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Association of vesicular arbuscular mycorrhiza with different fruit crops

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Abstract
Fifteen fruit crops viz. Mango, Papaya, Guava, Lemon, Bael, Orange, Pomegranate, Jackfruit, Aonla, Sweet orange, Banana, Custard apple, Sapota, Ber and Tamarind were investigated for arbuscular mycorrhizal association by observing the morphological characteristics of isolated VAM spore. All fruit species were infected by vesicular arbuscular mycorrhiza as found by Trypan blue staining procedure. The results indicated that VAM spore isolated by using wet-sieving and decanting method, only one type of spore *i.e., Glomus* was associated with all the fruit species of different locations. The shape of *Glomus* sp. was globose to sub-globose elongated, irregular some time and colour was light yellow to bright orange and brown black to dark black at maturity. The rhizosphere soil collected from Sweet Orange showed maximum number of spores *i.e.* 39 per 100g soil whereas, in Mango maximum per cent root colonization (34.8%). The correlation analysis among physico-chemical properties with spore population showed significant positive correlation with organic matter, available macronutrient N and K, whereas soil pH and available micro-nutrient *i.e.* Cu, Fe, Mn showed negatively significant correlation with mycorrhizal spore count.

Keywords: Fruit crops, physico-chemical properties, mycorrhiza, root colonization, correlation etc.

1. Introduction

The cultivation of fruit crops has a significant impact on the overall well-being of humans and the state of the nation. A high source of important vitamins, minerals as well as high in fiber, fruits are an excellent food choice. Fruits also include a variety of antioxidants that are beneficial to one's health, such as flavonoids It is generally agreed that the per capita production and consumption of fruits can be used to determine the overall standard of living of a population. Chemical fertilizers are used to achieve an optimum plant growth and obtain good fruit yield. However, the excess use of chemical fertilizers negatively affects viable soil microorganisms, especially mycorrhizal fungi. VAM fungi are probably the most ubiquitous soil microbe that can colonize 80% of terrestrial plant species consisted of many fruit crops. Mycorrhizal colonization increased seedling survival, enhanced growth, fruit yield and quality, uniformity of fruit crops, and earlier and increased flowering as well as induced resistance to abiotic and biotic stresses. (Meena kumari *et al.*, 2017) [8]. In marginally poor soils and under environmental stress mycorrhizal fungi can promote plant root growth. The production of high-quality fruit trees with balanced mineral nutrient is possible due to Mycorrhizal inoculation. Vesicular arbuscular mycorrhiza is the most abundant kind of mycorrhiza describe as a universal plant symbiosis. Vesicular arbuscular mycorrhizae are ubiquitous plant root symbiont considered as "keystone mutualists" in terrestrial ecosystem forming (O'Neill *et al.*, 1995) [11]. Mycorrhiza play crucial role in natural ecosystem as well as agriculture ecosystem, hence looking forward the advantages of mycorrhizal association with different fruit crops and its correlation with soil pH, EC, soil type, percent organic matter, available nutrients etc., the study was undertaken to know the mycorrhizal species associated with different fruit crops.

2. Materials and Methods

2.1 Collection of rhizosphere soil samples of fruit crops

Soil and root samples of fruit trees were collected by digging approximately 15-25 cm deep from different locations of Nagpur District of Maharashtra as depicted in Table 1.

Rhizosphere soil of Mango and Papaya was collected from Vihirgaon village and soil sample of Guava rhizosphere was collected from College of Agriculture, Nagpur Maharashtra. The fruit crop of Lemon, Bael, Orange and Pomegranate, the soil samples were collected from Wayagaon village and for the fruit crop Jackfruit, Aonla, Sweet orange and Banana were collected from Akola, Kargaon and Ghoturli village of Umred Taluka of Nagpur District. Sapota, Ber and Tamarind rhizospheric soil sample was collected from village Channa and Pohara of Kuhi Taluka of Nagpur District. Whereas, the rhizosphere soil from Custard apple fruit crop was collected from Regional Fruit Research Station, Taluka Katol, District Nagpur Maharashtra.

