RESEARCH PAPER

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Seasonal variation in three leguminous tree seedlings associated with AM Fungi

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Three important leguminous plants *Tamarindus indica* L., *Dalbergia sisso* Roxb., *Cassia nodosa* Roxb., their roots and soil samples were screened for their AM fungal association. Percentage of root colonization and spore number of these plants were co related to each other. Higher spore number was recorded in *Cassia nodusa* Ham., during December and April compared to *Tamarindus indica* L., *Dalbergia sisso* Roxb in soil depth between 16-24 cm. On contrast to this per cent root colonization significantly increased during April in *Dalbergia sisso* Roxb. , *Tamarindus indica* L., followed by *Cassia nodosa* Ham. The results revealed that both root colonization sporulation declined from September to April in the examined tree species.

Key words : Leguminous trees, Per cent root colonization, Spore number, Arbuscular mycorrhizal fungi (AMF)

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INTRODUCTION

Legumes are among the three largest families of flowering plants. The flowering plants are of greatest importance to agriculture world belonged to the orders Gramineae (cereals and grasses) and Leguminosae (legumes or the bean family). The Leguminosae consist of about 750 genera and 19,000 species of herbs, shrubs, trees, and climbers. This large family is divided into three subfamilies—the Mimosoideae, Caesalpinoideae, and Papilionoideae. *Tamarindus indica* L. is a multipurpose tropical fruit tree used primarily for its fruits, which are eaten fresh or processed, used as a seasoning or spice, or the fruits and seeds are processed for non-food uses. The species has a wide geographical distribution in the subtropics and semi-arid tropics and is cultivated in numerous regions. *Cassia nodosa* Roxb. is used as a traditional laxative throughout, bark and seeds are also used as antipyretics. However, it was noted that emesis may be observed. Tannin or dyestuff. The bark has been used for tanning leather, but the amount of tannin is comparatively low. The wood is used for general construction, furniture and cabinet making.

Arbuscular mycorrhiza (AM) Fungi are geographically ubiquitous and occur over a broad ecological range. They are common forms of mutualistic symbiotic association with agricultural crops (Bagyaraj, 2006). Many Horticultural crops, weeds grasses and forest tree species have also been reported to form AM fungal association (Smith and Read, 1997). The AM symbiosis influences several aspects of plant physiology, such as plant rooting, closing of the nutrient cycles, nutrient acquisition, and plant protection. The primary effect of the AM symbiosis is to increase the supply of mineral nutrients to the plant, particularly those nutrients whose ionic forms have a poor mobility rate or those present in low concentration in the soil solution. This situation mainly concerns phosphate, ammonium, zinc and copper. The processes of nutrient transport in AM systems have been reviewed recently. It has also been recognized that AM colonization affects a wide range of morphological parameters in developing root systems, with greater root branching as the most commonly described effect.

It is now well known that AM fungi play a vital role in plant growth, especially plants growing with nutrient deficient soils, since naturally occurring AM fungi are well adapted to the conditions of their natural occurrence due to their long process of evolution, they can be of immense

use for various plants. AM fungi benefit the host plant primarily by increasing the capacity of the root system to absorb and translocate phosphorus and minor elements through an extensive network of hyphae external to the root (Cuenca *et al.,* 2007). Less emphasis has so far been laid on seasonal fluctuation of their occurrence with roots of three leguminous plants therefore in investigation three leguminous trees were selected *viz., Tamarindus indica* L. *Dalbergia sissoo* Roxb. and *Cassia nodosa* Ham. growing in botanical garden. The present study is a setup in this direction.

RESEARCH METHODOLOGY

Research work was undertaken in three different places of University Botanical Garden. One twenty four soils were sampled to a depth from 0-32 cm by pushing a soil tube vertically into the soil. Each soil column was cut into 8 equal segments, which were stored individually in polythene bags at 4^0C until the AMF spores were extracted with in 15 days.

These samples were processed in the laboratory to quantity AMF spores and mycorrhization of roots. The AMF species were identified on the basis of spore morphology following the manual of Schenck and Perez (1990).

Sporulated soil samples were processed following wet sieving and decanting technique. To assess the degree of mycorrhizal colonization, thoroughly washed roots of all the three plants were stained in 0.05% Traypan blue in lactophenol after treating them in hot 10% KOH aqueous solution following the technique (Phillips and Hayman. 1970). The stained roots were cut into I cm segments, which were randomly picked up and examined under stereomicroscope for mycorrhizal association. The root colonization was quantified following Nicolson's formula (1967) as follows:

$$\text{Root colonazation (\%)} = \frac{\text{Number of root segments colonized}}{\text{Total number of root segments examined}} \text{x100}$$

Seasonal effect on AMF colonization or sporulation was computed by taking average of values of four months (June-September) for rainy, five months (October-February) for winter, and three months (March-May) for summer seasons.

