# Analysis of Vesicular Arbuscular Mycorrhiza Associated with Medicinal Plants in Uttarakhand State of India

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Abstract: This research work analyzes Vesicular Arbuscular Mycorrhiza (VAM) associated with medicinal plants i.e. *Catharanthus roseus*, *Ocimum* species and *Asparagus racemosus* in Himalayan region of Uttarakhand state of India. Soil samples were collected from various locations spread at different altitudes and environmental conditions. The soil and root samples were collected and observed at regular time intervals for a span of three years. In total 16 species of VAM were detected from these three medicinal plants. Approximately more than fifty percent of total species were identified as species of *Glomus*. Species of *Acaulospora* and *Sclerocystis* were isolated from different soil types of Uttarakhand region, but were never recorded as a dominant species. The average number of spores in ascending order obtained from different districts are 90.19 (Pauri), 93.93 (Almora), 118.4 (Haridwar), 119.92 (Udham Singh Nagar), 125.90 (Dehradun) per 10 g of soil. This research work reflected a trend representing decrease in the richness and diversity of VAM fungi with the increasing altitude. VAM fungal spores were primarily isolated as chlamydospores and few as sporocarps. Spores were found in higher abundance from sandy loam soils followed by clay and loam soils. The percentage of root colonization levels ranged from 58.22% to 65.43% for *Catharanthus roseus*, from 76.88% to 95% for *Ocimum spp*. and from 57.21% to 63.32% for *Asparagus racemosus*.

**Key word:** Medicinal plants • Vesicular Arbuscular Mycorrhiza • *Catharanthus roseus* • *Ocimum* and *Asparagus racemosus* 

# INTRODUCTION

A mycorrhiza is a symbiotic (generally mutualistic but occasionally weakly pathogenic) association between a fungus and the roots of a vascular plant. In a mycorrhizal association, the fungus colonizes the host plants' roots, either intracellularly as in arbuscular mycorrhizal fungi (AMF), or extracellularly as in ectomycorrhizal fungi. They are an important component of soil life and soil chemistry. Mycorrhiza is neither the fungus nor the root, but rather the structure formed from these two partners. Since the association is mutualistic, both organisms benefit from the association [1]. The fungus receives carbohydrates (sugars) and growth factors from the plant, which in turn receives many benefits, including increased nutrient absorption [2]. In this association, the fungus takes over the role of the plant's root hairs and acts as an extension of the root system [3]. The potential for manipulating mycorrhizal associations to increase productivity in

plantation forestry, or plant establishment during ecosystem recovery after severe disturbance, are the focus of major research initiatives [4-6]. There is also much interest in their potential utilization in medicinal, agricultural and horticultural crops [7,8]. Vesicular Arbuscular Mycorrhiza (VAM) is present in most medicinal plants, agronomic and vegetable crops. This type is characterized by the presence of arbuscules in the region of the root cortex; vesicle may or may not be present; and they function as reserve organs and also for fungal multiplication. The mechanisms of increased absorption are both physical and chemical. Mycorrhizal mycelia are much smaller in diameter than the smallest root and thus can explore a greater volume of soil, providing a larger surface area for absorption. Also, the cell membrane chemistry of fungi is different from that of plants (including organic acid excretion which aids in ion displacement). Mycorrhizae are especially beneficial for the plant partner in nutrient-poor soils.

VAM fungal infection increases the uptake of Nitrogen [9]. Researchers have paid much attention to the leguminous plants for VAM fungal research on nutrition. It is found that VAM fungi improve phosphorous nutrition of the host that results in better nodulation, nitrogen fixation and growth [10]. For high phosphorous requirement for nodulation, legume species extremely rely on VAM fungal infection at low phosphorous levels.

The Himalayan state of Uttarakhand is very rich in medicinal and aromatic plants [11]. The medicinal plants in the region occur naturally and most of propagate vegetatively by underground them rhizomes, stems and bulbs or corms. Medicinal plants play crucial role for existence of life on the earth. Out of approximately 2,50,000 higher plant species on earth, more than 80,000 are medicinal [12]. This herbal wealth is used by both developing and developed countries for their health care. A bulk of rural population relies on drug resources of plant origin. Locally collected plants are sold, where they are exploited commercially for preparation of medicines.

