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Research Article

Diversity of AM fungi in certain ornamental plants growing at different sites of Allahabad, Uttar Pradesh, India.

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Abstract: Use of flowers in Indian cultural and religious purposes has a central place in people's daily lifestyle. In India, increasing demand and supply of various types of flowers opened a vast scope for the production of flowers. In the present study, five important ornamental plants were collected from five different sites of Allahabad, Uttar Pradesh for the survey purpose to investigate arbuscular mycorrhizal status and diversity. All the ornamental plant species were found to be mycorrhizal but the magnitude of infection varied with the sites, plants species as well as stages of plant growth. A total of 38 species belonging to four different genera of AM fungi were isolated. *Glomus* was recorded as the most dominant genus with 22 species, followed by *Acaulospora* with 14 species, sporocarp of *Sclerocystis sinuosa* and *Scutellospora* with a single unidentified species. Study of Shannon Weiner diversity index, Species richness and Species evenness also carried out in all the five sites. Diversity of all the sites showed less variation indicating a stable and a diverse fungal community.

Key words: AM fungi, Diversity, *Glomus*, Ornamental plants

Introduction

Ornamental plants make a harmonious relationship between people and nature by associating the beauty and utility and make the environment beautiful and refreshing. They have a very significant economic and emotional value by displaying their lovely flowers. Flowers are one of nature's most gorgeous gifts to people. In Indian civilization use of flowers have a central place in people life as they have the language of their own. Flowers provide an everlasting impression to people by conveying different feelings and thoughts and are inseparable from the social fabric of people life. So, the international trade of floriculture getting boom day by day and there is a large demand of cut-flowers in global trade. Some major cut flowers of Indian and global markets are marigold, rose, chrysanthemum, gladiolus, tulip, carnation, orchids, etc. These ornamental plants have received an impetus worldwide. So, the growth improvement and flower production are desirable. But the cultivation of cut flowers at the commercial level is very costly for the growers because it requires high doses of fertilizers and heavy irrigation. Chemical fertilizer plays an essential role however, at the same time it also causes a set of environmental pollution and degradation of the natural environment.

In view of the above facts, there is need to focus on the potential use of biological tools such as Arbuscular Mycorrhizal (AM) fungi and plant associations to improve quality and quantity of plant resources. The microbial population are the key component of soil plant system as they ensure the adequate level of production and improve the plant growth by providing the enhanced P uptake and other essentials nutrients. About 80% of terrestrial plant species are known to form AM symbiosis. They pay a significant contribution to shaping the plant community structure through the enhanced supply of minerals and water.

To explore the potentiality of AM fungi in improving the production of cut flowers it is essential to know their mycorrhizal status and host plants dependency on them. In this regard, the present work has been undertaken to study the mycorrhization, diversity, species richness and evenness of AM fungi associated with some ornamental plants cultivated at different sites of Allahabad.

Materials and Methods

Sample Collection

For the isolation of AMF spores, during each sampling 100g soil was collected randomly in sterile polythene bags from each plant growing site. Root samples of the plants were also collected for the estimation of mycorrhizal infection. A set of five individuals per plant species was collected and mixed. Samples were brought back to the laboratory and the roots of the plants along with the fine roots present in the rhizospheric soils were washed with tap water and processed for the determination of root colonization. Soil samples were air dried in

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shade at room temperature and sieved for the estimation of AMF spore population and diversity.

Analysis of soil samples

Collected soil samples were air dried, sieved and packed in a polybag. Estimation of physico-chemical properties like EC, pH, phosphorus, potassium, nitrogen and organic carbon were done from Motilal Nehru Farmers Training Institute, IFFCO Phulpur, Allahabad, Uttar Pradesh. Soil characteristics of different sites are presented in **Table 1**.

