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Fungal Population Dynamics and Diversity of AMF species in Sewage Irrigated Agricultural fields of Ganga-Yamuna River Basin

Dheeraj Pandey[a], Ifra Zoomi[a], and Harbans kaur kehri[a*]

[a]Sadasivan Mycopathology Laboratory, Department of Botany, University of Allahabad, Allahabad -211002, India.

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Corresponding Author Email: kehrikaurhk@gmail.com

Abstract

In the present scenario most of the drainage and industrial wastewater pollute the river water. The agricultural field around river basin is irrigated with these metals polluted water. Polluted water contains organic pollutants and inorganic heavy metals like Zn, As, Cu, Pb, Cd, Hg, Ni, and Cr. Higher concentrations of these metals may reduce the microorganisms and plant diversity of this catchment area. Therefore, the present study has been undertaken to understand the effect of heavy metals and seasonal variation on microorganisms. A total of 10 fungal genera were isolated from both the studied site in which *Aspergillus niger, Aspergillus* sp. 2 and Sterile mycelia are dominating species in all season. Arbuscular mycorrhizal fungi were found to be associated with all the host plants however, percentage of association varies from site to site, 40.3 to 83.3% at site-I and 36.7 to 96.7% at site-II. AM spore population ranges 38.7 to 75.3 at site-I and 22.3 to 65.7 at site-II. Genus *Glomus* and *Acaulospora* were recorded as the dominant AM fungi in this survey. Maximum density showed by *Acaulospora foveata, Acaulospora bireticulata* and *Glomus etunicatum* respectively from site-I and site-II.

Keywords

River basin, Pollution, Soils, AM fungi.

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INTRODUCTION

Sewage forming is being practiced as one of the convenient methods of disposal of the urban sewage in the country. However, information regarding repercussions of the sewage irrigation on the soil and crops is limited. In Allahabad, more than 20 drainage outlets are there whose water is dumped in Ganga and Yamuna and this polluted water is used to irrigate the agricultural fields. A number of heavy metals are present in this water e.g. Zn, As, Cu, Pb, Hg, Ni, Cr etc. These heavy metals are non-biodegradable and due to bio-magnification process they persist for a longer time in soil. Higher concentrations of these metals may become toxic to the microorganisms and to the plant diversity of this catchment area (Rajbanshi, 2009; Sa'idi, 2010; Wuana and Okieimen, 2011).

There is an urgent need to focus on the potential role of biological remediation where soil microorganisms especially filamentous fungi, AM fungi and bacteria may play a great role. These microorganisms have developed their own mechanisms to cope up with such pollutants. AM fungi act as barrier between heavy metal pollutants and roots of the plants (Kehri

et al. 2014; Akhtar et al. 2017). Moreover, these microbes are known to occur in a wide variety of ecosystems including many stressful environments. However, several edaphic factors as well as plant species affect their population and diversity. Therefore, it is important to study the diversity and population dynamics of these filamentous fungi and AM fungi as they are better adapted to survive under stressed conditions prevailing there than the exotic species. In view of this the present study has been undertaken to investigate the abundance and diversity of these microbes in sewage polluted agricultural fields of Ganga-Yamuna catchment area.

MATERIALS AND METHODS

Study Site

The study sites are situated at eastern part of Yamuna basin Phoolbagia, Naini, Allahabad (site – I) and Daraganj Ganga basin area (site–II) (25.15°N 82.58°E) (Fig. 1). The area under study has tropical climate with three main seasons, summer (March-June), monsoon (July-October) and winter (November-February). The source of contamination is sewage water and Ganga-Yamuna river water. The soil of Daraganj is sandy with silt deposited and Phoolbagia, Naini soil was blackish loamy.

Sampling procedure

The organized sampling procedure was followed to access the uniform and homogenous diversity of fungi. Samples were collected from the same site three times in a year i.e. during summer, monsoon and winter. From each five patches of pure vegetation the quadrat of 1 m^2 was defined randomly. Five samples from each quadrat were drawn from corners and center of quadrat. Samples were collected at 5-15 cm depth after removing the top ~ 1 cm soil. Thus, in all 25 samples were collected in zip-lock sterilized polybags and brought to the laboratory for further examination. 50 g soil from each core sample was taken and mixed to form a composite sample of 1 Kg. This composite sample was sent to Motilal Nehru Farmer's Training Institute, IFFCO, Phulpur, Allahabad, India for the physico-chemical characterization. Plant roots and rhizospheric soils were utilized for the study of mycorrhization and establishment of trap culture.

Determination of spore population

Three replicates of 10 g air dried soil samples were taken for the determination of spore population. The soil was dissolved in water and stirred thoroughly. The suspension was immediately passed through 750, 500, 250- and 60-micron sieves. The spores were extracted by the method of Gerdemann and Nicolson (1963) with some modifications. The content from all the sieves was filtered on filter paper (Whatman paper No. 42) and further, enumeration of spore population by the method given by Gaur and Adholeya (1994).

