# BIODIVERSITY OF ARBUSCULAR MYCORRHIZAL FUNGI ASSOCIATED WITH VARIOUS MEDICINAL PLANTS OF REWARI DISTRICT OF HARYANA, INDIA

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**Abstract.** This study investigated the biodiversity of Arbuscular Mycorrhizal (AM) fungi associated with thirty-three medicinal plant species belonging to twenty-one families in district Rewari, Haryana (India). The results showed significant variation in AM spore density and root colonization among plant species. Forty-one AM fungal species were identified, belonging to six genera, with *Glomus, Gigaspora, and Acaulospora* being the most predominant. *Sclerocystis* and *Entrophospora* were the least among the recovered AMF spores. The results revealed that the number of AM spores in the plant's rhizosphere was unrelated to the percentage of AM root colonization. The highest percentage of root colonization was reported in *Cannabis sativa* (90.91%), while *Eucalyptus maculates* (30%) had the minimum colonization. The highest AM spore density was found in the rhizospheric soil sample of *Ocimum sanctum* (583.2±11.95), while the lowest was found in *Nerium oleander* (17.6±1.52). The study confirmed that AM fungal diversity varies among plant species, with *Tinospora cordifolia* exhibiting the highest AM spore species richness followed by *Artemisia arborescens* and the least in *Calotropis procera*. In conclusion, these fungal communities play a vital role in plant health and growth, showcasing a symbiotic relationship shaped by local soil and environmental factors. Understanding this diversity can guide sustainable cultivation and conservation of medicinal plants, supporting regional biodiversity and ecological balance.

**Keywords:** AM fungi; biodiversity; medicinal plants; rhizosphere; root colonization.

## INTRODUCTION

India boasts a rich heritage of traditional medicine, being one of the eight global centers of origin and diversification for domesticated plant species [55]. For centuries, various plant species have been utilized in Indian traditional medicine, renowned for their therapeutic properties. Haryana, a state in northern India, is endowed with a diverse range of medicinal plants [38, 56].

Medicinal plants have long been a rich source of bioactive compounds for treating various diseases [13, 16, 56, 57]. These plants harbor unique microbiomes, comprising diverse microorganisms that interact with plant-derived secondary metabolites [3, 53, 54]. Rhizospheric microorganisms play a vital role in maintaining plant health by enhancing nutrient acquisition, suppressing pathogens, facilitating nutrient exchange, and promoting abiotic stress tolerance [11, 30, 47]. Arbuscular mycorrhizal fungi (AMF) are key functional microorganisms that form symbiotic relationships with plant roots, enhancing nutrient uptake, particularly phosphorus and nitrogen, as well as other essential micronutrients [8, 23, 31, 37, 46, 52]. By promoting plant growth and health, AMF contribute significantly to the overall well-being of medicinal plants [23, 35, 36].

The symbiotic relationship between arbuscular mycorrhizal fungi (AMF) and medicinal plants enhances nutrient availability and translocation, ultimately influencing plant growth and productivity [40, 42, 51, 58]. Both abiotic and biotic factors, including environmental conditions, cultivation practices, and interactions with other microorganisms, impact the consistency of bioactive compounds in medicinal plants [27, 43]. Notably, AMF colonization can increase the production of secondary metabolites, such as terpenoids, alkaloids, and phenolics, which are valuable for their medicinal properties [29, 59, 61]. The association of AMF with medicinal plants has been documented in various studies, highlighting the importance of understanding AMF biodiversity in plants [34, 49]. Given the potential benefits of AMF-plant symbiosis, further research on AMF diversity associated with medicinal plants is essential for optimizing their utilization and conservation. This diversity is influenced by various factors, including soil type, host plant species, physico-chemical properties of the soil, and the presence of other microorganisms. Assessing AMF biodiversity in ecological sites typically involves evaluating root colonization and spore abundance. Additionally, AMF biomass, comprising spores, extraradical and intraradical hyphae, and colonized root fragments, plays a significant role in determining agricultural performance [2, 11, 12, 24].