2.2 Maintenance and preservation of roots

Collected root samples from different host were thoroughly washed in tap water to remove soil particles. Selected and cleaned roots were fixed in 4% formaldehyde/acetic acid solution (Johansen, 1940) [5].

2.3 Isolation and identification of Vesicular Arbuscular Mycorrhiza

Mycorrhized roots from different fruit crops viz., Mango, Papaya, Guava, Lemon, Bael, Orange, Pomegranate, Jackfruit, Anola, Sweet orange, Banana, Custard Apple, Sapota, Ber and Tamrind were isolated by wet sieving and decanting method (Gerdemann and Nicolson, 1963) [2] in 500µm, 104 µm and 45 µm sieve as shown in plate 1 and stained with Trypan blue (Phillips and Hayman, 1970) [12] as shown in plate 2. The spore was identified as mycorrhizal spore based on morphological characters and published literature.

2.4 Soil Analysis

The rhizosphere soil analysis was carried out by adopting standard methods as suggested by different researchers. Soil pH was determined by using an electrode pH meter following the method of Jackson (1973) [4]. Electrical conductivity (EC) of the soil, expressed in dS m^{-1}, was estimated using a conductivity meter as per Jackson (1973) [4]. Organic matter content in the soil was estimated by the titration method developed by Walkley and Black (1934) [20]. Available nitrogen (kg ha^{-1}) was determined by the alkaline potassium permanganate method following Subbiah and Asija (1956) [18], whereas available phosphorus (kg ha^{-1}) was estimated by the Olsen method using 0.5 M $NaHCO_3$ at pH 8.5, followed by calorimetric measurement with a spectrophotometer as described by Olsen and Sommer (1982) [10]. Available potassium (kg ha^{-1}) was extracted with neutral normal ammonium acetate and measured using a flame photometer following Jackson (1973) [4]. Available Sulphur (mg kg^{-1}) was analysed by the turbidimetric method suggested by Chesnin and Yien (1951) [1]. For micronutrients such as available Zn, Fe, Mn, and Cu (mg kg^{-1}), the DTPA extractable method was employed and estimated using an atomic absorption spectrophotometer as recommended by Lindsay and Norvell (1978) [6].

3. Results and Discussions

3.1 Isolation, identification, Percent root colonization and spore density of VAM, isolated from different fruit crops

The rhizosphere soil samples collected from different fruit crops were isolated by wet sieving and decanting method to identify the genus by studying their spores' type, colour and shape, like wise root colonization percentage and spore density were also studied as predicted in Table 2 and Plate 3. The *Glomus* type of spore was observed in rhizosphere soil of fruit crops in all the locations which was found singly and in cluster. Regarding colour of spore, dark orange brown colour was observed in the isolate collected from Papaya, Guava, Anola and Sweet Orange rhizosphere soil. Dark brown colour of VAM was observed in Sapota and Tamrind. Brown colour was observed in Mango, black colour spore seen at maturity in rhizosphere soil of Bael. Orange brown coloured spore observed in Lemon, Custard apple, Orange and Ber. Blackish brown colour was seen in pomegranate. Orange colour spore was observed in Banana. Spore was also found with one straight to recovered funnel shape subtending hyphae. The colour of hyphae was observed as Orange to brown. Regarding shape of mycorrhizal spore, it was globose to sub-globose in maximum isolates of fruit crops. Irregular shape was observed in Pomegranate and typical globose to sub-globose with elongated vesicles were observed in the roots of fruit crops. Based on the morphological characters of arbuscular mycorrhizal spore was identified to be *Glomus* sp. as given in descriptions of INVAM, 2019. The spore colour was determined according to INVAM colour chart showed spore was light yellow brown or bright yellow and transparent to translucent when young and became black brown to black at maturity. Similar result found in Manoharachary (2004) [7], Trappe (1982) [19], who reported the spore colour, size, shape, wall characteristics and nature of spores. Selvam and Mahadevan (2002) [14] reported wide variety of VAM species in over burden dumps and found *Glomus mosse* was the dominant VAM fungus. Singh and Tyagi. (1989) [17] reported the predominance of *Glomus* type of spores in the rhizosphere soil of both Kinnow as well as rough lemon seedlings.