Determination of mycorrhizal infection (MI)

1.5 g fresh roots were cut into 1 cm length and treated with freshly prepared 10 % KOH and heated in hot air oven at 90 °C for 2-3 hours (based upon the root hardness). Alkalinity of root bits removed by washing with tap water several time. The root bits were treated in 0.2 % sodium hypochlorite for two minutes (if pigmented). The root bits were further acidified with 1 N HCl and then stained in 0.1% trypan blue for 12 h at room temperature. Excess stain was removed by destaining with lactic acid, glycerol and water (1:1:1) (Phillips and Hayman, 1970). The mycorrhizal infection (MI) was examined through the gridline intersect method of Giovannetti and Mosse (1980).

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

Multiplication of AM fungi

For the multiplication of AM fungi trap culture was established by the method given by (Walker, 1999) with some modifications. The collected soil samples were mixed with equal amount of autoclaved coarse sand and filled in pots. Seeds of the *Sorghum bicolor* (sudan grass) were sown and pots were kept in semi controlled condition in greenhouse for four months and after four months the above ground parts of *Sorghum* plants were removed.

Isolation and Identification of filamentous soil fungi

Potato dextrose agar (PDA) medium was used for the isolation of fungi by using a dilution plate method (Timonin, 1980). 10 g of each soil sample was dissolved in 100 ml of sterile double distilled water and serially diluted upto dilution 10^{-3}. 0.1 ml of serially diluted (10^{-3}) aliquots was spread on the PDA plate. The plates were incubated at 25±2 °C for 48 h. After incubation distinct colonies were counted and identified on the basis of macromorphological and micromorphological characteristics using well stained slides under high power compound

microscope with the help of "A manual of soil fungi" (Gilman, 1945).

Identification of AM fungal spores

Collected spores from trap culture were separated and mounted in PVLG reagent. Gentle pressure was applied on coverslips to rupture the spores for details of wall layers and then baked in oven at 50 ºC for 12 h to reduce the depth of mounting medium and water content. The microscopic characters of spore were studied under the compound microscope. The AM fungal species were identified and named according to the "Phylogeny and taxonomy of Glomeromycota" (Schüßler and Walker, 2010). The identification were further confirmed with the species description of INVAM. (http://invam.wvu.edu/).

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

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

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

AM species diversity was calculated by following formula:

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

Pi = proportion of individual of species i

Simpson index of dominance= $\Sigma (Pi)^2$

Pi = proportion of individual of species i

Determination of Arsenic accumulation in Soil

For determination of Arsenic (As) in soil of survey sites, soil was well digested according to microwave assisted acid digestion by Bezzi et al. (2011). Arsenic content in soil sample analysed by ICP-MS (model-ARCOS, stimultaneous ICP Spectrometer, SPECTRO Analytical Instrument GmbH Germany).

Results and Discussion

The data of analysis of all the soil samples collected from both the sites selected for the present study has been presented in Table – 1. The elemental analysis of the soil samples showed that the soils were slightly alkaline, having pH 7.6 and 7.8 at site I and site II respectively. Cadmium was present beyond the permissible limit at both the sites, however, metals like Nickel, Copper, Lead, arsenic were present below the permissible limits. The organic carbon was high which ranges 1.60% - 1.62% at site I and site II respectively.

As for as vegetation is concerned, low plant diversity was recorded at both the sites. In all, five plants were selected from the sites, out of which three plants *viz., Croton banpandium*, *Cynodon* sp., *Parthenium heterophorus* were common at both the sites while *Coriandrum sativum* and *Lycopersicon esculantum* were present at site-I only whereas *Momordica charantia* and *Solanum xanthocarpum* were present at site II only.

Data of the arbuscular mycorrhizal intensity in the roots of the plants growing at the selected sites in different seasons have been presented in Tables 3 & 4. All the plants showed the mycorrhizal association in their roots, however, the intensity of the mycorrhizal association varied with the plant species, site and the seasonal variation. Mycorrhizal intensity ranged from 40.3% to 83.3% at site I and 30.0% to 96.7% at site II. Maximum infection was recorded in *Coriandrum sativum* (83.3%) and *Momardica charantica* (96.7%) at site I and site II respectively while minimum infection was recorded in *Parthenium heterophorus* (40.3%) and *Solanum xanthocarpum* (30.0%) at site I and site II respectively. Seasonal changes affected the intensity of mycorrhizal infection in the roots, maximum intensity was recorded during summer season while minimum in winter.

Data on spore population has been presented in tables 3 & 4. AM spore population ranged from 38 to 75 spores/10 g air dried soil at site I while 22 to 65 spores/ 10 g air dried soil at site II. Minimum spores' population was recorded in the rhizosphere soil of *Parthenium heterophorus* (33 spores / 10 g air dried soil) at site I and in *Solanum xanthocarpum* at site II (22 spores / 10 g dried soil). Likewise, maximum spore population was recorded in the rhizospheric sample of *Coriandrum sativum* (75 spores) and *Momardica charantica* (65 spores) at site I and site II

respectively. However, significant variation was not recorded in AM population. Maximum population was recorded in summer while minimum in winter season irrespective of the plant species. In the present study seasonal variation also found to affect the AM spore population as shown in fig.2 and 3.

