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Microbial Inoculant for Seed Germination and Seedling Growth of *Acacia mangium* Willd

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ABSTRACT

Microbial inoculants (MI), a biofertilizer, composed of many different beneficial microorganisms in a solution; was applied to test its efficacy on seed germination and seedling growth of *Acacia mangium* in the nursery conditions. 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. Proper control was maintained without MI inoculation. Most of the parameters studied (seed germination, shoot and root lengths, dry weights of shoot and root, collar diameter, phyllode number etc) were found maximum in 2% of MI. Although the highest vigor index, volume index and sturdiness (6308, 2425 and 70.1, respectively) were recorded in 2% of MI, the highest quality index (0.74) was recorded in 5% of MI. The nodule number was highest at a very low (0.5%) concentration of MI, but it normally decreased with the increase of concentrations. Total pigment content in phyllode was recorded highest (99.22 $mg.L^{-1}$) in 2% of MI. Therefore, MI influences seed germination and seedling growth of *A. mangium* 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.

KEYWORDS: Microbial inoculant, *Acacia mangium*, germination, seedling growth, nodulation status, phyllode's pigment.

INTRODUCTION

Mangium (*Acacia mangium* Willd.) is one of the most widely used fast-growing, moderate-sized, evergreen, multipurpose tree species under the family Leguminosae (Mimosoideae). The species is extensively spread throughout the world, and is native to Queensland, Australia, the south western portion of New Guinea, and the Molluca Islands of eastern Indonesia [1, 2, 3]. The species is used in plantation forestry programme throughout Asia, the Pacific and the humid tropics. Since its successful introduction in Sabah, Malaysia, in the mid 1960s, it has been widely introduced into many countries including Indonesia, Malaysia, Papua New Guinea, India, Bangladesh, Sri Lanka, China, Thailand, Vietnam and Philippines while naturalized in Brazil and Puerto Rico [4, 5]. The wood makes attractive furniture and cabinets, door frames, window parts moldings and sliced veneer. It is an excellent fuel wood and also used for pulp and paper, particle board and timber and the leaves are eaten by livestock [1, 3, 5, 6,]. The tree is used as ornamental, shade, windbreaks and nurse tree [2, 4, 7]. The plant is also known as soil improver and very useful for the rehabilitation of degraded lands because of its rapid growth, inherent ability to fix nitrogen and tolerance of a wide range of soils and environments [1, 8]. In Bangladesh, the species has already been proven successful for afforestation, reforestation, agroforestry and social forestry programs [9, 10].

Due to the rapid growth and tolerance of very poor soils, *A. mangium* is playing an increasingly important role in efforts to sustain a commercial supply of tree products whilst reducing pressure on natural forest ecosystems [1, 8, 11]. In Bangladesh, many organizations are also producing *A. mangium* seedlings in the nursery to fulfill the high demand in the plantation programs. As the plants are grown mostly in unfavorable soil and environmental conditions, beneficial soil microorganisms such as microbial inoculant can play an influential 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 by Dr. Teruo Higa, Professor of Horticulture at the University of Ryukyus, Okinawa, Japan, in the early 1980s [12]. Photosynthetic bacteria (*Lactobacillus spp.*, *Streptococcus spp.*), lactic acid bacteria (*Rhodopseudomonas spp.*), actinomycetes (*Streptomyces spp.*), yeast (*Saccharomyces spp., Candida spp.*) and beneficial fungi (*Aspergillus spp.*, *Penicillium spp.*) are

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the main species comprising the MI. Into the inoculant, the microorganisms are added 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 × 106 to 1 × 108 mL^{-1} [13]. MI can be applied in the soils to increase the diversity of beneficial microorganisms. It has been used with considerable success to improve soil quality and yield of crops, particularly in nature farming and organic farming systems [13]. It can also improve photosynthesis and fruit yield of crops and vegetables [13, 14]. Although *A. mangium* is used for a 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 the germination of seed and the growth of seedlings of *A. mangium* and also to find out the best concentration of MI for ensuring maximum germination and seedling development in the nursery conditions.

