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Study on the diversity of Arbuscular Mycorrhizal (AM) Fungi in some medicinal plants of Meghalaya

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Abstract

Mycorrhiza is an intimate association between the branched, tubular filaments (also known as hyphae) of a fungus and the roots of higher plants. They are found in 80% of vascular plant species and also some bryophytes. AM fungi have gained significant attention in recent years because of their role in soil fertility, nutrient uptake, and bio-control of plant diseases and growth of plants. Meghalaya is home to very rich floral diversity because of its favourable climatic conditions, including the availability of a wide variety of medicinal and aromatic plants. We have studied the association of mycorrhiza in five medicinal plants *Aloe vera, Catharanthus roseus, Costus igneus, Prunella vulgaris* and *Ageratum conyzoids* and observed that AM spore population is estimated highest in the plant *Prunella vulgaris* (62, 50gm^{-1} soil) with highest root colonization (88%) followed by *Catharanthus roseus* (56, 50gm^{-1} soil) with root colonization (76%) *Ageratum conyzoides* (50, 50gm^{-1} soil) with root colonization (64%) *Costus igneus* (38, 50gm^{-1} soil) with root colonization (52%) and *Aloe barbadensis* (25, 50gm^{-1} soil) with root colonization (29%). *Gigaspora* is found to be dominant AM strain in all the selected medicinal plants.

Key words : Medicinal plants, diversity, Arbuscular Mycorrhizal (AM) fungi, root infection, *Gigaspora*.

Mycorrhiza is an intimate association between the branched, tubular filaments (also known as hyphae) of a fungus and the roots of higher plants. Mycorrhiza as a mutualistic symbiosis between plant and fungus localized in a root or root like structures in which energy moves primarily from plant to fungus and inorganic resources move from fungus to plant[3]. "Mycorrhiza" literally means "fungus root". They are found in 80% of vascular plant species and also some bryophytes[11]. AM (arbuscular mycorrhizal) fungi have gained

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significant attention in recent years because of their role in soil fertility, nutrient uptake, bio-control of plant diseases and growth of plants. The diversity and community composition of AMF in different ecosystems and plant communities in India have received increasing interest over the past few decades[17].

Vesicular Arbuscular Mycorrhizae (VAM) or Arbuscular Mycorrhizae (AM) are mutualistic symbioses formed between the roots of most plants and fungi in the order Glomales. Currently, Glomales are placed in class Zygomycetes. VAM or more commonly, AM are Endomycorrhizae, formed by non-septate zygomycetes of phycomycetous fungi in the roots of the members of most of the families of angiosperms as well as gymnosperms, pteridophytes and bryophytes[31].

In case of Arbuscular Mycorrhiza (AM), the major benefit is probably an enhanced of uptake of phosphorus from soil. Ectomycorrhiza and Ericoid mycorrhiza are also found to facilitate nutrient uptake. Mycorrhizal plants are more efficient in use of mineral nutrients, by making fuller use of soil mineral reserves. The arbuscules are the main transfer site of mineral nutrients from the fungus to the plant and of carbon compounds to the fungus. Mycorrhizal fungi provide protection to plants against the attack of soil borne plant pathogens and also against heavy metals[5, 14,16, 20].

Meghalaya is home to very rich floral diversity because of its favourable climatic conditions, including the availability of a wide variety of medicinal and aromatic plants. In different medicinal plants, the occurrence of AMF has been studied previously by many researchers[25, 30, 32]. Several studies show that AM fungi can improve water and nutrient uptake abilities[13,17,31,33,39]. AM also enhance the survival capacity of host plant[1,2,6,10]. AMF improve the quality of soil by influencing its structure and texture, and hence, plant health. The primary advantage of VAM fungus to plants is improved uptake of poorly mobile ions from the soil. According to a plausible theory, the exterior hyphae and plant roots create a framework for aggregation, while bacterial polysaccarides bind the soil particles together for the improved soil stabilisation caused by VAM fungus[35,36,41].

Morton and Benny[23] proposed two clades of AM fungi, one consisting of *Gigaspora* and *Scutellospora* species and the other consisting of *Glomus*, *Sclerocystis*, *Acaulospora* and *Enterophospora*. This strategy still needs to be refined, though. One of the main limitations when researching the taxonomy of AM fungi is the obligate biotrophic nature of these organisms. When AM fungi associate with living roots, they produce hyphae, arbuscules, vesicles, and spores both inside and outside the cortex of the roots. Instead of vesicles, fungi in the family Gigasporaceae AM generate auxiliary cells[8,19].

