Chapter: 5

5.0: Occurance of AM Fungi in Rhizosphere of Lemon and Fig

The mycorrhizal fungi attach to the surface of the root and penetrate in or around the inside of the root cells. Then they send their filaments (called mycelium) into the surrounding soil, effectively extending the plant's roots and root absorbing capacity from ten to several thousand times far beyond what the plant can do alone and thus improving plant establishment and productivity.

Several kilometers of these ultra-fine filaments can be present in less than a thimbleful of soil. Mycorrhizal fungi supply the water and nutrients needed by the plant for establishment and survival and, in return, receive from the plant roots sugars and other compounds needed by the fungus. Mycorrhizal filaments are much smaller than roots, so they can easily penetrate into smaller spaces between soil particles.

In the small nooks and crannies of the soil these tiny filaments release powerful enzymes that dissolve tightly bound minerals like phosphorus, sulfur, iron and all the major and minor nutrients used by plants. The nutrients are organically assimilated by the mycorrhizae and become readily available for use by the plants.

With the exception of plants that form other type of mycorrhiza (ecto-, ericoid and orchid mycorrhiza) and the relatively few species that are never mycorrhizal. In most plants AM development is normal (Smith and Smith 2011). The non-mycorrhizal condition is found naturally only under extreme soil conditions (distributed or waterlogged soils). Colonization of roots by AM fungi involves subtle signaling between the symbionts, leading to expresses of key genes and tightly programmed cellular events.

Dependence on arbuscules for definitive identification of AM root and failure to recognize the common occurrence and importance of intercellular coiled hyphae as alternative AM structures has led to underestimation of the number of plant species that form arbuscular mycorrhizae in nature. The function symbiosis is accomplished by presence of hyphal coils arbuscular and intermediate structutre.

Brundret (2004) provided a new classification scheme for categories, subcategories and morphotypes where mycorrhizal association are classified on anatomical criteria the main categories of vesicular-arbuscular mycorrhizal associations (VAM) are linear (arum type) or coiling (paris type). Goto *et al*. (2010) checklist seventy-nine species of AMF explored from the semiarid Caatinga biome of Northeast Brazil. Blaszkowski *et al*. (2010) examined 19 samples of roots and 14 of rhizosphere soils of uncultivated species and described sporocarps and spores of *Ambispora gerdemanni* and *Glomus badium* from Europe and Poland.

On the basis of nrLSU gene sequences together with developmental pattern, Kaonongbua *et al*. (2010) taxonomically integrated genus Kuklospora into genus Acaulospora changing Kuklospora kentinensis to Acaulospora colombiana and Acaulospora kentinensis, they added a new species Acaulospora colliculosa Kaonongbua, J.B. Morton and Bever sp. nov. (Mycobank MB515732) from a tallgrass prairie of North America.

Vaingankar and Rodrigues (2011) added a new species *Acaulospora soloidea* Vaingankar and B.F. Rodrigues, sp. nov. (MB 518836) from the laterite soils of Goa. It has unique wall with fibrillose, hairy outgrowths and appear like the rays of the sun when mounted in water or PVLG.

On the basis of coenocytic hyphae AM fungi had long been recognized as a member of Zygomycota, recently on the basis of molecular phylogenetic studies AM fungi has been separated from poly-phyletic Zygomycota. A new Glomeromycota has been proposed for AM

fungi. Nowdays INVAM (International Collection of VAM Fungi) and BEG (Banque Europeennee des Glomales) are monitoring pyllogenetic classification. Molecular techniques are concluding that the group may be much more diverse than was previously thought. There is recent trend of sequence data of AM fungi for identification, still the conventional morphological observations are important and should not be neglected for identification.

IN VAM website present classification of Schubler *et al*. (2001) in which Glomeromycota is divided in four orders and 10 families.

GLOMEROMYCOTA C. Walker & Schuessler

Glomeromycetes Cavalier-Smith

Archaeosporales C. Walker & Schuessler

Ambisporaceae C. Walker, Vestberg & Schuessler

Ambispora Spain, Oehl & Sieved.

Archaeosporaceae J.B. Morton & D. Redecker emend. Oehl & Sievered.

Archaeospora J.B. Morton & D. Redecker

Interaspora Oehl & Sieverd.

Geosiphonaceae Engler. & E. Gilg emend. Schuessler

Geosiphon (Kutz.) F. Wettst.

Diversisporales C. Walker & Schuessler

Acaulosporaceae J.B. Morton & Benny

Acaulospora Gerd. & Trappe emed. S.M. Berch

Kuklospora Oehl & Sieverd.

Diversisporaceae C. Walker & Schuessler

Diversispora C.Walker & Schuessler

Otospora Oehl, J. Palenzuela & N. Ferrol

Entrophosporaceae Oehl & Sievered.

Entrophospora R. N. Ames & R.W. Schneid. Emend. Oehl & Sieverd.

GigasporaceaeJ.B. Morton & Benny

Gigaspora Gerd. & Trappe emend. C. Walker & F.E. Sanders

Scutellospora C. Walker & F.E. Sanders

Pacisporaseae C. Walker, Blaszk., Schuessler & Schwarzott

Pacispora Oehl & Sieverd.

Glomerales J.B. Morton & Benny

Glomeraceae Piroz. & Dalpe

Glomus Tul.& C. Tul.

Paraglomerales C. Walker & Schuessler

Paraglomaceae J.B. Morton & D. Redecker

Paraglomus J.B. Morton & D. Redecker

The term mycorrhizae denote "fungus roots". It is a symbiotic association between host plant and certain group of fungi in the root system, in which the fungal partner is benefited by obtaining its carbon requirements from thephotosynthates of the host and the host in turn is benefited by obtaining the much needed nutrients especially phosphorus, calcium, copper etc., which are otherwise inaccessible to it, with the help of the fine absorbing hyphae of the fungus. Based on

the type of association formed by the ubiquitous fungi two broad groups, viz., Ectomycorrhizae and Endomycorrhizae have been recognized. Endomycorrhizal fungi grow intra and intercellularly forming special fungal structures in the cortical region of the host. These fungal organisms are further divided into three subgroups. They are Ericoid,orchid and the ubiquitous and large group of arbuscular mycorrhizal (AM) fungi. Ericoid mycorrhizal fungi have septate hyphae and colonize plants belonging to Ericaceae, Whereas, orchid mycorrhizal fungi are aseptate with clamp connections, and colonize orchidaceous plants. The mycorrhizal fungi having aseptate hyphae are grouped under vesicular arbuscular mycorrhizal fungi (AMF) (Shivaprasad and Sulochana 2000).