MATERIALS AND METHODS

Collection of seeds and soils

The experiment was conducted in the nursery of the Institute of Forestry and Environmental Sciences; University of Chittagong, Bangladesh (lies approximately at the intersection of $91^0$50´E and $22^0$30´N) (Figure 1). The seeds of *A. mangium* were collected from the seed orchard division of Bangladesh Forest Research Institute (BFRI) where the source of seed was a mother tree of around 15 years old. The soils collected from the degraded hills of the University Campus was sieved well (<3mm) and mixed thoroughly with decomposed cow dung in a ratio of 3:1. The brown hill soils are sandy loam to sandy clay loam, moderately to strongly acid and poorly fertile with pH <5.5, organic matter <2.0%, BSP <40%, CEC <10 me/100g [15]. The white polybags of 15 cm x 10 cm in size were filled with the prepared mixture, while to reduce the rate of evaporation as well as to provide a source of organic matter, a thin layer of coconut husk was added to each bag as a top layer.

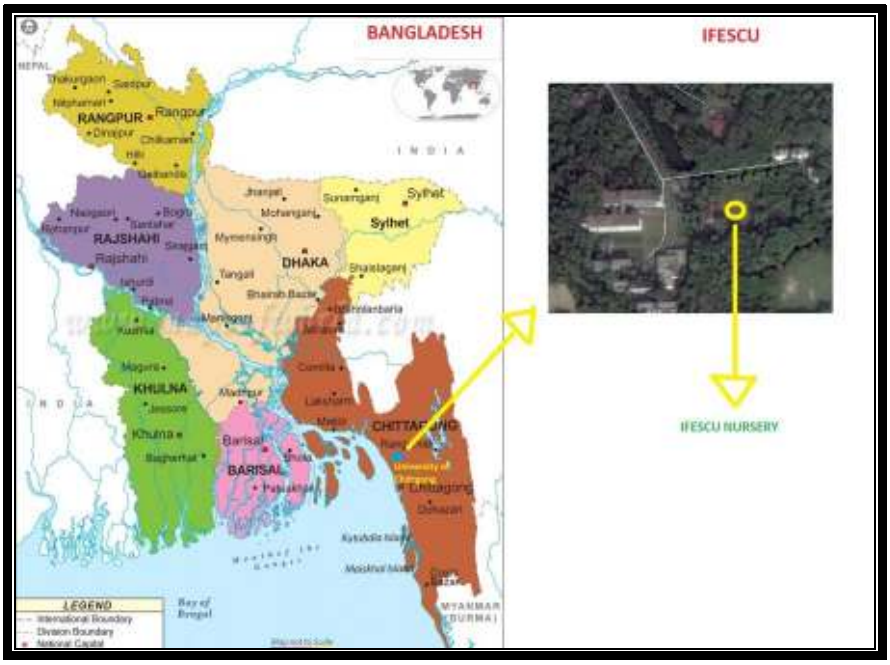

Figure 1: Map showing the location of the nursery of IFESCU (Institute of Forestry and Environmental Sciences, University of Chittagong) in Bangladesh where the experiment was conducted.

Treatment design

There were seven treatments including control and 25 replications for each treatment. Seeds were sown in polybags 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 each treatment, 50 mL of MI of required concentration was poured in each polybag soils before a week of sowing the seeds while another 50 mL was poured after a week of sowing the seeds. Four seeds were sown in each polybag to observe the influence of MI on germination in the nursery conditions (temperature, 28^0C; 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. Shoot and root lengths, collar diameter, phyllode number, fresh shoot and root weights, dry shoot and root weights and nodulation status were the recorded parameters. Shoots and roots were oven dried at 75ºC for 48 hr for recording dry weights. 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 as germination percent X seedling total length i.e. total shoot and root length [16]. 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.* [17] to quantify seedlings morphological quality. The formula for calculating quality index is as follow:

$$QI = T_{dw} / \left(\frac{H}{D_c} + \frac{S_{dw}}{R_{dw}} \right)$$

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). Dividing shoot height or shoot length (cm) with collar diameter (cm) of the seedling, sturdiness was obtained.

