

**ASSESSMENT OF AMF ASSOCIATION AND ITS EFFECTS ON
AMARANTHACEAE PLANTS**

Project submitted

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Submitted by

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CERTIFICATE

This is to certify that this project work entitled “ **Assessment of *AMF* Association and its Effects on *Amaranthaceae* Plants**” is a bonafide piece of project work done by Hanza M. H (Reg.no: **200021023425**) 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

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DECLARATION

I hereby declare that this project entitled “**Assessment of *AMF* Association and its Effects on *Amaranthaceae* Plants**” 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.

HANZA M. H

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1.INTRODUCTION

1.1 Mycorrhizal fungi and types

The plant-fungal symbiosis that exists on earth is called arbuscular mycorrhiza. Mycorrhiza is a term used to describe the function of fungi in a plant's rhizosphere, or root system. It is crucial to soil biology, chemistry, and plant nutrition.

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).

Ectomycorrhizae and endomycorrhizae are the two primary forms of mycorrhizae. They are divided into groups based on where the fungi colonise the plants. Ectomycorrhiza, also known as EcM, typically forms linkages between Ascomycota, Basidiomycota, and Zygomycota-class fungi and woody plants (such as beech, birch, willow, oak, pine, fir, and spruce). Ectomycorrhizas are made up of a mantle or hyphal sheath surrounding the root tip and a Hartig net of hyphae enclosing the plant cells in the root cortex (Martin et al., 2016).

More than 80% of plant groups include endomycorrhizae, which includes greenhouse and crop plants like fruits, flowers, grasses, and vegetables. Endomycorrhizal connections are characterised by the fungus' creation of vesicles and arbuscules as well as their penetration of the cortical cells. Endomycorrhizae come in a variety of forms, including as arbuscular, arbutoid, ericoid, monotropoid, and orchid mycorrhizas.

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. 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.

1.2 The beneficial effects of Arbuscular mycorrhiza to plants

Symbiosis with arbuscular mycorrhizal fungi has a variety of consequences on plants. Due to their greater growth, immunity, and resistance (Ghorsli 2002; Jeffries et al., 2003; Garg and Chandel, 2011; Smith and Smith, 2012; Begum et al., 2019), they are better able to withstand drought, and flooding, soil salinization, and heavy metal contamination. Mycorrhizal fungi improve water absorption and mineral nutrient delivery. Plants can obtain a wider range of nutrients because to the extensive network of extraradical hyphae that arbuscular mycorrhizal fungus create. As a result, plants colonised by AMF often have higher nutritional statuses (P, N, and other nutrients), which increases plant biomass. Plant exudation patterns may change when AMF have colonised a plant, which may have an impact on the macrofaunal and microbial populations in the rhizosphere. Glomalin, a glycoprotein that is released by the extra radicle hyphae of endomycorrhizae, aggregates soil particles, increasing the amount of water-stable aggregates and enhancing soil structure. Mycorrhizae are beneficial for the plant's defence against bacterial and fungal infection. This could be as a result of the plant receiving more nutrition, which has improved its health and strengthened its resistance to the intruder. The disease resistance of mycorrhizal plants is typically higher, particularly against microbial soil-borne diseases. They are also resistant to water stress, heavy metals, and salt. Fixation of ambient CO₂ by host plants may be impacted by mycorrhizal fungus by increasing the "sink effect" and moving photo assimilates 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)

1.4 Non mycorrhizal (NM) plant groups

All vascular plant species, including the majority of crops, make up about 29% of the population but do not support AM symbiosis. Thought to be non-host plants, however, can occasionally be colonised by AM fungus and exhibit rudimentary AM (RAM) traits. Proteaceae, Amaranthaceae, Chenopodiaceae, Carophyllaceae, Brassicaceae and some genera in the families of Fabaceae and Cactaceae, as well as a number of significant agricultural crops and weeds, are generally regarded as NM plants. The knowledge on the inoculation of AMF in Amaranthaceae plants is less.

SIGNIFICANCE

Weeds are a significant component of agroecosystems' interactions with arbuscular-mycorrhizal fungus (AMF). The character of weed communities in agroecosystems may be affected by AMF in a number of ways, including by altering the relative abundance of mycotrophic weed species (AMF hosts) and non-mycotrophic species (AMF non-hosts). In the current study, a variety of weed plants were collected from several locations with different soil types in order to assess the Amaranthaceae family, which has been reported as non-mycorrhizal, for symbiotic association with mycorrhizae.

OBJECTIVES

1. Taxonomic identification of plants used for the study of mycorrhiza
2. Study the root colonization of AM fungi associated with the Amaranthaceae plants collected from different sites.
3. Root initiation effect of AMF in selected plants
- 4 Check the AMF-inoculated seed germination rates in a few Amaranthus plants

2. REVIEW OF LITERATURE

2.1 Occurrence of mycorrhizal fungi

In an arbuscular mycorrhiza, also known as an AMF, the symbiotic fungus penetrates the cortical cells of the roots of a vascular plant to create arbuscules. According to Nathalie Diagne et al. (2020), the majority of terrestrial plants form symbiotic relationships with arbuscular mycorrhizal fungus (AMF). By increasing the root area's capacity to absorb nutrients, these soil bacteria improve plant nutrient intake. To complete its life cycle, the symbiont is given plant sugars in exchange. AMF also aids in the resistance of plants to biotic and abiotic stressors, including salinity, drought, extremely high or low temperatures, heavy metals, illnesses, and infections.

Several workers have published excellent general reviews about occurrence and the distribution of arbuscular mycorrhizal fungi. The ubiquity of mycorrhizas in the British flora is illustrated by a research by Harrison M. J. (1997), which found that 100% of gymnosperms, 70% of pteridophytes, and 80% of angiosperm species were able to form mycorrhizal relationships. Mycorrhizal associations come in a variety of morphological and physiological forms, and their structures and functions are influenced by the symbionts that are present.

AMF has colonised more than 80% of the examined plants growing on mining sites, according to a study by Wang, F. (2017), and there are numerous AMF species and a significant AMF diversity present in different mining-impacted locations.