5.1A: Characteristics of Arbuscular Mycorrhizae

AM fungi are ubiquitous in nature and colonize most of the cultivated crops except members of Chenopodiaceae, Cruciferaceae and Caryophyllaceae. They are obligate symbionts and colonize the cortical region of the very fine absorbing roots of host plants. All soil fungi that form arbuscule are placed under order Glomales. The order Glomales belongs to class Zygomycetes of the subdivision Zygomycotina. Of the six recognized genera, *Glomus*, *Sclerocystis*, *Enterophosphora* and *Acaulospora* form both vesicles and arbuscules. *Gigaspora* and *Scutellospora* do not have the intraradicalvesicles. They posses the following characteristic features in general;

5.1B: Vesicles and arbuscules

AM fungi have aseptate mycelium and upon colonization do not change the morphology of plant roots. On root colonization the AM fungi produce two specialized structures known as vesicles and arbuscules in the cortex region of root. Arbuscules are complex structures similar to haustoria produced within the host cells. They serve as sites of nutrient exchange between host and the fungus. The vesicles are terminal; ovate to globose structures that containdrops of yellow

oil. It is reported that vesicles are thin walled, act as temporary food storage organs, but when vesicles remain thickwalled they might function as resting spores.

Arbuscular mycorrhizal fungi are always found associated with a host plant. All efforts to culture the fungi on laboratory media have been found to be futile. The fungus produces resting spores (chlamydospores) in soil which vary in size ranging from 60-200 μm and are recoveredfrom soil by wet sieving. They produce typical AM infection in axenic cultures of hosts. AM fungi are found associated with cortical tissues of very fine feeder roots. No visible tissue damage could be produced. The external mycelium of the fungus is comprised of coarse hyphae and a tuft of much finer thin walled absorption hyphae.

The absorption hyphae are important structures with regard to plant nutrition. It comprises of strategically placed network of additional absorbing surface which enable the plant to tap soil nutrients and water beyond the depletion zone, which are otherwise inaccessible to plant roots. Nutrient ions taken up by the hyphae are transported through the hyphae and released in the root cells by means of arbuscules.

5.1C: Beneficial attributes of AMF to plants

It is a well documented fact that the host plant is enormously benefited due to AM association. This includes the enhanced uptake of phosphorus and other nutrients, imparting resistance to stressconditions such as drought, high salt concentration and heavy metal toxicity, assisting in the enhanced nitrogen fixation by leguminous plants, resistance to soil borne diseases, improved water relations, early establishment and growth of nursery seedlings, preventing soilerosion etc.Irrespective of their type, the most important role of AM fungi is to obtain nutrients from the soil surface in tropics are not deficient and transfer them to their hosts. Although all the soils in tropics are not deficient in phosphorus, its unavailability due to phosphorus binding capacityis found to be a major constraint. This is true in the case of many agricultural fields in India, particularly the acidic soils of Kerala.

The presence of AM fungi in roots of crops grown in such soils is very useful with reference to their phosphorous uptake. Mycorrhizal structures effectively take up phosphorus from lower concentration at which normal plant roots fail to absorb.Further, the AM fungi increase the total surface area of absorptive system of plant and explore the soil by the external hyphae beyond the root hairs and phosphorus depletion zone. Absorbed phosphorus is converted to polyphosphate granules in the external hyphae and passed to the finger like arbuscules for transfer to the host. The same mechanism also helps the uptake of potassium, zinc, iron, copper, magnesium and calcium. Effect of AM in aiding the uptake of nutrients, particularly phosphorus, has been well documented in studies conducted in cassava, black pepper, cashew and legumes grown in phosphorus fixing soils of Kerala.

AM fungi play an important role in the water economy of plants. Their association improves the hydraulic conductivity of the root at lower soil water potentials and this improvement is one of the factorscontributing towards better uptake of water by plants. Also, leaf wilting after soil drying, did not occur in mycorrhizal plants until soil water potential was considerably lowered (approx. 1.0 M. Pa) Leaflets of *Leucaena* plants inoculated with AMF mycorrhizae did not wilt at a xylem pressure potential as low 10 as 2.0 MPa.

Colonization by AM fungi can improve drought resistance of plants. This has been demonstrated in green house studies in cultivated crops such as wheat, onion, capsicum and red clover as well as several other plant species.

5.1D: AM fungal inoculum

Pure culture of AM fungi is still considered as a big challenge for microbiologists. The pre-symbiotic growth of AM Fungi is characterized by formation of running hyphae. After some weeks without additional host partner, growth of germinated AM propagules stops. Several factors such as nutrition (Hepper, 1983), chemical treatments (Gianinazzi-Pearson *et al.*, 1989),

and genetical factors (Bianciotto and Bonfante, 1993) have been studied to explain the lack of pure growth of the extraradical phase of AM fungi. Low amounts of natural or synthesized flavonoids may stimulate the fungal growth (Chabot *et al.*, 1992b). Otherwise, certain amino acids (Hepper and Jacobsen, 1983), volatile compounds, particularly carbon dioxide and root exudates, promote hyphal elongation (Carr*et al.*, 1985; Elias and Safir, 1987; Bécard and Piché, 1989b). Hildebrant *et al.* (2002) mentioned the presence of slime-forming bacteria identified as *Paenibacillus validus* on surface sterilized spores of *Glomus intraradices.* These bacteria stimulated the growth of *G. Intraradices* up to spore formation in the absence of any plant tissue. Similar results on presymbiotic sporulation have been found with other *Glomus* sp. The authors explain this unusual sporulation by possible utilization by the fungi of a chemical component (or components) secreted by the bacteria. Recently Buee *et al.* (2000) isolated a semi-purified fraction called "Branched factor" from 8 mycotrophic plant species that were very active in starting the germination and nuclear division of *Gigaspora* sp. The richness of the intraradical forms of AM in lipid contents may actually support the growth irrespective of the decrease in metabolic activity of the root explants by both surface sterilization and the shearing process. The results confirm that the quality rather than the quantity of root exudates is more involved in the stimulation of hyphal elongation (Carr*et al.*, 1985; Elias and Safir, 1987).

5.1E: Monoxenic cultures

All types of AM propagules (isolated spores or vesicles, mycelia, sheared mycorrhizal roots) are virtually able to initiate AM symbiosis. Chlamydospores of *Glomus* sp.(Mosse and Hepper, 1975; Mugnier and Mosse, 1987) and non-sporocarpica zygospores of *Gigaspora margarita* (Bécard and Fortin, 1988; Bécard and Piché, 1989a; Diop *et al*. 1992) are obviously the preferred inoculum starter even though dormancy andstrain mutation may occur under greenhouse conditions. The preference is due to availability of facility to recover,sterilize and to germinate these propagules. Considering the inability of some AM fungi to produce spores, the use of

intraradical forms of AM fungi seems to be a good opportunity to establish AM symbiosis. As intravesicles in mycorrhizal roots act as reserves and propagules, they have a higher inoculum potential than other AM propagules such as spores and hyphae (Magrou, 1946).

