Occurrence and Distribution of Arbuscular Mycorrhizal Fungi in Citrus Aurentifolia from Amravati Region

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Abstract

Arbuscular mycorrhizal fungus is a key component of soil, which associate with root and rhizosphere soil in symbiotic association. In the present work, five soil samples were collected from five different sites of Amravati region (Maharashtra). For isolation of AM spores, sieves and decanting method were used and observed that all the collected sample were infected by AM fungi but there population were 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, Entrophospora, Gigaspora, Glomus*

KEYWORDS: Citrus, Rhizosphere soil, AM spore.

Introduction: There is a long history of citrus cultivation in different geographical and climatic regions of the world. It is one of the most popular and extensively cultivated commercial fruit crop among the tropics and subtropics grown in width and length of the world.USA ranks first followed by Spain, India, Italy and Japan. Standing third in position, with respect to the international trade after banana and mango it has become an important horticulture crop of present day. Citrus is successfully planted in both temperate and humid climate of Assam and Meghalaya, subhumid climate of Maharashtra and arid climate of Rajasthan. In other state like Madhya Pradesh, Gujarat, Bihar, Orissa. In India total area under citrus is around 2,00,000 hectares. The *Citrus* belt of Vidarbha is well known for its good quality oranges and taste throughout the nation and abroad. The major producing district of Vidarbha is Amravati which contributes 71.65% share of total citrus cultivation. The orange yield of the region is 4, 91,405 tones, of which Amravati district contributes 66.72% (Dawne, 2007).

Citrus is a mycorrhizotrophic plant and all the species are AM dependent which fails to grow and establish in absence of AM fungi (Menge *et.at.*1978, Vinayak K and D.J. Bagaraj, 1990) Mycorrhizal *Citrus* has greater productivity and plant growth mainly due to increased phosphorus nutrition and other elements such as Cu, Zn, Mg and Fe (Hattings and Gerdemann, 1975). The varied beneficial effects of AMF association on the growth of citrus plants is well documented (Timmer *et.al*,1978, Krikun and Levy 1980). The frequency and abundance of citrus species varies from nation to nation and its application as fresh consumption or processed food products also change invariably.

In India there are 26 states involved in Citrus production but 9 states cover 70% area in which total production is 89% *National Research Center for Citrus Nagpur* (NRCC). Maharashtra is the second largest producer of citrus after Andhra Pradesh in the country and contributes to about 18.9% of the total production of citrus in the country. The state produces 1.41m.MT of citrus from an area of 0.28 m. ha. Having productivity of 5.1 MT/ha. The production of citrus is concentrated in the

belts of Amravati, Nagpur, Akola and Aurangabad. Maharashtra produces 12% of the total production of lime/lemon in the country and is the third largest producer in the country.

Importance of Citrus:

Citrus is a medicinal fruits it contain Vitamin "C", Vitamin "B6", Vitamin "A", Vitamin "E", Niacin, Thiamine, riboflavin, Copper, Calcium, Iron, Magnesium, Potassium, Zinc, phosphorous and Protein. Lemon fruit contain Flavonoids which having cancer fighting properties. It also prevents the diabetes, constipation, high blood pressure, fever, indigestion etc.

Nutritional Value:

The lemon contain approximately 50ml of juice, these juice contain 8% citric acid, (*Penniston, Nakada, Holmes, Assimos, 2008)*. Lemon fruit contain Carbohydrate 9.32g, Sugars 2.50g, Dietary Fiber 2.8g, Fat 0.30g, Protein 1.10g, Vitamin C 53g (64%) along with Thiamin 0.040mg (3%), Riboflavin 0.020mg (2%), Niacin 0.100mg (1%), Pantothenic acid 0.190mg (4%), Vitamin B6 0.080mg (6%), Folate 11µg (3%), Calcium 26mg (3%), Iron 0.60mg (5%), Magnesium 8mg (2%), Phosphorous 16mg (2%), Potassium 138mg (3%) and Zinc 0.06mg (1%).

Role of Mycorrhiza:

Arbuscular Mycorrhizal Fungi, which belong to phylum Glomeromycota *(Schubler et al., 2001),* Arbuscular mycorrhizal 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 *(K.Prasad, 2004)* and registered more species at 15cm. depth**.** (*Charles et.al*. 2008). 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)***.** 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 H. E., 1991).* In the present study, AMF status of *Citrus aurentifolia* from the different villages of Amravati district were investigated and compared

Materials and Method:

Soil and Root Sample:

The Rhizosphere soil and root samples of *Citrus aurantifolia* from five different villages of Amravati district were collected in sterile polythene bags. The collection was carried out in the month of February 2017 from Mardi, Amravati, Chandur Railway, Malkhed and Phora. All soil samples were dried and stored at 4^0C.The rhizosphere soil along with terminal feeder roots were taken from 15-20cm depth and preserved it in the laboratory for further investigation.