RESULTS AND ANALYSIS

Number of spores changed little with soil depth to 16-24 cm, but often declined with further increase in depth on each of the four soils examined (Table 1). The highest spore density was found in the dry seasons (summer and winter) coincident with the lack of flowering and fruiting of the growth season in all the experimental plants.

It is evident from Table 2 that mycorrhizal root colonization and sporulation varied to a great extent. The colonization was 19.5% and spore density 79.3/50 g soil during the month of June, which showed an increasing trend through September with highest colonization 56%

Table 1: Showing the recovering of AMF spores in three plants grown in university botanical garden

Plants	Depth of soil Sample (cm)	No of spores recovered/g.dried soil Time of sample			
		June 2005	Aug. 2005	Dec. 2005	April 2005
Tamarindis indica L.	0-5	18+_5.4	13+6.7	22+3.8	19+3.9
	6-15	31±4.2	36±4.6	66±5.4	73±5.6
	16-24	26±7.8	31±3.5	50±6.4	51±4.8
	25-32	11±6.5	9±7.8	18±2.3	18±2.2
Dalbergia sisso Roxb.	0-5	14±3.8	17±4.5	19±8.9	22±6.8
	6-15	35±6.6	38±3.9	56±3.5	68+4.6
	16-24	23±2.5	30±6.7	49±7.2	50±7.8
	25-32	12±5.3	13±7.6	16±5.6	17±3.7
Cassia nodusa Ham.	0-5	28±6.4	24±8.4	30±8.7	40±6.5
	6-15	39±1.0	37±9.3	56±4.6	76±5.2
	16-24	23±4.4	25±6.3	30±7.3	62±9.1
	25-32	10±2.3	8±7.5	18±2.2	41±5.4

± Standard error, * Each one is the mean value of 12 samples

Table 2: Showing monthly change in AMF root colonization of three leguminous tree growing in Botanical garden of Karnataka University

Plants	Months	AMF% Colonization	No. of Colonization AMF Spores/50g soil
Tamarindus Indica L.	June	19.7 ± 2.1	79.4
	July	44.2 ±5.0	76.5
	August	56.1 ±6.2	105.7
	September	46.8 ±4.3	107.6
	October	33.4 ±1.4	61.3
	November	27.1 ±2.2	66.4
	December	16.7 ±5.1	39.8
	January	13.4 ±6.0	52.9
	February	14.0 ±8.4	41.6
	March	24.1 ±8.4	62.8
	April	59.4 ±6.1	71.7
	May	25.7 ±3.2	67.6
Cassia nodosa Roxb.	June	26.4 ±2.2	76.4
	July	61.5 ±4.3	92.5
	August	58.2 ±5.1	97.2
	September	46.1 ±0.0	107.0
	October	37.2 ±4.2	64.7
	November	34.4 ±5.3	71.1
	December	16.7 ±5.1	39.8
	January	24.3 ±2.0	43.4
	February	18.1 ±3.2	58.2
	March	26.5 ±1.2	51.3
	April	41.6 ±6.2	63.4
	May	31.4 ±1.0	55.2
Cassia nodosa Roxb.	June	23.4 ±5.2	62.5
	July	58.5 ±3.3	83.9
	August	49.6 ±2.4	98.2
	September	44.7 ±1.5	104.6
	October	36.0 ±4.2	68.7
	November	28.0 ±3.3	67.8
	December	18.0 ±7.2	41.3
	January	15.0 ±4.4	53.4
	February	20.0 ±2.5	47.4
	March	30.0 ±5.3	59.3
	April	72.0 ±0.9	74.4
	May	31.0 ±2.1	63.2

± Standard error , * Each one is the mean value of 12 samples

and the spore density 509/105 g soil during the month of August. Both colonization and sporulation started to decline from September onwards with highest decrease (12.8% colonization and 47.3 spores/100 g soil, respectively) (59%) during April and sporulation (78.5% spores/50g soil) during May. In the present study, four plants *viz.,* a herb, shrub, twiner and tree has been selected. These plants are medicinally important and *Dalbergia sisso* Roxb. plant is commercially important timber tree. These plants are grown in University Botanical Garden.

