"STUDIES IN ARBUSCULAR MYCORRHIZAL (AM) FUNGI ASSOCIATED WITH SOME TREE SPECIES FROM MOLLEM AND DHARBANDODA FOREST AREAS OF GOA."

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By

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DEDICATED TO MY PARENTS

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CERTIFICATE

I certify that the thesis entitled **"STUDIES IN ARBUSCULAR MYCORRHIZAL (AM) FUNGI ASSOCIATED WITH SOME TREE SPECIES FROM MOLLEM AND DHARBANDODA FOREST AREAS OF GOA."** submitted by Shri. UDAY C. GAUNKAR, is a record of research work done by his during the period from 1999-2002 when he worked under my supervision. The thesis has not formed the basis for award of any Degree, Diploma, Associate-ship or Fellowship to Mr. UDAY C. GAUNKAR.

I affirm that the thesis submitted by Shri. UDAY C. GAUNKAR incorporates the independent research work carried out by him under my supervision.

(B. F. RODRIGUES)
(Signature of Guide)

DECLARATION

I herby declare that the Ph.D. thesis entitled **"STUDIES IN ARBUSCULAR MYCORRHIZAL (AM) FUNGI ASSOCIATED WITH SOME TREE SPECIES FROM MOLLEM AND DHARBANDODA FOREST AREAS OF GOA."** submitted to Goa University, forms an independent work carried out by me in the Department of Botany, Goa University, under the supervision of Dr. B. F. Rodrigues, Reader, Department of Botany, Goa University and the thesis has not formed previously the basis for the award of any Degree, Diploma, Associate-ship or other similar titles.

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INTRODUCTION

The rhizosphere and rhizoplane are inhabited by large number of micro-organisms whose activities are of great relevance to plant growth. Among these, the micro-organisms which form relationship in the endorhizosphere are well placed to influence plant behaviour. Doing so, they become an integral part of roots and in consequence considerably modify the activities of these absorbing organs. Of the various plant-microbe interactions the most prevalent and the widespread type of association is the "Mycorrhiza". Although seven types of mycorrhizae are recognized *viz.*, ectomycorrhizae, arbuscular, ericoid, arbutoid, monotropoid, orchid and E-strain mycorrhizas, the most prevalent and wide spread type of mycorrhiza in the plant kingdom are the arbuscular mycorrhizae (Peterson and Farquhar,1994). More than 80% of plant species including most agricultural, horticultural, plantation crops (Maronek, 1981; Barea *et al.*, 1993) and forest trees (Grove and LeTacon, 1993; Haselwandter and Bowen, 1996) are capable of forming arbuscular mycorrhiza.

It is now well known that these organisms are ubiquitous in distribution found in nearly all ecosystems throughtout the world ranging from the artic to the tropical rain forest (Bledose *et al.*, 1990) and in diverse habitats from aquatic to desert ecosystems (Peat and Fitter, 1993; Dhillion *et al.*, 1995). Disturbed habitats may support relatively little natural colonization by mycorrhizae (Hayman, 1982). In fact the underground parts of large majority of plants growing under natural condition do not exist simply as roots but as arbuscular mycorrhiza, and is a determining factor for plant establishment and survival in many ecosystems.

Mycorrhiza, are critical components of the root soil interface. These fungi are present quite consistently on the root surfaces or in the tissues or cells of the roots of many species so that dual organs of consistent morphological and histological patterns are formed. They conform to a number of common kinds the world over, and in them the fungus and the host co-exist in a physiologically, ecologically and reproductively active state for long periods, a state called mutualistic symbiosis. **Table 1** summarizes the characteristics of the common kinds of mycorrhizae. These symbiotic associations that participate in the uptake of phosphorus are involved in pioneer colonization of nutrient deficient sites (Harley, 1970).

Arbuscular mycorrhizal fungi belong to Class Zygomycetes in the order Glomales (Motan and Benny, 1990), form characteristic structures *viz.*, arbuscules and vesicles within the host roots, dimorphic branching of extramatrical hyphae (Mosse, 1963; Nicolson, 1959) and production of large numbers of chlamydospores and azygospores in the soil. Arbuscular mycorrhiza develops when a hypha from a spore or an already colonized root contacts a suitable host root (Powell, 1976). The development of arbuscular mycorrhizal fungi in root can be divided into four stages (Tommerup and Briggs, 1988).

a) Spore germination and hyphal growth from colonized propagules of arbuscular mycorrhizal fungi.
b) Growth of hyphae through soil to host roots. The mycelial system surrounding the roots is dimorphic (Mosse, 1959; Nicolson, 1967).
c) Penetration and successful initiation of colonization in roots. Hyphae penetrate mechanically and enzymatically into cortical cells (Kinden and

Table1: Characteristics of important kinds of mycorrhizas in their mature state (Harley, 1989).

Character	Kinds of mycorrhiza						
	Arbuscular	**Ecto**	**Ectendo**	**Arbutoid**	**Monotropoid**	**Ericoid**	**Orchid**
Fungi							
Septate	-	+	+	+	+	+	+
Aseptate	+	(+)	-	-	-	-	-
Hyphae enter cells	+	-	+	+	+	+	+
Fungal sheath present	-	+	+ or -	+	+	-	-
Hartig net formed	-	+	+	+	+	-	-
Hyphal coils in cells	+	-	+	+	-	+	+
Haustoria							
Dichotomous	+	-	-	-	-	-	-
Not dichotomous	-	-	-	-	+	-	+ or –
Vesicles in cells/tissues	+ or -	-	-	-	-	-	-
Host Taxon	Bryo, Pterido, Gymno, Angio	Gymno, Angio, (Pterido)	Gymno, Angio.	Ericales	Monotrop-aceae	Ericales	Orchid-aceae

Legend: + = Present - = Absent (·) = rare

Brown, 1975). At the point of penetration, hypha may or may not form appressoria (Abbott, 1982).

d) 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 intra-cellular hyphae. They are responsible for storage and vegetative reproduction.

Six genera *viz., Acaulospora*, *Entrophospora*, *Gigaspora*, *Glomus*, *Sclerocystis* and *Scutellospora* are currently recognized. Among these, *Gigaspora* and *Scutellospora* do not form vesicles inside host roots. Being obligate symbionts, arbuscular mycorrhizal fungi are unable to grow in pure cultures. Attempts to cultures them on artificial media have met with little or no success (Hepper, 1984; Tommerup, 1988; St. Arnaud *et al.*, 1996).

The role of arbuscular mycorrhizal fungi in phosphorus acquisition of plants has been well documented for more than two decades. In general, most large growth enhancement effects of root colonization with mycorrhizal fungi are caused by increases in phosphorus absorption, particularly from sparingly soluble phosphorus sources (Bolan *et al.*, 1987). When root exploration is restricted upto 80% of the plant phosphorus can be delivered by the external arbuscular mycorrhizal fungal hyphae to the host plant over a distance of more than 10 cm from the root surface (Li *et al.*, 1991).

Besides this increase in spatial availability of phosphorus, effective phosphorus acquisition by the external hyphae is related to

a) Formation of polyphosphates in the hyphae, and thus, maintaining low internal phosphate (Pi) concentration.

(b) The small hyphal diameter leading to a relatively larger soil volume delivering phosphorus per unit surface area compared to the root surface (Jungk and Claassen, 1989) and a correspondingly 2-6 times higher phosphorus influx rate per unit length of hyphae (Jakobsen *et al.*, 1992).

With respect to phosphorus nutrition, the growth response to arbuscular mycorrhizal colonization depends on soil and plant factors which determine the phosphorus acquisition of the host plant, extent of phosphorus deficiency-induced root response and the phosphorus status of the soil. With increasing soil phosphorus, the growth enhancement effect of arbuscular mycorrhizal fungi declines and may either be abolished or lead to growth depressions. When arbuscular mycorrhizal fungi improve phosphorus nutrition of the host plant there may be a corresponding increase in nodulation, nitrogen fixation and growth (Robson *et al.*, 1981).

Arbuscular mycorrhizal fungi have been shown to help plant to acquire other macronutrients like N, K, Ca, Mg, S (Azcon,1994; Marschner and Dell, 1994) and micronutrients such as iron, copper, Manganese, Zinc, Boron (Bethenfalvay and Franson, 1989; Kothari *et al.*, 1991; Tobar *et al.*, 1994). The high efficiency in nutrient uptake by mycorrhizal roots is mainly due to the activity of the hyphal network developing from the root into the surrounding soil which can absorb these nutrients, transport them to the root and release them into the host cells. So mycorrhizal roots have a much greater absorbing potential surface than non- mycorrhizal roots, since they

can exploit nutrients in the soil beyond the depletion zone which forms at the root surface.

Arbuscular mycorrhizal fungi have also been shown to increase water uptake and/ or otherwise alter the plants physiology to reduce stress response to drought and salinity. (Nelsen, 1987; Weissenhorn *et al.,* 1993, Subramanian *et al.,* 1995). There are evidences that arbuscular mycorrhizal fungi decrease metal accumulation in plants growing in polluted soils and thus protect the host against the phytotoxic metal effects (Arines *et al.,* 1989; Leyval *et al.,* 1991; Weissenhorn *et al.,* 1993).

The improved nutrient uptake and better water utilization in endomycorrhizal plants reduce the transplant shock, quick recovery after temporary wilting and survival after transplanting (Biermann and Linderman, 1983; Michelsen and Rosendahl, 1990). Hormone production is affected by arbuscular mycorrhizal formation (Allen *et al.,* 1980) and the resultant changes in hormone quilibrium leads to development modification in arbuscular mycorrhizal plants *viz.,* a higher production of floral buds, retarded leaf fall in deciduous woody plants and alteration in partitioning of root and shoot biomass (Gianinazzi-Pearson,1989). An interesting arbuscular mycorrhizal effect is the increase in resistance and tolerance of arbuscular mycorrhizal plants to soil Pathogens (Menge, 1982; Liu, 1995) and pests such as nematodes (Smith, 1988; Price *et al.*, 1995). Increase in synthesis of secondary metabolites like lignin, ethylene, phenols as well as phytoalexins in mycorrhizal plants may contribute to these protective effects (Dehne, 1982 & 1986; Morandi *et al.,* 1984; Morandi and Gianinazzi-Pearson, 1986).

Changes in density and composition of the rhizosphere mycoflora due to arbuscular mycorrhizal colonization are well documented (Secilia and Bagyaraj, 1988; Linderman, 1988; Paulits and Linderman, 1991). In legumes there is increased nodulation and nitrogen fixation in mycorrhizal root system as a result of plant's improved P nutrition (Barea *et al.,* 1987; Patterson *et al.,* 1990; Reinhard *et al.,* 1994). The improved N, P and micronutrient nutrition of plants by arbuscular mycorrhizal fungi causes secondary effects on the absorption of mobile ions like K, S and HNO_3, and there is an overall modification in the cation-anion balance. Early insight into the role of arbuscular mycorrhizal fungi suggests the role in enhancing soil aggregation, reduction in soil erosion and increased water holding capacity (Thomas *et al.,* 1993; Tisdal, 1994).

A certain number of management practices can affect mycorrhizal development and function. Heavy fertilization and use of pesticides, especially biocides and fungicides can greatly reduce arbuscular mycorrhizal fungal population and mycorrhizal colonization levels (Douds *et al.,* 1993; West *et al.,* 1993; Udaiyan *et al.,* 1995.) Others like disinfectants, micropropagation techniques or the use of soilless potting mixes completely eliminate arbuscular mycorrhizal fungi and cause stunting or transplantation problems for mycorrhizal dependent plant species (Niemi and Vestberg, 1992).

Many ecosystems are in various state of decline by erosion, low productivity and poor water quality caused by forest clearing, intensive agricultural production and continued use of land resources for the purpose that are not sustainable (Allen *et al.,* 1995). The biological diversity of these systems is being altered. Sieverding (1989) has pointed out that when soils with perennial plant species are cleared for cultivation,

species of arbuscular mycorrhizal fungi belonging to *Sclerocystis* tend to disappear. Biological diversity apart from animals has been mainly concentrated on plant diversity, but little is known about the richness of the mycorrhizal fungi with which plants are associated. Eventhough, mycorrhizae regulate plant diversity they do not follow the patterns of plant diversity (Allen *et. al.*, 1995).

The State of Goa with an area of approximately 3702 sq. km is one of the smallest State of India (15^0 48' 00" N and 14^0 53' 54" N Latitude and 74^0 20' 13" E and 73^0 40' 33" E Longitude) lies in the central portion of Western Ghats which extend from the Tapti River (Gujarat) in the north down to the peninsular tip of South India and is one of the bio-diversity hot-spots in the world. In Goa, the environment as a whole has suffered, mainly due to urbanization ans so called "development". Severing the close links between plant and soil microorganisms has contributed to degradation of many ecosystems. The re-establishment of functional ecosystems presumes knowledge of micro-elements of the system and therefore they must be studied to understand the ecosystem changes. No effort seems to be directed to isolate and identify the native arbuscular mycorrhizal fungi in the forest areas of Goa.

Although arbuscular mycorrhizal fungi can be used to enhance plant productivity, many concepts of arbuscular mycorrhizal fungal application cannot be adequately assessed or manipulated in a rational way for field application without the knowledge of their taxonomy and ecology. Also the potential importance of plant and soil inhabiting arbuscular mycorrhizal fungi to natural ecosystem and low cost agriculture and horticulture justifies the persuit of an understanding their taxonomy and

ecology. But much still remain to be learnt about these wide spread association. Hence, the present investigation was undertaken with the following objectives.

1. To study the dynamics of root colonization and to determine spore density of arbuscular mycorrhizal fungi, occurring in Mollem and Dharbandoda forest areas.

2. To study the arbuscular mycorrhizal fungal diverity in the rhizosphere soils of various plant species of Mollem and Dharbandoda forest areas.

3. To study the ecology and taxonomy with respect to edaphic factors of arbuscular mycorrhizal fungi associated with some plant species from Mollem and Dharbandoda forest area.

4. To study seasonal variations of arbuscular mycorrhizal fungi with respect to root colonization and spore density.

5. To study the effect of selected arbuscular mycorrhizal fungi on growth and productivity of selected forest tree species.

REVIEW OF LITERATURE

Arbuscular mycorrhizae are fungus-root symbiosis that occurs in the vast majority of plants have existed since the Devonian period and might have been essential for the evolution of land plants (Pirozynski and Malloch, 1975).

Research on arbuscular mycorrhizal association from the initial comprehensive description (Gallaud, 1905) entered a lag phase, until workers in the 1950's demonstrated convincingly that arbuscular mycorrhizae could enhance plant growth (Nicolson, 1967). During this period research was confined to reports on the range of plants forming mycorrhizal associations and the taxonomic position of the symbiotic fungi. However, these observations established that these associations were widespread. Janse (1896) undertook the first broad scale survey in Java, showing that great majority of tropical plants formed mycorrhiza. Stahl (1900) first categorized plant families into obligately, facultatively and non-mycorrhizal. Gallaud (1905) differentiated arbuscular mycorrhizae from orchid and ericoid mycorrhizae and described that arbuscules and vesicles are essential for the further understanding of mycorrhizal relationship. It is practically impossible to review all the voluminous literature available with regard to various aspects of arbuscular mycorrhizae. Hence, this review is focused on ecology, taxonomy and arbuscular mycorrhizal fungal benefits to the host plants.

ECOLOGY

A. OCCURRENCE AND DISTRIBUTION OF ARBUSCULAR MYCORRHIZAL FUNGI

Several workers have published excellent general reviews about mycorrhizae (Gerdemann, 1975; Hayman, 1978; Smith, 1980; Mosse *et al.*, 1981) and also reviewed on specific aspects of the symbiosis such as nitrogen uptake (Bowen and Smith, 1981), and nutrient translocation (Rhodes and Gerdemann, 1980).

Mycorrhizal ecologists by the early 1980's believed that plants and their mycorrhizal status are relatively well known. Brundrett (1991) reviewed the mycorrhizal status of arbuscular mycorrhizal plants in natural ecosystems. Since then several studies have appeared widening the knowledge of arbuscular mycorrhizal fungi and their distribution in natural ecosystems. Koske *et al.*, (1992) and Gemma *et al.*, (1992), have reported the mycorrhizal status of Hawaiian angiosperms and pteriophytes. Ueda *et al.*, (1992) found arbuscular mycorrhizal association in twenty six of the thirty three species of medicinal plants they examined.

In India, occurrence of arbuscular mycorrhizal association has been studied by different workers in various ecosystems *viz.*, subtropical evergreen forests (Sharma *et al.*, 1984), Seasonally dry tropical and subtropical forests (Parameswaran and Augustine, 1988; Jha *et al.*, 1988; Raman *et al.*, 1992; Bhat Narayana, 1993; Appaswamy and Ganapathy, (1995); Mohankumar and Mahadevan, 1987; Muthukumar *et al.*, 1996; Santhaguru *et al.*, 1995), Semiarid and arid (Mukerji and Kapoor, 1986; Rachel *et al.*, 1989 & 1991; Tarafdar and Rao, 1990; Neeraj *et al.*, 1991; Gupta and Mukerji, 1996),

Edaphic vegetation *viz.*, Tropical plains (Raghupathi and Mahadevan, 1993); Red laterite soils (Parvathi *et al.*, 1984; Vijayalakshmi and Rao, 1988); Red sandy loam soils (Ammani *et al.*, 1994); Dry saline soils (Kannan and Lakshminarasimhan, 1988; Janardhanan, *et al.*, 1994); Dune vegetation (Mohan and Natarajan, 1988; Mohankumar *et al.*, 1988; Beena *et al.*, 1997; Jaiswal and Rodrigues, 2001); Wet coastal vegetation (Sengupta and Chaudhari, 1990; Mohankumar and Mahadevan, 1986); Terrestrial wetlands (Bagyaraj *et al.*, 1979; Chaubal *et al.*, 1982; Raghupathy *et al.*, 1990; Dharmarajan *et al.*, 1993, Gupta and Ali 1993).

However, as further ecosystem types are explored for arbuscular mycorrhizal associations, surprises still appear (Allen, 1996). Arbuscular mycorrhizal association can also be found in unexpected plant groups and habitats. *viz.*, in aquatic plants (Khan, 1993); floating *Typha* mats (Stenlund and Charvat, 1994), parasitic plants (Palacios-Mayorga and Perez-Silva, 1993) and in Proteaceae (Bellgard *et al.*, 1994).

B. ROLE OF ARBUSCULAR MYCORRHIZAL ASSOCIATION

The role of arbuscular mycorrhizal fungi in stimulating plant growth through enhanced nutrient and water uptake is now widely recognised. Mineral nutrients especially phosphorus appear to be the major constrain for plant growth in natural ecosystem (Brundrett, 1991). But plants have developed strategies to ensure nutrient uptake and conservation through mycorrhization. Most plants in natural ecosystems are often less efficient in absorbing nutrients from soils than more opportunistic ruderal species which have low nutritional requirements. (Cardus, 1980; Chapin *et al.*, 1986; Chapin, 1988). Plants in natural ecosystems are adapted to low nutrient levels and have

less and slow growth rates which results in less demand for nutrients. Byalis (1975) has pointed out that plant species with poor development of root hairs tend to be mycotrophic *i.e.* dependent upon mycorrhizal fungi for nutrient uptake. These plants in natural ecosystem further conserve resources within long-lived shoot and root structures, efficiently reclaim minerals from senescing tissues (Boerner, 1986; Chapin, 1988) and establish carefully regulated mycorrhizal associations (Brundrett and Kendrick 1990).

The principal benefit of arbuscular mycorrhizal symbiosis for higher plants is an increased supply of phosphorus which is taken up by hyphae outside the root, translocated to internal fungal structures, and ultimately released cortical cells of the roots. As the phosphorus level of the soil or growth medium is increased, mycorrhizal colonization is reduced. This apparently results from physiological changes accompanying high concentrations of phosphorus in plant tissue, rather than a direct effect of phosphorus on growth of mycorrhizal fungus (Sanders, 1975; Ratnayake *et al.*, 1978). Newman *et al.*, (1992) demonstrated that nutrients could be transferred from dying roots to living plants through mycorrhizal links resulting in a preferential cycling of nutrients. It is known that some species in nature fail to grow in the absence of arbuscular mycorrhizal association. (Janos, 1980). In some cases this is because of an ineffective coarse root system (Byalis, 1975). The presence of arbuscular mycorrhizal propagules in the most undisturbed natural ecosystem, ensure mycorrhization of these plants, thus facilitating the capture of nutrients and survivability to such species contributing to the maintenance of the diversity (Read, 1993).

C. FACTORS INFLUENCING ARBUSCULAR MYCORRHIZAL FUNGI

I. Season

Seasonal changes in mycorrhizal colonization and spore numbers have been recorded in deciduous forest (Brundrett and Kendrick, 1988; Mayer and Godoy, 1989; Brundrett and Abbott, 1994), grasslands (Gay *et al.*, 1982); salt marshes (Lee and Koske, 1994); tropical forests (Louis and Lim, 1987; Jha *et al.*, 1992) arid and dry land (Allen, 1983; Meney *et al.*, 1993) communities. In India, seasonal changes in mycorrhizal colonization and spore numbers have been recorded in semievergreen forest, mixed deciduous forest, Teak forest and scrub jungle (Mohankumar and Mahadevan, 1986).

Large variations in arbuscular mycorrhizal colonization levels among seasons may occur because of rapid root growth or turnover of roots by plants during periods when soil moisture and temperature are favourable (Brundrett, 1991). Generally for plants with short lived roots, the root length colonized by arbuscular mycorrhizal fungi increase rapidly when roots grow and decrease when roots senesces (Land and Schonbeck,1991). But these changes are gradual in many species (especially perennial) because they have long lived roots (Brundrett, 1991). The species with long lived roots may function as keystone mutualist (Brundrett, 1991), benefiting all host plants by allowing arbuscular mycorrhizal fungi to penetrate within them (Brundrett and Kendrick 1990a)

Seasonal fluctuations in arbuscular mycorrhizal spore numbers have been attributed to germination activities (Gemma and Koske, 1988), soil micro- and macro-faunal activities (Rabatin and Stinner, 1988; Mc Gee and Baczocha, 1994) and destruction of arbuscular mycorrhizal spores by soil fungi and other parasites (Ross and Rottencutter 1977; Lee and Koske, 1994a). The variation in arbuscular mycorrhizal fungal spores to initiate mycorrhization may also contribute to seasonal changes, as newly formed spores require a period of dormancy (Tommerup, 1983; Gemma and Koske, 1988). The spore numbers generally decline during periods of mycorrhizal formation and increases during periods of root senescence (Brundrett, 1991).

II. Climatic factors

a. Rainfall and Relative Humidity

Literature on ecological studies related to the effect of climatic factors on arbuscular mycorrhizae is scarce. Michelini *et al.*, (1993) found significant relationship between arbuscular mycorrhizal fungal colonization and rainfall in *Citrus*. According to Hayman (1974) seasonal changes, which includes changes in both climatic and edaphic factors play a key role in controlling sporulation of arbuscular mycorrhizal fungi. The number of mycorrhizal spores and extent of colonization decreased especially during rainy season. Braunberger *et al.*, (1994) found that "false break" (rain during summer) decreased mycorrhizal colonization and proportion of root length colonized by arbuscular mycorrhizal structures. Udaiyan *et al.*, (1996) reported a positive relation between arbuscular mycorrhizal fungal spore numbers and Relative Humidity in *Acacia farnesiana*, but a negative relationship in *Acacia planiforms*.

b. Temperature

It has been shown that temperature significantly influences arbuscular mycorrhizal fungal colonization and sporulation both under field and glasshouse conditions. Higher root colonization is generally known to occur during higher temperature (Furlan and Fortin, 1973). Increased temperature is known to decrease the lag phase of colonization. Jarstfer and Sylvia, 1993) noted decreased sporulation under high temperatures. Schenck and Schroder (1974) observed maximum arbuscule development in soyabean near 30°C, but mycelial colonization was greater between 28-34°C. Daniels and Trappe (1980) observed that the optimum temperature for germination of *Glomus* and *Acaulospora* species were found 20-25°C, whereas, *Gigaspora* had much higher optima. These studies indicate that increased soil temperature fastens the development of arbuscular mycorrhizal fungi

The temperature effect may explain the slow development of colonization in temperate soils (Black and Tinker, 1979) where soil temperature is low compared to tropical soils. Since many species of arbuscular mycorrhizal fungi are worldwide in distribution, it is possible that strains and species may be temperature adapted. Schenck *et al.*, (1975) found that two isolates of *Glomus mosseae* from Florida germinated best at 34°C, whereas one from Washington had an optimum of 20°C. McGee *et al.*, (1987) have shown that propagules of some isolates of arbuscular mycorrhizal fungi can survive in dry soil temperatures upto 70°C and subsequently colonize roots of ephemeral plants following rain.

Many reports have shown that isolates may differ in their optimum temperature for germination, root colonization and spore production. (Graham *et al.*, 1982; Tommerup, 1983a). Though, there have been a number of studies on the effect of temperature on the mycorrhizal formation (Farlan and Fortin, 1973; Hayman, 1974; Smith and Bowen, 1979), only a few have considered temperatures of 30°C and above. Smith and Roncadori (1986) showed that although optimum root growth response in cotton occurred at 30°C, maximum root colonization occurred at 36°C indicating complex interactions between survival and germination of propagules and plant process such as root growth.

c. Edaphic factors

i) Water

The arbuscular mycorrhizal colonization has been found in plants around the world over a wide range of soil water content like in xerophytes of arid regions (Khan, 1974; Mukerji and Kapoor, 1986), wet soil of marshes (Chaubal *et al.*, 1982; Ragupathy *et al.*, 1990; Rickerl *et al.*, 1994), free floating (Bagyaraj *et al.*, 1979) and submerged aquatic plants (Clayton and Bagyaraj, 1984).

Water logging may substantially reduce the number of spores in mangrove soils and may abolish mycorrhizal colonization (Mohankumar and Mahadevan, 1986). Water logging may inhibit mycorrhizal formation through lack of aeration as oxygen is necessary for fungal growth (Crawford, 1992). Excessively high soil water potential reduced arbuscular mycorrhizal colonization (Khan, 1974). The distribution of spores in

the rhizosphere and arbuscular mycorrhizal colonization of roots is affected by soil moisture and seasons (Khan, 1974). Khalil and Loyanachan, (1994) reported a higher arbuscular mycorrhizal fungal spore populations in poorly drained soils compared to well drained and moderately drained soils. Water has been shown to affect arbuscular mycorrhizal fungal sporulation. Non-saturated and non stressed water conditions are best for spore production both in high and low phosphorus conditions (Nelson and Safir, 1982). Water activity has been found to be the important determinant of arbuscular mycorrhizal fungal spore germination *in vitro* (Douds and Schenck, 1991).

In natural ecosystems rain may stimulate germination of indigenous mycorrhizal fungi (Wilson, 1984). In regions characterized by hot dry summer and cool winter growing season plants die during summer and re-establish with onset of winter rains. However rains during summer results in a decrease of mycorrhizal propagules (Braunberger *et al.*, 1994). Tommerup (1987) indicated that spores of arbuscular mycorrhizal fungi can probably survive for atleast 20 years in dry soils, but only for 2 years in moist soils. Similarly, mycorrhizal roots remained viable for 6 months when stored in dry conditions (Tommerup and Abbott, 1981) but lost viability in moist soils (Tommerup, 1983). Mc Gee *et al.*, (1987) observed the survival of arbuscular mycorrhizal propagules in an Australian soil reaching 70°C during summer. Jha *et al.*, (1992) reported a positive relation between soil moisture and root colonization levels but a negative relationship between soil moisture and spore numbers.

ii) pH

The soil pH is one of the important factors considered by several authors (Abbott and Robson, 1991) in the mycorrhizal study. The study on soil pH can be subdivided into effects on spore germination (Daniels and Trappe, 1980), spore production (Kruckelmann, 1975; Read *et al.,* 1976) and effects on plant growth and nutrients uptake (Lambert and Cole, 1980).

Natural soils of the world cover a pH range of 2.8 to >10 (Bass Becking *et al.,* 1960). Daft *et al.,* (1975) reported considerable arbuscular mycorrhizal colonization in plants growing in a mine spoils of pH 2. Sparling and Tinker (1978) found no obvious effect of pH on mycorrhizal colonization in three grassland sites at pH 4.9, 5.9 and 6.2. Previous studies indicate some evidence for differences in adaptations of strains and species of arbuscular mycorrhizal fungi to soil pH. Lambert and Cole (1980) reported that an isolate of *Gigaspora gigantea* failed to colonize at low pH and six isolates of *Glomus tenue* differed in their ability to form mycorrhizas at low pH. Janarthanan *et al.,* (1994) reported that the typical arbuscular mycorrhizal fungi occur at extreme alkaline pH (10.5) which occurs in natural soils of saline regions. Peat and Fitter (1993) indicated that mycorrhizal dependent plant species occur at higher pHs than infrequently mycorrhizal species.