According to Oehl, et al. (2010), some AMF species had distinctive characteristics for particular soil types or particular land uses, and as a result, they each had their own niche. Soil type and land use intensity had a significant impact on AMF diversity and community composition. Some AM fungi, in contrast, may be referred to as "generalists" because they were found in all of the soil types examined, regardless of the level of land usage. Of the 61 AMF species that were observed, an estimated 53% could be categorised as "specialists" because they were (almost) exclusively found in particular soil types and/or under specific land use intensities. The remaining 28% appeared to be "generalists," and the remaining

19% could not be categorised. As well as having a significant impact on the composition of AMF communities, soil type and land use intensity.

The mycorrhizal status and vertical fungal distribution of the AMF spores found in the rhizospheric soil samples of four species of Chenopodiaceae at five different depths in two saline of central Argentina were initially reported by Becerra et al. (2014). Root colonisation was either low (0–50%), moderate, or absent. There were discovered 19 morphologically unique AMF species. Certain AMF species sporulated mostly in the deep soil layers depending on the host plant. Arbuscular mycorrhizal fungi are mostly moved by wind and water, soil shifting brought on by erosion, as well as by torrential rains and plant remains. All types of soil are colonised by endomycorrhizal fungus, and the type of soil affects both their abundance and the range of AMF species

2.2 Factors affecting AMF growth

According to Jamiokowska et al. (2018) Vascular plants depend on the soil-dwelling arbuscular mycorrhizal fungus. There are numerous advantages for all parties involved when arbuscular mycorrhizal fungus, plants, and soil microbes interact. The efficiency of mycorrhizal fungi is, however, influenced by both biotic and abiotic factors, including soil physico-chemical characteristics, water and biogenic element availability, agricultural techniques, and climatic circumstances. The species of arbuscular mycorrhizal fungus must first be adapted to the changing environment. The biological activity of the soil is greatly influenced by the structure and compactness of the soil. The mobility of ions in soil dilutions and their uptake by plants and soil microorganisms are significantly influenced by soil pH reaction.

According to a review by Entry et al. from 2002, arbuscular mycorrhizae form best at temperatures around 30°C for the majority of fungal host species. The temperatures influencing fungal germination, photosynthesis, and carbon transfer to roots determine the ideal range for the intact symbiosis.

The effects of inoculating different crops with different arbuscular mycorrhizal fungus (AMF) species in different types of soil were examined by Kim et al. (2017), who also chose AMF species that are appropriate for domestic, environmentally friendly farming. The AMF species utilised had different effects

on plants. While AMF had only marginal influence on the growth of red pepper and leek, *Scutellospora heterogama*, *Acaulospora longula*, and *Funneliformis mosseae* had a favorable impact on the host's growth in carrot. The inoculation with AMF stimulated the development of sorghum and carrot. The findings of this study reveal the nature of the interaction between soil, plants, and AMF; as a result, this study has significant ramifications for the use of AMF in environmentally friendly agriculture in the future.

2.3 AMF Diversity in plant families

A survey of the arbuscular mycorrhizal (AM) status of plants in the four vegetation types of forest, grassland, scrub, and cultivated land or plantation was conducted in the Western Ghats region of Southern India. 174 of the 329 analysed species were mycorrhizal. 81 species, including ones from numerous families thought to be non-mycorrhizal, such as Amaranthaceae, Capparaceae, Commelinaceae, Cyperaceae, and Portulacaceae, had AM association recorded for the first time (Muthukumar and Udaiyan, 2000).

Sanon, A. et al. (2009) did a study with the objective of determining whether the invasive plant *Amaranthus viridis* affected soil microbiological and chemical properties and to assess the impact of these modifications on native plant growth. The findings indicated that *A. viridis* invasion increased soil nutrient availability, bacterial population, and microbial activity. On the other hand, *A. viridis* invasion severely inhibited the growth of Acacia species as well as AM fungus and rhizobial development. Aside from inhibiting rhizobial growth, *Amaranthus viridis* aqueous extract also shown antibacterial activity.

Wang et al., 20 21 examined the diversity and AMF species composition of co-occurring mycotrophic and potential non-mycotrophic plants in two wetlands. According to the findings, putative non-mycotrophic plants frequently had AMF hyphae and vesicles but not arbuscules.

3. MATERIALS AND METHODS

3.1 Study area and plant materials collected

The study was done in the areas of Deshabhimani, Unichira, Aluva, Elamakkara, and Thrikkakkara in the Ernakulam district. The plants were gathered from various sections of these locations. The soil type includes loamy soil, dry soil, and fertile soil. Due to its proximity to the sea, Ernakulam experiences a tropical climate. Between 18.0°C in the winter and 36.°C in the summer, the average maximum temperature might vary. The region receives 2882 mm of rain annually. Soil pH varies based on the kind of soil. *Amaranthus spinous*, *Amaranthus viridis*, *Amaranthus dubius*, *Aerva lanata*, and other *Amaranthaceae* species were gathered over the summer and used in this study.

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 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

Isolation of AMF spores from rhizosphere soil followed the wet sieving and decanting method (Gerdemann and Nicolson 1963). Identification of AM fungal spores was done based on size, shape, color, wall structure following standard websites and taxonomic manuals (International Culture Collection of Vesicular-Arbuscular Mycorrhizal Fungi; <http://invam.caf.wvu.edu>), <http://www.amf-phylogeny.com>, <http://www.zor.zut.edu.pl/Glomeromyca>, Techniques in mycorrhizal research, VA Mycorrhiza and Handbook of AMF.

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.

3.5 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 Root initiation study

Three distinct plants from the Amaranthaceae family were chosen to grow on water mixed with VAM in order to study the impact of mycorrhizal fungi on root development. The chosen plants were *Amaranthus viridis*, *Alternanthera sessilis*, and *Aerva lanata*. Each of the three plants was given space to grow on 250 ml of water that contained 2.5g of VAM, the control was kept without VAM. Observations and data were gathered after one week by contrasting the number of roots, grown in VAM and in conditions without VAM.