A total of 20 species were recorded belonging to three different genera of AM fungi in association with the vegetables and wild plants at survey sites. *Glomus* and *Acaulospora* were recorded as the most dominant genera. *Glomus* with 7 species viz. *Glomus mosseae* (Nicolson and Gerd.) Gerdemann and Trappe, *G. etunicatum* Becker and Gerdemann, *G. intraradices* Schenck and Smith, *G. geosporus* (Nicol. and Gerd.) C. Walker, *G. constrictum* Trappe, *G. occultum* Walker, *G. versiforme* (P. Karsten) S. M. Berch, *G. ambisporum* G. S. Sm. & N. C. Schenck and two unidentified species viz. *Glomus* sp.1, *Glomus* sp.2 followed by *Acaulospora* with 7 species, viz. *A. foveata* Trappe & Janos, A. *lacunosa* Morton, A. *bireticulata* Rothwell and Trappe, *A. longula* Spain and Schenck, *A. scrobiculata* Trappe, *A. denticulata* Sieverding and Toro, *A. trappei* Ames and Linderman and three unidentified species viz. *Acaulospora* sp. 1 to 3 whereas Scutellospora with a single unidentified species. Maximum density showed by *Acaulospora foveata, Acaulospora bireticulata* from site-I and *Glomus etunicatum* respectively from site-II. Most abundant AM fungus at site-I is *Acaulospora foveata* with maximum diversity and frequency. Followed by *Acaulospora bireticulata*. Shanon-Weiner diversity index and Simpson index of dominance indicate that rhizosphere of *Coriandrum sativum* is most diversified and contains maximum types and number of AM spores followed by *Croton banpandium, Lycopersicon esculentum, Cyanodon* sp*., Parthenium hysterophorus*. (Table 5 and Fig. 4).

Maximum abundance of AM fungi at site II are *Glomus etunicatum* and frequency of *Glomus etunicatum* found double in comparison to *Glomus occultum*. Maximum Shanon-Weiner diversity index and Simpson index of dominance in *Croton banpandium* followed by *Cyanodon* sp., *Momordica charantia, Parthenium hysterophorus, Solanum xanthocarpum. Scutellospora* sp. found in rhizospheric soil of *Parthenium hysterophorus* only (Table 6, Fig. 5 and Plate 2). The rhizospheric soil of *Coriandrum sativum* and *Croton banpandium* showed maximum Shanon-Weiner diversity and Simpson dominance index and *Parthenium hysterophorus* and *Solanum xanthocarpum* showed a minimum.

The diversity of filamentous fungi was quite low, only 10 genera have been isolated from both the sites. Site-I had more diversity in comparison to site-II. *Aspergillus fumigatus, Alternaria tenius, Curvularia* sp*., Cunninghamella verticillata, Fusarium* sp*., Rhizopus* sp*., Scopulariopsis* sp., *Trichosporium* sp. were present only at site I, whereas *Aspergillus niger, Aspergillus* sp.2, and sterile mycelia were dominant at both the sites and in all the seasons (Table 2).

Filamentous fungi showed resistance against the heavy metal pollution and they have developed many types of mechanisms like absorption, accumulation, methylation etc. to provide assistance for bioremediation (Siddiquee et al, 2015). Likewise, AM fungi also promote plant establishment by increasing resistance to environmental stresses, enhancing plant nutrient, acquisition and improving soil quality (Hildebrandt, Regvar and Bothe, 2007). Different species of *Glomus* and *Acaulospora* have been found abundantly at both the sites. The possible reason might be wide niche, stress tolerant mechanism and wide range of host preference (Srivastva and Kehri, 2018). These fungi may be utilized in future for the bioremediation of highly polluted agricultural fields with heavy metals.

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Table 1: Physico-chemical properties of the Agriculture soils

Site	pH (H_2O)	EC (ds cm^{-1})	Organic Carbon (%)	Cd (%)	P_2O_5 (ppm)	K_2O (ppm)	Ni (ppm)	Cu (ppm)	Pb (ppm)	As (ppm)
I	7.8	3.08	1.62	7.69	391.86	32.86	137	110	136	6.67
II	7.6	3.22	1.60	8.3	255.34	36.66	79.20	73.33	110	6.69

Figure 1: Map showing survey sites.

Table 2: Diversity and frequency of filamentous fungi at site -I and site -II.

