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Diversity of arbuscular mycorrhizal fungi in the rhizosphere of *Angelica glauca* and *Valeriana jatamansi* in NW Himalaya, India

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Abstract. *Tapwal A, Kumar A, Sharma S. 2023. Diversity of arbuscular mycorrhizal fungi in the rhizosphere of* Angelica glauca *and* Valeriana jatamansi *in NW Himalaya, India. Asian J For 7: 89-98.* The diverse mycorrhizal association helps to conserve plant biodiversity, ecosystem function, and the accumulation of pharmaceutically important compounds in medicinal plants. Climate change may have an impact on plant diversity as well as on associated microbiota. The mycorrhizal association and diversity of Arbuscular Mycorrhizal Fungi (AMF) in the rhizosphere of two important medicinal plants of the North-Western (NW) Himalayas were explored during different seasons in two distant locations. The endomycorrhizal association in *Angelica glauca* Edgew. and *Valeriana jatamansi* Jones was confirmed by morpho-anatomical characterization of the roots. Microsclerotia, vesicles, and intracellular hyphal coils were found in the roots of both medicinal plant species. The research revealed 24 AMF representing eight genera in the rhizosphere of *A. glauca* and 19 AMF representing seven genera in the rhizosphere of *V. jatamansi*. The AMF colonization varied between 55.63-86.34% in the roots of *A. glauca* and 55.23-78.74% in *V. jatamansi*. The Spore Density (SD) in the rhizosphere soil of selected medicinal plants was highest during the winter season. The rhizosphere soil of *A. glauca* exhibited a rich diversity of AM fungi during the rainy season. On the other hand, in various seasons and locations, the maximum diversity of AM fungi was observed during the summer season in *V. jatamansi*. The genera–*Glomus* and *Acaulospora* had the highest species in both study sites.

Keywords: AMF, *Angelica glauca*, mycorrhiza, Northwest Himalaya, *Valeriana jatamansi*

INTRODUCTION

India is endowed with a rich wealth of medicinal plants. Although various medicinal plants are found throughout the country, Indian Himalayan Region is highly significant concerning varietal richness. *Angelica glauca* Edgew and *Valeriana jatamansi* Jones (Indian valerian) are two valuable medicinal plants in the family Apiaceae and Caprifoliaceae, respectively. These families were native to the North-Western (NW) Himalayas and are in high demand in the local market and the herbal and pharmaceutical sectors. *A. glauca* is found in the Himalayan northern temperate to alpine zones in the altitudinal range of 2,000-4,000 masl (Butola and Badola 2004). *V. jatamansi* is found in the North-Western Himalayan region at elevations of 3,000 masl but also reported between 1,500-1,800 meters above sea level (masl) from Khasi and Jaintia Hills (Bhardwaj et al. 2021).

Angelic acid, valeric acid, and Angeline resin are bitter furocoumarins found in the roots of *A. glauca* (Blake 2004; Butola and Vashistha 2013). They treat dyspepsia, infantile atrophy, gastric disorders, dysentery, constipation, menorrhagia, rinderpest, etc. (Joshi 2016). The essential oil of *A. glauca* has antibacterial, antifungal, and radical scavenging activity (Irshad et al. 2011). The *V. jatamansi* also contains valepotriates, non-glycosidic iridoid esters, monoterpenoids (Baby et al. 2005), and acetoxy isovaleric acids, which improve the therapeutic potential of the plant (Kaur et al. 1999). This medicinal herb is also known to have antihypertensive, anticancer, antidyspeptic, analgesic, antidepressant, cytotoxic, antimicrobial, antifungal, antibacterial, anticonvulsive, antispasmodic, laxative, carminative, anti-insomniac, and other pharmacological properties (Yang et al. 2005; Dinda et al. 2009; Dhiman et al. 2020).

The over-extraction of these plants from wild habitats to meet the increasing demand of the pharmaceutical industry is causing a threat to their genetic diversity. Although to meet the ever-increasing industrial need, numerous medicinal plants, including *A. glauca* and *V. jatamansi*, are currently in cultivation. However, the cultivated medicinal plants produce lower-quality secondary metabolites than their in-situ wild equivalents. Therefore, incorporating mycorrhizal fungi during medicinal plant cultivation may enhance their vegetative growth, tolerance to harsh environmental conditions, and secondary metabolite accumulation (Vierheilig et al. 2000; Karagiannidis et al. 2011).

Around 80% of plant species on the earth are known to be associated with arbuscular mycorrhizal fungi (AMF) (Remy et al. 1994; Wang and Qui 2006; Kivlin et al. 2015). Soluble or volatile exudates containing secondary metabolites like flavonoids and phenolics attract the AMF to young roots (Giovannetti and Sbrana 1998). Therefore, before the cultivation of medicinal plants through the artificial inoculation of AMF, assessing the dominant mycorrhizal mycobiota in the selected medicinal plant's rhizosphere region is preferable. Many researchers have

observed the diversity of AMF in the rhizospheres of medicinal and aromatic plants (Koul et al. 2012; Zeng et al. 2013; Song et al. 2019; Kumar and Tapwal 2022). The AMF association has been reported with most medicinal and aromatic plants, and it was observed that host and climatic conditions greatly influenced their diversity. For example, in different locations of Uttarakhand, Gaur and Kaushik (2011) found 16 AMFs associated with *Catharanthus roseus* (L.) G.Don, *Ocimum sanctum* L., and *Asparagus racemosus* Willd.. Ghosh and Verma (2015) evaluated the AMF diversity in the rhizosphere of 54 medicinal plants growing in Purulia's Gar-Panchakot hills, finding greater diversity in the rainy season than in the summer and winter. Verma et al. (2019) studied AMF diversity in the rhizosphere soil of seven ethnomedicinal plants from the Western Himalayas and recorded the association of 23 AMFs.

