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Influence of Arbuscular Mycorrhizal Fungi (AMF) on seedling growth responses of *Cedrela serrata* and *Dipterocarpus tuberculatus*

ISHWORANI CHONGTHAM* AND RADHA RAMAN PANDEY
Department of Life Sciences, Manipur University, Canchipur, Imphal 795 003, Manipur, India

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An investigation was carried out to identify suitable arbuscular mycorrhizal fungi (AMF) as bioinoculant for increasing the seedling growth and survival of two forest trees i.e. *Cedrela serrata* and *Dipterocarpus tuberculatus* in nursery conditions. Four different AMF species isolated from the rhizosphere soils of studied tree species grown in natural forests of Moreh, along the Indo-Burma border of Manipur, North East (NE) India, were used for inoculation. All the test AMF bio-inoculants significantly (P<0.05) enhanced the plant height, shoot collar diameter, root length, dry biomass and tissue P content in both the tree seedlings compared to uninoculated ones. *C. serrata* seedlings positively responded to highest biomass, percent colonization, tissue P content and mycorrhizal inoculation effect (MIE %) when inoculated with *Funneliformis geosporum*. In contrast, in *D. tuberculatus*, the plant height, biomass, AM spore density, and seedling quality index were recorded to be maximum in *Cetrospora armeniaca* inoculated treatments. Thus, the present findings suggest that indigenous AM fungi can enhance the survival rate and productivity of woody tree species under nursery conditions.

Key words: AMF species, nutrient use efficiency, seedling growth and quality index

INTRODUCTION

Raising healthy tree seedlings in the nurseries is becoming increasingly important as the natural forests are diminishing rapidly, and the areas of plantations are expanding worldwide. The tropical forests require extensive rehabilitation by planting healthy seedling stocks with high survival rates (Banerjee *et al*. 2013). The major obstacle in the production of tree seedlings in the tropics is their slow growth and high mortality rate in the nurseries (Tawaraya and Turjaman, 2014). The AMF (Phylum Glomeromycota) are obligate symbionts that colonize the roots of the majority of land plants including the woody trees and facilitate their growth, nutrient uptake and tolerance to various biotic and abiotic stresses (Pandey *et al*. 2016). Generally, the tropical and subtropical trees are highly dependent on mycorrhizal symbiosis because of the low phosphorus (P) availability in the soils (Muthukumar and Udayan, 2010). Recent reviews have reported the beneficial effect of AMF inoculation – in various tree seedlings and has resulted in increased seedling growth, survival rate and enhanced biomass production (Banerjee *et al*. 2013; Graham *et al*. 2013). The enhanced root colonization by AMF in inoculated tree seedlings is found to be directly correlated with plant growth variables and their nutrient uptake efficiency (Dutt *et al*. 2013).

Before the application of AMF inoculation in nurseries, understanding of the mycorrhizal association in a particular tree species (habitat- and tree-specific fungi) is pre-requisite, as the mycorrhizal dependency differs among plant species and AM fungi. Moreover, the seedlings of indigenous tree species for planting approaches need to be capable of withstanding harsh conditions of the land and must be selected according to their adaptation to the native soil and the environmental conditions. It has been suggested that the application of native AMF species during seed germination and at the early stage of plant growth can produce more quality seedlings than the non-native species. However, instead of a wide occurrence of

*Corresponding author: ishwo.mu@gmail.com

different AMF species in the forest ecosystem, little is known in India about their role in the growth enhancement of tree seedlings for reforestation of degraded lands in the subtropical regions.

The tropical deciduous and subtropical semi-evergreen forests trees such as *Cedrela serrata* Royle. (Family Meliaceae) and *Dipterocarpus tuberculatus* Roxb. (Family Dipterocarpaceae) are one of the most important multipurpose tree species growing luxuriantly in North East (NE) India as they are implicated in solving global problems such as deforestation and degradation of lands, greenhouse effect, *etc.* Moreover, the studies on the effect of native AMF species on growth-promoting abilities of indigenous tree seedlings in NE India are scarce (Jha *et al*. 2012; Pandey *et al*. 2016). Therefore, an attempt has been made to evaluate the influence of four native AMF species on seedling growth performances of *C. serrata* and *D. tuberculatus* under nursery conditions of Manipur.

