Journal of Medicinal Plants Studies
www.PlantsJournal.com

E- ISSN: 2320-3862
P- ISSN: 2394-0530
JMPS 2015; 3(2): 25-35

Received: 21-01-2015
Accepted: 05-02-2015

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Association and root colonization of some medicinal plants with Arbuscular Mycorrhizal Fungi

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Abstract
Twelve medicinal plants belonging to eight different families were selected to study the prevalence of AMF colonization. The plant roots and their respective rhizospheric soil were collected from the Garden of Medicinal Plants, University of North Bengal for AMF analysis and spore assessment per 100 gm of soil. The result showed variation in both AMF colonization and spore percentage. All medicinal plant species studied were found to be colonized by AMF. Highest percent colonization was evident in *Justicia adhatoda* (95±2.00). Highest spore count was found in *Abroma augustum* (197.4±9.31). Spore characteristics were studied and tried to identify upto species level based on the available standard keys. Histopathological studies revealed presence of abundant vesicles, thin branched as well as coiled arbuscules along with extra and intra radical hyphae. The present study revealed that the genus of *Glomus*, *Gigaspora*, *Acaulospora* were more predominant. *Scutellospora* and *Entrophospora* are least amongst the recovered AMF spores.

Keywords: Arbuscular mycorrhizal fungi (AMF), medicinal plant, endosymbiont, root colonization

1. Introduction
A substantial part of the soil microbial communities belongs to the arbuscular mycorrhizal (AM) fungi, an ancient group of fungi belonging to the phylum *Glomeromycota.* Arbuscular Mycorrhizal Fungi is the mother of plant root endosymbiosis that establish symbiotic relationships with plants and play an essential role in plant growth, disease protection, and overall soil quality. These are obligate and probably the most widespread terrestrial symbiosis that is formed by almost 80% of land plant species, mainly herbaceous ones [1]. Mycorrhizal symbiosis, helps to improve plant health by interacting with root pathogens leading to suppression in severity of diseases, improves supply of water and nutrients such as nitrogen and phosphate to the host plant. There is increasing evidence that AMF have a range of other effects for example protection against plant parasite, water stress tolerance alleviation of salt stress and in the sustainable maintenance of plant health and soil fertility [2]. AMFs are also considered to have potential to be used as biofertilizers. It has been seen that the occurrence of AMF is affected by the climate, soil type and among different host plant [3].

The occurrence of AMF in different medicinal plants have been reported previously by many researchers [4, 5, 6]. A large number of Indian medicinal plants have been reported by different researchers to harbor AMF [7, 8, 9].With the discovery of research in medicine, it was found that the plant contained abundant of principle active metabolites which can be used to treat and cure numerous diseases. Medicinal plant finds good application in varied industries including pharmaceutical, herbal, agricultural, food as well as cosmetic industries. AMF affects secondary metabolism and active ingredients production of plants, so it influences the quality of herbal medicines, thus improving the total yield. Considering their importance, their population in the rhizosphere of 12 medicinal plant viz. *Ambroma augusta*, *Artemisia vulgaris*, *Barleria lupulina*, *Barleria strigosa*, *Caesalpinia bonduc*, *Cassia alata*, *Coffea arabica*, *Eupatorium adenophorum*, *Gymnema sylvestre*, *Justicia adhatoda*, *Plumbago zeylanica* and *Vitex negundo* belonging to eight different families have been studied including their morphological characteristics and biodiversity.

Table 1: Medicinal uses of selected plant species

Sl no	Name	Family	Uses
1	*Ambroma augusta*	Sterculiaceae	The used parts are young leaves, bark and roots. It is used in uterine disorders, diabetes, rheumatic joint pain, gonorrhea, bronchitis, mental disease, back pain, skin disease, fever and indigestion. Methanolic extract have showed significant anti-inflammatory property [10, 11].
2	*Artemisia vulgaris*	Asteraceae	It is antibacterial, antifungal, antineoplastic, blood cleanser and mild sedative. It has been used as tonics, antimalarials, antidiabetics, anthelmintic, in treating wounds, bronchitis, ulcers and tuberculosis in traditional Anatolian medicine [12, 13].
3	*Barleria lupulina*	Acanthaceae	It has anti-inflammatory and analgesic properties, stops bleeding from wounds. Fresh leaves are used to remove warts [14].
4	*Barleria strigosa*,	Acanthaceae	Leaf juice is given in diarrhea. Fruit is prescribed in gingivitis as an expectorant. Powdered seeds are emetic and expectorant. Rootpaste is used as antidote for snake bite [15].
5	*Caesalpinia bonduc*,	Caesalpiniaceae	It has been reported to have anti-asthmatic, anti-tumor, anti-oxidant, antidiabetic, anti-bacterial, anxiolytic, immunomodulatory, anti-filarial, anti-inflammatory, hypoglycemic activity [16].
6	*Cassia alata*,	Caesalpiniaceae	Used for treatment of constipation, inguinal hernia, intestinal parasitosis, syphilis and diabetes [17, 18].
7	*Coffea arabica*,	Rubiaceae	It is a rich source of antioxidants, used to treat flu, asthma, bronchitis, headaches, jaundice, sores, malaria.
8	*Eupatorium adenophorum*	Asteraceae	It has uses like antiseptic, antidote, emetic, diuretic, purgative and stops bleeding.
9	*Gymnema sylvestre*	Apocynaceae	It is used to treat diabetes, jaundice, cough and snakebites [19].
10	*Justicia adhatoda*	Acanthaceae	It is used in chronic bronchitis, cold and cough, asthma, leucoderma, rheumatism, diarrhea, dysentery, piles, indigestion [10].
11	*Plumbago zeylanica*	Plumbaginaceae	It has analgesic antibacterial antifungal, inflammatory, larvicidal, anti diabetic, immunosuppressive activities, its roots are also used as expectorant, anti-rheumatic, anti-scabies, appetite stimulant, anti diarrhea, anti-periodic, anti-malarial [20].
12	*Vitex negundo*	Lamiaceae	It is used for treating stored garlic against pests and as a cough remedy in the Philippines. The leaves have anti-inflammatory and analgesic properties [21].

