Studies on Arbuscular Mycorrhizal (AM) fungal diversity in selected medicinal plants from Goa

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DECLARATION BY STUDENT

I hereby declare that the data presented in this Dissertation report entitled, "**Studies on Arbuscular Mycorrhizal (AM) fungal diversity in selected medicinal plants from Goa**" is based on the results of investigations carried out by me in the Botany Discipline at the School of Biological Sciences and Biotechnology, Goa University under the Supervision of Prof. B. F. Rodrigues and the same has not been submitted elsewhere for the award of a degree or diploma by me. Further, I understand that Goa University or its authorities will be not be responsible for the correctness of observations / experimental or other findings given the dissertation.

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COMPLETION CERTIFICATE

This is to certify that the dissertation report **"Studies on Arbuscular Mycorrhizal (AM) fungal diversity in medicinal plants from Goa"** is a bonafide work carried out by **Mr. Prabal Pandurang Prabhu** under my supervision/mentorship in partial fulfilment of the requirements for the award of the degree of Masters of Science in the Discipline Botany at the School of Biological Sciences and Biotechnology, Goa University.

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Mr. Prabal Pandurang Prabhu

INDEX

LIST OF TABLES

LIST OF PLATES

INTRODUCTION

India is a place of great bio-diversity with its rich source of medicinal plants distributed among the different geographical and ecological environments in terms of species variety, and genetic diversity within the country. It has a rich history concerning the use of traditional medicine from herbal and non-herbal sources that are well-documented and practiced comprehensively. In India, traditional healers are reported to use more than 2,500 plant species and 100 plant species as a regular source of medicines (Pie, 2001). To establish efficient strategies for producing biomass and getting high-quality material, studies on the biology, ecology, and agricultural technology of medicinal plant species are essential.

The traditional Indian medical system provided the best treatments for a variety of illnesses, including jaundice, rheumatoid arthritis, bronchial asthma, diabetes, *etc* (Thakur and Pathak, 2018). Natural medications are said to be safer for people and the environment than synthetic ones, and some of them can be quite effective in the treatment of cancer (Nema *et al.*, 2013).

Medicinal plants are significant for pharmacological research and drug development because they can be used as starting materials for the manufacture of medications or as models for pharmacologically active chemicals, in addition to their direct usage as therapeutic agents (Mukherjee, 2003). Plants owe their medicinal properties to secondary metabolites which synthesize and have contributed to more than 7,000 different compounds which are used today as heart drugs, laxatives, anti-cancer agents, hormones, antibiotics, analgesics, anesthetics, ulcer compounds and antiparasitic compounds (Biradar and Reddy, 2007). Medicinal plants are a rich source of bioactive compounds (Toussaint *et al.*, 2007), and these are thought to be safe for human beings and the environment compared to synthetic medicines for the treatment of cancer and many other diseases (Nema *et al.*, 2013).

The following categories of plant species recognized by the IUCN include extinct, extinct in the wild, severely endangered, endangered, vulnerable, near threatened, least concern, data

deficient, and not evaluated, and rare species are those with limited populations that are not currently vulnerable or endangered but are still at risk (Singh *et al.*, 2006). Due to the growth in demand, several medicinal plant species have moved into the threatened category. These species need to be evaluated as endangered or even critically endangered, or they need to be given legal protection (Mehta *et al.*, 2020).

Although contemporary society has advanced, man still relies heavily on plants and their by-products. Due to the extensive use of herbal medications, the cultivation of medicinal and aromatic plants has been boosted to meet the rising demand. As a result, researchers are working to improve the production of medicinal plants using suitable and effective soil bacteria found in the rhizosphere of such plants. Many soil bacteria collaborate with plants in a symbiotic relationship, But the AM fungi because of their superior impacts on plant development and are associated with 80% of all terrestrial plant species (Kumar *et al.*, 2019).

To supply the need for medicinal plants, large-scale production of medicinal plants employing contemporary farming techniques is being practiced in Asian nations. The development and quality of medicinal plants are being hampered by plant diseases and pests. Moreover, overuse of pesticides may lower the quality of plant-based medicines. Thus, it is necessary to create a novel technology for the growth of therapeutic plants (Vasudha *et al.*, 2013).

The medicinal plants that associate with different microorganisms can be developed into biocontrol and biofertilizer agents. Hence, it is essential to identify, characterize, and utilize rhizosphere bacteria related to medicinal plants (Vasudha *et al.*, 2013). The study of rhizosphere bacteria from significant medicinal plants is particularly essential since they are well known to affect plant development, produce metabolites that are vital for industry, and enhance the quality of medicinal products (Bafana and Lohiya, 2013).

Arbuscular mycorrhizal (AM) fungi

Arbuscular mycorrhizal (AM) fungi form mutualistic symbiotic relationships with plant roots of more than 80% of land plants including many important crops and forest tree species (Lee *et al.*, 2013). They are an ancient group of fungi that are members of the phylum Glomeromycota, and are obligate biotrophs. As per the classification system by Redecker *et al.*, (2013) it contains 25 species and four orders *viz.*, Glomerales, Archaeosporales, Paraglomerales, and Diversisporales. Beneficial services offered by these AM fungi are numerous (Thapa *et al.*, 2015). These fungi play a crucial role in the complex networks of below- and above-ground biotic interactions (Turrini and Giovannetti, 2012). They are essential endosymbionts that effectively contribute to plant production and ecological health. They are crucial for improving crops in a sustainable way (Gianinazzi *et al.*, 2010).

From the origin of terrestrial plants, AM fungi have been discovered near the roots of higher plants. In natural ecosystems, AM fungi make up a large portion of the rhizosphere microflora and are crucial to the restoration of nutrient cycling (Peterson *et al.*, 1985). They alter the composition and operation of plant communities, serving as helpful markers of ecological change (Douds and Miller, 1999). AM fungi through their hyphae nourished the early terrestrial plants. Symbiotic associations between fungi and roots of medicinal plants are very much significant in terms of nutrition supply, plant protection, growth, and yield of the medicinally important plants (Dotzler *et al.*, 2009; Bonfante and Selosse, 2010).

The presence of AM fungi in therapeutic plants has been frequently observed. Nevertheless, depending on the host plant species, growth season, soil characteristics, regional climate, and environmental conditions, the variety of AM fungal species and the degree of colonization in the rhizosphere of medicinal plants may vary (Solaiman and Anawar, 2015). Many studies

show that mycorrhizal medicinal plants often contain more nutrients and develop more successfully than non-mycorrhizal plants (Karagiannidisa *et al.*, 2011).

Development of AM fungi

AM fungus invades inside the root to establish symbiosis, which involves morphological changes in both plant cells and fungal hyphae. Pre-symbiosis reactions for both symbionts are triggered by reciprocal recognition through diffusible chemicals generated by plant roots and sprouting fungal spores, which enables successful symbiotic association. During pre-symbiosis communication, AM fungi create entrance sites such as appressoria and hyphopodia on the surface of the host root or entry coils (EC) in the epidermal or hypodermal cells, which they use to enter the root and create the intraradical mycelium. Hyphae can occasionally penetrate the root through the root hairs or between the two epidermal cells (Dickson, 2004).

The emergence of AM fungal spores and the development of hyphae can be induced by the production of strigolactones by the host roots making the branching fungal hyphae move in the direction of the host root (Waters *et al.*, 2017).

In the *Arum*-type, hyphae spread between cells in the root cortex to create tiny, branching trees that are known as arbuscules. The thick, coiled hyphae of the *Paris*-type can give rise to highly branched arbuscule-like formations as they develop intra-cellularly (Dickson, 2004). . The fungal mycelium colonizes the roots to form a common mycorrhizal network (CMN) (Figueiredo *et al.*, 2021).

Invading the root cortical cells, the fungus creates specialized structures such as arbuscules and vesicles. The vesicles might be the intercalary or terminal, function as propagules, or transform into intra-radical spores (Rodrigues, 2016). Several AM fungal species also produce vesicles, which are lipid-rich, large, spherical intraradical cells with a reserve function. Vesicle

colonization of roots is correlated with active nutrient absorption and root development (Reinhard and Miller, 1990). The AM fungi's spores are multi-nucleate resting structures with thick walls. Host root exudates can speed up germination. With the right soil matrix, temperature, carbon dioxide concentration, pH, and P content AM fungal spores germinate (Figueiredo *et al.*, 2021).

