

Effect of AM fungi on seedlings of *Tamarindus indica* L. and *Azadirachta indica* Juss for integrated nursery stock

KRISHNA H. WADDAR AND H.C. LAKSHMAN

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See end of the article for authors' affiliations

Correspondence to :

KRISHNA H.

WADDAR

Department of Botany,

Microbiology

Laboratory, Karnataka

University,

DHARWAD

(KARNATAKA)

INDIA

SUMMARY

The health of nursery plants and their productivity are directly linked to soil quality which is intern, is dependent upon the availability of diverse biota supporting stable and healthy ecosystem. The arbuscular mycorrhizal (AM) fungi constitute one of the important bioinoculants directly involved in improving plant growth under reduced fertilizer input. In the present investigation, the quantification of mycorrhization was undertaken on two tree species of *Tamarindus indica* L. and *Azadirachta indica* Juss plants growing in north Kanara district of Dharwad. Mycorrhizal dependency was scanned on these tree species. Most dominant indigenious AM fungal strains were identified. Green house potted experiments were conducted on plant root length, shoot dry weight, leaf area and number of leaves with inoculation of AM fungi, phosphate dosage supply and AM fungi treatment. The result revealed that AM fungi with low phosphate supply, significantly improved seedlings of both tree species at nursery level compared to non-inoculated (control) plants.

Key words :

Tamarindus

indica L. and

Azadirachta

indica Juss,

Arbuscular,

Mycorrhizal fungi,

Dependency,

Phosphate dosage

Tropical forests form a source of rich biological diversity with vast array of plants ranging from minute microorganisms to large trees associated beneficially regulating the community and ecosystem functioning. Symbiotic association between microorganisms and higher plants is common and is of great ecological importance in natural and man made biological systems (Lakshman, 2009). The mycorrhizal association plays an important role in P cycling and uptake of phosphate by plants. Mycorrhizal plants are therefore, adapted to cope with nutrient deficient situations or prevent pathogenesis by other organisms.

Mycorrhizal fungi will not prove effective with all plants when the plants have little or no mycorrhizal dependency (Harley, 1989). Variation in mycorrhizal dependency limits the potentiality of the fungus in improving the plant growth performances (Bagyaraj, 2006). The degree to which the tree depends on the fungal symbiont, varies with the inherent nature of tree species and the prevailing environmental conditions, particularly soil fertility. Forest tree species commonly have low rooting densities in soil compared agriculture species. This limits their absorbing ability of immobile soil nutrients (Harley and Smith, 1983). The importance of mycorrhizal association to forest species is therefore related to the extension of absorptive

area beyond the depletion zone, volume of soil explored in search of nutrients and effective up take and transport of nutrients.

The important of mycorrhiza in natural ecosystem is well recognized and studies were conducted with mycorrhizal fungi to improve early tree growth. The indigenous populated AM fungi may be suitable for inoculation purposes (Grove and Le Tacon, 1993; Lakshman, 2008), account of their broad host compatibility, wide distribution and efficiency in improving nutrient absorption and growth. Mycorrhizal fungi may particularly be important for the establishment and early growth of the trees in regions where growth and nutrient uptake are seasonal. Research in this field indicated that improved growth can be obtained with many economically important tree species of forest by inoculating the seedlings with specific mycorrhizal fungi.

The objective of this study is to determine the mycorrhizal dependence of both of these hard wood species which are the essential components of the habitats for the human beings, particularly growing in North Canara district of Western Ghats of Karnataka.

MATERIALS AND METHODS

In the present study was undertaken at North Canara district of Karnataka. Its

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geographical location lies in between $74^{\circ} 35' - 78^{\circ} 0.5'$ N altitude and $14^{\circ} 27' - 14^{\circ} 98'$ E longitude, occupying an area about 1,320.1 sq.km². South western part of Western Ghats exhibits remarkable diversity in vegetation due to variability in topography and rainfall. Rhizosphere soil samples of the trees were collected up to a depth of 15 cm. Four samples were collected for a plant growing at different localities. For quantitative studies, at least 4 sub-samples were replicated per site around a plant. The collected samples were kept in polythene bags, labeled, brought to the laboratory and stored at 4°C for analysis. The sub-samples were mixed thoroughly and 50g of the composite soil was kept for spore isolation. The mycorrhizal spores were recovered by wet-sieving and decanting method following the technique of (Gerdemann and Nicolson, 1963). The total spore count and number of individual spores of each sample were determined and frequency of spore occurrence was calculated as according to following formula :

$$\text{Frequency (\%)} = \frac{\text{No. of sampling in which particular AMF was recorded}}{\text{Total no. of sampling made}} \times 100$$