Measurement of pigment contents

Chlorophyll-a, chlorophyll-b, and carotenoid were determined in different treatments from the fresh phyllode of seedlings. Ten discs of phyllode were cut with a cork borer (inside diameter of 5 mm), weighed immediately, and dipped in 100% acetone of 5 mL in test tube with stopper. After 24 hr of incubation, the supernatant-colored solution from the top was decanted into a 25 mL volumetric flask. The phyllode 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. The supernatant-colored solution from the top was again decanted to the same volumetric flask very carefully. The process was repeated until the phyllode 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. With a spectrophotometer (Spectronic-20), the measurement of chlorophyll-a, chlorophyll-b, and carotenoid were made at 662 nm, 644 nm, and 440.5 nm respectively. The pigment contents in the extract were calculated using the following formulae [18, 19].

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

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

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

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; F_W the fresh weight of phyllode disc (g); and E is the absorbance at a particular wavelength (662, 644 and 440.5 nm).

Statistical analysis

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

RESULTS AND DISCUSSION

Germination and seedling growth

The highest (75%) seed germination was recorded in 2% of MI, while the lowest (58%) was recorded in control treatment (without inoculation). Shoot (49.2 cm) and root (34.9 cm) lengths were also highest in 2% of MI (Table 1). Collar diameter was maximum (7.02 mm) in 2% of MI and was significantly (*P*=0.018) varied from control. Seedlings treated with MI had more phyllode compared to the control (Table 1).

Table 1. Influence of microbial inoculant on germination, shoot and root lengths, collar diameter and phyllode number of *Acacia mangium* in the nursery.

Microbial inoculant (%)	Germination (%)	Length (cm)			Collar dia. (mm)	Number of phyllode
		Shoot	Root	Total		
Control	58^{c}	36.5^{b}	25.2^{b}	61.7^{c}	5.47^{b}	14.9^{b}
0.1	60^{b}	41.2^{ab}	29.8^{b}	71.0^{b}	5.98^{b}	15.7^{b}
0.5	67^{b}	43.4^{a}	31.4^{a}	74.8^{b}	6.26^{a}	16.6^{a}
1	72^{a}	47.1^{a}	33.6^{a}	80.7^{a}	6.79^{a}	18.1^{a}
2	75^{a}	49.2^{a}	34.9^{a}	84.1^{a}	7.02^{a}	19.7^{a}
5	73^{a}	42.7^{ab}	32.3^{a}	75.0^{b}	6.53^{a}	17.5^{a}
10	64^{b}	38.8^{b}	27.1^{b}	65.9^{c}	6.11^{a}	16.3^{b}
P value	<0.001	<0.001	<0.001	<0.001	0.018	0.029
F value	128.57	54.89	74.82	115.63	5.94	4.73

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).

Both fresh (16.9 g) and dry (4.28 g) shoot weights were maximum in 2% MI inoculated treatment and they were significantly (*P*=0.023 and *P*=0.025, respectively) varied from control. Both fresh and dry root weights were also maximum (6.7 g and 2.23 g, respectively) in 2% of MI (Table 2). Although maximum vigor index, volume index and sturdiness (6308, 2425 and 70.1, respectively) were recorded in 2% of MI (Table 2 and Figure 2), the maximum quality index (0.74) was recorded in 5% of MI (Figure 3). 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 [20, 21]. Because, better root development (with respect to root length and diameter) means higher capacity and chance of plant to uptake nutrient elements from buck of soil, that is from longer depth and distance in the soil, ultimately higher growth and biomass production in the plant. Such promotion might also be due to the biological active substances in the inoculant [22], such as indole acetic acid (IAA) and gibberellins produced by *Lactobacillus spp.*, *Rhodopseudomonas spp.*, *Aspergillus spp.* and *Saccharomyces spp.* which enhance plant growth [23]. Secretion of growth enhancing substances by the microbes may also be responsible for better root and shoot growth as well as higher number of phyllode in the treated seedlings. However, excessive secretion of the chemicals might cause toxicity, resulting growth reduction, which was probably happened for the seedlings treated with higher concentrations of MI.

