Available online at www.ijpab.com

DOI: http://dx.doi.org/10.18782/2582-2845.7768

ISSN: 2582 – 2845

Ind. J. Pure App. Biosci. (2019) *7*(6), 386-401

Research Article

Isolation and Identification of Arbuscular Mycorrhizal (AM) Fungi from The Root Zone Soils of 11 Different Fruit Crops Grown in the Central Campus, MPKV, Rahuri

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Received: 23.09.2019 | Revised: 29.10.2019 | Accepted: 7.11.2019

ABSTRACT

The aim of the present study was to isolate AM fungi from the root zone soils of 11 different fruit crops grown in the central campus, MPKV, Rahuri.

The chemical properties of the root zone soils of the collected sites which included, soil pH, organic carbon, available nitrogen, available phosphorus and micro-nutrients (Fe, Mn, Zn and Cu). As the soil pH is concerned, majority of soils are slightly alkaline (7.1-8.0). Strongly alkaline soil pH (8.58) was recorded in (Block- E, Survey no. 84) and neutral soil pH (7.40) in (Block- E, Survey No. 92).

Soil analysis for organic carbon content showed low to moderate status. The highest organic carbon status was recorded in Block: HF Survey no. 57 (0.66 %) and lowest in Block: E Survey no.78 and survey no. 94 and Block : B Survey no.24 (0.20 %).

The available nitrogen content in the present study ranged from 110 and 225 kg ha^{-1} i.e. very low to low nitrogen content as per six tier rating. The least and highest values of available phosphorus ranged between 10 and 28 kg ha^{-1}. Soil samples showed low to moderate ratings as per six tier rating.

In the present study, micronutrients viz. Fe, Mn, Zn and Cu were determined from 77 root zone soil samples collected. 83.12 per cent of soil samples showed deficit in Fe content, 100 per cent soil samples were deficit in Mn content, 81.82 per cent of soil samples showed deficit in Zn content, where as 3.99 per cent of soil samples showed deficit in Cu content.

A total of 11 AM fungal species were isolated from 77 different root zone soil samples belonging to five different genera viz., Glomus, Aculospora, Gigaspora, Sceutellospora and Rhizophagus. Glomus was the dominant genus followed by Aculospora, Sceutellospora, Rhizophagus and Gigaspora. Glomus mosseae was the most dominant species was recorded. A number of spore morphotypes were detected at each site, according to shape, colour and size. All the spores belonged to Glomerales and Diversisporales orders of AM fungi. A total of 4,243 spores of AMF were wet sieved from the soil samples collected.

Keywords: *AM fungi, Chemical properties, Micronutrients and Root zone soil.*

Cite this article: Raut, S., Deokar, C., Navale, A., & Dahatonde, J. (2019). Isolation and Identification of Arbuscular Mycorrhizal (AM) Fungi from the Root Zone Soils of 11 Different Fruit Crops Grown in the Central Campus, MPKV, Rahuri, *Ind. J. Pure App. Biosci. 7*(6), 386-401. doi: http://dx.doi.org/10.18782/2582-2845.7768

INTRODUCTION

The microbial communities have become an integral part of the biosphere. They play a key role in maintaining the biological equilibrium in biosphere. The living components developed certain relationships such as symbiotic, mutualistic and antagonistic. These relationships helped to maintain biological equilibrium of nature. The word Mycorrhiza (Greek: mykes = mushroom; rhiza = root) was coined by Albert Bernard Frank in 1885. The German Forest Plant Pathologist, to describe the mutual association of two different organisms, plant and fungus, which benefit from each other in a mutualistic symbiosis under ideal conditions, i.e. the plant provides carbohydrates for the fungus, which in turn makes nutrients available for the plant (Harley, 1959).

AM fungi are strictly obligate biotrophs feeding on the products of photosynthesis of their host. As AM fungi are cosmopolitan in distribution, not only bound to particular group of plants but can be found extensively associated with Pteridophytes, Gymnosperms and Angiosperms. They occur in all types of habitats including even sand dunes (Sarmah et al., 2001).

The AM fungi act as biofertilizers, bioregulators and bio protectors (Mulongoy et al., 1992).Various factors like change in pH, temperature, soil moisture content and soil depth etc., influence the distribution of AM fungi.

Facilitated nutrient uptake, particularly with respect to immobile nutrients, such as phosphorus, is believed to be the main benefit of the mycorrhizal symbiosis for plants (Miller & Jastrow, 1990). AMF hyphae produce a cell surface glycoprotein called glomalin, which improves soil aggregation and build up a macro porous structure of soil that allows penetration of water and air, and prevents erosion (Wright & Upadhyaya, 1998). The fungi also help in the uptake of micronutrients like Zn and Cu (Li et al., 1991). Further, the association also provides extraordinary support to the plants during unfavorable environmental stress like drought, increased level of salt, heavy metal toxicity and also during pathogen's attack (Harley & Smith, 1983; Kothari et al., 1991).

AM fungi enable the host plant to access nitrogen in an organic form that would be unavailable (Mukopadhyay & Maiti, 2009). The hyphae of AM fungi have capacity to extract nitrogen and transport it from the soil to the plant. AM fungi improves growth, nodulation and nitrogen fixation in legume-Rhizobium symbiosis. AMF association has tendency to supply more than 50 per cent of nitrogen required by plant (McFarland et al., 2010).