For all AM propagules, proper selection and efficiency of sterilization process are keys of the success of axenic or monoxenic AM fungal cultures. Isolated spores are often surface sterilize using the two-steps procedure of Metz *et al.* (1979) as modified by Bécard and Fortin (1988). AM sheared inocula are surface sterilized according to Diop*et al. (*1994) method. Then, vesicles are easily isolated by lacering from heavily colonized roots. Basically, the surface sterilization involves dipping in chloramines T (2%) solution with traces of a surfactant (Tween 20/80) and antibiotics such as Streptomycin sulphate or gentamycin. Sterilized AM propagules must be stored at 4°C until use.

Developing countries will benefit more through AM symbiosis using the monoxenic system, which is cheaper than greenhouse culture (Diop *et al.* 1994a).

Mycorrhizae provide many other benefits to plants. The fungal filaments take up and store water decreasing drought stress during dry periods. Plant roots are too thick to access the small pores that retain large amounts of water in the soil. The much thinner mycorrhizal hyphae easily penetrate into smaller spaces between soil particles and supply essential water during periods of moisture deficit.

The synthetic media used for micropropagation of plants do not contain AM fungi. Such micropropagated plants establish mychrrhizal association only after their transplant in the field soil.However an early inoculation of this plant with specific AM fungi bring modifications in root morphology improve plant performance and provide same benefits to micropropagated host as in the case of micropropagated plants (Lovato *et al*.1995).

During the last decade lot of information have been generated on mychirrhizal association in microrpropagated plants especially in fruit crops and some other crops for their better survival and improved growth.Many micropropagated plants have shown positive response when inoculated with AM fungi. Mychorrhizal fungal inoculation has also been found to influence root morphology and increase in l production of lateral roots in microrpropagated grapevine (Schellenbaum *et al*. 1991). Inoculation with *Glomus mosseae* induced more branched root system in micropropagated plum root stock (Giovennathi *et al*. 1996).

The application of AM fungi also offers an opportunity to reduce fertilizer as well as to limit use of pesticides (Hooker *et al*. 1994b). AM inoculation reduces acclimatization period by about 8 weeks as reported in *Anthylis systitoides*.

In addition symbiotic association between AM fungus and roots of micropropagated plantlets enhances the ability of later to establish and cope with stress situations arising as a result of certain alterations in environmental conditions encountered as a result of certain alterations in environmental conditions encountered as a result of their shift from in vitro to in vivo conditions.

The establishment of the symbiosis with the root system of micropropagated plants gives positive effects on survival and growth rates after acclimatization. The purpose of this investigation was to evaluate the influence of mycorrhiza on plant survival and on growth responses of two different species of micropropagated plants.

5.2: Results and Discussion

5.2A: Effect of AM fungi on seedling height and number of leaves in Maize Plant

To see the effect of AM fungi,the maize seeds were grown in normal soil used as control and maize seeds were grown in sterilised soil with AM.The results on seedling height and number of leaves were recorded after 7 days(Fig.5.1).It was observed that the seedling height and number of leaves were more in seedlings containing AM which proves AM fungi is beneficial for growth of plants (Plate 5.9, A-F).

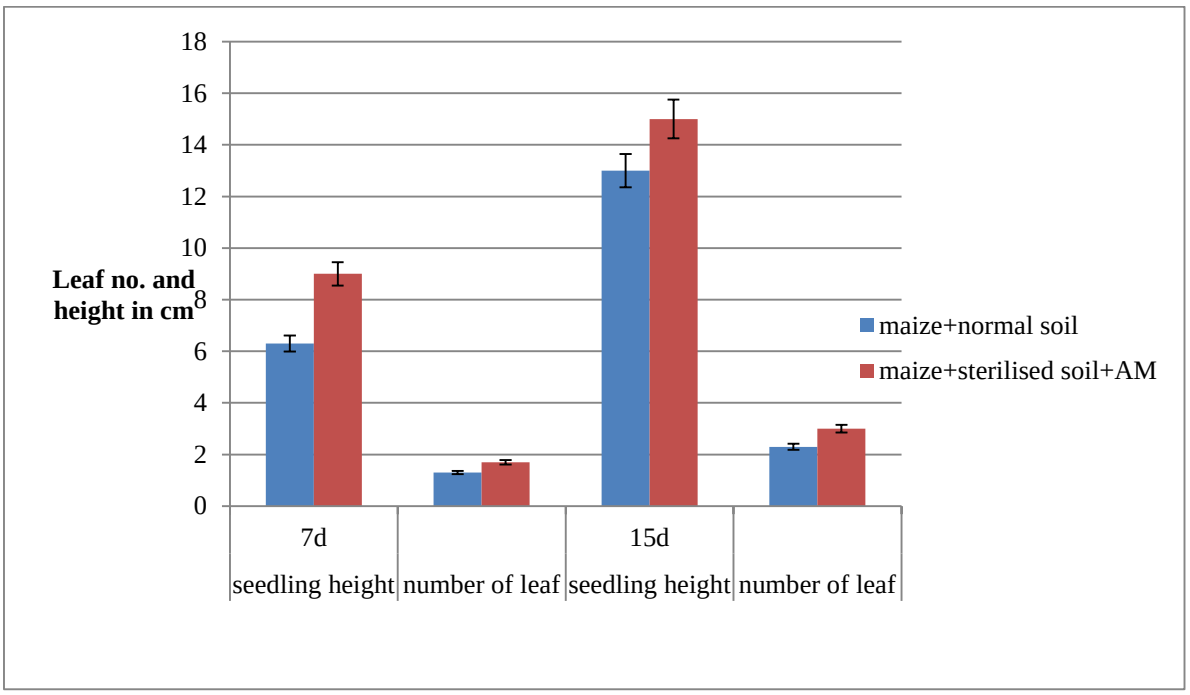

Figure 5.1: Effect of AM fungi on seedling height and number of leaves in maize plants

5.2B: Effect of AM fungi on maize plant biomass

In this the Maize seeds were grown in pots with AM and without AM.After one month seedlings were taken for fresh weight and dry weight.For dry weight the seedlings were kept in hot air oven for 48 hour at 80ºC ± 5 ºC.The fresh weight and gry weight of leaf+stem and root with AM and without AM were taken in three samples .It was observed that the samples containing AM fungi obtained highest fresh weight and dry weight(Fig.5.2).This proves that AM fungi is beneficial for growth of plants.