Three medicinal plants viz. Catharanthus roseus Linn. Ocimum spp. and Asparagus racemosus Willd. were considered for research work, due to their abundance and richness in Uttarakhand state. Moreover, these plants have tremendous medicinal values. The roots of Catharanthus roseus Linn. are sedative, tranquiliser, stomachic and are used as tonic. The leaves of Catharanthus roseus Linn, in form of an infusion are administered in menorrhagia and their juice is good for wasp-stings. It is also used as remedy for diabetes. An extract from the Catharanthus roseus Linn. plant demonstrates growth inhibitory effect in certain human tumours. The plant of Ocimum spp. is considered expectorant, stomachic, diuretic, antiseptic and cardiac stimulant and its decoction is used in catarrh, croup and bronchitis. The roots of Asparagus racemosus Willd. are refrigerant, demulcent, diuretic, aphrodisiac, antiseptic, antidiarrhoeal, antidysentric and galactagogue [11]. The medicinal value of these plants has been utilized since Vedic period. Therefore, this research was carried out to study the VAM fungi related to these medicinal plants.

The medicinal plants from some of these areas have been extensively studied. However, not enough survey has so far been conducted on the mycorrhizal association of medicinal plants. Therefore, an intensive survey of different areas has been conducted in a preliminary attempt to observe the mycorrhizal associations of these plants. The medicinal plants in the present survey were found to be VA mycorrhizal, despite the fact that they have an active principle in them, which is responsible for their medicinal value. Around eighty percent of medicinal plants used worldwide for domestic use, sale and export are harvested in wild state from their natural habitats [13]. Despite of enormous research data available on microbiological aspects and their ecology in diversified habitats in India, there is practically no data on the taxonomy and ecology of vesicular arbuscular mycorrhizal fungi of medicinal plants of Uttarakhand state. However, some randomized information gathered in some areas of Uttarakhand state has been analyzed by some researchers. In order to fill up this lacunae, efforts have been made to understand the existing knowledge of these fungi as well as other aspects like (i) Quantitative and qualitative composition of VAM fungi (ii) Distribution pattern of VAM fungi (iii) Colonization in roots of Catharanthus roseus Linn. Ocimum spp. and Asparagus racemosus Willd. medicinal plants.

## **MATERIALS AND METHODS**

The present research work investigates rhizosphere soil samples and plant samples of three medicinal plant species. The samples were collected from different habitats of Uttarakhand state of India. The study was started in December, 2005 and soil samples were initially collected during January-February 2006. After initial collection, the samples were collected regularly at an interval of 2-3 months.

The soil samples for the present investigation were collected from different parts of Uttarakhand state *viz*. Pauri Garhwal, Haridwar, Dehradun, Almora and Udham Singh Nagar districts. The collection sites were chosen such that the samples represented the complete state in terms of its major division of Garhwal and Kumaon region; and the different height of Himalayan ranges.

Fine roots of plants along with soil samples were collected from these sites. The roots were preserved and later on stained for determination of percent mycorrhizal colonization. Sterlized polythene bags were taken to the site for soil sample collection. Rhizosphere soil samples were collected at the depth of 4-16 cm. These samples were naturally air dried for further experimental analysis.

VAM propagules are isolated from soil using wet sieving and decanting technique. This technique is used to remove the clay and sand fractions of the soil while retaining spores and other similar sized soil and organic matter particles on sieves of various diameters. The VAM fungal spores are analyzed qualitatively by identifying them for their genera and species. The VAM fungal spores collected on filter paper (Whatman filter paper No.1) after wet sieving and decanting technique were observed under Stereoscopic binocular. These spores were picked through needle and mounted in lactophenol on slide. As an alternative, Polyvinyl lactic acid was also used as mounting medium. All slides with spores on mounting medium were observed cautiously under high power research microscope for isolation into genera and followed by species identification. VAM spores were identified using standard monographs given by [14-19] and INVAM (http://www.invam.caf.wvu.edu).

Method of Phillip and Hayman [20] was employed for root staining to find root colonization. The stained roots were examined under the microscope. To observe hyphae, vesicles and arbuscules under light microscope the root pieces were mounted on glass slide temporarily in lactophenol or permanently in polyvinyl alcohol. The cover slip was pressed gently to make the roots flattened and sealed with any of the adhesive materials such as DPX, quick fix or nail polish. The percentage of root colonization is obtained by applying following formula

 $%Colonization = \frac{Total\ no.of\ root\ segments\ colonized}{Total\ no.of\ root\ segments\ examined} X100$ 

#### RESULTS AND DISCUSSION

Periodical survey of various places such as Pauri, Haridwar, Dehradun, Almora and Udham Singh Nagar was undertaken to collect and identify different VAM species associated with medicinal plants. Rhizosphere soil samples collected from various localities revealed presence of several species of different genera. The VAM species identified were: Glomus aggregatum; Glomus fasciculatum; Glomus geosporum; Glomus monosporum; Glomus mosseae; Glomus claroideum; Glomus etunicatum; Glomus coronatum; Glomus intraradices; Glomus macrocarpum; Gigaspora margarita; Gigaspora rosea; Gigaspora gigantea; Sclerocystis sinuosa; Acaulospora scrobiculata; Acaulospora laevis.

