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Interaction and Biodiversity of Vesicular Arbuscular Mycorrhizal Fungi Associated with Some Medicinal Plants of Mid-Mountain Range of Himachal Pradesh

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ABSTRACT

The present investigation was focused on the study of endomycorrhizal status of some medicinally important plants growing in mid mountain range of Himachal Pradesh. Twenty one plants, belonging to fifteen families were examined for mycorrhizal status and diversity in natural habitat. A total of twenty four different vesicular arbuscular mycorrhizal (VAM) species, belonging to all the six genera of VAM fungi e.g.*Glomus, Acaulospora, Gigaspora, Sclerocystis, Scutellospora* and *Entrophospora* were isolated. It was found that mycorrhizal root colonization ranged from 33.93±4.2% to 89.62±10.0% and highest spore number was observed in *Rosmarinus officinalis* (841±25.9). VAM root colonization was observed in terms of presence of mycelium, vesicles and arbuscules in the cortical region of root.

Key words: VAM, mycelium, vesicles, arbuscules, Himachal Pradesh, medicinal plants.

INTRODUCTION

On our planet earth, we feel blessed to be surrounded by natural resources which benevolent God has provided us in abundance. Among all natural resources, one, that is rendering its valuable support to sustain human race, is plantation. Plants are one of the most important sources of medicine. Medicinal plants are important for the socio- economic upliftment of human being. Almost every civilization has a history of medicinal plant use. They have been subject of man's curiosity since time immemorial.

One of the richest reservoirs of biological diversity in the world is, the Indian Himalayan Region (IHR). Himachal Pradesh is a store house of biological wealth having potential for cultivation of herbs particularly medicinal and aromatic plants. Medicinal plants play an important role in the life of people living in rural and urban areas of Himachal Pradesh. Not only their primary health care need and dietary traditions are based on medicinal plants, but these also contribute towards the rural economy. With the increasing national and global trade in medicinal plants, this invaluable resource has come under pressure. This led to cultivation of these medicinal plants to support the increasing demand. About 85% of the world's flowering plants and trees, on which human life depend, form close root association with micro-organisms like fungi, mycorrhiza and bacteria. Mycorrhizal fungi are a key member of soil micro-biota and conduct activities which are crucial to plant establishment, development, nutrition and health.

VAM are recognized as most common type of mycorrhiza with diverse host range (Gerdemann, 1968). VAM fungi are known to be helpful in phosphorus uptake (Schweiger *et. al.,* 2007), bioremediation (Li *et al.*, 2006), enhancing plant growth (Javot *et al.,* 2007), Protection against toxicity (Aggarwal *et al.,* 1999), drought tolerance (Auge and Moore, 2005), increase in photosynthetic activity (Bethlenfolvay *et al.,* 1988) and fertility of soil (Charles *et al*., 2006). The study of endomycorrhizal biodiversity on some medicinal plants is, therefore, necessary from efficient utilization and conservation point of view. Considering the importance of medicinal plants in Himachal Pradesh, an investigation was carried out to study the endomycorrhizal status of these medicinal plants and to select the predominant VAM fungi for future inoculation studies for production of quality seedling of important plants in nurseries and their better survival in adverse conditions.

MATERIAL AND METHODS

Study Site:

Root and soil samples of medicinally important plants were collected from mid-mountain or inner Himalayan region of Himachal Pradesh during 2007-2008.

Collection of soil sample:

It was done by digging out a small amount of soil close to plant's roots, up to the depth of 15-30 cm and these soil samples were kept in sterilized polythene bags at 10˚C for further processing.

Isolation and quantification of VAM fungi:

Isolation of VAM spores was done by using 'Wet Sieving and Decanting Technique' of Gerdemann and Nicolson (1963). 50 gram of soil was mixed in water in a small plastic container having a capacity of about 1000 ml. The soil was thoroughly mixed with water and allowed to settle down overnight. The water was decanted on a series of sieves in the following order 150µm, 120µm, 90µm, 63µm, 45µm. from top to bottom on which spore were trapped. The trapped spores were then transfered to the Whatmann filter paper No.1 by repeated washing with water and were counted. The quantification of VAM spores were done by 'Grid Line Intersect Method' (Adholeya and Gaur, 1994). The spores were picked by hypodermic needle under stereo binocular microscope. The spores were mounted on polyvinyl lactic acid alcohol (PVLA) for further studies.

