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ARBUSCULAR MYCORRHIZAL STATUS OF MEDICINAL PLANTS : A FIELD SURVEY OF AM FUNGAL ASSOCIATION IN SHRUBS AND TREES.

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ABSTRACT

Twenty medicinal plants belonging to 16 angiospermic families were surveyed for the occurrence of arbuscular mycorrhizal fungi. All the plants surveyed were colonised with arbuscular mycorrhizae. The colonization in shrubs and trees ranged from 47% to 98% and 29% to 85% respectively, whereas the spore density in the rhizosphere soil showed variation from 12 spores/100g soil to 530 spores/100g soil in shrubs and 13 spores/100g soil to 464 spores/100g soil in tree species. Species composition of AM fungi revealed the presence of four genera *viz.*, *Acaulospora*, *Glomus*, *Sclerocystis* and *Scutellospora* in the rhizosphere soil of the medicinal plants studied.

INTRODUCTION

Arbuscular mycorrhizal fungi are ubiquitous in both natural and man-made ecosystems (Hayman, 1982). These fungi benefit the plant by improving the supply of nutrients, especially phosphorus and other minerals such as Zn, Cu, K and Ca (Cooper & Tinker, 1978). Besides direct nutritional advantage, arbuscular mycorrhizae are accredited with other benefits to the host plants such as ability of AM fungal roots to overcome water stress by stomatal regulation (Levy & Krukum, 1980), increasing disease resistance by depressing root penetration and larval development of nematodes (Sikora, 1978), tolerance to toxic heavy metals, drought, high soil temperature, adverse pH etc. Mycorrhizal inoculation also stimulates rooting (Barrow *et al*, 1977) growth and transplant survival (Bryan & Kormanik, 1977). Hence AM fungi are gaining importance in agro-forestry, agricultural, horticultural sectors and more recently in the field of ethnobotany for the commercial cultivation of medicinal plants in order to enhance plant growth and productivity.

Earlier reports on the occurrence of AM fungi in medicinal plants are mostly on rhizomes (Taber & Trappe, 1982; Selvaraj *et al.*, 1986). Nasim (1990), reported AM fungal association in a few medicinal perennial herbs. Later, Udea *et al.*, (1992)

reported AM fungi in 26 of the 33 species of medicinal plants they examined. Gautum and Sharma, (1996) surveyed AM fungal association in 21 medicinal plant species belonging to different angiospermic families from the forest areas of Madhya Pradesh. Lakshaman, (1997) screened 35 medicinal plants for AM fungal colonization from Sirsi area of North Canara district, Karnataka. More recently, Rani and Bhaduria, (2001) reported AM fungal association in some medicinal plants growing on alkaline soil of Mainpuri district, Uttar Pradesh. Selvaraj *et al.*, (2001) have documented AM association in *Cichorum intybus* L., while Muthukumar *et al.*, (2001) have extensively surveyed AM fungal status of 60 medicinal plants from Maruthumalai hills, Western Ghats, Southern India.

India is bestowed with natural plant wealth encompassing the Western Ghats, which is one of the hot spots of biodiversity in the world. In this paper we report the incidence of AM fungi in medicinal shrubs and trees from Goa region (Latitudes 15°48′ 00″ to 14°43″ 54″ and Longitude 74°20′ 13″ to 73°40′33″ E), which lies in the central portion of Western Ghats.

MATERIALS AND METHODS

The samples were collected from two places in South Goa. The cultivated plant species were sampled from Quepem (Flat topped hills) area whereas the wild plant species were sampled from the adjoining Western Ghat region *viz.*, Netravali (High hills).

Quepem - This area comprises of very shallow, well-drained, brown, loam to sandy clay loam surface soil and dark brown loam to clay loam sub soil with 1-5% slope.

Netravali - This area comprises of very moderately shallow, well drained to somewhat excessively drained, dark reddish brown, clay loam surface soil and reddish brown, clay subsoil with cambic horizon and more than 35 % coarse fragments with 8 - 30 % slope.

Root and rhizosphere soil samples of 20 medicinal plants belonging to 16 families (Table I A & B) were collected during August 2001, packed in polyethylene bags and transported to laboratory. For each plant species, three sub-samples were randomly collected. While sampling, care was taken to trace back the roots of the selected plant species. Root samples were freshly processed, whereas, the soil samples were stored at 4°C until analysed.

