

Microbial inoculant influences the germination and growth of *Albizia lebbeck* seedlings in the nursery

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Microbial inoculants (MI), a biofertilizer, composed of many different beneficial microorganisms has positive role on seed germination and growth of plants. In the present study, its efficacy on seed germination and seedling growth of *Albizia lebbeck* in the nursery was studied. The seeds were sown in polybags filled with a mixture of forest soil and cow dung (3:1) and treated with 0.1%, 0.5%, 1%, 2%, 5% and 10% concentrations of MI. Most of the parameters studied (seed germination, shoot and root lengths, dry weights of shoot and root, collar diameter, leaf number etc) were found maximum in 2% of MI. Although the highest vigor index, volume index and quality index (7053, 3738 and 1.106, respectively) were found in 2% MI, but the highest sturdiness (65.95) was found in 1% MI solution. The nodule number was higher at a very low (0.5%) concentration of MI but it normally decreased with the increase of concentration. Total pigment content in leaf was recorded highest (112.86 mg.L⁻¹) in 2% of MI. Therefore, MI influences seed germination and seedling growth of *A. lebbeck* and the low concentration (2%) of the inoculant can be recommended for getting maximum seed germination and seedling growth of the species studied.

Key words: *Albizia lebbeck*, germination, microbial inoculant, seedling growth

A*lbizia lebbeck* (L.) Benth [Kalo sirish in Nepali, Kala koroi in Bengali], is a moderate to large deciduous tree with a straight bole and broad crown under the family Leguminosae (Mimosoideae). The species is widely spread in the world, and is native to Asia, Africa and Northern Australia. It grows naturally in Nepal, Bangladesh, Myanmar and Pakistan and has been cultivated in tropical and subtropical regions in Northern Africa, the West Indies, South America, and south Asia. Extensive plantations had been established in Nepal and in South India (Luna, 1996; Kumar *et al.*, 2010; Elzaki *et al.*, 2012; Missanjo *et al.*, 2013). The wood is excellent for furniture and timber for general uses, post, piles, fuel wood and charcoal. The tree is used as ornamental and nurse tree for tea gardens, coffee and cocoa orchards (Mishara *et al.*, 2010; Missanjo *et al.*, 2013; Shaikh *et al.*, 2014). It is a soil improver because of its inherent ability to fix nitrogen (Qadri and Mahmood, 2005). The leaves are excellent fodder for livestock. The species is also very useful for the rehabilitation of degraded lands because of its rapid growth, ability to fix nitrogen and tolerance for a range of soil

conditions (Luna, 1996; Qadri and Mahmood, 2005; Mishara *et al.*, 2010; Bobby *et al.*, 2012; Shaikh *et al.*, 2014). The plant has already been proven successful for afforestation, reforestation, social forestry and agroforestry programs in Bangladesh (Zabala, 1990; Dey, 2006).

To fulfill the high demand, many organizations are producing *A. lebbeck* seedlings in the nursery in Bangladesh to supply those in the plantation programs. Because the plants are grown mostly in unfavorable soil conditions, beneficial soil microorganisms can play a significant role in early establishment and better growth of the inoculated seedlings under field conditions.

The microbial inoculant (MI) in this study, with the commercial name “Effective Microorganisms” or EM was developed at the University of Ryukyus, Okinawa, Japan, in the early 1980s by a distinguished professor of horticulture, Dr. Teruo Higa (Kyan *et al.*, 1999). The main species comprising MI are lactic acid bacteria (*Rhodopseudomonas* spp.), photosynthetic bacteria (*Lactobacillus* spp., *Streptococcus*

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spp.), yeast (*Saccharomyces* spp., *Candida* spp.), Actinomycetes (*Streptomyces* spp.) and beneficial fungi (*Aspergillus* spp., *Penicillium* spp.). The microorganisms are added into the inoculant in the manufacturing process and can survive in the inoculant liquid at pH 3.5 or below.

The density of most of the above mentioned microbes is in the range of 1×10^6 to 1×10^8 mL⁻¹ (Xu, 2000). MI can be applied as inoculant to increase the microbial diversity of soils. It has been used with considerable success to improve soil quality and yield of crops, particularly in nature farming and organic farming systems (Xu, 2000). Inoculation of MI culture can improve photosynthesis and fruit yield (Xu, 2000; Wang *et al.*, 2000). Although *A. lebbeck* is used for wide range of purposes and even planted intensively in the field, the initial growth potential under the influences of MI was not studied. Therefore, the aim of this study was to observe the effectiveness of MI on germination of seed and the growth of seedlings of *A. lebbeck* and also to find out the best concentration of MI solution for ensuring maximum seedling development in the nursery.

