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Arbuscular mycorrhizal fungi associated with some pteridophytes from western ghat region of Goa

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Abstract: Commonly occurring pteridophytes from two sites namely Mollem and Chorlem located in western ghats region of Goa were selected for the present study. All the pteridophytic species examined during the study, exhibited the presence of arbuscular mycorrhizal association. The highest arbuscular mycorrhizal root colonization (75%) was recorded in *Pityrogramma calomelanos* whereas the highest spore density (155 spores 100 g⁻¹ rhizosphere soil) was recorded in *Adiantum lunulatum*. A fairly good diversity of AM fungi was observed in the rhizosphere of pteridophytes of this region. A total of 17 AM fungal species belonging to five genera namely *Acaulospora, Glomus, Gigaspora, Sclerocystis* and *Scutellospora* were recorded.

Resumen: Para el presente estudio se seleccionaron pteridofitas cuya presencia es común en dos sitios, Mollem y Chorlem, localizados en la región de Goa en los Gates Occidentales. Todas las especies de pteridofitas examinadas durante el estudio mostraron la presencia de asociaciones micorrizicas arbusculares. En *Pityrogramma calomelanos* se registró la mayor colonización por micorrizas arbusculares (75%), mientras que la mayor densidad de esporas (155 esporas 100 g⁻¹ de suelo de la rizosfera) fue registrada en *Adiantum lunulatum*. Se observó una diversidad bastante alta de hongos MA en la rizosfera de las pteridofitas de esta región. Se registraron en total 17 especies de hongos MA pertenecientes a cinco géneros: *Acaulospora, Glomus, Gigaspora, Sclerocystis* y *Scutellospora*.

Resumo: No estudo presente foram seleccionadas Pteridófitas ocorrendo vulgarmente nas Gates ocidentais da região de Goa em duas estações localizadas em Mollem e Chorlem. Todas as espécies de Pteridófitas examinadas durante o estudo, exibiram a presença de associações micorrizicas arbusculares associadas. A colonização radicular mais elevada (75%) foi registrada em *Pityrogramma calomelanos* enquanto que a maior densidade de esporas (155 esporas por 100g de solo da rizosfera) foi encontrada em *Adiantum lunulatum*. Uma razoavelmente boa diversidade de fungos micorrízicos arbusculares (AM) foi observada na rizosfera das Pteridófitas desta região. Foram registrados um total de 17 fungos AM pertencendo a cinco géneros *Acaulospora, Glomus, Gigaspora, Sclerocystis* e *Scutellospora*.

Key words: *Acaulospora, AM fungi, Glomus, Gigaspora, pteridophytes, root colonization, Sclerocystis, spore density, Scutellospora.*

Introduction

Pteridophytes constitute a significant and important group in the plant kingdom as early land plants. They show various adaptations as they have evolved to fill different habitats thereby providing an ecological niche for many microorganisms like AM fungi that abound the soil. The earliest reports on mycorrhizal colonization in pteridophytes were mostly based on the samples removed...
from herbarium specimens (Boullard 1957). Later, Newman & Reddell (1987) examined 180 field specimens of fern species for mycorrhizal colonization. Mycorrhizal surveys were also reported from New Zealand ferns (Cooper 1976), and Ontario ferns and its allies (Berch & Kendrick 1982). More recently Gemma & Koske (1990) and Gemma et al. (1992) reported mycorrhizal status of Hawaiian pteridophytes.

In India, Mishra et al. (1980) and Ragupathy & Mahadevan (1993) have documented studies on arbuscular mycorrhizal colonization of pteridophytes. Work on arbuscular mycorrhizal status of pteridophytes from western ghats of Southern India has also published. Raja et al. (1995) reported AM fungal association in 43 pteridophytes from the Nilgiris and Kodaikanal hills of western ghats. Recently, Muthukumar & Udaiyan (2000) reported the arbuscular mycorrhizae of pteridophytes growing in different habitats and substrata. They surveyed 71 pteridophytic species belonging to 30 families from six different localities in western ghats of Southern India.

The state of Goa lies in the heart of western ghats, which is one of the hotspots of biodiversity. The major portion of the slopes of western ghat belt falls in this region (Rao 1985), encompassing luxuriant forest with good diversity of pterido-

Table 1. List of pteridophytes collected for the study.

<table>
<thead>
<tr>
<th>Name of pteridophyte</th>
<th>Family</th>
<th>Habitat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sellaginella sp.</td>
<td>Selaginellaceae</td>
<td>Soil</td>
</tr>
<tr>
<td>Lygodium flexuosum L.</td>
<td>Lygodiaceae</td>
<td>Soil</td>
</tr>
<tr>
<td>Lindsaea heterophylla Beddome</td>
<td>Lindsaeaceae</td>
<td>Soil</td>
</tr>
<tr>
<td>Pteris vittata L.</td>
<td>Pttridaceae</td>
<td>Rock</td>
</tr>
<tr>
<td>Adiantum lunulatum Burm.</td>
<td>Adiantaceae</td>
<td>Soil</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Site – Chorlem</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Athyrium hohenackeranum (Kunze) Moore</td>
<td>Athyraceae</td>
<td>Soil</td>
</tr>
<tr>
<td>Blechnum orientale L.</td>
<td>Blechnaceae</td>
<td>Soil</td>
</tr>
<tr>
<td>Gleichenia dichotoma Wild.</td>
<td>Gleicheniaceae</td>
<td>Soil</td>
</tr>
<tr>
<td>Pityrogramma calomelanos Link.</td>
<td>Hemionitidaceae</td>
<td>Soil</td>
</tr>
<tr>
<td>Christella dentata (Forssk.)</td>
<td>Thelepteridaceae</td>
<td>Soil</td>
</tr>
<tr>
<td>Brownsey &amp; Jermy.</td>
<td></td>
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</tr>
</tbody>
</table>

phytic flora (Tewari 1995). The present paper is an attempt to enumerate the status of arbuscular mycorrhizal fungi associated with the pteridophytes of western ghat region of Goa.

**Materials and methods**

**Sample collection**

Five root samples and rhizosphere/substratum samples for each of the ten commonly occurring pteridophytic species were randomly collected during September 2000, from two sites in western ghat region of Goa namely, Mollem and Chorlem (Table 1). The plants were completely uprooted for collection of samples. Samples were placed in the polyethylene bags, labelled and then transported to the laboratory. Root samples were freshly processed, whereas rhizosphere soil samples were stored in deep freezer at 4°C until they were analyzed.

**Estimation of root colonization**

The root samples were stained by using modified procedure for staining roots (Koske & Gemma 1989). Root samples of each pteridophyte species were washed gently under tap water and cleared in 2.5% KOH, acidified in 5 N HCl and stained in lactoglycerol with 0.05% Trypan blue. The stained roots were examined under compound microscope (x40 – x100). Hundred root segments for each sample were randomly selected for microscopic observation and the degree of colonization was estimated using slide method (Giovannetti & Mosse 1980).

**Isolation and quantification of spores and sporocarps**

For isolation of spores/sporocarps, wet sieving and decantating procedure (Gerdemann & Nicolson 1963) was followed. For this, 100 g of rhizosphere soil/substratum was dispersed in 1000 ml of water and the coarse particles were allowed to settle for 15-20 seconds. The soil suspension was then decanted through stacked sieves kept in descending order of pore size (500 μm – 45 μm). The above step was repeated twice so that the majority of spores were recovered from the soil. The debris on the sieves was then collected in the beaker and filtered through Whatman no. 1 filter paper. The filter paper was kept in Petridish and care was taken to see that it remains moist. The contents
were then examined for spores and sporocarps under stereomicroscope, and the quantification of spores and sporocarps was carried out using procedure described by Gaur & Adholeya (1994).

Identification of AM fungi

Diagnostic slides with spores/sporocarps were prepared using polyvinyl alcohol lactoglycerol (PVLG) as mountant. Both broken and unbroken spores were observed. Spores were examined using compound microscope (x40 – x100). The genera and the species of AM fungi were identified using bibliographies provided by Morton & Benny (1990), Schenck & Perez (1990), Walker & Vestberg (1998) and Wu (1993). Names and epithet of AM fungi are enlisted according to Walker & Trappe (1993).

Identification of pteridophytes

Identification of pteridophytes was carried out using 'The Manual of Pteridophyte Flora of Western Ghat, South India' (Manickam & Irudayaraj 1992).

Statistical analysis

Standard deviation was calculated for mean spore density and mean root colonization. Pearson’s one tailed correlation test was performed for both AM fungal parameters (mean spore density and mean root colonization). Analysis of variance (ANOVA) was also performed to study the influence of each host type of the colonization of AM fungi. Prior to ANOVA test, the root colonization values were subjected to square root transformations in order to fit in the statistical package (mstact) format.

Results

In the present study, the survey of pteridophytes for AM fungi showed variability in colonization and spore density. All the pteridophytes selected for study exhibited the presence of AM fungal association. Hyphal and vesicular stages of colonization were seen in all the pteridophytes. However, arbuscular colonization was seen only in Lindsaea heterophylla (Table 2).

In Mollem area, mean root colonization and mean spore density of AM fungi in pteridophytes showed no correlation (r = 0.008, P > 0.05, n = 4). Here, the highest root colonization and spore density was recorded in Lindsaea heterophylla (50%) and Adiantum lunulatum (155 spores 100 g⁻¹ rhizosphere soil), respectively (Fig. 1). However, in Chorlem area, the mean root colonization and spore density of AM fungi exhibited a weak negative correlation (r = -0.45, P > 0.05, n = 4) with highest root colonization and spore density recorded in Pityrogramma calomelanos (75%) and Christella dentata (116 spore 100 g⁻¹ rhizosphere soil) respectively (Fig. 2). The host species significantly influenced root colonization (r) and spore density (s) in Mollem (C.Dr = 0.092 & C.Ds = 5.40; P < 0.05) as well as Chorlem (C.Dr = 0.589 & C.Ds = 5.83; P < 0.05) area.

### Table 2. Type of colonization by AM fungi in pteridophytes.

<table>
<thead>
<tr>
<th>Name of pteridophytes</th>
<th>Hyphal colonization</th>
<th>Arbuscular colonization</th>
<th>Vesicular colonization</th>
<th>Presence of spores</th>
<th>Presence of sporocarps</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sellaginella sp.</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Lygodium flexuosum</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Lindsaea heterophylla</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Pteris vittata</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Adiantum lunulatum</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Athyrium hohenackeranum</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Blechnum orientale</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Gleichenia dichotoma</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Pityrogramma calomelanos</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Christella dentata</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

+ = present; — = absent.
During the course of study, a total of 17 AM fungal species belonging to five genera namely Acaulospora, Glomus, Gigaspora, Sclerocystis and Scutellospora were recorded. Glomus was most dominant among all the genera (Table 3). Out of the ten pteridophyte species studied, sporocarpic species of AM fungi were recorded in six pteridophytes (Tables 2 & 3). The species composition of AM fungi showed variability in the rhizosphere soil of the pteridophytes selected for study. In Mollem area the species richness of AM fungi ranged from 2-3 species/pteridophyte whereas in Chorlem area it ranged from 2-5 species/pteridophyte (Table 3).