This study aimed to investigate the diversity of indigenous arbuscular mycorrhizal (AM) fungi associated with commonly grown medicinal plants in the Rewari district of Haryana (India). By isolating, identifying, and classifying these fungi, the research sought to contribute to the development of future inoculants that could enhance seedling production and improve plant resilience in challenging environments. Understanding the predominant AM fungi in this region can inform strategies for optimizing plant growth, improving crop yields, and promoting sustainable agriculture practices [29].

## MATERIALS AND METHODS

**Sample Collection.** Seasonal field trips were conducted from 2022 to 2024 to collect soil and fine root samples from medicinal plants in the Rewari district of Haryana, India. Plants were randomly selected for


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sampling from various areas. Soil samples and fine roots were collected from the rhizospheric zone by excavating a small amount of soil adjacent to the plant roots at a depth of 15-30 cm. The collected samples were stored in sterilized polythene bags at 5°C ± 10°C for further laboratory analysis.

**Arbuscular Mycorrhizal Spore Isolation, Quantification, and Identification.** AM spores were isolated using the wet sieving and decanting technique [20]. A composite soil sample (50 g) was suspended in water and stirred, then allowed to settle overnight. The supernatant was decanted onto a series of sieves (150 µm, 120 µm, 90 µm, 60 µm, and 25 µm) to capture spores. After repeated washing, the retained spores were transferred to Whatman filter paper No. 1 and subsequently extracted using a hypodermic needle. Spores were examined under a stereo-binocular microscope and preserved in polyvinyl lactic acid alcohol (PVLA).

AM spores were quantified using the gridline intersect method under a stereo-binocular microscope at 40-60× magnification [19]. Spore identification was based on morphological characteristics, including hyphal attachment, wall ornamentation, wall layer thickness, and spore color. The identification of AM fungal spores was confirmed by comparing the observed characteristics with descriptions provided in the identification manual by Schenck and Perez [41].

**Colonization of AM roots.** The extent of arbuscular mycorrhizal (AM) fungal colonization in roots was evaluated using the rapid clearing and staining method [38]. Root segments (1 cm) were randomly selected (n = 15-30) and subjected to a series of treatments. Initially, the segments were cleared in 10% KOH for 24 hours, followed by acidification with 1% HCl for 20 minutes. Subsequently, the roots were stained with trypan blue for 24 hours and destained with lactophenol to remove excess stain. The stained root segments were then mounted in a 1:1 lactic acid-glycerol solution and examined for AM colonization. The root slide technique was employed to assess the percentage of root colonization [21]. Percent root colonization was calculated by the formula given below:

Percentage of AM root colonization = (No. of root segments with infection / Total no. of segments) × 100

**Soil Analysis.** Soil samples were analyzed for various physicochemical properties. The pH was determined using a standard pH meter. Total nitrogen content was estimated using the modified Kjeldahl method [25]. Available phosphorus was measured using the ammonium molybdate spectrophotometric method [10]. Soil organic carbon was determined using the method of Walkley and Black [50]. Potassium content was analyzed using a flame photometer.

**Determination of AM diversity index.** Similarly, spore diversity index was calculated using Shannon-Wiener index ($H'$), Simpson index ($D$), and evenness ($E$) formulas [15]:

$$H' = -\sum_{i=1}^{S} p_i \ln p_i$$

$$D = 1 - \sum_{i=1}^{S} p_i^2$$

$$E = \frac{H'}{H_{\max}} = \frac{H'}{\ln S}$$

where: $p_i$ is the proportion of the $i^{\text{th}}$ species, calculated as $ni/N$, $ni$ is the number of individuals of the species $i$, $N$ is the total number of individuals of the sample or across all species, and $H_{\max}$ is the maximum possible diversity ($\ln S$) where $S$ is the total number of identified species.

## RESULTS

**Chemical Analysis of Soil.** The analyzed soil exhibited the following physicochemical properties: pH, electrical conductivity (EC), organic carbon (OC), available phosphorus (P), potassium (K), and nitrogen (N), as presented in Table 1.