2. Materials and Methods

2.1. Selection of plants

Twelve medicinal plants were selected from the medicinal plant garden of University of North Bengal for present study which include *Ambroma augusta*, *Artemisia vulgaris*, *Barleria lupulina*, *Barleria strigosa*, *Caesalpinia bonduc*, *Cassia alata*, *Coffea arabica*, *Eupatorium adenophorum*, *Gymnema sylvestre*, *Justicia adhatoda*, *Plumbago zeylanica* and *Vitex negundo*.

2.2. Collection of root samples

The feeder roots of each plant species were collected from plants immediately after digging the plant from the ground.

2.3. Collection of soil samples

100 gm of soil sample were collected from the vicinity of the root region of each of the plant species and collected in polythene bags, labeled then refrigerated at 4 ^{0}C for further use.

2.4. Collection of AMF spores from soil samples

Wet sieving and decanting method [22] was followed for the isolation of spores for which 100 g of soil was homogenized with 1-2 Liters of water mixing in a bucket. The bucket was left for an hour and when the soil particles settled down the top water was sieved through a sieving set having pore size marked as BS-60, BS-80, BS-100, BS-150, BS-170, and BS-200 piled one above another. The size of the pores were fine enough to remove the larger particles of organic matter, but coarse enough to allow the desired spores to pass through. From each tier the spores was collected with a fine brush and suspended in sterile water on a petriplate, the heavier particles were allowed to settle for a few seconds and the excess water decanted through the sieve and spores were collected with fine brush then according to their size and colours kept in different petriplates. With the help of a simple microscope (4X) parasitized spores, plant debris etc. were separated and clean spores were mounted on slide with a drop of PVLG (Polyvinyl alcohol + lactic acid + Glycerol) and covered with cover slip and gently pressed and observed under dissecting microscope (Leica DM LS2).

2.5. Spore count

AMF spores were isolated from 100 gm of soil by Wet sieving and decanting method [22], the collected spores were spread on filter paper placed in a petriplate and counted under microscope and expressed under 100 gm of dry soil.

2.6. Assessment of Root colonization in plant roots

Root colonization of twelve medicinal plants was done following the method of Philip and Haymann [23]. Roots were gently washed under tap water to remove soil and cut into small pieces of 1cm and were then treated with 2% NaOH in a glass beaker, kept in water bath at 100 ^{0}C for 1 hour, NaOH was decanted off followed by rinsing with distilled H_20 thrice on a fine sieve or using a mesh and forceps. The roots were then kept in 1% HCl for 30 mins. HCl was decanted and roots were covered with 0.05% cotton blue in lacto glycerol (1:1:1 lactic acid, glycerol and water) in beaker and incubated overnight. The root samples were mounted on slides, a drop of lactophenol was put on the slide and covered with cover slip and gently pressed and to check the root colonization by AMF observed under dissecting microscope. Each plant root was studied from minimum three sites to find out the average percentage of root colonization. The root colonization percentage was calculated by the following formulae:

$$\text{Root colonization percentage} = \frac{\text{No. of infected root fragments}}{\text{No. of total root fragments}} \times 100$$

2.7 Identification of VAM fungi

The identification of arbuscular mycorrhizal fungi was done following the monograph and the manual of VAM Fungi [24].

3. Result and Discussion

The present study showed that all the plants under study exhibited root colonization by arbuscular mycorrhizal fungi since both arbuscles and vesicles were found to be present in the roots.

Table 2: Spore count per 100 gm of soil and root colonization percentage12 medicinal plants

Sl. No.	Plant Name	Of Colonization (40x)	Spore Per 100 gm Of Soil.
1	*Ambroma augusta*	83±08.86	197.40±9.31
2	*Artemisia vulgaris*	63±10.54	128.80±6.05
3	*Barleria lupulina*	87±08.20	140.20±8.28
4	*Barleria strigosa*	57±03.70	142.80±6.53
5	*Cassia alata*	51±01.67	58.80±6.61
6	*Caesalpinia bonduc*	67±06.50	83.60±6.58
7	*Coffea arabica*	71±05.02	143.20±8.70
8	*Eupatorium adenophorum*	56±01.22	187.00±7.10
9	*Gymnema sylvestre*	59±01.64	53.00±5.24
10	*Justicia adhatoda*	95±02.00	181.80±5.16
11	*Plumbago zeylanica*	74±08.41	169.00±8.41
12	*Vitex negundo*	75±01.51	192.60±9.55

Nutrient transport occurs through symbiotic structures inside the plant root cells known as arbuscles. Arbuscles were abundant in case of *Caesalpinia bonduc*, *Plumbago zeylanica*, *Vitex negundo* whereas vesicles were abundant in case of *Abroma augusta*, *Cassia alata*, *Coffea arabica*, *Gymnema sylvestre*. Histopathological studies showed that highest root colonization percentage is evident in *Justicia adhatoda* (95±2.00), followed by *Barleria lupulina* (87±8.20) and