MATERIALS AND METHODS

Materials:-

Laboratory instruments

Different laboratory instruments used during the course of investigation were pH meter, electronic weighing balance, water bath, sieves (500 µm - 250 µm - 125 µm -105 µm - 75 µm – 45 µm) spectrophotometer, stereoscopic zooming microscope, compound microscope, microscope attached with digital camera, etc.

Glassware

Different types of Borosil make glassware, *viz.*, petri dishes, glass slides, cover slips, pipettes, conical flasks and beakers of various capacities, glass rods, volumetric flasks, funnels, measuring cylinders of different capacities *etc.* were used.

Chemicals

The laboratory grade standard and pure chemicals used for study were $HgCl_2$, KOH, potassium dichromate, concentrated hydrochloric acid (HCL), phosphoric acid (H_3PO_4), sodium fluoride (NaF), ferrous sulphate, boric acid, potassium permanganate, sodium hydroxide, sulphuric acid (H_2SO_4), Darco-G 60, sodium bicarbonate, ammonium molybdate, stannous chloride solution *etc.*

MATERIALS AND METHODS

Isolation and identification of AM fungi

Study area: Central Farm Mahatma Phule Krishi Vidyapeeth, Rahuri

Mahatma Phule Krishi Vidyapeeth, Rahuri is the premier Agricultural University established

on March 29, 1968. Location of the campus is between 19^o 47' N to 19^o 57' N latitude and between 74^o 19' N longitude (Plate 1).

The annual maximum and minimum temperature ranges between 30 and 40^oc and 10 to 20^oc during summer and winter respectively. It is about 525 M above MSL with average rainfall of 460 mm. The main horticultural fruit crops cultivated are mango, guava, grapes and pomegranate .The Jurisdiction of MPKV, Rahuri extends over Western Maharashtra consisting of 10 districts *viz.*,Jalgaon, Dhule, Nandurbar, Nashik, Ahmednagar, Pune, Solapur, Satara, Sangali and Kolhapur. The total Area of central campus at Rahuri is 3548.30 ha which includes six blocks *viz.*, A, B, C, D, E, F and Horticulture farm (Plate 2).

Selected fruit crops for sampling

Table 1: List of fruit crops for sampling from orchards at central campus, MPKV, Rahuri

Sr. No.	Fruit Crop	Botanical Name	Number of orchards
1	Mango	*Mangifera indica*	31
2	Lemon	*Citrus limon*	04
3	Sweet orange	*Citrus sinensis*	04
4	Grapes	*Vitis vinefera*	11
5	Pomogranate	*Punica granatum*	05
6	Sapota	*Manilkara achrus*	12
7.	Guava	*Psidium guajava*	05
8.	Onla	*Embilica officinalis*	02
9.	Ber	*Zizipus maurantiana*	01
10.	Fig	*Ficus carica*	01
11.	Custard Apple	*Annona reticulata*	01
	Total no. of orchards :		77

Collection of samples (soil and root)

The soil samples for the present investigation were collected from root zone soil along with root system intact by digging the root zone area of eleven fruit crops from horticulture farm and Chief Scientist (seeds) Farm at central campus, MPKV, Rahuri. Random sampling method was employed. The soil and root samples were collected at a depth of 10-15 cm deep after scrapping away the top litter layer. The individual sample of each crop plant was collected. Representative soil samples were collected in sterilized polythene bags, labeled and stored at 20^0 C in the Department laboratory. The soil samples were air dried in shade and kept in polythene bags for further use. The roots were preserved and later on stained for determination of per cent mycorrhizal colonization.

Chemical properties and available nutrient status of soils collected from root zone of selected fruit crops from central campus MPKV, Rahuri

Estimation of soil pH

Soil pH (1:2.5) was estimated by potentiometry method (Jackson, 1973)

1. Weighted 20g air-dry soil into beaker and add 50 mL distilled water. Stirred at regular intervals for one hour.
2. The pH meter was calibrated using pH 7 buffer solution. Then the pH meter was standarized with known pH of buffer solutions 4.0 and 9.2.
3. Measured the pH of the sample suspension, stirring the suspension well just before introducing the electrodes. pH value determined from the automatic display of the pH meter.
4. Rinsed the electrodes after each determination with water carefully but do not blot them dry with filter paper before the next determination. Standardize the glass electrode after every ten determinations.

Estimation of organic carbon

Organic carbon of soil was estimated by Wet oxidation method (Nelson & Sommers, 1982)

One gram of soil sample was finely ground and passed through 0.5 mm sieve and placed into 500 mL conical flask. 10 mL of 1 N potassium dichromate solution was pipetted using 10 mL pipette and the flask was swirlled gently. 20 mL of concentrated hydrochloric acid was added by measuring cylinder. The flask was swirlled by hand for a minute and set aside on asbestos pad for exactly half an hour. At the end of half an hour, 200 mL of distilled water was added; add 10 mL of H_3PO_4 and 0.2 g of NaF and 3-4 drops of ferroin indicator. Titrate the contents of the flask against 0.5 N ferrous sulphate solution till the colour changes from brown-green- blue to finally red. Run the blank determination in the same manner.

Estimation of available nitrogen content

Available nitrogen content in the soil was estimated by alkaline permanganate method (Subbiah & Asija, 1956)

20 g of sieved soil (2 mm) was transferred, into one-liter round bottom flask. Little distilled water added with the help of jet in such a way that the particles of soil do not remain stuck to the sides of the flask. 2 to 3 glass beads were added to prevent bumping and 1 mL of liquid paraffin to prevent frothing. 100 mL of potassium permanganate and 100 mL of sodium hydroxide solution were added to the flask. Both the solutions were prepared fresh. Distillate was collected in a beaker containing 20 mL of boric acid working solution. Approximately 150 mL of distillate was collected. The distillate was titrated with standard 0.02 N H_2SO_4 till the colour changed from green to red and the burette reading was recorded. Blank was carried out without soil.

