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Biodiversity of Vesicular Arbuscular Mycorrhiza Associated with *Catharanthus roseus*, *Ocimum* spp. and *Asparagus racemosus* in Uttarakhand State of Indian Central Himalaya

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Abstract: This study deals in bio-diversity of Vesicular Arbuscular Mycorrhiza (VAM) associated with prominent medicinal plants i.e., *Catharanthus roseus*, *Ocimum* species and *Asparagus racemosus* in Central Himalayan region of India. Soil samples were collected from 104 locations spread at different altitudes and climatic conditions. The samples were regularly collected and observed at regular time intervals for a period of three years. In total 16 species of VAM were detected from these three medicinal plants. Approximately more than 50% 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 study reflected a trend indicating decrease in the richness and diversity of vesicular arbuscular mycorrhizal fungi with the increasing altitude. VAM fungal spores were primarily isolated as chlamydospores and few as sporocarps. 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 percentage of root colonization levels ranged 58.22-65.43, 76.88- 95 and 57.21- 63.32 for medicinal plants *Catharanthus roseus*, *Ocimum* spp. and *Asparagus racemosus*, respectively.

Key words: Biodiversity of vesicular, arbuscular mycorrhiza, *Catharanthus roseus*, *Ocimum* sp., *Asparagus racemosus*

INTRODUCTION

Mycorrhiza is a symbiotic mutualistic relationship between special soil fungi and fine plant roots; it 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. The fungus receives carbohydrates (sugars) and growth factors from the plant which in turn receives many benefits including increased nutrient absorption. In this association, the fungus takes over the role of the plant's root hairs and acts as an extension of the root system (Kaushik, 2000). 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 (Abad and Khara, 2007; Munir and Malkawi, 2004; Abas-Ali *et al*., 2007; Mishra and Dubey, 2006). There is also much interest in their potential utilization in medicinal, agricultural and horticultural crops (Jamshaid *et al*., 1999; Adriano-Anaya *et al*., 2006). 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 medicinal plants play a vital role for existence of the life on the earth. Out of approximately 2,50,000 higher plant species on earth, more than 80,000 are medicinal (Joy *et al*., 2001). India is one of the world's 12 biodiversity centres with the presence of over 45,000 different plant species. Biodiversity of India is

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unmatched due to the presence of 16 different agro-climatic zones, 10 vegetation zones, 25 biotic provinces and 426 biomes (habitats of specific species). Among these about 15,000-20,000 plants have superior medicinal significance. However, traditional communities for their medicinal values have used only 7,000-7,500 species. The history of medicine in India can be traced to the remote past in Vedic period. The major sources for the drugs used for the treatment of human ailments are the plants or their products. Among the various *samhitas*, *Charak samhita* and *Susrut samhita* are well known to possess many formulations based on plants alone or in combination with animal products or organic substances. This is a well-known fact that the plants based medicine does not cause side effects as do the other system of medicine. Moreover, the people are using many formulations of plants and their products since long and the traditional information was inherited from generation to generation and many of the uses have been made commercial (Tiwari, 2006). However, in order to avoid any toxic effect of traditional medicines time and duration of dose should be chosen carefully to avoid any damaging effect (Karim *et al*., 2011).

The Himalayan state of Uttarakhand is very rich in medicinal and aromatic plants (Kaushik and Dhiman, 2000). The medicinal plants in the region occur naturally and most of them propagate vegetatively by underground rhizomes, stems and bulbs or corms. This herbal wealth is being used not only by developing countries but also by developed countries for their health care systems. A bulk of our rural population relies on drug resources of plant origin. Locally collected plants are sold, where they are exploited commercially for preparation of medicines.

Based on the richness of medicinal plants in Uttarakhand state, three medicinal plants viz. Sadabahar (*Catharanthus roseus* Linn.), Tulsi (*Ocimum* sp.) and Shatavar (*Asparagus racemosus* Willd.) were considered for the present research work. Sadabahar, Tulsi and Shatavar were identified for research purpose since the medicinal uses of these plants are tremendous. The roots of Sadabahar are sedative, tranquiliser and stomachic and are used as tonic. The leaves of Sadabahar 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 Sadabahar plant has shown growth inhibitory effect in certain human tumors. The plant of Tulsi is considered expectorant, stomachic, diuretic, antiseptic and cardiac stimulant and its decoction is used in catarrh, croup and bronchitis. The roots of Shatavar are refrigerant, demulcent, diuretic, aphrodisiac, antiseptic, antidiarrhoeal, antidysentric and galactagogue (Kaushik and Dhiman, 2000). The medicinal value of these plants has been utilized since Vedic period. Since these plants have great medicinal values, this research was carried out to study their associated VAM fungi and various factors, which affected their growth.

