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Colonization and Diversity of AM Fungi Associated with Some Plants of Abandoned Cropland in J. P. University Campus, Chapra Bihar

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Abstract: **The present study was to analyse the mycorrhizal diversity in AM fungi associated with 12 plants of J. P. University Campus, Chapra. Arbuscular mycorrhizal fungal colonization ranged from 44.4 – 100 % . The highest infection was found in *Parthenium hysterophorus L.* and lowest in *Ricinus communis L* . 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 lemon (L.)* (10/ 100 gm of soil). A total of 25 AM fungal species were isolated which belongs to five genera (*Acaulospora, Entrophospora, Glomus, Scutellospora* and *Sclerocystis*) and among them *Glomus* was dominant genera. The maximum species richness (SR) was recorded in *Ricinus communis L.* (13) while the lowest (2) appeared in *Citrus lemon (L.)* . The highest Shannon wiener diversity (H) was recorded in *Ricinus communis L.* (2.036) and lowest H was recorded in *Clerodendrum infortunatum Linn*. (0.875). The maximal Simpson's diversity index (D) was recorded in *Ricinus communis L.* (0.86) and minimal D was recorded in *Citrus lemon (L.)* (0.29). The maximum species evenness (E_H) was recorded *Azadirachta indica A*. (0.92) and minimum E_H was recorded in *Citrus lemon (L.)* (0.63).**

Keywords: **Arbuscular mycorrhiza, Root colonization, Species richness, Shannon -Wiener index, Simpson 's diversity index, Species evenness.**

1. INTRODUCTION

In nature arbuscular mycorrhizal (AM) fungi, the most ubiquitous symbiotic soil microbes. Reports suggest that are 80% of plant species forms mycorrhizas [1]. In general, AM fungi and host plants are reciprocal symbionts. The symbiosis improves the nutrient uptake and provides protection from pathogens, while the AM fungi receive carbohydrates [2], [3], [4]. Arbuscular mycorrhizal (AM) fungi are principal component of the rhizosphere microflora in natural ecosystems. The symbiotic associations are present in two thirds of plant families and occur in most terrestrial ecosystems [5]. AM fungi can generate glomalin, which contributes to the formation and stablization of soil aggregates through a “gluing” action [6], [7] . Therefore, symbiotic associations play a critical role in the restoration and recontruction of fragile vegetation or degraded ecosystem [8] , [9] and in preservation of plant biodiversity and ecosystem functioning [10]. The major benefit of mycorrhiza is its greater soil exploration and increasing uptake of P, N, K ,Zn, Cu, S, Mg, Ca, and Mn [11]. The induced disease resistance of plant by AM fungal has become a hot spot in chemo – ecological study and in biocontrol of plant disease [12]. Development of AMF may depend on the edaphic conditions [13] ,[14]or climatic conditions [15] ,[16]. Recent findings suggest that adaptation of AMF to abiotic factors such as temperature and nutrient availability can strongly influence the effect of AMF symbiosis on plant growth [17] , [18]. Any disturbance on this relationship may

cause changes in terms of decreased population status and diversity of these mycorrhizal fungi. When a soil is put to agricutural use it undergoes a series of physical , chemical and microbiological changes . AM fungi can also improve the plant growth and survival in soils contaminated by heavy metals [19] and salt [20] . Mycorrhizal root colonization help to induce earlier flowering and increase flower number in horticultural crops [21], [22] , [23] , [24]. Most previous studies indicated that AM fungi had no host specificity [25], [26], [27]. However, some studies showed that the sporulation and community composition of AM fungi have been found to be host dependent [28], [29].

The basic aims of the present study were i) to investigate the changes of AM fungal colonization, sporulation, species composition and diversity and ii) to understand whether there was host preference of AM fungi of the twelve vegetative plant species in the J. P. University Campus, Chapra.