Estimation of available phosphorus

Determination of available phosphorus from soil was done by Olsen P method (Watanable & Olsen 1965)

Weighted 5 g of sieved soil (2mm), into a 250 mL conical flask. A pinch of phosphorus free Darco-G 60 and 50 mL of 0.5 N sodium bi carbonate solution (soil:solution-1:10) was added. Shaked the contents for 30 minutes. The contents were filtered using Whatman No.42 filter paper. 5 mL of the filtrate was pipetted into 25 mL volumetric flask and added 5 mL of ammonium molybdate solution. Mixed well until the evolution of CO_2 ceases. 10 mL of distilled water was added about washing the neck of the flask to remove the adhering molybdate. 1 mL of working stannous chloride solution was added and maked the volume to the mark with distilled water. The blank was runned with similar determination and colour intensity was measured at 882 nm on spectrophotometer.

Estimation of micronutrients

Available micronutrients (Fe, Mn, Zn& Cu) from soil were estimated by DTPA extractant (Atomic Absorption spectrophotometer) (Lindsay & Norvell 1978)

The content of soil were estimated by using Atomic absorption spectrophotometer with appropriate hallow cathode lamps (Lindsay & Norwvell, 1978).

20 g sieved (2 mm) air-dried soil was weighted in 150 mL conical flask. Extracting solution (DTPA) in proportion of 1:2 (soil: DTPA extracting solution) was added and shaked the sample for 2 hours on a horizontal shaker. Suspension was then filtered by gravity through Whatman no. 42 filter paper. The digested material was directly fed to Atomic absorption spectrophotometer (AAS) with appropriate hallow cathode lamps (Fe, Mn, Zn & Cu) with suitable dilutions and concentration of these elements was recorded in ppm by referring standard curve.

Isolation of AM fungi

The extraction of AM fungal spores and sporocarps from root zone soil was done by wet sieving and decanting method (Gerdemann & Nicloson, 1963). The procedure is as follows,

1. A quantity of hundred (100) grams of root zone soil was suspended in 1000ml of tap water. The mixture was

stirred for 10-15 seconds. Heavier coarse particles were allowed to settle in water for 1-2 minutes.

2. The supernant was decanted through sieves stacked in descending order of mesh size (500µm-250 µm -125 µm - 105 µm -75 µm - 45µm).

The above steps were repeated twice to ensure that the majority of spores were extracted from the sampled rhizosphere soil.

1. The contents left on each sieve were backwashed by water to remove turbidity of spores.
2. The residue (soil particles and spores) from each sieve was collected separately in beakers.
3. The residue was filtered through Whatman No.1 filter paper.
4. The filter paper was placed on petri dish and care was taken to see that it remained moist.
5. The contents of the Whatman No.1 filter paper were examined for spores and sporocarps under stereomicroscope.

3 Identification of AM fungi

The isolated AMF spores and sporocarps were picked up using a dissecting needle under a dissecting microscope and mounted in polyvinyl alcohol-lactoglycerol (PVLG) with Meltzer‘s reagent on a glass slide for identification. Taxonomic identification of spores was done upto species level based on size, colour, spore wall ornamentation, presence or absence of flexible inner walls and shape and size of subtending hypha of the spores.

Taxonomic identification of spores was carried out by matching the descriptions provided by The International Culture Collection of Vesicular Arbuscular Mycorrhizal Fungi (INVAM) (http://www.invam.caf.wvu.edu). The identification was done according to their spore morphology and wall characteristics. The photographs of AMF spores were taken with the help of Olympus imaging corp., digital camera model no. E-330 attached with Olympus CX31 microscope.

RESULT AND DISCUSSION

Isolation and identification of Arbuscular Mycorrhizal (AM) fungi.

Isolation

Collection of samples (soil and roots)

The soil samples for the present investigation were collected from root zone soil along with the root system intact by digging the rhizosphere area of eleven fruit crops from Horticulture Farm and Chief Scientist (seeds) Farm at the central campus, MPKV, Rahuri. Random sampling method was employed. The soil samples were collected from a depth of 10-15 cm deep after scrapping away the top litter layer. The individual sample of each crop plant was collected. Representative soil samples were collected in the sterilized polythene bags, labeled and stored at 20^0C in the Departmental laboratory. The soil samples were air dried in shade and kept again in polythene bags for further use. The roots were preserved and later on stained for determination of percent mycorrhizal colonization. Similar root and soil samples collection was carried carried out by Gashua (2015) and Sunita (2017).

Soil parameters

The collected soil samples from 77 orchards were analyzed for soil pH, organic carbon, available nitrogen, available phosphorus and micro-nutrients (Fe, Mn, Zn and Cu).

The data on chemical properties of the root zone soil samples of different fruit crops collected from 77 different places is presented in Table 2 and 3. The physicochemical properties of the rhizosphere soil of the collected sites included the soil pH, organic carbon, available nitrogen, available phosphorus (Table 2) and micro-nutrients (Fe, Mn, Zn and Cu) (Table 3). The soil provides the physical support needed for the anchorage of the root system of plant and also serves as the reservoir of air, water and nutrients which are essential for plant growth.