**Table 1**. Physical and chemical characteristics of soil collected

| Sr. No. | Soil parameter | Value |
|---|---|---|
| 1 | pH | 8.21 |
| 2 | Total nitrogen | 0.182 (%) |
| 3 | Available phosphorus | 18.66 Kg/hectare |
| 4 | Available potassium | 270.48 Kg/hectare |
| 5 | Electrical conductivity (EC) | 0.264 ppt |
| 6 | Organic carbon | 1.26 (%) |

Soil analysis from various locations in the Rewari district revealed a predominantly silty sand texture, with an alkaline pH ranging from 7.56 to 8.21. The soil was generally calcareous in nature. Electrical conductivity (EC) values varied between 0.1 and 1.26 $dSm^{-1}$, indicating suitability for normal plant growth in most areas, although soil from protected fields exhibited moderate salinity. Furthermore, the soil organic matter content was found to be very low.

**Plant Identification and Significance.** A total of 33 plant species were collected from different areas and identified by the Herbarium of the Botany Department, Kurukshetra University. These species belonged to 21 families (Table 2).

**Root Colonization by Arbuscular Mycorrhizal Fungi.** All plant species examined in this study exhibited root colonization by arbuscular mycorrhizal (AM) fungi, characterized by the presence of hyphae, vesicles, and arbuscules. The intensity of arbuscular colonization was relatively lower compared to mycelium and vesicles. Arbuscules, which facilitate nutrient transport, were abundant in 16 plant species, while vesicles were predominant in 6 plant species (Table 2). Notably, some plant samples lacked arbuscular structures in their root systems.


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**Table 2.** Occurrence and distribution of AMF species among selected medicinal plants of Rewari district of Haryana

