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Mycorrhizal association and root colonization of *Citrus reticulata*, *Citrus medica* and *Citrus limonia* grown in Darjeeling hills and foot hills

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Diversity of AM fungi associated with root colonization of *Citrus reticulata*, *Citrus medica* and *Citrus limonia* were studied. Arbuscular mycorrhizal fungi were isolated from the rhizosphere of these plants growing in different regions of Darjeeling hill areas such as Mirik, Kalimpong, Bijanbari, Kurseong as well as foot hills using wet sieving and decanting method. Microscopical observation revealed the presence of different AM spores. Presence of *Gigaspora* sp. was profuse in monsoon and winter season whereas during the summer, *Glomus* sp. flourished in all the locations. *Scutellospora* sp., *Acaulospora* sp. and *Entrophospora* sp. were also present in all the citrus species. Histopathological variations have also been observed among them. Microscopical observation revealed the presence of vesicles, arbuscules and dark septate endophyte (DSE). DSE was abundant in root tissues of *C. limonia* in comparison with C. *reticulata* and *C. medica*.

Key words: AM fungi, mandarin, dark septate endophyte, *Citrus reticulata*, *Citrus medica,Citrus limonia*

INTRODUCTION

The Eastern Himalaya and North-Eastern states are considered as the original homeland of citrus in India. In Darjeeling, *C. reticulata*, *C. medica* and *C. limonia* is extensively grown for its fruit. *C. medica* and *C. limonia* are widely used as rootstocks to raise grafted plants. Roots of plants are an important factor for providing nourishment to the plants. Arbuscular mycorrhizal fungi is one of the constituents of soil microbial constituent which helps in absorption of nutrients from the soil and protects the plants against root rot disease thereby improving the health and subsequently quality fruits are obtained.

Arbuscular Mycorrhizal Fungi (AMF) has 90% symbiotic association with higher plants (Chakraborty and Chakraborty, 2012). Arbuscules, vesicles and hyphae in the roots and spores with hyphae in the soil rhizosphere are formed by AMF. This hyphal network of AMF with plant roots augment the roots in the soil surface thereby causing an improvement in plant growth (Bowles *et al*., 2016) as well as translocation of various nutrients (Rouphael, *et al*., 2015). The root hairs of citrus plants are very short so they need mycorrhiza to help in absorbing adequate water and nutrition (Ortas, 2012). Molecular detection of AMF and their role in symbiosis and crop protection have been documented (Chakraborty, 2019). They also help in reducing biotic and abiotic stresses. AMF utilization is highly encouraged in modern global agricultural system in order to reduce the use of synthetic fertilizers and chemicals so as to have healthy crops and vegetables (Begum *et al*., 2019). The efficacy of AM fungi as a vital component of sustainable crop production systems, and it's prospective for exploitation as an on-farm agro-put has recently been reviewed (Rodrigues and Rodrigues, 2020). In the present study attempts have been made to document diversity of AM fungi associated with root colonization of *Citrus reticulata*, *Citrus medica* and *Citrus limonia* grown in Darjeeling hills and foot hills and exploittaion of dominant AMF along with other

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bioinoculants for improvement of health status of mandarin plants.

MATERIALS AND METHODS

Plant Material

Two months old nursery grown mandarin (*Citrus reticulata*) seedlings obtained from IARI Kalimpong, Nirmaldass Orchard Gurung Brothers Nursery Baramangwa Busty, Darjeeling, Bijanbari, and Mirik were used for experimental purposes. One year old *C. limonia* seedlings were obtained from Citrus Dieback Research Station (CDRS), Kalimpong and *C. medica* from Padmaja park, University of North Bengal. The selected seedlings initially maintained in 6" dia plastic pots and watered regularly for proper growth. After one year of growth, seedlings were transferred in the earthenware pots (12" dia). These were kept in Glass House conditions and after two years seedlings were planted in the experimental field. Suitable management practices were adopted in the field throughout the years (Fig. 1).

Isolation of AMF

Arbuscular mycorrhizal fungal spores from the three citrus species *viz.* Darjeeling mandarin (*Citrus reticulata*), Lime (*C. medica*) and Rangpur lime (*C. limoni*) were isolated from different regions of Darjeeling hill areas, Mirik[27° 04'04.74" N 88°11'27.66" E], Kalimpong [27°03'31.34"N 88°28'-00.05"E], Bijanbari[26°53'09.47"N 88°10'58.05" E], Kurseong [26°53'54.34"N 88°16'38.59" E], and foothills[26° 42'36.03" N 88°21'05.20" E] by wet sieving and decanting method (Gerdemann and Nicolson, 1963). Approximately 250 g of soil was suspended in 1 L water. Heavier particles were allowed to settle for a few seconds and the liquid was decanted through sieves of decreasing size (BS 60, BS 80, BS 100, BS 150 and BS 200). Pores are fine enough to remove the larger particles of organic matter, but coarse enough to allow the desired spores to pass through. The suspension that passed through these sieves was saved and stirred to resuspend all particles. The heavier particles were allowed to settle for a few seconds and the liquid decanted again through the sieve and spores collected by fine brushes and were kept in different petri plates according to their size and colours. Moreover for further observations or purification of AMF spores sucrose gradient centrifugation method was used. In sucrose gradient centrifugation (Daniels and Skipper, 1982), spores and minimal amount of organic particles were further purified by suspending sieving in 40% sucrose solution and centrifuging at 2000 rpm (approximate 370 x g) for 1 minute. The supernatant (with spores) was passed through a sieve of 400 mesh and rinsed with distilled water to remove sucrose residue. Their average spore population were determined.