Materials and methods

Collection of seeds and soils

The experiment was carried out in the nursery of the Institute of Forestry and Environmental Sciences; University of Chittagong, Bangladesh (lies approximately at the intersection of 91°50'E and 22°30'N) (Fig. 1). The seeds of *A. lebbeck* were collected from the seed orchard division of Bangladesh Forest Research Institute (BFRI) where the source of seed was a mother tree of 25 years old. The soils collected from the degraded hills of the University Campus was sieved well (<3 mm) and mixed thoroughly with decomposed cow dung in a ratio of 3:1. The brown hill soils (Rashid, 1991), are sandy loam to sandy clay loam, moderately to strongly acid and poorly fertile with pH <5.5, organic matter <2.0%, CEC <10 me/100 g, BSP <40% (Osman *et al.*, 2001). The white polybags of 15 cm x 10 cm in size were filled with the prepared mixture and a thin layer of coconut husk was added to each bag as top layer to reduce evaporation and to supply organic matter.

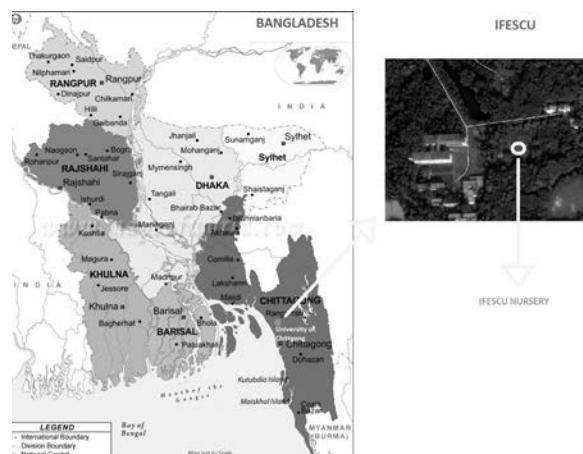


Fig. 1: Map showing the location of the nursery of IFESCU (Institute of Forestry and Environmental Sciences, University of Chittagong) in Bangladesh where the experiment was conducted.

Treatment design

The experiment was conducted in 03 March to 02 August 2015. There were seven treatments including control and 25 replications for each treatment. Seeds were sown in polybags (filled with soil and cow dung) with no added MI but water only for control treatment. Other treatments included; sowing the seeds in polybags with the concentrations of 0.1%, 0.5%, 1%, 2%, 5% and 10% of MI, respectively. For preparing 0.1% of MI solution, 0.1 mL stock solution of MI was added with 99.9 mL of water while for preparing 0.5% of MI solution, 0.5 mL stock solution of MI was added with 99.5 mL of water and the same formula was used for preparing the other solutions. For each treatment, 50 mL of MI of required concentration was poured in each polybag (filled with soil and cow dung) before a week of sowing the seeds while another 50 mL was poured after a week of sowing the seeds. Five seeds were sown in each polybag to observe the influence of MI on germination in the nursery conditions (temperature, 28°C; humidity, 75%). After completion of germination, only one seedling (the best one) per polybag was managed to observe growth performance and nodulation status of the seedlings. Partial shade and cover was ensured using polythene sheet on the nursery roof to protect the seedlings from strong sunlight and rains.

Growth measurement

Germination was recorded daily from the date of seed sowing to the last of germination. The seedlings were allowed to grow altogether for five months from the time of seed sowing. After five months, five representative seedlings from each treatment were selected for measuring growth parameters. The recorded parameters were shoot and root lengths, collar diameter, leaf number, fresh shoot and root weights, dry shoot and root weights, and nodulation status. For recording dry weights, shoots and roots were oven dried at 80°C for 48 hr. To assess the seedling vigor, total height (from the soil surface to seedling tip) of each seedling in each sub-plot was measured using a ruler to the nearest 0.1 cm. Vigor index was calculated according to Abdulbaki and Anderson (1973) as germination percent \times seedling total length i.e. total shoot and root length. Volume index was obtained by multiplying shoot height or shoot length (cm) with the square of collar diameter (mm^2) of the seedling. Quality index was developed following Dickson *et al.* (1960) to quantify seedlings morphological quality. The formula for calculating quality index is as follow:

$$QI = T \text{ dw} / \left(\frac{H}{D \text{ c}} + \frac{S \text{ dw}}{R \text{ dw}} \right)$$

where, QI is quality index, T dw is total dry weight (g), H is seedling height or shoot length (cm), D c is collar diameter (mm), S dw is shoot dry weight (g), R dw is root dry weight (g). Sturdiness was obtained by dividing shoot height or shoot length (cm) with collar diameter (cm) of the seedling.

Measurement of pigment contents

The pigment contents (chlorophyll-a, chlorophyll-b, and carotenoid) were determined from the fresh leaves of seedlings in different treatments (Wettstein, 1957; Khan, 2012). Ten leaf discs were cut with a cork borer (inside diameter of 5 mm), weighed immediately after cutting, and dipped in 100% acetone in 5 mL in test tube with stopper. After 24 hr of incubation, the supernatant-colored solution from the top was decanted carefully into a 25 mL volumetric flask. The leaf discs were then crushed with a blunt glass rod gently and 5 mL fresh acetone was added to the test tube and left for 15 min. Then the supernatant-colored solution from the top was again decanted to the same volumetric flask very

carefully, avoiding the fragmented plant tissues. The process was repeated until the leaf fragments became colorless. Finally, the volume was made up to 25 mL with fresh acetone and the measurement was taken immediately after preparation of the solution. The measurement of chlorophyll-a, chlorophyll-b, and carotenoid were made at 662 nm, 644 nm, and 440.5 nm respectively, with a spectrophotometer (Spectronic-20). The pigment contents in the extract were calculated following the formula of Wettstein (1957).

$$C_a = \frac{(9.784E662 - 0.99E644) \times V \times d}{1000 F_w} \quad (1)$$

$$C_b = \frac{(21.426E644 - 4.650E662) \times V \times d}{1000 F_w} \quad (2)$$

$$C_c = \frac{[4.695E440.5 - 0.268(C_a + C_b E662)] \times V \times d}{1000 F_w} \quad (3)$$

where, C_a is the chlorophyll-a (mg.L^{-1}); C_b the chlorophyll-b (mg.L^{-1}); C_c the carotenoid (mg.L^{-1}); V the total volume (25 mL); d the dilution factor; FW the fresh weight of leaf disc (g); and E is the absorbance at a particular wavelength (440.5, 644 and 662 nm).

Statistical analysis

All data were analyzed statistically using computer software SPSS (Version 20, SPSS Incorporation, Chicago, USA). Possible significant variations among the treatments were explored by Duncan's Multiple Range Test (DMRT). Prior to statistical analysis, the normality of each data set was tested using an Anderson-Darling test, in case a transformation was necessary. Log base 10 transformation was done where required. Means were separated by MS Excel (MS Office 2010).

Results and discussion

Germination and seedling growth

The highest (77%) seed germination was recorded in 2% of MI, while the lowest (61%) was recorded in control treatment (without inoculation). Shoot (54.0 cm) and root (37.6 cm) lengths were also highest in 2% of MI (Table 1). With the application of 2% of MI, shoot length increment was 33% compared to control (Fig. 2). Collar diameter was maximum (8.32 mm) in 2% of MI and was significantly ($P=0.015$) different

Table 1: Influence of microbial inoculant on germination, shoot and root lengths, collar diameter and leaf number of *Albizia lebbeck* in the nursery

Concentration of MI (%)	Germination (%)	Length (cm)			Collar dia. (mm)	Number of leaf
		Shoot	Root	Total		
Control	61 _c	40.6 _b	27.8 _b	68.4 _b	6.41 _b	16.8 _b
0.1	65 _b	45.3 _{ab}	31.2 _{ab}	76.5 _{ab}	6.95 _b	17.6 _b
0.5	72 _a	48.4 _a	33.5 _a	81.9 _a	7.42 _a	18.0 _a
1	74 _a	52.3 _a	35.7 _a	88.0 _a	7.93 _a	19.6 _a
2	77 _a	54.0 _a	37.6 _a	91.6 _a	8.32 _a	20.2 _a
5	68 _b	47.1 _{ab}	32.3 _{ab}	79.4 _{ab}	7.47 _a	18.4 _a
10	65 _b	42.9 _b	29.4 _b	72.3 _b	6.94 _b	17.2 _b
P value	<0.001	0.002	<0.001	<0.001	0.015	0.006
F value	137.18	16.37	74.82	104.51	6.35	9.21

Note: a-c = Mean values with different lowercase superscripts in a column are significantly different at $P<0.05$, according to Duncan's Multiple Range Test (DMRT).

from the control. Seedlings treated with MI had more leaf compared to the control (Table 1).