Isolated fungi	Distribution							
	Site – I			Site - II			Frequency (%)	
	I	II	III	I	II	III	(Site-I)	(Site-II)
Aspergillus niger Van Tieghem	+	+	+	+	+	+	100	100
A. flavus Link	+	+	+	+	-	+	66.7	66.7
A. terreus Thom	-	+	+	-	+	+	66.7	66.7
A. fumigatus Fresenius	+	+	+	-	-	-	100	-
***Aspergillus* sp. 1**	+	+	+	+	+	+	66.7	100
***Aspergillus* sp.2**	+	+	+	+	+	+	100	100
Alternaria tenuis Nees	+	+	-	-	-	-	66.7	-
***Aternaria* sp.**	+	+	-	+	+	-	66.7	66.7
***Curvularia* sp.**	+	+	-	-	-	-	66.7	-
Cunninghamella verticillata Plain	-	+	-	-	-	-	33.3	-
Fusarium oxysporum Emen. Snyder & Hansen	+	+	+	+	+	-	100	66.7
***Fusarium* sp.**	+	+	-	-	-	-	66.7	-
Penicillium chrysogenum Thom	+	-	+	+	+	+	66.7	100
***Penicillium* sp.1**	+	+	+	+	-	+	100	66.7
***Penicillium* sp.2**	+	+	+	-	+	+	100	66.7
***Rhizopus* sp.**	+	-	+	-	-	-	66.7	-
***Scopulariopsis* sp.**	-	+	-	-	-	-	33.3	-
Sterile mycelia	+	+	+	+	+	+	100	100
***Trichosporium* sp.**	-	+	-	-	-	-	33.3	-

I – October, II – January, III – June, F – frequency

Table 3: Seasonal variation in AMF spore population and Mycorrhizal infection in five host at site-I.

Plant	Family	Spore population (SP) (/ 10 g soil)			Mycorrhizal Infection (% MI)		
		I	II	III	I	II	III
Croton banpandium	Euphorbiaceae	56	54	69	73.3	60	70
Coriandrum sativum	Apiaceae	-	65	75	-	80	83.3
Cyanodon sp.	Poaceae	38	39	38	70	60	70
Lycopersicon esculentum	Solanaceae	-	69	52	-	70	66.7
Parthenium hysterophorus	Asteraceae	33	40	40	40.3	56.7	60

I –Sept.-Oct. II – Dec.-Jan. III – May-June

Table 4: Seasonal variation in AMF spore population and Mycorrhizal infection in five host at site-II.

Plant	Family	Spore population (SP) (/ 10 g soil)			Mycorrhizal Infection (% MI)		
		I	II	III	I	II	III
Croton banpandium	Euphorbiaceae	37	43	45	63.3	50	70
Cyanodon sp.	Apiaceae	37	43	55	60	63.3	83.3
Momordica charantia	Cucurbitaceae	60	-	65	70	-	96.7
Parthenium hysterophorus	Asteraceae	34	44	43	40	56.7	66.6
Solanum xanthocarpum	Solanaceae	22	27	37	30	36.7	43.3

I –Sept.-Oct. II – Dec.-Jan. III – May-June

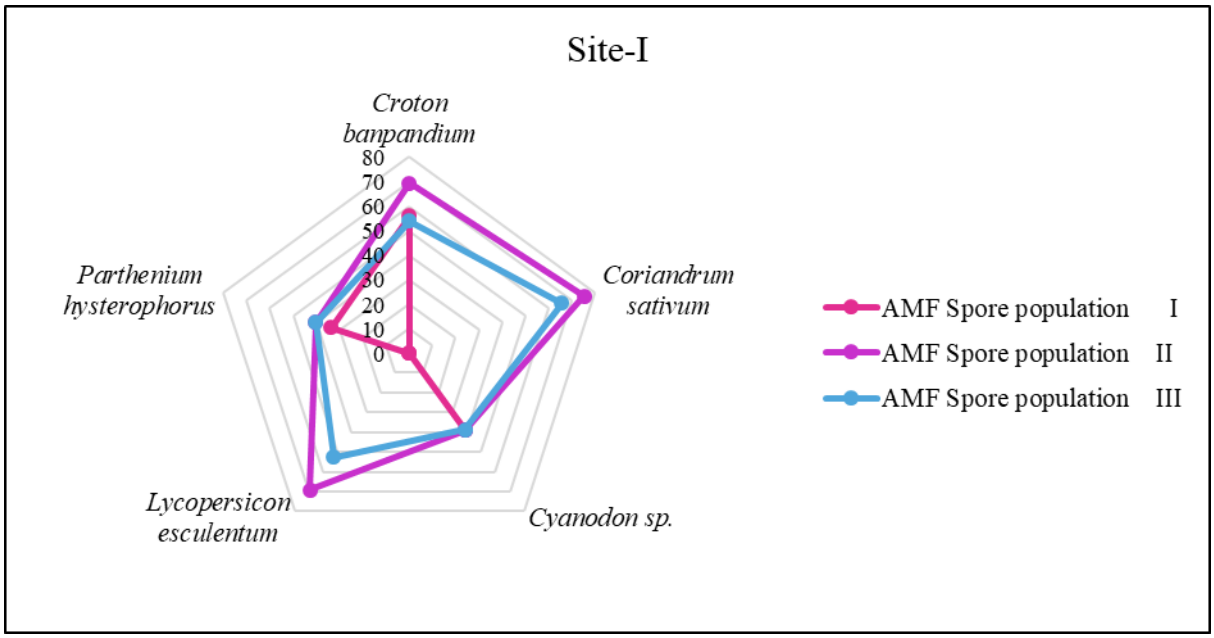

Figure 2; Effect of seasonal variation on AM spore population in relation with the host plant at site -I.