The Himalayan states of India are very rich in medicinal and aromatic plants. These medicinal plants occur naturally and most of them propagate vegetatively by under ground rhizomes, stems, bulbs or corms. This herbal wealth is being used not only by developing countries but also by developed countries for their health care systems. 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 (Kaushik and Dhiman, 2000). The medicinal plants from some of these areas have been extensively studied (Joy *et al*., 2001). 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 80% of medicinal plants used worldwide for domestic use, sale and export are harvested in wild state from their natural habitats. As markets increase for medicinal plant products, wild populations are being depleted, often at the expense of local livelihoods. Ticktin (2004), an ecologist, suggests that local experimentation in management techniques (through participatory research) with harvesters is essential to curb this overexploitation which threatens both forests and the people dependent upon them. Several projects have been developed to determine local sustainable harvesting levels for all types of forest products, using a combination of traditional and scientific methods for both harvesting and monitoring.

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 researchers have analyzed some randomized information gathered in some areas of Uttarakhand State. 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 (1) Quantitative and qualitative composition of VAM fungi (2) Distribution pattern of VAM fungi and (3) Colonization in roots of Sadabahar, Tulsi and Shatavar 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.

Sites: 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. 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. Sterilized 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.

Isolation of VAM spores from soil: Various techniques are used to recover Vesicular Arbuscular Mycorrhizal propagules from soil. There are primarily two types of techniques viz., 1 Wet sieving and decanting technique; 2 Density gradient technique. The most basic of these is 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. On the other hand, density gradient centrifugation is also now commonly used technique for extraction of VAM spores. In this research work, wet sieving and decanting technique was used for extraction of spores.

Qualitative analysis of VAM fungi: 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.

Identification of VAM spores: VAM spores were identified using standard monographs given by Hall and Fish (1978), Hall (1984, 1987), Gerdemann and Trappe (1974); Schenck and Perez (1989) and INVAM.

Root staining for VAM fungi colonization: Method of Phillips and Hayman (1970) was employed for root staining. 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.

Percentage of root colonization: The percentage of root colonization is obtained by applying following formula:

$$\%\text{Colonization} = \frac{\text{Total No. of root segments colonized}}{\text{Total No. of root segments examined}} \times 100$$

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. 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 from different medicinal plants collectively from diverse sites of every district was evaluated and analyzed. Figure 1 shows the average number of spores observed for all medicinal plants together at each district from year 2006 to 2008. 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. These results have been averaged out district and year wise for all plants collectively in Fig. 1. The average number of spores of

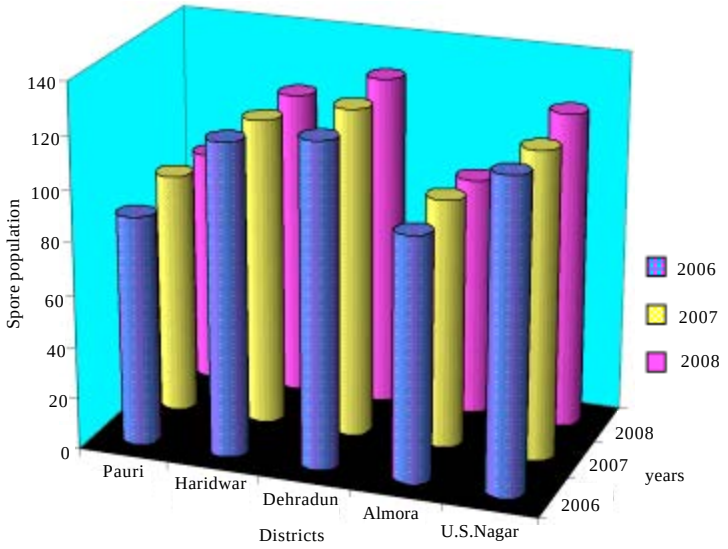

Fig. 1: Average number of total VAM fungal spores isolated from the soil samples of different districts in Uttarakhand state in different years (2006 to 2008)

VAM fungi was highest at Dehradun district followed by Haridwar, Udham Singh Nagar, Pauri and Almora which can be clearly observed collectively from Fig. 1. 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*.