Morphological characterization of AMF spores

With the help of a simple microscope (20X) parasitized spores, plant debris etc were separated. Spores were sonicated at 30 Hz for two minutes to remove the debris adhered to the spores then clean spores were stained with Melzar's reagent (50% aqueous solution of chloral hydrate with 2.5-3.75% potassium iodide and 0.75-1.25% iodine) and studied microscopically. For further use, the AMF spores were stored in Ringer's Solution (8.6g NaCl, 0.3g KCl, 0.33g $CaCl_2$ in 1 L of boiled distilled water) at -15ºC to -20 ºC or in sterile distilled water. Identification of genera and species was done microscopically using the specific spore characters such as size, colour, shape, wall structure, surface ornamentation and bulbous suspensor by using identification manuals (Trappe, 1982; Schenck and Perez, 1990) and using the help of standard keys (Walker, 1992) and website of INVAM.

Histopathological studies of mandarin roots

Fungal association of AM fungi within the root tissues was observed according to Philips and Hayman (1970). Young roots from mandarin plants were dug out manually. Roots were cut into 1cm or smaller pieces and washed in tap water gently to free them from soil particles. It was boiled in 2% KOH in hot water bath for 1 hour. The KOH was decanted and the roots washed with water for 2-3 times. 1% HCL was added and kept for 30 minutes. After decanting the HCL the sample was washed thrice in tap water and cotton blue, lactic acid and glycerol was added in the ratio 1:1:1 to stain the internal structures of AMF inside the root segments i.e. arbuscules, vesicles, auxiliary cells, and boiled in water bath for 1 hour. The excess stain was decanted and sample placed in 50% glycerol for destaining. The roots were then crushed under pressure in slide and covered with cover slip for microscopic observation. Percent root colonization was determined following the method of Giovanetti

Table 1: Percentage of spores in 100gm soil from different locations of mandarin orchards during summer season

Name of AMF	Location				
	Kalimpong 27°03'31.34"N 88°28'00.05"E	Mirik 27°04'04.74" N 88°11'27.66"E	Bijanbari 26°53'09.47"N 88°10'58.05"E	Kurseong 26° 53'54.34"N 88°16'38.59"E	Foothills 26°42'36 .03 " N 88°21' 05. 20"E
Glomus mosseae	26±0.41	23±0.70	18±1.41	30±0.94	36±0.52
Glomus aggregatum	06±0.43	ND	02±0.72	23±0.42	08±0.41
Glomus fasciculatum	08±0.92	05±0.42	08±0.44	16±0.32	08±0.70
Glomus badium	07±0.71	13±0.61	12±0.47	ND	07±0.73
Glomus constrictum	ND	ND	08±0.38	ND	09±0.92
Glomus versiforme	05±0.44	03±0.64	04±0.70	ND	ND
Gigaspora gigantea	19±0.94	18±0.79	18±0.51	12±0.62	06±0.40
Gigaspora margarita	12±0.47	14±0.71	11±0.87	10±0.71	08±0.73
Acaulospora bireticulata	05±0.42	08±0.75	03±0.48	04±0.74	10±0.38
Acaulospora capsicula	09±0.39	03±0.47	14±0.53	ND	04±0.71
Scutellospora rubra	02±0.44	13±0.45	01±0.272	05±0.84	04±0.42
Sclerocystis sp.	01±0.27	ND	01±0.22	ND	ND

±=SE ; ND = Not detected

and Mosse (1980). Since variations in vesicles, hyphal branching patterns, structure of hyphae and staining intensity of hyphae are different for each genus, it is possible to identify Glomeromycotan fungi upto genus level but it is difficult to separate species.

RESULTS

Diversity of AM Fungi in Citrus reticulata, Citrus medica and Citrus limonia

Among the AM fungi, *Gigaspora* sp. especially *Gi. gigantea* and *Gi. margarita* was found to be the dominant genus in mandarin rhizosphere during the winter season; but during the summer season, *Glomus* sp. dominated the spore population from all the soil samples collected from different mandarin orchards. Among the *Glomus* species, *G. mosseae*, *G. fasciculatum* and *G. aggregatum* were dominant. *Scutellospora* and *Acaulospora* were the less common genus found in the soil with a few occurance of *Entrophospora* sp., *Gi. gigantea*, *G. mosseae*, *G. fasciculatum*, *Scutellospora rubra* and *Acaulospora* sp. were the most common spore found throughout the year in all the soil samples.*G. mosseae*, *G. fasciculatum* and *G. aggregatum* were the most dominant spoes found in the soil from Kurseong whereas *Gi. gigantea* and *G. mosseae* were dominant spores in soil from Kalimpong, Mirik and Bijanbari (Fig. 2).

