Growth Improvement of Seedlings of A Tropical Forest Species, Khair (*Acacia catechu* Willd.), Using Beneficial Microbial Inoculant (Effective Microorganisms)

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Abstract. Microbial Inoculant (EM) was used to find out their effectiveness on the growth and development of seedlings of a tropical forest species, Khair (*Acacia catechu* Willd.), in the nursery. Effective Microorganisms (EM) are a combination of many different beneficial microorganisms in a solution. The seedlings were grown in a mixture of forest top soils and cowdung (3:1) kept in polybags. The EM solution at different concentrations (0.1, 0.5, 1, 2, 5 and 10%) was added before and after a week of sowing the seeds. Germination as well as physical growth parameters like, shoot and root length, vigor index, collar diameter, leaf number, fresh and dry weight of shoot and root and total biomass increment were measured. The nodulation status influenced by EM was also observed along with the measurement of chemical parameters like chlorophyll a, chlorophyll b and carotenoid. Both germination and the measured physical parameters were found significantly higher in seedlings treated with different concentrations of EM solution in comparison with control. Maximum growth was observed at 2% concentration followed by 5% and 1%. Nodulation was higher at 0.1% concentration of EM but it normally decreased with the increase of concentrations. Although there was a higher amount of pigments in leaves of the treated seedlings than the control but the variations recorded with respect to chlorophyll a, chlorophyll b and carotenoid were not significantly higher in most of the treatments. The results of the present study indicate that the Microbial Inoculant (EM) technology might be useful to improve the growth of seedlings in the nursery. These also indicate that the associated beneficial organisms along with the polybag soils might be of value in improving the degraded soil or poor field soil for better nutrient uptake and water uptake during the initial growth of transplanted seedlings.

Key words: Khair (*Acacia catechu* Willd.), Microbial Inoculant (EM), Germination and seedling growth, Pigment concentration of leaf, Nodulation status.

Introduction

Khair (*Acacia catechu* Willd.) is an important multipurpose tree species under the family Leguminosae (Mimosoideae). It is a small to moderate size deciduous tree with a light feathery crown. *Acacia catechu* is found chiefly in greater parts of India, West Bengal, Assam, Mayanmar, Bangladesh and some other tropical countries of the world. It is essentially a tree of dry regions. Although, it develops to its maximum size in localities with heavy rainfall, in dry situations it grows where few other trees can survive. It grows commonly on sandy and gravelly alluvium or gravelly loam. The tree is capable of growing on very poor shallow soils even in the rocky out-crops and those which are composed of calcareous soil. Khair is a strong light demander and does not tolerate heavy shade (Luna, 1996).

Khair grows naturally in different parts

of Bangladesh and it can also be planted under different plantation programs like, Agroforestry, Community Forestry, Social Forestry, Village and Farm Forestry Programs in different tropical countries of the world. It is widely used for its medicinal value and to produce khair for chewing pan (*Piper betle*) and preparing dye. To fulfil the demand in the plantation programs, many organizations are producing Khair seedlings in the nursery in Bangladesh. Artificially, as the plants are grown in unfavorable soil conditions, beneficial soil microorganisms like EM can play a significant role in early establishment and better growth of the inoculated seedlings under field conditions.

Effective microorganisms (EM) can be applied as inoculant to increase the microbial diversity of soils and plants. It has been used with considerable success to improve soil quality, soil health and growth, yield and quality of crops particularly in nature farming and organic farming systems (Xu, 2000). The inoculation of EM cultures to the plant ecosystem can improve the photosynthesis and fruit yield of plants (Xu, 2000; Wang *et al.* 2000). The objective of this study is to observe the effectiveness of EM inoculant on germination and the growth of Khair seedlings and also to find out the best concentration of EM solution for ensuring maximum seedling development in the nursery.

Materials and Methods

The experiment was carried out in the nursery of the Institute of Forestry and Environmental Sciences, University of Chittagong, Chittagong, Bangladesh. The seeds of *Acacia catechu*, used in this experiment were collected from the seed orchard division of Bangladesh Forest Research Institute (BFRI). The soils, collected from the forest floor of the University Campus was sieved well (<3mm) and mixed with decomposed cow dung in a ratio of 3:1. Polybags of 15cm x 10cm (6"x 4") in size were filled with the prepared mixture and a layer of coir (1 cm) was provided in each of the polybags as a top layer of the polybag media to reduce the evaporation and to supply a source of organic matter. There were seven treatments including control and 12 replications for each treatment. Seeds sown in polybag not added EM but water only (Control) and seeds sown in polybag soil added with 0.1%, 0.5%, 1%, 2%, 5% and 10% concentrations of EM solution. 50 ml solution of EM was mixed with the soils before one week of sowing the seeds and another 50 ml was applied after one week of sowing the seed in the polybag. Three seeds were sown in an individual polybag to observe the influence of EM on germination and after completing germination only one seedling per polybag was maintained to observe the physical and chemical growth parameter and nodulation status of seedlings. Partial shade and covering was provided over the nursery to protect the seedling from strong sunlight and rains.