AM fungi and plants were said to coexist 400 million years ago (Selosse *et al.*, 2015). All terrestrial environments, including tropical to temperate forests, alpine, dunes, deserts, grasslands, aquatic plants, and agroecosystems, as well as metal-polluted soils, have been observed to harbour AM fungus associations (Kehri *et al.*, 2018). AM fungal symbiotic association is a perfect example of a mutualistic relationship that regulate plant growth and development. They can enhance soil properties and hence promote plant growth under both stress-free and demanding conditions (Navarro *et al.*, 2014).

AM fungi improve the nutritional value of crops by influencing and increasing the synthesis of carotenoids and certain volatile chemicals (Hart *et al.*, 2015). Zeng *et al.*, (2014) reported that *Glomus versiforme* boosted the amounts of sugars, organic acids, vitamin C, flavonoids, and minerals, which in turn improved the quality of the citrus fruit. An increased accumulation of anthocyanins, chlorophyll, carotenoids, total soluble phenolics, tocopherols, and other mineral elements was reported due to mycorrhizal symbiosis (Baslam *et al.*, 2011).

Due to the huge demand, some of the medicinal plant species have become endangered while some are endemic, and thus protecting these species is important. Inoculating the medicinal plant species with efficient and indigenous AM fungal inoculum during the early phase of growth will help better development, greater nutrient uptake, and higher secondary metabolite production in a relatively short amount of time and at a reduced cost. Plants colonized by AM fungi are more resilient than non-mycorrhizal plants to a variety of biotic and abiotic stresses,

including dehydration, toxic metals, diseases that affect the roots, high soil temperatures, and salty soils (Khaliel *et al.*, 2011).

In the present investigation, AM fungal diversity, spore density, and colonization status were studied in 10 medicinal plants from Goa. The detailed aims and objectives of the present work are as follows:

1. Isolation of AM fungal spores occurring in the rhizosphere soils of the selected medicinal plants.
2. Determination of percent colonization, spore density, species richness, relative abundance and isolation frequency of AM fungi associated with the selected medicinal plants.
3. Preparation of trap and pure cultures using a suitable host plant.
4. Identification of the AM fungal species.
5. Physico-chemical characteristics of soils.

REVIEW OF LITERATURE

Khade and Rodrigues (2001) investigated AM fungi status in 25 tree species belonging to 18 families from Mollem forest area of Western Ghats of Goa and reported that AM colonization in the tree species varied with family and host genera. Highest root colonization was observed in *Macaranga peltata, Xylia xylocarpa, Zanthoxylum rhetsa* and *Randia ruglosa.* AM fungi belonging to five genera *viz., Acaulospora*, *Gigaspora*, *Glomus*, *Sclerocystis* and *Scutellospora* were recorded. *Glomus* was dominant genus. The study concluded that spore density was higher than in mixed deciduous, evergreen and semi-evergreen forest ecosystem of the Western Ghats in Southern India.

Khade *et al.,* (2002) examined 20 medicinal plants which belonged to 16 angiosperm families for AM colonization. All the plant species recorded 100% colonization. The AM fungi species belonged to 4 genera *viz., Acaulospora*, *Glomus*, *Sclerocystis,* and *Scutellospora*. *Glomus* was dominant. Higher number of AM fungal species was recorded in trees than shrubs.

Radhika and Rodrigues (2007) surveyed 36 medicinal plant species belonging to 25 families for AM colonization from North and South Goa. Of these 30 species recorded AM colonization. Forty-two AM fungal species belonged to five genera *viz., Glomus*, *Acaulospora*, *Scutellospora*, *Gigaspora* and *Ambispora*. *Glomus* was the dominant genera with *G. fasciculatum* being the dominant species.

Gupta *et al.,* (2009) recorded highest number of AM colonization and spore density in *Andrographis paniculata* and *ocimum sanctum* from MRDC (Medicinal Research and Development centre) Pantnagar, Uttarakhand.

Singh *et al.,* (2011) surveyed 69 plant species which belonged to 26 families for AM fungal colonization and spore density. Maximum root colonization and spore density was recorded in *Cynodon dactylon* and least root colonization was recorded in *Achyranthes aspera.*

Garampalli and Sunilkumar (2011) analysed 25 medicinal plants from Mysore district of Karnataka. They recorded highest root colonization and spore density in *Morus alba* and lowest in *Ipomoea muricata*.

Koul *et al.*, (2012), studied AM fungal association in plants from Gwalior-Chambal region of Madhya Pradesh and concluded that 110 plant species belonging to 54 families recorded AM association. Six AM species were associated with *Aloe vera* and highest spore density was recorded in *Aloe vera* (166.22 spores/100g soil) and lowest in *Abutilon indicum* (5.33 spores/100g soil).

Pawaar and Kakde (2012) studied the prevalence of AM fungi in some medicinal plants growing in tribal belt and around Mumbai. Out of 15 plant species, 11 were found to be mycorrhizal. They recovered AM spores belonging to five genera.

Garampalli *et al.*, (2012) surveyed 46 medicinal plant species (both wild and cultivated) from Western Ghats of Karnataka to determine AM colonization, spore density, diversity. They recorded AM colonization in all the species. The spore density ranged from 15-520 spores/ 100g of soil. Among 36 identified AM fungal taxa, the genus *Glomus* was dominant followed by *Acaulospora, Gigaspora,* and *Scutellospora.*

Chaubey *et al.*, (2013) studied microbial succession and restoration of degraded ecosystem at Northern Coalfield Limited, Singrauli. They observed that microbial biomass increased along with the age of plant. *Dalbergia sissoo* was the most promising species in terms of root colonization and AM spore density than the other species.

Mohan and Sandeep (2015) reported that AM fungi inoculated plants showed better growth and biochemical parameters like chlorophyll content and root P-uptake over non- inoculated plants. Plants inoculated with *Glomus fasciculatum* recorded maximum growth as well as increased chlorophyll and nutrient content.

Babu *et al.*, (2017) investigated 40 medicinal plants for AM association from Pookode lake area in Wayanad. Highest AM fungal colonization was recorded in *Centella asiatica* (80%) and maximum spore density in *Leucas aspera*. *Glomus fasciculatum* was the dominant AM species.

Santoshkumar *et al.*, (2018) investigated AM fungal status in some medicinal plants growing at Kondrangi hills, Eastern Ghats of Dindugul district in Tamil Nadu. In all, 20 AM fungal species belonging to 7 genera were identified. *Glomus* was the dominant genera. Highest AM fungal colonization was recorded in *Plumbago zeylanica.*

Kumar *et al.*, (2019) revealed that number of AM spores in the rhizosphere soil of plant is not correlated to AM colonization. Highest root colonization was reported in *Ricinus communis* while the highest spore density was recorded in *Mimosa pudica.*

Ghosh *et al.*, (2020) investigated the AM status of plant community in dry mixed deciduous and Sal forest from South-West Bengal. They reported maximum AM colonization, and spore density in mixed forest species than in Sal dominated forest.

Thapa *et al.*, (2015) studied AM colonization in 12 medicinal plants from Medicinal Plant Garden, University of North Bengal. They reported highest root colonization in *Justicia adhatoda* and highest spore count in *Abroma augustum*.

Urcoviche *et al.*, (2014) studied AM fungal diversity in medicinal and seasonal plants from Paranaense University – UNIPAR, Umuarama. They recorded highest spore density was during the month of June and reported G*lomus* as the dominant genus.

Panneerselvam (2014) studied the occurrence of AM fungal diversity in 20 medicinal plants from Vriddhachalam, Cuddalore district in Tamil Nadu. He reported highest AM colonization in *Justicia gendarussa* and lowest in *Naregamia alata*. The maximum spore density was recorded in *Maranta arundinaceae* and minimum in *Achyranthes aspera*. *Glomus* was found to be dominant genus.

MATERIALS AND METHODS

Study area

The root and soil samples of 18 medicinal plants were selected from six different talukas of Goa *viz.*, Quepem, Sanguem, Ponda, Pernem, Mormugao, and Bardez. Herbs, shrubs, and tree species were selected for the study.

Sample collection

The collection of root samples of medicinal plants and rhizosphere soil was undertaken in the study area from August to December 2021. After bringing the samples to the laboratory, roots were analyzed for AM colonization. Around 500 grams of rhizosphere soil sample of each host plant species were collected, labelled, air-dried, and then stored in plastic bags at 4^0C for further processing.

