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Arbuscular Mycorrhizal Fungal Diversity of Rhizosphere Soil And Root Colonization In Some Plants of J. P. University Chapra, Campus Bihar

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Abstract

The present study was to analyse the occurrence of mycorrhizal diversity in 12 plants of J.P. University Campus ,Chapra. Arbuscular mycorrhizal fungal colonization ranged from 44.4 – 100 % . The highest infection was found in *Azadirachta indica A. Juss*. and lowest in *Ricinus communis* L. All the plant species had VAM fungal infections with hyphae, vesicles, arbuscular and hyphal coil of the total species. AM fungal spore population with a range of 10 – 155 in 100 g of rhizosphere soils was detected. The maximum spore population was observed in the species, *Clerodendrum infortunatum Linn. (* 155 / 100 gm of soil) and minimum in *Citrus lemo*n (L.) (10 / 100 gm of soil). Totally 25 AM fungal species were isolated which belongs to five genera (*Aculospora, Glomus, Scutellospora, Sclerocystis* and *Entrophospora*) and among them *Glomus* was dominant genera.

Keywords:- Arbuscular mycorrhiza, Root colonization, Soil nutrients ,Spore density.

Introduction

Arbuscular mycorrhizal (AM) fungi are the most ubiquitous occurring in all natural ecosystems in most climatic zones throughout the world. They play a crucial role in plant nutrient uptake, water relation, ecosystem establishment, plant diversity, and the productivity of plant (Parwaara et al. 2012). The mycorrhizal fungal association occur in more than 80% of plant species in the 90% of plant families (Wang et al. 2006). Mycorrhizal fungal colonization are present in angiosperm, gymnosperm, pteridophytes and some bryophytes (Smith et al. 1997). AM fungi are most important factor of rhizosphere microflora in natural ecosystem and play a main role in restoration of nutrient cycling in local ecosystem (Peterson et al. 1985). The major benefit of mycorrhiza is its greater soil exploration and increase in uptake of P, N, K, Zn, S, Fe, Mg, Ca and Mn (Sundar et al. 2010) . The AM fungal colonization not only improves the growth but also enhances the active principle content of the medicinal plants (Zubek et al. 2009) . Development of AMF may depend on the edaphic condition (He et al. 2002; Morammad et al. 2003) or climatic condition (He et al. 2002: Muthukumar et al. 2002). Recent findings suggest that adaptation of AMF to abiotic factors such as temperature and nutrient

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availability can strongly influence the effect of AMF symbiosis on plant growth (Johnson 2010; Antunes et at.2011). Sedges are among the primary colonizers of disturbed areas like mine spoil (Louis 1990), volcanic substrates (Gemma et al. 1990), and fallowland (Khun 1991), which are either devoid of or contain very little VAM. Mycorrhizal fungi are benefited with carbon substrates from plants and in turn the plants are provided with nutrients especially phosphorus compound from soil solution through the hyphal net work of the fungi apart from increased absorptive surface area of the roots (Janos 1980; Loyanachan 2000; Ravarkar et al. 2000). Any disturbance on this relationship may cause change in terms of decreased population status and diversity of these mycorrhizal fungi.When a soil is put to agricultural use it undergoes a series of physical, chemical and microbiological changes. One of the most important of which is the changes that affect the root- inhabiting microorganism and poor plant growth (Bellard 1994 ; Rolden et al. 1997). In tropical countries after many years of cropping yields become very low due to loss of soil fertility and it is common to abandon field as fallowland and appear to be most rational method to regenrate soil fertility (Duponnois et al. 2001 ; Greenland et al. 1959). The mycorrhizal fungi as one of the important biological component of soil have been reported to play important role in the regeneration of the abandoned forests because of their symbiotic association with the plant roots. Few horticultural crops and flowers have been used as host plants in several experimental tests as potential target plants for practical use of mycorrhizal inoculation (Chang 1994 ; Lovato et al. 1995 ; Sramek et al. 2000). Arbuscular mycorrhizal root colonization helps to induce earlier flowering and increase flower number in horticultural crops (Sohn et al. 2003 ; Nowak 2004 ; Gaur et al. 2005 ; Usha et al. 2005). Therefore, the aim of the present study was to examine the abundance of AMF in rhizospheric soil and with roots of fallowland plants growing in the Campus of J. P. University, Chapra.