Distribution of Different VAM Fungi at Different Sites in Uttarakhand State of Indian Central Himalaya: Many soil samples were collected from different regions of Uttarakhand state. These samples showed the presence of VAM fungal spores. 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 (Fig. 1).

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 (Table1).

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 was examined at different ecological environment and geographical areas.

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 (Mosse and Hayman, 1980). A similar observation was made by Grime *et al*. (1987a, b), 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 topsoil 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

Table 1: List of collection sites and respective soil type

Collection site	District	Soil type
Lansdown (A)	Pauri	Sandy loam
Patisain (B)	Pauri	Sandy loam
Kyunkaleshwar (C)	Pauri	Sandy loam
Manglaur (D)	Haridwar	Clay
Narsan (E)	Haridwar	Clay
Patanjali (F)	Haridwar	Loam
Rishikesh (G)	Dehradun	Sandy loam
Dakpathar (H)	Dehradun	Sandy loam
Tigerfall (I)	Dehradun	Loam
Someshwar (J)	Almora	Sandy loam
Jalna (K)	Almora	Sandy loam
Gangolihat (L)	Almora	Sandy loam
Pantnagar (M)	Udham Singh Nagar	Loam
Gadarpur (N)	Udham Singh Nagar	Clay
Nanakmatta (O)	Udham Singh Nagar	Clay

Table 2: Species diversity of VAM fungi

Collection site	Soil sample count	%age of VAM infection	Species diversity	Mean of species diversity (%)
A	50	100	6	12.0
B	55	100	8	14.5
C	25	97	5	20.0
D	40	100	7	17.5
E	40	100	4	10.0
F	45	97.9	6	13.3
G	35	100	8	22.9
H	35	100	5	14.3
I	50	100	9	18.0
J	50	100	7	14.0
K	30	98	5	16.7
L	30	100	7	23.3
M	45	99	9	20.0
N	45	100	6	13.3
O	45	100	8	17.8

(Mosse and Bowen, 1968; Hayman and Stovold, 1979; Sylvia, 1986). They also observed that spore number was much higher in the cultivated soil as compared to non-cultivated soils. But in contrast to this observation Phosri *et al.* (2010) noticed lesser mycorrhizal formation in cultivated soil due to negative effects of fertilizers on mycorrhizal formation. However, in agreement to our results Wilberforce *et al.* (2003) noticed that due to the absence of host trees, abandoned agricultural land may often lack any natural mycorrhizal inoculum, while pathogens may be present.

Maximum species diversity was observed for habitats I and M. The highest numbers of soil samples were collected from site I (i.e., 50). The minimum mean species diversity was found at site E. Few sites did not show the existence of VAM fungi in all the samples collected. These habitats had all types of soil i.e., loam, sandy loam and clay. The mean species diversity of each habitat gives an indication of the species richness associated with habitat. However, species diversity gives a better indication of the difference in species diversity between the various habitat classifications. Species diversity of each habitat has been given in Table 2.

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. Overall, a total 16 species of VAM were detected from three medicinal plants. Table 3 represents distribution of *Glomus* species of VAM fungi for all 104 collection sites. The *Glomus* species that were observed are *Glomus fasciculatum*, *Glomus claroideum*, *Glomus aggregatum*, *Glomus mosseae*, *Glomus monosporum*, *Glomus geosporum*, *Glomus etunicatum*, *Glomus coranatum*, *Glomus intraradices*, *Glomus macrocarpum*. However, Table 4 demonstrates distribution of *Gigaspora*, *Acaulaspora and Sclerocystis* species of VAM fungi at different collection sites. The observation for each site has been categorized as absent, poorly present, moderately present and frequently present. Approximately more than 50% 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*, *Glomus 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. Allen (1987) and Trappe (1988) also found that the availability of arbuscular mycorrhizal propagules may be low at high altitudes because AM spores are soil- and animal-dispersed. Additionally, the number of fungal species in soil generally decreases along the altitude gradient (Bissett and Parkinson, 1979; Kernaghan and Harper, 2001). Contradictorily, Newman and Reddell (1988) observed that the number of tree species showed a highly significant decline with increasing altitude but no significant relationship to

