (9.22 cm^2), while the seedlings germinated from the seeds osmoprimed at 5% for 3 days showed the lowest leaf area (5.80 cm^2). Although the leaf area of seedlings osmoprimed at 10% and 20% for 3 days and non-primed seeds were on par, these were found to be on par with leaf area of seeds osmoprimed at 15% for 3 days.

The number of leaves per seedling also showed significant variation due to PEG priming treatments (F = 3.15, p = 0.01). At 90^{th} day after transplanting the maximum number of leaves were produced by seeds subjected to PEG priming at 20% for 3 days (13.33) followed by seeds subjected to PEG priming for 5% for 3 days (12.33), wherein the former had a significant increase over the control (8.33) and the latter was found to be on par with the control. The least number of leaves were produced from seeds subjected to osmopriming at 5% for 6 days. At 180 days after transplanting also, the variation in leaf number was highly significant in the seedlings germinated from PEG mediated osmoprimed seeds (F=6.97, p=0.01). Leaf number was the highest in the seedlings germinated from seeds osmoprimed at 5% for 3 days (17.25), and lowest in the seedlings germinated from seeds osmoprimed at 5% for 6 days (10.50). The number of seedlings PEG primed at 5, 10, 15 and 20% for 3 days were found to be on par and performed superior over

control. Even though, the seeds osmoprimed at 15 and 20% for 3 days having higher leaf number than the non-primed seeds, the values were at par.

The effect of osmopriming on the root parameters and total seedling length of sandal seedlings presented in Table 29. The results indicated that the root length showed significant variation due to different osmopriming treatments at 90 DAT ($F = 3.46$, $p = 0.02$). The root length of the control seedlings (4 cm) was found to be greater than that of the osmopriming. Although the non-primed seeds performed better over the primed seeds, the root length of the seeds osmoprimed at 5% and 20% for 3 days were found to be on par with the control. In addition, the number of lateral produced by the seedlings exhibited no significant variation due to the different treatments in osmopriming ($F = 0.79$, $p = 0.52$). The number of lateral roots produced by different osmopriming treatments were found to be on par with that of the non-primed seeds. At 180 DAT, the average root length was the highest in the seeds subjected to PEG priming at 10% for 3 days (7.35 cm) and seedlings from seeds osmoprimed at 5% and 10% for 6 days recorded the lowest root length (5.35 cm) and the seedlings primed at 3, 6, 9 and 12 days were on par with respect to root length. The variations in root length ($F=5.99$, $p=0.01$) were highly significant in osmoprimed seeds, while the variations in number of lateral roots were not significant ($F=1.28$, $p=0.30$).

The seedling length showed significant variation due to osmopriming ($F = 10.81$, $p = 0.01$) ($F=26.76$, $p=0.01$) at 90 and 180 days after transplanting. Total length of seedling in different osmopriming treatments varied from 16.9 cm in seeds osmoprimed at 15% for 3 days to 20.56 cm in seeds osmoprimed at 5% for 3 days where the latter was found to be on par with the seedling length of the control (18.56cm).

At 180th day after transplanting, the highest seedling length was recorded by seedlings from seeds subjected to PEG priming at 10% for 3 and 6 days (28.15 cm) which was on par with that of the seeds primed at 5% for 3 days (27.10 cm). Whereas, the seeds primed at 15% for 3 days were found to be on par with that of the non-primed seeds.

Table 28. Effect of osmopriming on the shoot growth attributes of sandal seedlings at 90 and 180 days after transplanting

Osmopriming		Shoot height (cm)		Collar Girth (mm)		Number of leaves		Leaf Area (cm^2)	
Duration (Days)	Concentration (%)	90 DAT	180 DAT	90 DAT	180 DAT	90 DAT	180 DAT	90 DAT	180 DAT
3	5	16.56 ± 0.31^{a}	20.90 ± 0.33^{a}	2.64 ± 0.16	7.11 ± 0.03^{ab}	12.33 ± 0.33^{ab}	17.25 ± 0.63^{a}	2.17 ± 0.49^{c}	5.80 ± 0.38^{c}
	10	14.77 ± .45^{b}	20.80 ± 0.30^{a}	2.53 ± 0.30	7.43 ± 0.07^{a}	9.33 ± 2.33^{bc}	16.50 ± 1.55^{a}	6.98 ± 1.11^{a}	9.22 ± 0.79^{a}
	15	13.84 ± 1.66^{b}	19.10 ± 0.11^{b}	2.17 ±0.10	7.52 ± 0.13^{a}	11 ± 0.57^{bc}	15.75 ± 1.11^{ab}	5.06 ±0.82^{ab}	6.89 ± 0.39^{bc}
	20	13.84 ± 0.41^{b}	18.10 ± 0.16^{c}	2.14 ± 0.17	6.68 ± 0.15^{bc}	13.33 ± 1.76^{a}	16.00 ± 1.47^{ab}	5.90 ± 0.82^{a}	8.35 ± 0.53^{ab}
6	5	13.17 ± 0.38^{b}	17.87 ± 0.22^{cd}	2.44 ± 0.31	5.89 ± 0.30^{d}	7.33 ± 0.88^{c}	10.50 ± 0.65^{c}	3.11 ± 1.01^{bc}	6.58 ± 0.49^{c}
	10	14.27 ± 0.57^{b}	17.27 ± 0.16^{d}	2.26 ± 0.17	5.40 ± 0.11^{e}	8.33 ± 0.88^{bc}	11.00 ± 0.71^{c}	2.30 ± 0.50^{c}	5.46 ± 0.51^{c}
	Control	14.16 ± 1.04^{ab}	18.50 ± 0.41^{bc}	1.79 ± 0.03	6.33 ± 0.14^{cd}	8.33 ± 1.52^{bc}	13.00 ± 1.41^{bc}	6.04 ± 1.14^{a}	8.44 ± 0.58^{ab}
Values within the same column with similar superscripts are homogenous									

Table 29. Effect of osmopriming on the root growth attributes and seedling length of sandal seedlings at 90 and 180 days after transplanting

Osmopriming		Root Length (cm)		Number of Lateral Roots		Seedling Length	
Duration (Days)	Concentration (%)	90 DAT	180 DAT	90 DAT	180 DAT	90 DAT	180 DAT
3	5	4.00 ± 0.36^{ab}	6.20 ± 0.07^{bcd}	5.66 ± 0.34	1.75 ± 0.48	20.56 ± 0.33^{a}	27.10 ± 0.37^{a}
	10	2.43 ± 0.38^{b}	7.35 ± 0.12^{a}	5.33 ± 0.68	3.25 ± 0.25	17.2 ± 1.45^{b}	28.15 ± 0.31^{a}
	15	3.06 ±0.34^{b}	6.60 ± 0.77^{ab}	5.66 ±0.20	3.75 ± 0.63	16.9 ±0.66^{b}	25.70 ± 0.84^{b}
	20	3.96 ± 0.27^{ab}	5.47 ± 0.11^{cd}	4.66 ± 0.41	2.75 ± 0.63	17.80 ± 0.41^{b}	23.57 ± 0.11^{c}
6	5	3.26 ± 0.18^{b}	5.35 ± 0.10^{d}	5.66 ± 0.38	3.25 ± 0.75	16.43 ± 0.38^{b}	23.22 ± 0.26^{c}
	10	3.83 ± 0.18^{b}	5.35 ± 0.06^{d}	5.00 ± 0.15	2.25 ± 0.95	17.80 ± 0.57^{b}	22.62 ± 0.16^{c}
	Control	4.10 ± 0.78^{a}	6.35 ± 0.13^{bc}	7.00 ± 2.00	3.25 ± 0.25	18.26 ± 1.80^{a}	24.85 ± 0.26^{b}
Values within the same column with similar superscripts are homogenous							

The fresh weight of the different components of sandal seedlings at 90 and 180 DAT are presented in the Table 30. The leaf weight of the sandal seedlings at 90 DAT showed significant variation due to osmopriming treatments ($F = 2.57$, $p = 0.04$).The highest leaf weight was produced by seedlings from seeds subjected to PEG priming at 5% for 3 days (0.31 g) and it was par with osmopriming at 10% for 3 days (0.28 g), 5% for 6 days (0.28 g) as well as with that of the non-primed seeds (0.27 g). The trend in the variation of shoot weight due to different osmopriming treatments were found to be synonymous to that of the leaf weight. Contradictory to the results of leaf weight, the shoot weight produced by the seeds subjected to osmopriming treatments performed better than non-primed seeds whereas no statistically significant variation was recorded in the root weight of the seedlings due to osmopriming treatments ($F = 0.39$, $p = 0.86$). The root weight produced by the seeds exposed to different osmopriming treatments were found to be on par with that of the control. Variation in total fresh weight of the seedlings due to osmopriming similar to that of root weight having non-significant variation among treatments ($F= 2.27$, $p=0.90$). Although the total fresh weigh recorded highest value in seedlings osmoprimed at 5% for 3 days, it was found to be on par with the total fresh weight of seedlings primed at 10% for 3 days and seedlings primed at 5 and 10% for 6 days. The results were indicating that osmopriming could not impart considerable increment in the fresh weight of the sandal seedlings at 90 days after transplanting.

With regard to the variations in fresh weight of the seedlings at 180 DAT, the leaf ($F=18.16$, $p=0.01$), shoot ($F=15.79$, $p=0.01$) and root fresh weight ($F=3.36$, $p=0.01$) of sandal seeds subjected to different osmopriming treatments had high statistical significance. Although, the average leaf weight was found to be highest in seedlings subjected to osmopriming at 5% for 3 days (1.12 g) similar to that at 90 DAT, the leaf weight of the non-primed seeds (0.85 g) was found to be superior over the rest of the osmopriming treatments and the values were found to be on par with each other at 180 DAT. Contrary to the results of leaf weight, the shoot weight of the seeds subjected to different osmopriming treatment was superior over the non-primed seeds, where the later recorded the least value of shoot weight indicating that the effect of osmopriming was found to be beneficial in increasing the shoot weight at both the intervals. The seedlings subjected to PEG priming at 10% for 3 days recorded the highest shoot weight (0.70 g) whereas the seedlings of control recorded the lowest value (0.57 g). The seeds subjected to osmopriming at 5% and 15% for 3 days

and seeds subjected to osmopriming at 5% for 6 days were found to be on par with each other at 180 DAT.

Contrary to the results obtained at 90 DAT, the highest average value of root weight was recorded in seeds subjected to PEG priming at 20% for 3 days (0.44 g) at 180 DAT which was found to be on par with seeds osmoprimed at 5 and 15% for 3 days as well as seeds primed at 10% for 6 days. The seeds subjected to osmopriming at 5, 15 and 15% for 3 days and 10% for 6 days were found to be on par with that of the control seedlings at 180 DAT. During the observations made at 6th month, variations in total fresh weight of the sandal seedlings among different osmopriming treatments exhibited highly significant variations (F= 11.22, p=0.01) where the total fresh weight ranged from 1.76 g in seedlings osmoprimed at 10% for 6 days to 2.16 g in seedlings osmoprimed at 5% for 3 days. It can be interpreted from the results that osmopriming with PEG for 3 days imparted a significant increase in the total fresh weight (2.16 g) of the sandal seedlings compared to control (1.82 g).

The results of the effect of osmopriming on the seedling dry weight of sandal seedlings at 90 and 180 DAT are presented in Table 31. Analysis of variance indicated that variation in leaf dry weight due to different osmopriming treatments was significant at 90 (F = 4.17, p = 0.01) and 180 DAT (F=39.40, p=0.01). During 3rd month, the highest leaf weight (0.08 g) was obtained from seeds subjected to PEG priming at 5% and 20% for 3 days while the minimum dry weight was recorded in seeds osmoprimed at 10% for 3 days. The variation in shoot dry weight (F = 0.75, p =0.61) and root dry weight (F = 0.90, p = 0.51) of the sandal seedlings due to osmopriming the treatments followed a similar trend. Significant variation was not observed in both the characteristics due to priming treatments. The values of shoot and root dry weight of sandal seedlings subjected to different osmopriming treatments were on par with that of control. Simultaneously the total dry weight of the seedlings also exhibited non-significant variation due to osmopriming treatments (F = 1.35, p = 0.29) at 90 days after transplanting.

Table 30. Effect of osmopriming on the fresh weight of sandal seedlings at 90 and 180 days after transplanting

Osmopriming		Fresh Weight (g)							
		Leaf		Shoot		Root		Total	
Duration (Days)	Concentration (%)	90 DAT	180 DAT	90 DAT	180 DAT	90 DAT	180 DAT	90 DAT	180 DAT
3	5	0.31 ± 0.01^{a}	1.12 ± 0.04^{a}	0.31 ± 0.01	0.62 ± 0.01^{b}	0.10 ± 0.01	0.42 ± 0.01^{ab}	0.65 ± 0.03	2.16 ± 0.05^{a}
	10	0.28 ± 0.01^{ab}	0.85 ± 0.01^{b}	0.28 ± 0.01	0.70 ± 0.01^{a}	0.10 ± 0.01	0.39 ± 0.01^{bc}	0.61 ± 0.06	1.94 ± 0.02^{b}
	15	0.24 ±0.02^{b}	0.83 ± 0.01^{b}	0.24 ±0.01	0.68 ± 0.01^{a}	0.09 ±0.01	0.41 ± 0.01^{abc}	0.53 ± 0.00	1.92 ± 0.02^{b}
	20	0.23 ± 0.01^{a}	0.76 ± 0.01^{bc}	0.23 ± 0.01	0.62 ± 0.01^{b}	0.10 ± 0.01	0.44 ± 0.01^{a}	0.51 ± 0.03	1.82 ± 0.02^{bc}
6	5	0.28 ± 0.01^{ab}	0.81 ± 0.00^{bc}	0.28 ± 0.01	0.69 ± 0.01^{a}	0.11 ± 0.0	0.38 ± 0.01^{c}	0.57 ± 0.03	1.89 ± 0.02^{b}
	10	0.25 ± 0.01^{b}	0.72 ± 0.01^{c}	0.25 ± 0.01	0.62 ± 0.01^{b}	0.11 ± 0.01	0.42 ± 0. 01^{ab}	0.60 ± 0.01	1.76 ± 0.01^{c}
	Control	0.27 ± 0.00^{ab}	0.85 ± 0.06^{b}	0.16 ± 0.00	0.57 ± 0.01^{c}	0.09 ± 0.01	0.40 ± 0.01^{bc}	0.53 ± 0.02	1.82 ± 0.07^{bc}
Values within the same column with similar superscripts are homogenous									

Table 31. Effect of osmopriming on the dry weight of sandal seedlings at 90 and 180 days after transplanting

Osmopriming		Dry Weight (g)							
		Leaf		Shoot		Root		Total	
Duration (Days)	Concentration (%)	90 DAT	180 DAT	90 DAT	180 DAT	90 DAT	180 DAT	90 DAT	180 DAT
3	5	0.08 ± 0.01^{a}	0.26 ± 0.01^{a}	0.06 ± 0.01	0.21 ± 0.01^{a}	0.06 ± 0.01	0.12 ± 0.00^{bc}	0.21 ± 0.02	0.58 ± 0.02^{a}
	10	0.02 ± 0.01^{c}	0.21 ± 0.01^{b}	0.07 ± 0.05	0.18 ± 0.02^{bc}	0.05 ± 0.0	0.14 ± 0.02^{ab}	0.15 ± 0.04	0.54 ± 0.15^{b}
	15	0.07 ±0.01 ^{ab}	0.19 ± 0.01^{bc}	0.06 ±0.01	0.20 ±0.01^{a}	0.04 ±0.01	0.15 ± 0.01^{a}	0.19 ± 0.01	0.54 ± 0.19^{b}
	20	0.08 ± 0.01^{a}	0.14 ± 0.00^{d}	0.07 ± 0.01	0.16 ± 0.00^{bc}	0.04 ± 0.01	0.10 ± 0.01^{c}	0.20 ± 0.01	0.39 ± 0.20^{d}
6	5	0.04 ± 0.01^{bc}	0.19 ± 0.00^{c}	0.04 ± 0.01	0.14 ± 0.01^{c}	0.04 ± 0.01	0.12 ± 0.01^{abc}	0.14 ± 0.02	0.45 ± 0.14^{d}
	10	0.05 ± 0.01^{abc}	0.20 ± 0.00^{bc}	0.06 ± 0.01	0.16 ± 0.01^{bc}	0.04 ± 0.01	0.09 ± 0.01^{c}	0.16 ± 0.01	0.45 ± 0.16^{c}
	Control	0.06 ± 0.01^{ab}	0.26 ± 0.01^{a}	0.05 ± 0.00	0.20 ± 0.01^{a}	0.06 ± 0.00	0.15 ± 0.01^{ab}	0.17 ± 0.00	0.60 ± 0.01^{a}
Values within the same column with similar superscripts are homogenous.									

Contrary to the results of 3rd month, the shoot (F=6.49, p=0.01), root (F=5.90, p=0.01) and total dry weight (F=27.52, p=0.01) were all found to be highly significant at 6th month. It is evident from the results that on the 180th day after transplanting, the seeds primed at 5% for 3 days and non-primed seeds recorded the highest average leaf (0.26 g), shoot (0.20 g), root (0.15 g) and the total dry weight (0.60 g). The results indicated that the osmopriming treatments could not impart a considerable increase in the dry matter accumulation of the sandal seedlings over the control seeds. Eventhough, the non-primed seeds exhibited a superior performance over the seeds subjected to osmopriming treatments, the seedling dry weight of seeds subjected to PEG priming at 5% for 3 days were found to on par with the non-primed seeds.