Bueno de Mesquita et al. (2018) analyzed 177 plant species, including *Angelica grayi* (J.M.Coult. & Rose) J.M.Coult. & Rose, and observed that AMF, Dark Septate Endophytes (DSE), or both colonized 86% of the plants. The most prevalent AMF genera were *Acaulospora* and *Entrophospora*, but *Archaeospora*, *Claroideoglomus*, and *Glomus* were also recorded. Although the AMF relationship has been thoroughly researched with numerous medicinal and aromatic plants, little material is accessible concerning *A. glauca* Edgew. and *V. jatamansi* Jones. In the current study, we identified 24 and 19 AMF from the rhizosphere of *A. glauca* and *V. jatamansi*, respectively. In addition, the diversity indices of AMF in rhizosphere soil and root colonization were also investigated.

MATERIALS AND METHODS

Sample collection

Rhizosphere soil and roots of *A. glauca* and *V. jatamansi* were collected from two sites viz.: Site-I: Dhrudi (31°14′49.55″ N and 077°28′50.64″ E, 2547 masl) in Shimla district, and Site-II: Chhikkadhar (32°12′02.13″ N and 077°15′24.44″ E, 2964 masl) in Kullu District of Himachal Pradesh, India (Figure 1). The samples were collected during the rainy, winter and summer, seasons from the rhizosphere of five plants and prepared one composite sample. Three composite samples were collected from each site in each season. The soil pH and EC ranged from 6.4-6.9 and 168.2-188.6 (dS/m), respectively.

Arbuscular mycorrhizal spore isolation and identification

Wet sieving and decanting were followed to extract AM spores (Gerdemann and Nicolson 1963). First, 20 g soil was air-dried, suspended in 1000 mL water, agitated for 10 minutes, and undisturbed for 1 hour to allow heavier particles to settle down. Next, the soil suspension was decanted through a succession of sieves with pore sizes of 700 µm, 250 µm, 75 µm, and 40 µm in descending order of pore size. The material retained on the second, third, and fourth sieves was collected in Petri dishes and examined using a stereomicroscope (Nikon SMZ 1500). Finally, the AMF was identified by recording morphological features under the Nikon E-400 microscope.

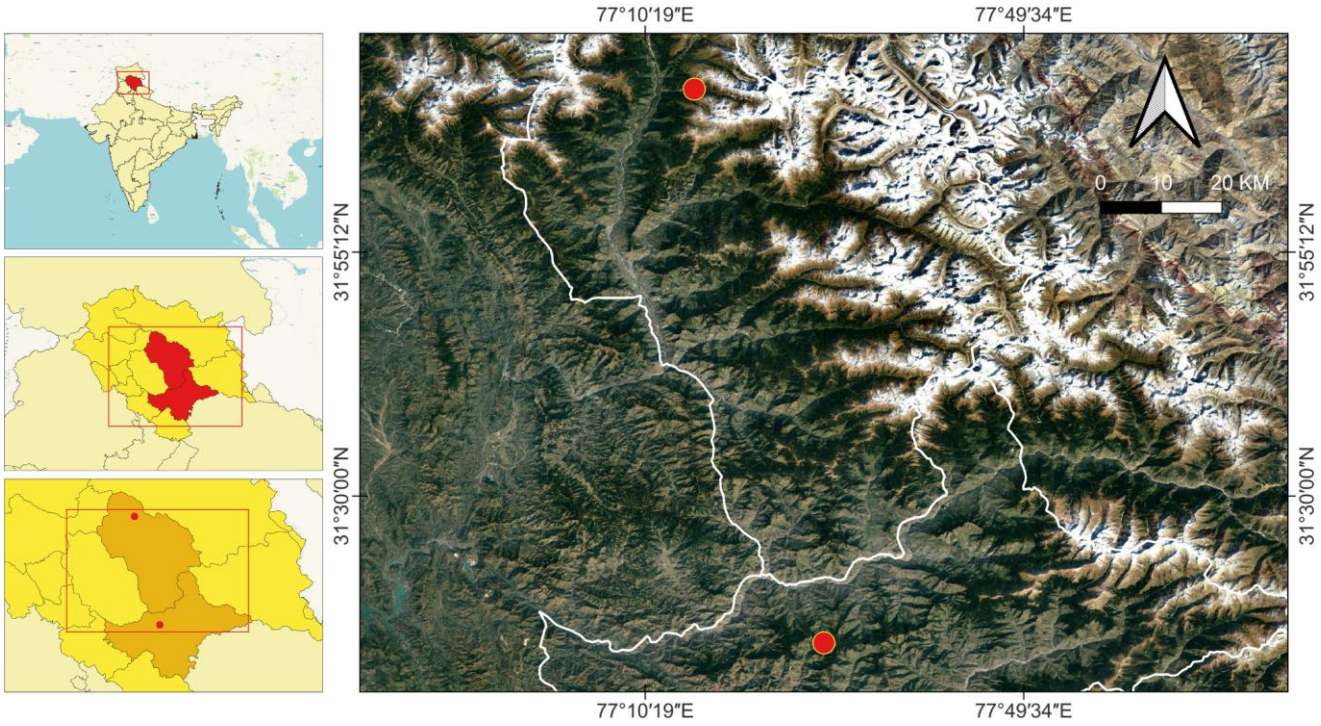

Figure 1. Map of study area in Shimla (31°14′49.55″N, 077°28′50.64″ E) and Kullu (32°12′02.13″N, 077°15′24.44″E) District, Himachal Pradesh, India

