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Diversity of arbuscular-mycorrhizal fungi in the agricultural fields of Kanhauri village, district Rewari, Haryana, India

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Abstract
The Arbuscular Mycorrhizal Fungal diversity was studied in seven plants from the agricultural fields of Kanhauri village, district Rewari, Haryana. The rhizospheric soils and roots were collected from the plants of *Brassica juncea*, *Allium cepa*, *Chenopodium album*, *Argemone mexicana*, *Parthenium hysterophorus*, *Solanum xanthocarpum* and *Cannabis sativa* in the month of March 2021. Rhizospheric soils were screened for the presence of Arbuscular Mycorrhizal fungal spores by wet sieving and decanting method of Gerdeman and Nicolson (1963). The roots were checked for percent root colonization by Phillips and Hayman method (1970). Based on morphological characters of spores, species belonging to eight genera (*Racocetra, Dentiscutata, Funeliformis, Rhizophagus, Glomus, Acaulospora & Sclerocystis*) were identified. Maximum spore density, species richness & root colonization were observed in *Solanum xanthocarpum*. *Rhizophagus* was the dominant genera and *Funneliformis mosseae* had the highest isolation frequency. Root colonization in the plants was in the form of hyphae, arbuscules, vesicles and auxillary cells. The soils are sandy loam with a pH of 6.69, total Nitrogen 0.21%, available phosphorus 22.7Kg/hectare, Potassium 27ppm and organic matter 2.24%.

Keywords: am fungi, *Rhizophagus, Solanum xanthocarpum, Acaulospora, Dentiscutata, Racocetra*

Introduction
Arbuscular Mycorrhiza (AM), a mutualistic symbiosis between fungi and higher plants, is one of the most ubiquitous soil beneficial organisms. Mycorrhizal fungi have multiple ecological functions including improving the absorption of mineral nutrients and water to their host plants (Smith and Read 2008) [16], improving plants tolerance to environmental stresses such as drought, salinity and heavy metals (Kaya et al. 2009) [10], and maintaining soil structure in agricultural soils that is important for land sustainability (Jeffries et al. 2003). Environmental conditions could affect population, diversity, and distribution of AM fungi (Brundrett 1991) [4]. For example, climate and edaphic factors, and physicochemical edaphic factors have been related to sporulation and colonization of AM fungi (Panwar et al. 2011) [12]. In agricultural ecosystem, in particular, the population and diversity of AM have been reported to be influenced by land management (Oehl et al. 2003; Kabir 2005) [11, 9]. As no records exist of AM fungal diversity in this district of Haryana, the work was undertaken.

Materials and Methods
Sampling: Samples were collected during the month of March, 2021 from the agricultural fields of village Kanhauri, district Rewari, Haryana. The roots and rhizosphere soils were collected in the plastic bags. The soil samples were stored in refrigerator until analysis.

Spore extraction: Gerdeman and Nicolson method (1963), was followed for extraction of spores. 100 grams of root zone soil sample was taken and mixed with 1L of water The suspension was passed through 500µm, 250µm, 150µm, 75µm, 25µm sieves and the water was allowed to flow for half an hour mildly so that hyphae and sporocarp do not break. The residue in the respective sieves were collected in beakers carefully with approximately 100ml of water. This water which contained spores and sporocarps was filtered on a circular Whatman filter paper, taken in Petridish and observed under Motic stereozoom microscope for AM fungal spores.

Spore Quantification
Spore Density-Spores in the 100g of soil were collected in five Petri dishes and the spore number was counted by the method of Gaur and Adholeya, (1994) [5]. The spores were picked up using needle. The spores were mounted on glass slide in polyvinyl lacto Glycerol (PVLG) and covered with cover slips. The slides were heated at 40-50°C temperature so that the air bubbles are removed and the spores appear clear. This is then sealed with Dibutyl Pthalate Xylene (DPX) which makes the slide semi-permanent.

Identification of AMF based on morphological characters: The spores were identified on the basis of morphological characters including attachment of hyphae, ornamentation of wall, thickness of wall layers and colour of spore. The identification of arbuscular mycorrhizal fungal spores was done with the help of websites www.invam.caf.wvu.edu and www.zor.zut.edu.