In the present study, it was established that 99.5% of the sites accounted for VAM fungal spores. In Catharanthus roseus, presence of Glomus fasiculatum and Glomus mosseae were found to be dominant. However, Glomus aggregatum and Glomus fasiculatum were predominantly present and associated with all the Ocimum species. Glomus coranatum, Glomus mosseae and Sclerocystis species were found to be abundantly associated with Asparagus racemosus. The number of spores of VAM fungi isolated from different sites for the medicinal plant Catharanthus roseus, Ocimum spp. and Asparagus racemosus are given in Table 1. The number of spores ranged from 52 to 197 per 10 g of soil considering all medicinal plants individually under study. The average number of spores from 24 sites contained more than 120 spores per 10 g of soil, whereas 38 and 25 sites contained 100-120 and 80-100 spores per 10 g of soil respectively, while 17 sites contained less than 80 spores per 10 g of soil.

VAM Fungi **Distribution:** Many soil samples were collected from different regions of Uttarakhand state. These samples showed the presence of VAM fungal spores (Table 1). VAM fungi are well distributed throughout Uttarakhand. Maximum numbers of spores were isolated from Dehradun district followed by Udham Singh Nagar, Haridwar, Pauri and Almora. Soil samples collected from hilly terrain showed fewer VAM spores. The occurrence of spores at higher altitudes (above 1700 m) was qualitatively and quantitatively inferior as compared to regions at lower altitude regions.

Soil samples collected from cultivated habitats of Dehradun, Pantnagar, Haridwar, Pauri and Almora had larger number of spores in comparison to un-cultivated sites such as Kyunkaleshwar and Dehradun forests. The study reveals that VAM fungal spores are in abundance in cultivated soil as compared to non-cultivated soils. Species richness of VAM fungi was highest at Dehradun district followed by Haridwar, Udham Singh Nagar, Pauri and Almora.

This study describes the distribution of VAM fungi in the rhizosphere soil of medicinal plants. Both plants and rhizosphere soils were collected during a three year period (2006-2008), at different sites and during different seasons. The average number of spores isolated for different medicinal plants collectively from year 2006 to 2008, from diverse sites has been shown in tabular form

Table 1: Average number of spores per 10 g of soil for host plant Catharanthus roseus, Ocimum spp. and Asparagus racemosus at different sites.

		Avg Spore no.	Avg Spore no.	Avg Spore no.	us at different sites.
Locality	District	in Catharanthus roseus	in <i>Ocimum spp</i> .	in Asparagus racemosus	Overall Avg. no. of spores
Dhari	Pauri	82	102	98	94
Srinagar	Pauri	67	69	74	70
Srikot	Pauri	91	99	92	94
Pauri	Pauri	99	127	110	112
Ghurdauri	Pauri	65	85	72	74
Khandusain	Pauri	92	115	99	102
Kotdwar	Pauri	81	93	72	82
Safdarkhal	Pauri	79	124	85	96
Minthi	Pauri	63	91	71	75
			120	108	
Doggada	Pauri	102 102		99	110
Kaliasaur	Pauri		123		108
Satpuli	Pauri	102	115	104	107
Lansdown	Pauri	118	115	112	115
Buvakhal	Pauri	79	108	95	94
Jwalpadevi	Pauri	69	79	74	74
Patisain	Pauri	99	115	104	106
Gumkhal	Pauri	78	98	82	86
Dandapani	Pauri	79	77	66	74
Kyunkaleshwar	Pauri	52	71	63	62
Kandolia	Pauri	64	94	79	79
Nagdev	Pauri	94	109	103	102
Dhumakot	Pauri	75	70	62	69
Nainidanda	Pauri	79	88	88	85
Kalagarh	Pauri	116	118	108	114
Chilla	Pauri	91	98	87	92
Binsar	Pauri	64	71	72	69
Bhagwanpur	Haridwar	105	109	89	101
Laksar	Haridwar	131	145	102	126
Jwalapur	Haridwar	109	119	93	107
Khanpur	Haridwar	122	117	106	115
Roorkee	Haridwar	124	139	115	126
Manglaur	Haridwar	114	118	92	108
Bahadrabad	Haridwar	110	99	79	96
Narsan	Haridwar	131	139	126	132
Patanjali	Haridwar	157	163	154	158
Kankhal	Haridwar	153	138	132	141
Sultanpur	Haridwar	127	142	118	129
Pathri	Haridwar	147	137	109	131
Jhabreda	Haridwar	111	111	87	103
Landora	Haridwar	107	117	91	105
Pirankaliyar	Haridwar	109	104	81	98
Tapovan	Dehradun	112	113	93	106
Mussorie	Dehradun	98	112	114	108
Rishikesh	Dehradun	159	151	143	151
Chakrata	Dehradun	159	171	156	162
Dakpathar	Dehradun	162	158	151	157
Sahastradhara	Dehradun	120	134	109	121
Jollygrant	Dehradun	98	117	109	108
Ballupur	Dehradun	109	106	82	99
Tigerfall	Dehradun	123	134	97	118
Khoonigarh	Dehradun	98	118	72	96
Lakhamandal	Dehradun	125	133	102	120
Rajpur	Dehradun	121	130	94	115
Raipur	Dehradun	132	138	87	119
Pathribagh	Dehradun	142	159	101	134