3.7 Seed germination study

The seeds of various plants in the Amaranthaceae family were chosen and allowed to germinate in a germination tray in order to determine the mycorrhizal effect on germination. The experiment was conducted in the lab with six replicates. In a 50:50 mixture of soil and VAM, 10 seeds each of *Gomphrena globosa*, *Alternanthera bettzickiana*, and *Amaranthus dubius* were planted, and 10 seeds of each of the same plants were planted in soil without VAM. Every 10 days, observations and outcomes were recorded..

3.7 Data Analysis

Several statistical tools were used to assess the data, Graphs, tables and calculator.net was used to get the mean and standard deviation.

4. RESULTS

4.1 Taxonomic Identification of Plants

The following plants are collected and taxonomically identified noting the morphological characters and by local names (Figure 1).

1. *Aerva lanata*

Local name - Mountain knotgrass

Family - Amaranthaceae

Parts utilized - leaves, stem and roots.

Description: An annual with a branched, slightly woody root system. Most of the stems are drooping, spreading, and widely dispersed; they can grow up to 6 feet in length. The alternating, oval, and 0.5 to 1.5 in long sessile leaves. They develop from two-lobed, white papery stipules with red bases on the plant. The leaf axils are where the small clusters of two or three flowers grow. The pink, green, or lifeless white flowers are about 2.5 mm long.

2. *Amaranthus viridis*

Local name - green amaranth

Family - Amaranthaceae

Parts utilized - leaf, stem

Description: Annual plant, upright, light green stem has a height range of 60 to 80 cm. The leaves are oval in shape, approximately 3-6 cm long by 2-4 cm wide, with long petioles of around 5 cm, and several branches grow out from the base of the plant. The plant develops terminal panicles that have a few branches and tiny green blooms with three stamens.

2. *Amaranthus spinosus*

Local name - spiny amaranth

Family - Amaranthaceae

Parts utilized - leaves, stem

Description : *Amaranthus spinosus* is a 1.5 m tall, erect, multi-branched annual herb. The stem is cylindrical, tough, frequently reddish, and smooth. Simple, alternating leaves that are up to 9 cm long and have long petioles that are frequently diamond-shaped and lightly haired on the central veins below.

4. *Alternanthera sessilis*

Local name - sessile joyweed

Family - Amaranthaceae

Parts utilized - leaves and shoots.

Description : This herb is perennial and has prostrate stems that frequently root at the nodes and very rarely ascend. Petioles 1–5 mm long, leaves obovate to broadly elliptic, rarely linear-lanceolate, 1–15 cm long, 0.3–3 cm wide, glabrous to sparsely villous. Flowers are in sessile spikes, and the bract and bracteoles are shining white, 0.7-1.5 mm long, and glabrous. The sepals are equal in length, 2.5-3 mm, and the outer ones are 1-nerved or noticeably 3-nerved at the base. There are 5 stamens, 2 of which are sterile in the wild.

5. *Amaranthus dubius*

Local name - Red spinach

Family - Amaranthaceae

Parts utilized - Leaves and stem

Description : It grows usually to be 80–120 centimetres (31–47 inches) in size. It comes in both red and green variants, as well as others that have several colours. Erect annual plant that can grow to a height of 150 cm; stems that are thin to stout, branching, and either glabrous or, notably in the inflorescence, covered in small to somewhat long hairs. Petiole up to 8.5(-12) cm long; lamina oblong or rhomboid-ovate; leaves simple, spirally arranged, without stipules, axillary and terminal inflorescence, the terminal one up to 25 cm long, spikelike or paniculate Flowers are unisexual and subsessile.

6. *Alternanthera bettzickiana*

Local name - calico-plant

Family - Amaranthaceae

Parts utilized - Leaves

Description : The plant is frequently used as a decorative edging plant. Its cultivar "Red," which is native to South America, resembles several *Alternanthera dentata* and *Alternanthera brasiliana* types in appearance.

Despite typically being less than 1 metre tall as a cultivated plant, it is an upright, sprawling herbaceous plant that can grow up to 3 metres tall. Although they are beautifully hirsute when young, the plant's stems, which can be red, green, or purple, eventually become glabrescent.

7. *Gomphrena globosa*

Local name - Globe amaranth

Family - Amaranthaceae

Parts utilized - flowers, roots, stem and seeds

Description: An annual plant, globe amaranth grows to a height of 30-90 cm. Along the reddish stems, the leaves are placed in an opposing pattern and are plain with complete margins. The plant consistently produces thick, globular flower clusters on long stalks throughout the summer and autumn. Erect, robust, and branching stem covered in grey strigose hairs. Inconspicuous flowers are accompanied by papery, colourful bracts. Bracts are 3-5 mm long, elliptical with an acuminate apex, and they are available in white, pink, and violet colours.

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. The following two types of spores belonging to *Glomus sps* are observed during study (Figure 1).

Glomus sp. The spores found were round to oval in shape, the colour of the spores ranged from light yellow, brown to reddish brown and shiny black.

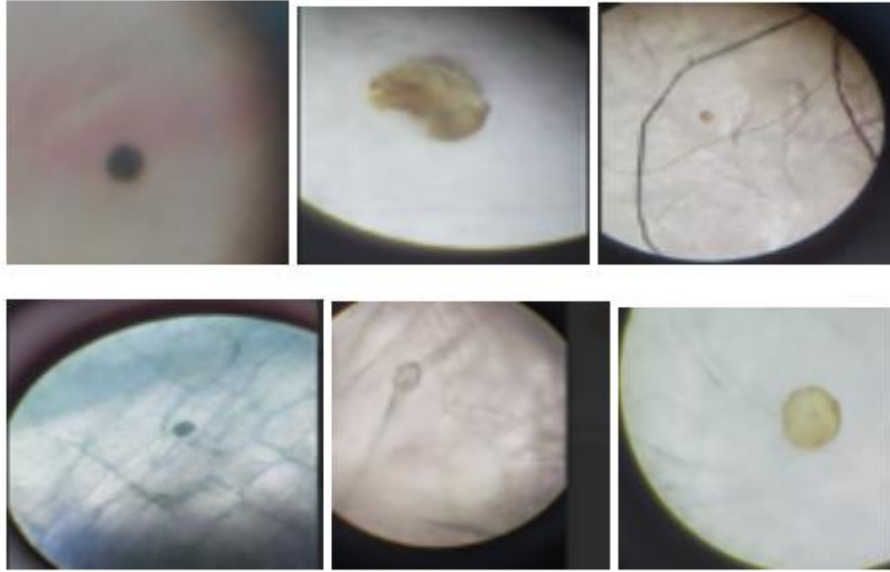


Fig. 1 Mycorrhizal spores observed during the study

4.3 Distribution and Root colonisation of AMF

A total 6 weedy species belonging to Amaranthaceae family were collected and examined from various fields during summer season for percent AM root colonization and spore identification. The percentage of root colonization was compared among different plant species in Amaranthaceae.