In *C. medica* the association of *Glomus* sp. was more abundant than when compared to *C. reticulata*. Over all spore count showed four different kinds of *Glomus* species to be dominant in all the soil samples. Among them *G. fasciculatum* and *G.mosseae* were most abundant. The genus *Acaulospora* comprises of *A. bireticulata, A. capsicula* and *A. delicata*. Among *Gigaspora*, species of *Gi. gigantea* and *Gi. margarita* are common and few unidentified spores of *Acaulospora*. *Scutellospora* are fewer in comparison to others and presence of *Sclerocystis* was rare (Fig.3).

In *C. limonia* also, the association of *Glomus* sp. was more dominant. *G. fasiculatum, G. mosseae, G. ambisporum, G. multicaule, Gi. gigantea, Gi. margarita, Gi. albida,A.cavernata, A. bireticulata* and *Scutellospora* sp. are some of the common spores found in *C. limonia* (Fig.4).Among the AM fungi, *Glomus mosseae* could be determined as the most predominant, followed by other genera such as *Gigaspora*, *Acaulospora* and *Scutellospora*. Percentage of AM spores determined from different regions showed maximum of different *Glomus* sp., followed by *Gigaspora* sp., *Acaulospora* and *Scutellospora* during the summer months. Presence of *Gigaspora* sp. was profuse in monsoon and winter season but their spore popu

Table 2: Percentage of spores in 100gm soil from different locations of mandarin orchards during monsoon season

Name of AMF	Location				
	Kalimpong 27°03'31.34"N 88°28'00.05"E	Mirik 27°04'04.74" N 88°11'27.66" E	Bijanbari 26°53'09.47"N 88°10'58.05"E	Kurseong 26° 53'54.34"N 88°16'38.59"E	Foothills 26°42'36.03" N 88°21'05.20"E
Glomus mosseae	10±0.61	18±0.55	12±1.24	14±1.15	23±1.73
Glomus aggregatum	08±0.74	ND	02±1.15	08±1.73	08±0.49
Glomus fasciculatum	15±0.92	09±0.44	14±0.95	18±1.15	10±0.46
Glomus badium	04±0.23	05±0.63	12±0.99	02±0.98	07±0.63
Glomus constrictum	ND	ND	08±0.69	ND	09±0.59
Glomus versiforme	03±0.41	02±0.58	04±0.63	ND	ND
Gigaspora gigantea	29±0.42	23±0.77	18±0.57	30±1.15	13±1.12
Gigaspora margarita	16±0.67	15±0.22	11±0.58	10±1.73	11±0.98
Acaulospora bireticulata	04±0.38	12±0.94	03±0.73	04±0.69	10±0.69
Acaulospora capsicula	07±0.47	03±0.72	02±0.77	ND	04±0.95
Scutellospora rubra	03±0.44	13±0.15	13±1.15	14±0.63	05±0.73
Sclerocystis sp.	01±0.22	00±0.98	01±0.32	ND	ND

±=SE ; ND = Not detected

lation decreased in summer season. During the summer, *Glomus* sp. flourished in all the locations.This was evident as the spores of *Gigaspora* sp. was less common in the plant rhizosphere of the foothills as compared to the hills. Spore population of *Glomus* sp. were found to be maximum in *C. limonia* and *C. medica*. However,*Gigaspora* sp. was evident in *C. limonia*but their population was less. *C. medica* showed variation in spore diversity.

Percentage population of AMF spores

According to seasonal variation the colonization percentage of *Glomus* sp. was maximum in summer season but *Gigaspora* sp. was found in abundance during moonsoon and winter Results have been presented in Tables 1, 2 and 3. Percentage population per 100 gm of soil of dominant AMF spores of *Citrus reticulata*; *C. limonia* and *C. medica* plants grown in foot hills have been illustrated in Fig. 5.

Histopathology and root colonization in citrus plants

Citrus reticulata

Microscopic observations on histopathological studies of *C. reticualta* showing vesicles, hyphae and arbuscules have been presented in Fig. 6 (A-L). About 72-75 percent root colonization was found

Table 3: Percentage of spores in 100gm soil from different locations of mandarin orchards during winter season