4.5.8. Effect of osmopriming on the growth analysis indices of sandal seedlings

Table 32 presents the result of growth analysis indices of sandal seedlings such as specific leaf area, specific leaf weight, leaf area ratio and leaf weight ratio. At 90 days after transplanting, the specific leaf area of the sandal seedlings was the highest in seedlings from seeds osmoprimed at 10% concentration for 3 days (155 $cm^2 g^{-1}$) and the lowest value was recorded in seeds subjected to PEG priming at 5% for 3 days (26.56 $cm^2 g^{-1}$) indicating a highly significant variation due to priming. The non-primed seeds recorded a significantly higher specific leaf area of (101.06 $cm^2 g^{-1}$) over the seeds subjected to different osmopriming treatments except the osmopriming at 10% for 3 days. Although, the highest specific leaf area recorded in seeds osmoprimed at 10% for 3 days, the specific leaf weight was the lowest.

At 180 DAT, specific leaf area (F=14.75, p=0.01) and specific leaf weight (F=11.60, p=0.01) exhibited highly significant variation. The highest specific leaf area was observed in the seedlings from seeds subjected to osmopriming for 3 days at 20% concentration (61.02 $cm^2 g^{-1}$). However, the seeds subjected to PEG priming at 5% for 3 days as well as 10% for 6 days recorded lower values of specific leaf area compared to control. On the contrary the seeds subjected to PEG priming at 5% (0.05 g cm^{-2}) recorded the highest value for specific leaf weight whereas, the seeds primed at 20% for 3 days (0.02 g cm^{-2}) recorded the lowest value.

Table 32. Effect of osmopriming on the growth analysis indices of sandal seedlings at 90 and 180 days after transplanting

Osmopriming		Specific Leaf Area ($cm^2 g^{-1}$)		Specific Leaf Weight ($g\ cm^{-2}$)		Leaf Area Ratio ($cm^2 g^{-1}$)		Leaf Weight Ratio	
Duration (Days)	Concentration (%)	90 DAT	180 DAT	90 DAT	180 DAT	90 DAT	180 DAT	90 DAT	180 DAT
3	5	26.56 ± 6.28^{c}	22.30 ± 1.03^{d}	0.04 ± 0.00^{a}	0.05 ± 0^{a}	10.54 ± 2.64^{c}	9.89 ± 0.64^{d}	0.39 ± 0.00^{a}	0.44 ± 0.01^{a}
	10	155 ± 51.09^{a}	43.27 ± 4.78^{b}	0.01 ± 0^{c}	0.02 ± 0^{cd}	53.74 ± 13.25^{a}	17.02 ± 1.40^{b}	0.16 ± 0.03^{b}	0.40 ± 0.02^{b}
	15	65.54 ± 7.14^{b}	35.99 ± 2.58^{bc}	0.01 ± 0.00^{bc}	0.03 ± 0^{bc}	26.33 ± 2.81^{bc}	12.89 ± 1.19^{cd}	0.40 ± 0.01^{a}	0.36 ± 0.01^{c}
	20	70.33 ± 6.85^{b}	61.02 ± 4.89^{a}	0.01 ± 0.00^{bc}	0.02 ± 0^{d}	28.94 ± 3.04^{bc}	21.05 ± 1.56^{a}	0.41 ± 0.01^{a}	0.34 ± 0.01^{c}
6	5	67.63 ± 12.79^{b}	35.00 ± 1.91^{bc}	0.01 ± 0.05^{bc}	0.03 ± 0^{bc}	22.71 ± 8.39^{bc}	14.65 ± 1.16^{bc}	0.32 ± 0.07^{a}	0.41 ± 0.01^{ab}
	10	44.3 ± 12.46^{b}	27.66 ± 2.50^{cd}	0.02 ± 0.01^{ab}	0.03 ± 0^{b}	14.21 ± 3.95^{bc}	12.14 ± 1.11^{cd}	0.33 ± 0.05^{a}	0.44 ± 0.01^{ab}
	Control	101.06 ± 24.67^{b}	33.09 ± 3.15^{bc}	0.01 ± 0.01^{bc}	0.03 ± 0^{bc}	34.67 ± 5.40^{ab}	14.03 ± 0.97^{bc}	0.37 ± 0.07^{a}	0.43 ± 0.01^{ab}
Values within the same column with similar superscripts are homogenous									

The leaf area ratio of the seedlings recorded a definite trend in variation due to osmopriming treatments (F = 4.55, p=0.01) at 90 days after transplanting. The seeds osmoprimed at 10% for 3 days recorded the highest leaf area ratio (53.74 $cm^2 g^{-1}$) and the lowest value was recorded in seeds osmoprimed at 5% for 3 days (10.54 $cm^2 g^{-1}$). All the other treatments recorded values which were on par with that of non-primed seeds. The leaf area ratio (F=9.74, p=0.01) at 180 DAT of the seedling from different osmopriming treatments followed a similar pattern as that of the specific leaf area at 180 DAT where the seeds subjected to PEG priming at 20% recorded the highest leaf area ratio (21.05) and the seeds primed at 5% (9.89) recorded the lowest value.

The leaf weight ratio significantly varied due to priming treatments at 90 DAT. The highest leaf weight ratio was recorded in seeds subjected to PEG priming at 20% for 3 days and the smallest ratio was recorded in seeds subjected to PEG priming at 10% for 3 days. As far as the leaf weight ratio (F=9.19, p=0.01) is concerned, at 180 DAT the seeds primed at 5% of PEG for 3 days recorded the highest value (0.44) which was found to be on par with the values of seeds primed at 5 and 10% for 6 days as well as with the non-primed seeds.

The effect of osmopriming on the root: shoot ratio is presented in Table 37. The root: shoot ratio of the sandal seedlings recorded a non-significant variation due to osmopriming treatments at 90 DAT (F = 1.22, p = 0.35). The ratio of the seedlings obtained from seeds subjected to different osmopriming treatments were on par with that of the value recorded by the control seedlings. Whereas, the values of root: shoot ratio (F=2.62, p=0.04) were observed to have significant variation at five per cent level at 180 DAT. The ratio was highest in the seedlings osmoprimed at 5% for 6 days (0.85), which was found to be on par with the values of seeds subjected to PEG priming at 5, 10 and 20% for 3 days as well as with the non-primed seeds.

Significant variation was observed in the vigour index I (F = 57.88, p=0.01) and vigour index II (F = 3.25, p = 0.03) of the sandal seedlings due to different osmopriming treatments. The seeds subjected to different osmopriming treatments recorded significantly higher values over the non-primed seeds. A similar pattern observed in case of vigour index II where the vigour index values of the treatments were significantly higher than that of the vigour index of the non-primed seeds (Table 34). The results revealed that the seedling primed at 5% for 3 days recorded the highest value of vigour index I (1604.20) and the seedlings of control recorded the lowest value (840.26). Similarly the vigour index II was found to be the highest in seedlings primed at 5% for

3 days (0.16) and the lowest in the seedlings of control (0.08). Hence, it can be concluded that six osmopriming treatments could impart a significant increase in the vigour of seedling growth.

Table 33. Effect of osmopriming on the root: shoot ratio of sandal seedlings at 90 and 180 days after transplanting

Osmopriming		Root : Shoot Ratio	
Duration (Days)	Concentration (%)	90 DAT	180 DAT
3	5	1.05 ± 0.24	0.56 ± 0.02^{b}
	10	0.91 ± 0.29	0.79 ± 0.11^{ab}
	15	0.73 ± 0.14	0.77 ± 0.10^{ab}
	20	0.62 ± 0.18	0.65 ± 0.06^{ab}
6	5	0.98 ± 0.13	0.85 ± 0.06^{a}
	10	0.76 ± 0.11	0.56 ± 0.05^{b}
0 (Control)		1.20 ± 0.02	16.99 ± 0.52^{e}
Values within the same column with similar superscripts are homogenous			

Table 34. Effect of osmopriming on the vigour indices of sandal seedlings

Osmopriming		Vigor Index I	Vigor Index II
Duration (Days)	Concentration (%)		
3	5	1604.20 ± 27.14^{a}	0.16 ±0.02^{a}
	10	1307.20 ± 51.73^{b}	0.12 ± 0.04^{abc}
	15	1318.20 ± 15.60^{bc}	0.14 ± 0.01^{ab}
	20	1388.40 ± 32.47^{b}	0.15 ± 0.01^{ab}
6	5	1183.20 ± 22.67^{d}	0.10 ± 0.01^{bc}
	10	1257.92 ± 10.79^{cd}	0.12 ± 0.01^{abc}
0 (Control)		840.26 ± 47.82^{e}	0.08 ± 0.08^{c}
Values within the same column with similar superscripts are homogenous			

Table 35 shows the effects of osmopriming on absolute growth rate (F=1.00, p=0.46), relative growth ratio (F=1.00, p=0.46) and net assimilation rate (F=2.75, p=0.05) of sandal seedlings at 180 DAT. The absolute growth rate showed similar values in all osmoprimed seedlings and non-primed seedlings (0.001 cm day^{-1}) obviously indicating that these were all on-par with each other. Similarly, the relative growth rate also showed similar values in both osmoprimed and non-primed seedlings (0.01 g g^{-1} day^{-1}) indicating that the osmopriming treatments could not contribute to the relative growth rate of the seedlings. The values of net assimilation rate also showed that seedlings osmoprimed at 10% for 3 days had highest values

(0.05 g cm^{-2} day^{-1}) whereas, the other treatments recorded values slightly lower than and identical to the value of non-primed seeds.

Table 35. Effect of osmopriming on the growth analysis indices of sandal seedlings

Osmopriming		Absolute Growth Rate (cm day^{-1})	Relative Growth Rate (g g^{-1} day^{-1})	Net Assimilation Rate (g cm^{-2} day^{-1})
Duration (Days)	Concentration (%)			
3	5	0.001 ± 0	0.01 ±0	0.02 ±0.^{b}
	10	0.001 ± 0	0.01 ±0	0.05 ±0^{a}
	15	0.003 ± 0	0.01 ±0	0.03 ±0^{b}
	20	0.001 ± 0	0.01 ±0	0.02 ±0^{b}
6	5	0.002 ± 0	0.01 ±0	0.03 ±0^{ab}
	10	0.003 ± 0	0.01 ±0	0.02 ±0^{b}
0 (Control)		0.004 ± 0	0.01 ±0	0.03 ±0^{ab}
Values within the same column with similar superscripts are homogenous				

4.5.9 Effect of osmopriming on the chlorophyll content of sandal seedlings

The variation in chlorophyll content of the leaves at 180 DAT also showed significant difference due to osmopriming (F=115.26, p=0.01). The average chlorophyll content was lowest in seeds osmoprimed for 3 days at 20% (14.40 mg g^{-1}) and the highest chlorophyll content was recorded in the non-primed seeds (26.90 mg g^{-1}). The only treatments that were on par were the seeds osmoprimed at 15% for 3 days and seeds osmoprimed at 5% for 6 days.

Table 36. Effect of osmopriming on the chlorophyll content of sandal seedlings

Osmopriming		Chlorophyll ($mg\ g^{-1}$)
Duration (Days)	Concentration (%)	
3	5	24.90 ± 0.49^{b}
	10	22.93 ± 0.21^{c}
	15	18.20 ± 0.20^{d}
	20	14.40 ± 0.20^{f}
6	5	18.26 ± 0.76^{d}
	10	15.93 ± 0.46^{e}
0 (Control)		26.90 ± 0.41^{a}
Values within the same column with similar superscripts are homogenous		

4.5.10. Effect of chemical priming on growth attributes and biomass production of sandal seedlings

The effect of chemical priming with $MnSO_4$ on the growth and biomass production of sandal seedlings are presented in Tables 37 to 41. The results indicated that the variation shown due to chemical priming in the shoot height was highly significant at 90 (F=5.88, p=0.01) and at 180 DAT (F=14.49, p=0.01). The highest shoot height was recorded by seeds subjected to chemical priming at 1 M for 3 days (16.76 cm) and the shoot height was the lowest in seeds primed at 0.4 M for 3 days (12 cm). Shoot height of the seeds subjected to chemical priming at 0.4 M for 9 days was found to be on par with that of the seeds subjected to chemical priming at 1 M for 3 days. The results of the shoot height on 180 DAT clearly indicates that the shoot height of the sandal seedlings found to increase with increase in the concentration of $MnSO_4$ for a priming period of 3 days with the maximum shoot height (21.87 cm) obtained from seeds primed at 1 M for 3 days, after which a decreasing trend was followed. It is also evident that, except the seeds primed at 1 M for 3 days and 0.6 M for 9 days, every other treatments belonging to chemical priming has recorded shoot height which was on par with the shoot height of the non-primed seeds. Similar observations were recorded in the collar girth of sandal seedlings from seeds subjected to various chemical priming treatments attributing to a highly significant variation in collar girth at 90 (F=5.39, p=0.01) as well as at 180 DAT (F=21.06, p=0.01). It is evident from the results that at 90 DAT chemical priming with $MnSO_4$ at 1 M concentration at all the four durations imparted an increase in the collar girth of the seedlings. On the other hand, at 180 DAT, the collar girth of the seedlings were found to decrease constantly with an increase in the priming concentration as well

as duration with maximum collar girth recorded in seeds primed at 0.4 M for 3 days (9.07 mm) and the minimum being observed from seeds primed at 1 M for 9 days. However, the collar girth of the primed seeds were found to be higher than that of the non-primed seeds.

The results of the effect of chemical priming on the leaf area and leaf number of sandal seedlings indicated that the leaf area in sandal seedlings showed highly significant variation ($F = 3.16$, $p = 0.01$) while the variation was significant at 5% level ($F = 2.36$, $p = 0.03$) in leaf number per seedling at 90 DAT. The highest leaf area was recorded in seeds subjected to chemical priming at 0.9 M for 9 days (6.94 cm^2) while seeds subjected to chemical priming at 0.4 M for 9 days (2.05 cm^2) recorded the lowest value. The treatment which produced highest leaf area was found to be on par with seeds primed at 0.6m for 9 days, seeds primed at 0.6 M, 0.8 M and 1 M for 3 days, seeds primed at 0.4 M and 0.8 M for 6 days as well as with the leaf area of non-primed seeds. During the 6^{th} month after transplanting, the leaf area of the sandal seedlings which recorded a leaf area of 7.04 cm^2 in seeds primed at 0.4 M for 3 days was found to attain a peak value of 14.73 cm^2 in seeds primed with $MnSO_4$ at 0.4 M for 6 days beyond which a highly significant ($F= 12.26$, $p=0.01$) decrease in the leaf area of the seedlings were noticed with an increase in the priming duration. In addition, the leaf area of the non-primed seeds were found to be on par with the leaf area produced by most of the chemical priming treatments except chemical priming at 0.8 M for 3 days, 0.4 M for 6 days and 1 M for 6 days.

The highest average value of leaf number was recorded in seeds chemical primed at 0.8 M for 9 days (13.33) whereas the lowest record of leaf number was observed in seeds subjected to chemical priming at 1 M for 9 days (7), except which all other treatments recorded a higher leaf number over control (8.33) ($F=3.16$, $p=0.03$). On the other hand, besides the very high significant ($F=5.61$, $p=0.01$) variation in leaf number when observed at 180 DAT due to chemical priming treatments, no definite pattern could be extracted. The duration of the priming process did not affect the number of leaves produced per plant whereas, the leaf number of the primed seeds were found to be higher than that of the leaf number produced by non-primed seeds (13) except the seeds chemical primed at 1 M for 6 days (11) as wells as for 9 days (9.50).

