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Arbuscular Mycorrhizal biodiversity associated with *Citrus aurentifolia* from Amravati region

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ABSTRACT

Arbuscular Mychorrhizai fungus is a key component of soil, which associate with root and rhizosphere of soil and create symbiotic association. In the present work, five soil samples were collected from five different sites of Amravati region (Maharashtra) for isolation and identification of AM spores, for this sieves and decanting method were used and observed that all the collected sample were infected by AM fungi but there population varied according to the soil sample, the Chandur Railway and Mardi site having maximum population of Am fungi but the Malkhed site contain very less population. The isolated spore belongs to five Genera which are *Acaulospora, Entrophosphora, Gigospora, Glomus and Scutelospora*. *Glomus* species was observed high amount.

Key words: Citrus plant, Rhizosphere soil, AM spore, AM species.

INTRODUCTION

Arbuscular Mycorrhiza is fungus-root symbiosis that occurs in the vast majority of plants have existed since the Devonian period and might have been essential for the evolution of land plants. Fungal species involved in the formation of arbuscular mychorhiza (AM) with higher plants are worldwide distributed in all terrestrial ecosystems. During the past decade, it has been established that AMF influences soil fertility and thus the growth and development of plant and therefore, these can be an alternative to rising agriculture and fertilizer costs. AMF form a key functional group of soil biota that can contribute towards the ecosystem sustainability and plant productivity (Urcoviche *et al.* 2014). AMF, which belong to phylum Glomeromycota (Schubler *et al*., 2001), Arbuscular mycorrizal fungi (AMF) propagule composition has an important effect on root colonization *(*Klironomos and Hart, 2002*)*. The occurrence of AMF at four soil depths i.e. 8, 15, 23 and 30cm. were studied by and registered more species at 15cm. depth. (Charles *et al*., 2008). The ability of soil to

support AMF population decreased significantly with increased soil depth and the involvement of factor other than soil P^H and moisture content in AMF distribution (Shukla *et al*., 2013).

Arbuscular Mycorrhiza are associate with the root of majority of the land plants, the most important role of Arbuscular Mycorrhiza is that they uptake phosphorus, which is a limiting nutrient in most of the soils (Yao *et al*., 2001; Koide and Schreiner, 1992) and nitrogen (N) and also water from the soil and transport them to the plant root. AM fungi not only uptake the nutrient from the soil but also it enhance the productivity of plant by suppressing plant disease *(*Khaosaad *et al*., 2007*)*, controlling nematode infection *(Elsen et al., 2008)*, stimulation of phytohormones production (Martínez-Medina *et al*., 2011), improve soil texture (Wu *et al*.,2008) and plant tolerance to stress conditions including drought (Pinior *et al*., 2005) and salinity (Hajiboland *et al*., 2010*).* The Biotic and Abiotic factor are greatly affected the diversity and distribution of Arbuscular mycorrhiza *(Mohammad et al. 2003).* The recent experimental study showing that AMF can grow and form spores in vitro, if provided with a carbon source and stimulated by particular bacterial strains (Hildebrandt *et al*., 2006). On global basis, Mycorrhiza occurs in 83% dicot and 79% monocot, whereas all gymnosperms are having Mycorrhizal colonization (Wilcox, 1991). Am fungi are fatty acid heterotrophs(Wewer, 2014) that depend on host deliverd organic carbon (C) in the form of fatty acids (Bravo, 2017, Keymer *et al.* 2017) in order to complete their life cycle.

MATERIALS AND METHODS

Sampling:

The Rhizosphere soil samples of *Citrus aurentifolia* were collected in sterile polythene bags. The collection was carried out in the month of February 2013 from Mardi, Amravati, Chandur Railway, Malkhed and Phora. All soil samples were dried and stored at 4^0C.

Quantitative and Qualitative Estimation of AM fungi:

Different methods are used for counting AM fungal spores. The procedure describe by *(Gaur and Adholeya, 1994)* was used for counting Am spores as it is a simplified method for counting Am fungal spores. In the present study, the wet sieving and decanting technique was used (Gerdemann and Nicolson, 1963) for isolation of AM fungi. Isolated fungi were scanned and mounted on slide in Polyvinyl Lactic Acid as mounting medium. The AM fungi were identified by using standard manual of *(*Schenck and perez, 1990*)* keys of (Morton and Benny, 1990*)* and of (Mehrotra and Baijal, 1994). The Isolated AM fungi were identified by morphology of spores especially on the basis on their wall layer.

Observation:

Qualitative Analysis of AM fungi:

Root and rhizosphere soil sample of *Citrus aurentifolia* from five different sites of Amravati were collected in sterile polythene bags during the month of February 2013.