Terminal feeder roots were collected from four different portions of the root system of a plant. 'The root samples were preserved in labeled plastic vials containing FAA (90:5:5). Mycorrhizal colonization was measured following the method of Philips and Hayman (1970). Grid-line intersect method (Glovannetti and Moss, 1980) was employed for the estimation of root colonization. Seeds were scarified with 10% H₂SO₄ for 20 minutes followed by successive rinses with sterile water. The seeds surface was sterilized with 4% Sodium hypochlorite followed by successive rinses with sterile water. The seeds were placed in small plastic pots containing sterilized sand and germinated in dark at 25°C. On germination, the seedlings were thinned to one plant per earthen pot measuring 25x30cm in size and each pot filled with 4 kg of sterilized (1:3) sandy loam soil.

Very frequently observed AM fungi in the rhizosphere were selected for inoculum and multiplication programme. *Sorghum vulgare* plants were used as host for large scale production of inoculums. Inoculums of the AM species were multiplied, air-dried and kept at 4°C. Preliminary sets of experiments were conducted on the germinating seedlings to find out the radically infecting and promising species for further inoculation studies. Ten gram of inoculums of the frequently observed fungi was placed at a layer 5 cm below the germinated seeds of the selected plants. Four replicates of each fungal species were kept at 29 + 2°C. Colonization percentage was calculated

after 60 and 120 days of inoculation. Thus, the experiment consisted of an un inoculated plants, mycorrhizal treated plants and P added mycorrhizal and non-mycorrhizal plants. The pots were watered daily with deionized water and 10ml for each P.F. Hoagland nutrient solution without P was added twice in a week.

AM fungi inoculated seedlings were harvested after 180 days of inoculation. The rate of root colonization and number of AM spores in each earthen pot were determined. The plants were harvested, separated into root and shoot and weighed separately. Growth parameters, like root length, leaf area and leaf number were determined. Dry weights of the harvested plants were estimated and after drying the plant material to constant weight fit a hot air oven at 70°C. From these data, the following parameters were determined.

$$\text{Root/Shoot length} = \frac{\text{Root dry weight}}{\text{Shoot dry weight}}$$

$$\text{Specific root length} = \frac{\text{Root weight}}{\text{Root biomass}}$$

$$\text{Mycorrhizal dependency} = \frac{\text{Dry wt. of M. plant} - \text{Dry wt. of NM plant}}{\text{Dry wt. of plant}} \times 100$$

The dried materials were powdered and the fractions collected by passing through 0.2mm sieve were kept for plant nutrient analysis.

RESULTS AND DISCUSSION

Preliminary inoculation experiments with the most frequent fungi in the rhizosphere of the selected two trees revealed that the fungi differed in their potential to colonize plant roots under similar conditions. The root colonization was found to be very high with *Glomus fasciculatum* compared to the other two fungi on seedlings of both plants (Fig. 1). *G.fasciculatum* was found to be the efficient fungus for colonization on both *Tamarindus indica* L. and *Azadirachta indica* Juss. Differential responses of AM fungi within host suggest that under certain conditions selection should occur to favour certain host-fungus combinations.

The host plants exhibited consistent difference in colonization when inoculated with *G. fasciculatum*. Colonization rate of 74% was observed in *Tamarindus indica* L. compared to *Azadirachta indica* Juss where it was 58% after 180 days of treatment. As the host plants showed very different root colonization, the effectiveness of fungus species in producing the growth enhancement may also vary accordingly. A reduction in root colonization was observed among the host plants by P addition (47% and 51% for *Tamarindus indica* L. and *Azadirachta*