MATERIALS AND METHODS

Experimental site and soil

This study was conducted in the greenhouse of Manipur University Campus, Imphal, India, (Location: 24°452 N, 93° 552 E; 795 MSL)The mean minimum and maximum temperatures of the experimental site during the study period, i.e., June 2017 to June 2018, ranged from 11.6 to 27.5 °C, while the relative humidity (RH) varied from 84 to 93.3%. The soil used in the present experiment was sandy loam in texture, collected from a natural forest situated in Moreh at Indo-Burma border of Manipur, India. The soil was sieved to remove the pebbles and root fragments, and autoclaved thrice at 121°C for 1 hr with 24 hr gap between each sterilization process. Soil properties were assessed using standard procedures (**Jackson, 1971)**, which revealed: 68.5 kg ha^{-1} total N, 88.3 kg ha^{-1} available P, 135.8 kg ha^{-1} exchangeable K and 1.61% of OC. The pH and EC of the test soils were 6.2 and 0.16 d Sm^{-1}, respectively.

Preparation of AM fungal inocula

Four dominant AMF species i.e., *Dentiscutata erythropus* (C. Walker & D. Redecker), *Funneliformis geosporum* (T.H. Nicolson & Gerd., C. Walker & A. Schüßler), *Gigaspora gigantea* (Gerd. & Trappe) and *Cetrospora armeniaca* (Oehl, F.A. Souza & Sieverd.) isolated from the rhizosphere soil samples each of *C. serrata* and *D. tuberculatus* were selected and mass multiplied by using *Sorghum vulgare* L. as a host plant in trap pots measuring to 12 cm height and 24 cm diameter. After 120 days of plant growth, roots of the host were assessed for AM colonization levels and spore density in inoculum soil (Muthukumar *et al*. 2001). The percent root colonization in *S. vulgare* by AMF ranged from 75 to 90%, with a spore population of 40 spores per 10g soil**.** For mycorrhizal treatment, 100g of inoculum consisting of soil along with AMF spores and chopped colonized roots of *S. vulgare* were prepared for each test pot.

Experimental set up and AM inoculation

The pot experiments consisted of completely randomized block design (2×5×5) with two tree species, *C. serrata* (CS) and *D. tuberculatus* (DT), and five inoculation treatments (C-Control, T1- *Dentiscutata erythropus*, T2- *Funneliformis geosporum*, T3- *Gigaspora gigantea* and T4- *Cetrospora armeniaca*) individually, with five replicates of each. Mature uniform seeds of *C. serrata* and *D. tuberculatus* were collected from each of a single mother tree growing in natural forest stand, subjected to heat treatment with boiled water at 70°C for one min., then allowed to cool down and further soaked in water for 2–3 days. The seeds of individual trees were sown in trays containing autoclaved soils. The seeds of *C. serrata* germinated within 10–15 days, while *D. tuberculatus* seeds took 20–25 days to germinate. After two months, the uniform young plantlets of each tree species were transferred (1 seedling/ bag) individually into polythene bags (30x15 cm) filled with 4 kg of sterilized soil and sand mixture (1: 1 v/v). Before transplanting of seedling, 100g of each test AMF species inoculum was placed at a depth of 5 cm in respective treatment pots. For the control pots, no AM inocula were added. All the planted seedlings were kept in the greenhouse, watered regularly to maintain the moisture at field capacity.

Harvest and measurement

After one year of the growth period, the plants were harvested carefully from the pots, and

different growth parameters viz., shoot and root length (cm), and shoot collar diameter (cm) were determined. The tissue samples, i.e. shoots (leaves and stems) and roots, were placed separately in labeled paper envelopes and oven-dried at 80 °C for 48 hr to determine the dry weights. The percent root colonization and spore population were estimated as described by Muthukumar *et al*. (2001). The P content of the oven-dried shoot and root material was determined by using the Molybdenum blue method after triple acid digestion (Jackson, 1971). Nutrient use efficiency was calculated as shoot biomass produced per unit of nutrient content (Koide, 1991). Mycorrhizal Inoculation Effect (MIE %) and Seedling Quality Index (SQI) was calculated to access the seedling growth using the method described by Sumana and Bagyaraj (2003) and Turjaman *et al*. (2008), respectively.