The root samples were washed with water, cleared with 10% KOH, acidified in 1N HCl and then stained in lactoglycerol trypan blue (0.05%) according to Phillips and Hayman, (1970). Quantification of AM fungal colonization was carried out using the slide method (Giovannetti & Mosse 1980). For isolation of AM fungal spores/ sporocarps, wet sieving and decanting method proposed by Gerdemann and Nicolson (1963) was followed and quantification of spore density was carried out after the procedure given by Gaur and Adholeya (1994). Intact and unparasitized spores were used for the quantification of spore density and taxonomy of AM fungi. Arbuscular mycorrhizal fungi were identified using bibliographies provided by Morton and Benny (1990); Schenck and Perez (1990); Walker and Trappe (1993) and Wu (1993).

Identification of plant species was

Table IA. List of medicinal shrubs.

S.N.	Name of the plant	Family	Status	Plant part used
1	*Adhatoda zeylanica Medikus*	Acanthaceae	Wild	Leaves
2	*Crossandra infundibuliformis* (L.) Nees.	Acanthaceae	Cultivated	Leaves and flowers
3	*Rauvolfia serpentina* (L.) Benth. ex Kruz.	Apocynaceae	Wild	Root and bark
4	*Calotropis gigantea* (L.) R. Br.	Asclepiadaceae	Wild	Latex
5	*Chromolaena odorata* (L.) R. King & H. Robinson	Asteraceae	Wild	Leaves
6	*Carica papaya* L.	Caricaceae	Cultivated	Unripe fruit & latex
7	*Ricinus communis* L.	Euphorbiaceae	Wild	Leaves
8	*Ixora coccinea* L.	Rubiaceae	Cultivated	Corolla tube
9	*Citrus medica* L	Rutaceae	Cultivated	Root
10	*Datura metel* L.	Solanaceae	Wild	Leaves & flowers

Table IB. List of medicinal trees.

S.N.	Name of the plant	Family	Status	Plant part used
1	*Alstonia scholaris* (L.) R. Br.	Apocynaceae	Wild	Bark
2	*Holarrhena pubescens* (Buch.-Ham.) Wallich ex Don	Apocynaceae	Wild	Bark (latex)
3	*Anacardium occidentale* L.	Anacardiaceae	Cultivated	Seed (oil)
4	*Tamarindus indica* L.	Ceasalpiniaceae	Wild	Leaves & Bark
5	*Erythrina variegata* L.	Fabaceae	Wild	Bark
6	*Strychnos nux-vomica* L.	Loganiaceae	Wild	Stem bark
7	*Garcinia indica* Choisy	Clusiaceae	Wild	Fruits
8	*Murraya koenigii* (L.) Sprengel	Rutaceae	Cultivated	Bark, root
9	*Sapindus laurifolius* Vahl	Sapindaceae	Wild	Bark, fruit
10	*Microcos paniculata* L.	Tiliaceae	Wild	Root and Leaves

carried out using flora of Goa, Diu, Daman, Dadra and Nager Haveli (Rao 1985) and flora of Central Tamil Nadu (Mathew 1991).

Standard deviation was calculated for mean root colonization and mean spore density. Pearson's correlation test was performed to assess the relationship between AM fungal root colonization levels and spore numbers in the rhizosphere soil. Prior to correlation analysis, data for root colonization was subjected to arcsine transformations whereas data for spore density was subjected to log transformations.

RESULTS

Data on root colonization and spore population of AM fungi is presented in Table II (A & B). It is observed that, arbuscular mycorrhizal fungi colonized all the medicinal plant species selected for study. Three stages of root colonization *viz.*, hyphal, arbuscular and vesicular

Table IIA. List of medicinal shrubs.