Percentage root colonization

Root samples were washed with tap water, sliced into 1 cm lengths, clarified in 10% KOH for 1 hour at 90°C, acidified with 1% HCl, and stained with trypan blue. The grid line intersects method assessed root colonization (Phillips and Hayman 1970; Giovannetti and Mosse 1980). The formula determined the colonization of the roots:

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

Diversity indices: The following formulas were used to determine diversity indices:

$$\textbf{Relative Abundance} = \frac{\text{Numbers of spores of a species}}{\text{Total number of spores}} x\ 100$$

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

Shannon-Wiener Index of Diversity (Shannon 1948) $= -\sum \text{Pi ln Pi}$

Where, Pi = ni/N, where ni is the spore number of a species, and N is the total number of identified spore samples).

Simpson's Index of Dominance (Simpson 1949) = 1-($\sum$n(n-1)/N(n-1)

Evenness (Pielou 1966)$= \frac{H'}{H'max}$

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

$$\textbf{Sorenson's coefficient (Sørensen 1948)} = \frac{2j}{(a+b)}$$

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

The Pearson correlation coefficient was computed using Microsoft Excel, and the relationship between spore density and root colonization, relative abundance, and isolation frequency was determined.

RESULTS AND DISCUSSION

The endomycorrhizal association in *A. glauca* and *V. jatamansi* was confirmed by morpho-anatomical characterization of the roots. Microsclerotia, vesicles, and intracellular hyphal coils were all found in the roots of both species; however, arbuscular were only found in *A. glauca* (Figures 1 and 2). Vesicles are terminal swellings of hyphae that form inter-and intracellularly, with sizes ranging from 5 to 10µm. In the roots of *A. glauca*, AMF colonization ranged from 55.63 to 86.34%, while in the roots of *V. jatamansi*, AMF colonization ranged from 55.23 to 78.74%. In the terse, maximum root colonization was recorded in the rainy season and minimum in the winter at both study sites of these medicinal plants. In addition, dark septate hyphae were seen in the cells of both plants. They formed the microsclerotia or moniliform cells, which were light to dark brown in color, thick-walled, and ranging in diameter from 1-2 µm.

The presence of 24 AMF representing eight genera was identified in the rhizosphere soil of *A. glauca*. *Glomus* had the most species (6), followed by *Acaulospora* and *Funneliformis* (Table 1; Figure 4). Data from this study revealed that Site-I had 7 AMF and Site-II had 6 AMF genera. *Gigaspora* and *Halonatospora* were only found at site I, while *Entrophospora* was only in site II. In all seasons, four AMFs were found: *Funneliformis constrictus*, *F. mosseae*, *Glomus aggregatum*, and *Rhizophagus clarus*. *V. jatamansi*'s rhizosphere soil comprised 19 AMF from seven genera. With nine species, *Acaulospora* was the most common genus, followed by *Glomus* and *Funneliformis* (Table 2, Figure 4). The site-by-site assessment, Sorenson's Index indicated that in rainy (23%), winter (50%), and in summer season 47% of AMF were shared by both sites of *A. glauca*. In the case of V. jatamansi, both sites shared 57% of AM fungi in the rainy season, 14% in winter, and 58% in summer. *Claroideoglomus* and *Rhizophagus* were only found at site I, while *Oehlia* and *Scutellospora* were only at site II. The remaining genera were found at both sites. In all seasons, four AMFs were found: *Acaulospora foveata*, *Acaulospora laevis*, *F. constrictus*, and *Glomus rubiforme*.

The ecological measures like relative abundance and isolation frequency of AMF in the rhizosphere of *A. glauca* and *V. jatamansi* were also studied. The data analysis showed that in *A. glauca*, the *Glomus macrocarpum* species had the highest relative abundance (29.41%) at site I, where the RA (%) ranged from 2-29.41%. At site II, the relative abundance was between 1.92-26.27%, with the highest percentage (29.41%) recorded for *F. constrictus*. On the other hand, the relative abundance of AMF in the rhizosphere of *V. jatamansi* varied between 4.16-25.94% at site I, with the highest RA (25.94%) recorded for *F. mosseae*. At site II, the relative abundance ranged from 1.88 to 22.99%, with the highest value (22.99%) also recorded for *F. mosseae*. Both study sites of medicinal plants showed isolation frequencies ranging from 25 to 100%. At site I of *A. glauca*, the AMF, including *F. constrictus*, *G. ambisporum*, and *G. macrocarpum*, had the highest isolation frequency (100% IF), while at site II, it were *C. etunicatum*, *F. constrictus*, *F. mosseae*, *G. aggregatum*, *G. ambisporum*, and *G. rubiforme*. Additionally, at site I of *V. jatamansi*, the highest IF (100%) was recorded for *F. mosseae*, *F. constrictus*, *G. aggregatum*, and *R. intraradices*. In contrast, at site II, the highest IF (100%) was recorded for *F. contrictus*, *F. mosseae*, and *R. rubiforme* (Tables 1 and 2).

