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Research Article

OCCURRENCE OF AM FUNGI IN RHIZOSPHERE SOILS OF ENDEMIC AND ENDANGERED MEDICINAL PLANTS

***MD Khamar Jahan , M. Bavaji and A. Sreeramulu**
Department of Botany, S.V.University , Tirupat-517502, Andhra Pradesh , India
**Author for Correspondence*

ABSTRACT
The arbuscular mycorrhizal fungi status of ten medicinal plant species were investigated. The result indicated that all the ten selected plant species observerd. AM fungal association in the roots and spore population in the rhizosphere soil. However, maximum percent root colonization of AM fungi was observed in *Rhynchosia heynei* (75 %) followed by others, while minimum in *Pimpinella tirupatiensis* (20%). *Rhynchosia heynei* (152) showed more spore density whereas less in *Pimpinella tirupatiensis* (21). In the present study, percentage of AMF colonization was noticed less in summer, moderate in winter and highest in rainy season. Similarly the highest number of AMF spores was found in rainy season while moderate in winter and least in summer. Total 12 species were isolated belonging to four genera in which *Glomus sp* were found dominant followed by *Acaulospora, Sclerocystis* and *Entrophosphora* .

Key Words: *Medicinal Plants, Root Colonization, AM fungi*

INTRODUCTION
Arbuscular mycorrhizal fungi are soil microbes , forming obligate symbiotic associationship with the roots. The AM fungi colonizing in roots and surrounding soil, forms an important link between the plant and its below ground environment. AM fungal infection leads to the formation of hyphae, vesicles and arbuscules inside the root cortex. Arbuscular mycorrhizal fungi (AMF) are associated with almost all the plants in nature (Hayman , 1982) and are ubiquitous. AMF represent an important component of the soil microbial community that can significantly affect plant growth and soil stability. Since this interactions are able to alter ecosystem productivity and plant diversity (Gange *et al*., 1999). AM fungal association has been reported from time to time in different host plants growing in phosphorus deficient soils (Bagyaraj, 1986). But less data is available on AM fungal association with the medicinal plants , which are important sources of many drugs . In the present investigation an attempt has been made to study AMF colonization in medicinal plants growing in Tirumala hill region of Andhra Pradesh.

MATERIALS AND METHODS
Roots and rhizosphere soil samples of ten plant species viz , of *Tephrosia calophylla ,Rhynchosia heynei , Indigofera barberi ,Rhynchosia beddomei , Saphora interrupta ,Cerapegia spiralis , Pimpinella tirupatiensis , Leucas indica , Plumbago indica ,Boswellia ovalifoliolata* were collected from Tirumala hill region of Chittoor District ,Andhra Pradesh , India. Roots and rizosphere soil samples of individual plants were used for experimental purposes and for each species three plants were used. The roots of the sample plants were carefully dug up along with their rhizophore soil in triplicate and transported to the laboratory in polythene bags. Plant roots were gently washed under tap water to remove soil particles. These roots were fixed in FA (formalin, acetic acid, rectified spirit, 5:5:90). These roots were cut into 1cm and cleared in 10% KOH by simmering at 90^{o}C for 1 hour. The KOH cleared roots were stained in lactophenol following the procedure of Phillips & Hayman (1970). The segments were examined under microscope for mycorrhizal infection. .Root colonization was measured according to the method of Giovannetti and Mosse (1980). The percentage of root colonization were calculated using the following formula:

$$\text{Percent root colonization} = \frac{\text{Number of arbuscular mycorrhizal positive segments}}{\text{Total number of segment studied}} \times 100$$

The collected sample soil near the different root system were collected and an aqueous suspension passed through different sieves (50m, 100m, 200m, 300m and 400m mesh) to collect spores of different sizes. The number of spores and mycelial fragments was estimated using wet sieving and decanting method (Gerdmann & Nicolson 1963). To know the relationship between the soil properties with AM colonization different physical and chemical properties of soil were analyzed. Soil sample were analyzed following the standard procedures for pH, moisture, nitrogen (Subbaiah and Asija ,1956), phosphorus,and potassium(Jackson 1973). Spores were identified using a manual (Schenck and Perez 1990).

RESULTS AND DISCUSSION

All the ten plant species studied, exhibited AM fungal association. AMF colonization in roots and the spore population in the rhizosphere soil samples of all ten plant species showed wide variation under different seasons . The percent of root colonization and spore number associated with medicinal plants are presented in Table 1. The level of AM fungal association depends on root morphology, metabolism and rate of plant growth (Warner and Mosse 1980). Percent root colonization and mycorrhizal spore counts steadily increased in rainy season. The reports of Raghupathy and Mahadevan (1993) also showed highest percent root colonization during rainy season. In the present study, the percent root colonization recorded was higher in rainy season than in winter and summer. The maximum percent root colonization (75%) was recorded in *rhynchosia heynei* whereas minimum percent root colonization (15%) in were observed *pimpinella tirupatiensis* during rainy season. The highest spore count was seen in rhizosphere soil of *rhynchosia heynei* (152) followed by *sophora interrupta* (136) and least spore population was observed in *pimpinella tirupatiensis* (40). Spores observed in the soil are g*lomus, acaulospora, sclerocystis and Entrophosphora*, *Glomus* was found to be dominant.