2. MATERIALS AND METHODS

Root sampling: In the months of rainy season of 2015 – 2017 fine roots of plants growing in the campus of J. P. University were collected from different plants such as *Calotropis gigantea (L.) Ait.* , *Ziziphus mauritiana Lamm*., *Croton sparciflorus (L.)*, *Parthenium hysterophorus L.* , *Dalbergia sissoo Rox. Ex D*c. etc . Roots were collected randomly from a depth of 0- 30 cm . After bringing these plant samples to laboratory the roots were separated and further processing was done.

Estimation of root colonization: Roots were washed throughly to remove attached soil particles. The cleaned roots were cut into 1 cm long piece and were fixed in formalin acetic acid (FAA) according to the procedure described by [30] . The roots were boiled in 10% KOH for 1 hr, 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, vesicles, arbuscules, or hyphal coil in each segment. The percentage of AM fungal colonization was assessed by using the formula :

$$\textbf{* Percentage of colonization} = \frac{\text{Number of root segments infected}}{\text{Total number of root segments observed}} \times 100$$

Spore extraction : Separation of AM fungal spores from rhizospheric soil of each plant was done by using wet sieving and decanting method proposed by [31] from the 100 gm of soil sample. Soil samples were collected randomly using three replicates . 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 sieves. 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 alcohol lactophenol (PVLG) on a glass slide and identified under a compound microscope (Olympus BX41) and photographed (Nikon eclipse 200). ldentification was based on spore morphology and sub cellular characters [32].

Data analysis *:* Spore density was calculated from direct count of spores. Species richness was defined as the number of AM fungal species soil sample (12), Shannon – Weiner diversity index (H') was calculated according to the formula :

$H' = -\sum Pi\ (In\ Pi)$ where Pi is the proportion of individual species that contributes to the total number of individuals, Simpson's diversity index was calculated according to the formula :

$D = 1- \sum n\ (n-1) / N\ (N-1)$ where n is the number of individuals of a given species and N is the total number of individuals in a community and Species evenness (E_H) was calculated according to the formula :

$\sum H = H / H'\ max = H / InS$, $H'\ max = InS$ where S = Total number of species in a community.

3. RESULTS AND DISCUSSION

The rate of AM fungal colonization in root and endo- mycorrhizal spore density in rhizosphere of herbaceous vegetation showed wide range of variation in J. P. University Campus, Chapra Bihar. Colonization was characterized by the presence of hyphae, arbuscules, vesicles and hyphal coil. AM fungal colonization varied with species . The vegetative plant

species along with their AMF characterization are presented in Table 1. Percentage colonization was maximal for *Parthenium hysterophorus L.* and *Lantana camara Var.* (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 dominated genus followed by *Acaulospora* (8 species), *Scutellospora* (1 species), *Sclerocystis* (1 species) and *Entrophospora* (1species). *Glomus* species has been recorded as dominant mycorrhizal genus [33]. 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 was recorded in *Citrus lemon (L.)* (10/ 100 gm of soil) belongs to the family *Rutaceae*. Twelve plant species of J. P. University of Chapra, Campus were analyzed for mycorrhizal density, diversity, species richness, species evenness etc. in the present study . AM diversity recorded in *Ricinus communis L.* (13) and minimum in the plant species of *Citrus lemon (L.)* (2). The maximum species richness(SR) was recorded in *Ricinus communis L.* (13) while the lowest (2) appeared in *Citrus lemon (L.)* . The highest Shannon Wiener diversity (H) was recorded in *Ricinus communis L.* (2.036) and lowest H was recorded in *Clerodendrum infortunatum Linn*. (0.875). The maximal Simpson′s diversity index (D) was recorded in *Ricinus communis L.* (0.86) and minimal D was recorded in *Citrus lemon (L.)* (0.29). The maximum species evenness (E_H) was recorded *Azadirachta indica A*. (0.92) and minimum E_H was recorded in *Citrus lemon (L.)* (0.63) (Table 2).

