ISOLATION AND IDENTIFICATION OF MYCORRHIZAL SPORE FROM RHIZOSPHERIC SOIL OF SOME MEDICINAL PLANT IN VAISHALI DISTRICT

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ABSTRACT

Arbuscular Mycorrhizal fungi are ancient, widespread and form association with many plant species. The fungus- plant relationships are usually described as mutually beneficial. The present study aims at isolation and identification of some mycorrhizal fungal spores from some medicinal plant of some regions of Vaishali district of Bihar during 2009 – 2011. Random collection of rhizospheres soil samples from Karnpura, Mahnar,Sarai, Bidupur and Diyararegions of Vaishali district was done. Wet sieving and decanting method were employed for the isolation and mycorrhizal spores. Collected rhizospheric soil was suspended in water and passed through sieves of different sizes of 500μm, 250μm, 100μm and 38μm. Spores concentrations of different size, shape, colour and hyphal attachment were examined under stereo microscope. The spore density seemed to be dominated mainly by species of *Glomus*. However, *Gigaspora, Acaulospora* and *Scutellospora* too were identified at a comparatively lesser percentage.

INTRODUCTION

The term mycorrhiza was coined by A.B. Frank, a scientist in Germany, more than 100 years ago (Habte, 2000). It literally means Fungus- root and describes the mutualistic association existing between a group of soil fungi and higher plant (Habte, 2000). AM fungi are considered to be obligate biotrophs as they are unable to grow and reproduce in regions of the soil where the host plant roots are absent (Meyer, 2007). Mycorrhiza refers to an association or symbiosis between plants and fungi that colonize the cortical tissue of roots during periods of active growth (Sylvia et. al., 1998). Depending on the individual AM fungi and conditions, many plant species show large positive growth responses to AM colonization. These responses are usually due to more efficient acquisition of soil nutrients, especially P, from the relatively extensive mycorrhizosphere compared with the rhizosphere of non – mycorrhizal (NM) control plants (Smith et. al., 1979; Facelli et. al., 1999). However, some plant species show little or no growth increase; other – including wheat – can show growth depression at least during vegetative stage when colonized (Graham & Abbott, 2000, Zhu et. al., 2001a). In nature, more than eighty percent of angiosperms and almost all gymnosperms are known to have mycorrhizal associations.

Material and Method

Wet sieving and decanting method are the commonly used method for spore isolation (Gerdemann and Nicolson, 1963; Walker et. al., 1982). Wet sieving and decanting are a simple method which used sieve of various size to separate spores and other similar size particles from sand and clay (Daniels and Skipper, 1982). The various steps involved in

wet sieving and decanting method were as follows: -

1. First 10 g soil sample was taken and dissolved in 100 ml distilled water in conical flask.
2. Then conical flask was shaken for 30 min.
3. After that the conical flask was kept in undistributed condition for 30 min.
4. The heavier particles were allowed to settle down.
5. Suspension was decanted through a 500μm sieve to remove organic matter and roots.
6. This suspension was decanted through 250 μm, 100μm and 38μm sieve consequently.
7. The entire residue was collected on 38μm sieve.
8. After settlement residue was dissolved in distilled water and filtered through filter paper.
9. This paper was spread in Petridis and a residue present in filter paper was taken and mounted on a slide and was examined.

Observation

Periodical survey of various places such as Karnpura, Mahnar,Sarai, Bidupur and Diyararegion was undertaken to collect and identify different AM fungi Genera and species associated with medicinal Plants. Rhizosphere soil samples collected from various localities revealed presence of several species of different genera on the basis of resemblances the AM fungi identified as *Gigaspora spp., Acaulospora spp*. and *Glomus spp*. Shown as in plate under: -

