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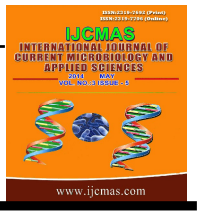

Original Research Article

Arbuscular mycorrhizal fungi associated with rhizosphere soils of brinjal cultivated in Andhra Pradesh, India

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Keywords

Brinjal, mycorrhizae, rhizosphere, root colonization

A B S T R A C T

Mycorrhizae are highly evolved and have non-pathogenic symbiotic association between roots of most vascular plants and certain specialized soil fungi that colonize the cortical tissues of roots during periods of active plant growth both in natural environment and in cultivation. The present study is aimed to assess the association of arbuscular mycorrhizal fungi in brinjal crop along with arbuscular mycorrhizal (AM) fungal population density in the rhizosphere soils, investigate for qualitative composition of AM fungal species and per cent root colonization. The results showed that the number of AM fungal propagules in brinjal crop collected from different localities varied from 635 to 1325 spores per 100gm soil. Due to the widespread nature of AM fungi, they occurred in almost all the soil samples but with a variation both in number and type of spores and sporocarps. Altogether, 20 AM fungal species were isolated belonging to the genera of *Glomus*, *Acaulospora*, *Gigaspora*, *Sclerocystis* and *Entrophospora*. *Glomus* was observed to be predominant followed by *Acaulospora* in the rhizosphere soils of brinjal. The spore distribution, density and the composition of AM fungi were observed to be influenced by environmental and physico-chemical factors. The AM spore population, percentage of root colonization and distribution varied by the seasonal fluctuations in moisture, temperature, pH and soil mineral nutrient status such as N, P, K, Zn, Fe, etc. The data showed that nitrogen deficient soils appeared to have more number of AM fungal propagules. The soils having high levels of phosphorus and potassium content harboured least number of AM fungal spore population while, low levels favoured more spore density. Similarly low levels of zinc, copper and manganese were favourable for more fungal occurrence and distribution. However, presence of high levels of iron favoured more AM fungal spore occurrence.

Introduction

Mycorrhizae are highly evolved and have non-pathogenic symbiotic association between roots of most vascular plants and cortical tissues of roots during periods of active plant growth both in natural environment and in cultivation.

The arbuscular mycorrhizal (AM) type of symbiosis is very common as the fungi involved can colonize a vast taxonomic range of both herbaceous and woody plants, indicating lack of general host specificity among this type. However, it is important to distinguish between specificity, innate ability to colonize, ineffectiveness, amount of colonization and effectiveness, plant response to colonization. AM fungi differ widely in the level of colonization they produce in a root system and in their impact on nutrient uptake and plant growth.

In the tropics, many crops are grown in infertile acid soils, where low level of available phosphorus frequently limits their establishment. In such soils, an efficient mycorrhizal association can increase phosphorus uptake and crop yield.

Mycorrhizal plants exhibit greater uptake of phosphorus and trace elements when these nutrients are sparingly soluble in soils (Powell and Bagyaraj, 1984; Abbot et al., 1992). They can also show improved resistance to drought, environmental stress and also fight against the root borne pathogens (Allen and Boosalis, 1983).

As per National Horticulture Database (2010), India stands second in the world in vegetable production (88.62 million tons) after China. Vegetable production has increased nearly three times in the last 50 years. A large area being covered with vegetable crops resulting in increased yield and thus created a better socio-economic status for farmers. Vegetables like tomato, brinjal, cabbage, cauliflower, radish and onion are now produced throughout the year. The projected demand for vegetables in India is 88.6 tons in 2001-02, 160 tons in 2007-08, and 185 tons for 2011-12, thus showing a growth rate increase of 10.9%[4] (Venugopal et al., 2011). The challenge now is to grow more food of high nutritive quality. The solution lies in developing and adopting hi-tech agriculture to improve the productivity in eco-friendly manner.