The spore density (SD) in the rhizosphere soil of selected medicinal plants was highest during the winter, i.e., 2.14 and 3.25 in *A. glauca* and 2.64 and 1.65 in *V. jatamansi* at Site-I and Site-II, respectively. At the same time, the minimal SD in *A. glauca* rhizosphere soil was measured during the rainy season at Sites I (1.03) and II (0.5). In comparison, a minimum (1.09) SD was reported at

Site-I in the summer and rainy season (0.96) at site II of *V. jatamansi* (Figure 5).

In both sites, the Shannon-Wiener index of AMF diversity in the rhizosphere soil of *A. glauca* was highest during the summer season (1.81) and lowest in the rainy season (1.71) at Site-I, while it was maximum in the rainy season (1.97) and lowest in winter (1.55) at Site-II. On the other hand, the Shannon-Wiener index of AMF diversity in the rhizosphere soil of *V. jatamansi* was recorded as highest (2.02) during summer and minimum (1.57) in rainy season at Site-I, whereas higher (1.96) in rainy and lower (1.65) in summer season at Site-II (Figure 6).

In the rhizosphere of *A. glauca*, there was maximum (0.82) dominance of species (*G. ambisporum*) in the winter and minimum (0.76) in the rainy season at Site-I. In comparison, high dominance (0.85) of species (*F. constrictus*) was recorded in the rainy season and lowest in winter at site II. The higher dominance (0.85) of species was recorded in the summer season (*F. constrictus*) and lowest in the rainy season at Site-I of *V. jatamansi*, while higher dominance (0.87) of species (*Funneliformis mosseae*) recorded in rainy and low (0.80) in winter season at Site-II (Figure 7).

At both sites, AMF evenness in the rhizosphere soil of *A. glauca* was highest (0.91 and 0.96) in the winter season and lowest 0.75 and 0.79 at Site-I and II, respectively, in the summer season. In the case of *V. jatamansi*, it was highest in the winter (0.94) and lowest in the summer (0.84) at Site-I, whereas it was maximum in rainy (0.94) and minimum in the winter (0.83) season at Site-II (Figure 8).

Table 3 and Figure 9 show the results of an analysis of the data for correlations between root colonization, relative abundance, and diversity indices of selected plants. Data analysis of *A. glauca* root colonization demonstrated a substantial negative correlation with Spore Density (SD) and Isolation Frequency (IF) at both sites. The relative abundance was also found to have a positive correlation with the IF and SD while a negative correlation with the diversity index (H'). In the case of *V. jatamansi*, root colonization is negatively correlated to spore density at both sites. The correlation of relative abundance with SD is positive and negative with H' at both study sites. But the correlation between all the variables is statistically non-significant ($\alpha = 0.05$).

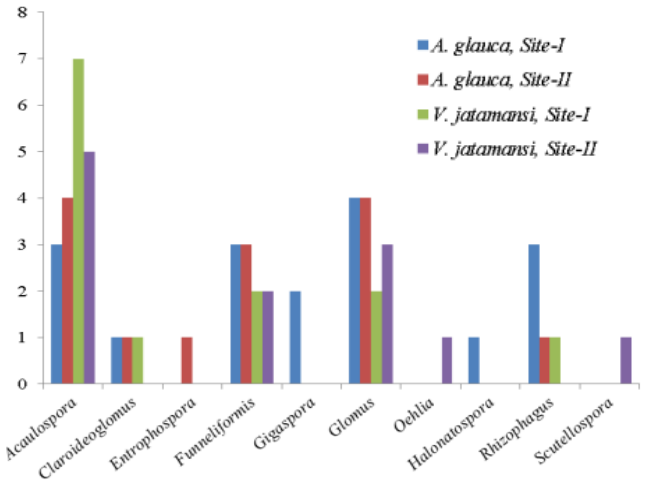

Figure 4. Species representation of AMF

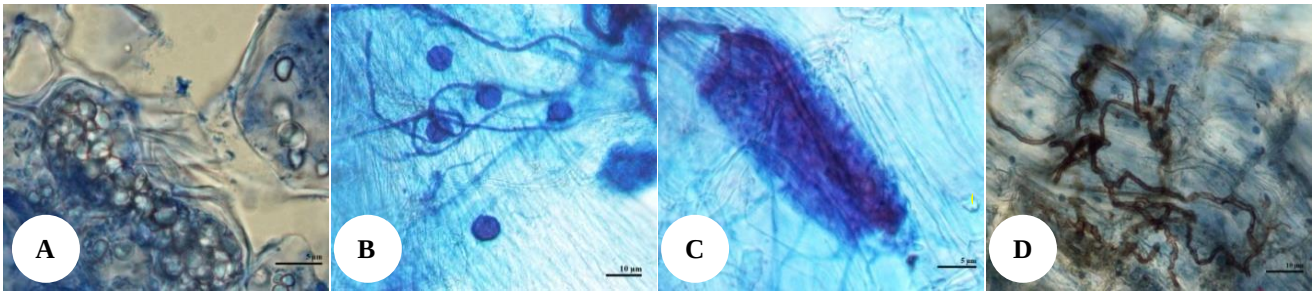

Figure 2. Mycorrhizal association and dark septate hyphae in the roots of *A. glauca*. A. Clusters of microscleridia in the cortical cells, B. Vesicles with extraradical hyphae, C. Arbuscules, D. Dark septate hyphae; scale bar: a, d – 50µm, b, c – 10µm