It has been demonstrated that arbuscular mycorrhizal (AM) fungi are essential for nitrogen cycling and can lessen nutrient losses following rain-induced leaching episodes. This was further supported by the discovery of spores from various VAM fungus species in the soils of the rhizosphere. The uptake of N, P, and, most noticeably, P were all greatly boosted by VAM injection[7,37]. Plants benefit from AM fungus because they help them produce

and endure in mineral-rich environ-ments. Therefore, under nutritional conditions, roots that colonised AM fungi may have a better uptake of immobile micro- and macronut-rients[4,9,18,21,26,27].

Collection site :

The composite samples were collected from various parts of Meghalaya – Baridua (26.10125° N, 91.84647° E), Cherrapunji (25.2702° N, 91.7323° E), NEHU campus (25.6131° N, 91.8981° E), during the month of January-March, 2023. The temperature of the regions ranges from a minimum of 18° C and a maximum of 30° C (**Figure: 1**)

Collection of rhizospheric soil and root sample :

Aloe vera, Catharanthus roseus, Costus igneus, Prunella vulgaris and *Ageratum conyzoids's* rhizospheric soil samples were aseptically taken from a depth of 5-10 cm below the surface. During the sampling process, the plant's fine roots were also gathered. To avoid contaminating the soil, the sampling was done in polypropylene plastic containers. After being properly transferred to the laboratory and kept there at 4° C for further analysis following collection from the appropriate areas. The microbiology lab at the University of Science and Technology of Meghalaya's Department of Botany is where the laboratory tests were carried out.

Isolation of AM Fungal spores :

Fity gm of rhizospheric soil from the samples were first weighed. From these 50gm samples, the spores were collected. AMF soil sample were isolated by wet-sieving and decanting method[12]. After measuring 50gm of soil in the weight machine, the soil is homogenized in beaker with around 1000ml of water and mixed very carefully with the help of a glass rod for 15 minutes and kept in a safe place in laboratory for 1 hour in order to allow the heavier soil particles to settle down in the container. During this time of mixing, the spores move to the upper surface of the water with the foaming of soil particles. After 1 hour, the soil suspension was decanted through a series of sieving method using different measurement of the sieves such as 1st position (250µm), 2nd position (125µm), 3rd position (60µm), and 4th position (37µm). The first sieve is used for separating the debris particles of the soil. The remaining sieve's suspensions were transferred to separate beaker (100ml) by using wash bottle with minimal amount of distilled water or tap water.

After transferring into the beaker (2nd, 4th respectively) the suspensions were filtered using Whattman's filter paper. Then the filter paper was transferred to the large petridishes (around 12-15cm) and observed forspores under stereo-microscope. The spores were separated from the debris in the filter paper by using bamboo needle and the spores were mounted on clean slides using a drop of PVLG (Polyvinyl alcohol + Lactic acid + Glycerol) or PVLG + Melzer's reagent (1:1) and observed under compound microscope followed by the methods of Schenck and Peter (1990) and carefully covered with the cover slip and care was taken to protect from air bubbles (**Photoplate 1**).