Estimation of root colonization

Phillips and Hayman (1970) method was employed for the assessment of AM colonization. The root segments of 1cm were thoroughly washed with tap water, then placed in the test tube containing 10% KOH and hydrolyzed for 30-45 minutes at 90^0C. The hydrolyzed root segments were then washed 3-4 times with water and acidified in 5N HCl for 3-5 minutes. Later the root segments were stained in 0.5% Trypan blue in lactophenol for 1hr. The stained root segments were then temporarily mounted on the glass slide using polyvinyl alcohol lacto-glycerol (PVLG). All the slides were examined under a compound microscope (Olympus BX41) and scored for AM colonization. A root segment was considered mycorrhizal if it showed the presence of hyphae and vesicles and/ or arbuscules.

Estimation of percent root colonization was carried out using the following formula:

$$\text{Percent root colonization} = \frac{\text{Number of root segments colonized}}{\text{Total number of root segments observed}} \times 100$$

Isolation of AM fungal spores

The AM spores from the rhizosphere soil were isolated using the wet sieving and decanting method (Gerdemann and Nicolson, 1963). For this, 100g of soil was suspended in a beaker containing tap water. The mixture was stirred for 10-15 seconds and kept undisturbed for 2 min, to settle the heavier particles. The soil water mixture was decanted through the stacked sieves with the coarse sieve on top and the fine sieve at the bottom. The range of sieves used was 60µ, 100µ, 150µ and 240µ. The suspension from each sieve was collected separately in a beaker. It was then filtered separately through Whatman No. 1 filter paper. The filter papers were then placed in separate Petri plates and the spores were isolated under a stereomicroscope (Olympus SZ) using a fine needle. This process was repeated in triplicate and the spore population was quantified as the number of AM spores per 100g of soil.

Identification of AM spores

Clean and intact spores were mounted on a slide using a drop of PVLG (Polyvinyl alcohol lacto-glycerol) and covered with a cover slip, gently pressed and sealed with DPX medium, and later observed under a compound microscope.

Taxonomic identification of spores was carried out by matching the descriptions provided by (Blaszkowski, 2012; Schenck and Perez, 1990; Rodrigues and Muthukumar, 2009). Identification was based on spore colour, size, shape, hyphal attachment, structure, and general nature.

Soil analysis

Samples collected from different sites were air-dried in the shade and sieved through a 2mm sieve to separate gravels and unwanted residues. Soil analysis was carried out at the soil testing and analysis laboratory, at Directorate of Agriculture Ela Old Goa. The soil was analyzed for pH, Electrical conductivity (EC), organic carbon (OC), available Potassium (K), and Phosphorus (P). The pH of the soil samples was determined in 1:2 soil water suspension. Electrical conductivity was measured at room temperature using a Conductivity meter. The rapid titration method proposed by Walkley and Black (1934) was used to estimate OC. Bray and Kurtz (1945) method was employed to analyze available P and available K content was estimated using Olsen's method (Olsen *et al.,* 1954).

Preparation of trap cultures

Trap cultures were prepared for the multiplication of AM spores by using sterile sand and rhizosphere soil (1:1). *Eleusine coracana* was used as a host. Hoagland's solution (minus P) was added bi-weekly to the pots. The trap cultures were maintained for around 2 months in the polyhouse. Later, the roots of these plants were examined for colonization, and sand-soil substrate was analyzed for fresh spores.

Preparation of Polyvinyl alcohol lacto-glycerol (PVLG)

Polyvinyl alcohol - 16.6g

Lactic acid – 100ml

Distilled water – 100ml

Glycerin – 10ml

16.6g polyvinyl alcohol was dissolved in 100 ml distilled water by heating at 90°C. After cooling 100ml lactic acid and 100ml glycerin were added.

Preparation of Trypan blue stain

Trypan blue – 0.05g

Lactic acid – 50ml

Glycerin – 10ml

Distilled water – 40ml

0.05g of Trypan blue powder is dissolved in 40ml distilled water and then 50ml of lactic acid and 10ml of glycerin are added.

Preparation of Hoagland's Solution (Hoagland and Aron, 1950)

Macronutrients

KNO_3 - 10.11g in 100ml

$MgSO_4.7H_2O$ – 24.65g in 100ml

Ca (NO_3) – 16.41g in 100ml

$MgCl_2$ – 20.33g in 100ml

$FeSO_4.7H_2O$ – 0.557g in 50ml

Na_2EDTA – 0.745g in 50ml

(50ml Na_2EDTA + 50ml $FeSO_4.7H_2O$) 100ml

Micronutrients

H_3BO_3 – 0.715g

$MnCl_2.H_2O$ – 0.452g

$ZnSO_4.7H_2O$ – 0.055g

$CaSO_4.5H_2O$ – 0.020g

$NaMoO_4$ – 0.725g

Dissolve in 250ml of distilled water

Makeup to 1L with distilled water

RESULTS AND DISCUSSION

Soil analysis

The results of the physicochemical analysis of the soil samples from the study sites are depicted in **Table 1**. The soils varied in pH, EC, OC, P, K, B, and S. The pH values of the soils ranged from acidic (5.1) to alkaline (7.5). EC was highest in soils of *Leucas aspera* (0.87 m.mhos/cm) and lowest in *Phyllanthus emblica* (0.13 m.mhos/cm) soils. The OC was observed to be highest in *Microcos paniculata* (5%) and lowest in *Datura stramonium* (0.14%) soils from Site V. The P content was highest in the soils of *Justicia adhatoda* (751.12Kg/h) and lowest in *Phyllanthus emblica* (52.67Kg/h). K content was highest in soils of *Justicia adhatoda* (1370.88Kg/ha) and lowest in *Datura stramonium* (153.22 Kg/ha) from Site VI. Boron was found to be highest in soils of *Justicia adhatoda* (2.09 ppm) and lowest in *Datura stramonium* (0.02 ppm) from site V. Sulphur was found to be high in *Leucas aspera* (87.8 ppm).

Soils of some plant species showed higher nutrient level than others. According to Vyas and Gupta (2014), soil pH has little or direct impact on the diversity pattern of AM in a natural and agricultural ecosystem.

A total of 10 medicinal plants belonging to 10 families were identified from 8 study sites **(Table 2) (Plate 1 and 2)**. These include *Rauvolfia serpentina, Gloriosa superba, Datura stramonium, Microcos paniculata, Piper nigrum, Leucas aspera, Justicia adhatoda, Boerhaavia diffusa, Hemidesmus indicus, Phyllanthus emblica* that were sampled for AM diversity.

The study revealed that all the plant species analysed recorded AM colonization and showed the presence of hyphae, vesicles and/or arbuscules **(Plate 3 and 4)**. The highest AM fungal root colonization was recorded in Rauwolfia serpentina (100%) from sites I and II, followed by site III (96.67%). Whereas, Aiswarya *et al.* (2017) reported 31%, Halder *et al.,* (2016) reported 33.33%, Koul *et al.,* (2012) reported 35%, Radhika and Rodrigues (2010) reported 40%, and Hussain and Srinivas (2013) reported 55% AM fungal root colonization in *R. serpentina.*

The least root colonization was recorded in *Piper nigrum* (48.33%). Similarly, Rajkumar *et al.,* (2012) reported 48% colonization in *P. nigrum.*

In *Gloriosa superba*, from site I recorded 65% root colonization followed by site II (58%) and site IV (51%). An earlier study reported (21%) root colonization in *G. superba* (Jothi *et al.,* 2017).

In *Datura stramonium,* site V recorded 66.67%, site VII recorded 65%, site VI recorded 56.67% root colonization, and 8 AM species in the rhizosphere. Kumar *et al.,* (2019) reported 18.74% root colonization and recorded 5 AM species in *D. stramonium.*

In *Leucas aspera,* root colonization was found to be 51.67%. Earlier, Koul *et al.,* (2012) reported 54%, and Jothi *et al.,* (2017) reported 44% colonization in *L. aspera.*

In the present study, *Justicia adhatoda* recorded 76.67% AM colonization. Earlier, Garampalli *et al.,* (2012) reported 93%, Jothi *et al.,* (2017) reported 22%, Kumar *et al.,* (2019) reported 81.25%, and Thapa *et al.,* (2015) reported 95% colonization in the same species.