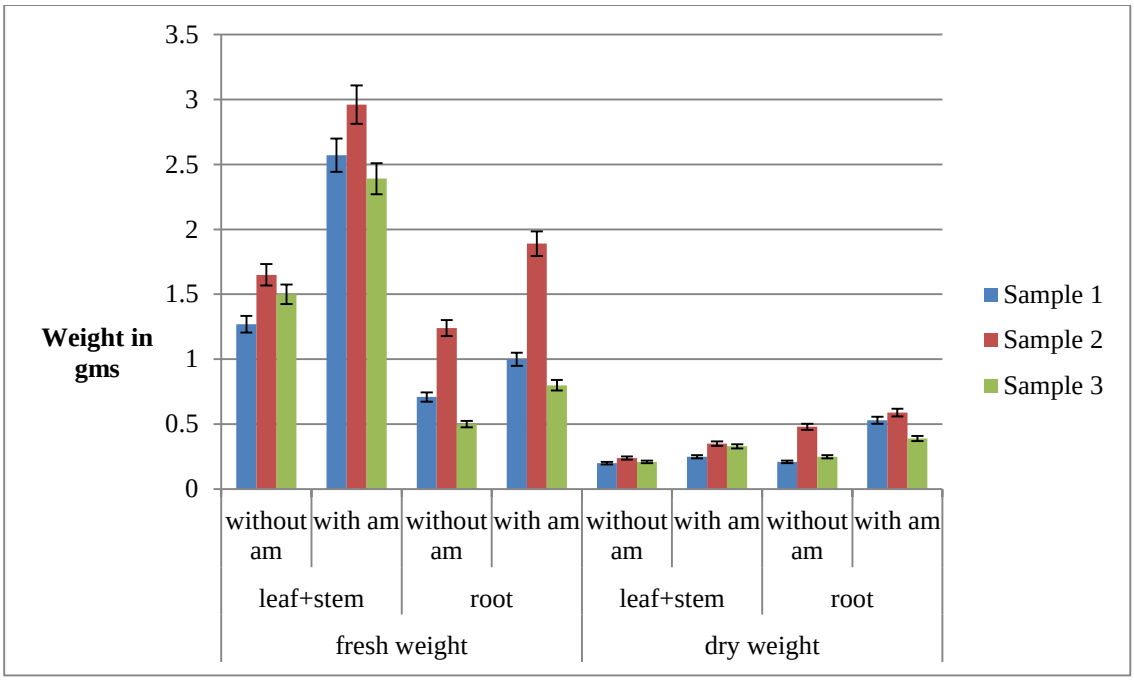

Figure5.2:Effect of AM fungi on fresh weight and dry weight in maize plant

5.2C: Physical parameters of soil samples

The soil samples were analysed in lemon and fig tree roots for different parameters which is described in Table:5.1.

Table 5.1 : General information and physical parameter of soil samples which were collected from Lemon and Fig tree roots.

Sr.No.	Parameter	Lemon plant field	Fig plant field
1.	Soil samples collection from	Khedoi (Kutch)	Ratnal (Kutch)
2.	Farm/plot no.	696/1	26/1
3.	TC/Non TC plants	Non TC (Kagdi)	Non TC(Poona red)
4.	Fruit Harvesting time	Feb. to April	Nov. to March
5.	Any Diseases	Sukara	Tambera
6.	Average fruit harvesting from per plant/ season	80-120 kg/plant/season	50-80 kg/plant/season
7.	Mother Plant collection from	Local field (by grafted)	From Poona(by grafted)
8.	Farmer's Name	Mr. Praveen Singh T. Jadeja 9979667627	Mr. Rahul Gala 9375050000

Results from Table 5.2. Describe about the fresh weight and dry weight of soil samples collected from rhizospheric part of two different plants.

- In case of lemon plants all 10 samples obtained 25 gm were taken and lowest dry weight obtained was 22.34 and highest 24.27.Sample 3 obtained highest percent moisture ie. 97.08%, (Plate 5.1, A-I).
- In case of fig plant all 10 samples obtained 25 gm were taken and lowest dry weight obtained was 23.69 and highest 24.27.Sample 4 obtained highest percent moisture ie. 94.76%.

Table 5.2 Percentage moisture in collected soil samples (20 samples from lemon and fig cultivation area

Lemon soil samples				Fig soil samples			
Sr. No.	Fresh Weight (gm)	Dry Weight (gm)	% Moisture	Sr. No.	Fresh Weight (gm)	Dry Weight (gm)	% Moisture
1	25	23.11	4.32	1	25	22.37	4.47
2	25	23.89	4.18	2	25	23.05	4.33
3	25	24.27	4.12	3	25	23.34	4.28
4	25	24.14	4.14	4	25	23.69	4.22
5	25	24.21	4.13	5	25	23.00	4.34
6	25	24.25	4.12	6	25	23.62	4.23
7	25	21.45	4.66	7	25	23.02	4.34
8	25	23.50	4.25	8	25	23.42	4.26
9	25	23.70	4.21	9	25	22.65	4.41
10	25	22.34	4.47	10	25	23.29	4.29

Note : All 20 soil samples were kept in Oven for 3 days at 75 degree centigrade

Table 5.3 Field data of Lemon plantation

Sr. No.	Plant code No.	Age of Plant (Year)	Soil pH	Location (GPS)		Remarks
				Latitude	Longitude	
1	I	5	7.2	23˚ 07'N	69˚ 90'E	All soil samples were collected from different places of
2	II	6	7.4	23˚15'N	69˚78'E	
3	III	7	7.5	23˚05'N	69˚65'E	
4	IV	4	7.6	23˚00'N	69˚61'E	
5	V	5.5	7.6	23˚15'N	69˚51'E	
6	VI	6.5	7.7	23˚07'N	69˚61'E	
7	VII	7.5	7.7	23˚05'N	69˚63'E	

8	VIII	8	7.6	23˚01'N	69˚68'E	Lemon orchard
9	IX	7.5	7.7	23˚11'N	69˚61'E	
10	X	4.5	7.8	23˚15'N	69˚80'E	

All the samples collected from field of lemon had basicpH(7.2-7.8) in different age group of lemon plants. Plate 1 shows mature trees of lemon in the field. From this field soil samples were collected.

