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EFFECTS OF FOUR ARBUSCULAR MYCORRHIZAE ON *ACACIA MANGIUM* WILD. SEEDLINGS IN LATERITIC SOIL

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SUMMARY

***Acacia mangium* is a fast growing leguminous tree having multipurpose uses, grows in nutrient poor degraded soil. The timber is used for furniture, agricultural implements, as fuel wood, in manufacturing charcoal and activated carbon. Arbuscular mycorrhizal (AM) association plays an unique role in nutrient uptake, specially phosphate and other less mobile nutrients in nutrient poor soils. Three indigenous and one introduced (*Glomus mosseae*) AM fungi were inoculated with *A. mangium* in a P-deficient red lateritic soil and growth and physiological parameters were recorded. Treatment with AM increased shoot height (59% to 112.5%); leaf area (131.7 % to 168.3 %); biomass (104.8% to 132.1%); chlorophyll content (40% to 60%) and insoluble carbohydrate (33% to 52%) over control. NPK content were also increased significantly. Root colonisation by AMF ranged from 50% to 65%. One indigenous AM species *Paraglomus occultum* was observed most efficient followed by *Acaulospora delicata* and *Glomus moseae*. This experiment reveals that arbuscular mycorrhizae has large applied value with adequate and efficient AMF inoculum and has potential to replace the need of fertilizer application in afforestation in poor soils.**

***Key words: Acacia mangium,* arbuscular mycorrhizae, carbohydrates, chlorophyll, plant growth, root colonization%.**

INTRODUCTION

Acacia mangium a leguminous tree indigenous to north-eastern Australia and south-east Asia is now widely used in plantation programs throughout Asia and the Pacific for soil reclamation, sustainable commercial supply of wood for various end uses and to reduce pressure on natural forest. It is a fast growing multipurpose tree, capable of growing in nutrient poor degraded soil (Duguma 1995). The timber is used for furniture, agricultural implements, as fuel wood, sawdust for mushroom culture, in manufacturing charcoal and activated carbon. It is also used as ornamental shade tree and in agroforestry for increasing soil fertility (Pinyopusarerk *et al.* 1993). It prefers humid tropical climates, can tolerate maximum monthly temperature range 25-32^{0}C and requires monthly rainfall above 100 mm (Pinyopusarerk *et al.* 1993). The seedlings of *A. mangium* grow better with phosphate fertilizer (Manubag *et al.* 1995). In arid and semi-arid regions, low soil P content poses problems in survival and growth of *A. mangium*. Applications of chemical P fertilizer to plantations of *A. mangium* are therefore required and may not be economical in large-scale plantations.

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In recent years arbuscular mycorrhizal (AM) association is gaining importance for its beneficial role in nutrient uptake, specially phosphate and other less mobile nutrients (Miller and Jastrow 1994). AM enhances water uptake particularly in nutrient deficient and dry soils (Aúge 2001). AM hyphae procure nutrient form beyond the nutrition depletion zone of roots (Li *et al.* 1991). AM fungal mycelia are able to absorb water from lower water potential than the roots (Bethlenfalvay *et al.* 1988). AM fungal symbiosis enhances the ability of the plant to acclimatize better to stressed conditions (Sylvia and Williams 1991). AM fungi are obligate symbiont and use upto 20% of total photosynthates produced by host plant. Dependency on AM varies with the P requirement of plant. The cost-benefit ratio in the symbiosis is generally high in nutrient poor soils. Introduction of AM is likely to be particularly important in disturbed arid and semi-arid habitats, which have generally limited naturally occurring AM (Mason and Wilson 1994). The present study was carried on to find out the impact of three indigenous and one introduced AM fungi inoculation on growth and physiological parameters of *A. mangium* in a P-deficient red lateritic soil.