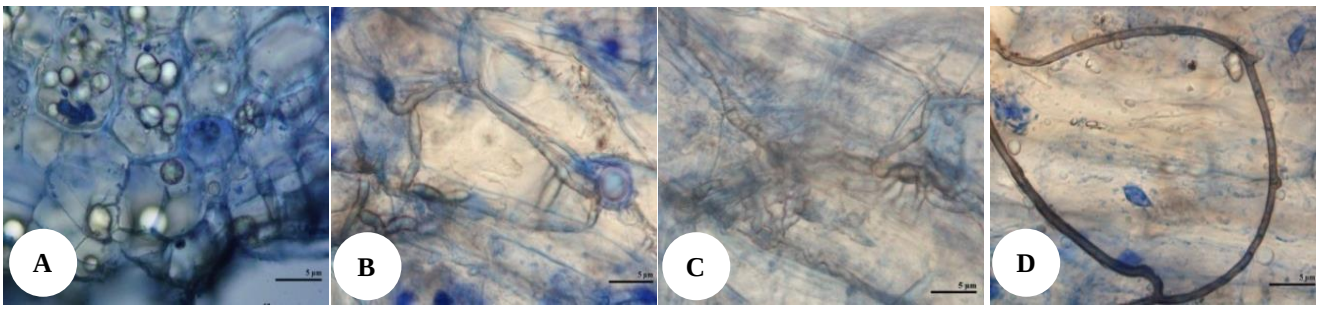

Figure 3. Mycorrhizal association and dark septate hyphae in the roots of *V. jatamansi*. A. Clusters of microsclerotia in the cortical cells, B. H-shaped hyphae, C. Intracellular hyphal coils, D. Dark septate hyphae; scale bar: –10µm