The laboratory studies indicate a good germination of arbuscular mycorrhizal spores requires a pH range of 6 to 7, although there are cases of germinating at pH 5 and below as well as at pH 8 and above (Siqueira *et al.,* 1982). The pH optima for spore

germination may probably differ with each arbuscular mycorrhizal fungal species and to environments to which each is indigenous (Gerdman and Trappe, 1974; Green *et al.*, 1976). It has been established that spore germination is pH sensitive, but different species have different pH optima (Robson and Abbott, 1989). Hepper and Smith (1976) showed that arbuscular mycorrhizal fungal spore germination on agar is sensitive to metals such as Manganese, Copper and zinc, whose activities in soil solutions is dependent of pH.

iii) Nutrients

a) Nitrogen:

In general, nitrogen is very important nutrient which limits plant growth. Plants require nitrogen in large amounts for their growth. There have been a few studies on the effects of nitrogen on arbuscular mycorrhizal fungi compared to phosphorus. Studies indicate that nitrogen supresses root colonization (Mosse and Phillips 1971; Menge, 1984). Hepper (1983) demonstrated that increased application of NO_3 increased the levels of root colonization in lettuce. In contrast, Chambers *et al.*, (1980) reported that both NH_4+ and NO_3^- depressed arbuscular mycorrhizal formation and suggested the effect to a drop in rhizosphere pH. Sylvia and Neal (1990) suggested that plants nitrogen stress like phosphorus stress promotes mycorrhization. Thomson (1986) reported that pH modification by nitrogen sources influenced mycorrhization. Jha *et al.*, (1992) found that soil N was positively related to arbuscular mycorrhizal colonization and negatively to spore numbers. Like phosphorus, the effect of soil nitrogen on arbuscular mycorrhizal colonization and spore number can vary with plant species (Muthukumar *et al.*, 1994a; Udaiyan *et al.*, 1996).

b) Phosphorus

A relationship exists between the extent of mycorrhizal colonization and the concentrations of soil phosphorus. The sites with large amount of soil P may have high levels of colonization and large spore numbers. In contrast, sites with small amount of phosphorus may have low levels of colonization or spore numbers (Hayman, 1978; Gianinazzi-Pearson *et al.*, 1980; Jeffries *et al.*, 1988). Positive (Jha *et al.*, 1992; Udaiyan *et al.*, 1996) and negative (Bolgiano *et al.*, 1983; Boerner, 1986; Morita and Konishi, 1989) relations have been found between the amount of extractable soil phosphorus and arbuscular mycorrhizal colonization.

The root colonization of arbuscular mycorrhizal fungi are adversely affected by application of phosphorus through inhibition of spore germination (Miranda and Harris, 1994), depressing the development of arbuscles, vesicles, internal and external hyphae and penetration points (Menge *et al.*, 1978; Schwab *et al.*, 1983; Miranda and Harris, 1994: Suriyapperuma and Koske, 1995). However arbuscular mycorrhizal fungi vary in their sensitivity to soil phosphorus (Trouvelot *et al.*, 1987; Lamar and Davey 1988).

The important factor for controlling arbuscular mycorrhizal colonization has been as plant tissue phosphorus (Sylvia and Neal, 1990; Menge *et al.*, 1978). This conclusion has been extended to changes in root membrane permeability and to the availability and quantity of root exudates (Ratnayake *et al.*, 1978; Graham *et al.*, 1981; Bolan *et al.*, 1984). They however, reported that increasing soil phosphorus gradually from a severely

deficient condition could increase mycorrhizal colonization before the expected decrease occurred as soil phosphorus become sufficient. This increase in arbuscular mycorrhizal colonization due to small amounts of phosphorus application suggest that mycorrhization may to certain extent depend on soil phosphorus and not totally on plant phosphorus (Graham *et al.*, 1981). Sanders and Fitter (1992a), however, have found no relationship between plant phosphorus and arbuscular mycorrhizal colonization in grassland species. Sylvia and Neal (1990) reported that root colonization by arbuscular mycorrhizal fungi was not affected when plants were deficient in nitrogen, but when nitrogen was sufficient, phosphorus addition suppressed root colonization. But the response of arbuscular mycorrhizal colonization and spore number to soil phosphorus can vary with host species (Muthukumar *et al.*, 1994a).

c) Potassium

There are very less reports on role of potassium. Daniels and Trappe, (1980) has reported that the potassium has no effect on arbuscular mycorrhizal fungi. But Ebbers *et al.*, (1987) reported significantly positive correlation between spore abundance and available soil potassium in prairie drop seed (*Sporobolus heterolepsis*). Zahka *et al.* (1995) indicated that concentration of soil potassium was a key predictor for variations observed in colonization levels and for the occurrence of arbuscules in the root cortical cells.

d) Other nutrients

The role of other nutrients on root colonization and spores abundance of arbuscular mycorrhizal fungi is little known compared nitrogen, phosphorus and potassium. Micronutrients like copper, zinc and manganese are reported to inhibit arbuscular mycorrhizal fungi at low concentration and arbuscular mycorrhizal fungal colonization levels and suggested a combined effect of Zn-pH leading to this inhibition. In contrast, McIlween and Cole (1978) reported the stimulatory effect of zinc on spore germination at low concentrations. Sreenivasa and Bagyaraj (1988) reported that the sub-optimal levels of zinc, copper and manganese would enhance root colonization and sporulation of *Glomus fasciculatum* associated with Rhodes grass (*Chloris gayana*).

TAXONOMY

Taxonomy of Glomalean fungi is less than 30 years old, starting with the formal Linnaean classification by Gerdman and Trappe (1974). Approximately 150 species (Schenck and Perez, 1990) have been described based on morphological features of spores. More than 65 of these had been described by the year 1983. But since then the taxonomical concepts about arbuscular mycorrhizae have advanced (Walker, 1983; Morton, 1987) and only a few have been redescribed using modern concepts and terminologies (Koske and Walker, 1985; Walker and Koske, 1987). Subsequently, the matching of new collections with old descriptions has led to innumerable difficulties in their identification.

Problems in Glomalean Taxonomy

Spores are the most retrievable part of the fungal organisms, because each can be manipulated as descrete object. Most taxa described in the first classification were sporocarpic (Gerdemann and Trappe, 1974) because they were more easily detected in soil sievings. As additional procedures were introduced (Daniels and Skipper, 1982), more non-sporocarpic species were discovered. The presence of spore propagules in field soil sample is unpredictable even when most plants were mycorrhizal (McGee, 1989). Modification of collection procedures is often necessary when spores are present for each set of circumstances. In addition, soil samples collected from field usually contains spores of different arbuscular mycorrhizal fungal species. Molina *et al.*, (1978) recovered an average of two to five species from *Festuca* plants in western United States and species mixtures are common even in extreme soil conditions.

Spores of arbuscular mycorrhizal fungi are recovered under a dissecting microscope, but characters observable at this level often overlap among different species and even among different genera. In nature most spores are either deteriorated or modified in same way to cause misinterpretation of characters, properties of their occurrence (Morton, 1993). It is true that all structural components of arbuscular mycorrhizal fungal spores for taxonomic decisions are succeptible to alterations or deterioration by a wide range of biotic or abiotic agents in the soil environment. The assumption that field collected spores possesses intact informative character is erroneous. Many species-level characters like spore wall are exposed to soil environment (Morton and Benny, 1990). They may be ephemeral and therefore absent in field collected

specimens. So collections of spores from pot cultures are essential for identifications or characterization of Glomalean fungi.

The taxonomy of Glomalean fungi is further plagued by inaccurate descriptions and the type method in the present form has failed to provide a fixed reference point at study (Morton, 1993). New taxa are in danger of redundant and inconsequential unless a high priority is placed on re-evaluation of known taxa. A number of taxa have been inadequately described. New structures or new properties of existing structures are often missed if more obvious diagonistic features are present. Hence, as Walker (1992) stated "The identification of the fungi in Glomales is a difficult and specialized task".

ROLE OF MYCORRHIZAE

Mycorrhiza constitutes the most striking example of symbiosis in plant kingdom. Mycotrophy represents a specialized mode of tree nutrition, the significance of which has been realized during the last few decades. They help in the faster uptake and translocation of water and nutrients, particularly phosphorus, nitrogen and potassium, besides other elements like zinc, calcium *etc.* Plants equipped with mycorrhizae are better adapted to withstand drought and invasion by pathogenic organisms.

Nutritional benefits

a) Nitrogen

In arbuscular mycorrhizal research on nitrogen nutrition, the most attention has been paid to legumes. When arbuscular mycorrhiza improves the phosphorus nutrition of the host plant there may be corresponding increase in nodulation, nitrogen fixation and growth (Robson *et al.*, 1981). In view of the high phosphorus requirements for nodulation, many legume species growing on low phosphorus soils are highly dependent on arbuscular mycorrhizal colonization. However, the symbioses impose a strong competition for photosynthates, usually at expense of root growth. Accordingly, the beneficial effects on nitrogen fixation are either confined to, or at least most distinct, in low phosphorus soils. The ability of mycorrhizal plants to utilize nitrogen sources has been attributed to an indirect effect associated with improved phosphorus nutrition. Some studies have demonstrated that the arbuscular mycorrhizal fungi were able to metabolise inorganic nitrogen (Ames *et al.*, 1983; Smith *et al.*, 1985) The presence (Coxwell and Johnson, 1985) and absence (Rose and Youngberg, 1981) of the involvement of arbuscular mycorrhizal on the nitrogen nutrition of the host plants have been reported. Studies have suggested that mycorrhizal plants can derive nitrogen from both organic as well as inorganic sources that are not available to non-mycorrhizal plants (Ames *et al.*, 1984; Barea *et al.*, 1987 & 1989). Johansen *et al.*, (1992 & 1994) recently reported the uptake of N^{15} from labeled ammonium salts by the external arbuscular mycorrhizal fungal hyphae. The existence of inter-plant hyphal bridges between individual plants permits transfer of nutrients such as nitrogen in a non-legume and legume combination (Newman *et al.*, 1992).

b) Phosphorus

In general large growth enhancement effects of root colonization with mycorrhizal fungi are occurred by increase in phosphorus absorption, particularly from sparingly soluble phosphorus sources (Bolan *et al.*, 1987). The low diffusion rate of phosphorus in soils limits its uptake by plants root system (Silberbush and Barber, 1983). When root uptake is restricted, upto 80% of the plant phosphorus can be delivered by the extrametrical arbuscular mycorrhizal hyphae to the host over a distance of more than 10 centimeters from the root surface (Li *et al.*, 1991). Convincing experimental evidences is still lacking for the speculation that arbuscular mycorrhizal plants obtain phosphorus from sources that are not available to non-mycorrhizal plants (Bolan, 1991). With increasing levels of soil phosphorus, the mycorrhizal response on plant growth declines and may either be abolished or lead to growth depressions (Peng *et al.*, 1993). The shift in root length: shoot dry weight ratio is a typical response to improved P nutrition in both mycorrhizal and non-mycorrhizal plants. In mycorrhizal plants, the phosphorus concentrations per unit dry weight are higher and thus the phosphorus use efficiency is lower than non-mycorrhizal plants. (Koide, 1991; Marschner and Dell, 1994).

c) Potassium and other nutrients

Very little is known on the role of mycorrhiza in uptake of K, Ca, Mg and S. Although for arbuscular mycorrhizae, there are many results on effects of colonization on concentrations and amounts of K in shoots, these results are inconsistent and difficult to interprete (Sieverding and Toro, 1988). The ability of the extramatrical arbuscular mycorrhizal fungal hyphae to uptake and transport potassium has also been demonstrated

in compartmented pots (George *et al.*, 1992). Significant differences in growth response of soyabean to different geographical isolates of *Glomus mosseae* seemed to be more related to improved potassium rather than phosphorus nutrition of the host (Bethlenfalvay *et al.*, 1989). The hyphal uptake of calcium (Rhodes and Gerdmann, 1978) and SO_4-S (Cooper and Tinker, 1978) has been shown through supplying radio isotopes (^{45}C, $^{35}SO_4$). The uptake and transport rate of calcium is very low compared to phosphorus.

There are numerous reports on the enhancement of zinc and copper uptake by arbuscular mycorrhizal colonized plants. At least in part, this enhancement can be attributed to uptake and transport in external hyphae to the host plant (Kothari *et al.*, 1990 & 1990a). The hyphal contribution of *Glomus mosseae* to the uptake of zinc ranged from 16-25% compared to 13-20% for phosphorus in maize grown in calcareous soil (Kothari *et al.*, 1991). In the same soil, Li *et al.*, (1991a) demonstrated that the delivery of copper from the hyphal compartment ranged from 52 to 6% of the total copper uptake under restricted rooting space. In contrast, manganese uptake and concentration in plants are either unaffected but more often are lower in arbuscular mycorrhizal plants (Lambert and Weidensaul, 1991). The decrease in concentration of manganese in plants is most likely an indirect effect caused by arbuscular mycorrhizal induced changes in the rhizosphere micro-organisms in general and particularly the decline in population of manganese reducers (Kothari *et al.*, 1991 & 1991a). The role of arbuscular mycorrhizal fungi on boron nutrition of the host plant is either lacking or inconclusive. Abuscular mycorrhizal fungi may decrease boron concentration in host plants (Kothari *et al.*, 1991 & 1991a). Plants have varying mechanisms for mobilizing, chelating and reducing ferric (Fe) in

order to facilitate uptake (Marshner, 1986). Treeby (1992) indicated that arbuscular mycorrhizal fungi may facilitate the iron uptake in acidic but not in alkaline soils.

d) Non-nutrient benefits

Many experiments have shown that the rate of photosynthesis is higher in mycorrhizal plants compared to non-mycorrhizal plants (Allen *et al.*, 1981; Kucey and Paul, 1982; Snellgrove *et al.*, 1986). The rate of photosynthesis *in vitro* can be limited by phosphorus availability (Lewis, 1986; Sivak and Walker, 1986). The direct role of phosphorus in photosynthesis and in subsequent mobilization or storage of photosynthates has now been clearly demonstrated. But the differences in the sensitivity of photosynthetic mechanism of plant species to phosphorus deficiency (Dietz and Foyer, 1986), may be a possible basis for differences in response to mycorrhizal colonization.

Mycorrhizal plants may be drought tolerant (Allen and Allen, 1986; Osonubi, 1989) but it has been difficult to distinguish direct mycorrhizal effect on water relations from those mediated via improved mineral nutrition. The increased growth of mycorrhizal plants under drought conditions than non-mycorrhizal plants has been attributed to increased stomatal conductance (Osonubi, 1989; Subramanian *et al.*, 1995) and increased root conductivity provided by increased surface area of mycorrhizal hyphae (Read and Boyd, 1986). These reported changes in mycorrhizal plants under drought conditions could be either due to the secondary response to better phosphorus nutrition (Michelsen and Rosendahl, 1990; Osonubi *et al.*, 1990) or mediated via direct mycorrhizal effect (Henderson and Davies, 1990). The relief of nutrient stress might also

be allowed by increased rates of root growth and more efficient extraction of water from soil profile (Fitter, 1985). The improved water availability reduces severe drought stress symptoms such as proline accumulation (Levy and Krikun 1980; Nemec and Meredith, 1981; Cooper, 1984).

Colonization by arbuscular mycorrhizal fungi enhances hormone accumulation in host tissues with changes in the levels of cytokinin, abscissic acid and gibberellin like substances (Baas and Kuiper 1989; Danneberg *et al.*, 1992). Isoflavanoids and phytoalexins which are inhibitory to pathogenic fungi have been isolated from mycorrhizal plants (Morandi *et al.*, 1984; Morandi and Gianinazzi-Pearson, 1986).

CHAPTER – 1

OCCURRENCE AND DISTRIBUTION OF ARBUSCULAR MYCORRHIZAL (AM) FUNGI IN MOLLEM AND DHARBANDODA FOREST AREAS.

INTRODUCTION

Arbuscular mycorrhizal (AM) fungi are soil borne organisms which establish an obligate mutalistic association with the roots of about 90% of the terrestial plants, making them the largest widespread plant symbiosis on earth. (Grandmougin-Ferjani *et al.*, 1999). Approximately 6,000 species of vascular plants have been examined for mycorrhizas (Newman and Reddell, 1987; Trappe, 1987). Plant species within a family not only vary in their extent of mycotrophy, but also contain non-mycorrhizal species. Many reports are required to determine if a species is inconsistently mycorrhizal (Trappe, 1987) because very less is known about the occurrence of species with low or highly variable levels of mycorrhizas, parasitic plants are usually non-mycorrhizal (Lesica and Antibus, 1985; Harley and Harley, 1987).

Arbuscular mycorrhizal fungi can be found in most ecosystems throughout the world ranging from the Artic to the Tropical Rain Forests (Janos, 1980; Beldose *et al.*, 1990). Arbuscular mycorrhizal fungi play a vital role in natural ecosystems like Tropical forests by influencing the plant composition and succession (Janos, 1980a; Giovannetti and Gianinazzi-Pearson, 1994). Besides this, increase uptake of nutrients especially phosphorus (Gray and Gerdemann, 1969), nutrient cycling (Eason and Newman, 1990), influencing the soil microbial population and exudates in the mycorrhizosphere (Linderman, 1988) and establishment of seedlings or contribute to the growth of shaded understorey (Hogberg *et al.*, 1999) are the major attributes of arbuscular mycorrhizal fungal association.

Knowledge of the diversity of arbuscular mycorrhizal fungal communities is a pre-requisite in managing these microorganisms in natural communities. Any change in the richness of population of arbuscular mycorrhizal fungi or in their functional diversity could have important consequences for the equilibrium of natural plant community structure (Read, 1991; Martins, 1993). Further, mycorrhizal associations are potential factors that determine plant diversity in ecosystems. They can probably modify the structure and functioning of plant community in a complex and unpredictable way (Grime *et al.*, 1987; Read, 1992).

Several studies have reported arbuscular mycorrhizal association with tree species from India. Sharma *et al.*, (1984) have studied arbuscular mycorrhizal association in trees from subtropical evergreen forest of Northeast India. Thapar and Khan (1985) have studied the distribution and frequency of arbuscular mycorrhizal fungal spores associated with rhizosphere soil of forest trees in India. Records exists on arbuscular mycorrhizal status of tree species from Arid zone in Rajasthan (Tarafdar and Rao, 1990), hard wood tree species from North India, Dehra Dun (Thapar and Vijayan, 1990), tree species of Manandur forest of Tamil Nadu (Raman *et al.*, 1992), legume trees (Santhaguru *et al.*, 1995) and tree species from Rayalaseema forest in Andhra Pradesh (Vijaya *et al.*, 1995).

Arbuscular mycorrhizal status of tree species from Western Ghats of Southern India has also been documented (Mohankumar and Mahadevan, 1987). Kandaswamy *et al.*, (1988), carried out an intensive survey for the prevalence of arbuscular mycorrhizal fungi in forest tree species occurring at different altitudes in Western Ghats

of Nilgiri District, Southern India. Later, Mohankumar and Mahadevan (1988 & 1989) carried out ecological studies on arbuscular mycorrhizal fungal association with plant species from Kalakad Reserve forest area located in the Western Ghats, Tamil Nadu. They have investigated the influence of edaphic factors and seasonal variation on the distribution of arbuscular mycorrhizal fungi in 7 well defined ecosystems *viz.*, evergreen, semi evergreen, mix deciduous, teak forest, scrub-jungle and grassland ecosystem at high and low altitudes. Ragupathy and Mahadevan (1993) conducted extensive survey of mycorrhizal status of angiosperms in India. They have reported the presence of arbuscular mycorrhizal association in 51% of the 733 angiosperms screened. However, they did not to quantify the presence of internal arbuscular mycorrhizal fungal structures. More recently Muthukumar *et al.*, (2000) surveyed arbuscular mycorrhizal fungal association in 4 vegetation types *viz.*, Forest, Grassland, Scrub and Cultivated lands. They have also reported the arbuscular mycorrhizal fungal association in medicinal plants of Maruthamalai hills, in Western Ghats of Southern India (Muthukumar *et al.*, 2001).

No work on arbuscular mycorrhizal association in tree species from the Western Ghats region of Goa State has been reported so far. In the present chapter, the occurrence and distribution of arbuscular mycorrhizal fungi with respect to root colonization, spore density and species richness in some dominant tree species from Mollen and Dharbandoda forest areas have been studied.

MATERIALS AND METHODS

STUDY AREA

The area covered by Collem range, which includes Dharbandoda and Mollem forest areas is about 5486.14 hectares, which occupies dense forest in the northern parts of Sattari taluka and most parts of Sanguem taluka. Two areas *viz.*, Mollem and Dharbandoda were selected for studying the occurrence of arbuscular mycorrhizal fungi in tree species. These areas are located between 15^{O} 15' 34" N – 15^{O} 31'45"S Latitude and 74^{O} 21' 00" E – 74^{O} 09'45" W Longitude **(Fig. 1)** and encompasses rich forest varying from moist deciduous to semi-evergreen under humid tropical climate with the highest altitude about 891 m above mean sea level **(Plate I)**.

SAMPLING TECHNIQUES

Root and rhizosphere soil samples from 28 tree species belonging to 22 families were collected during June 1998 - May 2000, packed in polyethylene bags and transported to laboratory. For each tree species, three sub-samples were randomly collected. Root samples were freshly processed, whereas, the soil samples were stored at 4 ^{O}C until analyzed. While collecting root samples, the roots were dug and traced back to the plant, which ensured that the roots belonged to the intended species.

PLANT NOMENCLATURE

Identification of tree species was carried out using floras (Rao, 1985 & 1986; Mathew, 1991; Mohanan and Henry, 1994 and, Naithani *et al.*, 1997).

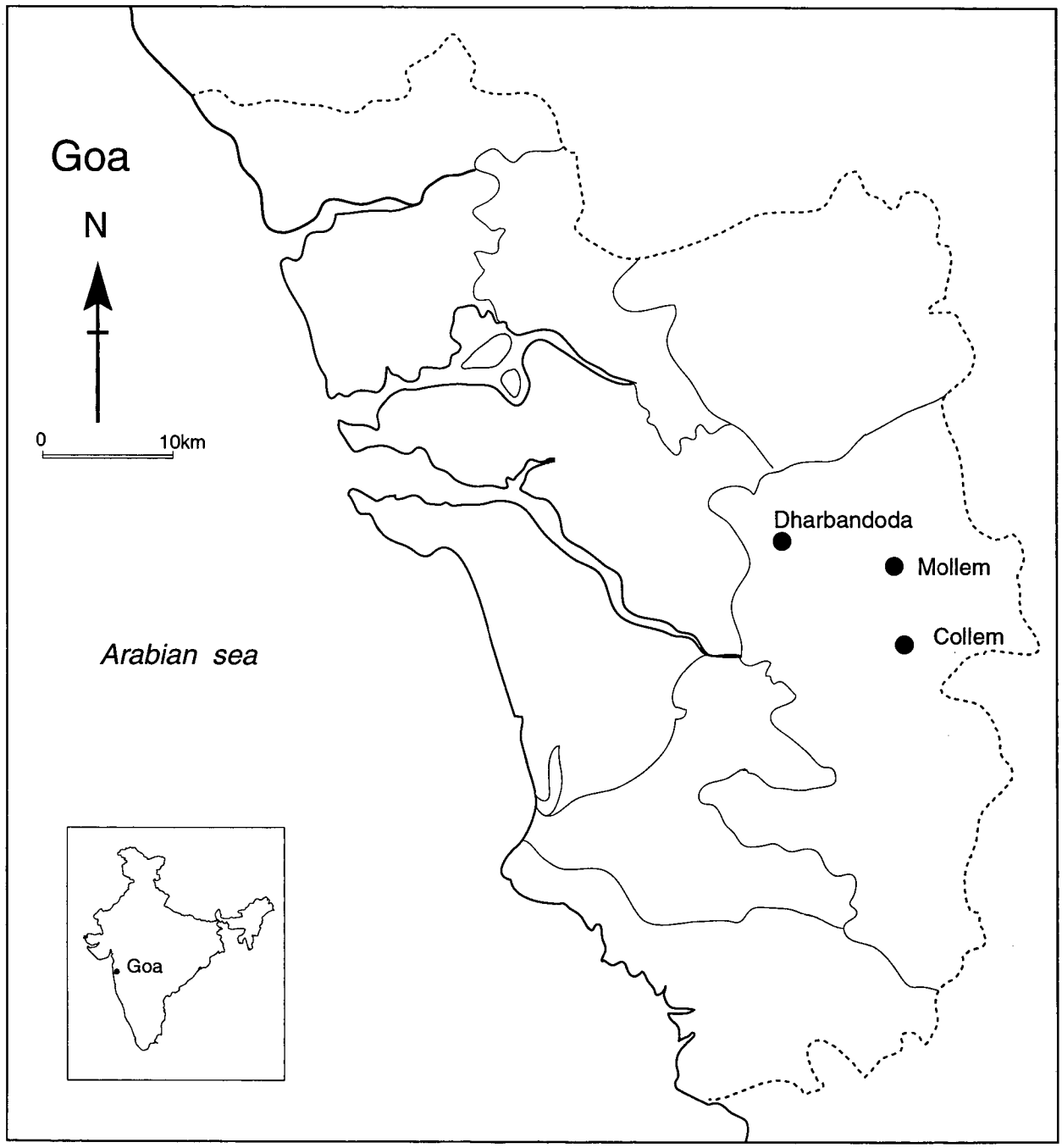


Figure 1 Map of Goa Showing location of study site

PLATE- I

GENERAL VIEW OF VEGETATION COVER OF

a. Mollem forest.

b. Dharbandoda forest.


a


b

SOIL ANALYSIS

For soil analysis, samples were collected from a depth of 0-25 cm. From the rhizosphere of each tree species, three sub samples were collected and mixed to form a composite sample. All the composite samples were brought to the laboratory in polyethylene bags, passed through 2mm sieve to remove the larger soil particles and were processed separately. Each composite sample was processed three times to get the mean value.

Soil pH was measured after dilution with distilled water (1:1 w/v soil: water) soon after the samples were brought to the laboratory. Electrical Conductivity (EC) was determined in 1:1 water: waste extracts (Bower and Wilcox, 1965). Soil nitrogen was determined by micro-Kjeldahl method (Jackson, 1971). Soil phosphorus was determined by molybdenum blue method (Jackson, 1971), while available phosphorus (P) was determined by using Olsen's method (Olsen *et al.*, 1954). Potassium was estimated after extraction in ammonium acetate solution (Jackson, 1971). Organic carbon was estimated by Walky and Black's rapid titration method (Walkey and Black, 1934).

RECOVERY OF SPORES

Hundred grams of air dried composite sample was assayed for spore count using modified wet sieving and decanting procedure (Gerdemann and Nicolson, 1963), the details of which are as follows:

- 100g of soil was added to 1 litre of tap water in a beaker.

- Large soil clumps were broken using a glass rod and the mixture was stirred for 10-15 seconds.

- The course particles were allowed to settle in the water for 2 minutes.

- The suspension was then decanted through a series of stacked sieves with mesh sizes ranging from 500μm – 38μm and the remains on the sieves were washed into beakers.

- Steps 1-4 were repeated twice to increase the likelihood that a majority of the spores in the soil had been removed.

- The suspension from each of the beakers was then filtered separately through a Whatman No.1 filter paper. Each of the filter paper containing spores and debris was then transferred separately in a petriplate.

- The suspension from each of the beakers was then filtered separately through a Whatman No.1 filter paper. Each of the filter paper containing spores and debris was then transferred separately in a petriplate.

- For each sample steps 1-7 were repeated 3 times.

- Arbuscular mycorrhizal spores were observed under Leica Stereomicroscope. The observed spores from the filter papers were picked up using a wet needle and were used either for developing pot cultures or for identification.

ESTIMATION OF ROOT COLONIZATION

Root samples were washed thoroughly under tap water. Feeder roots were cut into 1cm fragments. The composite sample of each species was cleared in 10% KOH in an autoclave at 121^0C for 15-25 minutes. Cleared roots were thoroughly washed with tap

water and acidified with 5% HCl. Later, the roots were stained with 0.05% trypan blue (Phillips and Hayman, 1970). Stained roots were mounted in glycerol and observed under Leica compound microscope. The quantification of arbuscular mycorrhizal fungal structures *viz.*, hyphae, arbuscules and vesicles and the total root length colonization was carried out by using Gridline intersection method (McGonigle *et al.*, 1990) as described below.

Examination of roots at x 200 magnification under compound microscope:

The roots from a sample are mounted on microscope slides with coverglass.

The field of view is moved across the slide, and a hairline graticule inserted into the eyepiece acts as the line of intersection with each root.

