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Diversity of arbuscular mycorrhizal fungi and root colonization in *Polygonatum verticillatum*

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Abstract. *Kumar A, Tapwal A. 2022. Diversity of arbuscular mycorrhizal fungi and root colonization in* Polygonatum verticillatum. *Nusantara Bioscience 14: 53-63.* Diversity of arbuscular mycorrhizal fungi (AMF) associated with *Polygonatum verticillatum* (L.) All. was investigated in two sites of Himachal Himalaya, India. A total of 15 AMF species were isolated and identified from the rhizosphere soil of *P. verticillatum*. The spore density was 1.48 ± 1.91 and 3.99 ± 3.78 per 20 grams of rhizosphere soil at the site I and site II, respectively. Mycorrhizal colonization in the roots of *P. verticillatum* was recorded at 46.12 and 52.23 percent at the site I and site II, respectively. In addition, the mycorrhizal structures like darkly stained endophytic hyphae, coiled intracellular hyphae, Y-shaped hyphae, and 'H' connection (Arum type) were recorded.

Keywords: AM fungi, Astavarga, Mahameda, *Polygonatum verticillatum*, root colonization

INTRODUCTION

Polygonatum verticillatum (L.) All., commonly known as Whorled Solomon's Seal, belong to the family Asparagaceae. *Polygonatum* comprises 71 species (Zhao et al. 2017), mostly distributed in temperate regions. It is found in India, Pakistan, Nepal, Afghanistan, Bhutan, Korea, Russia, and in moderate climate zones of North America and Europe (Saboon et al. 2016). Its distribution is also recorded in subtropical and boreal zones of the northern hemisphere (Meng et al. 2014). In India, *P. verticillatum* distribution is reported from Kashmir to Sikkim as undergrowth in the temperate forests at an altitude range of 2000-3000 m (Pandey et al. 2006; Bisht et al. 2011).

Polygonatum verticillatum is locally known as Salam-mishri, 'Mahameda' in Ayurveda, and Tridanti, Devamani, and Vasuchhidra in Sanskrit. *Polygonatum verticillatum* has been used as a folklore remedy and is a member of the 'Astavarga' group of medicinal plants in Ayurvedic medicines. Astavarga is a group of eight astounding medicinal plants and is known for jeevaniya (vitality promoting), vaysthapan (restoring of youthful condition), body nourishment, antioxidant, and reviving properties (Buchake et al. 2010). Charak Samhita, an ancient Ayurvedic literature, has described its noteworthy role in curing cough, dyspnea, consumption, cardiac problems, and voice disorders (Baliga et al. 2013). β-sitosterol, 2-hydroxybenzoic acid (Khan et al. 2013; Sagar 2014; Saboon et al. 2016), santonin, diosgenin (Khan et al. 2015), and quinine present in *P. verticillatum* exhibit a variety of pharmaceutical properties like antioxidant activities, anti-inflammatory activity, anticancer activity (Patra et al. 2018), antimalarial activities (khan et al. 2012) antipyretic, analgesic, diuretic, sex stimulant (Wujisguleng et al. 2012; Akhtar et al. 2013; Sharma and Samant 2014; Razzaq et al. 2015) and energizer, etc. (Tariq et al. 2015; Saboon et al. 2016; Virk et al. 2016). Rhizomes of this species improve liver health and cure throat pain, headache, eye diseases, gastric troubles, epilepsy, high blood pressure, etc. (Tiwari and Chaturvedi 2016).

Plants have long been used as a source of medicinal bioactive substances to treat a variety of diseases (Gouda et al. 2016; Chalo et al. 2017; Dhakal et al. 2021). Medicinal plants accumulate an array of unique bioactive secondary metabolites that confer high specificity to the associated microorganisms in their distinctive micro-biome (Qi et al. 2012). The microorganisms present in the rhizosphere of plants play a vital role in supporting plant's health by improving plant nutrition (Jacoby et al. 2017), suppressing pathogen outbreaks (Pieterse et al. 2014), nutrient exchange and modulation abiotic stress tolerance (Baum et al. 2015; Cheng et al. 2019) such as drought, low temperature, and salinity (Sun et al. 2021). Among them, arbuscular mycorrhizal fungi (AMF) are the important functional groups that promote plant growth, provide nutrition, and improve health (Giovannini 2020). AMF facilitate nutrient uptake, mainly phosphorus, nitrogen (Campo et al. 2020), potassium, sulfur, copper, zinc, calcium, etc. (Avio et al. 2006; Prasad et al. 2017; Liu et al. 2018; Wang et al. 2018) and enhance the availability of nutrients as well as their translocation (Rouphael et al. 2015). The abiotic factors viz. light, temperature, humidity, soil fertility, and cultivation techniques influence the consistency of active ingredients in medicinal plants, as do biotic factors such as herbivory, disease-related stimuli, and reciprocal symbioses with *Rhizobium* bacteria and mycorrhizal fungi (Szakiel et al. 2011). In host plant, the AMF enhances the content of secondary metabolites (Zubek et al. 2015; Johny et al. 2021) like terpenoids,