Table 1: Continued

Locality	District	Avg Spore no. in Catharanthus roseus	Avg Spore no. in <i>Ocimum spp</i> .	Avg Spore no. in Asparagus racemosus	Overall Avg. no. of spores
Lachhiwala	Dehradun	112	123	83	106
Kalsi	Dehradun	105	102	75	94
Anarwala	Dehradun	129	138	96	121
Sinola	Dehradun	131	144	112	129
Kisanpur	Dehradun	172	197	138	169
Doiwala	Dehradun	154	159	113	142
FRI	Dehradun	184	170	153	169
Bhanoli	Almora	110	111	73	98
Jainti	Almora	104	124	78	102
Someshwar	Almora	69	74	73	72
Chaukutia	Almora	114	119	97	110
Bhikiasain	Almora	79	71	57	69
Sult	Almora	82	86	54	74
Bhatraujkhan	Almora	101	122	59	94
Marchula	Almora	92	109	102	101
Manila	Almora	92 89	92	86	89
			67		
Dunagiri	Almora	61		73	67
Pandhuka	Almora	89	106	102	99
Majkhali	Almora	97	109	112	106
Binsar	Almora	81	88	68	79
Jalna	Almora	102	125	109	112
Sheraghat	Almora	98	95	65	86
Takula	Almora	64	75	68	69
Gananath	Almora	108	125	112	115
Kaparkhan	Almora	69	83	67	73
Binta	Almora	87	97	98	94
Gangolihat	Almora	102	117	111	110
Katpuriya	Almora	102	105	114	107
Sitlakhet	Almora	110	120	112	114
Upal	Almora	67	77	78	74
Gwalakhot	Almora	93	100	98	97
Kosi-Katarmal	Almora	107	123	118	116
Kausani	Almora	87	96	99	94
Jageshwar	Almora	107	128	116	117
Ranikhet	Almora	87	108	99	98
Dwarahat	Almora	82	87	95	88
Khatima	Udham Singh Nagar	112	123	89	108
Rudrapur	Udham Singh Nagar	142	145	121	136
Pantnagar	Udham Singh Nagar	169	175	142	162
Sitarganj	Udham Singh Nagar	104	92	86	94
Kichha	Udham Singh Nagar	115	123	98	112
Gadarpur	Udham Singh Nagar	132	138	96	122
Bazpur	Udham Singh Nagar	119	122	89	110
Kashipur	Udham Singh Nagar	143	151	102	132
Jaspur	Udham Singh Nagar	129	135	111	125
Tanakpur	Udham Singh Nagar	132	123	99	118
Nanakmatta	Udham Singh Nagar	114	118	86	106
Doraha	Udham Singh Nagar	110	105	79	98
Negigarhi	Udham Singh Nagar	142	162	104	136

Table 2: Average number of Spores District and Plant Wise

	Pauri	Haridwar	Dehradun	Almora	US Nagar	Average Plant wise
Catharanthus roseus	83.92	123.8	130.71	91.034	127.92	111.48
Ocimum spp.	99	126.47	138.43	101.34	131.69	119.39
Asparagus racemosus	87.65	104.93	108.57	89.41	100.15	98.14
Average District Wise	90.19	118.4	125.90	93.93	119.92	