In *Hemidesmus indicus*, 65% root colonization was recorded. Earlier studies by Aishwarya *et al.*, (2017) reported 25%, Radhika and Rodrigues (2010) reported 34%, while Garampalli *et al.*, (2012) reported 62% root colonization in *H. indicus*.

In *Phyllanthus emblica* 60% root colonization was recorded. Earlier studies by Hussain and Srinivas (2013) reported 20%, Koul *et al.*, (2012) reported 79% colonization in *P. emblica*.

The highest spore density was recorded in *R. serpentina* from Site I (378.67 spores/100g soil). While Aiswarya *et al.*, (2017) reported 431spores/100g, Koul *et al.*, (2012) reported 15.33 spores/100g, and Hussain and Srinivas (2013) reported 45.33 spores/100g. The least spore density was recorded in *Piper nigrum* (128 spores/100g soil). Garampalli *et al.*, (2012) reported 69 spores/100g of rhizosphere soil in *P. nigrum*.

All the plant species showed the presence of hyphal, vesicular and/or arbuscular colonization. The shape of the vesicles varied from elliptical, round, globose, oval, and elongated. Similar observations have been reported earlier (Kumar *et al.*, 2019).

AM fungal taxa consist of a specific multidimensional niche determined by the plant species present at the site and edaphic factors like pH, moisture content, soil nutrients, etc. (Ahulu *et al.*, 2006, Jothi *et al.*, 2017). As a result, considerable variation in the composition of AM fungal taxa is observed. Aishwarya *et al.*, (2017) predicted that root colonization and sporulation variation is based on host-symbiont specificity. According to Chakraborty *et al.*, (2019), the degree of mycorrhizal colonization can vary from plant to plant and even in individual plants. The variation may be caused due to root exudates available in the rhizosphere soil that might stimulate the AM spores to germinate and colonize (Hussain and Srinivas, 2013, Tester *et al.*, 1987).

AM fungal sporulation depends on various host fungi, environmental factors, soil organisms and growing season. According to Khade and Rodrigues (2008), the germination potential of AM fungal species is known to vary at different times of the year.

In the present study, increased spore densities did not increase colonization rates and *vice versa*. A similar observation was reported in earlier studies (Rajkumar *et al*., 2012; Koul *et al*., 2012; Kumar *et al*., 2019; Chauhan *et al*., 2013).

Table 2: AM fungal colonization and spore density in selected medicinal plants.

Sr no.	Site	Plant species/ Family	Habit	Type of colonization	Colonization (%)	Spore density (spore/100g soil)
1	I	*Rauvolfia serpentina* (Apocynaceae)	Under Shrub	H, V, A, HC, HS	100.00± 0.00	378.66± 9.79
2	II			H, V, A, HC, HS	100.00± 0.00	361.33± 45.39
3	III			H, V, A, HC, HS	96.67± 1.15	329.33± 24.47
4	I	*Gloriosa superba* (Colchicaceae)	Climber	H, V	65.00± 2.64	365.33± 45.56
5	II			H, V	58.33± 2.51	278.67± 7.05
6	IV			H, V	51.67± 1.52	296.00± 18.90
7	V	*Datura stramonium* (Solanaceae)	Herb	H, V, A, HS	66.67± 1.52	292.00± 17.67
8	VI			H, V, A, HS	56.67± 2.08	234.67± 22.43
9	VII			H, V, A, HS	65.00± 2.00	262.67± 13.13
10	II	*Microcos paniculata* (Malvaceae)	Tree	H, V, A	55.00± 1.73	217.33± 33.65
11	VIII	*Piper nigrum* (Piperaceae)	Climber	H, V, A	48.33± 3.05	128.00± 12.85
12	VIII	*Leucas aspera* (Lamiaceae)	Herb	H, V, A, HS	51.67± 1.52	222.67± 45.04
13	IV	*Justicia adhatoda* (Acanthaceae)	Tree	H, V, A	76.67± 3.05	173.33± 5.33
14	III	*Boerhaavia diffusa* (Nyctaginaceae)	Herb	H, V, A	56.67± 1.15	150.67± 19.91
15	I	*Hemidesmus indicus* (Apocynaceae)	Shrub	H, V, A, HS	65.00± 3.60	153.33± 7.42
16	IV	*Phyllanthus emblica* (Phyllanthaceae)	Tree	H, V, A, HS	60.00± 2.64	168.00± 18.33

Legend: H=Hyphae, V= Vesicles, A= Arbuscules, HS= Hyphal swelling, HC= Hyphal coils
Sites: I – Taleigao, II – Sanguem, III – Quepem, IV – Ponda, V – Vagator, VI – Anjuna, VII – Velsao, VIII – Pernem. All values are mean of three replicates.

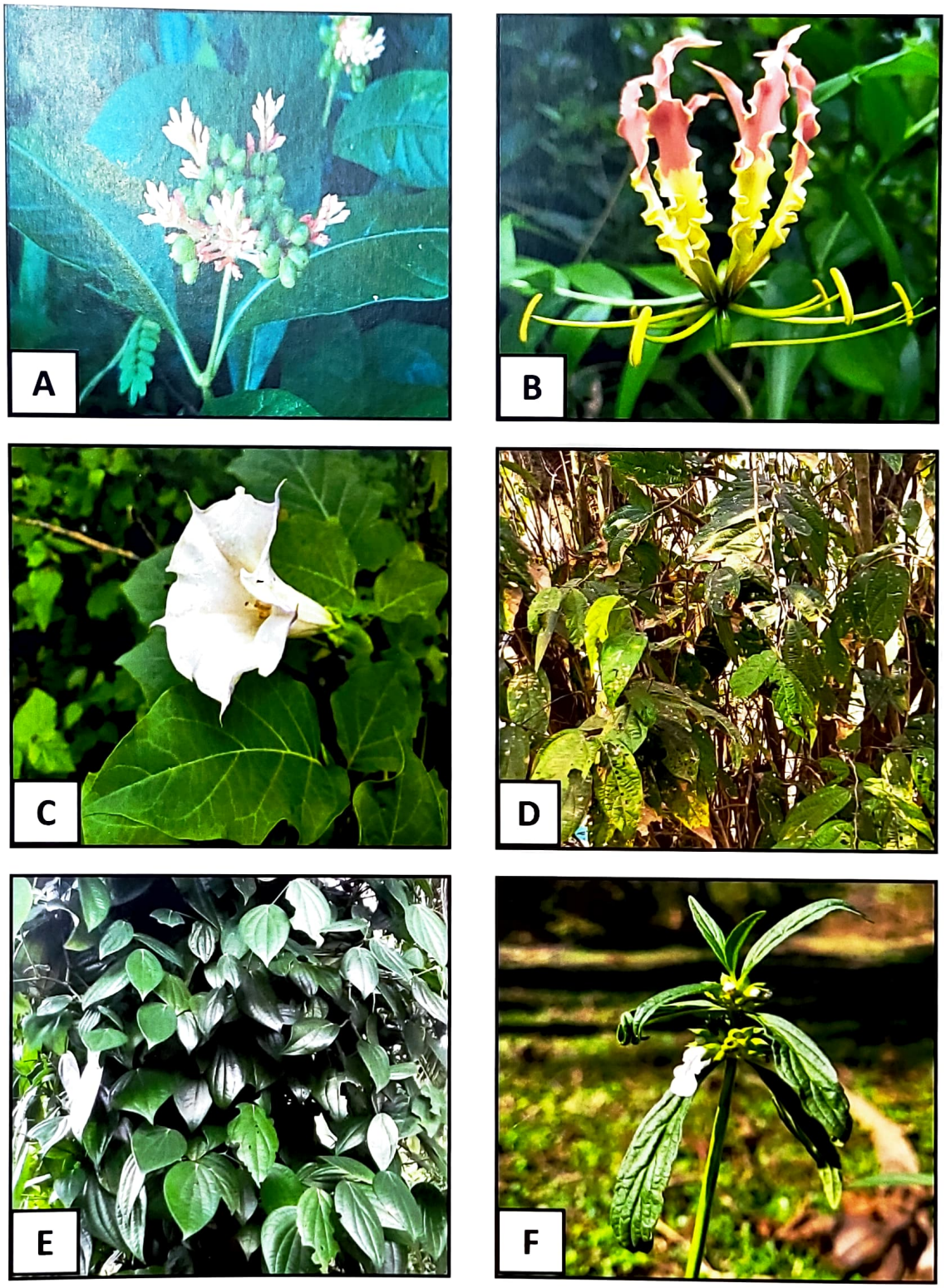

PLATE 1 - Plant diversity. **A.** *Rauvolfia serpentina*, **B.** *Gloriosa superba*, **C.** *Datura stramonium*, **D.** *Microcos paniculate*, **E.** *Piper nigrum*, **F.** *Leucas aspera.*