Determination of AMF Spore Population

AMF spore population was determined in 10 g air dried soil in triplicates for each sample by wet sieving and decanting method (Gerdemann and Nicolson, 1963). Soil solution was passed through the sieves of 500 µm, 210 µm, 150 µm, 90 µm and 60 µm in descending order. AMF spores were transferred on filter papers, which were counted under a stereoscopic binocular. Number of spores were expressed as the mean of three replicates.

Identification of AM Fungi

AMF spores were mounted in polyvinyl lactoglycerol (PVLG) and PVLG + Melzer's reagent (1:1 v/v) and identified up to the species level using the synoptic keys of Trappe (1982), Schenck and Perez (1987) and INVAM species guide (http;//invam.wvu.edu).

Assessment of AM fungal Colonization in the Roots

Intensity of AM colonization in the root samples was determined by the method of Phillips and Hayman (1970). For the quantification of AM colonization, 100 root bits were mounted on slides (10 per slide) and examined under a compound microscope (CH20*i*, Olympus). Mycorrhizal intensity in the roots was expressed in the terms of percent root bits infected and was calculated as follows:

$$\text{Mycorrhizal intensity} = \frac{\text{Number of root bits infected}}{\text{Total number of root bits examined}} \times 100$$

Frequency, Abundance, Density, Diversity, Richness and Evenness of AM Fungi species

Species frequency, abundance, density, diversity, richness and evenness of AM fungi were expressed as follows:

$$\text{Frequency} = \frac{\text{Number of sites/plants at which AMF sp. occurred at once X 100}}{\text{Total number of sites/plants}}$$

$$\text{Abundance} = \frac{\text{Total number of AMF sp. at all sites/plants}}{\text{Number of sites/plants where the AMF sp. occurred at once}}$$

$$\text{Density} = \frac{\text{Total number of AMF sp. at all sites/plants}}{\text{Total number of sites/plants}}$$

AMF species diversity was calculated by following formula:

Shannon-Weiner diversity index = $- \sum_{i=1}^{s} (Pi \ln Pi)$

Pi = proportion of individual of species i

Species richness is calculated by Margalef's indices of richness = (S-1)/ln N

Species evenness is calculated by

$$\text{Pielou's index} = \frac{H'}{H'max}$$

H´ = Shannon-Weiner diversity index

H´max = maximum Shannon-Weiner diversity index

Results and Discussion

The arbuscular mycorrhizal status in terms of mycorrhizal infection in roots and spore population in the rhizospheric soils were expressed. The root and soil samples of five ornamental plants *viz*., *Tagetes erecta* L., *Calendula officinalis* L., *Aster amellus* L., *Dahlia pinnata* Cav. and *Rosa indica* L. belonging to two different familes *i.e.* Asteraceae and Rosaceae were collected at different stages of growth *viz*., vegetative, budding and flowering from different ornamental sites of Allahabad. These sites were Roxburgh Botanical garden, Company garden, Anand Bhawan campus, Sam Higginbottom Institute of Agriculture, Technology and Sciences (SHIATS) campus and Jhunsi nursery **(Plate-1)**.

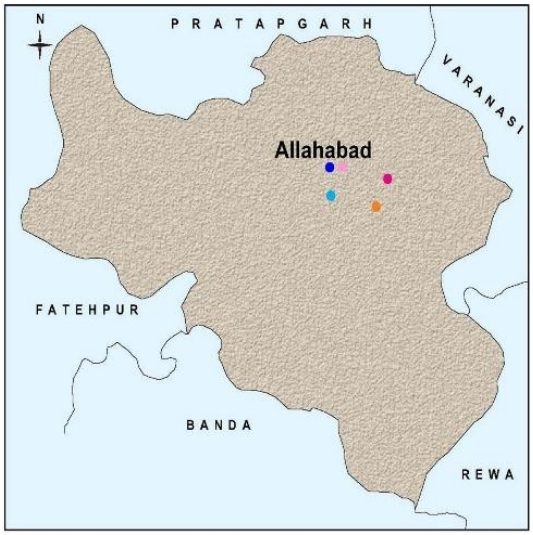

Plate 1. Map of Allahabad Showing Survey Sites

- Roxburgh Botanical Garden
- Anand Bhavan
- Company Garden
- SHIATS
- Jhunsi Nursery

All the ornamental plants showed mycorrhizal infection in their roots. However, the magnitude of infection and spore population varied with the sites, plant species and stages of plant growth. Minimum root bit infection was recorded at the vegetative stage while maximum at flowering stage. The average range of infection varied from 19.3% to 55.7%. Maximum infection was recorded in Marigold at SHIATS while minimum in Rose at Jhunsi nursery **(Fig. 1 and Plate 2)**.