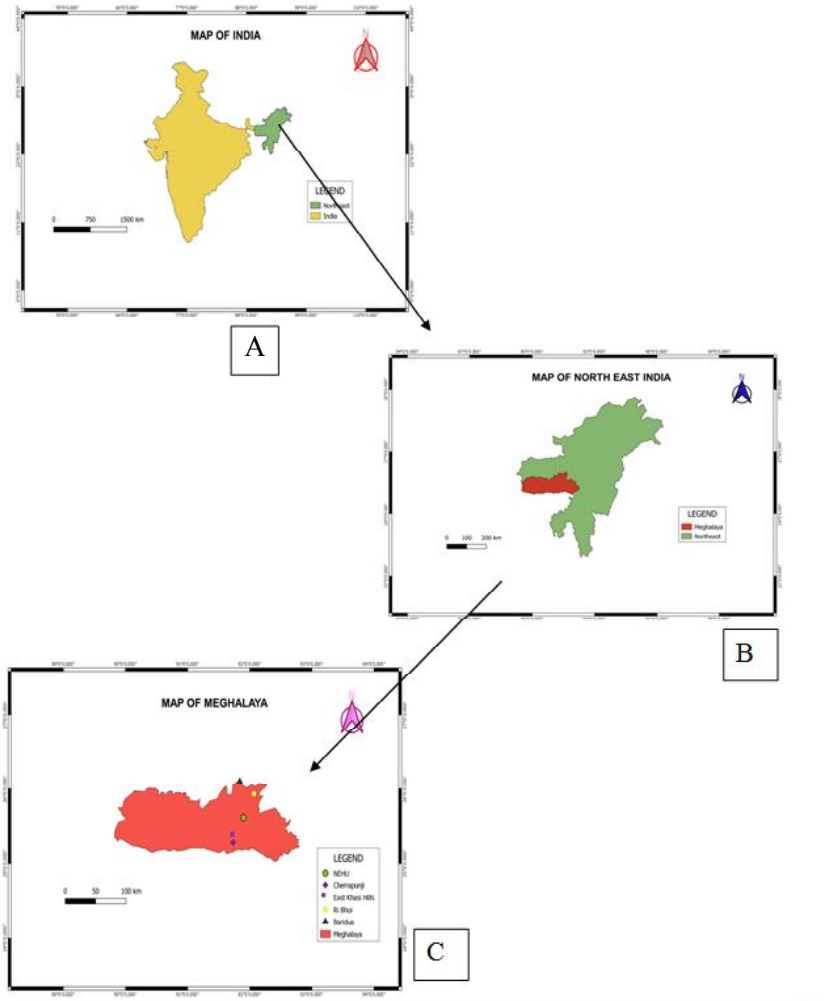


Figure 1: Collection site (A) Map of India showing North-East region (B) The North-East region highlighting the state of Meghalaya (C) The state of Meghalaya highlighting the location points of the soil collection areas. **(Courtesy: Google source)**

Enumeration and identification of AM Fungal spores :

The AMF spores were then counted under a light microscope, and the number of spores on each slide was totalled. A high-quality photograph of the spores was then taken using a camera at various resolutions. After the spores had been counted, they were classified based on their size, shape, colour, wall structures, surface ornamentation, presence or absence of bulbous suspensors, hyphal attachment to the wall and its structure, and type—straight, curved, or recurved—among other characteristics. The identification was carried out using the manual identification

handbook of arbuscular mycorrhizal fungi 32 and the online descriptions provided by the International Culture Collection of Vesicular Arbuscular Mycorrhizal Fungi (http://invam.caf.wvu.edu), AMF phylogeny (www.amf-phylogeny.com)[25,32].

Estimation of root colonisation (%) :

Using a needle and forceps, the fine roots of each plant were removed from the soil sample. Before the root colonisation test, the obtained fine roots were carefully cleaned with tap water to remove any soil or other adhering materials and then preserved in FAA (Formalin-Aceto-Alcohol) solution according to a slightly modified version of the procedure[22]. After being well cleaned with tap water, the root segments are chopped into small pieces (about 1 cm) using fresh blades and placed in a conical flask filled with 100 ml of 10% KOH. The flask is then autoclaved at 15 lb pressure for 15 minutes. Following autoclaving, the liquid was removed from the flask and the root segments were separated by sieving and washed twice or three times with tap water. The root segments were rinsed with tap water to get rid of any remaining KOH after 5 minutes. The segments were then bleached in alkaline H_2O_2 for 10 to 30 minutes before being rinsed with tap water once more. The roots were acidified for 10–20 minutes in 20–30ml of 1% HCl. Then, for 10 to 60 minutes, they were stained with 0.05% trypan blue. The root segments were then de-stained and kept in acidic glycerol until their mycorrhizal structures were examined. Four to five pieces of each treated root segment from each plant were put on glass slides and covered with cover slips. Percentage of root infection was calculated by the following formula:

$$\% \text{ of root colonisation} = \frac{\text{Number of segments showing infection}}{\text{Total number of segments studied}} X\ 100$$

Maintenance and storage of AM fungal spores :

The AM fungal spores can be maintained and stored by applying two methods for short term (for temporary maintenance and storage) and for long term (for permanent maintenance and storage). In the present project work, the temporary method was used by using the Ringer solution (NaCl-8.6g, KCl-0.3g, $CaCl_2$-0.3g and distilled water-1000ml).