Root Colonization of AM Fungi was done by Philips and Hayman method, 1970 and percentage of root colonization was calculated by Read *et al*., 1976 [14].
Root samples were cut into 1cm bits, washed in water & treated with 10%KOH at 90º C for 1 hour in oven. After removing from KOH solution, the roots were washed 2-3 times thoroughly with tap water and kept in 5%HCl for 5 minutes. Then the root segments were stained with 0.05 % trypan blue and kept overnight. Root samples were mounted in PVLG and observed under Labomed trinocular microscope.
Percentage of mycorrhizal root colonization was calculated using the following formula.

$$\text{Percentage root colonization} = \frac{\text{Number of root segments infected by AM fungi}}{\text{Total number of segments scored}} \times 100$$

Diversity measures used to describe AM Fungi
Spore density (SD): The number of spores in 100gm soil

Species richness (SR): Number of identified AMF species per soil sample

$$\text{IF (Isolation Frequency)} = \frac{\text{The number of soil samples in which AMF species occurred}}{\text{The total number of soil samples}} \text{ x } 100$$

Soil Analysis: Soil pH was taken by pH meter, Total nitrogen was determined by the modified Kjeldhal method (Jackson, 1973) [7], Phosphorus by Ammonium molybdate spectrophotometric method (Bray's method, 1945), Soil Organic carbon by Black and Walkey's method (1934) [17], Potassium by flame photometer, Copper, Iron, Lead & Zinc were estimated by Atomic Absorption Spectrophotometer (at SAIF, IIT, Bombay).

Results

Table 1: Spore density, Species richness, Root colonization & Mycorrhizal Spore types

S. No.	Name of the Plant	Spore Density/100gm soil	Species Richness	Root colonization %				Mycorrhizal Spore types
				H	A	V	%	
1	*Brassica juncea*	2050± 13.50	6	++	--	++	70	*Glomus macrocarpum, Funeliformis mosseae,Rhizophagus intraradices, Dentiscutata nigra, Rhizophagus irregularis, Rhizophagus fasciculatus*
2	*Allium cepa*	750± 10.5	8	++	--	+++	80	*Racocetra corolloidea, Denticutata nigra Funeliformis mosseae,Rhizophagus intraradices, Rhizophagus irregularis,Gigaspora gigantea, Glomus macrocarpum, Acaulospora bireticulata*
3	*Chenopodium album*	445 ±7.09	4	++	--	+++	80	*Racocetra corolloidea, Denticutata nigra Funeliformis mosseae, Rhizophagus irregularis*
4	*Cannabis sativa*	260 ±11.6	5	++	++	+++	100	*Racocetra corolloidea, Denticutata nigra Funeliformis mosseae, Rhizophagus intraradices, Rhizophagus fasciculatus*
5	*Argemone mexicana*	430 ±12.53	4	++	++	++	60	*Funeliformis mosseae, Rhizophagus intraradices,, Glomus macrocarpum, Rhizophagus fasciculatus*
6	*Partheniu m hysteroph orus*	214 ±4.33	5	++	++	+++	90	*Funeliformis mosseae, Rhizophagus intraradices, Rhizophagus fasciculatus, Acaulospora bireticulata, Denticutata nigra*
7	*Solanum xanthocarpum*	5075 ±7.0	9	++	--	++	94	*Racocetra corolloidea, Denticutata nigra Funeliformis mosseae Rhizophagus intraradices, Rhizophagus irregularis, Rhizophagus fasciculatus,, Glomus macrocarpum, Acaulospora bireticulata, Sclerocystis coremoides*

Physico- chemical characteristics of the Soil

Table 2

S. No	Soil parameter	value
1	Texture	Sandy Loam
2	pH	6.69
3	Total Nitrogen	0.21%
4	Available Phosphorus	22.7Kg/hectare
5	Potassium	27%
6	Organic matter	2.24%
7	Cu	0.00031%
8	Fe	2.2%
9	Pb	ND
10	Zn	0.0013%