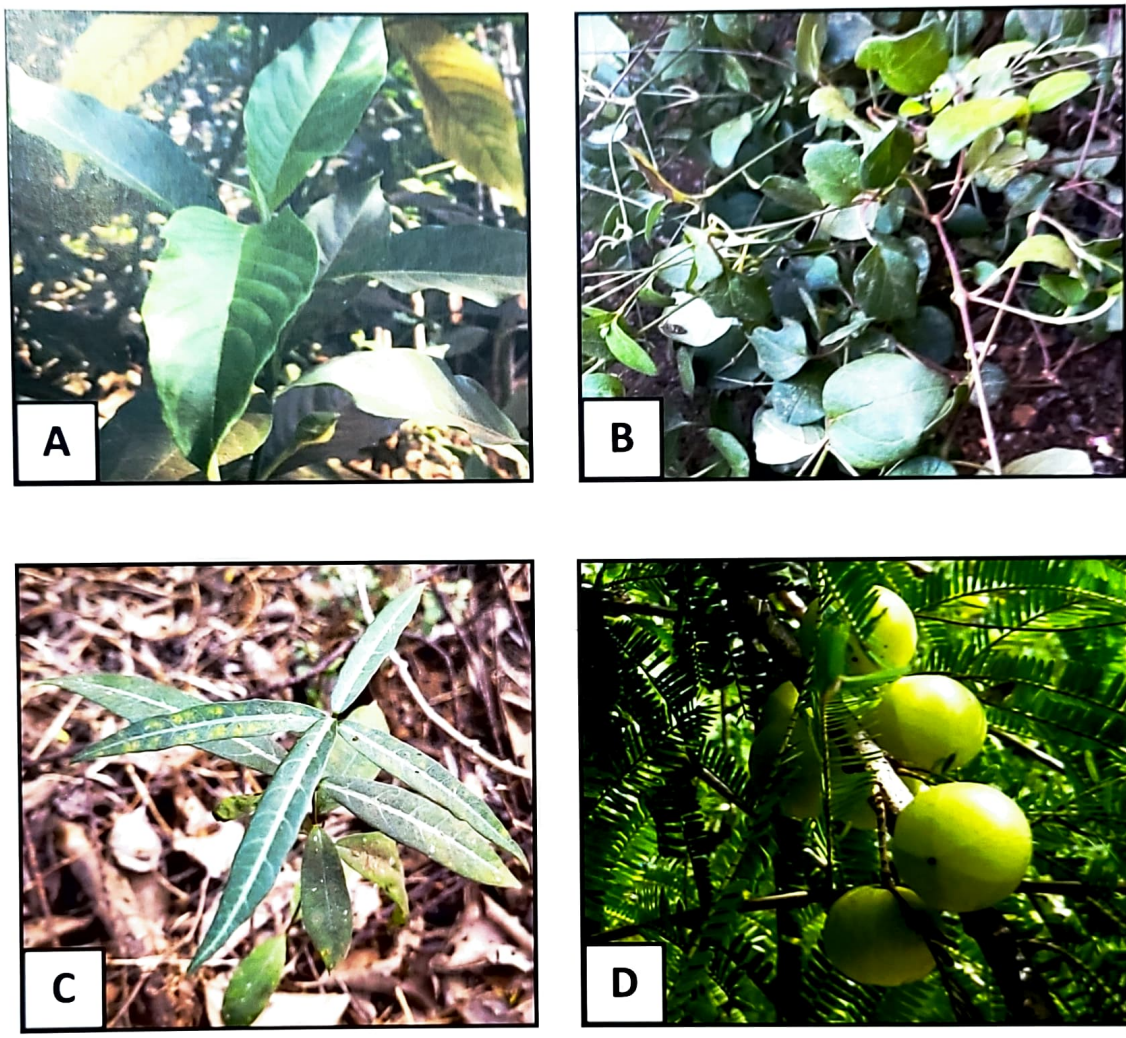

PLATE 2 - Plant diversity. **A.** *Justicia adhatoda*, **B.** *Boerhaavia diffusa*, **C.** *Hemidesmus indicus*, **D.** *Phyllanthus emblica*.

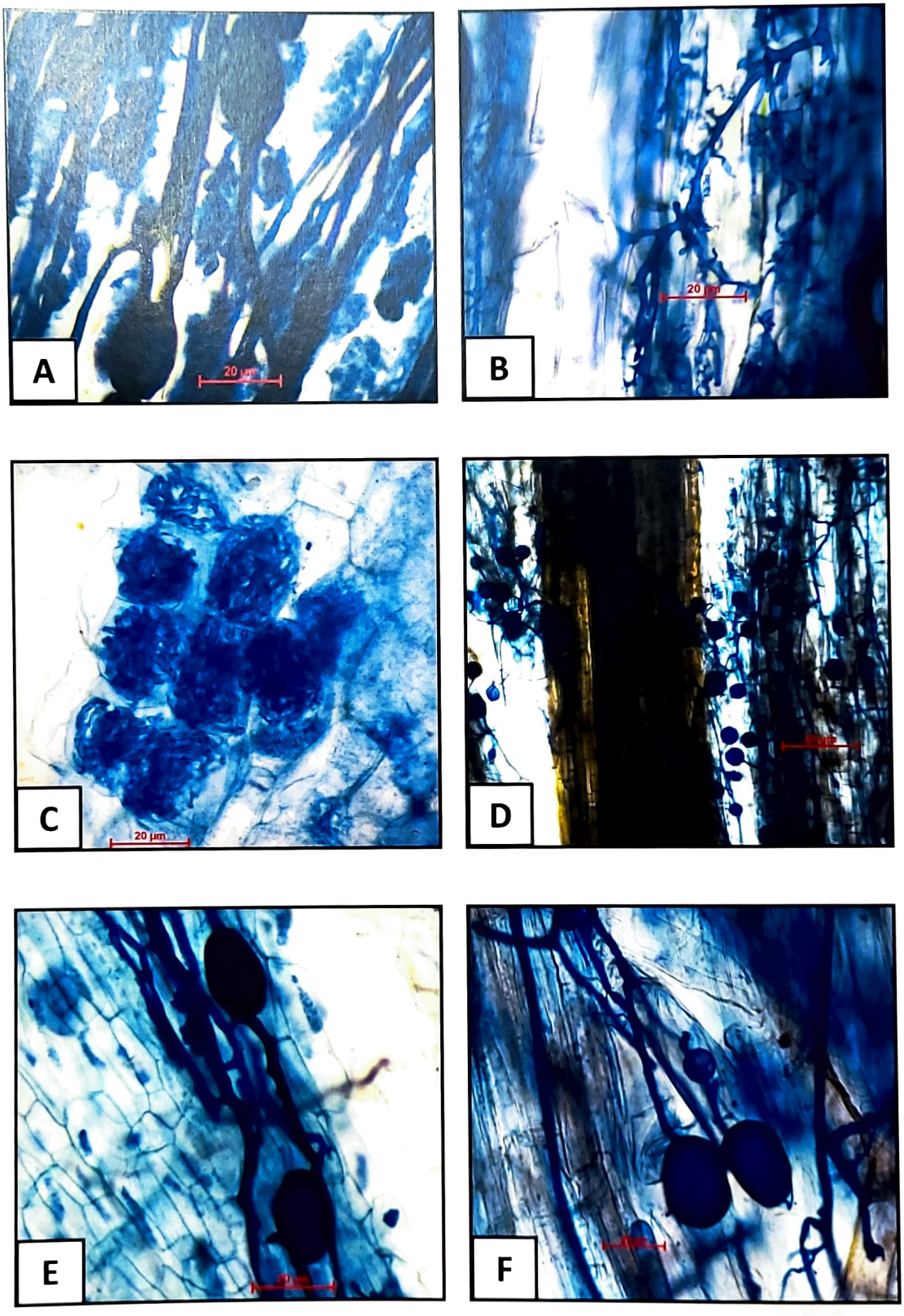

PLATE 3 – AM fungal root colonization **A.** Hyphal and vesicular colonization in *Rauvolfia serpentina,* **B.** Hyphal swelling in *Justicia adhatoda,* **C.** Hyphal coils in *Rauvolfia serpentina,* **D.** Hyphal and vesicular colonization in *Phyllanthus emblica* **E.** Vesicles in *Rauvolfia serpentina,* **F.** Vesicles in *Leucas aspera.*