It is now recognized that AM fungi can be harnessed in order to improve productivity in agriculture, fruit culture, and forestry by reducing the input of fertilizers and/or by enhancing plant survival, thus offsetting ecological and environmental concerns. For this reason, studies on mycorrhizae gained importance due to its practical use as a low input technology for managing soil fertility and plant nutrition.

Brinjal belonging to Solanaceae family is chosen for the study, as this is a commonly consumed vegetable. Commercially it is less expensive and economically more important.

The ability of AM fungus association with most of the terrestrial plants is well documented. However, there are only a few reports on the occurrence and distribution of AM fungus association in the selected vegetable crop. Hence, the present study is aimed to assess the association of AM fungi in brinjal crop along with AM fungal population density in the rhizosphere soils collected from different locations, investigate for qualitative composition of AM fungal species and per cent root colonization.

In the present study, brinjal crop commonly grown, in and around Hyderabad viz., Uppal (S1), Shameerepet (S2), Alwal (S3), Tenugudem (S4), Shamshabad (S5), Ghatkesar (S6), National Institute of Nutrition campus (S7), Kanchanbagh (S8), Medchal (S9)

and Rajendranagar (S10) were surveyed for arbuscular mycorrhizal (AM) fungal association.

Materials and Methods

In the present study, brinjal crop commonly grown, in and around Hyderabad viz., Uppal (S1), Shameerepet (S2), Alwal (S3), Tenugudem (S4), Shamshabad (S5), Ghatkesar (S6), National Institute of Nutrition campus (S7), Kanchanbagh (S8), Medchal (S9) and Rajendranagar (S10) were surveyed for arbuscular mycorrhizal (AM) fungal association.

Sampling of rhizosphere soils

The rhizosphere soil samples of tomato were collected randomly with the help of a widger by lifting up gently a block of soil with the plant roots intact. Later the root system along with the sticky soil around was carefully removed and placed in a polythene bag. These samples were quickly transported to the laboratory and moisture content was determined immediately.

Soils for further analysis were wrapped up in separate polythene bags and stored at 4^{o}C. They were air dried and analysed for total nitrogen, phosphorus and potassium.

Isolation and quantitative estimation of AM fungal spores

Extramatrical chlamydospores, azygospores and zygospores produced by AM fungi were assayed for spore count using wet sieving and decanting method (Gerdemann and Nicolson, 1963) and quantification of spore density of AM fungi was carried out using method described by Gaur and Adholeya (1994).

100 gm of composite air dried rhizosphere soil sample from brinjal cultivated fields was mixed in water thoroughly and the decantant was passed through the sieves of various sizes (420µm, 250µm, 105µm, 75µm and 53µm) by placing the sieves one below the other in descending order of pore sizes from 420µm to 53µm.

The debris retained on the sieves was carefully collected on synthetic fibered white cloth and kept in a petridish divided into compartments with the glass marker for easy counting of spores. The petridish was observed under stereo-binocular dissecting microscope. AM fungal propagules existing both as spores and sporocarps counted by scanning each filter cloth sieved under 420µm, 250µm, 105µm, 75µm and 53µm. Total AM propagules were calculated for 100 gm of moisture free soil.

The AM fungal spores were mounted in lactophenol, placed coverslip and observed under compound microscope. The spores were identified upto species level using standard keys of Morton and Redecker (2001a, b) and Schenck and Perez (1990) and the spores observed in each field were recorded.

Soil analysis

The rhizosphere soils of brinjal were analysed for the following physico-chemical factors like soil texture and soil type by following Robinson's pipette method (Piper, 1944), soil pH was read with global pH meter (with double distilled water mixed in the 1:2.5 soil water suspension), moisture content by oven drying method at 105^{o}C for 24 hours, available nitrogen by micro-kjeldahl method, available phosphorus (P) and potassium by Jackson (1973) method.

