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Survey of VA - mycorrhizae in agroforestry and its implications on forest trees

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During the last two decades, different aspects of mycorrhizal associations on crops as well as forest plants have been studied extensively, in different geographical and agroclimatic conditions (Harley 1989). It is suggested that mycorrhizal fungi form symbiotic relationship with plants and are highly potential for the improvement of over all growth of plant even in infertile soil, P deficient adverse soil facilitate revegetation of waste lands and heavy metal toxicity (Gianinazzi *et al.* 1995). Their effects are mutualistic and benefiting each of the partners (Allen 1992). Plants are mainly benefited by way of increased quality of nutrients, which they are unable to absorb P without the help from the fungi (Sanders & Sheikh 1983). There are reports on the survey of forest trees (Bhadauria & Yadav 1999) that hosts form vesicular-arbuscular mycorrhizae. But, very little work has been directed towards determining whether these fungi influence the growth of forest tree seedlings (Matosevic *et al.* 1997). Therefore, this study was conducted to explore the potential use of mycorrhizal fungi in the production of timber plant *Terminalia tomentosa.*

The present study was carried out during the month of October 1998 in an area of deciduous forest. Geographical location of the study area is between (15°20′ to 15°28′ North latitude and 75°27′ to 75°30′ East longitude) in Dharwad district of Karnataka. Rhizospheric soil and roots of each tree species were collected in triplicate. The roots were washed and cleared in 10% KOH at 90°C for 1 h and stained with 0.05% Trypan blue (Phillips & Hayman 1970). The per cent of colonization was determined by gridline intersect method (Giovanentti & Mosse 1980). The mycorrhizal spores in the soil were assessed by wet-sieving and decanting method (Gerdeman & Nicolson 1963). Different VA-mycorrhizal fungal spores were identified using the keys (Schenck & Perez 1990). Prior to spore enumeration from the soil one part was used for analysis of soil characteristics by the standard analytical methods (Jackson 1973).

The agroforestry soil was red laterite, E.C. 0.1 m mohs cm^{-1}, pH 7.3, P 0.98 mg kg^{-1}, N 9.52 mg g^{-1}, K 34.73 mg kg^{-1} and organic matter 2.13%.

Greenhouse pot experiments were conducted to know the effect of *Glomus mosseae* on *Terminalia tomentosa.* Earthen pots measuring (35 x 25 cm) were filled with 8 kg of sterilized forest soil (three parts of forest soil mixed with one part of sand). Used soil was sterilized in 5% Methyl bromide. Seeds of *Terminalia tomentosa* were surface sterilized with 2% sodium hypochlorite and sown in experimental pots. Roots pieces and soil mixture from pot culture of *Pennisetum typhoideum* infected with the tested VA-mycorrhizal fungi *Glomus mosseae* used as the mychorrhizal inoculum 89-319 of spores and 200 g highly colonized root bits were added 1 cm below the sown seeds of *Terminalia tomentosa.* Experiments with mycorrhizal inoculum with and without superphos-

phate; 750 mg kg-1 to 1500 mg kg-1 rock phosphate of 1400 mg kg-1 and 2800 mg kg-1 was given. Six replications were maintained for each treatment with control. All the pots were watered on alternate day. Percentage of VAM colonization, spore population and plant height was recorded. Plants were harvested 360 days after inoculation.

Forty-five tree species representing twenty-three angiospermic families were colonized with VA-mycorrhizae. There were no obvious differences in VAM structures observed with regard to sites or sampling season. Coarse aseptate hyphal coils were seen in roots of the outer cortical layers. Hyphal constructions and arbuscules occurred in macerated root samples. Elongate vesicles were formed terminally on inter and intracellular hyphae and sometime occurred in groups. Mycorrhizal infections of all the plants were classified in three categories (Table 1). Higher degree of VAM colonization 48-96 per cent was recorded in twenty-three plants. Moderate colonization 31-47 per cent was seen in fourteen plants and lower per cent of colonization 10-19 per cent was determined in eight plants. Similarly all the rhizospheric soil samples of examined plants possessed a good number of VAM spores ranging from 51-108 per 100 g soil, expect *Grevillea robusta, Pterocarpus marsupium, Azadirachta indica, Coreya arboreya, Acacia melanoxylon and Casuarina equisetifolia* had lower number of spores from 36-49 spores per 100 g soil. Therefore, the per cent of VAM colonization did not have correlation with spore population. Isolation of VAM spores from respective rhizospheric soil indicated that altogether six genera of VAM fungi were recorded. These were *Acaulospora, Gigaspora, Glomus, Sclerocystis* and *Scutelospora*. Among *Sclerocystis* one species was represented namely *Coremiodies*. But *Glomus* was represented by six species namely; *Aggregatum, Etunicatum, Fasciculatum, Microcarpum, Mosseae, Tubeforme*. Among these *Glomus fasciculatum and Glomus mosseae*, were found to be the most dominated among the recovered spores.