Table 5.4 :Field data of Fig plantation

Sr. No.	Plant code No.	Age of Plant (Year)	Soil pH	Location (GPS)		Remarks
				Latitude	Longitude	
1	I	8	7.6	23.22'N	69.84'E	All soil samples were collected from different places of Fig orchard.
2	II	9	7.7	23.18'N	69.81'E	
3	III	7	7.7	23.25'N	69.61'E	
4	IV	10.5	7.8	23.27'N	69.75'E	
5	V	10	7.7	23.31'N	69.51'E	
6	VI	7.5	7.7	23.21'N	69.78'E	
7	VII	6.5	7.8	23.19'N	69.81'E	
8	VIII	7.5	7.7	23.11'N	69.41'E	
9	IX	8.5	7.9	23.08'N	69.40'E	
10	X	6	7.8	23.16'N	69.83'E	

All the samples collected from field of fig plantation, obtained slightly alkaline pH (7.6-7.9) in different age (6-10.5 years) of fig plants. The plate 1 shows mature tree of fig plants in field of different code (I-X). From the field where plantation of fig were done soil samples were collected (Plate 5.3, A-J).

Plate :5.1

Plate 5.1 (A – D) : Different codes (I-IV) indicate mature lemon trees present in Khedoi village of Kutch region.

Plate :5.2

Plate 5.2 (A – F) : Different codes (V-X) indicate mature lemon trees present in Khedoi village of Kutch region.

Plate 5.3

Plate 5.3 (A – F): Different codes (I-VI) indicates mature fig trees present in Ratnal village of Kutch region.

Plate 5.4

Plate 5.4 (A – D): Different codes (VII-X) indicates mature fig trees present in Ratnal village of Kutch region.

Table 5.5:Isolation of AM spores in 100 gm soil sample (number of spores) of Lemon orchards.

Sr.No.	Plant Code	Date of Isolation	No. of AM Spores in 100 gm soil
1	I	26-11-2015	45
2	II	26-11-2015	21
3	III	26-11-2015	91
4	IV	26-11-2015	57
5	V	27-11-2015	55
6	VI	27-11-2015	33
7	VII	27-11-2015	75
8	VIII	27-11-2015	93
9	IX	27-11-2015	135
10	X	27-11-2015	270

In lemon plants the AM spores were isolated from 100 gm soil samples, in which highest 270 spores were collected in sample no.X (Plate 5.5, A-D).

Table 5.6:Number of spores per 100 gm soil from Fig orchards

Sr.No.	Plant Code	Date of Isolation	No. of AM spores in 100 gm soil
1	I	28-11-2015	65
2	II	28-11-2015	81
3	III	28-11-2015	105
4	IV	29-11-2015	25
5	V	29-11-2015	80
6	VI	29-11-2015	100
7	VII	29-11-2015	80
8	VIII	30-11-2015	50
9	IX	30-11-2015	60
10	X	30-11-2015	35

In fig plants the AM spores were isolated from 100 gm soil samples, in which highest 105 spores were collected in sample no.III.