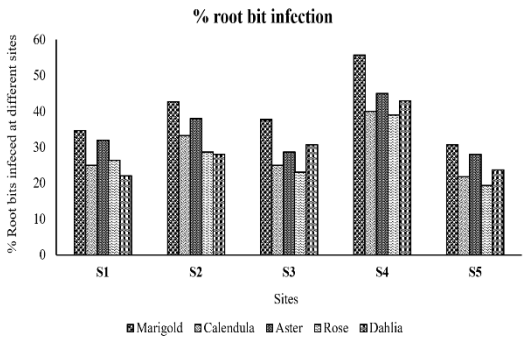

Figure 1. Showing % root bits infected in different ornamental plants from different ornamental sites

S1- Roxburgh Botanical Garden
S2- Company Garden
S3- Anand Bhawan Campus
S4- SHIATS
S5- Jhunsi Nursery

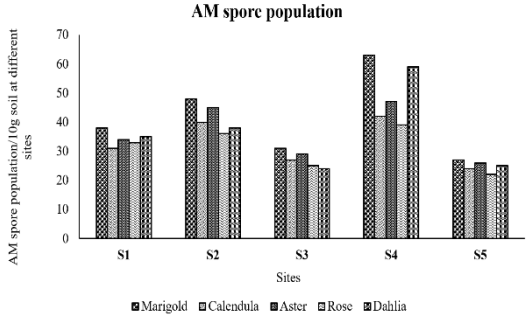

Figure 2. Showing AMF spore population in different ornamental plants from different ornamental sites

S1- Roxburgh Botanical Garden
S2- Company Garden
S3- Anand Bhawan Campus
S4- SHIATS
S5- Jhunsi Nursery