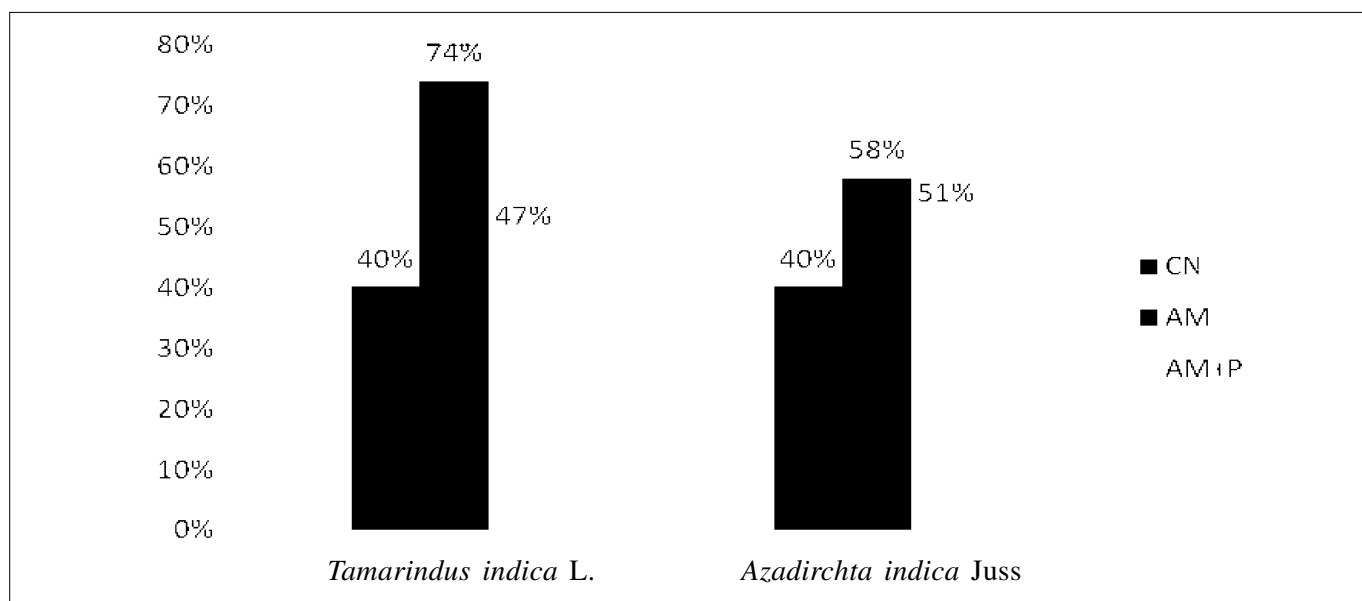


Fig. 1 : Effect of AM fungi on per cent colonization, of *Tamarindus indica* and *Azadirachta indica*, after 180 days under green house condition
CN-Control AM-Arbuscular Mycorrhizal fungi, P-phosphorus

indica Juss, respectively) after 180 days of inoculation. The relatively low mycorrhizal infection in plants grown with added P may suggest the negative effect of Pin mycorrhizal development as indicated by Miller and Jackson (1998). However, there are also reports of high AM infection as well as enhanced P uptake at high concentrations of added P (Amijee *et al.*, 1989).

AM inoculation has increased the growth characteristics of the selected plants. Increase of over 56% and 47% dry weight was observed in AM inoculated. *Tamarindus indica* L. and *Azadirachta indica* Juss, respectively (Table 1). Similar findings have observed by the improvement in growth by AM inoculation. A positive correlation between total biomass production and colonization percentage was recorded in the present study. However, differences in effectiveness were observed among the AM fungi on inoculation to the host plants. Although AM fungal species are not considered to have any specificity towards different taxa of hosts under favourable conditions, there are many differences in their

effectiveness in a particular host (Kothari and Singh, 1996).

Addition of P to a moderate level will not inhibit AM development and it was reported that at very low P level, mycorrhizal association would not enhance the host growth. However, root colonization of the endophytes were consistently affected by P fertilization in both hosts initially, but after 180 days significant differences could not be observed. Similarly significant influence on dry matter yield was not observed among the host plants after P addition. This type of relationship between host and endophyte was earlier reported by Rachel *et al.* (1993). But in non-mycorrhizal (NM) plants, addition of P has resulted in an increase of 20% over dry weight.