Plate: 5.5

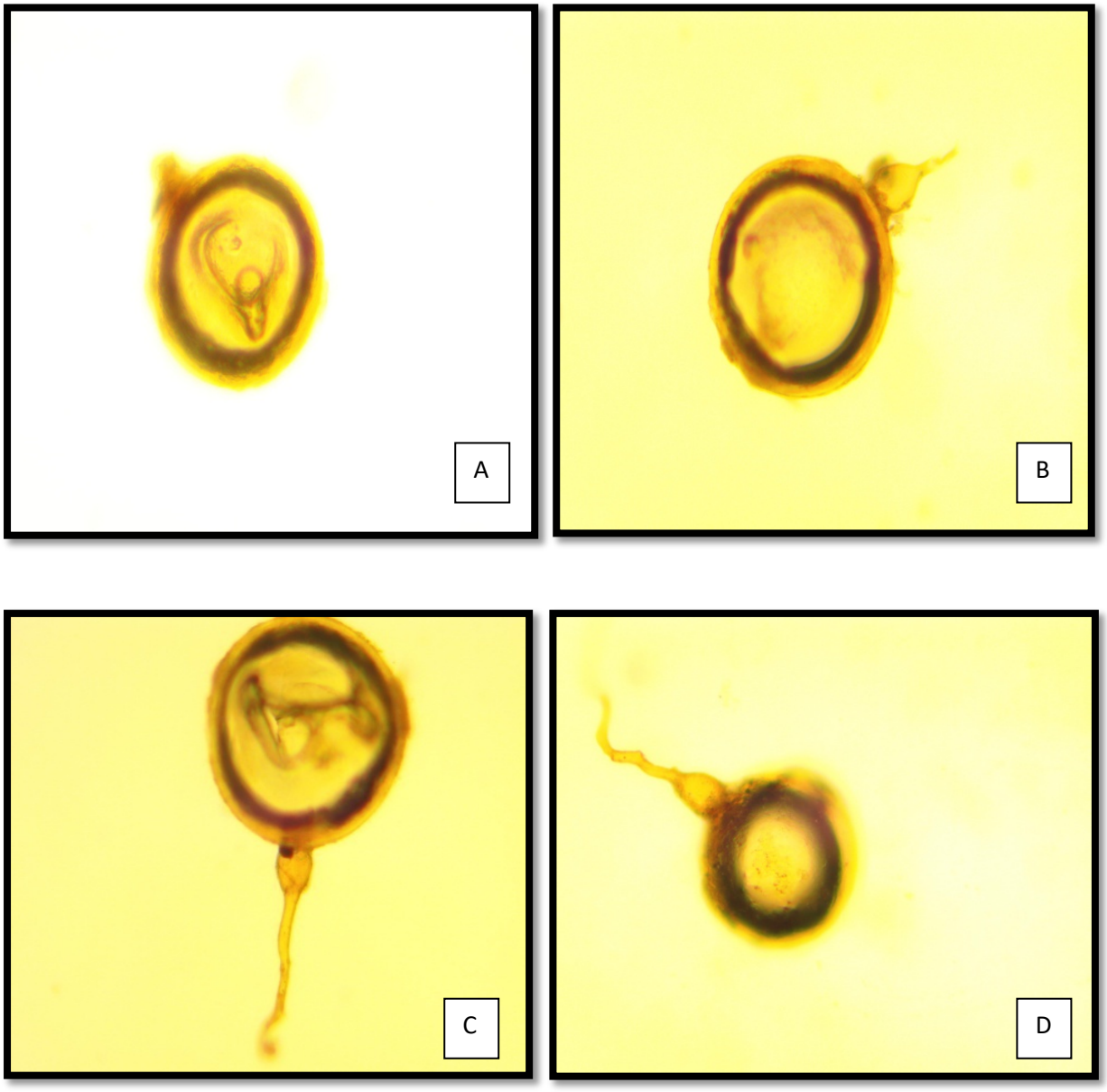

Plate 5.5:(A-D) Photographs of AM spores of *Gigaspora*species

Plate :5.6

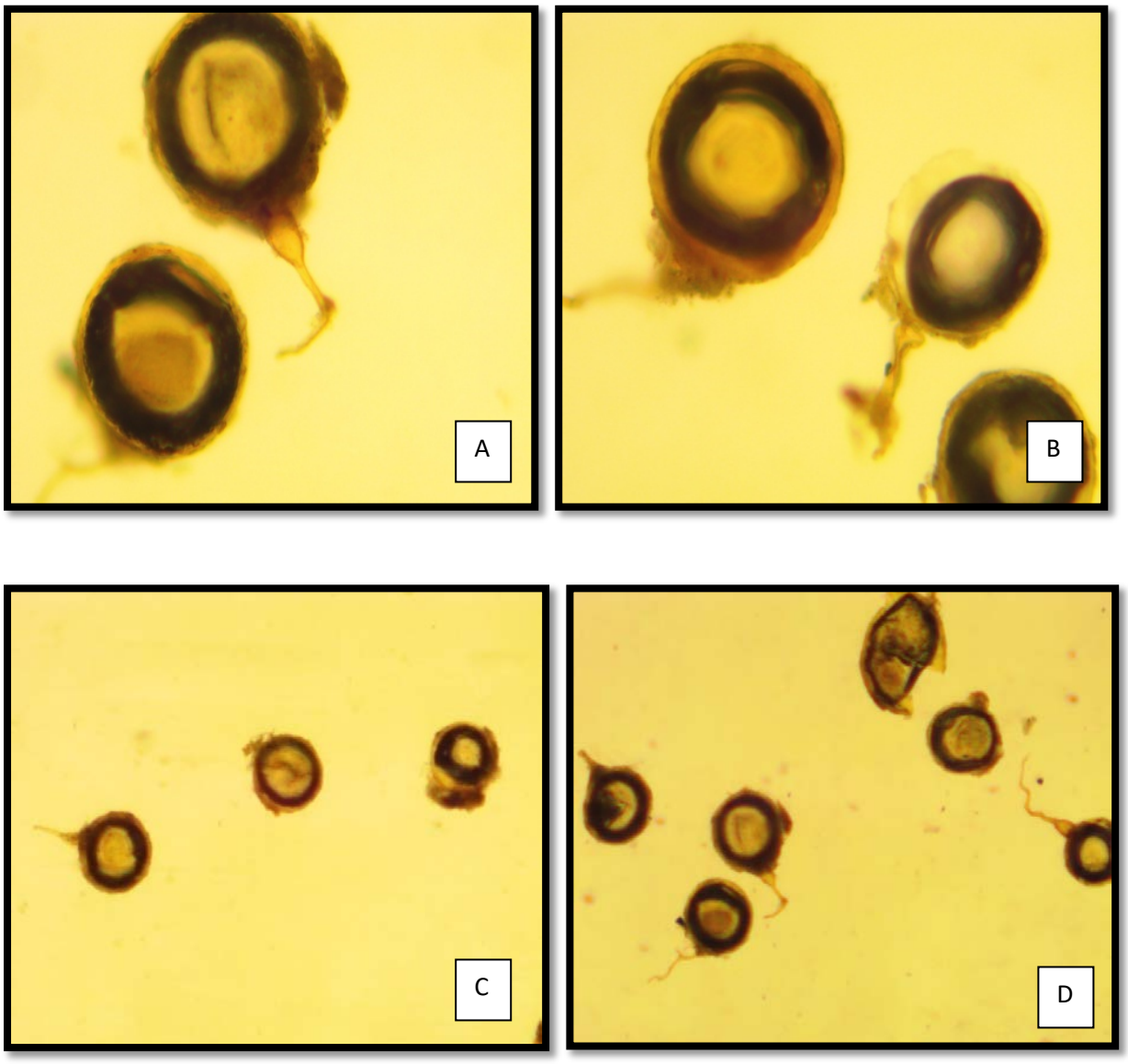

Plate 5.6: (A-D) Photographs of spores of *Gigaspora* species

Plate : 5.7

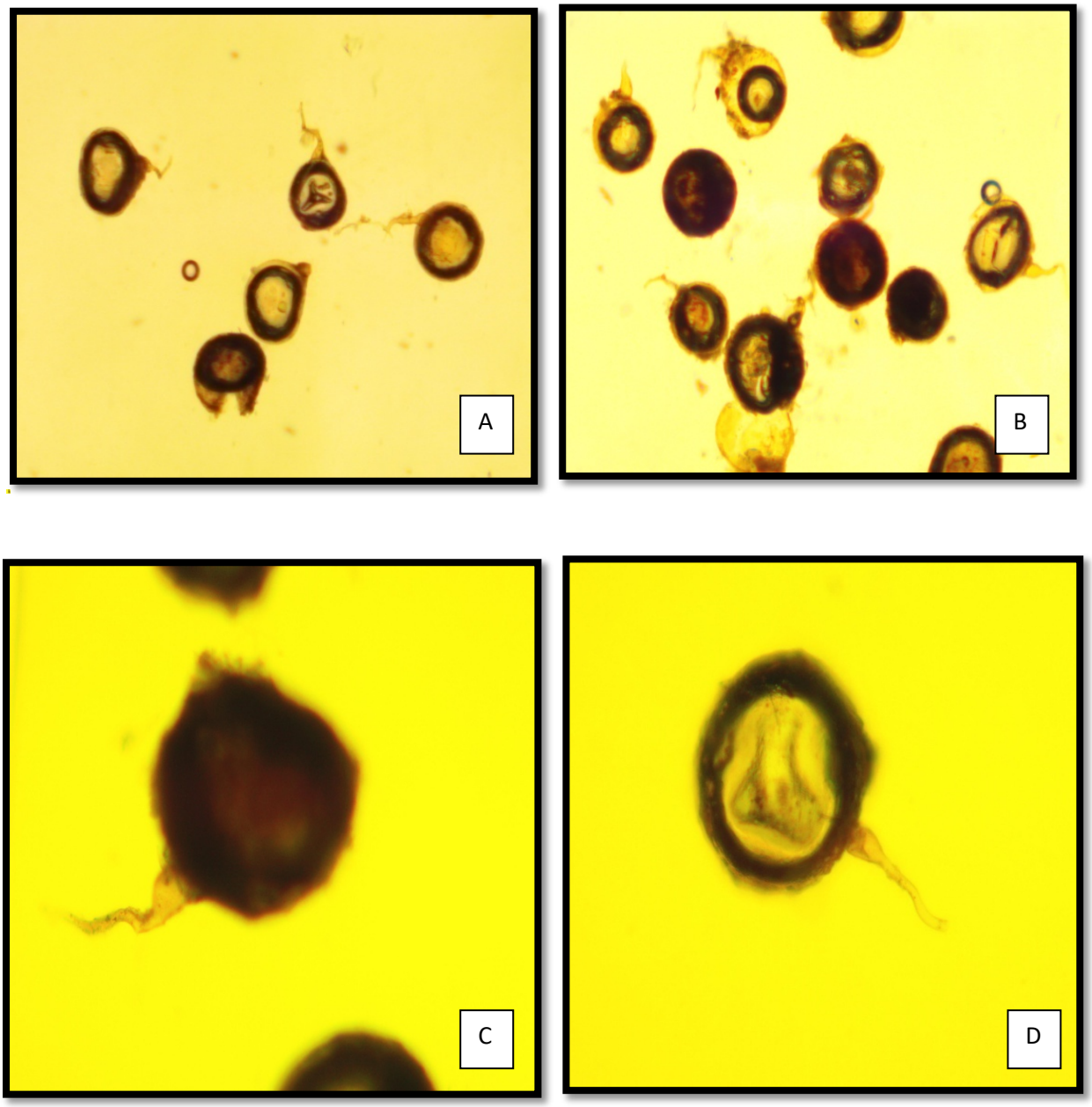

Plate 5.7:(A-D) Photographs of spores of *Gigaspora* species.

***Gigaspora albida* N.C. Schenk & G.S. Smith**

Spores found singly in soil, dull white coloured with light greenish yellow ting. globose to subglobose. 200-280 µm in size. Spore wall three layered, L1 hyaline to pale yellow, adherent; L2 thickened at maturity by multilayering and L3 thin walled. Sporogenous cell 32-45 µm in size, auxiliary cells aggregates of 4-20 are formed with narrow projections.