Table 3: Distribution of *Glomus* species of VAM fungi

Locality	*Glomus fasciculatum*	*Glomus claroideum*	*Glomus aggregatum*	*Glomus mosseae*	*Glomus monosporum*	*Glomus geosporum*	*Glomus etunicatum*	*Glomus coranatum*	*Glomus intraradices*	*Glomus macrocarpum*
Dhari	++	-	++	+	-	+	++	+	-	-
Srinagar	+	-	+	-	-	+	+	-	+	-
Srikot	-	-	-	+	-	-	-	-	-	+
Pauri	+	+	+	+	-	+	-	+	+	++
Ghurdauri	-	-	+	+	-	-	-	+	-	-
Khandusain	+	-	-	+	-	+	-	+	-	-
Kotdwar	+	+	-	-	+	+	+	+	-	+
Safdarkhal	-	-	+	+	-	-	+	-	-	+
Minthi	-	-	-	+	-	+	-	-	-	-
Doggada	+	-	-	-	-	+	-	-	-	+
Kaliasaur	++	-	+	-	+	-	-	-	-	+
Satpuli	-	+	+	+	+	-	+	-	-	-
Lansdown	+	-	+	+	-	+	++	+	-	-
Buvakhal	+	-	-	+	-	+	-	-	+	-
Jwalpadevi	+	-	-	-	-	+	-	+	-	+
Patisain	-	-	+	-	-	-	-	-	-	-
Gumkhal	-	-	+	++	-	-	-	-	-	+
Dandapani	+	-	+	-	-	-	-	-	-	+
Kyunkaleshwar	+	-	-	-	-	-	-	-	-	+
Kandolia	++	-	+	+	-	+	-	+	-	-
Nagdev	-	-	+	-	-	+	-	-	-	-
Dhumakot	-	-	++	-	+	-	-	-	+	-
Nainidanda	-	-	-	+	-	-	+	-	-	-
Kalagarh	++	+	+	+	+	++	-	-	-	-
Chilla	-	-	-	+	-	-	-	-	-	+
Binsar	+	-	-	-	-	+	-	-	-	-
Bhagwanpur	++	-	+	+	-	+	-	-	-	++
Laksar	+	-	+	-	+	-	-	+	-	-
Jwalapur	-	-	+	-	-	+	-	-	-	-
Khanpur	-	-	+	-	+	-	-	-	-	-
Roorkee	++	+	++	-	++	+	-	+	-	+
Manglaur	+	-	+	+	-	+	+	+	-	+
Bahadrabad	-	-	+	+	-	-	-	-	-	+
Narsan	-	-	+	-	+	-	-	+	-	-
Patanjali	+++	+	++	-	-	+	-	+	-	++
Kankhal	+	-	-	+	-	+	-	-	-	-
Sultanpur	-	+	-	+	-	-	-	-	+	-
Pathri	-	-	+	-	-	-	-	-	-	-
Jhabreda	+	-	-	+	+	-	-	-	-	-
Landora	+	-	+	-	-	+	-	-	-	+
Pirankaliyar	++	-	+	+	-	+	-	-	+	-
Tapovan	+++	+	+	-	-	-	-	+	-	-
Mussoorie	+	-	+	++	+	+	+	-	-	+
Rishikesh	++	+	++	-	+	+	-	-	+	+
Chakrata	-	-	+	++	-	+	-	-	-	-
Dakpathar	+++	+	+++	+	+	++	-	+	+	-
Sahastradhara	-	-	+	+	+	-	-	+	-	+
Jollygrant	+	-	+	+	++	-	-	-	-	++
Ballupur	-	+	+	-	-	+	+	-	-	-
Tigerfall	+	-	-	+	+	-	-	+	-	-
Khoonigarh	+	-	-	++	-	-	-	-	+	+
Lakhamandal	+	+	-	+	-	-	-	+	-	-
Rajpur	++	-	+	++	-	-	-	+	-	-
Raipur	+	+	-	-	-	+	-	-	+	-
Pathribagh	-	+	+	+	-	-	-	-	-	+
Lachhiwala	-	-	++	-	+	+	-	-	-	-
Kalsi	++	-	+	+	-	-	-	+	+	-
Anarwala	-	-	+	-	-	+	-	-	-	-
Sinola	+	-	-	-	-	+	-	-	-	+
Kisanpur	-	+	-	-	-	++	-	-	-	+
Doiwala	-	-	-	+	+	-	-	-	-	+
FRI	+++	+	+++	++	+	+	-	++	+	++
Bhanoli	-	-	+	+	-	-	+	-	-	+
Jainti	-	-	+	-	-	+	-	-	-	-