Quantification Analysis:

The isolated AM fungi from each soil samples were varied according to the soil sample. Soil PH, soil moisture, micronutrients and soil depth all are major factor which effect on AM fungi population. In the present study, found that *Glomus* species in high amount in all the five samples total 18 species, out of 18 species *Glomus aggregatum and Glomus fasciculatum* observed in maximum site then after *Glomus albidum, Glomus arborense, Glomus fecundisporum, Glomus flavisporum, Glomus fulvum, Glomus geosporum, Glomus globiferum, Glomus glomerulatum,* Glomus halon, *Glomus leptotichum, Glomus manihot, Glomus maculosum, Glomus microaggregatum, Glomus verseforme and Glomus pulvinatum* also isolated and identified then *Acaulospora* with 8 species and *Enthrophosphora* and *Gigospora* found very less in number, *Enthrophosphora* Am fungi observed only at site number 1, 3, 5 and *Gigaspora* found in site 1 and 4. The total numbers of AM fungi were recorded during the study in soil sample showing in the Table2.

Quantification analysis show the number of species found in all the sites, one the basis of the above observation following graph is formed, it show the number of spore in each 100g of soil sample.

Table-1. Showing the site number of collected samples and identifies species.

Sr. no.	Site no.	Genera	Species identified
1	S1, S2, S3, S4, S5	*Acaulospora*	*Acaulospora appendicular*
2	S1, S4, S5	*Acaulospora*	*Acaulospora delicate*
3	S2, S4,S5	*Acaulospora*	*Acaulospora bireticulata*
4	S1,S2,S3,S4,S5	*Acaulospora*	*Acaulospora denticulate*
5	S3	*Acaulospora*	*Acaulospora foveta*
6	S2, S4	*Acaulospora*	*Acaulospora lacunose*
7	S1, S3	*Acaulospora*	*Acaulospora laevis*
8	S3	*Acaulospora*	*Acaulospora tuberculata*
9	S5	*Entrophosphora*	*Entrophosphora colombiana*
10	S1, S3	*Entrophosphora*	*Entrophosphora infrequens*
11	S1, S4	*Gigaspora*	*Gigaspora decipiens*
12	S1, S2 ,S4,S3	*Glomus*	*Glomus aggregatum*
13	S1,S2, S4	*Glomus*	*Glomus albidum*
14	S1	*Glomus*	*Glomus arborense*
15	S1, S2, S3, S4, S5	*Glomus*	*Glomus fasciculatum*
16	S1, S3	*Glomus*	*Glomus fecundisporum*
17	S1, S4,S5	*Glomus*	*Glomus flavisporum*
18	S2,S4,S5	*Glomus*	*Glomus fulvum*
19	S2,S4,S5	*Glomus*	*Glomus geosporum*
20	S3,S2	*Glomus*	*Glomus globiferum*
21	S5	*Glomus*	*Glomus citriculata*
22	S3	*Glomus*	*Glomus glomerulatum*
23	S2,S4,S5	Glomus	Glomus halon
24	S1,S3	*Glomus*	*Glomus hoi*
25	S3	*Glomus*	*Glomus leptotichum*
26	S1,S4,S5	*Glomus*	*Glomus manihot*
27	S5	*Glomus*	*Glomus maculosum*
28	S3,S2	*Glomus*	*Glomus microaggregatum*
29	S5	*Glomus*	*Glomus verseforme*
30	S3	*Glomus*	*Glomus pulvinatum*
31	S1,S2,S3,S4,S5	*Scutelospora*	*Scutelospora nigra*
32	S5	*Scutelospora*	*Scutelospora clavispora*