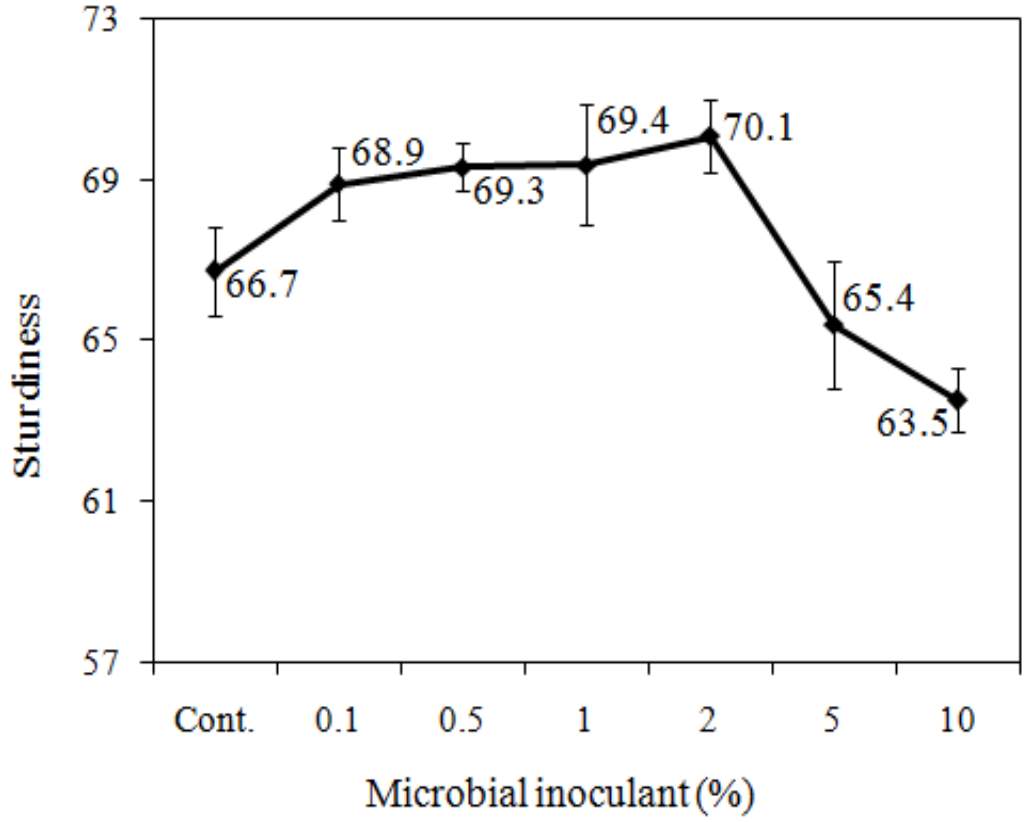

Figure 2. Influence of microbial inoculant on sturdiness of *Acacia mangium*.

Microbial inoculant is being applied in Japan, China, Philippines, Thailand, Vietnam, United States, Brazil, France and many other countries of the world. Application of MI can play a role in enhancing germination, growth, and yield of different agricultural crops and vegetables [24, 25, 26, 27]. MI with organic fertilizer and other chemicals is also reported to enhance germination, growth, and yield of various grains [13, 28, 29].