***Gigaspora gigantean* (T.H. Nicolson & Gerd.)Gerd.& Trappe**

Spores found singly in soil, bright greenish yellow to bright orange in colour, globose to subglobose, rarely irregular. 240-400µm in size. Spore wall three layered, L1 adherent and pale yellow, L2 laminate, thickening as the spore wall is differentiating at germination. Subtending hypha shows sporogenous cell (38-54 µm), auxillary cells aggregates of 4-20 are formed with narrow projection. Immature spores often are salmon-coloured to orange with opaque content.

(Plate 5.5 (A-D), 5.6 (A-D) and 5.7(A-D).

Plate :5.8

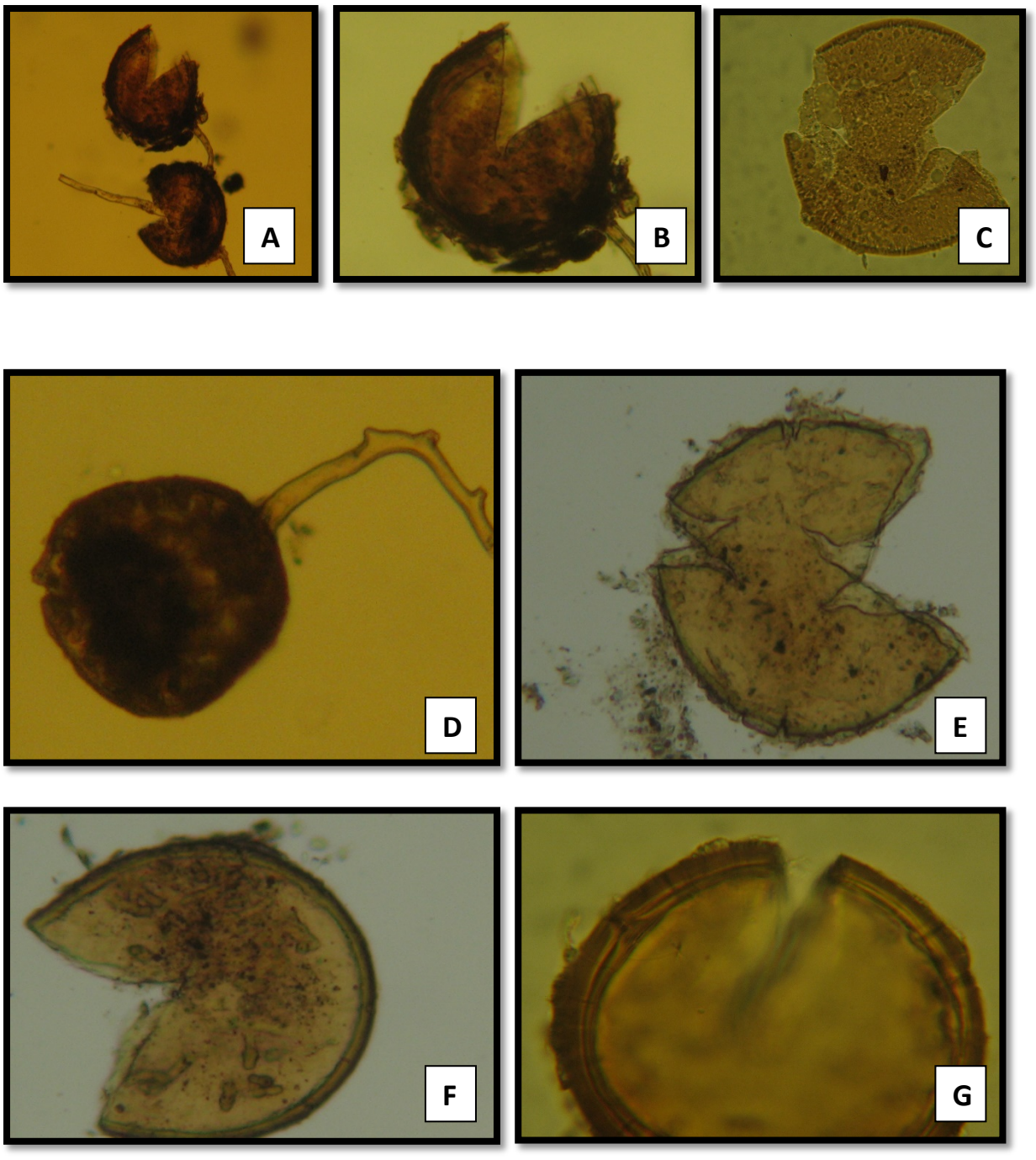

Plate5.8:(A-G) Photographs of spores of *Glomus* species.

