STATUS OF MYCORRHIZAL COLONISATION ON DIFFERENT MEDICINAL PLANTS COLLECTED FROM ERNAKULAM DISTRICT

Project submitted

TO

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Submitted by

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DEPARTMENT OF BOTANY

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CERTIFICATE

This is to certify that this project work entitled "**Status of Mycorrhizal Colonisation of different Medicinal Plants collected from Ernakulam District**" bonafide piece of project work done by Dilsa Sidhique (Reg.no:200021023408) in the Department of Botany, Bharata Mata College, Thrikkakara under my guidance and supervision for the award of Degree of Bachelor of Science in Botany during the academic year 2020-2023. This work has not previously formed the basis for the award at any other similar title of any other university or board.

Place : Thrikkakara   Dr Shahina N K

Date :

Department of Botany

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DECLARATION

I hereby declare that this project entitled "**Status of Mycorrhizal Colonisation of different Medicinal Plants collected from Ernakulam District**" is the result of work carried out by me under the guidance of Dr. Shahina N.K Department of Botany, Bharata Mata College, Thrikkakara. This work has not formed on the basis for the award at any other similar title of any other university of board.

Dilsa Sidhique

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INTRODUCTION

1.1 Mycorrhizal fungi and types

Mycorrhizae are soil fungi that have a mutualistic connection with plant roots. The plant supports the fungus by providing the carbohydrates required for fungal growth, while the fungus helps the plant by increasing its root surface area and delivering inorganic nutrients like phosphate (Bowles et al., 2016). More than 83% of dicotyledonous plants and 79% of monocotyledonous plants have mycorrhizal fungi, which are found in the majority of plants and are associated with all gymnosperms (Begum et al., 2019).

The two main types of mycorrhizae are ectomycorrhizae and endomycorrhizae. On the basis of where the fungi colonise the plants, they are categorised into categories. Ectomycorrhiza, also known as EcM, often creates connections between woody plants (including beech, birch, willow, oak, pine, fir, and spruce) and Ascomycota, Basidiomycota, and Zygomycota-class fungi. According to Martin et al. (2016), ectomycorrhizas are made up of a mantle or hyphal sheath that surrounds the root tip and a Hartig net of hyphae that encloses the plant cells in the root cortex.

Endomycorrhizae, comprising greenhouse and crop plants like fruits, flowers, grasses, and vegetables, comprise more than 80% of plant groups. The formation of vesicles and arbuscules by the fungus as well as its penetration of the cortical cells are characteristics of endomycorrhizal connections. the endomycorrhizae

The majority of terrestrial plants can establish a mutualistic relationship with arbuscular mycorrhiza fungi (AMF). When soil spores germinate and infect roots, arbuscule forms appear inside the infected roots (Figure 1). Arbuscules are where the exchange of nutrients between plants and fungi occurs. Another characteristic of this interaction is the presence of a sizable mycorrhizal network around the root system. Glomeromycota and Mucoromycota form the distinctive structures known as arbuscules and vesicles in arbuscular mycorrhizae (Quilambo, 2003). Arbuscules may be Arum type or paris type.

igure 1. Diagram of a longitudinal section of a root showing the characteristic structures of rbuscular mycorrhizal fungi.
ıdapated from M. Brundrett.(57))

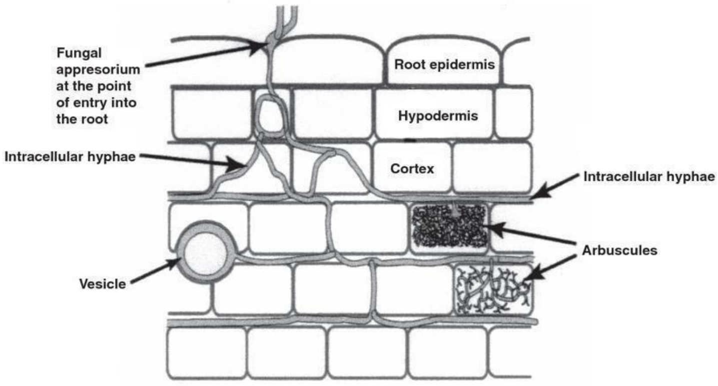


Figure 1 Arbuscular Mycorrhiza

1.2 The beneficial effects of Arbuscular mycorrhiza to plants

There are many effects on plants from symbiosis with arbuscular mycorrhizal fungus. They can better withstand drought, flooding, soil salinization, and heavy metal contamination thanks to the increased growth, immunity, and resistance (Ghorsi 2002; Jeffries et al., 2003; Garg and Chandel, 2011; Smith and Smith, 2012; Begum et al., 2019).

Enhance nutrient uptake and increase biomass: Mycorrhizal fungi increase absorption of water and transportation of mineral nutrients Arbuscular mycorrhizal fungi build a vast network of extraradical hyphae that enables plants to access a larger variety of nutrients. Because of this, AMF-colonized plants frequently exhibit a greater nutritional status (P, N, and other nutrients), which raises plant biomass

Alter soil condition: After AMF have colonised a plant, plant exudation patterns may alter, affecting the macrofaunal and microbial communities in the rhizosphere. The extraradicle hyphae of endomycorrhizae release a glycoprotein called glomalin that aggregates soil particles, increasing the quantity of water-stable aggregates and improving soil structure.

Defense against pathogen: The plant's defense against bacterial and fungal infection is aided by the presence of mycorrhizae. This might be because of the plant's increased nourishment, which has made it healthier and more resistant to the invader.

Stress tolerance : Mycorrhizal plants frequently have higher disease resistance, especially against microbial soil-borne infections.They also resistant to salt, heavy metal and water stress.

Atmospheric CO2 fixation: Mycorrhizal fungi may affect the amount of atmospheric CO_2 fixation by host plants by increasing the "sink effect" and transporting photoassimilates from the aerial parts to the roots.

1.3 Factors affecting Arbuscular Mycorrhizal Fungal growth

The majority of the variables affecting AMF development are abiotic. It addresses variables including temperature, soil pH, humidity, light intensity, and the presence of plant nutrients. Low pH may have a deleterious impact on the activity and growth of AMF. The volume of water available can also influence the growth of mycorrhizal because AMF are bacteria that are highly sensitive to low oxygen availability. The continuous cultivation of soil may also have an effect on the amount of AMF spores (Jamiołkowska et al., 2018). AMF growth is influenced by temperature as well. Whether temperatures are unusually high or low, the amount of AMF in the soil declines. It requires a temperature that is optimum for growth. High levels of nitrogen in the soil, drought, nutrient scarcity, and trace metal concentrations can all have an impact on mycorrhizal fungus.Besides this. According to research, soil disturbance reduces species diversity, AMF spore density, and extraradical mycelium length when compared to undisturbed soil (Boddington and Dodd, 2000).

1.4 Identification of Mycorrhiza from the plant roots

Studies on the ecology of AMF used soil-collected spores. The spore size, shape, number of cells, thickness of the spore wall, color, and surface ornamentation etc used to distinguish the mycorrhiza. Spore data gathered from soil, however, may not accurately represent the diversity and functionally active AMF invading plant roots. Thus, it is essential for the ecological research of AMF species that these species be identified within plant roots, so presently the identification of AMF within plant roots has been made possible by the development of appropriate primers and methods (Lee et al., 2013).