Materials and methods

Study area

Chapra city is located 25.77 latitude and 84.72 longitude and it is situated at an elevation of 61 meter above sea level. It is in 25.77 ^{0}N and 84.72 ^{0} E. Chapra is situated on the bank of the Saryu river and the Gangas river flow parallel to the Saryu.

Collection of the samples

In the month of rainy season of (2015 – 2017) fine roots of plant growing in the campus of J. P. University were collected from different plants such as *Calotropis*, *Ziziphus*, *Croton*, *Parthenium* , *Dalbergia* etc . Roots were collected randomly from a depth of 0- 30 cm . After bringing these plants to laboratory the roots were seprated and analyzed when fresh.

Estimation of root colonization

Roots were washed throughly to remove attached soil particles. The cleaned roots were cut into 1cm long piece and were fixed in formalin acetic acid (FAA) according to the procedure described by Phillips and Hayman (1970) . The roots were boiled in 10% KOH for 1hr, acidified with 5N HCI and stained for 24 hr with 0.5 % tryphan blue. Each root was divided into 12 1cm long segments, which were then cleaned, stained and were arranged on slides. The slides were observed under compound microscope to score for any structures associated with mycorrhizal fungi like hyphae,

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vesicles, arbuscules, or hyphal coil in each segment. The percentage of AM fungal colonization was assessed by using the formula :

*** Percentage of colonization** = Number of root segments infected x 100
Total number of root segments observed

Spore separation and quantification

Separation of AM fungal spores from rhizospheric soil of each plant was done by using wet sieving and decanting method proposed by Gerdmann and Nicolson (1963) from the 100 gm of soil sample. Soil samples were collected randomly . All the samples were sieved (< 2mm mesh size) to remove stones, coarse roots and other litter, and fine roots were collected from each sample. The root soil mixture was vigorously mixed with a glass rod for 30 seconds. The suspension was passed through 250µm, 150µm, 98µm and 75µm seives. The material remaining on the sieve was washed into beakers. After settlement of the heavier particles , the supernants was filtered through gridded filter papers. Each filter paper was spread on to a glass plate and scanned under stereo microscope (Olympus SZ2-ILST). Intact and crushed spores were counted. AM fungal spores from the filter paper were picked up using a wet needle and mounted in Polyvinyl alcohal lactophenol (PVLG) on a glass slide and identified under a compound microscope (Olympus BX41) and photographed (Nikon eclipse 200). Identification was based on spore morphology and sub cellular characters (Schenck and Perez 1990).

Results and discussion

The present investigation is the first report for occurrence of arbuscular mycorrhizal fungal diversity in some plant specices of J. P. University Campus, Chapra Bihar. Physico – chemical properties of soil are presented in Table 1. Soils were basic (PH = 7.60) . Colonization was characterized by the presence of hyphae, arbuscules , vesicles and hyphal coils. AM fungal colonization varied with species and the situation of their occurrence. The vegetative plant species along with their AMF characterizations are noted in Table 2. Percentage colonization was maximal for *Azadirachta indica A. Juss*. (100 %) and minimal for *Ricinus communis L.* (44.4 %). Both Arum and Paris type morphologies were observed. Twenty five AM fungal species of five genera, viz, *Glomus* , *Acaulospora*, *Scutellospora*, *Sclerocystis* and *Entrophospora* were recovered from rhizosphere soils of study sites. *Glomus* (14 species) was the dominant genus followed by *Acaulospora* (8 species), *Scutellospora* (1 species), *Sclerocystis* (1 species) and *Entrophospora* (1 species) (Table 3) . *Glomus* species was recorded as dominant mycorrhizal genus (Nisha et al. 2010) . The highest spore density was recorded in the plant species of *Clerodendrum infortunatum Linn*. (155/ 100 gm of soil) belongs to the family *Lameaceae*. The lowest spore density recorded in *Citrus lemon (L.)* (10 / 100 gm of soil) belongs to the family *Rutaceae*. Since 12 plants of J.P. University of Chapra, Campus were analysed for the mycorrhizal density and diversity. The major population of *Glomus* species was recorded followed by *Aculospora*, *Entrophospora*, *Sclerocystis* and *Scutellospora* (Koul et al. 2012 ; Nisha et al. 2010 ; Parwaara et al. 2012; Peterson et al. 1985 ; Phillips et al. 1970 ; Porter et al. 1987 ;Rajkumar et al 2012) . The mycorrhizal association changed among various plant species and there