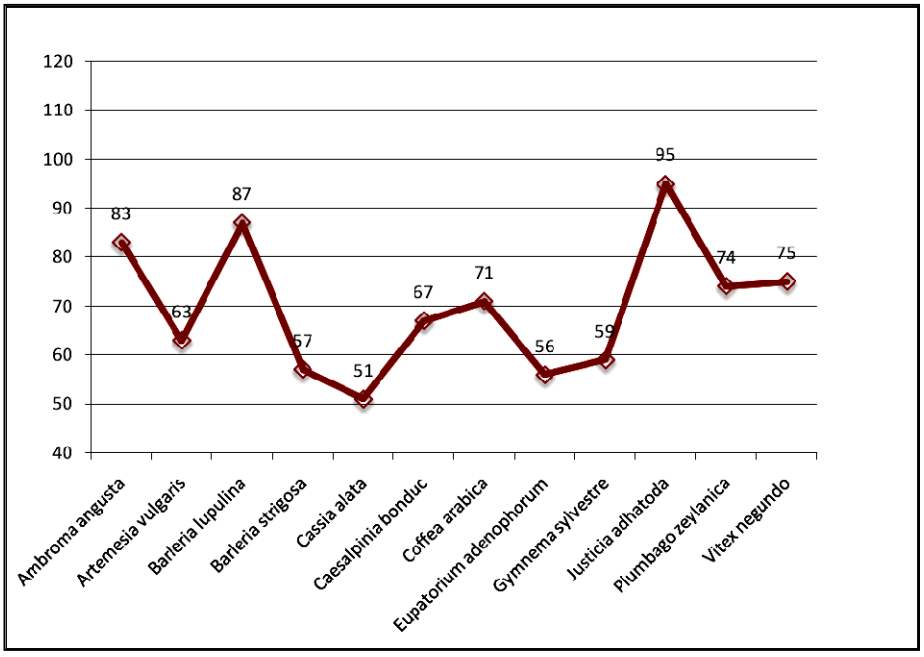

Fig 1: Percentage of AMF colonization in 12 medicinal plants

Abroma augusta (83±8.86) whereas least colonization was observed in case of *Cassia alata* (51±1.67), followed by *Eupatorium adenophorum* (56±1.22) and *Barleria strigosa* (57±3.70). Abundance of AMF as well as their spores may depend on the physical characteristics of soil. Rhizospheric soil is beneficial to microorganisms as it contains many types of root exudates that may be beneficial to them. In this study significant variation has been observed in percentage of root colonization among different species of plant which might be due to some components of the rhizospheric soil that might have favored AMF growth as also reported by other researchers [25]. Various toxic metabolites are exudated by the roots which in turn attracts the AM fungi by producing the easily oxidisable compounds, thereby resulting in the increased colonization physiological difference between species leading to the variation in root colonization [26].

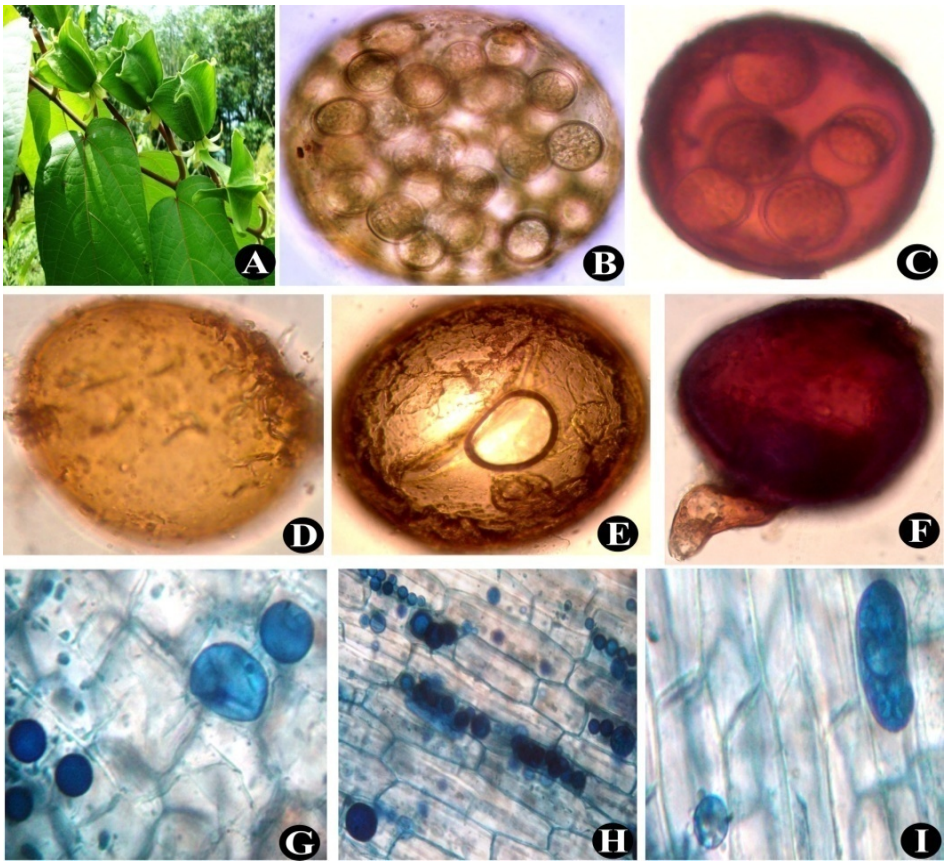

Fig 2: *Abroma augusta*(A); Sporocarp of *Glomus* sp. (B); Sporocarp of *Scutellospora* sp. (C); *Glomus* sp. (D); Mature *Gigaspora* sp. (E); *Glomus fasciculatum* (F); Vesicles and Arbuscules (G) to (I)

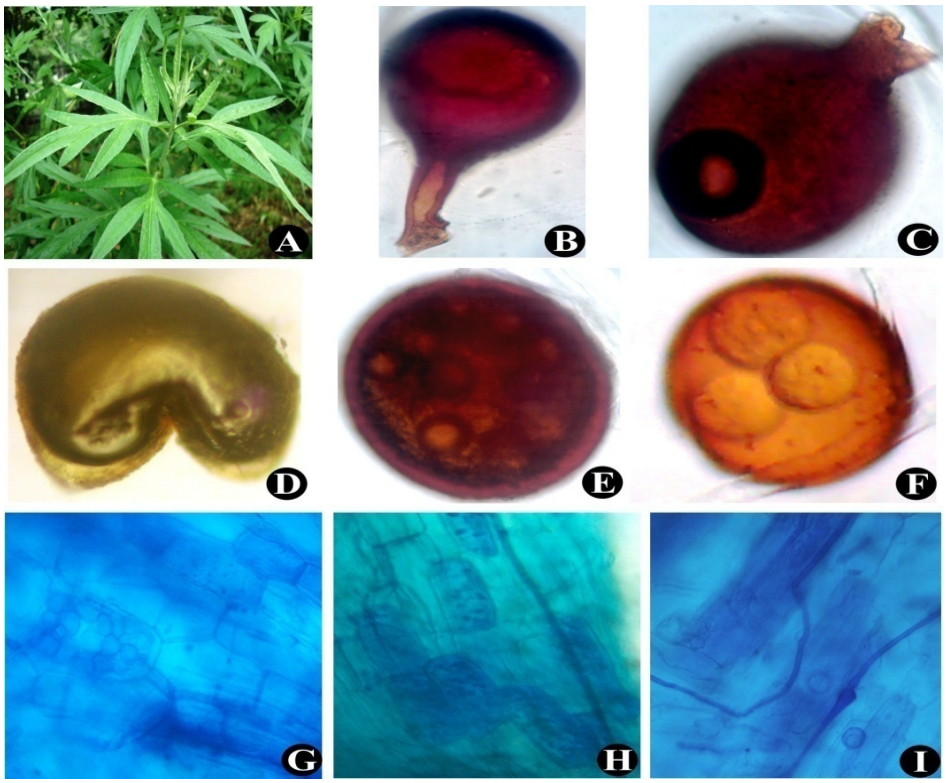

Fig 3: *Artemisia vulgaris*(A); *G. constrictum* (B); *Glomus* sp. (C); Ruptured spore of *Gigaspora* sp. (D); Sporocarp of *Glomus* sp. (E); Sporocarp of *Glomus* sp. (F); Arbuscules and Intraradical hyphae (G) to (I)