At each intersection, there are six possible mutually exclusive outcomes (p, q, r, s, t and u).

p no fungal structures,

q arbuscules,

r mycorrhizal vesicles,

s arbuscules and mycorrhizal vesicles,

t mycorrhizal hyphae but no arbuscules or mycorrhizal vesicles, and

u hyphae not seen to be connected to arbuscules or mycorrhizal vesicles.

Examine 150 intersections for each root sample, scoring each intersection in one category only form: p, q, r, s, t and u.

Where a total of G (= p + q + r + s + t + u) were inspected.

The percentage of root length colonized by arbuscules, and the percentage of root length colonized by mycorrhizal vesicles, is calculated as

Arbuscular Colonization (AC) = 100 (q + s/G).

Vesicular Colonization (VC) = 100 (r + s/G).

The percentage of root length colonized by hyphae is calculated as:

Hyphal Colonization (HC) = 100 {(G-p)/G}

POT CULTURES

Trap cultures were established by mixing field collected soil along with fresh roots and autoclaved sand (1:2) (v/v). This mixture was placed in pots and seeded with host species. The hosts used in the study included *Eleusine coracana* L., *Allium cepa* L. and *Coleus sp.* To establish single species pot cultures, 50 to 60 intact spores of identical morphology were placed near the roots of one-week-old seedlings of the above host plant species. The pot cultures were maintained for three to five months. Plants were harvested and the soil was wet sieved to collect spores. The spores isolated from the pot cultures were later used for confirming the identified spores recovered during the study period.

ESTIMATION OF SPORE DENSITY

Estimation of spore density was carried out as per the procedure given by Gaur and Adholeya (1994) which is described below in detail.

- A circular filter paper (Whatman No. 1, size 11cm diameter) is taken and folded into two equal halves.

- This is followed by a second fold, resulting into four equal quadrants.

- The paper is reopened; two lines are drawn along the two folds to divide the filter paper in four equal quadrants.

- Vertical lines are drawn on one half of the filter paper so as to divide it into approximately 15 columns with each column about 0.5 cm apart. Each column is then numbered and the direction of counting marked by an arrow.

- The filter paper is then folded in such a manner that the marked portion becomes the receiving surface for the sample during filtration. Thus, the spores are collected only on the marked surface of the filter paper and rest of the filter paper is retained without spores.

- This filter paper with the sample spores is spread in a bigger petriplate (about 120 mm in diameter).

- If there are clusters of spores on filter paper they can be spread apart by the pressure of water from a pointed wash bottle with a very fine edge (a needle/ syringe could be used).

- Care is taken that the spores do not go off the filter paper during spreading. Some spores do come over the unmarked side of the filter paper, but these could be easily counted.

- Focus the two lines under the stereomicroscope. Follow the space in between the two lines vertically as shown in the diagram and count the spores. Thus, by moving the petriplate the spores can be counted in every space between the two lines and since the lines are numbered and the direction set, it is easy to keep track of each spore on the filter paper.

Standard deviation was calculated for mean root colonization and mean spore density.

IDENTIFICATION OF ARBUSCULAR MYCORRHIZAL FUNGAL SPORES

Isolated arbuscular mycorrhizal fungal spores were mounted in Polyvinyl Lacto Glycerol (PVLG) and stained with or without Melzers reagent were examined for various characteristics using compound microscope (40x – 400x) and identified using standard keys (Schenck and Perez, 1990; and Wu, 1993). Only healthy, intact and unparasitised spores were used for the quantification of spore density and taxonomy of arbuscular mycorrhizal fungi.

RESULTS AND DISCUSSION

Soil analysis results revealed that the soils were acidic with pH ranging from 5.28 to 5.29. There was no much difference in the pH of Mollem (5.28) and Dharbandoda (5.29) forests with pH values being given in parenthesis. Electrical Conductivity (EC) ranged from 0.09 to 0.57 M mhos/cm. The Electrical Conductivity (EC) was found to be high at Mollem (0.57 M mhos/cm) than at Dharbandoda (0.09 M mhos/cm). Total nitrogen ranged from 0.25 to 0.44%. Similarly, total phosphorus varied from 0.11 to 0.20%, while total potassium ranged from 0.16 to 0.17 kg/hectare. Organic carbon ranged from 2.10 to 2. 68%. Organic carbon was found to be much higher at Mollem (2.68%) than at Dharbandoda (2.10%). Available phosphorus ranged from 11.6 Kg/hectare to 49.2 kg/hectare. Available phosphorus was higher in Mollem (49.2 kg/hectare) than in Dharbandoda soils (11.6 Kg/hectare). Similarly available

potassium was higher in Mollem soils (423.2kg/hectare) as compared to Dharbandoda forest soils (397.7kg/hectare) **(Table 2)**.

Twenty-eight tree species belonging to twenty-two families from both the sites were taken for the study. It was observed that all the twenty-eight tree species undertaken in the present study were found to be mycorrhizal. The mycorrhizal colonization was characterised by intraradical and extrametrical hyphae, intracellular hyphal coils, vesicles and/or arbuscules **(Plate II)**.

In Mollem forest, the total root colonization by native arbuscular mycorrhizal fungi in all the tree species ranged from 21.43% to 80.15% with average total root colonzation being 46.67%. The hyphal colonization was recorded in all the 28 tree species with maximum hyphal colonization recorded in *Lagerstroemia lanceolata* (60.89%) belonging to family Lythraceae and minimum hyphal colonization recorded in *Terminalia paniculata* (16.82%) belonging to family Combretaceae. Arbuscular colonization was recorded in 7 tree species, which accounts for only 25% of the total tree species undertaken for the study. Among these, maximum arbuscular colonization was recorded in *Ervatamia heyneana* (7.15%) belonging to family Apocynaceae while minimum arbuscular colonization was recorded in *Strychnos nux-vomica* (1.55%) belonging to family Loganiaceae. Vesicular colonization was recorded in all the 28 tree species. Maximum vesicular colonization was recorded in *Hoppea wightiana* (22.16%) belonging to family Dipterocarpaceae while minimum vesicular colonization was recorded in *Terminalia bellirica* (1.80%) belonging to family Combretacae. Maximum percent total root colonization was recorded in *Lagerstroemia lanceolata*

PLATE- II

ARBUSCULAR MYCORRHIZAL ASSOCIATION IN TREE SPECIES AT MOLLEM AND DHARBANDODA FOREST.

a) & b) **Vesicles and hyphae (x 100)**
c) – e) **Vesicles of various shapes.**

a
b
c
d
e

Table 2: Soil Characteristics of Mollem and Dharbandoda forest areas.

Site	pH	EC M mhos/cm	Total N_2 (%)	Total P_2O_5 (%)	Total K_2O Kg/hacter	Available phosphorus Kg/hac	Available K Kg/hac	Organic carbon (%)
Mollem	5.28 ± 0.17	0.57 ± 0.04	0.44 ± 0.03	0.20 ± 0.02	0.17 ± 0.02	49.20 ± 1.83	423.2 ± 12.82	2.68 ± 0.04
Dharbandoda	5.29 ± 0.45	0.09± 0.02	0.25 ± 0.05	0.11 ± 0.02	0.16 ± 0.02	11.66 ± 1.45	397.7 ± 15.28	2.10 ± 0.02

± Indicates Standard deviation.

All values are mean of three readings.

(80.15%) while, minimum percent root colonization was recorded in *Terminalia paniculata* (21.43%). **(Table 3)**.

In Dharbandoda forest, the total root colonization by native arbuscular mycorrhizal fungi in all the tree species ranged from 31.84% to 82.19% with average total root colonzation being 53.20%. The hyphal colonization was recorded in all the 28 tree species. Maximum hyphal colonization was recorded in *Terminalia paniculata* (73.74%) belonging to family Combretaceae while minimum hyphal colonization was recorded in *Buchanania cochinchinensis* (29.62%) belonging to family Anacardiaceae. Arbuscular colonization was recorded in 9 tree species, which accounts for only 32.14% of the total tree species undertaken for the study. Among these, maximum arbuscular colonization was recorded in *Terminalia bellirica* (5.88%) belonging to family Combretacae while minimum arbuscular colonization was recorded in *Microcos paniculata* (1.79%) belonging to family Teliaceae. Vesicular colonization was recorded in all the 28 tree species. Maximum vesicular colonization was recorded in *Phyllanthus emblica* (21.25%) belonging to family Euphorbiaceae while minimum vesicular colonization was recorded in *Helicteres isora* (1.65%) belonging to family Sterculiaceae. Maximum percent total root colonization was recorded in *Terminalia paniculata* (82.19%) while, minimum percent root colonization was recorded in *Buchanania cochinchinensis* (31.84%) **(Table 4)**.

Eighty six percent (24 out of 28) tree species from Mollem had functional mycorrhizas. The incidence of non-functional mycorrhizas was recorded in *Mallotus stenathus, Ixora notoniana, Feronia elephantum* and *Terminalia bellirica.* While

Table 3: Colonization patterns in some tree species from Mollem forest.

Sr. No.	Name of Species	Hyphal colonization (%)	Arbuscular colonization (%)	Vesicular colonization (%)	Total colonization (%)
1.	*Lagerstroemia lanceolata* Wall.ex Wt.& Arn.	60.89 ± 10.55	3.45 + 1.22	15.81 + 3.17	80.15 ± 6.51
2.	*Ficus tomentosa* (Roxb. Ex Willd).	51.82 ± 8.13	3.80 ± 0.00	14.57 ± 3.13	70.19 ± 6.23
3.	*Leea indica* (Burm.f.) Merr.	40.77 ± 8.10	6.76± 1.53	18.84 ± 4.12	66.37± 5. 82
4.	*Hoppea wightiana* Wall. ex Wt.& Arn	40.27 ± 3.12	2.53 ± 0.00	22.16 ± 3.81	64.96 ± 7.00
5.	*Careya arborea* Roxb.	39.43 ± 5.18	4.12 ± 0.00	13.60 ± 2.23	57.15 ± 4.64
6.	*Holarrhena pubescens (Buch.-Ham.) Wallich ex Don*	31.48 ± 6.11	5.68 ± 0.00	16.13 ± 3.86	53.29 ± 4.23
7.	*Phyllanthus emblica* L.	29.13 ± 2.87	2.24 ± 0.81	20.86 ± 5.39	52.23 ± 5.00
8.	*Mallotus stenathus* Muell. Arg.	45.53 ± 7.55	0.00 ± 0.00	5.92 ± 1.22	51.45 ± 3.93
9.	*Xylia xylocarpa* Taub.	30.12 ± 8.91	3.11 ± 0.81	17.40 ± 3.87	50.63 ± 4.04
10.	*Bombax ceiba* L.	39.36 ± 8.91	2.14 ± 0.00	8.05 ± 2.23	49.55 ± 3.20
11.	*Strychnos nux-vomica* L.	30.19 ± 5.53	1.55 ± 0.02	15.76 ± 3.97	47.50 ± 3.60
12.	*Microcos paniculata* L.	25.16 ± 6.11	3.65 ± 0.00	16.8 ± 4.12	45.61 ± 5.02
13.	*Helicteres isora* L.	32.98 ± 8.33	5.67 ± 0.00	6.97 ± 2.84	45.52 ± 5.20
14.	*Glochidion ellipticum* Wight.	34.52 ± 6.45	4.19 ± 0.00	5.45 ± 1.18	44.16 ± 3.53
15.	*Maesa indica* (Roxb.) A. DC.	35.75 ± 5.83	2.18 ± 0.00	6.57 ± 1.32	44.50 ± 3.24
16.	*Terminalia crenulata* Roth	34.43 ± 9.33	5.43 ± 0.00	3.84 ± 2.57	43.70 ± 3.80
17.	*Ixora notoniana* Wallich ex Don	23.55 ± 3.19	0.00 ± 0.00	17.83 ± 5.12	41.38 ± 3.54
18.	*Ervatamia heyneana* (Wall) Cooke.	26.16 ± 4.91	7.15 ± 2.73	6.73 ± 2.21	40.04 ± 3.13
19.	*Alstonia scholaris* (L.) R.Br.	33.28 ± 4.67	2.91 ± 0.00	3.62 ± 1.55	39.81 ± 4.52
20.	*Dillenia pentagyna* Roxb.	33.67 ± 7.32	1.85 ± 0.00	4.45 ± 2.84	39.97 ± 2.33
21.	*Dalbergia paniculata* Roxb.	24.38 ± 4.58	3.44 ± 0.00	11.57 ± 3.82	39.59 ± 4.34
22.	*Cassia fistula* L.	18.92 ± 3.87	4.16 ± 1.33	15.34 ± 5.63	38.42 ± 4.43
23.	*Buchanania cochinchinensis* (Lour.) Aimeida.	21.22 ± 3.91	2.25 ± 0.00	12.46 ± 4.19	36.43 ± 4.02
24.	*Feronia elephantum* Corr. Serr.	27.81 ± 4.12	0.00 ± 0.00	9.17 ± 3.18	36.98 ± 4.53
25.	*Syzygium cumini* (L.) Skeels	30.38 ± 6.55	4.53 ± 0.00	2.36 ± 1.46	36.55 ± 2.84
26.	*Terminalia bellirica* Roxb.	33.67 ± 7.12	0.00 ± 0.00	1.80 ± 0.81	35.47 ± 2.54
27.	*Acacia nilotica* (L.) Del.	27.73 ± 5.84	1.63 ± 0.00	4.48 ± 2.27	33.84 ± 2.93
28.	*Terminalia paniculata* Roth.	14.51 ± 4.18	2.31 ± 0.00	4.61± 2.91	21.43 ± 1.81
	Average	**29.17 ± 6.11**	**3.61 ± 1.25**	**10.82 ± 3.07**	**46.67± 4.14**

Legend:

± Indicates Standard deviation.

Table 4: Colonization patterns in some tree species from Dharbandoda forest.

Sr. No.	Name of Species	Hyphal colonization (%)	Arbuscular colonization (%)	Vesicular colonization (%)	Total colonization (%)
1.	*Lagerstroemia lanceolata* Wall.ex Wt.& Arn.	38.71 ± 7.22	3.41 ± 0.00	5.25 ± 2.31	47.37 ± 8.77
2.	*Ficus tomentosa* (Roxb. Ex Willd).	33.97 ± 8.28	0.00 ± 0.00	14.51± 3.48	48.48 ± 9.44
3.	*Leea indica* (Burm.f.) Merr.	32.80 ± 7.88	2.81 ± 0.00	14.35± 3.13	49.96 ± 6.80
4.	*Hoppea wightiana* Wall. ex Wt.& Arn	45.81 ± 12.71	4.27 ± 2.11	7.07 ± 2.89	57.15 ±12.77
5.	*Careya arborea* Roxb.	50.72 ± 12.82	3.12 ± 0.00	8.79 ± 2.63	62.63 ± 7.16
6.	*Holarrhena pubescens (Buch.-Ham.) Wallich ex Don*	37.53 ± 7.21	4.18 ± 0.00	10.84 ± 3.62	52.55 ± 4.77
7.	*Phyllanthus emblica* L.	38.25 ± 6.68	0.00 ± 0.00	21.25 ± 10.59	59.50 ± 9.52
8.	*Mallotus stenathus* Muell. Arg.	47.61 ± 6.17	3.43 ± 1.43	4.57 ± 2.17	55.61 ± 9.09
9.	*Xylia xylocarpa* Taub.	44.49 ± 6.73	2.72 ± 0.00	5.31 ± 1.32	52.52 ± 6.61
10.	*Bombax ceiba* L.	38.01 ± 5.20	1.83 ± 0.00	7.32 ± 2.11	47.16 ± 9.07
11.	*Strychnos nux-vomica* L.	33.66 ± 5.80	2.11 ± 1.88	15.28 ± 4.88	51.05 ± 7.70
12.	*Microcos paniculata* L.	48.52 ± 752	1.79 ± 0.83	7.39 ± 2.57	57.70 ± 2.50
13.	*Helicteres isora* L.	49.73 ± 6.80	0.00 ± 0.00	1.65 ± 0.51	51.38 ± 5.93
14.	*Glochidion ellipticum* Wight.	44.93 ± 5.24	3.18 ± 0.00	9.93 ± 3.63	58.04 ± 6.22
15.	*Maesa indica* (Roxb.) A. DC.	31.73 ± 4.26	1.89 ± 0.00	16.64 ± 4.36	50.08 ± 6.44
16.	*Terminalia crenulata* Roth	51.45 ± 13.11	3.89 ± 1.79	7.63 ± 2.19	62.97 ± 9.88
17.	*Ixora notoniana* Wallich ex Don	41.24 ± 6.52	0.00 ± 0.00	13.35 ± 3.66	54.59 ± 3.96
18.	*Ervatamia heyneana* (Wall) Cooke.	41.07 ± 12.88	1.82 ± 0.87	16.71 ± 4.43	61.42 ± 3.11
19.	*Alstonia scholaris* (L.) R.Br.	45.42 ± 6.18	4.35 ± 0.00	9.91 ± 2.84	55.43 ± 5.16
20.	*Dillenia pentagyna* Roxb.	31.33 ± 14.19	2.62 ± 1.55	21.03 ± 3.71	64.98 ± 1.92
21.	*Dalbergia paniculata* Roxb.	38.41 ± 7.14	0.00 ± 0.00	9.14 ± 2.76	47.55 ± 7.79
22.	*Cassia fistula* L.	37.16 ± 6.16	1.23 ± 0.00	3.08 ± 1.05	41.47 ± 6.37
23.	*Buchanania cochinchinensis* (Lour.) Aimeida.	25.89 ± 5.12	3.73 ± 0.00	2.22 ± 1.11	31.84 ± 5.22
24.	*Feronia elephantum* Corr. Serr.	27.87 ± 5.61	4.59 ± 0.00	8.97 ± 2.83	41.43 ± 7.19
25.	*Syzygium cumini* (L.) Skeels	31.10 ± 6.02	2.79 ± 0.00	8.62 ± 2.21	42.51 ± 7.56
26.	*Terminalia bellirica* Roxb.	38.12 ± 6.33	5.88 ± 2.20	3.14 ± 1.07	47.14 ± 9.71
27.	*Acacia nilotica* (L.) Del.	40.69 ± 12.53	0.00 ± 0.00	13.85 ± 3.53	54.54 ± 6.73
28.	*Terminalia paniculata* Roth.	73.74 ± 14.81	2.18 ± 1.86	6.27 ± 2.33	82.19 ± 6.21
	Average	**40.62 ± 8.11**	**3.11 ± 1.61**	**9.78 ± 2.99**	**53.20± 6.88**

Legend:

± Indicates Standard deviation.

seventy nine percent (22 out of 28) tree species from Dharbandoda had functional mycorrhizas. The incidence of non-functional mycorrhizas was recorded in *Ficus tomentosa, Phyllanthus emblica, Helicteres isora, Ixora notoniana, Dalbergia paniculata, Dalbergia paniculata* and *Acacia niotica*. **(Table 5 & 6)**.

The spore density in rhizosphere soils at Mollem varied from 48.84 spores $100g^{-1}$ of soil to 1235.08 spores $100g^{-1}$ of soil. Average spore density was found to be 261.74 spores $100g^{-1}$ of soil. The highest spore density was recorded in *Glochidion ellipticum* (1235.08 spores $100g^{-1}$ of soil) belonging to family Euphorbiaceae, while lowest spore density was recorded in *Careya arborea* (48.84 spores $100g^{-1}$ of soil) belonging to family Lecythidaceae. **(Table 6).**

The spore density in rhizosphere soils at Dharbandoda varied from 732.73 spores $100g^{-1}$ of soil to 2450.128 spores $100g^{-1}$ of soil. The average spore density was founded to be 1417.49 spores $100g^{-1}$ of soil. The highest spore density was recorded in *Ervatamia heyneana* (2450.12 spores $100g^{-1}$ of soil) belonging to family Apocynaceae, while lowest spore density was recorded in *Ficus tomentosa* (732.73 spores $100g^{-1}$ of soil) belonging to family Moraceae **(Table 6).**

At Mollem, the maximum frequency of occurrence was recorded in *Acaulospora scrobiculata* (53.57%) while, mimimum frequency of occurrence was recorded in *Acaulospora gerdmannii* (3.57%). At Dharbandoda, the maximum frequency of occurrence was recorded in *Acaulospora spinosa* (39.28%) while

Table 5: Spore density in some tree species from Mollem forest.

Sr. No.	Name of Species	Family	*Spore Density $100g^{-1}$ soil
1.	*Lagerstroemia lanceolata* Wall.ex Wt. & Arn.	Lythraceae	428.54 ± 40.00
2.	*Ficus tomentosa* (Roxb. Ex Willd).	Moraceae	188.14 ± 17.50
3.	*Leea indica* (Burm.f.) Merr.	Leaceae	1008.51 ± 99.50
4.	*Hoppea wightiana* Wall. ex Wt.& Arn	Dipterocarpaceae	136.43 ± 11.90
5.	*Careya arborea* Roxb.	Lecythidaceae	48.84 ± 5.20
6.	*Holarrhena pubescens (Buch.-Ham.) Wallich ex Don*	Apocynaceae	92.47 ± 8.00
7.	*Phyllanthus emblica* L.	Euphorbiaceae	144.55 ± 14.20
8.	*Mallotus stenathus* Muell. Arg.	Euphorbiaceae	260.98 ± 24.60
9.	*Xylia xylocarpa* Taub.	Mimosaceae	120.43 ± 11.50
10.	*Bombax ceiba* L.	Bombacaceae	228.42 ± 20.80
11.	*Strychnos nux-vomica* L.	Loganiaceae	52.59 ± 4.80
12.	*Microcos paniculata* L.	Tiliaceae	52.59 ± 5.60
13.	*Helicteres isora* L.	Sterculiaceae	592.97 ± 49.00
14.	*Glochidion ellipticum* Wight.	Euphorbiaceae	1235.08 ± 129.00
15.	*Maesa indica* (Roxb.) A. DC.	Myricinaceae	112.46 ± 10.88
16.	*Terminalia crenulata* Roth	Combretaceae	176.38 ± 16.90
17.	*Ixora notoniana* Wallich ex Don	Rubiaceae	88.70 ± 7.80
18.	*Ervatamia heyneana* (Wall) Cooke.	Apocynaceae	548.51 ± 6.10
19.	*Alstonia scholaris* (L.) R.Br.	Apocynaceae	448.16 ± 4.00
20.	*Dillenia pentagyna* Roxb.	Dilleniaceae	64.52 ± 5.70
21.	*Dalbergia paniculata* Roxb.	Fagaceae	376.61 ± 36.70
22.	*Cassia fistula* L.	Ceasalpinoideae	84.50 ± 7.70
23.	*Buchanania cochinchinensis* (Lour.) Aimeida.	Anacardiaceae	64.50 ± 2.60
24.	*Feronia elephantum* Corr. Serr.	Rutaceae	196.63 ± 18.70
25.	*Syzygium cumini* (L.) Skeels	Myrtaceae	58.15 ± 4.60
26.	*Terminalia bellirica* Roxb.	Combretaceae	104.96 ± 9.50
27.	*Acacia nilotica* (L.) Del.	Leguminosae	156.48 ± 13.60
28.	*Terminalia paniculata* Roth.	Combretaceae	248.00 ± 22.80
Average			**261.74 ± 7.47**

Legend:

- Mean of three readings.

± Indicates Standard deviation.

Table 6: Spore density in some tree species from Dharbandoda forest.

Sr. No.	Name of Species	Family	*Spore Density $100g^{-1}$ soil
1.	*Lagerstroemia lanceolata* Wall.ex Wt.& Arn.	Lythraceae	738.26 ± 45.30
2.	*Ficus tomentosa* (Roxb. Ex Willd).	Moraceae	732.73 ± 43.65
3.	*Leea indica* (Burm.f.) Merr.	Leaceae	815.67 ± 30.39
4.	*Hoppea wightiana* Wall. ex Wt.& Arn	Dipterocarpaceae	979.32 ± 32.62
5.	*Careya arborea* Roxb.	Lecythidaceae	991.64 ± 21.24
6.	*Holarrhena pubescens (Buch.-Ham.) Wallich ex Don*	Apocynaceae	1055.67 ± 62.37
7.	*Phyllanthus emblica* L.	Euphorbiaceae	1178.78 ± 66.90
8.	*Mallotus stenathus* Muell. Arg.	Euphorbiaceae	1077.98 ± 62.94
9.	*Xylia xylocarpa* Taub.	Mimosaceae	1207.73 ± 35.69
10.	*Bombax ceiba* L.	Bombacaceae	1209.59 ± 99.95
11.	*Strychnos nux-vomica* L.	Loganiaceae	1256.67 ± 47.39
12.	*Microcos paniculata* L.	Tiliaceae	1314.04 ± 77.23
13.	*Helicteres isora* L.	Sterculiaceae	1317.05 ± 62.25
14.	*Glochidion ellipticum* Wight.	Euphorbiaceae	1860.71 ± 48.55
15.	*Maesa indica* (Roxb.) A. DC.	Myricinaceae	1884.08 ± 51.39
16.	*Terminalia crenulata* Roth	Combretaceae	1996.50 ± 96.53
17.	*Ixora notoniana* Wallich ex Don	Rubiaceae	749.07 ± 31.39
18.	*Ervatamia heyneana* (Wall) Cooke.	Apocynaceae	2450.12 ± 90.35
19.	*Alstonia scholaris* (L.) R.Br.	Apocynaceae	2043.85 ± 35.29
20.	*Dillenia pentagyna* Roxb.	Dilleniaceae	1253.18 ± 52.91
21.	*Dalbergia paniculata* Roxb.	Fagaceae	1763.04 ± 53.23
22.	*Cassia fistula* L.	Ceasalpinoideae	1345.42 ± 77.13
23.	*Buchanania cochinchinensis* (Lour.) Aimeida.	Anacardiaceae	2122.47 ± 61.34
24.	*Feronia elephantum* Corr. Serr.	Rutaceae	1009.65 ± 23.25
25.	*Syzygium cumini* (L.) Skeels	Myrtaceae	893.13 ± 69.67
26.	*Terminalia bellirica* Roxb.	Combretaceae	1048.81 ± 79.07
27.	*Acacia nilotica* (L.) Del.	Leguminosae	1537.42 ± 62.14
28.	*Terminalia paniculata* Roth.	Combretaceae	1351. 03 ± 82.16
Average			**1417.49 ± 57.23**

Legend:

- Mean of three readings.

± Indicates Standard deviation.

mimimum frequency of occurrence was recorded in 2 Arbuscular mycorrhizal fungal species *viz., Glomus hoi and G. vessiforme* (7.14%) **(Table 7)**.

Except for the genera *Entrophospora*, rest of the five genera *viz., Scutellospora, Sclerocystis, Glomus, Gigaspora* and *Acaulospora* were recovered which were common to both Mollem and Dharbandoda forest areas. At Mollem, a total of 30 Arbuscular mycorrhizal species were identified. The identified species includes *Acaulospora* (5), *Gigaspora* (2), *Glomus* (19) *Sclerocystis* (3) and *Scutellospora* (1) with the number of species given in parenthesis. While at Dharbandoda, a total of 25 arbuscular mycorrhizal species were identified. The identified species includes *Acaulospora* (5), *Gigaspora* (2), *Glomus* (14) *Sclerocystis* (3) and *Scutellospora* (1) with the number of species given in parenthesis **(Fig. 2 & 3)**.