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was a significant difference in root colonization. In our study the maximum root infection was observed in *Azadirachta indica A. Juss* (100 %) belongs to the family *Meliaceae* and lowest root infection was recorded in *Ricinus communis L.* (44.4 %) belongs to the family *Euphorbiaceae.* This may be due to the fungal toxic substance present in root tissue that reduces the mycorrhizal association (Tester et al. 1987) . Newsham et al. (1995) suggested that overall functionally in AM communities remain fairly constant regardless of species variation. Variation in AM fungal root colonization and spore density was observed . AM fungal colonization is known to depend on soil moisture and phosphorus availability (Ruotsalainen et al. 2002; Wang et al. 2010) , and physiology , growth rate and turnover of plant roots (Lugo et al. 2003). Variation in spore density at the study sites might be due to environmental fluctuation playing a key role in influencing AM symbiosis . Zhao (1999) reported seasonality, edaphic factors , age of host plants and dormancy might be factors contributing to variation in spore density . Stutz et al. (2000) reported that *Glomus* species are known to be widely distributed and commonly found in different ecosystems and geographical regions.

Table 1 : Physico – Chemical properties of different rhizosphere soils of J. P. University Chapra.

Parameters	pH	EC (m mohs/ cm)	Organic carbon (%)	Macro and Micro nutrients						
				N (g/ kg)	P (g/kg)	K (g/kg)	Zn (ppm)	Cu (ppm)	Mn (ppm)	Fe (ppm)
	7.60	0.10	0.61	0.09	0.01	0.41	0.99	0.78	3.88	8.10
	Basic	Normal	Normal	Low	Normal	High	Normal	Normal	Normal	Normal

Table 2: Showing rhizosphere soil samples and root colonization of some plant species of J. P. University Chapra, Campus Bihar.

Table:- 2 AM association with roots and AM Spores present in rhizospheric soil.

Sr no	Plant name	Type of colonization	% Colonization	Spore density per 100 gm soil
1	*Calotropis gigantea (L.) Ait.*	H, V, A, HC	77.7 ± 20.9 ±12.10	78.33
2	*Citrus lemon (L.)*	H, V, A ,HC	97.2 ± 4.8 ± 2.8	10
3	*Croton sparciflorus (L.)*	H, V, A ,HC	72.2 ±4.84 ±2.8	113.33

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4	*Azadirachta indica A. Juss.*	H, V, A ,HC	100 ±0 ± 0	88.33
5	*Clerodendrum infortunatum Linn.*	H, V, A ,HC	52.7 ± 4.7 ± 2.7	155
6	*Ziziphus mauritiana Lamm.*	H, V, A	100 ± 0 ± 0	93.33
7	*Parthenium hysterophorus L.*	H, V, A ,HC	63.8 ± 4.7 ± 2.7	125
8	*Dalbergia sissoo Rox. Ex Dc.*	H, V, A	72.2 ± 4.8 ±2.8	73.33
9	*Phoenix dactylifera L.*	H, V, A ,HC	47.16 ± 17.3 ±10	38.33
10	*Lantana camara Var.*	H, V, A ,HC	63.8 ± 4.7 ± 2.7	48.33
11	*Ricinus communis L.*	H, V, A ,HC	44.4 ±4.84 ±2.8	86.66
12	*Psidium guajava L.*	H, V, A ,HC	94.4 ± 9.64 ± 5.5	128.33

H – Hyphae, A – Arbuscular, V – Vesicule, HC – Hyphal Coil

Table 3 : Showing the AM Fungal spores isolated from the rhizosphere soil samples of J. P. University Campus, chapra.

S no.	AM Fungal genra	AM Fungal species
1	*Aculospora*	*Aculospora spinosa, Aculospora denticulata, Aculospora laevis, Aculospora morrowiae, Aculospora scrobiculata, Aculospora spinosa, Aculospora undulata, A. (uni)- Aculospora (unidentified)*
2	*Entrophospora*	(unidentified)
3	*Glomus*	*Gloumus aggegatum, Glomus aureum, Glomus claroideum, Glomus clarum, Glomus dimorphicum, Glomus etunicatum, Glomus fasiculatum, Glomus geosporum, Glomus hoi, Glomus intraradices, Glomus mosseae, Glomus macrocarpum, Glomus multicaule, Glomus pustulatum*
4	*Scutellospora*	*Scutellospora reticulata*
5	*Sclerocystis*	*Sclerocystis rubiformis*