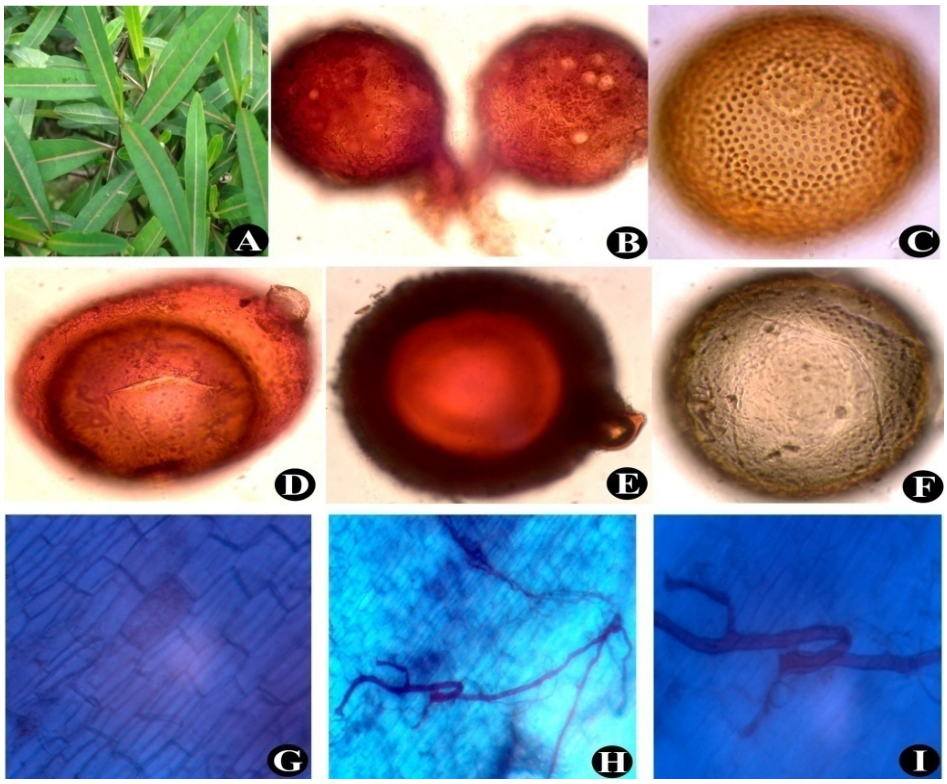

Fig 4: *Barleria lupulina* (A); *Glomus fasciculatum* (B); *Acaulospora* sp.(C); *Glomus* sp. (D); *Scutellospora* sp. (E); Mature *Gigaspora* sp. (F); Arbuscules and Intraradical hyphae (G) to (I)

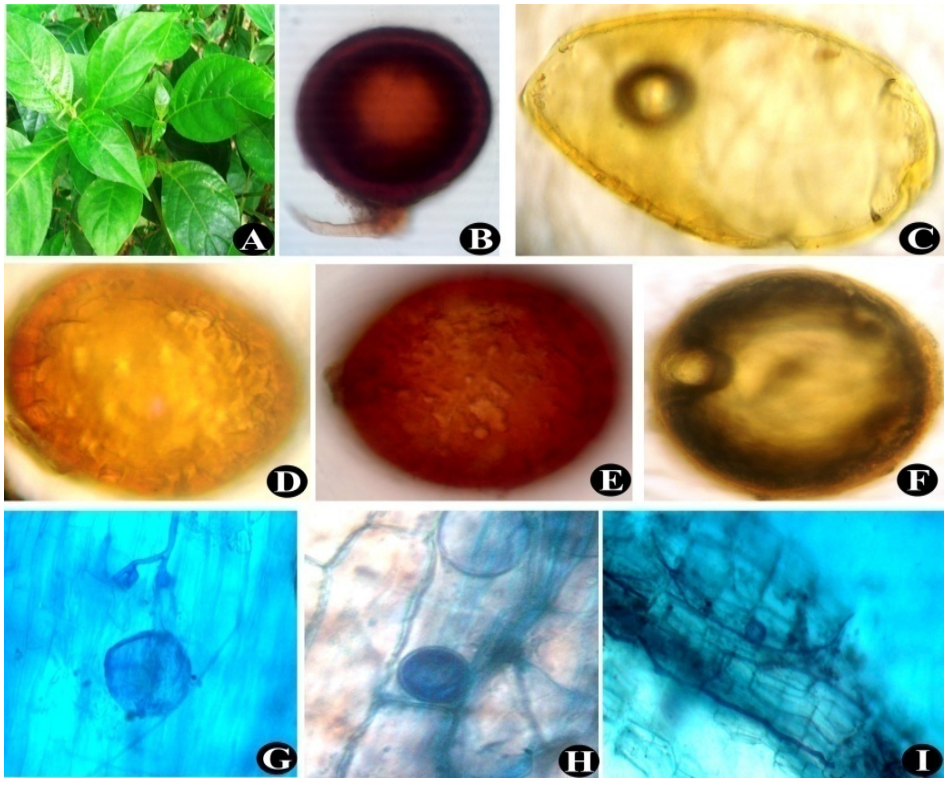

Fig 5: *Barleria strigosa* (A); *Glomus mosseae* (B); *Glomus* sp. (C); *Glomus* sp. (D); *Scutellospora* sp. (E); Mature *Gigaspora* sp. (F); Vesicles and Intraradical hyphae (G) to (I)