**Discussion**

The present study is the first report on arbuscular mycorrhizal status of pteridophytes from western ghat region of Goa. Our study recorded the absence of correlation between root colonization and spore density of AM fungi which is in accordance with earlier report on AM fungal association in pteridophytes from western ghats, Southern India (Muthukumar & Udaiyan 2000). A possible reason for the lack of correlation may be due to the fact that sporulation of AM fungi is dependent on wide range of environmental factors (Muthukumar et al. 2001).

The spore density range recorded in our study is much higher (10-155 spore 100 g⁻¹ rhizosphere soil) than reported by Muthukumar & Udaiyan (2000) in the rhizosphere soil of pteridophytes from western ghats, Southern India (2.35-39.52 spore 100 g⁻¹ rhizosphere soil). Also, our study recorded the presence of 17 AM fungal species belonging to five genera, which differs from their findings that reported the presence of 8 AM fungi belonging to four genera. Comparatively higher spore density and recovery of relatively higher number of AM fungi from the rhizosphere of pteridophytes, in the present study may be attributed to low host specificity of AM fungi and varying eco-edaphic factors along the different regions of the western ghats.

In our study the composition of AM fungi varied in the rhizosphere soil of the pteridophytes from the same area and is in agreement with the findings of Zhao & Zhao (1999).

The present work, mainly documents AM fungal status of terricolous (soil) pteridophytes and...
Table 3. Diversity of AM fungi associated with pteridophytes.

<table>
<thead>
<tr>
<th>AM fungal species</th>
<th>Sel</th>
<th>Lyg</th>
<th>Lin</th>
<th>Pte</th>
<th>Adi</th>
<th>Ath</th>
<th>Ble</th>
<th>Gle</th>
<th>Pit</th>
<th>Chr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acaulospora foveata Trappe &amp; Janos</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
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<tr>
<td>Glomus claroideum Schenck &amp; Smith emend. Walker &amp; Vestberg</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
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<tr>
<td>Glomus etunicatum Becker &amp; Gerdemann</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
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<tr>
<td>Glomus fasciculatum (Thaxter) Gerdemann &amp; Trappe emend. Walker &amp; Koske</td>
<td>-</td>
<td>-</td>
<td>+</td>
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<td>-</td>
<td>+</td>
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<tr>
<td>Glomus formosanum Wu &amp; Chen</td>
<td>+</td>
<td>-</td>
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<tr>
<td>Glomus hoi Berch &amp; Trappe</td>
<td>-</td>
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<tr>
<td>Glomus macrocarpum (Tul.) Tul. Var. geospora Nicolson &amp; Gerdemann</td>
<td>-</td>
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<tr>
<td>Glomus multicaulis Gerdemann &amp; Bakshi</td>
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<td>-</td>
<td>+</td>
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<tr>
<td>Glomus sp.</td>
<td>-</td>
<td>-</td>
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<td>-</td>
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<td>-</td>
<td>-</td>
<td>+</td>
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<tr>
<td>Gigaspora albida Schenck &amp; Smith</td>
<td>-</td>
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<td>-</td>
<td>-</td>
<td>+</td>
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</tr>
<tr>
<td>Gigaspora decipiens Hall &amp; Abbott</td>
<td>-</td>
<td>-</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
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<td></td>
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<tr>
<td>Gigaspora margarita Becker &amp; Hall</td>
<td>+</td>
<td>-</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Sclerocystis rubiformis Wu &amp; Chen</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Sclerocystis sinuosa Iqbal &amp; Bushra</td>
<td>-</td>
<td>-</td>
<td>+</td>
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<td>-</td>
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<tr>
<td>Sclerocystis taiwanensis Wu &amp; Chen</td>
<td>-</td>
<td>+</td>
<td>-</td>
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<td>+</td>
<td>-</td>
<td>-</td>
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<td>+</td>
<td></td>
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<tr>
<td>Scutellospora gregaria (Schenck &amp; Nicolson) Walker &amp; Sanders</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Scutellospora reticulata (Koske, Miller &amp; Walker) Walker &amp; Sanders</td>
<td>-</td>
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<td>-</td>
<td>+</td>
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</tr>
</tbody>
</table>

Species richness of AM fungi: 3 2 2 2 3 2 3 3 5 3

Sel - Selaginella sp., Lyg - Lygodium flexuosum, Li - Lindsaea heterophylla, Pte - Pteris vittata, Adi - Adiantum lunulatum; Ath - Athyrium hohenackeranum. Moore, Ble - Blechnum orientale, Gle - Gleichenia dichotoma, Pit - Pityrogramma calomelanos, Chr - Christella dentata.

Further studies on arbuscular mycorrhizal association with the epiphytic and other lithophytic pteridophytes in this region needs to be undertaken. Such studies would enable us to better understand the role of arbuscular mycorrhizal fungi in this fascinating group of plant kingdom.

Acknowledgement

The first author (Ms. Sharda W. Khade) would like to thank the Planning Commission, New Delhi, for financial assistance.
References


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.

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

Table IB. List of medicinal trees.

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

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.

<table>
<thead>
<tr>
<th>S.N.</th>
<th>Name of the plant</th>
<th>Family</th>
<th>% Root colonization</th>
<th>Spore density / 100 g rhizosphere soil types of propagules</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Type of colonization</td>
</tr>
<tr>
<td>1</td>
<td><em>Adhatoda vasica</em> (Linn.) Nees in Wall.</td>
<td>Acanthaceae</td>
<td>HVA</td>
<td>6.9 ± 4.3</td>
</tr>
<tr>
<td>2</td>
<td><em>Crossandra infundibuliformis</em> (L.) Nees.</td>
<td>Acanthaceae</td>
<td>HVA</td>
<td>47 ± 4.2</td>
</tr>
<tr>
<td>3</td>
<td><em>Rauvolfia serpentina</em> (L.) Benth. ex Kruz.</td>
<td>Apocynaceae</td>
<td>HVA</td>
<td>68 ± 5.7</td>
</tr>
<tr>
<td>4</td>
<td><em>Calotropis gigantea</em> (L.) R. Br.</td>
<td>Asclepiadaceae</td>
<td>HV</td>
<td>86 ± 8.8</td>
</tr>
<tr>
<td>5</td>
<td><em>Chromolaena odorata</em> (L.) R. King &amp; H. Robinson</td>
<td>Asteraceae</td>
<td>HVA</td>
<td>69 ± 4.9</td>
</tr>
<tr>
<td>6</td>
<td><em>Carica papaya</em> L.</td>
<td>Caricaceae</td>
<td>HVA</td>
<td>78 ± 5.6</td>
</tr>
<tr>
<td>7</td>
<td><em>Ricinus communis</em> L.</td>
<td>Euphorbiaceae</td>
<td>HVA</td>
<td>60 ± 4.5</td>
</tr>
<tr>
<td>8</td>
<td><em>Ixora coccinea</em> L.</td>
<td>Rubiaceae</td>
<td>HVA</td>
<td>98 ± 8.9</td>
</tr>
<tr>
<td>9</td>
<td><em>Citrus medica</em> L.</td>
<td>Rutaceae</td>
<td>HVA</td>
<td>69 ± 5.2</td>
</tr>
<tr>
<td>10</td>
<td><em>Datura metel</em> L.</td>
<td>Solanaceae</td>
<td>HVA</td>
<td>59 ± 6.4</td>
</tr>
</tbody>
</table>

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

Table IIB. List of medicinal trees.

<table>
<thead>
<tr>
<th>S.N.</th>
<th>Name of the plant</th>
<th>Family</th>
<th>% Root colonization</th>
<th>Spore density / 100 g rhizosphere soil types of propagules</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Type of colonization</td>
</tr>
<tr>
<td>1</td>
<td><em>Alstonia scholaris</em> (L.) R. Br.</td>
<td>Apocynaceae</td>
<td>HV</td>
<td>40 ± 3.1</td>
</tr>
<tr>
<td>2</td>
<td><em>Holarrhena pubescens</em> (Buch.-Ham.) Wallich ex Don</td>
<td>Apocynaceae</td>
<td>HV</td>
<td>85 ± 7.4</td>
</tr>
<tr>
<td>3</td>
<td><em>Anacardium occidentale</em> L.</td>
<td>Anacardiaceae</td>
<td>HV</td>
<td>71 ± 7.2</td>
</tr>
<tr>
<td>4</td>
<td><em>Tamarindus indica</em> L.</td>
<td>Ceasalpinaceae</td>
<td>HVA</td>
<td>60 ± 6.5</td>
</tr>
<tr>
<td>5</td>
<td><em>Erythrina variegata</em> L.</td>
<td>Fabaceae</td>
<td>HV</td>
<td>69 ± 5.6</td>
</tr>
<tr>
<td>6</td>
<td><em>Strychnos nux-vomica</em> L.</td>
<td>Loganiaceae</td>
<td>HV</td>
<td>71 ± 5.5</td>
</tr>
<tr>
<td>7</td>
<td><em>Garcinia indica</em> Choisy</td>
<td>Clusiaceae</td>
<td>HVA</td>
<td>29 ± 1.2</td>
</tr>
<tr>
<td>8</td>
<td><em>Murraya koenigii</em> (L.) Sprengel</td>
<td>Rutaceae</td>
<td>HVA</td>
<td>68 ± 4.9</td>
</tr>
<tr>
<td>9</td>
<td><em>Sapindus laurifolius</em> Vahl</td>
<td>Sapindaceae</td>
<td>HVA</td>
<td>50 ± 5.2</td>
</tr>
<tr>
<td>10</td>
<td><em>Microcos paniculata</em> L.</td>
<td>Tiliaceae</td>
<td>HV</td>
<td>82 ± 7.7</td>
</tr>
</tbody>
</table>

*aMean 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.

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

and presence of genus Gigaspora in their studies, while Gautam 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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OCCURRENCE OF ARBUSCULAR MYCORRHIZAL FUNGI IN TREE SPECIES FROM WESTERN GHATS OF GOA, INDIA

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Received June 2001

KHADE, S. W. & RODRIGUES, B. F. 2003. Occurrence of arbuscular mycorrhizal fungi in tree species from Western Ghats of Goa, India. We surveyed the prevalence of arbuscular mycorrhizal (AM) fungi in Mollem forest area of Western Ghats of Goa. A total of 25 tree species belonging to 18 families was screened. AM colonisation in the tree species varied with family and host genera. The highest mean root colonisation (100%) was recorded in Macaranga peltata, Xylia xylocarpa, Zanthoxylum rhetsa and Randia ruglosa. Maximum mean spore density of 745 spores 100 g⁻¹ rhizosphere soil was recorded in Leea indica. A total of 18 AM fungi belonging to five genera, namely, Acaulospora, Gigaspora, Glomus, Sclerocystis and Scutellospora was found to be associated with the tree species studied.