TABLE l: AM association with roots and AM Spores present in rhizospheric soil .

Sr no	Plant name	Colonization (%)	Spore density per 100 gm soil	AM diversity
1	*Calotropis gigantea (L.) Ait.*	97.7 ± 4.8 ±2.8	78.33	*A. lae, A. spinosa, G. clar, G. fasi, G. intr, G. macro*
2	*Citrus lemon (L.)*	72.2 ± 4.8 ± 2.8	10	*G. fasi, S. reti*
3	*Croton sparciflorus (L.)*	94.4 ± 9.64 ± 5.5	113.33	*G. clar, G. etuni, G. fasi, G.geo, G. intr, G. macro, G. moss, G. multi, S. rubi*
4	*Azadirachta indica A. Juss.*	63.8 ± 4.7 ± 2.7	88.33	*G. clar, G. fasi, G. intra*
5	*Clerodendrum infortunatum Linn.*	77.7 ± 20.9 ± 12.10	155	*G. clar, G. fasi, G. intra*
6	*Ziziphus mauritiana Lamm.*	72.2 ± 4.8 ±2.8	93.33	*Entro, G. agg, G. clar, G. clarum, G. fasi, G. intra, G. moss*
7	*Parthenium hysterophorus L.*	100 ± 0 ± 0	125	*Entrpo, G. agg, G. clar,G. fasi, G. intra, G. lae*
8	*Dalbergia sissoo Rox. Ex Dc.*	63.8 ± 4.7 ± 2.7	73.33	*G. agg, G. clar, G. fasi, G. intr, G. macro, G. moss G. hoi*
9	*Phoenix dactylifera L.*	47.16 ± 17.3 ± 10	38.33	*A. lae, G. clar,G. dimor, G. fasi, G. intra, A. (unidentified)*
10	*Lantana camara Var.*	100 ± 0 ± 0	48.33	*A. deli , A. undu, A. morr, A. (unidentified) , G. aur, G. clar, G. etun, G. fasi, G. intra, G. macr, G. hoi*