Assessment of AM infection in roots and percentage of root colonization

For assessing the amount of AM infection, roots of brinjal crop collected from different locations were checked for AM root colonization. The plants in the natural fields were carefully uprooted to obtain lateral roots and rootlets intact. Well-developed roots with rootlets were cut by taking three replicates of each sample. These were kept in polythene bags and immediately transferred to the laboratory. Root system was thoroughly washed under running tap water and cut into bits of approximately 1cm length. 10 to 15 root bits were selected randomly and were gently washed in water to remove soil particles. Washing was done with much care, so that the mycelium and spores, which are attached to the root, would not be washed away. Cleaned roots were fixed in FAA solution (Formalin : Acetic acid : Alcohol in 5:5:90 ratio) (Kormanik et al., 1980).

Cleaning and staining of the roots was done by following Phillips and Hayman technique (1970). The roots fixed in FAA solution were washed thoroughly, transferred into 10% KOH solution and boiled at 90^{o}C for 1 hour. After cooling the root segments were washed in distilled water and acidified by immersing in 1 N HCl for few minutes. Later they were stained with 0.5% trypan blue in lactophenol. Excess stain was removed by keeping the stained roots in lactophenol for 2 minutes. The stained root segments were mounted on slides and observed under microscope for mycelium, arbuscules and vesicles. The infection, colonization and establishment were studied. The percentage of VAM infection was estimated by the root slide technique (Read et al., 1976). All infected and uninfected segments were counted. The percentage of root infection was calculated as

$$Per\ cent\ root\ colonization = \frac{Total\ number\ of\ root\ segments\ colonized}{Total\ number\ of\ root\ segments\ observed} X\ 100$$

Results and Discussion

Ecology of AM fungi

Brinjal plants belonging to Solanaceae family were screened for the presence of AM fungi. The data of fungal propagules isolated from the soil samples of these plants collected from different locations is given in Table 1. The soil samples collected from ten different locations screened were found to be AM fungal associated. All the samples collected from the soil irrespective of the location showed percentage root colonization (Table 1). The data indicates the number of AM fungal propagules in different crops collected from different locations varied from 635 to 1325 spores per 100gm soil.

Among all the ten soil samples surveyed, soils from NIN garden harboured more number of AM propagules 1325 per 100 gm soil while, soil samples from Kanchanbagh area harboured least number 635 spores per 100 gm soil. In all, twenty AM fungal species were isolated representing five genera namely *Acaulospora, Glomus, Sclerocystis, Gigaspora* and *Entrophospora* (Table 2). *Glomus* represented by seven species, *Acaulospora* by six species, *Sclerocystis* by four species, *Gigaspora* by two species and *Entrophospora* by only one species. Out of these twenty AM fungal species, the present soils are dominated by *Glomus* species followed by *Acaulospora*.

Physico-chemical factors of the Soil

The data of physico-chemical characteristics of the rhizosphere soil samples of brinjal collected from different places in relation to number of propagules is presented in Table 1 and Figure 1. All the soils investigated in the present study were of red sandy loam type and received no fertilizers. These soils formed good habitats for AM fungal colonization. The soils had a pH range between 6.9 and 7.5. The soils with pH neutral to slightly alkaline harboured more number of propagules.

Moisture content of the samples ranged from 22% to 30%. Maximum number of spores (1325 per 100g soil) was recorded in samples containing 30% moisture content while, less number (635 per 100g soil) were observed in samples with 22% moisture. Thus low moisture content suppressed the AM spore population.

The data of the rhizospere soils showed that nitrogen deficient soils appeared to have more number of AM fungal propagules. Soils with low nitrogen content of 700mg kg^{-1} soil had AM fungal propagules of 1325 per 100g. While soil samples with high nitrogen (830 mg kg^{-1}) had less spore density of 635 per 100 g. These soil samples were studied for their phosphorus content and it was observed that soils containing 3.3 to 3.4 mg/kg phosphorus had maximum number of AM fungal spores whereas the soils having least number of AM fungal spores showed high levels of phosphorus content i.e., 4.3 mg per kg.

High levels of potassium harboured least number of AM spores compared to soils containing low levels of potassium. Hence, it was evident that low levels of potassium favoured more AM fungal spore association. Similarly, soils with low levels of zinc, copper and manganese were favourable for more AM fungal occurrence and distribution. However, presence of high levels of iron favoured more of AM fungal spore occurrence.