Key words: AM fungi - Acaulospora - Gigaspora - Glomus - root colonisation - Sclerocystis - Scutellospora - spore density - tree species


Introduction

Arbuscular mycorrhizal (AM) fungi can be found in most ecosystems throughout the world ranging from the Artic to the tropical rain forests (Janos 1980a, Beldose et al. 1990). AM fungi play a vital role in natural ecosystems like tropical forests by influencing the composition and succession of plants (Janos 1980b, Giovanetti & Gianinazzi-Pearson 1994). Besides this, increase uptake of nutrients, especially phosphorus (Bolan et al. 1987, Jayachandran et al. 1989), nutrient cycling (Newman & Eason 1989) and exudates in the mycorrhizosphere (Linderman 1988) are major attributes of AM fungal association.

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AM association with forest tree species has been studied in India, covering various areas including subtropical evergreen forest and arid zone (Sharma et al. 1984, Thapar & Khan 1985, Tarafdar & Rao 1990, Thapar & Vijayan 1990, Raman et al. 1992, Santhaguru et al. 1995, Vijaya et al. 1995). The Western Ghats of southern India has also been the focus of AM studies by many workers. Kandasamy et al. (1988) carried out an intensive survey of the prevalence of AM fungi in forest tree species occurring at different altitudes in the Western Ghats of Nilgiri District. Ecological studies on AM fungal association with plant species from Kalakad forest reserve located in the Western Ghats, Tamil Nadu has been documented (Mohankumar & Mahadevan 1988, 1989). The authors investigated the influence of edaphic factors and seasonal variation on the distribution of AM fungi in six well-defined ecosystems, namely, evergreen, semi-evergreen, mixed deciduous, teak forests, scrub-jungle and grassland, at high and low altitudes. Muthukumar and Udaiyan (2000) have documented AM fungal association in four vegetation types, namely, forest, grassland, scrub and cultivated lands. AM fungal association in medicinal plants of Maruthamalai Hills in Western Ghats has also been studied (Muthukumar et al. 2001).

The state of Goa (14° to 16° N latitude and 73° to 75° E longitude) lies in the central portion of Western Ghats that extends from the Tapti river (Gujarat) in the north down to the peninsular tip of south India. It is one of the biodiversity hotspots of the world. No work on AM association in tree species from this portion of Western Ghats has been reported so far. In this paper, we report the occurrence and distribution of AM fungi in tree species from Mollem forest area located in the Western Ghats of Goa.

Materials and methods

Mollem forest, the largest sanctuary in Goa region, was selected for studying the occurrence of AM fungi in tree species. It is located at latitude 15° 29' N and longitude 74° 13' E (Figure 1). This sanctuary is spread over an area of 240 km² and encompasses rich forests varying from moist deciduous to semi-evergreen with the highest altitude of about 891 m above mean sea level. The climate of the tract is tropical with three main seasons, namely, monsoon (June till October), winter (November till January) and summer (February till May). The annual rainfall recorded is 5569 mm with maximum humidity of 96%. The mean maximum and minimum temperatures recorded are 37.2 and 15.9 °C respectively. The soil is moderately drained, gravelly with silty clay loam texture with pH of 5.4 to 6.2 and low in nutrients especially available phosphorus (16 kg ha⁻¹) and total nitrogen (0.24%). The forest tree species are more than 200 years old with an average bole diameter of 4.2 m. Terminalias are the dominant canopy species with Careya arborea, Lagerstroemia lanceolata, Dillenia pentagyna, Microcos paniculata, Strychnos nux-vomica and Calycoperis floribunda being the frequently occurring species in the forest extension areas.
Figure 1 The study site of Mollem forest in Western Ghats of Goa

Root and rhizosphere soil samples of 25 tree species belonging to 18 families were randomly collected from forest extension area of Mollem between October and November 2000. For each tree species, three plants were sampled. During sampling, care was taken to trace back the feeder roots of the selected tree species. Samples were packed in polyethylene bags and transported to the laboratory. 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 1 N HCl and then stained in lactoglycerol trypan blue (0.05%) according to Phillips and Hayman (1970). Quantification of AM fungal colonisation was carried out by using the slide method (Giovannetti & Mosse 1980). Presence of arbuscules, vesicles and hyphae connecting to the fungal structures were taken into consideration while estimating the degree of root colonisation of AM fungi. 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 as described by Gaur and Adholeya (1994). Intact and unparasitised spores were used for the quantification of spore density and taxonomy of AM fungi. AM fungi were identified according to their spore morphology and wall characters (Morton & Benny 1990, Schenck & Perez 1990, Walker & Trappe 1993, Wu 1993).

Identification of tree species was carried out based on Rao (1985) and Mathew (1991). Standard deviation was calculated for mean root colonisation and mean spore density. Pearson's correlation test was performed to assess the relationship between levels of AM fungal root colonisation and number of spores in the rhizosphere soil. Data on root colonisation was arcsin transformed whereas spore numbers were log transformed prior to correlation analysis.

Results

The results of the analysis of roots and rhizosphere soil samples indicate the widespread occurrence of AM fungal association in different tree species occurring in Mollem forest (Table 1, Figure 2). All the tree species selected for the study were found to exhibit AM fungal colonisation. Hyphae and vesicles were predominant AM fungal structures whereas the arbuscules were observed in root samples of seven tree species. In the present study, mean root colonisation and mean spore density varied with families and host genera. An average root colonisation of 50.7% was recorded; the mean root colonisation levels ranged from 17 to 100%. Maximum AM fungal root colonisation was recorded in *Macaranga peltata*, *Xyloxy Roxb.* *xyloroxb.* *Zanthoxylum rhetsa* and *Randia ruglosa*. A colonisation of 17% was recorded in *Dillenia pentagyna*, *S. nux-vomica* and *Lagerstroemia lanceolata*.

An average of 220 spores 100 g⁻¹ soil was recorded in the rhizosphere soil of the tree species studied. AM fungal spores ranged between 18 spores 100 g⁻¹ soil for *S. nux-vomica* and 745 spores 100 g⁻¹ soil for *Leea indica* and were not correlated with mean root colonisation (r = 0.255; p > 0.05).

Species composition of AM fungi in rhizosphere soil of different tree species revealed the presence of 18 species belonging to five genera, namely, *Acaulospora* (5), *Glomus* (8), *Gigaspora* (1), *Sclerocystis* (3) and *Scutellospora* (1) with the species number given in parenthesis (Table 2, Figure 2).
Table 1  Arbuscular mycorrhizal status of tree species in Mollem forest, Western Ghats of Goa

| Tree species                      | Family           | Root colonisation of AM fungi | *Mean root colonisation (%) | *Mean spore density 100 g⁻¹ soil  
|----------------------------------|------------------|-------------------------------|-----------------------------|-----------------------------  
| Alstonia scholaris (L.) R.Br.     | Apocynaceae      | Hypha + Arbuscule + Vesicle - | 40 ± 3.50 (39.22)           | 460 ± 40.55 (2.66)           
| Ervatamia heyneana (Wall.) Cooke.| Apocynaceae      | Hypha + Arbuscule + Vesicle - | 42 ± 3.10 (40.39)           | 273 ± 26.90 (2.43)           
| Holarrhena antidysenterica Wall ex A. DC. | Apocynaceae | Hypha + Arbuscule + Vesicle - | 67 ± 5.85 (54.93)           | 350 ± 37.15 (2.54)           
| Buchanania cochinchinensis (Lour.) Almeida. | Anacardiaceae | Hypha + Arbuscule + Vesicle - | 18 ± 0.08 (25.10)           | 72 ± 8.02 (1.85)             
| Lamnea coromandelica (Houtt.) Merrill. | Apocynaceae      | Hypha + Arbuscule + Vesicle - | 25 ± 1.50 (29.99)           | 78 ± 6.92 (1.89)             
| Calycophyllum floribunda (Roxb.) Lamk. | Combretaceae     | Hypha + Arbuscule + Vesicle - | 52 ± 4.80 (46.14)           | 500 ± 51.25 (2.69)           
| Terminalia paniculata Roth.      | Combretaceae     | Hypha + Arbuscule + Vesicle - | 50 ± 5.50 (44.90)           | 550 ± 56.03 (2.74)           
| Terminalia crenulata Roth.       | Combretaceae     | Hypha + Arbuscule + Vesicle - | 84 ± 8.31 (66.41)           | 340 ± 2.58 (5.23)            
| Dillenia pentagyna Roxb.         | Dilleniaceae     | Hypha + Arbuscule + Vesicle - | 17 ± 1.20 (24.34)           | 27 ± 1.72 (1.43)             
| Macaranga flavida (Roxb.) Mull. Arg. | Euphorbiaceae   | Hypha + Arbuscule + Vesicle - | 100 ± 11.73 (89.98)         | 70 ± 5.85 (1.84)             
| Phyllanthus emblica L.           | Euphorbiaceae    | Hypha + Arbuscule + Vesicle - | 25 ± 3.10 (29.99)           | 575 ± 58.20 (2.75)           
| Lea indica (Burm. f.) Merrill.   | Euphorbiaceae    | Hypha + Arbuscule + Vesicle - | 52 ± 4.50 (46.14)           | 745 ± 70.15 (2.87)           
| Caryya arborea Roxb.             | Euphorbiaceae    | Hypha + Arbuscule + Vesicle - | 50 ± 5.28 (44.99)           | 42 ± 3.67 (1.62)             
| Asacia pinnata (L.) Willd.       | Mimosaceae       | Hypha + Arbuscule + Vesicle - | 66 ± 5.55 (66.41)           | 20 ± 1.52 (1.30)             
| Sterculia mucosa L.              | Loganiaceae      | Hypha + Arbuscule + Vesicle - | 12 ± 1.25 (24.34)           | 18 ± 1.88 (1.25)             
| Lagerstroemia lanceolata Wall. ex Wt. & Arn. | Lythraceae     | Hypha + Arbuscule + Vesicle - | 17 ± 0.99 (24.34)           | 5 ± 3.95 (1.71)              
| Xyia xylocarpa Taub.             | Mimosaceae       | Hypha + Arbuscule + Vesicle - | 100 ± 11.32 (89.98)         | 240 ± 23.11 (2.38)           
| Ziziphus rugosa Lamk.            | Rhamnaceae       | Hypha + Arbuscule + Vesicle - | 60 ± 5.58 (50.76)           | 42 ± 3.85 (1.62)             
| Randia rugosa (Thw.) Hook. f.    | Rubiaceae        | Hypha + Arbuscule + Vesicle - | 100 ± 3.33 (89.98)          | 630 ± 62.25 (2.79)           
| Zanthoxylum rhoia (Roxb.) DC.     | Rutaceae         | Hypha + Arbuscule + Vesicle - | 100 ± 12 (89.98)            | 27 ± 1.72 (1.43)             
| Helicteres isora L.              | Sterculiaceae    | Hypha + Arbuscule + Vesicle - | 17 ± 1.80 (24.34)           | 25 ± 1.72 (1.39)             
| Microcospaniculata L.            | Tiliaceae        | Hypha + Arbuscule + Vesicle - | 33 ± 2.8 (35.05)            | 70 ± 8.10 (1.84)             
| Gymnema arborea Roxb.            | Verbenaceae      | Hypha + Arbuscule + Vesicle - | 82 ± 7.93 (64.88)           | 200 ± 19.02 (2.50)           
| Ficus sp.                        | Urticaceae       | Hypha + Arbuscule + Vesicle - | 33 ± 2.48 (35.05)           | 30 ± 4.15 (1.47)             
| Hopea wightiana Wall ex Wt. & Arn. | Dipterocarpaceae | Hypha + Arbuscule + Vesicle - | 20 ± 1.82 (26.56)           | 69 ± 5.80 (1.83)             