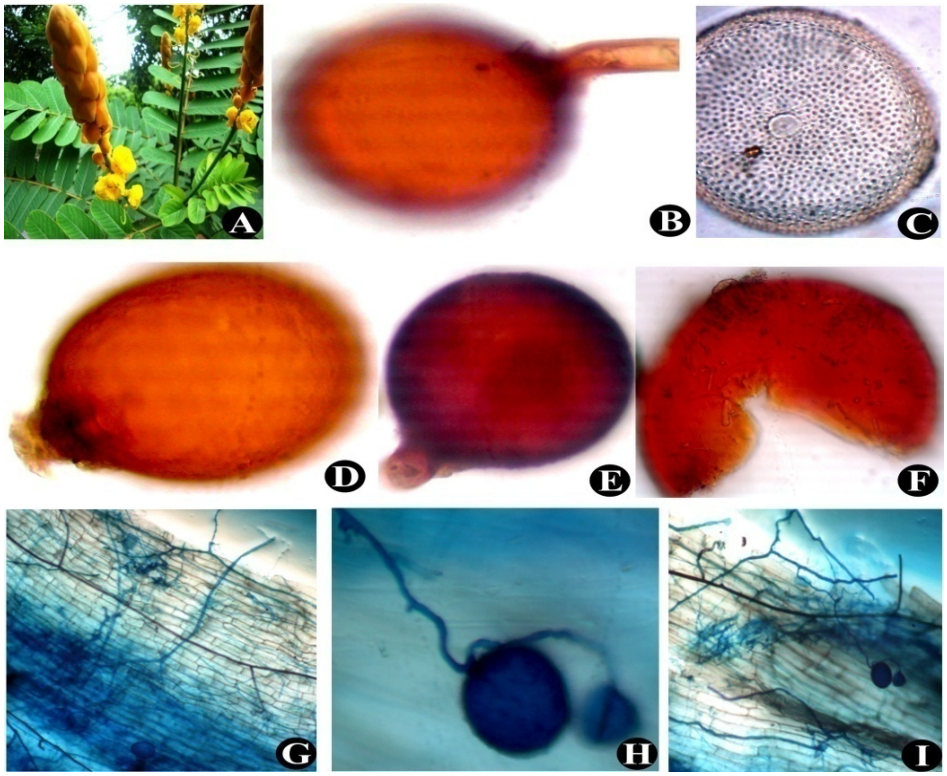

Fig 6: *Cassia alata* (A); *Glomus mosseae* (B); *Acaulospora* sp.(C); *Glomus* sp. (D); *Glomus constrictum* (E); Ruptured spore of *Scutellospora* (F); Intraradical hyphae and vesicles (G) to (I)

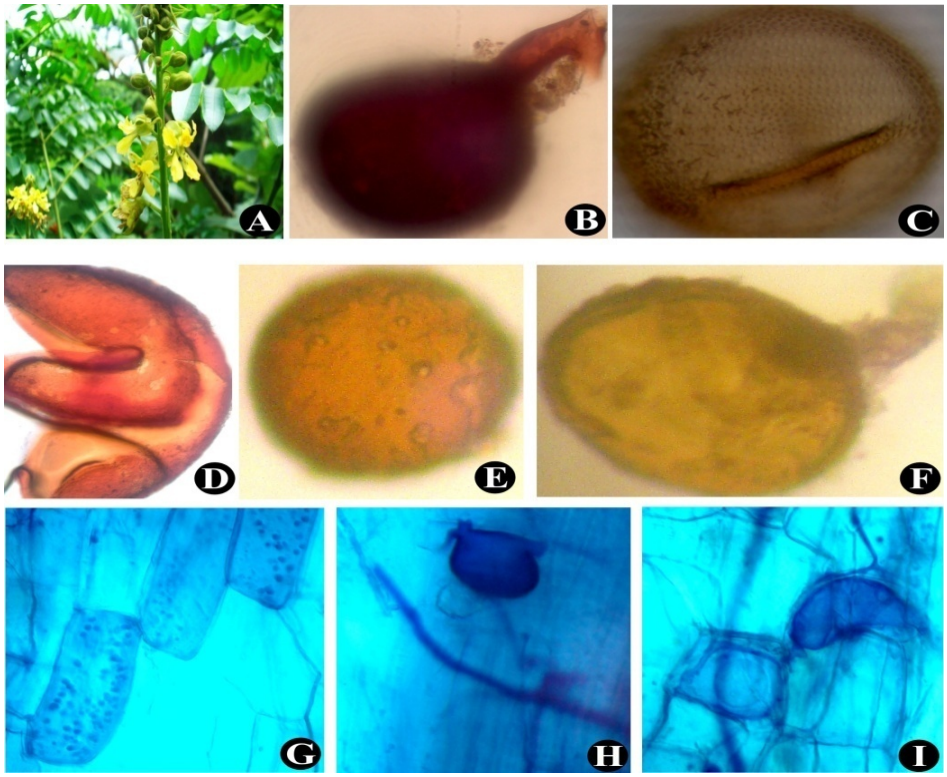

Fig 7: *Caesalpinia bonduc* (A); *Glomus mosseae* (B); *Acaulospora* sp. (C); Ruptured spore of *Scutellospora* sp. (D); *Glomus* sp. (E); *Glomus* sp. (F); Juvenile arbuscules, Vesicles and Intraradical hyphae (G) to (I)