Fungal root colonisation and spore density:

The infection of AM fungi in roots were studied by the method of root colonisation[22]. The main infections are characterised as hyphal attachment, vesicles and arbuscules. In the present project work, the hyphal attachment, vesicles, arbuscules and AM fungal spores are found. The result of root colonisation (%) and spore density have been shown in Table-1 and Table-2. The spore density is different for the different samples of 50gm of soil.

AM Fungal spore density :

The species richness is calculated by total number of spores of a particular species from identified species of AM fungi. The number of times a species occurs is called occurrence frequency. AM spore population

Table-1. AM root colonisation and spore density of the studied medicinal plants

Sl. No.	Plants studied	Spore density (in 50gm^{-1} of soil)	Root colonisation (%)
1	*Aloe barbadensis*	25±0.3	29.41
2	*Catharanthus roseus*	56±0.4	76.47
3	*Costus igneus*	38±0.3	52.94
4	*Prunella vulgaris*	62±0.2	88.23
5	*Ageratum conyzoides*	50±0.3	64.70

Table-2. Relative abundance of isolated AM Genera in the studied medicinal plants

Name of the AM Genera	Plants studied				
	Aloe barbadensis	*Catharanthus roseus*	*Costus igneus*	*Prunella vulgaris*	*Ageratum conyzoides*
Glomus sp.	46%	52%	47%	58%	34%
Gigaspora sp.	21%	22%	18%	23%	42%
Acaulospora sp.	18%	14%	24%	11%	8%
Scutellospora sp.	15%	12%	11%	8%	16%