Both P fertilization and AM inoculation influenced the growth of the roots irrespective of the host plant. Morphological modification of the root system due to mycorrhizal colonization has been reported earlier (Schellenbawn *et al.*, 1991) which includes an increase in the number of laterals of all orders, but with a decrease

Table 1 : Effect of phosphorus (P) and AMF on growth parameters of selected plants for 180 days

Frowth parameters	<i>Tamarindus indica</i>				<i>Azadirachta indica</i>			
	Control	P	AMF	AMF+P	Control	P	AMF	AMF+P
Root length (cm/Plant)	3.86	3.94	3.19	3.29	15.14	21.13	16.11	16.71
Dry wt. of shoot (g/plant)	1.99	3.17	3.15	2.16	7.22	8.93	13.05	12.61
Dry wt. of root (g/plant)	0.98	1.48	1.22	1.32	3.89	4.87	4.36	4.93
Leaf area (cm ² /plant)	17.58	22.45	28.72	31.07	56.14	73.52	81.37	94.15
Leaf number/plant 1.151.11	9.72	11.05	14.12	15.19	9.51	13.23	15.12	15.11
C.D. (P=0.05%)	1.09	1.15	1.11	2.05	5.32	4.00	7.04	7.13

Table 2 : Effect of phosphorus (P) and AMF on growth parameters of selected plants for 180 days

Treatments	<i>Tamarindus indica</i>			<i>Azadirachta indica</i>		
	N%	P%	K%	N%	P%	K%
Control	2.72	0.05	1.29	2.39	0.19	1.32
P	2.93	0.18	1.53	3.71	0.37	1.29
AMF	3.10	0.21	1.72	3.92	0.41	1.93
AMF+P	2.81	0.24	1.58	2.86	0.47	1.42
C.D. (P=0.05%)	2.10	0.09	0.07	2.0	0.10	0.11

Table 3 : Effect of phosphorus (P) and AMF on root/shoot ratio (R/S), specific root length (SRL) and mycorrhizal dependency (MD)

Treatments	<i>Tamarindus indica</i>			<i>Azadirachta indica</i>		
	N%	P%	K%	N%	P%	K%
Control	0.57	2.88	-	0.53	4.17	-
P	0.69	2.83	-	0.51	4.19	-
AMF	0.37	2.77	31.4	0.33	3.66	41.06
AMF+P	0.47	2.58	31.62	0.37	3.52	39.10
C.D. (P=0.05%)	0.06	1.08	11.0	0.13	1.14	10.00

in average length of root axes. In host plants, mycorrhizal inoculation increased the total root length and decreased the specific root length compared to the non-mycorrhizal plants, indicating an additional benefit of mycorrhizal colonization (Table 1).

A major influence of AM inoculation on growth was apparent by the increase in leaf area. Total leaf area showed an increase of more than 50% in inoculated plants. Schwob *et al.* (1998) has observed enhanced leaf area development by AM inoculation. The leaf expansion was stimulated relatively more than biomass accumulation by the fungal symbiont. Leaf number was also higher in inoculated plants. AM stimulated increase in leaf area was further enhanced at P addition, but it has not affected the dry mass of the plant.

The beneficial effect of AM symbiosis on plant growth has largely been attributed to higher uptake of P. The significantly higher P status observed in the mycorrhizal plants was due to the increased absorption of P from the soil (Table 2). The enhanced photosynthetic rate associated with increased P uptake may be attributed directly to the growth improvements of the plants (Schwob *et al.*, 1998, Lakshman, 2009). The P status of the NM plants were clearly inferior to that of mycorrhizal plants. Addition of P to the soil of NM plants had significantly increased the internal P concentration. Significantly lower internal P concentration in NM plants was observed by Neeraj *et al.*, 1990 and Lakshman, 1996). Marked increase in N and K contents were also noticed in all the inoculated plants and the enhancement was higher in *Tamarindus indica* L. and *Azadirachta indica* Juss.

Mycorrhizal symbioses enhanced both root and shoot

growth and resulted in significantly lower root/shoot (R/S) ratio for mycorrhizal plants than NM plants (Table 3). The P deficient plant lacking AM symbiosis tends to have high R/S ratio, usually associated with nutrient stressed condition. The mycorrhizal fungus by reducing the root branches induces changes in root architecture, which make the plant less capable of independent nutrient absorption (Lakshman, 1996; Hetrick *et al.*, 1988). This will reduce the R/S ratio and consequently the plant becomes more dependent on the endophyte association. The considerably higher shoot and total biomass indicated the variations in enhancement of the above ground growth compared to the growth of the root.

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Authors' affiliations:

H.C. LAKSHMAN, Department of Botany, Microbiology Laboratory, Karnataka University, DHARWAD (KARNATAKA) INDIA

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