1.5 Studies on mycorrhizal association in various plant groups

Arbuscular mycorrhizal fungi have been found in a wide variety of plant communities, including forests, deserts and mangroves. Glomeromycota, often known as arbuscular mycorrhizal fungi, are among the most important fungi seen in plants; they create mutualistic interactions with the roots of over 90% of plant species. According to studies conducted thus far in the Western Ghats region , Glomus species were dominating in the soils of the root zones of several tree species (Rajkumar et al., 2012). Similar to this, *Glomus etunicatum* was discovered in the cashew tree's root zone soil by Lakshmipathy et al. in 2004.

The actions of mycorrhizal fungi are shown to affect a wide range of plant species, including some with significant economic value. More research has been done on the prevalence of AMF in relation to crop plants, cereal crops, vegetable plants etc but there has been less research done on medicinal plants in relation to AM colonisation.

REVIEW OF LITERATURE

2.1 Occurrence and Role of AMF

The interactions between weeds and arbuscular mycorrhizal fungi (AMF) should undoubtedly be considered in this assessment. AMF, which have symbiotic interactions with the majority of vascular plants, are known to have a significant impact on a variety of elements of plant biology . Plant roots are invaded by arbuscular mycorrhizal fungi (AMF), which increase plant productivity and enhance soil carbon sequestration. It is found that the majority of terrestrial plants, including many important agricultural species, rely on arbuscular mycorrhizal (AM) fungus for nitrogen uptake. Through a special plant-fungal interface, the intraradical mycelium of the host root takes nutrients from the soil, transfers them to the extraradical mycelium of the fungus, and then exchanges the nutrients for carbon from the host.

The development of mycorrhizae by plant seedlings was studied by Brundrett (2017) after either being inoculated with isolates of vesicular-arbuscular mycorrhizal (VAM) fungus or after growth in whole cores of natural habitat soil containing both VAM and ECM fungi. Three forms of mycorrhizal associations dominated the plant kingdom: VAM alone (56% of species), ECM and VAM combined (16% of species), or non-mycorrhizal roots (25% of species). ECM is frequently formed more quickly than VAM in plants with dual connections for ECM and VAM. The vast and diverse Papilionaceae, Myrtaceae, and Anthericaceae families are the exception to the rule that plants within a family and the majority of genera have stable mycorrhizal associations.

A thorough analysis of the mycorrhizal literature reveals information about mycorrhizas and other nutritional adaptations for 336 plant groups, which comprise 99% of flowering plants. Arbuscular (AM), orchid, ectomycorrhizal (EM), ericoid mycorrhizal (EM), and nonmycorrhizal (NM) roots are all found in 74%, 9%, 2%, 1%, and 6%, respectively, of Angiosperm species. Arbuscular mycorrhizae make up 72% of vascular plants' mycorrhizal systems, ectomycorrhizae make up 2%, ericoid mycorrhizae make up 1.5%, and orchid mycorrhizal make up 10%(Brundrett, 2009).

2.2 Mycorrhizal association in Plants

The coevolution of roots and mycorrhizas of land plants was assessed in a study by Brundrett (2002) he observed that mycorrhizal plants have their maximum exodermis suberization and root cortical thickness, whereas nonmycorrhizal plants frequently have roots that are finer, have more roots hairs, and have much more advanced chemical defences.

Researchers examined the colonisation and spore population in soil samples from the rhizosphere of several medicinal plants in the Paithal Hills, Western Ghats of Kannur District, Kerala, India (Santhoshkumar, S., and Nagarajan, N. (2020). The Malvaceae plant *Sida acuta* (11%) had the lowest AM fungal infection rate, whereas the Euphorbiaceae plant *Euphorbia hirta* (78%) had the highest. The plant species with AM infection is *Azadirachta indica* (25%) infected. Meliaceae with *Acalypha indica* (38%) Euphorbiaceae, *Alternanthera sessilis* 33% (Amaranthaceae), *Bauhinia purpurea* 25% (Caesalpiniaceae), *Eclipta prostrate* 26% (Asteraceae), *Ficus bengnalensis* 22% (Moraceae), *Impatiens balsamina* 35% (Balsaminaceae), *Ipomoea obscura* 27% (Convolvulaceae), *Mimosa pudica* 34% (Mimoceaese), *Mangifera indica* 37% (Anacardiaceae), *Phyllanthus amarus* 32% (Phyllanthceae), *Tridax procumbens* 38%.

Another study conducted in Bangladesh by Islam et al., 2022, revealed that the prevalence of mycorhiza in each family varied from plant to plant. The Asteraceae plant *Xanthium strumarium* has the highest root colonisation (981.0%). *Rorippa nasturtium, Brassica oleracla var. botrytis* (Brasicaceae), *Punica granatum* (Lythraceae), *Tecoma capensis* (Bignoniacea), *Spinacia oleracia* (Chenopodiaceae), and *Chenopodium album* (Goosefoot) were among the plants whose roots did not contain mycorrhiza. The results of the soil investigation show that the rhizospheric soils lacked nutrients that would be conducive to mycorrhizal symbiosis with plants.

The major plant and grass species in the Rajshahi BCSIR forest of Bangladesh were examined for colonisation status and rhizosphere soil characteristics by (Halder et al., 2016). Six dominating plants, including *Psidium guajava*, *Swietenia mahagoni, Artocarpus heterophyllus*, *Manihot esculenta, Acalypha indica*, and *Fragaria ananassa*, as well as two grass species, *Digitaria sanguinalis* and *Cynodon dactylon*, had their roots sampled, and soil samples were taken from the rhizosphere zone. *Manihot esculenta* (85%) and *Swietenia mahagoni* (78.1036%) had the highest colonisation rates, while Acalypha indica (2.19%) had the lowest rates. *Psidium guajava*, *Swietenia mahagoni*, and *Manihot esculenta* were among the investigated species that have mycorrhizal structure, such as arbuscles and vesicles.

The arbuscular mycorrhizal association of 47 angiosperms from the defunct limestone mine in Coimbatore, India, was examined a study by Logaprabha and Tamilselvi (2015.) Only *Achyranthes aspera, Aerva lanata, Amaranthus viridis, Alternanthera pungens, Carex speciosa,* and *Argemone mexicana* (Papavaraceae) (out of a total of 41 plants) demonstrated mycorrhizal connection. *Boerhaavia diffusa, Commelina bhengalensis,* and *Trianthema portulacastrum* are a few common non-mycorrhizal plants that have been linked to the AM fungus. The calcareous, sandy loam soils in the study area had a pH that was relatively alkaline and few readily available nutrients. There were found to be ten AMF spores in all, including species from the genera Acaulospora, Gigaspora, and Glomus. Glomus was the most frequent genus in the study region.

