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ARBUSCULAR MYCORRHIZAL FUNGAL DIVERSITY ASSOCIATED WITH *CATHARANTHUS ROSEUS* ALONG AN ALTITUDINAL GRADIENT IN SHIMLA HILLS

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ABSTRACT

Physiological performance of plants, particularly plant productivity and crop quality depend on the rhizospheric characteristic feature, an area of huge relevance to plants, producers, consumers and environmental health. Among the rhizosphere components, arbuscular mycorrhizae fungi (AMF) are one of the most common types of symbiotic associations between some rhizosphere microorganisms and plant roots. The present study was conducted to investigate the comparative analysis of fungal status in rhizosphere of *Catharanthus roseus* from three selected sites in Shimla (HP). AM root colonization ranged from 53 to 63.3% and AM spore count in rhizospheric soil varied from 85 to 115. Maximum AM colonization and spore count were observed at Site-III (2300mts) i.e., 63.3 and at Site-I (2200mts) i.e., 115, respectively. Fourteen different of species of AM fungi belonging to five genera i.e., *Glomus, Acaulospora, Sclerocystis, Endogone, Dentiscutata* were reported. The study confirmed the diversity of AMF in *C. roseus* along different altitude.

KEY WORDS: Arbuscular mycorrhizae fungi, *Catharanthus roseus,* Shimla, Rhizosphere

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INTRODUCTION

Traditional systems of medicine have established the landmark health care throughout the world since the earliest days of humanity, and still continue to be widely practiced with greater extend universally. Plants have provided a source of stimulus for novel drug compounds, as plant derived medicines have made large beneficence to human health welfare and well being. The Himalayas have a great abundance of traditional medicinal knowledge. Several studies have been accomplished for the use of medicinal plants in the northwestern Himalayan region and particularly the state of Himachal Pradesh.[1] The climatic conditions prevailing in the region maintain a supreme habitat for the natural growth of different medicinal plants and herbs. The secondary metabolites are also known to play a vital role in the modification of plants depending on the environment and also represent an important agent of pharmaceuticals.[2] Elicitation is a process of enhancing the synthesis of secondary metabolites by the plants to ensure their survival, persistence and competitiveness.[3] Elicitors can be biotic and abiotic elicitors. Biotic elicitors basically alienated from bacteria, fungi, viruses and plant cell wall components. Arbuscular Mycorrhizal Fungi (AMF) in the roots of several medicinal plants help the plants to acquire mineral nutrients from the soil.[4] The inoculation of AMF and other soil favoring microorganisms significantly increased the biomass of different medicinal plants.[5] The main advantage of mycorrhiza to the host plants is the extension of the penetration zone of the root fungus system. AMF has been found to stimulate growth, improve pathogen resistance, devote to design proper soil structure as well as influence the level of secondary metabolites in plants.[6] In this study, we analyzed the relationship between the biodiversity of AMF in the rhizospheric soil of *Catharanthus roseus,* an important medicinal plant, along an altitudinal gradient considering the altitude as possible interrelated factors.

MATERIALS AND METHODS

Sampling area

The samples were collected from three sites from each study area: I (Boileauganj; 2000mts), II (Summer hill; 2200mts) and III (Chauda Maidan; 2300mts) (Fig. 1) in Shimla district, Himachal Pradesh. Shimla is located in the south-western ranges of the Himalayas at 31.61°N 77.10°E. Its average altitude is of 2,206 metres (7,238 ft) above mean sea level. The climate of Shimla is generally sub-temperate, semi-humid characterized by cold winter with mild summer and moderate rainfall.

Figure 1
Map of sampling area

Collection of soil samples

Soil samples along with secondary and tertiary roots of three individuals were collected from the rhizospheric soil of *Catharanthus roseus.* Soil samples were air dried and stored at 4ºC for processing. The soil samples were analysed for soil physio-chemical parameters, isolation of Arbuscular Mycorrhizal (AM) spores and studying mycorrhizal root colonization.