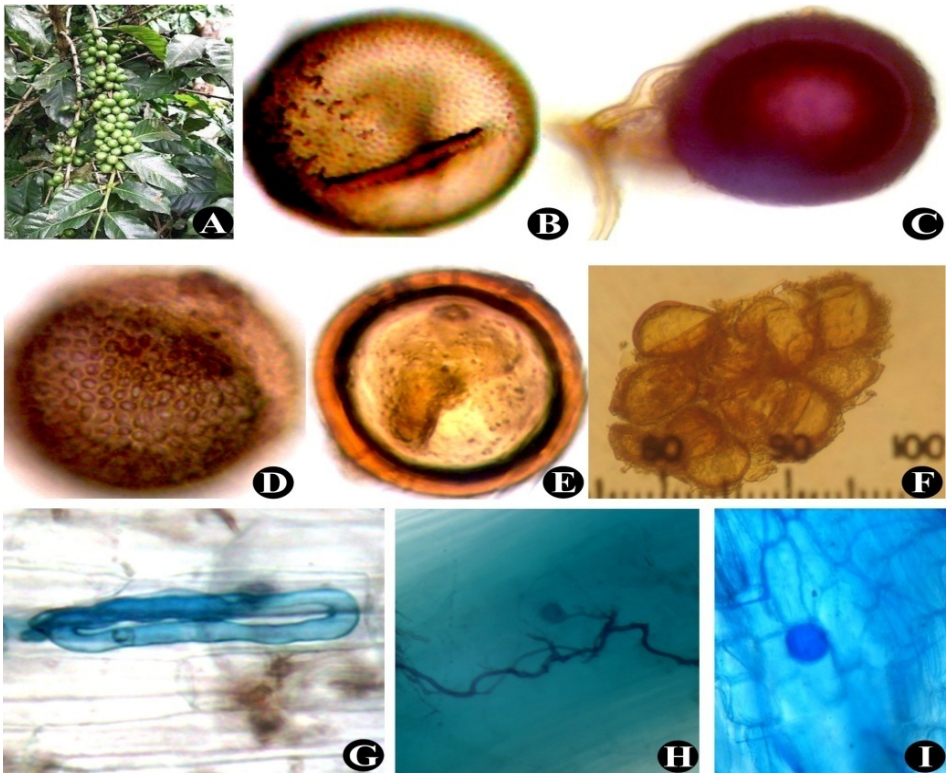

Fig 8: *Coffea arabica* (A); *Acaulospora spinosa* (B); *Glomus fasciculatum* (C); *Acaulospora* sp. (D); *Glomus* sp. (E); Sporocarp of *Glomus* sp. (F); Mature arbuscules, Intraradical hyphae and Vesicles (G) to (I)

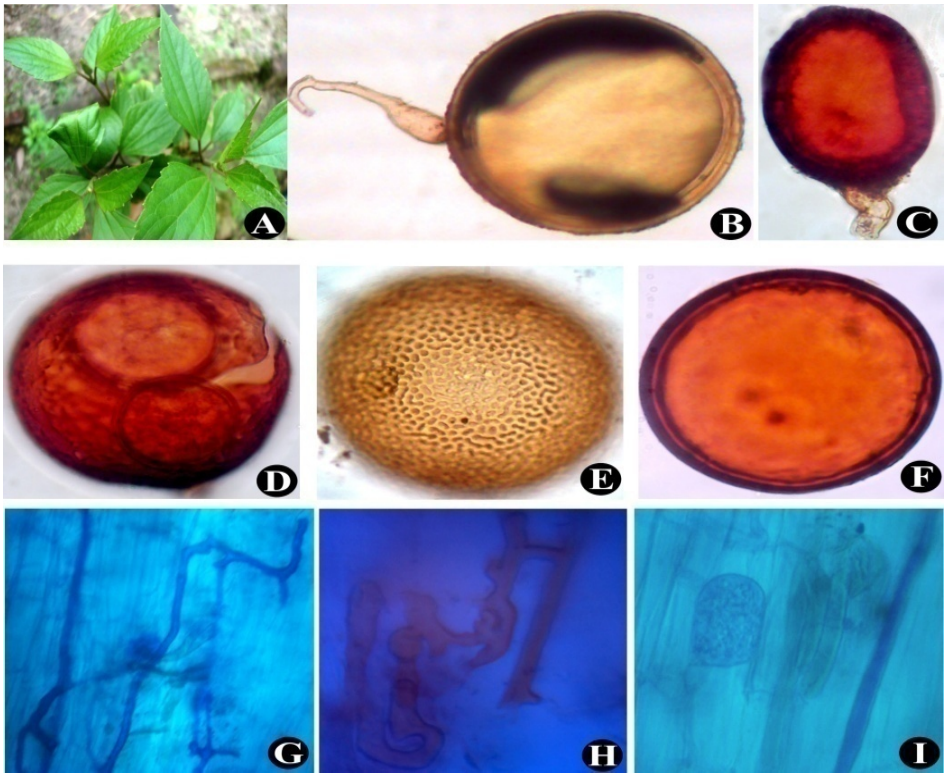

Fig 9: *Eupatorium adenophorum* (A); *Gigaspora margarita* (B); *Glomus* sp.(C); Ruptured spore of *Scutellospora* sp. (D); *Acaulospora bireticulata* (E); *Glomus* sp. (F); Intraradical hyphae and juvenile arbuscules (G) to (I)

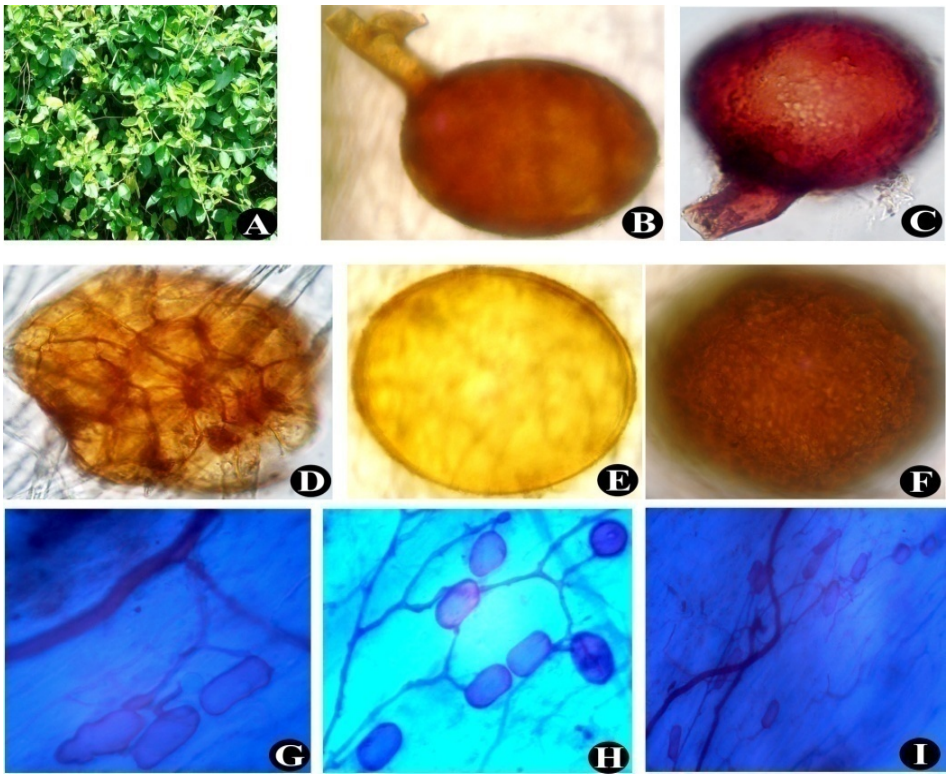

Fig 10: *Gymnema sylvestre* (A); *Glomus mosseae* (B); *Glomus constrictum* (C); Sporocarp of *Glomus* sp. (D); Juvenile spore of *Gigaspora* sp. (E); *Scutellospora* sp. (F); Vesicles and Intraradical hyphae (G) to (I)