AM fungal root colonization in natural field conditions

The percentage of AM fungal root colonization of brinjal from all the selected locations in natural field soils is presented in Table 1. From the data, it is clear that the percentage of root colonization varied in the samples of brinjal collected from different locations. Percentage root colonization was observed to be maximum (90%) in the samples of NIN campus and minimum (54%) in Kanchanbagh samples. The root samples of NIN campus were observed to be heavily colonized by vesicles (Fig 2).

In the present study, the population dynamics of AM fungi was determined by collecting the resting spores from different soils in and around Hyderabad. Due to the widespread nature of AM fungi, they occurred in almost all the soil samples but with a variation both in number and type of spores and sporocarps irrespective of the locality.

Altogether, 20 AM fungal species were isolated from ten different soils belonging to the genera of *Glomus*, *Acaulospora*, *Gigaspora*, *Sclerocystis* and *Entrophospora*. Among the soil samples, the samples collected from NIN garden had more number of AM propagules followed by Shamshabad soil samples, may be due to low nutrient status and average amounts of moisture content.

Table.1 Number of AM fungal propagules in relation to physico-chemical factors in the rhizosphere soil samples of brinjal

Factors	*Soil samples*									
	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
AM fungal propagules/100g soil	1100	860	1125	740	1200	850	1325	635	1150	700
AM infection (%)	78	63	80	60	82	65	90	54	80	60
Sand %	50	50	60	50	60	50	60	50	65	50
Silt %	35	35	25	40	30	35	30	30	20	40
Clay %	15	15	15	10	10	15	10	20	25	10
pH	6.9	7.0	7.2	6.8	7.5	7.2	7.4	7.2	7.5	7.2
Moisture %	25	24	27	24	30	24	30	22	29	23
N (mg/kg)	720	760	710	790	700	810	700	830	710	790
P (mg/kg)	3.5	3.8	3.3	4.0	3.4	3.8	3.4	4.3	3.3	4.0
K (mg/kg)	460	510	480	510	450	510	450	550	460	520

S1- Uppal, S2- Shameerpet, S3- Alwal, S4- Tenugudem, S5- Shamshabad, S6- Ghatkesar, S7- NIN campus, S8- Kanchanbagh, S9- Medchal, S10- Rajendranagar.

Table.2 Arbuscular mycorrhizal fungal spore distribution in the rhizosphere soils of brinjal collected from different locations

AM fungal species	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
Glomus mosseae	+	+	+	+	+	+	+	+	+	+
G. aggregatum	+	-	+	+	-	-	+	-	+	-
G. geospora	-	-	+	-	+	-	+	+	+	-
G. reticulatum	+	+	+	-	-	+	+	-	-	+
G. fasciculatum	+	+	+	+	+	-	+	+	+	-
G. constrictum	+	+	+	-	+	+	+	-	+	+
G. epigeum	-	-	+	+	-	-	-	-	+	-
Acaulospora laevis	+	-	-	+	+	-	-	+	+	-
A. foveata	-	-	+	-	-	-	+	-	+	-
A. mellea	+	+	-	-	+	+	+	-	-	-
A. morroweae	-	-	+	-	+	-	-	-	-	+
A. rehmii	+	-	-	+	-	+	+	+	+	-
A. nicolsonii	+	-	-	+	+	-	+	-	+	+
Sclerocystis sp.	-	+	-	-	-	+	+	-	+	+
S. sinuosa	-	+	+	-	+	-	+	+	-	+
S. pakistanica	+	-	-	-	+	+	-	-	+	+
S. microcarpa	-	-	-	-	-	-	+	+	-	-
Enterophospora sp.	+	+	+	+	-	-	-	+	-	-
Gigaspora sp.	-	+	-	-	+	+	-	+	+	+
Gigaspora margarita	-	-	+	+	-	+	+	+	-	-

S1- Uppal, S2- Shameerpet, S3- Alwal, S4- Tenugudem, S5- Shamshabad, S6- Ghatkesar, S7- NIN campus, S8- Kanchanbagh, S9- Medchal, S10- Rajendranagar.