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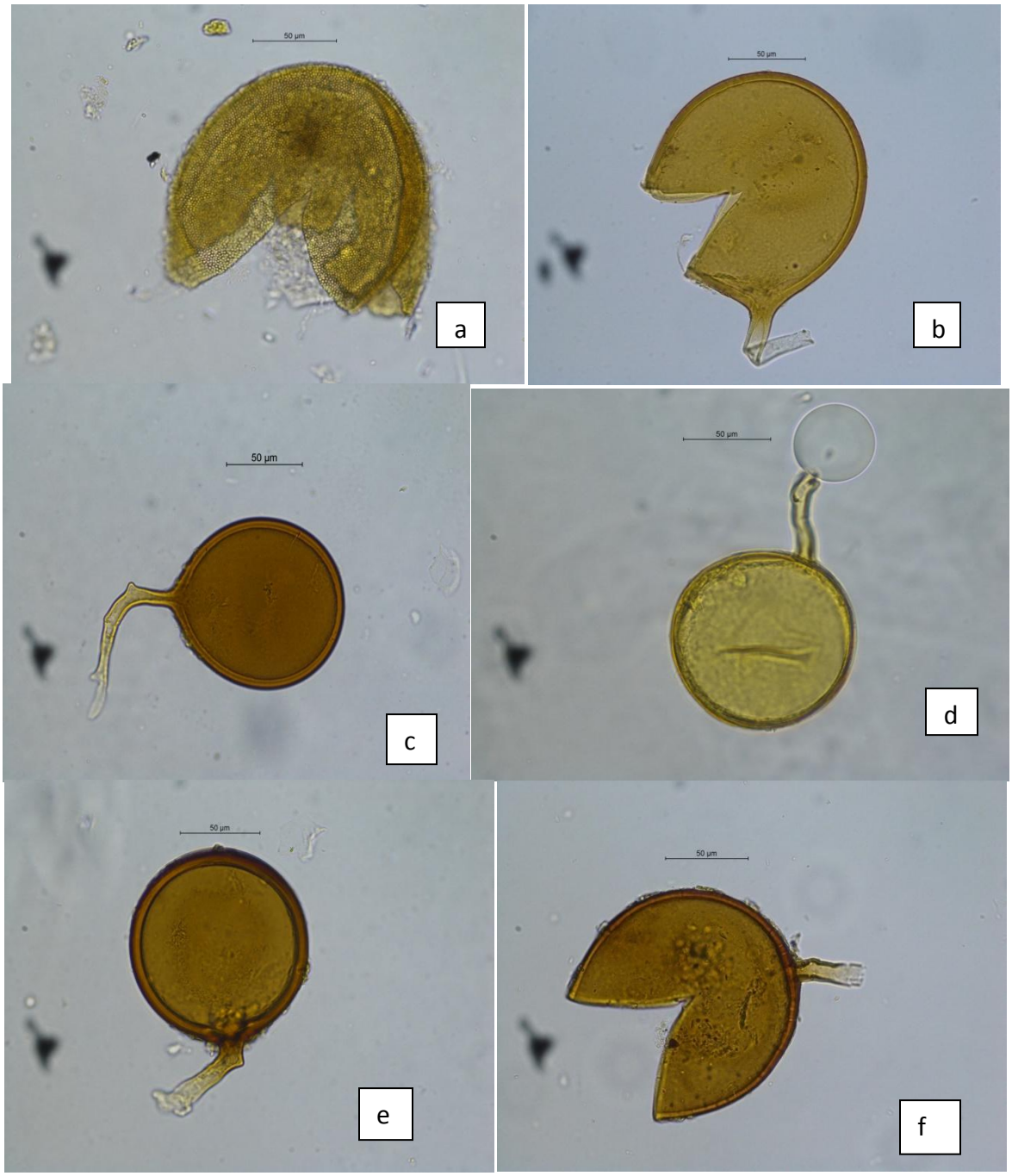

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Fig - a = *Aculospora spinosa* of *Ricinus communis L.*, b = *Glomus fasiculatum* of *Calotropis gigantea (L.) Ait.*, c = *Glomus aureum* of *Lantana camara Var.*, d = *Glomus claroideum* of *Parthenium hysterophorus L.* , E = *Glomus dimorphicum of Psidium guajava L.*, f = *Glomus fasiculatum of Ricinus communis L.*