*Mean of three independent observations  
*Values in parentheses are arcsin transformed for root colonisation  
b Values in parentheses are log transformed for spore density
Figure 2  AM fungi in tree species of Mollem forest
Table 2  List of AM fungi associated with tree species of Mollem forest, Western Ghats of Goa

<table>
<thead>
<tr>
<th>Arbuscular mycorrhizal fungal species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acaulospora foveata Trappe &amp; Janos</td>
</tr>
<tr>
<td>Acaulospora scrobiculata Trappe</td>
</tr>
<tr>
<td>Acaulospora spinosa Walker &amp; Trappe</td>
</tr>
<tr>
<td>Acaulospora leavis Gerd. &amp;Trappe</td>
</tr>
<tr>
<td>Acaulospora rugosa Morton</td>
</tr>
<tr>
<td>Glomus fasciculatum (Thaxter) Gerd. &amp; Trappe emend. Walker &amp; Koske</td>
</tr>
<tr>
<td>Glomus hoi Berch &amp;Trappe</td>
</tr>
<tr>
<td>Glomus macrocarpum (Tul.)Tul. var. geospora Nicol. &amp; Gerdemann</td>
</tr>
<tr>
<td>Glomus maculosum Miller &amp; Walker</td>
</tr>
<tr>
<td>Glomus multicarpellum Gerd. &amp; Bakshi</td>
</tr>
<tr>
<td>Glomus geosporum (Nicolson &amp; Gerd.) Walker</td>
</tr>
<tr>
<td>Glomus globiferum Koske &amp; Walker</td>
</tr>
<tr>
<td>Glomus monosporum Gerd. &amp;Trappe</td>
</tr>
<tr>
<td>Gigaspora margarita Becker &amp; Hall</td>
</tr>
<tr>
<td>Sclerocystis rubiformis Wu &amp; Chen</td>
</tr>
<tr>
<td>Sclerocystis sinuosa Gerd. &amp;Bakshi</td>
</tr>
<tr>
<td>Sclerocystis taianumensis Wu &amp; Chen</td>
</tr>
<tr>
<td>Scutellospora coralloidea Gerd. &amp;Trappe</td>
</tr>
</tbody>
</table>

Discussion

Majority of temperate, subtropical and tropical plant communities are colonised by AM fungi (Trappe 1987). Besides confirming the ubiquitous nature of AM fungi, the present study also indicates the widespread association of this fungi with tree species of Mollem forest located in the Western Ghats of Goa.

Our study reports the presence of only AM fungal association with tree species. This is in accordance with the approach taken by several workers who reported that majority of vascular flora have mycorrhizal association with AM fungi (Trappe 1987, Berliner & Torrey 1989, Brundrett 1991, Fontenla et al. 1998). A possible reason for this widespread association could be low host specificity of AM fungi, which is of advantage for the plant species in natural communities. This low specificity of AM fungi towards the host increases the chance of infection by a compatible fungus and facilitate its integration into the potentially enormous nutrient catchment provided by the AM fungal mycelium (Francis & Read 1994).

However, unlike in the above mentioned studies, our results did not show the occurrence of non-mycorrhizal species.

Our study reports the predominance of AM hyphae and vesicles in the roots of forest trees. AM endophytes, which are involved in intracellular association, expend more energy on hyphal growth during mycorrhizal formation, and, therefore, receive long-term benefits throughout the process (Brundrett & Kendrick 1990). There is evidence that some plant species in deciduous forest community have roots which maintained living cortex for up to two to 10 years without undergoing secondary growth and still contained inactive hyphae and vesicles of AM fungi (Brundrett & Kendrick 1988). These species with long-lived roots may function as keystone mutualists, benefiting the mycorrhizal hosts (Gilbert 1980).
Predominance of vesicles in the roots of the forest tree species studied indicates that conditions were favourable for their formation and that mycorrhizal strategies of plants might be correlated with environmental conditions.

Presence of arbuscules was recorded in the roots of only seven out of the 25 tree species studied. A possible reason for this small number of arbuscules could be that most of the roots were in an inactive stage at the time of sampling. Arbuscules, being ephemeral structures, may be absent if the roots are inactive (Brundrett 1991). In addition, co-existing plants in natural communities may avoid competition for the nutrients by having roots that are active at different times of the year (Veresoglou & Fitter 1984, Fitter 1986).

Arbuscules are the main sites for host-fungus nutrient exchange and their presence is normally used to designate AM association (Smith & Gianinazzi-Pearson 1988). Nevertheless, the presence of AM fungal hyphae and vesicles have been used as evidence of AM association (Brundrett 1991). However, they are unreliable indicators since they also occur in the senescent roots of non-host species and rhizome scale leaves (Hirrel et al. 1978, Staz & Sakai 1984). Thus, in ecosystems surveys, it may be best to define AM colonisation levels as a proportion of the plant's root system that, when susceptible to colonisation, supports an active colonisation with arbuscules (Brundrett & Kendrick 1988). This requires prior understanding of host-root phenology or, alternatively, collection of root samples throughout the year (Brundrett 1991).

The average root colonisation (50.7%) recorded in the present study is in agreement with the findings of Mohankumar and Mahadevan (1988). The range of root colonisation of AM fungi (17 to 100%) in our study is also in accordance with the earlier reports on AM association of plants from Western Ghats (12 to 90%) by Muthukumar et al. (2001).

Large variation in spore numbers (18 to 745 spores 100 g⁻¹ rhizosphere soil) recorded in the rhizosphere soil of different tree species in the present study can be attributed to several reasons. Firstly, the occurrence of several AM fungi in the soils or within roots suggests that interspecific competition between them is possible (Brundrett & Kendrick 1990). Secondly, subsequent variation in the timing of spore production occurs among AM fungi associated with host plants, suggesting that competition between fungi and environmental factors probably also influence spore production in natural communities (Gemma & Koske 1988). Also, peak period of spore production is generally thought to coincide with the period of fungal resource remobilisation from senescing roots (Sutton & Barron 1972) and is greatest in natural communities when root activity is interrupted by a long dry season (Janos 1980b).

Spore density range recorded in the present study is much higher than the figures reported by Mohankumar and Mahadevan (1988) in mixed deciduous, evergreen and semi-evergreen forest ecosystems of Kalakad forest reserve in Western Ghats (79 to 130 spores 100 g⁻¹ rhizosphere soil) and by Raman et al. (1992) in Mamandur forest of Tamil Nadu (30 to 301 spores 100g⁻¹ rhizosphere soil) in southern India. The occurrence of higher spore density in this study compared with other localities in the Western Ghats could be due to varying ecoedaphic factors.
In our study no definite correlation could be established between AM fungal root colonisation and spore numbers. Our results are in agreement with observations made by Rani et al. (1995) but contrast with the findings of Muthukumar et al. (2001), who have reported positive correlation between the two in medicinal plants from Maruthamalai hills in Western Ghats. The poor correlation between spore density and root colonisation could be because sporulation of AM fungi is dependent on a wide range of environmental factors (Muthukumar et al. 2001) and their germination potential varies at different times of the year (Tommerup 1983, Gemma & Koske 1988). Also, soils in an ecosystem often contain low numbers of living spores of AM fungi (Brundrett & Kendrick 1988, Janos 1980b) and these spores may not function as propagules if they are quiescent or have an innate period of dormancy. Thus, initiation of AM colonisation in occurring ecosystem suggests that pre-existing network of hyphae is often the main AM inoculum (Brundrett 1991).

Our study recorded the presence of 18 AM fungal species from rhizosphere soil of 25 tree species from Mollem forest. Johnson et al. (1991) reported similar findings in their study of plant and soil controls of mycorrhizal fungal communities. They reported the presence 12 to 22 different species of AM fungi per study site. However, Muthukumar et al. (2001) reported only 35 AM fungal species from the rhizosphere soil of about 329 plant species from Western Ghats. Recovery of relatively higher number of species in the present study is in agreement with Francis and Read (1994) who have reported that high species diversity, characteristic of phosphorus-deficient grassland ecosystem dominated by plant species with arbuscular mycorrhizae, may be attributed to a low level of host specificity.

AM fungi belonging to the genus *Glomus* were the most representative types in our study. The predominance of this genus in tropical soils has been reported by other workers (Thapar & Khan 1985, Raghupathy & Mahadevan 1993).

Much attention is focused on the conservation of forest macro flora and fauna compared with the vast world of microbes. Nevertheless, there is an urgent need for detailed inventory of these microbes including AM fungi. In addition, measures for their conservation at ecosystem levels are pertinent (Bisht et al. 1995). Western Ghats of Goa has rich biodiversity. Thus, more extensive sampling over a longer period is required to determine the species diversity of AM fungi (Walker et al. 1982). AM fungi are the key links in regulating the patterns of energy and nutrient flux in terrestrial ecosystem which often transcend the pattern of community distribution and species abundance in the biosphere (Fahey 1992). The present work is just one step in this direction and contributes data necessary for further studies on AM fungi from this region of which very little has been explored.

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Prospects in Reclamation of Iron ore Mine Waste Lands: Role of Arbuscular Mycorrhizal (AM) Fungi and Inoculation Procedures

*Rodrigues B.F., Khade Sharda W., Bukhari Mehtab, Jaiswal Varsha & Uday Gaonkar

INTRODUCTION

Mining is one of the most common activities of ancient and modern world. Mining is regarded as the second largest industry after agriculture and has played a vital role in the development of civilization from ancient days. Most valuable materials for man such as metals, chemicals, fuels for energy, rocks and stones for building comes from mining (Trivedy, 1990). Land surfaces are inevitably disturbed in seeking to win ores from the earth. Mechanization and improved technology has brought increasingly large tracts of land into state of disturbance. With increasing demands, land has been constantly exploited for raw materials from the natural environment. Land is not a resource, which automatically renews itself like rainfall and sunlight. It is a finite resource, being diminished by the spread of industry and urbanization (Coleman, 1979).