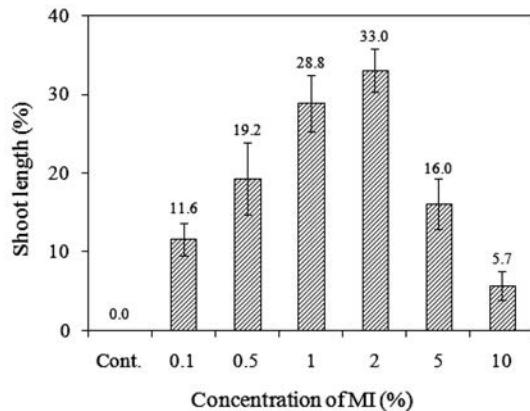


Fig. 2: Influence of microbial inoculant on shoot length increased to decreased (%) with respect to control in *Albizia lebbeck*. Cont. = Control.

Both fresh (21.4 g) and dry (6.95 g) shoot weights were maximum in 2% of MI. Both fresh and dry root weights were also maximum (9.1 g and 3.01

g, respectively) in 2% of MI and were significantly ($P<0.001$ and $P=0.026$, respectively) varied from control (Table 2). Though maximum vigor index, volume index and quality index (7053, 3738 and 1.106, respectively) were found in 2% of MI (Table 2 and Fig. 3), the maximum sturdiness (65.95) was found in 1% of MI treatment (Fig. 4). The total dry biomass increased gradually with the increase of the concentrations of MI up to 2% while decreased gradually from its maximum point as the concentrations of MI increased above 2%. Increased biomass production might be due to the better root development in the treated seedlings (Khan et al., 2011, 2014). Such promotion might also be due to the biological active substances in the inoculant (Lim et al., 1999), such as indole acetic acid (IAA) and gibberellins produced by *Lactobacillus* spp., *Rhodopseudomonas* spp., *Aspergillus* spp. and *Saccharomyces* spp. which enhance plant growth (Chowdhury et al., 1994). However, germination rate was below 80% which was probably due to the quality of seeds as well

Table 2: Influence of microbial inoculant on fresh and dry weights of shoot and root, vigor index and volume index of *Albizia lebbeck*

Concentra- tion of MI (%)	Fresh weight (g)			Dry weight (g)			Total dry biomass increment (%)	Index	
	Shoot	Root	Total	Shoot	Root	Total		Vigor	Volume
Control	14.9 _b	5.9 _b	20.8 _b	4.48 _b	1.89 _c	6.37 _b	00.00	4172 _c	1668 _c
0.1	15.7 _b	6.7 _b	22.4 _b	4.72 _b	2.25 _b	6.97 _b	+9.42	4973 _b	2188 _b
0.5	16.5 _b	7.3 _a	23.8 _{ab}	4.65 _b	2.37 _b	7.02 _b	+10.20	5897 _a	2665 _a
1	19.3 _a	8.0 _a	27.3 _a	5.88 _a	2.58 _a	8.46 _a	+32.81	6512 _a	3289 _a
2	21.4 _a	9.1 _a	30.5 _a	6.59 _a	3.01 _a	9.60 _a	+50.71	7053 _a	3738 _a
5	20.7 _a	8.8 _a	29.5 _a	6.29 _a	2.92 _a	9.21 _a	+44.58	5399 _b	2628 _a
10	17.6 _b	7.8 _a	25.4 _{ab}	5.16 _a	2.57 _a	7.73 _a	+21.35	4700 _b	2066 _b
P value	<0.001	<0.001	<0.001	0.017	0.026	0.021	--	<0.001	<0.001
F value	146.12	114.09	127.65	6.19	4.83	5.27	--	57.65	88.34

a-c = Mean values with different lowercase superscripts in a column are significantly different at $P<0.05$, according to Duncan's Multiple Range Test (DMRT).

as the environmental factors regulating seed germination.