Name of AMF	Location				
	Kalimpong 27°03'31.34"N 88°28'00.05"E	Mirik 27°04'04.74" N 88°11'27.66"E	Bijanbari 26°53'09.47"N 88°10'58.05"E	Kurseong 26°53'54.34"N 88°16'38.59"E	Foothills 26°42'36.03" N 88°21'05.20"E
Glomus mosseae	14 ±0.48	23±0.87	18±1.43	30±1.94	36±1.45
Glomus aggregatum	06±0.41	ND	02±0.45	23±0.89	08±0.81
Glomus fasciculatum	18±0.92	05±0.83	08±0.46	16±0.97	08±0.40
Glomus badium	06 ±0.76	13±0.75	12±0.70	ND	07±0.31
Glomus constrictum	ND	ND	08±0.75	ND	09±0.94
Glomus versiforme	03 ±0.61	03±0.42	04±0.40	ND	ND
Gigaspora gigantea	21 ±0.94	18±0.55	18±0.71	12±0.94	06±0.47
Gigaspora margarita	12±0.74	14±0.47	11±0.45	10±0.68	08±0.71
Acaulosporabireticulata	05±0.57	08±0.65	03±0.41	04±0.49	10±0.67
Acaulospora capsicula	05 ±0.65	03±0.58	14±0.11	ND	04±0.48
Scutellospora rubra	09 ±0.44	13±0.70	01±0.27	05±0.43	04±0.95
Sclerocystis sp.	01±0.12	ND	01±0.32	ND	ND

±= SE ; ND = Not detected

in 2-3 year old seedlings while 85-89 percent colonization was observed in 7-8 years old trees. Abundant filamentous structures known as extraradical hyphae (Fig. 6 A & B) and intraradical hyphae (Fig 6 C) were observed. They comprise the fungal thallus (body) in the soil. These Extraradical hyphae bear profuse spores. Mature spores were found attached to the roots. The hyphae penetrated the root surface at entry points. Extrametrical hyphae with single oil-filled spores were observed. Presence of profuse arbuscules (Fig. 6 G & H) were observed. Oval shaped and flattened vesicles (Fig. 6 E & I) present in abundance. Subglobose oil-filled structures (Fig. 6 D) have a thicker wall than typical vesicles suggesting they may be intraradical spores. These vesicles took dark stain. Vesicles are formed by hyphal swellings that may be both inter or intra cellular. Cells of mandarin root are very small so it is quiet difficult to observe the hyphal pattern, structure of arbuscules and infection peg. But after extensive study, young and mature arbuscules and thick irregularly coiled hyphae (Fig. 6 K) were observed. Both arum and paris type of hyphae are present that suggests the presence of both *Glomus* and *Gigaspora* infestation. Arbuscules were visible only under high magnification (100x). Dark Septate Endophyte (DSE) were rarely present in root cortex (Table 4). DSE also formed arbuscules which are called sclerotia. These dark septate hyphae and

Fig. 1 : Maintenance of *Citrus reticulata*saplings in glass house

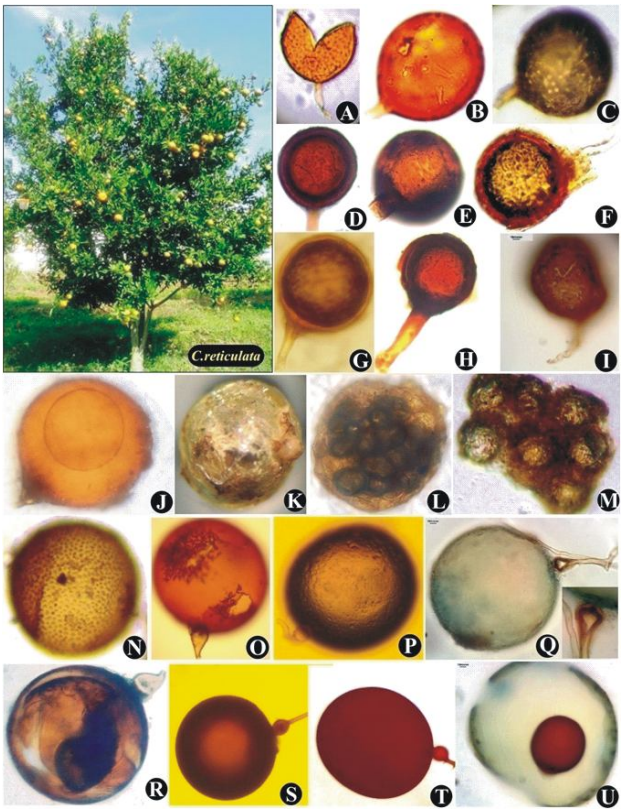

Fig. 2 : Microscopic observations of AMF spores obtained from *C. reticulata* plants. (A)Broken spore of *Glomus* sp., (B)*G. ambisporum*,(C) *G. mosseae*, (D) *G. fasciculatum*, (E) *G.const-rictum*, (F) *G. ambi-sporum*, (G) *Glomus* sp.,(H) *G. badium*, (I) *G. mosseae*,(J) *Glomus* sp, (K) *Gigaspora* sp., (L&M) *Sclerocystis sinuosum*, (N) *Acaulospora* sp.,(O) *Gi. marga-rita*,(P)*Gi.gi-gantea*, (Q)*Gi. gigantea*, (R) *Gigaspora* sp., (S&T) *S. reticulata*, (U) *S. Rubra*