Mycorrhizal Percent Colonization :

The grid line intersect method of Giovannetti and Mosse (1980) was used for quantifying AM percent colonization. The Percent root colonization was calculated by the formula

$$\text{Percent infection} = \frac{\text{Total number of infected roots intersecting grid line}}{\text{Total numbers of roots intersecting grid line}} \times 100$$

Quantitative and Qualitative Estimation of AM fungi:

Spore Count

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. Five different rhizosphere soil samples were collected from five different sites of Amravati. The soil was dried at room temperature. 100g of soil sample was mixed in 500ml distilled water. After 1 to $1^{1/2}$ hrs the contents of the beaker were decanted through the series of sieves which were arranged in a descending order as mesh no.0.037 mm (400), 0.053mm (300), 0.075mm (200) and 0.150mm (100). The process was repeated for 4 to 5 times. AM spores retained on the sieves were carefully collected into beaker. A circular filter paper was taken and folded into four equal quadrants. The paper was opened two lines were drawn along the two folds divide the filter into four quadrants. Vertical lines were drawn on one half of the filter paper so as it to divide it into approximately into 15 columns about 0.5 cm apart. Each column was then numbered and the direction of counting was marked by an arrow. The filter paper was then folded in such a manner that the marked portion becomes the receiving surface for the sample during filtration. The filter paper along with sample spore was spread in a bigger petridish. If there were clusters of spores on filter paper they were spread apart by the pressure of water from a pointed wash bottle with a very fine edge needle or syringe. Precautions were taken so that the spores do not go off the filter paper during spreading. The collected spore on petridish was observed under the stereo binocular dissecting microscope. The spores were counted in every space between the two lines, therefore quantification of spores from each soil sample were easy.

Isolation of Mycorrhizal Spores:

In the present study, the wet sieving and decanting technique was used *(Gerdemann and Nicolson, 1963).* 100gm of soil was taken and mixed in 500ml of lukewarm water in a large beaker until all soil aggregates disperse to leave uniform suspension. Heavier particles were allowed to settle down. Sieves were arrange in descending order Mesh no. 400 (0.037mm), 300(0.053mm), 200(0.075mm) and 100(0.150mm). The 0.15mm sieve was used for removal of large organic matter and roots. The content of beaker were decanted through the sieve, process was repeated 4-5 times. The residues retains on the sieves were carefully collected on Whatman filter paper No.1 then Am fungal spores were scanned and mounted on slide in polyvinyl lactic acid as mounting medium.

Taxonomy of AM fungi- 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 layers.

Observation:

Qualitative Analysis of AM fungi:

Root and rhizosphere soil sample of *Citrus aurantifolia* from five different sites of Amravati were collected in sterile polythene bags.

Table-1: Showing Site Number, Genera and identified species

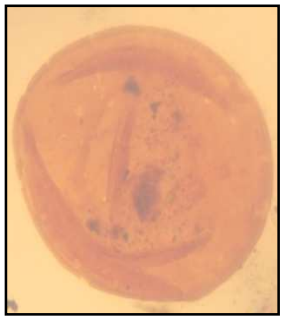
Sr. no.	Site no.	Genera	Species identified
1	S1, S2, S3, S4, S5	*Acaulospora*	*Acaulospora appendicula*
2	S1, S4, S5	*Acaulospora*	*Acaulospora delicate*
3	S2, S4,S5	*Acaulospora*	*Acaulospora bireticulata*
4	S1,S2,S3,S4,S5	*Acaulospora*	*Acaulospora denticulate*
5	S3	*Acaulospora*	*Acaulospora foveata*
6	S2, S4	*Acaulospora*	*Acaulospora lacunose*
7	S1, S3	*Acaulospora*	*Acaulospora laevis*
8	S3	*Acaulospora*	*Acaulospora tuberculata*
9	S5	*Entrophosphora*	*Entrophospora colombiana*
10	S1, S3	*Entrophosphora*	*Entrophospora 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	S3	*Glomus*	*Glomus glomerulatum*
22	S2,S4,S5	Glomus	Glomus halon
23	S1,S3	*Glomus*	*Glomus hoi*
24	S3	*Glomus*	*Glomus leptotichum*
25	S1,S4,S5	*Glomus*	*Glomus manihotis*
26	S5	*Glomus*	*Glomus maculosum*
27	S3,S2	*Glomus*	*Glomus microaggregatum*
28	S5	*Glomus*	*Glomus verseforme*
29	S3	*Glomus*	*Glomus pulvinatum*
30	S1,S2,S3,S4,S5	*Scutelospora*	*Scutellospora nigra*
31	S5	*Scutelospora*	*Scutellospora clavispora*