The maximum spore density of mycorrhiza was observed in sweet orange *i.e.* 39 no. of spores/100g of soil, whereas the minimum was recorded in Ber *i.e.*, 11. The percent root colonization was maximum (34.8%) in Mango and minimum root colonizations was 15.4% in Pomegranate. Nair and Girija (1988) [9] also found that VAM colonization in roots of fruit species varied from 19.9% to 70.6%. Hasan and Khan (2004) [3] carried out a survey for mango nurseries and orchards of different age groups in Uttar Pradesh, India and reported greater colonization in nurseries (22.5-31.7%) than in orchards (11.6-27.6%). The spore population of young orchards was 190.0-314.4 and 64.0-90.9 in old orchards.

3.2 Assessment of physico-chemical properties of mycorrhizal rhizosphere soil of different fruit crops

The physico-chemical properties of mycorrhizal soil of different fruit crops collected from different location presented in Table 3. In case of soil type, all locations showed clay soil type except mycorrhizal soil collected from village Akola and Kargaon from Jackfruit and Aonla fruit crop where the soil type was loamy. Regarding pH of soil, it was in the range of 7.45±1.03 to 8.10±1.06 and in Ber collected from Channa village it was 8.10±1.06 which was maximum as compared to the other mycorrhizal samples collected from other locations. The physical property E.C (dSm^{-1}) was recorded in the range of 0.083±0.03 to 0.201±0.04 and in Pomegranate it was 0.201±0.04 dSm^{-1}, which was maximum as compared to other location's mycorrhizal soil samples. Organic matter is known to influence arbuscular mycorrhizal fungi, the percent organic matter in all the soil sample was in the range of 1.15±0.17 to 1.50±0.21%. The maximum percent organic matter was recorded in sweet orange and minimum in Ber. The chemical properties like available nutrient of nitrogen, phosphorus, potash, Sulphur, copper, zinc, iron and magnesium were studied to know the correlation of all physical and chemical properties on mycorrhizal population Table 4. Available Nitrogen varied from 156±27.7 to 326±29.7 across different locations where as minimum (156±27.7) being in Ber and maximum (326±29.7) in Sweet Orange. Phosphorus Availability varied from 9.6±0.35 to 18.8±0.56 in different locations whereas minimum (9.6±0.35) in Guava and maximum (18.8±0.56) in Mango. Available potassium varied from 155±31.5to 341±41.7, available Sulphur ranged from 11.5±1.47 to 27.9±1.35 across different locations where as minimum (11.5±1.47) in Bael and maximum (27.9±1.35) in Jackfruit. Available Copper ranged from 1.19±0.16 to 3.47±0.14 and available Zinc was minimum (0.65±0.16) in sweet orange and maximum (1.82±0.19) in Orang. Available Iron ranged from 3.25±0.36 to 5.24±0.61 in Nagpur region whereas, Manganese ranged from 2.37±0.14 to 4.72±0.14. Similar result found in Shivakumar (2013) [16] reported that physico-chemical properties of soil of study site were analysed i.e. soil pH ranged from 6.1 to 7.8, the EC ranged from 0.11-0.20 and the OM% ranged from 1.18 to 1.40%. Macronutrients such as N, P, K. ranged from 71.4 to 81.9, 5 to11 and187 to 262kg/acre, respectively. Micronutrient such as Cu, Zn, Fe and Mn ranged from 2.18 to 3.62, 0.76 to 1.1, 5.18 to 7.2 and 3.16 to 4.16 Kg/acre, respectively.