{S1-Chandur railway, S2- Malkhed, S3-Mardi, S4-Amravati, S5- Pohra}

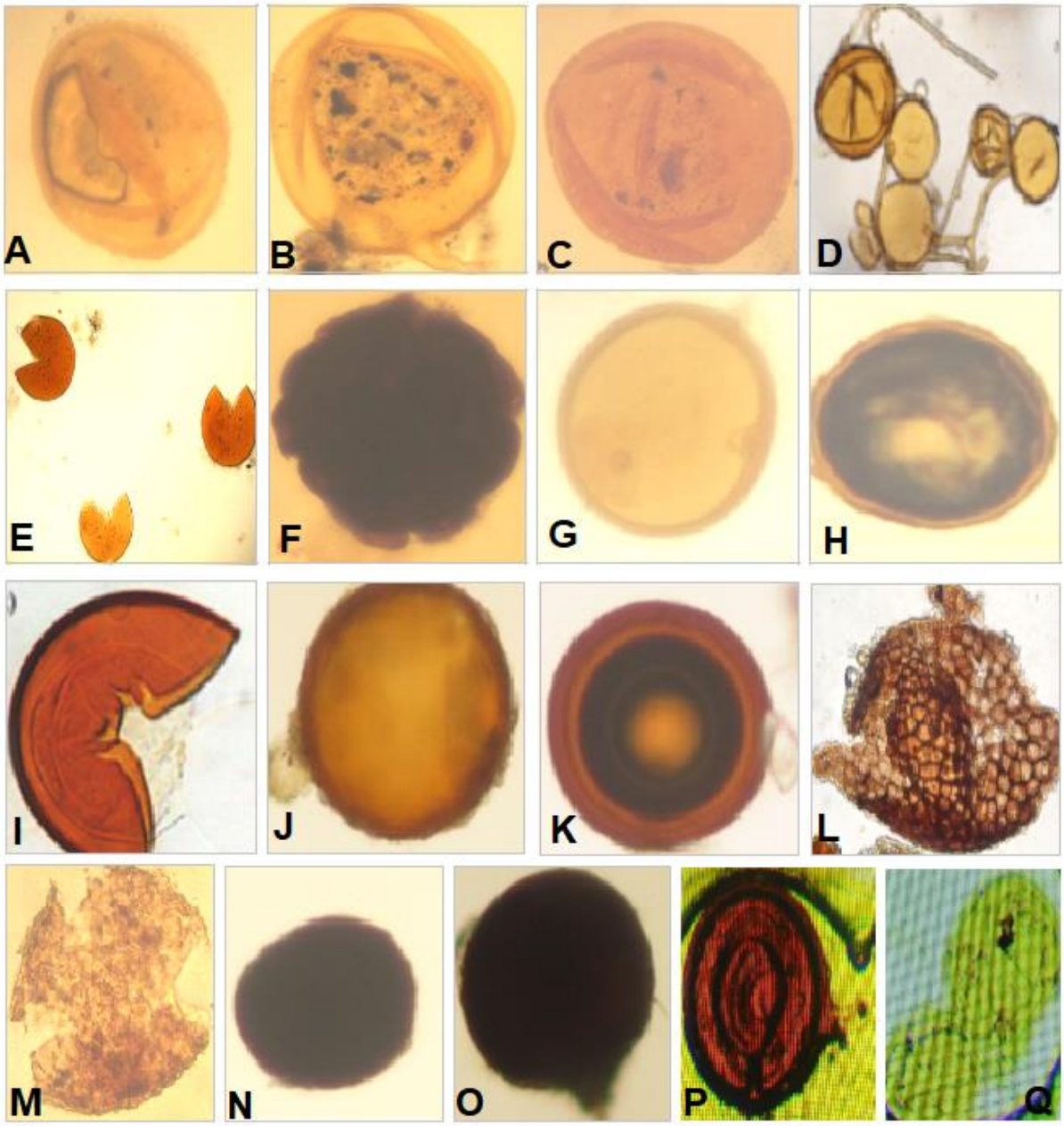

Figure 1 : A, B *Glomus fasciculatum;* **C,D,E:** *G.aggregatum;* **F***: Glomus citriculata;* **G:** *G. dominikii;* **H**- *G.glomerulatum;* **I**- *G.tuberculatum;* **J**: *G.albidum;* **K :**- *A.foveata;* **L, M:** *A. bireticulata;* **N**: *A.sporocarpa;* **O:** *Scutellospora nigra.;* **P :***E.infraquens;* **Q**: *E.colombiane*

Table-2. Total number of AM fungi species as follow

Serial No.	Name of AM Genra	No. of spores found in each Sites Number					No. of Species
		S.1	S.2	S.3	S.4	S.5	
1	*Acaulospora*	4	4	5	5	4	8
2	*Glomus*	8	8	9	8	9	19
3	*Gigospora*	1	-	-	1	-	1
4	*Enthrophosphora*	1	-	1	-	1	2
5	*Scutelospora*	1	1	1	1	2	2