alkaloids, and phenolics (Yadav et al. 2013; Zeng et al. 2014), cyclohexanone derivatives, apocarotenoids, phytoalexins, triterpenoids and glucosinolates (Zubek et al. 2010, 2013; Singh et al. 2013). *Polygonatum verticillatum* is little explored for the mycorrhizal association. The present study investigated mycorrhizal colonization and diversity of AMF associated with P. verticillatum in two distant sites in Himachal Himalaya, India.

MATERIALS AND METHODS

Study area

Two sites with temperate climates were selected to collect soil and root samples. The study areas include Chikkadhar, Kullu District, India (Site I) (32°12'02.13"N, 077°15'24.44" E; 2,964 m asl), and Jani, district Kinnaur (Site II) (31°29'13.1"N, 78°04'39.3"E; 2,686 m asl) in Himachal Himalaya, India. Soils up to the depth of 0-30 cm were collected in sterile polyethylene bags and carried to the laboratory.

Isolation and identification of AMF

Ten single soil samples from each sampling site were combined to form a composite sample. Isolation of AMF spores from rhizosphere soil followed the wet sieving and decanting method (Gerdemann and Nicolson 1963). Air dried soil sample (20 g) was suspended in 1000 mL water for 1 hour, and then the suspension was decanted through a series of sieves ranging from 40 µm to 700 µm arranged in descending order of pore size. The sieved material collected from sieves was observed under a stereomicroscope (Nikon SMZ 1500), and the spores were isolated using a hypodermal needle. The spore population was expressed in terms of the number of spores per 20 g of dry soil.

Taxonomic identification of AM fungal spores was done based on size, shape, color, wall structure, number of wall layers, surface ornamentation, hyphal attachments, and the presence or absence of bulbous suspensor under NIKON E-400 microscope 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.p1/Glomeromyca, Techniques in mycorrhizal research, VA Mycorrhiza and Handbook of AMF.

Assessment of root colonization by AMF

Root staining

This study used trypan blue as a stain to process roots for AMF colonization assessment. Roots were first washed thoroughly with tap water, cut into 1 cm lengths, and cleared in 10% (w/v) KOH for 1 hour at 90 °C, acidified with 1% HCl and stained with 0.05% trypan blue overnight, and then finally de-stained with lactic acid-glycerin (1:1 by volume) at room temperature (Phillips and Hayman 1970).

Assessment of root colonization-Grid-line intersect method

The gridline intersects method assessed root colonization (Giovannetti and Mosse 1980). This method is used to estimate both the proportion of infected roots and their total length. For each sample, 100 root segments were chosen at random. The roots (50 segments at a time) were evenly spread out in a Petri dish (10.2 X 10.2 cm^2) with gridlines marked on the bottom to make 0.5-inch squares. Vertical and horizontal gridlines were scanned with a stereomicroscope (Nikon SMZ 1500), and the presence or absence of colonization was noted at each location where the roots intersected a line. The root segments were spread out and examined three times. Finally, the percentage of colonization was computed by dividing the total number of colonized root segments by the total number of investigated root segments. Intersections between gridlines and roots are designated as either mycorrhizal or non-mycorrhizal. The mycorrhizal structures like hyphae, arbuscules, and vesicles were observed in root segments under a microscope. The following formula determined the percentage of root colonization:

$$\text{Root colonization (\%)} = \frac{\text{Total no. of colonized root segments}}{\text{Total no. of root segments investigated}} x\ 100$$

Diversity and data analysis

Some important ecological diversity indicators, such as spore density, relative abundance, isolation frequency, Shannon-Wiener index of diversity (H'), Simpson's index of dominance (D), Evenness (E), and Sorenson's coefficient (Cs) can be used to describe AMF community structure. According to Kavitha and Nelson (2013), spore density, to some extent, represents the biomass of AMF species. Relative abundance is defined as a percentage of the spore number of a species, which indicates the ability of distinct AMF species to sporulate. The percentage of soil samples in which a species existed, which revealed the extent of dispersion of a particular AMF species in an ecosystem, is described as isolation frequency. Finally, the Shannon-Wiener index of diversity reflected the degree of diversity.