(S1-Chandur railway, S2- Malkhed, S3-Mardi, S4-Amravati, S5-pohra

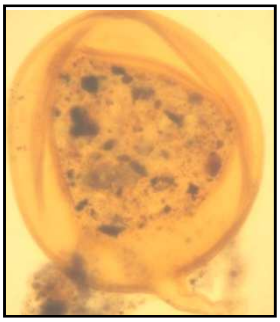
The collected soil sample from all the site showing diversity in AM fungi the observed genera were as *Acaulospora* with eight species *A. appendiculata, A. delicata, A. denticulata and A. laevis, A. bireticulata, A. foveata, A. lacunosa* and *A. tuberculata, . Enthrophospora*, *Gigaspora* and *Scutellospora* were observed in very lesser number such as *E. colombiana* and *E. infrequens, G. decipiens* and *S. clavispora* and *S. nigra.* The *Glomus* genera was found most abundantly with many species such as *G.aggregatum, G. albidum, G. arborense, G. fasciculatum, G.*

fecundisporum, G. flavisporum, G.fulvum, G.geosporum, G. globiferum, G. glomerulatum, G. halon, G. hoi, G. leptotichum, G.manihotis, G. maculosum, G. microaggregatum, G. verseforme and *G. Pulvinatum*

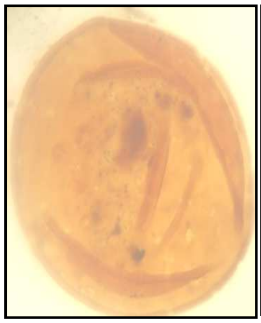
Photo Plate 1:



Glomus fasciculatum

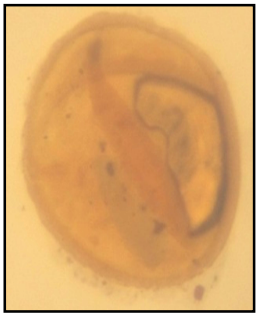


Glomus fasciculatum



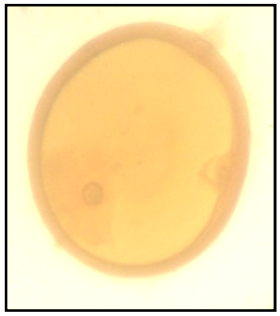
Glomus fasciculatum

Glomus aggrigatum

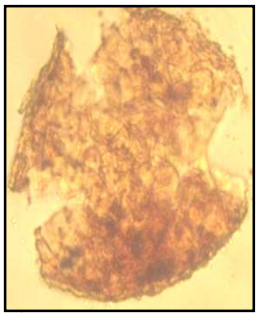


Glomus aggrigatum

Glomus geosporum



Glomus geosporum



Glomus maculosum

Quantitative analysis of AM Fungi -

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 affect on AM fungi population. In the present study, *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 *Enthrophospora* and *Gigospora* found very less in number, *Enthrophospora* Am fungi observed only at site number S-1, S-3, S-5 and *Gigaspora* found in site S-1 and S-4. The total numbers of AM fungi were recorded during the study in soil sample showing in the Table 2.