The effect of diverse edaphic factors was observed on the occurrence and distribution of AM fungal species through these studies. AM fungal species were found to be distributed well over different soil

samples. However, their population varied significantly. In the present study, it was observed that 100 % of the sites accounted for the presence of AM fungi.

Effect of soil properties on the distribution of AM fungi:

The chemical characteristics of soil samples from all the 77 study sites are presented in Tables 4.1. It is seen that majority of the soils are slightly alkaline (7.1-8.0). Higher (strongly alkaline) soil pH (8.58) was recorded in mango fruit crop (Block- E, Survey no. 84) and low value (neutral) of soil pH (7.40) in mango fruit crop (Block- E, Survey No. 92). All the root samples collected from 77 locations were found to be infected with AM fungi irrespective of soil pH.

4.1.1.2.2 Organic carbon

Soil analysis for organic carbon (%) shows low to moderate organic carbon content. The highest organic carbon was recorded in grape fruit crop (Block: HF Survey no. 57) i.e., 0.66 per cent and lowest in mango fruit crop (Block: E Survey no.78 and survey no. 94) and guava fruit crop (Block: B Survey no.24) *i.e.*, 0.20 per cent, respectively. Majority of the soil samples showed low to moderate range of organic carbon (%).

4.1.1.2.3 Available Nitrogen

The soil analysis showed that very low status of available nitrogen content in soil was seen from mango fruit crop (Block: A, Survey no.137, Block: E, Survey no. 78 and 94) and guava fruit crop (Block: B, Survey no.24) *i.e.*, 110 kg ha^{-1}. Low status of available nitrogen content was found in soil from ber fruit crop (Block: HF, Survey no.165) 225 kg ha^{-1}. The remaining fruit crops showed nitrogen content in the range of 110 and 225 kg ha^{-1} *i.e.*, very low to low status of available nitrogen content as per six tier rating.

4.1.1.2.4 Available Phosphorus

The above soil analysis data has shown low status of available phosphorus found in soil samples (10 kg ha^{-1}). High status of available phosphorous content in soil was found in sapota fruit crop (Block: HF, Survey no. 45) *i.e.*, 28 kg ha^{-1}. The low and high status of available phosphorus ranged between 10 kg ha^{-1} and 28 kg ha^{-1}. Soil samples showed low to moderate ratings as per six tier rating.

4.1.1.2.5 Micronutrients

In the present study, micronutrients *viz.*, Fe, Mn, Zn and Cu were estimated from 77 root zone soil samples collected. 83.12 per cent of soil samples showed deficit in Fe content, 100 % soil samples were deficit in Mn content, 81.82 per cent of soil samples showed deficit in Zn content, where as 3.99 % of soil samples showed deficit in Cu content (Table 3.1).

The spore distribution, spore density and the composition of AM fungi were observed to be influenced by environmental and chemical factors of soil. The AM spore population, percentage of root colonization and distribution was affected by pH and soil mineral nutrient status such as N, P, K, Zn, Fe, etc. The earlier studies carried out by Khade & Rodrigues (2009), Gaur (2011) and Patale (2018) also showed a similar trend.

4.1.2 Identification of AM fungi.

4.1.2.1 AM fungal species identified from each sample site

The isolation and identification of AM fungi in the rhizosphere soils of selected fruit crops from central campus MPKV, Rahuri was done. Altogether, 11 AM fungal species were isolated from seventy seven different rhizosphere soil samples belonging to five different genera *viz.*, *Glomus*, *Aculospora*, Gigaspora, *Sceutellospora* and *Rhizophagus* (Table 4). *Glomus* was the dominant genus followed by *Aculospora*, *Sceutellospora*, *Rhizophagus* and *Gigaspora*. *Glomus mosseae* the most dominant species was recorded. Similar work of AM fungi isolation and identification was done by several researchers who isolated and identified the AM fungal species. Kavita & Nelson (2013) described seven species of *Glomus* from sunflower rhizosphere. Similarly, in this study also four species of *Glomus* were isolated and identified.

The present findings were in agreement with Charoenpakdee et al. (2010) who identified thirty-four morphospecies of AMF using spore characteristics. *Acaulospora* and *Glomus* occurred most frequently and

overall, were the most prevalent, containing 16 and 10 species respectively. There were 5 species in *Scutellospora*, 2 species in *Gigaspora* and 1 species in *Entrophospora* across 10 sampling sites. Also, Kumar Vinod et al. (2016) isolated and identified thirteen taxa of AM fungi from 105 rhizosphere soils and root samples of litchi (*Litchi chinnensis* Sonn.) trees. Among these, 8 species belonged to the genus *Glomus*, 2 species to *Aculospora* and one species each to *Rhizophagus*, *Entrophagus* and *Sceutellospora. Glomus* was observed to be predominant followed by *Aculospora in* the rhizosphere soil of lichi.

Similarly, D'Souza (2012) recovered twenty eight species of AM fungi of five genera, *viz. Glomus*, *Acaulospora*, *Scutellospora*, *Gigaspora* and *Entrophospora* from rhizosphere soils of seventeen mangrove species of eight families at seven riverine and fringe habitats in Goa. *Glomus* (16 species) was the dominant genus followed by *Acaulospora* (6 species), *Scutellospora* (4 species), *Gigaspora* (1 species) and *Entrophospora* (1 species). *Glomus* was the dominant genus, three species of which were sporocarpic forms. Motha (2014) isolated and identified 20 AM fungal species from ten different rhizosphere soils of brinjal in Andhra Pradesh, India belonging to the genera of *Glomus*, *Acaulospora*, *Gigaspora*, *Sclerocystis* and *Entrophospora. Glomus* was observed to be predominant followed by *Acaulospora* in the rhizosphere soils of brinjal.