3.3 Correlation of physico-chemical properties with mycorrhizal spore count:

The population of vesicular arbuscular mycorrhizal spore is influenced by the physico-chemical properties of rhizosphere soil. Soil pH sensitive to AM spore helps in the stabilization of OM% in soil and VAM helps in uptake of available nutrients in soils and supply to plants. Hence effects are made to study the correlation between physico-chemical properties with AM spore count of rhizosphere soil collected from fruit species of different locations as predicted in Table 4. The mycorrhizal spore counts i.e., 0.959, 0.955 and 0.953, respectively showed significant positive correlation with soil physico-chemical properties like organic matter, available macrounutrient N and K. Whereas soil pH and available micro-nutrient i.e., Cu, Fe, Mn showed negatively significant correlation i.e.,-0.836,-0.706,-0.917 and-0.913 with mycorrhiza spore count. The same results are observed by Shivakumar (2013) [16] reported Pearson correlation followed to edaphic factors such as available K on AM spore density revealed a positive correlation and negative correlation of Cu, Zn and P on AM spore population. Wani and Konde (1996) [21] reported a negative correlation between organic carbon and available phosphorous in the soil. AM spore population was positively and significantly correlated with organic carbon and available N but negatively and significantly correlated with available K, Cu and Zn in the soil. The correlation between AM spore population and available K, Fe and Mn were found to be negative and non-significant reported by Sharma and Sharma (2006) [15].

Table 1: Collection of rhizosphere soil from different fruit crop for the isolation of Vesicular Arbuscular Mycorrhiza (VAM)

Sr. No.	Common name	Botanical name	Location		GPS Coordinates	Age of Tree
			Taluka	Village		
1.	Mango	*Mangifera indica*	Nagpur	Vihirgaon	21°05'20.1"N 79°09'56.2"E	15
2.	Papaya	*Carica papaya*	Nagpur	Vihirgaon	21°05'32.7"N 79°09'39.6"E	3
3.	Guava	*Psidium guajava*	Nagpur	College of Agriculture, Nagpur	21°08'39.3"N 79°04'23.3"E	6
4.	Lemon	*Citrus Limon*	Umred	Wayagaon	20°52'57.5"N 79°17'03.2"E	7
5.	Bael	*Aegle marmelos*	Umred	Wayagaon	20°52'46.9"N 79°17'24.6"E	17
6.	Orange	*Citrus sinensis*	Umred	Wayagaon	20°52'18.7"N 79°16'50.2"E	6
7.	Pomegranate	*Punica granatum*	Umred	Wayagaon	20°52'49.3"N 79°17'17.6"E	5
8.	Jackfruit	*Artocarpus heterophyllus*	Umred	Akola	20°55'38.8"N 79°18'39.0"E	2
9.	Aonla	*Phyllanthus emblica*	Umred	Kargaon	20°47'13.6"N 79°25'11.1"E	24
10.	Sweet orange	*Citrus limetta*	Umred	Ghoturli	20°53'04.5"N 79°17'50.5"E	8
11.	Banana	*Musa paradisiaca*	Umred	Ghoturli	20°52'47.0"N 79°17'27.5"E	2
12.	Custard apple	*Annona squamosa*	Katol	Regional Fruit Research Station, Katol	21°15'07.4"N 78°36'33.6"E	9
13.	Sapota	*Manilkara zapota*	Kuhi	Channa	20°59'40.8"N 79°31'24.5"E	20
14.	Ber	*Ziziphus mauritanaia*	Kuhi	Channa	21°00'03.2"N 79°31'01.2"E	11
15.	Tamarind	*Tamarindus indica*	Kuhi	Pohara	21°00'17.2"N 79°31'07.3"E	27

Table 2: Morphological characteristics of vesicular arbuscular mycorrhiza in rhizosphere soil of different fruit crops