In all, a total of 31 arbuscular mycorrhizal fungal species were identified from both Mollem and Dharbandoda forest. A total of 6 arbuscular mycorrhizal species *viz., Acaulospora gerdmannii, Glomus ambisporum, G. clarum, G. dimorphicum, G. etunicatum* and *G. intrardix* were found restricted to the rhizosphere soils of Mollem forest, whereas *Acaulospora rugosa* was restricted to the rhizosphere soils of Dharbandoda forest while. A total of 24 arbuscular mycorrhizal species *viz., Acaulospora foveata, A. laevis, A. scrobiculata, A. spinosa, Gigaspora decipiens, G. margarita, Sclerocystis rubiformis, S. sinuosa, S. taiwanensis, Scutellopsora corolloidea. Glomus australe, G. caledonium, G. claroideum, G. fasciculatum, G. geosporum, G. globiferum, G. heterosporum, G. hoi, G. monosporum, G. macrocarpum, G. mosseae, G. pustulatum G. pansihalos* and *G. versiforme* were found

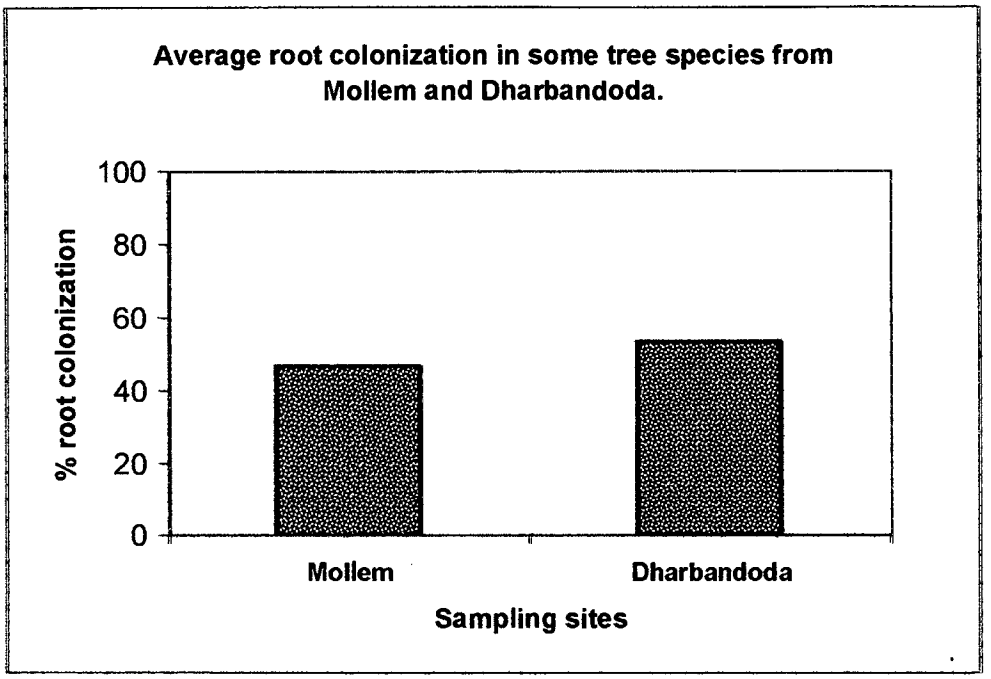

Average root colonization in some tree species from Mollem and Dharbandoda.
% root colonization
100
80
60
40
20
0
Mollem
Dharbandoda
Sampling sites

Fig. 2

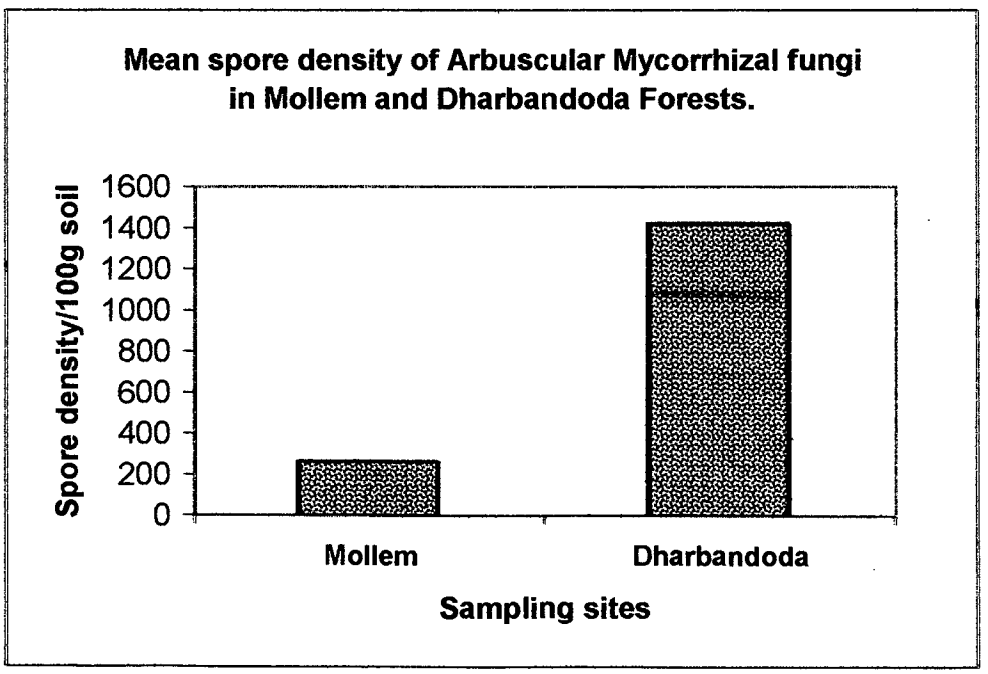

Mean spore density of Arbuscular Mycorrhizal fungi in Mollem and Dharbandoda Forests.
Spore density/100g soil
1600
1400
1200
1000
800
600
400
200
0
Mollem
Dharbandoda
Sampling sites

Fig. 3

Table: 7 Frequency of occurrence of AM species in rhizosphere soils of various tree species at Mollem and Dharbandoda forest.

Sr.No	Name of AM species	Frequency of occurrence of AM fungi (%)	
		Mollem	Dharbandoda
1.	*Acaulospora foveata*	32.14	17.85
2.	*A. gerdmannii*	3.57	--
3.	*A. laevis*	10.71	21.42
4.	*A. rugosa*	-	14.28
5.	*A. scrobiculata*	53.57	32.14
6.	*A. spinosa*	25.00	39.28
7.	*Gigaspora decipiens*	10.71	14.28
8.	*G. margarita*	10.71	10.71
9.	*Glomus ambisporum*	32.14	--
10.	*G. australe*	17.85	14.28
11.	*G. caledonim*	17.85	17.85
12.	*G. claroideum*	25.00	25.00
13.	*G. clarum*	17.85	--
14.	*G. dimorphicum*	10.71	--
15.	*G. etunicatum*	25.00	--
16.	*G. fasciculatum*	39.28	28.57
17.	*G. geosporum*	21.42	21.42
18.	*G. globiferum*	25.00	21.42
19.	*G. heterosporum*	25.00	21.42
20.	*G. hoi*	14.28	7.14
21.	*G. intraradix*	25.00	--
22.	*G. macrocarpum*	17.85	10.71
23.	*G. monosporum*	32.14	17.85
24.	*G. mosseae*	42.85	28.57
25.	*G. pansihalos*	10.71	10.71
26.	*G. pustulatum*	14.28	10.71
27.	*G. versiforme*	7.14	7.14
28.	*Sclerocystis rubiformis*	10.71	10.71
29.	*S. sinuosa*	35.71	21.42
30.	*S. taiwanensis*	7.14	17.85
31.	*Scutellospora corolloidea*	14.28	10.71
Average		**21.18**	**18.13**

to be common in the rhizosphere soils of both Mollem and Dharbandoda forests **(Table 7)**.

At Mollem, maximum arbuscular mycorrhizal species were recorded in *Ervatamia heyneana* (19) while minimum arbuscular mycorrhizal species (1) were recorded in *Maesa indica* with the number of species given in parenthesis **(Table 8)**. At Dharbandoda, maximum arbuscular mycorrhizal fungal species were recorded in *Terminalis crenulata* (16), while minimum arbuscular mycorrhizal fungal species (1) were recorded in *Feronia elephantum* with the number of species given in parenthesis **(Table 9)**.

Electrical Conductivity (EC) at both the sites was low thereby indicating that there was no likelihood of salinity problems. High amount of organic carbon was recorded at both the sites. Available phosphorus was found to be low at both the sites, which justifies the importance and role of arbuscular mycorrhizal fungi at such sites.

The incidence of mycorrhizae in trees in the present study (100%) is higher than that reported by Raman *et al.*, (1992) who have reported 19 out of a total of 25 tree species to be mycorrhizal from the Manandur forest of Tamil Nadu. The Genus *Glomus* was found to be dominant in both Mollem and Dharbandoda forest areas. Thaper *et al.*, (1985) reported six arbuscular mycorrhizal fungal species from the soils of Dehra Dun, of which *Glomus* was dominant.

Table: 8 Arbuscular mycorrhizal fungal species diversity in some tree species from Mollem forest.

Sr. No.	Name of Species	AM species
1.	*Lagerstroemia lanceolata* Wall.ex Wt.& Arn.	*Glomus australe, G. ambisporum, G. caledonium, G. fasciculatum, Acaulospora foveata, A. scrobiculata Sclerocystis sinuosa.*
2.	*Ficus tomentosa* (Roxb. Ex Willd).	*Glomus clarum, G. etunicatum, Acaulospora spinosa.*
3.	*Leea indica* (Burm.f.) Merr.	*Glomus claroideum, Sclerocystis sinuosa.*
4.	*Hoppea wightiana* Wall. ex Wt.& Arn	*Glomus fasciculatum, G. geosporum, Acaulospora scrobiculata.*
5.	*Careya arborea* Roxb.	*Acaulospora foveata, A. spinosa, Gigaspora decipiens, Glomus ambisporum, G. intrardix, G. fasciculatum, G. geosporum, G. pustulatum G. globiferum, G. monosporum, G. mosseae, G. pansihalos, Sclerocystis sinuosa.*
6.	*Holarrhena pubescens (Buch.-Ham.) Wallich ex Don*	*Acaulospora scrobiculata, A. spinosa, Glomus etunicatum, G. hoi, G. monosporum, G. macrocarpum, G. mosseae, G. versiforme, G. fasciculatum.*
7.	*Phyllanthus emblica* L.	*Glomus ambisporum, G. hoi.*
8.	*Mallotus stenathus* Muell. Arg.	*Glomus ambisporum, Scutellospora corolloidea.*

Contd.

9.	*Xylia xylocarpa* Taub.	*Acaulospora foveata, A. spinosa, A. scrobiculata, Gigaspora margarita, Glomus etunicatum, Glomus australe, G. fasciculatum, G. geosporum, G. globiferum, G. heterosporum, G. monosporum, G. mosseae, G. pustulatum, G. intrardix.*
10.	*Bombax ceiba* L.	*Glomus dimorphicum, G. intraradices, Acaulospora foveata, A. scrobiculata.*
11.	*Strychnos nux-vomica* L.	*Acaulospora foveata, A. laevis, A. scrobiculata, A. spinosa, Gigaspora decipiens, Glomus ambisporum, G. etunicatum, G. claroideum, G. globiferum, G. heterosporum, G. monosporum, G. pansihalos, G. geosporum, Sclerocystis sinuosa, S. taiwanensis, Scutellopsora corolloidea.*
12.	*Microcos paniculata* L.	*Glomus claroideum, G. macrocarpum.*
13.	*Helicteres isora* L.	*Glomus monosporum, G. heterosporum.*
14.	*Glochidion ellipticum* Wight.	*Glomus microcarpum, G. monosporum, G. mosseae, G. pustulatum, Acaulospora scrobiculata, Sclerocystis sinuosa.*
15.	*Maesa indica* (Roxb.) A. DC.	*Glomus pustulatum.*
16.	*Terminalia crenulata* Roth	*Acaulospora scrobiculata, Glomus ambisporum, G. intradix, G. caledonium, G. fasciculatum, G. geosporum, G. monosporum, G. mosseae, Sclerocystis rubiformis, S. sinuosa.*
17.	*Ixora notoniana* Wallich ex Don	*Glomus pansihalos, G. hoi, Acaulospora laevis.*

Contd.

18.	*Ervatamia heyneana* (Wall) Cooke.	*Acaulospora gerdmannii, A. foveata, A. laevis, A. scrobiculata, A. spinosa, Gigaspora margarita, Glomus ambisporum, G. clarum, G. dimorphicum, G. etunicatum, G. intrardix, G. Australe, G. caledonium, G. fasciculatum, G. globiferum, G. mosseae, Sclerocystis rubiformis, S. sinuosa* and *Scutellopsora corolloidea.*
19.	*Alstonia scholaris* (L.) R.Br.	*Glomus australe, G. claroideum.*
20.	*Dillenia pentagyna* Roxb.	*Glomus fasciculatum. G. globiferum, Sclerocystis taiwanensis.*
21.	*Dalbergia paniculata* Roxb.	*Glomus heterosporum, Acaulospora scrobiculata.*
22.	*Cassia fistula* L.	*Glomus claroideum, G. clarum, G. mosseae, Acaulospora scrobiculata.*
23.	*Buchanania cochinchinensis* (Lour.) Aimeida.	*Acaulospora foveata, A scrobiculata, Gigaspora decipiens, Glomus ambisporum, G. clarum, G. etunicatum, G. intradix, G. caledonium, G. fasciculatum, G. globiferum, G. heterosporum, G. monosporum, G. macrocarpum, G. mosseae, Sclerocystis rubiformis, S. sinuosa.*
24.	*Feronia elephantum* Corr. Serr.	*Glomus mosseae, Acaulospora foveata.*
25.	*Syzygium cumini* (L.) Skeels	*Glomus heterosporum, Acaulospora scrobiculata.*
26.	*Terminalia bellirica* Roxb.	*Glomus etunicatum, G. clarum.*
27.	*Acacia nilotica* (L.) Del.	*Glomus mosseae, G. claroideum.*
28.	*Terminalia paniculata* Roth.	*Acaulospora foveata, A. scrobiculata, A. spinosa, Gigaspora margarita, Glomus dimorphicum, Glomus australe, G. claroideum, G. fasciculatum, G. globiferum, G. heterosporum, G. hoi, G. macrocarpum, G. mosseae, G. versiforme Sclerocystis sinuosa, Scutellopsora corolloidea.*

Table 9: Arbuscular mycorrhizal fungal species diversity in some tree species from Dharbandoda forest.

Sr. No.	Name of Species	AM species
1.	*Lagerstroemia lanceolata* Wall.ex Wt.& Arn.	*Glomus caledonius, G. fasciculatum, Acaulospora foveata.*
2.	*Ficus tomentosa* (Roxb. Ex Willd).	*Glomus globiferum, G. geosporum, Acaulospora spinosa.*
3.	*Leea indica* (Burm.f.) Merr.	*Glomus heterosporum, G. hoi, Acaulospora scrobiculata, A. spinosa.*
4.	*Hoppea wightiana* Wall. ex Wt.& Arn	*Glomus claroideum, Acaulospora spinosa.*
5.	*Careya arborea* Roxb.	*Acaulospora foveata, A. scrobiculata, A. spinosa, Gigaspora decipiens, Glomus australe, G. fasciculatum, G. globiferum, G. mosseae, G. pustulatum, Sclerocystis rubiformis, S. sinuosa.*
6.	*Holarrhena pubescens (Buch.-Ham.) Wallich ex Don*	*Acaulospora rugosa, A. laevis, A. scrobiculata, Glomus australe, G. fasciculatum, G. geosporum, G. heterosporum, G. mosseae, Sclerocystis sinuosa, Scutellopsora corolloidea.*
7.	*Phyllanthus emblica* L.	*Glomus heterosporum, G. claroideum.*
8.	*Mallotus stenathus* Muell. Arg.	*Glomus claroideum, G. monosporum, Acaulospora spinosa, Gigaspora decipiens.*

Contd.

19.	*Alstonia scholaris* (L.) R.Br.	*Glomus macrocarpum, G. pustulatum, Acaulospora scrobiculata.*
20.	*Dillenia pentagyna* Roxb.	*Glomus versiforme, Acaulospora laevis.*
21.	*Dalbergia paniculata* Roxb.	*Glomus mosseae, Scutellospora coralloidea.*
22.	*Cassia fistula* L.	*Glomus monosporum, Gigaspora margarita, Sclerocystis sinuosa.*
23.	*Buchanania cochinchinensis* (Lour.) Aimeida.	*Acaulospora scrobiculata, A. spinosa, A. laevis, Glomus caledonium, G. claroideum, G. fasciculatum, G. globiferum, G. hoi, G. macrocarpum, G. mosseae, G. pansihalos, Sclerocystis rubiformis, S. taiwanensis, Scutellopsora corolloidea.*
24.	*Feronia elephantum* Corr. Serr.	*Glomus hoi.*
25.	*Syzygium cumini* (L.) Skeels	*Glomus geosporum, G.globiferum.*
26.	*Terminalia bellirica* Roxb.	*Glomus fasciculatum, G. claroideum, Acaulospora scrobiculata*
27.	*Acacia nilotica* (L.) Del.	*Glomus caledonium, G. hoi, Gigaspora decipiens.*
28.	*Terminalia paniculata* Roth.	*Acaulospora rugosa, Acaulospora foveata, A. laevis, A. scrobiculata. A. spinosa, Gigaspora decipiens, Glomus geosporum, G. hoi, G. macrocarpum, G. mosseae, G. Caledonium, G. pustulatum Sclerocystis sinuosa, S. taiwanensis.*

Contd.

9.	*Xylia xylocarpa* Taub.	*Acaulospora scrobiculata, A. spinosa, Glomus claroideum, G. fasciculatum, G. geosporum, G. globiferum, G. macrocarpum, Sclerocystis taiwanensis.*
10.	*Bombax ceiba* L.	*Glomus claroideum. G .pustulatum, Acaulospora rugosa, Sclerocystis taiwanensis.*
11.	*Strychnos nux-vomica* L.	*Acaulospora rugosa, A. spinosa, Gigaspora margarita, Glomus caledonium,G. fasciculatum, G. geosporum, G. globiferum, G. mosseae.*
12.	*Microcos paniculata* L.	*Glomus australe, Gigaspora decipiens, Acaulospora scrobiculata.*
13.	*Helicteres isora* L.	*Glomus mosseae, Acaulospora foveata.*
14.	*Glochidion ellipticum* Wight.	*Glomus caledonium, G. globiferum, Sclerocystis taiwanensis.*
15.	*Maesa indica* (Roxb.) A. DC.	*Glomus geosporum, G.heterosporum, Acaulospora laevis*
16.	*Terminalia crenulata* Roth	*Acaulospora foveata, A. laevis, A. scrobiculata, A. spinosa, A. rugosa, Gigaspora decipiens, Glomus claroideum, G. fasciculatum, G. heterosporum, G. monosporum, G. mosseae, G. pansihalos, G. versiforme Sclerocystis sinuosa, S. taiwanensis, Scutellopsora corolloidea.*
17.	*Ixora notoniana* Wallich ex Don	*Glomus australe, G. monosporum, sclerocystis sinuosa, Acaulospora spinosa.*
18.	*Ervatamia heyneana* (Wall) Cooke.	*Acaulospora foveata, A. laevis, A. scrobiculata, A. spinosa, Gigaspora margarita, Glomus caledonium, G. fasciculatum, G. geosporum, G. heterosporum, G. monosporum, G. mosseae, G. pansihalos, Sclerocystis rubiformis, S. sinuosa.*

Certain arbuscular mycorrhizal fungal species may prefer certain habitats as previous studies have clearly demonstrated that arbuscular mycorrhizal fungal community composition is influenced by temperature (Koske, 1987), soil moisture (Anderson *et al.,* 1984), soil nutrients and pH (Porter *et al.,* 1987; Johnson *et al.,*1991) and, plant community composition (Mc Graw and Hendrix, 1984).

The present study differs from other investigations on arbuscular mycorrhizal fungi in the quantification of arbuscular mycorrhizal structures within roots. Most studies on mycorrhizae in tree species have not distinguished between active and inactive mycorrhizae or different internal arbuscular mycorrhizal structures. These studies have merely quantified total mycorrhizal colonization (Mukerji and Kapoor, 1986; Ragupathy *et al.,* 1990; Raman *et al.,* 1992; Ragupathy and Mahadevan, 1993; Gupta and Mukerji, 1996) eventhough the presence of arbuscules is an indication of mutualism between plants and arbuscular mycorrhizal fungi (Smith and Smith, 1989; Read, 1991; Koske *et al.*, 1992)

Although the present study good amount of spore density and percentage root colonization was recorded at the sites shows, higher spore density and higher percentage root colonization was recorded at Dharbandoda forest as compared to Mollem forest.

Great diversity exists within fungi responsible for arbuscular mycorrhizal formation in plants in majority of terrestial ecosystems. Conservation and efficient utilization of arbuscular mycorrhizal fungal biodiversity are of crucial importance for

sustainable plant production systems (Giovannetti and Gianinazi-Pearson, 1994). As Hendrix (1993) pointed out that failing to maintain plant and in turn the arbuscular mycorrhizal fungal diversity would change the nature of phyto- and myco-biont association where pathogenicity will replace mutualism. Studies on the distribution and mycorrhizal status of plants in nature will enable us to understand the influence of these mycobionts on plant species diversity and distribution.

CHAPTER – 2

TAXONOMY OF ARBUSCULAR MYCORRHIZAL FUNGI OCCURRING IN MOLLEM, COLLEM AND DHARBANDODA FOREST AREAS.

INTRODUCTION

Taxonomic groupings of Glomalean fungi are mainly based on stable and discrete morphological characters of fungal mycelium and spores (Morton and Benny, 1990).. Based on variations in spores, about 150 species have been recognized (Schenck and Perez, 1990). Various taxonomical parameters *viz.*, shape, colour, and size of spores and sporocarps, characteristics of peridium, spore walls number, colour, thickness and ornamentation, hyphal attachment, shape and type of occlusions, *etc.* are taken into consideration while identifying arbuscular mycorrhizal fungal species.

Until recently, the order Endogonales (Zygomycotina) has consisted of only one family, the Endogonaceae (Benjamin, 1979; Morton, 1988). Six genera (*Acaulospora* Gerdemann and Trappe emend. Berch, *Entrophospora* Ames and Schneider, *Gigaspora* Gerdemann and Trappe, Glomus Tulasne and Tulasne, *Sclerocystis* Berkeyley and Broome and *Scutellospora* Walker and Sanders were included whose members formed an arbuscular mutualistic symbiosis with many terrestrial plant families (Trappe, 1987) and one Genus (Endogone Link:Fr.) whose members were saprobic (Gerdemann and Trappe, 1974) or formed putative ectomycorrhizal associations (Chu-Chou and Grace, 1979; Fassi *et al.*, 1969). Pirozynski and Dalpe (1989) described a new family Glomaceae containing two Genera namely *Glomus* and *Sclerocystis*. Morton and Benny (1990) emended the family Glomaceae and erected a new order Glomales and two new families Acaulosporaceae and Gigasporaceae **(Table 10).**

Table 10: Classification of Arbuscular Mycorrhizal fungi.

Old Classification (Gerdemann and Trappe,1974) (Benjamin, 1979, Warcup,1990)	**Present Classification (Morton and Benny, 1990)**
Order: Endogonales	1. Order: Endogonales
Family: Endogonaceae	Family: Endogonaceae
Genera: ***Endogone***	Genera: ***Endogone***
Sclerogone	***Sclerogone***
Glomus	
Sclerocystis	2. Order: Glomales
Acaulospora	Suborder: Glomineae
Entrophospora	i)Family: Glomaceae
Gigaspora	Genera: ***Glomus***
Scutellospora	***Sclerocystis***
	ii)Family: Acaulosporaceae
	Genera: ***Acaulospora***
	Entrophospora
	Suborder: Gigasporineae
	Family: Gigasporaceae
	Genera: ***Gigaspora***
	Scutellospora

CLASSIFICATION OF ARBUSCULAR MYCORRHIZAL (AM) FUNGI (ZYGOMYCETES) (MORTON AND BENNY, 1990).

Order: Glomales

Fungi, forming arbuscular endomycorrhizae in mutualistic symbiosis with living plants. Sexual reproduction rare. Asexual reproduction by spores resembling chlamydospores and azygospores. Spores formed singly, but also in aggregates or in sporocarps.

I) Suborder: Gigasporineae

Only arbuscules formed in mycorrhizal roots; Azygospores produced on the apex of a sporogenous cell of a fertile hypha; auxiliary cells formed.

I A) Family: Gigasporaceae

Germ tubes produced directly through the wall or from a germination shield.

I Aa) Genus: ***Gigaspora***

Germ tubes produced directly through spore wall; inner flexible wall groups absent; auxiliary cells finely papillate or echinulate.

Ab) Genus: ***Scutellospora***

Germ tubes from germination shield; inner flexible wall groups always present; auxiliary cells knobby, broadly papillate or smooth.

II) Suborder: Glomineae

Arbuscules and vesicles formed in mycorrhizal roots. Spores produced terminally or laterally on or within fertile hyphae.

II A) Family: Glomaceae

Chlamydospores formed apically from fertile hyphae.

II Aa) Genus: ***Sclerocystis***

Fruiting body of a sporocarp composed of spores with lateral walls adherent to one another; connecting hyphae embedded in a central hyphal plexus; chlamydospores in a single layer except at the base; base composed of a sterile hyphae.

II Ab) Genus: ***Glomus***

Fruiting structure of a sporocarp not formed as mentioned above; spores also produced singly or in loose to tight aggregates in soil less commonly in roots.

II B) Family: Acaulosporaceae

Azygospores formed or within the neck of a sporiferous saccule.

II Ba) Genus: ***Acaulospora***

Spores arise laterally from the neck of a sporiferous saccule.

II Bb) Genus: ***Entrophospora***

Spores formed in the neck of the sporiferous saccule.

Increasing attention is given to taxonomic character of spore and sporocarp ontogeny in descriptions of new species or studies of the established ones (Morton and Benny, 1990; Walker, 1992). Ontogeny diversity has been considered diagnostic in the differentiation of two genera, *Acaulospora* and *Entrophospora (*Ames and Schneider, 1979).

The present chapter describes the morphological features of the various AM fungal spores recovered and identified from the 3 forest sites *viz.*, Mollem, Dharbandoda and Collem.

MATERIALS AND METHODS

Rhizosphere soil samples from the selected host plants were collected from all the three sites. Spores of arbuscular mycorrhizal fungi were extracted from 100g soil using wet sieving and decanting procedure (Gerdemann and Nicolson, 1963). Spores were separated using sieves having mesh sizes ranging from 38µm to 500µm. Intact spores were picked up using a wet-needle and observed under Leica Stereomicroscope. Only healthy spores were mounted in Polyvinyl Lacto Glycerol (PVGL), (Koske and Tessier, 1983) with or without Melzer's reagent and examined under a compound microscope (40x-1000x).

Spores of arbuscular mycorrhizal fungi were identified using the standard keys (Schenck and Perez, 1990; Wu, 1993). Spores in the rhizosphere soils were multiplied using *Eleusine coracana* (L.) Gaertn., *Allium cepa* L. and *Coleus sp.* as host plants. The spores isolated from the pot cultures were later used for confirming the earlier identification.

Voucher specimens are deposited in the Department of Botany, Goa University. Photographs of the identified spores were taken under Leica compound microscope WILD MP 32.

RESULTS AND DISCUSSION

A total of 33 arbuscular mycorrhizal fungal species belonging to 5 genera *viz.*, *Acaulospora* (6), *Gigaspora* (2), *Glomus* (19), *Sclerocystis* (3) and *Scutellopsora* (3) with the number of species given in parenthesis have been identified and described in detail in the present chapter.

Recently Walker and Vestberg, (1998) considered the spores of *Glomus maculosum, Glomus multisubtensum* and *Glomus fistulosum* to be the synonyms of *Glomus claroideum.* All the specimens that had been determined as *G. fistulosum, G. claroideum, G. maculosum* along with *G. multisubtensum* had similar morphological characteristics and they can be considered as conspecific. However, based on minor morphological differences, they cannot be treated as different species. As *Glomus claroideum* was the

earliest published, therefore takes precedence over all others that are considered synonymous.

Almeida and Schenck, (1990) transferred most of the species of *Sclerocystis* (Except *Sclerocystis coremioides*) to *Glomus* based on spore ontogeny. However, Wu (1993a) argued that distribution of shared characters is justified to group all species with highly organized sporocarp into *Sclerocystis.*

The spores and sporocarps of *Sclerocystis sinuosa* exhibited wide range of morphological variations. The heterogenous spores with uneven wall thickness and indistinct sinuous features have been illustrated by Muthukumar *et al.*, (2000) and Wu and Chen (1993). Almeida and Schenck (1990) noted a great variation in the sporocarp size and peridial nature in *Sclerocystis sinuosa.* They also considered *Sclerocystis pakistanica* synonymous to *Sclerocystis sinuosa* as the descriptions of the two species were almost identical except for the sporocarp size and nature. Observations made in the present study are in conformity with the Almeida and Schenck, (1990), Wu and Chen (1993) and Muthukumar *et al.,* (2000) with regards to the existence of variations in *Sclerocystis sinuosa.*

The spores and sporocarps of *Sclerocystis taiwanensis* in the present investigation fit well into description s of Wu and Chen (1987). Spores of *Sclerocystis taiwanensis* resemble *Sclerocystis clavispora* and *Sclerocystis microcarpus in lacking* a peridium and

the spore wall thickness at the apex. Almeida and Schenck (1990) considered *Sclerocystis microcapra* to be a synonym of *Sclerocystis clavispora.* While, Wu (1993b) reported the occurrence of smaller sporocarps of *Sclerocystis clavispora.* Various spore shapes in *Sclerocystis taiwanensis* has been reported from India by Muthukumar *et al.,* (1997).

DISTINCT MORPHOLOGICAL FEATURES OF THE IDENTIFIED AM FUNGAL SPECIES.