The State of Goa with an area of approximately 3702 sq. km lies on the West Coast of India between 15°48'00"N and 14° 53'54" N Latitude and 74°20'13"E and 73°40'33"E Longitude. In Goa, mining commenced in 1910. However, commercial exploitation began in 1947 when the erstwhile Portuguese Government granted over 700 concessions all over Goa. During those days the concept of pollution and conservation were not given due weightage and the people welcomed widespread mining activities as an additional source of income and employment. In a way, therefore the growth of mining has proceeded simultaneously with the growth of agriculture.

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and population. Goa has been a prime exporter since 1950, as much as 300 million tons of iron ore has been exported. Present production of iron ore is of the order of 15 million tons per year which constitutes 40% of the total iron ore production in the country and 50% of its export. The estimated reserves of iron ore as on today is around 400 million tons and is expected to last for another 25-30 years at the present rate of mining.

The mining operations are such that, two classes of wastes are produced viz., (i) piles of surface overburden waste rock and lean ore, which constitutes the reject dumps, and (ii) a fine grained waste resulting from the ore beneficiation process and deposited in large man made basins called tailing ponds. The latter kind of waste materials are termed as tailings.

It is true that in the process of mining mineral resources from the earth, disturbance to environment and ecosystem is unavoidable. In other words, mining is to some extent an unavoidable destructive process. Though, there are problems of mine wastes in terms of erosion, environmental pollution, damage to adjoining agricultural fields, forests, etc., many a times they are exaggerated. These hazards are within measurable limits and can be easily ameliorated to a significant extent by extensive research and planning to control the impacts.

MINING AND ITS IMPACT ON ENVIRONMENT

In terms of employment and foreign exchange earnings, mining industry plays an important role in Goa's economy. On the other hand, however, the wastes produced by mining activities are likely to pose a serious threat to the environment if proper measures are not taken to re-establish vegetation at the mining sites.

The tailings occupy large segments of the landscape in the vicinity of the mine and diminish the aesthetic quality of the natural landscape. The tailing basins may occupy up to 40% of a mine site land area (Shetron and Duffek, 1970). Essentially open cast mining involves excavation and movements of large volumes of earth's crust. A ton of iron ore mined for instance produces 2 to 3 tons of waste. Dean and Havens (1971) estimated that the total tonnage of such wastes in United States covers about 200 million acres. The annual accumulation exceeds one billion tons, which are distributed over an area of approximately 2 million acres. In the Western States, nearly one half million tons are being produced daily (Neilson and Peterson, 1972). The excavation of iron ore exposes large chunks of earth's crust to the atmosphere that intrude upon the landscape.

Mining accounts for a substantial proportion of the loss of land of primary production. In India, 7,85,000 hectares of land is reported to be under mining operations (Baliga, 1985). Discriminate mining since 1961 has destroyed 50,000 hectares of forest in Goa and it is estimated that during all these years as much as 900 to 1000 million tons of waste rock, low grade ores and slimes have been accumulated near mining areas. The waste materials consist mainly of laterites, quartzites, manganiferous and other types of clays, slimes, etc.

With the present annual production of 15 million tons of iron ore, it is expected that 40-50 million tons of wastes have to be stored per year, and approximately 150 million cubic meters of gases to be discharged from pits to the drainage system. Mine waste dumps are biggest man-made hillocks, volume and height of such dumps increases every year. Most of the waste dumps
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rise up to 50-60 meters high with 50° -55° angle of repose. These being unconsolidated are prone to slumps and slides due to heavy monsoon rains.

Damage to environment by mining activity has been caused largely by reject dumps pumping out of muddy waters from the working pits including those where the excavation have gone below the water level, and slimes from the beneficiation plant. The damage is more conspicuous during monsoon, when the rainwater carries out the washed out materials from the mine waste dumps to the adjoining agricultural fields and water streams. The slimes and silt which enter the agricultural fields get hardened on drying, thus making aeration and root penetration difficult. Indiscriminate dumping of rejects has rendered over 10,000 hectares of agricultural land infertile, pollute the springs and wells, and also cause silting of waterways especially during monsoon. Such silting of waterways over the years have caused flooding of adjacent fields and inhabited areas during the dry seasons. The noise created due to the blasting operations, movement of heavy vehicles, operation of heavy machineries and dumping of iron ore poses a constant problem in the surrounding areas. Dust from blasting, crushing and transportation is the major cause of air pollution around the neighbouring villages of mining areas, sometimes reaching miles away with increasing wind velocities. Diseases such as silicosis, tuberculosis and allergic diseases like asthma are frequently common in inhabitants of the area and the mineworkers. The dust has its effects on nearby communities, industrial machinery and demanding effects on vegetation by blocking plant pores and hampers photosynthesis.

The most common hazards of open cast mining of iron ore have been the defacing of landform by development of depression and elevation or sloppy terrain. It also leads to large-scale deforestation, destruction of wild life and natural resources resulting in a fragile ecosystem lacking in flora and fauna. In the process of mining the topsoil is removed, leaving bare rock, thereby making it hard for vegetation to become re-established. Normally, natural processes would gradually recolonise the mine sites and spoil heaps building up the soil and reclothing the landscape with vegetation. However this can take a long time. Meanwhile, the unprotected surface is subjected to erosion leading to the clogging rivers and lakes with silt. Thus the common approach towards stabilization i.e. establishment of a permanent cover of vegetation involves not merely growing plants. But it necessitates bringing into a plant community that will maintain itself indefinitely without further attention or artificial aid such as irrigation. Such a performance could be achieved most advantageously, by selecting species adapted to growth, spread and reproduction under the inimical conditions. Most of the plants, which are desirable for the revegetation of these lands, are dependent on "Mycorrhizal Fungi".

ECOSYSTEM, PLANT SUCCESSION AND MINING

The vegetation together with the soil in which it has its roots, the associated fauna, and the environment that surrounds them form a closely interrelated and interdependent to the ecosystem. Although ecosystems are sensitive to the outside influences, they are self-sustaining. Once properly established, they need no further support. This is because of natural cycling of accumulated materials, which maintain the vegetation and the other organisms with it. After a major disturbance, vegetation slowly and gradually develops over a period of time: a process termed plant succession.
If the above two properties (self-sustaining and capacity to develop) of the ecosystem are considered, then one may presume that after mining disturbance, there is no need of any revegetational efforts i.e., a self-sustaining vegetation cover will develop naturally. But the process of natural succession will take many years.

The aim of revegetation of mining sites is to achieve vegetation cover within a few years, so that the subsequent succession may take place at a rapid pace. Hence, it is obvious that one must look for the appropriate treatments and management strategy so that useful vegetation can be established quickly economically leading to a self-sustaining ecosystem.

**CHARACTERISTICS OF MINE WASTES**

For reclamation of any degraded area, knowledge of physico-chemical parameters of degraded and undegraded area in the locality is essential. However, the exact assessment of these parameters over the entire area is not easy, as the constitution of the soil varies even at the close proximity of the sampling sites due to the random dumping of the top soil overburden, rock waste and due to interaction of various factors.

Soil texture is used extensively as a guide to evaluate soil water storage, water availability, surface erosion, land stability and chemical properties (Shetron and Trettin, 1984). Natural soil consists of an inorganic framework of sand, silt and clay particles, intimately mixed with organic material. The physical analysis of iron ore wastes reveals that the tailings and rejects have high bulk and particle density which is normally the characteristic feature of metalliferous mine waste (Rodrigues and Bukhari, 1996 & 1997) (Table 1 & 2). The bulk density of natural soils fall within the range of 1.0 – 1.5 g cm\(^{-3}\) (Williamson et al., 1982) and particle density 2.63 g cm\(^{-3}\) (Waddington, 1969). Bulk density is a useful measure of compaction to root penetration. Surface accumulation of fines in slim dams may give a bulk density as high as 7.5 g cm\(^{-3}\) with low infiltration (Ruschen et al., 1974).

Cation exchange capacity (CEC) is important as it is a measure of total exchangeable cations (calcium, magnesium, potassium and sodium) in soil materials (Black, 1968). Low water holding capacity of the rejects and tailings can be attributed to the poor soil texture, structure and organic matter content which are known to be responsible for improving water holding capacity.

Maclean and Dekker, (1976) studied the pH of different wastes and reported large variations mainly among different sites ranging from pH 1.5 to above 10. Varying soil pH changes the concentration of many nutrients and toxic ions in soil solutions as well as the concentrations of nutrient ions (Russell, 1973). In solutions of acid soils, there are often higher concentrations of aluminium and manganese, and lower concentrations of calcium, magnesium and molybdenum compared to that in alkaline soils (Porter et al., 1987).

Nutrient deficiencies are widely reported as a major limitation, particularly in terms of a complete lack of organic matter and nitrogen in mining wastes. Smith and Bradshaw, 1983 showed that micro-nutrient deficiencies are frequently encountered in the mine wastes. Wong (1983) showed that the tailings were alkaline, lacking in organic matter and nitrogen, but metals such as Fe, Zn, Cu, Mn, Mg and Ca.
Table—1  Some properties of iron ore mine rejects in Goa. (Rodrigues and Bukhari, 1997)

<table>
<thead>
<tr>
<th>Property</th>
<th>Mean (S.D.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>6.02 (0.18)</td>
</tr>
<tr>
<td>EC (mS/cm)</td>
<td>0.051 (0.012)</td>
</tr>
<tr>
<td>Total N</td>
<td>93.2* (N.A.)</td>
</tr>
<tr>
<td>Available N</td>
<td>3.8* (N.A.)</td>
</tr>
<tr>
<td>P</td>
<td>1.5 (N.A.)</td>
</tr>
<tr>
<td>SO₄²⁻</td>
<td>&lt;0.1 (N.A.)</td>
</tr>
<tr>
<td>Ca</td>
<td>1.76 (0.80)</td>
</tr>
<tr>
<td>Mg</td>
<td>0.92 (0.55)</td>
</tr>
<tr>
<td>K</td>
<td>0.76 (0.26)</td>
</tr>
<tr>
<td>Na</td>
<td>2.60 (0.54)</td>
</tr>
<tr>
<td>Cu</td>
<td>&lt;0.05 (N.A.)</td>
</tr>
<tr>
<td>Fe</td>
<td>&lt;0.1 (N.A.)</td>
</tr>
</tbody>
</table>

Concentrations in μg g⁻¹ oven dry spoil.
N.A. = Not applicable.
S.D. = Standard deviation.
EC = Electrical conductivity.
* = Mean of two replicates taken from bulked samples.