Gaur & Kaushik (2011) isolated a total of 16 species of AM fungi from three medicinal plants of which more than fifty per cent of the total species identified belonged to the genus *Glomus*. Ajaz et al. (2017) identified arbuscular mycorrhizal fungi by considering spore morphology and wall characteristics.

4.1.2.2 Morphology of AM fungal spores identified

The number of spore morphotypes detected at each site according to shape, colour and size are presented in Table-5 .Most of the morphotypes were common to all sites and few were specific. All spores belonged to Glomerales and Diversisporales orders of AM fungi. A total of 4,243 spores of AMF were wet sieved from soil samples collected from eleven different fruit crops cultivated in seventy seven localities of central campus, MPKV, Rahuri. Spore morphology-based identification of AM fungal species indicated the dominance of *Glomus* species in the soils used in this study. Members of other genera were also detected, including those of *Aculospora*, *Gigaspora*, *Sceutellospora* and *Rhizophagus*. The predominant AM fungal morphotypes recognized in these soils included *Glomus mosseae, Glomus aggregatum, Glomus fasciculatum, Glomus epigaeum, Rhizophagus intraradices, Gigaspora albida, Rhizophagus irregularis* and minor species identified in these soils included *Aculospora scrobiculata Aculospora denticulate, Sceutellospora arenicola and Sceutellospora heterogama.*

Table 2: Chemical properties and available N and P content of soils collected from root zone of selected fruit crops from central campus MPKV, Rahuri

Sr. No.	Fruit crop	Block	Survey No.	pH (1:2.5)	Organic carbon (%)	Available nitrogen content (kg ha^{-1})	Available Phosphorus (kg ha^{-1})
1	Mango	HF	28	7.79	0.22	118	10
2		HF	29	7.62	0.30	150	14
3		HF	30	7.62	0.28	135	12
4		HF	37	7.81	0.45	143	14
5		HF	39	7.96	0.25	120	10
6		HF	69	7.68	0.39	130	12
7		HF	73	7.59	0.45	168	20
8		HF	74	7.93	0.25	135	11
9		HF	166	7.90	0.28	130	13
10		HF	168	7.95	0.34	150	15

11		A	136	8.20	0.37	162	16
12		A	137	8.12	0.24	110	10
13		B	8	7.73	0.48	163	18
14		B	71	8.04	0.32	155	15
15		B	72	8.00	0.27	125	12
16		E	75	8.02	0.39	138	12
17		E	78	8.09	0.20	110	10
18		E	79	8.16	0.32	147	16
19		E	80	8.20	0.48	170	19
20		E	81	7.92	0.25	128	10
21		E	82	8.03	0.28	130	13
22		E	83	8.11	0.33	140	14
23		E	84	8.58	0.62	195	22
24		E	90	7.45	0.39	160	17
25		E	91	7.62	0.52	178	20
26		E	92	7.40	0.45	160	17
27		E	93	7.41	0.21	115	10
28		E	94	8.16	0.20	110	10
29		E	102	8.09	0.25	135	12
30		E	103	7.62	0.32	140	15
31		E	119	7.93	0.32	145	16
32	Lemon	HF	30	7.65	0.21	115	10
33		HF	39	7.79	0.61	190	20
34		HF	40	7.92	0.40	172	18
35		HF	166	7.84	0.35	135	15
36	Sweet Orange	HF	30	7.76	0.40	162	17
37		HF	39	7.98	0.22	112	10
38		HF	40	7.96	0.34	150	14
39		HF	166	7.85	0.43	138	13
40	Grape	HF	27	7.84	0.61	190	23
41		HF	27	7.65	0.27	135	12
42		HF	27	7.79	0.35	162	18
43		HF	27	7.41	0.30	134	11
44		HF	27	7.45	0.66	213	28
45		HF	27	7.92	0.48	180	19
46		HF	27	7.94	0.26	125	11
47		HF	27	7.99	0.24	120	11
48		HF	27	7.74	0.52	170	21
49		HF	27	7.55	0.26	130	11
50		HF	27	7.84	0.38	155	18
51	Pomogranate	HF	40	7.73	0.59	188	22
52		HF	43	7.76	0.50	175	20
53		HF	72	8.10	0.30	140	11
54		B	12	8.21	0.32	150	15
55		E	70	8.29	0.38	166	18
56	Sapota	HF	41	7.75	0.54	185	22
57		HF	42	7.78	0.35	152	17
58		HF	43	7.90	0.32	135	11
59		HF	45	7.76	0.34	155	28
60		HF	47	7.96	0.47	172	20
61		HF	49	7.64	0.45	168	20
62		E	63	8.14	0.45	135	17
63		E	64	8.29	0.34	138	15
64		E	73	7.96	0.47	170	18
65		E	75	7.95	0.43	166	16
66		E	76	8.00	0.50	180	20
67		E	93	8.16	0.62	210	25
68	Guava	HF	25	7.78	0.60	190	20
69		HF	26	7.98	0.34	160	18
70		HF	44	8.21	0.50	183	20

71		HF	67	7.85	0.33	150	14
72		B	24	7.98	0.20	110	10
73	Onla	HF	43	7.90	0.36	150	16
74		D	72	8.22	0.50	198	15
75	Ber	HF	165	7.55	0.69	225	10
76	Fig	HF	169	7.96	0.24	125	19
77	Custard Apple	HF	72	8.10	0.32	145	14

2.1 Soil pH

Rating of pH.