GENUS : *ACAULOSPORA*

Acaulospora foveata **Trappe & Janos,** ***Mycotaxon*** **15:** 515-522,1982 **(Plate III , Fig. a)**

Spores formed singly in soil, yellowish brown to reddish brown, globose to subglobose, 185-195 (-410) x 215-240 (-480) µm. Spore surface uniformly pitted with round to oblong or occasionally irregular depressions 4-8 (-12) x 4-16 µm, with rounded bottoms, separated by ridges 1-12 µm broad. Outer spore wall yellowish or reddish brown, 11-15 µm thick, with an adherent but separable, hyaline inner layer 3 µm thick. Spore contents of small hyaline guttules.

Acaulospora gerdemannii **Schenck & Nicolson,** ***Mycologia*** **71:**178-198. 1979.

Spores formed singly in the soil, sessile, spherical, 200-250 µm diam. with globular contents and double walls. Outer wall 1.0-1.5 µm thick, brown and with cerebriform folds up to 10-12 µm tall; outer wall becoming brittle with age; inner wall 1.0-1.5 µm thick,

pliable, hyaline with its surface ornamented with an alveolate reticulum; alveoli 7.5-10 µm in width, outer wall readily separating from the inner wall on older spores. Hyaline below the spore attachment 10-12 µm wide, frequently giving rise to numerous finely branched hyphae forming nonlobed vesicles in vesicular arbuscular mycorrhizae.

Acaulospora laevis **Gerdemann & Trappe,** ***Mycologia Memoir No.*** **5:** 76, 1974 **(Plate III, Fig. b)**

Spores formed singly in soil, sessile, dull yellow to yellow-brown to red-brown in colour, smooth, globose to subglobose, 119- 125 (-300) x 119-130 (-520) µm in diam. Spore wall consisting of 3 layers; A rigid, yellow-brown to red-brown outer wall 2-4 µm thick, and two hyaline inner membranes. Spore contents globose to somewhat polygonal.

Acaulospora rugosa **Morton,** ***Mycologia*** **78:** 641-648, 1986.

Spores formed singly in soil, sub-hyaline to straw-coloured, mostly globose to sub-globose (49-) 102-112 (-118) µm in diam. Spore wall structure consisting of 5 walls in 3 groups (A, B & C). Wall group A with two walls (1 & 2); hyaline outer wall 1-1.5 µm thick in lactophenol based mountants, often forming folds 2 (-10) µm deep surrounding intact spores, separating readily from wall 2 in crushed spores; in water, walls 1 & 2 adherent, appearing wrinkled or rugose; wall 2 pale yellow, laminated, 1.2-3 µm thick. Wall group B a semirigid hyaline wall (wall 3), 1-1.3µm thick. Wall group C two hyaline walls (4 & 5); wall 4 membranous; wall 5 in water rigid, 1-2.5 µm thick, in PVGL of indeterminate thickness, exhibiting some elasticity.

PLATE- III

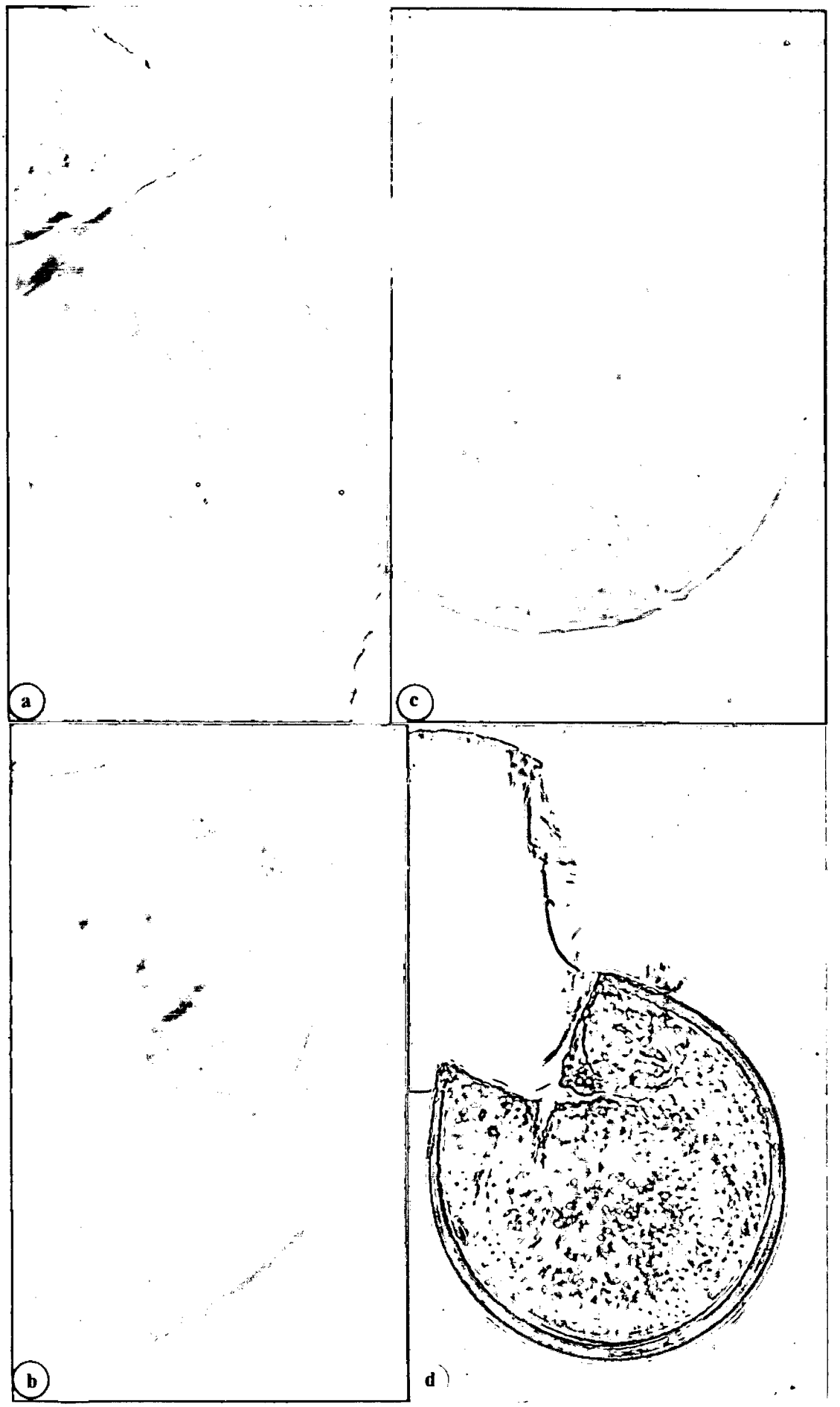
SPORES OF ARBUSCULAR MYCORRHIZAL FUNGI

a) *Acaulospora foveata* Trappe & Janos (400x).

b) *Acaulospora laevis* Gerdemann & Trappe (100x).

c) *Acaulospora scrobiculata* Trappe (100x).

d) *Acaulospora spinosa* Walker & Trappe (100x).


a
c
b
d

Acaulospora scrobiculata **Trappe,** ***Mycotaxon*** **6:** 359-366, 1977 **(Plate III, Fig. c)**

Spores formed singly in soil, hyaline to light brown, globose to broadly ellipsoid, 100-149 (-240) x 100-154 (-220) µm. Spore surface evenly pitted with depressions 1-1.5 x 1-3 µm, separated by ridges 2-4 µm thick, the mouths of the depressions circular to elliptical or occasionally linear to Y-shaped. Spore wall 4-8.5 µm thick, consisting of four layers; a rigid, pitted, outer layer 3-6 µm thick and 3 inner smooth layers (<1µm). Spore contents of small, relatively uniform guttules.

Acaulospora spinosa **Walker & Trappe,** ***Mycotaxon*** **12:** 515-521, 1981 **(Plate III, Fig. d)**

Spores formed singly in soil, dull yellowish brown to dark reddish brown, globose to subglobose, 100-240 (-298) x 100-225 (-335) µm. Spore surface ornamented with crowded blunt spines 1-4 µm high, 1 µm in diam. at the polygonal base, tapering to 0.5 µm at the tip, sometimes adhering in lines to form an irregular, partial reticulum on parts of the spore surface. Spore wall 3 layered; outer layer yellowish brown to reddish brown, 4-10 µm thick including spines, enclosing 2 membranous hyaline walls, each 0.2- 1 µm thick.

GENUS : ***GIGASPORA***

Gigaspora decipiens **Hall & Abbott,** ***Trans. Br. Mycol. Soc.*** **83:** 203-208, 1984.

Spores found singly in the soil, terminally on a sporogenous cell; white to golden yellow, globose to subglobose (350-) 399.7 (435) x (350) 402.9 (-430) µm. Spore wall structure of three walls (1-3) in one group. Wall-1 hyaline unit wall 2-6 µm thick. Wall-2 robust

laminated (20-) 36.2 (-45) µm thick, staining purple in Melzers reagent. Wall-3 a papillate wall formed during germination, 2-4 µm thick, extending 0.5-6 µm into the lumen of the spore. Sporogenous cell yellowish brown, subtending hyphae (10-) 14.1 (-20) µm in diam. and germination directly through the spore wall.

***Gigaspora margarita* Becker & Hall, *Mycotaxon* 4:** 155-160, 1976 **(Plate IV, Fig. a & b)**

Spores formed singly in soil, globose to subglobose, 260-384 (-480) x 270-396 (-480) µm. Spore wall smooth, composed of 4-8, rarely 10, fused laminations; spore wall 5-24 µm thick, each lamination 1.5-4 µm thick. Contents of the spores white, composed of many small oil droplets. Sporogenous cell 27-51 (-58) µm broad borne on a subtending hypha; subtending hypha generally septate below the suspensor like cell.

GENUS : *GLOMUS*

***Glomus ambisporum* Smith & Schenck, *Mycologia* 77:** 566-574, 1985.

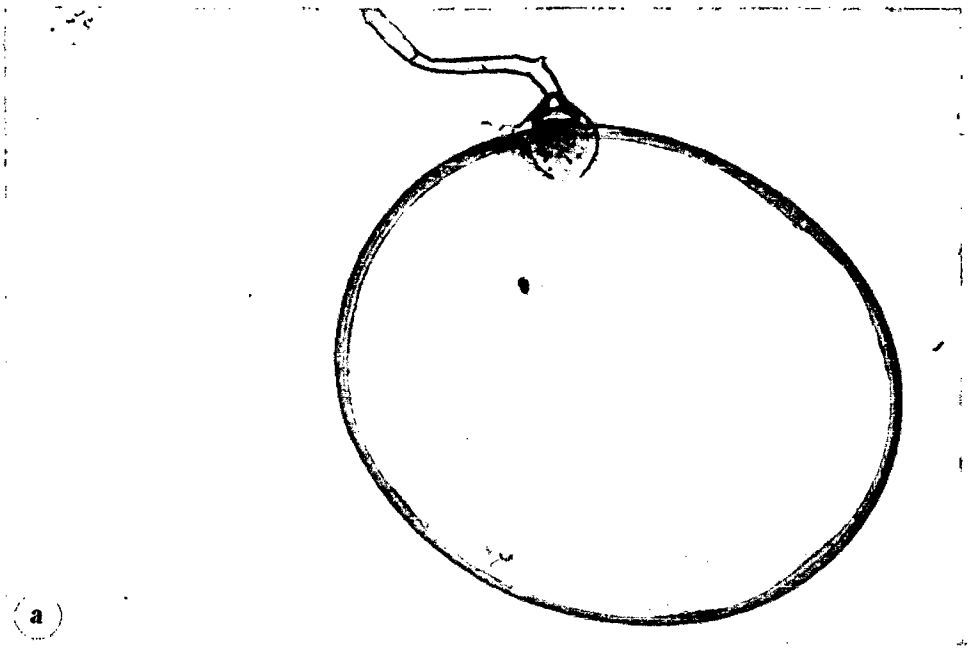
Spores produced solely in sporocarps in soil or aggregated around roots, dark brown to black, globose to subglobose (98-) 120 (-166) x (95-) 105 (-157) µm diam. Spore wall structure of three walls (1-3) in one group. Wall-1, reticulate, ephemeral, sub hyaline, (2-) 2.8 (-4) µm thick. Wall-2, dark brown to black, (3-) 5-(14) µm thick, laminated. Wall-3, membranous 1 µm thick. Subtending hyphae straight (10-) 15 (-20) µm wide and (10-) 21 (-24) wide at the point of attachment with the spore. Hyphal wall yellow –brown, 5 µm thick and pore 2 µm wide.

PLATE- IV

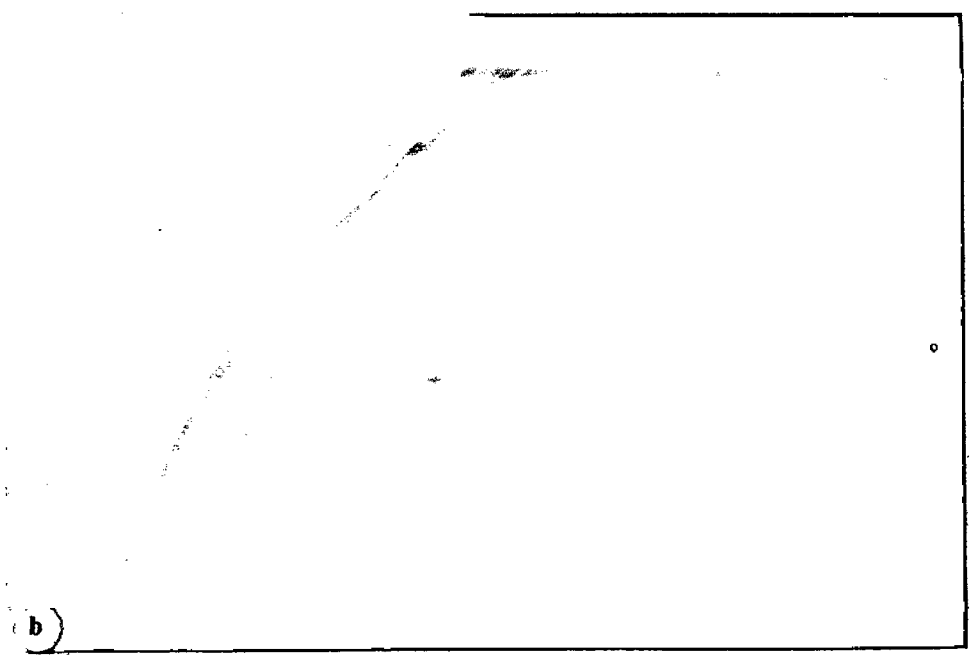
SPORES OF ARBUSCULAR MYCORRHIZAL FUNGI

a) ***Gigaspora margarita*** Becker & Hall **(100x)**

b) ***Gigaspora margarita*** Becker & Hall **spore showing wall layers (400x).**



(a)



(b)

***Glomus australe* (Berkeley) Berch, *Can. J. Bot.* 13:**2608-2617, 1983.

Sporocarp is reniform. Peridium is upto 0.5 mm thick and composed of interwoven hyphae 12-18 µm diam. Spores form in loose clusters (120-) 160 (-180) µm and have two wall layers; the outer is hyaline or pale yellow, approximately 4 µm thick; the inner is light or dark brown, 7 (-15) µm thick. The subtending hypha at the point of attachment to the spore is broad (20-25 µm) and thick walled. The inner wall continues into the subtending hypha for 2-40 µm and may extend into the central branched hypha of the cluster. The pore in the subtending hypha is open.

***Glomus caledonium* (Nicolson and Gerd.) Trappe and Gerdmann**, *Mycologia,* **60:** 318, 1968 **(Plate V, Fig. a & c)**

Spores found singly or in sporocarps, subglobose, with a pallid peridium of hyaline thin-walled hyphae 8-25 µm diam. and a light known gleba. Spores dull yellow to brown, generally globose to subglobose 130-279 x 120-27 µm. Spore wall 6-10(-16) µm thick, composed of hyaline outer layer 1-4 (-8) µm thick and yellow to brown inner layer 4-8 (-10) µm thick. Spore contents separated from attached hypha by a thin, yellow, curved wall formed at the hyphal attachment or occasionally as much as 15 µm down the attached hypha from the point of attachment.

Glomus claroideum **Schenck & Smith emend Walker & Vestberg** Annals of Botany 82: 601-624. (1998) **(Plate V, Fig. c, Plate VI, Fig. c)**

= ***Glomus claroideum*** **Schenck & Smith** *Mycologia* **74**: 84 (1982).

= ***Glomus maculosum*** **Miller & Walker**. *Mycotaxon* **25**: 218 (1986).

=***Glomus fistulosum*** **Skou & Jakobsen**, *Mycotaxon* **36**: 274 (1989).

= ***Glomus multisubtensum*** **Mukerji, Bhattarcharjee & Tewari** *Tr. Br. Myco. Soc.* **81**: 3 (1983).

Sporocarps unknown. Spores formed singly or in the loose clusters in the soil, hyaline to pale straw-colored, globose- subglobose, 95-220 μm in diam. Spore wall (4.6-) 6-15 (-19.6) μm thick, of three walls in two wall groups (A & B). Wall group A of an outer, thin, hyaline unit wall (wall 1), 0.3-1.0 μm thick, tightly adherent to wall 2, a brittle pale straw-coloured laminated wall, 4-13 μm thick, with 4-16 laminae. Inner wall group (group B, Wall 3), membranous (<1μm). Wall 3 may bear domed, scalloped ingrowths, 6-15 μm diameter and up to 12 μm deep. Spore contents of crowded oil droplets. Spores formed on one to three subtending hypha; subtending hypha concolorous with spore wall 2, straight to sharply recurved, parallel sided, sometimes constricted at the spore base, 5-13 (-25) μm long and 5-13 μm wide proximally, and 5-7 μm wide at the point of connection to a thin walled hyaline parent hypha. Walls of the subtending hypha 1.5-3 μm thick proximally, tapering distally to 1μm.

PLATE- V

SPORES OF ARBUSCULAR MYCORRHIZAL FUNGI

a) & C) *Glomus caledonium* (Nicolson and Gerd.) Trappe and Gerdmann (100x).

b) *Glomus claroideum* Schenck & Smith emend Walker & Vestberg (100x).

d) *Glomus clarum* Nicolson and Schenck (100x).

a
c
b
d

Glomus clarum **Nicolson and Schenck**, *Mycologia,* **LXXI(1):** 173-198, 1979. **(Plate V, Fig. d)**

Spores found singly or in clusters in the soil, hyaline, globose to subglobose, 68-290 µm in diam. Spore wall complex, hyaline, becoming yellow with age. Walls 7-31µm in diam., consisting of an inner (2-9 µm) and outer wall (5-20 µm) that do not separate readily; inner wall of several (2 to 5 layers) of 0.5-2.0 µm; subtending hypha 15-18 µm wide with thick walls (7-39 µm) extending upto 400 µm below the spore wall. Pore opening to the chlamydospore 3-5 µm in width with a bulging septum in the pore separating the pore contents from the subtending hyphae.

Glomus dimorphicum Boyetchko and Tewari ***Can. J. Bot.,*** 64: **90-95, 1986 (Plate VI, Fig. a)**

Single spores yellow to reddish brown, globose to subglobose, 90-300 µm. Spore wall made up of 3 walls organized in 2 wall groups; wall 1 hyaline, laminate, 2-8 µm thick, ephemeral; wall 2 light yellow to reddish brown, laminate 2-8 µm thick; wall 3 light yellow to reddish brown membranous, 1 µm thick. Subtending hypha straight to slightly curved, light yellow to light brown; wall 1.5-9 µm thick and consisting of wall 1-3. Point of attachment occasionally possessing a septum, often occluded, flared or cylindric, 10-34 µm thick.

PLATE- VI

SPORES OF ARBUSCULAR MYCORRHIZAL FUNGI

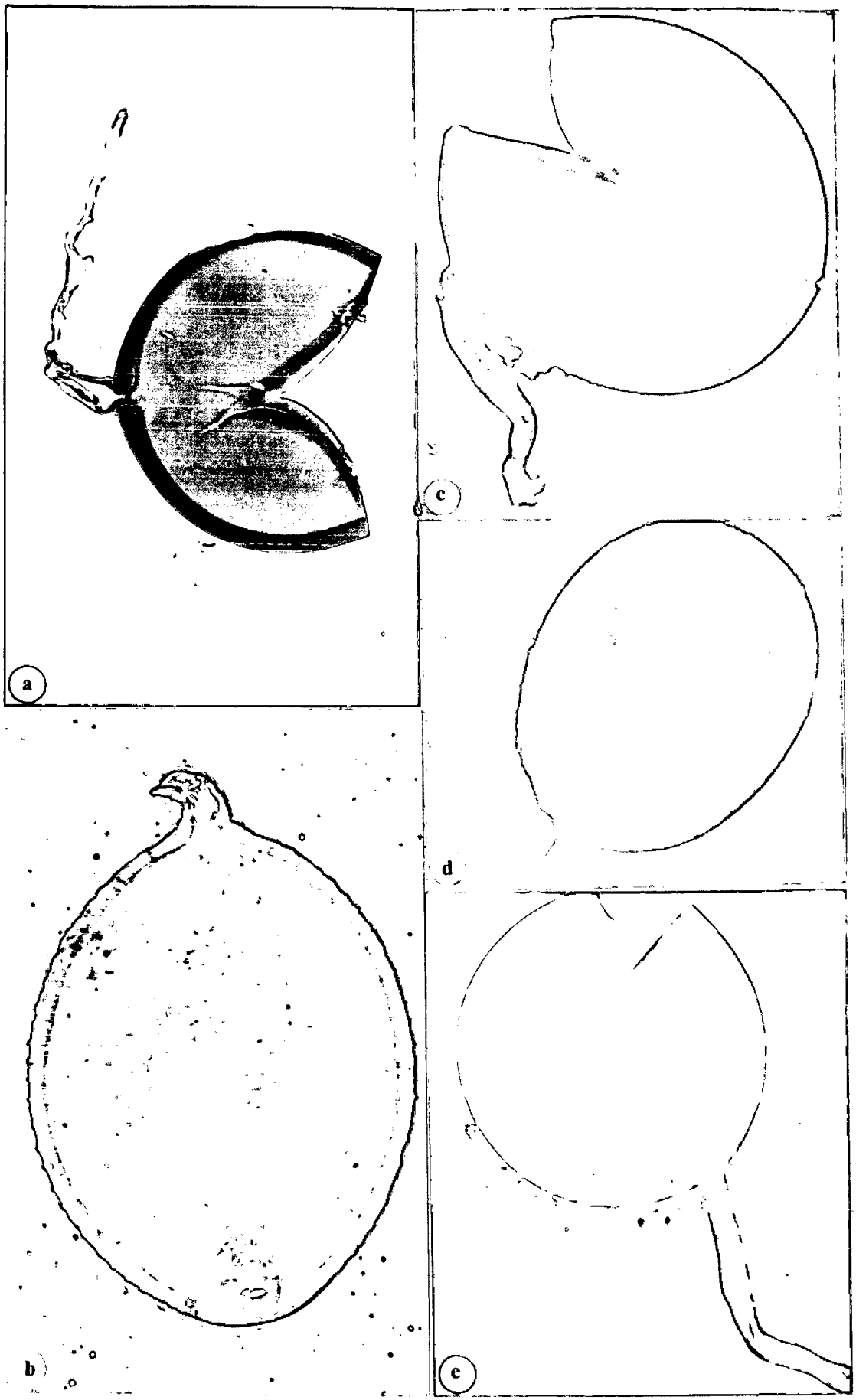
a) ***Glomus dimorphicum*** Boyetchko and Tewari **(100x).**

b) ***Glomus monosporum*** **Gerdemann & Trappe (100x).**

c) ***Glomus claroideum*** **Schenck & Smith emend Walker & Vestberg (100x).**

d) ***Glomus pustulatum*** **Koske, Friese, Walker & Dalpe (100x).**

e) ***Glomus versiforme*** **(Karsten) Berch (100x).**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Glomus etunicatum **Becker & Gerdemann.** ***Mycotaxon*** **6:** 29-32, 1977.

Spores found singly in soil, globose to subglobose; (95-) 120.9 (-145) x (95-) 117.6 (-140) µm, yellow to yellow brown. **Spore wall structure of two walls (1 & 2) in one group. Wall** – 1 evanescent (1-) 1.3 (-2) µm thick. Wall – 2 laminated (4 -) 5.2 (-8) µm thick. Subtending hypha (3-) 4.8 (-8) with (4 -) µm diam., with (4-) 7.6 (- 1) µm at the point of attachment with the spore. Spores often occur inside dead root pieces.

Glomus fasciculatum **(Thaxter) Gerdemann & Trappe emend. Walker & Koske,** ***Mycotaxon*** **30:** 253-262, 1987 **(Plate VII, Fig. a)**

Spores formed singly or in loose aggregation in soil, pale yellow to pale yellow-brown, globose to subglobose. Spore wall structure of 3 walls in one group. Wall 1 a smooth hyaline unit wall, 0.2-1(-1.8) µm thick. Wall 2 pale yellow to pale yellow-brown, laminated (2-) 5-10 (-14.3) µm thick. Wall 3, a hyaline membranous wall, 0.1-0.9 µm thick. Subtending hypha flared, straight or slightly constricted proximally, (3.5-) 9-15 (-19) µm broad at the spore base. Pore open or closed by thickening of wall 2.

Glomus geosporum **(Nicolson and Gerdemann) Walker,** ***Mycotaxon*** **15:** 49 - 61, 1982.

Sporocarps unknown. Spores formed singly in soil, light yellow-brown to dark yellow-brown, globose to subglobose, 110-225 (-290) µm. Spore walls 4-6 (-18) µm in thickness, 3 - layered with a thin hyaline, tightly adherent outer wall (<1µm), a yellow- brown to red-brown laminated middle wall and a yellow to yellow-brown inner wall (<1µm) that forms a septum. Spore contents granular in appearance, cut off by a thick septum that protrudes

slightly into the subtending hypha. Spores with one straight to recurved, simple to slightly funnel shaped subtending hypha upto 200 µm long, 10-18 (-24) µm diam., with yellow to dark yellow-brown wall thickening that extends 30-100 µm along the hypha from the spore base.

***Glomus globiferum* Koske & Walker, *Mycotaxon* 26:** 133-142, 1986.

Sporocarps unknown. Spores formed singly in the soil, or in pairs or triplicates adhering to each other by common peridial hyphae; orange- brown to rich red –brown, to fuscous-black; globose to subglobose; (150-) 220 (-260) x (150-) 225 (-270) µm, excluding peridium. Peridium of loosely interwoven hyaline to yellow–brown, coenocytes or sparsely septate, thin walled hypae, (5-) 12 (-50) µm broad, bearing numerous terminal or intercalary globose to ovoid, pale yellow-brown or hyaline vesiculate swellings. Vesiculate swellings (12-) 25 (-65) x (12-) 35 (-75) um, with walls (1-) 1.5 (-2.5) µm thick, consisting of a thin, hyaline outer unit wall, and a thicker, slightly coloured inner laminated wall. Spore wall structure of four walls (1-4) in two groups (A, B). Group A, consisting of 3 or 4 tightly adherent walls. Wall-1 hyaline to pale yellow-brown unit wall, (0.5-) 0.5 (-3) µm thick. Wall-2 orange-brown to red-brown, laminate (6-) 10 (-30) µm thick with up to 9 sub equal laminations that are often indistinct. Group B consisting of a single, hyaline membranous wall (wall-3) 1 µm thick. Subtending hyphae straight or recurved; usually constricted proximally but occasionally straight or funnel shaped (15) 15 (-27) µm wide at the point of attachment with the spore, (18 -) 19 (-37) µm at the sides point, with the wall

(2-) 3 (-8) µm thick. At the spore base; subtending hyphae usually appears to be inserted into the spore wall.

Glomus heterosporum **Smith & Schenck,** ***Mycologia*** **77:** 566-574, 1985.

Spores produced in sporocarps, light to dark brown, obovoid to ellipsoid, occasionally globose, (99-) 114 – 206 x (61-) 111-201 µm. Spores with two distinct walls. Inner wall laminate brown 3-10 µm thick. Outer wall smooth, evanescent, hyaline, 2-7 µm thick. Spore contents hyaline, non-globular and separated from hyphal attachment by a septum. Hypha at the point of attachment is 5-15(-31) µm wide. Spores frequently with multiple hyphal attachments.

Glomus hoi **Berch & Trappe,** ***Mycologia*** **77:** 654-657, 1985 **(Plate VII, Fig. b)**

Spores borne singly in soil, globose to subglobose, (50-) 80-120 (-140) µm, light brown. Wall of spores composed of two distinct, separable layers; outer layer orange- yellow, 2-6(-8) µm thick, with an outer surface that fractures and sloughs; inner layer hyaline – light yellow, membranous 1µm thick. Subtending hypha (5-) 8-12 (-13) µm wide, with a single wall 2.5-5 µm thick. Pore in the subtending hypha occluded by a fine curved septum at or somewhat below its point of attachment to the spore.