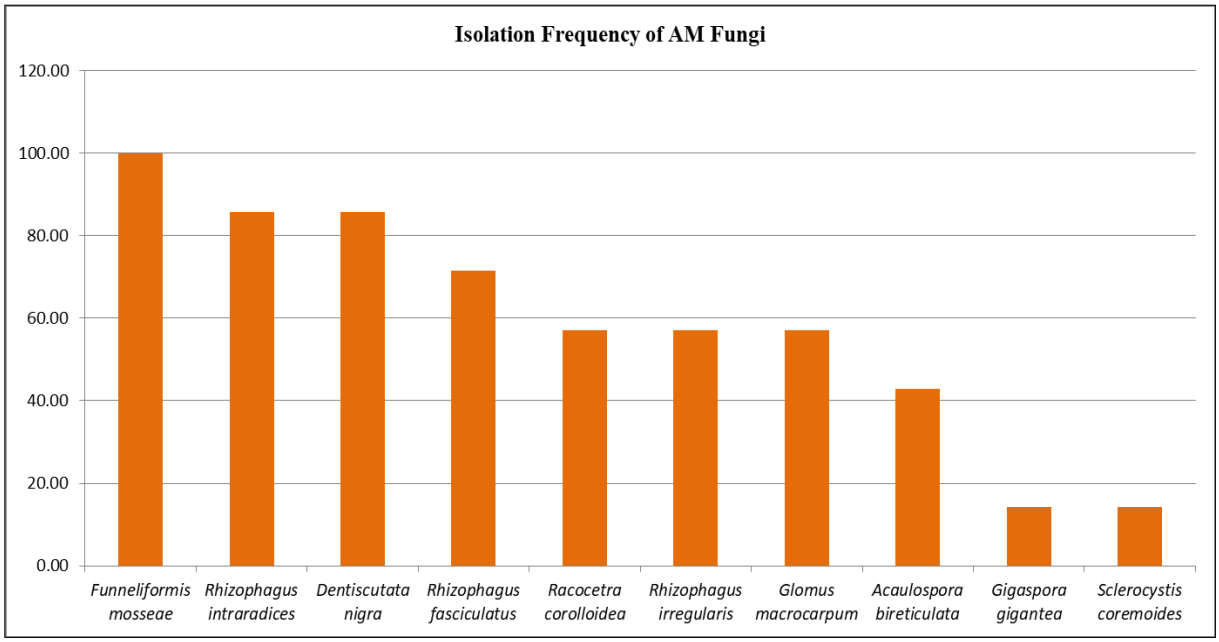

Fig 1

Species descriptions based on morphology of spores and sporocarps isolated from soils
The descriptions are based on the observations of several spores from the soil samples

Funneliformis mosseae

Fig 2: Spore and Sporocarp of *Funneliformis mosseae* Sporocarp produce spores in clusters of 2-10 surrounded by a tight peridium. Spore colour: Light yellow to brown. Shape: Globose to sub globose Spore wall: three-layered. Subtending hypha flared to funnel-shaped

Rhizophagus irregularis

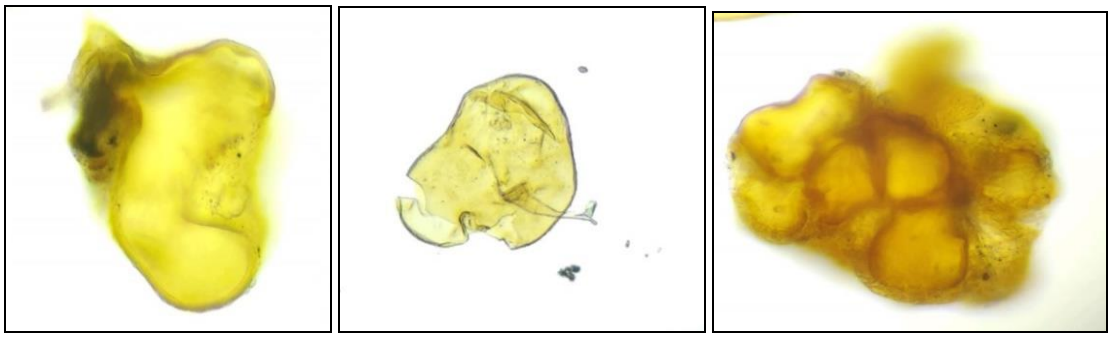

Fig 3: Spores and Sporocarp of *Rhizophagus irregularis*

Sporocarp: Spores are produced in clusters which is covered by the peridium, made up of dense layer of hyphae.Spores: borne singly and in loose aggregates of a variable number of spores. Spore colour: Hyaline to yellow brown. Shape: Globose, subglobose, ovoid, oblong, or irregular enough to sometimes appear knobby. Spore wall: Three layered. Hypha is cylindrical to slightly flared.