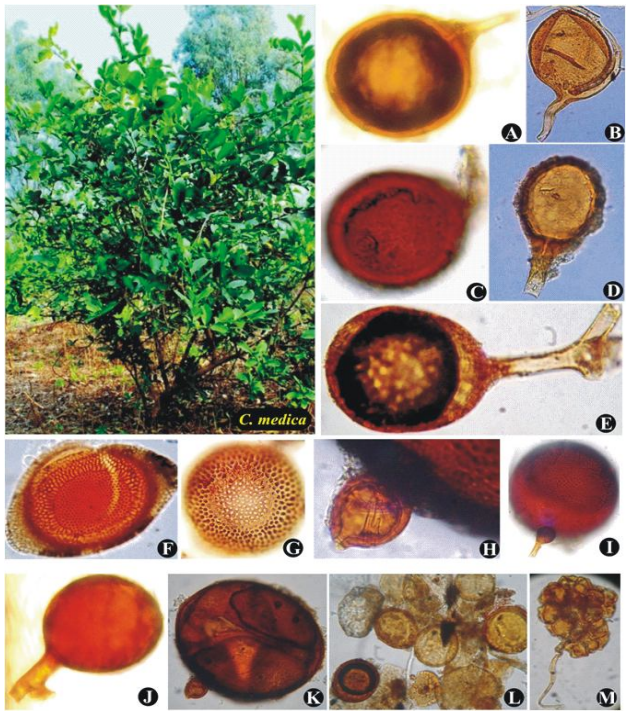

Fig. 3 : Microscopic observations of AMF spores obtained from *C. medica* plants. (A)*Glomus*sp., (B)*G. mosseae*, (C)*Glomus* sp., (D)*G. mosseae*,(E)*G. ambisporum*,(F)*Entrophospora* sp. (G)*A. bireticulata*; (H)Close up of bulbous suspensor of *Gi. albida*, (I)*Gi. albida*,(J)*Glomus* sp.,(K)*Gi. gigantea*, (L)*Rhizophagus aggregatus*, (M)*Sclerocystis sinuosum*

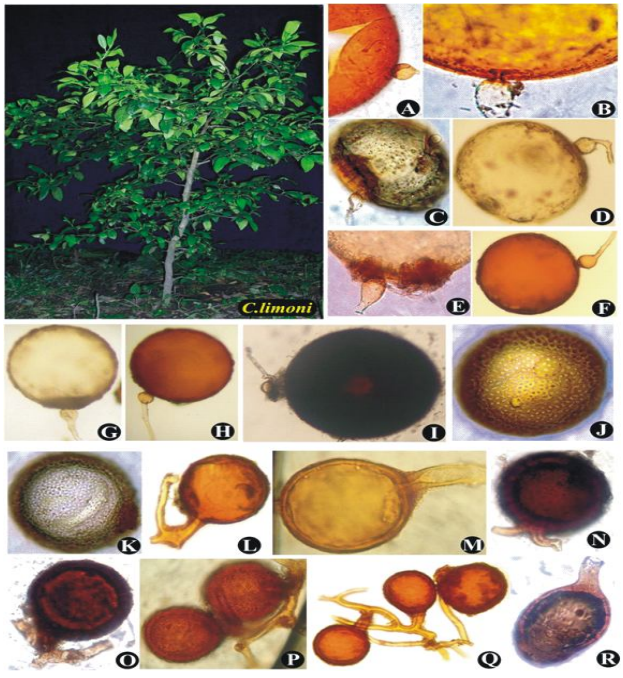

Fig. 4 : Microscopic observations of AMF spores obtained from *C. limonia* plants.(A)Broken spore of *Gi. decipiens*, (B) Sporogenous cell of *Gi. gigantea*, (C)*Gi. margarita*,(D)*Gi. Decipiens*, (E)Bulbous suspensor of *Gi. albida*, (F & H)*Scutellospora reticulata*,(G)*Gi. margarita*, (I)*Gigaspora* sp.,(J)*A. bireticulata*, (K)*A. cavernata*, (L)*Glomus* sp.,(M) *G. mosseae*, (N)*G. multicaule*, (O)*G.ambi-sporum*, (P)*Glomus* sp.,(Q)*G. fasciculatum*,(R)*Glomus* sp.

Table 4: AMF and Dark septate endophytic (DSE) associations of *Citrus reticulata*, *Citrus medica* and *Citrus limonia* plants

Citrus plants		No. of spores / 100gm soil	Percent root colonization	No. of Vesicles/ root	Vesicle / cm root	DSE
C. reticulata	Kalimpong[a]	136	76	78	04	+++
C. reticulata	Mirik	142	84	82	06	++
C. reticulata	Bijanbari	117	69	54	01	++
C. reticulata	Kurseong	103	52	36	01	+
C. medica	site 1[b]	139	87	48	04	+++
C. medica	site 2	95	86	66	06	+++
C. medica	site 3	108	59	46	04	++
C. limonia	site 1[c]	115	88	72	05	+++
C. limonia	site 2	89	63	57	03	++
C. limonia	site 3	93	75	63	02	+++

[a] Darjeeling hills ; [b] Foot hills ; [c] Foot hills

the sclerotia formed do not take the blue stain.