Germination was recorded daily from the date of seed sowing and continued up to germination of the last seed. The seedlings were allowed to grow altogether for three months from the time of seed sowing. After three months, five representative seedlings from each treatment were selected for measuring physical parameters. The recorded parameters were, shoot and root length, collar diameter, leaf number, fresh shoot and root weight, dry shoot and root weight, nodule number and their fresh and dry weight etc. For recording dry weight, shoots and roots was oven dried at 70°C for 48 hours and its weight was measured until the constant weight was obtained. Other than the above parameters, some parameters such as vigor index, total dry biomass increment (%), number of nodule increased/ decreased (%), fresh and dry weight of nodule increased/

decreased (%) etc were also calculated.

The pigment content (Chlorophyll a, chlorophyll b and carotenoid) of the fresh leaves of seedlings of different treatments were determined with the leaves collected from the second, third and fourth from the top. Ten leaf discs were cut with a cork borer (inside diameter 5mm), weighed immediately after cutting and dipped in 5 ml 100% acetone in test tube with stopper. After 24 hours of incubation, the supernatant colored solution from the top was decanted carefully in 25ml volumetric flask. The leaf discs were then crushed with a blunt glass rod gently and 5ml fresh acetone was added to the test tube and left for 15 minutes. Then the supernatant 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 measurement was taken immediately after the preparation of 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 concentrations of pigments in the extract were calculated by following the formula of Wettstein (1957).

All the data were analyzed statistically by using the computer software package SPSS and they were subjected to analysis by DMRT.

Results and Discussion

Germination and morphological growth parameters of seedlings

The effects of EM on the germination and morphological growth parameters of seedlings like, shoot length, root length, total length, collar diameter and number of leaf are shown in Table 1. The highest germination (65%) was observed in 5% EM solution followed by 64% germination in 2% and 61% germination in 1% concentration of EM solution. Highest shoot growth (55.8 cm) was observed in 2% concentration of EM solution whereas highest root growth (32 cm) was found in 5% concentration. Collar diameter was highest (5.15 mm) in 2% concentration of EM solution followed by 1% and 5% concentrations and was significantly ($P<0.05$) different from control and 0.1%, 0.5% and 10% concentrations. EM treated seedlings showed more leaves compared with control (Table 1). Vigor index was highest (5597) in 5% concentration of EM solution followed by 2% and 1% concentrations and was

Table 1. Influence of different concentrations of Effective Microorganisms (EM) on germination, shoot and root length, vigor index, collar diameter and leaf number of Khair (*Acacia catechu*) after 3 months of sowing the seeds in the nursery.

Concentration of (EM (%)	Germination of (%)	Length (cm)			Vigor index	Collar dia. (mm)	Number of compound leaf
		Shoot	Root	Total			
Control	48 b *	45.4 b	22.6 c	68.0 b	326 c	3.85 b	26 c
0.1	53 b	48.3 ab	26.7 b	75.0 ab	3975 bc	3.94 b	29 b
0.5	57 a	51.6 ab	27.2 b	78.8 ab	4492 b	4.27 b	32 b
1	61 a	53.2 a	29.4 a	82.6 a	5039 a	4.83 a	34 b
2	64 a	55.8 a	31.3 a	87.1 a	5574 a	5.15 a	41 a
5	65 a	54.1 a	32.0 a	86.1 a	5597 a	4.60 a	33 b
10	55 b	52.7 a	28.1 b	80.8 ab	4444 b	4.19 b	30 b

*- Means followed by the same letter (s) in the same column do not vary significantly at $P<0.05$, according to Duncan's Multiple Range Test (DMRT).

significantly (P<0.05) different from control and 0.1%, 0.5% and 10% concentrations of EM solution (Table 1).

