Plant Archives Vol. 3 No. 2, 2003 pp. 167-174 *ISSN 0972-5210*

Arbuscular Mycorrhizal (AM) Status of Tropical Medicinal Plants : A Field Survey of Arbuscular Mycorrhizal Fungal Association in Herbs

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Abstract

The status of arbuscular mycorrhizal (AM) colonization and spore density was critically examined in twenty medicinal herbs belonging to fourteen angiospermic families. All the plant species examined were colonized by arbuscular mycorrhizal fungi having colonization levels ranging from 10% in *Achyranthus aspera* belonging to family Amaranthaceae to 94% in *Justicia procumbens* belonging to family Acanthaceae.

Spore number ranged from 10 spore/100g rhizosphere soil in *Ludwigia linifolia* belonging to family Onagraceae to 382 spores/100g rhizosphere soil in *Leucas aspera* belonging to Lamiaceae. A total of twenty arbuscular mycorrhizal fungal species belonging to four genera viz., *Acaulospora, Gigaspora, Glomus* and *Scutellospora* were recorded.

Key words : Arbuscular mycorrhizal (AM) fungi, medicinal plants.

Introduction

India has efficient history of use of plants in the indigenous system of medicines, (Ayurveda, Unani and Sidha), and the use of medicinal treatment dates back over 5000 years. India officially recognizes over 2500 plants having medicinal values, and it has been estimated that over 6000 plants are used as traditional folk and herbal medicines (Huxley, 1984).

The World Health Organization (WHO) has compiled a list of 20,000 medicinal plants used in different parts of the globe (Gupta and Chadha, 1995). Because of increasing importance of herbal medicines, and India being a varitable reservoir of medicinal plants, it is very appropriate and necessary to make concerted and serious efforts to improve the quality and quantity of herbal medicines.

In addition to the conventional methods of improving the growth and yield of medicinal plants *viz.*, growing under appropriate soil and climatic condition and supplying suitable plant nutrients, N, P, K and other major and minor elements, another method that is advocated presently is by harnessing useful and appropriate soil

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microorganisms present in the rhizosphere of medicinal plants (Sen, 1998).

Arbuscular mycorrhizal fungi by virtue of their symbiotic associations with roots of most vascular plants are among the most significant microbes in terrestrial ecosystems. They offer good scope for their use in plant growth improvement because of their nutrient mobilization capacity and moisture retention capacity. Mycorrhizae are not only more efficient in utilizing available nutrients from the soil (Bowen and Smith, 1981), but also are involved in transfer of nutrients from components of soil minerals and organic residues to solution and in nutrient cycling in an ecosystem (Jeffries and Barea, 1994). The inoculation of different species of arbuscular mycorrhizal fungi *viz., Glomus fasciculatum, Glomus mosseae* and *Glomus macrocarpum* have been found to increase the yield and protein content of the crop (Mathur and Vyas, 1990; Sivaprasad *et al,* 1990). Mycorrhizal plants needs less fertilizer and can withstand heavy metal and acid pollution better (Crush and Hay, 1981).

The occurrence of arbuscular mycorrhizal fungi in medicinal plants has reported earlier by Taber and Trappe, (1982), Nasim, (1990) and Udea *et al* (1992). Recently Muthukumar and Udaiyan (2001), Selvaraj *et al* (2001) and Rani and Bhaduria, (2001) have reported the occurrence of arbuscular mycorrhizal fungi in medicinal plants from India. In the present study an attempt was made to record the occurrence of arbuscular mycorrhizal fungi in some of the medicinal herbs from Goa.

Materials and Methods

The samples were collected from Quepem situated in Sanguem, South Goa (15° 12' 45" N latitude and 74° 4' 15" E longitude). This area comprises of very shallow, well drained, brown, loam surface soil and dark brown loam to clay loam sub soil with 1-5% slope.

