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Studies on AM fungal association with *Vigna radiata* (L.) R. Wilczek from Telangana, India

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AM fungi are the plant root obligate symbiotic associates with greater ability of soil exploration and increasing nutritional uptake and phosphorus. 70-80% of crop plants naturally associated with AM fungi in phosphorus deficient crop soils. AM fungi are known to increase plant growth, yield, tolerate abiotic, biotic stress besides increasing immunity of plant. AM fungi are present in rhizosphere soil in abundance and produce external and internal hyphae, vesicles and arbuscules inside the root cortex, besides hydrophobic glycoprotein and glomalin. AM fungi increases soil binding capacity and acts as a bio fertilizer. Legumes relatively have "P" requirement for nodule development and nitrogen fixation. *Vigna* crop is grown throughout the country. Therefore, AM fungi have been isolated from non-rhizosphere and rhizosphere soil supporting *Vigna* plantation. Data on AM fungal spore count morpho-taxonomy of AM fungi, percentage, root colonization, impact of pH, orgnic carbon and phosphorous are evaluated. The data also has indicated that *Acaulospora bireticulata , Acaulospora elegans, Acaulospora foveata, Acaulospora laevis, Acaulospora nicolsonii, Acaulospora spinosa, Entrophospora infrequens, Rhizophagus fasciculatus, Funneliformis caledonium, Gigaspora rosea, Glomus macrocarpum, Glomus botryoides, Scutellospora arenicola, Scutellospora minuta, Zygosporium rostratum* are the AM fungi associated with *Vigna radiata.*

Key words: AM fungi, arbuscules, hyphae, soil, rhizosphere, *Vigna*

INTRODUCTION

AM fungi are the plant root obligate symbiotic associates with greater ability of soil exploration and increasing nutritional uptake and phosphorus. 70-80% of crop plants naturally associated with AM fungi in phosphorus deficient crop soils. AM fungi are known to increase plant growth, yield, tolerate abiotic, biotic stress besides increasing immunity of plant. AM fungi are present in rhizosphere soil in abundance and produce external and internal hyphae, vesicles and arbuscules inside the root cortex, besides hydrophobic glycoprotein and glomalin. AM fungi increases soil binding capacity and acts as a bio fertilizer. Legumes relatively have "P" requirement for nodule development and nitrogen fixation. (Bagyaraj *et al*. 1979; Gupta and Mukerji, 2006; Harley and Smith, 1983; Harikumar, 2015; Janaki rani and Manoharachary, 1994; Lakshman *et al*. 2006; Manimegalai *et al.* 2011; Suresh and Selvaraj, 2006).Some legumes have been worked out for AM fungal association (Arumugan, 2010; Jalander and Mamatha, 2015; Navnita *et al.* 2016; Pellegrino and Bedini, 2014; Ray and Valsalakumar, 2010; Sujata and Sharma, 2015; Valsalakumar *et al.* 2007). However, *Vigna radiata* associated with AM has not been worked out extensively for the AM fungal association and their role in plant rowth. Therefore the present paper deals with AM fungal association, role of some ecologcal function and impact of AM fungi on plant growth of *V. radiata* from Telangana.

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MATERIALS AND METHODS

Vigna radiata (L.) R. Wilczek Mung bean or green gram has long been a food crop in Asia. It is native to India and now widespread throughout the trop-

ics, It is less known as a useful green manure crop. An upright annual legume ranging in height from 15 cm to 1 m; average height of mature plant, 0.9 m. Branches freely, but not heavily foliaged. Leaves, stems and pods are slightly hairy. Junctions of branches and stems are stipuled. The first flowers appear seven to eight weeks after planting and the crop reaches maturity in 12 to 14 weeks. Pods borne at top of plant. Seeds, green and almost globular. Pods clothed in long, spreading, deciduous silky hairs. The seed is reported to contain 24% crude protein, 53% carbohydrates, and 2% fat etc.