MATERIALS AND METHODS

The experiment was carried out in a net house of Vidyasagar University, Midnapore (22°30/N latitude and 87°19/E longitude), West Bengal, India. Here the dry summer, (March to June) has an average temperature of 30^{0}C with a maximum of 42^{0}C. In winter the average temperature is 18^{0}C (range 11^{0}C to 26^{0}C). The soil is acid lateritic with low silica/sesquioxide ratio. The soil used in the experiment was a denuded top soil up to 30 cm depth having 35% coarse sand, 30% sand, 20% silt and 15% clay. A chemical analysis of soil following standard techniques (Jackson 1973) indicated pH 5.6, available nitrogen 0.0064%, available phosphorus 0.0021%, available potassium 0.0028% and organic matter 0.4%.

Starter culture of *Glomus mosseae* was procured from the Department of Agricultural Microbiology, University of Agricultural Sciences, Bangalore and multiplied on *Sorghum vulgare* in sterilized sand soil (1:1 v/v) mixture (Sylvia 1994). The final inoculum contained 60 spores g^{-1}. The local strain of *Acaulospora delicata, Glomus aggregatum* and *Paraglomus occultum* were isolated from indigenous soil (Gerdeman and Nicholson, 1963) and pure cultured and multiplied similarly. Local AM species were identified according to manual of Schenck and Perez (1990) and INVAM photo guide (http://www.invam.caf.wvu.edu). The inoculum contained 6.3, 6.2 and 6.0 spores g^{-1}. Inocula of the two AM fungi weighing 20 g were used for the experiments.

Soil used for the experiment was sterilized by spraying 38% formaldehyde solution (1:4 v/v) in water was applied at the rate of 20ml per kg soil. The soil was sealed airtight with a plastic sheet for 3 days and then opened to aerate for 15 days. Polypots of 25 x 10 cm size were filled with 3kg soil in each. Seeds of *A. mangium* were surface sterilized with 0.5% NaOCl solution and sown at 3 seeds per pot. AM inocula were placed 4 cm below the seeds. Control pots were treated with 1g charcoal powder and 20g heat-killed AM inoculum. Seven replicates were maintained for each treatment. Plants were watered as required. Hoagland's nutrient solution without phosphorus was added 10 ml/ pot at 30 days interval from 90 days onward. The treatments were: (i) control (sterilized soil) (ii) sterilized soil + *Acaulospora delicata* (iii) sterilized soil + *Glomus aggregatum* (iv) sterilized soil + *G. mosseae* and (v) sterilized soil + *Paraglomus occultum*.

Growth performance of *A. mangium* plants was analysed in terms of the shoot height, leaf area and total biomass. Data were recorded from 240 days old uniform grown seven plants of each treatment. Shoot height was measured from root collar to terminal bud. Leaf area were marked and measured on graph paper. Root and shoot biomass was determined after drying at 80°C for 72h. Extraction and estimation of leaf chlorophyll from mature leaf was done as per the method of Arnon (1949). Chlorophyll content was measured at 90, 180 and 240 days old plant. Soluble and insoluble carbohydrate contents from leaves were determined following the method of McCready *et al*. (1950). For measurement of both chlorophyll and carbohydrate mature leaves were taken from middle portion of seedlings. Root colonization percentage was evaluated after treating a root sample with 10% KOH solution and staining with tryphan blue (Phillips and Hayman 1970). Colonization percentage

was obtained according to the formula: Colonisation % = Number of infected root segments (1cm.) × 100/ Number of total root segments observed. Total N content in shoot was measured by Kjeldahl method, available P in shoot was measured by ammonium molybdate method and K was determined by flame photometer using K filter (Allen 1974).

Statistical analysis of the data was done in terms of least significant difference (LSD), which was calculated at 95% confidence limits (Panse and Sukhatme 1967).