Glomus intraradix **Schenck & Smith,** ***Mycologia*** **74:**77-92, 1982.

Spores found frequently within roots and infrequently in loose clusters in the soil. Spores yellow brown, globose to subglobose; (60-) 81.9 (-94) x (58-) 77.8 (-94) µm diam. Spore

wall structure of 2 walls (1 & 2) in one group. Wall - 1 hyaline, ephemeral (2-) 3.1 (-6) µm thick; wall - 2 laminated (2-) 3.3 (-6) µm thick. Subtending hyphae (4-) 5.3 (-6) µm diam with (4 -) 6.9 (-10) µm diam. at the point of attachment. Walls of the spores extending into the hyphal attachment forming an apparent tubaeform flaneup at the juncture of hyphal attachment. Reaction of walls in Melzers reagent not distinctive.

Glomus macrocarpum **Tulasne & Tulasne Can. J. Bot. 61:** 2608-2617, 1983 **(Plate VII, Fig. c & d)**

Sporocarps are fragmentary. Spores yellow to brown, usually longer than wide, subglobose or globose, (90-) 120 (-140) x (70-) 110 (-130) µm. Spore wall is composed of two distinct layers; outer layer is thin, 1-2 µm and hyaline; inner wall layer is yellow, 6-12 µm thick, with laminations. Spore taper to the point of attachment of the single persistent hypha. Hyphal width at this point varies from 12-18 µm. The inner wall at maturity thickens to occlude the pore of the attached hypha, and the wall thickening continues into the subtending hypha for up to 90 µm from the spore. Infrequently, the pore seems to be closed by a septum that is thinner than the normal occluding wall 2-thickening. Spores characteristically bear a straight, long subtending hypha, which may extend up to 100 µm before branching or breaking.

PLATE- VII

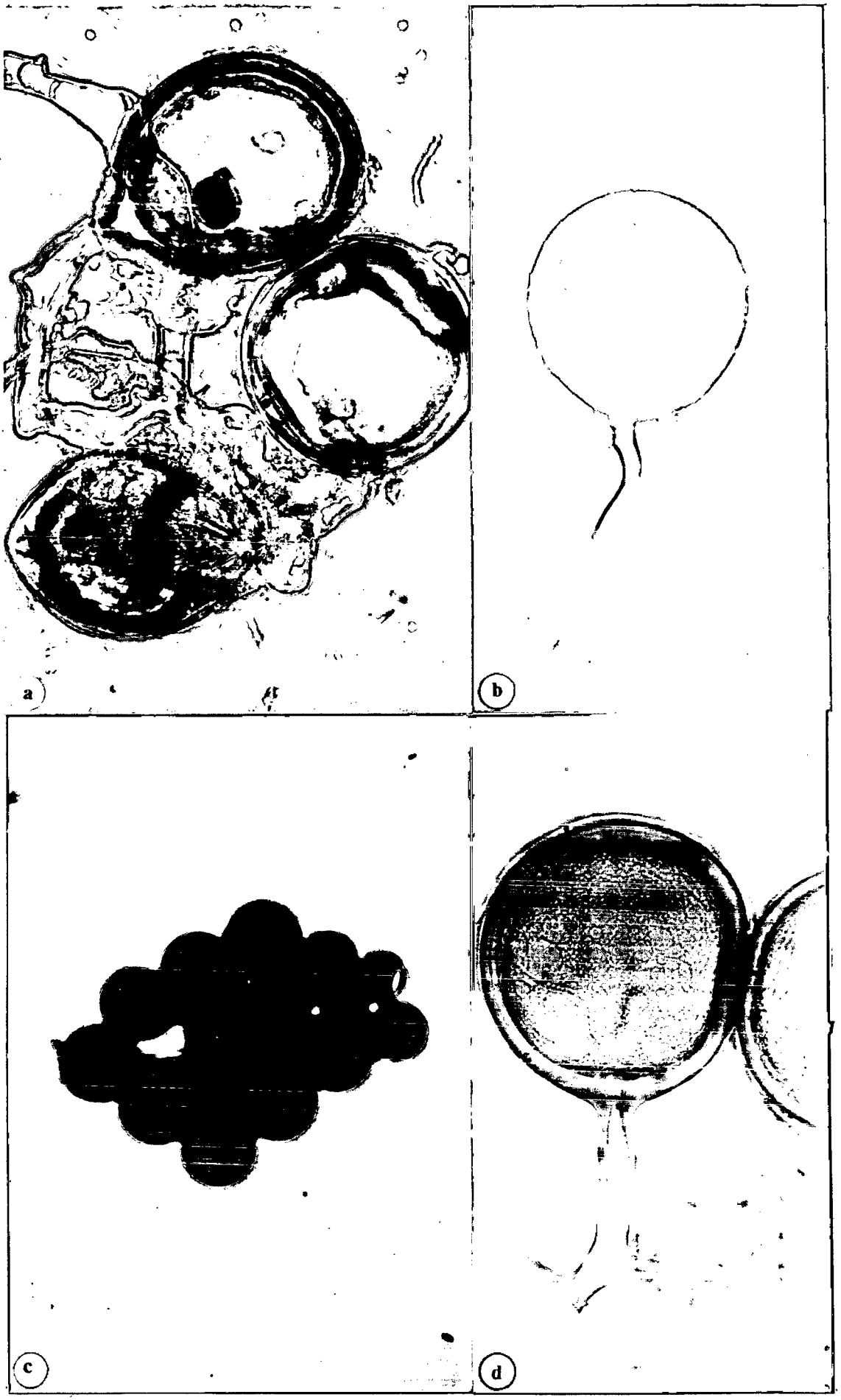
SPORES OF ARBUSCULAR MYCORRHIZAL FUNGI

a) *Glomus fasciculatum* (Thaxter) Gerdemann & Trappe emend. Walker & Koske (400x).

b) *Glomus hoi* Berch & Trappe (100x).

c) *Glomus macrocarpum* Tulasne & Tulasne (100x).

d) *Glomus macrocarpum* Tulasne & Tulasne (400x).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Glomus monosporum **Gerdemann & Trappe,** ***Mycologia Memoir No.*** **5:**76, 1974

(Plate, VI Fig. b)

Sporocarps globose to ellipsoid, containing mostly 1, occasionally two or rarely three spores. Peridium of branched interwoven, thin walled hyphae. Spores globose to subglobose or rarely ellipsoidal, dull brown; (140-) 140 (-330) µm diam. Spore wall structure of two wall (1 & 2) in one group. Wall-1 hyaline 0.5 µm thick. Wall –2 dull brown laminate (4-) 8.5 (-10) µm thick with minute, abundant to scattered acidulation's that protrude into the outer wall; thickening of the inner wall extending into the subtending hyphae. Subtending hyphae straight or strongly recurved, (8-) 10 (-12) µm wide and appressed to the spore walls.

Glomus mosseae **(Nicol. & Gerd.) Gerdemann & Trappe,** ***Mycologia Memoir No.*** **5:**76, 1974

Sporocarps rare, three to four spored when present. Peridium partly enclosing chlamydospores. Spores pale yellow to yellow – brown, globose to subglobose; (144-) 184.6 (-260) x (143-) 181.2 (-255) µm diam. with one distinct funnel shaped attachment. Spore wall structure of two walls (1 & 2) in one group. Wall – 1 hyaline 0.5-2 µm thick unit wall. Wall – 2 laminated (2 -) 4.4 (-7) µm thick. Subtending hyphae (10-) 9.3 (-15) diam., and (18-) 23.4 (-35) diam., at the point of attachment with the spore. Spore contents separated from the subtending hyphae by a curved septum extending (20-) 39.03 (-75) into the subtending hyphae. Reaction of wall layers to Melzers reagent not distinctive.

Glomus pansihalos **Berch & Koske,** ***Mycologia*** **78:** 832-836, 1986.

Sporocarps irregularly ellipsoid, peridium absent. Spores globose, subglobose (108-) 130 – 155 (-200) x (110-) 120 – 130 (-180) µm. Yellowish brown to dark brownish-orange. Wall composed of a single group, hyaline or light yellow 3-8 (38) µm, laminate, subtending hypha flared, straight or constricted, 10-20µm at point of attachment, widening to 17-20µm, yellow brown, paler than spore wall, pore in subtending hypha occluded.

Glomus pustulatum **Koske, Friese, Walker & Dalpe,** ***Mycotaxon*** **26:**143-149, 1986. **(Plate VI Fig. d)**

Sporocarps formed singly in soil, pale yellow to yellow brown or orange-brown, globose to irregular (43-) 86 – 140 x (60) –86 (-140) µm. Spore wall three in one group, wall-1 yellow brown to orange brown 1-5µm; wall-2 pale yellow to yellow brown laminated, 3-10µm thick; wall-3 a thin hyaline membranous wall 21 µm thick. Subtending hyphae straight or recurved, pale yellow to yellow brown. Pore closed by ingrowth of wall-2.

Glomus versiforme **(Karsten) Berch,** ***Can. J. Bot.,*** **61:** 2608-2617, 1983. **(Plate VI, Fig. e)**

Sporocarps enclosing five to fifteen spores, reddish brown, peridium absent, spores orange brown, globose to subglobose; (130-) 147.7 (-172) x (121-) 143.9 (-164) µm diam. Spore wall structure of three walls (1-3) in one group, wall-1 hyaline unit wall (1-) 2.2 (-3) µm thick. Wall-2 laminated (10-) 10.8 (-12) µm thick and wall-3 a unit wall 0.5 to 1µm thick, tightly adherent to wall-2 often difficult to see. Subtending hyphae straight (9-) 11.9 (-18)

µm diam., and (12-) 14.1 (-18) µm at the point of attachment. Pore occluded by thickening of wall-2. Reaction of walls to Melzers reagent not distinctive.

GENUS : *SCLEROCYSTIS*

***Sclerocystis rubiformis* Gerdmann & Trappe *Mycologia Memoir No.* 5:** 76, 1974 **(Plate VIII, Fig. a)**

Sporocarps dark brown, subglobose to ellipsoid, 180 x 180 – 375 x 675µm. Spores obovoid to ellipsoid, or subglobose, 37 - 125 x 29 – 86 µm with a small pore opening into the thick walled subtending hypha. Spore wall laminate 3 - 7.6 µm thick, up to 13.5µm thick at spore base. Perforated projections on the inner surface. A variable stalk like projections protrudes near the base of some spores.

***Sclerocystis sinuosa* Gerdemann & Bakshi, *Trans. Br. Mycol. Soc.*, 66:** 340-343, 1976 **(Plate VIII, Fig. c & d)**

= ***Sclerocystis pakistanica* Iqbal & Bushra, *Trans. Br. Mycol. Soc. Japan* 21:** 57-63, 1980.

Sporocarps reddish-brown to dull brown, globose to subglobose, 220-650 µm in diam. Peridium tightly enclosing a sporocarp, composed of thick walled interwoven hyphae. Peridial hyphae sinuous. Sometimes sinuous feature may be indistinct. Chlamydospores globose, subglobose, obovoid, clavate or irregular, 45-150 x 30-83 µm, walls single with

walls evenly thickened or unevenly thickened, usually thickened at the apex or lateral side, (1.5-) 5-22.5 (-30) µm, with 1-2 attached hyphae.

***Sclerocystis taiwanensis* Wu & Chen, *Trans. Br. Mycol. Soc. R.. O. C.* 2:** 73-83, 1987 **(Plate VIII, Fig. b & c)**

Sporocarps reddish brown, brown or dark brown, globose to subglobose, 200-300 x 180-280 µm, with chlamydospores formed radially in a single, tightly packed layer around a central plexus of hyphae. Peridium lacking. Chlamydospores clavate, cylindro-clavate, 40-57 (-105) x 22-28 (-55) µm, with or without a septum at the spore base. Chlamydospore wall laminate or single, with a hyaline separable outer layer (1µm thick), yellow -brown inner layer (4-) 12-15 (25) µm thick at the apex, 1.5-3 (-5) µm thick at sides, generally thickest at the apex.

GENUS : *SCUTELLOSPORA*

***Scutellospora coralloidea* (Trappe, Gerdemann & Ho) Walker & Sanders, *Mycologia* 77:** 702-720. 1985 **(Plate IX, Fig. a)**

Spores formed singly in soil, terminally on a bulbous suspensor like cell. Spores globose to subglobose, 308 – 393 x 294-420 (-454) µm, dark brown. Spore wall structure of 3 walls (1-3) in two groups (A & B). Group A with an outer ornamented unit wall (wall-1) tightly adherent to an inner laminated wall (wall-2); wall 1 brittle, dark brown, 3-5 µm thick excluding widely spaced patch like warts; warts hyaline to pale yellow, 1-2 µm high,

PLATE- VIII

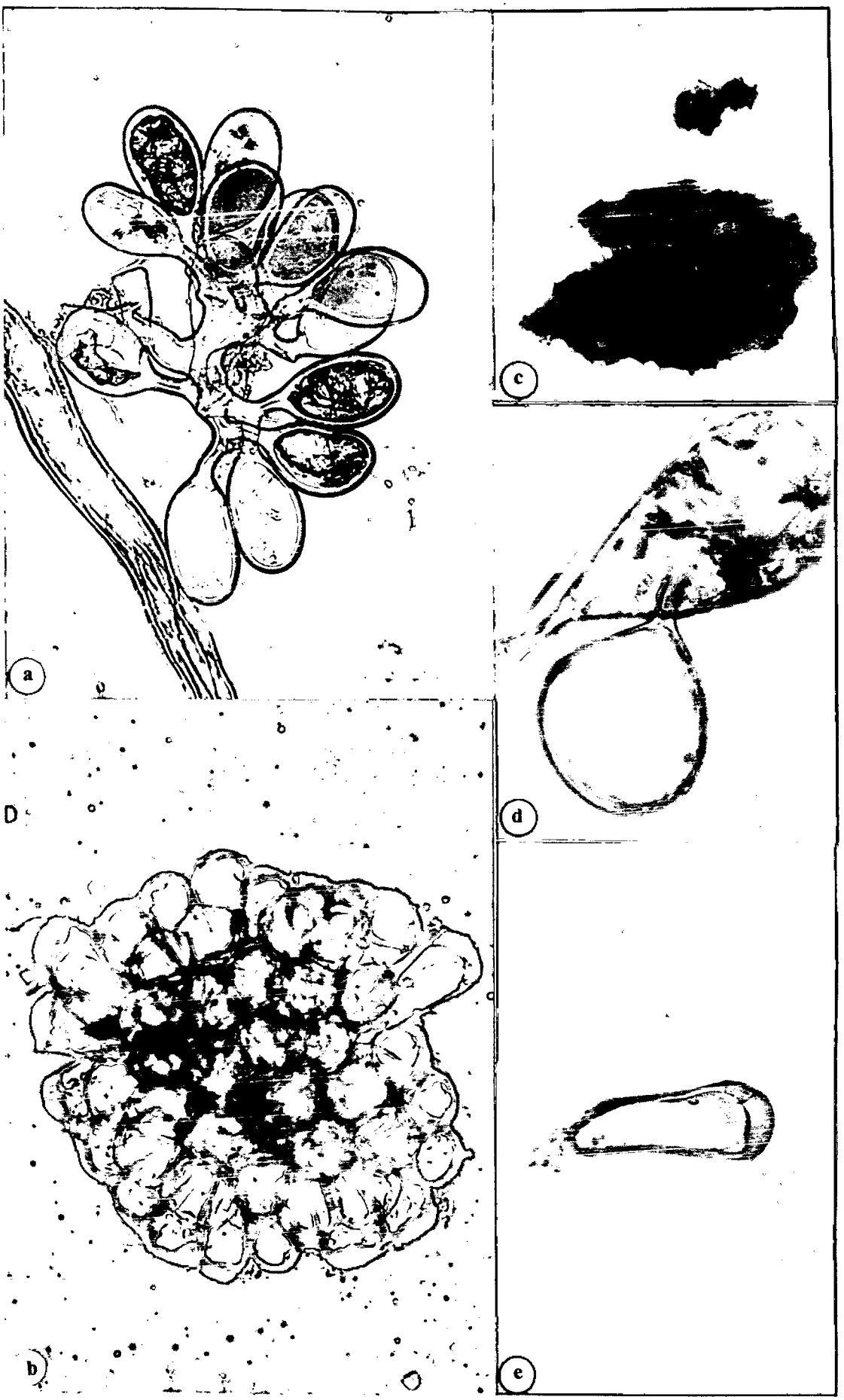
SPORES OF ARBUSCULAR MYCORRHIZAL FUNGI

a) *Sclerocystis rubiformis* Gerdmann & Trappe (100x).

b) Sporocarp of *Sclerocystis taiwanensis* Wu & Chen (100x).

c) & d) *Sclerocystis sinuosa* Gerdemann & Bakshi (100x).

e) *Sclerocystis taiwanensis* Wu & Chen (100x).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2-12 µm across, with subangular margins; wall 2 brittle, yellow brown to orange brown 4.5-8 µm thick. Group B consisting of a hyaline, membranous wall (wall-3) 0.5 µm thick, enclosing spore contents. Sporogenous cell pale brown, 55-64 µm wide, with a peg-like, narrow, yellow-pale brown hypha up to 20 x 5 µm. Sporogenous cell borne terminally on a septate or aseptate subtending hypha.

Scutellospora gregaria **(Schenck & Nicolson) Walker & Sanders,** ***Mycologia*** **77:** 702-720. 1985 **(Plate VIII, Figs. b - d)**

Spores found singly in the soil, terminally to somewhat eccentrically on a bulbous sporogenous cell. Spores red brown to dark brown, globose to subglobose, 250-334 (-448) x 250-334 (-480) µm. Spore wall structure of four walls (1-4) in two groups (A & B). Wall group A composed of three closely appressed walls, an outer unit wall (wall-1) and two laminated walls (wall-2 & wall-3); wall-1 brittle, brown, (1-3) µm thick excluding the closely packed warts situated on its outer surface, warts pale brown, 1- 2 (-5) µm high with rounded tips, 2-7 (-10) µm diam at the base, crowded together in groups; wall-2 brittle, yellow (3-5) µm thick; wall-3, brittle, pale yellow to nearly colourlesss, (5-13) µm. thick. Group B of a single membranous hyaline wall (wall-4) 1-2 µm thick. Sporogenous cell pale brown, 39-45 (-80) µm wide, with 1 or 2 thick or thin walled hyaline projections, borne terminally. Germination shield globose to subglobose 135 x 144 µm diam.

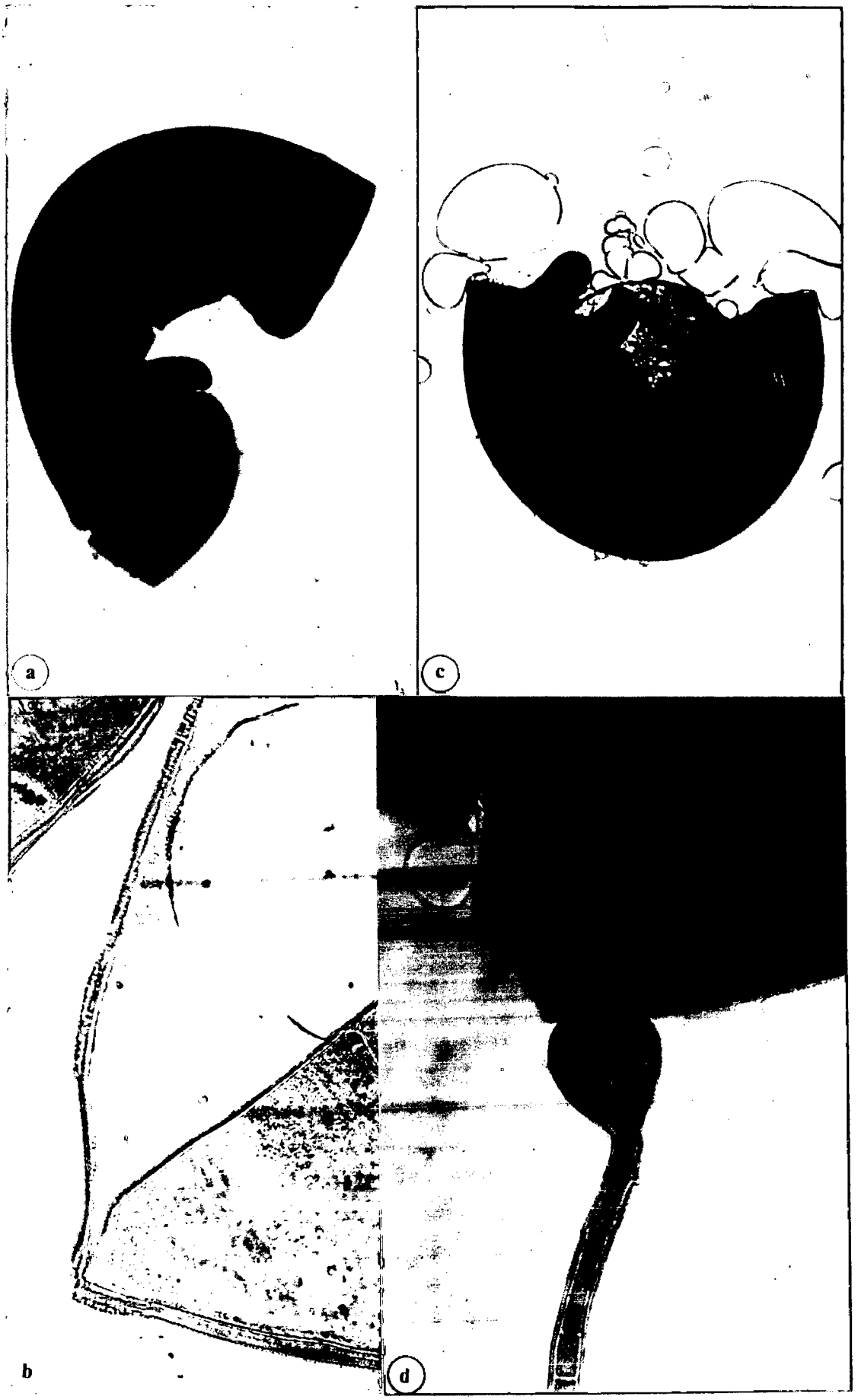
PLATE- IX

SPORES OF ARBUSCULAR MYCORRHIZAL FUNGI

a) *Scutellospora coralloidea* (Trappe, Gerdemann & Ho) Walker & Sanders (100x).

b) & d) *Scutellospora gregaria* (Schenck & Nicolson) Walker & Sanders (400x).

c) *Scutellospora gregaria* (Schenck & Nicolson) Walker & Sanders (100x).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Scutellospora pellucida **(Nicol. & Schenck) Walker & Sanders,** ***Mycotaxon*** **27:** 219-235, 1986.

Spores found singly in the soil, terminally on a bulbous sporogenous cell. Spores hyaline to pale grey, glistening with oil droplets, globose ellipsoid, or irregular, (58-) 180-220 (-410) μm diam. Spore wall structure of six walls (1-6) in three groups (A, B & C). Group A of a brittle, hyaline unit wall (wall-1) (1-2) μm thick, closely appressed to an inner brittle, hyaline, laminated wall (wall-2) (2-) 5 (-18) μm thick. Group B of a hyaline membranous (wall-3) less than 1 μm thick, closely adherent to two hyaline unit walls (wall 4 and 5) measuring 1-2 μm each. Group C consists of a single amorphous wall (wall-6) (2-5) μm thick. Sporogenous cell (10-) 18 –51 μm broad, borne terminally on septate subtending hyphae. Germination shield oval to spherical, (60-) 60 (-110) x (110-) 110 (-130) μm, margins with invaginations.

CHAPTER- 3

SEASONAL VARIATIONS IN ARBUSCULAR MYCORRHIZAL FUNGI IN SELECTED TREE SPECIES FROM MOLLEM AND DHARBANDODA FOREST AREAS.

INTRODUCTION

Ubiquitous occurrence and distribution of arbuscular mycorrhizal fungi forming a significant part of every natural and cultivated ecosystem play a major role in plant species diversity and survival (Bergelson and Crawley, 1988). Spatio-temporal variations in mycorrhizal colonization have been studied in several plant communities (Rabatin, 1979; Gay *et al.,* 1982; Allen 1983; Louis and Lim, 1987; Brundrett and Kendrick, 1988; Van Duin *et al.,* 1989; Mayer and Godoy, 1989; Reinhardt and Miller, 1990; Sanders, 1990; Sanders and Fitter, 1992a). These studies have either found (Rabatin, 1979; Gay *et al.,* 1982; Sanders and Fitter, 1992) or failed to find a consistent pattern of arbuscular mycorrhizal fungal development in various habitats studied. Similar variations in arbuscular mycorrhizal fungal spore population have been noted in different habitats (Giovannetti, 1985; Gemma *et al.,* 1989; Beena *et al.,* 1997; Muthukumar, 1998).

The presence of fungal structures within roots at appropriate time during the season is essential for the effective functioning of the symbiosis. As arbuscules are the site of nutrient transfer from the fungus to the host (Smith, 1993) their abundance should correspond with episodes of nutrient acquisition by the plants. In contrast, the abundance of hyphae and vesicles within roots and spores in the soil indicates the period of a significant carbon drain from the host (Abott and Gazey, 1994.)

Arbuscular mycorrhizal development within plant roots and subsequent sporulation may be dependent on climatic and edaphic factors (Fitter, 1985; Sanders, 1990; Muthukumar *et al.,* 1994). But the direct influence of these factors on

mycorrhizal fungi is difficult due to their intimate association with a host in nature. Most of the green house and pot culture studies have focused attention only on selected factors. Unfortunately, these studies though provide a basic understanding, are potentially less valuable under natural conditions where the response is governed by multiplicity of interacting factors (St. John and Coleman, 1983). Earlier studies have emphasized the role of pH (Hayman and Tavares, 1985), moisture, fertility and physico-chemical characteristics of the soil (Hayman, 1982) in the symbiotic relationship between the host and the fungi.

The present chapter deals with studies on seasonal variations of arbuscular mycorrhizal fungi with respect to root colonization, spore density and species richness in selected tree species from Mollem and Dharbandoda forest sites.

MATERIALS AND METHODS

STUDY SITE

In the present study, two forest areas *viz.*, Mollem and Dharbandoda were selected. These areas are located between 15^{O} 15' 34" N – 15^{O} 31'45"S Latitude and 74^{O} 21' 00" E – 74^{O} 09'45" W Longitude and encompasses rich forest varying from moist deciduous to semi-evergreen under humid tropical climate.

SAMPLING TECHNIQUES

Six quadrates each with an area of 100 x 100 sq. mt. were randomly laid at both the sites. In all a total of eight tree species *viz., Terminalia crenulata* Roth, *Terminalia*

paniculata Roth, *Strychnos nux-vomica* L, *Buchnania cochinchinensis* (Lour) Almeida, *Careya arborea* Roxb., *Ervatamia heyneana* (Wall) Cooke., *Xylia xylocarpa* Taub and *Holarrhena pubescens* (Buch.-Ham.) Wallich ex Don common to all the six quadrates were selected for the study.

PLANT NOMENCLATURE

Plants selected in the present study were identified using floras (Rao, 1985 and 1986; Mathew, 1992; Mohanan and Henry, 1994; and Naithani *et al.*, 1997).

CLIMATIC DATA

Climatic data was obtained from Meteorological Department for a period of one year *i.e.*, from February 2000 – January 2001 during which the present study was undertaken. The mean value was obtained for rainfall, relative humidity and, maximum and minimum temperature for pre-monsoon (March), monsoon (July) and post-monsoon (November).

SOIL ANALYSIS

For analyzing soil pH, EC, Total N, P and K content, the soil samples were collected from a depth of 0-25 cm from eight different locations for each quadrate at the study site and were brought to the laboratory in polyethylene bags. Samples from each quadrate were passed through 2mm sieve to remove the larger soil particles and were mixed thoroughly to obtain a composite sample. Later, each composite sample was

processed three times to get the mean value. This was repeated for all the six quadrates and the average mean value was obtained for each quadrate.

Soil pH was measured after dilution with distilled water (1:1 w/v soil: water) soon after the samples were brought to the laboratory. Electrical Conductivity (EC) was determined in 1:1 water: waste extracts (Bower and Wilcox, 1965). Soil nitrogen was determined by micro-Kjeldahl method (Jackson, 1971). Soil phosphorus was determined by molybdenum blue method (Jackson, 1971). Soil Potassium was estimated after extraction in ammonium acetate solution (Jackson, 1971).

Roots and rhizosphere soil samples were collected at the end of second month of each season (March, July and November). Rhizosphere soil samples were excavated from a depth of 0-25cm, placed in plastic bags, labeled and stored at 4^0 C until processed. While collecting soil samples, soil was dug at three places for each plant so as to cover the entire rhizosphere. For each plant species, rhizosphere soil samples were collected randomly from 3 individuals of the species. Later, a composite sample was made for each species and worked out. This was carried out for all the six quadrates during pre-monsoon (March), monsoon (July) and post-monsoon (November) for a period of one year. The roots were dug and traced back to plant, which ensured that the roots belonged to the intended plant species.