In the district of Kasur, a thorough examination into the connection between Curcuma longa weeds and vesicular arbuscular mycorrhizal fungus (VAM) has been conducted. The arbuscular mycorrhizal associations of 14 weed species—including *Sonchus aspera L., Chenopodium album L., Rumex dentatus L., Ageratum conyzoides L., Convolvulus arvensis L., Cynodon dactylon Pers., Oxalis corniculata L., Malva parviflora L., and Malvastrum coromandelianum* L. were observed. The infection affected *S. aspera* L. the most (81.2%), followed by *C.*

dactylon (70.1%), *O. corniculata* (69.3%), *M. Coromandelianum* (68.2%), and *P. minor* (66.5%). However, *T. resupinatum* (7.3%) and *Ageratum conyzoides* L. (6.5%) were not particularly well colonised (Tahira et al., 2013).

Twelve medicinal plants from eight different families were used to examine the prevalence of AMF colonisation(Thapa et al., 2015). The plant roots and related rhizospheric soil for the AMF analysis and spore assessment per 100 g of soil were donated by the University of North Bengal's Garden of Medicinal Plants. According to the findings, there were differences in AMF colonisation and spore percentage. The presence of AMF was found in every type of medicinal plant that was investigated. The highest colonisation rate was seen in *Justicia adhatoda* (952.00).

The primary factor in how medicinal plants adapt to their surroundings is mycorhizal fungus. Studies have shown that AM fungus helped medicinal plants grow and absorb nutrients, which accelerated the buildup of active compounds and improved resilience to abiotic challenges like drought, low temperature, and salinity. Rui-Ting et al (2021) observed that *Piriformospora indica,* an AM-like fungus that can be grown in a lab without using roots, has previously demonstrated effects on medicinal plants that are comparable to those of AM fungi. These fungi offer fresh mechanistic routes to the synthetic production of therapeutic plants packed with components that are in high demand on the global market. This paper gives a general overview of the variety of AM fungi that live in the rhizosphere of medicinal plants, analyses how they work, and discusses potential future areas of research using *P. indica.*

SIGNIFICANCE

Medicinal plants have been used in medicine since the dawn of mankind. Research has been conducted worldwide to support their efficacy, and some of the findings have prompted the creation of plant-based medicines. Studies have shown that AM fungus helped medicinal plants grow and absorb nutrients, which accelerated the buildup of active compounds and improved resilience to abiotic challenges like drought, low temperature, and salinity. These fungi offer fresh mechanical routes for the artificial production of therapeutic plants that are rich in components.

The current study examines the relationship between mycorrhiza and numerous medicinal plants growing in distinct Ernakulam district soils. The research aids in gaining a fundamental understanding of mychorrhizal interaction and how it affects different medicinal plants. Additionally, assist in gathering knowledge about the therapeutic plant's qualities.

OBJECTIVES

1. Taxonomic identification of plants used in the study of mycorrhiza
2. Study the root colonization of AM fungi associated with the plants collected from three different sites.
3. Isolation and identification of AM fungal spores from the rhizosphere soil

MATERIALS AND METHODS

3.1 Study area and plant materials

The study was carried out in Aluva, Tripunithura and Pallikara which are places located in Ernakulam district. The plants were collected from the different locations of these places . The soil type varies with respect to places. Mainly alluvial soil, black cotton soil and laterite soil is seen in these places. These are urban areas with a temperature range from 27°-30°c. The humidity ranges from 70- 80%. The place receives rainfall of 2882 mm. The pH of the soil may varies according to the different types of soil. The plants that are used of this study were belongs to different families. They are *Euphorbia hirta, Acalypha indica, Phyllanthus niruri, Ricinus communis, Euphorbia maculata* plants belongs to Euphorbiaceae family, *Spermacoci exilis* and *Acmella cilia* members of the Asteraceae family and the *Spermacoci exilis* member of Rubiaceae

these are the plant that is used in the study of mycorrhizal association in the plants. All the plants that are collected for this study are the weed plant that is mostly grown along with any other plants. Also, they have high medicinal values

(Table 1).

Table.1 The common names, locations and medicinal uses of plants collected

Sl. No	Vernacular names	Location	Medicinal use
1	Chithirappala	Pallikkara	Female diseases, childhood worm infestations, respiratory conditions (cough, bronchitis, and asthma), diarrhoea, digestive issues, jaundice, and tumours are all treated with *euphorbia hirta.*
2	Keezharnelli	Tripunithura, Mulanthuruthy , Puthiyakavu	internal use for jaundice, gonorrhea, frequent menstruation, and diabetes and topical use as a poultice for skin ulcers, sores, swelling, and itchiness.

3	Kuppameni	Aluva ,BMC campus , Neduvannoor	It has the capability to serve as anthelmintic, anti-inflammation, antibacterial, anti-cancer, anti-diabetes, anti-
			hyperlipidemic, anti-obesity, anti-venom, hepatoprotective, hypoxia, and wound healing medicine.
4	Aavanakku	Pukkattupadi,Edathala,Ashokapuram	It is a traditional treatment for arthritis, back pain, muscle aches, bilharziasis, chronic back and sciatic pain, chronic headaches, constipation, placenta evacuation, gallbladder discomfort, menstrual cramps, rheumatism, insomnia, and sleeplessness.
5	Spotted spurge	Aluva ,BMC campus , Neduvannoor	It is an annual herb that is commonly used for the treatment of diarrhea, hemoptysis, hematuria, and sore swollen .
6	Tooth ache plant	Aluva, Pallikkara, Tripunithura	The flower heads are often used to cure toothaches, throat infections, and gum disease. Fresh or dried flowers are also frequently used to assist relieve tooth discomfort.Additionally, the leaves can be utilised to treat bacterial and fungus skin conditions..
7	False buttonweed	Aluva,Pallikkara,Tripunithura	The treatment of malaria, boils and other skin diseases.
8	Kayyonni	Aluva,Pallikkara,Tripunithura	It is frequently used to treat liver issues, fever, gastrointestinal problems, respiratory problems (including asthma), and respiratory tract problems.

3.2 Field Sampling:

Various locations of Ernakulam district were sampled for soil, plant roots, and the rhizosphere between the months of February and March 2023. Samples of each plant and soil were taken in order to further process and isolate AM spores and study the colonisation of mycorrhizal roots.

3.3 Soil pH analysis

The pH is a negative logarithm of the amount of active hydrogen ions (H+) in the soil solution. Because soil pH affects the availability of nutrients to the crop, it is a fairly straightforward but crucial measurement for soils. The microbial population in the soil is also impacted. In the pH range of 5.5 to 6.5, the majority of the nutrients can be found. The pH was determined (soil-water suspensions) with the help of a pH meter.

3.4 Isolation and identification of AMF

The isolation and identification of spores were done by plate count method: was first used by Mosse and Bowen (1968).10 grams of soil was added to 90 ml of distilled water and 10 ml was promptly transferred onto a filter paper by using a pipette. The filter paper was viewed under a dissecting microscope. Morphological identification of spores up to genus level was based on spore size, color, the thickness of the wall layers, and the subtending hyphae by the identification manual [http://schuessler.userweb.mwn.de/amphylo] and the website of the international collection of vesicular and AM fungi (http://invam.wvu.edu).