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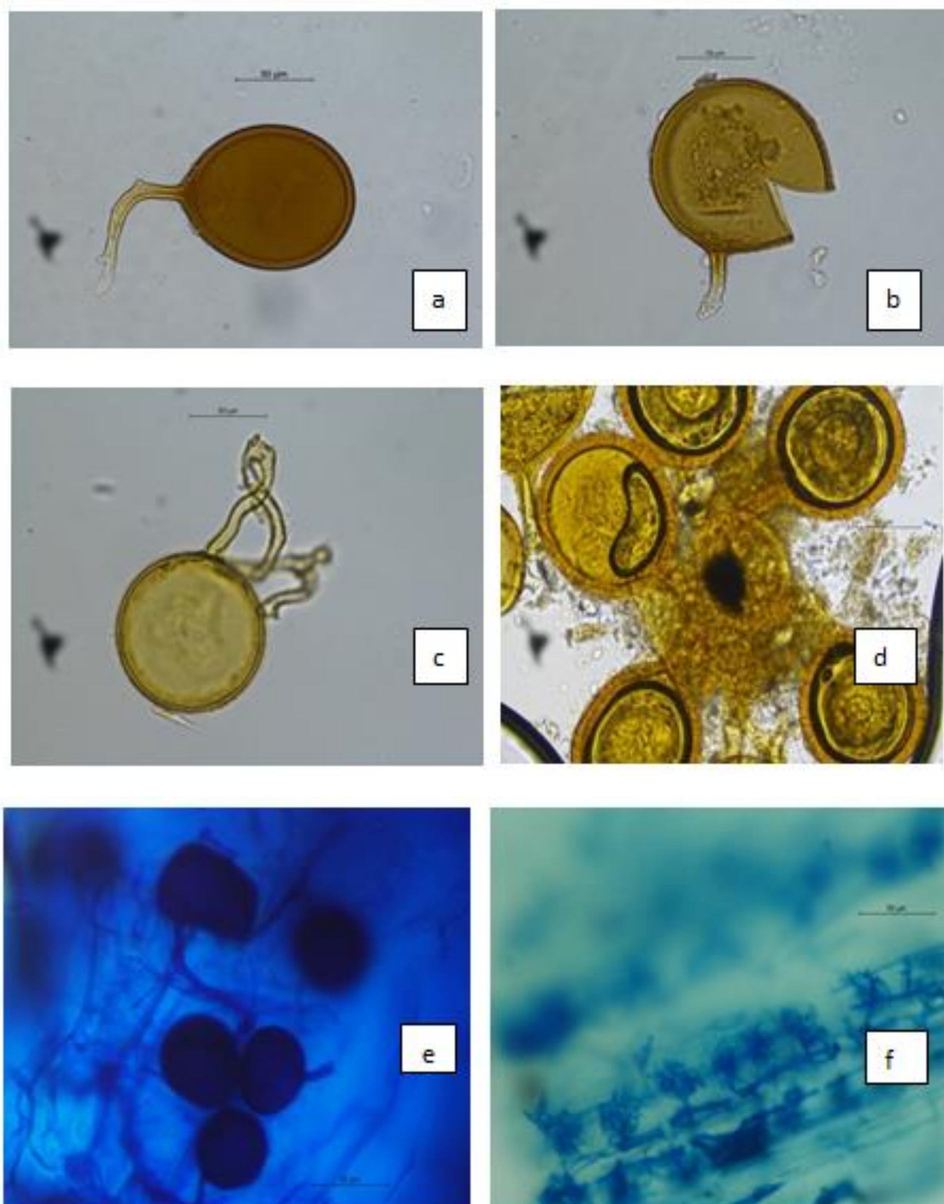
11	*Ricinus communis L.*	44.4 ±4.84 ±2.8	86.66	*A. dent, A. scro, A. spi, G. agg, G. clar, G. dimor, G. etun, G. fasi, G. geo, G. hoi, G. intr, G. moss, G. pust*
12	*Psidium guajava L.*	52.7 ±4.7 ± 2.7	128.33	*G. cla, G. claru, G. dimor, G. etu, G. fasi, G. geo, G. intra, G. moss*

A.spi - Aculospora spinosa, A. dent- Aculospora denticulata, A. lae- Aculospora laevis, A. morr – Aculospora morrowiae, A. scro- Aculospora scrobiculata, A. Spi – Aculospora spinosa, A. undu- Aculospora undulata, A. (uni)- Aculospora (unidentified), Entrophospora, G. agg – Gloumus aggegatum, G. aur- Glomus aureum, G. clar- Glomus claroideum, G. claru – Glomus clarum, G. dimor- Glomus dimorphicum, G. etu – Glomus etunicatum, G. fasi- Glomus fasiculatum, G. geo- Glomus geosporum, G. hoi- Glomus hoi, G. intra- Glomus intraradices, G. moss- Glomus mosseae, G. macr- Glomus macrocarpum, G. multi- Glomus multicaule, G. pust- Glomus pustulatum, S. reti- scutellospora reticulata, S. rubi- Sclerocystis rubiformis.

TABLE 2: Shannon diversity (H), Simpson's diversity (D), Species richness (S), and Species evenness (E_H) of different plant species.