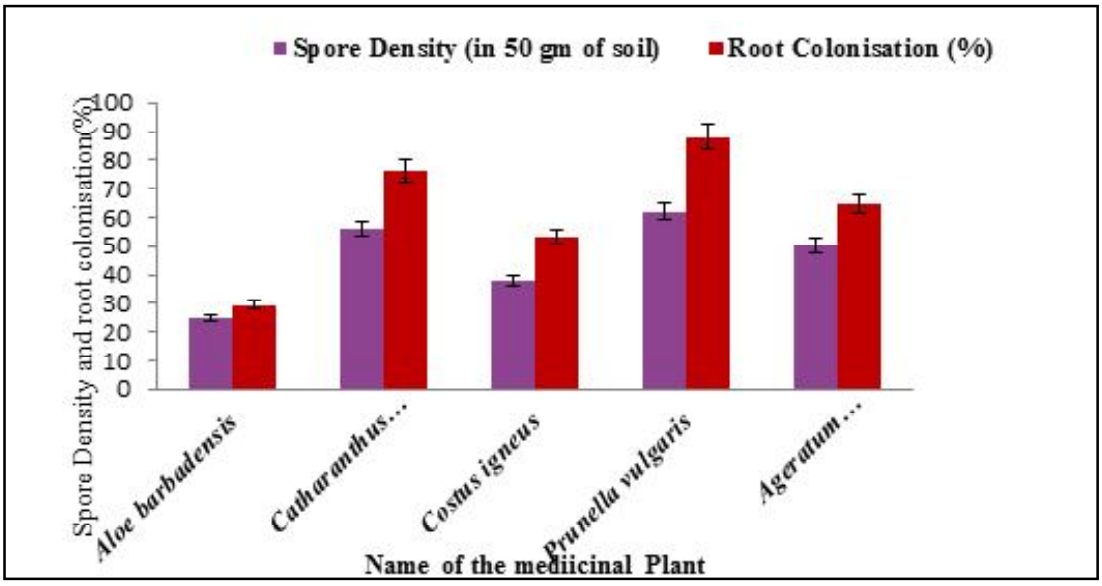


Figure 2. AM Spore density and Root colonization in the studied plants

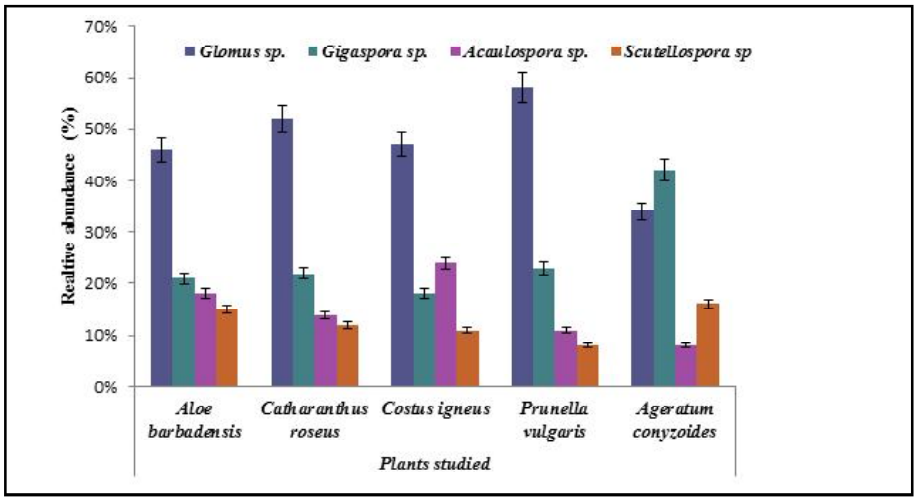
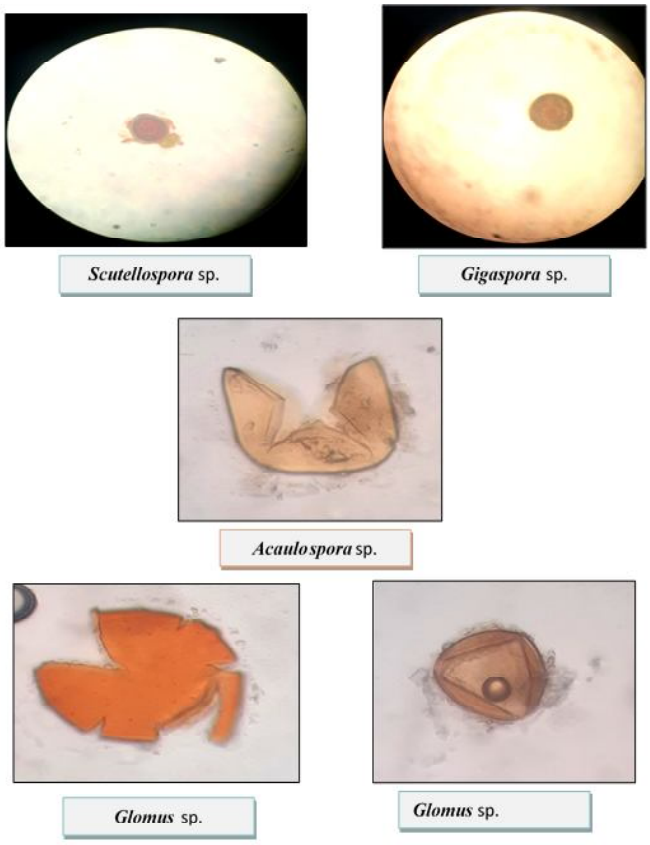
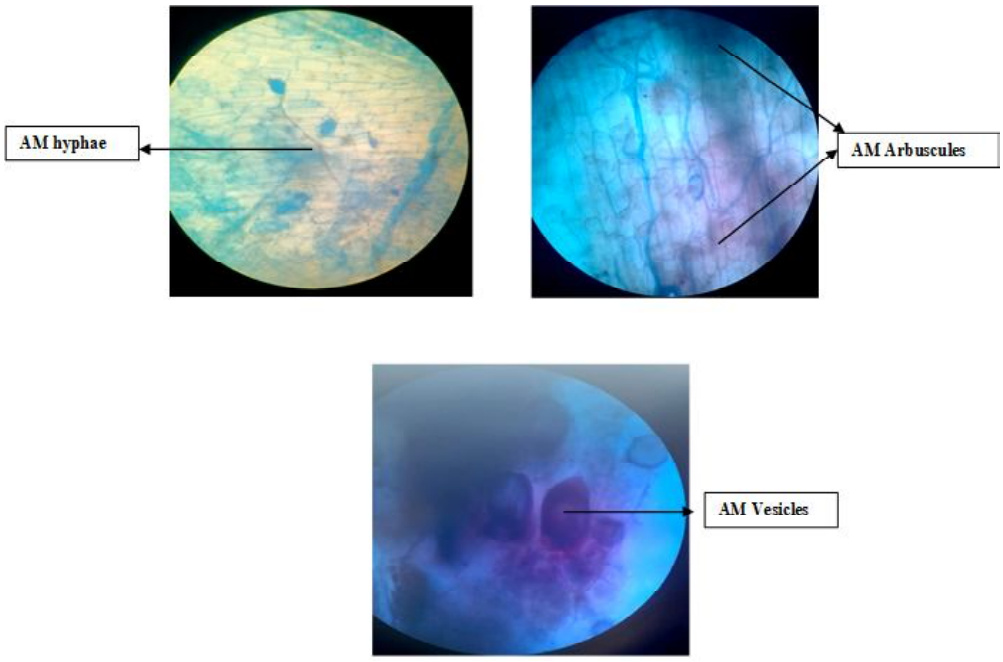