Random soil samples (Rhizosphere and Non-Rhizosphere soil) were collected during 2014-15 in monsoon and winter season supporting *Vigna radiata* crop from nearby localities of Osmania University area, Bhadrachalam and Eturnagaram. The soil samples were collected with sterilized trovel upto 6.0 cm after scraping away of upper 0.5 inch soil. Healthy plants were pulled out and rhizosphere soil adhered to roots was also collected along with non-rhizosphere soil with sterilized trovel after scraping away of an inch of surface soil. These were brought to the laboratory in an insulated box and analysed for AM fungal numbers on the same day of collection along with other parameters.

Analysis of Soil Samples for Physico-chemical parameters

pH is one of the important parameter to determine the chemical nature of soil sample. 50 g of dried soil was mixed with 50 ml of water (1:1) thoroughly with the help of a glass rod, and then it was allowed to settle for some time. The pH of the supernatant was measured using pH meter, N, P, K, organic carbon , moisture contents and others were estimated as per Piper (1944) and Gangopadhyay and Das (1984).

Measurement of AM Fungal root colonization

The roots of *Vigna* were collected after 60 days washed to remove all the soil and dirt and preserved in FAA Farmalin acetic acid, prepared roots for observation. Root segments (1cm long) stained in 0.1 % trypan blue were mounted on glass slides, examined under microscope The presence or absence of AM colonization (Arbuscules/vesicles/hyphae) was scored (Giovanetti and Mosse, 1980). The percentage of root colonization was calculated using the following formula.

Isolation and spore count of AM fungi from Non-rhizosphere and rhizosphere soil

The isolation of spores of AM fungi was carried out by wet sieving and decanting method Gerdemann and Nicolson (1963) from 100 g rhizosphere and non-rhizosphere soil.
Whatsman filter paper No.1 with diameter of 11 cm was used for estimation of the spore count filter paper divided in to four equal quadrants. Vertical lines were drawn on each quadrant of the filter paper so as to divide it into approximately 16 columns about 1mm apart at the centre. Filter paper containing spores and debris was spread on a large petri-plate and observed under a binocular microscope. All spores present in each segment of the filter paper between number lines were counted by moving the petri-plate. Numerous spores were observed and intact spores were picked up using a fine needle and mounted in a drop of PVLG on a clean glass slide for identification as Gaur and Adholeya (1994) and Phillips and Heyman (1970).

Identification of AM Fungi

All arbuscular mycorrhizal fungal spores were mounted in PVLG and observed under trinocular research microscope (Vierheilig *et al.* 1998). The previously prepared permanent slides were stained using Lactophenol and spores were distinguished and later photographed with digital camera. The morphological characters such as spore size, shape, colour, number of wall layers, ornamentation, mantle on the spores, visible spore contents, shape of the subtending hyphal attachment, germination shield, soporiferous saccule etc. were observed under trinocular research microscope. These characteristics were compared with the characters given in the manual for identification of AM fungi by Schenck and Perez (1990).Separate keys were also used to identify species of each genus (Gerdemann and Trappe, 1974; Hall and Fish 1979; Mosse and Bowen, 1968, Nicolson and Gerdemann, 1978; Silva *et al.* 2005; Shubler and Walker, 2010; Trappe 1982; Wu, 1993).

Root Colonization

Inoculum productions: spores of *Rhizophagus*

fasciculatus were washed in sterilized water and 20 spores were added to 100 gr of sterilized soil in a small pot. Triplicates were maintaning. Inoculum was raised in such soils supporting maize plants. After 45 days the root bits and soil having spores was used as inculum Mosse (1962). Later the seeds *V.radiata* were put into such pots having AM fungal inoculum. Parameters like fresh weight and growth of shoot, root, leaves, pods and hight were estimated.

RESULTS AND DISCUSSION

AM fungal propagules and percentage of root colonization in rhizosphere and non-rhizosphere soil supporting *Vigna radiata* and percentage of root colonization are recorded after 60 days (Table 1). Rhizosphere soil samples collected during winter season have shown the presence of more AM fungal propagules supporting *Vigna radiata* in all the three places of collection, than normal soils. similarely root colonization was more in winter season rather than in monsoon. This clearly indicates that moisture plays an important role.