Isolation and quantification of AM spores

AM spores were extracted from the collected soil samples by using 'Wet Sieving and Decanting Technique[7] and quantified by 'Grid Line Intersect Method' to obtain viable and debris free AM spores.[8] The photographs of the counted spores were taken with the help of Nikon coolpix S6200 with an adapter tube.

Identification of AM fungi

Identification of AM spores was done on the basis of colour, size, shape, wall structure, surface ornamentation of spores, size of hyphae, bulbous suspensor, number and arrangements of the spores in the sporocarps. These AM spores were identified by using the key.[9]

Estimation of AM root colonization studied by "Rapid Clearing and Staining Method".[10]

Physico-chemical analysis of soil

Soil samples were analyzed for following physicochemical parameters (Table 1).

Table 1
Physio-chemical properties of soil

S.No.	Area	Altitude (mts)	Parameter					
			pH	EC (ds/m)	TDS (mg/l)	AK (kg/ha)	AN (kg/ha)	OC (%)
1	BoileauGanj	2000	6.31±0.26	0.09±0.01	0.08±0.01	856.96±0.88	1401.8±1.71	1.59±0.03
2	SummerHill	2200	6.69±0.15	0.07±0.003	0.05±0.001	892.33±1.85	1222.1±0.63	0.55±0.02
3	ChaudaMaidan	2300	7.18±0.09	0.09±0.01	0.08±0.01	802.5±1.04	1069.1±1.61	2.49±0.59

EC –Electric conductivity,TDS-Total dissolved solids,
AN- Available Nitrogen, AK –Available potassium,
OC- Organic Carbon

Statistical Analysis

Ecological measures of diversity used to describe the structure of AMF communities included spore density, species richness, relative abundance ,isolation frequency, Shannon -Wiener index of diversity, Simpson's index of dominance (Table 2).[11] The Pearson correlation coefficient was used to determine the relationship between spore density and species richness, relative abundance and isolation frequency. The data was analysed with the help of SPSS software version 20.

Table 2
Diversity measures used to describe AM communities

Spore density (SD)	The number spores in 50gm soil
Species richness (SR)	Number of identified AMF species per soil sample
Relative abundance (RA)	$\frac{Spores\ number of\ a\ species\ (genus) \times 100}{Total\ number\ of\ identified\ spore\ samples}$
IF (Isolation Frequency)	$\frac{The\ total\ soil\ samples\ in\ which\ AMF\ species\ occured \times 100}{The\ total\ number\ of\ soil\ samples}$
Simpson's index of dominance	D=Σ(ni/N)2
Shannon-Wiener index of Diversity(H')	H'= -ΣPi lnPi

Pi is the relative abundance of each identified species per sampling site calculated by the following formula, Pi=ni/N,where ni is the spore numbersof a species and N is the total number of identified species per sampling sites

RESULT AND DISCUSSION

Diversity of AM fungal spores and Colonization with respect to altitudinal variation