Identification of VAM fungi:

For identification of VAM spores the following criteria was used like conventional morphological characters i.e. colour, size, shape, wall structure, surface ornamentations of spores and size of subtending hyphae, bulbous suspensor, the number and arrangement of the spores in the sporocarps. These VAM spores were identified by using the keys of Walker (1983); Schenk and Perez (1990); Morton and Benny (1990); Mukerji (1996); Morton and Redecker (2002).

Colonization of VA Mycorrhiza:

It was studied by 'Rapid Clearing and Staining Method' by Phillips and Haymann (1970). The root segment was washed with water to remove soil particles. It was then cut into 1cm small pieces. Root segment was washed with water and placed in 10% KOH solution at 90˚C for half an hour or for 24 hours at room temperature. The KOH was decanted and root was washed with water till the brown colour is cleared. Then these segments were acidified with 1% HCl for 3-5 minutes. After this, the root segment was submerged in 0.5 % Trypan blue for 24 hours. After 24 hours the segment was destained with Lacto phenol. The root was examined in lactic acid or lactic acid: glycerol (1:1) solution. The percentage mycorrhizal root colonization was calculated by following formula:

$$\textbf{Percentage root colonization} = \frac{\text{Number of roots with infection} \times 100}{\text{Total number of root segments studied}}$$

RESULTS AND DISCUSSION

Status of endomycorrhizal fungi associated with medicinally important plants of mid mountain region of Himachal Pradesh was determined. A total of twenty one plants, belonging to fifteen families (13 dicot and 2 monocot) were studied (Table 1).

Table 1. Distribution of some studied medicinal plants of Himachal Pradesh

	Group	
	Dicot	**Monocot**
Families	**13**	**2**
Genera	**18**	**2**
Species	**19**	**2**

In last couple of years, a number of reports have been focused on the study of biodiversity of VAM fungi. Mehrotra (2007) studied the diversity of VAM fungi in India and proved that *Glomus* species to be most common species. Singh and Jamaluddin (2007) reported three VAM fungi genera with eight species on *Vitex negundo*. Castillo *et al*. (2006) also studied the diversity of VAM fungi in evergreen forest, deciduous forest and grassland ecosystem of Southern Chill.

In the present investigation mycorrhizal root colonization was observed in the form of presence of mycelium, vesicles and arbuscules. Mycelia of different kinds like H-shaped, Y-shaped, coiled and parallel mycelia were obserbed in the root segments of various plants. Various types of vesicles i.e. round, oval, beaked and elongated were present in the cortical cells. Among the all twenty one plant studied, two plants were having only mycelium, three with mycelium and vesicles, six with mycelium and arbuscules and the ten plants with mycelium, vesicles and arbuscules.

It is envisaged from the observation that the mycorrhizal root colonization ranged from the minimum 33.93±4.2 % to maximum 89.62±10.0 % . The minimum root colonization was observed in *Swertia paniculata* and the maximum in *Hedychium spicatum* (Table 1). Variation in the degree of infection among different plants with in a family was observed by Thapar *et al*. (1992). Similarly, the range of variation in the percentage of VAM root colonization may be due to the effect of host chemicals on growth of VAM fungi (Rahman *et al.* 2003).

The range of sporulation also varied from lowest 50 ± 2.6 to highest 841 ± 25.9. The minimum spore count was observed in *Prunus amygladus* and the maximum spore number in *Rosmarinus officinalis* (Table 2).

The variation in the spore number resulted that the multiplication of spores depends upon species to species level. Muthukumar and Udaiyan (2001) reported that the plant senescence trigger the sporulation of VAM fungi. Similar were the observation of Allen (1991).

It is evident from Table 1 that rate of colonization could not be correlated with the spore number. Similar were the observation made by Scheltema *et al*. (1987). In the present study for e.g. *Hedychium spicatum* showed maximum (89.62 ± 10.0) VAM root colonization, but had fewer numbers of spores (88.3 ± 1.5), while *Citrus medica* possessed more spores number (143 ± 1.4), but had lower rate of root colonization (43.74 ± 1.2). As VAM fungal sporulation is dependent on a wide range of host fungal and environment factors, spore number in natural soil are not always correlated with root colonization level.