Further the Rhizosphere soil has supported more AM fungal propagules than non-rhizosphere soil, which may be due to influence of root exudates, age of the plant and soil conditios. pH range of 7.6 – 8.0 did not show any impact on AM fungal propagules and percentage of root colonization by the AM fungi, respectively indicating that pH may be a decisive factor. The physico-chemical factors of the soil samples collected in monsoon and winter season did not differ much and have been shown in Table 2. The data clearly indicates that the 'P' ranged from 31 to 52 Kg/hectare, thus falls under limited levels. However, the carbon content is around 0.5 to 1% all the soils investigated which are sandy loam soils. A total of 15 Arbuscular mycorrhizal fungi have been reported from both Non-rhizosphere and Rhizosphere soils, of which 11 have been reported only from rhizosphere soil (Table 3).

However soils from both Non-rhizosphere soil and rhizosphere soils supporting *Vigna* at OU campus have horboured 5 species while rhizosphere soil, Non-rhizosphere soils of Bhadrachalam and Eturnagaram have shown the presence of around 10-11 fungal species.

Pot culture experiment indicate that Mycorrhizal

Table 1: Physico-chemical properties of Rhizosphere soil of *Vigna* plant from three localities

Parameter	O.U Campus Hyderabad	Bhadrachalam	Eturnagaram
pH	7.64	7.62	8.45
Organic Carbon %	1.0	0.50	0.60
Nitrogen(N) kg/hectare	290	238	456
Phosphorous (P) kg/hectare	31	39	52
Potash (K_2O) kg/hectare	238	291.4	446.60
Water Holding Capacity	42	63	68
% of Moisture	6	7.6	8.5

Fig. 1 : *Vigna radiata* in the fields of study area

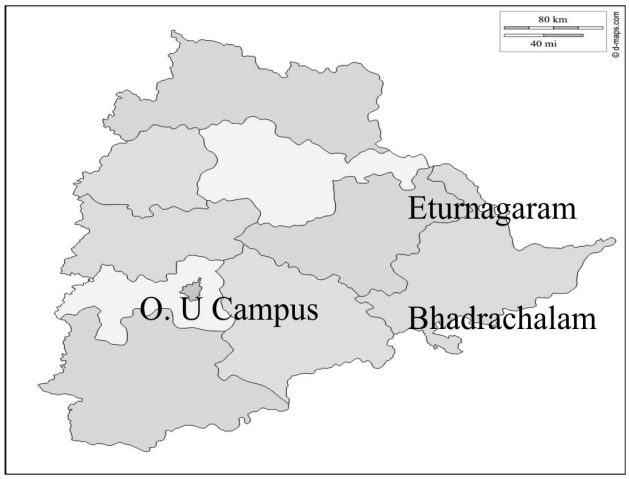

Fig. 2 : Sampling areas in Telangana

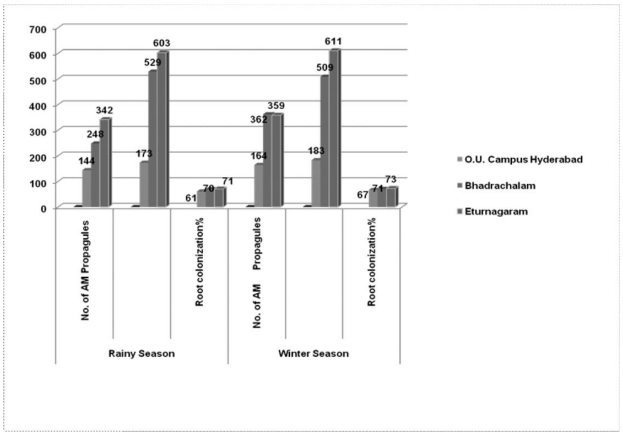

Fig. 3 : Number of AM propagules and percentage of root colonization

Table 2: Number of AM propagules and percentage of root colonization

Locality	Rainy Season No. of AM Propagules		Root colonization%	Winter Season No. of AM Propagules		Root colonization%
	NRS	RS		NRS	RS	
O.U. Campus Hyderabad	144	173	61	164	183	67
Bhadrachalam	248	520	70	362	509	71
Eturnagaram	342	603	71	359	611	73