A microscopic examination of root segments indicated the presence of hyphae inside the cells. Plant species along with their AMF characterizations are presented in the Table 1.

Except for two species *Amaranthus dubius* and *Alternanthera betzickiana* of the family Amaranthaceae all others were having mycorrhizal associations. All the mycorrhizal species show hyphal presence in their roots. Maximum hyphae, Vesicles and arbuscules were observed in *Gomphrena globosa* followed by *Amaranthus spinosus* (Figure 1).

There was a great variation in the distribution of hyphae, arbuscules and vesicles between various locations. (Figure 2) *Aerva lanata* displayed poor mycorrhizal connection in roots below 60% colonisation in all three locations. For *Amaranthus viridis*, the vesicle were more than that of hyphae and arbuscules. Vesicles were comparatively less in all locations. In *Amaranthus spinosus*, more number of hyphae compared to arbuscules and vesicles. Comparing Location 1 to the other two locations, there was a significant difference in the number of vesicles in *Alternanthera*

sessilis. *Gomphrena glabose* had a nearly same distribution of hyphae, arbuscules, and vesicles. pH had no effect on colonisation rate

Table 1 Shows the Mean standard deviation of AMF and pH

Plant	Hyphae	Arbuscules	Vesicles	Ph
<i>Aerva Lanata</i>	40 ±4.7	26.6±7.2	13.3±2.7	4.63 ±0.07
<i>Amaranthus viridis</i>	43.3±11.8	70 ±4.7	36.6 ±7.2	6.61 ±0.06
<i>Amaranthus spinosus</i>	70 ±4.7	50 ±4.7	33.3±2.7	6.1 ±0.1
<i>Alternanthera sessilis</i>	86.6 ±2.7	90±0	73.3±21.7	6.29 ±0.06
<i>Amaranthus dubius</i>	0	0	0	5.5 ±0.07
<i>Alternanthera bettzickiana</i>	0	0	0	5.3±0.09

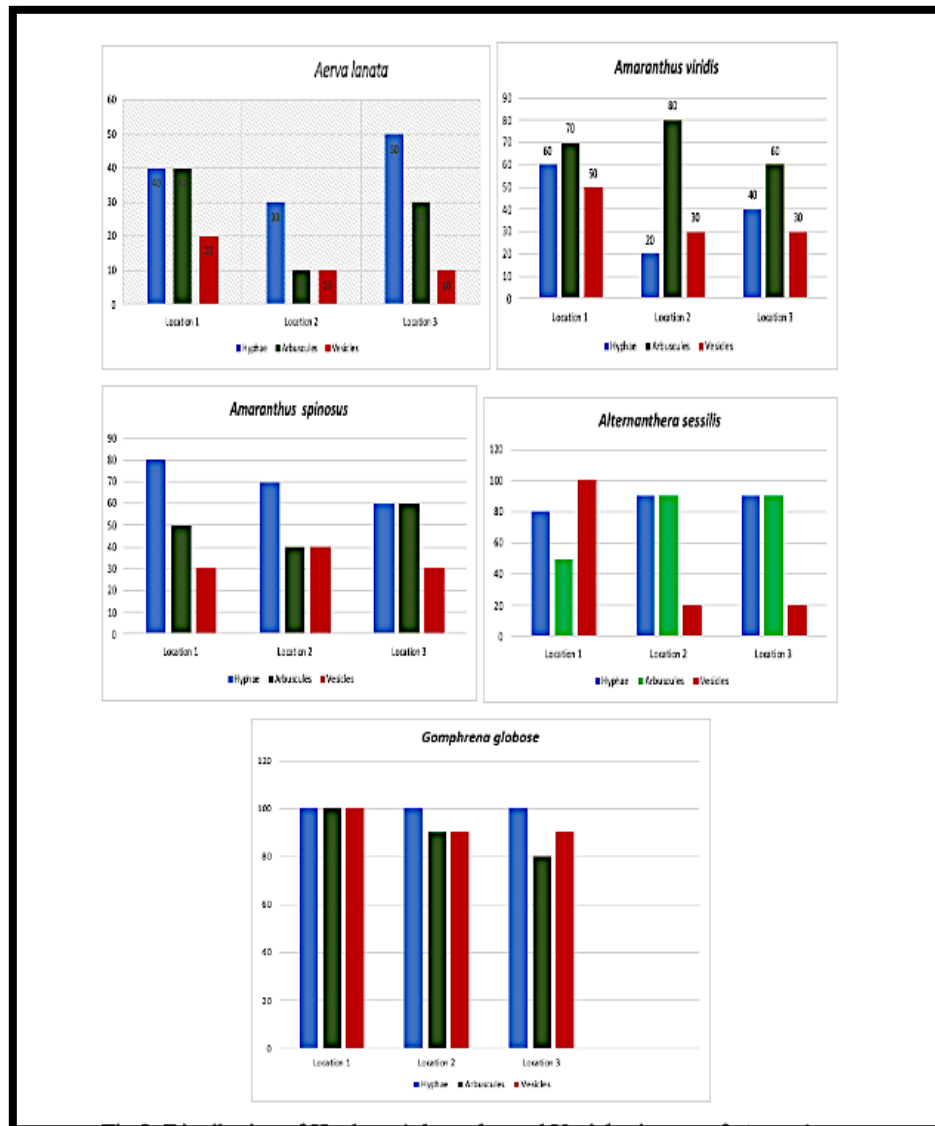


Fig 2. Distribution of Hyphae, Arbuscules and Vesicles in root of *Aerva lanata*, *Amaranthus viridis*, *Amaranthus spinosus*, *Alternanthera sessilis* and *Gomphrena globose* collected from various soils

The highest root colonization was observed in *Gomphrena globosa* (100%) followed by *Alternanthera sessilis* and lowest colonisation in *Aerva lanata*. *Amaranthus dubius* and *Alternanthera bettzickiana* did not show any AMF association.