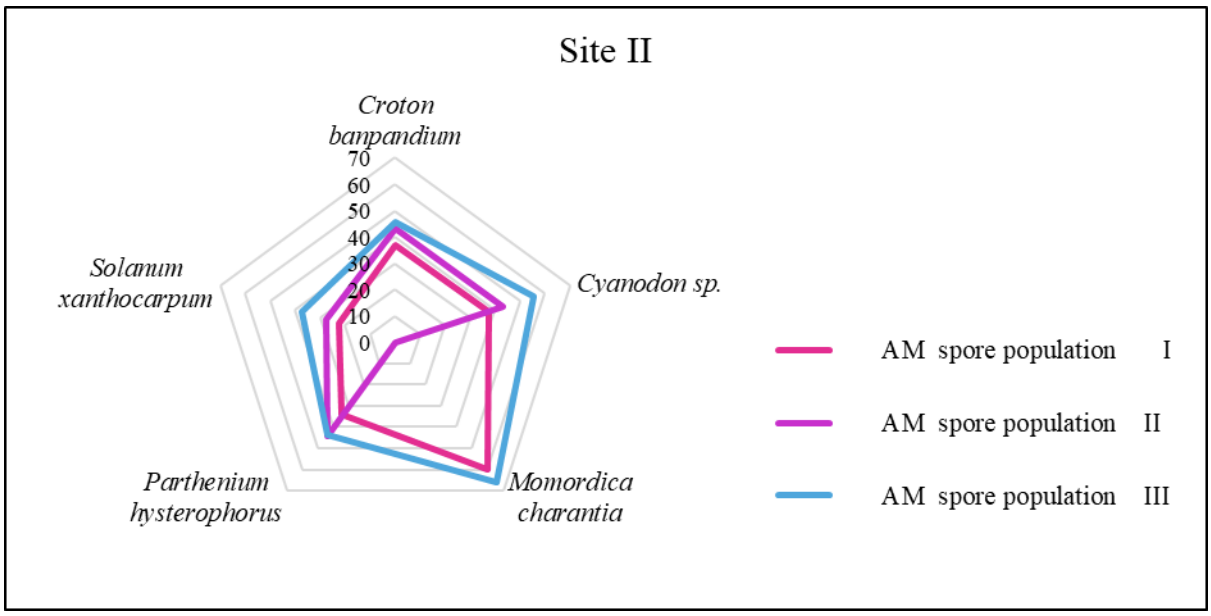

Figure 3: Effect of seasonal variation on AM spore population in relation with the host plant at site –II.