S.N.	Name of the plant	Family	% Root colonization		Spore density / 100 g rhizosphere soil types of propagules		
			Type of colonization	* Total colonization	spores	sporocarps	Total spore density*
1	*Adhatoda vasica* (Linn.) Nees in Wall.	Acanthaceae	HVA	6.9 ± 4.3	528	2	530 ± 44.0
2	*Crossandra infundibuliformis* (L.) Nees.	Acanthaceae	HVA	47 ± 4.2	58	–	58 ± 4.8
3	*Rauvolfia serpentina* (L.) Benth. ex Kruz.	Apocynaceae	HVA	68 ± 5.7	76	–	76 ± 6.1
4	*Calotropis gigantea* (L.) R. Br.	Asclepiadaceae	HV	86 ± 8.8	200	–	200 ± 25.67
5	*Chromolaena odorata* (L.) R. King & H. Robinson	Asteraceae	HVA	69 ± 4.9	10	2	12 ± 0.85
6	*Carica papaya* L.	Caricaceae	HVA	78 ± 5.6	240	6	246 ± 18.5
7	*Ricinus communis* L.	Euphorbiaceae	HVA	60 ± 4.5	62	–	62 ± 5.7
8	*Ixora coccinea* L.	Rubiaceae	HVA	98 ± 8.9	354	–	354 ± 37.8
9	*Citrus medica* L.	Rutaceae	HVA	69 ± 5.2	88	–	88 ± 8.5
10	*Datura metel* L.	Solanaceae	HVA	59 ± 6.4	18	–	18 ± 1.2

*Mean value of three readings. ± Indicates Standard deviation.
H = Hyphal colonization; A = Arbuscular colonization; V = Vesicular colonization.

Table IIB. List of medicinal trees.

S.N.	Name of the plant	Family	% Root colonization		Spore density/ 100 g rhizosphere soil		
			Type of coloni-zation	* Total coloni-zation	Type of propagules Spores	Sporo-carps	Total spore density*
1	*Alstonia scholaris* (L.) R. Br.	Apocynaceae	HV	40 ± 3.1	350	8	358 ± 38.44
2	*Holarrhena pubescens* (Buch. -Ham.) Wallich ex Don	Apocynaceae	HV	85 ± 7.4	464	–	464 ± 42.36
3	*Anacardium occidentale* L.	Anacardiaceae	HV	71 ± 7.2	22	–	22 ± 1.5
4	*Tamarindus indica* L.	Ceasalpiniaceae	HVA	60 ± 6.5	224	6	230±24.66
5	*Erythrina variegata* L.	Fabaceae	HV	69 ± 5.6	70	–	70 ± 7.5
6	*Strychnos nux-vomica* L.	Loganiaceae	HV	71 ± 5.5	126	4	13 ± 0.5
7	*Garcinia indica* Choisy	Clusiaceae	HVA	29 ± 1.2	58	–	58 ± 4.6
8	*Murraya koenigii* (L.) Sprengel	Rutaceae	HVA	63 ± 4.9	100	2	102±11.8
9	*Sapindus laurifolius* Vahl	Sapindaceae	HVA	50 ± 5.2	44	–	44 ± 3.7
10	*Microcos paniculata* L.	Tiliaceae	HV	82 ± 7.7	52	2	54 ± 6.0

*Mean value of three readings. ± Indicates Standard deviation.
H = Hyphal colonization; A = Arbuscular colonization; V = Vesicular colonization.

colonization were recorded (Table II A & B). An average root colonization of 70.30% was recorded in shrubs, whereas the highest and lowest root colonization was recorded in *Ixora coccinea* (98%) and *Crossandra infundibuliformis* (47%) respectively. In tree species, the average root colonization recorded was 62% with the highest and the lowest being recorded in *Holarrhena pubescens* (85%) and *Garcinia indica* (29%) respectively.

Arbuscular mycorrhizal spore populations also showed variation in the rhizosphere soil of the shrubs and tree species. An average spore density of 164.40 spores/100g soil was recorded in the shrubs, whereas AM fungal spores in the rhizosphere soil of shrubs ranged from as low as 12 spores/100g soil in *Chromolaena odorata* to as high as 530 spores/100g soil in *Adhatoda zeylanica*. An average spore density of 141.50 spores/100g soil was recorded in tree species. Here, the maximum spore density was recorded in *Holarrhena pubescens* (464 spores/100g soil) and the minimum spore density was recorded in *Strychnos nux-vomica* (13 spores/ 100g soil). However, no significant correlation was observed between the extent of root colonization and spore density of AM fungi in both shrubs and trees.