In the present investigation, biodiversity of AMF (AM colonization, AM spore and AM diversity) associated with *C. roseus* medicinal plants collected from three different sites of district Shimla Himachal Pradesh were studied. A total of 4640 spores were sieved from rhizospheric soil of study sites. In this investigation the species richness was maximum at Boileauganj (SR- 7%, SD-550/50gm soil) (Table 4). However the spore density was minimum when compared to Summerhill (SR-6%, SD 595/50gm soil). Spore density and spore richness were found to be less in Chauda maidan (SR 4%, SD-360/gm soil). Corrrelation analysis demonstrated that spore density of AMF species was positively correlated with species richness (SPSS software version 20.0, r=.823) (Fig. 4(a)). Based on the relative abundance and isolation frequency it was observed *Glomus macrocarpum* (Fig.2 (i)) (was dominant (18.62% of RA) followed by *Glomus mosseae* (Fig.2 (j)) (16.16% of RA) and *Glomus geosporum* (Fig.2 (g)) (14.00% of RA). However, *Glomus mosseae* contributed to greater isolation frequency (70%) and widely distributed followed by *Glomus geosporum* (60%), *Glomus macrocarpum* (50%).Correlation analysis revealed that there was a positive correlation betweeen relative abundance and relative frequency (SPSS software version16.0,(r=.924) (Fig.4 (b)). This finding suggested that AMF colonization widely varied among same medicinal plant *C. roseus* collected from different study sites. Shannon-Wiener Index of diversity (H') and Sampson's index of dominance (D) showed a greater diversity (Table 4). Variation in spore density and percentage colonization encompassed by different sampling sites assigned to host specificity, edaphic and climatic conditions.[12] Table 5 reveals that percentage of AMF colonization of *C. roseus* varied to some extent at different study sites. Highest infection rate of 63% was recorded at Boileauganj and lowest infection rate of 52% at Chauda maidan. In case of AM root colonization, root samples of *C. roseus* medicinal plant collected from three different sites of Shimla showed less range of variation as showed in Table 7. AMF root colonization ranged from 53±8 to 63.3±0.08%. It is speculated from the result (Table 5) that maximum AMF root colonization was found at Chauda maidan (63.3±0.08) and least was observed at Boileauganj (53±8). Similiarly, at Summerhill (63±0.08) AMF colonization were found. This result illustrated that mycorrizal infection varied within the same family (Apocynaceae). The population of AM spore in rhizospheric soil of *C. roseus* of three different areas varied to some extent. The density of AM spore varied from85±3.54 and 115± 5.0. The maximum number of population was recorded in the rhizospheric soil of Summerhill 115± 5.0.and it is followed by Summerhill 104±9.62, 103±12.04. The minimum number of AM spore population was recorded at Chauda maidan 96.0±9.62 and fillowed by (85±3.54). Different AM spores isolated from rhizosphere of studied medicinal plant *C. roseus.* It can be envisaged from Table 6 that fourteen different species of AM fungi belonging to five genera i.e., *Glomus, Acaulospora, Endogone, Dentiscutata, Sclerocystis* were screened from rhizospheric soil of different sites. *Glomus* was found to be dominant genera (7 species) followed by *Acaulospora* (4 species), *Sclerocystis, Dentiscutata, Endogone* (1 species). AM fungi belonging to genus *Glomus* are most dominant. Among different species of *Acaulospora*, the most frequent and most abundant species reported in medicinal plants at different sites was *A. laevis* (4) (Fig.2 (c)) followed by *A. foveata* (3) (Fig.2 (b)). Similarly, among *Glomus* species, *G. geosporum* was reported in seven sites followed by *G. mosseae* (3). Endogone species reported at eight sites. In case of *Sclerocystis* (5), *Dentiscutata* (1), recognised in medicinal plants *were Sclerocystis ceremoides* (5) (Fig. 2 (c)) and *Dentiscutata nigra* (1).Along the altitudinal gradient, fourteen species were found (Table 6). The highest mean value of spore density was 7.66spore/500 ml of rhizospheric soil. The highest absolute values (7.66spore/500ml) for AMF richness (fourteen species) were found at the lowest altitude (2000 mts). The lowest values were found at highest altitudes (2300 mts). In addition, the *Glomus geosporum* (Fig 2 (g)), *Endogone* species (Fig 2 (h)) and *Dentiscutata nigra* (Fig 2 (e)) were three species found at all altitudinal level along the three studied gradient. The abundance of *Glomus geosporum* clearly decreases with increasing altitude.

Table 3
Relative abundance (RA) and Isolation frequency of AM rhizosphere soil Catharanthus roseus

S. No	AMF SPECIES	Relative Abundance (RA)%	Isolation Frequency (IF)%
1	*Glomus geosporum*	14.00	60
2	*Acaulospora foveata*	11.85	40
3	*Glomus mosseae*	16.16	70
4	*Acaulospora laevis*	7.02	20
5	*Endogone species*	8.40	30
6	*Glomus intraradices*	14.90	60
7	*Glomus macrocarpum*	18.62	50
8	*Acaulospora tuberculata*	11.91	40
9	*Acaulospora delicate*	12.71	50
10	*Dentiscutata nigra*	9.37	30
11	*Sclerocystis ceremoides*	11.85	40
12	*Glomus scintillans*	6.35	30
13	*Glomus reticulate*	3.23	20
14	*Glomus taiwanense*	0.54	10