Sr. No.	Ratings	pH (1:2.5)
1	Extremely acidic	<4.5
2	Strongly acidic	4.6-5.5
3	Moderately acidic	5.6-6.5
4	Slightly acidic	6.6-6.9
5	Neutral	7.0
6	Slightly alkaline	7.1-8.0
7	Moderately alkaline	8.1-9.0
8	Strongly alkaline	9.1-10.0
9	Very strongly alkaline	10.1-11.0

Patil & Mali (1999)

2.2 Organic carbon

Six tier ratings of organic carbon and available nutrients.

Sr. No.	Ratings	Organic carbon (%)	Available Nutrients (kg $ha^{-1)}$		
			N	P	K
1	Very Low	<0.20	<140	<7	<100
2	Low	0.21-0.40	141-280	7.1-14	101-150
3	Moderate	0.41-0.60	281-420	14.1-21	151-200
4	Moderately high	0.61-0.80	421-560	21.1-28	201-250
5	High	0.81-1.0	561-700	28.1-35	251-300
6	Very High	>1.0	>700	>35	>300

Bangar & Zende (1978)

Table 3: Status of micro-nutrients (Fe, Mn, Zn and Cu) of soils collected from root zone of selected fruit crops from central campus MPKV, Rahuri

Sr. No.	Fruit crop	Block	Survey No.	Available micro-nutrient (mg kg^{-1})			
				Fe	Mn	Zn	Cu
1	Mango	HF	28	2.67	1.81	0.44	0.55
2		HF	29	2.05	1.95	0.42	0.20
3		HF	30	2.28	1.95	0.43	0.22
4		HF	37	2.92	1.42	0.48	0.55
5		HF	39	2.66	1.81	0.45	0.55
6		HF	69	2.18	1.68	0.46	0.22
7		HF	73	2.06	1.45	0.45	0.50
8		HF	74	3.30	1.68	0.57	0.97
9		HF	166	2.75	1.98	0.43	0.25
10		HF	168	2.48	1.81	0.41	0.20
11		A	136	4.65	1.21	0.63	0.95

12		A	137	4.68	1.25	0.63	0.93
13		B	8	2.11	1.26	0.41	0.37
14		B	71	2.89	1.81	0.47	0.80
15		B	72	2.85	1.42	0.46	0.75
16		E	75	4.79	1.95	0.68	0.92
17		E	78	1.64	1.95	0.30	0.26
18		E	79	2.18	1.68	0.42	0.85
19		E	80	2.05	1.45	0.45	0.34
20		E	81	4.32	1.98	0.55	0.55
21		E	82	4.37	1.82	0.52	0.95
22		E	83	4.45	1.82	0.56	0.74
23		E	84	4.22	1.66	0.51	0.85
24		E	90	4.32	1.25	0.57	0.68
25		E	91	4.35	1.25	0.55	0.75
26		E	92	4.25	1.82	0.57	0.76
27		E	93	4.33	1.66	0.57	0.78
28		E	94	4.53	1.24	0.60	0.75
29		E	102	4.66	1.25	0.60	0.93
30		E	103	4.10	1.98	0.55	0.95
31		E	119	4.32	1.45	0.67	0.80
32	Lemon	HF	30	2.25	1.60	0.58	0.85
33		HF	39	2.65	1.82	0.53	0.48
34		HF	40	2.48	1.65	0.51	0.14
35		HF	166	2.05	1.55	0.40	0.17
36	Sweet Orange	HF	30	2.28	1.62	0.43	0.68
37		HF	39	2.45	1.82	0.42	0.89
38		HF	40	2.28	1.85	0.43	0.81
39		HF	166	2.18	1.83	0.38	0.65
40	Grape	HF	27	1.80	1.90	0.32	0.85
41		HF	27	1.94	1.32	0.34	0.31
42		HF	27	2.21	1.62	0.46	0.65
43		HF	27	2.52	1.16	0.48	0.48
44		HF	27	2.74	1.39	0.50	0.14
45		HF	27	4.39	1.28	0.57	0.82
46		HF	27	4.42	1.21	0.58	0.89
47		HF	27	4.59	1.18	0.60	0.71
48		HF	27	4.44	1.90	0.58	0.73
49		HF	27	4.45	1.51	0.59	0.93
50		HF	27	4.42	1.64	0.53	0.80
51	Pomogranate	HF	40	4.45	1.52	0.56	0.77
52		HF	43	4.49	1.73	0.59	0.73
53		HF	72	4.45	1.24	0.56	0.80
54		B	12	4.41	1.89	0.51	0.83
55		E	70	4.45	1.30	0.56	0.89
56	Sapota	HF	41	4.52	1.79	0.60	0.77
57		HF	42	4.43	1.96	053	0.82
58		HF	43	4.57	1.94	0.60	0.85
59		HF	45	4.37	1.04	0.51	0.85
60		HF	47	4.68	1.60	0.60	0.88
61		HF	49	4.48	1.02	0.59	0.87
62		E	63	3.77	1.05	0.51	0.80
63		E	64	4.32	1.34	0.59	0.75