Table 3: Continued

Locality	*Glomus fasciculatum*	*Glomus claroideum*	*Glomus aggregatum*	*Glomus mosseae*	*Glomus monosporum*	*Glomus geosporum*	*Glomus etunicatum*	*Glomus coranatum*	*Glomus intraradices*	*Glomus macrocarpum*
Someshwar	+	-	+	-	-	+	-	-	-	+
Chaukutia	+	-	-	-	-	+	-	-	-	-
Bhikiasain	-	-	+	+	-	-	+	-	-	-
Sult	-	-	+	+	-	-	-	-	-	-
Bhatraujkhan	++	-	+	-	-	-	-	-	-	+
Marchula	-	-	+	-	-	+	-	-	+	-
Manila	+	-	+	+	-	+	-	-	-	-
Dunagiri	+	-	+	-	+	-	-	+	-	-
Pandhuka	++	-	-	-	-	+	-	-	+	+
Majkhali	+	-	-	-	-	-	-	-	+	-
Binsar	++	-	+	+	-	-	-	-	-	-
Jalna	-	-	+	-	-	+	-	+	-	-
Sheraghat	-	-	+	-	-	-	-	-	-	+
Takula	+	-	+	-	-	-	-	-	-	+
Gananath	-	-	+	-	-	+	-	-	-	-
Kaparkhan	+	-	+	-	-	++	-	-	+	-
Binta	++	-	-	+	+	-	+	-	+	-
Gangolihat	-	-	+	-	-	-	-	+	-	-
Katpuriya	-	-	-	+	-	+	-	-	-	-
Sitlakhet	+	-	-	-	-	-	-	-	-	+
Upal	-	-	+	+	-	-	-	-	-	-
Gwalakhot	+	-	-	-	-	+	-	+	-	+
Kosi-Katarmal	++	-	+	+	+	-	+	+	-	+
Kausani	+	-	++	-	-	-	-	+	-	-
Jageshwar	+++	-	++	+	-	+	-	+	-	-
Ranikhet	-	-	+	++	-	+	-	-	-	-
Dwarahat	-	-	+	-	-	-	+	-	-	-
Khatima	-	-	-	+	-	+	-	-	+	+
Rudrapur	+	-	-	-	-	+	-	+	-	+
Pantnagar	++	+	++	+++	-	+	-	++	+	+
Sitarganj	-	-	+	-	+	-	-	-	-	-
Kichha	++	-	+	-	++	+	-	-	+	-
Gadarpur	+	-	+	-	-	-	-	-	-	+
Bazpur	+	-	+	-	-	++	-	++	-	-
Kashipur	++	+	-	++	+	-	-	-	+	-
Jaspur	-	++	-	-	-	+	+	-	-	+
Tanakpur	-	-	+	+	-	-	-	-	-	-
Nanakmatta	+	-	++	++	+	-	-	+	-	-
Doraha	+	-	++	-	-	+	-	-	-	-
Negigarhi	-	-	+	-	-	+	-	-	-	+

-, +, ++ and +++ denotes absent, poorly present, moderately present and frequently present, respectively

mycorrhiza. Similarly, Allison and Goldberg (2002) noticed that the community-level responses to mycorrhizal colonizations along environmental gradients are not necessarily the same as the responses found at species-level which is due to changes in species composition along the gradients and their different responses to mycorrhiza.