Relative abundance (RA), isolation frequency (IF), Shannon-Wiener index of diversity (H'), Simpson's index of dominance (D), Evenness (E), and Sorenson's coefficient (Cs) were calculated as outlined by Dandan and Zhiwei (2007).

$$RA = \frac{\text{Spore numbers of a species}}{\text{Total spore number}} x\ 100$$

$$IF = \frac{\text{Number of soil samples where species occurred}}{\text{Total number of soil samples}} x\ 100$$

$$H' = -\sum Pi \ln Pi$$

Pi = ni/N,

Where, ni is the spore number of a species, and N is the total number of identified spore samples.

$$D = \sum (ni/N)^2$$

$$Evenness = \frac{H'}{H'max}$$

H'max is the maximal H' and is calculated by the following formula:

$H' = \ln S$,

Where, S is the total number of identified species per sampling site

$$\text{Sorenson's coefficient (Cs)} = \frac{2j}{(a+b)}$$

Where, a or b is the total number of species per sampling site, and j is the number of species common to both sites.

RESULTS AND DISCUSSION

Root colonization

Fungal structures like thin intraradical hyphae and coiled intracellular hyphae in cortical root cells confirm the mycorrhizal association. The fungal hyphae were Y-shaped and exhibited an arum type of mycorrhizal infection. The intraradical hyphae in cortical root cells were darkly stained, thin-walled, about 3.28 µm in width, Y-shaped septate branches, and showed 'H' connections between parallel strands of hyphae. This type of hyphal proliferation in the root cortex is known as the Arum type (Brundrett 2004). These characters can be used as diagnostic features to identify the genus of AMF in mycorrhizal roots (Morton and Bentivenga 1994). These mycorrhizal characteristics are exhibited by the *Glomus* species (Souza 2015). The roots collected from study sites showed the presence of dark septate endophytic and intracellular coiled hyphae. The mycorrhizal status of roots collected from both study sites is given in Table 1. Distinct AM vesicles and arbuscules were not recorded, but dark septate hyphae could be seen prominently in the cortical root cells (Figure 3).

The colonization by AMF in the roots of *P. verticillatum* was recorded at 46.12% and 52.23%, respectively, in Site I (Chikadhar, Kullu District) and site II (Jani, Kinnaur District). The AM fungal root colonization is influenced by soil moisture, soil texture (Herold et al. 2014; Sharma and Kothamasi 2015), humidity, temperature (Urcoviche et al. 2014; Bhardwaj and Chandra 2018), seasonal periods, and host plant (Carrenho et al. 2007; Haichar et al. 2016; Guyonnet et al. 2017), soil pH and available nutrients (N and P) (Khanam et al. 2006; Liu et al. 2016). Most medicinal plants' roots and agronomic and vegetable crops exhibit endomycorrhizal association (Gaur and Kaushik 2011). Thangavelu and Raji (2016) reported 10.38-84.55% root colonization in five pot-grown species of *Asparagus*, and the extent of root colonization by AMF varies in different plant species. Yaseen et al. (2016) recorded root range colonization in 20-80% of 20 medicinal plants from Charsadda, Khyber Pakhtunkhwa (Pakistan). At the same time, 48-100% root colonization was recorded in 46 medicinal plant species in the Western Ghats of Karnataka region (Rajkumar et al. 2012). Tapadar et al. (2017) recorded 82.98% root colonization in *Smilax perfoliata*. According to Johny et al. (2021), root colonization amounts vary between AMF and plant species. *Rhizophagus irregularis* exhibited maximum AMF root colonization (43 ± 1.00%) in Ashwagandha, followed by *Claroideoglomus claroideum* (34 ± 4.33%). Root colonization by *R. irregularis* was greatest in marigolds (73 ± 2.88%). *Claroideoglomus etunicatum* and *C. claroideum* displayed the highest affinity to plants, with root colonization rates of 73 ± 5.77 and 72 ± 2.88%, respectively, in licorice. Sinegani and Yeganeh (2016) studied the symbiosis of 48 medicinal plant species with AMF in the semi-arid regions of Iran. They reported percent root range colonization in 32.37% to 77.26%.