Sr. No.	Fruit Plant	Morphological Characteristics			No. of spore/100 g soil	Root Colonization %
		Types of Spores	Colour	Shape		
1.	Mango	*Glomus* sp.	Brown	Globose	26	34.8
2.	Papaya	*Glomus* sp.	Dark orange brown	Sub-globose	20	28.1
3.	Guava	*Glomus* sp.	Dark orange brown	Sub-globose	34	29.1
4.	Lemon	*Glomus* sp.	Orange brown	Globose	18	22.5
5.	Bael	*Glomus* sp.	Black	Globose	23	22.0
6.	Orange	*Glomus* sp.	Orange brown	Globose	18	25.6
7.	Pomegranate	*Glomus* sp.	Black brown	Irregular	22	15.4
8.	Jackfruit	*Glomus* sp.	Black brown	Sub-globose	17	24.5
9.	Aonla	*Glomus* sp.	Dark orange brown	Sub-globose	13	18.9
10.	Sweet orange	*Glomus* sp.	Dark orange brown	Globose	39	23.5
11.	Banana	*Glomus* sp.	Orange	Globose	19	21.4
12.	Custard apple	*Glomus* sp.	Orange brown	Globose	21	24.3
13.	Sapota	*Glomus* sp.	Dark brown	Globose	19	27.7
14.	Ber	*Glomus* sp.	Orange brown	Globose	11	16.8
15.	Tamarind	*Glomus* sp.	Dark brown	Sub-globose	14	20.3

Table 3: Physico-chemical properties of mycorrhizal rhizosphere soil of different fruit crops

Sr. No.	Fruit crops	Physico-chemical Properties of Soil											
		Soil Type	pH	EC (dSm^{-1})	OM%	N (kg/ha)	P (kg/ha)	K (kg/ha)	S (mg/kg)	Cu (mg/kg)	Zn (mg/kg)	Fe (mg/kg)	Mn (mg/kg)
1.	Mango	Clay	7.58±1.01	0.083±0.03	1.41±0.22	281±17.6	18.8±0.56	282±26.4	17.3±1.05	2.35±0.14	1.16±0.09	3.64±0.77	3.15±0.13
2.	Papaya	Clay	7.72±1.03	0.091±0.01	1.33±0.17	222±31.5	11.3±0.49	255±24.2	18.6±2.18	2.56±0.16	1.21±0.12	4.23±0.27	3.54±0.13
3.	Guava	Clay	7.53±1.03	0.150±0.04	1.47±0.19	281±31.9	9.6±0.35	312±28.1	22.5±1.19	1.19±0.16	0.72±0.16	3.44±0.49	2.95±0.17
4.	Lemon	Clay	7.52±1.03	0.139±0.04	1.27±0.16	197±24.7	15.6±0.43	217±33.7	13.8±1.41	2.65±0.24	0.78±0.11	4.66±0.44	4.21±0.21
5.	Bael	Clay	7.64±0.99	0.128±0.05	1.37±0.21	261±21.1	12.1±0.72	268±17.5	11.5±1.47	1.98±0.21	0.93±0.11	3.78±0.44	3.21±0.19
6.	Orange	Loamy	7.82±1.04	0.111±0.07	1.25±0.23	198±24.6	13.4±0.77	219±36.8	21.9±2.30	2.27±0.07	1.82±0.19	4.66±0.44	2.37±0.14
7.	Pomegranate	Clay	7.64±0.91	0.201±0.04	1.36±0.21	176±13.5	18.5±0.78	155±31.5	18.2±2.15	3.47±0.14	0.83±0.17	5.20±0.73	4.56±0.14
8.	Jackfruit	Clay	7.86±1.04	0.094±0.03	1.24±0.18	185±33.8	10.5±0.76	193±16.6	27.9±1.35	2.64±0.11	0.86±0.08	4.98±0.72	4.26±0.21
9.	Aonla	Clay	7.95±1.02	0.135±0.02	1.16±0.19	176±13.5	18.5±0.78	155±31.5	18.2±2.15	3.47±0.14	0.83±0.17	5.20±0.73	4.56±0.15
10.	Sweet orange	Clay	7.45±1.03	0.171±0.03	1.50±0.21	326±29.7	15.8±0.84	341±41.7	16.5±1.72	1.21±0.05	0.65±0.16	3.25±0.36	3.25±0.18
11.	Banana	Clay	7.80±1.02	0.149±0.01	1.29±0.20	201±33.2	11.4±0.88	232±44.5	12.3±1.29	3.78±0.17	1.04±0.14	4.50±0.55	3.56±0.16
12.	Custard apple	Clay	7.71±1.03	0.150±0.01	1.33±0.16	235±23.3	14.4±0.25	261±19.4	15.1±2.57	2.15±0.15	0.84±0.19	3.97±0.69	3.52±0.13
13.	Sapota	Loamy	7.76±1.05	0.159±0.02	1.31±0.20	206±17.6	13.8±0.90	219±36.8	19.9±1.89	3.19±0.07	0.96±0.13	4.47±0.54	3.98±0.11
14.	Ber	Clay	8.10±1.06	0.172±0.05	1.15±0.17	156±27.7	13.2±0.65	159±23.3	17.4±1.80	2.49±0.22	0.91±0.13	5.24±0.61	4.72±0.14
15.	Tamarind	Clay	7.90±1.01	0.123±0.01	1.21±0.22	181±27.4	14.7±0.59	179±21.6	23.3±1.32	2.33±0.14	0.73±0.14	5.12±0.65	4.28±0.17