RESULTS AND DISCUSSION

Shoot height was maximum in *Paraglomus occultum* treatment followed by *A. delicata* treatment throughout the experiment. In other two AMF treatment growth pattern was almost similar. *Paraglomus occultum* increased shoot height by 112.5%, *A. delicata* by 89.6%, *G. mosseae* by 69.4% and *G. aggregatum* by 59% over control (Table1). All AMF treatments increased leaf area significantly (P<0.001) higher over control by 131.7% to 168.3%. Total biomass production was maximum by inoculation with *Paraglomus occultum* followed by *A. delicata* treatment and significantly higher than *G. aggregatum* treatments (P<0.01). AMF inoculation increased biomass 104.8% to 132.2% over control (P<0.001).

Soluble carbohydrate content (Table 1) was minimum in *A. delicata* followed by *Paraglomus occultum* inoculated plants and was significantly lower than *G. aggregatum* treatment (P<0.01). Among the AMFs, it was observed maximum in *G. aggregatum* treatment. AM inoculation decreased soluble carbohydrate content 23.9% to 40.3% than control which was found highly significant (P<0.001).

Insoluble carbohydrate (Table 1) was maximum in *A. delicata* treatment followed by *Paraglomus occultum* and *G. mosseae* and minimum in *G. aggregatum* treatment. Insoluble carbohydrate was increased by 33.1% to 51.9% in AMF treatments over control that was highly significant (P<0.001). *A. delicata* increased significantly more than other three AMF treatments (P<0.001).

Nitrogen content in *A. delicata* and *Paraglomus occultum* treated plants was recorded significantly (P<0.05) higher than other two treatments. In general, there was significant increase (47.4 - 92.3%) in N content in AM treatments over control. P content was found maximum in *Paraglomus occultum* treated plants which was significantly higher than plants treated with *G. mosseae* and *G. aggregatum* (P<0.01). *A. delicata* enhanced shoot P content significantly higher than *G. aggregatum* (P<0.01). Overall rise in P content in AM

Table 1. Effect of three indigenous (*A. delicata, G. aggregatum and Paraglomus occultum*) and one introduced (*G. mosseae*) AM fungi on growth parameters soluble and insoluble carbohydrate and N, P and K content in leaves of *A. mangium* plants (240 days old).

Treatments		Shoot height (cm)	Leaf area (cm^2)	Root weight (g)	Shoot weight (g)	Total Biomass (g)	Soluble carbohydrate (mg g^{-1} fw)	Insoluble carbohydrate (mg g^{-1} fw)	N (%)	P (%)	K (%)
Control		14.4±2.1	8.2±2.1	1.0	1.3	2.30	20.1	50.2	1.56	0.10	0.11
A. Delicata		27.3.±1.8	22.0±1.2	1.40	3.82	5.22	12.0	76.3	2.80	0.16	0.15
G. aggregatum		22.9±2.0	19.0±0.6	1.38	3.3	4.71	15.3	66.8	2.40	0.12	0.15
G. mosseae		24.4± 0.8	20.1±1.5	1.46	3.42	4.88	14.0	67.2	2.30	0.14	0.16
Paraglomus occultum		30.6±2.1	21.6±0.9	1.32	4.02	5.34	13.2	70.0	3.00	0.18	0.17
L.S.D.	P<0.05	6.9	4.0	0.27	0.50	0.91	1.6	3.0	0.28	0.036	0.034
	P<0.01	9.3	5.4	0.38	0.71	1.26	2.3	4.3	0.40	0.051	0.048
	P<0.001	12.5	7.3	0.56	1.03	1.68	3.3	6.2	0.58	0.074	0.069

treatments was significantly higher than control (P<0.001). Shoot K content in AMF treatments followed the trend as P and was recorded significantly higher over control (P<0.01) (Table 1).

The enhancement of chlorophyll content (Fig. 1) in AMF inoculated plants over control was visible at 90 days onward. At 240th day, it was maximum under *A. delicata* followed by *Paraglomus occultum* treatment. All AMF inoculation significantly enhanced chlorophyll content by 40% to 60% over control. Root colonization (Fig. 2) by AMF ranged from 48% to 65% and was maximum in *Paraglomus occultum* treatment.