But the influence of MI on forest crops has not been studied widely. From this study, it has been observed that soil amended with different concentrations of MI can also enhance the seed germination and improve the seedling growth of forest crops. Mridha [30], Khan [19], and Khan *et al*. [20, 21, 31] also reported enhanced seed germination rate and seedling growth with the application of low concentrations of the inoculant. However, as the concentration of inoculant increased, seed germination rate and seedling growth was depressed, which may be due to the perniciousness exerted by the higher concen-trations of the inoculant [21, 32].

Table 2. Influence of microbial inoculant on fresh and dry weights of shoot and root, vigor index and volume index of *Acacia mangium.*

Microbial inoculant (%)	Fresh weight (g)			Dry weight (g)			Total dry biomass increment (%)	Index	
	Shoot	Root	Total	Shoot	Root	Total		Vigor	Volume
Control	10.9[b]	3.6[b]	14.5[b]	2.73[b]	1.21[c]	3.94[b]	00.00	3571[c]	1092[c]
0.1	11.7[b]	4.5[b]	16.2[b]	2.97[b]	1.52[b]	4.49[b]	+13.96	4260[b]	1473[b]
0.5	12.8[b]	5.4[ab]	18.2[ab]	3.23[b]	1.84[b]	5.07[b]	+28.68	5012[b]	1701[b]
1	15.6[a]	6.2[a]	21.8[a]	3.91[a]	2.07[a]	5.98[a]	+51.78	5810[a]	2172[a]
2	16.9[a]	6.7[a]	23.6[a]	4.28[a]	2.23[a]	6.51[a]	+65.23	6308[a]	2425[a]
5	16.2[a]	6.5[a]	22.7[a]	4.05[a]	2.17[a]	6.22[a]	+57.87	5475[a]	1821[a]
10	14.5[a]	5.7[a]	20.2[a]	3.63[a]	1.90[a]	5.53[a]	+40.36	4218[b]	1448[b]
P value	0.023	<0.001	<0.001	0.025	<0.001	<0.001	--	<0.001	<0.001
F value	5.17	66.79	35.98	5.16	113.65	59.41	--	127.49	134.06

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).

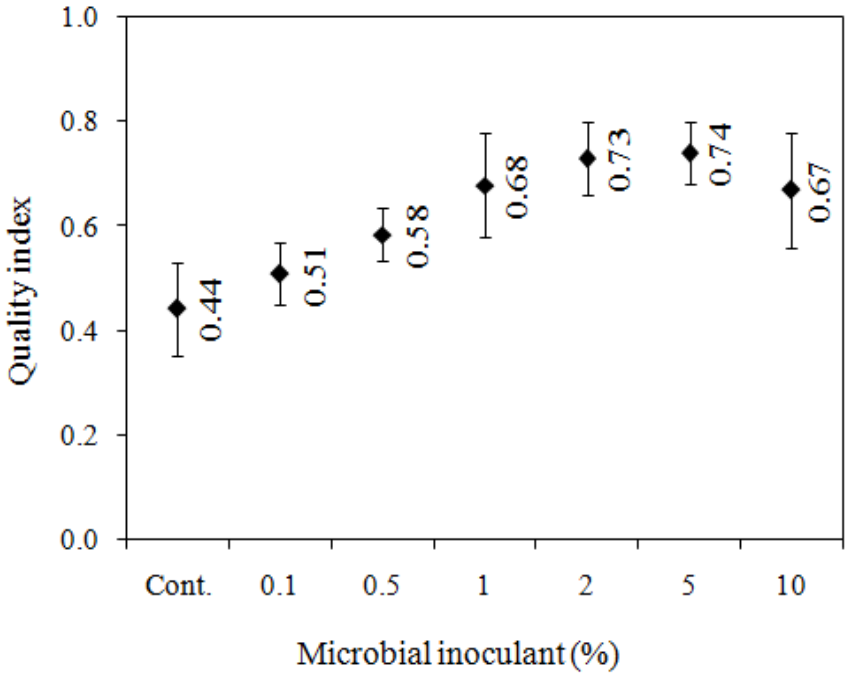

Figure 3. Influence of microbial inoculant on quality index of *Acacia mangium*.