Table 3: Average number of Spores District and Year Wise

	Pauri	Haridwar	Dehradun	Almora	US Nagar	Average Year Wise
2006	88.2	120.4	124.8	94.2	120.2	109.56
2007	92.4	117.8	125.9	95.7	118.4	110.04
2008	89.98	117	127.01	91.89	121.17	109.41
Average District Wise	90.19	118.40	125.90	93.93	119.92	

(Table 2 and 3), whereas this data is also represented district wise graphically in Figure 1. The data corresponding to plants *Catharanthus roseus*, *Ocimum spp.* and *Asparagus racemosus* individually have been presented in tabular form, whereas the same data district wise has been shown graphically in Figures 2, 3 and 4 respectively.

VAM fungal spores were found to be well distributed in soil samples obtained from diverse places of Uttarakhand state, ranging from hilly terrains of Pauri and Almora to plane areas of Haridwar, Dehradun and Udham Singh Nagar. The qualitative and quantitative variation in VAM fungal spores were examined at different ecological environment and geographical areas.

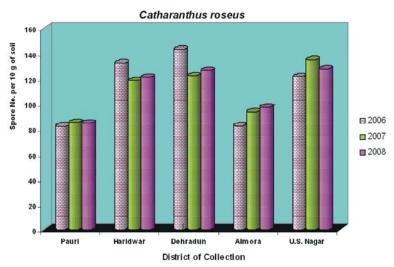


Fig. 1: Average number of VAM fungal spores isolated from the soil samples of *Catharanthus roseus* from different districts in Uttarakhand from year 2006 to 2008.

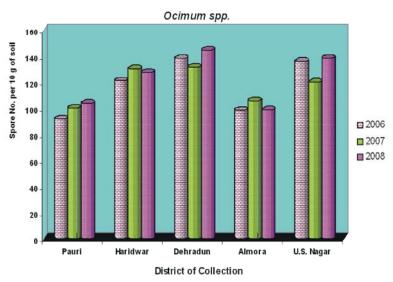


Fig. 2: Average number of VAM fungal spores isolated from the soil samples of *Ocimum spp.* from different districts in Uttarakhand state in different years (2006 to 2008).

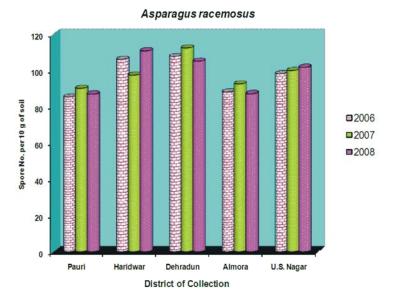


Fig. 3: Average no. of VAM fungal spores isolated from the soil samples of *Asparagus racemosus* from different districts in Uttarakhand state in different years (2006 to 2008).

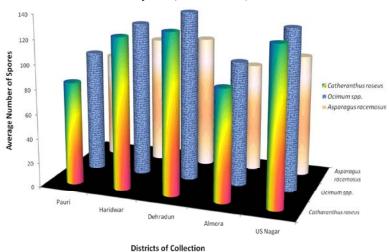


Fig. 4: Average number of spores isolated from the soil samples of medicinal plants from different districts of Uttarakhand state.

The medicinal plant roots of natural as well as cultivated plants were found to be heavily colonized by VAM fungi during the period of active growth. Mycorrhizal colonization was more frequent in forest areas than in the cultivated fields. It is likely that fertilizer application to cultivated land reduces VAM species [21]. A similar observation was made by Grime *et al.* [22, 23], who worked on the mechanisms of floristic diversity with reference to mycorrhizae.

Present research revealed more abundance of VAM fungal spores in cultivated soils than non-cultivated soils. Maximum numbers of spores were isolated from undisturbed natural vegetation sites, followed by cultivated and lastly non-cultivated and barren areas.

The potential reason for maximum number of spores availability in undisturbed natural vegetation is that spores keep multiplying in association with plants and remain in soil for isolation later on. Whereas, in cultivated habitat the top soil is disturbed each time as some fresh crop is sown. Previously, several researchers also reported that quantitative spore population differed in cultivated and non-cultivated soils [24-26]. They also observed that spore number was much higher in the cultivated soil as compared to non-cultivated soils.