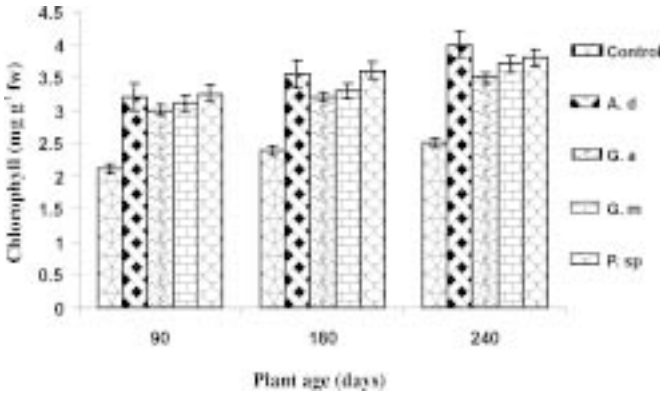

Fig. 1. Effect of three indigenous [*A. delicata* (A. d), *G. aggregatum* (G. a) and *Paraglomus* sp. (P. sp.)] and one introduced [*G. mosseae* (G. m)] AM fungi on chlorophyll content in leaves at three different age of *A. mangium* plant. (Vertical bars indicate standard error).

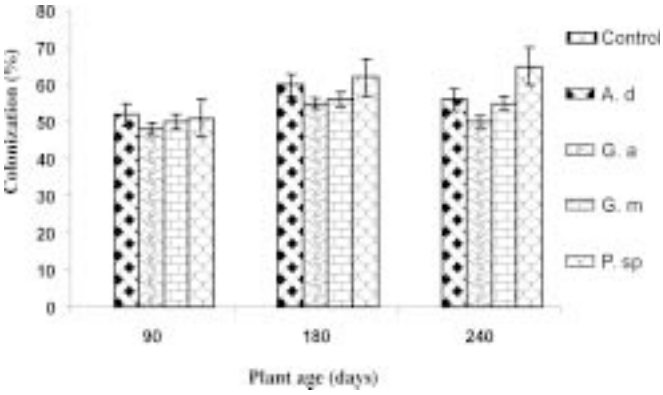

Fig. 2. Effect of three indigenous [*A. delicata* (A. d), *G. aggregatum* (G. a) and *Paraglomus* sp. (P. sp.)] and one introduced [*G. mosseae* (G. m)] AM fungi on root colonization % at three different age of *A. mangium* plant. (Vertical bars indicate standard error).

This experiment further emphasized the applied value of arbuscular mycorrhizae and showed that treatment in nursery beds with adequate and efficient AMF inoculum has potential to replace the need of fertilizer application (Elsheikh and Mahamedzein 1998). Similar results were reported in *Acacia auriculiformis* and *A. mangium* (Mizoguchi 1992, Habte and Soedarjo 1996), but the dependency on AM was not observed so high as this experiment. Severe P deficiency in lateritic soil may have increased the dependency. Steady increasing growth trend in AM inoculated *A. mangium* seedlings upto final harvest was in tune with Mizoguchi (1992). AM-inoculation exhibited larger leaf area as compared to noninoculated controls, thereby maximizing the area available for CO_2 assimilation (Wright *et al.* 1998). The elevated uptake of nutrient also boosts up the rate of photosynthesis (Smith and Read 1997), especially in P deficient area (Brundrett 1991). Arbuscular mycorrhizae is known to enhance vigour and tolerance to adverse conditins of plants (Sharma *et al.* 1999) which is mainly due to changes of biochemical constituents (Dumas *et al.* 1990) AM has been found to influence chlorophyll contents (Phrike *et al.* 2002). The level of carbohydrate in shoot and root was also affected in AM plants (Bass and Lambers 1988). A plant may be associated with a number of AM species at a time but all may not be equally effective (Estaún 1987). Among a number of rhizospheric AMF species, one or two may be most effective to a particular host (Mathur and Vyas 2000). Hence, selection of the proper AM fungi for inoculation to nursery-raised seedlings is important.

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