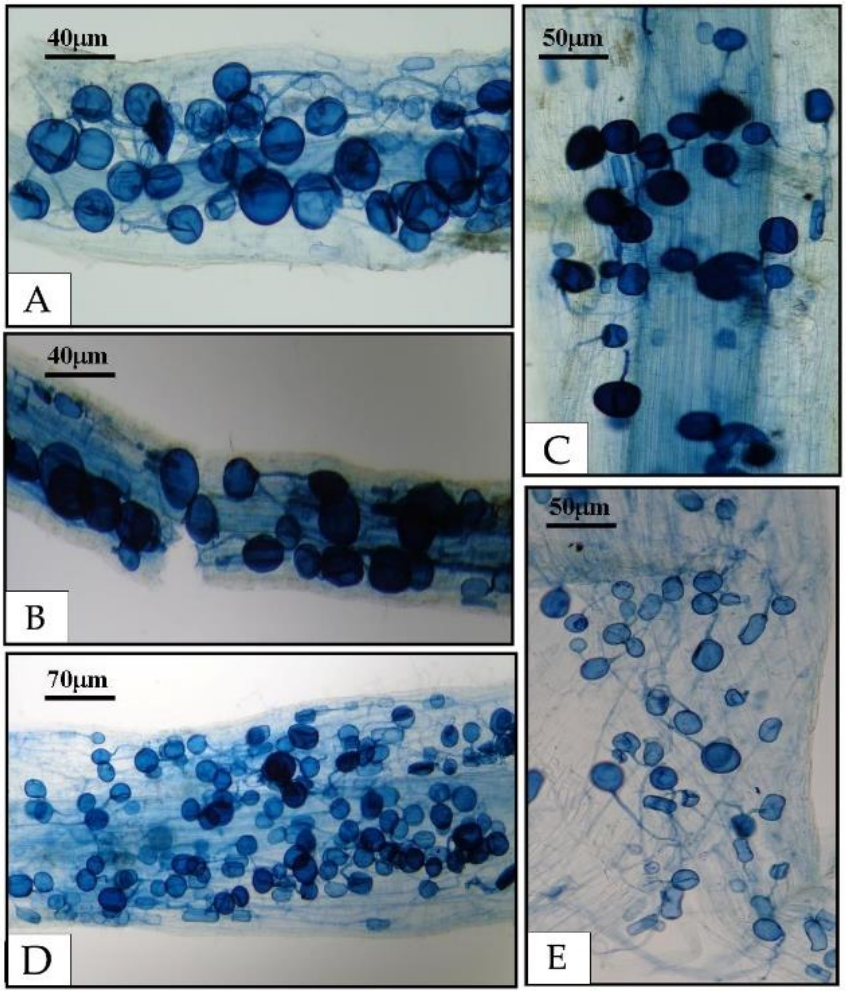

Plate 2. Root bits infection in the roots of ornamental plants collected from different sites of Allahabad, Uttar Pradesh.

A- Roxburgh Botanical Garden
B- Company Garden
C- Anand Bhavan Campus
D- SHIATS
E- Jhunsi Nursery

In the same way, the range of AMF spore population varied from 22.0 to 63.0 spores/10g air dried soil. Minimum spore population was recorded at vegetative stage while the maximum at flowering stage. Maximum spore population was recorded in Marigold at SHIATS while minimum in Rose at Jhunsi nursery **(Fig. 2 and Plate 3)**