The diversity of AM fungi in shrubs and trees is reported in Table III. A total of 16 AM fungi belonging to four genera *viz. Acaulospora, Glomus, Sclerocystis* and *Scutellospora* (Plate 1) were reported from the rhizosphere soil of the medicinal plants studied. However, comparatively higher numbers of AM fungal species were recorded in trees (12) than in shrubs (8) with the number of AM fungi given in parenthesis.

DISCUSSION

The present study extends the list of mycorrhizal plants used for medicine and documents their AM fungal association. Our study revealed higher root colonization of AM fungi in medicinal plants which is in agreement with findings of Srivastava and Basu, (1995) and Lakshaman, (1997).

Arbuscular mycorrhizal spore populations reported during our study is below the spore density range (200 spores/100g soil - 8900 spores/100g soil) as reported by Gautum and Sharma, (1996) in medicinal plants from forest areas of Madhya Pradesh.

In the present study no definite correlation could be established between AM fungal root colonization and spore numbers. Our results are contradictory to the findings of Muthukumar *et al.*, (2001) who have reported positive correlation between the two in medicinal plants from Maruthamalai hills in Western Ghats of Southern India. Poor correlation between spore density and root colonization could be due to the fact that sporulation of AM fungi is dependent on wide range of environmental factors (Muthukumar *et al.*, 2001)

In our study AM fungi belonging to genus *Glomus* were the most representative type in the rhizosphere soil of medicinal plants. Lakshaman (1997) and Selvaraj *et al.*, (2001) have also reported the dominance of genus *Glomus* in rhizosphere soil of medicinal plants. However, our study differs with the findings of Lakshaman (1997) and Muthukumar *et al.*, (2001) who have reported the absence of genus *Sclerocystis*

Table III. List of arbuscular mycorrhizal fungi in medicinal plants.

Sr. no	Arbuscular mycorrhizal fungi	Shrubs	Trees
I	***ACAULOSPORA sp.***		
1	*A. morrowae* Spain & Schenck.	+	–
2	*A. scrobiculata* Trappe.	–	+
3	*A. spinosa* Walker & Trappe.	+	+
II	***GLOMUS sp.***		
1	*G. aggregatum* Schenck & Smith	+	–
2	*G. fasciculatum* Gerd. & Trappe emend. Walker & Koske	+	+
3	*G. heterosporum* Smith & Schenck.	–	+
4	*G. glomerulatum* Sieverding.	–	+
5	*Glomus macrocarpum* Tulasne & Tulasne	–	+
6	*Glomus maculosum* Miller & Walker	–	+
7	*G. monosporum* Gerdemann & Trappe.	–	+
8	*G. mosseae* Nicolson & Gerdemann	+	–
9	*G. versiforme* (Karsten) Berch.	+	–
III	***SCLEROCYSTIS sp.***		
1	*S. microcarpa* Iqbal & Bushra	–	+
2	*S. sinuosa* Gerdemann & Bakshi	–	+
3	*S. taiwanensis* Wu & Chen.	+	+
IV	***SCUTELLOSPORA sp.***		
1	*Scutellospora gregaria* (Schenck & Nicolson) Walker & Sanders	+	+

and presence of genus *Gigaspora* in their studies, while Gautum and Sharma (1996) have also reported the presence of genus *Gigaspora* and *Sclerocystis* in medicinal plants from forests of Madhya Pradesh.

As plants continue to be an important resource material for therapeutic agent both in developed and developing countries, measures for their protection, conservation and commercial cultivation are suggested. In addition to the conventional methods of improving growth and yield of medicinal plants *viz.*, growing them under appropriate climatic conditions and supplying suitable plant nutrients, an alternative method is by harnessing useful micro-organisms present especially in the rhizosphere and rhizoplanes of medicinal plants (Sen, 1998).

Our study is a brief report on field survey of AM fungi in medicinal plants and throws light on the wide array of AM fungi. A fairly good levels of AM fungal spore population present in the rhizosphere.soil, suggests further studies towards utilization aspects of these fungi for commercial cultivation of medicinal plants.

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