Nodulation status

Although most of the parameters were highest in 2% of MI, the highest (112) nodule number was in 0.5% of MI while the lowest (75) was in 10% of MI (Table 3). Both fresh and dry nodule weights were maximum (2.07 g and 0.53 g, respectively) at 0.5% of MI and lowest (1.36 g and 0.33 g, respectively) were in 10% of MI. The nodule dry weight increment rate was positive in 0.1% and 0.5% of MI while negative in all other treatments, in comparison with control (Table 3). The number of nodule in soybean root was also not changed significantly due to application of low concentrations of MI [33], while decreased in higher concentrations in *Albizia procera* and *Dalbergia sissoo* [20, 31], The higher concentrations of MI solution in the substratum may affect the growth of plants because of secretion of toxic metabolites.

Table 3. Influence of microbial inoculant on nodule number and their fresh and dry weights of *Acacia mangium*.

Microbial inoculant (%)	Nodule				
	Number	Weight (g)		Weight increased or decreased (%)	
		Fresh	Dry	Fresh	Dry
Control	104[a]	1.89[a]	0.47[a]	0.00	0.00
0.1	109[a]	1.95[a]	0.50[a]	+3.17	+6.38
0.5	112[a]	2.07[a]	0.53[a]	+9.52	+12.77
1	101[a]	1.78[a]	0.45[a]	-5.82	-4.26
2	97[b]	1.62[b]	0.40[b]	-14.29	-14.89
5	86[b]	1.51[b]	0.37[b]	-20.11	-21.28
10	75[c]	1.36[c]	0.33[c]	-28.04	-29.79
***P* value**	<0.001	<0.001	<0.001	--	--
***F* value**	140.23	96.75	77.42	--	--

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).

Content of phyllode pigments

Influence of MI on the content of pigments (Chlorophyll-a, chlorophyll-b, and carotenoid) in phyllode were also determined (Table 4). Chlorophyll-a was maximum (49.23 $mg.L^{-1}$) in 2% of MI inoculated treatment while lowest (33.49 $mg.L^{-1}$) in control. Whereas the content of chlorophyll-b was maximum (13.06 $mg.L^{-1}$) in 2% of MI, the content of carotenoid was maximum (37.19 $mg.L^{-1}$) in 5% of MI. Total pigment was recorded highest (99.22 $mg.L^{-1}$) in 2% of MI and was significantly ($P<0.001$) varied from control. The results are in agreement with the findings of Wang *et al.* [14], Xu

[13], Mridha *et al.* [32], and Khan *et al*. [19, 20] that low concentrations of inoculant with organic fertilizer promote root growth and enhance photosynthetic efficiency and yield of seedlings.

Table 4. Influence of microbial inoculant on pigment contents in fresh phyllodes of *Acacia mangium*.

Microbial inoculant (%)	Pigment contents (mg.L^{-1})				Total pigment increment (%)
	Chlorophyll-a	Chlorophyll-b	Carotenoid	Total	
Control	33.46^{c}	8.52^{b}	26.73^{c}	68.71^{c}	0.00
0.1	38.62^{b}	9.84^{b}	30.92^{b}	79.38^{b}	+15.53
0.5	40.23^{b}	10.31^{a}	32.45^{ab}	82.99^{b}	+20.78
1	44.71^{a}	11.71^{a}	35.27^{a}	91.69^{a}	+33.44
2	49.23 ^{a}	13.06^{a}	36.93^{a}	99.22^{a}	+44.40
5	45.37^{a}	12.12^{a}	37.19^{a}	94.68^{a}	+37.80
10	39.35^{b}	10.18^{ab}	29.31^{b}	78.84^{b}	+14.74
P value	<0.001	0.013	<0.001	<0.001	--
F value	63.57	6.68	68.49	93.75	--

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).

CONCLUSION

Microbial inoculant influences seed germination and seedling growth of *A. mangium* 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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