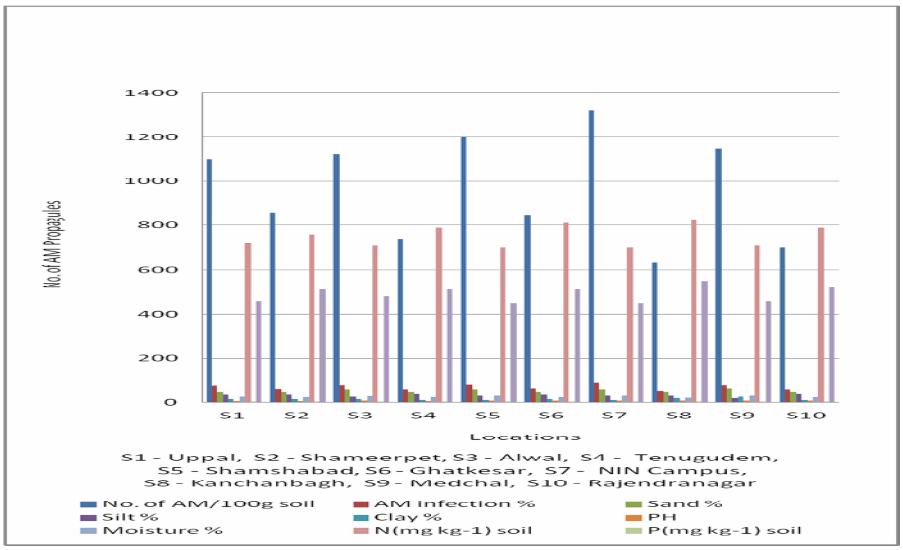

Fig.1 Number of AM propagules in relation to physico-chemical factors in the rhizosphere soil samples of brinjal

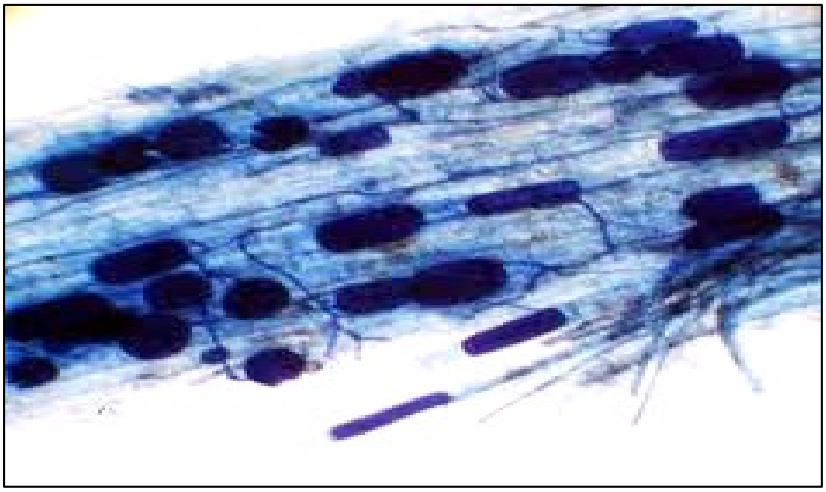

Fig.2 Root colonization showing vesicles in different shapes and hyphae

Among the 20 AM fungal species isolated from the rhizosphere soils of brinjal crop, *Glomus* was predominant followed by *Acaulospora*. The results are very much supportive of the earlier studies (Singh et al., 2000; Whipps, 2004). Earlier reports also revealed the predominance of the above AM fungal genera in the rhizosphere soils of different plant cultivars (Gerdemann and Trappe, 1974; Hall and Abbott, 1984; Hindumathi and Reddy, 2011).