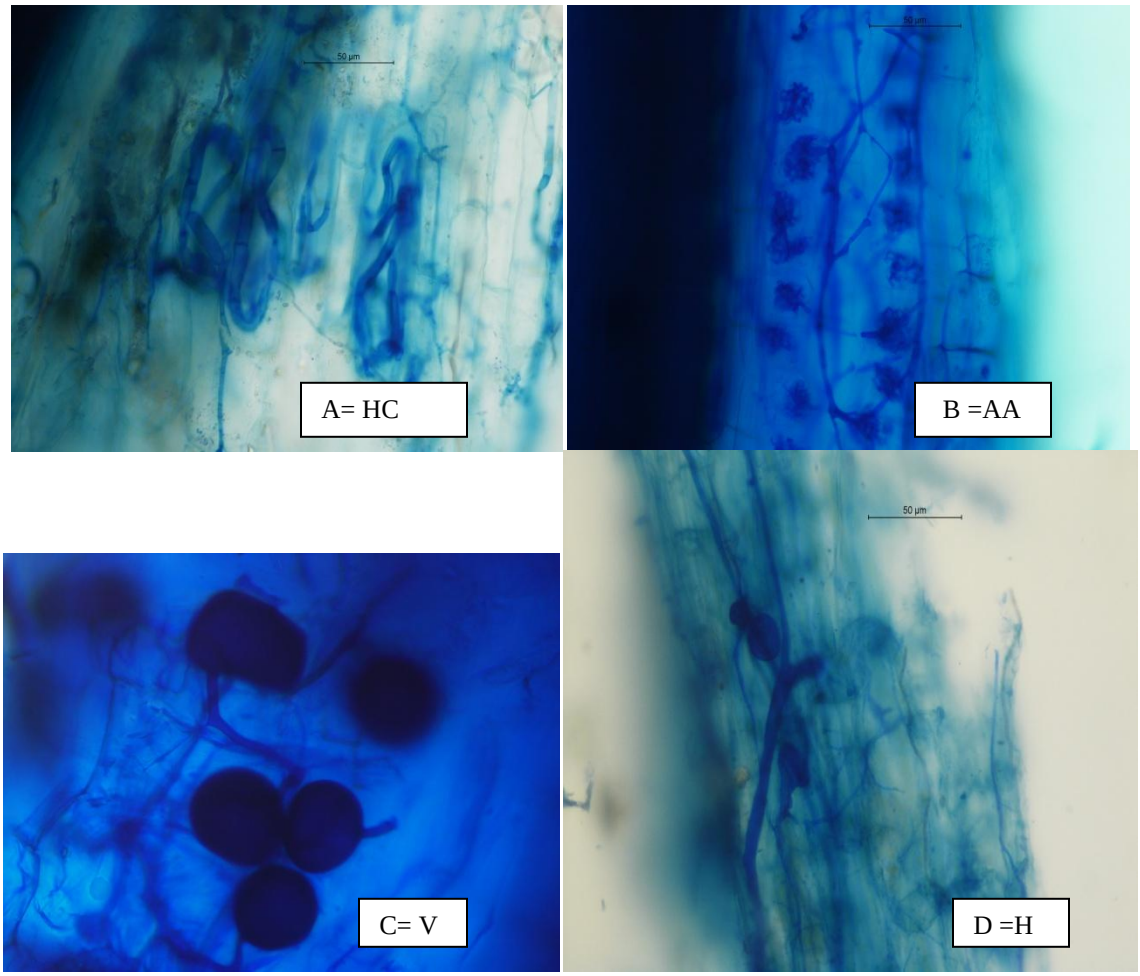

Fig - A= *Calotropis gigantea* (L.) hyphal coil (HC), B= *Parthenium hysterophorus L.*Arum arbuscules (AA), C = *Lantana camara* Var. Vesicles (V), D= hyphae (H)

In the present study , the composition and diversity of the AM fungi composition were described based on morphological species. The results indicated that *Glomus* was the dominated genus , followed by *Aculospora* . *Aculospora and Glomus* species usually produce more spores than *Scutellospora, Entrophospora*, and *Sclerocystis* species in the same environment (Bever et al. 1996 ; Suresh et al 2010). This may be explained by the difference in development. *Aculospora* and *Glomus*