Table 37. Effect of chemical priming on the shoot growth attributes of sandal seedlings at 90 and 180 days after transplanting

Chemical priming		Shoot height (cm)		Collar Girth (mm)		Number of leaves		Leaf Area (cm^2)	
Duration (Days)	Concentration (M)	90 DAT	180 DAT	90 DAT	180 DAT	90 DAT	180 DAT	90 DAT	180 DAT
3	0.4	12.00 ± 0.86^{d}	18.87 ± 0.20^{bcd}	2.58 ± 0.07^{bcd}	9.07 ± 0.18^{a}	10.33 ± 1.20^{bcd}	16.00 ± 0.81^{bcd}	2.77 ± 0.73^{bc}	7.04 ± 0.75^{cd}
	0.6	13.10 ± 0.80^{cd}	19.07 ± 0.33^{bcd}	3.01 ± 0.10^{ab}	8.02 ± 0.06^{b}	11.33 ± 0.66^{abc}	18.25 ± 3.03^{abc}	5.65 ± 1.46^{ab}	8.05 ± 0.33^{c}
	0.8	13.50 ± 0.28^{cd}	19.12 ± 0.23^{bcd}	2.57 ± 0.20^{bcd}	8.15 ± 0.02^{b}	11.66 ± 0.33^{abc}	19.50 ± 1.70^{ab}	5.83 ± 1.08^{ab}	13.85 ± 0.99^{a}
	1.0	16.76 ± 1.21^{a}	21.87 ± 0.20^{a}	2.79 ± 0.15^{abc}	8.08 ± 0.11^{b}	14.00 ± 2.08^{a}	19.00 ± 1.87^{ab}	6.31 ± 1.49^{a}	10.78 ± 1.11^{b}
6	0.4	13.83 ± 0.16^{cd}	19.17 ± 0.08^{bc}	1.72 ± 0.27^{g}	7.21 ± 0.05^{c}	9.00 ± 0.57^{cd}	17.25 ± 1.03^{abcd}	4.68 ± 0.78^{abc}	14.73 ± 1.14^{a}
	0.6	14.40 ± 0.55^{bc}	18.65 ± 0.37^{bcd}	1.73 ± 0.08^{g}	7.43 ± 0.14^{c}	9.66 ± 1.20^{bcd}	15.00 ± 1.58^{bcde}	$2.87 \pm 0..83^{bc}$	6.64 ± 0.77^{cd}
	0.8	14.46 ± 0.37^{bc}	18.82 ± 0.24^{bcd}	2.42 ± 0.13^{cde}	7.38 ± 0.11^{c}	13.00 ± 1.52^{ab}	13.50 ± 1.55^{cdef}	3.69 ± 1.09^{abc}	8.75 ± 0.96^{bc}
	1.0	13.76 ± 0.17^{cd}	18.37 ± 0.31^{bc}	1.91 ± 0.07^{fg}	6.43 ± 0.10^{d}	13.00 ± 1.52^{ab}	11.00 ± 0.70^{ef}	2.83 ± 0.48^{bc}	5.46 ± 0.72^{d}
9	0.4	16.03 ± 0.61^{ab}	19.12 ± 0.22^{bcd}	2.2 ± 0.13^{defg}	5.98 ± 0.10^{e}	10.00 ± 0.57^{bcd}	19.00 ± 0.70^{ab}	2.05 ± 0.85^{c}	7.77 ± 0.57^{cd}
	0.6	14.10 ± 0.47^{bc}	19.42 ± 0.77^{b}	2.03 ± 0.13^{efg}	5.27 ± 0.06^{g}	10.00 ± 0.00^{bcd}	15.00 ± 0.81^{bcde}	6.78 ± 0.96^{a}	8.24 ± 0.57^{c}
	0.8	13.40 ± 0.05^{cd}	18.77 ± 0.13^{bcd}	2.05 ± 0.15^{efg}	5.55 ± 0.18^{fg}	7.00 ± 0.57^{d}	22.00 ± 2.34^{a}	6.94 ± 1.71^{a}	8.64 ± 0.79^{bc}
	1.0	14.20 ± 0.47^{bc}	18.35 ± 0.11^{d}	2.07 ± 0.18^{efg}	5.67 ± 0.11^{ef}	11.66 ± 1.66^{abc}	9.50 ± 0.64^{f}	2.74 ± 0.84^{bc}	6.36 ± 0.38^{cd}
	Control	14.16 ± 1.04^{ab}	18.5 ± 0.20^{cd}	1.79 ± 0.03^{g}	6.33 ± 0.14^{d}	8.33 ± 1.52^{cd}	13.00 ± 0.71^{def}	6.04 ± 1.14^{ab}	8.43 ± 0.58^{bc}
Values within the same column with similar superscripts are homogenous									

With regard to the root length, statistically significant variation was not observed due to the effect of treatments at 90 DAT ($F=1.75$, $p=0.11$) whereas it recorded significant variation at 180 DAT ($F=50.82$, $p=0.01$). During the 3rd month after transplanting, the root length obtained as a result of different chemical priming treatments were found to be on par with that of the non-primed seeds indicating that chemical priming did not induce significant increase in the root length of the sandal seedlings. Although the root length of the seedlings showed highly significant variation at 180 DAT, the seeds subjected to different chemical priming treatments for a duration of 9 days recorded values lower than the non-primed seeds. Contradictory to the highly significant variation observed in number of lateral roots produced among the treatments, the highest lateral root number was recorded by the non-primed seeds (7 nos.). Hence, it can be concluded that the chemical priming of sandal seeds negatively impacted the production of lateral roots in sandal seedlings. On the contrary, the number of lateral roots produced by seedlings did not vary significantly due to chemical priming treatments when observed at 180 DAT ($F=0.91$, $p=0.54$). The number of lateral roots produced by all chemical priming treatments as well as the non-priming treatments were found to be on par.

The seedling length at 90 DAT exhibited significant variation ($F=4.06$, $p=0.01$) due to different treatments with the highest average seedling length (19.93 cm) recorded in seeds subjected to priming at 1 M concentration for 3 days which was found to be on par with the seedling length produced by the seeds subjected to chemical priming at 0.4 M and 0.8 M for 9 days. Keeping these treatments aside, the seedling length obtained from non-primed seeds were higher over that produced by the rest of the chemical priming treatments. The seedling length ($F=98.86$, $p=0.01$) of the sandal seedlings at 180 DAT showed very high significant variation among treatments. The variation in the seedling length of sandal were greatly influenced by chemical priming treatments such that every chemical primed seeds recording a higher seedling length compared to control and the highest being obtained from seeds subjected to chemical priming at 1 M for 3 days (29.1 cm). Eventhough the chemical priming treatments were found to be superior over control, a significantly greater seedling length were produced by seeds primed for a duration of three days and the length decreased with increase in priming duration.

Table 38. Effect of chemical priming on the root growth attributes and seedling length of sandal seedlings at 90 and 180 days after transplanting

Chemical priming		Root Length (cm)		Number of Lateral Roots		Seedling Length	
Duration (Days)	Concentration (M)	90 DAT	180 DAT	90 DAT	180 DAT	90 DAT	180 DAT
3	0.4	3.33 ± 0.33^{b}	8.00 ± 0.04^{a}	2.83 ± 0.16^{cdef}	7.44 ± 0.06	14.83 ± 1.01^{e}	26.87 ± 0.24^{b}
	0.6	3.66 ± 0.33^{b}	8.07 ± 0.04^{a}	3.26 ± 0.26^{bcde}	7.64 ± 0.13	16.36 ± 0.94^{cde}	27.15 ± 0.33^{b}
	0.8	4.66 ± 0.88^{ab}	7.3 ± 0.14^{b}	3.83 ± 0.16^{abc}	7.53 ± 0.06	17.33 ± 0.44^{bcde}	26.42 ± 0.29^{bc}
	1.0	3.33 ± 0.66^{b}	7.22 ± 0.06^{b}	3.16 ± 0.16^{bcde}	7.48 ± 0.16	19.93 ± 1.37^{a}	29.1 ± 0.23^{a}
6	0.4	4.00 ± 1.00^{b}	7.25 ± 0.08^{b}	3.26 ± 0.26^{bcde}	8.13 ± 0.09	17.1 ± 0.37^{bcde}	26.42 ± 0.10^{bc}
	0.6	4.00 ± 0.57^{b}	6.82 ± 0.08^{c}	1.73 ± 0.03^{f}	8.14 ± 0.05	16.13 ± 0.54^{de}	25.47 ± 0.45^{d}
	0.8	4.66 ± 0.33^{ab}	$6.52\ 0.09^{d}$	2.46 ± 0.08^{def}	8.29 ± 0.07	16.93 ± 0.29^{bcde}	25.35 ± 0.26^{de}
	1.0	4.00 ± 0.57^{b}	6.35 ± 0.15^{de}	2.11 ± 0.06^{ef}	8.09 ± 0.12	15.88 ± 0.11^{de}	24.72 ± 0.37^{de}
9	0.4	6.00 ± 0^{ab}	6.47 ± 0.13^{d}	3.36 ± 0.47^{bcd}	8.15 ± 0.0	19.4 ± 0.65^{ab}	25.6 ± 0.35^{cd}
	0.6	5.33 ± 1.45^{ab}	6.12 ± 0.07^{e}	4.73 ± 0.33^{a}	8.22 ± 0.06	18.83 ± 0.56^{abc}	25.55 ± 0.15^{d}
	0.8	4.33 ± 1.20^{b}	6.05 ± 0.02^{e}	2.5 ± 0.96^{def}	8.36 ± 0.09	15.9 ± 1.00^{de}	24.82 ± 0.13^{de}
	1.0	5.00 ± 0.57^{ab}	6.17 ± 0.04^{e}	1.86 ± 0.23^{f}	8.27 ± 0.12	16.06 ± 0.39^{de}	24.52 ± 0.15^{e}
	Control	4.10 ± 0.78^{a}	6.35 ± 0.13^{de}	7.00 ± 2.00^{bc}	6.34 ± 0.14	18.26 ± 1.80^{abcd}	24.85 ± 0.25^{de}
Values within the same column with similar superscripts are homogenous							

The effect of chemical priming on the fresh weight of leaf ($F = 3.52$, $p = 0.01$), shoot ($F = 11.49$, $p = 0.01$), and root ($F = 16.19$, $p = 0.01$) and total fresh weight ($F=17.37$, $p=0.01$) of the sandal seedlings due to different chemical priming treatments exhibited significant variation at 90 DAT (Table 39). While the seeds subjected to chemical priming at 0.8 M for 9 days recorded the highest value of leaf weight (0.29 g), a steep decrease was observed in the shoot weight (0.21 g) and root weight (0.12 g) of these seeds whereas the seeds subjected to chemical priming at 1 M for 3 days exhibited superior results in the fresh weight (Leaf weight=0.28 g, shoot weight=0.27 g and root weight = 0.27 g) of the seedlings over the other treatments and control. The total fresh weight of the seedlings subjected to chemical priming varied from 0.48 g in seeds primed at 0.8 and 1 M for 6 days to 0.83 g in seeds primed at 1 M for 3 days. The total fresh weight of the seedlings subjected to chemical priming at 0.4 M for 3 days was found to be on par with the seeds primed at 0.6, 0.8 and 1 M for 6 days, seeds primed at 0.4, 0.6 and 1 M for 9 days as well as with control. This indicated that the seeds subjected to chemical priming at 1 M for 3 days has positively benefitted the growth of sandal seedlings.

The fresh weight of the leaf ($F=99.50$, $p=0.01$), shoot ($F=42.90$, $p=0.01$), root weight ($F=15.04$, $p=0.01$) and total fresh weight ($F= 51.08$, $p=0.01$) exhibited significant variation among treatments due to chemical priming at 180 DAT. According to the results, the highest average value of leaf weight was observed in seeds primed at 0.4 M for 3 days (1.03 g) and the lowest was observed in seeds primed at 0.8 M for 6 days (0.32 g), whereas the shoot weight recorded the highest value in seeds primed at 0.8 M for 6 days (0.61 g) and the lowest value was recorded in seeds primed at 0.4 M for 3 days (0.36 g). On the other hand, the root weight was highest in seeds primed at 0.8 M for 8 days (0.49 g). Hence, the results indicate that the fresh weight of the seedlings were independent of the chemical priming treatments. Even though the non-primed seedlings recorded the highest total fresh weight (1.82 g), the seedlings subjected to chemical priming at 0.4 M for 3 days recorded value (1.75 g) which was comparative to that of the non-primed seeds and the values were on par. Overall, the chemical priming could not benefit to improve the fresh weight of the seedlings when compared to non-primed seeds.

Table 39. Effect of chemical priming on the fresh weight of sandal seedlings at 90 and 180 days after transplanting

Chemical priming		Fresh Weight (g)							
		Leaf		Shoot		Root		Total	
Duration (Days)	Concentration (M)	90 DAT	180 DAT	90 DAT	180 DAT	90 DAT	180 DAT	90 DAT	180 DAT
3	0.4	0.27 ± 0.01^{abc}	1.03 ± 0.02^{a}	0.21 ± 0^{cd}	0.36 ± 0.00^{f}	0.12 ± 0.01^{cde}	0.36 ± 0.01^{c}	0.60 ± 0.04^{a}	1.75 ± 0.02
	0.6	0.26 ± 0.00^{abc}	0.62 ± 0.01^{d}	0.23 ± 0^{bc}	0.35 ± 0.01^{f}	0.16 ± 0.03^{bc}	0.41 ± 0.01^{b}	0.65 ± 0.02^{ab}	1.39 ± 0.02
	0.8	0.27 ± 0.01^{abc}	0.55 ± 0.01^{e}	0.26 ± 0^{ab}	0.47 ± 0.03^{d}	0.18 ± 0.01^{b}	0.47 ± 0.00^{a}	0.71 ± 0.06^{def}	1.49 ± 0.02
	1.0	0.28 ± 0.00^{ab}	0.50 ± 0.00^{e}	0.27 ± 0^{a}	0.53 ± 0.01^{bc}	0.27 ± 0.00^{a}	0.47 ± 0.00^{a}	0.82 ± 0.04^{cde}	1.51 ± 0.01
6	0.4	0.25 ± 0.02^{abc}	0.41 ± 0.01^{f}	0.19 ± 0^{de}	0.36 ± 0.01^{f}	0.1 ± 0.00^{efg}	0.45 ± 0.01^{b}	0.54 ± 0.04^{g}	1.19 ± 0.01
	0.6	0.24 ± 0.00^{bcd}	0.36 ± 0.01^{fg}	0.18 ± 0^{de}	0.50 ± 0.01^{cd}	0.15 ± 0.00^{bcd}	0.57 ± 0.01^{c}	0.48 ± 0.03^{fg}	1.23 ± 0.01
	0.8	0.24 ± 0.00^{cd}	0.32 ± 0.01^{g}	0.17 ± 0^{e}	0.61 ± 0.00^{a}	0.07 ± 0.00^{g}	0.48 ± 0.00^{c}	$0.57 \pm 0.0.04^{def}$	1.30 ± 0.02
	1.0	0.20 ± 0.01^{d}	0.39 ± 0.00^{f}	0.17 ± 0^{e}	0.36 ± 0.01^{f}	0.10 ± 0.01^{efg}	0.47 ± 0.00^{b}	0.54 ± 0.02^{efg}	1.27 ± 0.02
9	0.4	0.23 ± 0.02^{cd}	0.41 ± 0.01^{f}	0.18 ± 0^{de}	0.41 ± 0.01^{e}	0.13 ± 0.00^{cde}	0.54 ± 0.00^{b}	0.50 ± 0.02^{efg}	1.23 ± 0.01
	0.6	0.26 ± 0.00^{abc}	0.49 ± 0.01^{e}	0.19 ± 0^{de}	0.61 ± 0.01^{a}	0.11 ± 0.00^{def}	0.41 ± 0.01^{b}	0.56 ± 0.02^{efg}	1.52 ± 0.01
	0.8	0.29 ± 0.00^{a}	0.69 ± 0.01^{c}	0.21 ± 0^{cd}	0.49 ± 0.01^{d}	0.12 ± 0.01^{def}	0.49 ± 0.01^{a}	0.62 ± 0.04^{efg}	1.67 ± 0.03
	1.0	0.23 ± 0.01^{cd}	0.72 ± 0.00^{c}	0.19 ± 0^{de}	0.41 ± 0.01^{e}	0.08 ± 0.00^{fg}	0.36 ± 0.01^{c}	0.50 ± 0.03^{efg}	1.50 ± 0.03
	Control	0.27 ± 0.00^{abc}	0.85 ± 0.06^{b}	0.16 ± 00^{e}	0.57 ± 0.00^{b}	0.09 ± 0.01^{efg}	0.40 ± 0.00^{b}	0.53 ± 0.02^{efg}	1.82 ± 0.01
Values within the same column with similar superscripts are homogenous									

The results of effect of chemical priming on the leaf dry weight (F = 3.13, p=0.01), shoot dry weight (F = 2.51, p = 0.02), root dry weight (F = 3.60, p=0.01) and the total dry weight (F = 5.64, p=0.01) of the seedling displayed a significant variation among treatments at 90 DAT. Chemical priming of sandal seeds at 0.6 M for 3 days has resulted in the maximum dry weight of leaf (0.11 g), shoot (0.07 g), root (0.08 g) and the seedling (0.26 g) whereas the seeds chemical primed at 1 M for 6 days recorded the least values of the leaf (0.05 g), shoot (0.05 g) and root (0.04 g) dry weight. Meanwhile the seeds subjected to chemical priming at1 M for 3 days which recorded very high values of the fresh weight recorded a significantly lower value of the seedling dry weight which is shown in Table 39.

The analysis of variance confirmed that the dry weight of the leaf (F=10.30, p=0.001), shoot (F=7.34, p=0.001), root (F=3.46, p=0.002) and the whole seedling (F=14.90, p=0.001) of sandal exhibits significant variation at 180 DAT. The results presented in Table 40 revealed that the highest dry weight of different plant parts were recorded in seeds subjected to chemical priming at 0.8 M for 9 (0.75 g) days followed by seeds subjected to chemical priming at 1 M for 3 days (0.66 g) indicating that the chemical priming of the sandal seeds using $MnSO_4$ at greater duration has contributed to an increase in the dry matter accumulation whereas the priming at lower durations could not contribute to significant increase in the dry matter production when compared to the control.

4.5.11. Effect of chemical priming on the growth analysis indices of sandal seedlings

The specific leaf area of sandal seedlings resulted due to chemical priming exhibited very high significance among the treatments at 90 (F=3.75, p=0.002) and 180 DAT (F=10.84, p=0.001). The peak value of specific leaf area was recorded in seeds primed at 0.6 M for 9 days (106.15 $cm^2 g^{-1}$) which was on par with seeds primed at 0.8 M for 9 days (89.83 $cm^2 g^{-1}$), 1 M for 3 days (76.97 $cm^2 g^{-1}$) and non-primed seeds (101.06 $cm^2 g^{-1}$). At 180 DAT, specific leaf area recorded the highest value in seeds primed at 0.4 M for 6 days (68.41 $cm^2 g^{-1}$) while the lowest value was recorded in seeds primed at 0.4 M for 3 days (26.54 $cm^2 g^{-1}$).