Shetron (1983) reported that in iron ore tailings the organic matter and nitrogen are essentially non-existent, phosphorus levels are low; Ca, Mg, K and metal range in availability; have alkaline pH and low cation exchange capacity.

MYCORRHIZAL FUNGI

In 1842, Vittadini proposed that tree rootlets are nourished by certain fungal mycelia, which mantle them, as observed by him more than a decade earlier. This hypothesis was elaborated to a theory of mutualistic symbiosis by Bernhard Frank (1885) who coined the term “mycorrhiza” to denote the symbiotic association formed by fungal mycelia with plant roots (Gr. myces = fungus; rhizo = roots). The concept of fungus-root symbiosis has since been a subject of extensive research. Though the word was introduced in 1885, mycorrhizae itself, of course are millions of years older. It is generally believed that Arbuscular mycorrhizal (AM) fungi evolved early in the history of vascular plants (Trappe, 1987, Morton, 1990). Despite of their geological age (Birch, 1986; Pirozynsky and Dalpe, 1989) and their crucial role in origin of plants very little is known about their phylogenetic origin (Sancholle and Dalpe, 1993). The members of Glomales are believed to have been present as early as the Cambrian period (Pirozynsky and Dalpe, 1989). They played a pivotal role in the origin of the terrestrial flora. Early Devonian plants showed the presence of the endosymbionts represented by non-septate mycelia, coiled hyphae, irregularly shaped thin walled spherical structures resembling the vesicles. Arbuscule like structures are recently been reported from plants preserved in Rhynie Chart (Remy et al., 1995). In their symbiotic habit, the mycorrhizal fungi constitute a special group among root inhabiting fungi.
Table—2. Some properties of iron ore mine tailings in Goa. (Rodrigues and Bukhari, 1996).

<table>
<thead>
<tr>
<th>Properties</th>
<th>Mean (S.D.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>6.48 (0.07)</td>
</tr>
<tr>
<td>EC (mS/cm)</td>
<td>0.065 (0.018)</td>
</tr>
<tr>
<td>Total N</td>
<td>60.3* (N.A)</td>
</tr>
<tr>
<td>Available N</td>
<td>1.7* (N.A.)</td>
</tr>
<tr>
<td>P</td>
<td>1.9 (N.A.)</td>
</tr>
<tr>
<td>SO₄²⁻</td>
<td>&lt;0.1 (N.A)</td>
</tr>
<tr>
<td>Ca</td>
<td>2.34 (0.57)</td>
</tr>
<tr>
<td>Mg</td>
<td>0.75 (0.17)</td>
</tr>
<tr>
<td>K</td>
<td>0.71 (0.30)</td>
</tr>
<tr>
<td>Na</td>
<td>4.85 (2.94)</td>
</tr>
<tr>
<td>Cu</td>
<td>&lt;0.05 (N.A.)</td>
</tr>
<tr>
<td>Fe</td>
<td>&lt;0.1 (N.A.)</td>
</tr>
</tbody>
</table>

Concentrations in μg.g⁻¹ oven dry spoil.
N.A. = Not applicable.
S.D. = Standard deviation.
EC = Electrical conductivity.
Mean of two replicates taken from bulked samples.

ADVANTAGES OF MYCORRHIZAL FUNGI

Mycorrhizal association is a universal phenomenon throughout the plant kingdom and is beneficial and even indispensable for the life and healthy growth of the host plants. Because almost all plant species of natural vegetation and the agricultural crop plants of the tropics live in association with fungi, it should be possible to increase productivity through manipulation of mycorrhizal systems.

Absence of suitable mycorrhizal fungi is one of the main reasons for the failure of reforestation programmes. Tree species, especially exotics, fail to establish on afforestation sites in the absence of their fungal symbionts. Repeated attempts for the last twenty years to raise pine plantation in Puerto Rico failed due to lack of ectomycorrhizae on their roots. The pine seedlings would grow to 5-30 cm in height, then become chlorotic, and die. Transfer of mycorrhizal soil from successful plantations in the mainland to Puerto Rico nurseries helped successful establishment of pine plantations on this island. The inoculated plants of slash pine grew healthy and reached a height of 2.0-2.5 m in three years. Similarly, success in raising plantations in South Africa was achieved only when ectomycorrhizal inoculum from South African nurseries was introduced in South African nurseries. Soil from South African pine nurseries was used as soil inoculum in Kenya and this resulted in large scale successful pine
Normal growth occurs only when mycorrhizal fungi present in the site colonize and establish mycorrhiza on the roots. It may be possible to grow mycotrophs without its fungal symbiont if nursery plants are given a high dose of fertilizers. However, such seedlings when transplanted in the field where mycorrhiza is absent and nutrient is discontinued for economic reasons, the growth of plants is retarded appreciably in many cases.

Mycorrhizal plants are more efficient to draw nutrient from soils, particularly soils poor in available phosphorus as compared to non-mycorrhizal plants. Mycorrhizae benefit their hosts in a variety of ways. They increase the absorptive surface of colonized roots through inducing profuse branching. The extramatrical hyphal growth of these fungi can explore large soil areas for acquisition of nutrients from the soil. The hyphae of mycorrhizal fungi are able to mobilize nutrients from the substrates where such nutrients are in unavailable form for absorption by the plant roots. The fungal hyphae assimilate nutrients from such substrates through their active metabolic process and these assimilated nutrients are then transported to the plant roots.

Besides, direct nutritional advantages, mycorrhizae have also been accredited with other benefits to the host plants such as ability of arbuscular mycorrhizal roots to overcome water stress by stomatal regulation in Citrus (Levy and Krukum, 1980). Mycorrhizal inoculation also stimulates rooting (Barrow and Roncadri, 1977) growth and transplant survival (Bryan and Kormanik, 1977) of cutting and seedlings raised in sterilized nursery media. It also increases disease resistance by depressing root penetration and larval development of nematodes (Sikora, 1978). In addition to this, mycorrhizal plants have shown to have greater tolerance to toxic heavy metals, to drought, to high soil temperature, to saline soil, to adverse soil pH than the non-mycorrhizal plants (Schenck, 1984). Arbuscular mycorrhizal fungi also bind soil into semi-stable aggregates, thus improving the structure of the soil. Because of these attributes, mycorrhizae are now considered important in the establishment of plants in inhospitable sites like mine wastelands.

Apart from nutrient benefits to plants derived from mycorrhizal symbiosis, it is known to impart disease resistance in plants. In Pinus echinatus, mycorrhizal roots resist infection due to Phytophthora cinnamomi, where as uninfected roots are highly infected. This is based on the hypothesis that the rhizosphere and the sheath surface of mycorrhizal roots possess microflora different from this in non-mycorrhizal roots. In order to derive maximum benefits from mycorrhizae, it is necessary that nursery raised seedlings have optimum level of mycorrhization on their roots for which introduction of suitable mycorrhizal species at nursery stage should form an integral part of nursery management practices.

**BROAD CLASSIFICATION OF MYCORRHIZAE**

Based on colonization anatomy, two major groups of mycorrhizae have been recognized. Viz., Ectomycorrhizae and Endomycorrhizae.

**Characteristics of Ectomycorrhizae**

1. Prevalent in the temperate regions, in forest and ornamental tree species.

2. Characterized by the presence of a mantle of fungal tissue around the host rootlet, and intercellular penetration of the rootlet cortex (Hartig net), and not intracellular.
3. Fungi belong to Basidiomycetes, which form sporophores and release air-borne spores. The inoculum thus is easily spread by wind from one location to another.

4. Host plant roots with ectomycorrhizal colonization are short, swollen, dichotomously branched with distinctive colours viz., white, black, orange, yellow or olive green.

5. It is fairly easy to isolate a number of ectomycorrhizal fungi in pure culture by using routine microbiological techniques and grow them saprophytically. Thus, it is possible to produce inoculum on a large scale.

Characteristics of Endomycorrhizae

1. These fungi are prevalent in the tropics.

2. The colonization is both intercellular and intracellular. In this case, there is no 'Hartig net' and no 'mentle'.

3. Endomycorrhizal fungi cannot be cultured in vitro conditions. The production of endomycorrhizal inoculum requires the growth of a susceptible host plant. The inoculum has to be produced under sterile glasshouse conditions.

Classification of Endomycorrhizae

The Endomycorrhizae are again classified into 4 major groups viz., i) Arbuscular mycorrhizae (AM); ii) Arbutoid mycorrhizae; iii) Ericoid mycorrhizae, and iv) Orchid mycorrhizae.

Arbuscular Mycorrhizae (AM) Fungi:

The arbuscular mycorrhizal fungi are non-septate and ubiquitous. Despite their near omnipresence, the AM fungi have, until recently, received very little attention, because the AM fungi can neither be cultured in the absence of a living root nor isolated on agar plates by standard microbiological techniques. It is now well established that many plants cannot grow adequately without AM fungi, especially in phosphate-deficient soils. Arbuscular mycorrhizal fungi are characterized by the presence of arbuscules and vesicles.

Arbuscules are similar to haustoria, developed by repeated dichotomous branching of hyphae that enter in the cortical cells. Each arbuscular tip sometimes appears to be surrounded by a cloud of granular material. They remain viable or active only for a short period i.e. 4-15 days. Their main function is nutrient transfer between symbionts. The cause of their destruction is digestion of the fungal cell wall by host chitinase activity.

Vesicles develop as terminal or intercalary swellings of the inter- or intra-cellular hyphae. They may be spherical or oval or lobed. Vesicle size and shape usually depends on the host conditions. They are known to contain oil droplets. When young, they have thin walls and contain homogenous protoplasm. They remain thin walled and function as storage organs. Later they develop into thick walled chlamydospores functioning as reproductive structures.
Arbutoid Mycorrhizae

This type of colonization is suggested to be a transition between ectomycorrhizae and endomycorrhizae. It is hence, sometimes called as ectendomycorrhiza. This type of colonization is characterized by intracellular penetration. There is also a Hartig net and occasionally a fungal sheath. It is structurally intermediate between ectomycorrhizae and endomycorrhizae. Due to this type of colonization, root dimorphism might occur, with colonized roots remaining shorter. Only a few fungal species are known to form arbutoid colonization viz., *Amanita*, *Cortinarius* and *Boletus*.

Ericoid Mycorrhizae

This type of colonization is prevalent in the members of the family Ericaceae and hence the term Ericoid mycorrhizae. In this type of colonization, the fungal hyphae penetrate the epidermal and cortical cells and the fungus ramifies within each cell to form a coil or knot of filaments occupying much of the volume within the cells. The stele is not invaded. Hyphal knots can be readily detected at 200 to 400x magnification when observed under a microscope after staining with 0.05% trypan blue stain. Presently only one fungal species viz., *Pezizella ericae* is known to cause this type of colonization.