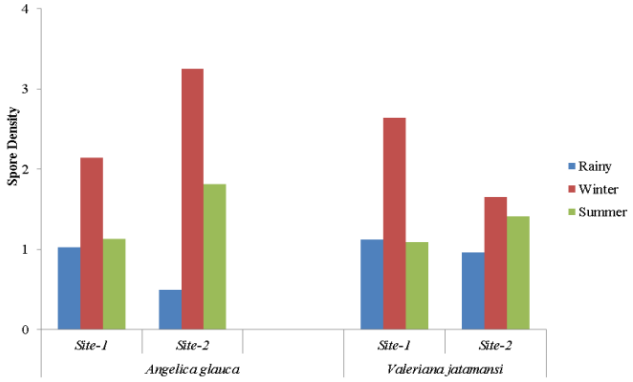

Figure 5. Spore density of AMF

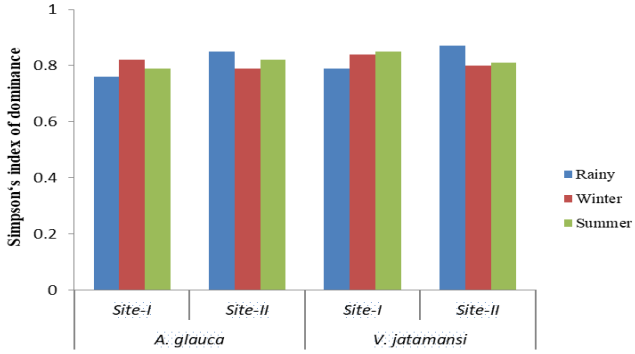

Figure 7. Simpson's Index dominance of AMF

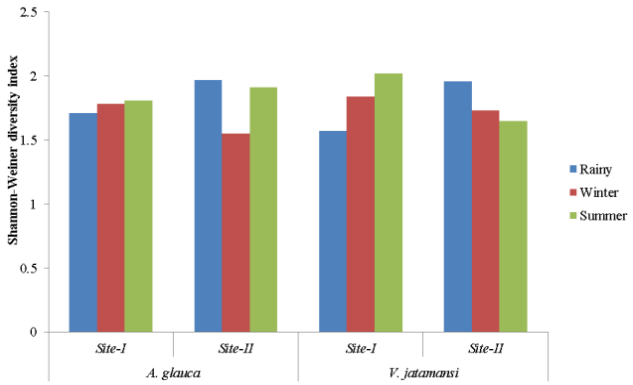

Figure 6. Shannon-Weiner Diversity Index of AMF

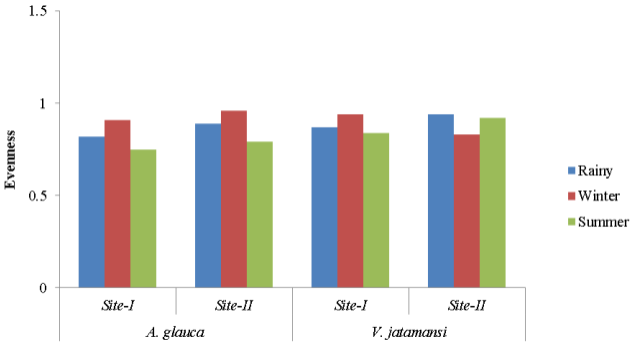

Figure 8. Evenness index of AMF

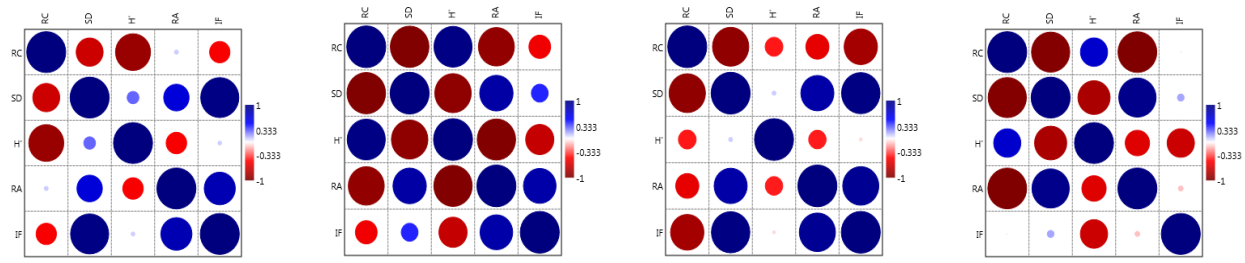

Figure 9. Pearson correlation between different variables (A. Site-I and B. Site-II of *A. glauca*; C. Site-I and D. Site-II of *V. jatamansi*). Note: RC: Root Colonization; SD: Spore Density; H': Shannon-Weiner Diversity Index; RA: Relative Abundance; IF: Isolation Frequency

Table 1. Occurrence, relative abundance, and isolation frequency of AMF in the rhizosphere of *A. glauca*

Name of AMF	Site	Seasonal presence of AMF			RA	IF
		Rainy	Winter	Summer	(%)	(%)
Acaulospora alpina Oehl, Sýkorová & Sieverd.	I	–	–	–	--	--
	II	–	–	+	1.92	25
Acaulospora foveata Trappe & Janos	I	–	+	+	6.5	62.5
	II	+	–	–	3.84	50
Acaulospora lacunosa J.B. Morton	I	–	+	+	3.5	37.5
	II	+	–	+	3.84	50
Acaulospora laevis Gerd. & Trappe	I	–	–	–	--	--
	II	+	–	+	3.84	50
Acaulospora pustulata Palenz., Oehl, Azcón-Aguilar & G. A. Silva	I	–	–	+	2.0	25
	II	–	–	–	--	--
Claroideoglomus claroideum (N.C. Schenck & G.S. Sm.) C. Walker &A. Schüßler	I	+	–	–	9.09	50
	II	–	–	–	--	--
Claroideoglomus etunicatum (W.N. Becker & Gerd.) C. Walker & A. Schüßler	I	–	–	–	--	--
	II	–	–	+	9.61	100
Funneliformis caledonius (T.H. Nicolson & Gerd.) C. Walker & A. Schuessler	I	–	–	+	2.0	25
	II	–	–	–	--	--
Funneliformis constrictus (Trappe) C. Walker & A. Schüßler	I	–	+	+	24.0	100
	II	+	+	+	26.27	100
Entrophospora sp.	I	–	–	–	--	--
	II	–	–	+	1.92	25
Funneliformis geosporum (T.H. Nicolson & Gerd.) C. Walker & A. Schluessler	I	–	–	–	--	--
	II	+	–	+	5.76	62.5
Funneliformis mosseae (T.H. Nicolson & Gerd.) C. Walker & A. Schuessler	I	+	+	+	23.37	91.66
	II	+	+	+	21.15	100
Gigaspora albida N.C. Schenck & G.S. Sm.	I	+	–	–	6.06	50
	II	–	–	–	--	--
Gigaspora decipiens I.R. Hall & L.K. Abbott	I	+	–	–	6.06	25
	II	–	–	–	--	--
Glomus aggregatum N.C. Schenck & G.S. Sm.	I	+	+	+	7.13	58.33
	II	+	–	–	7.69	100
Glomus ambisporum G.S. Sm. & N.C. Schenck	I	–	+	+	33.0	100
	II	+	–	+	18.26	100
Glomus glomerulatum Sieverd.	I	–	–	–	--	--
	II	–	+	+	8.88	75
Glomus macrocarpum Tul. & C. Tul.	I	+	–	–	29.41	100
	II	–	–	–	--	--
Glomus microcarpum Tul. & C. Tul.	I	+	–	–	6.06	25
	II	–	–	–	--	--
Glomus rubiforme (Gerd. & Trappe) R.T. Almeida & N.C. Schenck	I	–	–	–	--	--
	II	–	+	–	24.61	100
Halonatospora pansihalos (S.M. Berch & Koske) Błaszkowski, Niezgoda, B.T. Goto & Kozłowska	I	+	–	–	6.06	25
	II	–	–	–	--	--
Rhizophagus clarus (T.H. Nicolson & N.C. Schenck) C. Walker & A. Schuessler	I	–	+	+	7.0	50
	II	+	+	+	8.96	75
Rhizophagus irregularis (Błaszk., Wubet, Renker & Buscot) C. Walker & A. Schüßler	I	–	–	+	2.0	25
	II	–	–	–	--	--
Rhizophagus fasciculatus (Taxt.) C. Walker & A. Schüßler	I	–	–	+	6.0	50
	II	–	–	–	--	--