The variation in specific leaf weight was significant at 5% level at 90 DAT (F=2.36, p=0.03) whereas the variations recorded were highly significant at 180 DAT (F=6.44, p=0.01). At the 3rd month, seedlings exposed to chemical priming at 1 M for 6 days recorded the

Table 40. Effect of chemical priming on the dry weight of sandal seedlings at 90 and 180 days after transplanting

Chemical priming		Dry Weight (g)							
		Leaf		Shoot		Root		Total	
Duration (Days)	Concentration (M)	90 DAT	180 DAT	90 DAT	180 DAT	90 DAT	180 DAT	90 DAT	180 DAT
3	0.4	0.05 ± 0.00^{de}	0.26 ± 0.01^{ab}	0.06 ± 0^{bcd}	0.17 ± 0.01^{def}	0.05 ± 0.00^{bcd}	0.16 ± 0.02^{abc}	0.17 ± 0.00^{de}	0.60 ± 0.02^{bc}
	0.6	0.11 ± 0.02^{a}	0.16 ± 0.01^{e}	0.07 ± 0^{abcd}	0.16 ± 0.01^{ef}	0.08 ± 0.00^{a}	0.13 ± 0.00^{c}	0.26 ± 0.03^{a}	0.46 ± 0.00^{f}
	0.8	0.09 ± 0.00^{abc}	0.23 ± 0.01^{bc}	0.07 ± 0^{abc}	0.21 ± 0.00^{bc}	0.06 ± 0.00^{abc}	0.18 ± 0.00^{ab}	0.23 ± 0.01^{ab}	0.64 ± 0.01^{bc}
	1.0	0.08 ± 0.00^{abcd}	0.27 ± 0.01^{ab}	0.07 ± 0.01^{abcd}	0.22 ± 0.01^{b}	0.05 ± 0.00^{bcd}	0.17 ± 0.01^{abc}	0.20 ± 00^{bcd}	0.66 ± 0.01^{b}
6	0.4	0.1 ± 0.00^{ab}	0.21 ± 0.011^{cd}	0.04 ± 0.01^{d}	0.16 ± 0.00^{def}	0.07 ± 0.00^{ab}	0.15 ± 0.01^{bc}	0.21 ± 0.02^{bcd}	0.53 ± 0.00^{de}
	0.6	0.06 ± 0.00^{bcde}	0.25 ± 0.01^{abc}	0.06 ± 0^{bcd}	0.21 ± 0.01^{bc}	0.05 ± 0.00^{bcd}	0.12 ± 0.01^{c}	0.18 ± 0.00^{cde}	0.58 ± 0.02^{cd}
	0.8	0.07 ± 0.00^{bcde}	0.17 ± 0.00^{e}	0.07 ± 0^{abcd}	0.19 ± 0.00^{bcde}	0.03 ± 0.01^{d}	0.13 ± 0.01^{c}	0.17 ± 0.01^{cde}	0.49 ± 0.01^{ef}
	1.0	0.05 ± 0.00^{e}	0.19 ± 0.00^{de}	0.05 ± 0^{bcd}	0.19 ± 0.00^{bcde}	0.04 ± 0.00^{cd}	0.15 ± 0.02^{bc}	0.14 ± 0.00^{e}	0.535 ± 0.02^{de}
9	0.4	0.08 ± 0.00^{abcde}	0.19 ± 0.01^{de}	0.09 ± 0^{a}	0.14 ± 0.01^{f}	0.04 ± 0.01^{cd}	0.14 ± 0.01^{bc}	0.22 ± 0.00^{abc}	0.48 ± 0.03^{ef}
	0.6	0.06 ± 0.00^{cde}	0.25 ± 0.00^{ab}	0.06 ± 0^{bcd}	0.19 ± 0.00^{bcde}	0.05 ± 0.00^{bcd}	0.20 ± 0.01^{a}	0.18 ± 0.00^{cde}	0.65 ± 0.02^{bc}
	0.8	0.07 ± 0.00^{abcde}	0.28 ± 0.01^{a}	0.08 ± 0^{ab}	0.25 ± 0.01^{a}	0.05 ± 0.01^{bcd}	0.21 ± 0.01^{a}	0.21 ± 0.01^{bcd}	0.75 ± 0.02^{a}
	1.0	0.05 ± 0.00^{de}	0.21 ± 0.00^{cd}	0.06 ± 0^{bcd}	0.18 ± 0.00^{cde}	0.04 ± 0.00^{cd}	0.13 ± 0.01^{c}	0.16 ± 0.01^{e}	0.53 ± 0.02^{de}
	Control	0.06 ± 0.01^{bcde}	0.25 ± 0.01^{ab}	0.05 ± 0^{cd}	0.19 ± 0.00^{bcd}	0.06 ± 0.00^{abc}	0.14 ± 0.00^{bc}	0.17 ± 0.00^{cde}	0.60 ± 0.01^{bcd}
Values within the same column with similar superscripts are homogenous									

highest value (0.06 g cm^{-2}) of specific leaf weight, whereas the rest of the treatments found to be on par with the specific leaf weight of non-primed seeds. The specific leaf weight was found to be the highest in seeds subjected to chemical priming at 0.6 M for 6 days (0.04 g cm^{-2}) and was the lowest in seeds primed at 0.4 M for 6 days (0.01 g cm^{-2}) during 6th month.

During 3rd month, leaf area ratio exhibited highly significant variation (F = 3.15, p=0.006) recording the highest value in seeds primed at 0.8 M for 9 days (37.51 cm^2 g^{-1}) and the lowest being recorded by seeds subjected to chemical priming at 0.04 M for 9 days (9.53 cm^2 g^{-1}). Leaf area ratio exhibited a large variation (F=11.37, p=0.01) at 180 DAT in the values ranging from 10.37 in seeds primed at 1 M for 6 days to a peak value of 27.58 in seeds primed at 0.4 M for 6 days. Both the highest (27.58 cm^2 g^{-1}) and lowest values (10.37 cm^2 g^{-1}) of leaf area ratio was found to have highly significant difference from the leaf area ratio of the non-primed seeds (14.03 cm^2 g^{-1}).

On the contrary, the statistically significant variation was not observed in the leaf weight ratio of sandal seedlings due to chemical priming treatments at 90 DAT (F=0.95, p=0.51) as well as 180 DAT (F=1.68, p=0.10). The leaf weight ratio of the sandal seedlings due to different chemical priming treatments were found to be non-significant and were on par to each other as well as with control.

The effect of chemical priming on the root: shoot biomass ratio at 90 DAT and 180 DAT are presented in Table 42. While the root: shoot biomass ratio recorded a non-significant variation at 90 DAT (F=1.58, p=0.15), it recorded a highly significant variation at 180 DAT (F=3.04, p=0.01). During the 6th month, the root: shoot biomass ratio was found to be the highest in seeds primed at 0.6 M for 9 days which was found to be on par with seeds primed at 0.4 M and 0.8 M for 9 days, 0.4 M and 1 M for 6 days as well as with the seeds primed at 0.4 M, 0.6 M and 0.8 M for 3 days.

Table 41. Effect of chemical priming on the growth indices of sandal seedlings at 90 and 180 days after transplanting

Chemical priming		Specific Leaf Area (cm^2g^{-1})		Specific Leaf Weight (gcm^{-2})		Leaf Area Ratio (cm^2g^{-1})		Leaf Weight Ratio	
Duration (Days)	Concentration (M)	90 DAT	180 DAT	90 DAT	180 DAT	90 DAT	180 DAT	90 DAT	180 DAT
3	0.4	47.58 ± 7.84^{cd}	26.54 ± 2.01^{e}	0.02 ± 0^{b}	0.03 ± 0.00^{ab}	15.66 ± 3.36^{de}	11.58 ± 0.93^{de}	0.32 ± 0.02	0.43 ± 0.03
	0.6	51.67 ± 3.59^{bcd}	49.27 ± 2.60^{bc}	0.02 ± 0^{b}	0.02 ± 0.00^{de}	21.11 ± 3.41^{bcde}	17.49 ± 0.57^{bc}	0.41 ± 0.05	0.36 ± 0.02
	0.8	59.17 ± 6.33^{bcd}	58.35 ± 3.55^{ab}	0.01 ± 0^{b}	0.02 ± 0.00^{de}	24.36 ± 3.70^{abcde}	21.65 ± 1.61^{b}	0.40 ± 0.02	0.37 ± 0.02
	1.0	76.97 ± 13.22^{abc}	40.75 ± 5.86^{cd}	0.01 ± 0^{b}	0.02 ± 0.00^{bcd}	30.54 ± 7.13^{abcd}	16.20 ± 1.62^{cd}	0.38 ± 0.02	0.40 ± 0.02
6	0.4	46.40 ± 5.50^{cd}	68.41 ± 2.94^{a}	0.02 ± 0^{b}	0.01 ± 0.00^{e}	21.59 ± 2.30^{bcde}	27.58 ± 2.26^{a}	0.47 ± 0.06	0.40 ± 0.02
	0.6	43.25 ± 10.98^{cd}	27.13 ± 4.41^{e}	0.03 ± 0^{b}	0.04 ± 0.00^{a}	15.94 ± 4.58^{de}	11.24 ± 1.05^{e}	0.36 ± 0.03	0.42 ± 0.0
	0.8	54.03 ± 21.35^{bcd}	50.80 ± 7.54^{bc}	0.01 ± 0^{b}	0.02 ± 0.00^{de}	21.06 ± 6.56^{bcde}	17.92 ± 2.64^{bc}	0.41 ± 0.03	0.35 ± 0.01
	1.0	56.85 ± 8.28^{bcd}	28.89 ± 4.25^{de}	0.06 ± 0^{a}	0.03 ± 0.00^{abc}	19.08 ± 2.22^{cde}	10.37 ± 1.68^{e}	0.34 ± 0.03	0.35 ± 0.01
9	0.4	23.53 ± 9.37^{d}	39.77 ± 3.74^{cde}	0.02 ± 0^{b}	0.02 ± 0.00^{de}	9.53 ± 4.19^{e}	16.18 ± 0.94^{cd}	0.39 ± 0.02	0.41 ± 0.02
	0.6	106.15 ± 9.47^{a}	32.41 ± 2.38^{de}	0.01 ± 0^{b}	0.03 ± 0.00^{abc}	37.51 ± 4.40^{a}	12.65 ± 0.62^{de}	0.35 ± 0.01	0.39 ± 0.01
	0.8	89.83 ± 12.49^{ab}	31.21 ± 3.69^{de}	0.01 ± 0^{b}	0.03 ± 0.00^{abc}	32.65 ± 3.73^{abc}	11.58 ± 1.14^{de}	0.36 ± 0	0.37 ± 0.01
	1.0	47.16 ± 12.79^{cd}	29.53 ± 2.53^{de}	0.02 ± 0^{b}	0.03 ± 0.00^{abc}	17.74 ± 6.40^{cde}	12.05 ± 1.32^{de}	0.35 ± 0.03	0.40 ± 0.01
	Control	101.06 ± 24.67^{a}	33.09 ± 3.15^{de}	0.01 ± 0^{b}	0.03 ± 0.00^{bcd}	34.67 ± 5.40^{ab}	14.03 ± 0.97^{de}	0.37 ± 0.07	0.42 ± 0.01
Values within the same column with similar superscripts are homogenous									

Table 42. Effect of chemical priming on the root: shoot ratio of sandal seedlings at 90 and 180 days after transplanting

Chemical priming		Root : Shoot Ratio	
Duration (Days)	Concentration (M)	90 DAT	180 DAT
3	0.4	0.84 ± 0.08	0.98 ± 0.12^{ab}
	0.6	1.14 ± 0.08	0.85 ± 0.09^{abc}
	0.8	0.86 ± 0.15	0.87 ±0.04^{abc}
	1.0	0.89 ± 0.2	0.75 ± 0.06^{bcd}
6	0.4	2.77 ± 1.61	0.92 ± 0.11^{abc}
	0.6	0.91 ± 0.14	0.57 ± 0.06^{d}
	0.8	0.44 ± 0.20	0.68 ± 0.06^{cd}
	1.0	0.81 ± 0.01	0.81 ± 0.09^{abcd}
9	0.4	0.45 ± 0.15	0.98 ± 0.05^{ab}
	0.6	0.85 ± 0.14	1.07 ± 0.03^{a}
	0.8	0.69 ± 0.18	0.83 ± 0.08^{abcd}
	1.0	0.62 ± 0.04	0.70 ± 0.08^{cd}
	(0) Control	1.21 ± 0.02	0.74 ± 0.03^{bcd}
Values within the same column with similar superscripts are homogenous			

The vigour index I (F=180.53, p=0.01) and vigour index II (F=98.42, p=0.01) have recorded highly significant variation due to chemical priming (Table 43). The highest vigour index I was recorded in seedlings subjected to chemical priming at 1 M for 3 days (1714.26) and the lowest being recorded in seeds primed at 0.8 M for 9 days (21.14) indicating that the chemical priming treatments at 3 days could only impart an increase in the vigour of seedling growth. The seeds subjected to chemical priming at 0.6 M for 3 days (0.22) recorded the peak value of vigour index II. In general, most of the chemical priming treatments were found unsuitable to increase the vigour of seedling growth of sandal.

The results of the effect of chemical priming on the growth indices such as absolute growth rate (F=2.96, p=0.01), relative growth rate (F=0.93, p=0.53) and net assimilation rate (F=1.58, p=0.15) of the sandal seedlings are presented in Table 44. The absolute growth rate of the sandal seedlings recorded the highest value in seedlings subjected to chemical priming at 0.8 M for 9 days (0.008 cm day^{-1}). Most of the treatments resulted in similar growth rate of the seedlings. The relative growth rate and net assimilation rate of the seedlings subjected to chemical priming were at par.

Table 43. Effect of chemical priming on the vigour indices of sandal seedlings

Chemical priming		Vigor Index I	Vigor Index II
Duration (Days)	Concentration (M)		
3	0.4	1285.60 ± 87.86^{c}	0.15 ± 0.00^{c}
	0.6	1418.50 ± 82.27^{bc}	0.22 ± 0.02^{a}
	0.8	1525.33 ± 38.80^{b}	0.20 ± 0.01^{a}
	1.0	1714.26 ± 118.16^{a}	0.17 ± 0.00^{b}
6	0.4	205.20 ± 4.50^{ef}	0.02 ± 0.00^{ef}
	0.6	64.53 ± 2.16^{fg}	0.01 ± 0.00^{ef}
	0.8	67.53 ± 1.16^{fg}	0.01 ± 0.00^{ef}
	1.0	42.40 ± 0.29^{g}	0.00 ± 0.00^{f}
9	0.4	310.40 ± 10.41^{e}	0.03 ± 0.00^{e}
	0.6	37.66 ± 1.13^{g}	0.00 ± 0.00^{f}
	0.8	21.14 ± 1.34^{g}	0.00 ± 0.00^{f}
	1.0	21.37 ± 0.52^{g}	0.00 ± 0.00^{f}
	(0) Control	840.26 ± 47.82^{d}	0.08 ± 0.08^{d}
Values within the same column with similar superscripts are homogenous			

Table 44. Effect of chemical priming on the growth analysis indices of sandal seedlings

Chemical Priming		Absolute Growth Rate (cm day^{-1})	Relative Growth Rate (g g^{-1} day^{-1})	Net Assimilation Rate (g cm^{-2} day^{-1})
Duration (Days)	Concentration (M)			
3	0.4	0.003 ± 0^{ab}	0.01 ± 0	0.03 ± 0^{ab}
	0.6	0.002 ± 0^{b}	0.00 ± 0	0.02 ± 0^{b}
	0.8	0.005 ± 0^{ab}	0.01 ± 0	0.03 ± 0^{ab}
	1.0	0.004 ± 0^{ab}	0.01 ± 0	0.03 ± 0^{ab}
6	0.4	0.002 ± 0^{b}	0.01 ± 0	0.02 ± 0^{ab}
	0.6	0.002 ± 0^{b}	0.01 ± 0	0.03 ± 0^{ab}
	0.8	0.002 ± 0^{b}	0.01 ± 0	0.03 ± 0^{ab}
	1.0	0.002 ± 0^{ab}	0.01 ± 0	0.04 ± 0^{a}
9	0.4	0.002 ± 0^{b}	0.01 ± 0	0.02 ± 0^{ab}
	0.6	0.002 ± 0^{b}	0.01 ± 0	0.03 ± 0^{ab}
	0.8	0.006 ± 0^{a}	0.01 ± 0	0.03 ± 0^{ab}
	1.0	0.002 ± 0^{b}	0.01 ± 0	0.03 ± 0^{ab}
	(0) Control	0.004 ± 0^{b}	0.01 ± 0	0.03 ± 0^{ab}
Values within the same column with similar superscripts are homogenous				

4.5.11. Effect of chemical priming on the chlorophyll content of sandal seedlings

The highly significant (F=223.45, p=0.01) variation observed in Chlorophyll content of the leaves followed a non-uniform trend, however the seeds subjected to different chemical priming treatments for a period of 9 days exhibited a significantly lower chlorophyll content compared to control (Table 45). The chlorophyll content was the highest in the seedlings subjected to chemical priming at 0.8 M for 3 days.

Table 45. Effect of chemical priming on the shoot growth attributes of sandal seedlings

Chemical priming		Chlorophyll (mg g^{-1})
Duration (Days)	Concentration (M)	
3	0.4	25.20 ± 0.52^{e}
	0.6	25.00 ± 0.52^{e}
	0.8	40.46 ± 0.66^{a}
	1.0	22.46 ± 0.27^{f}
6	0.4	33.80 ± 0.25^{b}
	0.6	31.46 ± 0.26^{c}
	0.8	35.13 ± 0.57^{b}
	1.0	35.03 ± 0.26^{b}
9	0.4	25.16 ± 0.58^{e}
	0.6	22.20 ± 0.60^{f}
	0.8	16.96 ± 0.52^{h}
	1.0	18.70 ± 0.36^{g}
	(0) Control	26.90 ± 0.41^{d}
Values within the same column with similar superscripts are homogenous		

4.6 Results of Z- test

The Z- test was conducted to understand the significant difference in the growth attributes of sandal seedlings at 90 and 180 DAT. The results are presented in the Table 46. The results indicate that all the growth parameters except leaf fresh weight of the seedlings exhibit very high significant variation among treatments at 90 DAT and 180 DAT due to different priming treatments.