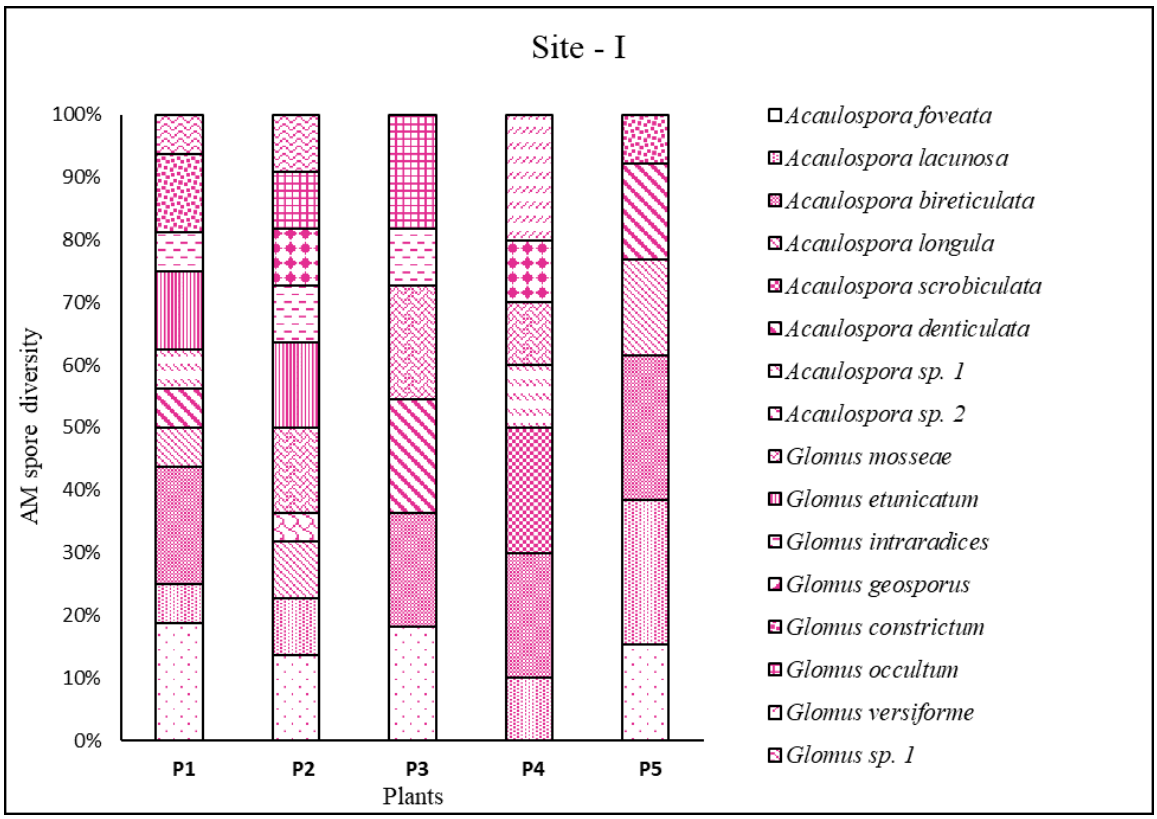

Figure 4: Diversity of AM fungi at site-I.

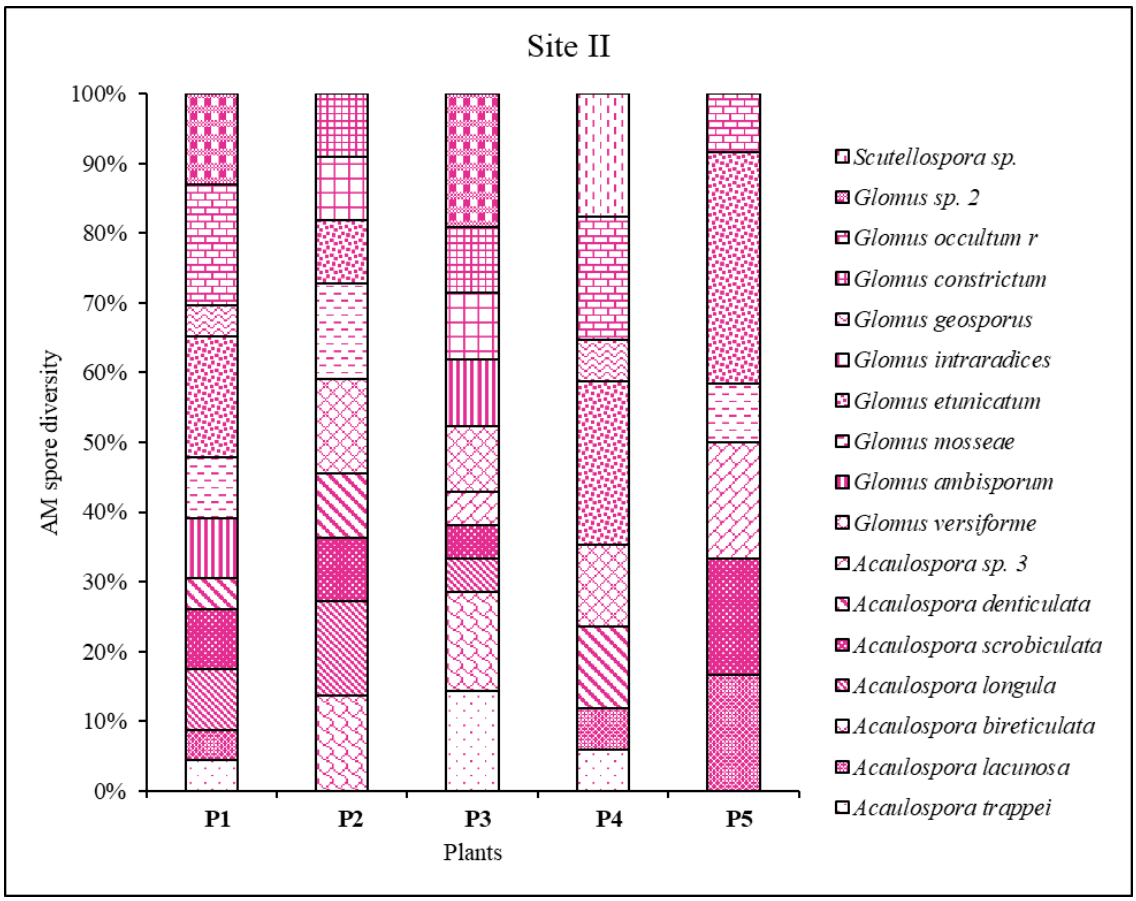

Figure 5: Diversity of AM fungi at site-II.

Table 5: Diversity of AM fungi at site -I.