Fresh and dry matter production

Both fresh and dry shoot weights were maximum (5.64 g and 2.85 g respectively) in 2% concentration of EM solution and were significantly (P<0.05) different from control. Both fresh and dry root weights were also maximum (2.47 g and 1.58 g respectively) in 2% concentration of EM solution and were significantly (P<0.05) different from control. In all the cases, lowest growth were observed in control treatments (Table 2). Total dry biomass increment (%) was highest in 2% concentration of EM solution followed by 1% and 5% concentrations and was positive for all the treatments compared with control. The increased biomass production (shoot and root) in the treated seedlings recorded in the present study might be due to the better root development, which promoted dry matter weight. Such promotion might be due to the biological active substances in EM (Lim *et al.*, 1999) and by the production of growth enhancing components such as IAA and gibberelins which may have positively influenced plant growth (Chowdhury *et al.*, 1994).

Effective microorganisms (EM) inoculants are being applied in Japan, USA, France, China, Brazil, Thailand and many other countries of the world. Application of EM solution can play a role in enhancing germination, growth and yield of various agricultural crops and vegetables (Iwaishi, 2000; Shin *et al.*, 1995; Vongprachanch, 1995; Zacharia, 1995). EM solution with organic fertilizers and other chemicals is also reported to enhance germination, growth and yield of different agricultural crops (Ahmed *et al.*, 1995; Anuar *et al.*, 1995; Xu, 2000). But the influence of EM on the forest crop has not been studied widely (Pandey *et al.*, 2001). From this study on forest crop, it has also been observed that soil amended with different concentrations of EM provided the higher growth, but with the increase of EM concentration above 2%, seedling growth gradually decreased. Mridha *et al.* (1999) also reported that at lower concentration of EM, germination was enhanced by EM treatment, however, as the concentration of EM increased, germination decreased.

Table 2. Influence of different concentrations of Effective Microorganisms (EM) on germination, shoot and root length, vigor index, collar diameter and leaf number of Khair (*Acacia catechu*) after 3 months of sowing the seeds in the nursery.

Concentration of (EM (%)	Fresh weight (g)			Dry weight (g)			
	Shoot	Root	Total	Shoot	Root	Total	Total dry biomass increment (%)
Control	4.47 b *	1.52 b	5.99 b	2.25 b	0.82 b	3.07 b	00.00
0.1	4.73 ab	1.84 ab	6.57 b	2.43 ab	1.05 ab	3.48 b	+ 13.36
0.5	5.08 ab	2.19 a	7.27 ab	2.58 ab	1.24 a	3.82 ab	+ 24.43
1	5.29 a	2.36 a	7.65 a	2.66 a	1.46 a	4.12 a	+34.20
2	5.64 a	2.47 a	8.11 a	2.85 a	1.58 a	4.43 a	+ 44.30
5	5.31 a	2.23 a	7.54 a	2.72 a	1.33 a	4.05 a	+ 31.92
10	5.15 ab	2.04 ab	7.19 ab	2.68 ab	1.24 ab	3.92 ab	+ 27.69

*- Means followed by the same letter (s) in the same column do not vary significantly at P<0.05, according to Duncan's Multiple Range Test (DMRT).

Nodulation status of seedling

Nodule number was highest (57) in 0.1% concentration of EM solution followed by 53 in

Table 3. Influence of different concentration of Effective Microorganisms (EM) on number and their fresh and dry weight of nodule of Khair *(Acacia catechu)* after 3 month of sowing the seeds in the nursery.

		Nodule				
Concentration of (EM (%)	Number	Weight (g)		Number increased / Decreased (%)	Weight increased / decreased (%)	
		Fresh	Dry		Fresh	Dry
Control	53 a *	0.62 a	0.16 a	00.00	00.00	00.00
0.1	57 a	0.71 a	0.20 a	+ 7.55	+ 14.52	+ 25.00
0.5	52 a	0.68 a	0.18 a	-1.89	+9.68	+12.5
1	49 b	0.61 a	0.17 a	-7.55	-1.61	+ 6.25
2	45 b	0.56 ab	0.15 ba	-15.09	-9.68	- 6.25
5	44 b	0.52 ab	0.14 ab	-16.98	-16.13	- 12.50
10	33 c	0.43 b	0.12 b	-39.62	-30.65	- 25.00

*-Mean followed by the same letter (s) in the same column do not vary significantly at P<0.05, according to Duncan's Multiple Range Test (DMRT).

control, 52 in 0.5% concentration and 49 in 1% concentration. Nodule number was lowest (32) in 10% concentration of EM solution. Both fresh and dry weight of nodule was also maximum (0.71 g and 0.2 g respectively) at 0.1% concentration and lowest (0.43 g and 0.12 g respectively) in 10% concentration of EM solution. The rate of nodule increment was positive in case of 0.1% concentration of EM solution and negative for all other treatments compared with control (Table 3). Fresh weight increment rate of nodule was positive in 0.1% and 0.5% concentrations and dry weight increment rate of nodule was positive in 0.1%, 0.5% and 1% concentrations and negative for other treatments with respect to control (Table 3). These results support the findings of Thach, *et al.* (1999) that the increase in number of nodules in soybean roots was not significant due to application of EM.