Root colonization of VAM fungi: The medicinal plants were studied for mycorrhizal colonization. Selective root colonization photomicrographs in medicinal plants *Catharanthus roseus*, *Ocimum* spp. and *Asparagus racemosus* are shown from Fig. 2 to 7. Slides for *Catharanthus roseus* root colonization are shown in Fig. 2 and 3. *Catharanthus roseus* showed a marginally higher percent mycorrhizal colonization of 58.22 to 65.43% with respect to *Asparagus racemosus*. Vesicles and intracellular hypha can be clearly observed from these figures. Among three medicinal plant studied, *Ocimum sanctum* showed highest colonization that ranged from 76.88 to 95%. Figure 4 and 5 demonstrate root colonization with vesicles and intercellular hypha for *Ocimum* sp. Figure 6 and 7 represent root colonization showing vesicles and hyphae of *Asparagus racemosus*. It was observed that for medicinal plant *Asparagus racemosus* root colonization ranged from 57.21 to 63.32% which was minimum among three plants.

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* sp. and *Asparagus racemosus* differ in the manner and extent with which root colonization rate occurs and also differ in their capacity to form propagules.

Table 4: Distribution of *Gigaspora, Acaulaspora and Sclerocystis* species of VAM fungi

Locality	*Gigaspora margarita*	*Gigaspora rosea*	*Gigaspora gigantea*	*Acaulaspora scrobiculata*	*Acaulaspora laevis*	*Sclerocystis sinuosa*
Dhari	+	+	-	-	+	-
Srinagar	-	+	++	+	-	+
Srikot	++	-	+	+	-	-
Pauri	-	+	+	++	+	-
Ghurdauri	+	-	-	-	-	-
Khandusain	-	-	-	+	-	+
Kotdwar	-	+	+	-	+	++
Safdarkhal	+	-	-	-	+	-
Minthi	+	-	+	-	+	-
Doggada	+	-	+	+	-	-
Kaliasaur	-	-	-	+	+	+
Satpuli	-	+	-	+	-	-
Lansdown	+	+	++	-	-	-
Buvakhal	+	-	-	-	-	-
Jwalpadevi	-	-	-	-	+	-
Patisain	+	-	-	-	+	-
Gumkhal	-	-	-	-	-	-
Dandapani	-	-	+	-	+	-
Kyunkaleshwar	-	-	-	+	-	+
Kandolia	+	+	-	-	-	-
Nagdev	-	-	-	+	-	-
Dhumakot	+	+	-	-	-	-
Nainidanda	-	+	-	-	-	-
Kalagarh	+	++	+	-	-	-
Chilla	-	-	+	+	-	-
Binsar	-	+	+	-	-	+
Bhagwanpur	+	+	-	-	+	-
Laksar	+	++	+	-	-	-
Jwalapur	+	-	+	-	-	-
Khanpur	-	+	-	-	-	+
Roorkee	+	+	-	+	-	-
Manglaur	-	-	-	+	+	-
Bahadrabad	++	+	+	-	+	-
Narsan	-	+	-	-	-	+
Patanjali	+	+	-	-	-	-
Kankhal	+	++	-	+	-	-
Sultanpur	-	-	-	-	-	-
Pathri	+	+	+	-	-	-
Jhabreda	-	-	-	-	+	-
Landora	-	-	-	-	-	-
Pirankaliyar	+	++	-	-	-	-
Tapovan	+	+	+	-	-	-
Mussoorie	+	-	+	-	-	+
Rishikesh	++	-	+	+	-	-
Chakrata	+	+	-	+	-	+
Dakpathar	++	++	+	-	-	-
Sahastradhara	-	+	-	-	+	-
Jollygrant	+	-	-	-	+	-
Ballupur	+	+	-	+	-	++
Tigerfall	+	-	-	-	-	-
Khoonigarh	-	+	++	-	-	-
Lakhamandal	-	+	-	-	-	-
Rajpur	+	+	-	-	-	-
Raipur	-	+	-	-	-	-
Pathribagh	-	+	-	+	-	-
Lachhiwala	-	-	-	+	-	-
Kalsi	+	++	-	-	+	-
Anarwala	++	-	-	-	-	+
Sinola	-	-	+	-	-	-
Kisanpur	+	+	+	-	-	-
Doiwala	-	+	-	+	+	-
FRI	+	+	++	+	+	-
Bhanoli	-	-	+	+	-	+
Jainti	+	-	-	-	-	-
Someshwar	+	-	-	-	-	-