Effect of physico-chemical factors of the soil

The spore distribution, density and the composition of AM fungi were observed to be influenced by environmental and physico-chemical factors. The AM spore population, percentage of root colonization and distribution is affected by the seasonal fluctuations in moisture, temperature, pH and soil mineral nutrient status such as N, P, K, Zn, Fe, etc. Earlier studies carried

out by Bagyaraj and Sreeramulu (1982), Reddy et al (2006), Reddy et al (2007), Hindumathi and Reddy (2011) in chilli, sorghum, mungbean and soybean also showed similar trend. Mahesh and Selvaraj (2008) studied the occurrence and distribution of AM fungi in non-polluted and polluted soil samples and found that non-polluted soil contains more number of AM propagules and sandy loam soils with nutrient deficiency also supported the maximum number of AM propagules which is in unison with our present study.
Vesicular arbuscular mycorrhizal (VAM) fungi are an important group of soil borne microorganisms that contribute substantially to the establishment, productivity and longevity of natural or man-made ecosystems (Harley and Smith, 1983).

In the present study, the mycorrhizal spore population in rhizosphere soil as well as the percentage of mycorrhizal infection in plant roots fluctuated with the changes in physico-chemical factors of the soil. The results are in concurrent with the earlier findings of Kehri and Chandra (1988) and Reddy et al (2007).

Light textured sandy loam soil with neutral to slightly alkaline pH, low moisture percentage favoured extensive mycorrhizal root association (Sreeramulu and Bhagyaraj, 1986). In our study, the soil pH ranged from 6.9 to 7.5, which was closer to neutral to slightly alkaline and had more number of AM fungal propagules. The importance of soil moisture on the AM fungal distribution was reported by Saif and Khan (1977). Maximum number of spores 1325 per 100g soil was observed at moisture level 30%. The least number of spores i.e. 635 per 100g were observed when the moisture was 22%. Thus, decrease in soil moisture content was found to have a negative effect on spore number. Our results show that, the soils with low moisture percentage suppressed the AM spore population. This can be explained by the fact that mycorrhizal spores and their effect to promote plant growth are affected by decrease in the soil moisture. An optimum level of moisture content between 25 to 30% seems to be ideal for AM association. Thus, our results are in agreement with the above cited references.
In the present study, the rhizosphere soils having low levels of nitrogen like 700mg kg^{-1} soil contained 1325 AM fungal propagules per 100 gm soil and 90% root colonization, but soil sample with nitrogen content 830 mg kg^{-1} had only 635 spores per 100g soil and 54% root colonization. Thus, it clearly indicates that high levels of nitrogen reduces the number of AM propagules and per cent root infection which is on par with the research work published by Azcon-Aquilar and Barea, 1982.

The phosphorus deficiency in semi-arid region soils is the rule of AM plant association (Williams et al., 1974). Mosse (1981) observed that high levels of free phosphorus in the soil decreases the mycorrhizal development, and Ojala et al (1983) recorded decrease in mycorrhizal dependency of the plant with increase in available phosphorous in the soil. Our study also showed the same trend that, samples with more phosphorous content (4.3 mg/kg) had least number of AM propagules compared to the soil having less phosphorus (3.3-3.4 mg/kg) which harboured maximum number of AM spores.

As per our experimental results of the soil samples, high levels of potassium in the soil had least number of AM spores

compared to samples with low potassium levels. Our findings are coinciding with the observations made by Barea et al (2002), Corriveau et al (2007) and Suresh et al (2000) that low levels of potassium favoured more number of AM fungal association than high levels of potassium in the soil.

Similar trends were noticed that low levels of Zn, copper and manganese and high levels of iron favoured AM fungal propagules. These observations are in agreement with the earlier reports (Solis-Dominguez et al., 2011; Barea et al., 2002; Brundrett, 2002; Garg and Chandel, 2010; Guether et al., 2009).

In the present study, genus *Glomus* was represented by more number of species compared to the other genera suggesting thereby its preponderance. This study also provides a clear understanding of the ecology of AM fungi.

Due to the widespread nature of AM fungi, they occurred in almost all the soil samples but with a variation both in number and type of spores and sporocarps irrespective of the locality. The genus *Glomus* was represented by more number of species compared to the other genera suggesting thereby its preponderance. This study also provides a clear understanding of the ecology of AM fungi.

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