Soil pH varied with locations and no distinct correlations were observed among the soil pH and root colonization and the number of vesicles, arbuscules etc.

Table 2 Root colonisation percentage of AMF

Plant	Rate of root colonisation
<i>Aerva Lanata</i>	43.3 ±2.7
<i>Amaranthus viridis</i>	76.6 ±7.2
<i>Amaranthus spinosus</i>	66.6 ±7.2
<i>Alternanthera sessilis</i>	93.3 ±5.4
<i>Amaranthus dubius</i>	0
<i>Alternanthera bettzickiana</i>	0
<i>Gomphrena globosa</i>	100

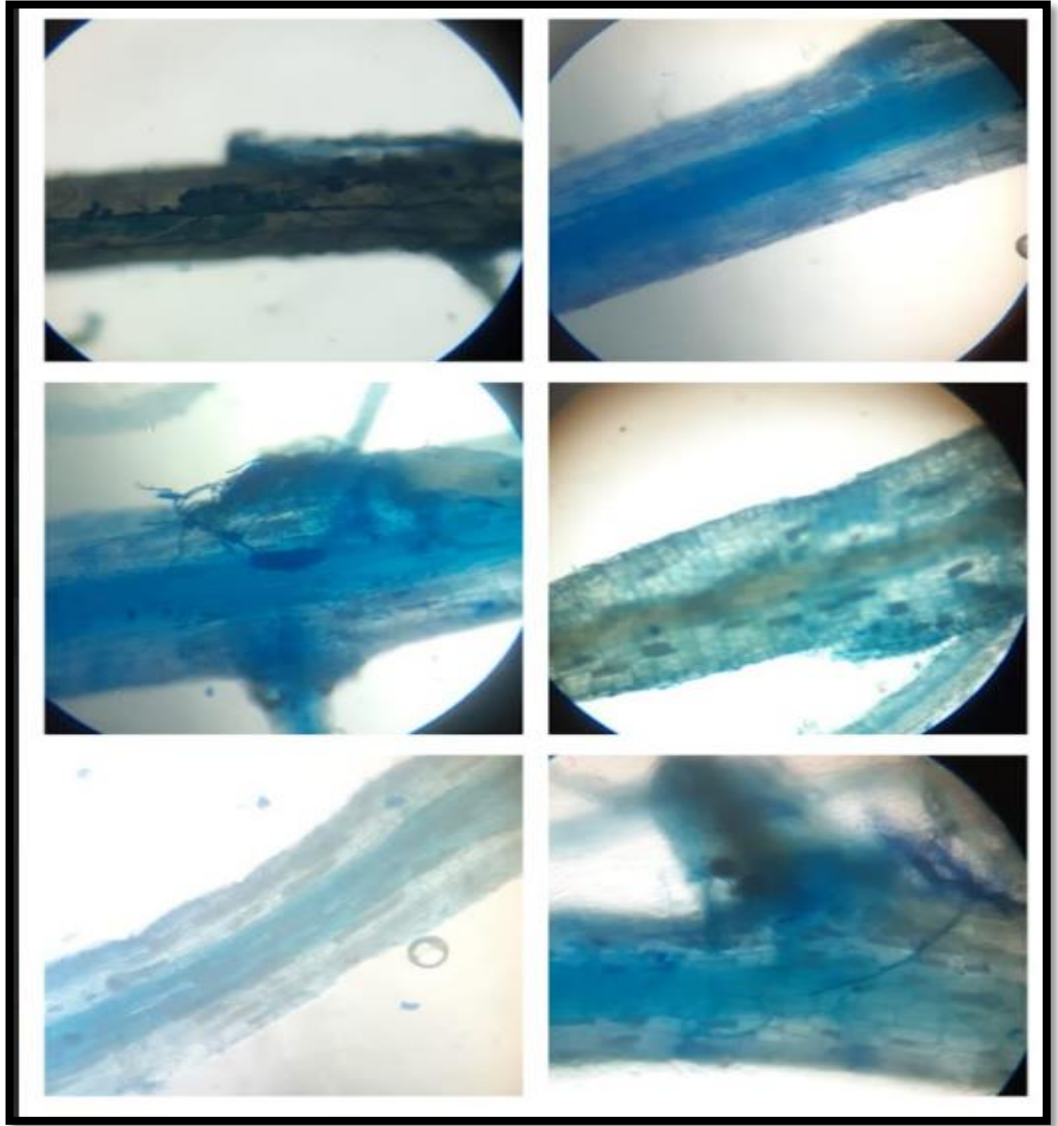


Fig: 3 Mycorrhizal association in roots of various plants studied

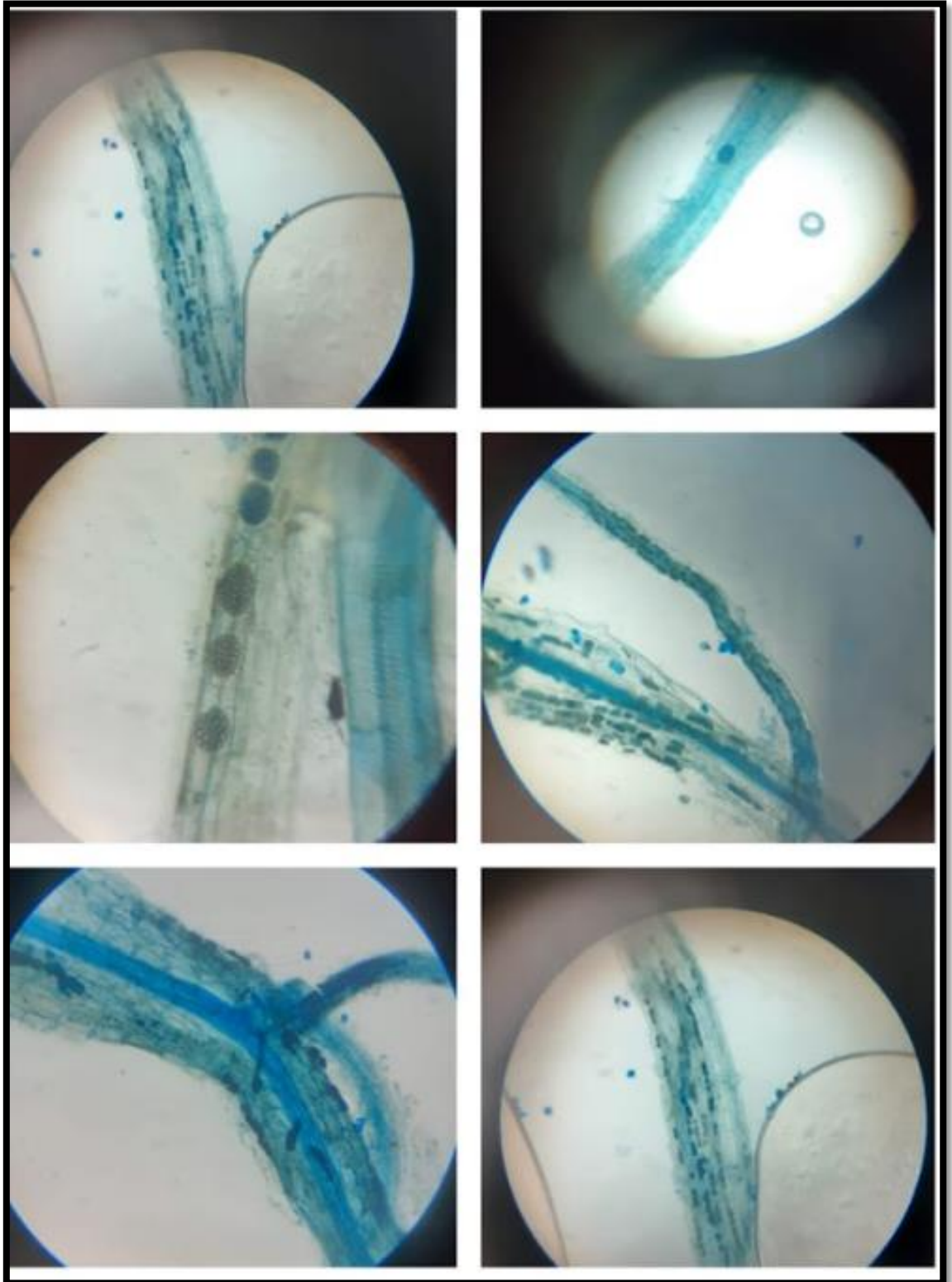


Fig: 3 Mycorrhizal association in roots of various plants studied

4.4. Root initiation study

Amaranthus viridis, *Alternanthera sessilis*, and *Aerva lanata* were chosen to grow on water mixed with VAM in order to study the impact of mycorrhizal fungi on root development. The Maximum number of roots were observed in plants kept with VAM, except in *Alternanthera sessilis*, same was the observation in root length (Table 3, Figure 4,5)

Table 3. The Mean Standard deviation of Root number and root length with and without VAM

Plant Name	Water + VAM		Water only	