Statistical analysis

The statistical analysis was performed for all the plant growth and mycorrhizal parameters in two studied tree species using a one-way analysis of variance (ANOVA) (SPSS version 9, SPSS Inc., Chicago, Illinois) and the means were separated by Duncan's multiple range test ($P<0.05$).

RESULTS AND DISCUSSION

The present findings revealed that all the four examined AMF species significantly ($P<0.05$) enhanced the different growth parameters of both tree seedlings compared to uninoculated controls (Table 1). At harvest, the effect of inoculation with *F. geosporum* was found to be most effective and revealed significant ($P<0.05$) increase in the majority of the growth variables such as shoot and root lengths and the dry mass of *C. serrata* seedlings compared to other treatments (Table 1). The shoot collar diameter was maximum in *D. tuberculatus* seedlings. The earlier studies also reported that inoculation with native AM fungi improves the total plant growth and nutrient uptake efficiency in some tropical woody trees (Jha *et al*. 2012).The percent root colonization in seedling roots of both the species varied significantly ($P<0.05$), and ranged from 24–49% in different AMF treatments (Table 1).

However, maximum root colonization was observed in *C. serrata* (49%) inoculated with *F. geosporum.* These results are in conformity with the earlier findings of Sumana and Bagyaraj (2003) who recorded 43% and 42% AM colonization in *Casuarina equisetifolia* and *Azadirachta indica* seedlings, respectively ~~while~~ inoculated with *F. geosporum* under nursery conditions. The AM spore density was highest in soils of *D. erythropus* inoculated with *C. serrata* (50 spores per 25g). In contrast, in *D. tuberculatus* seedlings, spore population was maximum in *C. armeniaca* treated pots (23 spores per 25g (Table 1). Banerjee *et al*. (2013) also observed a higher population of AMF (115–180 spores per 25g dry soil) following inoculation in *Tectona grandis* and *Azadirachta indica* plants, respectively, after six months of pot trials with. Urgiles *et al*. (2009) reported increased AM colonization and seedling growth of *Cedrela montana* and *Heliocarpus americanus* in the nursery. They highlighted the importance of AM fungi to achieve high-quality seedling production. Inoculation with AM fungi at the seedling stage also promoted the growth responses, nutrient uptake, and survival rates of several host tree species viz., *Ploiarium alternifolium, Calophyllum hosei, Casuarina equisetifolia, Azadirachta indica,* and *Tectona grandis* (Muthukumar and Udaiyan, 2010). The shoot and root P contents were significantly ($P<0.05$) increased in *C. serrata* seedlings inoculated with *F. geosporum*, followed by *C. armeniaca*. In contrast, least P content was recorded in uninoculated plants (Table 2).

However, roots of *D. tuberculatus* seedlings colonized by *C. armeniaca* recorded maximum P content. Nutrient-use efficiency was higher in uninoculated controls (Table 2). Similar results have been reported in earlier studies (Wulandari *et al.* 2014). Banerjee *et al.* (2013) reported that AM fungal colonization induces vigorous growth of seedlings and also increases the tissue nutrient contents and their uptake efficiency. The inoculation of AM fungi is advantageous for mycotrophic plant species in various ways. AM inoculated seedlings can increase root length and density in response to P deficiency and therefore, root colonization also increases under nutrient-deficient soil condition (Graham *et al*. 2013).

The microbial inoculation effect and SQI of *C. serrata* and *D. tuberculatus* seedlings in different

Table 1: Effect of native AM fungal inoculation on plant growth and mycorrhizal parameters in seedlings of selected tree species under nursery condition.