Table 4
Diversity measurement of AMF community

Factors	Boileauganj			Summer Hill			Chauda Maidan		
	I	II	III	I	II	III	I	II	III
Sporedensity(SD)	550	515	310	525	290	595	360	390	450
Species richness(SR)	7	5	3	5	2	6	4	3	3
Shannon-Wiener index of diversity (H')	0.8198	0.7062	0.6180	0.1860	1.0600	1.0314	3.3440	1.0614	1.0232
Sampson's index of dominance (D)	0.1200	0.1387	0.1421	0.5084	0.5444	0.2597	0.1998	0.2280	0.1836

Table 5
Mycorrhizal studies of AMF roots of Catharanthus roseus

S. No	Study Area	AMF spore count	AM colonization in root samples (%)	No. of Arbuscules per root samples
1	BOILEAUGANJ (2000mts)	101±5.79	63±0.08	6
		102±16.81	59±0.08	7
		104±4.30	53±8	4
2	SUMMERHILL (2200mts)	115±5.0	63±0.08	3
		104±9.62	59.9±0.08	5
		103±12.04	60±0.1	6
3	CHAUDA MAIDAN (2300mts)	85±3.54	58±0.09	2
		98.0±7.58	57.3±0.08	5
		96.0±9.62	63.3±0.08	4