	Root number	Root length (cm)	Root number	Root length
<i>Aerva lanata</i>	12.3 ±0.5	9 ±0.4	3 ±0.4	4.8±0.3
<i>Alternanthera sessilis</i>	16.2 ±0.3	7.3±0.7	40 ±0.9	8. ±0.7
<i>Amaranthus viridis</i>	29 ±0.4	7.6±0.9	1 ±0.4	1.1±0.5

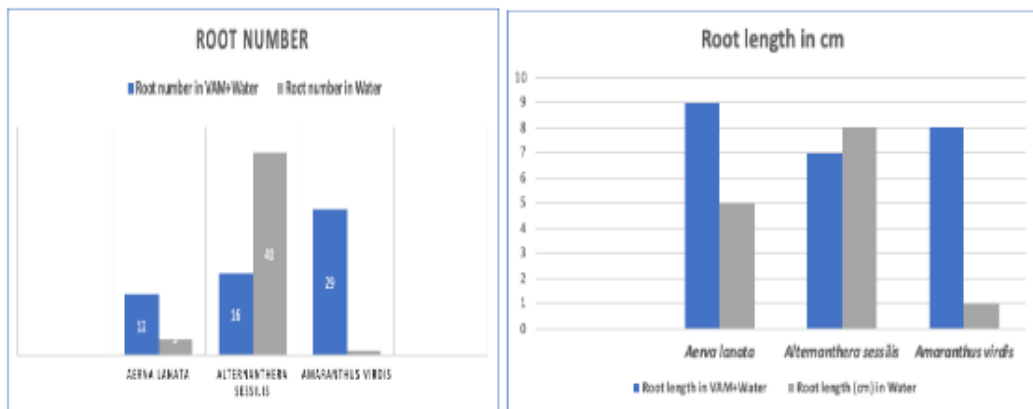


Fig 4 The comparison of Root number and root length with and without VAM



Aerva lanata- in water



Water+ VAM



Alternanthera sessilis - in water



Water+ VAM



Amaranthus viridis - in water



Water+ VAM

Fig 5 Photos of root initiation study in plants

4.5 Seed germination study

Seed germination study was conducted in three types of plants *Amaranthus dubius*, *Gomphrena globosa* and *Alternanthera bettzickiana* (Figure 6). Figure 11 shows the germination percentage of seeds (mean of 3) after 10 days. *Gomphrena globosa* has a slightly higher percentage of growth in VAM than without VAM. *Amaranthus dubius* and *Alternanthera bettzickiana* had less growth in VAM. But after 20 days more of *Gomphrena globosa* plants germinated without VAM. In *Amaranthus dubius* also more plants were germinated without VAM but in *Alternanthera bettzickiana* the germination rate was almost equal.