Treatments †	Plant growth parameters #					AMF #	
	Plant height (cm plant $^{-1}$)	Shoot collar diam. (cm plant $^{-1}$)	Root length (cm plant $^{-1}$)	Dry weight (g plant $^{-1}$) Shoot	Root	Total colonization (%)	Spore density (25g dry soil)
Cedrela serrata							
C	16.6±0.4 ^{a}	1.3±0.1 ^{a}	14.2±0.8 ^{a}	1.7±0.2 ^{a}	0.9±0.1 ^{a}	00.0±0.0 ^{a}	00.0±0.0 ^{a}
T1	24.4±0.6 ^{b}	1.5±0.1 ^{b}	21.6±1.0 ^{b}	2.3±0.3 ^{ab}	1.9±0.3 ^{b}	42.8±2.9 ^{c}	50.3±2.4 ^{d}
T2	66.7±1.0 ^{e}	2.3±0.1 ^{d}	54.0±2.7 ^{e}	6.2±0.7 ^{c}	4.6±0.2 ^{d}	49.4±2.1 ^{c}	42.6±2.9 ^{c}
T3	40.4±0.6 ^{c}	1.8±0.2 ^{c}	34.7±1.7 ^{c}	3.3±0.3 ^{b}	2.5±0 .2 ^{b}	35.7±2.9 ^{b}	27.6±3.5 ^{b}
T4	50.2±1.1 ^{d}	1.6±0.1 ^{b}	44.1±1.2 ^{d}	3.0±0.4 ^{b}	3.3±0.3 ^{c}	32.5±0.9 ^{b}	30.0±1.0 ^{b}
F - statistics (df =4,20)	621.23***	156.80***	97.34***	16.74***	34.72***	26.26***	43.19***
Dipterocarpus tuberculatus							
C	10.0±0.1 ^{a}	1.5±0.1 ^{a}	7.0±0.6 ^{a}	1.0±0.1 ^{a}	1.3± 0.1 ^{a}	00.0±0.0 ^{a}	00.0±0.0 ^{a}
T1	17.8±0.2 ^{bc}	2.8±0.1 ^{bc}	14.8±0.6 ^{b}	2.8±0.2 ^{bc}	3.1±0.2 ^{b}	27.7±0.6 ^{b}	15.7±3.2 ^{bc}
T2	19.6±0.4 ^{c}	3.0±0.1 ^{c}	16.1±0.6 ^{bc}	2.8±0.3 ^{bc}	3.5±0.2 ^{b}	37.7±1.0 ^{c}	19.6±1.2 ^{c}
T3	16.8±0.2 ^{b}	2.7±0.1 ^{b}	14.5±0.2 ^{b}	2.4±0.1 ^{b}	3.0±0.2 ^{b}	24.5±1.5 ^{b}	12.0±0.6 ^{b}
T4	18.8±0.4 ^{c}	2.9±0.1 ^{bc}	16.34±0.7 ^{c}	3.1±0.1 ^{c}	4.3±0.1 ^{c}	33.4±3.4 ^{c}	23.0±3.1 ^{d}
F - statistics (df =4,20)	190.97***	46.35***	48.21***	25.88***	35.43***	24.28***	12.15***

*** Significant at P<0.001. 'ns' non-significant
†C= Control, T1= *Dentiscutata erythropus*, T2= *Funneliformis geosporum*, T3= *Gigaspora gigantea* and T4= *Cetrospora armeniaca*
#Means ± standard error in a column for mycorrhizal fungi followed by a different letter(s) are significantly different according to Duncan's multiple range test (P< 0.05).

Table 2: Tissue P concentration and nutrient-use efficiency in shoot and root biomass of seedlings of selected tree species inoculated with different AM fungi under nursery condition.

Treatments †	Phosphorus concentration (%) #		Nutrient use efficiency (μg mg-1 #)
	Shoot	Root	
Cedrela serrata			
C	0.12±0.02 ^{a}	0.10±0.02 ^{a}	13.8±0.28 ^{a}
T1	0.17±0.02 ^{a}	0.13±0.02 ^{a}	13.7±0.28 ^{a}
T2	0.26±0.03 ^{b}	0.21±0.04 ^{b}	08.5±0.22 ^{a}
T3	0.16±0.03 ^{a}	0.14±0.03 ^{a}	09.4±0.16 ^{a}
T4	0.19±0.01 ^{a}	0.17±0.01 ^{ab}	12.5±0.16 ^{a}
F - statistics (df =4,20)	4.79**	2.94*	1.18ns
Dipterocarpus tuberculatus			
C	0.09±0.02 ^{a}	0.13±0.02 ^{a}	19.7±0.26 ^{b}
T1	0.12±0.02 ^{ab}	0.16±0.02 ^{ab}	10.0±0.14 ^{a}
T2	0.14±0.02 ^{ab}	0.20±0.01 ^{b}	12.0±0.09 ^{a}
T3	0.11±0.02 ^{a}	0.14±0.03 ^{a}	10.7±0.21 ^{a}
T4	0.18±0.04 ^{b}	0.21±0.03 ^{b}	12.8±0.19 ^{a}
F - statistics (df =4,20)	2.54*	3.23*	4.35*

*, ** Significant at P<0.05 and P<0.01. 'ns' non-significant
†C= Control, T1= *Dentiscutata erythropus*, T2= *Funneliformis geosporum*, T3= *Gigaspora gigantea* and T4= *Cetrospora armeniaca*
#Means ± standard error in a column for mycorrhizal fungi followed by a different letter(s) are significantly different according to Duncan's multiple range test (P<0.05).