64		E	73	4.52	1.52	0.60	0.76
65		E	75	4.13	1.86	0.51	0.77
66		E	76	4.23	1.02	0.52	0.78
67		E	93	4.22	1.65	0.52	0.72
68	Guava	HF	25	4.20	1.51	0.50	0.69
69		HF	26	4.32	1.02	054	0.63
70		HF	44	4.25	1.51	0.50	0.62
71		HF	67	4.46	1.60	059	0.80
72		B	24	4.55	1.04	0.60	0.86
73	Onla	HF	43	4.20	1.69	0.52	0.70
74		D	72	4.55	1.96	0.60	0.97
75	Ber	HF	165	4.54	1.28	0.60	0.87
76	Fig	HF	169	4.38	1.64	0.54	0.83
77	Custard Apple	HF	72	4.11	1.51	0.48	0.70

3.1 Micronutrients

Critical limit of available micronutrients (mg kg^{-1})

Content	Micronutrients (mg kg $^{-1}$)			
	Fe	Mn	Zn	Cu
Deficit	< 4.5	< 2.0	< 0.6	< 0.2

Katkar & Patil (2010)

Table 4: AM fungal species identified from each sample site of selected fruit crops from orchards at central campus, MPKV, Rahuri

Sr. No.	Fruit crop	Block	Survey No.	AM fungal spores
1	Mango	HF	28	*Glomus mosseae, G. epigaeum*
2		HF	29	*Glomus fasciculatum, G. aggregatum, G. epigaeum*
3		HF	30	*Glomus mosseae, G. epigaeum*
4		HF	37	*Glomus mosseae, G. fasciculatum*
5		HF	39	*Glomus aggregatum, G. epigaeum*
6		HF	69	*Glomus mosseae*
7		HF	73	*Glomus mosseae, G. fasciculatum , G. aggregatum*
8		HF	74	*Glomus mosseae, G. epigaeum*
9		HF	166	*Glomus fasciculatum*
10		HF	168	*Glomus mosseae, G. fasciculatum*
11		A	136	*Glomus fasciculatum, G. aggregatum, G. epigaeum*
12		A	137	*Glomus aggregatum, G. epigaeum*
13		B	8	*Glomus mosseae, G. aggregatum*
14		B	71	*Glomus aggregatum, G. epigaeum*
15		B	72	*Glomus mosseae, G. epigaeum*
16		E	75	*Glomus mosseae*
17		E	78	*Glomus mosseae, G. fasciculatum*
18		E	79	*Glomus fasciculatum , G. aggregatum, G. epigaeum*
19		E	80	*Glomus mosseae, G. fasciculatum, G. aggregatum*
20		E	81	*Glomus epigaeum*
21		E	82	*Glomus mosseae*
22		E	83	*Glomus mosseae, G. aggregatum*
23		E	84	*Glomus fasciculatum, G. aggregatum, G. epigaeum*
24		E	90	*Glomus mosseae, G. fasciculatum*

25		E	91	*Glomus mosseae, G. aggregatum, G. epigaeum*
26		E	92	*Glomus mosseae, G. epigaeum*
27		E	93	*Glomus mosseae*
28		E	94	*Glomus fasciculatum*
29		E	102	*Glomus mosseae*
30		E	103	*Glomus fasciculatum*
31		E	119	*Glomus mosseae, G. fasciculatum*
32	Lemon	HF	30	*Glomus mosseae, Aculospora scrobiculata*
33		HF	39	*Glomus aggregatum, Aculospora scrobiculata*
34		HF	40	*Glomus mosseae, G. aggregatum*
35		HF	166	*Glomus mosseae, Aculospora scrobiculata*
36	Sweet Orange	HF	30	*Glomus mosseae, G. aggregatum*
37		HF	39	*Glomus aggregatum*
38		HF	40	*Glomus mosseae, G. aggregatum*
39		HF	166	*Glomus mosseae*
40	Grape	HF	27	*Glomus mosseae, G. aggregatum, Gigaspora albida*
41		HF	27	*Glomus aggregatum*
42		HF	27	*Glomus mosseae, Gigaspora albida*
43		HF	27	*Gigaspora albida*
44		HF	27	*Glomus mosseae, G. aggregatum, Gigaspora albida,*
45		HF	27	*Glomus mosseae, G. aggregatum*
46		HF	27	*Glomus aggregatum, Gigaspora albida*
47		HF	27	*Glomus mosseae, Gigaspora albida*
48		HF	27	*Glomus mosseae, G.aggregatum, Gigaspora albida*
49		HF	27	*Glomus mosseae, Gigaspora albida*
50		HF	27	*Glomus aggregatum*
51	Pomogranate	HF	40	*Glomus mosseae, G. aggregatum*
52		HF	43	*Glomus mosseae, Gigaspora albida*
53		HF	72	*Glomus aggregatum*
54		B	12	*Glomus mosseae*
55		E	70	*Glomus mosseae, G. aggregatum*
56	Sapota	HF	41	*Glomus mosseae, Rhizophagus intraradices, Rhizophagus irregularis*
57		HF	42	*Glomus mosseae*
58		HF	43	*Rhizophagus intraradices*
59		HF	45	*Glomus mosseae*
60		HF	47	*Glomus mosseae, Rhizophagus intraradices*
61		HF	49	*Glomus mosseae*
62		E	63	*Glomus mosseae, Rhizophagus intraradices*
63		E	64	*Rhizophagus irregularis*
64		E	73	*Glomus mosseae, Rhizophagus intraradices*
65		E	75	*Rhizophagus intraradices , Rhizophagus irregularis*
66		E	76	*Glomus mosseae, Rhizophagus intraradices*
67		E	93	*Glomus mosseae, Rhizophagus irregularis*
68	Guava	HF	25	*Rhizophagus intraradices, Rhizophagus irregularis*
69		HF	26	*Glomus mosseae.*
70		HF	44	*Glomus mosseae, Rhizophagus intraradices*