Diversity of AMF associated with *Polygonatum verticillatum*

15 AMF species belonging to 7 genera, viz., *Glomus*, *Funneliformis*, *Claroideoglomus*, *Acaulospora*, *Rhizophagus*, *Gigaspora*, and *Scutellospora*, were identified from the rhizosphere soil collected from site 1. Among these, *Glomus* was the dominant genus represented by 5 species, followed by *Acaulospora* (3 spp.) and *Funneliformis* (2 spp.). The species of *Glomus* are: *G. ambisporum*, *G. glomerulatum*, *G. microcarpum*, *G. macrocarpum*, *G. aggregatum*. *Acaulospora* was represented by *A. laevis*, *A. spinosa*, *A. rehmii*, and *Funneliformis* by *F. Geosporum* and *F. constrictus*. The rest of the genera were represented by one species each. Only 3 species representing two genera, i.e., *G. glomerulatum*, *F. geosporum*, and *F. constrictus*, were recorded from site II. Although much literature is available on the diversity of AMF associated with medicinal plants, *P. verticillatum* is little explored in this aspect. Gaur and Kaushik (2011) have isolated 16 AMF from three medicinal plants, i.e., *Catharanthus roseus*, *Ocimum* species, and *Asparagus racemosus* in the Uttarakhand state. Thangavelu and Raji (2016) recorded a dual association of AM and DSE in five pot-grown species of *Asparagus* (*A. aethiopicus*, *A. densiflorus*, *A. setaceus*, *A. racemosus*, and *A. umbellatus*). The spores of 15 AMF species from 10 genera were isolated by Zubek et al. (2012) from rhizosphere soils of the following medicinal plant species, i.e., lemon balm (*Melissa officinalis*), sage (*Salvia officinalis*), and lavender (*Lavandula angustifolia*). Verma et al. (2019) investigated AM diversity in seven ethnomedicinal plants from the Western Himalayas and recorded 23 AMF, where *Glomus* was dominating genus. Kumar et al. (2019) investigated 22 medicinal plants from the Hamirpur district of Himachal Pradesh for AM association, identified 43 AMF from their rhizosphere soils, and reported *Glomus* and *Acaulospora* as dominant genera. In Zhangzhou (China), 66 species of AM fungi have been found in the rhizosphere of 20 medicinal plants, with *Glomus* as the most common genus (Jiang et al. 2012). From the southern region of Fujian (China), 91 AM fungi species were isolated from medicinal plants' rhizosphere, with *Glomus* as the predominant genus (Jiang 2012).

Twenty-six species of AM fungi were isolated from the rhizosphere of *Begonia fimbriata* (Su et al. 2018), belonging to the genera *Acaulospora*, *Glomus*,

Scutellospora, and *Gigaspora*. Song et al. (2019) studied the diversity of AMF of *Sophora flavescens*, and 220 AMF species were detected, representing 8 families and 14 genera. *Glomus*, *Septoglomus*, *Rhizophagus*, *Kamienskia*, and *Sclerocystis* were the dominant AMF genera in the rhizosphere of *S. flavescens*. Koul et al. (2012) recorded 42 species of AMF in the rhizosphere of medicinal plants in India, where six AM fungal species were found in *Aloe vera*, five in *Artemisia annua*, and one in marigolds. Sundar et al. (2011) studied the association of AMF with three medicinally important plants, viz. *Eclipta prostrata*, *Indigofera aspalathoides*, and *I. tinctoria* were collected from three different localities of Kanyakumari (South India), identified 21 AMF, and recorded *Glomus* as the dominant genus. According to the classification of Krüger et al. (2012), there are 11 AMF families, 17 AMF taxa, and about 230 AMF species, where *Glomus* is the most diverse genus, exhibiting around 93 morphospecies. In our study, *Glomus* was recorded dominant genus; it conformed with studies conducted with other medicinal plants (Guadarrama and Álvarez-Sánchez 1999; Muthukumar and Udaiyan 2000; Hijri et al. 2006).

Occurrence, Relative abundance, and Isolation frequency of AMF species are given in Table. 2. A total of 15 AM fungi were identified from the site I (Chikkadhar) and 3 species from site II (Jani). Just three species were reported to be shared by both study sites. Relative abundance (RA) was found in the range of 1.51% (*A. laevis*, *A. rehmii*, *A. spinosa*, *Scutellospora gregaria*, *Scutellospora* sp. and Unknown sp.) to 34.84 % (*G. ambisporum*) in the site I and 11.11% (*F. constrictus*) to 69.44% (*G. glomerulatum*) in site II. Similarly, isolation frequency (IF) varied from 33.33% (*A. laevis*, *A. rehmii*, *A. spinosa*, *F. geosporum*, *S. gregaria*, *Scutellospora* sp. and Unknown sp.) to 100% (*C. etunicatum*, *F. constrictus*, *G. ambisporum*, *G. glomerulatum*, *G. microcarpum*, and *Rhizophagus intraradices*) from the site I and it was ranging from 0-100% (maximum 100% was found in *F. constrictus* and *G. glomerulatum*), while the majority of the AMF species were found to have zero values at site II.