3.5 Assessment of root colonization

Fixed root pieces were washed with distilled water. After that, root pieces were selected and cut into small segments (about 1 cm). Root segments were put in a beaker containing enough 10% KOH solution, covered, and heated at 90˚C in the water bath for 2 hrs. KOH was poured off and washed with distilled water three times. Roots were acidified with 1% HCl for 3 min. Root pieces were stained with trypan blue solution for 15–20 minutes and subsequently, the root was de-stained at room temperature in glycerol/ water. After destaining, these rootsegments were examined under the microscope to observe mycorrhizal root colonization. The extent of VA mycorrhizal colonization was estimated by the percentage of root length colonization examined for each sample at least 10 root segments and calculated by the following formula (Phelps and Hayman, 1970).

$$\text{Root colonization (\%)} = \frac{\text{No: of roots with AM}}{\text{Total No: of roots studied}} \times 100$$

3.6 Morphological Identification of AMF Spores : The identification of arbuscular mycorrhizal fungi is carried out based on the similarity of spore morphological characteristics including color, spore shape, size and ornament, The stages of AMF identification are as follows:

1. Color of spores: using the standard color chart that is commonly used. The colors of mycorrhizal spores range from hyaline yellow, greenish yellow, brown, reddish brown to blackish brown.
2. Spore shape: in general, the shape of the spores is globe, sub globose, oval.
3. Spore size, Has ornament and spore contents such as bulbous, spines, fine threads such as hair, spines, and others.

Identification of AMF which has abundant spores and is found in the rhizosphere of all observed horticultural plants is as follows:

Glomus sp. The spores found were round to oval in shape, the color of the spores ranged from light yellow, brown to reddish brown and shiny black. Spore walls are slippery. Pass the 125 µm and 250 µm sieve . The genus forms long infection units with "H" connections between parallel strands, intraradical vesicles, and arbuscules that stain dark with Trypan blue. Phylogenetically, the genus is several times polyphyletic.

3.7 Data Analysis

The data were analysed using various statistical tools, Mean and standard deviation were calculated using an online tool calculator.net.

RESULT

Eight common medicinal plant species from three different families were examined in order to determine the mycorrhizal relationship. Two plants are Asteraceae, two plants are Euphorbiaceae, and one plant is from Rubiaceae.

4.1 Taxonomic Identification of Collected Medicinal Plants

The family, genus name and species name of collected medicinal plants (Figure1) were identified and recorded based on morphological observation. The detailed description is as follows.

1. *Euphorbia hirta*

Local name: Asthma-plant

Family: Euphorbiaceae

Parts utilized: leaves, stem and roots

Description: Herbs that are 20–35 cm tall, upright, and pubescent. Petiole up to 3 mm long, leaves decussate, 1-2.5 x 0.7-1.5 cm, broadly oblong to ellipticlanceolate, base obliquely truncate, border serrulate, apex acute, hispid on both sides. Cyathia formed axillary clusters that were either solitary or double. Minute, around 1 mm long involucre with 5 crimson glands. 4-6 ebracteolate male flowers. Female florets are laterally pendulous and have twofid styles. pubescent, 1.5–2 mm-wide capsule. Seeds are tiny, scarlet, fourangled, and finely ridged.

2.Phyllanthus niruri

Common name- Gale of the wind

Family- Euphorbiaceae

Useful parts- stem and leaf of plant

Description: It is a small deciduous tree with a crooked trunk and spreading branches and leaves, closely set along the branchlets, light-green, glabrous, imbricate when young. Flowers are greenish yellow in axillary, fruit 1.3-1.6 cm, fleshy, glabrous and pale yellow. Its habitat is similar to the one of P. amarus . This plant is largely used for medicinal purposes in several countries.

4.Acalypha indica

Common name- Indian Mercury

Family- Euphorbiaceae

Useful parts- stem, leaf and flower of plant

Description: *Acalypha indica* is an annual herb. Stipules are triangular, the petiole is thin and 1.5–3.5 cm long, and the leaf blade is rhombic-ovate or subovate, membranous, (minute, long, soft, straight hairs along the veins, basecuneate, margin-serrate, and apex–acute. Flowers grow on upright, axillary spikes that are longer than the leaves but lack a stalk. Male flowers are little and dense in the distance. A noticeable semicupular leaf-like, serrated, green bract that is about 7mm long covers each female flower as it is dispersed along the inflorescence axis. Fruit is a capsule.

4. *Ricinus communis*

Common name- Castor oil plant

Family- Euphorbiaceae

Useful parts- leaves and root of the plant

Description : *Ricinus communis* is a sturdy shrub or small tree that can reach a height of 4 m and has a gently woody stem. The stems are round, smooth, and frequently crimson, and they have a clear sap. The leaves alternate and are plain. The centres of the leaf blades have long purple stalks attached to them. These are huge, star-shaped, and have finely toothed margins. They are shiny dark green or reddish in colour, with a paler underside, and when crushed, possess an odour. The flowers are arranged in terminal spike clusters with reddish female flowers (1–7) at the top and creamy male flowers (3-5) at the bottom of the inflorescence. The fruit is a big, oval, shiny, bean-like capsule with three lobes that is spiky, green to reddish purple in colour.

5 . *Euphorbia maculata*

Common name- Spotted spurge

Family- Euphorbiaceae

Useful parts- stem , leaf and root of the plant

Description : *C*an grow up to 30 centimetres in height. Rarely longer than 45 cm , the stems are arranged in a carpet along the ground. Up to 3 centimetres long and placed in opposite pairs, the leaves are oval but somewhat elongate. The cyathia, which are the only bisexual reproductive organs found in this genus of plants, are very small and have four white, occasionally pink, petal-like appendages. The leaves frequently have a scarlet spot in the centre, giving rise to the common name "spotted spurge."The leaves are stipulated, alternately borne, and occasionally opposite. Most of them have simple leaves, but some can have palmately complex leaves.

6. *Spermacoce exilis*

Common name-Pacific false buttonweed

Family- Rubiaceae

Useful parts- stem and leaves of the plant

Descripion: Annual herb up to 0.3 m tall with a creeping to slightly ascending growth habitat . Green, smooth leaves are ovate or elliptic to oblong with entire leaf margin, base cuneate, apex acute, glabrous, subsessile; stipule bristles subulate . White, short tubular flowers are arranged in a cluster between the uppermost pair of leaves. The petal-like lobes are triangular and about the same length as the tube. Bracts and bracteoles subulate. Calyx lobes 2, 1-2 mm long, lanceolate. Corolla white, c. 2 mm long. Capsule c. 1 mm long, truncate, compressed, pubescent. Seeds oblong, reticulate.