Table 46. Results of Z- test

Parameters	Z-test value	p- value
Shoot height	15.22	<0.01
Collar girth	35.70	<0.01
Leaf area	9.27	< 0.01
Leaf number	14.23	<0.01
Root length	16.90	< 0.01
Root number	10.20	<0.01
Seedling length	17.71	<0.01
Leaf fresh weight	1.63	0.10
Shoot fresh weight	18.61	< 0.01
Root fresh weight	35.69	<0.01
Total fresh weight	42.31	<0.01
Leaf dry Weight	22.66	<0.01
Shoot dry Weight	25.62	< 0.01
Root dry Weight	20.00	<0.01
Total Dry Weight	27.27	<0.01

4.7. Relationship among the seed biochemical constituents and seedling attributes of sandal due to different priming techniques

Correlation matrix was laid out among the seed biochemical constituents and seedling attributes of sandal for different priming techniques and the results are presented in the following sections.

4.7.1. Correlation between biochemical constituents of hydroprimed seeds with the seedling growth attributes in sandal

Table 47 depicts the results of correlation analysis between biochemical constituents of hydroprimed seeds with the seedling growth attributes of sandal at 90 and 180 DAT. The carbohydrate content of the seeds recorded a positive significant correlation with shoot height (r=0.832), leaf area (r=0.723) and leaf number (r=0.723) of sandal seedlings at 90 DAT. A highly significant positive correlation with these attributes were also recorded at 180 after transplanting also indicating that the total carbohydrate strongly contributes to the leaf area, leaf number and shoot height of the sandal seedlings. Significant positive correlation in the parameters like root length (r=0.708), number of lateral roots (0.882), shoot fresh weight (r=0.716) and leaf dry weight (0.715) with carbohydrate content was observed at 180 DAT.

The correlation among the protein content of the seed and the seedling growth attributes of sandal seedlings was mostly negative in hydropriming. There existed significant negative

correlation among shoot height(r=-0.707), leaf area (r=-0.712), and leaf dry weight (r=-0.739) and protein content at 90 DAT, whereas, at 180 DAT number of lateral roots (r=-0.807) also was negatively correlated with protein content. Leaf dry weight did not show any significant correlation with protein content at 180 DAT.

The correlation among the crude fat of the seeds and the seedling growth attributes were mostly positive at both intervals. During the 3rd month, the root length (r=0.958) and shoot dry weight (r=0.849) recorded strong positive correlation indicating that the crude fat greatly contributes to an increase in root length of the seedlings subjected to hydropriming. Meanwhile the crude fat content was strongly correlated with root weight as well as seedling dry weight revealing that the increase in crude fat content leads to a significant increase in the root weight and seedling dry weight of sandal.

Table 47. Correlation between biochemical constituents of hydroprimed seeds with seedling growth attributes

Growth parameters	Days after transplanting					
	90			180		
	Carbohydrate	Protein	Crude Fat	Carbohydrate	Protein	Crude Fat
Shoot Height	0.832*	-0.707*	0.693	0.854**	-0.818*	0.607
Collar Girth	0.170	-0.039	-0.417	-0.089	0.088	-0.459
Leaf Area	0.723*	-0.712*	0.648	0.917**	-0.806*	0.547
Leaf Number	0.729*	-0.595	0.514	0.863**	-0.684	0.609
Root Length	0.331	-0.403	0.958**	0.708*	-0.386	0.489
No. of Lateral Roots	0.615	-0.416	0.499	0.882**	-0.807*	0.625
Leaf Fresh Weight	-0.200	0.143	0.760*	0.494	-0.330	-0.154
Shoot Fresh Weight	-0.351	0.394	0.366	0.716*	-0.605	-0.021
Root Fresh Weight	0.060	-0.162	0.114	0.538	-0.409	0.944**
Leaf Dry Weight	0.489	-0.739*	0.660	0.715*	-0.610	-0.073
Shoot Dry Weight	0.534	-0.224	0.849**	0.352	-0.321	0.945**
Root Dry weight	0.222	-0.231	0.517	0.607	-0.676	0.823*

** - highly significant at 5% level of significance
* - significant at 5% level of significance

4.7.2. Correlation between biochemical constituents of bioprimed seeds with the seedling growth attributes in sandal

The results of correlation analysis between carbohydrate content of seeds subjected to biopriming and the seedling growth attributes differed from that of hydropriming. From Table 48 it can be concluded that every seedling growth attributes except leaf number, root length and shoot dry weight are negatively correlated to carbohydrate at 90 DAT, although the correlation was not significant. The shoot height (r=-0.466) and root dry weight (r=-.415) showed significant negative correlation with carbohydrate content of the seeds at 90 DAT whereas, leaf number (r= .387) showed a positive correlation and the degree of correlation was less compared to hydropriming. The degree of correlation increased with period and at 180 DAT, correlation existed among more seedling parameter. On the contrary, the root length which recorded a positive correlation with carbohydrate at 90 DAT exhibited a highly significant negative correlation at 180 DAT. The shoot dry weight also recorded similar correlation as that of root length. The parameters like shoot height, root length, number of lateral roots, leaf fresh weight, shoot fresh weight, leaf dry weight, shoot dry weight and root dry weight of the seedlings were negatively correlated with carbohydrate content of the primed seeds while leaf number showed positive correlation and the correlation coefficients were slightly higher compared to initial values. There was no significant correlation recorded between the protein content and the seedling growth attributes at 90 DAT. But at 180 days after transplanting, the root length, shoot fresh weight and leaf fresh weight, leaf dry weight and shoot dry weight recorded significant negative correlation with the protein content of the seeds. The correlation was weak between the crude fat content of the seeds and the seedling growth attributes at 90 and 180 DAT. At 90 DAT, the leaf number and leaf weight displayed a significant positive and weak correlation whereas at 180 DAT, the leaf number recorded slightly higher positive significant correlation while the root weight recorded a negative significant correlation with crude fat content of the seeds.

Table 48. Correlation between biochemical constituents of bioprimed seeds with seedling growth attributes in sandal

Growth parameters	Days after transplanting					
	90			180		
	Carbohydrate	Protein	Crude Fat	Carbohydrate	Protein	Crude Fat
Shoot Height	-0.466**	-0.004	0.247	-0.603**	-0.271	0.158
Collar Girth	-0.146	-0.110	0.307	0.057	0.152	-0.118
Leaf Area	-0.122	-0.006	0.155	-0.324	0.04	0.231
Leaf Number	0.387*	-0.051	0.363*	0.459**	0.341	0.367*
Root Length	0.212	0.288	-0.093	-0.560**	-0.447*	0.163
No. of Lateral Roots	-0.342	-0.186	0.317	-0.626**	-0.349	0.169
Leaf Fresh Weight	-0.212	-0.195	0.422*	-0.504**	-0.364*	-0.146
Shoot Fresh Weight	-0.123	0.041	0.177	-0.470**	-0.587**	-0.186
Root Fresh Weight	-0.19	0.092	0.349	-0.238	-0.140	-0.447*
Leaf Dry Weight	-0.311	-0.247	0.347	-0.523**	-0.548**	-0.292
Shoot Dry Weight	0.028	0.064	0.083	-0.501**	-0.399*	-0.247
Root Dry weight	-0.415*	-0.290	0.163	-0.381*	-0.272	-0.127

** - significant at 1% level of significance
* - significant at 5% level of significance

4.7.3. Correlation between biochemical constituents of osmoprimed seeds with the seedling growth attributes in sandal

The results of the correlation between the biochemical constituents of osmoprimed seeds and the seedling growth attributes showed only meager correlations at 90 DAT (Table 49). Correlation analysis indicated that the shoot fresh weight (r=0.690) recorded a significant positive correlation with carbohydrate content whereas, the shoot height (r=0.593) was the only parameter which recorded a positive significant correlation with protein. On the other hand, the crude fat displayed a negative correlation with the growth attributes of the seedlings wherein the leaf number recorded significant negative correlation and shoot height (r=-0.812) recorded a highly significant strong negative correlation indicating that the shoot height of the seedlings marked significant decrease with an increase in the crude fat content at 90 DAT. At 180 DAT, the leaf (r=0.590), shoot (r=0.881) and root dry weight (r=0.630) recorded a highly significant strong positive correlation with seed carbohydrate content concluding that carbohydrate greatly contributes to an increase in the shoot dry weight of the seedlings. Meanwhile, leaf fresh weight of the seeds was

the only parameter showed some degree of correlation with protein content (r=0.612) and it was positive. Contrary to the results of carbohydrate and protein the crude fat recorded to exhibit highly significant strong negative correlation with leaf number, shoot height, number of lateral roots produced, leaf weight, shoot weight, leaf dry weight and root dry weight indicating that the seedlings obtained from the seeds subjected to osmopriming were negatively affected with an increase in the crude fat content in the seed.

Table 49. Correlation between biochemical constituents of osmoprimed seeds with seedling growth attributes in sandal

Growth parameters	Days after transplanting					
	90			180		
	Carbohydrate	Protein	Crude Fat	Carbohydrate	Protein	Crude Fat
Shoot Height	0.453	0.593*	-0.812**	0.535	0.322	-0.808**
Collar Girth	-0.022	-0.182	0.039	-0.542	-0.254	0.400
Leaf Area	0.022	-0.020	-0.167	-0.537	-0.220	0.174
Leaf Number	-0.167	0.383	-0.632*	0.439	0.452	-0.827**
Root Length	-0.228	-0.04	-0.127	0.487	0.108	-0.633*
No. of Lateral Roots	0.101	0.224	-0.034	0.539	0.257	-0.777**
Leaf Fresh Weight	0.284	-0.165	-0.356	0.480	0.612*	-0.711**
Shoot Fresh Weight	0.690*	-0.101	-0.469	0.544	0.530	-0.783**
Root Fresh Weight	0.162	-0.562	0.032	0.179	-0.156	0.073
Leaf Dry Weight	-0.021	0.513	-0.299	0.590*	0.491	-0.798**
Shoot Dry Weight	0.184	-0.378	-0.395	0.881**	0.070	-0.574
Root Dry weight	0.354	0.060	-0.569	0.630*	0.358	-0.713**

** - significant at 1% level of significance
* - significant at 5% level of significance

4.7.4. Correlation between biochemical constituents of chemically primed seeds with the seedling growth attributes in sandal

The Table 50 presents the result of correlation analysis between the biochemical constituents of seeds subjected to chemical priming and the seedling growth attributes. The results revealed that the seedling growth attributes of sandal showed negligible correlation with the biochemical constituents of seeds with a few exceptions. The carbohydrate content did not show any significant correlations at 90 DAT, whereas, it showed a highly significant negative correlation with leaf area (r=-0.644) and a significant negative correlation with leaf number (r=-0.471) at 180 DAT. Meanwhile, the seedling growth was least affected by an increase or decrease in the protein content at 90 DAT as well as at 180 DAT. The shoot height (r=-0.467) recorded a significant

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negative correlation with crude fat at 90 DAT whereas it recorded a significant positive correlation with the shoot fresh weight and leaf dry weight at 180 DAT.

Table 50. Correlation between biochemical constituents of sandal seeds primed with $MnSO_4$ with seedling growth attributes

Growth parameters	Days after transplanting					
	90			180		
	Carbohydrate	Protein	Crude Fat	Carbohydrate	Protein	Crude Fat
Shoot Height	-0.192	0.044	-0.467*	-0.209	0.241	-0.369
Collar Girth	-0.106	0.270	-0.068	-0.018	0.101	-0.326
Leaf Area	-0.073	0.059	0.264	-0.644**	0.396	0.112
Leaf Number	-0.306	0.365	0.102	-0.471*	0.028	0.002
Root Length	-0.219	0.404	0.019	0.084	0.101	0.109
No. of Lateral Roots	-0.124	-0.346	-0.178	-0.104	0.227	-0.205
Leaf Fresh Weight	-0.095	0.166	0.228	0.109	0.262	-0.047
Shoot Fresh Weight	0.046	0.326	0.213	0.449*	-0.122	0.615**
Root Fresh Weight	-0.068	0.199	-0.128	0.025	0.249	-0.481*
Leaf Dry Weight	-0.310	-0.011	0.164	0.392	0.022	0.519**
Shoot Dry Weight	-0.235	0.291	-0.059	-0.055	0.084	0.322
Root Dry weight	-0.164	-0.208	0.353	0.248	0.176	0.153

** - significant at 1% level of significance
* - significant at 5% level of significance

4.8. Principal Component Analysis to group the treatment combinations

Principal Component Analysis, a dimension reduction technique was conducted for the 12 seedling growth attributes of sandal at 90 and 180 DAT. The principal component are extracted based on the Eigen values criteria. From the present study, 12 inter-dependent variables transformed to two independent principal components which showed Eigen value greater than 1 were extracted. In both the cases, the first component accounted for the 99 percentage of the variance and the second component accounted for what was left over. Conjointly the two principal components explained 100 per cent of the cumulated variability for the characters studied at 90 as well as 180 DAT which is presented in Tables 51 and 52.

Table 51. Total variance explained by different principal components at 90 days after transplanting

Principal Component	Eigen Value	Percent Variability	Cumulative Percent Variability
PC1	4.64	99.00	99.00
PC2	1.79	1.00	100.00

Table 52. Total variance explained by different principal components at 180 days after transplanting

Principal Component	Eigen Value	Percent Variability	Cumulative Percent Variability
PC1	5.23	99.00	99.00
PC2	1.74	1.00	100.00

Table 53 represents the factor loadings of different seedling growth attributes of sandal at 90 DAT. Growth attributes of the seedlings such as leaf area, root number, collar girth, leaf weight, shoot weight, root weight, leaf dry weight and root dry weight had high loadings on the first principal component (PC1). The second principal component was found to have high loading from attributes like leaf number, shoot height, root length, and shoot dry weight. The characters like root number, collar girth, leaf weight, shoot weight, root weight and root dry weight was found to have negative loading on PC2. Amidst the two principal components, PC1 can be explicated as the biomass traits factor, because it and PC2 can be interpreted as the seedling length factor.

Table 53. Factor Loadings of Different Growth Attributes at 90 DAT

Sl No.	Variables	PC1	PC2
1	Shoot height	0.251	0.330
2	Collar Girth	0.340	-0.259
3	Leaf Area	0.286	0.267
4	Leaf Number	0.261	0.404
5	Root Length	0.142	0.331
6	Root Number	0.286	-0.128
7	Leaf Weight	0.380	-0.280
8	Shoot Weight	0.273	-0.217
9	Root Weight	0.355	-0.164
10	Leaf Dry Weight	0.311	0.120
11	Shoot Dry Weight	0.165	0.483
12	Root Dry Weight	0.318	-0.253

Figure 13 represents the distribution of seedling growth attributes with respect to the principal component 1 and principal component 2. It was observed that PC1 was showing high loading for leaf area, leaf dry weight, root weight and root dry weight whereas shoot dry weight was positioned away from PC1 in the graph. The position of leaf area from the two components

had no much difference. Meanwhile, the PC2 had high factor loading for leaf number, shoot height, and root length which were contributing greatly to the seedling length of sandal.

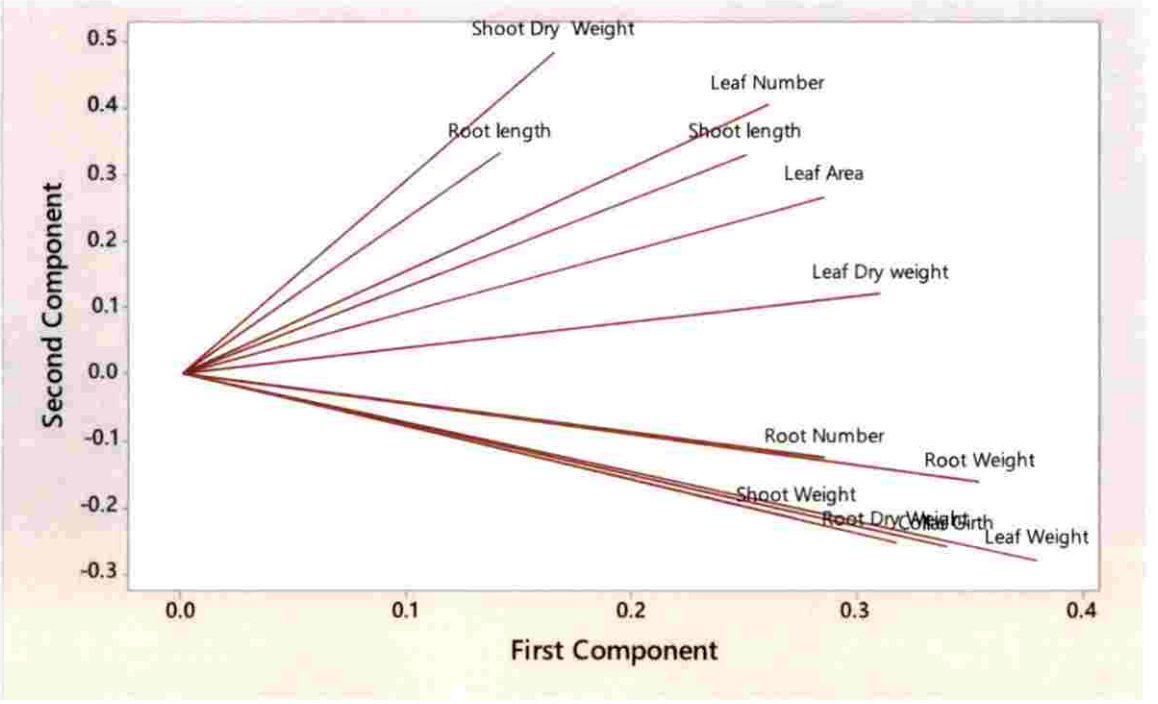

Figure 13. Distribution of seedling growth attributes based on PC1 and PC 2 at 90 DAT

Table 54 depicts the factor loadings of different seedling growth attributes of sandal at 180 DAT. Contrary to the results obtained at 90 DAT, the growth attributes of the seedlings which had high loadings on PC1 were root number, collar girth, leaf weight, shoot weight, root weight, leaf dry weight, shoot dry weight and root dry weight. The second principal component was found to have high loading from attributes like leaf area and leaf number. The characters like root length, leaf weight, shoot weight, root weight and shoot dry weight was found to have negative loading on PC2. Amidst the two principal components, PC1 can be explicated as the biomass traits factor, because it and PC2 can be interpreted as the seedling growth factor.