The AM spore density was recorded at 1.48±1.91 and 3.99±3.78 per 20 grams in the rhizosphere soil of P. verticillatum at sites I and II, respectively (Figure 1). Low spore density may be due to the harsh environmental conditions of the study sites. Climatic and edaphic factors (Antunes et al. 2012; Sivakumar 2013; Nouri et al. 2014) significantly impact the population dynamics of AMF; rapid changes in soil nutrients can affect the AMF association and spore numbers (Khanam et al. 2006). Environmental variations, host phenology, interspecific competition, and regional spatial dynamics can influence AM fungal communities' population, distribution, and composition in various ecosystems (Öpik et al. 2006; Melo et al. 2019). Information on the AMF spore density in the rhizosphere of *P. verticillatum* is not available. Still, it was recorded in the range of 3.01 to 2860 spores by many researchers with other medicinal plants, e.g., 10 spores in *Ludwigia linifolia* and 382 spores/100 g of soil in *Leucas aspera* (Bukhari et al. 2003); 84 spores/100g in *Withania coagulans* and 147 spores in *Mitragyna parvifolia* (Panwar and Tarafdar 2006); spore density ranged from 47.53 in *Datura stramonium* to 177.4 in *Mimosa pudica* per 50 g of soil sample (Kumar et al. 2019); 27 spores/10 g of soil in the rhizosphere of *Adhatoda vasica* and 196 spores in *Zanthoxylum acanthopodium* (Singh et al. 2017); 3.01 spores density per 50 g in *Peumus boldus* and 37.30 in *Matricaria chamomilla* (Urcoviche et al. 2014); AM fungal spore density varied from 270 (*Leonurus heterophyllus*) to 2860 (*Lophatherum gracile*) per 100 g soil (Wang and Jiang 2015). Bhat et al. (2014) isolated and identified 151 spores/100g and 24 spores/100g of natural soil of *C. roseus* at two sites, and *Glomus* was found to be the predominant genus in the rhizosphere of both sites. Garampalli et al. (2012) investigated the arbuscular mycorrhizal status of 46 medicinal plant species of herbs and shrubs in the western ghats of Karnataka region, which recorded spore density ranged from 15 to 520 per 100 g of soil.

Glomus glomerulatum, *F. geosporum*, and *F. constrictus* were common to both sites, and Sorenson's Coefficient (Table 3) was found to be 0.33. The diversity indices are shown in Figure 2.

Table 1. Status of AMF association in *Polygonatum verticillatum*

Site	Root colonization (%)	AMF identified	AM structures observed		
			Arbuscules	Vesicles	Hyphae
Chikkadhar	46.12	*Glomus* (5spp.), *Acaulospora* (3 spp.), *Funneliformis* (2 spp.), *Claroideoglomus* (1 sp.), *Rhizophagus* (1 sp.), *Gigaspora* (1 sp.), *Scutellospora* (1 sp.), Unknown (1 sp.)	- (Arum type)	-	+ +
Jani	52.23	*Glomus* (1 spp.), *Funneliformis* (2 spp.)	- (Arum type) Intracellular hyphal coils present	-	++

Note: -: Absent, ++: Moderate

Table 2. Occurrence, relative abundance, and Isolation frequency of AMF species in rhizosphere soil of *Polygonatum verticillatum* (L.) All