Note: Site-I: Dhrudi, Site-II: Chhikkadhar, RA: Relative Abundance, IF: Isolation Frequency, +: Present, −: Absent, --: Not applicable

Table 2. Occurrence, relative abundance, and isolation frequency of AMF in the rhizosphere of *V. Jatamansi*

Name of AMF	Site	Seasonal presence of AMF			RA	IF
		Rainy	Winter	Summer	(%)	(%)
Acaulospora alpina Oehl, Sýkorová & Sieverd.	I	–	–	+	4.16	50
	II	–	–	–	--	--
Acaulospora excavate Ingleby & C. Walker	I	–	–	–	--	--
	II	–	+	–	1.88	25
Acaulospora foveata Trappe & Janos	I	–	+	+	4.41	62.5
	II	+	+	+	9.84	6.66
Acaulospora lacunosa J.B. Morton	I	–	+	+	6.16	75
	II	+	–	+	7.63	50
Acaulospora laevis Gerd. & Trappe	I	+	+	+	7.25	75
	II	+	–	+	7.77	50
Acaulospora mellea Spain & NC. Schenck	I	–	–	+	2.08	25
	II	–	–	–	--	--
Acaulospora rehmii Sieverd. & S. Toro	I	–	–	+	2.08	25
	II	–	–	–	--	--
Acaulospora rugosa J.B. Morton	I	+	–	–	11.11	50
	II	–	–	–	--	--
Acaulospora spinosa C. Walker & Trappe	I	–	–	–	--	--
	II	–	+	–	3.77	50
Claroideoglomus etunicatum (W.N. Becker & Gerd.) C. Walker & A. Schüßler	I	–	–	+	4.16	50
	II	–	–	–	--	--
Funneliformis constrictus (Trappe) C. Walker & A. Schüßler	I	+	+	+	25.68	100
	II	+	–	+	19.82	100
Funneliformis mosseae (T.H. Nicolson & Gerd.) C. Walker & A. Schüßler	I	+	+	–	25.94	100
	II	+	–	+	25.99	100
Glomus aggregatum N.C. Schenck & G.S. Sm.	I	+	–	+	16.54	100
	II	–	–	–	--	--
Glomus macrocarpum Tul. & C. Tul.	I	–	–	–	--	--
	II	+	+	–	11.71	75
Glomus rubiforme (Gerd. & Trappe) R.T. Almeida & N.C. Schenck	I	+	+	+	14.24	75
	II	+	–	+	19.96	100
Glomus versiforme (P. Karst.) S.M. Berch	I	–	–	–	--	--
	II	+	+	–	17.77	87.5
Oehlia diaphana (J.B. Morton & C. Walker) Błaszk., Kozłowska, Niezgoda, B.T. Goto & Dalpé	I	–	–	–	--	--
	II	–	+	–	3.77	25
Rhizophagus intraradices (N.C. Schenck & GS Sm.) C. Walker & A. Schüßler	I	–	+	+	14.35	100
	II	–	–	–	--	--
Scutellospora sp.	I	–	–	–	--	--
	II	–	+	–	5.66	75

Note: Site-I: Dhrudi, Site-II: Chhikkadhar, RA: Relative Abundance, IF: Isolation Frequency, +: Present, –: Absent, --: Not Applicable

Table 3. Pearson correlation of root colonization and relative abundance with diversity indices

	SD		H'		IF	
	Site-I	Site-II	Site-I	Site-II	Site-I	Site-II
Angelica glauca						
Root colonization	-0.69	-0.98	-0.89	0.97	-0.52	-0.55
Relative Abundance	0.65	0.85	-0.52	-0.98	0.79	0.83
Valeriana jatamansi						
Root colonization	-0.93	-0.98	-0.45	0.69	-0.85	0.025
Relative Abundance	0.84	0.95	-0.44	-0.62	0.92	-0.12

Note: at $\alpha = 0.05$

Discussion

The diversity and population of AMF in the rhizosphere of medicinal plants play a vital role in their growth and accumulation of secondary compounds of therapeutic and pharmacological value. *A. glauca* and *V. jatamansi*, two important medicinal plants of temperate Himalaya, were investigated for the mycorrhizal association in roots and diversity of AMF in the rhizosphere soil. The distribution of AMF has not exhibited a consistent trend in the selected sites, but the genus *Glomus* and *Acaulospora* had the highest number of species in both study sites. The *Glomus* species are most widely distributed and considered a cosmopolitan presence in many ecosystems (Sýkorová et al. 2007). Their wide adaptability of sporulation patterns in varied environmental conditions adds to their wide distribution in different geographical regions (Stutz et al. 2000). They dominate habitats in various climatic conditions, from tropical to cold temperate regions (Suresh and Nelson 2015). Previously, the genus *Glomus* was reported to be dominant with numerous medicinal plants (Selvaraj et al. 2001). *Acaulospora* species are regarded as facultative symbionts with a wide host range. They are also suited to various soil conditions and can be found in various nutrient-rich soils (Shepherd et al. 1996, Straker et al. 2010). The comparatively low abundance of *Acaulospora*, which is more frequent in acidic soils, could potentially be due to high soil pH (Wang et al. 2019). The study also identified AMF genera with low species abundance. That suggests these species are likely to be weak competitors in colonizing the roots of selected medicinal plants, resulting in a lower frequency of occurrence. Major genera's occurrence may be attributed to their high competitive interaction and adaptability, allowing them to develop better than other AMFs (Singh et al. 2010).