Table 4: Continued

Locality	*Gigaspora margarita*	*Gigaspora rosea*	*Gigaspora gigantea*	*Acaulaspora scrobiculata*	*Acaulaspora laevis*	*Sclerocystis sinuosa*
Chaukutia	-	-	-	-	-	-
Bhikiasain	+	+	-	+	+	-
Sult	-	+	+	+	-	-
Bhatraujkhan	+	-	+	-	-	-
Marchula	-	-	-	-	+	-
Manila	+	-	+	-	+	+
Dunagiri	-	-	_	+	+	+
Pandhuka	+	-	+	+	-	-
Majkhali	-	+	+	+	-	-
Binsar	-	+	+	-	-	+
Jalna	++	-	+	-	-	-
Sheraghat	-	-	-	-	-	-
Takula	+	-	-	-	-	-
Gananath	-	-	++	+	-	-
Kaparkhan	+	-	-	-	-	-
Binta	-	+	-	+	-	-
Gangolihat	-	+	-	+	+	-
Katpuriya	+	-	+	-	-	-
Sitlakhet	+	-	+	-	-	+
Upal	+	-	-	+	-	-
Gwalakhot	-	-	+	-	+	-
Kosi-Katarmal	++	-	++	+	-	-
Kausani	-	-	-	+	-	-
Jageshwar	+	++	-	-	-	+
Ranikhet	+	+	-	-	-	+
Dwarahat	+	-	+	+	+	-
Khatima	-	-	+	-	+	-
Rudrapur	-	++	+	-	-	-
Pantnagar	++	+	-	+	+	+
Sitarganj	+	-	+	-	-	-
Kichha	+	+	+	+	-	-
Gadarpur	-	-	-	+	-	+
Bazpur	-	+	-	-	-	-
Kashipur	+	+	-	-	-	-
Jaspur	+	+	++	-	-	-
Tanakpur	++	+	-	-	+	-
Nanakmatta	+	-	-	-	-	-
Doraha	-	-	-	-	-	-
Negigarhi	+	+	-	-	-	-

-, +, ++ and +++ denotes absent, poorly present, moderately present and frequently present respectively

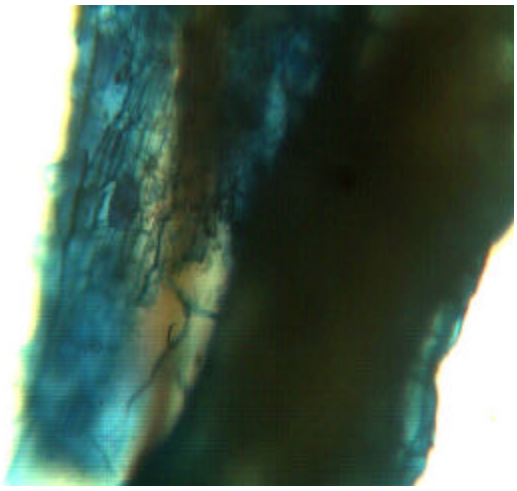

Fig. 2: Root colonization of *Catharanthus roseus* showing vesicles and intracellular hypha