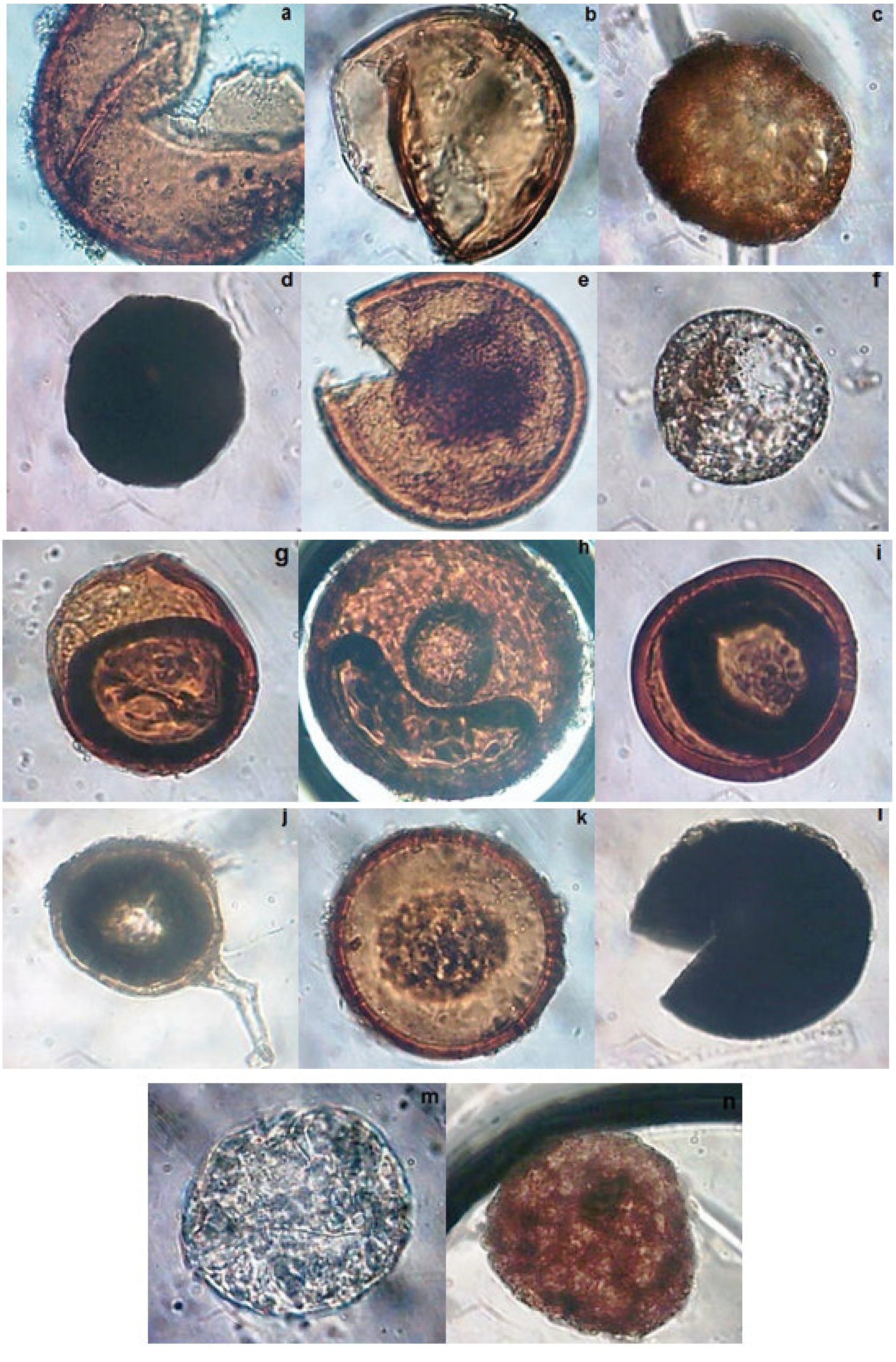

Figure 2
Diversity of AMF spores (a) *Acaulospora delicate*; (b) *Acaulospora foveata*; (c) *Acaulospora laevis*; (d) *Acaulospora tuberculata*; (e) *Dentiscutata nigra*; (f) *Endogone* species (g) *Glomus geosporum* (h) *Glomus intraradices;* (i) *Glomus macrocarpum*; (j) *Glomus mosseae*; (k) *Glomus reticulata*; (l) *Glomus scintillans*; (m) *Glomus taiwanense*; (n) *Sclerocystis ceremoides*

Table 6
Specific density of AMF spores along altitudinal gradient

S. No	AMF SPECIES	BOILEAUGANJ (2000mts)	SUMMERHILL (2200mts)	CHAUDA MAIDAN (2300mts)
1	*Glomus geosporum*	7.66±1.15	1.33±0.88	1±0.57
2	*Acaulospora foveata*	2±1	0	1.66±0.88
3	*Glomus mosseae*	1±10.57	0	0
4	*Acaulospora laevis*	2.33±0.33	0	0
5	*Endogone species*	7±1.52	7±1.76	1.33±0.66
6	*Glomus intraradices*	0	0	2.66±1.45
7	*Glomus macrocarpum*	0.66±0.66	1.66±0.88	1.33±0.88
8	*Acaulospora tuberculata*	0	3±1	0.33±0.33
9	*Acaulospora delicata*	1±1	1.66±1.66	0
10	*Dentiscutata nigra*	2.33±2.33	0	0
11	*Sclerocystis ceremoides*	4.33±3.38	5.33±1.20	0
12	*Glomus scintillans*	0.33±0.33	3±1	0
13	*Glomus reticulate*	4±2.08	2.33±1.45	4±.57
14	*Glomus taiwanense*	3±0.57	1.33±0.33	2±1

Data are mean values (n=4-5) and standard deviations of spore number for each AMF species (number of spores for each AMF per 500 ml of rhizospheric soil)

Table 7
Natural occurrence of AM spores with Catharanthus roseus
(1.Glomus geosporum 2. Acaulospora foveata 3. Glomus mosseae 4. Acaulospora laevis 5. Endogone species 6. Glomus intraradices 7. Glomus macracarpum 8. Acaulospora tuberculata 9. Acaulospora delicata 10. Dentiscutata nigra 11.Sclerocystis ceremoides 12.Glomus scintllans 13.Glomus reticulata 14.Glomus taiwanense)

Site	AMF species													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
BOILEAUGANJ (2000mts)	+	-	-	+	+	-	+	-	+	-	+	+	-	+
	+	+	+	+	+	-	-	-	-	-	-	-	+	+
	+	+	-	+	+	-	-	-	-	+	+	-	+	+
SUMMERHILL (2200mts)	-	-	-	-	+	-	+	+	-	-	+	+	+	+
	+	-	-	-	+	-	+	+	-	-	+	+	+	-
	+	-	-	-	+	-	-	+	+	-	+	+	-	-
CHAUDA MAIDAN (2300mts)	+	+	-	-	+	-	+	+	-	-	-	-	+	+
	-	-	-	-	+	+	+	-	-	-	-	-	+	+
	+	-	-	+	-	+	-	-	-	-	-	-	+	+