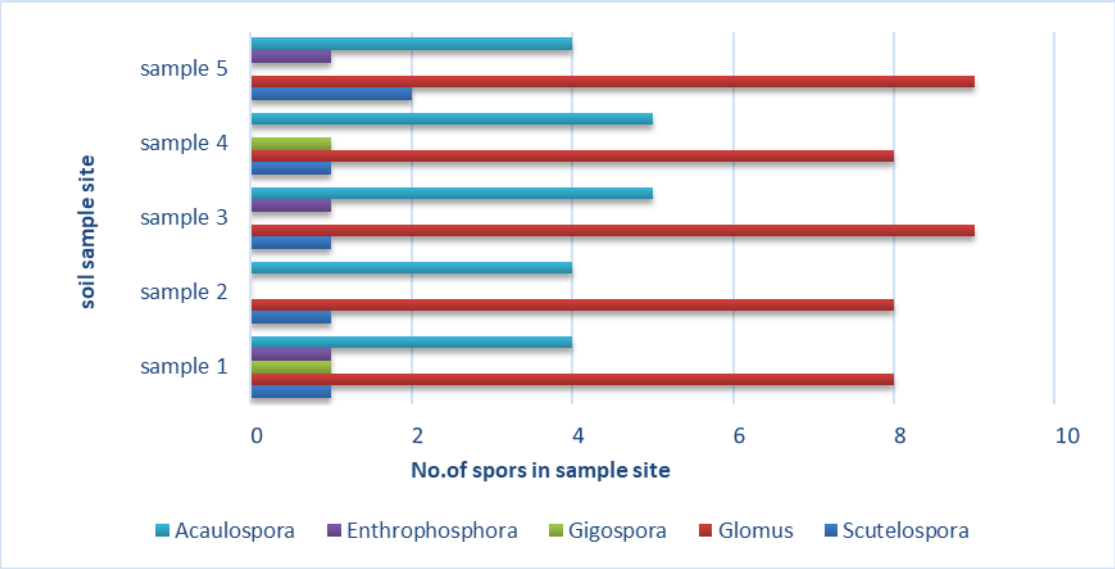

Fig:2. Showing the number of spores found in each soil sample and identified spores in each sample.

Result and Discussion:

An extensive field investigation was carried out in Amravati region, Maharashtra (India), on a *Citrus* plant which belongs to Rutaceae family. The association of AM fungi with *Citrus aurentifolia* plant and their colonization and population in the Rhizosphere is presented in (Table 1). All the sites having low to moderate AM population but in site1 (Chandur railway) maximum population were observed with maximum AMF colonization. More than 102 AM species have so far been reported from India (Manoharachary,C. ,2005). AMF colonization is about 20 % of the fine root segments in *Citrus volkameriana*. A total of 32 AMF species were isolated from rhizospheric soil. Maximum species belongs to *Glomus* (Fig 1-10) and *Acaulospora* (Fig 11-14) isolated species were 19 & 8 in numbers, remaining belongs to *Enthrophosphora, Gigospora* and *Scutelospora* which were 2, 1 & 2 in numbers they were isolated and identified on the basis of their morphological characteristics. Seasonal variation is a major factor in biodiversity of AM fungus was quite evident from the fluctuations of spores in soils (Sampath Kumar, 2001). *Glomus* species were the most commonly found in all type of different soil (Panneerselvam and Thamizhiniyan, 2011; Camprubí and Calvet,1996; Beena *et al.*, 2000; Bhuvaneswari, 2010; Unegbu *et al.*, 2016) and in present work also the major isolated species was belongs to Glomus. *Acaulospora appendicula* and *A. denticulata* were also observed in all five sites. *Gigaspora* spore was frequently observed in the two soils sites (Chandur railway, Amravati), but they were apparently from only one species. The soil sample of village Malkhed (s-2)contains least number of AM spores, the possible reason behind these variation season, age of plant, soil P^H, salinity etc. (Abbott and Robson, 1991; Johnson *et al.*, 1992), whereas Mardi(S3) and Amravati sites (S4)contain the widest variety of AM species. All the five sites of soils exhibit different physico-chemical and microbiological characteristics. Citrus plant mostly contain *Glomus* and *Acaulospora* species in major quantity but absence of other genera or they may be present in low rate is not surprising because sometimes they are not detected at the time of survey of Am fungi, in present survey also found that *Glomus* and *Acaulospora* were present in high rate, (Singh *et al.*,2008). A total 32 species were isolated from all the sites. 19 species belonging to *Glomus*, 8 species from *Acaulospora*, *Enthrophosphora* and *Scutelospora* both were having 2 species and only one species i*n Gigospora. G. fasciculatum* was found in all the five site and *G. aggregatum* found in four site. Similarly *A. appendicula* and *A. denticulate* were found in all the sites.

CONCLUSION

Arbuscular Mycorrhiza is associate with the soil and root of major land plants. AMF is an ecofriendly it increase the soil fertility by up taking phosphorus from the soil and improve plant productivity. AMF is a natural tool so, it is necessary to retain the AM population in soil. In the present study, only few site

having high AM population and that site productivity was good the only reason that site uptake phosphorus compound from the soil, these compound concentration in soil is low but it is soluble phosphorus.

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