For soil analysis, samples were collected from a depth of 0-25cm from five different locations of Quepem and were brought to the laboratory in polyethylene bags. Samples were passed through 2mm sieve to remove the larger soil particles and were mixed thoroughly to obtain a composite sample. Later, the composite sample was processed three times to get the mean value. Soil pH was measured after dilution with distilled water (1:1 w/v soil: water) soon after the samples were brought to the laboratory. Electrical Conductivity (EC) was determined in 1:1 water: waste extracts (Bower and Wilcox, 1965). Total nitrogen was determined by micro-Kjeldahl method (Jackson, 1971). Total phosphorus is estimated by ammonium molybdate method (Jackson, 1971). Total potassium was determined by flame photometric method (Jackson, 1971). Available phosphorus was determined by Olsen's method (Olsen, 1954).

Twenty medicinal herbs belonging to fourteen angiospermic families were surveyed for arbuscular mycorrhizal fungal association. Randomly selected root samples were cut into 1cm segments, cleared with 10% KOH and stained with 0.05% trypan blue in lactophenol (Phillips and Hayman, 1970). The degree of colonization was calculated using slide method (Giovannetti and Mosse, 1980). Hundred grams of rhizosphere soil sample were assayed for spore count using wet sieving and decanting procedure (Gerdemann and Nicolson, 1963). In each case three replicates were taken. Estimation of spore density was carried out

as per the procedure given by Gaur and Adholeya, (1994). Identification of arbuscular mycorrhizal fungal species was carried out by using the manual for identification (Schenck and Perez, 1990) matching original descriptions and those provided by the International Collection of Vesicular Arbuscular Mycorrhizal fungi (http://invam.caf. wvu.edu). Plants collected in the present study were identified using floras (Matthew, 1991; Mohanan and Henry, 1994 and Naithani *et al,* 1997).

Standard deviation was calculated for mean root colonization and mean spore density and Pearson's correlation was used to understand the relationship between arbuscular mycorrhizal root colonization levels and spore numbers in the rhizosphere soil. Frequency of occurrence was calculated by using the formula given below.

$$\text{Frequency (\%)} = \frac{\text{Number of samples in which AM species occurred}}{\text{Total number of samples studied}} \times 100$$

Results and Discussion

Soil analysis results are depicted in Table 1. Soil was highly acidic and was found to be deficient in available phosphorus. Results on mycorrhizal colonization and spore number are represented in Table 2. It is well known that mycorrhizal colonization is heaviest on infertile soil. Low levels of phosphorus can themselves lead to an increased intensity of colonization (Mosse, 1973).

Arbuscular mycorrhizal colonization was recorded in all the plant species examined in the study. However, the extent of colonization exhibited variations (Table-2). The results in the present study are contradictory to those of Mohankumar and Mahadevan (1984) who reported absence of arbuscular mycorrhizae in the medicinal plant but are in agreement with those of Taber and Trappe, (1982), Lakshman, (1997), Srivastava *et al* (1995), Rani and Bhaduria, (2001) and Muthukumar and Udaiyan, (2001) who have reported arbuscular mycorrhizal colonization in medicinal plants. The variations in extent of mycorrhizal colonization among different plant species observed in the present study confirm earlier findings of Manjunath and Bagyaraj, (1982) and Gerdemann, (1965), who have stated that the extent to which plants respond to arbuscular mycorrhizal colonization varies with plant species. According to Tommerup, (1992) the fungi vary in their colonization patterns due to differences in rate of intra-radical growth, amount of hyphae per entry point, and growth of external mycelium along roots before entry points are formed.

The mycorrhizal colonization in the present investigation was characterized by the presence of hyphae, hyphal coils, vesicles and/or arbuscules. The absence of arbuscules in some plant species suggests that the hyphal coils may serve the function of arbuscules. Barker *et al* (1998) reported that in Paris type of arbuscular mycorrhizal colonization, growth into the root is slow, being primarily intra-cellular and the fungus forms coils inside each cell with rare of minimally

Table 1 : Soil characteristics of study site-Quepem.

Soil Parameters	Value
pH	5.2
Electrical conductivity (mmhos/cm)	0.49
Total nitrogen (mg 100 g^{-1})	400
Total phosphorus (mg 100 g^{-1})	168
Total potassium (mg 100 g^{-1})	160
Available phosphorus (mg 100 g^{-1})	0.31

Table 2 : Percent root colonization, spore density and arbuscular mycorrhizal (AM) fungal diversity in some medicinal herbs from Quepem-Goa.