| Sr. No. | Botanical name | Family | Type of infection (M) | (A) | (V) | AMF spore no. / 50 g soil | AM Colonization (%) | Mycorrhizal spore type |
|---|---|---|---|---|---|---|---|---|
| 1. | *Achyranthus aspera* L. | Amaranthaceae | + | + | - | 109.4±4.1 | 80 | 5,11,26,31 |
| 2. | *Aloe vera* (L.) *Burm.* F. | Asphodelaceae | + | - | - | 44±2.35 | 50 | 1,13,25,35 |
| 3. | *Amaranthus viridis* L. | Amaranthaceae | + | + | + | 162±5.29 | 70 | 3,6,11,19,30,34,37,39 |
| 4. | *Artemisia arborescens* L. | Asteraceae | + | - | - | 143.8±2.95 | 41.67 | 4,7,12,19,18,28,30,36 |
| 5. | *Asparagus officinalis* L. | Asparagaceae | + | + | - | 51±2.91 | 66.67 | 2,4,13,19 |
| 6. | *Azadirachta indica* A. Juss. | Meliceae | + | - | - | 155.2±4.87 | 62.5 | 1,6,7,11,25,37, |
| 7. | *Calotropis procera (*Aiton) W.T. Aiton | Apocynaceae | - | + | - | 123.2±1.3 | 40 | 1,7,19 |
| 8. | *Cannabis sativa* L. | Cannabaceae | + | - | - | 164.4±5.22 | 90.91 | 11,29,31,34,37,39 |
| 9. | *Chenopodium album* L. | Amaranthaceae | + | + | - | 159.2±6.61 | 40 | 13,16,32,36,38 |
| 10. | *Coccinia grandis* (L.) Voigt | Cucurbitaceae | + | - | - | 62.6±2.41 | 60 | 20,23,35 |
| 11. | *Datura metel* L. | Solanaceae | + | + | - | 45.8±1.3 | 70 | 2,7,11,22,35 |
| 12. | *Eucalyptus maculates* Smith. | Myrtaceae | + | - | - | 72.2±4.02 | 30 | 2,4,8,24,38 |
| 13. | *Glycyrrhiza glabra* L. | Fabaceae | + | + | + | 204.8±6.87 | 70 | 1,8,10,23,31,33,34,36,41 |
| 14. | *Heliotropium curassavicum* L. | Boraginaceae | - | + | + | 416.2±5.72 | 40 | 5,11,22,37 |
| 15. | *Justicia adhatoda* L. | Acanthaceae | + | - | - | 45.4±2.97 | 60 | 6,13,17,26 |
| 16. | *Mansoa alliacea* (Lam.) A.H. Gentry | Bignoniaceae | + | - | - | 50±4.32 | 50 | 4,6,23,36 |
| 17. | *Musa paradisiaca* L. | Musaceae | + | - | - | 46.6±2.88 | 45.45 | 4,7, 28 |
| 18. | *Nerium oleander* L. | Apocynaceae | + | - | - | 17.6±1.52 | 40 | 3,7,18,25,29,40 |
| 19. | *Ocimum sanctum* L. | Lamiaceae | + | + | - | 583.2±11.95 | 60 | 1,4,8,28,33 |
| 20. | *Phyllanthus emblica* L. | Phyllanthaceae | + | + | + | 68.2±4.76 | 70 | 14,24,29,31 |
| 21. | *Phyllanthus niruri* L. | Phyllanthaceae | + | - | - | 54±3.54 | 84.62 | 2,6,22,25,30 |
| 22. | *Pluchea lanceolata* (DC.) C.B. Clarke | Asteraceae | + | - | - | 24.6±1.34 | 60 | 7,13,34 |
| 23. | *Pongamia pinnata* (L.) Pierre | Fabaceae | + | + | - | 89±3.39 | 50 | 4,6,17,29,33,35 |
| 24. | *Prosopis cineraria* (L.) Druce | Fabaceae | + | + | - | 226.6±5.59 | 72.73 | 15,19,22,24,26 |
| 25. | *Punica granatum* L. | Punicaceae | + | + | - | 62.8±3.56 | 45.45 | 20,26 |
| 26. | *Rauwolfia serpentina* (L.) Benth. ex Kurz | Apocynaceae | + | - | - | 88±3.94 | 80 | 3,11,14,17,22,39 |
| 27. | *Sapindus saponaria* L. | Sapindaceae | + | + | + | 236.2±8.64 | 54.55 | 7,12,21,35,43 |
| 28. | *Syzygium cumini* (L.) Skeels | Myrtaceae | + | - | - | 53.6±1.52 | 60 | 15,19,22,30,35 |
| 29. | *Tecoma stans* (L.) Juss. ex Kunth | Bignoniaceae | + | - | - | 55.4±5.59 | 60 | 3,7,12,19,25,27,33,36 |
| 30. | *Tinospora cordifolia* (Willd.) Hook. f. & Thomson | Menispermaceae | + | - | - | 168±8.15 | 90 | 7,8,15,24,26,29,33,34,37 |
| 31. | *Vachellia nilotica* (L.) P.J.H. Hurter & Mabb. | Fabaceae | + | + | - | 74.6±1.67 | 38.46 | 10,25,27,29,35 |
| 32. | *Vitex negundo* L. | Verbenaceae | + | - | + | 79.8±7.79 | 66.67 | 8,9,16,23,30,34,37 |
| 33. | *Withania somnifera* (L.) Dunal | Solanaceae | + | + | - | 82.8±4.82 | 60 | 12,20,27,34,36,41 |

Each value is a mean of five replicates, ±: Standard deviation, A: Arbuscule, V: Vesicle, M: Mycelium, +: present, --: absent

The percentage of root colonization by arbuscular mycorrhizal (AM) fungi varied significantly among 33 medicinal plant species, ranging from 30% in *Eucalyptus maculata* to 90.91% in *Cannabis sativa* (Table 2). *Tinospora cordifolia* showed 90% colonization. The variation in root colonization may be attributed to differences in rhizospheric soil characteristics and root exudates that support AM fungal growth. The degree of root colonization was influenced by host plant species, soil properties, and environmental factors, but no correlation was found between root colonization and spore density or diversity.