In the present study, the percent root colonization recorded was higher in rainy season than in winter and summer. Least activity ofAM fungi in other seasons than rainy season might be due to reduced translocation of carbohydrates towards root. Raman and mahadevan, (1987) also found out that during rainy season the heat is reduced and there by the drought stress also increases, thereby increasing the root colonization. The spore population was observed to vary from during rainy, winter and summer seasons respectively. Spore population were observed maximum in rainy season when cpompared to winter and summer seasons. The results were same with the results of Sangeeta Kaushal (2000) who obtained maximum spore population in rainy season.

The physico-chemical characteristics of rhizosphere soil have been presented in Table 2. The population of AM fungi in diffent soils was determined in terms of resting spores and sporocarps in the soil. The data documents that the AM fungi are widespread in all the soils investigated but varied both in number and the type of spore and sporocarps. No correlation was found between pH and spores numbers. A range of moisture between 11% to 20% has been observed and this range has positive effect on spore distribution .It is a well- known fact that the nutritionally deficient soils and in particular phosphorus, harbor more AM fungi. The author's findings also support this and are corroborated by the findings of Manoharachary *et al.,(* 1998). It is important to note that all the plants screened were mycorrhizal. Colonization rates and spore densities also differed among the different localities and plant species . But in our present investigation all the collected plant species were mycorrhizal, but colonization rate, spore abundance and species richness greatly diverse in nature. As the spores represent the dormant state of the fungus, the physiologically active state is most likely the mirror image of the seasonal spore counts. This factor also can effect on spore number estimation as well as species diversity.

Table 1: AM fungal root colonization percent and spore population in the rhizosphere soil of Medicinal plants

S.No.	Name of the plant	Colonization %			Spore population (100g of soil sample)			AM fungal species
		R	W	S	R	W	S	
1	*Tephrosia calophylla* Fabacceae	35	27	20	90	83	71	*Acaulospora leaevis, Glomus fasciculatum,Glomus fistulosum*
2	*Rhynchosia heynei* Fabacceae	75	65	52	152	140	130	*Acaulospora leaevis,Glomus fistulosum ,Glomus austral, Sclerocystis pachycaulis*
3	*Indigofera barberi* Fabacceae	43	34	27	95	86	79	*Acaulospora bireticulata , Glomus fasciculatum ,Glomus fistulosum*
4	*Rhynchosia beddomei* Fabaceae	33	26	23	80	72	60	*Acaulospora nicolsonii, Glomus fistulosum , Glomus fasciculatum, Sclerocystis pachycaulis,*
5	*Saphora interrupta* Fabaceae	64	50	46	136	120	105	*Acaulospora leaevis, Acaulospora bireticulata, , Glomus fistulosum , Sclerocystis pachycaulis,*
6	*Cerapegia spiralis* Asclepiadaceae	28	25	23	50	33	26	*Acaulospora leaevis, Glomus fistulosum , Glomus fasciculatum*
7	*Pimpinella tirupatiensis* Apiaceae	20	17	15	21	17	12	*Glomus fasciculatum , Acaulospora nicolsonii*
8	*Leucas indica* Labiatae	48	37	30	100	86	73	*Acaulospora bireticulata, Glomus mosseae, Glomus fasciculatum , Sclerocystis pakistanica,*

All Vaules are Mean of Three Replications

Table 2: Physicochemical analysis in the rhizosphere soil samples of medicinal plants

S.NO.	Name of the plant	Rhizosphere soil samples				
		pH	Moisture %	Nitrogen mg kg-1	Phosphorus mg kg-1	Potassium mg kg-1
1	*Tephrosia calophylla*	7.9	15	830	*9.8*	*415*
2	*Rhynchosia heynei*	8.0	20	950	7.6	489
3	*Indigofera barberi*	7.6	16	810	8.9	456
4	*Rhynchosia beddomei*	7.7	13	816	10.0	490
5	*Saphora interrupta*	7.8	20	880	8.0	410
6	*Cerapegia spiralis*	7.9	12	780	9.6	520
7	*Pimpinella tirupatiensis*	8.o	11	820	11.0	570
8	*Leucas indica*	7.6	16	870	8.4	600
9	*Plumbago indica*	7.6	18	960	7.9	480
10	*Boswellia ovalifoliolata*	7.9	17	920	8.0	498

All vaules are mean of three replications

In the present results the genus *glomus* and *acaulospora* are the dominant mycospecies in the roots of medicinal plants, the possible reasons for the predominance of *glomus sp*. are that spores of *glomus* species have different temperature and pH preferences for germination (Wang *et al*.1997) and *acaulospora* species are often associated with acidic soils (Morton 1986; Abbott and Robson 1991). Dominance of genus glomus from medicinal plants has been reported earlier by Selvaraj *et al. (2001).* In the results examined it was found that there was no correlation between root colonization and spore number in soil. This confirmed the earlier works done by Mohan , *et al*., (2005) and Govinda rao *et al*., (1989). The results obtained from the study suggests that the colonization percentage and number of spore AM spores differ with different ten medicinal plants. This confirms that the medicinal plants of Tirumala hill region are colonized by arbuscular mycorrhizal fungi. It is also apparent that rainy season may considered as the best season for the propagation of plants by the application of AMF as bio-inoculants even for the plants of rare and threatened species.

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