71		HF	67	*Glomus mosseae*
72		B	24	*Rhizophagus intraradices*
73	Onla	HF	43	*Glomus epigaeum*
74		D	72	*Glomus mosseae*
75	Ber	HF	165	*Glomus mosseae, Aculospora denticulate, Sceutellospora arenicola, S. heterogama*
76	Fig	HF	169	*Glomus mosseae*
77	Custard Apple	HF	72	*Glomus mosseae, Aculospora denticulate*

Table 5: Morphology of AM fungal spores identified from root zone soil samples of selected fruit crops from orchards at central campus, MPKV, Rahuri

Sr. No.	Spore type	Shape	Spore diameter (µm)	Colour of wall	Number of wall layer
1.	*Glomus mosseae*	Globose-elliptical	105-310 x 110-305	Brownish yellow	Double layered
2.	*Glomus aggregatum*	Globose-subglobose	67-90	Brown	Double layered
3.	*Glomus fasciculatum*	Globose-subglobose	75-150 x 35-100	Light brown	Single layered
4.	*Glomus epigaeum*	Globose-subglobose	80-150	Pale yellow to deep yellow	Double layered
5.	*Aculospora denticulate*	Globose-subglobose	112-149	Red brown	1-4 layered
6.	*Aculospora scrobiculata*	Globose-subglobose	100-240 x 100-220	Greenish yellow	Four layered
7.	*Gigaspora albida*	Globose	232-252x 234-250	Greenish yellow	One-six layered
8.	*Sceutellospora arenicola*	Subglobose to irregular	160-270	Orange- brown	Double layered
9.	*Sceutellospora heterogama*	Globose-subglobose	150-220	Yellow brown-red brown	Four layered
10.	*Rhizophagus irregularis*	Globose-subglobose, ovid, oblong or irregular	70-165	Pale yellow to yellow brown	Three layered
11.	*Rhizophagus intraradices*	Globose-subglobose	40-140	Yellow brown	Three layered

CONCLUSION

The present investigation was carried out with a view to isolate and identify AM fungi collected from root rhizosphere soils of eleven different fruit crops from 77 orchards at central campus, MPKV, Rahuri, Maharashtra.

The spore distribution, spore density and the composition of AM fungi were observed to be influenced by environmental and physico-chemical factors of soil. The AM spore population and distribution were affected by pH and soil mineral nutrient status such as organic carbon, available N, available P, Fe, Mn, Zn and Cu.

Eleven AM fungal species were isolated from seventy seven different rhizosphere soil samples belonging to five different genera *viz.*, *Glomus*, *Aculospora*, *Gigaspora*, *Sceutellospora* and *Rhizophagus*. *Glomus* was the dominant genus followed by *Aculospora*, *Sceutellospora*, *Rhizophagus* and *Gigaspora*. *Glomus mosseae* was the most dominant species recorded.

All the isolated spores belonged to Glomerales and Diversisporales orders of AM fungi. A total of 4,243 spores of AMF were wet sieved from soil samples.The spore morphology-based identification of AM fungal

species indicated the dominance of *Glomus* species in soils used in this study. Members of other genera were also detected, including those of *Aculospora, Gigaspora, Sceutellospora* and *Rhizophagus.*The predominant AM fungal morphotypes recognized in these soils included *Glomus mosseae, Glomus aggregatum, Glomus fasciculatum, Glomus epigaeum, Rhizophagus intraradices, Gigaspora albida, Rhizophagus irregularis* and minor species identified in these soils included *Aculospora scrobiculata Aculospora denticulate, Sceutellospora arenicola and Sceutellospora heterogama.*

1. AM fungal species belonging to five different genera *viz., Glomus, Aculospora, Gigaspora, Sceutellospora* and *Rhizophagus* were recovered from the rhizosphere soil samples.
2. The most dominant genus recorded in the present study is *Glomus* (4) followed by *Aculospora* (2), *Sceutellospora* (2) *Rhizophagus* (2) and *Gigaspora* (1).
3. The organic carbon, available nitrogen and available phosphorus showed positive correlation with root colonization while available phosphorus showed negative correlation with number of spores.
4. The micronutrients Fe and Zn showed non-significant positive correlation with root colonization (%) whereas Mn and Cu showed non-significant negative correlation with root colonization (%). Micronutrients Fe, Zn and Cu showed significantly positive correlation with IP (per 100 g of soil) while Mn showed significantly negative correlation with IP (per 100 g of soil).Micronutrient Zn showed positive but non-significant correlation with no. of spores (per 100 g of soil), whereas Fe, Mn and Cu showed negative but non-significant correlation with no. of spores (per 100 g of soil).
5. The isolation frequency was highest in *Glomus mosseae* (68.83 %) followed by *Glomus aggregatum* (33.76 %), *Sceutellospora arenicola* and *Sceutellospora heterogama* (01.29 %) respectively.

Acknowledgement

I express the depth of gratitude to, my Research Guide and Chairman of my Advisory Committee Dr. C.D. Deokar, Professor, College of Agriculture, Dhule . I am obligated to all the researchers and scientists whose literature helped me throughout the course of the investigation and to prepare this manuscript properly.

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