AM fungi colonized plant roots, forming arbuscules, vesicles, and various mycelial forms (Y-shaped, H-shaped, coiled, and parallel) and vesicle shapes (rectangular, columnar, round, globose, oval, and beaked), highlighting the diversity of AMF structures within the plant roots [7]. Notably, mycelium was absent in *Calotropis procera* and *Heliotropium indicum*, while vesicles were present in *Amaranthus viridis, Glycyrrhiza glabra, Heliotropium curassavicum, Phyllanthus emblica, Sapindus Saponaria*, and *Vitex negundo*. Arbuscular-type infections were observed in 16 plant species, including *Achyranthus aspera, Amaranthus viridis, Asparagus officinalis, Calotropis procera, Chenopodium album, Datura metel, Glycyrrhiza glabra, Heliotropium curassavicum, Ocimum sanctum, Phyllanthus emblica, Pongamia pinnata, Prosopis cineraria, Punica granatum, Sapindus Saponaria, Vachellia nilotica*, and *Withania somnifera*. The degree of AMF root colonization varied significantly among plant species, ranging from 30% in *Eucalyptus maculata* to 90.91% in *Cannabis sativa*.

AM spore density also varied significantly, with the highest density in *Ocimum sanctum* (583.2 ± 11.95 spores/50g soil) and the lowest in *Nerium oleander*


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(17.6 ± 1.52 spores/50g soil) (Table 2). Spore population varied among plant families, with Lamiaceae, Boraginaceae, and Sapindaceae having higher spore counts.

**Diversity of Arbuscular Mycorrhizal Fungi.** A comprehensive analysis of arbuscular mycorrhizal (AM) fungi revealed a diverse range of species associated with various plant hosts (Table 3, 4 & 5). A total of six genera, namely *Acaulospora, Entrophospora, Glomus, Gigaspora, Funneliformis*, and *Sclerocystis*, were identified, encompassing 41 distinct AM fungal species (Table 4). Notably, the genus *Acaulospora* emerged as the dominant group, comprising 19 species, followed by *Glomus* with 13 species, *Funneliformis* with 2 species, *Sclerocystis* with 3 species, *Entrophospora* with 1 species, and *Gigaspora* with 3 species.

**Table 3**. List of AMF species isolated from medicinal plants of Rewari region (Haryana, India)

| Sr. No. | Isolated AMF species | Sr. No. | Isolated AMF species |
|---|---|---|---|
| 1 | *Acaulospora appendiculata* Spain, Sieverding & Schenck | 22 | *Funneliformis mosseae* (Nicol. & Gerd.) Walker & Schüßler |
| 2 | *Acaulospora capsicula* Błaszk., Blanke & Renker | 23 | *Gigaspora rosea* Nicolson & Schenck |
| 3 | *Acaulospora cavernota* Trappe & Gerdemann | 24 | *Gigaspora* sp.1 |
| 4 | *Acaulospora columbiana* Sieverding & Toro | 25 | *Gigaspora sp.2* |
| 5 | *Acaulospora foveata* Trappe & Janos | 26 | *Glomus aggregatum* Schenck & Smith |
| 6 | *Acaulospora lacunosa* Morton | 27 | *Glomus aureum* Nicol. ex Gerd. |
| 7 | *Acaulospora laevis* Gerdemann & Trappe | 28 | *Glomus constrictum* Trappe |
| 8 | *Acaulospora delicata* Walker, Blaszk. & Diederichs | 29 | *Glomus fasciculatum* (Thaxter) Gerdemann & Trappe |
| 9 | *Acaulospora denticulata* Sieverding & Toro | 30 | *Glomus formosanum* Koske & Walker |
| 10 | *Acaulospora gerdemanni* Schenck & Nicolson | 31 | *Glomus glomerulatum* Sieverd. |
| 11 | *Acaulospora mellea* Spain & Schenck | 32 | *Glomus* sp.1 |
| 12 | *Acaulospora scrobiculata* Trappe | 33 | *Glomus* sp.2 |
| 13 | *Acaulospora sporocarpia* Berch & Fortin | 34 | *Glomus* sp.3 |
| 14 | *Acaulospora* sp.1 (unidentified) | 35 | *Glomus* sp.4 |
| 15 | *Acaulospora* sp. 2 (unidentified) | 36 | *Glomus* sp.5 |
| 16 | *Acaulospora* sp. 3 (unidentified) | 37 | *Glomus* sp.6 |
| 17 | *Acaulospora* sp.4 (unidentified) | 38 | *Glomus* sp.7 |
| 18 | *Acaulospora* sp.5 (unidentified) | 39 | *Sclerocystis coremoides* Berk. & Broome |
| 19 | *Acaulospora* sp.6 (unidentified) | 40 | *Sclerocystis* sp.1 |
| 20 | *Entrophospora* sp.1 | 41 | *Sclerocystis* sp.2 |
| 21 | *Funneliformis intraradices* (N.C. Schenck & G.S. Smith) Walker & Schubler | | |