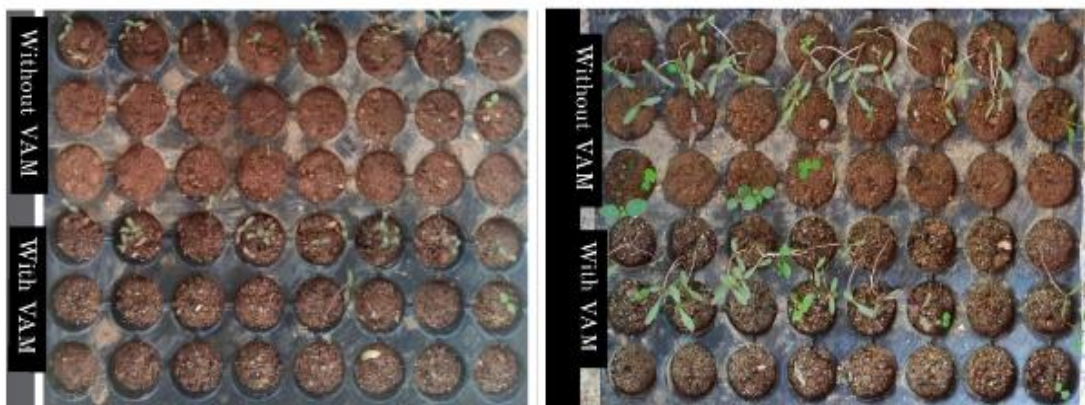


Fig: 6 Experimental study of seed germination

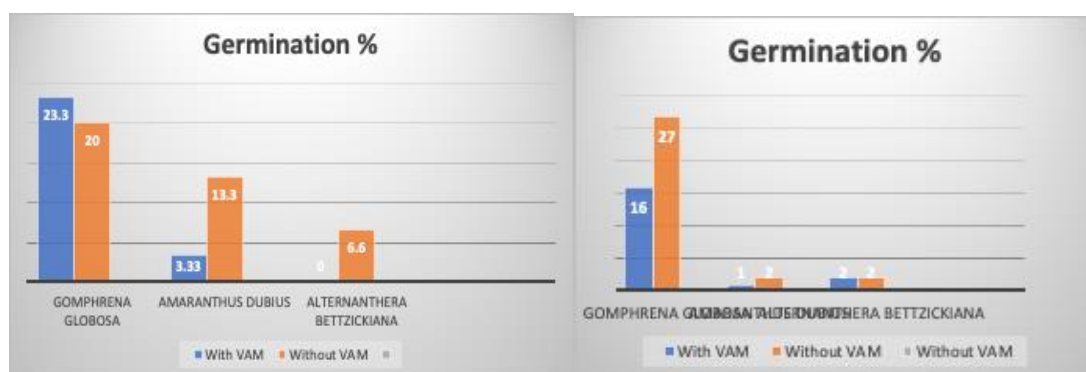


Fig 8 The germination percentage of seeds after 10 days and 20 days

5. DISCUSSION

More than 80% of the plants have arbuscular mycorrhizal fungus present. However, the majority of studies indicate that Amaranthaceae are not mycorrhizal. In the present study observed 8 plants of Amaranthaceae for AMF association, except for *Amaranthus dubius* and *Alternanthera bettzickiana* all others were having mycorrhizal association. Root colonisation found maximum in *Gomphrena globosa*, and many coiled running hyphae with appressorium were observed in *Amaranthus viridis* roots. Similar observations were seen in studies of Shwetha & Lakshman (2011). The present study observed maximum % of AMF in *Alternanthera sessilis* (93.3 ± 5.4). *Amaranthus spinosus* was observed 66.6 ± 7.2 %. But according to Niemira, B. A et al., (1996) % of AMF in *Amaranthus spinosus* was 5% and that of *Alternanthera sessilis* was 64%. This may be due to the influence of environment, soil condition and host cross-type.

The present study on seed germination, it was observed that there is no role for AMF in seed germination of plants in Amaranthaceae. The growth rate of *Gomphrena globosa* is somewhat higher with VAM than without VAM. Less growth was observed in VAM for *Amaranthus dubius* and *Alternanthera bettzickiana*. However, more *Gomphrena globosa* plants germinated without VAM after 20 days. The germination and early growth of weedy species can be significantly impacted by AMF, and some of these effects may indicate that AMF is acting in a parasitic or antagonistic manner towards plants (Jordan et al., 2000).

6. CONCLUSION AND SCOPE OF THE STUDY

Six weedy species belonging to the Amaranthaceae family were collected from various fields during the summer season and identified taxonomically. The roots were examined for per cent AM root colonization. The percentage of root colonization was compared among different plant species in Amaranthaceae. Maximum colonisation was observed in *Gomphrena* globose followed by *Alternanthera sessilis*, *Amaranthus viridis*, *Amaranthus spinosus* and *Aerva lanata*.

The influence of mycorrhizal fungi on root development was tested in *Amaranthus viridis*, *Alternanthera sessilis*, and *Aerva lanata* except for *Alternanthera sessilis*, plants maintained with VAM showed the maximum number of roots and root length.

According to the current study on seed germination, AMF plays no part in the seed germination of plants in the Amaranthaceae family.

Our findings suggest that not all Amaranthaceae species can be categorised as non-mycorrhizal, even if plants can grow without symbionts in some situations. The dynamics, variety, and productivity of plant communities can be impacted by AMF. This study suggest further studies on AMF effects on weed functional ecology.