+ = Present - = absent

From the above results (Table 7) it is clear that biodiversity of arbuscular mycorrhizal fungi deviate from in same plant collected from different sampling sites. A decreasing inclination of hyphal and arbuscular colonization with increasing altitude was observed.[13] Well-developed AM colonization of root reinforces the transportation of organic nutrients from hosts to AMF, thereby recovering hyphal extension and sporulation. Accordingly, in the present study, higher density of AM fungal spores and hyphae was detected at lower altitudes (Boileauganj, 2000 mts). It revealed that soil phosphatase activity is strengthened by an increase in AM colonization, and that AMF may generate extracellular phosphatise.[14] by promoting mineralization of organic phosphorus for host plant uptake. Additionally, the hyphae of AMF provided an elevated area for intercommunication with other plant growth-promoting rhizobacteria, and therefore substitute an important pathway for the translocation of energy-rich plant assimilates to the soil.[15] The dominant AM fungal species (i.e*., G.geosporum, G. Mosseae and G. intraradices*) identified in the present study, are also members of Glomeraceae family.[16] Previously reported that AM fungal species belonging to *Glomeraceae* family recurrently domineer in agricultural soils. Soil properties are heavily affected by altitudinal gradients and this may affect the distribution of AM fungal species.[17] In fact, we perceived a marked variation in soil factors such as OC and AN between different sites of *C. roseus* located at different altitudes; these variations significantly influenced the AM fungal species community structure. Although prominent variations in soil pH were recorded between the different altitudes in our study, the distribution of AM fungal species are certified to be extremely sensitive to soil pH.[18] Different environmental factors both colonization and number of AMF. Altitude is considered as important factor for acting on diversity. Generally, an increase in altitude is tag along a decrease in temperature affecting disparately both fungi and especially AMF.[19] Mycorrhizal inoculation may undeviatingly enhance root water uptake providing requisite water to preserve physiological activity in plants, distinctively under severe drought conditions.[20] Mycorrhizal inoculation also boost the root P uptake, particularly under dry soil conditions.[21] Mycorrhizae, therefore, are feasible to be important for increasing P redemption during drought periods.[22] Moreover, improved drought tolerance and better drought retrieve by mycorrhizal plants has been associated to improved P uptake.[23].Both mycorrhizal types can occur, with a wide variation in degree of colonization, throughout the litter and the soil organic and mineral horizons, partially to a depth of 35 cm.[24] The arbuscular mycorrhizal fungi diversity impact the constitution of the plant community.[25] The presence of different AM fungi can hence influence competitive interactions among plant species and so esteem the plant community composition.[26] Recently, it was showed that AMF abundance, species richness and species diversity varied among different sampling sites, as some of the sites were irrigated and some were non irrigated.[27] AMF spore diversity, density and richness were similar among seasons.[28] Similar the AM colonization and diversity of medicinal plant *C. roseus* from district Shimla (HP) were investigated in the present study. According to research, we could conclude that the biodiversity of AM fungi was abundant, *Glomus* was the dominant genus. The degree of colonization and the spore density discrete markedly among plant species. Taking in account the potential application of AM fungi on medicinal plants, it seems that more attention should be paid to the superlative AM fungi during the process of their cultivation, especially mycorrhizal performance (e.g., improving growth, increasing secondary metabolite production).

CONCLUSION

In conclusion, this study clearly demonstrated that AM fungal root colonization, hyphal extension and sporulation, as well as fungal species diversity associated with *Catharanthus roseus* showed decreasing trends with increase in altitude. Consequently, we have also revealed the AM fungal species distribution pattern is also significantly influenced by other environmental variables such as AK, pH, and AN. Therefore, in order to maximize the benefits of AMF in sustainable *C. roseus*, we propose that organic agricultural practice be employed to improve the microecology of *Catharanthus roseus.*

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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