Plants inoculated with mycorrhiza showed significant increase in the growth over non-mycorrhizal plants. When the plants treated with the fertilizer rate 750 mg kg-1 (SP) and 2,800 mg kg-1 (RP), mycorrhizal plants showed four and half fold increase over non-mycorrhizal plants (Table 2). Examination of roots both during early and at the end of the experiment showed that all non-mycorrhizal plants were free of infection, whereas the inoculated plants had normal VA-mycorrhizal colonization in rock phosphate treated plants. Mycorrhizal plants had higher percent of phosphorus over non-mycorrhizal plants after six months growth. There was reduced spore number with increased dosage of super phosphate treatment. This work clearly demonstrates that the occurrence of VA-mycorrhizal fungi are obiqutous, and its importance in growth and P nutrition of agroforestry trees. Out of fortyfive trees, twenty-three plants had 48-96% higher mycorrhizal colonization. Despite the non-patchy composition of understory in the present agroforestry plant community, it is reasonable to assess the mycorrhizal association most common in the shrubs, semi-hard and woody trees (Table 1). The fungus involved in mycorrhizae has often have a fairly wide host range with non-specificity.

At present there is little evidence that connections occur in sufficient abundance to be ecologically significant. However, based on this study it is clear that mycorrhizal colonization rate is independent of plant age. *Grevillea robusta* and *Santalum album* were reported to be non-mycorrhizal (Harley & Smith 1983) but in the present study, these two families were found to be mycorrhizal. Possibly, this could be expected, this agroforestry had tropical soil with low available phosphorus and generally plants are heavily colonized by VA-mycorrhizae. VA-mycorrhiza (*Glomus mosseae*) inoculation on seedling of *Terminalia tomentosa* showed moderate growth and phosphorus nutrition over the non-mycorrhizal plants. But there was a significant growth of the seedlings as indicated by shoot height, per cent of colonization spore population, and P uptake on additional rock phosphate treatment with mycorrhiza. These results are in confirmity with earlier workers (Reena & Bagyaraj 1990).

In the pot experiments, dry matter production and P uptake was increased in the plants treated with rock phosphate with *Glomus mosseae*. Therefore, it must not be assumed from these results that *Glomus mosseae* is necessarily a more efficient VAM that those occurring naturally in the soils. Weight of mycorrhizal roots formed by the indigenous mycorrhiza (*Glomus mosseae*) decreased colonization and spore number, when the plants treated with increased superphosphate. The predominance of hyphae, arbuscules or vesicles in roots of mycorrhizal plants significantly affect inorganic chemical analysis of plant (*Terminalia tomentosa*) tissue, since these fungal structures have specific functions in mobilization and storage of various elements.

Table 1. Effective moderate and lower colonization of VA-mycorrhizae in the agroferestry tree species of Dharwad Karnataka.