Orchid Mycorrhizae

This type of mycorrhiza has been proposed as one of the most complex of the symbiotic interaction. Members of the genera *Neottia*, *Limodoron*, *Epipogon* and *Vanilla* are dependent on mycorrhizae for growth. The entry of the fungal hyphae is a must for further growth of the seedlings. The fungal members which exhibit this type of colonization include *Fomes*, *Corticum* and *Rhizoctonia*.

STAGES OF DEVELOPMENT OF AM FUNGI

Arbuscular mycorrhizal fungal spores occur in physiologically inactive stages in soil. The spore germinate, grows and multiplies in the presence of actively growing roots of plants. The development of AM fungi in roots can be divided into four stages (Tommerup and Briggs, 1988):

- Spore germination and hyphal growth from infective propagules of AM fungi.
- Growth of hyphae from soil to host roots. The mycelial systems surrounding the roots are dimorphic (Mosse, 1959; Nicolson, 1967).
- Penetration and successful initiation of colonization in roots. Hyphae penetrates mechanically and enzymatically into cortical cells (Kinden and Brown, 1975). At the point, penetrating hyphae may or may not form appresoria (Abbott, 1982).
- Spread of colonization and development of internal hyphal system, arbuscules, which bifurcate inside a cell and bring about nutritional transfer between two symbionts and vesicles which, develop as terminal or intercalary swellings in inter- or intracellular hyphae. They are responsible for storage and vegetative reproduction.
CURRENT STATUS OF AM FUNGI

Endomycorrhizae produced by the nonseptate fungi are commonly called as "Arbuscular Mycorrhizal (AM) Fungi". These fungi belong to the Family Endogonaceae of the Order Endogonales and Class Zygomycetes (Trappe and Schenck, 1982). It includes six genera viz., Acaulospora, Gigaspora Entrophospora, Glomus, Sclerocystis and Scutellospora.

Benjamin (1979) placed eight genera of endogonaceous fungi under Endogonaceae in a single family Endogonaceae. Morton and Benny (1990) divided this order in two independent Orders viz., Endogonales and Glomales on the basis of their spore structure and mycorrhiza development (Table 1).

<table>
<thead>
<tr>
<th>Table-3: Classification of AM fungi.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Old Classification (Gerdemann &amp; Trappe, 1974; Benjamin, 1979; Warcup, 1990)</strong></td>
</tr>
<tr>
<td>Order: Endogonales</td>
</tr>
<tr>
<td>Family: Endogonaceae</td>
</tr>
<tr>
<td>Genera: Endogone</td>
</tr>
<tr>
<td>Sclerogone</td>
</tr>
<tr>
<td>Glomus</td>
</tr>
<tr>
<td>Sclerocystis</td>
</tr>
<tr>
<td>Acaulospora</td>
</tr>
<tr>
<td>Entrophospora</td>
</tr>
<tr>
<td>Gigaspora</td>
</tr>
<tr>
<td>Scutellospora</td>
</tr>
<tr>
<td>(ii) Family: Acaulosporaceae</td>
</tr>
<tr>
<td>Genera: Acaulospora</td>
</tr>
<tr>
<td>Entrophospora</td>
</tr>
<tr>
<td>Gigaspora</td>
</tr>
<tr>
<td>Scutellospora</td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>

Taxonomy of Glomales is based on the structure of their spores/sporocarps (Morton, 1990a). Some authors lay more emphasis on the wall structure, which also is a valid criterion (Walker, 1983, 1992). Glomales are divided into sub-orders, Glomineae and Gigasporineae (Table 3). The genera included in this order are placed under three families (Morton and Benny, 1990). Till 1990, about 150 species of fungi are genuine and validly published. Schenck and Perez (1990) have scientifically done the accumulation of the data on identification of AM fungi.

TYPES OF ECTO- AND ENDO-MYCORRHIZAL INOCULA FOR NURSERIES

The following types of inocula being employed for introduction of ecto- and endomycorrhizal fungi in nurseries are described below:
I. SOIL INOCULUM

Soil inoculum consists of mycorrhizal roots, spores, chlamydospores, hyphae, hyphae strands, rhizomorphs and other propagules of mycorrhizal fungi. This method has the disadvantage of transferring inadvertently pathogenic fungi to the nursery and the planting site. This is particularly objectionable if the transport is made between countries and it is not permissible under quarantine regulations. Also the soil inoculum is bulky, messy, and it is difficult to transport to the nursery and involves cost of transport. Finally, the same soil inoculum introduced into the same host species may induce different growth responses due to different fungal symbionts.

The endomycorrhizal inoculum can be chopped finely using hand tools. This inoculum is usually air dried to about 5-20% moisture and resembles granular fertilizer in its consistency.

II. INOCULATION WITH SPORES

The spores of ectomycorrhizal fungi are easy to collect and store, cheap to transport and can be taken to long distances. Spore inoculum is commonly used in case of ectomycorrhizal fungi belonging to class Gasteromycetes, the members of which produce fruiting bodies in the form of puff balls and truffles. At maturity, these fruiting bodies are full of powdery mass of spores. These fruiting bodies can be stored at 4-6 °C for 6-12 months without losing their viability. The following methods are being used for spore inoculation of nursery soils.

(a) Spore Soil Mix

In this, approximately 2 µg of spore powder (containing approximately 1x10¹² spores) is thoroughly mixed with one kg of sterilized or fumigated soil and this spore mix is used as inoculum. For inoculation of nursery beds or potting mixture, 1 kg of soil spore mix is evenly spread over one square meter of nursery bed soil and the soil is then thoroughly racked up to uniformly distribute the spore inoculum in the soil.

(b) Seed Encapsulation with Spores

Slurry is made by suspending spores in water and the sticker is added to the slurry. The seeds are then dipped in the slurry and kept overnight before sowing in nursery beds.

(c) Spore Pellets

In Philippines, inoculum of ectomycorrhizal fungi in the form of tablets have been prepared under the name "MYCOGROE". These tablets consist of basidiospores of ectomycorrhizal fungi combined with soil as carrier and palletized in a tabletting machine. Pellets containing arbuscular mycorrhizal inoculum have been successfully used to inoculate plants. These pellets can be prepared by mixing 20 parts mycorrhizal inoculum (finely ground roots, soil and spores, pot culture), one part sedimentary-loess clay (mean particle size 16 µ) and one part tertiary sedimentary clay (mean particle size 2-6 µ). Add water until malleable and then roll into pellets.

(d) Mycorrhizal Beads

In Philippines, scientists have produced ecto-mycorrhizal beads, under the trade name "MYCORRHIZAL BEADS" by entrapping mycelia of *Pisolithus tinctorius* grown in liquid
fermenters in calcium alginate beads. The entrapped mycelial inoculum has many advantages over solid medium inoculum. With this technique, the mycelium is produced in 1-2 weeks while in solid technique, several months are required to produce the inoculum.

(e) Pure Culture Inoculum

It is easier to produce ectomycorrhizal inoculum on a large scale using routine microbiological techniques. However, for arbuscular mycorrhizal (AM) fungi, large scale production of pure inoculum has been a major drawback as these fungi are obligate symbionts and hence requires the presence of a living host plant root and cannot be cultured using routine microbiological techniques. This process is time consuming and labour expensive. An extensive research is required to make pure cultures at reasonable time and cost.

INOCULATION PROCEDURES FOR NURSERIES

I. Broadcast Inoculations

This involves spreading a known quantity of mycorrhizal inoculum over a given area of soil surface and then mixing the inoculum into the soil to a depth of 10-20 cm before seeding. Several inocula viz. duff (consists of mycorrhizal tree roots and fungal propagules), sporocarps and spores, and pure culture vegetative mycelium have been applied in this manner to obtain mycorrhizal seedling in nurseries.

II. Banding of Inoculum Below Seeds

This involves placing the inoculum below the seed in a layer or band. This facilitates concentration of inoculum near developing roots. The major advantage of this method is that it requires only one third as much inoculum as the broadcast method. In addition, it saves time and labour. The only disadvantage being the need of an additional machine.

III. Slurry Dips

Slurries of mycorrhizal inoculum can be prepared by mixing the mycorrhizal inoculum with water, and a carrier such as clay or soil. The seedlings are inoculated by dipping them into the slurry prior to planting.

MYCORRHIZAL INOCULUM TECHNOLOGY

Successful production of mycorrhizal seedlings is dependent upon type and age of inoculum used, time of inoculation, inoculum density, inoculum placement and a number of host and fungus interactions. The mycorrhizal inocula consist of soil inoculum, mycorrhizal seedlings and roots, sporocarps and spores, and pure cultures of mycorrhizal fungi.

Seedlings can be inoculated at three different stages viz., i. before seeds are sown, ii. When seeds are sown and, iii. After seedlings emerge. The most efficient stage would be to inoculate the seedlings before or when the seeds are sown. An efficient time to inoculate cuttings is at the time of propagation. It also depends upon the economic considerations and on the ecology of mycorrhizal fungi. As regards to economics, the stage when the seeds are sown or cuttings are
propagated requires least amount of inoculum per volume of growing medium. Again, the newly developed rootlets of seedlings or cuttings are receptive to mycorrhizal colonizations, as they are non-lignified. The mycorrhizal fungi are also known to increase the rooting in cuttings and increase the root developments during propagation. It is also cost efficient to develop a mechanical inoculation system for use when seeds are sown or cuttings are propagated than any other stages. Inoculation at the time of transplantation is time consuming, requires more inoculum, and the introduced fungi must be compatible with the native microorganisms and climate conditions of the planting site.

ROLE OF AM FUNGI IN RECOVERY OF MINE WASTELANDS

Nicolson (1967) suggested that plant growth in industrial waste could be improved by incorporating AM fungi. Khan (1978) reported similar results for Australian coal spoils, noting that some members of Proteaceae were successful non-mycorrhizal invaders. However, species vary in their degree of dependency on mycorrhizal endophytes. Janos (1980) has explained that during succession, three main types characterize a range of ecological dependency: non-mycotrophs, facultative mycotrophs and obligate mycotrophs. In this case, obligate mycotrophs could fail to become established in sites of low inoculum density and may only become established after endophytes have colonized the area. If this is so, then these organisms are determinants of community composition during early succession and may in part, control the progress of succession (Reeves et al., 1979).