Table 3: List of species of AM fungi reported from rhizosphere soil of *Vigna* from three localities

Name of Genus	O.U. Campus Hyderabad	Bhadrachalam	Eturnagaram
Acaulospora bireticulata F.M. Rothwell & Trappe	+	+	+
Acaulospora elegans Trappe and Gerdemann	-	+	-
*Acaulospora foveata*Trappe & Janos	+	-	+
Acaulospora laevis Gerd. & Trappe	-	+	+
Acaulospora nicolsonii C.Walker, L.E. Reed & F. E. Sanders	-	+	+
Acaulospora spinosa Walker and Trappe	-	+	+
Entrophospora infrequens (I.R. Hall) R.N. Ames & R.W. Schneid	-	+	-
Rhizophagus fasciculatus (Thaxt.) C.Walker & A. Schubler	+	+	+
Funneliformis caledonium (T.H. Nicolson & Gerd.) C.Walker & A. Schubler	+	+	+
Gigaspora rosea Nicolson and Schenck	-	+	+
Glomus macrocarpum Tul. & C.Tul.	_	+	-
Glomus botryoides Rothwell and Victor	-	-	+
Scutellospora arenicola Koske & Halvorson	-	-	+
Scutellospora minuta (Ferrer & R.A. Herrera) C. Walker & F.E. Sanders	-	+	-
Zygosporium rostratum (Giesenh) Bunting & E.W. Mason	+	-	+

Note: + =Present, - =Absent

Table 4: Growth rate of plant parts

Increasing level of plant parts	Control	Mycorrhizal Inoculated
Fresh weight of Shoot -gm	1.023	2.63
Fresh weight of Root -gm	0.132	0.437
Length of Root -cm	5.46	6.19
Length of Shoot -cm	21.9	26.37
Number of leaves	36	54
Number of Pods	8	15
Plant height -cm	25.1	29.3

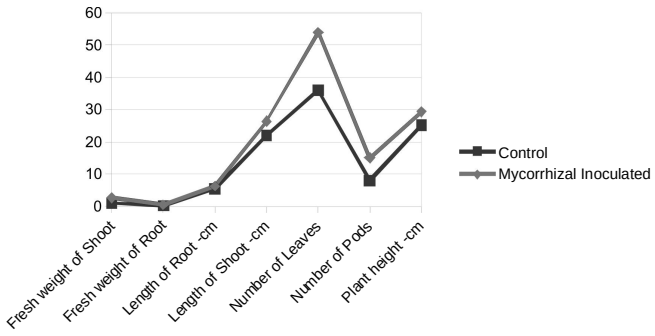

Fig. 4 : Growth rate of plant parts

inoculated 60 days old plants have shown more of press weight of shoot and root , increased root length, more number of leaves and plant height and yield than non inoculated control plants (Table 4). Similar results were obtained by Molla *et al.* (2010) while working on chick pea, cow pea, garden pea, ground nut and others. Further the role of mycorrhiza on growth of pulses has been eloberated by Arumugam *et al.* (2010), Bagayoko *et al.* (2000), Chandrashekar *et al.* (2014), Lalitha *et al. (*2011), Navnita *et al.* (2016), Pellegrino and Bedini (2014), Santhappan *et al.* (2015), Sujata and Sharma (2015), and Valsalakumar *et al.* (2007).

The present data clearly indicates the dependence of *Vigna radiata* on arbuscular Mycorrhizal fungi along with other beneficial microorganisms.

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