**Table 4**. Genus-wise distribution of AMF species isolated from rhizospheric soils

| Sr. No. | AMF Genus | Number of species | Percentage (%) |
|---|---|---|---|
| 1 | *Acaulospora* | 19 | 46.34 |
| 2 | *Glomus* | 13 | 31.71 |
| 3 | *Gigaspora* | 3 | 7.32 |
| 4 | *Sclerocystis* | 3 | 7.32 |
| 5 | *Funneliformis* | 2 | 4.88 |
| 6 | *Entrophospora* | 1 | 2.43 |
| **Total** | | **41** | **100** |

**Table 5**. Diversity indices of AMF associated with medicinal plants

| Plant species | Species richness (*S*) | Shannon index (*H'*) | Simpson index (*D*) | Evenness (*E*) |
|---|---|---|---|---|
| **0** | **1** | **2** | **3** | **4** |
| *Achyranthus aspera* | 4 | 1.21 | 0.31 | 0.87 |
| *Aloe vera* | 4 | 1.05 | 0.36 | 0.76 |
| *Amaranthus viridis* | 8 | 1.85 | 0.18 | 0.89 |
| *Artemisia arborescens* | 8 | 1.72 | 0.20 | 0.83 |
| *Asparagus officinalis* | 4 | 1.12 | 0.33 | 0.81 |
| *Azadirachta indica* | 7 | 1.68 | 0.19 | 0.86 |
| *Calotropis procera* | 3 | 0.92 | 0.42 | 0.84 |
| *Cannabis sativa* | 6 | 1.65 | 0.21 | 0.92 |
| *Chenopodium album* | 5 | 1.42 | 0.26 | 0.88 |
| *Coccinia grandis* | 3 | 0.85 | 0.45 | 0.77 |
| *Datura metel* | 5 | 1.36 | 0.27 | 0.85 |
| *Eucalyptus maculata* | 5 | 1.20 | 0.30 | 0.75 |
| *Glycyrrhiza glabra* | 9 | 2.02 | 0.15 | 0.92 |
| *Heliotropium curassavicum* | 4 | 1.10 | 0.34 | 0.79 |
| *Justicia adhatoda* | 4 | 1.08 | 0.35 | 0.78 |
| *Mansoa alliacea* | 4 | 1.02 | 0.37 | 0.74 |
| *Musa paradisiaca* | 3 | 0.80 | 0.47 | 0.73 |
| *Nerium oleander* | 6 | 1.48 | 0.23 | 0.83 |
| *Ocimum sanctum* | 5 | 1.55 | 0.22 | 0.96 |
| *Phyllanthus emblica* | 4 | 1.18 | 0.32 | 0.85 |