Citrus medica

Microscopic observations on histopathological studies of *C. medica* showing hyphae, vesicles and arbuscules have been presented in Fig.7(A-O). Presence of numerous extraradical and intraradical hyphae (Fig, 7, A-C) with spore (Fig. 7 G) and vesicles (Fig 7, D-F & I) are evident. Both intercellular and intracellular hyphae ramify along the whole length of the plant root. Mostly, *Arum*-type arbuscular mycorrhizal association observed. Lemon shaped vesicle (Fig.7 F) with vacuole and oil globules present. Appresoria with infection pegs were clearly seen. The regions along the root at which appresoria form and where hyphae enter the epidermis are referred to as entry points. Often an appressorium forms between epidermal cells (Fig. 7 H) and hyphae formed from the appressorium penetrate adjacent epidermal cells. Finely branched spreading arbuscules with thick trunk were present in abundance. Such straight intraradicular hyphae and fine spreading arbuscules are characteristic of *Glomus* species. Vesicles are globose shaped with presence of oil droplets and is the most characteristic cytological features of mature vesicles. Vesicles frequently enlarge to occupy the entire volume of the cell. The hyphal tip swells to form vesicles. A few DSE (Fig 7, M-O) and melanized hyphae with haustorium were also observed. These melanized hyphae run in parallel with intraradicular hyphae. Microsclerotia and sclerotia formed by melanized hyphae. Fine endophyte mycelium traversed the root length.Percent colonization is 76-78% in all the root samples observed.

Citrus limonia

Microscopic observations on histopathological studies of *C. limonia* showing different shapes of vesicles, hyphae, arbuscules and dark septate hyphae have been presented in Fig.8 (A-N). The root system showed diverse characters of colonization. Both Arum and Paris type hyphae are abundant in all the varieties. In Arum type association hyphae proliferated in the cortex longitudinally in the root system. Paris type hyphae have spread and formed several coiled structures. Both intra

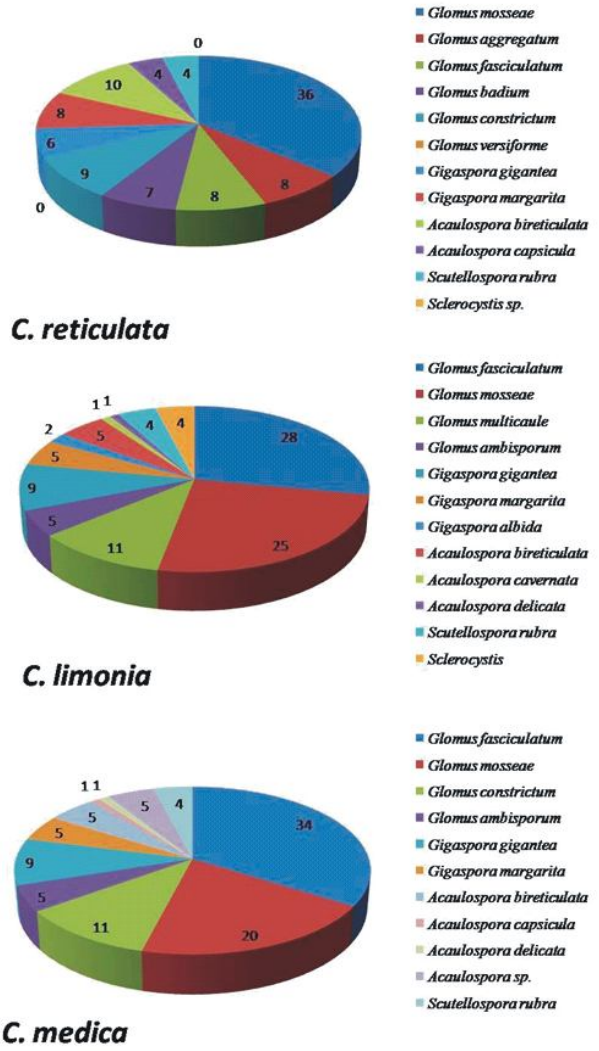

Fig. 5 : Percentage population per 100 gm of soil of dominant AMF spores of *Citrus reticulata*; *C. limonia* and *C. medica* plants grown in foot hills.

radical and extra radical hyphae (Fig. 8 A-C) are present. Profuse thin extraradical highly branched hyphae with single spores attached to roots (Fig.8 D-F). These thin hyphae are also known as "absorptive" hyphae. Oval shaped vesicles which are darkly stained and irregular shaped vesicles present in plenty (Fig. 8 G- H). Appressorium (Fig 8, I) , rectangular vesicles (Fig.8,J) and arbuscules (Fig. 8, K) are also present in a few number. Presence of such vesicles indicate the occurance of Acaulosporaceae spores. The colonization percent of Dark Septate Endophyte (DSE) in all root specimen of *C. limonia* (Fig. 8 L-N) were observed. The DSE with microsclerotia of different shapes and sizes were highly melanized Microsclerotia formed by DSE are evident which looks like cluster of grapes. Two types of microsclerotia were observed. One was linear with a single row of small grape-like struc