It is seen that iron ore mine rejects are poor in nutrients as indicated in Table 1. In a survey conducted at Sanquelim iron ore mines belonging to M/s Sesa Goa Limited, all the herbaceous plants growing on a 12 year oldreject dump showed AM fungal colonization (Rodrigues & Bukhari, 1997) (Table 4). In all, a total of 27 species of AM fungi belonging to five genera were recorded from the iron ore mines (Rodrigues, 2000) (Table 5) (Plate I). Glasshouse studies conducted to evaluate effect of two AM fungal species *(Glonus mosseae* Nicolson and *Gerdemann and Glomus fasciculatum* (Thaxter *sensu* Gerdemann) Gerdemann & Trappe) on biomass of nine tree species grown on mine

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Plant species</th>
<th>Family</th>
<th>Degree of root colonization (%)</th>
<th>Type of colonization</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Lygodium flexuosum (L.)Swartz.</td>
<td>Schizaeaceae</td>
<td>72</td>
<td>H A</td>
</tr>
<tr>
<td>2</td>
<td>Polygala elongata Klein ex Willd.</td>
<td>Polygalaceae</td>
<td>10</td>
<td>H A</td>
</tr>
<tr>
<td>3</td>
<td>Impatiens Kleinii W &amp; A.</td>
<td>Balsaminaceae</td>
<td>81</td>
<td>H V</td>
</tr>
<tr>
<td>4</td>
<td>Hydrocotyle asiatica L.</td>
<td>Apiaceae</td>
<td>42</td>
<td>H A V</td>
</tr>
</tbody>
</table>

Table—4: Degree of root colonisation (%) in some naturally occurring herbaceous plant species of iron ore mine wastelands of Goa. (Rodrigues and Bukhari, 1997a)

(Contd. . . )
   Rubiaceae 79  H  V

6. *Spermacte hispida* L.  
   Rubiaceae 37  H  V

   Asteraceae 87  H  A

8. *Parthenium hysterophorus* L.  
   Asteraceae 81  H  V

   Asteraceae 11  H  V

    Gentianaceae 40  H  V

    Convolvulaceae 96  H  A

12. *Lindernia crustacea* (L.) F.Muell  
    Scrophulariaceae 90  H  A

13. *Lindernia parviflora* (Rox.f.)Haines  
    Scrophulariaceae 60  H  A

    Scrophulariaceae 36  H  V

15. *Striga asiatica* (L.) Kuntze  
    Scrophulariaceae 93  H  V

    Scrophulariaceae 56  H  V

17. *Justicia procumbens* L.  
    Acanthaceae 23  H  A  V

18. *Comphrena celosioides* C.Martius  
    Amaranthaceae 62  H  A

    Araceae 74  H  V

    Eriocaulaceae 29  H  V

    Poaceae 29  H  V

22. *Heteropogon contortus* (L.) P. Beauv. Ex Roeme  
    Poaceae 30  H  A

23. *Schultes*  
    Poaceae 67  H  V

Legend:  
H = Hyphae;  
A = Arbuscules;  
V = Vesicles.
Plate I: 

a. Arbuscular Colonization (400x); b. Vesicular and hyphal Colonization (100x)

c. Surface ornamentation in *Acaulospora spinosa* spore (400x);

d. Spore of *Gigaspora margarita* (100x);

e. Spore of *Glomus macrocarpum* (400x);

f. Spore of *Scutellospora gregaria* (100x), and

g. Chlamydospores of *Sclerocystis taiwanensis* (400x)
rejects, revealed that the inoculated plants performed better than the uninoculated controls (Rodrigues, 1997). It was also noted that out of the nine tree species, *Glomus mosseae* Nicolson and Gerdemann appeared to be the best mycorrhizal inoculum for eight tree species (Table 6):

Table-5: Diversity of AM species found on iron ore mine reject dumps of Goa. (Rodrigues, 2000).

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>AM species</th>
<th>Sr. No.</th>
<th>AM species</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td><em>Glomus geosporum</em></td>
<td>16.</td>
<td><em>Glomus deserticola</em></td>
</tr>
<tr>
<td>2.</td>
<td><em>Glomus mossee</em></td>
<td>17.</td>
<td><em>Gigaspora albida</em></td>
</tr>
<tr>
<td>3.</td>
<td><em>Glomus fasciculatum</em></td>
<td>18.</td>
<td><em>Gigaspora margarita</em></td>
</tr>
<tr>
<td>4.</td>
<td><em>Glomus loi</em></td>
<td>19.</td>
<td><em>Gigaspora caudida</em></td>
</tr>
<tr>
<td>5.</td>
<td><em>Glomus aggregatum</em></td>
<td>20.</td>
<td><em>Acaulospora nicolsonii</em></td>
</tr>
<tr>
<td>7.</td>
<td><em>Glomus reticulatum</em></td>
<td>22.</td>
<td><em>Acaulospora birecticulata</em></td>
</tr>
<tr>
<td>8.</td>
<td><em>Glomus clarum</em></td>
<td>23.</td>
<td><em>Acaulospora laevis</em></td>
</tr>
<tr>
<td>10.</td>
<td><em>Glomus caledoniu</em></td>
<td>25.</td>
<td><em>Acaulospora foevata</em></td>
</tr>
<tr>
<td>12.</td>
<td><em>Glomus etinuualu</em></td>
<td>27.</td>
<td><em>Sutellosora gilmorei</em></td>
</tr>
<tr>
<td>13.</td>
<td><em>Glomus albidiu</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14.</td>
<td><em>Glomus monosporum</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15.</td>
<td><em>Glomus globiferum</em></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table-6: Effect of AM fungal species on dry weight (g) on various plant species grown on iron ore mine rejects. (Rodrigues, 1997).

<table>
<thead>
<tr>
<th>Plant species</th>
<th>Total dry wt. (g)</th>
<th>Control</th>
<th>G. F.</th>
<th>G. M.</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. retia</em></td>
<td></td>
<td>1.48(0.10)</td>
<td>2.28(0.14)</td>
<td>4.05(0.31)</td>
</tr>
<tr>
<td><em>C. indica</em></td>
<td></td>
<td>1.68(0.06)</td>
<td>1.93(0.43)</td>
<td>2.54(0.25)</td>
</tr>
<tr>
<td><em>C. ferruginea</em></td>
<td></td>
<td>0.22(0.03)</td>
<td>0.64(0.06)</td>
<td>1.00(0.13)</td>
</tr>
<tr>
<td><em>C. giganteum</em></td>
<td></td>
<td>0.12(0.00)</td>
<td>0.35(0.03)</td>
<td>0.38(0.03)</td>
</tr>
<tr>
<td><em>C. papouana</em></td>
<td></td>
<td>0.82(0.09)</td>
<td>1.37(0.10)</td>
<td>1.56(0.06)</td>
</tr>
<tr>
<td><em>C. labec</em></td>
<td></td>
<td>0.24(0.02)</td>
<td>0.84(0.17)</td>
<td>0.86(0.12)</td>
</tr>
<tr>
<td><em>C. tuficophila</em></td>
<td></td>
<td>0.31(0.03)</td>
<td>0.74(0.05)</td>
<td>0.75(0.09)</td>
</tr>
<tr>
<td><em>C. min</em></td>
<td></td>
<td>0.44(0.07)</td>
<td>0.89(0.10)</td>
<td>2.36(0.26)</td>
</tr>
<tr>
<td><em>C. stipa</em></td>
<td></td>
<td>0.35(0.04)</td>
<td>0.78(0.07)</td>
<td>0.43(0.07)</td>
</tr>
</tbody>
</table>

*Note:* G.F. = *Glomus fasciculatum* & G.M. = *Glomus mossee* Mean of 5 replicates. Figures in the brackets denote Standard deviation values.
CONCLUSION

"Mine land is a fascinating challenge because the pre-existing ecosystems are extinguished. It is a challenge to the biologists and engineers to replace them as they were. It is also a challenge to the soil scientists, ecologists and to the agriculturists to reconstruct an ecosystem from nothing at minimal cost.

Mine rejects are not true soils but are derived mostly from crushed bedrock and/or glacial deposits hence they are low in nutrients. In this relation, the role of microorganism in rehabilitation has received little attention than correction of nutritional deficiencies and imbalances, toxicity, moisture deficits and wind erosion.

Future revegetational research has to be oriented towards:

- Developing methods of maintaining inoculum level in soil.
- Developing techniques for introducing the endophytes in the soil.
- Naturally colonizing plant species should be given preference while considering revegetation strategies. Seedlings of such plant species should be inoculated with AM fungi in nursery stages and then transplanted to the target site. This would enhance plant growth and survival in the inhospitable sites.

In addition to this, alternative strategies such as reducing the angle of slope of reject dumps through terracing in order to improve water holding capacity, addition of organic materials like sewage sludge, sea weeds, green manure etc., would help to elevate the soil status and enhance mycorrhization in spoils. Removal and storage of topsoil for reuse would make reestablishment of vegetation relatively easier as topsoil contains organic matter, plant nutrients, seed propagules and useful microbes. This would also lead to increase the inoculum potential of AM fungi thereby helping in plant growth and survival.

Thus mining industry need not lead to degradation of environment if those who are involved in such programmes apply a combination of imagination, care and scientific skill.

REFERENCES


PAPERS PRESENTED:


*Presented paper (oral) entitled "Spatial variations in arbucular mycorrhizal (AM) fungi associated with Carica papaya L. in agro – based ecosystem of Goa" at the 29th Annual Meeting of the Mycological Society of India and National Symposium on Prospecting of Fungal Diversity and Emerging Technologies, held at Agharkar Research Institute, Puna, India, 6th-7th February 2003.

AWARD:

Dr. THRUMALARCHAR MERIT AWARD - for the best poster entitled "Arbuscular mycorrhizal (AM) status of pteridophytes from Western Ghats of Goa, India" at the at the 29th Annual Meeting of the Mycological Society of India and National Symposium on Prospecting of Fungal Diversity and Emerging Technologies, held at Agharkar Research Institute, Puna, India, 6th-7th February 2003.
27th Annual Meeting of
Mycological Society of India & International Symposium on
"FRONTIERS OF FUNGAL DIVERSITY AND DISEASES
IN SOUTH-EAST ASIA"

Organised by:
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U.P., India

We have great pleasure in certifying that Prof./Dr./Mr./Ms. Osharda M. Upadhye

participated in the deliberations of the
International Symposium and presented a paper (Key-Note Address/Lead Lecture/Oral/
Poster) entitled

in the I/II/III/IV/V technical session.

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February 6-7, 2003

Organised by
Agharkar Research Institute, Pune Maharashtra, India

We have great pleasure in certifying that Prof./Dr./Mr./Ms. K. Apple, S. W. participated in the deliberations of the National Symposium and delivered Lead Lecture/Oral/Poster Presentation entitled Spatial variation in Arbuscular Mycorrhizal (AM) fungi associated with Carica Papaya l. in the T2 technical session.

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Mycological Society of India

Sanjay K. Singh
Organising Secretary

V.S. Rao
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This is to certify that Dr. M.J. Thirumalachar Memorial Award 2002 for best Poster paper presentation is given to Ms. Sharda W. Khade & Dr. B.F. Rodrigues in the 25th Annual Meeting of the Mycological Society of India (MSI) and National Symposium on Prospecting of Fungal Diversity and Emerging Technologies held at Agharkar Research Institute, Pune, India, on 6th-7th February 2003.

Prof. D.J. Bhat
Secretary

"Arbuscular Mycorrhizal Status of Pteridophytes from Western Ghats of India"

Prof. K. Natarajan
President