Sr no	Plant name	Shannon diversity (H)	Simpson's diversity (D)	Species richness (S)	Species evenness (E_H)
1	*Calotropis gigantea (L.)*	1.379	0.7	6	0.87
2	*Citrus lemon (L.)*		0.29	2	0.63
3	*Croton sparciflorus (L.)*	1.505	0.84	9	0.68
4	*Azadirachta indica A. Juss.*	1.02	0.63	3	0.92
5	*Clerodendrum infortunatum Linn.*	0.875	0.54	3	0.79
6	*Ziziphus mauritiana Lamm*	1.512	0.74	7	0.77
7	*Parthenium hysterophorus L.*	1.253	0.61	6	0.699
8	*Dalbergia sissoo Rox. Ex Dc.*	1.465	0.71	7	0.75
9	*Phoenix dactylifera L.*	1.54	0.78	6	0.85
10	*Lantana camara Var.*	1.82	0.81	12	0.73
11	*Ricinus communis L.*	2.036	0.86	13	0.79
12	*Psidium guajava L.*	1.57	0.78	8	0.75

In the present study, the composition and diversity of the AM fungi composition were described based on morphological species. The result indicated that *Glomus* was the dominant genus, followed by *Acaulospora* . *Acaulospora* and *Glomus* species usually produce more spores than *Gigaspora* and *Scutellospora* species in the same environment [34], [35]. This may be explained by the difference in development. *Acaulospora* and *Glomus* species are thought to require less time to produce spores than *Gigaspora* and *Scutellospora* species. Furthermore , members of the *Gigasporaceae* typically establish an extensive mycellium in soil and produce fewer spores than those of the *Acaulosporaceae* and *Glomaceae* [36] , [37] . [38] reported that *Glomus* species are known to be widely distributed and commonly found in different ecosystem and geographical regions. The AMF community structure in herbaceous vegetative plants are first time described in present study. The results of soil analysis showed that pH, electrical conductivity of soil in vegetative ecosystem were 7.60 and $0.10dsn^{-1}$ respectively . Soil pH was reported to be optimal for mycorrhizal symbiosis [39]. AMF behaviour is affected by soil pH and influence mycorrhizal establishment and growth of plant [40]. The species that produce more spores had wider distribution which species with a smaller geographical range produced fewer spores. Spore production of AM fungi is known to vary greatly by ecosystem type and is affected by many environmental and biological factors [41].



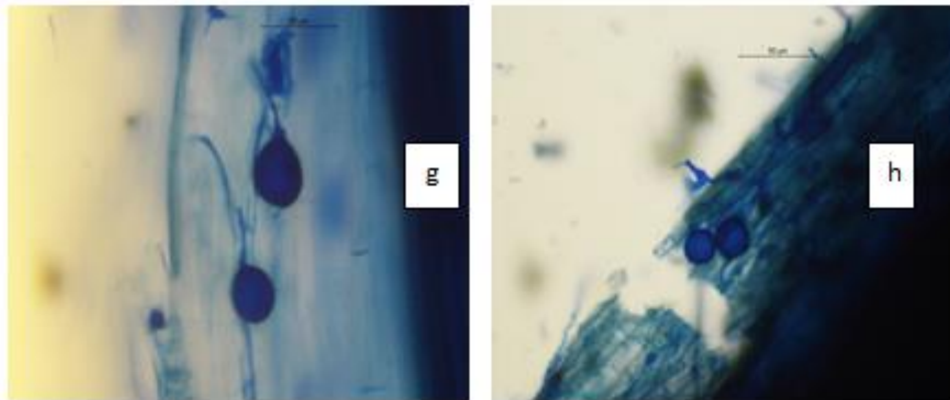


Fig. a = *Glomus aureum*, b = *Glomus fasiculatum* , c = *Glomus hoi*, d = *Sclerocystis rubiformis* , e = Vesicles, f = Arbuscules, g = Vesicles, h = Vesicles

4. CONCLUSION

The present study confirms the occurrence of AM Fungal in the herbaceous plants of J. P. University Campus, Chapra Bihar. All the plant species studied were mycorrhizal and the dominant genera were *Glomus* and *Acaulospora*. The maximum species richness , species evenness, Shannon- wiener diversity and simpson 's diversity index were recorded in *Ricinus communis L., Azadirachta indica A., Ricinus communis L.* and *Ricinus communis L.* .

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