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Index in Cosmos
March 2019 Volume 9 Number 3
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species are thought to require less time to produce spores than *Scutellospora*, *Entrophospora*, and *Sclerocystis*.Furthermore, membes of the *Gigasporaceae* typically establish an extensive mycellium in soil and produce fewer spores than those of the *Aculosporaceae and Glomaceae* (Hart et al. 2002 ; Piotrowski et al. 2004) . The result showed a strong symbiotic relationship between some plants and AM fungi, but significant differences were observed in the different plant species. As the studies have shown non random differences in distribution among different AM fungi species and genera in the field it is also likely that the preferances of different AM fungi for different host plants in our study might be reflected at the species or family level (Helgason et al 2002 ; Husband et al. 2002) . All 12 plants were reflected by AM fungi, but the degree of colonization and the spore density varied among plant species. This may be due to differences in the ability of AM species to sporulate (Turnau et al. 2001). Many AM species AM fungal spore density have been positively correlated with organic matter. Organic matter could enhance spore production (Douds et al.1997), extra radical proliferation of hyphae (Joner et al. 1995) , and improve AM colonization (Mouratov et al. 2002) . In addition, AM fungal hyphae grew best in soils with a high amount of organic matter (Frey et al. 1997). Soil pH causes sporulation , spore gremination (Wang et al. 1993), hyphal growth and root colonization (Medeiros et al. 1994) and reproduction and communitry structure of AM fungi (An et al. 2008) . From the research, we could conclude that the biodiversity of AM fungi was abundant , though *Glomus* was the dominant genus. The degree of colonization and spore density varied markedly among plant species. The reduction in AM fungal spore population with increasing soil depth can also be explained by fewer roots in deeper soil layer (Cuenca and Lovera 2010). AM fungal abundance was very sensetive to change in pH , the sporulation and germination of some AM fungal species decreased in alkaline soils. The availability of plant nutrients achieve the maximum amount in a pH range between 6.0 and 7.5 for most plants and an increase of soil pH value could limit the availability of nutrients (Asghar et al. 2008). According to Shi et al. (2007) , VAM is more likely to form spores when the condition prevails or host vegetation is stressed or disturbed. Padma and Kandaswamy (1990) recorded that nearly 80 % - 85% of P is made unavailable to plants due to fixation and immobilization. The root hair of the studied vegetative species was small and poorly developed. This feature is known to create potential plant mycotrophy, enhancing nutrient acquisition in stressed environments (Baylis 1975). AM association have been critical important in the evolution and sucession of land plants where plants have adopted to grow in soils of low nutrient status. Analysis of data revealed that N content of soil did not have any influence on either spore density in rhizosphere or root colonization by AMF. This is in contrast to Sharma et al. (2009) and Ghorbani et al. (2012) who observed comparatevely high AMF spore population supported by higher available N in soil. Koomen et al. (1987) reported that most AMF tested preferred a near neutral pH. Although Zubek et al. (2009) did not find a close correlation between AMF species richness and soil pH , they found the highest diversity in calcareous substrata. Moreover, higher P contents have been found have a negative impact on AMF colonbization in some cases (Duan et al. 2010 ; Entz et al. 2004), though not in other (Vosatka 1995 ; Ryan and Ash 1999; Zubek et al. 2012b). This shows that *Glomus* have a fairly high level of adaptation to a various environmental conditions (Puspitasari et al. 2012). When the soil pH , P and organic C increases the number and type of VAM will increase (Muzakir, 2011). AM fungi

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Index in Cosmos
March 2019 Volume 9 Number 3
UGC Approved Journal

are a great component of soil microbial biomass. This symbiosis benefits plant growth , particularly by enhancing phosphorus , water and mineral nutrient uptake (Lie et al. 1991). The hyphae of AM fungi play an important role in the formation and stability of soil aggregates and contribute to the composition of plants community structures (Smith and Read 1997). In the present work , there were large numbers of hyphae associated with plant roots. The VAM association were more in rainy season because roots showed better growth during this season and active root growth provided more entry points to VAM fungi (Bhaskaran and Selvaraj 1997; Allen et al. 1998).

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Index in Cosmos
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Index in Cosmos
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Index in Cosmos
March 2019 Volume 9 Number 3
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