Pigments concentration of fresh leaf

Effects of EM on the concentration of leaf pigments (chlorophyll a, chlorophyll b and carotenoid) were determined and the results are presented in Table 4. Chlorophyll a was highest (58.43 mg/L) in 5% concentration of EM solution and lowest (40.75 mg/L) in control treatment. Chlorophyll b was highest (41.37 mg/L) in 1% concentration followed by 39.14 mg/L in 5% concentration and 38.25 mg/L in 2% concentration of EM solution and was significantly (P<0.05) different from other treatments including control. Carotenoid was highest (47.25 mg/L) in 2% concentration of EM solution followed by 1% concentration. Total pigment was highest (139.47 mg/L) in 5% concentration of EM solution and was significantly (P<0.05) different from 0.1%, 0.5%, 10% concentrations and control. Total pigments increment (%) was positive for all the treatments compared with control. The present results are in agreement with the information given by Xu (2000), Wang *et al.* (2000) and Mridha *et al.* (2002), that EM applied with organic fertilizers has shown to promote root growth and activity, and to enhance photosynthetic efficiency and capacity, which resulted in increased yield.

So, from the present information it can be concluded that the low concentration of EM solution (2%) can be used and recommended for getting maximum seed germination and seedling development of Khair which may influence its better growth and production in the field. These technology may be applied with

Table 4. Influence of different concentration of Effective Microorganisms (EM) on pigments concentration in fresh leaves of Khair *(Acacia catechu)* after 3 month of sowing the seeds in the nursery.

Concentration of (EM (%)	Pigments concentration of leaf (mg/L)				
	Chlorophyll a	Chlorophyll b	Carotenoid	Total	Total pigment increment (%)
Control	40.75 c *	25.67 c	32.35 c	98.77 d	00.00
0.1	43.15 c	32.74 b	36.41 bc	112.3 c	+ 13.67
0.5	48.07 b	34.86 b	43.56 b	126.49 b	+ 28.00
1	47.45 b	41.37 a	46.71 a	135.53 a	+ 37.13
2	52.84 b	38.25 a	47.25 a	138.34 a	+39.97
5	58.43 a	39.14 a	41.90 b	139.47 a	+ 41.11
10	51.64 b	33.87 b	42.43 b	127.94 b	+ 29.46

*-Means followed by the same letter (s) in the same column do not vary significantly at $P<0.05$, according to Duncan's Multiple Range Test (DMRT).

other plants in nursery for the production of EM inoculated seedlings for better growth in the nursery as well as to transport beneficial soil microorganisms with the inoculated seedlings to the fields to improve the soil health for better growth and survival of transplanted seedlings under field conditions.

References

Ahmad, R., T. Hussain, G. Jilani, S.A. Shahid, S.N. Akhtar and M.A. Abbas. 1995. Use of Effective Microorganisms for sustainable crop production in Pakistan. Pp. 15-27. In: Sharifuddin, H.A.H., A.R. Anuar and M.F. Shahbudin, (eds). Effective Microorganisms (EM). Proceedings of the 2nd Conference on Effective Microorganisms (EM) held at Kyusei Nature Farming Center, Saraburi, Thailand. November 17-19. 1993.

Anuar, A.R., H.A.H. Sharifuddin, M.F. Shahbudin and A.R. Zaharah. 1995. Effectiveness of Effective Microorganisms (EM) on maize grown on sandy tin tailings. Pp. 42-54. In: Sharifuddin, H.A.H., A.R. Anuar and M.F. Shahbudin, (eds.) Effective Microorganisms (EM). Proceedings of the 2nd Conference on Effective Microorganisms (EM) held at Kyusei Nature Farming Center, Saraburi, Thailand. November 17-19. 1993.

Chowdhry, A.R., M.M. Hussain, M.S. Mia, A.M.M.S. Karim, J. Haider, N.I. Bhuyan and K. Shifuddin. 1994. Effect of organic amendments and EM on crop production in Bangladesh. Pp. 155-163. In: Parr, J.F., S.B. Hornick and M.E. Simpson, (eds.). Kyusei Nature Farming. Proceedings of the Second International Conference on Kyusei Nature Farming, Piracicaba, SP, Brazil. October, 7-11.1991.