***Glomus aggregatum* N.C. Schenck & G.S. Smith**

Loose cluster of spores found in sporocarp without a peridium, found in the roots and soil. Spores are globose, obovate to irregular in shape, 40-120µm in diameter and hyaline to pale yellow in color. The spore wall consists of 1-2 layers (L1, L2). The subtending hyphae usually straight or flared or constricted sometimes recurved; double subtending hyphae at the point of attachment were sometimes observed in samples collected from the region. The hyphal pore usually is open, sometimes closed by thin septum. (Plate 5.8A)

***Glomus fasciculatum* (Thaxt.)Gerd.& Trappe**

Zygospores found in loosely coherent spongy mass, Pale yellow to pale yellow-brown in colour, globose to subglobose, 60-110 µm in diameter. Spore wall consisting of three layers, L1 is hyaline producing a pinkish-red reaction in Melzer's reagent; L2 is thin light yellow-brown; L3 is thin flexible and continuous with the innermost layer of the subtending hypha. Subtending hypha is sylendrical to slightly flared.

***Glomus intraradices* N.C. Schenck & G.S. Smith**

Spores pale creame to yellow brown sometimes with a green tint, Globose, subglobose with some elliptical spores. Size ranging between 40-140µm. Spore wall three layered (L1, L2 and L3), L1 wall layer hyaline, L2 wall layer hyaline with age, this layer degrades with L1 and often found together as rough patches. L3 wall layer pale yellow-brown, sublayred (or laminae) that either remain adherent or separate with applied pressure. In juvenile spores, initial sublayer is thin and become thick due to formation of additional sublayers. Thickness varies from 3.2-12µm in mature spores. L3 layer simultaneously develop in subtending hypha. Subtending hypha is cylindrical, occationally slightly constricted and 11-18 µm thick.

***Glomus mosseae* (T.H. Nicolson & Gerd.) Gerd. & Trappe**

2-8 spores are surround by tight peridium to form sporocarp, spores found singly in soil spores straw coloured to dark orange-brown, majority are yellow-brown, globose to subglobose, 100-260 µm, spore wall three layered (L1, L3 and L3). Outer L1 is hyaline, mucilaginous, often degrading, sloughing in mature spores, middle L2 is hyaline, refractile fracturing into fragments, inner L3 layer is yellow brown to pale orange-brown, subtending hypha funnel-shaped with 16-32 µm length. (Plate 5.8 D).

Plate : 5.9

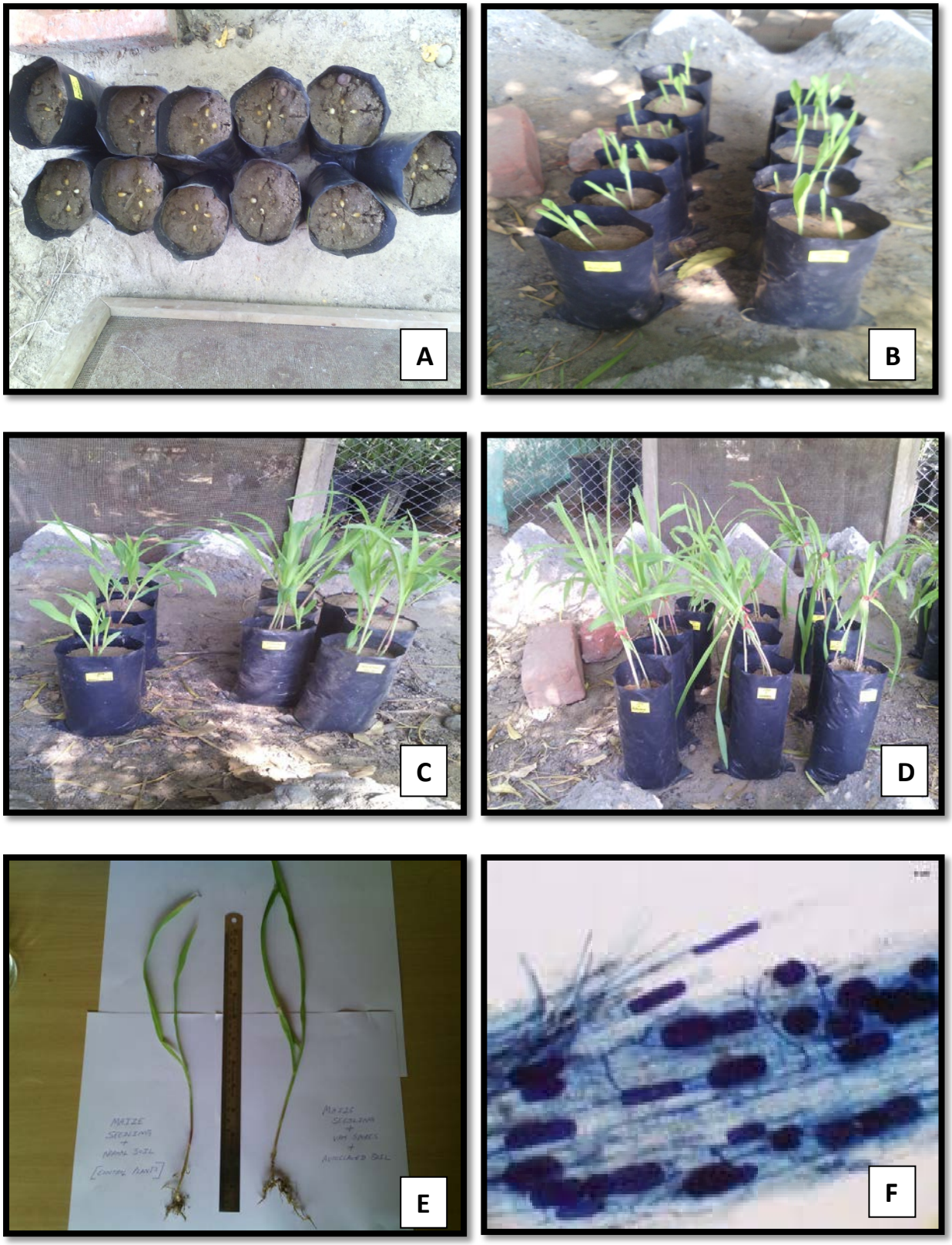

Plate 5.9 : A. Maize seed Sown with AM spores, B. Germinated Maize seeds with and without AM fungi, C,D. Seedling of Maize after 15 days, E. Seedlings of Maize showing effect of plant growth after AM interaction, F. Section of Maize root showing presence of arbuscules and vesicles.