Name of fungi	Site	Occurrence	% Freq.	D	A	RA (%)	IF (%)
Acaulospora laevis Gerd. & Trappe	I	+	7.14	0.33	1	1.51	33.33
	II	-	--	--	--	--	--
Acaulospora rehmii Sieverd. & S. Toro	I	+	7.14	0.33	1	1.51	33.33
	II	-	--	--	--	--	--
Acaulospora spinosa C. Walker & Trappe	I	+	7.14	0.33	1	1.51	33.33
	II	-	--	--	--	--	--
Claroideoglomus etunicatum (W.N. Becker & Gerd.)	I	+	21.42	1.33	1.33	6.06	100
C. Walker & A. Schüßler	II	-	--	--	--	--	--
Funneliformis constrictus (Trappe) C. Walker & A.	I	+	21.42	1.66	1.66	7.57	100
Schüßler	II	+	100	1.33	1.33	11.11	100
Funneliformis geosporum (T.H. Nicolson & Gerd.) C.	I	+	7.14	0.66	2	3.03	33.33
Walker & A. Schluessler	II	+	100	2.33	2.33	19.14	100
Glomus aggregatum N.C. Schenck & G.S. Sm.	I	+	14.28	0.66	1	3.03	66.66
	II	-	--	--	--	--	--
Glomus ambisporum G.S. Sm. & N.C. Schenck	I	+	21.42	7.66	7.66	34.84	100
	II	-	--	--	--	--	--
Glomus glomerulatum Sieverd.	I	+	21.42	3.33	3.33	15.15	100
	II	+	100	8.33	8.33	69.44	100
Glomus macrocarpum Tul. & C. Tul.	I	+	14.28	1.33	2	6.06	66.66
	II	-	---	--	--	--	--
Glomus microcarpum Tul. & C. Tul.	I	+	21.42	1.66	1.66	7.57	100
	II	-	--	--	--	--	--
Rhizophagus intraradices (N.C. Schenck&G.S. Sm.)	I	+	21.42	2	2	9.09	100
C. Walker & A. Schüßler	II	-	--	--	--	--	--
Scutellospora gregaria (N.C. Schenck & T.H.	I	+	7.14	0.33	1	1.51	33.33
Nicolson) C. Walker & F.E. Sanders	II	-	--	--	--	--	--
Scutellospora sp.	I	+	7.14	0.33	1	1.51	33.33
	II	-	--	--	--	--	--
Unknown sp.	I	+	7.14	0.33	1	1.51	33.33
	II	-	--	--	--	--	--

Note: Site I: Chikkadhar; Site II: Jani

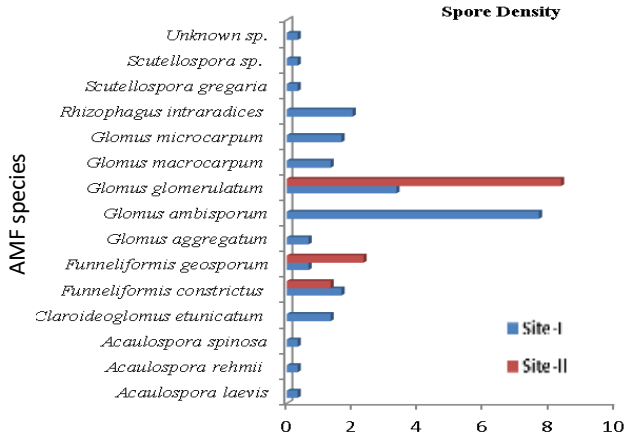

Figure 1. AMF spore density in the rhizosphere soil of *Polygonatum verticillatum*

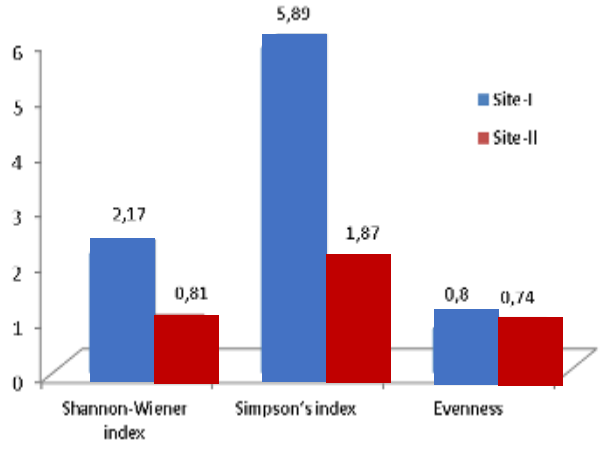

Figure 2. Diversity indices of AMF associated with *Polygonatum verticillatum*

The resources of many medicinal plants are suffering an unavoidable loss of resources due to damage to the natural habitat and long-term over-exploitation (Zhao et al. 2019; Ullah et al. 2020). *Polygonatum verticillatum* is one of many important medicinal plants, and the knowledge of its AMF association will be of immense importance. The potential AMF can be multiplied during cultivation trials for better active ingredient contents. According to the literature, plants produce significantly more secondary metabolites after AM fungal colonization (Oliveira et al. 2013; Zeng et al. 2013; Mechria et al. 2015; Pedone-Bonfim et al. 2015; Kapoor et al. 2017; Duc et al. 2021). Thus, the use of AM fungi could be employed as a strategy for crop biofortification (Antunes et al. 2011; Dutta and Neog 2016) because they influence soil fertility, plant nutrition, plant physiology, and secondary metabolism

(Wipf et al. 2014; Schweiger and Müller 2015; Cervantes-Gámez et al. 2016). Furthermore, AMF is essential for plant growth and health (Ban et al. 2017). As a result, it is critical to investigate the diversity of these fungi in the rhizosphere community structure of *P. verticillatum*. Several academics are working on identifying certain mycorrhizal fungus and their role in phytochemical production (Kumar et al. 2021).