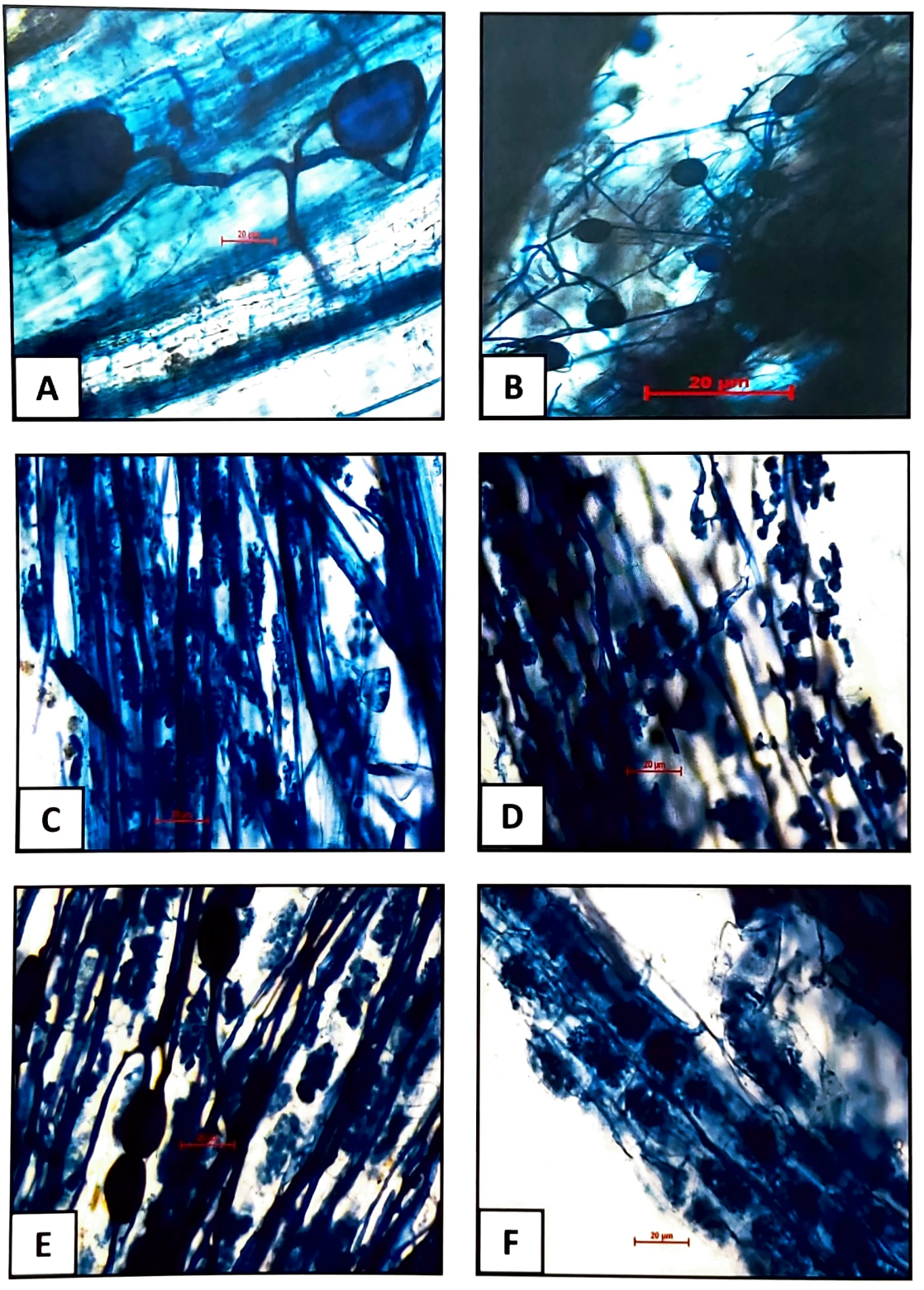

PLATE 4 - AM fungal root colonization observed in roots. **A.** Vesicles in *Hemidesmus indicus,* **B.** Vesicles in *Phyllanthus emblica,* **C, D & E.** Arbuscules in *Rauvolfia serpentina,* **F.** Arbuscules in *Leucas aspera.*

The highest species richness was recorded in *Rauvolfia serpentina* site I (8), while the least was recorded in *Leucas aspera* (3) and *Boerhaavia diffusa* (3) **(Table 3).**

The study revealed that several AM species were recorded in the rhizosphere of each plant species. This could be due to the interspecific competition between them (Brundett and Kendrick 1990) or competition between fungi and environmental factors which might influence spore production in natural communities (Gemma and Koske, 1988). Khade and Rodrigues (2008) suggested the influence of electrical conductivity on spore density.

A total of 18 AM fungal species belonging to five genera, *viz., Acaulospora, Glomus, Gigaspora, Scutellospora* and *Dentiscutata* were recovered from the rhizosphere soils of the study sites **(Table 4)**. *Glomus* (8) was the dominant genus, followed by *Acaulospora* (7), *Gigaspora* (1), *Scutellospora* (1), and *Dentiscutata* (1), with species number given in parenthesis. The diversity of AM fungal species is presented in **Plate 5, 6 and 7.** Various AM species were recorded in different plant species **(Table 3)**.

Maximum Relative Abundance was observed in *Glomus* sp. 2 (16.82%). In contrast, the least was seen in *Glomus* sp. 8 (0.05%). The highest isolation frequency was recorded in *Glomus* sp. 3 (83.33%), while least in *Acaulospora* sp. 1(4.16%) and *Glomus* sp. 7(4.16%) **(Table 4).**

Several studies have reported *Glomus* as a dominant genus in the soil types studied (Rajkumar *et al.,* 2012; Selvaraj *et al.,* 2001; Robinson-Boyer *et al.,* 2009; Koul *et al.,* 2012). This may be because *Glomus* species have different temperature and pH preferences for germination (Robinson- Boyer *et al.*, 2009). The genus *Acaulospora* is often found to be associated with acidic soils (Abbott and Robson, 1991). Rajkumar *et al.,* (2012) suggested that the climatic conditions of soil can be more influential on the distribution and abundance of mycorrhizal spores. They also indicated that the soil pH strongly influences the decline in colonization rates, spore density, and species richness.

Table 3: AM fungal species richness at the study sites.

Sr no.	Site	Plant species	Species richness	AM species
1	I	*Rauvolfia serpentina*	8	*A. dilatata, A. rehmii, A. scrobiculata, Glomus* sp. 1, *Glomus* sp. 2, *Glomus* sp. 3, *Glomus sp.* 5, *Scutellospora* sp.
2	II		6	*A. rehmii, A. laevis, Glomus* sp. 1, *Glomus* sp. 2, *Glomus* sp. 3, *Scutellospora* sp.
3	III		6	*A. dilatata, A. delicata, A. scrobiculata, Glomus* sp. 1, *Glomus* sp. 2, *Glomus* sp. 3
4	I	*Gloriosa superba L.*	6	*A. scrobiculata, Gigaspora* sp., *Glomus* sp. 1, *Glomus* sp. 2, *Glomus* sp. 5, *Glomus* sp. 7
5	II		5	*A. scrobiculata, Scutellospora sp., Glomus* sp. 1, *Glomus* sp. 2, *Glomus* sp. 5
6	IV		6	*A. dilatata, A. rehmii, A. scrobiculata, Glomus* sp. 1, *Glomus* sp. 2, *Glomus* sp. 5
7	V	*Datura stramonium*	4	*A. rehmii, A. scrobiculata, Glomus* sp. 2, *Glomus* sp. 3
8	VI		6	*A. dilatata, A. scrobiculata, A. laevis, Gigaspora* sp., *Glomus* sp. 2, *Glomus* sp. 3
9	VII		6	*A. dilatata, A. scrobiculata, A. laevis, Scutellospora* sp., *Glomus* sp. 3, *Glomus* sp. 5
10	II	*Microcos paniculata*	4	*A. rehmii, Glomus* sp. 1, *Glomus* sp. 3, *Glomus* sp. 4
11	VIII	*Piper nigrum*	4	*A. dilatata, A. scrobiculata, Glomus* sp. 1, *Glomus* sp. 3
12	VIII	*Leucas aspera*	3	*A. delicata, Glomus* sp. 1, *Glomus* sp. 3
13	IV	*Justicia adhatoda*	5	*A. undulata, A. rehmii, Glomus* sp. 1, *Glomus* sp. 3, *Glomus* sp. 8
14	III	*Boerhaavia diffusa*	3	*A. rehmii, A.* sp. 1, *Glomus* sp. 3, *Glomus* sp. 6,
15	I	*Hemidesmus indicus*	5	*A. dilatata, Glomus* sp. 1, *Glomus* sp. 3, *Glomus* sp. 4, *Scutellospora* sp.
16	IV	*Phyllanthus emblica*	5	*A. rehmii, Dentiscutata* sp., *Gigaspora* sp. *Glomus* sp. 2, *Glomus* sp. 3

Table 4: Relative abundance and Isolation frequency of AM species at the study sites.