Family and plant species	*Root colonization (%)	AM fungal structures	*No. of spores + sporocarps/100g rhizosphere soil	Identified arbuscular mycorrhizal (AM) fungal species
AMARANTHACEAE *Achyranthus aspera* L.	10 ± 0.8	HV	25 ± 2.0	*G. fasciculatum, G. macrocarpum, G. mosseae*
Amaranthus spinosus L.	25 ± 2	HVA	15 ± 1.3	*G. mosseae, G. geosporum, G. sinuosum.*
Celosia argentia L.	56 ± 4.5	HVA	21 ± 2.5	*A. Scrobiculata, A. spinosa, S. coralloidea.*
ACANTHACEAE *Andrographis paniculata* Nees.	69 ± 4.9	HVA	139 ± 13.6	*G. fasciculatum, G. mosseae, G. sinuosum.*
Asteracantha longifolia Nees.	30 ± 3.1	HV	80 ± 7.9	*G. taiwanensis, G. macrocarpum*
Justicia procumbens L.	94 ± 8.6	HVA	251 ± 24	*G. mosseae, S. gregaria, Gi. margarita.*
AIZOACEAE *Mollugo pentaphylla* L.	93 ± 10.1	HVA	72 ± 7	*G. sinuosum, G. mosseae.*
APIACEAE *Centella asiatica* L.	58 ± 4.7	HVA	28 ± 2.5	*G. geosporum, G. intraradices, G. mosseae, A. scrobiculata.*
APOCYNACEAE *Vinca rosea* L.	44 ± 4.2	HVA	98 ± 9.9	*G. fasciculatum, A. spinosa, S. gregaria, Gi. margarita, S. reticulata.*
ASTERACEAE *Vernonia cinerea* (L.) Less	61 ± 6.4	HVA	100 ± 10.2	*G. geosporum, A. laevis, A. bireticulata, S. gregaria.*
BALSAMINACEAE *Impatiens oppositifolia* L.	46 ± 3.2	HVA	60 ± 4.8	*A. spinosa, A. scrobiculata.*
Impatiens kleinii Wight & Arn.	78 ± 6.5	HVA	30 ± 3.1	*G. mosseae, A. spinosa, G. sinuosum, G. taiwanensis.*
CAESAL PINIACEAE *Cassia tora* L.	69 ± 4.8	HVA	91 ± 10	*Gi. albida, Gi. margarita, S. coralloidea, G. sinuosum, G. taiwanensis.*

Continued.....

Table 2 Continued....

GENTIANACEAE *Canscora diffusa* (Vahl) R. Br.	50±5.2	HVA	30 ± 3.5	*G. macrocarpum, A. spinosa, A. scrobiculata.*
LAMIACEAE *Leucas aspera* Spreng.	67±6.6	HVA	382 ± 39	*A. spinosa, A. scrobiculata, S. reticulata, G. taiwanensis.*
Ocimum tenuiflorum L.	20±1.8	HVA	51 ± 5.2	*G. sinuosum, G. macrocarpum*
MIMOSACEAE *Mimosa pudica* L.	86±8.2	HVA	224 ± 22.9	*G. fasciculatum, G. mosseae, G. sinuosum, S. gregaria.*
MUSACEAE *Musa paradisica* L.	66±6.9	HVA	20 ± 8	*G. macrocarpum, A. spinosa.*
ONAGRACEAE *Ludwigia linifolia* Roxb.	66±6.1	HVA	10 ± 0.9	*G. fasciculatum, G. taiwanensis.*
PEDALIACEAE *Sesamum mulayanum* Nair.	58±5.4	HVA	194 ± 19.2	*G. multicaule, G. microcarpum, Gi. margarita, G. clavisporum, G. coremioides.*

* Mean of three samples; ± - Standard deviation. H - Hyphal; V-Vesicular, A - Arbuscular colonization.

structured arbuscules.