Table 4: Correlation between physico-chemical properties of soil with AM spores collected from different fruit crops

	Spore count	pH	EC	OM%	Available N	Available P	Available K	Available S	Available Cu	Available Zn	Available Fe	Available Mn
Spore count	1											
pH	-0.836***	1										
EC	0.157	-0.061	1									
OM%	0.959***	0.119	-0.13	1								
Available N	0.955***	-0.856***	0.103	-0.283	1							
Available P	-0.039	-0.104	0.238	-0.304	0.119	1						
Available K	0.953***	-0.878***	0.124	-0.211	0.967***	-0.026	1					
Available S	-0.104	0.298	-0.261	0.532*	-0.224	-0.179	-0.239	1				
Available Cu	-0.706**	0.568*	-0.221	-0.018	-0.701**	0.003	-0.685**	-0.151	1			
Available Zn	-0.217	0.163	-0.332	0.323	-0.157	0.015	-0.082	0.087	0.15	1		
Available Fe	-0.917***	0.865***	-0.162	0.293	-0.97***	-0.057	-0.983***	0.283	0.678**	0.079	1	
Available Mn	-0.913***	0.863***	-0.175	0.326	-0.968***	-0.059	-0.978***	0.302	0.663**	0.138	0.997***	1

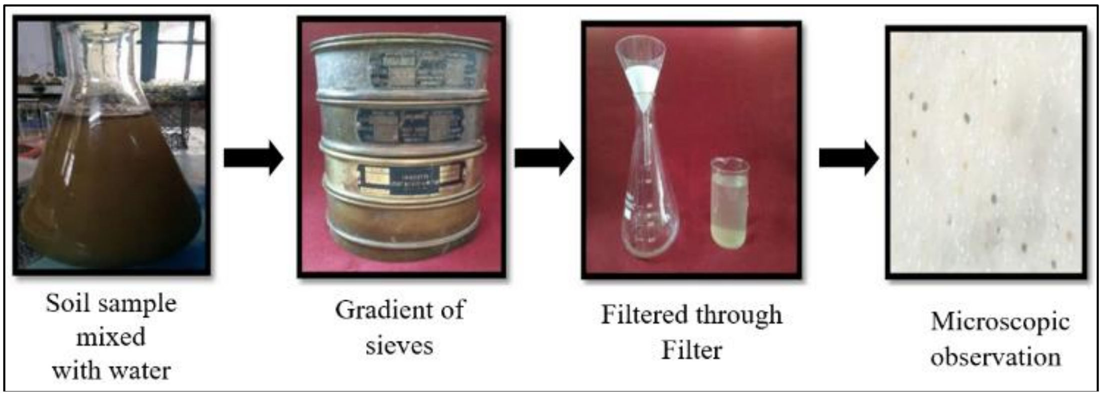

Plate 1: Wet Sieving and Decanting Method t