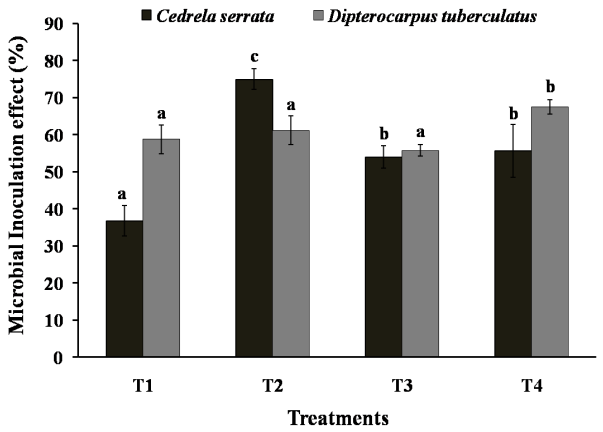

Fig. 1: MIE of *Cedrela serrata* and *Dipterocarpus tuberculatus* plants inoculated with different AM fungi under pot conditions using sterilized soil. (T1= *Dentiscutata erythropus*, T2= *Funneliformis geosporum*, T3= *Gigaspora gigantea* and T4= *Cetrospora armeniaca*) #Error bars indicate ± SE followed by the same letter (s) for the treatments do not differ significantly according to DMRT (P<0.05).

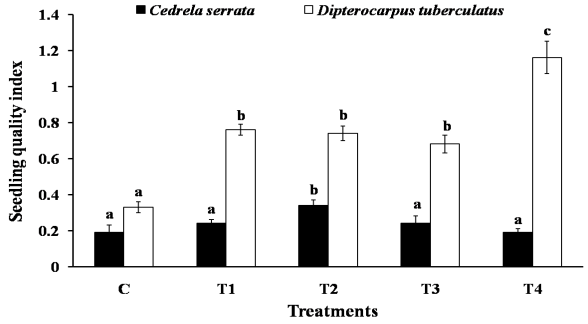

Fig. 2. SQI of *Cedrela serrata* and *Dipterocarpus tuberculatus* plants inoculated with native AM fungi under nursery conditions. (C= Control, T1= *Dentiscutata erythropus*, T2= *Funneliformis geosporum*, T3= *Gigaspora gigantea* and T4= *Cetrospora armeniaca*) #Error bars indicate ± SE followed by the same letter (s) for the treatments do not differ significantly according to DMRT (P<0.05).

AM treatments varied significantly (P<0.05) with each other (Fig. 1, 2). The highest MIE% was observed in *C. serrata* seedlings colonized by *F. geosporum* (75%), whereas the maximum SQI (116%) was recorded in *D. tuberculatus* plants inoculated with *C. armeniaca* compared to control plants. In this study, the plant species inoculated with different AM fungi had minimal nutrient use efficiency than the uninoculated controls, as reported earlier, showing better growth performance of AM inoculated seedlings.

This was probably due to more accumulation of nutrients during the early stage of seedling growth, as indicated by the improved seedling quality and microbial inoculation effect. For any plant species, if the nutrient concentration is sufficient to support maximum growth, an initial increase in nutrient content will produce a reduction in nutrient utilization efficiency. This would be of immense importance during seedling establishment, and survival after transplantation to nutrient stressed soils in the field.

Earlier studies have revealed that AM fungal inoculation can reduce the fertilizer requirement for seedling production (Tawaraya and Turjaman, 2014). Although, to reduce the cost and input of fertilizers for healthy seedling production, AM fungal inoculation can well replace the use of these fertilizers in nurseries and the field, without loss of efficiency of (Muthukumar and Udaiyan, 2010). Our results indicate that nursery grown seedlings of tree species are– highly dependent on AM fungi for improved growth responses without any fertilizer application.

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