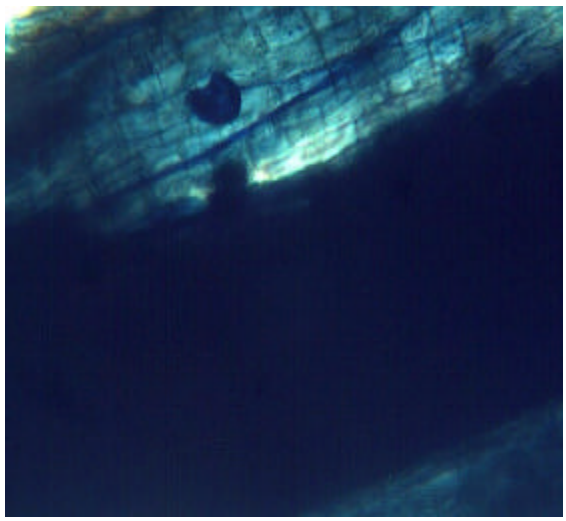

Fig. 3: Root colonization of *Catharanthus roseus*

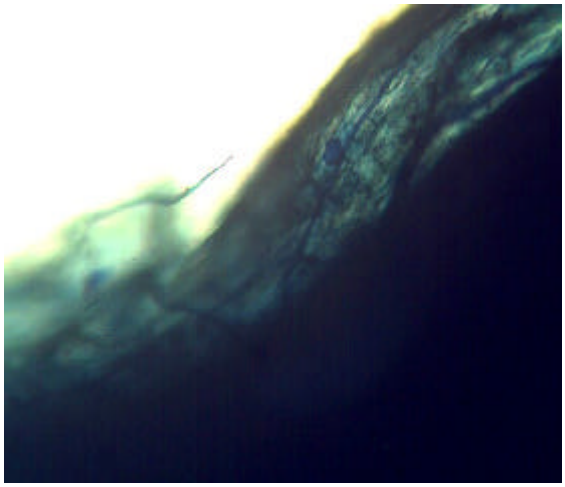

Fig. 4: Root colonization of *Ocimum* spp.

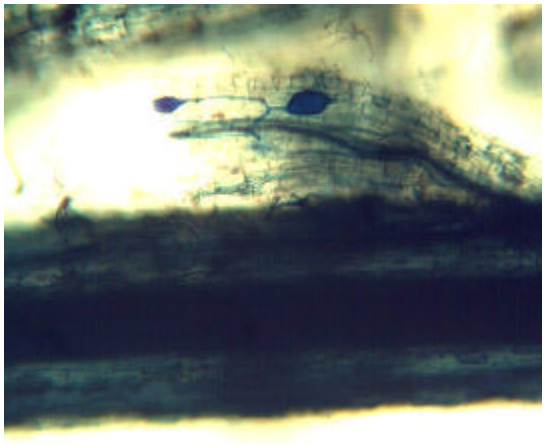

Fig. 5: Root colonization of *Ocimum* spp. showing vesicles and intercellular hypha

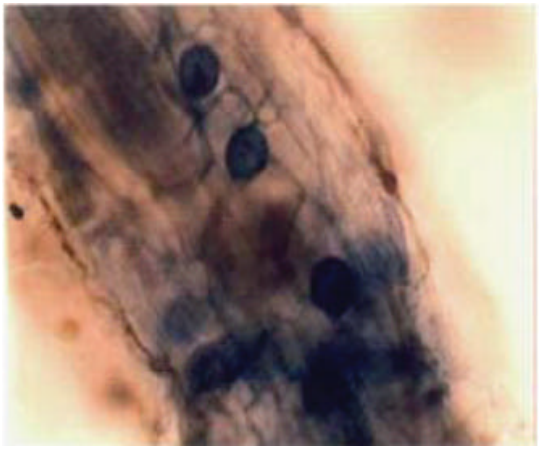

Fig. 6: Root colonization of *Asparagus racemosus* showing vesicles and hypha

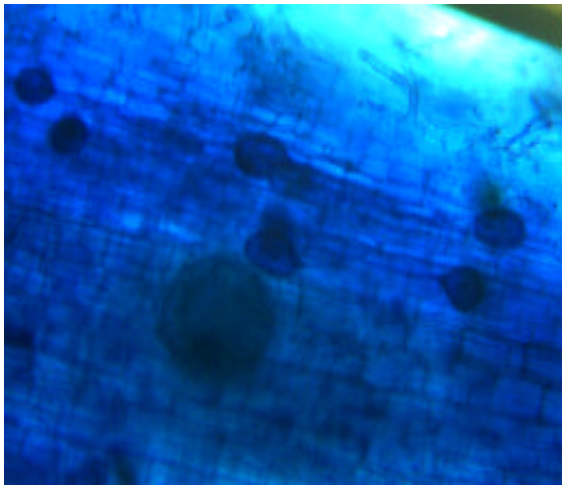

Fig. 7: Root colonization of *Asparagus racemosus* showing vesicles and hyphae

CONCLUSION

Based on the richness of medicinal plants in Uttarakhand state, the present research work considered three medicinal plants which are Sadabahar, Tulsi and Shatavar. 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* sp. 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. 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* sp. 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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