ESTIMATION OF ROOT COLONIZATION

Root samples were washed thoroughly under tap water. Feeder roots were cut into 1cm fragments. The composite sample of each species was cleared in 10% KOH

in an autoclave at 121^{0}C for 15-25 minutes. Cleared roots were thoroughly washed with tap water and acidified with 5% HCl. Later, the roots were stained with 0.05% trypan blue (Phillips and Hayman, 1970). Stained roots were mounted in glycerol and observed under Leica compound microscope. Percent root colonization was estimated by using Slide technique (Giovanetti and Mosse, 1980). For a sample to be regarded as possessing arbuscular mycorrhizal association, it had to be seen to have vesicles, arbuscules or both.

RECOVERY OF ARBUSCULAR MYCORRHIZAL FUNGAL SPORES AND THEIR IDENTIFICATION

Hundred grams of air dried composite sample was assayed for spore count using wet sieving and decanting procedure (Gerdemann and Nicolson, 1963). Spores were separated using sieves having mesh sizes ranging from 38 μm to 500 μm. Each composite sample was processed three times. Spores were observed under Leica stereomicroscope. Estimation of Spore density was carried out as per the procedure given by Gaur and Adholeya (1994). Only healthy spores were enumerated while broken and parasitised spores were discarded. Spores were mounted in Polyvinyl Lacto Glycerol (PVLG), (Koske and Tessier, 1983), examined for their various characteristics and identified using the standard keys (Schenck and Perez, 1990; Morton and Benny, 1990; Wu, 1993). Spores in the rhizosphere soils were multiplied using *Eleusine coracana* L., *Lycopersicum esculentum* Mill., *Allium cepa* L. and *Coleus sp.* as a host plants. The spores isolated from the pot cultures were later used for confirming the identification of the spores recovered from both the sites.

Relative abundance of spores and Frequency of occurrence for each of the arbuscular mycorrhizal fungal species was calculated for both the sites. Relative abundance and Frequency of occurrence was calculated using the formulas given below:

$$\text{Relative Abundance (\%)} = \frac{\text{Number of AM fungal spores of particular species}}{\text{Total number of AM fungal spores of all species}} \times 100$$

$$\text{Frequency of Occurrence (\%)} = \frac{\text{Number of soil samples that possess spores of particular AM species}}{\text{Total number of soil samples screened}} \times 100$$

RESULTS AND DISCUSSION

Results related to climatic data for the year 2000-2001 obtained from State Meteorological Department is depicted in **Fig. 4 & 5**. The data for Mollem and Dharbandoda forest areas during the study period (2000-2001) indicated that both these areas recorded an average rainfall of 3511.6 mm. The minimum and maximum temperature recorded was 19.8 °C and 33.6 °C respectively. The minimum and maximum humidity recorded at Mollem and Dharbandoda was 55% and 94% respectively.

Detailed seasonal information on climatic data for the study period is given in **Table 11.** It was observed that 90% of the rainfall occurred during monsoon (3154

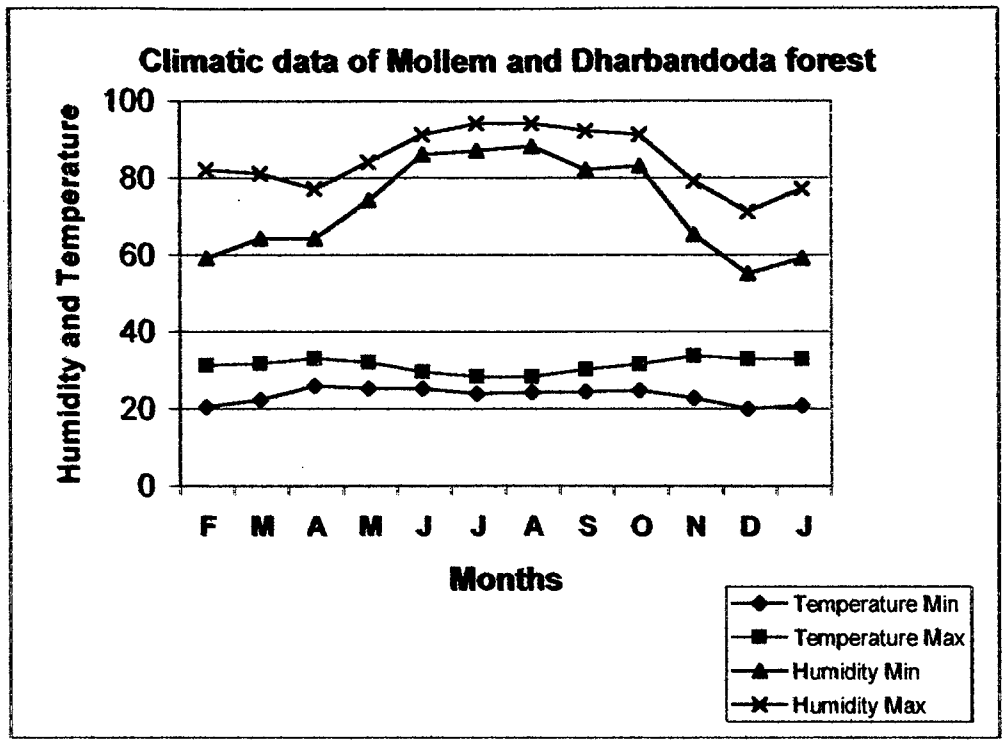

Climatic data of Mollem and Dharbandoda forest
Humidity and Temperature
100
80
60
40
20
0
F M A M J J A S O N D J
Months
Temperature Min
Temperature Max
Humidity Min
Humidity Max

Fig. 4

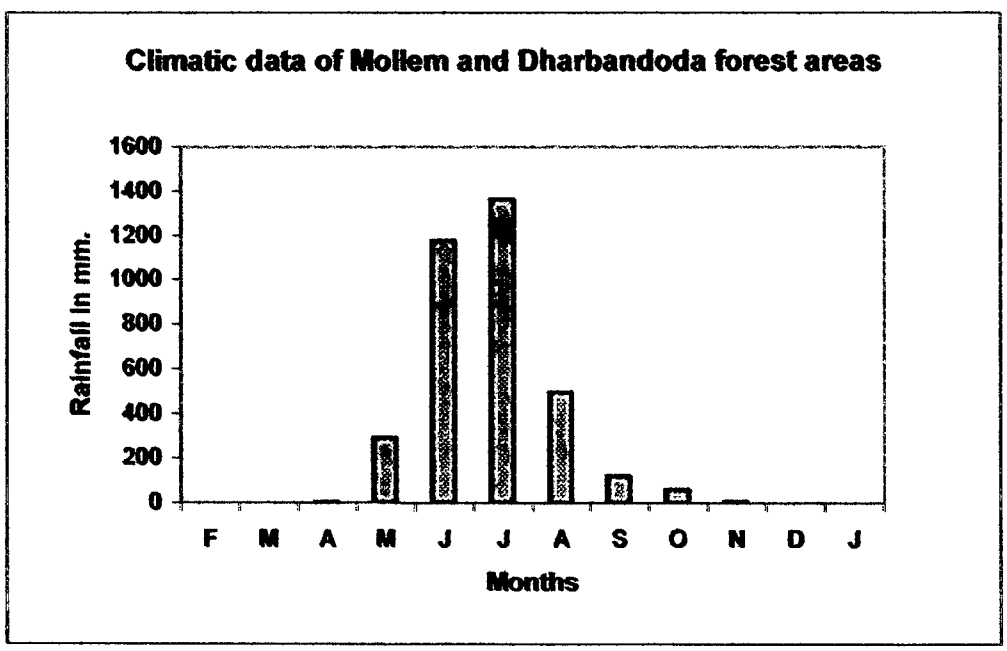

Climatic data of Mollem and Dharbandoda forest areas
Rainfall in mm.
1600
1400
1200
1000
800
600
400
200
0
F M A M J J A S O N D J
Months

Fig. 5

Table 11 : Meteorological data for Mollem and Dharbandoda for the year 2000- 2001.

Season	Rainfall in mm	Temperature °C		Humidity (%)	
		Min.	Max.	Min.	Max.
Monsoon	3154.00	23.9 (24.37 ± 0.52)	30.1 (29.00 ± 0.95)	82 (85.75±2.62)	94 (92.75 ± 1.50)
Pre-monsoon	291.00	20.4 (23.42 ± 2.57)	33.0 (31.97 ± 0.75)	59 (62.25±6.29)	84 (81.00 ± 2.94)
Post-monsoon	66.60	19.8 (21.95 ± 2.14)	33.6 (32.65 ± 0.86)	53 (65.5 ± 12.36)	91 (79.5 ± 8.38)

Source: Meteorological Department, Government of Goa.
Legend: Average values are indicated in parenthesis.
± Indicates Standard deviation.

mm), 8% of the rainfall occurred in pre-monsoon (291 mm) while only 2% of the rainfall occurred during post-monsoon (66.60 mm). Minimum temperature of 19.8°C and maximum temperature of 33.6°C was recorded during post monsoon season, while minimum relative humidity (53%) was recorded in post-monsoon season, while maximum relative humidity (94%) was recorded in monsoon season.

Soil analysis results revealed that the soils at both the sites were acidic with pH ranging from 5.12 to 5.76. Electrical Conductivity (EC) for both the sites ranged from 0.02 to 0.65 M mhos/cm. However, the Electrical Conductivity (EC) was found to be high at Mollem (0.44 - 0.65 M mhos/cm) than at Dharbandoda (0.02 – 0.13 M mhos/cm). Total nitrogen ranged from 0.15 to 0.48%. Similarly, total phosphorus varied from 0.06 to 0.22%, while total potassium ranged from 0.08% to 0.23% at both the sites. Total nitrogen, phosphorus and potassium, was higher in Mollem forest soils as compared to Dharbandoda forest soils **(Table 12 & 13)**.

Average spore density for Mollem (208.33 spores 100^{-1} soil) and for Dharbandoda (2744.63 spores 100^{-1} soil) and, average root colonization for Mollem (31.16%) and Dharbandoda (53.18%) were recorded during pre-monsoon season with values given in parenthesis. During pre-monsoon season, at Mollem, minimum spore density was recorded in *Terminalia paniculata* (132 spores 100^{-1} soil) and maximum spore density was recorded in *Xylia xylocarpa* (271.83 spores 100^{-1} soil) while at Dharbandoda, minimum spore density was recorded in *Careya arborea* (1930.83 spores 100^{-1} soil) and maximum spore density was recorded in *Ervatamia heyneana* (4248.33 spores 100^{-1} soil). During pre-monsoon season, at Mollem minimum percent

Table 12 : Some physical and chemical properties of soils from Mollem forest.

Season	pH*	E.C. * M mhos/cm	Total N_2* (%)	Total P_2O_5* (%)	Total K_2O* (%)
Pre-monsoon	5.12 ± 0.23	0.65 ± 0.23	0.45 ± 0.05	0.20 ± 0.08	0.18 ± 0.04
Monsoon	5.50 ± 0.22	0.44 ± 0.16	0.39 ± 0.02	0.19 ± 0.03	0.16 ± 0.02
Post-monsoon	5.22 ± 0.08	0.59 ± 0.05	0.48 ± 0.04	0.22 ± 0.04	0.18 ± 0.01

*Mean of six readings.
± Indicates Standard deviation.

Table 13 : Some physical and chemical properties of soils from Dharbandoda forest.

Season	pH*	E.C. * M mhos/cm	Total N_2* (%)	Total P_2O_5* (%)	Total K_2O* (%)
Pre-monsoon	5.55 ± 0.29	0.12 ± 0.05	0.33 ± 0.28	0.14 ± 0.02	0.17 ± 0.04
Monsoon	5.48 ± 0.32	0.13 ± 0.02	0.27 ± 0.13	0.11 ± 0.03	0.23 ± 0.11
Post-monsoon	5.76 ± 0.20	0.02 ± 0.00	0.15 ± 0.05	0.06 ± 0.02	0.08 ± 0.02

*Mean of six readings.
± Indicates Standard deviation.

root colonization was recorded in *Xylia xylocarpa* (27%) and maximum percent root colonization was recorded in *Terminalia crenulata* (37.33%) while at Dharbandoda, minimum percent root colonization was recorded in *Strychnos nux-vomica* (46%) and maximum percent root colonization was recorded in *Holarrhena pubescens* (64.66%) **(Table 14)**.

Average spore density for Mollem (125.33 spores 100^{-1} soil) and for Dharbandoda (1060.33 spores 100^{-1} soil) and, average root colonization for Mollem (36.83%) and Dharbandoda (68%) were recorded during monsoon season with values given in parenthesis. During monsoon season, at Mollem minimum spore density was recorded in *Careya arborea* (109.66 spores 100^{-1} soil) and maximum spore density was recorded in *Strychnos nux-vomica* (163.83 spores 100^{-1} soil) while at Dharbandoda, minimum spore density was recorded in *Strychnos nux-vomica* (705.83 spores 100^{-1} soil) and maximum spore density was recorded in *Careya arborea* (1940 spores 100^{-1} soil). During monsoon season, at Mollem minimum percent root colonization was recorded in *Terminalia paniculata* (33.33%) and *Ervatamia heyneana* (33.33%) and *Xylia xylocarpa* (33.33%) while, maximum percent root colonization was recorded in *Buchnania cochinchinensis* (45.83%). At Dharbandoda, minimum percent root colonization was recorded in *Terminalia paniculata* (60.33%) and maximum percent root colonization was recorded in *Buchnania cochinchinensis* (80.50%) **(Table 15)**.

Average spore density for Mollem (153.71 spores 100^{-1} soil) and for Dharbandoda (1248.23 spores 100^{-1} soil) and, average root colonization for Mollem (49.44%) and Dharbandoda (77.53%) were recorded during post-monsoon season with

Table 14: Mean spore density and percent root colonization for pre-monsoon season in some tree species from Mollem and Dharbandoda forest areas.

Sr. No	Tree species	Family	Mean spore density100g^{-1} soil*		Root colonization* (%)	
			Mollem	**Dharbandoda**	**Mollem**	**Dharbandoda**
1.	*Terminalia paniculata*	Combretaceae	132.00 ± 77.05	2060.50 ± 66.21	30.83 ± 17.49	49.66 ± 6.94
2.	*Terminalia crenulata*	Combretaceae	232.00 ± 16.77	4152.66 ± 64.95	37.33 ± 7.36	47.83 ± 8.97
3.	*Strychnos nux-vomica*	Loganiaceae	204.00 ± 19.86	2344.50 ± 98.11	33.50 ± 4.76	46.00 ± 9.33
4.	*Buchnania cochinchinensis*	Anacardiaceae	181.83 ± 11.80	2472.66 ± 57.46	33.66 ± 11.77	51.66 ± 8.57
5.	*Careya arborea*	Lecythidaceae	159.33 ± 13.16	1930.83 ± 62.47	33.16 ± 9.41	52.50 ± 7.12
6.	*Ervatamia heyneana*	Apocynaceae	246.00 ± 12.62	4248.33 ± 55.25	28.33 ± 7.65	59.66 ± 11.0
7.	*Xylia xylocarpa*	Mimosaceae	271.83 ± 15.21	2531.33 ± 43.38	27.00 ± 8.07	55.50 ± 8.14
8.	*Holarrhena pubescens*	Apocynaceae	238.00 ± 23.01	2216.66 ± 45.51	29.00 ± 11.25	64.66 ± 9.39
Mean			**208.33 ± 16.18**	**2744.63 ± 16.96**	**31.16 ± 3.85**	**53.18 ± 1.31**

* Values are mean of three readings.
± Indicates Standard deviation.

Table 15: Mean spore density and percent root colonization for monsoon season in some tree species from Mollem and Dharbandoda forest areas.

Sr.No	Tree species	Family	Mean spore density100g^{-1} soil*		Root colonization* (%)	
			Mollem	**Dharbandoda**	**Mollem**	**Dharbandoda**
1.	*Terminalia paniculata*	Combretaceae	111.16 ± 22.82	992.50 ± 38.58	33.33± 3.20	60.33 ± 6.47
2.	*Terminalia crenulata*	Combretaceae	116.66 ± 16.24	856.83 ± 44.79	36.66± 4.50	61.50 ± 6.77
3.	*Strychnos nux-vomica*	Loganiaceae	163.83 ± 21.77	705.83 ± 48.51	39.33± 6.80	71.66 ± 5.20
4.	*Buchnania cochinchinensis*	Anacardiaceae	140.50 ± 19.31	1260.16 ± 55.55	45.83± 2.78	80.50 ± 7.09
5.	*Careya arborea*	Lecythidaceae	109.66 ± 11.96	1940.00 ± 93.00	35.50± 4.50	60.83 ± 8.61
6.	*Ervatamia heyneana*	Apocynaceae	120.66 ± 12.30	848.83 ± 71.98	33.33± 3.77	65.66 ± 5.16
7.	*Xylia xylocarpa*	Mimosaceae	125.33 ± 12.51	876.66 ± 66.86	33.33± 5.04	72.16 ± 6.43
8.	*Holarrhena pubescens*	Apocynaceae	120.16 ± 9.68	1008.33 ± 62.84	34.83± 3.60	73.66 ± 7.96
Mean			**125.33 ± 15.82**	**1060.33 ± 20.25**	**36.83 ± 4.27**	**68.00 ± 1.20**

*Values are mean of three readings.
± Indicates Standard deviation.

values given in parenthesis. During post-monsoon season, at Mollem minimum spore density was recorded in *Terminalia paniculata* (118.66 spores 100^{-1} soil) and maximum spore density was recorded in *Strychnos nux-vomica* (190 spores 100^{-1} soil) while at Dharbandoda, minimum spore density was recorded in *Careya arborea* (904.83 spores 100^{-1} soil) and maximum spore density was recorded in *Terminalia paniculata* (1720.50 spores 100^{-1} soil). During post-monsoon season, at Mollem minimum percent root colonization was recorded in *Careya arborea* (39.11%) and maximum percent root colonization was recorded in *Strychnos nux-vomica* (65.22%) while at Dharbandoda, minimum percent root colonization was recorded in *Xylia xylocarpa* (70.83%) and maximum percent root colonization was recorded in *Terminalia paniculata* (82.33%) **(Table 16)**.

At both the sites, maximum average root spore density was recorded in pre-monsoon collection *i.e.* 208.33 spores/100g soil for Mollem and 2744.63 spores/100g soil for Dharbandoda site. While minimum average density was recorded in the monsoon collection at both the sites *i.e.* 125.33 spores/100g soil for Mollem and 1060.33 spores/100g soil for Dharbandoda site **(Fig. 6)**.

Root colonization was observed throughout the year. Average percentage root colonization was maximum in post-monsoon collections *i.e.* 49.44% for Mollem site and 77.53% for Dharbandoda site. Average percentage root colonization was lowest in pre-monsoon collections *i.e.* 31.16% for Mollem and 53.18% for Dharbandoda site **(Fig. 7)**.

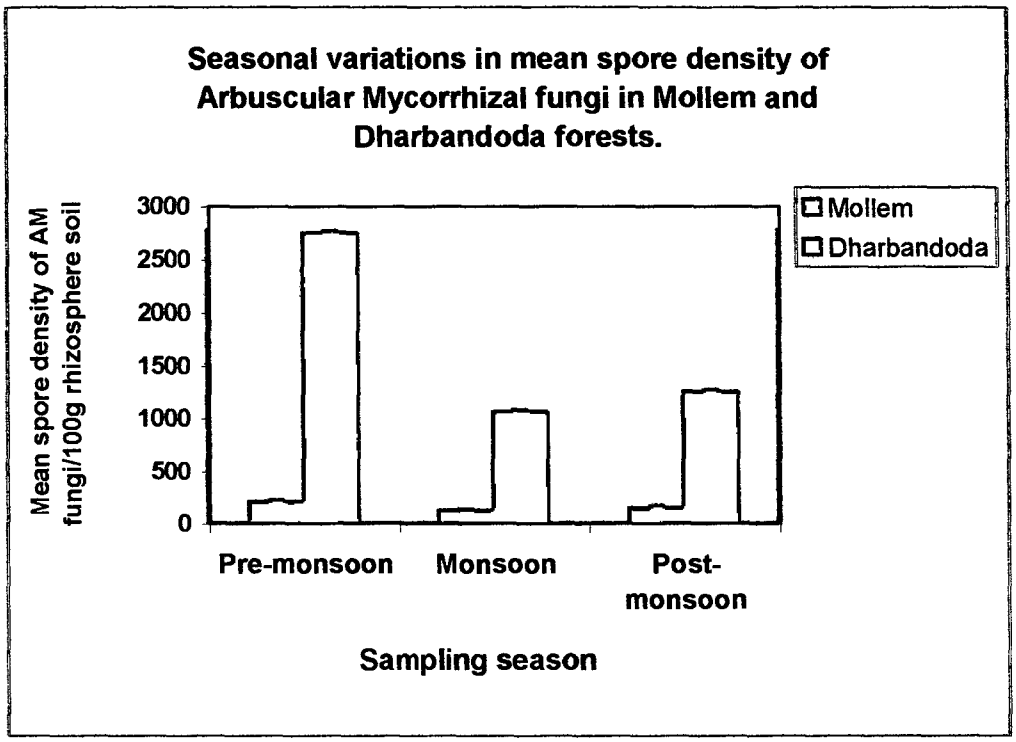

Seasonal variations in mean spore density of Arbuscular Mycorrhizal fungi in Mollem and Dharbandoda forests.
Mean spore density of AM fungi/100g rhizosphere soil
3000
2500
2000
1500
1000
500
0
Mollem
Dharbandoda
Pre-monsoon
Monsoon
Post-monsoon
Sampling season

Fig. 6

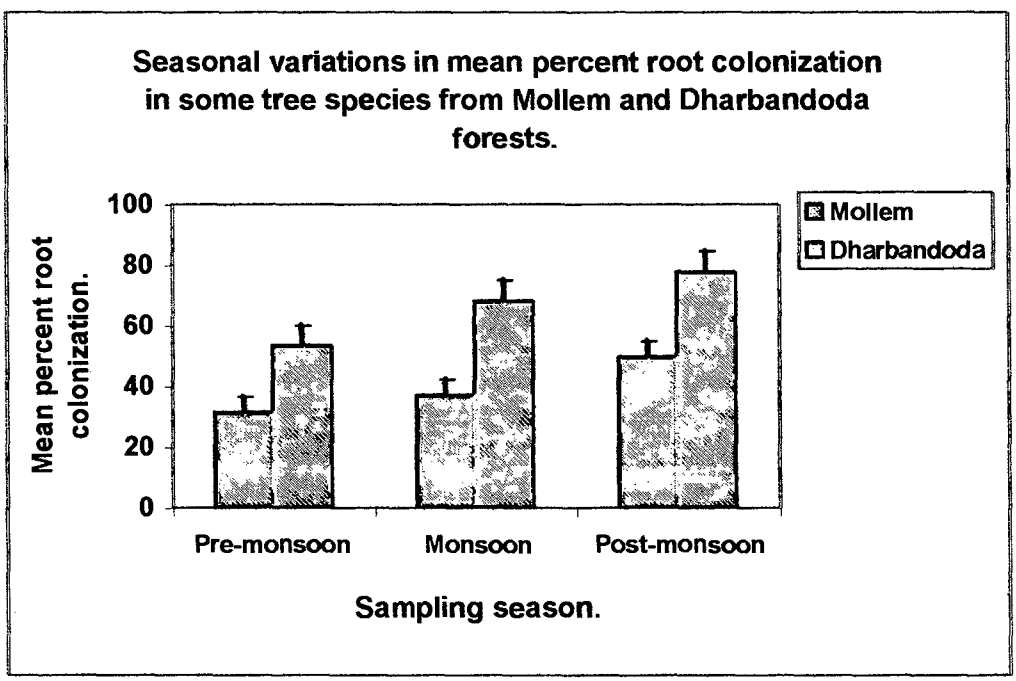

Seasonal variations in mean percent root colonization in some tree species from Mollem and Dharbandoda forests.
Mean percent root colonization.
100
80
60
40
20
0
Mollem
Dharbandoda
Pre-monsoon
Monsoon
Post-monsoon
Sampling season.

Fig. 7

In the present study variation in spore density and root colonization can be attributed to seasonal changes. Earlier studies also indicated that seasonal variations can have remarkable influence in the occurrence of arbuscular mycorrhizal spores (Nicolson & Johnston, 1979). Muthukumar *et al.*, (1998) suggested that the spore production of arbuscular mycorrhizal colonization was strongly influenced by environmental factors together with host species and soil types. Khan (1974) reported that the type of soil influenced the spread of arbuscular mycorrhizal fungi. Also, within environmental factors, seasonal variations play an important role in influencing mycorrhizal symbiosis. According to Hayman (1974) seasonal variations in both climatic and edaphic factors play a key role in controlling sporulation of arbuscular mycorrhizal fungi. The distribution of spores in the rhizosphere and arbuscular mycorrhizal colonization of roots is affected by soil moisture and seasons (Khan, 1974).

In the present study it was observed that when the number of spores in soil was high, the frequency of colonization was low and vice versa. Luis and Lim (1987) also observed that high root colonization followed high number of spores in the surrounding soil. Several factors could contribute to such a pattern including seasonal variation in the development of the host plant (Giovannetti, 1985) and seasonal nutrient availability (Louis and Lim, 1987).

The present study revealed that arbuscular mycorrhizal colonization was minimum in the pre-monsoon season (dry season). Newman *et al.* (1986) found a reduction in percentage of colonization intensity during dry season. Similar

Table 16: Mean spore density and percent root colonization for post-monsoon season in some tree species from Mollem and Dharbandoda forest areas.

Sr. No	Tree species	Family	Mean spore density100g^{-1} soil*		Root colonization* (%)	
			Mollem	**Dharbandoda**	**Mollem**	**Dharbandoda**
1.	*Terminalia paniculata*	Combretaceae	118.66 ± 10.29	1720.50 ± 62.24	54.33 ± 6.34	82.33 ± 3.44
2.	*Terminalia crenulata*	Combretaceae	188.66 ± 11.79	1648.50 ± 94.23	44 .61± 8.54	77.83 ± 6.91
3.	*Strychnos nux-vomica*	Loganiaceae	190.00 ± 18.41	1112.33 ± 63.08	65 22 ± 9.07	74.50 ± 6.74
4.	*Buchnania cochinchinensis*	Anacardiaceae	182.33 ± 10.98	1032.16 ± 86.34	47.56 ± 9.95	79.83 ± 3.43
5.	*Careya arborea*	Lecythidaceae	127.66 ± 14.55	904.83 ± 52.34	39.11± 9.35	77.50 ± 7.76
6.	*Ervatamia heyneana*	Apocynaceae	152.00 ± 16.05	1304.83 ± 48.03	54.23 ± 10.28	74.66 ± 10.25
7.	*Xylia xylocarpa*	Mimosaceae	177.83 ± 12.31	1140.16 ± 34.41	46.79 ± 12.89	70.83 ± 8.28
8.	*Holarrhena pubescens*	Apocynaceae	124.00 ± 11.59	1128.83± 65.01	43.71 ± 12.25	79.66 ± 6.05
Mean			**153.71 ± 13.24**	**1248.23 ± 19.56**	**49.44 ± 2.07**	**77.53 ± 2.32**

*Values are mean of three readings.
± Indicates Standard deviation.

observations were made by Chinnery *et al.*, (1987) in sugarcane in Barbados. Giovannetti (1985) recorded reduced arbuscular mycorrhizal colonization during summer drought. Redhead, (1975) found that arbuscular mycorrhizal colonization tended to decrease under low rainfall. Earlier studies have shown that rainfall or soil wetting could stimulate AM fungal spore germination (Gemma and Koske, 1988; Braunberger *et al.*, 1994), which is in accordance with present study.

The present study indicated that among climatic factors, temperature, rainfall and relative humidity played an important in root colonization and spore formation. The influence of temperature on arbuscular mycorrhizal colonization and sporulation has been widely reported (Allen, 1983). The influence of atmospheric temperature on mycorrhizal colonization and spore number may be due to its effects on soil temperature, moisture and host plant growth (Haugen and Smith, 1992).

It was also observed that the spore density was minimum in the monsoon season and gradually increased in post-monsoon (winter season) and was maximum in the pre-monsoon (summer season). Similar observations have been made by Bhaskaran and Salvaraj (1997) who reported minimum number of spores in November and thereafter the spore number gradually increased. Differences in host plants and soil fertility stimulate differential sporulation by arbuscular mycorrhizal fungi in the field (Schenck and Kinlock, 1980). The spore population is found to have seasonal variations and also varies according to different hosts and also different sites (Sparling and Tinker, 1975). Friese and Allen (1991) and, Sanders and Fitters (1992) suggested that the structure and intensity of arbuscular mycorrhizal colonization varies within plant population and

depends on factors such as type and spatial availability of inoculum, season, stage of plant development, susceptibility to inoculation and plant nutritional status.