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| 0 | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| *Phyllanthus niruri* | 5 | 1.60 | 0.21 | 0.99 |
| *Pluchea lanceolata* | 3 | 0.78 | 0.48 | 0.71 |
| *Pongamia pinnata* | 6 | 1.52 | 0.22 | 0.85 |
| *Prosopis cineraria* | 5 | 1.48 | 0.24 | 0.92 |
| *Punica granatum* | 2 | 0.52 | 0.58 | 0.75 |
| *Rauwolfia serpentina* | 6 | 1.70 | 0.20 | 0.95 |
| *Sapindus saponaria* | 6 | 1.66 | 0.21 | 0.93 |
| *Syzygium cumini* | 5 | 1.40 | 0.26 | 0.87 |
| *Tecoma stans* | 8 | 1.90 | 0.17 | 0.91 |
| *Tinospora cordifolia* | 9 | 2.10 | 0.14 | 0.96 |
| *Vachellia nilotica* | 5 | 1.25 | 0.29 | 0.78 |
| *Vitex negundo* | 7 | 1.75 | 0.18 | 0.90 |
| *Withania somnifera* | 6 | 1.58 | 0.22 | 0.88 |

The Shannon diversity index (*H'*) ranged from 0.52 to 2.10, evenness from 0.71 to 0.99, and dominance (D) from 0.14 to 0.58 (Table 5). The diversity of AM fungal spores varied significantly among plant species, with the value of index (*H'*) observed in *Tinospora cordifolia* (2.10) and *Glycyrrhiza glabra* (2.02), followed closely by *Artemisia arborescens*, *Amaranthus viridis*, and *Tecoma stans*, each exhibiting 8 species. In contrast, several plant species, including *Calotropis procera*, *Pluchea lanceolata*, *Punica granatum*, and *Musa paradisiaca*, displayed relatively low AM fungal spore diversity. The maximum *D* occurred in *Punica granatum*, and the minimum in *Tinospora cordifolia*. The term "evenness" essentially describes how evenly species abundance is distributed within a community. It reflects how individuals are distributed among species, impacting overall diversity. An ecosystem where each species has a similar number of individuals has higher evenness, signifying a balanced ecosystem. Even distribution of AM fungi was much prevailing in *Phyllanthus niruri* as Evenness (E) was measured to be highest as compared to *Pluchea lanceolata*.

## DISCUSSION

Ensuring the long-term availability of medicinal plants requires sustainable production methods. One promising approach is the integration of arbuscular mycorrhizal (AM) fungi into agricultural practices. These fungi form widespread symbiotic relationships with most land plants, including medicinal species [45]. By leveraging this symbiosis, the reliance on chemical fertilizers can be significantly reduced, promoting environmentally friendly agriculture.

The mutualistic association between AM fungi and host plants involves a complex series of interactions that benefit both partners. The effectiveness of this relationship hinges on the compatibility between the fungi and the plant, which directly impacts nutrient uptake and plant health [9, 22]. AM fungi enhance nutrient absorption by expanding the root system's surface area, facilitating greater nutrient uptake from the soil [26]. Additionally, these fungi contribute to plant resilience by promoting disease resistance and tolerance to abiotic stresses like drought and salinity [4].

In medicinal plants, AM fungi have been shown to boost the production of secondary metabolites, thereby increasing bioactive properties [32, 62]. This symbiotic relationship not only improves plant growth and stress resilience by enhancing nutrient availability, particularly phosphorus and other essential minerals, but also supports soil health. Consequently, this contributes to biodiversity and the stability of agricultural ecosystems.

Given the increasing challenges posed by climate change and soil degradation, utilizing AM fungi in agriculture could lead to more sustainable farming systems with reduced chemical inputs. Understanding AM fungal diversity and improving their application in crop management is crucial for achieving sustainable agricultural objectives [18]. By adopting these practices, we can move towards more resilient and environmentally friendly agricultural systems.

The physical characteristics of soil play a crucial role in determining the abundance of arbuscular mycorrhizal fungi (AMF) and their spores. The rhizospheric soil, rich in diverse root exudates, creates a conducive environment for microorganisms, including AMF, to thrive. Our study revealed notable differences in the percentage of root colonization among various plant species, suggesting that specific components in the rhizospheric soil may enhance AMF growth. This observation aligns with previous research highlighting the influence of rhizospheric factors on AMF development [47, 53].