Iwaishi, S. 2000. Effect of Organic Fertilizer and Effective Microorganisms on Growth, Yield and Quality of Paddy-Rice Varieties. Journal of Crop Production, 3(1): 269-273.

Lim, T.D., T.W. Pak and C.B. Jong. 1999. Yields of Rice and Maize as Affected by Effective Microorganisms. Pp. 92-98. In: Senanayake, Y.D.A. and U.R. Sangakkara, (eds.). Kyusei Nature Farming and Effective Microorganisms. Proceedings of the 5th International Conference on Kyusei Nature Farming and Effective Microorganisms for Agricultural and Environmental Sustainability. Bangkok, Thailand. October 23-26. 1997.

Luna, R.K. 1996. Plantation Trees. International Book Distributors. Dehra Dun, India. 975 pp.

Mridha, M.A.U., H.U. Chowdhuary, H.L. Xu. and H. Umemura. 1999. Influences of Effective Microorganisms on Seed Germination and Seedling Growth of Some Crop Plants. Nature Farming and Sustainable Environment, Vol. II. International Nature Farming Research Center, Atami, Japan, 1999. Pp. 125-136.

Mridha, M.A.U., B.M. Khan, M.K. Hossain, H. Tasnim and M.F Rahman. 2002. Effective Microorganisms (EM) for Neem tree. APANews, No. 20: 5-6.

Pandey, S.R., M.A.U. Mridha and M. K. Hossain. 2001. Effects of microbial inoculant (EM-Bokashi) on germination, growth and nodulation of *Albizia procera* (Roxb). (Accepted for the Proceedings of the 7th Conference on Effective Microorganisms, Bali, Indonesia.).

Shin, H.S., G.H. Ryu, J.O. Lee and K.H. Lee. 1995. Studies of Effective Microorganisms (EM) and problem weeds in paddy rice field of Korea. Pp. 82-84. In: Sharifuddin, H.A.H., A.R. Anuar and M.F. Shahbudin, (eds.) Effective Microorganisms (EM). Proceedings of the 2nd International Conference on Effective Microorganisms (EM) held at Kyusei Nature Farming Center, Saraburi, Thailand. November 17-19. 1993.

Thach, N.Q., C.A. Long, V.V. Liet, N.V. Trung, N. X. Thanh, T.V. Dich, N. Duong, N.K. Tuna, L.T.H. Xuan. and P.V. Dan. (1999). Preliminary Results of Effective Microorganisms (EM) Application in Vietnam. Pp. 254-260. In: Senanayake, Y.D.A. and U.R. Sangakkara, (eds.). Kyusei Nature Farming and Effective Microorganisms. Proceedings of the 5th International Conference on Kyusei Nature Farming and Effective Microorganisms for Agricultural and Environmental Sustainability. Bangkok, Thailand. October 23-26. 1997.

Vongprachanch, B. 1995. The use of EM fermented materials in farm trials. Pp. 90-94. In: Sharifuddin, H.A.H., A.R. Anuar and M.F. Shahbudin, (eds.) Effective Microorganisms (EM). Proceedings of the 2nd International Conference on Effective Microorganisms (EM) held at Kyusei Nature Farming Center, Saraburi, Thailand. November 17-19. 1993.

Wang, R., H.L. Xu, and M.A.U. Mridha. 2000. Effect of Organic Fertilizer and EM Inoculation on Leaf Photosynthesis and Fruit Yield and Quality of Tomato Plants. Journal of Crop Production, 3(1): 173-182.

Wettstein, D. 1957. Formula of chlorophyll determination. Exp. Cell Research, 12(3): 427-489.

Xu, H.L. 2000. Effect of a Microbial Inoculant, and Organic Fertilizer, on the Growth, Photosynthesis and Yield of Sweet Corn. Journal of Crop Production, 3(1): 183-214.

Zacharia, P.P. 1995. Studies on the application of effective Microorganisms (EM) in paddy, sugarcane and vegetables in India. Pp. 31-41. In: Sharifuddin, H.A.H., A.R. Anuar and M.F. Shahbudin, (eds.) Effective Microorganisms (EM). Proceedings of the 2nd Conference on Effective Microorganisms (EM) held at Kyusei Nature Farming Center, Saraburi, Thailand. November 17-19. 1993.