The most abundant endophyte recorded was Glomus fasciculatum, followed by Glomus aggregatum, Glomus macrocarpum and Glomus mosseae. Species of Acaulospora and Sclerocystis were isolated from different

soil types of Uttarakhand region, but were never recorded as a dominant species [27]. Overall, a total 16 species of VAM were detected from three medicinal plants. Approximately more than fifty percent of total species were identified as species of Glomus like Glomus fasciculatum, Glomus aggregatum etc. In Catharanthus roseus plant, Glomus fasciculatum, Glomus claroideum, Glomus aggregatum, Glomus mosseae and Glomus monosporum species were found. It was observed that in Catharanthus roseus, Glomus species were present dominantly. Glomus aggregatum, Glomus fasciculatum, Glomus geosporum, Glomus mosseae, Gigaspora margarita, Gigaspora rosea, Sclerocystis sinuosa and Acaulospora laevis were found in Ocimum species. Among all the species of Ocimum observed, Glomus aggregatum and Glomus fasciculatum were predominantly present. In plants of Asparagus racemosus, various species were isolated and identified. These species were recognized as Glomus etunicatum, Glomus coranatum, Glomus mosseae, fasciculatum, Gigaspora gigantea, Gigaspora margarita, Sclerocystis sinuosa and Acaulospora scrobiculata.

The study reflected a trend indicating decrease in the richness and diversity of vesicular arbuscular mycorrhizal fungi with the increasing altitude. It coincides with the fact that the flora richness and diversity also decreases with increase in geographical altitude. Very few VAM spores were found from the soil sample of Kyunkaleshwar due to high altitude (Table 1).

Root Colonization of VAM Fungi: The medicinal plants were studied for mycorrhizal colonization. It was observed that for medicinal plant Asparagus racemosus root colonization ranged from 57.21 to 63.32 percent. However, Catharanthus roseus showed a marginally higher percent mycorrhizal colonization of 58.22 to 65.43%. Among three medicinal plant studied, Ocimum sanctum highest colonization that ranged from 76.88 to 95%. Much is known and documented in earlier literature about the functioning of symbiosis between plant and VAM fungi, but the details of ecology of VAM fungi are not well documented in medicinal plants in general and that of Uttarakhand in particular. VAM fungi in medicinal plants Catharanthus roseus, Ocimum spp. and Asparagus racemosus differ in the manner and extent with which root colonization rate occurs and also differ in their capacity to form propagules.

## CONCLUSION

Based on the richness of medicinal plants in Uttarakhand state, the present research work considered three medicinal plants which are *Catharanthus roseus Linn. Ocimum spp.* and *Asparagus racemosus Willd.* On

the basis of study carried out to find the presence or absence of vesicular arbuscular mycorrhizal fungi in Uttarakhand region, it can be concluded that the VAM fungi are well distributed throughout various region of the state. All the plant species studied i.e. Asparagus racemosus, Catharanthus roseus and Ocimum spp. exhibited association with VA mycorrhizal fungi. Undistributed natural vegetation of these medicinal plants showed maximum number of spores in comparison to the cultivated ones. VAM fungal spores were primarily isolated as chlamydospores and few as sporocarps. In total 16 species of VAM were detected from these three medicinal plants. Approximately more than fifty percent of total species were identified as species of Glomus It was observed that in Catharanthus roseus, Glomus species were dominantly present. Glomus aggregatum, Glomus fasciculatum, Glomus geosporum, Glomus mosseae, Gigaspora margarita, Gigaspora rosea, Sclerocystis sinuosa and Acaulospora laevis were found in Ocimum species. Among all the observed species of Ocimum, Glomus aggregatum and Glomus fasciculatum were predominantly present. In Asparagus racemosus, various species such as Glomus etunicatum, Glomus coranatum, Glomus mosseae, Glomus fasciculatum, Gigaspora gigantea, Gigaspora margarita, Sclerocystis sinuosa and Acaulospora scrobiculata were observed. Interestingly, species of Acaulospora and Sclerocystis were isolated from different soil types of Uttarakhand region, but were never recorded as a dominant species. The isolated number of spores varied in count from 70 to 178 per 10 g of soil. VAM spores were found in higher abundance from sandy loam soils followed by clay and loam soils. The number of spores was minimum at higher altitude. The root colonization levels ranged from 58.22% to 65.43%, 76.88% to 95% and 57.21% to 63.32% for medicinal plants Catharanthus roseus, Ocimum spp. and Asparagus racemosus respectively. An inference was made out clearly that Ocimum spp. had highest levels of mycorrhizal root colonization percentage.

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