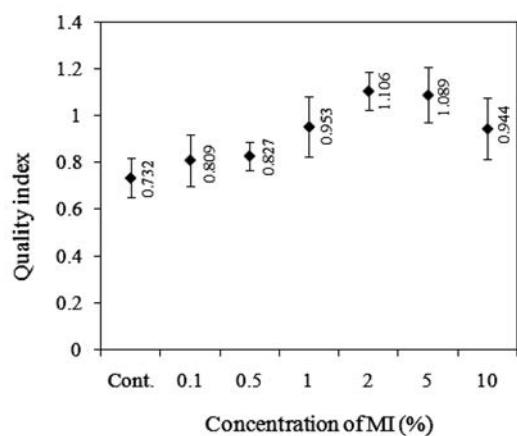


Fig. 3: Influence of microbial inoculant on quality index of *Albizia lebbeck*. Cont. = Control.

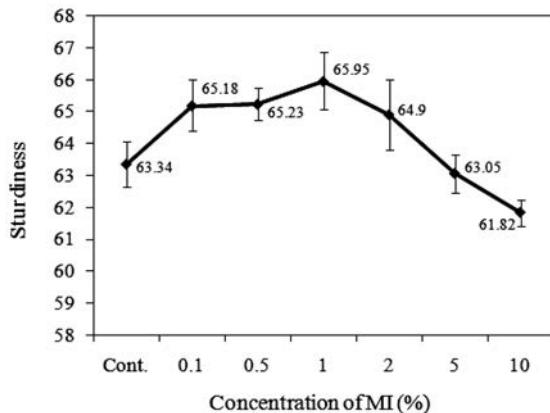


Fig. 4: Influence of microbial inoculant on sturdiness of *Albizia lebbeck*. Cont. = Control.

Microbial inoculants are being applied in Japan, China, Thailand, United States, France, Brazil and

many other countries of the world. Application of MI can play a role in enhancing germination, growth, and yield of various agricultural crops and vegetables (Vongprachanch, 1995; Zacharia, 1995; Iwaishi, 2000; Chowdhury et al., 2002). MI with organic fertilizer and other chemicals is also reported to enhance germination, growth, and yield of different grains (Ahmed et al., 1995; Anuar et al., 1995; Xu, 2000).

But the influence of MI on forest crops has not been studied widely (Khan et al., 2006, 2011). From this study, it has been observed that soil amended with different concentrations of MI can also improve the seedling growth of forest crops. Mridha (2005), Khan (2012) and Khan et al. (2011, 2014) also reported enhanced seed germination rate and seedling growth with the application of low concentrations of MI. Specially, applying 2% of MI enhanced seed germination and seedling growth at an optimum level which might be due to the presence of microbial population in such a density within this concentration which was capable to produce optimum level of growth enhancing hormones and other chemicals. However, as the concentration of MI increased, seed germination and seedling growth was depressed, which may be due to the perniciousness exerted by the higher concentrations of the inoculant (Mridha, 2005; Khan et al., 2014).

Nodulation status

Although most of the parameters were highest in 2% of MI, the highest (134) nodule number was in 0.5% of MI while the lowest (95) was in 10% of MI (Table 3). Both fresh and dry nodule weights were maximum (2.07 g and 0.67 g,

Table 3: Influence of microbial inoculant on nodule number and their fresh and dry weights of *Albizia lebbeck*

Concentration of MI (%)	Number	Nodule			Weight increased or decreased (%)
		Fresh	Dry	Fresh	Dry
Control	127 ^a	1.77 ^a	0.56 ^{ab}	0.00	0.00
0.1	131 ^a	2.01 ^a	0.65 ^a	+13.56	+16.07
0.5	134 ^a	2.07 ^a	0.67 ^a	+16.95	+19.64
1	121 ^a	1.89 ^a	0.61 ^a	+6.78	+8.93
2	116 ^b	1.75 ^a	0.54 ^{ab}	-1.13	-3.57
5	104 ^b	1.57 ^b	0.51 ^{ab}	-11.30	-8.93
10	95 ^c	1.42 ^b	0.44 ^b	-19.77	-21.43
P value	<0.001	0.013	0.019	--	--
F value	51.42	6.68	5.3	--	--