AM Fungi/plants	Diversity of AM fungi							
	P1	P2	P3	P4	P5	D	F	A
Acaulospora foveata **Trappe & Janos**	3	3	2	-	2	2.0	80	2.5
Acaulospora lacunosa **Morton**	1	2	-	1	3	1.4	80	1.8
Acaulospora bireticulata **Rothwell and Trappe**	3	-	2	2	3	2.0	80	2.5
Acaulospora longula **Spain and Schenck**	1	2	-	-	2	1.0	60	1.7
Acaulospora scrobiculata **Trappe**	-	-	-	2	-	0.4	20	2.0
Acaulospora denticulata **Sieverding and Toro**	1	-	2	-	2	1.0	60	1.7
Acaulospora **sp. 1**	1	-	-	1	-	0.4	40	1.0
Acaulospora **sp. 2**	-	1	-	-	-	0.2	20	1.0
Glomus mosseae **(Nicolson and Gerd.) Gerdemann and Trappe**	-	3	2	1	-	1.2	60	2.0
Glomus etunicatum **Becker and Gerdemann**	2	3	-	-	-	1.0	40	2.5
Glomus intraradices **Schenck and Smith**	1	2	1	-	-	0.8	60	1.3
Glomus geosporus **(Nicol. and Gerd.) C. Walker**	-	2	-	1	-	0.6	40	1.5
Glomus constrictum **Trappe**	2	-	-	-	1	0.6	40	1.5
Glomus occultum **Walker**	-	2	2	-	-	0.8	40	2.0
Glomus versiforme **(P. Karsten) S. M. Berch**	-	-	-	2	-	0.4	20	2.0
Glomus **sp. 1**	1	2	-	-	-	0.6	40	1.5
Total AMF spores	16	22	11	10	13			
Shanon-Weiner diversity index	2.187	2.264	1.768	1.887	1.738			
Simpson index of dominance	0.875	0.893	0.826	0.84	0.817			

P1- ***Croton banpandium,*** **P2-** ***Coriandrum sativum*****., P3-** ***Cyanodon*** **sp, P4-** ***Lycopersicon esculentum,*** **P5-** ***Parthenium hysterophorus***
D- Density, F- Frequency, A- Abundance

Table 6: Distribution of AM Fungi at site –II.

AM fungi/plants	Diversity of AM fungi							
	P1	P2	P3	P4	P5	D	F	A
Acaulospora trappei **Ames and Linderman**	1	-	3	1	-	1.0	60	1.7
Acaulospora lacunosa **Morton**	1	-	-	1	2	0.8	60	1.3
Acaulospora bireticulata **Rothwell and Trappe**	-	3	3	-	-	1.2	40	3.0
Acaulospora longula **Spain and Schenck**	2	3	1	-	-	1.2	60	2.0
Acaulospora scrobiculata **Trappe**	2	2	1	-	2	1.4	80	1.8
Acaulospora denticulata **Sieverding and Toro**	1	2	-	2	-	1.0	60	1.7
Acaulospora **sp. 3**	-	-	1	-	2	0.6	40	1.5
Glomus versiforme **(P. Karsten) S. M. Berch**	-	3	2	2	-	1.4	60	2.3
Glomus ambisporum **G. S. Sm. & N. C. Schenck**	2	-	2	-	-	0.8	40	2.0
Glomus mosseae **(Nicolson and Gerd.) Gerdemann and Trappe**	2	3	-	-	1	1.2	60	2.0
Glomus etunicatum **Becker and Gerdemann**	4	2	-	4	4	2.8	80	3.5
Glomus intraradices **Schenck and Smith**	-	2	2	-	-	0.8	40	2.0
Glomus geosporus **(Nicol. and Gerd.) C. Walker**	1	-	-	1	-	0.4	40	1.0
Glomus constrictum **Trappe**	-	2	2	-	-	0.8	40	2.0
Glomus occultum **Walker**	4	-	-	3	1	1.6	60	2.7
Glomus **sp. 2**	3	-	4	-	-	1.4	40	3.5
Scutellospora **sp.**	-	-	-	3	-	0.6	20	3.0
Total AMF spore	23	22	23	17	12			
Shanon-Weiner diversity index	2.269	2.177	2.203	1.956	1.676			
Simpson index of dominance	0.8847	.8843	.8798	.8443	.7917			

P1- ***Croton banpandium,*** **P2-*Cyanodon* sp., P3-*Momordica charantia,* P4-** ***Parthenium hysterophorus,*** **P5-** ***Solanum xanthocarpum***
D- Density, F- Frequency, A- Abundance