Rhizophagus intraradices / Glomus intraradices

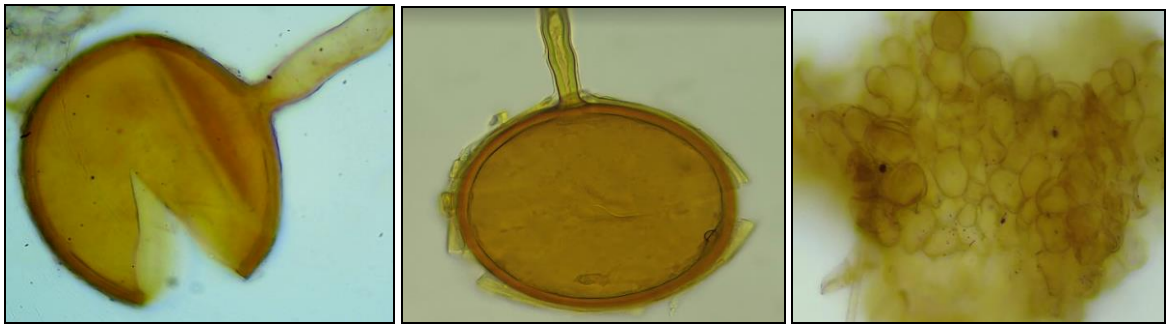

Fig 4: Spores and Sporocarp of *Rhizophagus intraradices*

Sporocarp: loose clusters of spores. Spores occur in loose aggregates or singly in the soil, and frequently are formed inside of roots. This is the reason this species is used for the mass production of spores for biofertilizers. Spore colour: white/ hyaline when young, pale cream to yellow brown when mature. Shape: Globose to subglobose. Spore wall: Three layered. Subtending Hypha is cylindrical to slightly flared, occasionally slightly constricted

Glomus macrocarpum

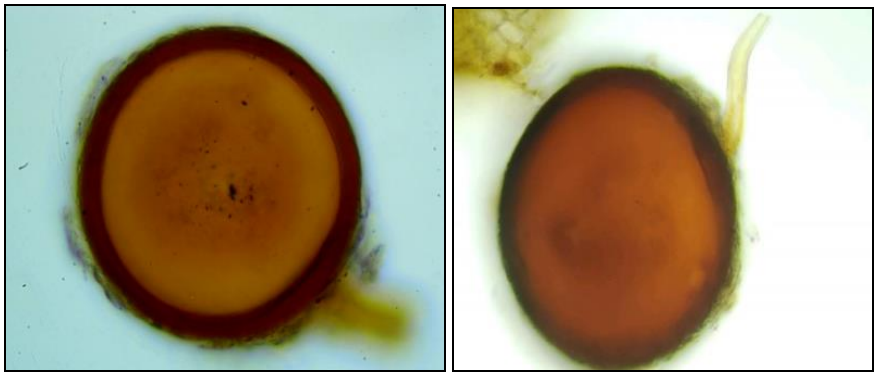

Fig 5: Spores of *Glomus macrocarpum*

Spores rarely single in the soil, usually in sporocarps containing 2-15 randomly distributed spores without peridium. Spore colour: Yellow to orange red. Shape: Globose to subglobose. Spore wall: 2 layered. Subtending Hypha yellow, straight or curved cylindrical to Flared. Wall of subtending hypha yellow, continuous with spore wall.

Glomus fasciculatum/ Rhizophagus fasciculatus

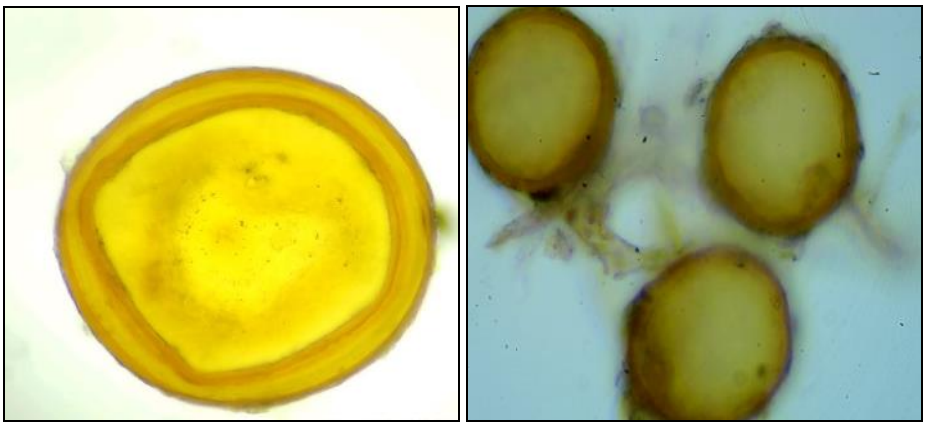

Fig 6: Spore and Loose sporocarp of *Glomus fasciculatum*

Spores single in the soil or in aggregates with 2-20 spores lacking a peridium.
Spore colour: Pale yellow to pale yellow-brown. Shape: Globose to sub globose. Spore wall: three layered. Subtending Hypha: Cylindrical to slightly flared.