REFERENCE

1. Becerra, A., Bartoloni, N., Cofré, N., Soteras, F., & Cabello, M. (2014). Arbuscular mycorrhizal fungi in saline soils: Vertical distribution at different soil depth. *Brazilian Journal of Microbiology*, 45, 585-594
2. Begum, N., Qin, C., Ahanger, M. A., Raza, S., Khan, M. I., Ashraf, M., ... & Zhang, L. (2019). Role of arbuscular mycorrhizal fungi in plant growth regulation: implications in abiotic stress tolerance. *Frontiers in plant science*, 10, 1068.
3. Begum, N., Qin, C., Ahanger, M. A., Raza, S., Khan, M. I., Ashraf, M., ... & Zhang, L. (2019). Role of arbuscular mycorrhizal fungi in plant growth regulation: implications in abiotic stress tolerance. *Frontiers in plant science*, 10, 1068.
4. Boddington, C. L., & Dodd, J. C. (2000). The effect of agricultural practices on the development of indigenous arbuscular mycorrhizal fungi. I. Field studies in an Indonesian ultisol. *Plant and Soil*, 218, 137-144.
5. Bowles, T. M., Barrios-Masias, F. H., Carlisle, E. A., Cavagnaro, T. R., & Jackson, L. E. (2016). Effects of arbuscular mycorrhizae on tomato yield, nutrient uptake, water relations, and soil carbon dynamics under deficit irrigation in field conditions. *Science of the Total Environment*, 566, 1223-1234.
6. Brundrett, M. C. (2009). Mycorrhizal associations and other means of nutrition of vascular plants: understanding the global diversity of host plants by resolving conflicting information and developing reliable means of diagnosis. *Plant and Soil*, 320, 37-77.
7. Entry, J. A., Rygielwicz, P. T., Watrud, L. S., & Donnelly, P. K. (2002). Influence of adverse soil conditions on the formation and function of arbuscular mycorrhizas. *Advances in Environmental Research*, 7(1), 123-138.
8. Garg, N., & Chandel, S. (2011). Arbuscular mycorrhizal networks: process and functions. *Sustainable Agriculture Volume 2*, 907-930.
9. Gorski, M. S. (2002). Studies on mycorrhizal association in some medicinal plants of Azad Jammu and Kashmir. *Asian J Plant Sci*, 1(4), 383-387.
10. Harrison, M. J. (1997). The arbuscular mycorrhizal symbiosis (pp. 1-34). Springer US.

11. Jamiołkowska, A., Księżniak, A., Gałązka, A., Hetman, B., Kopacki, M., & Skwaryło-Bednarz, B. (2018). Impact of abiotic factors on development of the community of arbuscular mycorrhizal fungi in the soil: a review. *International Agrophysics*, 32(1).
12. Jamiołkowska, A., Księżniak, A., Gałązka, A., Hetman, B., Kopacki, M., & Skwaryło-Bednarz, B. (2018). Impact of abiotic factors on development of the community of arbuscular mycorrhizal fungi in the soil: a review. *International Agrophysics*, 32(1).
13. Jeffries, P., Gianinazzi, S., Perotto, S., Turnau, K., & Barea, J. M. (2003). The contribution of arbuscular mycorrhizal fungi in sustainable maintenance of plant health and soil fertility. *Biology and fertility of soils*, 37, 1-16.
14. Jordan, N. R., Zhang, J., & Huerd, S. (2000). Arbuscular-mycorrhizal fungi: potential roles in weed management. *WEED RESEARCH-OXFORD-*, 40(5), 397-410.
15. Kim, S. J., Eo, J. K., Lee, E. H., Park, H., & Eom, A. H. (2017). Effects of arbuscular mycorrhizal fungi and soil conditions on crop plant growth. *Mycobiology*, 45(1), 20-24.
16. Lakshmiathy, R., Balakrishna, A. N., Bagyaraj, D. J., Sumana, D. A., & Kumar, D. P. (2004). Evaluation, grafting success and field establishment of cashew rootstock as influenced by VAM fungi.
17. Lee, E. H., Eo, J. K., Ka, K. H., & Eom, A. H. (2013). Diversity of arbuscular mycorrhizal fungi and their roles in ecosystems. *Mycobiology*, 41(3), 121-125.
18. Martin, F., Kohler, A., Murat, C., Veneault-Fourrey, C., & Hibbett, D. S. (2016). Unearthing the roots of ectomycorrhizal symbioses. *Nature Reviews Microbiology*, 14(12), 760-773.
19. Muthukumar, T., & Udaiyan, K. (2000). Arbuscular mycorrhizas of plants growing in the Western Ghats region, Southern India. *Mycorrhiza*, 9, 297-313.
20. Oehl, F., Laczko, E., Bogenrieder, A., Stahr, K., Bösch, R., van der Heijden, M., & Sieverding, E. (2010). Soil type and land use intensity determine the composition of arbuscular mycorrhizal fungal communities. *Soil Biology and Biochemistry*, 42(5), 724-738.
21. Phillips, J. M. and DS Hayman. 1970. *Improved procedures for clearing roots and staining parasitic and vesiculararbuscular mycorrhizal fungi for rapid assessment of infection. Trans. Br. Mycol. Soc*, 55, 158-161.

22. Quilambo, O. A. (2003). The vesicular-arbuscular mycorrhizal symbiosis. *African Journal of Biotechnology*, 2(12), 539-546.
23. Rajkumar, H. G., Seema, H. S., & Sunil Kumar, C. P. (2012). Diversity of arbuscular mycorrhizal fungi associated with some medicinal plants in Western Ghats of Karnataka region, India. *World journal of science and technology*, 2(1), 13-20.
24. Sadhana, B. (2014). Arbuscular Mycorrhizal Fungi (AMF) as a biofertilizer-a review. *Int. J. Curr. Microbiol. App. Sci*, 3(4), 384-400.
25. Shuab, R., Malla, N. A., Ahmad, J., Lone, R., & Koul, K. K. (2016). Arbuscular mycorrhizal fungal symbiosis with saffron (*Crocus sativus* L.) Plant. *J. New Biol. Rep*, 5(8).
26. Shwetha, C., & Lakshman, H. C. (2011). Association of arbuscular mycorrhizal fungi in some plants of amaranthaceae. *Karnataka Journal of Agricultural Sciences*, 24(3), 303-308.
27. Smith, S. E., & Smith, F. A. (2012). Fresh perspectives on the roles of arbuscular mycorrhizal fungi in plant nutrition and growth. *Mycologia*, 104(1), 1-13.
28. Wang, F. (2017). Occurrence of arbuscular mycorrhizal fungi in mining-impacted sites and their contribution to ecological restoration: Mechanisms and applications. *Critical Reviews in Environmental Science and Technology*, 47(20), 1901-1957.
29. Wang, Y., Li, Y., Li, S., & Rosendahl, S. (2021). Ignored diversity of arbuscular mycorrhizal fungi in co-occurring mycotrophic and non-mycotrophic plants. *Mycorrhiza*, 31, 93-102.