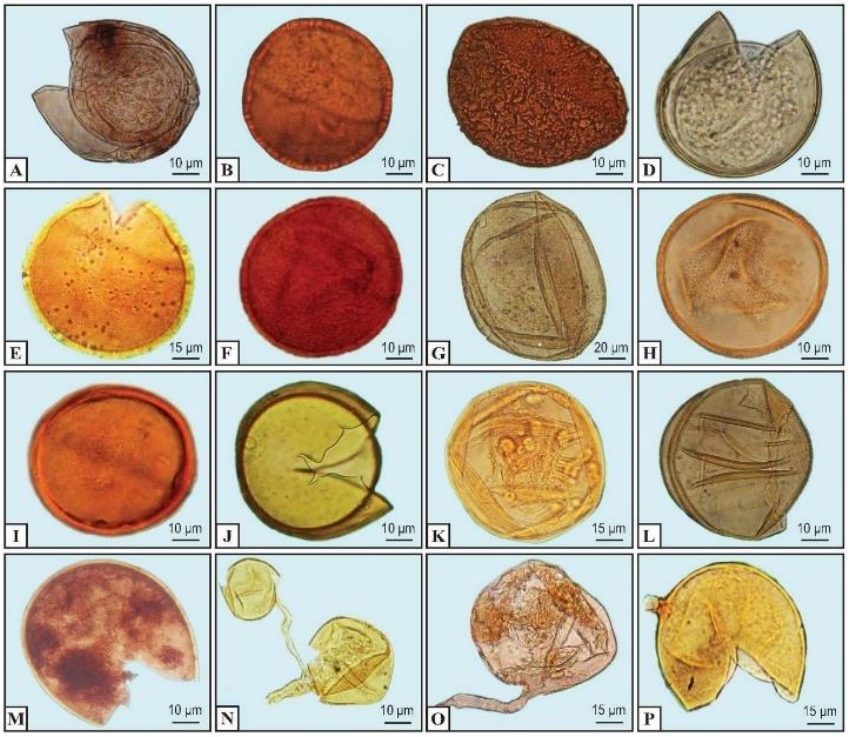

Plate 3. AM spores isolated from the rhizospheric soil of ornamental plants of different sites:
A. *Acaulospora colombiana* (Spain and Schenck) Kaonongbua, Morton and Bever **B.** *Acaulospora denticulata* Sieverding and Toro **C.** *Acaulospora foveata* Trappe and Janos **D.** *Acaulospora delicata* Walker, Pfeiffer and Bloss **E.** *Acaulospora lacunose* Morton **F.** *Acaulospora laevis* Gerdemann and Trappe **G.** *Acaulospora longula* Spain and Schenck **H.** *Acaulospora scrobiculata* Trappe **I.** *Acaulospora trappei* Ames and Linderman **J.** *Acaulospora* sp.1 **K.** *Acaulospora* sp.2 **L.** *Acaulospora* sp. 3 **M.** *Acaulospora* sp. 4 **N.** *Glomus aggregatum* Schenck and Smith emend. Koske **O.** *Glomus albidum* Walker and Rhodes **P.** *Glomus ambisporum* Smith and Schenck

Diversity, Density, Frequency, Abundance, Species richness and Species evenness at different survey sites of Allahabad

A variety of AMF spores were recovered from the rhizospheric soils of the plants from different survey sites and identified as mentioned above. A total of 38 species belonging to four different genera of AM fungi were isolated as the most dominant forms associated with the ornamental plants. *Glomus* was recorded as the most dominant genus with 22 species, *viz. G. aggregatum* Schenck and Smith emend. Koske, *G. albidum* Walker and Rhodes, *G. ambisporum* Smith and Schenck, *G. caledonium* (Nicolson and Gerd.) Trappe and Gerdemann, *G. claroideum* Schenck and Smith, *G. constrictum* Trappe, *G. deserticola* Trappe, Bloss and Menge, *G. fasciculatum* (Thaxt.) Gerdemann and Trappe, *G. fuegianum* (Speg.) Trappe and Gerdemann, *G. geosporum* (Nicolson and Gerd.) Walker, *G. gerdmanii* Rose, Daniels and Trappe, *G. intraradices* Schenck and Smith, *G. macrocarpum* Tul. and C. Tul., *G. mosseae* (Nicolson and Gerd.) Gerdemann and Trappe, *G. multicaule* Gerdemann and Bakshi, *G. occultum* Walker, *G. tortuosum* Schenck and Smith and five unidentified named *Glomus* sp.1 to 5 followed by *Acaulospora* with 14 species *viz. A. colombiana* (Spain and Schenck) Kaonongbua, Morton and Bever, *A. delicate* Walker, Pfeiffer and Bloss, *A. denticulata* Sieverding and Toro, *A. foveata* Trappe and Janos, *A. lacunosa* Morton, *A. laevis* Gerdemann and Trappe *A. longula* Spain and Schenck, *A. scrobiculata* Trappe, *A. trappei* Ames and Linderman, *A. tuberculata* Janos and Trappe and four unidentified named *Acaulospora* sp.1 to 4, sporocarp of *Sclerocystis sinuosa* Gerd. and Bakshi and *Scutellospora* with one unidentified species named *Scutellospora* sp. **(Table-2)**.

Table 1. Edaphic features of the soils of different sites of Allahabad included in the survey

Sites	Soil texture	pH	EC (m.mhos.cm^{-1})	Organic Carbon (%)	Nitrogen (%)	Phosphorus (Kg/ha)	Potassium (Kg/ha)
Anand Bhawan Campus	loam	7.8	1.53	0.69	0.57	33.00	242.0
Company Garden	loam	7.2	0.55	0.74	0.53	21.00	249.0
Jhunsi Nursery	loam	7.6	0.77	0.43	0.38	31.00	253.0
Roxburgh Botanical Garden	loam	7.6	0.74	0.56	0.35	26.00	247.0
SHIATS Campus	loam	7.4	0.42	0.97	0.41	25.00	239.0

Table 2. Diversity, density, frequency, abundance, species richness and species evenness of dominant AM fungi collected from different survey sites of Allahabad