Arbuscular mycorrhizal spore population also showed variation in the rhizosphere soil of the medicinal herbs (Table II). Minimum spore density was recorded in *Ludwigia linifolia* (10 spores/100g rhizosphere soil) belonging to family Onagraceae, while maximum spore density was recorded in *Leucas aspera* (382 spores/100g rhizosphere soil) belonging to family Lamiaceae. Variations in spore number have been reported earlier by Kruckelmann, (1975) who found significant differences in spore number in six different plant species growing in monoculture for sixteen years. The influence of host plant on incidence of arbuscular mycorrhizal fungi has also been observed by Schenck and Kinloch, (1980) on a woodland site newly planted with six agronomic crops and grown in monoculture for seven years. However, no significant correlation ($r=0.24$; $p<0.05$) was observed between spore density and root colonization of arbuscular mycorrhizal fungi in medicinal herbs. As arbuscular mycorrhizal fungal sporulation is dependent on a wide range of host fungal and environmental factors, spore numbers in natural soils are not always correlated with colonization levels.

Arbuscular mycorrhizal species belonging to four genera *viz., Acaulospora, Gigaspora, Glomus*

Table 3 : Percent frequency distribution of arbuscular mycorrhizal (AM) fungal species in some medicinal herbs, from Quepem-Goa.

Arbuscular mycorrhizal (AM) fungal species	Frequency (%)
Acaulospora bireticulata Rothwell & Trappe	5
Acaulospora laevis Gerdemann & Trappe	5
Acaulospora scrobiculata Trappe	25
Acaulospora spinosa Walker & Trappe	35
Gigaspora albida Schenck & Smith	5
Gigaspora margiarita Becker & Hall	20
Glomus clavisporum (Trappe) Almeida & Schenck, comb. nov.	5
Glomus coremioides (Berk. & Broome) Redecker et Morton, comb. nov.	5
Glomus fasciculatum (Thaxter) Gerd. & Trappe emend. Walker & Koske	25
Glomus geosporum (Nicol. & Gerd.) Walker	15
Glomus intraradices Schenck & Smith	5
Glomus macrocarpum Tul. & Tul.	25
Glomus microcarpum Tul. & Tul.	5
Glomus mosseae (Nicol. & Gerd.) Gerdemann & Trappe	40
Glomus multicaule Gerdemann & Bakshi	5
Glomus sinuosum (Gerd. & Bakshi) Almeida & Schenck, *comb. nov.*	35
Glomus taiwanensis (Wu & Chen) Almeida & Schenck, *comb. nov.*	25
Scutellospora corolloidea (Trappe, Gerdemann & Ho) Walker & Sanders	10
Scutellospora gregaria (Schenck & Nicol.) Walker & Sanders	20
Scutellospora reticulata (Koske, Miller & Walker) Walker & Sanders	10

and Scutellospora were recorded from rhizosphere soils of medicinal herbs. The number of arbuscular mycorrhizal fungal species ranged from 2 to 5 per plant species (Table 2). Among the various genera of arbuscular mycorrhizal fungi encountered, *Glomus* (55%) was most dominant genera as compared to *Acaulospora* (20%), *Gigaspora* (10%) and *Scutellospora* (15%). Dominance of genus *Glomus* from medicinal plants has been reported earlier by Lakshaman, (1997) and Selvaraj *et al* (2001). Frequency of occurrence of arbuscular mycorrhizal fungal species is represented in Table 3. Among the various arbuscular mycorrhizal fungal species reported *Acaulospora spinosa* (35%), *Acaulospora scrobiculata* (25%), *Gigaspora margarita* (20%), *Glomus fasciculatum* (25%), *Glomus mosseae* (40%), *Glomus sinuosum* (35%), *Glomus taiwanensis* (25%) and *Scutellospora gregaria* (20%) were the most frequently occurring species. Thus, presence of these fungi in medicinally important plants

suggests that these fungi are resistant to the active principal of these medicinally important plants (Iqbal *et al,* 1988).

With the increased global interest in medicinal herbs, the world market is growing faster than the supply. Therefore, to meet the demand of the herbal medicine, the answer lies only when the medicinal plants are cultivated commercially. In this respect, mycorrhiza can be used for commercial cultivation to harness the plant growth and development. However, there is a need to conduct qualitative and quantitative studies on the effect of arbuscular mycorrhizal fungi on medicinal plants and screening of suitable arbuscular mycorrhizal fungi for commercial cultivation of medicinal plants.

Acknowledgement

The first author Ms. Mehtab Jahan Bukhari would like to thank Shri Bhaskar Nayak, Principal, Govt. College of Arts, Science and Commerce, Quepem- Goa for providing laboratory facilities.

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