Proliferation of root system and luxuriant growth of plant occurring during the luxuriant growth of plant occurring during the rainy season could be attributed to high relative humidity (90%-95%) and leaching of mineral salts from soil. Increased level of mycorrhizal colonization in other plant species and sporulation during this season has been reported (Raghupathy and Mahadevan, 1993; Bhaskaran and Selvaraj, 1997; Allen *et al*. 1998). However, an abrupt fall in root colonization and sporulation

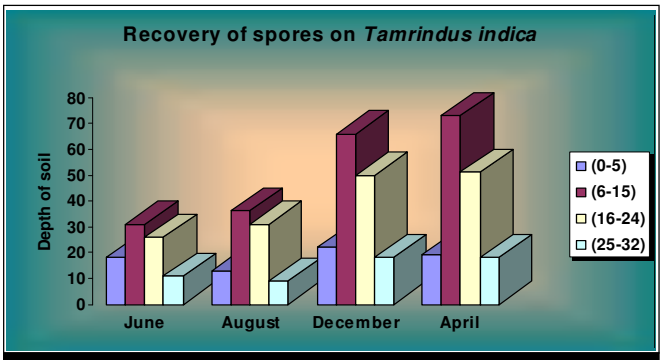

Fig. 1: Recovery of spores on *Tarindus indica*

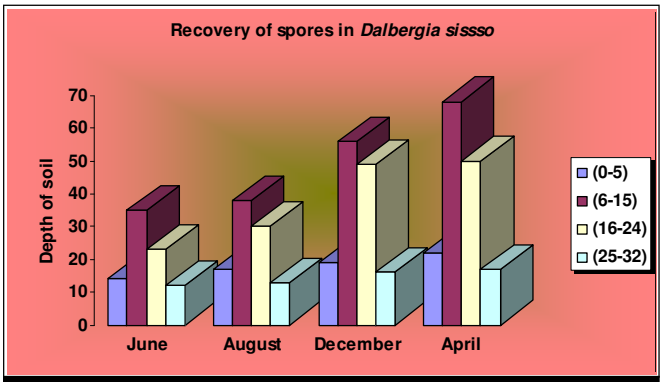

Fig. 2: Recovery of spores on *Dalbergia sissso*

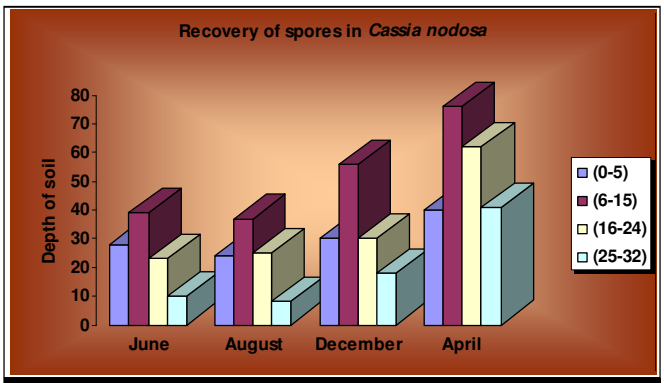

Fig. 3: Recovery of spores on *Cassia nodosa*

during the winter season could be ascribed to senescence in plants which resulted in reduced root exudation and to the fruiting stage during which most of the photosynthate was allocated to the aerial parts. It was urgently needed for the development of fruits rather than to the roots. Thus, reduced root exudation and limited carbohydrate allocation to the roots rendered AMF fungi to starve of carbon source, which might have adversely affected the root colonization and sporulation. Giovannetti (1985), Brundrett *et al.* (1985) and Abbot and Robson (1991), have also arrived at a similar conclusion. Immediately after the onset of summer, the dormancy was broken and plants started to bloom signaling the greatest metabolic activities resulting in profuse root exudation, which might have favored mycorrhizal root colonization and sporulation (Koske and Gemma, 1990; Bever *et al*,1996, Lakshman,1996). However, in the present study, *Tectona grandis* Roxb. *i.e.,* tree species, where spore population and per cent root colonization is more or less correlated with each other in all the three seasons. This may be due to plant growing at the very slow rate with continuous metabolitic activity colonized with indigenous AMF species.

The great agricultural and environmental importance of legumes, plus the ability of their rhizosphere system was able to harbour symbionts and other associated microbes of great relevance to plant productivity, make legumes target crops in sustainable agriculture. Current developments in the ecology, physiology, biochemistry, molecular biology, and biotechnology of microbe-plant relationships have given new insights into understanding the formation and functioning of the tripartite arbuscular-mycorrhizal and nitrogen-fixing symbioses of legumes.

Out of various AMF species identified *(Glamus mosseae. G. fasciculatum, Gigaspora margarita, G. gigantean* and *Acaulospora laevis). G.mosseae* was found to be the most predominant species as it occurred in 71.3% of the samples analyzed. This may be due to adaptation of this fungus to alkaline soils (Harley and Smith 1983). In conclusion physico chemical analysis studied soil is warranted. More studies are needed to co relate and compare.

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