Dentiscutata Nigra

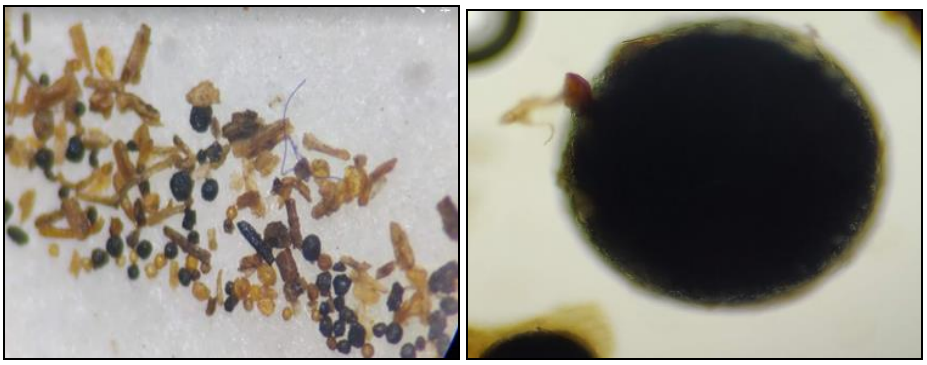

Fig 7: Spores of *Dentiscutata nigra*

Spore colour: Dark red-brown to black. Shape: Mostly globose. Spore Wall: Two layers, the outer being smooth and the inner layer with a complex ornamentation pattern.

Racocetra corolloidea

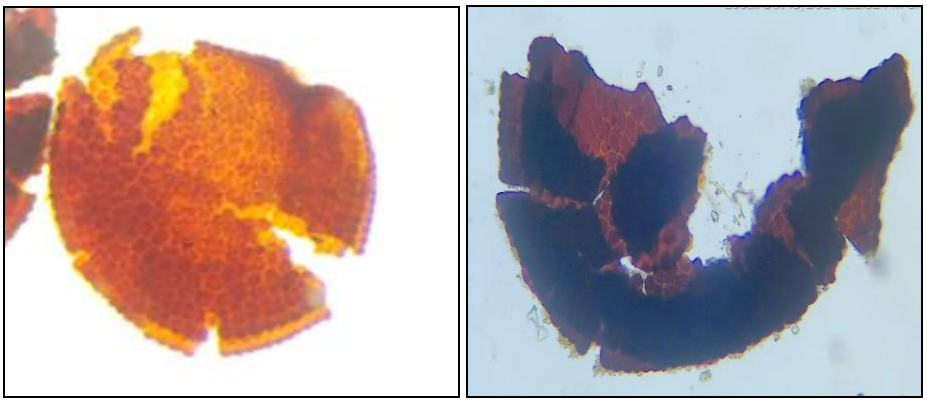

Fig 8: Spores of *Racocetra corolloidea*

Spores occur singly in soil. Spore colour: Orange-red to dark red brown.Shape: Globose to subglobose. Spore wall: Two-layered (L1, and L2) ornamented by flattened warts with angular margins.

Acaulospora bireticulata

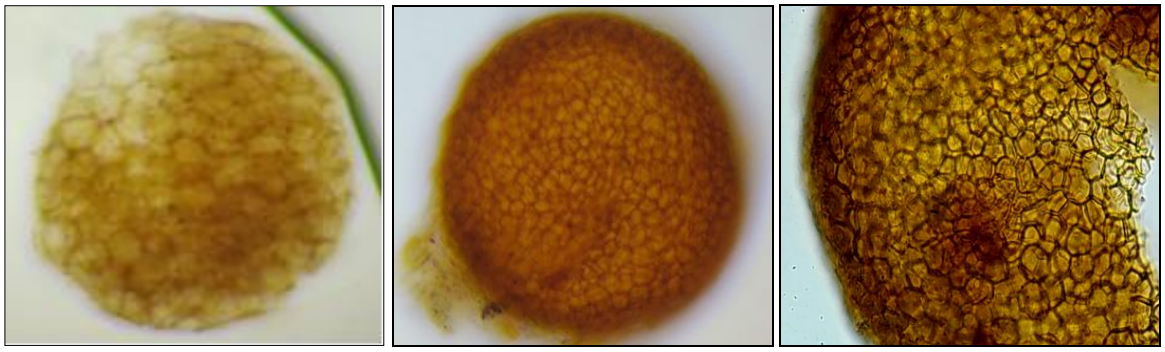

Fig 9: Spores of *Acaulospora bireticulata*

Spores formed singly in soil, sessile, develop laterally on the neck of a sporiferous saccule.Spore colour: Yellowish brown to brown. Shape: Globose to subglobose Spore wall: Spore wall three-layered. Spore surface ornamented with a polygonal reticulum network. Cicatrix: Not seen