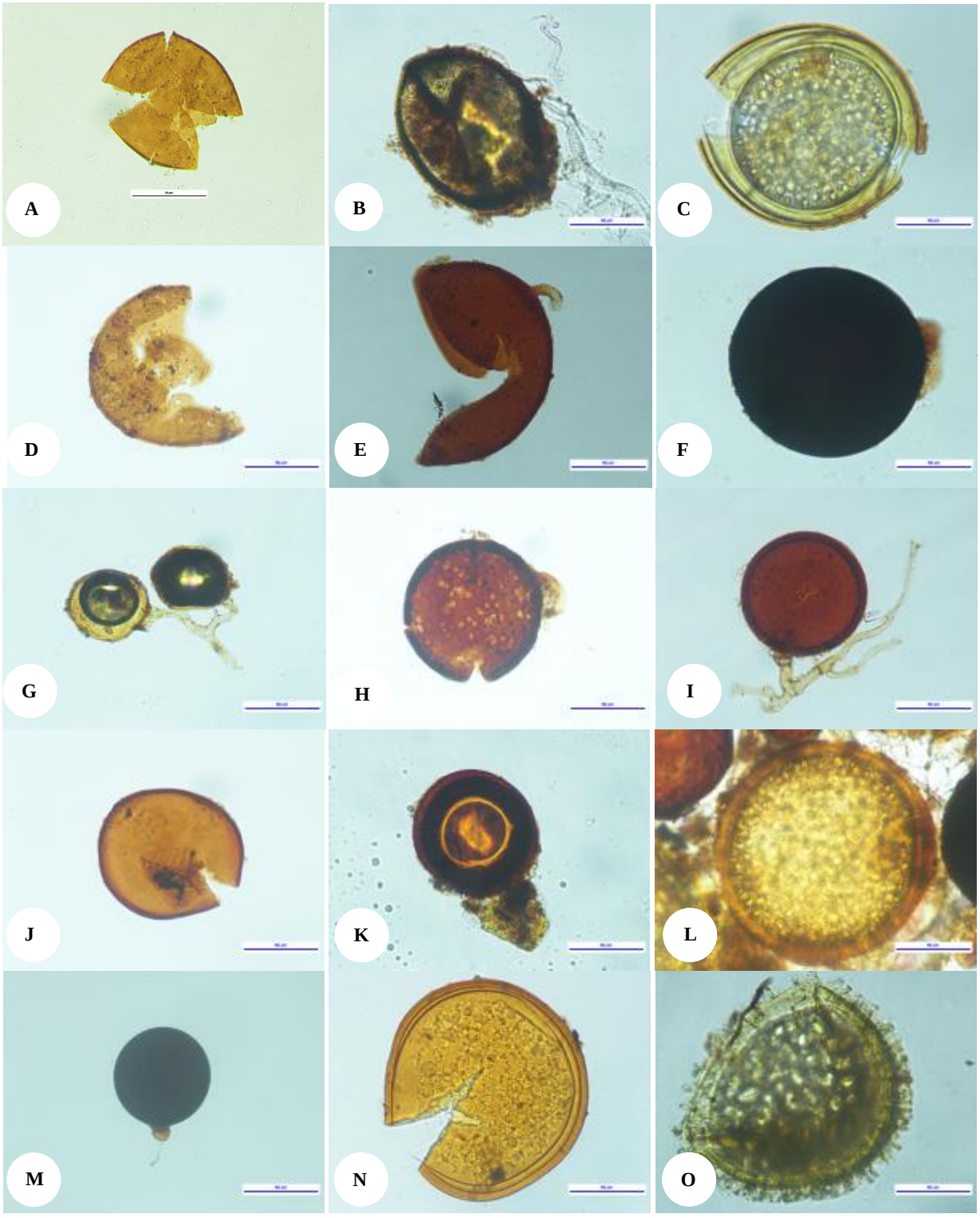

Figure 3. Diversity of AMF spores in the rhizosphere of *Polygonatum verticillatum* (L.) All. A. *Acaulospora laevis*; B. *A. rehmii*; C. *A. spinosa*; D. *Claroideoglomus etunicatum*; E. *Funneliformis constrictus*; F. *F. geosporum*; G. *Glomus aggregatum*; H. *G. ambisporum*; I. *G. glomerulatum*; J. *G. macrocarpum*; K. *G. microcarpum*; L. *Rhizophagus intraradices*; M. *Scutellospora gregariaI*; N. *Scutellospora* sp.; O. Unknown sp.