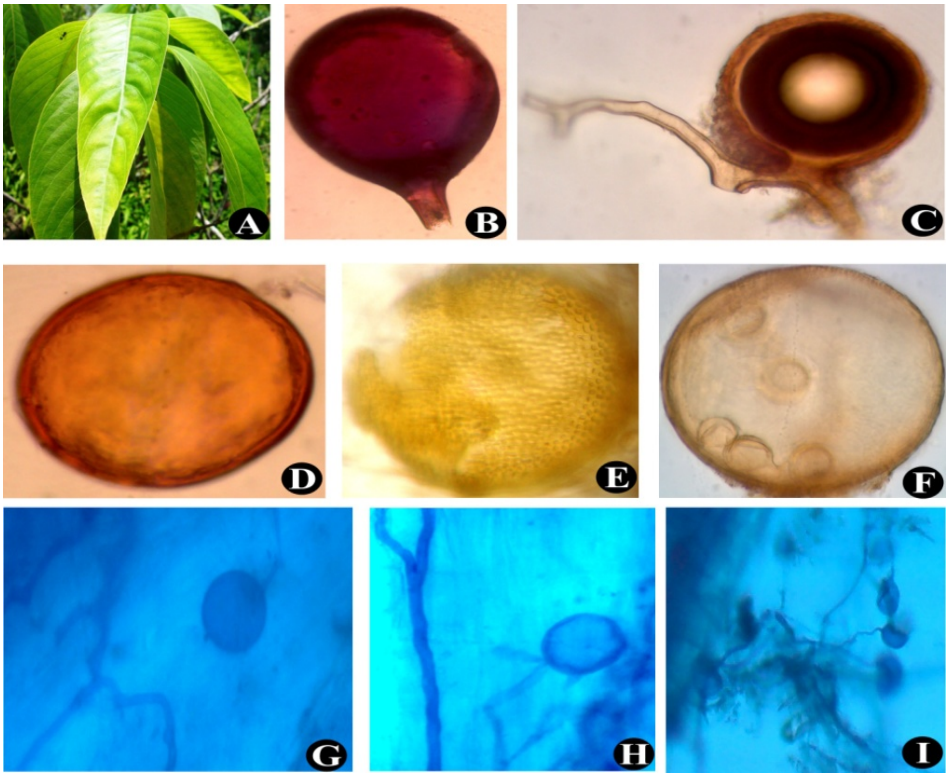

Fig 11: *Justicia adhatoda* (A); *Glomus constrictum* (B); *Glomus mosseae* with hyphal branching(C); *Glomus* sp. (D); *Acaulospora* sp. (E); An AMF spore sporocarp. (F); Intraradical hyphae and vesicles (G) to (I)

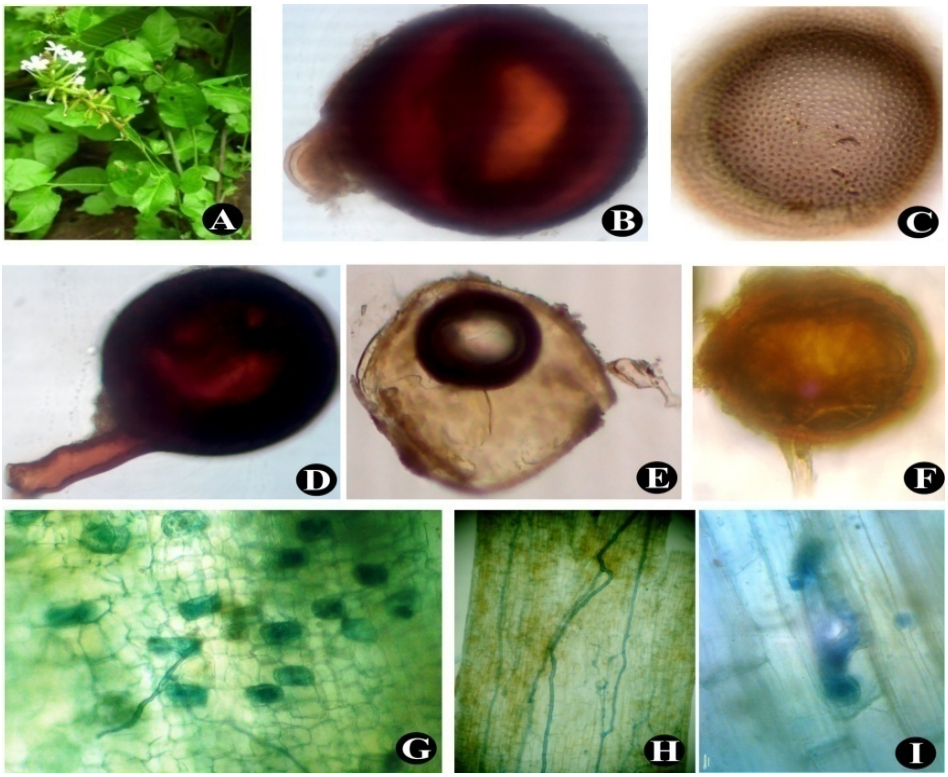

Fig 12: *Plumbago zeylanica* (A); *Glomus* sp.(B); *Acaulospora spinosa*(C); *Glomus constrictum* (D); Mature spore *Gigaspora* sp.(E); *Glomus* sp.(F); Vesicles, intraradical hyphae and arbuscule (G) to (I)