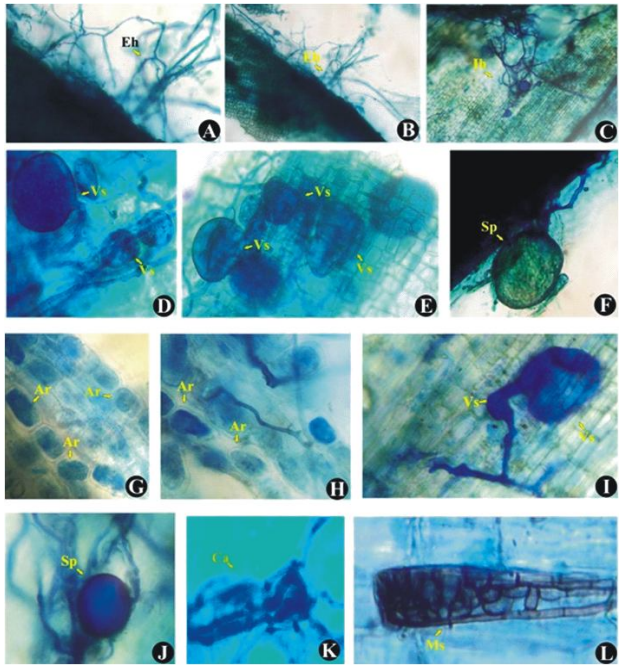

Fig. 6 : Histopathological study of *C. reticulata* showing vesicles, hyphae, arbuscules. (A-B) Extraradical hyphae, (C) Intraradical hyphae, (D) Oil-filled structure,(E) Oval vesicles, (F)Mature spore attached to hyphae, (G-H) Arbuscules,(I)Flattened vesicle, (J) Extraradicle spore, (K) Coiled hyphae, (L) Microsclerotia

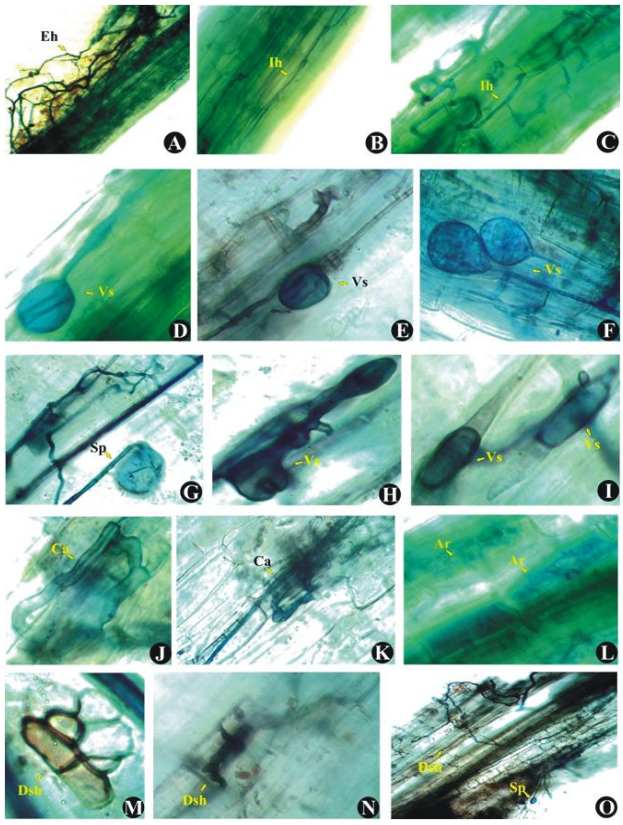

Fig. 7 : Histopathological study of *C. medica.*
(A-C) Extraradical, intraradical and interradical hyphae, (D-F) Vesicles, (G) Intraradical hyphae with spore, (H)Mature vesicle with infection nearby(I) Vesicles, (J-K) Coiled arbuscule (L) Arbuscules, (M-O)Dark septate hyphae