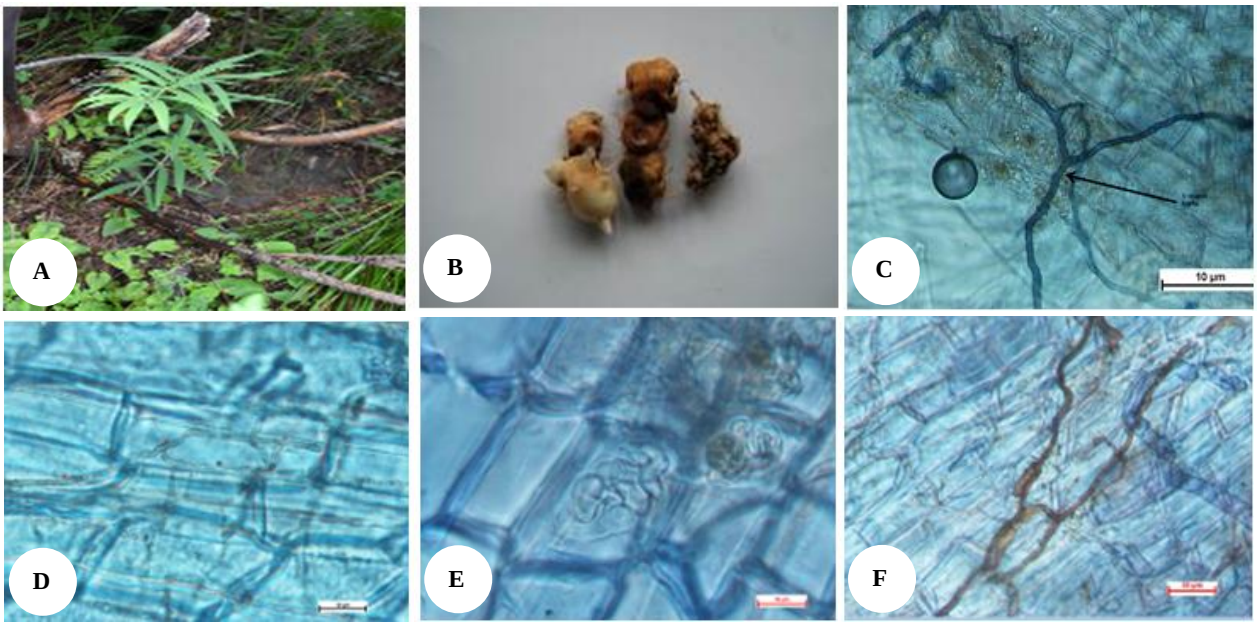

Figure 4. Status of the mycorrhizal colonization with roots of *P. verticillatum.* A. *P. verticillatum*; B. Rhizomes of *P. verticillatum*; C. Y-shapes intraradical hypha; D. H-shaped connection; E. Hyphal coils in the cortical cells; F. Dark septate hyphae

In conclusion, the present study is focused on the diversity and root colonization of AM fungi in the rhizospheric soil of a highly valuable medicinal plant, *P. verticillatum*, found in two temperate regions of the Himachal Himalaya. The plant is well-known for its pharmaceutical properties like antioxidant, anti-inflammatory, anti-cancerous, antimalarial, antipyretic, analgesic, diuretic, aphrodisiac, etc. An accurate and deep understanding of the rhizosphere microbiome is important because of its significant role in enhancing the therapeutic properties of medicinal plants. Here, we provide information on the spore density, root colonization, and diversity of AM fungi in the rhizosphere of *P. verticillatum.* The degree of colonization and spore density varied greatly between the study sites. The fungal genera *Glomus* and *Funneliformis* were the dominant genera at sites I and II, respectively. Considering the possible application of AM fungi in the future on such important medicinal plants, it appears that more attention should be paid to the dominant AM fungi in the association of medicinal plants for the process of in vitro cultivation and mycorrhizal performance so that growth and secondary metabolite production could be improved.

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