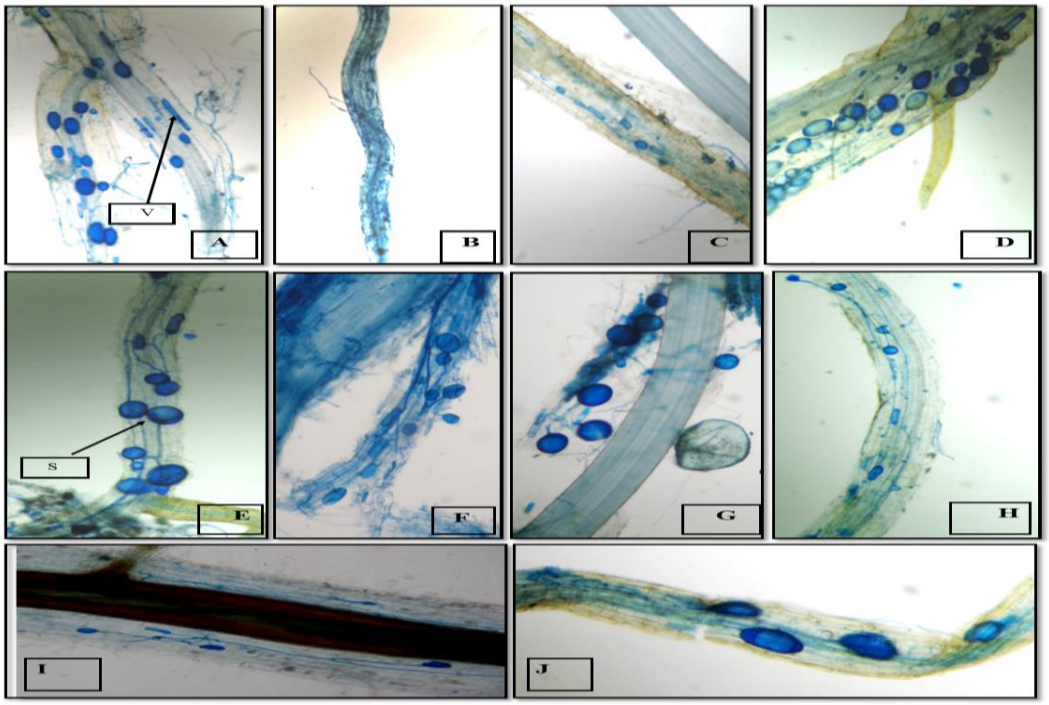

Plate 1: Showing mycorrhizal infection of host plants at site -I (A-E) and site -II (F-J), V= Vesicles, S=Spore

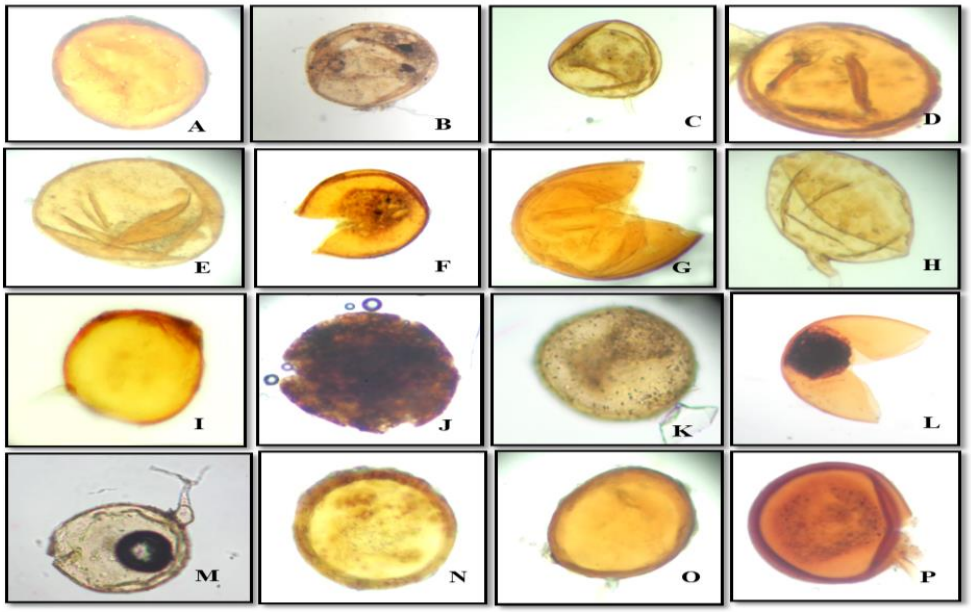

Plate 2: Showing AM fungi Spores of site –I (A-H) and site –II (I-P). **A** *Acaulospora foveolata* Trappe & Janos **B** *Glomus constrictum* Trappe **C** *Glomus occultum* Walker **D** *Acaulospora lacunosa* Morton **E** *Acaulospora denticulata* Sieverding and Toro **F** *Glomus* sp. **G** *Glomus intraradices* Schenck and Smith **H** *Glomus mosseae* (Nicolson and Gerd.) Gerdemann and Trappe **I** *Glomus geosporum* (Nicol. and Gerd.) C. Walker **J** *Acaulospora scrobiculata* **K** *Glomus mosseae* (Nicolson and Gerd.) Gerdemann and Trappe **L** *Acaulospora bireticulata* Rothwell and Trappe **M** *Glomus mosseae* (Nicolson and Gerd.) Gerdemann and Trappe **N** *Acaulospora scrobiculata* Trappe **O** *Glomus* sp.1 **P** *Glomus etunicatum* Becker and Gerdemann.

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