Sr. No.	AM Fungal Species	Relative Abundance (RA%)	IF%
1	*Acaulospora delicata*	1.66	20.83
2	*Acaulospora dilatata*	13.67	62.50
3	*Acaulospora rehmii*	7.71	54.16
4	*Acaulospora undulata*	0.64	12.5
5	*Acaulospora scrobiculata*	12.49	75.00
6	*Acaulospora laevis* (3)	2.62	16.66
7	*Acaulospora* sp. 1	0.10	4.166
8	*Glomus* sp. 1	13.55	66.66
9	*Glomus* sp. 2	16.82	62.50
10	*Glomus* sp. 3	16.50	83.33
11	*Glomus* sp. 4	4.82	33.33
12	*Glomus* sp. 5	2.36	29.16
13	*Glomus* sp. 6	0.85	12.50
14	*Glomus* sp. 7	0.96	4.16
15	*Glomus* sp. 8	0.05	8.33
16	*Gigaspora* sp.	3.00	37.50
17	*Scutellospora* sp.	1.82	54.16
18	*Dentiscutata* sp.	0.32	8.33

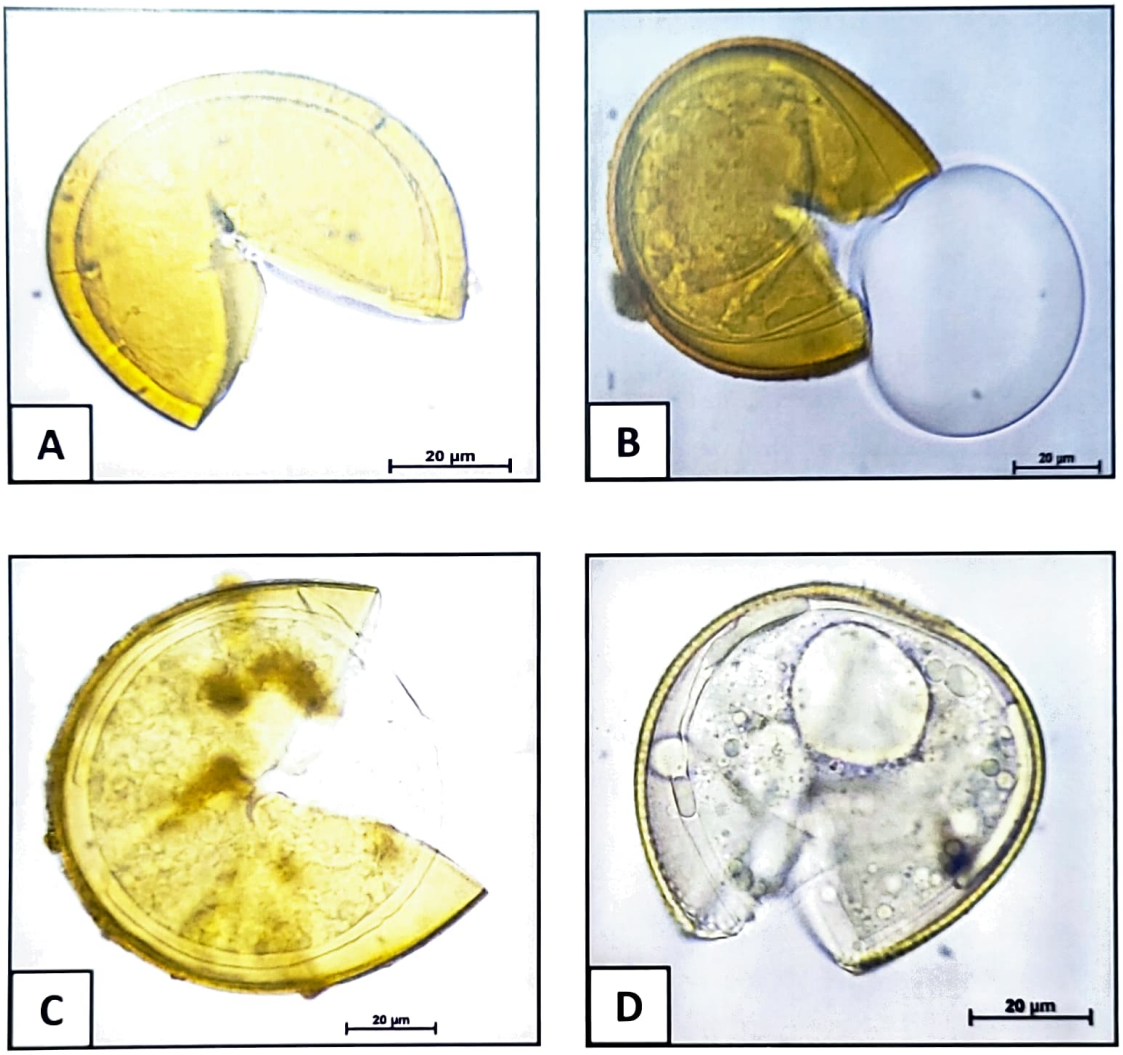

Plate 5: Spores of genus *Acaulospora* isolated from study sites A. *Acaulospora delicata*, **B.** *Acaulospora dilatata*, **C.** *Acaulospora rehmii*, **D.** *Acaulospora* . sp. 1 (unidentified).