Table 2. Mycorrhizal quantification and root colonization of medicinal plants

S.No.	Botanical Name	Local Name	Family	Presence of VAM	% VAM root colonization	VAM spore no./50 gm.soil
1.	*Swertia paniculata* Wall.	Chirayata	Gentianaceae	+ - +	33.93 ± 4.2	*81 ± 2.0
2.	*Achillea millefolium* Linn.	Gandana	Asteraceae	+ + -	44.12 ± 7.4	74 ± 5.2
3.	*Glaucium flavum* Crantz.	Yellow horned poppy	Papaveraceae	+ - +	71.38 ± 14.3	184 ± 7.2
4.	*Gentiana kurooa* Royle.	Karu	Gentianaceae	+ - -	61.21 ± 1.0	112.6 ± 4.1
5.	*Macuna prurita* (Linn.) Hook.	Kaunch	Papilionaceae	+ + +	86.10 ± 13.9	141.3 ± 3.0
6.	*Centratherum anthelminticum* (Willd.) Kunze.	Kalijiri	Asteraceae	+ + +	59.01 ± 8.7	178 ± 6.2
7.	*Digitalis lanata* Linn.	Tilpushpi	Scrophulariaceae	+ - +	72.57 ± 2.5	302 ± 5.0
8.	*Thallictrum rugosum* Ait.	Pillijari	Ranunculaceae	+ + +	66.66 ± 11.1	100.6 ± 3.0
9.	*Mentha spicata* Linn.	Sansfi	Liliaceae	+ - +	66.92 ± 11.7	112.6 ± 5.0
10.	*Asparagus officinalis* Linn.	Geranium	Geraniaceae	+ + +	72.96 ± 14.6	253.6 ± 4.9
11.	*Geranium wallichianum* Wall.	Lavender	Labiatae	+ + +	84.15 ± 8.9	137.5 ± 4.9
12.	*Lavendula angustifolia* Mill.	Mushak-Bala	Valarianaceae	+ + +	76.51 ± 4.7	246.5 ± 6.3
13.	*Valeriana jatamansi* Jones.	Rosmary	Labiatae	+ + +	88.07 ± 4.1	841 ± 25.9
	Rosmarinus officinalis Linn.					

14.	*Hedychium spicatum* Buch-Ham.	Kapur-	Zingeberaceae	+ + +	89.62±10.0	88.3 ± 1.5
15.	*Princepia utilis* Royle.	Bhekhra	Rosaceae	+ - -	57.90±4.9	153.6±6.0
16.	*Urtica dioica* Linn.	Bichubuti	Urticaceae	+ + -	56.24±5.5	142.6±8.3
17.	*Solanum surretence* Burm.f.	Laghu-kantkari	Solanaceae	+ + -	73.05±8.1	166.6±4.7
18.	*Citrus medica* Linn.	Nimbu	Rutaceae	+ - +	43.74 ± 1.2	143 ± 1.4
19.	*Prunus armeniaca* Linn.	Khumani	Rosaceae	+ - +	46.00 ± 0.9	109.6±4.9
20.	*Prunus amygladus* (Linn.) Batsch.	Badam	Rosaceae	+ + +	73.33 ± 6.6	50.0 ± 2.6
21.						

* Mean of three replicates; M = Mycelium; V = Vesicle; A =Arbuscule; + = Present; - =Absent.

Table 3 indicates that a total of twenty four VAM species, belonging to six genera of VAM fungi i.e. *Glomus, Acaulospora, Gigaspora, Scutellospora, Entrophospora* and *Sclerocystis* were isolated from mid mountain medicinal plants of Himachal Pradesh. A variety of spores were screened out from the rhizospheric soil of these plants. The abundance of *Glomus* spore was more predominant than any other VAM fungi. Twelve species of *Glomus*, four species of *Acaulospora*, three species each of *Gigaspora* and *Sclerocystis* and one species each of *Entrophospora* and *Scutellospora* were isolated.

The occurrence of VAM spore depends upon different environmental conditions, plant species and type of soil (Trimurtulu and Johri, 1998). Chaturvedi *et al.* (2007) reported that *Glomus* species favour neutral and alkaline soil, whereas *Acaulospora* species are associated with acidic soils. Similarly, Muthukumar and Udaiyan (2007) recorded that *Gigaspora* species predominate in soil with high sand content.