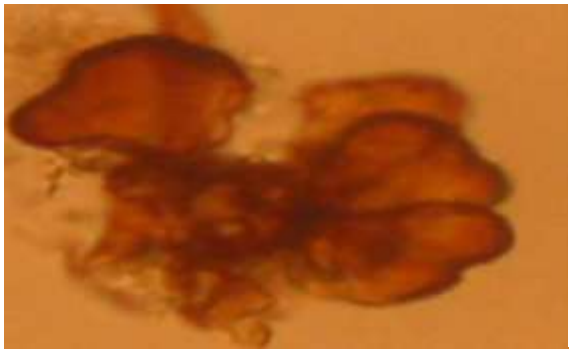

Fig. *Gigaspora spp.*

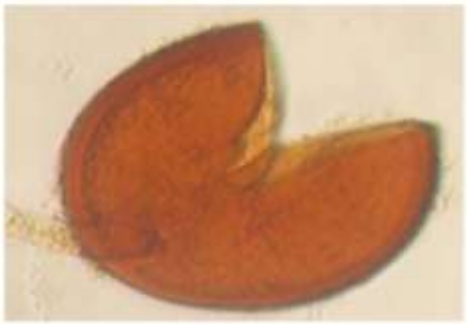

Fig. *Acaulospora spp.*

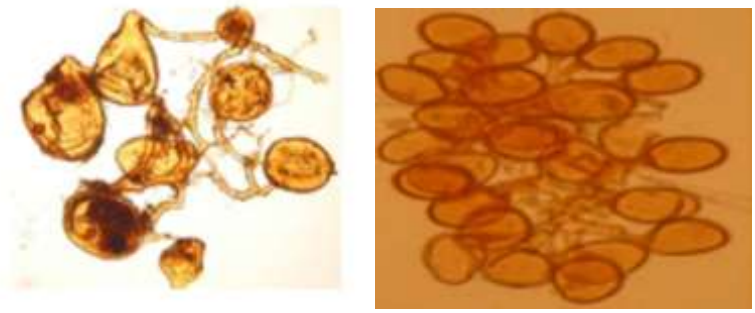

Fig. *Glomus spp.*Fig. *Glomus spp.*

In *Asparagus racemosus, Acaulospora spp*. and *Glomus spp*. Were found to be dominant. However, *Glomus spp*. and *Gigaspora spp*. Dominantly present in *Oscimum sanctum*. *Glomus spp*. and *Gigaspora spp*. are dominant in *Catharanthus roseus*.
The number of spore of AM fungi isolated from different sites of the medicinal plant *Asparagus rcemosus, Cataranthus roseus and Oscimun sanctum* are given in table below:-

Table shown: Average number of spores per 100 g of soil for host plant *Asparagus racemosus, Catharanthus roseus and Oscimum sanctum.*

Locality	Avg. No. of spore in *Asparagus racemosus*	Avg. No. of Spore in *Catharanthus roseus*	Avg. No. of Spore in *Oscimum sanctum*	Overall Avg. No. of Spore
Karnpura	**65**	**51**	**73**	**64**
Mahnar	**86**	**110**	**125**	**105**
Sarai	**78**	**112**	**105**	**100**
Bidupur	**86**	**110**	**121**	**106**
Diyara	**94**	**83**	**101**	**98**
Average Spores Area wise	**99**	**93.2**	**105**	**94.6**

The number of spores ranges from 53 to 130 per 100 g. of soil considering all medicinal plants individually under study. The average number of spores from five site contained more than 110 spores per 100 g. of soil. AM fungi distribution in many soil samples were collected from different regions of Vaishali, Bihar. These sample showed the presence of

AM fungal spores shown in table 5. AM fungi are well distributed throughout Vaishali district. Maximum number of spores were isolated from Mahnar. Soil samples collected from uncultivated habitat such as Manhar, Bidupurand Diyarahad large number of spores in comparison to cultivated sites such as Karnpura and Manhar. This study describes the distribution of AM fungi in the rhizosphere soil of medicinal plants. Both plant and rhizosphere soil were collected during a three-year period 2009-2011 at different sites and during different seasons. The average number of spores isolated for different medicinal plants collectively from year 2009 to 2011 from diverse sites has been shown in tabular form in below whereas this data is also represented area wise schematically in figure. The data corresponding to plants *Asparagus racemosus, Catharanthus roseus* and *Oscimum sanctum* individually have been presented in tabular form.

Table: Average number of spore Area wise and Year wise

Year	Karnpura	Manhar	Sarai	Bidupur	Diyara	Average year wise
2009	88.2	124.8	94.2	120.2	120.4	109.56
2010	92.4	125.9	95.7	118.4	117.8	110.04
2011	89.98	127.01	91.89	121.17	117	109.41
Average Spores Area wise	90.19	125.90	93.93	119.92	118.40	

RESULT AND DISCUSSION

Rhizospheric soil samples from five different locations were subjected to the recovery of AM fungal spores. The soil samples of different location showed different types of spores. All the recovered spores represent genera namely *Glomus,Acaulospora* and *Gigaspora*. The entire collected rhizosphere soil samples exhibited the presence of varied range of spore population in the soil profile.Highest spore number was observed in the rhizosphere soil of *Ocimum sanctum* L. Collected from Bidupur. Lowest spore was noticed in the rhizosphere soil of *Catharanthus roseus* L. Collected from Karnpura.Percent root colonization was observed in experimental plants grown with soil samples taken from various places. The total AM fungal spore r at different localities varied from ninety to one hundred eighteen per hundred grams of soil. The highest spore density was observed in the soil with rich organic matter and slight acidic soil compare to neutral and alkaline soils. There was a wide variation in spore number specially in *Glomus species* followed by *Acaulospora species.*

Mycorrhizal colonization was more frequent in forest areas than in cultivated fields. It is likely that fertilizer application to cultivated land reduce AM fungal spores like Karnpura. A similar observation was made by Grine et al. 1987, who worked on the mechanisms of floristic diversity with reference to mycorrhizae. The potential reason for maximum number of spore's availability in undisturbed natural vegetation is that spores keep multiplying in association with plants whereas, in cultivated habitat the top soil is disturbed each time as some fresh crop is sown. Previously, several researchers like Gaur and Kaushik2011, also reported that quantitative spore's population differ in cultivated and non-cultivated soil. This result suggests mycorrhiza plays vital role in plant fertilizing ability.

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