7 . *Eclipta alba*

Common name- False daisy (bhringraj)

Family- Asteraceae

Useful parts- roots and leaves of the plant

Description : It is an erect or prostrate, branched (occasionally rooting at nodes) annual herb upto 30-40 cm high.Stem is cylindrical or flat, rough due to appressed white hairs, nodes distinct and greenish occasionally brownish . Leaves are opposite, sessile to sub-sessile 2.0 to 6.2 cm long, 1.5-1.9 cm wide, oblong, lanceolate, sub-entire, acute to sub-acute and strigose with appressed hairs on both surfaces.Flowers are white, solitary or two on unequal axillary peduncles involucral bracts are about 8 in number, ovate, obtuse or acute and strigose with oppressed hairs.Disc flowers are tubular and corolla is often 4 toothed.Stamens are 5, filament epipetalous, free, anther united into a tube with base obtuse.Pistil is bicarpellary . Ovary is inferior and unilocular with one basal ovule.Fruit is achenial cypsela, one seeded, cuneate, with a narrow wing and brown in colour .

8 . Acmella ciliata

Common name- Fringed Pod Toothache Plant .

Family- Asteraceae

Useful parts - Flower and leaves of the plant

Description: Herbs, 30-80 cm tall and perennial. Green to purple stems that are typically decumbent to ascending and rooted at nodes. The leaf blade is broad to narrowly oval, 2.3-7.5 1-5.9 cm, glabrous to sparsely pilose on both surfaces, with a denticulate to coarsely dentate border and an acute apex. Decumbent position on the stem, capitulum radiating inflorescence. Flowers are either all yellow or all yellow and orange. The margin features fruits.

Figure 1. Plants collected and identified for study (a)*Euphorbia hirta* ,(b)*Phyllanthus niruri* ,(c) *Acalypha indica* ,(d) *Rucinus communis* ,(e) *Euphorbia maculata* ,(f) *Spermacoci exilis* ,(g) *Eclipta alba* , (h) *Acmella ciliata*

4.2 Morphological Identification of AMF Spores

The identification of arbuscular mycorrhizal fungi is carried out based on spore morphological characteristics; colour, pore shape, size and ornament. Three types of spores belonging to Glomus are observed during the study(Figure 2). The spores found were round to oval in shape, the colour of the spores ranged from light yellow, and brown to reddish brown and shiny black.

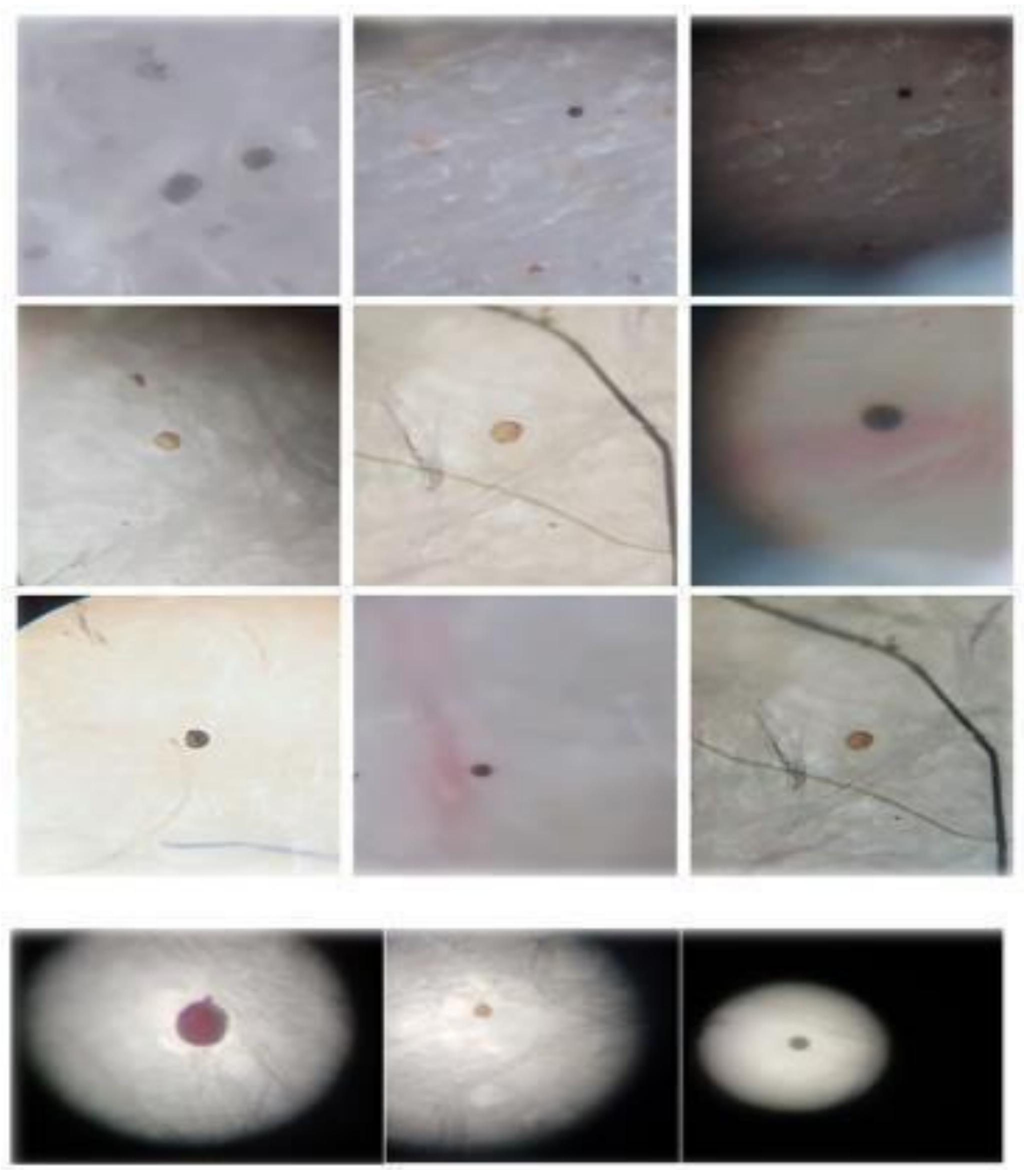


Figure 2. The different spores isolated from rhizosphere soil

4.2 Root colonization of AMF in plants

The presence of mycorrhizal fungus in plant roots has been detected using vesicles, arbuscular structures, and hyphal growth (Figure 3). Every plant showed signs of having VAM in its roots. The number and abundance of vesicles, arbuscules, and H hyphae varied amongst plants. Both *Arum*-type and *Paris* type of Arbuscular mycorrhiza were found in plant roots