It is confirmed from the result that VAM fungi colonized the most medicinal plants. Agricultural sustainability could be viewed as 'maximum plant production with minimum soil loss'. This type of study could be the beginning of further research pursuits that will utilize such symbiotic fungi to manipulate the host in different ways. The management of their population in the soil is an essential tool for overall plant health in the present scenario of sustainable crop productivity.

Table 3. Natural occurrence of VAM spores with medicinal plants of Himachal Pradesh (Tentative identification of VAM spores)

		Identification Code (I.C)																								
S No	Medicianl Plants	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
1.	*Swertia paniculata*	+	-	-	-	+	+	+	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+	+
2.	*Achillea millefolium*	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	+	+	-	-	-	-	-
3.	*Glaucium flavum*	-	-	+	+	+	-	-	-zs	+	-	+	-	-	-	-	-	-	-	-	-	-	+	-	-
4.	*Gentiana kurooa*	+	+	-	-	-	-	-	-	+	-	-	+	-	-	-	-	-	-	-	+	-	-	-	+
5.	*Macuna prurita*	-	-	-	+	-	-	-	-	+	-	+	-	+	+	+	+	-	-	+	-	-	-	-	-
6.	*Centratherum anthelminticum*	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	+	+	-	+	-
7.	*Digitalis lanata*	+	-	-	-	+	-	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-	+	+	+
8.	*Thallictrum rugosum*	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+	-	-	+	-	-	+	-	-
9.	*Mentha spicata*	-	-	-	+	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	+	-
10.	*Asparagus officinalis*	-	-	-	-	-	-	+	-	-	+	-	-	-	+	-	+	-	-	-	-	-	+	-	-
11.	*Geranium wallichianum*	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
12.	*Lavundula angustifolia*	-	-	+	-	-	-	-	-	-	-	-	-	+	-	-	-	+	-	-	-	+	-	-	-
13.	*Valeriana jatamansi*	-	-	-	-	-	-	-	+	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
14.	*Rosmarinus officinalis*	-	+	-	+	-	-	-	-	-	-	-	+	-	-	-	+	+	-	-	-	-	-	-	-
15.	*Hedychium spicatum*	+	-	+	-	-	-	-	+	-	-	+	-	+	-	-	-	-	-	-	-	+	-	-	
16.	*Princepia utilis*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+
17.	*Urtica dioica*	-	-	+	-	+	-	+	-	-	-	-	+	-	+	-	-	-	+	-	-	-	-	-	
18.	*Solanum surretense*	-	-	-	-	-	-	-	+	-	-	+	-	-	-	+	-	-	-	-	-	-	+	-	-
19.	*Citrus medica*	+	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	+	-
20.	*Prunus armeniaca*	-	-	-	+	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-
21.	*Prunus amygladus*	+	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	+	+	-	-	-	

I.C.

1.	*Acaulospora laevis*	Gerdemann & Trappe
2.	*A.margarita*	Becker & Hall
3.	*A.mellea*	Spain & Schenck
4.	*A.rehnii*	Sieverding & Toro
5.	*Glomus macrocarpum*	Tulasne & Tulasne
6.	*G.scintilans*	Gerdemann & Trappe
7.	*G.etunicatum*	Becker & Gerdemann
8.	*G.constrictum*	Trappe
9.	*G.intraradices*	Schenck & Smith
10.	*G.mosseae*	Nicolson & Gerdemann (Gerdemann & Trappe
11.	*G.pulvinatum*	Henning (Trappe & Gerdemann)
12.	*G.caledonium*	Nicolson & Gerdemann Trappe & Gerdemann
13.	*G.deserticola*	Trappe, Bloss & Menge
14.	*G.geosporum*	Nicolson & Gerdemann (Walker)
15.	*G.reticulatum*	Bhattacharjee & Mukerji
16.	*G.nigra*	Redhead
17.	*Gigaspora gregaria*	Schenck & Nicolson
18.	*G.margarita*	Becker & Hall
19.	*G.gigantea*	Nicolson & Gerdemann (Gerdemann & Trappe)
20.	*Sclerocystis sinuosa*	Gerdemann & Bakshii
21.	*S.duscii*	(Patouillard) VanHohn)
22.	*S.rubiformis*	Gerdemann & Trappe
23.	*Scutellospora* sp.	
24.	*Entrophospor*a sp.	

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