Tree species	Tree age (years)	Per cent VAM colonization M ± S.E.	Range	Spore number per 100 g soil M ± S.E.
Acacia leucophloea Willd.	8	64.5 ± 8.1	(0 - 81)	66.5 ± 3.1
Acacia melanoxylon R. Br.	11	53.2 ± 6.3	(0 - 73)	49.3 ± 9.4
Acacia arabica (LAM.) Willd.	5	79.4 ± 3.5	(0 - 92)	51.4 ± 5.4
Acacia catechu Willd.	6	34.5 ± 7.1	(90 - 53)	58.1 ± 6.2
Aegle marmelos Corr.	9	67.3 ± 5.1	(0 - 87)	53.4 ± 407
Albizzi lebbek Benth.	12	96.7 ± 7.4	(0 - 100)	81.5 ± 10.2
Anacardium occidentale Linn.	3	54.2 ± 3.3	(0 - 78)	94.2 ± 8.4
Anona squamosa Linn.	15	15.4 ± 5.1	(0 - 23)	71.3 ± 7.2
Anogeissus latifolia Wall.	13	49.1 ± 2.7	(0 - 69)	54.3 ± 8.4
Artocarpus integrifolia L.	4	51.5 ± 6.4	(0 - 70)	59.4 ± 6.2
Azadirachta indica Juss.	4	17.4 ± 4.6	(0 - 44)	46.5 ± 5.8
Bauhinia purpurea Linn.	5	47.5 ± 4.0	(0 - 66)	92.6 ± 4.1
Buahinia faveolata Dalz.	15	36.3 ± 5.5	(0 - 49)	56.1 ± 5.7
Calophyllum tomentosum Wight.	8	46.4 ± 3.1	(0 - 62)	114.4 ± 102
Careya arborea Roxb.	9	54.7 ± 2.1	(0 - 73)	47.3 ± 4.5
Cordia macleodii Hook.	7	47.6 ± 1.1	(0 - 65)	61.5 ± 6.1
Casuarina equisetifolia Forst.	11	14.4 ± 5.4	(0 - 19)	44.5 ± 8.1
Dalbrgia sisso Roxb.	3	31.2 ± 8.3	(0 - 44)	81.2 ± 6.1
Delonix regia (Hook) Raf.	5	19.5 ± 4.2	(0 - 58)	103.4 ± 4.7
Eucalyptus globulus Labill.	5	19.7 ± 3.4	(0 - 25)	36.3 ± 6.4
Glirricidia maculata Linn.	6	21.4 ± 2.7	(0 - 50)	64.3 ± 72
Grevillea robusta (A.) Cunn.	7	11.0 ± 5.2	(0 - 14)	43.1 ± 5.3
Hardwickia binata Rixb.	8	19.2 ± 3.3	(0 - 23)	57.61 ± 9.1
Mangifera indica Linn.	4	24.7 ± 3.5	(90 - 43)	76.6 ± 4.5
Michelia champaca Linn.	3	93.2 ± 4.3	(0 - 100)	59.4 ± 9.3
Mimusops elengi Linn.	7	31.5 ± 6.4	(0 - 47)	108.4 ± 5.2
Mitragyna parviflora Korth.	2	50.1 ± 6.4	(0 - 74)	82.7 ± 1.4
Peltophorum ferrugineum Benth.	5	43.0 ± 7.2	(0 - 89)	103.3 ± 3.2
Phyllanthus emblica L.	14	28.41 ± 8.1	(0 - 39)	93.2 ± 4.6
Pithecolobium dulce (Roxb).	8	39.2 ± 2.8	(0 - 46)	59.6 ± 6.1
Polyalthia longifolia Thw.	10	17.5 ± 5.1	(0 - 21)	86.3 ± 8.4
Pongamia glabra Vent.	3	37.6 ± 4.4	(0 - 53)	62.3 ± 5.5
Pterocarpus marsupium Roxb.	11	54.7 ± 4.3	(0 - 61)	43.5 ± 3.2
Syzygium cuminii (L.) Skeels.	9	48.2 ± 4.5	(0 - 67)	56.6 ± 3.2
Samanea saman Merr.	15	46.4 ± 6.1	(0 - 61)	91.4 ± 6.4
Santalum album Linn.	5	10.3 ± 4.3	(0 - 14)	102 ± 804
Syzygium jambolanum DC.	6	87.5 ± 8.2	(0 - 100)	83.7 ± 7.1
Terminalia chebula Retz.	7	58.4 ± 2.1	(0 - 66)	101.4 ± 10.2
Tamarindus indica Linn.	11	59.3 ± 3.6	(0 - 98)	108.5 ± 4.4
Tectona grandis Linn.	9	47.3 ± 5.0	(0 - 52)	71.5 ± 3.2
Terminalia bellerica Roxb.	7	125 ± 2.5	(0 - 100)	82.1 ± 14.3
Terminalia paniculata Roth.	8	2.1 ± 2.7	(0 - 57)	57.2 ± 8.0
Thespesia populnea Cav.	12	18.3 ± 7.1	(0 - 25)	97.4 ± 3.3
Xylia xylocarpa Roxb.	6	49.3 ± 3.2	(0 - 83)	77.3 ± 3.2
Zizyphus mauritaina Lamk.	5	51.2 ± 4.7	(0 - 77)	69.2 ± 8.1

Table 2. The effect of *Glomus mosseae* inoculation with super phosphate and rock phosphate treatment on growth, per cent colonization, spore population and P uptake in *Terminalia tomentosa* for 360 days.

Days after treatment inoculation	Plant height (cm) M ± S.E.	% VAM colonization	Spore 100 g^{-1} soil	% P in shoot
360 NM	7.7 ± 3.1	--	--	0.05
360 M[a]	29.4 ± 5.1	48.1 ± 4.5	49 ± 5.2	0.11
360 M[b]	22.5 ± 2.4	39.3 ± 3.6	46 ± 8.1	0.10
360 M[c]	24.3 ± 6.1	51.0 ± 4.7	98 ± 4.3	0.14
360 M[d]	32.4 ± 4.3	59.0 ± 2.5	105 ± 2.5	0.21
C.D. (P=0.05)	2.5 ± 2.0	11.0 ± 5.0	23.2 ± 1.1	0.0321

a: 750 mg sp kg^{-1}, b: 1500 mg sp kg^{-1}, c: 1400 mg sp kg^{-1}, d: 2800 mg sp kg^{-1}, Sp: super phosphate, Rp: rock phosphate, *not significant.
360NM: 360 days non-mycorrhizal plants.
360M: 360 days mycorrhizal plants.

Further research to clarify the basic physiology of this symbiotic relationship in trees is desirable to realize the full potential of VAM in agroforestry or forestry.

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