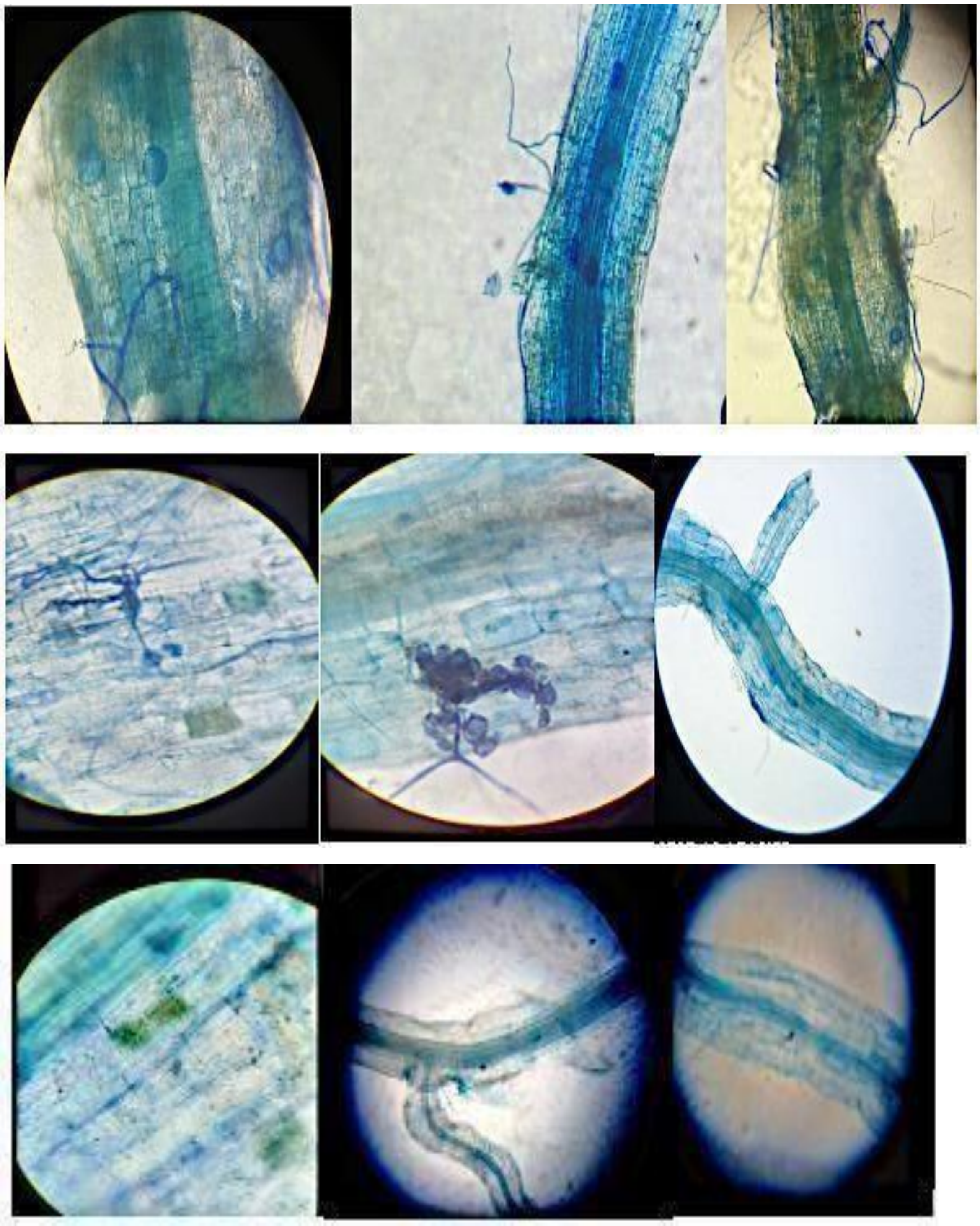


Figure 3. The diversity of AMF in the roots of medicinal plants

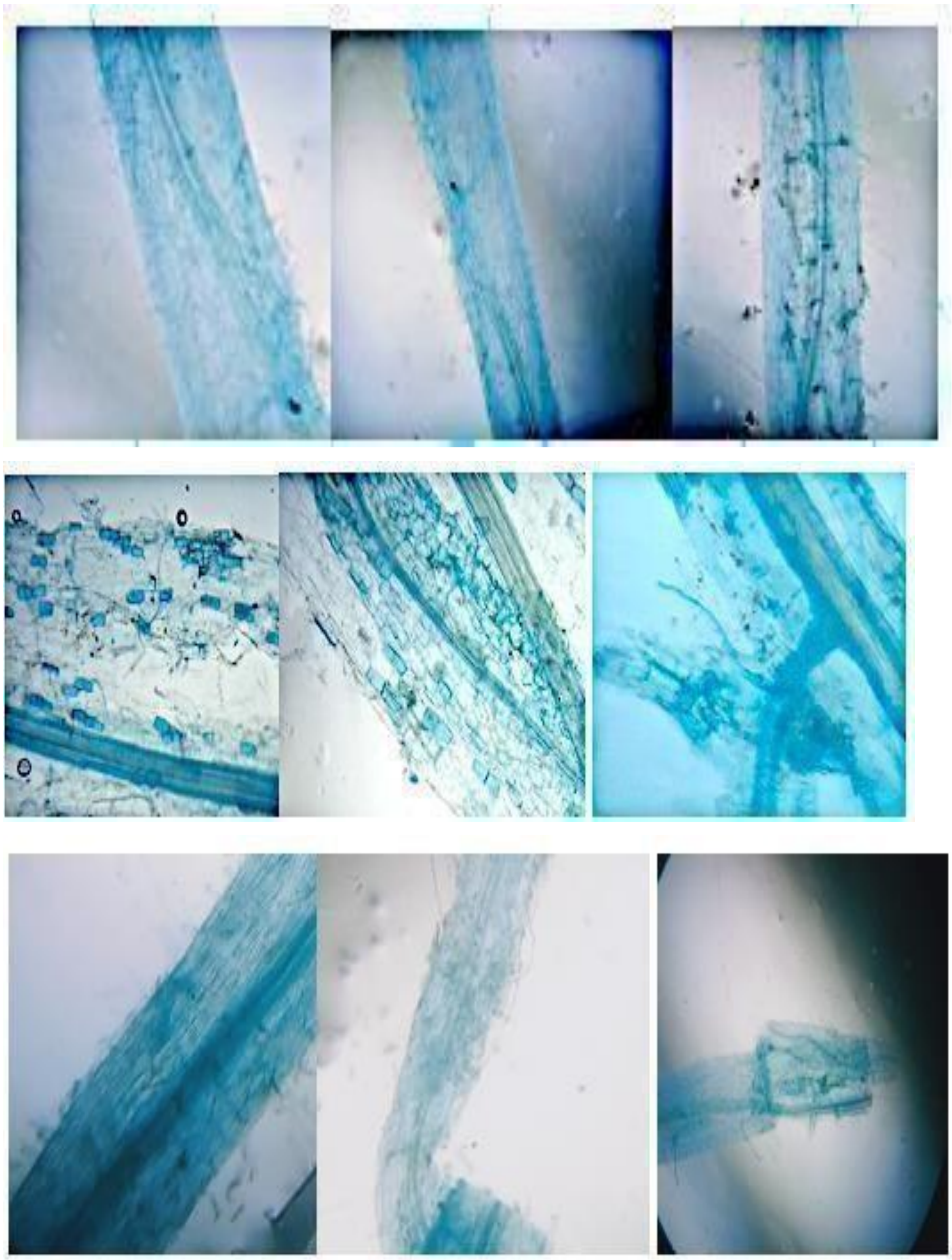
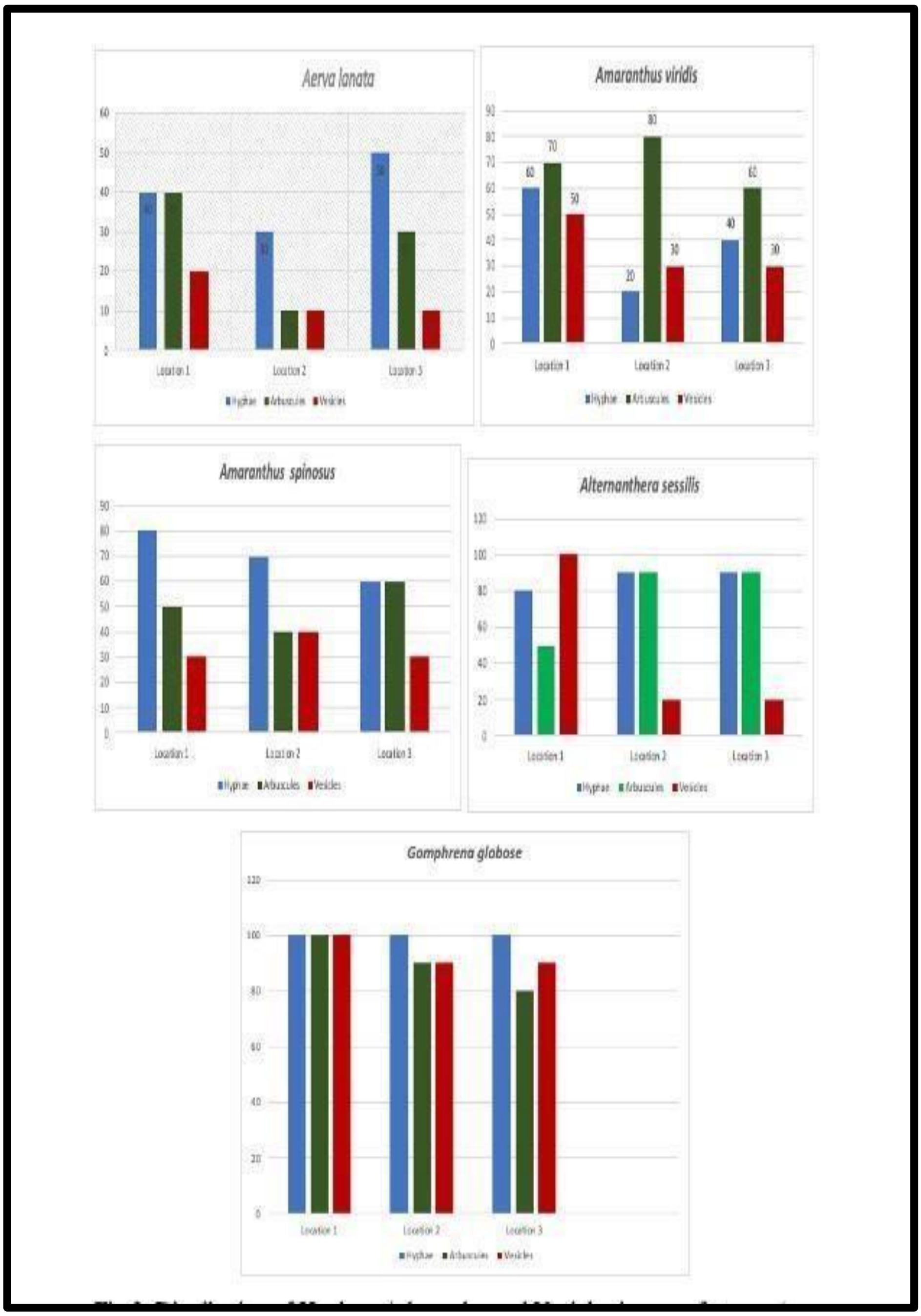