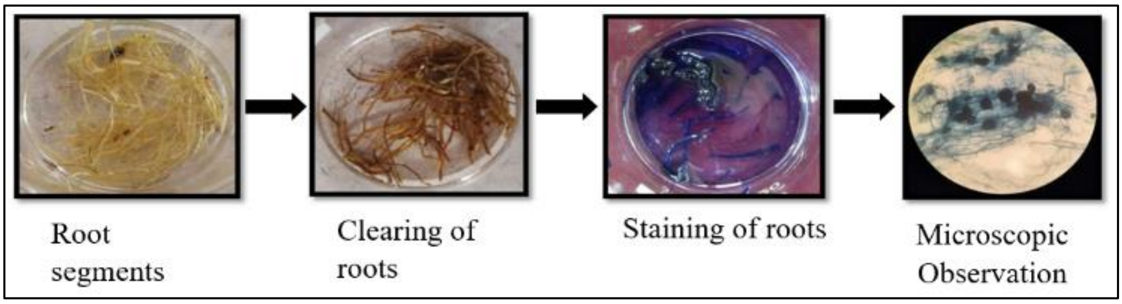

Plate 2: Staining of VAM

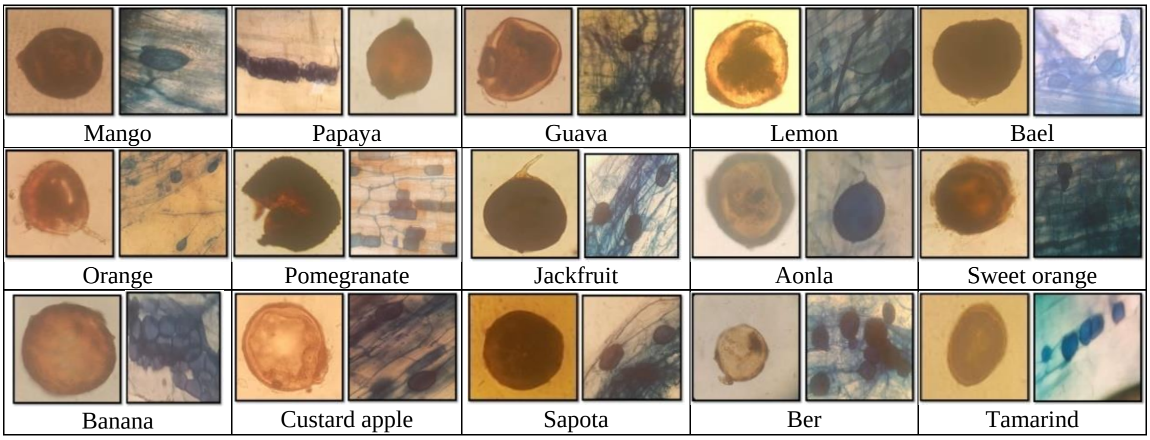

Plate 3: Isolation of *Glomus* sp. and mycorrhizal infection in roots of different fruit crops

4. Conclusion

The study on association of vesicular arbuscular mycorrhiza with different fruit crops exhibited the association of only one type of AM spore i.e., *Glomus* and shape was Globose to sub-globose elongated, irregular sometime and colour was light yellow to bright orange and brown black to dark black at maturity observed. Among different fruit crops sweet orange rhizosphere soil showed maximum number of AM spores due to high percentage of organic matter and available nitrogen and potash, thus organic matter and available nitrogen and potash play a significant correlation with mycorrhizal spore population.

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6. Conflict of Interest

The authors declare that there is no conflict of interest

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