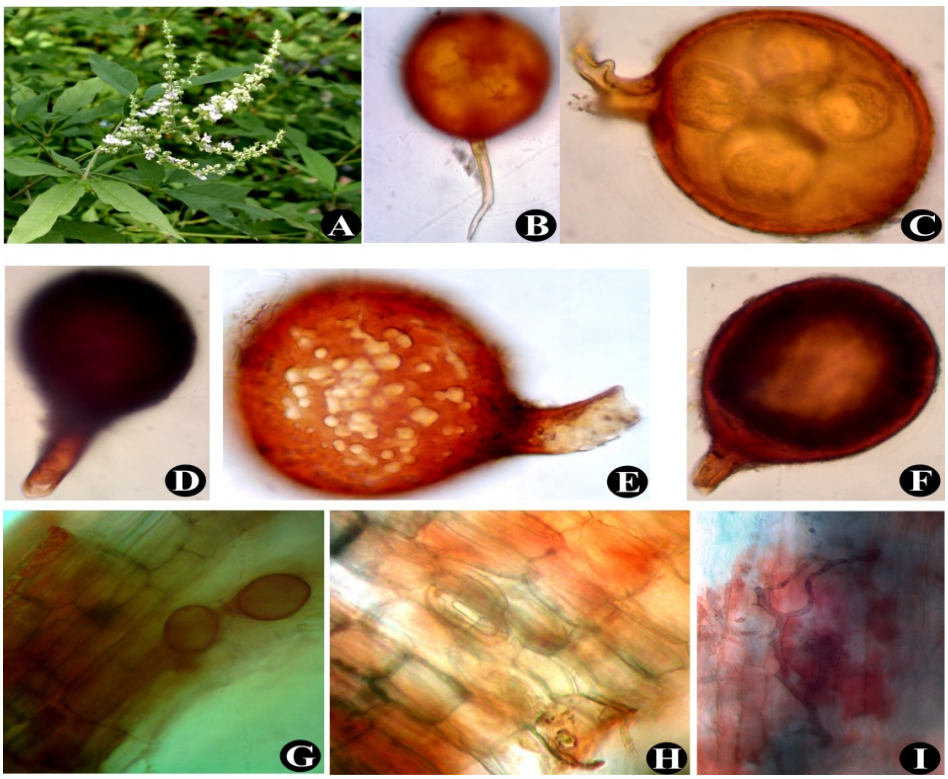

Fig 13: *Vitex negundo* (A); *Glomus mosseae* (B); Sporocarp of *Glomus* sp. (C); *Glomus constrictum* (D); *Glomus* sp. (E); *Glomus* sp. (F); Vesicles, arbuscules and Intraradical hyphae (G) to (I)

The identification of spores in this study was done following the monograph and the manual of Gerdemann and Trappe [24]. The semi-permanent slides were prepared using PVLG (Polyvinyl alcohol + lactic acid + Glycerol). The extracted spores from each of the soil samples were divided into groups of same characteristics for identification under dissecting microscope. Morphological features of isolated AMF spores were critically examined with special reference to variation in

size, wall thickness, shape, and color of spores. Population of spore number count revealed that spores number ranged from 53 to 197 per 100 g of rhizospheric soil. The maximum number of spore per 100 gm of soil was recorded in case of *Abroma augusta* (197.4±9.31) followed by *Vitex negundo* (192.6±9.55) and *Justicia adhatoda* (181.8±5.16) and minimum spore population was recorded in case of *Gymnema sylvestre* (53±5.24), *Cassia alata* (58.8±6.61) *and Caesalpinia bonduc* (83.6±6.58).

Table 3: Microscopic characters of AMF spores associated with medicinal plants

AMF	Spore size (µm)	Spore layer	Colour	Other descriptions
Glomus				
G. constrictum	110-130 x 150-160	2	Brownish orange to dark brown	Subtending hyphae straight or curved, usually markedly constricted at the spore base. Globose to subglobose, sometimes ovoid
G. fasciculatum	70-120	3	Pale yellow to bright brown	Spore layer continuous. Shape globose to subglobose
G. mosseae	200	3	Brown to orange-brown	Hyphae are double layered. Spore globose to sub-globose
Gigaspora				
Gigaspora sp.	250-270 x 265-370	2	Greenish yellow	Formed terminally or laterally on a bulbous sporogenous cell. Globose to subglobose
Acaulospora				
A. bireticulata	280-410	3	Brownish	Surface ornamentation is prominent. Spores are borne laterally from the neck of a sporiferous saccule. Globose
A. spinosa	140-220	3	cream to pale orange-brown	Globose or subglobose
Scutellospora				
***Scutellospora* sp.**	140-220	3	Dark orange-brown to red-brown	Germinal walls are formed completely separate from the spore wall. Globose to subglobose

Different variety of spores was isolated from different medicinal plants in this study. Detailed photographic illustrations of AM spores and their associations with root system have been presented in Fig (2-13). Microscopic characters of AMF spores that were found associated with medicinal plants are described in Table 3. In this study, the genus of *Glomus*, *Gigaspora*, *Acaulospora* were more predominant than other AMF as observed in different plants by other researchers [26, 27]. *Scutellospora* and *Entrophospora* were least amongst the recovered AMF spores with *Glomus* being present in every plant's soil sample. Presence of different types of *Glomus* can lead to an assumption that various species of *Glomus* might have developed a good adaptive mechanism of symbiosis with different host plants [26]. *Gigaspora gigantea* has been found to be present in *Eupatorium adenophorum*, *Barleria lupulina* and *Justicia adhatoda*. *Scutellospora* is found in case of *Eupatorium adenophorum, Vitex negundo and Sclerocystis* was present in *Cassia alata* and *Coffea arabica*. Of all the rhizosphere studied *Entrophospora* was rare and present only in *Gymnema sylvestre* which might be due to seasonal variation. The results of this study shows that biodiversity of arbuscular mycorrhizal fungi differ in different plants. It has been observed that the host plants controls the diversity of the arbucular mychorrhizal population through the difference in the effects on the hyphal growth and sporulation[28]. The diversity of AMF found in this study can be further utilize to evaluate their activity in the enhancement of growth of these medicinal plants.

4. Acknowledgement

Financial assistance from University Grants Commission, India is highly acknowledged.

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