Although characters were reduced to two components at 180 DAT similar to that of 90 DAT, the variation of the growth attributes at 6th month were greatly explained by PC1. It can be interpreted from the Figure 16, that the PC1 had high loading for characters which determined the seedling height as well as the biomass production. On the other hand, at 180 DAT PC2 had high loading for leaf parameters like leaf area and leaf number.

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Table 54. Factor Loadings of Different Growth Attributes at 180 days after transplanting

Sl No.	Variables	PC1	PC2
1	Shoot Height	0.381	0.035
2	Collar Girth	0.358	0.129
3	Leaf Area	0.224	0.347
4	Leaf Number	0.207	0.360
5	Root Length	0.383	-0.060
6	Root Number	0.164	0.010
7	Leaf Weight	0.144	-0.415
8	Shoot Weight	0.102	-0.616
9	Root Weight	0.180	-0.379
10	Leaf Dry Weight	0.358	0.055
11	Shoot Dry Weight	0.347	-0.150
12	Root Dry Weight	0.384	0.086

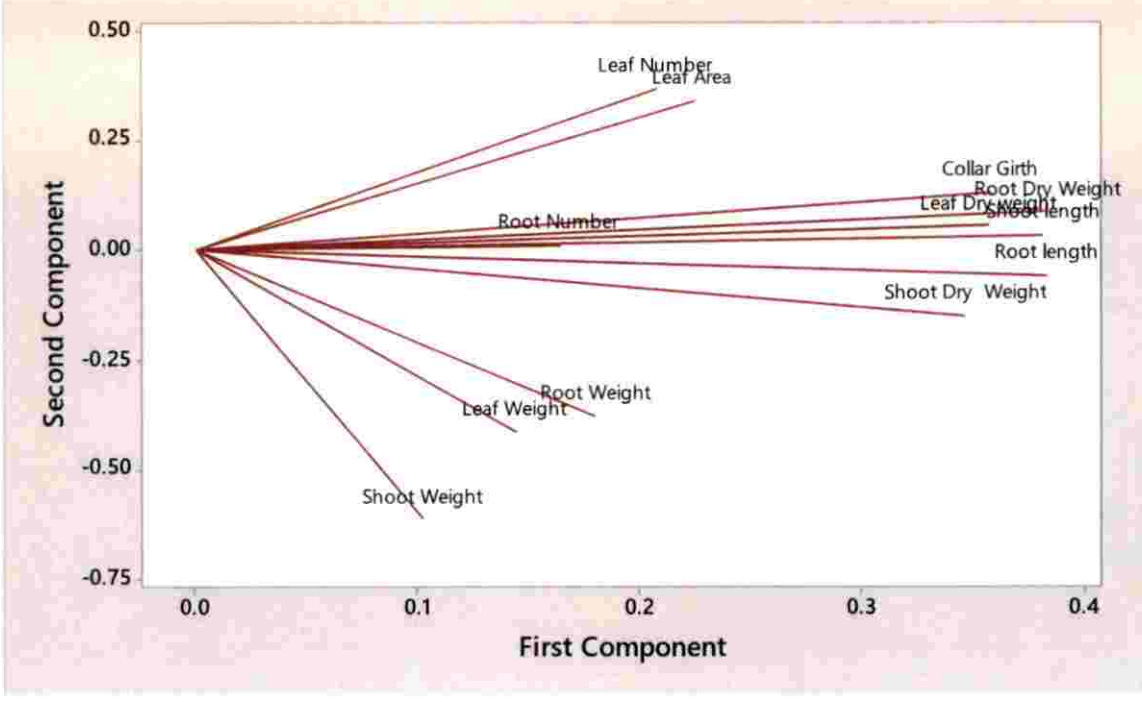


Figure 14. Distribution of seedling growth attributes based on PC1 and PC 2 at days after transplanting

4.9 Cluster Analysis

In order to group the treatment combinations, they were subjected to cluster analysis to identify the most superior treatment.

4.9.1 Cluster analysis of treatments based on seedling growth attributes at 90 days after transplanting

Hierarchical cluster analysis was conducted to group the 39 treatments (including control) which led to the formation of 16 clusters at 90 DAT. The cluster XV had the maximum number of treatments (5), followed by cluster VI and cluster VII (4). Cluster II, VII, X, XI and XIII contained only one treatment in each cluster (Table 55). Interpretation of cluster wise means for each attribute indicated that cluster XIV recorded the highest mean values for shoot height (17.91), root number (6.66), collar girth (3.05), leaf weight (0.32), root weight (0.23), leaf dry weight (0.09) and root dry weight (0.07). Mean values of leaf area (9.98) and shoot dry weight (0.09) were highest in cluster XIII whereas leaf number (13.00) recorded highest value in cluster X. Root length recorded highest mean value in cluster IX (4.73) and shoot weight was highest in cluster IV.

The treatments within a cluster are said to have minimal variation and the variations between the treatments in two different clusters will be greater. Hence from the table it can be interpreted that the treatments biopriming at 25% for 4 days (cluster III), biopriming at 100% for 6 days (cluster VIII), chemical priming at 0.4 M for 3 days (cluster X), biopriming at 50% for 4 days (cluster XI) and biopriming at 100% for 4 days (cluster XV) will exhibit large variation among themselves as well as the treatments belonging to other clusters. Limited variation was found to occur between seedlings of control as well as the seedlings subjected to osmopriming at 10% for 3 days.

4.9.2 Cluster analysis of treatments based on seedling growth attributes at 180 days after transplanting

Hierarchical cluster analysis was conducted to group the 39 treatments (including control) which led to the formation of 16 clusters at 180 DAT. The cluster VII had the maximum number of treatments (5), followed by cluster XVI (4). Cluster I, II and IX contained only one treatment in each cluster (Table 56). It can be revealed from the mean values of attributes in each cluster that, the treatments followed a trend which was contradictory to that found on 90th DAT.

Table 55. Clusters of seedling growth attributes of sandal at 90 days after transplanting

Cluster Number	Treatments
Cluster I	Hydropriming 3 days, Biopriming at 25% for 6 days and Chemical priming at 1 M for 3 days
Cluster II	Biopriming at 25% for 4 days
Cluster III	Biopriming at 25% for 2 days and Biopriming at 100% for 8 days
Cluster IV	Biopriming at 50% for 8 days and Biopriming at 75% for 8 days
Cluster V	Biopriming at 50% for 2 days, Chemical priming at 0.6 M for 9 days and Biopriming at 25% for 8 days
Cluster VI	Biopriming at 75% for 2 days, Chemical priming at 0.8M for 3 days, Chemical priming at 0.4 M for 6 days and Osmopriming at 15% for 3 days
Cluster VII	Biopriming at 100% for 2 days, Biopriming at 75% for 4 days, Chemical priming at 0.8 M for 9 days and Osmopriming at 20% for 3 days
Cluster VIII	Biopriming at 100% for 6 days
Cluster IX	Hydropriming 6 days and Chemical priming at 0.6 M for 3 days
Cluster X	Chemical priming at 0.4 M for 3 days
Cluster XI	Biopriming at 50% for 4 days
Cluster XII	Biopriming at 50% for 6 days, Chemical priming at 0.6M for 6 days and Chemical priming at 0.8 M for 6 days
Cluster XIII	Biopriming at 100% for 4 days
Cluster XIV	Chemical priming at 0.4 M for 9 days, Osmopriming at 5% for 3 days
Cluster XV	Biopriming at 75% for 6 days, Chemical priming at 1 M for 6 days, Chemical priming at 1 M for 9 days, Osmopriming at 5% for 6 days, Osmopriming at 10% for 6 days
Cluster XVI	Osmopriming at 10% for 3 days, Control

The mean values of attributes at 180 DAT revealed that cluster XII recorded the highest mean values for shoot height (23.92), root length (7.80), collar girth (8.60), leaf dry weight (0.35) and shoot dry weight (0.21) whereas cluster XIV recorded high mean values for leaf area (14.29) and root weight (0.44). Cluster II, IV, V and XI recorded highest mean values for leaf weight (0.82), root number (3.62), leaf number (21.37) and shoot weight (0.62) and shoot dry weight (0.21), respectively.

The grouping of treatments based on the seedling performance at 180 DAT indicated that hydropriming for 3 days (Cluster I), hydropriming for 6 days (Cluster II), biopriming at 50% for 4 days (Cluster IX) had the maximum variation due to the individual grouping these treatments. Contrary to the results of 90 DAT, the grouping of treatments at 180 DAT indicated that the seedlings of control recorded least variation with seedlings subjected to chemical priming at 0.8 M for 6 days and 0.6 M for 9 days. These results further indicate that during 3rd month control was found to be on par with one of the treatments which appeared to be superior in performance,

whereas at 6th month, the priming treatments performed better over control grouping it with poor performing treatments.

Table 56. Clusters of seedling growth attributes of sandal at 180 days after transplanting

Cluster Number	Treatments
Cluster I	Hydropriming 3 days
Cluster II	Hydropriming 6 days
Cluster III	Biopriming at 25% for 2 days and Biopriming at 25% for 6 days
Cluster IV	Biopriming at 50% for 2 days and Chemical priming at 0.8 M for 9 days
Cluster V	Biopriming at 75% for 2 days, Chemical priming at 0.6 M for 3 days and Chemical priming at 0.4 M for 9 days
Cluster VI	Biopriming at 100% for 2 days, Biopriming at 25% for 4 days and Osmopriming at 20% for 3 days
Cluster VII	Biopriming at 100% for 4 days, Chemical priming at 0.4 M for 3 days, Biopriming at 50% for 6 days, Chemical priming at 0.6 M for 6 days and Osmopriming at 15% for 3 days
Cluster VIII	Chemical priming at 0.8M for 6 days, Control and Chemical priming at 0.6 M for 9 days
Cluster IX	Biopriming at 50% for 4 days
Cluster X	Biopriming at 75% for 6 days and Osmopriming at 5% for 3 days
Cluster XI	Biopriming at 75% for 4 days, Biopriming at 25% for 8 days and Osmopriming at 10% for 3 days
Cluster XII	Biopriming at 75% for 8 days and Chemical priming at 1 M for 3 days
Cluster XIII	Biopriming at 25% for 8 days, Biopriming at 50% for 8 days and Biopriming at 100% for 8 days
Cluster XIV	Chemical priming at 0.8 M for 3 days and Chemical priming at 0.4 M for 6 days
Cluster XV	Hydropriming 9 days and Hydropriming 12 days
Cluster XVI	Chemical priming at 0.4 M for 9 days, Osmopriming at 10% for 6 days, Osmopriming at 10% for 6 days and Chemical priming at 1 M for 9 days

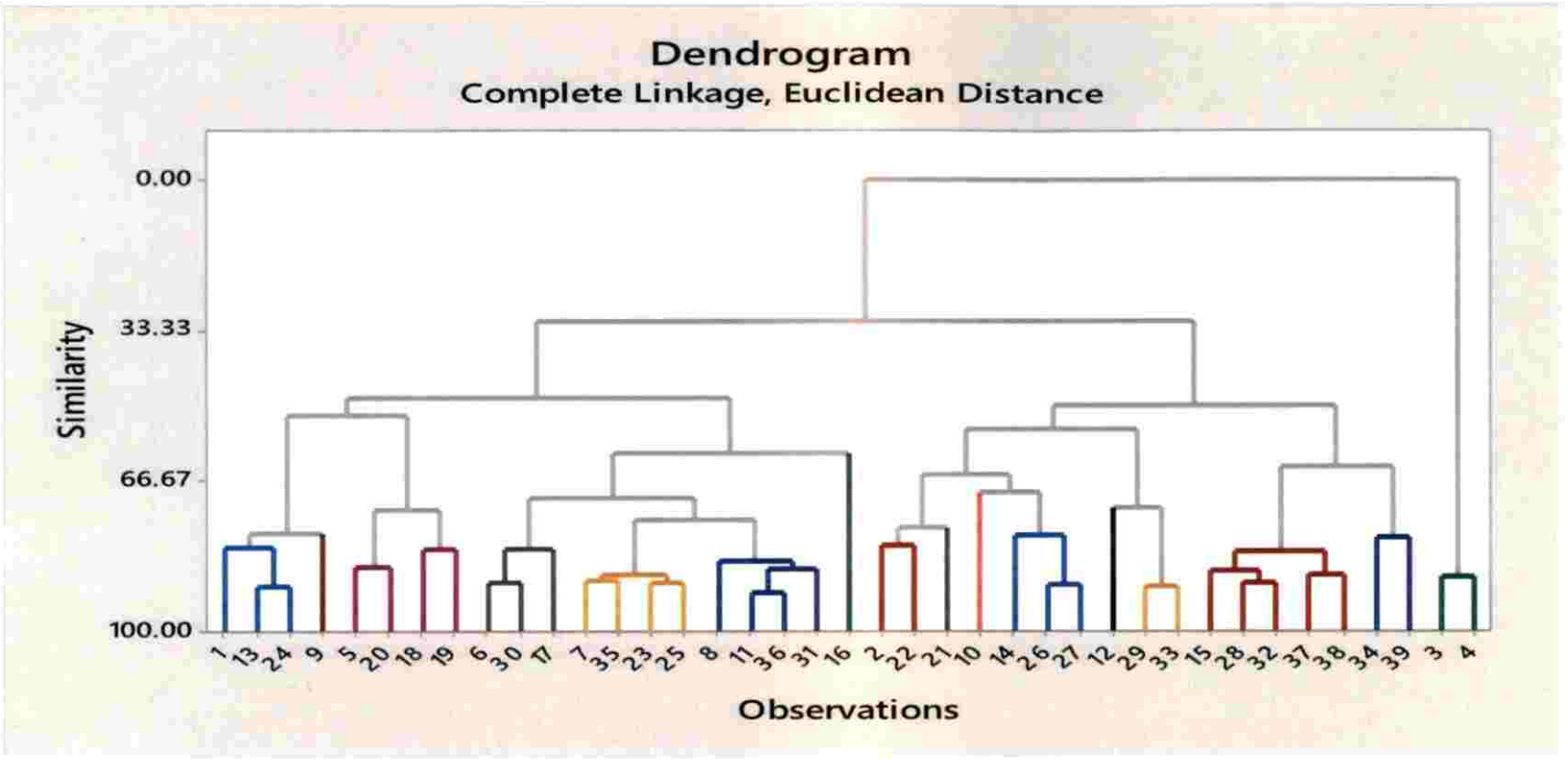


Figure 15. Dendrogram presnting the clustering pattern of seed priming treatments based on seedling performance at 90 days

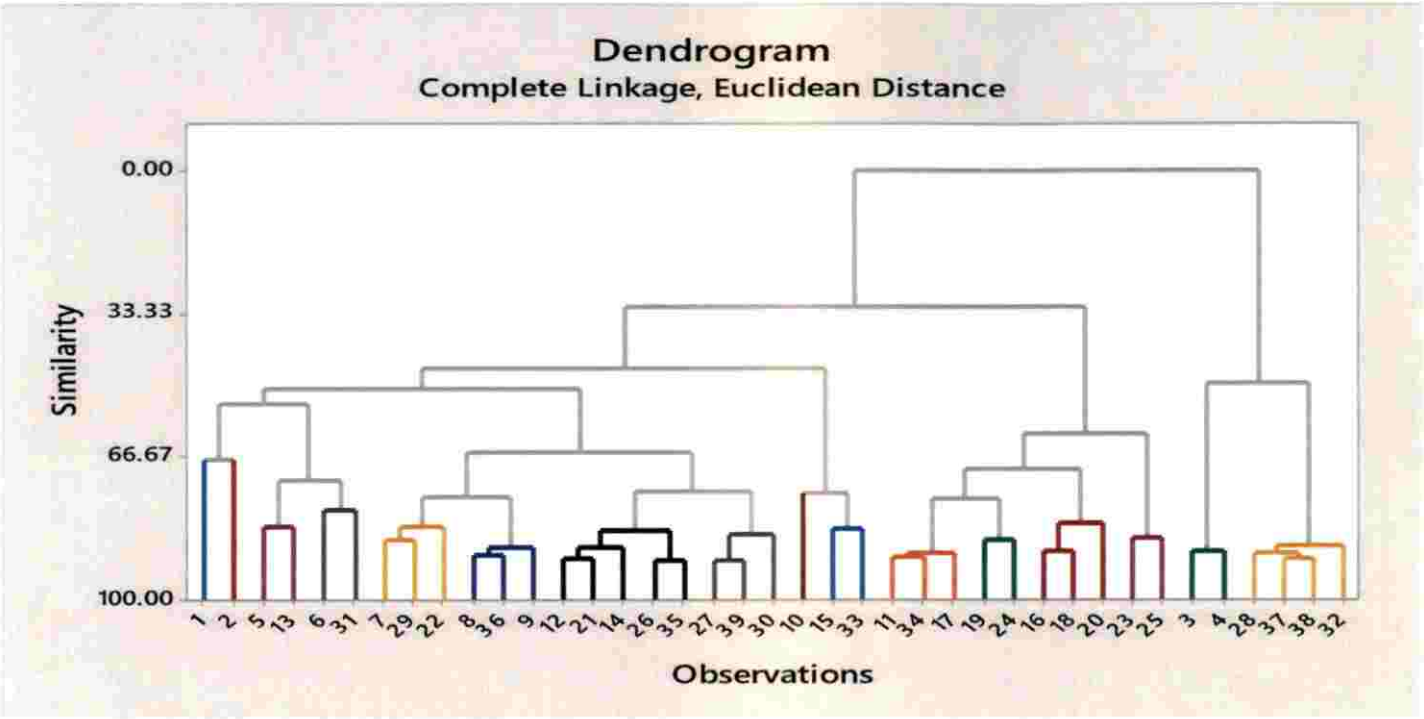


Figure 16. Dendrogram presnting the clustering pattern of seed priming treatments based on seedling performance at 180 days after transplanting

DISCUSSION

DISCUSSION

The results of the present investigation on "impact of seed priming techniques on germination and seedling performance in sandal (*Santalum album*, L.) are discussed in the following sections.