AM Fungi/Survey Sites	AM Fungi Diversity							
	S1	**S2**	**S3**	**S4**	**S5**	**D**	**F**	**A**
Acaulospora colombiana (Spain and Schenck) Kaonongbua, Morton and Bever	-	-	1	-	-	0.2	20	1
Acaulospora delicata Walker, Pfeiffer and Bloss	-	-	1	-	2	0.6	40	1.5
Acaulospora denticulata Sieverding and Toro	1	1	-	-	-	0.4	40	1
Acaulospora foveata Trappe and Janos	4	-	1	3	-	1.6	60	2.7
Acaulospora lacunosa Morton	-	1	2	1	1	1	80	1.25
Acaulospora laevis Gerdemann and Trappe	-	3	-	6	2	2.2	60	3.6
Acaulospora longula Spain and Schenck	-	3	-	-	-	0.2	20	3
Acaulospora scrobiculata Trappe	1	2	-	-	-	0.6	40	1.5
Acaulospora trappei Ames and Linderman	3	5	2	3	-	2.6	80	3.25
Acaulospora tuberculata Janos and Trappe	-	-	-	5	-	1	20	5
Acaulospora sp.1	2	1	-	-	-	0.6	40	1.5
Acaulospora sp.2	-	-	-	3	1	0.8	40	2
Acaulospora sp.3	1	-	1	-	1	0.6	60	1
Acaulospora sp.4	-	1	-	-	-	0.2	20	1
Glomus aggregatum Schenck and Smith emend. Koske	4	1	-	-	-	1	40	2.5
Glomus albidum Walker and Rhodes	-	-	2	-	-	0.4	20	2
Glomus ambisporum Smith and Schenck	-	2	-	-	-	0.4	20	2
Glomus caledonium (Nicolson and Gerd.) Trappe and Gerdemann	-	1	-	3	-	0.4	40	2
Glomus claroideum Schenck and Smith	2	-	-	1	-	0.6	40	1.5
Glomus constrictum Trappe	-	2	-	3	3	1.6	60	2.6
Glomus deserticola Trappe, Bloss and Menge	-	-	3	-	1	0.8	40	2
Glomus fasciculatum (Thaxt.) Gerdemann and Trappe	2	3	-	7	-	2.4	60	4
Glomus fuegianum (Speg.) Trappe and Gerdemann	-	-	1	-	-	0.2	20	1
Glomus geosporum (Nicolson and Gerd.) Walker	1	-	-	-	1	0.4	40	1
Glomus gerdmanii Rose, Daniels and Trappe	1	-	-	-	-	0.2	20	1
Glomus intraradices Schenck and Smith	-	1	2	4	-	1.4	60	2.3
Glomus macrocarpum Tul. and C. Tul.	-	1	-	-	-	0.2	20	1
Glomus mosseae (Nicolson and Gerd.) Gerdemann and Trappe	4	3	2	8	-	3.4	80	4.25
Glomus multicaule Gerdemann and Bakshi	-	-	-	1	1	0.4	40	1
Glomus occultum Walker	1	-	-	1	2	0.8	60	1.3
Glomus tortuosum Schenck and Smith	2	3	-	4	-	1.8	60	3
Glomus sp.1	-	1	-	3	-	0.8	40	2
Glomus sp.2	-	-	2	-	1	0.6	40	1.5
Glomus sp.3	2	-	-	-	-	0.4	20	2
Glomus sp.4	-	-	-	1	2	0.6	40	1.5
Glomus sp.5	1	-	-	-	-	0.2	20	1
Sclerocystis sinuosa Gerd. and Bakshi	-	-	-	2	-	0.4	20	2
Scutellospora sp.	-	-	1	2	-	0.6	40	1.5
Total no. of AMF spores	32	35	21	61	18			
Shanon diversity index	2.63	2.74	2.49	2.76	2.40			
Species richness	4.33	4.78	3.94	4.38	3.81			
Species evenness	0.86	0.86	0.93	0.83	0.92			