Sclerocystis coremioides

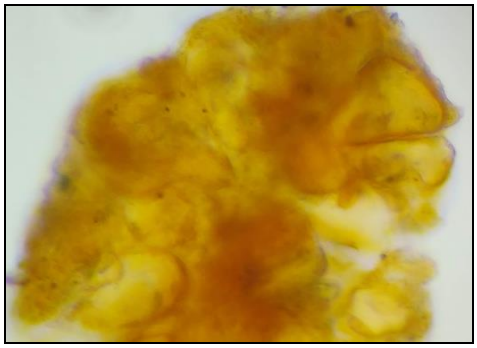

Fig 10: Sporocarp of *Sclerocystis*

This species produces sporocarps like a coremium; orange when immature, becoming orange-brown to dark orange-brown when mature. A dense layer of hyphae (=peridium), covers all spores and keeps them tightly packed. Clavate spores organized in a single layer from a central plexus of hyphae (Redecker et al. 2000) [15].

Gigaspora gigantea

Spores of *Gigaspora gigantea* species occur singly in soil, with bright greenish yellow to bright yellow-green color, globose to subglobose shape,rarely irregular. Spore with three-layered outer wall (L1, L2, and L3) (Bentivenga and Morton, 1995) [1].

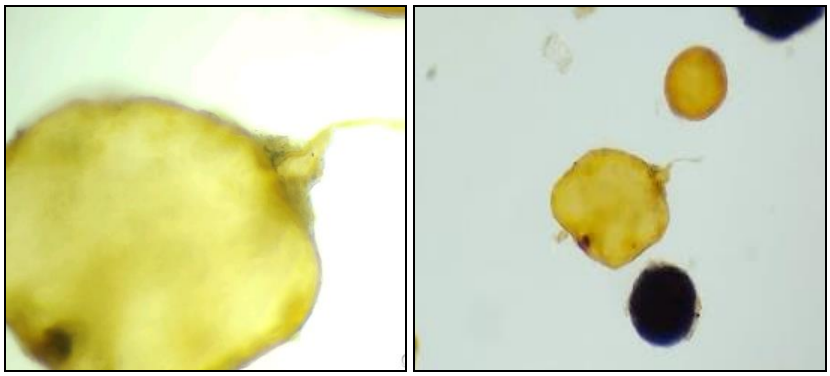

Fig 11: Spore of *Gigaspora gigantea*

Discussion

The agricultural soils of village Kanhauri are sandy loam. Irrigation is by canals and tube wells. The major crops cultivated are wheat, mustard, sorghum, pearlmillet, clusterbean, red gram and peas. Apart from these some farmers grow vegetables like onion, cucumber, ladyfinger, brinjal, tomatoes, spinach, fenugreek, bottle gourdand bittergourd. Cotton is grown by some. Common weeds that grow in the fields are *Argemone mexicana*,

Parthenium hysterophorus, Solanum xanthocarpum, Cannabis sativa, Calotropis procera, chenopodium album etc. The study of AM Fungi shows that *Solanum xanthocarpum* showed highest spore density in the rhizosphere soil, 5075 ±7.0spores/100g of soil and 94% root colonization followed by *Brassica juncea* having spore density of 2050±13.50/100g of soil and root colonization 70%. *Solanum xanthocarpum* is rarely found nowadays according to the farmers. AM Fungus *Funneliformis mosseae* showed highest isolation frequency of 100%. This genus was found in the rhizosphere of all the plants. The predominant genera in the soils were found to be *Funneliformis, Rhizophagus and Dentiscutata*.Bhardwaj et al 1997 also found that Glomus to be the predominant genera in the soils of Haryana.(The new name of *Glomus mosseae* is *Funneliformis mosseae* and new name of *Glomus intraradices* is *Rhizophagus intraradices*). The spore size of *Racocetra, Gigaspora, Glomus* and *Dentiscutata* ranged from 150 µ to 350 µ, which made the isolation and identification much easier.Each species has been described above with regard to their colour, shape, wall layers and hyphal attachment.

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