5.1 Effect of seed priming techniques on the germination attributes of *Santalum album*

Efficient seed germination is important for the propagation of crops. Germination parameters are considered to be the best indicators of how best a seed lot perform in the field. The distribution of seedlings in to strong and weak classes in terms of the time taken to achieve complete emergence is facilitated by the speed of germination and mean germination time which contributes to the seed vigour. Seed priming alters the germination process due to improved hydraulic activity (Bradford, 1986). Metabolic changes like cell cycle related events (De castro *et al*., 2000) and mobilization of seed reserves such as storage proteins (Job *et al*., 2000) may contribute to enhance germination under the process of priming.

The results of the present study indicated that the sandal seeds subjected to biopriming with *Pseudomonas fluorescens* at 100% for 8 days and the seeds subjected to chemical priming with 0.8 M of Manganese sulphate for 3 days recorded the highest germination percentage of 88 per cent among the 52 priming treatments. The germination percent exhibited by the seeds subjected to chemical priming at 0.4 M, 0.6 M and 1 M of manganese sulphate for 3 days were on par with the germination percentage of seeds subjected to biopriming with *Pseudomonas fluorescens* at 25%, 50% and 75% for 8 days. Besides, the higher germination per cent showed by biopriming treatments over control, the seeds subjected to biopriming for 4 days at different concentrations were on par with the best treatment. In spite of the highest germination percentage obtained in chemical primed seeds, the speed of germination of seeds exposed to different chemical priming treatments (highest value recorded 9.10) were lower than that of seeds subjected to biopriming (highest value recorded 12.19). The speed of germination of chemical priming treatments were remarkably higher than control when the seeds were kept for 3 days and found to be very low when compared to control with an increase in priming duration whereas the speed of germination of every biopriming treatments were found to be superior over control.

Although the seed exposed to various PEG 6000 priming treatments for 3 days and 6 days exhibited germination percentage comparative to the superior biopriming treatments, it tend to have a gradual decrease with an increase in the duration of priming whereas, a sudden decrease

was observed in the germination percentage of seeds subjected to different chemical priming treatments with an increase in the duration. The speed of germination recorded by the seeds subjected to PEG priming though higher than control, were lower than the biopriming and chemical priming treatments. The seeds subjected to hydropriming exhibited poor germination percentage and reduced germination speed when compared to control. These results can be further simplified as the percentage increase or decrease in germination by seed priming treatments over control. It is observed that the germination percentage in hydropriming treatments were reduced by 32.60 per cent when compared to the 46 percent germination in control which is in conformity with the results of Le prince *et al*., 1994, stating that a decrease in the germination of hydroprimed seeds may be attributed to the free radical accumulation which fastens the lipid peroxidation during hydration and subsequent drying.

The biopriming treatments recorded an increase in the a percent germination of sandal seeds which ranged from 44.93 to 91.30, where the results are in conformity with the findings of Rodriguez *et al*., 2015, they stated that biopriming of seeds of *Abies hickelii* with *Pseudomonas fluorescens* increased the germination percent by 91 per cent over control. Studies says that the increase in the germination of bioprimed seeds can be attributed to synthesis of plant or tree growth hormones, enhancement of nutrient availability, and provision of biological control attributable to the fructification of antibiotics or siderophores (Chanway *et al*., 2000 and Deepa *et al*., 2010). In addition to increased rate and uniformity of germination, biopriming protects seeds against the soil and seed-borne pathogens. Moreover, some bacteria used as biocontrol agents are able to colonize rhizosphere and support plant in both direct and indirect way after germination stage (Callan et al. 1997). Soaking of seeds in bacterial suspension initiates the physiological processes in the seed where plumule and radicle emergence is prevented (Anitha *et al.* 2012), until the seeds have temperature and oxygen after being sown.

The chemical priming with $MnSO_4$ for 3 days recorded a percent increase of 91.3% in germination over the germination recorded in control in the present study which is supported by the reports in safflower and maize (Muhammad *et al*., 2015), carrot (Muniavar *et al*., 2013) and calendula (Mirlotfi *et al*., 2015), the rate of emergence was decreased by 97.00% when compared to control which is also in conformity with previous studies which states that longer duration of priming negatively affects the seed germination. This can be due to increase in Mn toxicity

occurring the seed due to increased soaking period which prevents germination as well as further seedling growth as reported in wheat (Burke *et al.*, 1990).

Similarly the percentage increase in germination due to PEG priming was 69.56 percent which is in line with the results obtained in *Guazuma ulmifolia* (Tay and November, 2010) and *Mimosa bimucronata* (Brancalion *et al.*, 2008), loblolly and short leaf pines (Hallgren, 1989) and *Gmelina arborea* (Adebisi *et al.*, 2013). On the other hand, the percent decrease in germination was found to be 92.76% which is similar to the finding in watermelon by Armin et al., 2010 where, germination percentage of seeds was reduced due to PEG priming. This was assumed as a result of osmotic effects of PEG ions ascribed to the reduced water intake causing lower germination by seeds.

5.2 Effect of seed priming techniques on the electrical conductivity of seed leachates

One of the prime objective of seed priming is to lower the electrical conductivity of the seed leachates, which is associated with the loss of see viability and vigour. (Perry, 1977). Lower electrical conductivity of seeds indicate the precise integrity of cellular membranes during priming which reduces the leakage from the cells (Copeland and Mc Donald, 2001). Intense leakage solutes indicate the defective cell membrane of the non-viable and poor vigour of tree seeds (Smith *et al.*, 2001; Sukesh and Chandrashekhar, 2011).

Among the 52 treatments undertaken in the present study it was observed that the seeds subjected to hydropriming for 12 days recorded the highest EC (1.96 dS cm^{-1}) and the lowest value was recorded in seeds subjected to biopriming at 100% for 8 days (0.03 dS cm^{-1}). Hence, it can be concluded that the electrical conductivity of hydroprimed seeds were directly related to the increase in priming duration, which indicates that the disintegration cell membranes is triggered by soaking the seeds in water for longer duration. The result of the present study is supported by the finding of Rinku *et al.* (2017) where seeds subjected to hydropriming recoded higher conductivity in tomato than control.

The electrical conductivity of biopriming treatments varied from 0.03 dS cm^{-1} to 0.06 dS cm^{-1}. The results clearly depicted that the reduction in seed leakage resulting in better membrane integrity in sandal has been achieved through biopriming treatments. This can be attributed to the enzyme activity induced cell repair. This is line with the finding of Rinku *et al.*, 2017 where biopriming was found to have low electrical conductivity values when compared to hydropriming and control. Farooq *et al.*, (2011) stated that the priming is beneficial in all cases where the

electrolyte leakage was reduced compared to untreated seeds which seem due to better membrane repair during the re-drying process following priming.

The results of electrical conductivity of seed leachates of sandal due to osmopriming treatments were contradictory to the findings of Ashraf and Foolad, 2005, in which osmopriming with PEG is reported to reduce the solute leakage. The electrical conductivity of the PEG priming treatments were comparable to hydropriming treatments and an increase in concentration of PEG was inversely related to the electrical conductivity of the leachates due to a reduction of water uptake in the higher concentrations of PEG. The electrical conductivity of seeds exposed to osmopriming at 5% for days recorded an EC of 1.02 dS cm^{-1} while those seeds primed at 20% for 3 days recorded an EC of 0.46 dS cm^{-1}. In fact this trend was observed in each priming duration under osmopriming. This trend which occurs due to reduction of water uptake was observed in radish and eggplant (Rudrapal and Nakamura, 1988), corn (Sung and Chang, 1993) soybean (Senaratna and Mc Kersie, 1983) and Onion (Choudhery and Basu, 1988). In addition, the EC value was found to be increasing with increase in priming durations. This was in accordance with finding of Brancaloin *et al.*, 2010 in *Guazuma ulmifolia*.

Similar to the biopriming treatments the chemical priming treatments recorded lower values of EC, but were relatively greater than the biopriming treatments. The values ranged from 0.98 dS cm^{-1} in seeds chemical primed at 0.6 M for 9 days to 0.13 dS cm^{-1} in seeds primed at 0.4 dS cm^{-1} for 12 days. It was also observed that EC of the seeds exposed to chemical priming were decreasing with increasing duration. The lower EC recorded by chemical priming treatments in sandal seed leachates was in compliance with the finding of Mirlotfi *et al.*, 2015 in Calendula seeds in which chemical priming with $MnSO_4$ greatly lowered the EC of the seed leachates.

5.2 Effect of seed priming techniques seed biochemical composition

The biochemical constituents of seeds like carbohydrates, proteins and fats play a major role in enhancing germination and resultant seedling growth. Seeds accumulate the chemical energy produced during photosynthesis as seed reserves of multiple forms, including carbohydrates, lipids and proteins. The seed reserve material content is normally, correlated with germination percentages or speed of germination (Soriano *et al.*, 2014). For instance, the starch content was positively correlated with germination rate *Citrullus lanatus* seeds (Wang *et al.*, 2011) and the soluble sugars and proteins showed positive correlation with germination percentage of *Medicago truncatula* seeds (Vandecasteele *et al.*, 2011), *Pinus pinaster* (Wahid and Bounoua,

2013) respectively. However, no significant correlation observed between fat content and germination percentage of *Linum usitatissimum* seeds (Kanmaz and Ova, 2015), but fatty acid content was negatively correlated with germination percentage of *Gossypium spp.* seeds (Hoffpauir *et al.*, 1950). Jijeesh and Sudhakara (2016) obtained a high positive correlation for vigor index and biochemical constituents with crude oil and soluble and total carbohydrates in *Tectona grandis*. Collar diameter, number of lateral roots and seedling dry weight were also correlated with vigor index and biochemical constituents. Thus, the influence of seed reserves on germination depends on the amount of reserve and the plant species. High carbohydrate content in the seed is believed to contribute to the greater germination value of seeds (Sharma *et al.*, 2006). Sandal seeds in the present study recorded large variations in the biochemical constituents with different seed priming treatments. With regard to carbohydrate content, hydropriming induced a considerable decrease in the carbohydrate content of the seed which accounted to a 76.69 % reduction when compared to control. Although osmopriming contributed the greatest increase in carbohydrate in seeds, not every treatment could impart this increase which was similar in case of chemical priming treatments too, whereas, each biopriming duration and concentration could increase the carbohydrate content of the seeds. When compared to control, the percent increment in carbohydrate was 84.41% in osmopriming, 70.87% in biopriming and 63.10% in chemical priming.

The protein content of the seeds determines the speed germination and initial growth of seedlings. During seed development, storage proteins are synthesized in abundance and accumulate primarily in the protein storage vacuoles of terminally differentiated cells of embryo and endosperm. These proteins may play a role in equipping the seed for survival, maintaining a minimal level of hydration in the dry organism and preventing the denaturation of cytoplasmic components. They may also play a role during imbibition by controlling water uptake. It was observed that the protein was mainly mobilized and used during germination of the legume species *Dalbergia nigra* (Ataíde *et al.*, 2013). Seeds of *Helianthus annuus* (Erbas *et al.*, 2016) and *Sterculia urens* (Satyanarayana *et al.*, 2011) have high protein and oil content, which decreased drastically during germination of these two species. The stored proteins are the major source of reduced nitrogen to the growing seedlings. These stored proteins are found to increase during seed priming process. The results of the present study revealed that, in osmopriming treatments there was increase the protein content of the seeds. All the PEG priming treatments were superior to

control with a percent increase of 60% when compared to control seeds. Biopriming and osmopriming treatments were found to impart 40% increase in the protein content while few of these treatments were found to induce a reduction in protein content of the seeds. Hydropriming followed a different trend from the other priming treatments. Hydropriming for 12 days imparted a 40 per cent increase in the total protein content of the seeds compared to control seeds, whereas the rest of the hydropriming treatments were on par with control.

An increment in per cent crude fat compared to control was observed only in seeds exposed to biopriming at 75% for 6 days and seeds subjected to chemical priming at 0.8 M for 8 days (57%) whereas, rest of the priming treatments resulted in to a decrease in the crude fat compared to control (55.35%). The results of osmopriming treatments were contradictory to the fact that PEG priming promotes for an increase in the fat content of seeds (Inayat-ur-Rahman *et al.*, 2013). The increased carbohydrate and protein content in the seeds subjected to osmopriming can be due to the osmoconditioning of seeds which further the cell membrane integration leading to production of enzymes favouring carbohydrate and protein synthesis in the seeds (Sung and Chung, 1993).

A decrease in the reserve material of seeds subjected hydropriming can be attributed to the uncurbed water intake by the seeds during soaking (Taylor *et al.*, 1998). The results of per cent crude fat in seeds subjected to hydropriming were ascribed to the findings of study in Korean Soybean where the fat content remained unaltered after hydropriming (Oh *et al.*,1992). The decrease in the fat content of seeds subjected to osmopriming can be due to the conversion of lipids in to sugars which is common in seeds with greater oil content (Taiz and Zeiger, 2002). The greater carbohydrate and protein content in the PEG primed seeds can be due to the larger molecular size of PEG which retards the entry of PEG molecules in to cells preventing the deterioration of enzyme related activities with cells of seed.

5.4 Effect of seed priming techniques on the seedling growth attributes and biomass production in sandal at 90 DAT and 180 DAT.

A major advantage, besides enhanced and uniform germination, of seed priming is the promotion of faster growth of young seedlings leading to a reduced nursery period. The results of the experiments to study the effect of seed priming techniques on the seedling growth and biomass production in sandal seedlings at 90 and 180 days after transplanting have shown significant variations among the treatments (duration and concentration) within a priming a technique as well

as between the different priming techniques. With regard to the shoot and root growth parameters of the seedlings, it can be concluded that the benefits of priming had been accumulated on the seedlings emerged from the seeds subjected to biopriming with *Pseudomonas fluorescens* followed by chemical priming and osmopriming. Even though, the seedlings subjected to hydropriming were recording values of seedling growth attributes higher than that of control at 90 DAT, the growth was found to decrease gradually at 180 DAT on comparison with control. Contrary to this, the biopriming treatments recorded a steep increase in the growth of seedlings at 90 and 180 DAT. Although, the results of chemical priming treatments were comparable to the results of biopriming treatments, the values were slightly lower than that of the biopriming. The trend was similar to Osmopriming treatments. The seedling length was highest in seeds subjected to biopriming treatments for 8 days. Unlike the results exhibited by hydropriming, osmopriming and chemical priming, in which the growth was retarded with longer priming duration, the biopriming treatments exhibited the best growth from the seedlings subjected for longest duration. The highest seedling length obtained from the present study at 180 DAT was 33.10 cm from seedlings subjected to biopriming at 100% for 8 days which was 46.5% greater than the seedling length obtained from a similar study conducted by Das *et al.* (2013) in West Bengal in the absence of host plant, and 7.1% higher than the seedling length obtained in the presence of host plant.

Simultaneously, the fresh weight of the seedlings obtained from different priming treatments were comparable to that of the fresh weight of the seedlings of control at 90 DAT as well as 180 DAT. Meanwhile, the seedlings subjected to osmopriming at 5% for 3 days with PEG were shown to have a highly significant difference than the control (1.82 g) recording the highest seedling fresh weight (2.16 g) among all the treatments. On the other hand, highest dry weight was recorded in seedlings subjected to biopriming at 100% for 3 days (0.92 g) and the lowest value was recorded in seedlings hydroprimed for 9 days. Contradictory to the highest fresh weight recorded by the osmopriming treatments, the dry weight of the seedlings subjected to osmopriming were lower than the control. Chemical priming treatments were also recorded an increased initial growth which declined gradually by the 6th month. When compared with all the priming treatments it can be concluded that biopriming with *Pseudomonas fluorescens* at 100% for 8 days will be the best priming treatment for sandal to improve the biometric growth as well as the biomass on the basis of dry weight. These results were in accordance with the findings reported in sunflower (Moeinzadeh *et al.*, 2010), pea (Negi *et al.*, 2014) and *Acacia hickelii* (Rodriguez *et al.*, 2015).

Reports say that a partial or complete retrogression of the benefits acquired through seed priming can occur after the redrying of the seeds (Carpenter, 1989, Parera and Cantilife, 1992). This reversion, possibly may be due to the decline in cytoplasm viscosity caused by lowering the concentration of oligosaccharides in cells, thus aggravating the susceptibility of the seeds towards deterioration process (Buitink *et al.*, 2000, Brancalion *et al.*, 2010). Hence it can be concluded from the present study that these may be the reason for the inferior performance of the certain treatments when compared to control.

The highest seedling length obtained from seedlings subjected to biopriming were similar to the findings in soybean (Entesari *et al.*, 2013) which reported that the priming of seeds with bio-agents increased the mineral levels (N, P, K), chlorophyll biosynthesis and photosynthetic activity. Similarly the increased dry matter productivity by the seeds subjected to biopriming can be correlated to the increased seed protein content. Yadav *et al.* (2010) reported that the increased shoot height of the bioprimed seedlings are due to the early emergence of the seedlings, which was similar to the present study. Biopriming could reduce the seed germination period to 21 days over the reported 140 days of germination. Study by (Nezarat and Golami, 2009) revealed that the *Pseudomonas fluorescens* are capable of increased nitrogen fixing and phosphate solubalisation and production of growth promoting substances increasing the dry matter production of the seedlings.