D- Density **F**- Frequency **A-** Abundance
S1- Roxburgh Botanical Garden; **S2**- Company Garden; **S3**- Anand Bhawan Campus; **S4**- SHIATS; **S5**- Jhunsi Nursery

Density of *G. mosseae* was recorded to be maximum (3.4) while minimum of *A. colombiana*, *A. longula, Acaulospora* sp.4, *G. fuegianum, G. gerdmanii, G. macrocarpum* and *Glomus* sp. 5 (0.2). The frequency of AMF spores was found to be ranged from 20 to 80. The abundance of AMF spores was also recorded and found to be ranged from 1.0 to 4.25. Shannon-Weiner diversity index of AMF spores at different sites was analyzed and found to be maximum at SHIATS (2.76) and minimum at Jhunsi nursery (2.40) **(Fig. 3)**. AMF species richness at different sites was found to be ranged from 3.81 to 4.78 **(Fig. 4)** while the Pielou's index of AMF species evenness was found to be ranged from 0.83 to 0.93 **(Fig. 5) (Table-2)**.

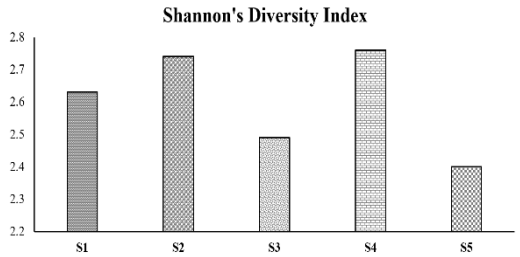

Figure 3. Showing Shannon's Diversity Index of AM fungi at different sites
S1- Roxburgh Botanical Garden
S2- Company Garden
S3- Anand Bhawan Campus
S4- SHIATS
S5- Jhunsi Nursery

AM fungi can be recognized as 'keystone mutualists' (Kumar *et al.*, 2010). In all terrestrial ecosystem, about 80% of plant taxa develop AM symbiosis

between plant roots and soil fungi (Smith and Read, 2008). Almost all the families of Angiosperms are reported to be colonized by AM fungi (Malloch *et al.*, 1980). Though they show little or no host specificity to the plant species (Ahulu *et al.*, 2007; Opik *et al.*, 2009; Santos-Gonzalez *et al.*, 2007) but from place to place their occurrence and distribution is diverse and varies.

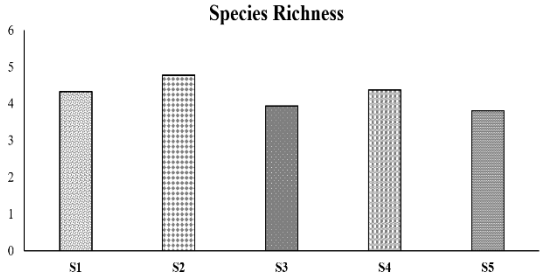

Figure 4. Showing Species Richness of AM fungi at different sites
S1- Roxburgh Botanical Garden
S2- Company Garden
S3- Anand Bhawan Campus
S4- SHIATS
S5- Jhunsi Nursery

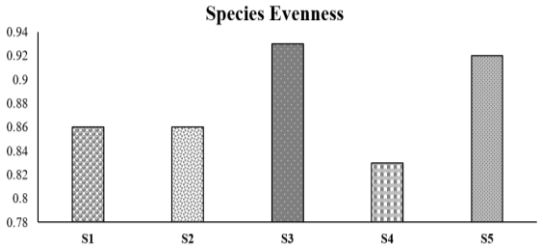

Figure 5. Showing Species Evenness of AM fungi at different sites
S1- Roxburgh Botanical Garden
S2- Company Garden
S3- Anand Bhawan Campus
S4- SHIATS
S5- Jhunsi Nursery

Mycorrhizal Status and Sites

At various surveyed sites, there were significant differences in spore population and mycorrhizal intensity in the roots. All the surveyed plants were colonized by AM fungi but the magnitude of colonization varied with the taxa as well as site. Maximum mycorrhization was recorded at SHIATS campus and minimum in Jhunsi nursery site.