Figure 3. Relative abundance of identified AM spores in the studied plants



PHOTOPLATE 1. Microphotographs of the identified AM spores



PHOTOPLATE 2. Microphotographs of the identified AM spores with their root colonization in the studied plant

is estimated highest in the plant *Prunella vulgaris* (62, 50gm^{-1} soil) with highest root colonization (88%) followed by *Catharanthus roseus* (56, 50gm^{-1} soil) with root colonization (76%), *Ageratum conyzoides* (50, 50gm^{-1} soil) with root colonization (64%), *Costus igneus* (38, 50gm^{-1} soil) with root colonization (52%) and *Aloe barbadensis* (25, 50gm^{-1} soil) with root colonization (29%) (Table-1, Figure 2, Photoplate 1 and 2).

Relative Abundance of isolated AM spores:

Gigaspora sp. showed highest relative abundance in *Ageratum conyzoides*(42%) followed by *Prunella vulgaris* (23%), *Catharanthus roseus* (22%), *Aloe barbadensis* (21%) and *Costus igneus* (18%).

Glomus sp. showed highest relative abundance in *Prunella vulgaris*(58%) followed by *Catharanthus roseus*(52%), *Costus igneus*(47%). *Aloe barbadensis* (46%) and *Ageratum conyzoides* (34%).

Acaulospora sp. showed highest relative abundance in *Costus igneus* (24%) followed by *Aloe barbadensis* (18%), *Catharanthus roseus* (14%), *Prunella vulgaris* (11%) and *Ageratum conyzoides* (8%).

Scutellospora sp. showed highest relative abundance in *Ageratum conyzoides* (16%) followed by *Aloe barbadensis* (15%), *Catharanthus roseus*(12%), *Costus igneus* (11%) and *Prunella vulgaris*(8%). (Table 2, Figure 3).

The identified species of AM fungi belong to mostly Glomeromycota and the genera are mainly *Glomus* sp., *Acaulospora* sp., *Gigaspora* sp. and *Scutellospora* sp. (Photoplate 1).

It is apparent from the study that *Aloe barbadensis* has less AM fungal spore population and has minimum percentage of root colonisation whereas *Prunella vulgaris* has the most AM spore population and maximum percentage of root colonisation. The extent of mycorrhizal infection in root systems is known to be influenced by environmental conditions and the physiological conditions of the plants viz. climate, soil type, pH, vegetation disturbances and the host plant root hairs[34,39].

Gigaspora sp. was the dominant genus followed by *Glomus* sp, *Acaulospora* sp. and *Scutellospora* sp. during the present study. The identified AM spores can be further used as an inoculum as a biofertilizer for the cultivation of various medicinal plants of NE India which will be more beneficial for the sustainable development of Socio-economic aspects of this region. So, future work can be carried out to further undergo single spore culture of these isolated strains on the endangered and rare medicinal plants of NE India for maintaining the Genebank of the medicinal plants.

AM fungi have been variously studied for their contribution in degraded land as well as their potential application in agriculturally cultivated plants for enhanced production. The present project work reveals the diversity, colonisation, occurence frequency and the morphology of the identified species of AM fungi in the rhizospheric soil of aloe vera, periwinkle, insulin plant, common self-heal plant and billygoat weed. Further investigation of differences in percentage colonisation might be attributed to the initial inoculum of the AM fungi in the field under natural conditions. The differences in root colonisation percentage and species richness might be an attribute to change in climatic conditions around the year. In addition, host dependence, age of the host plants and dormancy might play significant role in AM fungi establishment and diversity.

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