Figure 3. The diversity of AMF in the roots of medicinal plants



A maximum number of vesicles were observed in *Acmella ciliata* followed by *Euphorbia hirta* and *Eclipta alba* (Figure 4). The abundance of Arbusules was highest in both *Acalypha indica* a *Euphorbia macula* (Figure 5). *Euphorbia hirta* showed *the* hyphal distribution highest (Figure 6).

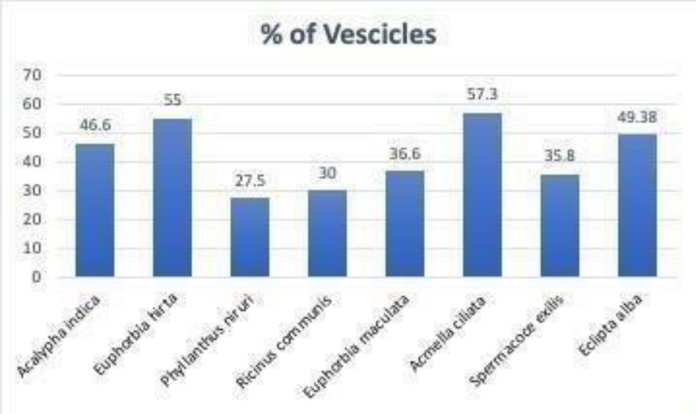


Figure 4 shows abundance of vesicles among studied plants(X axis=name of plants, Y axis=% of vesicles)

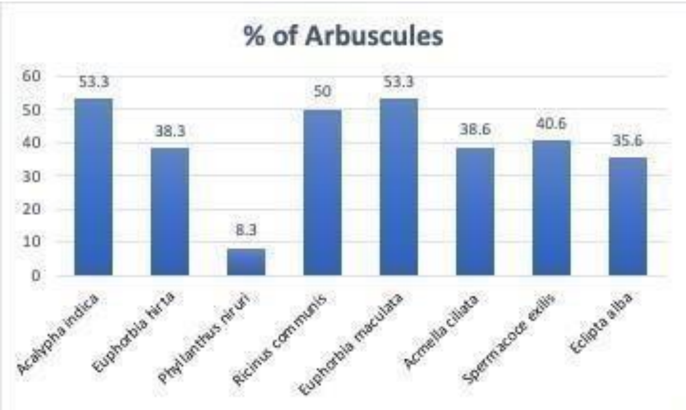


Figure 5 shows abundance of arbuscules among studied plants(X axis=name of plants, Y axis=% of arbuscules)

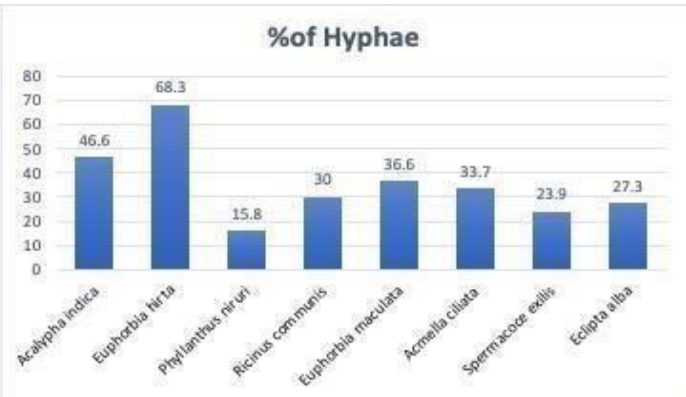


Fig 6 shows the abundance of H hyphae among studied plants(X axis=name of plants, Y axis=% of H hyphae)

The percentage of root colonization was compared among different plant species. Plants were found to be colonized with AMF, and the degree of percentage of colonization varied from 39.1 ±9.5 in *Ricinus communis* to 76.6 ±1.3 *Euphorbia hirta* Table 2, Figure 7.