1. The effectiveness of priming treatments to enhance the germination and seedling growth attributes of sandal can be presented in the order of:

   Biopriming at 100% for 8 days > Biopriming at 50% for 8 days > Biopriming at 75% for 8 days > Biopriming at 100% for 6 days > Biopriming at 75% for 6 days > Chemical priming at 1 M for 3 days > Biopriming at 50% for 4 days.

5.5 Effect of seed priming techniques on the chlorophyll content of the leaves in sandal

The role of seed priming in imparting an increase in the total chlorophyll content of leaves are least explored. From the present study, it can be concluded that the chlorophyll content of the leaves were found to be increased in case of seedlings subjected to chemical priming as well as biopriming, whereas the chlorophyll content was diminished in seedlings subjected to hydropriming and osmopriming. The highest content of chlorophyll was observed in the seedlings subjected to chemical priming with $MnSO_4$ for 3 days which can be attributed to the role of Mn in

chlorophyll production (Anderson and Pyliotis, 1996). The increased chlorophyll content in the leaves of seedlings subjected to biopriming may be attributed to the production of plant hormones which triggers the cell metabolic activities as reported in *Abies hickelii* (Rodriguez *et al.*, 2015). The reduction in chlorophyll content can be due to suppression of enzymes required for chlorophyll synthesis (El-Samad *et al.*, 2011).

5.6 Effect of seed priming techniques on the vigour indices of sandal seedlings

Seed vigour is an important component to decide the seed quality. Vigour index I is a measure of seedling length which can vary due to both internal and external factors of seed whereas, the vigour index II is a measure of the seedling dry matter which is an indicator of the seed reserves. It can be concluded from the present study that the two vigour indices of sandal seeds subjected to biopriming and osmopriming were found to be superior over control, whereas in case of the hydropriming and chemical priming treatments, the treatments carried out for 3 days only recorded higher vigour indices. An increase in the priming duration has caused a steep decrease in the vigour indices of these seeds. The variation in the vigour indices are also determined by the variation in the germination percentage recorded by seeds of different priming treatments. Although the seeds subjected to hydropriming recorded a lower germination rate compared to control, the vigour indices of these seeds were higher than control, which can be attributed to the slight increase in the dry weight of these seedlings.

The greater vigour index II in biopriming seeds can be due to the mobilization of seed reserves during priming which is in accordance with the findings reported in *Abies hickelii* (Rodriguez *et al.*, 2010) and maize (Kalaivani, 2010). The increase in vigour index of seeds due to osmopriming has been reported earlier in *Gmelina arborea* (Adebisi *et al.*, 2013).

5.7 Grouping of treatments using hierarchical cluster analysis

The cluster analysis to group the treatments on the basis of seedling performance resulted in the formation of sixteen clusters at 90 and 180 days after planting. The treatments within a cluster were found to exhibit similar characteristics in terms of the seedling growth of sandal. The principal component analysis conducted prior to the cluster analysis revealed that plant dry weight and shoot height were the most correlated seedling growth attributes. Similarly, the various groups obtained through cluster analysis also revealed that more weightage was given to the dry weight

and shoot height of the seedlings to group the treatments. The clustering of treatments based on the seedling performance at 90 DAT resulted in clusters of treatments from every priming treatments adopted in the study. The clusters formed on the basis of seedling growth at 90 DAT incorporated hydropriming and biopriming treatments within same cluster. This pattern could be observed in every cluster. Contradictory to these results, the clusters of treatments based on the seedling growth at 180 DAT resulted in more specific grouping of treatments. The hydropriming treatments were placed in distinct clusters and the treatments were inferior compared to control. The clustering at 180 DAT resulted in the grouping of the best treatments within the same clusters. Hence, the results of the cluster analysis were in accordance with the individual finding of the study to determine the best priming treatments which improved the seedling growth of sandal.

5.8 Effect of seed priming techniques on the germination and seedling growth attributes of the sandal seeds stored for one month

The lack of germination and further seedling growth in the seeds subjected to one month storage after priming can be due to the reversion of benefits obtained by priming during storage. The reasons for the reduced longevity of the primed sandal seeds can be attributed to decreased DNA repair activity during the seed hydration (Van Pij len *et al.*, 1996). This was also in compliance with the studies in sweet corn (Chiu *et al.*, 2002) and bitter gourd seeds (Yeh *et al.*, 2005) which indicated that the reduced storability of the primed seeds may be due to the decreased activity of antioxidant enzymes resulting an increase in the lipid peroxidation activity mediated by the Reactive Oxygen Species.

SUMMARY

SUMMARY

The present investigation on the impact of seed priming techniques on the germination and seedling growth attributes of sandal (*Santalum album* L.) was carried out at the College of Forestry, Vellanikkara, Thrissur, Kerala. The sandal seeds were collected from the Nachivayal Reserve Forest, Marayoor Provenance. The study analyzed the effect of different duration and concentration of priming agents viz. water (hydropriming), *Pseudomonas fluorescens* (biopriming), Polyethylene Glycol 6000 (Osmopriming) and $MnSO_4$ (chemical priming) on seed germination and subsequent seedling growth in sandal. Hydropriming was done at for four durations viz., 3, 6, 9 and 12 days. Biopriming was done using four different concentrations of *Pseudomonas fluorescens* (25, 50, 75 and 100%) for 2, 4, 6 and 8 days. Osmopriming was carried out at 5, 10, 15 and 20% concentrations of PEG 6000 for 3, 6, 9 and 12 days and the chemical priming was experimented at 0.4, 0.6, 0.8 and 1 M concentrations of $MnSO_4$ for 3, 6, 9 and 12 days. The non-primed seeds were kept as control. Primed seeds were stored for one day and one month after the completion of priming processes and the germination and seedling growth were observed. During the study period the seeds subjected to priming process and stored for one day only germinated and those stored for one month failed to germinate. The salient findings of the study are as follows:

1. Hydropriming of the sandal seeds could not improve the germination rate compared to control. Hence, distilled water cannot be used as an effective priming agent to obtain good germination.

2. Biopriming at different durations and concentrations of *Pseudomonas fluorescens* significantly increased the germination parameters of the sandal seeds. The highest germination percentage (88%) was recorded in the seeds subjected to biopriming at 100% for 8 days which was 91.3% higher compared to control (46%) and they recorded the highest germination speed also (12.10). The days for completion of the germination in the seeds subjected to biopriming at 50% and 100% for 8 days were 21 days whereas 56 days were taken to complete the germination in control seeds.
3. Osmopriming treatments recorded an initial increase in the germination up to 6 days and thereafter the germination was decreased. Osmopriming at four concentrations for 3 and 6 days duration increased the germination percentage of seeds whereas, the osmopriming for

the durations 9 and 12 days significantly reduced the germination percentage of the seeds compared to control. The highest germination recorded in osmopriming was 78% however; the seed germination was spread over a period of 62 days in osmopriming treatments wherein the control seeds took 56 days to complete germination.

4. The chemical priming with $MnSO_4$ at 0.4, 0.6, 0.8 and 1 M concentrations for 3 days recorded the highest germination percentage (88%) which was similar to that recorded during biopriming. However, the speed of germination was lower compared to that of the biopriming treatments. The seeds subjected to chemical priming at 0.4 M for 3 days recorded the lowest germination period among chemical priming treatments (26 days).
5. Electrical conductivity of the sandal seeds subjected to different priming treatments were measured which is a measure of membrane integrity. Electrical conductivity was the highest in the leachates of seeds hydroprimed for 12 days (1.96 dS cm^{-1}) and was the lowest in seeds subjected to biopriming (0.03 dS cm^{-1}). The leachate conductivity of the seeds subjected to osmopriming treatments (1.69 dS cm^{-1}) were comparable to that of the hydropriming treatments. Although the different concentrations and duration of MnSO4 reduced the leakage of solutes from the sandal seeds, the electrical conductivity was very higher than that of the biopriming treatments. Hence, biopriming treatments were the best in reducing the leakage of solutes from the cells leading to better membrane integrity and stability.
6. Biochemical analysis of the primed and non-primed seeds indicated that the hydropriming treatments significantly lowered the carbohydrate, protein and crude fat content of the seeds compared to control (1.03 mg g^{-1}, 0.05 mg g^{-1}, and 55.35%, respectively). The biopriming duration and concentrations increase the total carbohydrate (1.76 mg g^{-1}) and protein content (0.07 mg g^{-1}) of the seeds whereas, a reduction in crude fat (48.31%) was observed with priming. Similar trend of biopriming was observed during osmopriming treatments, however, these treatments recorded the highest carbohydrate (1.96 mg g^{-1}) and protein content (0.08 mg g^{-1}) of the seed compared to the other priming techniques. Chemical priming also contributed to an increase in carbohydrate and protein content, but was lower than that of biopriming and osmopriming treatments.
7. With regard to seedling growth and biomass production, hydropriming contributed the least towards the promotion of seedling growth of sandal. The seedling growth was best

enhanced by biopriming treatments resulting in the production of tallest seedlings (33.10 cm), collar girth (9.28 mm), number of leaves per plant (17.25), root length (8.35 cm) and seedling dry weight (0.92 g). The biopriming treatments were also found to improve the vigour index of the sandal seedlings when compared to control. Similarly, long duration of the biopriming treatments (8 days) were found to be superior to that at short duration (2, 4 and 6 days) and control.

8. In the Osmopriming, the seedling growth attributes could be studied only from the seeds subjected to different concentrations for 3 days and 5% and 10% for 6 days due to lower survival of the seedlings. The seedlings obtained from the seeds subjected to Osmopriming did not show significant increase in growth attributes of the seedlings compared to control whereas the maximum fresh weight (2.16 g) was recorded in the seedlings subjected to osmopriming. Besides the low rate of seedling growth, the different osmopriming treatments could significantly improve the vigour index of the seedlings over control.
9. The seedling growth attributes could be recorded only from the seeds subjected to chemical priming for the duration 3, 6 and 9 days. Analogous to the growth attributes recorded by the bioprimed seeds, the seedlings of chemical primed seeds were found to perform superior over control seedlings which could be ascribed to the increased chlorophyll content (40.46 mg g^{-1}) of the chemically primed seedlings. The seedlings subjected to chemical priming produced the seedlings with higher growth attributes next to biopriming. The vigour indices also recorded higher values in the chemical priming. The results of chemical priming indicated that chemical priming with $MnSO_4$ for 3 days only could enhance the germination and seedling growth of sandal.
10. The results of the correlation analysis between biochemical composition of seeds subjected to different priming treatments and their resultant seedling growth exhibited large variation among the priming techniques. The results revealed that the shoot growth attributes of hydroprimed seeds were positively correlated to the carbohydrate and crude fat content, whereas all the parameters recorded a negative correlation with the protein content. The dry matter production of the hydroprimed seedlings recorded significant positive correlation with crude fat.
11. Growth attributes of the seedlings subjected to biopriming recorded negative correlation to the seed reserve materials which was found to have strong negative correlation by 6th

month. Similarly seedlings subjected to osmopriming recorded significant positive correlation with carbohydrate content whereas a negative correlation was recorded with the total protein content and per cent crude fat. Meanwhile, the growth attributes of seedling recorded negative correlation with the carbohydrate content and crude fat whereas positive correlation was recorded with the protein content of the seeds.

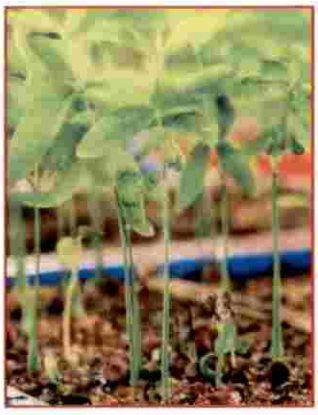
12. The better priming treatments effective to enhance the germination and seedling growth attributes of sandal can be presented in the order of:

    Biopriming at 100% for 8 days > biopriming at 50% for 8 days > biopriming at 75% for 8 days > biopriming at 100% for 6 days > biopriming at 75% for 6 days > chemical priming at 1 M for 3 days > biopriming at 50% for 4 days.

13. Cluster analysis of the treatments resulted in the grouping of best biopriming treatments in distinct adjacent groups.
14. With regard to the seeds subjected to different priming techniques and stored for one month, seeds failed to germinate in all priming methods.

Overall, it can be concluded that the biopriming at 100% for 8 days followed by 50% and 75% for 8 days were the best treatments to enhance the seed germination and seedling growth attributes of sandal. Groping of treatments by cluster analysis lead clustering of these treatments in the same cluster. Chemical priming at 0.4 M for 3 days is also a suitable priming treatment to enhance seed germination. Also, biopriming was found to be the cheapest priming method among the four selected priming methods in the study.

The results of the present study throw light on the effect different concentration and duration of the priming agent on germination and seedling growth of the sandal. The study also looks into the biochemical changes associated with seed priming in the seeds. Since the sandal seeds failed to germinate after one month of storage, the storage duration has to be standardized in the case of sandal seeds. Furthermore, the enzymatic activities involved in the priming process which triggered as well as reduced the growth performance of sandal seedlings must also be studied. A pictorial representation of the results of the present study is given in Figure 17.



•Highest germination in short duration treatments,
•Early emergence in seeds primed at 0.4 M for 3 days

•Tall seedlings
•Increase in seed reserve materials,
•Reduction in loss due to leakage
•Highest chlorophyll content and increased vigour

Chemical priming

Increased germination,
Slow emergence,
Highest increment in seed reserve materials.

•Greater loss due to leakage,
•Reduced dry weight accumulation.
•Seedling growth not improved.

Osmopriming

•Uniform and enhanced germination
•Early emergence
•Increase in seed reserve materials.
•Minimal loss due to leakage,

•Tallest seedlings
•Increase in leaf area and leaf number and dry weight
•Increased vigour
•Increased chlorophyll content

Biopriming

•Low germination, slow emergence
•Reduction in seed reserve material
•Heavy loss of food reserve due to leakage

•Poor seedling growth,
•Low chlorophyll content,
•Low vigour of the seedlings

Hydropriming

Figure 17. Pictorial representation of the effects of different priming treatments on the germination and seedling performances in sandal

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Impact of seed priming techniques on germination and seedling performance in sandal (*Santalum album*, L.)

By

CHITRA P (2017-17-005)

ABSTRACT OF THE THESIS

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VELLANIKKARA

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ABSTRACT

Santalum album L, known as the East Indian Sandalwood is a semi-root parasitic tree native to South India and it is one of the most precious and valuable among Indian forest trees. The poor rate of germination coupled with long germination period is the major constraints in the regeneration of sandal. Present study was conducted to assess the impact of seed priming techniques on the germination and seedling growth attributes of sandal at College of Forestry, Vellanikkara, Thrissur. The effect of different duration and concentration of priming agents viz. water (Hydropriming for 3, 6, 9 and 12 days), *Pseudomonas fluorescens* (Biopriming at 25, 50, 75 and 100% for 2, 4, 6 and 8 days), Polyethylene Glycol 6000 (Osmopriming at 5, 10, 15 and 20% for 3, 6, 9 and 12 days) and $MnSO_4$ (Chemical priming at 0.4, 0.6, 0.8 and 1.0 M for 3, 6, 9 and 12 days) on seed germination and subsequent seedling growth in sandal were studied. The non- primed seeds were kept as control. Primed seeds were stored for one day and one month after the completion of priming processes and the germination and seedling growth were observed. The germination was obtained only in the seeds stored for one day after priming process and the seeds stored for one month failed to germinate.

Results indicated that the hydropriming of the seeds could not improve the germination of the sandal seeds compared to control. Biopriming significantly increased the seed germination and the highest germination percentage (88%) was recorded in the seeds subjected to biopriming for 8 days at 100% concentration, which was 1.9 times higher compared to control. The highest germination recorded in osmopriming was 78%. The chemical priming with $MnSO_4$ at different concentrations for 3 days also recorded the higher germination (88%) comparable to biopriming.

Electrical conductivity was the highest in the leachates of seeds hydroprimed for 12 days (1.96 dS cm^{-1}) and was the lowest in seeds subjected to biopriming (0.03 dS cm^{-1}). The leachate conductivity of the seeds subjected to osmopriming treatments (1.69 dS cm^{-1}) was comparable to that of the hydropriming treatments. Although the different concentrations and duration of $MnSO_4$ reduced the leakage of solutes from the sandal seeds, the electrical conductivity was higher than that of the biopriming treatments. Hence, biopriming treatments were the best in reducing the leakage of solutes from the cells leading to better membrane integrity and stability. Biochemical analysis of the primed and non-primed seeds indicated that the hydropriming treatments recorded significantly lower carbohydrate, protein and crude fat content compared to control.

The biopriming and osmopriming treatments increased the total carbohydrate and protein content of the seeds whereas, a reduction in crude fat was observed. Chemical priming also increased the carbohydrate and protein content compared to control but was lower than that of seeds subjected to biopriming and osmopriming.

A better seedling growth was also obtained on biopriming seeds at 100% concentration of Pseudomonas fluorescens for 8 days, which resulted in tallest seedlings (33.10 cm) with collar girth of 9.28 mm, 17 leaves per plant, root length of 8.35 cm and seedling dry weight 0.92 g. The biopriming treatments improved the vigour index of the sandal seedlings compared to control. Seedlings obtained from chemical priming also performed better than control. Hydropriming and osmopriming could not enhance the growth and biomass production of the sandal seedlings compared to control. Overall it can be concluded that the better priming treatments to enhance the germination and seedling growth attributes of sandal was in the order: biopriming at 100% for 8 days >biopriming at 50% for 8 days >biopriming at 75% for 8 days >biopriming at 100% for 6 days >biopriming at 75% for 6 days > chemical priming at 1.0 M for 3 days >biopriming at 50% for 4 days. The results of the present investigation can be applied in forest nurseries to obtain increased and uniform germination of sandal seeds which can ensure the quality planting stock production.