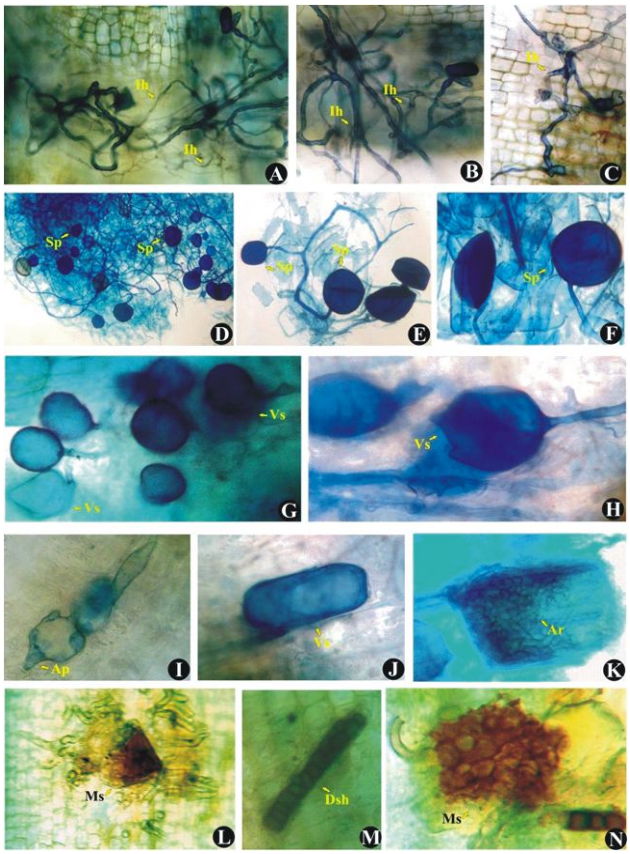

Fig. 8 : Histopathological study of *C. limonia* showing different shapes of vesicles, hyphae, arbuscules and dark septate hyphae.
(A-C) Extraradical hyphae,(D-F) Extraradical thin hyphae with single spores,(G-H) Oval shaped vesicles,(I) Appresorium, (J) Rectangular shaped vesicle,(K) Arbuscles, (L) Microsclerotia, (M) Dark septate hyphae , (N) Microsclerotia

ture (Fig.8 M) while the other was a cluster which more or less looked like *Sclerocystis* (Fig. 8 N). Mature microsclerotia did not show well developed zone of tissue. The central part was made up of pseudoparenchymatous tissue but he hyphal nature exists. Towards the outside of the microsclerotia the hyphae were more loosely arranged (Fig.8 L). Morphology of DSE of *C. limonia* differed from those found in *C. medica* roots. Percent colonization is 75-80 % in all the studied root samples. Few arbuscules (Fig. 8 K) composed of highly coiled hyphae also present.

DISCUSSION

Plant species differ in the pattern of AMF root colonization. Root colonization in citrus plants varied according to the age of the plant. Colonization percent was more in mature plants than young seedlings. Presence of abundant vesicles was evident (Allay *et al,* 2012).Diversity of AM fungi and their association with different plantation crops have also been demoonstrated by Chakraborty and De (2013).The vegetative thallus consists of arbuscules, intraradical vesicles, extraradical auxiliary cells, intraradical and extraradical hyphae. Root colonization with arbuscular mycorrhizal fungi and dark septate endophytes in tea plants have been demonstrated (Das Biswas *et al,* 2020). Both arum and paris type hyphae were abindant in all the tea varieties tested. The physical nature of arbuscules, vesicles, intraradical hyphae were studies extensively to determine the colonization impact of tea varieties. AM Fungi and dark septate fungal associations in medicinal and aromatic plants (Muthukumar *et al*, 2006) and in four ginger species (Surbala and Pandey, 2020) have also been reported. Arbuscules are finely branched structures in close contact with the cell plasma membrane, functioning in exchange of nutrients between host and fungal cells. Hyphae are important in nutrient acquisition and as propagules to initiate new root colonization. Vesicles are globose structures arising from swelling of the hyphae and filled with glycogen granules and lipids and are considered to be storage structures. AM fungi have weak cellulase and endopolygalacturonase activities which have the capacity to catalyze to the release of oligosaccharides or oligosaccharins from plant cell wall. The latter could trigger the colonization and spread of the fungus which are all controlled by the host. AM fungi effect the evolution of the plant, microbial communities and soil nutrient status. Hence, assessment of AM fungal diversity is essential if the benefits associated with the symbiosis are to be exploited.

Frequently observed is an increased uptake of less mobile nutrients, especially P, but also ammonium ($NH4^+$), Cu and zinc (Zn), potassium (K), calcium (Ca) and sulfur (S). AM also promotes symbiotic N fixation,even if, this may be related to improved P nutrition. Enhanced uptake of P is most often responsible for the growth increase of plants due to mycorrhization (Fitter *et al.* 2011). Up to 80% of the plant P, 60% of Cu and 25% of Zn can be delivered by external AMF hyphae extending as much as 12 cm from the root surface .The root system of citrus plants are not so deep and has less root hairs so they greatly depend on AMF to absorb nutrients (Wu *et.al*., 2017). This suggests that association of AM fungi in citrus roots promtes plant growth, improves fruit quality and enhance nutrition absorption. Direct, short-term AM influences such as patho

gen antagonism, alleviation of drought and heavy metal stresses, competition and enhancement of photosynthetic rates and phytohormone levels are well-established.

AM symbiosis increases resistance to biotic and abiotic stresses and reduces disease incidence, representing a key component of sustainable agriculture (Aliasgarzad *et al*, 2006; St-Arnaud and Vujanovic, 2007; Maya and Matsubara, 2013). Root colonization of rice plants with AM fungi and their use for induction of resistance against *Drechslera oryzae*causing brown spot disease has been demonstrated (Khati and Chakraborty, 2019). Activation of defense against fungal pathogen causing wilt root rot complex in *Citrus reticulata* using bioinoculants have also been documented (Chakraborty, 2019). It is evident that AM fungi impart beneficial effects to crops in terms of growth and productivity by increasing favourable microbial interaction and resistance to unfavourable biotic and abiotic conditions.

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