a-c = Mean values with different lowercase superscripts in a column are significantly different at $P<0.05$, according to Duncan's Multiple Range Test (DMRT)

respectively) at 0.5% of MI and lowest (1.42 g and 0.44 g, respectively) were in 10% of MI. The rate of nodule increment was positive in case of 0.1% and 0.5% of MI, while negative for all other treatments compared to control (Fig. 5). The nodule dry weight increment rate was positive for 0.1%, 0.5%, and 1% of MI while negative for all other treatments, with respect to control (Table 3). The number of nodule in soybean root was also not changed significantly due to application of low concentrations of MI (Thach et al., 1999) while decreased in higher concentrations in *Dalbergia sissoo* and *Acacia auriculiformis* (Khan et al., 2011, 2014). The higher concentrations of MI solution in the substratum may affect the growth of plants because of toxic effect of secretion of toxic metabolites.

Content of leaf pigments

Effects of MI on the content of leaf pigments (Chlorophyll-a, chlorophyll-b, and carotenoid) were also determined (Table 4). Chlorophyll-a was highest (56.12 mg.L⁻¹), in 2% of MI and lowest (37.39 mg.L⁻¹) in the control treatment. Chlorophyll-b was highest (15.67 mg.L⁻¹) in 2% of MI, followed by 14.98 mg.L⁻¹ in 5% and 13.59 mg.L⁻¹ in 1% of MI, and was significantly ($P<0.001$) varied from control. Carotenoid was maximum (41.07 mg.L⁻¹) in 2% of MI. Total pigment was recorded highest (112.86 mg.L⁻¹) in 2% of MI and was significantly ($P<0.001$) different from control. The results are in agreement with the findings of Xu (2000), Wang et al. (2000), Mridha et al. (2002), Khan et al. (2006, 2011) and Khan (2012) that low concentrations of MI

Table 4: Influence of microbial inoculant on pigment contents in fresh leaves of *Albizia lebbeck*

Concentration of MI (%)	Pigments concentration of leaf (mg.L ⁻¹)				Total pigment increment (%)
	Chlorophyll-a	Chlorophyll-b	Carotenoid	Total	
Control	37.39 _c	10.34 _b	28.41 _c	76.14 _c	0
0.1	41.51 _b	11.75 _{ab}	32.97 _b	86.23 _b	+13.25
0.5	43.34 _b	12.06 _{ab}	34.02 _b	89.42 _b	+17.44
1	50.02 _a	13.59 _a	37.18 _a	100.79 _a	+32.37
2	56.12 _a	15.67 _a	41.07 _a	112.86 _a	+48.23
5	54.17 _a	14.98 _a	40.84 _a	109.99 _a	+44.46
10	47.49 _b	12.32 _{ab}	31.49 _b	91.30 _b	+19.91
P value	<0.001	<0.001	<0.001	<0.001	--
F value	38.26	49.58	91.63	61.47	--

a-c = Mean values with different lowercase superscripts in a column are significantly different at $P<0.05$, according to Duncan's Multiple Range Test (DMRT)

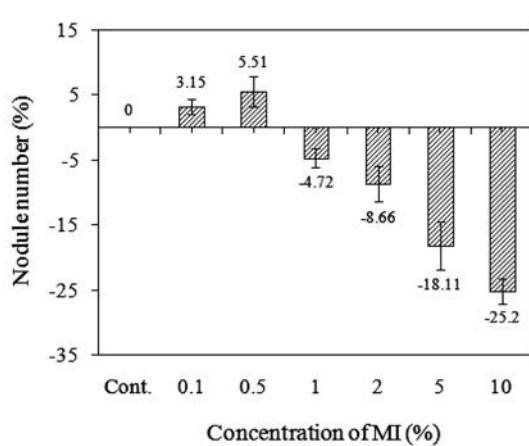


Fig. 5: Influence of microbial inoculant on nodule number increased to decreased (%) with respect to control in *Albizia lebbeck*. Cont. = Control.

with organic fertilizer promote root growth and enhance photosynthetic efficiency and yield of seedling.

Conclusion

Microbial inoculant influences seed germination and seedling growth of *A. lebbeck* and the low concentration (2%) of the inoculant can be recommended for getting maximum seed germination and seedling growth of the species in the nursery conditions that may also be effective for seedling development in the field.

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