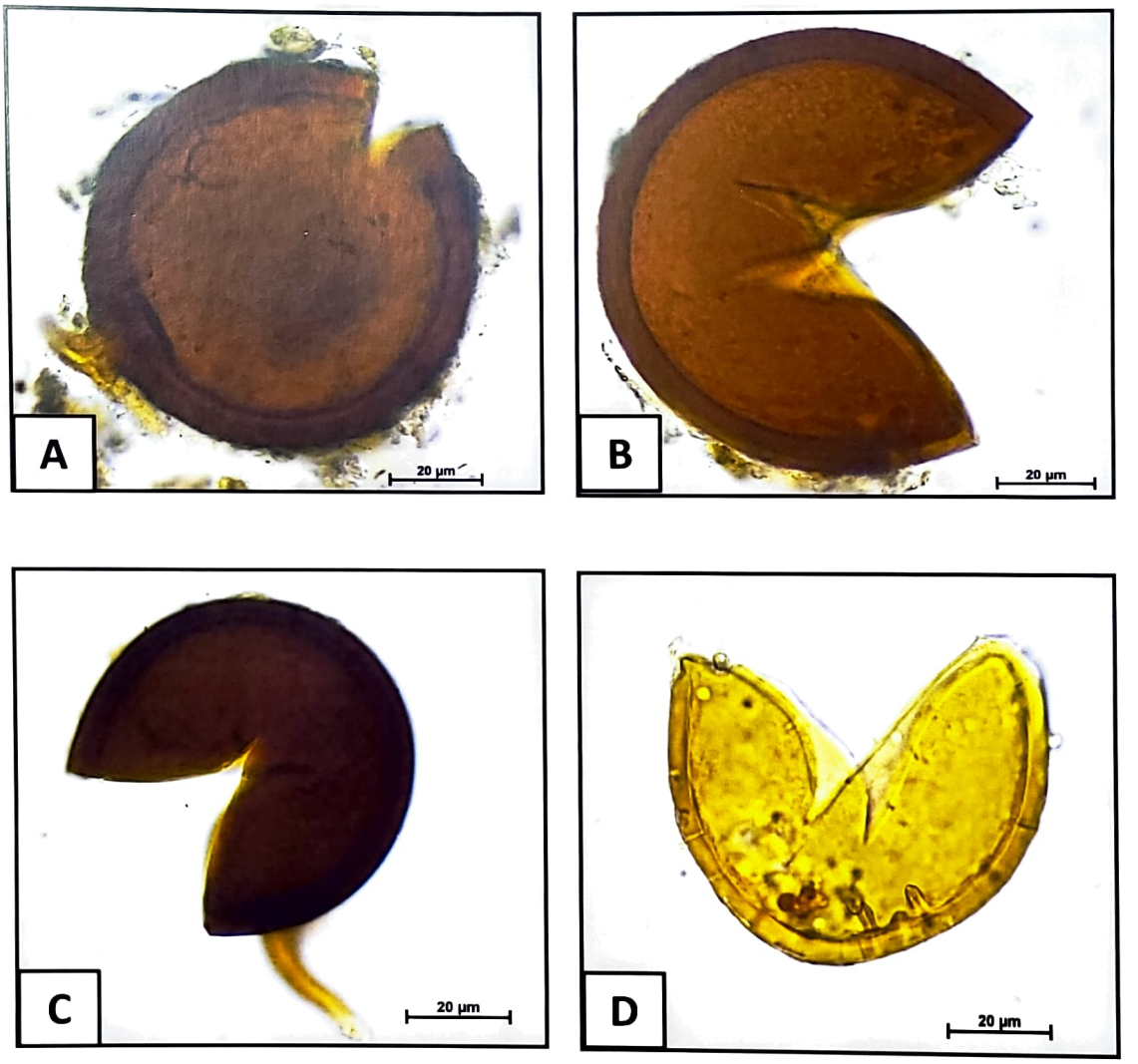

Plate 6: Spores of the genus *Glomus* isolated from study sites A. *Glomus* sp. 1 (unidentified), **B.** *Glomus* sp. 2 (unidentified), **C.** *Glomus* sp. 5 (unidentified), **D.** *Glomus* sp. 8 (unidentified).

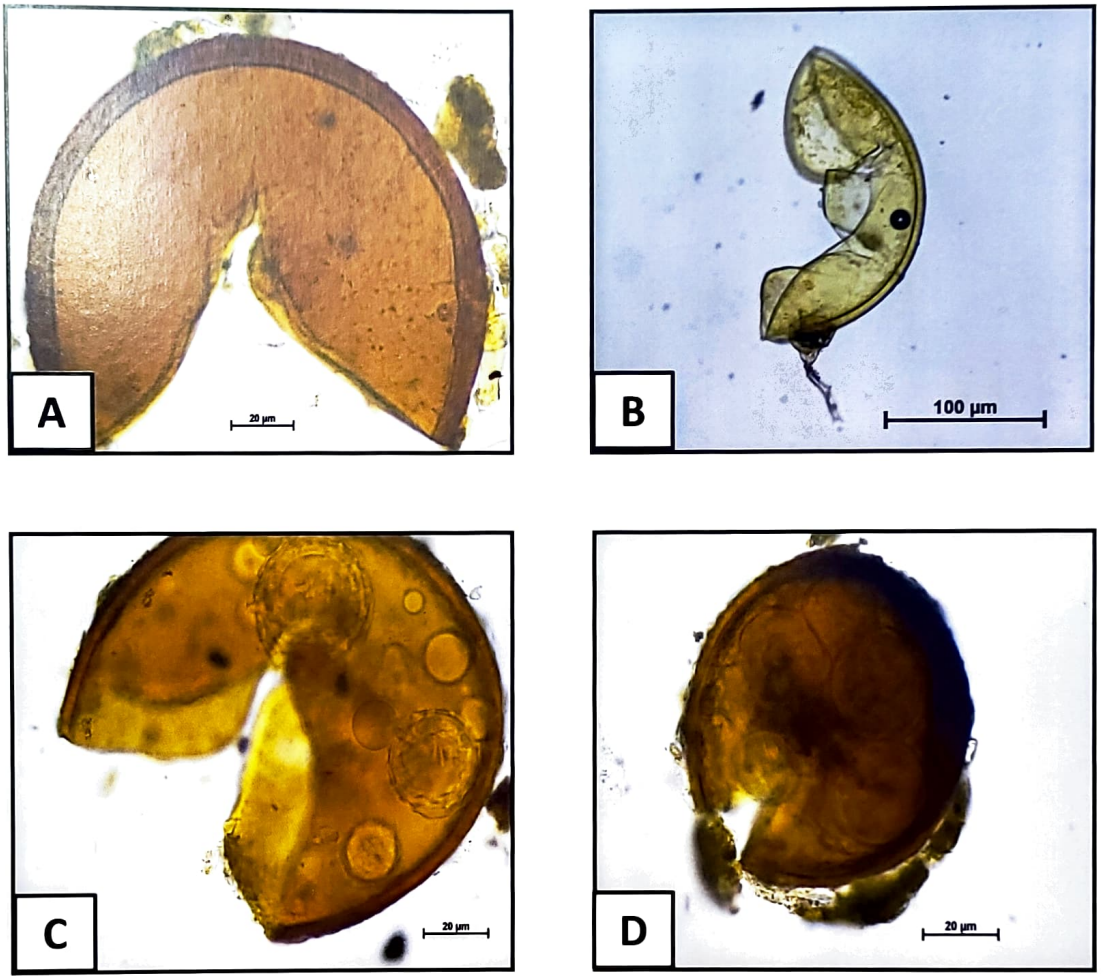

Plate 7: A – B: Spores of *Scutellospora* and *Gigaspora* genera isolated from study sites **A.** *Scutellospora* sp. (unidentified), **B.** *Gigaspora* sp.(unidentified), **C – D:** Spore in spore syndrome.

In the present study, AM fungal colonization was recorded in all the plants species studied from August to January, indicating that medicinal plants depend on AM fungal association for better growth and development.

In the present study, 5 out of 18 AM species were mass multiplied as monospecific cultures prepared from trap cultures (**Plate 8**). All the cultured AM fungal species recorded root colonization and adequate number of spores. (Stutz *et al.*, 2000) reported successive preparation of trap cultures can greatly assist in the assessment of AM species composition in natural habitats. However, these methods are the least artificial (live host plants are used) and thus help in understanding the biology of AM fungal lifecycle as well as in supporting production and storage of the inocula for a longer duration (Rodrigues K. M., 2017).

Plate 8: A-B. Trap cultures, **C.** Pure cultures.

CONCLUSION

A total of ten medicinal plants belonging to ten families from six talukas of Goa were undertaken in the present investigation. The study revealed that all the plant species were colonized by AM fungi. However, the degree and type of colonization, spore density and species richness differed among the plant species and study sites. It was observed that root colonization, spore density and species richness are correlated with climatic and edaphic factors of the study area and host dependence of AM fungal species. In the present study, AM spore density was found to be maximum in wildly growing medicinal plants compared to cultivated species.

Mycorrhizal symbiosis plays a fundamental role in shaping plant communities and terrestrial ecosystems and is of high value for the sustainability of the ecosystem. It has been observed that the host plants control the diversity of the AM population through various effects on hyphal growth and sporulation. Appropriate strategies can be drawn for the artificial inoculation of some of these indigenous AM fungi, making the re-establishment and regeneration attempt ecologically and economically viable even in an constrained ecosystem. Such approaches will increase the scope to manipulate the symbiosis in conservation schemes.

The abundance of *Glomus* and *Acaulospora* species in the soil makes it more favoured for mass multiplication. The efficient inoculum thus produced can be utilized to increase the growth and productivity of medicinal plants. Moreover, this investigation may also be important for studying the effect of different anthropogenic activities on AM fungi. From a practical point of view, using a species with widespread distribution implies that mycorrhizal inoculum produced with one or many species can be used under different soil and climatic conditions.

From the present study, it can thus be concluded that most or all medicinal plants harbour mycorrhizal associations. However, the diversity of AM fungi species differs in different medicinal plants, and the host plant and environmental factors control the extent of AM fungal colonization. The diversity of AM fungi recorded in this study can be further utilised to evaluate their activity in the enhancement of the growth of these medicinal plants.

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