Table-2 Number of AM fungi species

Sr.No.	Name of AM spore	No. of spores found in each Sites Number					No. of Species
		S1	S2	S3	S4	S5	
1	*Acaulospora*	04	04	05	05	04	08
2	*Glomus*	08	08	09	08	08	19
3	*Gigospora*	01	-	-	01	-	01
4	*Enthrophospora*	01	-	01	-	01	02
5	*Scutellospora*	01	01	01	01	02	02

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

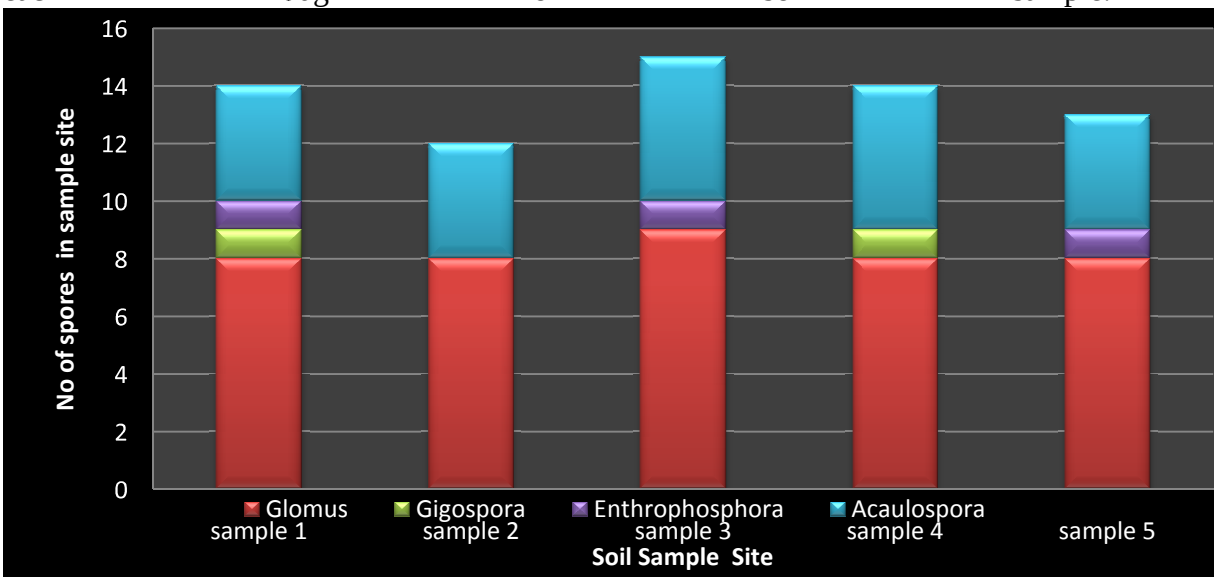


Fig: 1. showing the number of spores found in each soil sample and identified spores in each sample.

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 aurantifolia* 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 maximum population were observed with maximum AMF colonization. More than 102 AM species have so far been reported from India *(Manoharachary, C.2005).* In this study, AM fungi colonization in citrus root is less to moderate. A total of 32 AMF species were isolated and they belonging to five genera viz., *Acaulospora, Glomus, Enthrophospora, Gigospora* and *Scutellospora.* Maximum species belongs to *Glomus* and *Acaulospora* which are 19 & 08, remaining belongs to *Enthrophospora, Gigospora* and *Scutellospora* which are 02, 01 & 02 they were isolated and identified on the basis of their morphological characteristics.

Harley & Smith, 1983. Investigate, Occurrence of AM in all phyla of terrestrial plants from bryophytes to angiosperms. AM associations with plants are wide and geographically ubiquitous. AM fungi are found naturally in all terrestrial ecosystems. Several efforts have been made to mass multiply AM inoculate *(Raman et al 1994)*. Cultures of AM fungi on plants growing in disinfested soil have been the frequently used technique to increase propagule numbers *(Menge,1984).* AM fungi are present in Rhizosphere of all type of soil but seasonality were played important role for AM spore production and Mycorrhiza colonization, Other two has supported these view are *(Selvaraj & Baskaran,1996). Glomus* species were the most commonly found in all type of different soil *(M. Panneerselvam and P. Thamizhiniyan, 2011; Camprubí and Calvet,1996; Beena,K.R. et.al., 2000; Bhuvaneswari T.*,2010*)* and in present work also the major isolated species was *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 pH, salinity etc. *(Abbott, L. and Robson, A.,1991; Johnson, N. C., Tilman, D. and Wedin, D.,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. The number of spores in these Rhizosphere soils was similar to those detected in agricultural soils by other workers (*Hayman & Stovold,1979).*

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*, *Enthrophospora* and *Scutellospora* 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. denticulata* were found in all the sites.

Conclusion:

Arbuscular Mycorrhiza is associate with the soil and root of major land pants. AMF is an eco friendly it increases the soil fertility by up taking phosphorus from the soil and improves 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. Hence it can be concluded that, indigenous most dominant AMF species be utilize for growing healthy and disease free citrus root stock for better growth and yield.

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