Soil status of all the survey sites under study showed substantial variations in their pH, electrical conductivity as well as nitrogen, phosphorus, potassium and organic carbon contents. These fluctuations in soils might have been responsible for the variations in the mycorrhizal status of the ornamental plants at different sites.

Several workers reported the AM dependency on physico-chemical properties of soils such as pH (Read *et al.*, 1976; Raman and Sambandan, 1998), EC, organic carbon (Raman and Sambandan, 1998), total nitrogen (Beena *et al.*, 2000), phosphorus (Selvaraj *et al.*, 2001) and potassium levels (Schalamuk *et al.*, 2006). A negative relation was observed between soil phosphorus and AMF spores (Janaki and Manoharachary, 1994). High P in soil reduced the root colonization, spore production and hyphal growth (Abbot and Robson, 1991; Covacevich *et al.*, 2007). These reports are in conformity with the present findings.

Mycorrhizal status with different Ornamental Plants

The ornamental plants which are included in the survey were found to be colonized by AM fungi but the status of mycorrhization varied with the different ornamental plant species. Maximum AMF spore population and mycorrhizal association were recorded in marigold and minimum in rose. Many other workers also reported the AM fungal colonization in ornamental plants (Rekha *et al.*, 1987; Kumar *et al.*, 2012). Although AM fungi show little or no host specificity. It was found that some plant species have higher response to AM fungi while others are less responsive (Bagyaraj, 2011; Bagyaraj *et al.*, 2015). Various factors are responsible for AM fungi community composition that alter the soil and rhizospheric atmosphere including root exudates pattern, plant host morphology and supply of C to fungus in roots. Root exudates quality and quantity and the surface of root affect the number of infection sites and susceptibility of the roots to endophyte penetration. For AM symbiosis these factors might be stimulatory or inhibitory in nature and might be the cause of differential mycorrhizal status of diverse ornamental plants.

Mycorrhizal Status and Stages of Plant Growth

Colonization in the roots of the different plant species varied with the type of AMF propagules as well as the stages of plant growth *viz.*, vegetative, budding and flowering. Maximum AMF spore population and % root bit infection were recorded at flowering stage while minimum at vegetative stage in ornamental plants. Javaid *et al.* (2007) and Kumar *et al.* (2012) also reported maximum mycorrhization at flowering stage and minimum at seedling stage in the ornamental plants. Our findings also showed the similar trends.

Various factors are responsible for the development of infection and spore production. Besides these, the physiology and nutrient status of the host, root susceptibility to the penetration by the symbiont and root exudation pattern are the prominent ones (Jungk, 1994). Jakobsen and Nielson (1983) reported that because of the variation in these factors the mycorrhizal status of the plants also show variation with growth stages.

Mycorrhizal association and Diversity

Less variation in Shannon Wiener Diversity index study representing a stable and diverse AM fungal community (Fowler and Antonovics, 1981).

Brundrett and Kendrick (1990) and Radhika and Rodrigues (2010) reported that due to interspecific competition, variation in spore number and species in the rhizospheric soil and roots may possible or it may due to environmental factors which affect the spore production in natural communities (Gemma and Koske, 1988; Radhika and Rodrigues, 2010).

Glomus mosseae has been found abundantly in all the sites and frequency and density is also high. The possible cause might be because of its wider niche, strongest competitor among all and no host preference. Among all the sites no significant difference was found in species evenness. High diversity and ecosystem productivity are recognized from the high value of species richness and species evenness.

Conclusion

The present study concluded that all the ornamental plants showed mycorrhizal colonization but their dependency on AM fungi varied with the sites, plant species and stages of plant growth. Due to the wide occurrence of *Acaulospora* and *Glomus* species in the soil, it can be used for the mass multiplication and use as a microbial inoculant for the better growth of ornamental plants with the reduced cost of chemical fertilizer.

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