Moreover, plant roots release a range of metabolites, some of which can attract AMF by producing easily oxidizable compounds, thereby promoting colonization. The variation in root colonization among plant species can be attributed to these physiological differences, which affect the interaction between plant roots and AMF [49]. Understanding these dynamics is essential for elucidating the complex relationships between plants and AMF, and for optimizing their mutual benefits in various ecosystems.

The extent of arbuscular mycorrhizal (AM) root colonization is a key indicator of the symbiotic relationship between fungi and plant roots, with higher colonization levels typically associated with enhanced benefits for the plant. However, the degree of root colonization is influenced by a complex array of factors, including host plant species, seasonal variations, soil properties, and environmental conditions [1]. Seasonal fluctuations in spore production, nutrient availability, and changes in soil temperature and pH can all impact


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AM associations by modifying physiological processes [44].

Interestingly, our study revealed that the percentage of root colonization in the surveyed plants did not correlate directly with spore count or diversity. This finding suggests that the relationship between AM fungi and plant roots is more nuanced than previously thought, and that other factors may play a significant role in determining the efficacy of this symbiosis. Further research is needed to elucidate the complex interactions between AM fungi, plant roots, and environmental factors, and to explore the implications for plant growth and ecosystem functioning.

AM fungi form symbiotic relationships with plant roots, enhancing nutrient exchange and promoting mutual benefits. High levels of AM root colonization often correlate with improved plant growth [48]. Our findings are consistent with previous research on AM fungal diversity associated with medicinal plants in specific regions of Haryana and Himachal Pradesh, which reported similar trends in root colonization [6, 14].

Root colonization by mycorrhizal fungi can be influenced by various factors, including plant species, seasonal changes, soil properties, and environmental conditions. Research indicates that fluctuations in soil nutrients, spore production, and other environmental variables can affect the degree of root colonization [39, 44, 60]. Additionally, soil temperature and pH can influence AM associations by altering physiological processes.

Notably, our study found that that root colonization percentages in the studied plants didn't directly correspond to spore counts or diversity. This suggests that the relationship between AM fungi and plant roots is complex and influenced by multiple factors, warranting further investigation to understand the underlying mechanisms and implications for plant growth and ecosystem functioning. Our findings also showed significant variability in spore populations, implying that various factors contribute to spore abundance in the rhizosphere of medicinal plants. The intricate relationships between plant and root phenology, root production, and spore numbers underscore the complexity of interactions between plant roots and spore-forming microorganisms [17].

Our findings are consistent with previous research on the biodiversity of arbuscular mycorrhizal (AM) fungi, which has reported the dominance of *Acaulospora* and *Glomus* species [12, 34]. The absence of arbuscules in the root regime of seventeen distinct medicinal plant species suggests variability in the symbiotic relationships between AM fungi and host plants. Arbuscules are typically formed during the establishment of AM symbiosis, and their presence is indicative of a functional relationship between the fungi and the plants [35, 36].

Studies on AM fungi associated with medicinal plants have reported *Glomus* as a dominant genus in various regions, including India and China [28, 34]. The diversity of AM fungi can vary significantly depending on the host plant, geographical location, and environmental conditions [5]. According to the classification of Krüger *et al.* [33], *Glomus* is one of the most diverse genera, with approximately 93 morphospecies.

In conclusion, this study provides valuable insights into the diversity and root colonization of AM fungi in the rhizospheric soil of medicinal plants in the Rewari district of Haryana. By understanding the complex interactions between AM fungi and medicinal plants, we can unlock new opportunities for enhancing plant growth, productivity, and therapeutic potential. The findings of this research contribute to the development of sustainable agricultural practices and highlight the importance of rhizosphere microbiome in medicinal plant cultivation. Further exploration of these dynamics can lead to improved crop management strategies and enhanced pharmaceutical properties of medicinal plants.

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