Table 2 The diversity of AMF in different plants

Sl.No	Name of the Plant	Arbuscules (%)	vesicles (%)	H hyphae (%)	Percentage of Colonisation
1	*Acalypha indica*	53.3±2.7	46.6±2.7	46.6 ±5.4	76.6 ±7.2
2	*Euphorbia hirta*	38.3±5.9	55 ±8.4	68.3 ±7.5	76.6 ±1.3
3	*Phyllanthus niruri*	8.3 ±6.8	27.5 ±4.2	15.8±3.7	39.1 ±9.5
4	*Ricinus communis*	50 ±4.7	30 ±4.7	30 ±4.7	56.6 ±2.7
5	*Euphorbia maculata*	53.3 ±7.2	36.6 ±9.8	36.6 ±5.4	73.3 ±7.2
6	*Acmella ciliata*	38.6 ±5.0	57.3 ±5.9	33.7 ±4.1	66.8 ±2.1
7	*Spermacoce exilis*	40.6 ±7.9	35.8 ±10.8	23.9 ±7.7	52.3 ±1.9
8	*Eclipta alba*	35.6±8.9	49.38 ±8.6	27.3 ±0.9	65.4 ±6.3

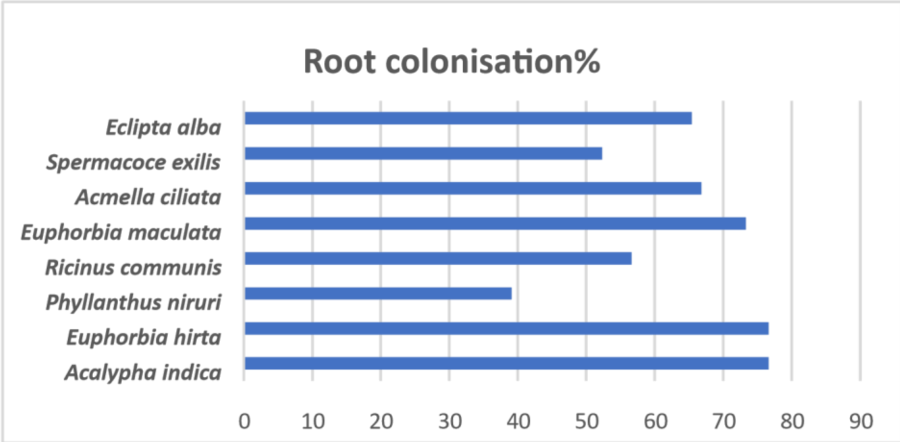


Figure 7. AMF Root colonisation percentage in plants

To check the influence of pH on root colonisation percentage, conduct a Pearson correlation coefficient analysis using the online tool of Social Science Statistics. The result showed a moderate positive correlation, which means there is a tendency for high X variable scores to go with high Y variable scores (and vice versa), ie. The root colonisation percentage is higher at higher pH (6.5)

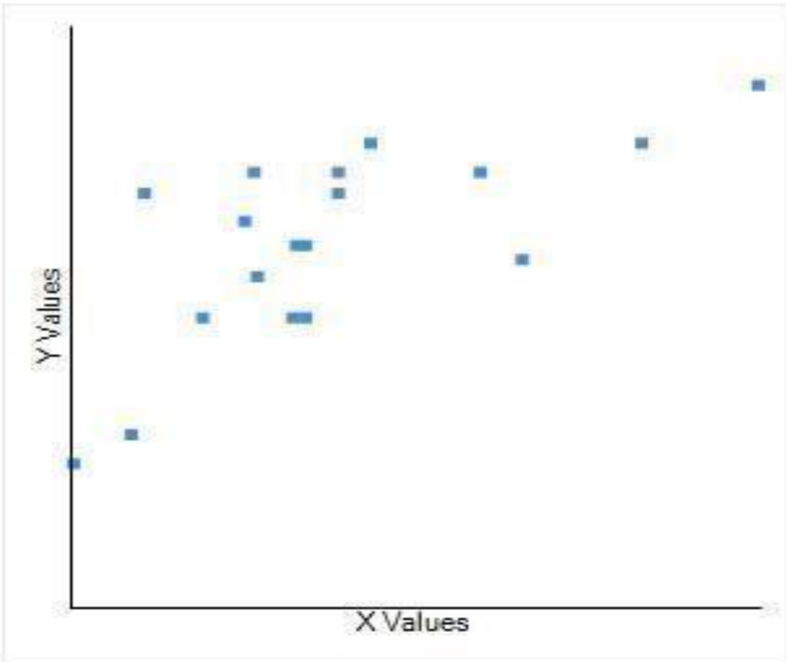


Figure 8. The correlation analysis of pH(X axis=) of soil and AMF root colonisation percentage (Y axis) .

DISCUSSION

Studies have shown that AM fungus supported the growth and nutrient uptake of medicinal plants, speeding the accumulation of active substances and enhancing resilience to abiotic challenges such salinity, low temperature, and drought. In the current experiment (Raei and Weisany, 2013). *Phyllanthus niruri* was the only plant to not show an average rate of (>60) AMF root invasion. All the other medicinal plants showed good amount of AMF colonisation. This may be due to different types of root flavonoids which increases their AMF associations and in turn their rapid growth.

The present study reveals two Glomus species. Similar findings are in conformity that the genus Glomus was predominance mycorrhizal species in the rhizosphere, as per a number of studies (Dhar and Mridha, 2006).

Examining the roots of the analysed plants revealed vesicles and arbuscles. The development, sporulation, and community organisation of AM can be influenced by a number of conditions.The root colonisation percentage is larger at higher pH, according to the results of the current investigation, which found a somewhat favourable link between pH and AMF root colonisation. According to Clark (1997), there is typically less root colonisation in low pH soils than in high pH soils.

CONCLUSION AND FUTURE SCOPE

The AMF species colonisation in eight medicinal plant species was investigated after their taxonomic identification from various locations of Ernakulam. They belong to three different families. From the present findings, it can conclude that root colonization and spore population was abundant in all the studied medicinal plant species, only one genus Glomus was dominant seen in all the rhizosphere soil. The colonisation of mycorrhizae in root varied according to locality and soil. There was a positive correlation between pH and root colonisation. The favourable pH showed maximum colonisation was around 6.5.

According to studies, mutualistic associations have a significant impact on medicinal plants by promoting plant development and enhancing the generation of secondary metabolites that are valuable for pharmacological uses.

Even though the mutualistic relationship between AMF was discovered in the current study, further research is necessary to understand how they improve secondary metabolites.

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