The diversity (Shannon-Weiner Diversity Index) of AMF in the rhizosphere soil of *A. glauca* and *V. jatamansi* was highest during the summer and lowest in the rainy season at Site-I. In comparison, rich diversity was recorded in the rainy season at Site-II. Both environmental factors and host plant species influence the diversity of soil fungal communities. The higher AMF diversity in the summer could be related to the harsh environmental conditions experienced by the host plant, which encourages the development of chlamydospores by AMF. Low moisture in the rhizosphere soil creates drought-like conditions, potentially affecting the composition and dominance of AMF populations. In addition, seasonal variations in AMF diversity were observed in selected medicinal plants and study sites. Seasonal variation has a substantial impact on the occurrence of AMF (Mallesha and Bagyaraj 1991). The host and seasons are major factors determining the spore density and species richness of AMF in natural settings (Su et al. 2011). AMF species' abundance is known to be affected by disturbance, sporulation efficiency, and dormancy (Walker et al. 1982; Zhao 1999).

Simpson's Index, relative abundance value, and percent isolation frequency were also used to describe the community structure of AM fungi associated with medicinal plants during different seasons, providing additional ecological diversity measures. These measures explain a more comprehensive understanding of the AM fungi community and their dynamics throughout the year. The data analysis revealed the maximum abundance and dominance of AM fungal genera (*Glomus* and *Funneliformis*) belonging to the order Glomarales in the rhizosphere soil of both medicinal plants (*A. glauca* and *V. jatamansi*). The predominance of Glomerales is due to their efficient sporulation and infective efficacy (Redecker et al. 2013), or it might also be due to the phenology of the host plants (Liu and Wang 2003; Bauer et al. 2020). It has been reported that low pH (5.5-6.5) favors the production of more spores by *Glomus* species (Wang et al. 1993). Moreover, several studies worldwide reported Glomerales members' predominance in medicinal plants' rhizosphere (Thapa et al. 2015; Wang and Jiang 2015; Verma et al. 2019; Kumar and Tapwal 2022). The species were found more evenly distributed in the winter season at both the sites of *A. glauca*. Whereas, in the case of *V. jatamansi*, the AMF was more equally spread in winter and summer at sites-I and II, respectively. As discussed earlier that several factors can influence the distribution and composition of arbuscular mycorrhizal (AM) fungi, including soil type, texture, temperature, moisture, host plant, disturbance, as well as nutrient availability (Hawkes et al. 2011; Martinez-Garcia et al. 2015; Bauer et al. 2020).

At both sites of selected plants, maximum spore density and low root colonization were observed during the winter season. Due to good vegetative growth in favorable environmental conditions, less spore density was reported on average throughout the rainy season. Root colonization is poor during cool and dry conditions, while sporulation is high (Moreira et al. 2006). Sporulation occurs during the dry season due to root senescence by the possibility of considerable root turnover, particularly in annuals or competitions (Sitienei et al. 2015). Spores germinate quickly during the wet season or disintegrate due to high moisture and may be destroyed by microbes, reducing their number (Guadarrama and Avarez-Sánchez 1999; Cuenca and Lovera 2010; Sitienei et al. 2015). The host species also influence AMF spores' density (Varela-Cervero et al. 2016); rather than the AMF species, the host plant species and environmental conditions control the spore abundance of AMF (Koske and Halvorson 1981). AMF spore output is known to vary considerably among ecosystems. It is regulated by various parameters, including habitat, host, fungus, and spore density, which tends to rise during root inactivity or senescence (Muthukumar et al. 2003). The uneven spatial distribution of AMF spores and the complex structure of the underground root component could be key variables impacting AMF spore density (Zhao et al. 2001). Earlier research has shown that environmental conditions and vegetation play a significant role in the makeup of AMF communities (Brundrett 1991). The density of propagules varies from plant to plant and site to site (Allen and Allen 1980).

Root colonization was highest during the rainy season and lowest during the winter at all *V. jatamansi* and *A. glauca* sampling sites. There may be a strong link between soil moisture and AMF root colonization. Zangaro et al.

(2013) also recorded higher mycorrhizal colonization in fine roots during spring and summer than in the fall and winter. Kumar et al. (2013) recorded similar results in the rainy season. Some researchers believe seasonal precipitation positively impacts root growth, leading to AMF spore germination and colonization (Oliveira 2001; He et al. 2002). The data analysis revealed that at sites I and II, there was a negative correlation between root colonization percentage and density in the selected medicinal plants (Figure 9). This could indicate that when the number of spores increases, root colonization decreases and vice versa. Radhika and Rodrigues (2010) and Urcoviche et al. (2014) have also recorded a negative correlation between AMF root colonization and spore density in medicinal plants.

In conclusion, the study was primarily focused on the seasonal diversity of AMF in the rhizosphere *A. glauca* and *V. jatamansi* and their root colonization by AMF. Furthermore, 24 (twenty-four) and 19 (nineteen) AMF were identified from the rhizosphere soil of *A. glauca* and *V. jatamansi*, respectively. *Glomus* and *Acaulospora* were the dominant AMF genera in both study sites. The degree of colonization of roots and spore density in rhizosphere soil varied in both study sites and during sampling seasons.

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