A total of thirty mycorrhizal fungal species from Mollem and twenty four species of mycorrhizal fungal species from Dharbandoda belonging to five genera *viz., Acaulospora, Gigaspora, Glomus, Sclerocystis* and *Scutellospora* were recorded.

Identified arbuscular mycorrhizal fungi include *Scutellospora coralloidea,. Sclerocystis sinuosa, Sclerocystis taiwanensis, Sclerocystis rubiformis Glomus ambisporum, Glomus fasciculatum, Glomus geosporum, Glomus heterosporum, Glomus hoi, Glomus macrocarpum, Glomus australe, Glomus caledonium, Glomus claroideum, Glomus clarum, Glomus dimorphicum, Glomus etunicatum, Glomus intraradix, Glomus monosporum, Glomus mosseae, Glomus pansihalos, Glomus pustulatum, Glomus versiforme, Glomus globiferum,Gigaspora decipiens, Gigaspora margarita, Acaulospora foveata,. Acaulospora gerdmannii, Acaulospora laevis, Acaulospora rugosa, Acaulospora scrobiculata, Acaulospora spinosa*e **(Table 17 & 18)**.

At Mollem, *Acaulospora scrobiculata* was the most frequently occurring arbuscular mycorrhizal fungi in all the seasons with frequency of occurrence being 87.50% in pre-monsoon, 75% in monsoon and 50% in post-monsoon. Among the other arbuscular mycorrhizal fungal species, the presence of *Scutellospora coralloidea, Sclerocystis taiwanensis, Glomus pustulatum, Glomus versiforme, Glomus monosporum, Glomus mosseae, Glomus intraradix, Glomus hoi, Glomus heterosporum,*

Glomus geosporum, Glomus globiferum, Glomus fasciculatum, Glomus etunicatum, Glomus caledonium, Glomus claroideum, Glomus ambisporum, Gigaspora margarita, Acaulospora spinosa, Acaulospora scrobiculata and *Acaulospora foveata* was recorded in all the three seasons. The more frequently occurring fungi in pre-monsoon collections include *Acaulospora scrobiculata* and *Glomus fasciculatum* each with 87.5% frequency of occurrence. *Acaulospora scrobiculata* and *Glomus mosseae* were the most frequently occurring fungi in monsoon collections with 75% frequency of occurrence while *Glomus fasciculatum* was the most frequently occurring fungi in post-monsoon collections each with 62.5% frequency of occurrence **(Table 17).**

At Darbandoda, *Acaulospora spinosa* was the most frequently occurring arbuscular mycorrhizal fungi in all the seasons with frequency of occurrence being 75% in pre-monsoon, 62.5% in monsoon and 37.5% in post-monsoon. Among the other arbuscular mycorrhizal fungal species, the presence of *Scutellospora rubiformis, Scutellospora sinuosa, Sclerocystis taiwanensis, Glomus pustulatum, Glomus mosseae, Glomus heterosporum, Glomus macrocarpum Glomus geosporum, Glomus globiferum, Glomus pansihalos, Glomus fasciculatum, Glomus caledonium, Glomus claroideum, Gigaspora decipiens, Acaulospora scrobiculata Acaulospora laevis* and *Acaulospora foveata* was recorded in all the three seasons. The more frequently occurring fungi in pre-monsoon collections includes *Acaulospora spinosa* and *Glomus fasciculatum* each with 75% frequency of occurrence. *Acaulospora spinosa* was the most frequently occurring fungi in monsoon collections with 62.5% frequency of occurrence while *Acaulospora scrobiculata* and *Glomus globiferum* were the most frequently occurring fungi in post-monsoon collections each with 50% frequency of occurrence **(Table 18).**

In Mollem, at species level *Acaulospora scrobiculata* and *Glomus fasciculatum* were most abundant in pre-monsoon collections each with a relative abundance of 6.93%. Three mycorrhizal fungi species *viz., Acaulospora scrobiculata, Glomus etinucatum* and *Glomus mosseae* were most abundant in monsoon collections each with a relative abundance of 8.21% while in post monsoon *Glomus fasciculatum* was the most abundant in post-monsoon collections with a relative abundance of 9.80% **(Table 19).**

In Darbandoda, at species level *Acaulospora spinosa* and *Glomus fasciculatum* were most abundant in pre-monsoon collections each with a relative abundance of 7.50%. *Acaulospora spinosa* was most abundant in monsoon collections with a relative abundance of 7.81% while in post monsoon *Acaulospora scrobiculata* and *Glomus globiferum* were the most abundant in post-monsoon collections with a relative abundance of 8.33% **(Table 20).**

Quantitative assessment of fungal diversity currently relies solely on enumeration of the spores of the organisms forming mycorrhizae in plant communities (Koske, 1987). Seasonal changes and phenological state of the host influence mycorrhizal colonization as well as sporulation (Giovannetti, 1985), although vegetative growth of the fungi and their capacity to sporulate are not necessarily correlated (Abbott and Robson, 1991). Thus, the absence of sporulation does not indicate the absence of fungal organism. The presence of spores does indicate,

however, that mycorrhizal biomass of detected organism has reached critical mass of sporulation and quantity of spores may provide some measure of fungal fitness.

Table 17: Seasonal variation of frequency of occurrence of AM species in rhizosphere soils of tree species in Mollem forest.

Sr.No.	Name of AM species	Seasonal Frequency of occurrence of AM fungi (%)		
		Pre-monsoon	Monsoon	Post-monsoon
1.	*Acaulospora foveata*	62.5	50.0	50.0
2.	*A. gerdmannii*	12.5	-	-
3.	*A. laevis*	25.0	25.0	-
4.	*A. rugosa*	-	-	-
5.	*A. scrobiculata*	87.5	75.0	50.0
6.	*A. spinosa*	75.0	37.5	37.5
7.	*Gigaspora decipiens*	37.5	-	-
8.	*G. margarita*	25.0	25.0	25.0
9.	*Glomus ambisporum*	62.5	25.0	37.5
10.	*G. australe*	25.0	37.5	-
11.	*G. caledonium*	37.5	25.0	25.0
12.	*G. claroideum*	25.0	25.0	25.0
13.	*G. clarum*	25.0	-	12.5
14.	*G. dimorphicum*	25.0	12.5	-
15.	*G. etunicatum*	75.0	62.5	12.5
16.	*G. fasciculatum*	87.5	37.5	62.5
17.	*G. geosporum*	37.5	25.0	37.5
18.	*G. globiferum*	75.0	50.0	25.0
19.	*G. heterosporum*	37.5	25.0	37.5
20.	*G. hoi*	25.0	12.5	12.5
21.	*G. intraradix*	50.0	37.5	37.5
22.	*G. macrocarpum*	25.0	25.0	-
23.	*G. monosporum*	75.0	62.5	50
24.	*G. mosseae*	62.5	75.0	25
25.	*G. pansihalos*	25.0	25.0	-
26.	*G. pustulatum*	37.5	25.0	12.5
27.	*G. versiforme*	25.0	12.5	12.5
28.	*Sclerocystis rubiformis*	37.5	25.0	-
29.	*S. sinuosa*	37.5	-	25.0
30.	*S. taiwanensis*	25.0	25.0	25.0
31.	*Scutellospora coralloidea*	25.0	25.0	25.0

Table 18: Seasonal variation of frequency of occurrence of AM species in rhizosphere soils of tree species in Dharbandoda forest.

Sr.No	Name of AM species	Seasonal Frequency of occurrence of AM fungi (%)		
		Pre-monsoon	Monsoon	Post-monsoon
1.	*Acaulospora foveata*	62.5	50.0	37.5
2.	*A. gerdmannii*	-	-	-
3.	*A. laevis*	50.0	37.5	37.5
4.	*A. rugosa*	-	50.0	37.5
5.	*A. scrobiculata*	62.5	50.0	50.0
6.	*A. spinosa*	75.0	62.5	37.5
7.	*Gigaspora decipiens*	25.0	25.0	12.5
8.	*G. margarita*	25.0	-	-
9.	*Glomus ambisporum*	-	-	-
10.	*G. australe*	25.0	-	37.5
11.	*G. caledonium*	62.5	25.0	25.0
12.	*G. claroideum*	62.5	50.0	37.5
13.	*G. clarum*	-	-	-
14.	*G. dimorphicum*	-	-	-
15.	*G. etunicatum*	-	-	-
16.	*G. fasciculatum*	75.0	50.0	37.5
17.	*G. geosporum*	50.0	50.0	25.0
18.	*G. globiferum*	37.5	37.5	50.0
19.	*G. heterosporum*	37.5	25.0	25.0
20.	*G. hoi*	25.0	25.0	-
21.	*G. intraradix*	-	-	-
22.	*G. macrocarpum*	37.5	25.0	25.0
23.	*G. monosporum*	25.0	50.0	-
24.	*G. mosseae*	62.5	25.0	12.5
25.	*G. pansihalos*	25.0	37.5	37.5
26.	*G. pustulatum*	37.5	25.0	25.0
27.	*G. versiforme*	12.5	-	-
28.	*Sclerocystis rubiformis*	25.0	25.0	25.0
29.	*S. sinuosa*	37.5	25.0	25.0
30.	*S. taiwanensis*	37.5	25.0	25.0
31.	*Scutellospora coralloidea*	25.0	25.0	-

Table 19 : Seasonal variation of Relative Abundance of AM species in rhizosphere soils of tree species in Mollem forest.

Sr.No.	Name of AM species	Seasonal variations in Relative Abundance of AM fungi (%)		
		Pre-monsoon	Monsoon	Post-monsoon
1.	*Acaulospora foveata*	4.95	5.47	7.84
2.	*A. gerdmannii*	0.99	-	-
3.	*A. laevis*	1.98	2.73	-
4.	*A. rugosa*	-	-	-
5.	*A. scrobiculata*	6.93	8.21	7.84
6.	*A. spinosa*	5.94	4.10	5.88
7.	*Gigaspora decipiens*	2.97	-	-
8.	*G. margarita*	0.99	2.73	3.92
9.	*Glomus ambisporum*	4.95	5.47	5.88
10.	*G. australe*	1.98	4.10	-
11.	*G. caledonium*	2.97	2.73	3.92
12.	*G. claroideum*	1.98	2.73	3.92
13.	*G. clarum*	1.98	-	1.96
14.	*G. dimorphicum*	1.98	1.36	-
15.	*G. etunicatum*	5.94	8.21	1.96
16.	*G. fasciculatum*	6.93	4.10	9.80
17.	*G. geosporum*	2.97	2.73	5.88
18.	*G. globiferum*	5.94	5.47	3.92
19.	*G. heterosporum*	2.97	2.73	1.96
20.	*G. hoi*	1.98	1.36	1.96
21.	*G. intraradix*	3.96	4.10	5.88
22.	*G. macrocarpum*	1.98	2.73	-
23.	*G. monosporum*	5.94	5.47	7.84
24.	*G. mosseae*	4.95	8.21	3.92
25.	*G. pansihalos*	1.98	2.73	-
26.	*G. pustulatum* —	1.98	2.73	1.96
27.	*G. versiforme*	1.98	1.36	1.96
28.	*Sclerocystis rubiformis*	2.97	2.73	-
29.	*S. sinuosa*	2.97	-	3.92
30.	*S. taiwanensis*	2.97	2.73	3.92
31.	*Scutellospora coralloidea*	2.97	2.73	3.92

Table 20 : Seasonal variation of Relative Abundance of AM species in rhizosphere soils of tree species in Dharbandoda forest.

Sr.No.	Name of AM species	Seasonal variations in Relative Abundance of AM fungi (%)		
		Pre-monsoon	Monsoon	Post-monsoon
1.	*Acaulospora foveata*	6.25	6.25	6.25
2.	*A. gerdmannii*	-	-	-
3.	*A. laevis*	5.00	4.68	6.25
4.	*A. rugosa*	-	6.25	6.25
5.	*A. scrobiculata*	6.25	6.25	8.33
6.	*A. spinosa*	7.50	7.81	6.25
7.	*Gigaspora decipiens*	2.50	3.12	2.08
8.	*G. margarita*	2.50	-	-
9.	*Glomus ambisporum*	-	-	-
10.	*G. australe*	2.50	-	6.25
11.	*G. caledonium*	6.25	3.12	4.16
12.	*G. claroideum*	6.25	6.25	6.25
13.	*G. clarum*	-	-	-
14.	*G. dimorphicum*	-	-	-
15.	*G. etunicatum*	-	-	-
16.	*G. fasciculatum*	7.50	6.25	6.25
17.	*G. geosporum*	5.00	6.25	4.16
18.	*G. globiferum*	3.75	4.68	8.33
19.	*G. heterosporum*	3.75	3.12	4.16
20.	*G. hoi*	2.50	3.12	-
21.	*G. intraradix*	-	-	-
22.	*G. macrocarpum*	3.75	3.12	4.16
23.	*G. monosporum*	2.50	6.25	-
24.	*G. mosseae*	6.25	3.12	2.08
25.	*G. pansihalos*	2.50	4.68	4.16
26.	*G. pustulatum*	3.75	3.12	4.16
27.	*G. versiforme*	1.25	-	-
28.	*Sclerocystis rubiformis*	2.50	3.12	4.16
29.	*S. sinuosa*	3.75	3.12	2.08
30.	*S. taiwanensis*	3.75	3.12	4.16
31.	*Scutellospora coralloidea*	2.50	3.12	-

however, that mycorrhizal biomass of detected organism has reached critical mass of sporulation and quantity of spores may provide some measure of fungal fitness.

CHAPTER- 4

OCCURRENCE AND DISTRIBUTION OF ARBUSCULAR MYCORRHIZAL FUNGI IN DISTURBED AND UNDISTURBED FOREST AREAS.

INTRODUCTION

Most plants in natural ecosystem have mycorrhizal associations, which involve three-way interaction between fungi, soil and plants (Brundrett, 1991). Consequently, the impact of soil disturbance on this association will depend on the nature of fungal propagules and changes to soil conditions, as well as the influence of any surviving vegetation. The influence of disturbance on symbiosis and secondary plant succession has been studied in variety of ecosystems including arid communities (Reeves *et al.,* 1979), Coal wastes (Daft and Nicolson, 1974; Daft *et al.,* 1975; Daft and Hacskaylo 1976; Khan, 1978), Mine spoils (Marx, 1975; Miller, 1979; Allen and Allen, 1980) and Sand dunes (Nicolson, 1960; Nicolson and Johnston, 1979; Koske and Halvorson, 1981; Sylvia, 1986; Gemma and Koske, 1988).

Most studies of mycorrhizal associations in highly disturbed habitat such as mine sites have found reduced levels of mycorrhizal propagules (Danielson, 1985; Jasper *et al.*, 1992; Pfleger *et al.*, 1994). Less severe forms of soil disturbance, including agricultural village, soil animal activities, fire and erosion can also have a detrimental effect on mycorrhizal associations (Habte *et al.*, 1988; O'Halloran *et al.*, 1986; Read and Birch, 1988; Vilarino and Arines, 1991).

Propagules of mycorrhizal fungi are consumed or parasitized by a wide range of microorganisms and larger animals (Brundrett, 1991). Dramatic changes to soil conditions such as temperature extremes and anaerobic conditions, which

may occurs in stockpiled top soil may themselves be responsible for the death of fungal structures and further attrition could be cause by the ageing of propagates, or by their premature germination (Malajczuk *et al.*, 1994). Typical changes that occur when soil is disturbed and stockpiled include the loss of organic matter and nutrients, as well as structural and biological components (Abdul-Karim and McRae, 1984; Danielson, 1985). There are also cases where the substrata to be revegetated (such as waste rock dump material) would initially be devoid of soil organism.

The capacity of propagules of mycorrhizal fungi to persist in soil without roots, tolerate disturbance, resist predation by soil organisms and dispersal in new location will determine the outcome of the disturbance on mycorrhizal associations, but are not well understood (Malajczuk *et al.*, 1994). In undisturbed natural communities, a network of hyphae is believed to be primarily responsible for the spread of mycorrhizal fungi to new roots (Brundrett, 1991; Read and Birch, 1988).

The present chapter gives a comparative account on the distribution and occurrence of arbuscular mycorrhizal fungi in disturbed and undisturbed forest areas.

PLATE- X

GENERAL VIEW OF DISTURBED AND UNDISTURBED SITE.

a) **Undisturbed forest site at Mollem.**

b) **Disturbed forest site at Collem.**


a


b

MATERIALS AND METHODS

STUDY SITE

Two sites *viz.*, Mollem and Collem, both forest areas situated in Sanguem taluka, North Goa were selected for the study. Mollem, which harbours wild life sanctuary and declared as reserve forest was selected as undisturbed forest while, Collem where iron ore mining operations are carried out was taken as disturbed site for the present study **(Plate X).**

SAMPLING TECHNIQUES

In all, six quadrats each of size 100 m x 100 m were laid each separated from the other by at least 50 metres. A total of eight tree species common to all the quadrats *viz.*, *Terminalia crenulata,* Roth, *Terminalia paniculata* Roth, *Strychnos nux-vomica* L, *Buchnania cochinchinensis,* (Lour) Almeida, *Careya arborea* Roxb, *Ervatamia heyneana,* (Wall) Cooke., *Xylia xylocarpa* Taub and *Holarrhena pubescens* (Buch.-Ham.) Wallich ex Don were selected for the study.

PLANT NOMENCLATURE

Plants selected in the present study were identified using floras (Rao, 1985 and 1986; Mathew, 1992; Mohanan and Henry, 1994; and Naithani *et al.*, 1997).

SOIL ANALYSIS

For analyzing soil pH, EC, Organic carbon, Available phosphorus, Total N, P and K content, the soil samples were collected from a depth of 0-25 cm from eight different locations for each quadrate at the study site and were brought to the laboratory in polyethylene bags. Samples from each quadrate were passed through 2mm sieve to remove the larger soil particles and were mixed thoroughly to obtain a composite sample. Later, each composite sample was processed three times to get the mean value. This was repeated for all the six quadrates and the average mean value was obtained for each quadrate.

Soil pH was measured after dilution with distilled water (1:1 w/v soil: water) soon after the samples were brought to the laboratory. Electrical Conductivity (EC) was determined in 1:1 water: waste extracts (Bower and Wilcox, 1965). Soil nitrogen was determined by micro-Kjeldahl method (Jackson, 1971). Soil phosphorus was determined by molybdenum blue method (Jackson, 1971). Potassium was estimated after extraction in ammonium acetate solution (Jackson, 1971). Organic carbon was detected by Walky and Black's rapid titration method (1963). Available phosphorus was estimated by Olsen's method.

ESTIMATION OF ROOT COLONIZATION

Rhizosphere soil and root samples were collected from all the eight tree species from each quadrat and worked out separately for spore density and root colonization. Rhizosphere soil samples were collected in the post-monsoon

season from the selected tree species at each site. For each tree species, rhizosphere soil samples were collected randomly from three individuals of the species. Later, a composite sample was made for each species and worked out. Rhizosphere soil samples were excavated from a depth of 0-25cm, placed in plastic bags, labeled and stored at 10^0 C until processed. While collecting soil samples, soil was dug at three places for each plant so as to cover the entire rhizosphere.

Root samples were brought to the laboratory and washed thoroughly under tap water. Feeder roots were cut into 1cm fragments. The composite sample of each species was cleared in 10% KOH in an autoclave at 121^0C for 15-25 minutes. Cleared roots were thoroughly washed with tap water and acidified with 5% HCl. Later, the roots were stained with 0.05% trypan blue (Phillips and Hayman, 1970). Stained roots were mounted in glycerol and observed under Leica compound microscope. Percent root colonization was estimated by using Slide technique (Giovanetti and Mosse, 1980). For a sample to be regarded as possessing arbuscular mycorrhizal association, it had to be seen to have vesicles, arbuscules or both.

RECOVERY OF ARBUSCULAR MYCORRHIZAL FUNGAL SPORES AND THEIR IDENTIFICATION

Hundred grams of air dried composite sample was assayed for spore count using wet sieving and decanting procedure (Gerdemann and Nicolson, 1963). Spores were separated using sieves having mesh sizes ranging from 38 μm to 500 μm. Each composite sample was processed three times. Spores were observed under

Leica stereomicroscope. Estimation of Spore density was carried out as per the procedure given by Gaur and Adholeya (1994). Only healthy spores were enumerated while broken and parasitized spores were discarded. Spores were mounted in Polyvinyl Lacto Glycerol (PVLG), (Koske and Tessier, 1983), examined for their various characteristics and identified using the standard keys (Schenck and Perez, 1990; Morton and Benny, 1990; Wu, 1993). Spores in the rhizosphere soils were multiplied using *Eleusine coracana* L., *Lycopersicum esculentum* Mill., *Allium cepa* L. and *Coleus sp.* as a host plants. The spores isolated from the pot cultures were later used for confirming the identification of the spores recovered from both the sites.

Relative abundance of spores and Frequency of occurrence for each of the arbuscular mycorrhizal fungal species was calculated for both the sites. Relative abundance and Frequency of occurrence was calculated using the formulas given below:

$$\text{Relative Abundance (\%)} = \frac{\text{Number of AM fungal spores of particular species}}{\text{Total number of AM fungal spores of all species}} \times 100$$

$$\text{Frequency of Occurrence (\%)} = \frac{\text{Number of soil samples that possess spores of particular AM species}}{\text{Total number of soil samples screened}} \times 100$$

RESULTS AND DISCUSSION

Soil analysis results indicated that the soil at Collem was less acidic having a pH of 5.70 with Electrical Conductivity (EC) (0.12 mhos/cm), total nitrogen (0.23%), total potassium (0.18%), total phosphorus (0.20%), Organic carbon (0.52%) and available phosphorus (5.81 Kg/ha). While Mollem soils had pH of 5.28 with Electrical Conductivity (EC) (0.57 mhos/cm), total nitrogen (0.048%), total potassium (0.18%), %), total phosphorus (0.22%), Organic carbon (2.68%) and available phosphorus (49.13 Kg/ha). **(Table 21).**

The average spore density at Mollem was 157.91 spores/100 g soils. Maximum spore number was recorded in *Terminalia crenulata* (188.83 spores/100g soil) while minimum spore number was recorded in *Terminalia paniculata* (119 spores/100g soil). Average root colonization at Mollem was 49.15%. Maximum root colonization was recorded in *Strychnos nux-vomica* (65.33%), while minimum root colonization was recorded in *Careya arborea* (39.50%) **(Table 22).**

The average spore density at Collem was 32.95 spores/100 g soils. Maximum spore number was recorded in *Careya arborea* (46 spores/100g soil) while minimum spore number was recorded in *Holarrhena pubescens* (17 spores/100g soil). Average root colonization at Collem was 37.22%. Maximum

Table 21 : Soil analysis data of disturbed (Collem) and undisturbed (Mollem) forest sites.

Site	pH	E.C. M mhos/cm	Total N_2 (%)	Total P_2O_5 (%)	Total K_2O (%)	Organic Carbon (%)	Available P Kg/ha
Mollem	5.28 ± 0.09	0.57 ± 0.05	0.48 ± 0.04	0.22 ± 0.04	0.18 ± 0.01	2.68 ± 0.04	49.13 ± 1.83
Collem	5.70 ± 0.28	0.12 ± 0.01	0.23 ± 0.04	0.20 ± 0.01	0.18 ± 0.03	0.52 ± 0.10	5.81 ± 0.85

± indicates Standard deviation.

All Values are mean of six readings.

Table 22 : Percent root colonization and mean spore density in some tree species from undisturbed forest site at Mollem .

Sr. No.	Tree species	Family	Root colonization▪ (%)	Spore density100g^{-1} soil▪
1	*Terminalia paniculata, Roth*	Combretaceae	49.25 ± 6.34	119.00 ± 6.29
2	*Terminalia crenulata, Roth*	Combretaceae	43.83 ± 8.54	188.83±13.19
3	*Strychnos nux-vomica L.*	Loganiaceae	65.33 ± 9.07	190.16±10.00
4	*Buchnania cochinchinensis, (Lour) Almeida.*	Anacardiaceae	47.50 ± 9.95	182.16±13.04
5	*Careya arborea, Roxb*	Lecythidaceae	39.50 ± 9.85	128.16± 6.61
6	*Ervatamia heyneana, (Wall) Cooke.*	Apocynaceae	54.33 ± 10.28	151.66±11.86
7	*Xylia xylocarpa, Taub.*	Mimosaceae	46.50 ± 12.89	179.66±14.00
8	*Holarrhena pubescens (Buch.-Ham.) Wallich ex Don*	Apocynaceae	47.00 ± 12.25	123.66± 8.54
Average			**49.15 ± 9.89**	**157.91±10.44**

▪ Values are mean of three readings. ± Indicates Standard deviation.

root colonization was recorded in *Buchnania cochinchinensis* (59.50%), while minimum root colonization was recorded in *Xylia xylocarpa* (19%) **(Table 23) (Fig. 8 & 9).**

At Mollem, *Acaulospora scrobiculata* and *Glomus fasciculatum* were the most frequently occurring arbuscular mycorrhizal fungi each with a frequency of occurrence of 62.50% while at Collem, *Acaulospora scrobiculata* was the most frequently occurring arbuscular mycorrhizal fungi with a frequency of occurrence of 37.50%. In all, a total of 18 arbuscular mycorrhizal fungal species were found to be common at both the disturbed and undisturbed sites. These include The more frequently occurring fungi in pre-monsoon collections include *Acaulospora scrobiculata, A. foveata, A. spinosa, Gigaspora decipiens, G. margarita, Glomus ambisporum, G. caledonium, G. claroideum, G. clarum, G. fasciculatum, G. geosporum, G. heterosporum, G. mosseae, G. pansihalos, G. versiforme, Sclerocystis rubiformis, S. sinuosa* and *Scutellospora coralloidea*. In all, a total of 12 arbuscular mycorrhizal fungal species were exclusively found in Mollem forest. These include *Acaulospora gerdmanni, A. laevis, Glomus australe, G. dimorphicum, G. etunicatum, G. globiferum, G. hoi, G. intraradix, G. macrocarpum, G. monosporum, G. pustulatum* and *Sclerocystis taiwanensis.* Similarly, a total of only 2 arbuscular mycorrhizal fungal species *viz., Scutellospora gregaria* and *S. pellucida* were exclusively found in Collem forest **(Table 24).**

In Mollem, at species level *Acaulospora scrobiculata* and *Glomus fasciculatum* were most abundant each with a relative abundance of 7.24%, while at

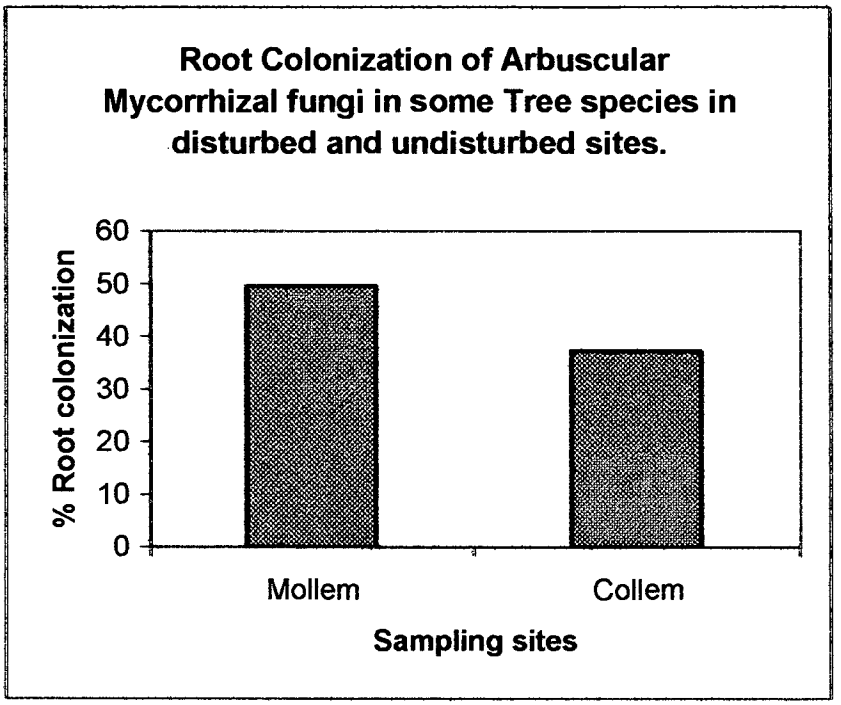

Root Colonization of Arbuscular Mycorrhizal fungi in some Tree species in disturbed and undisturbed sites.
% Root colonization
60
50
40
30
20
10
0
Mollem
Collem
Sampling sites

Fig. 8

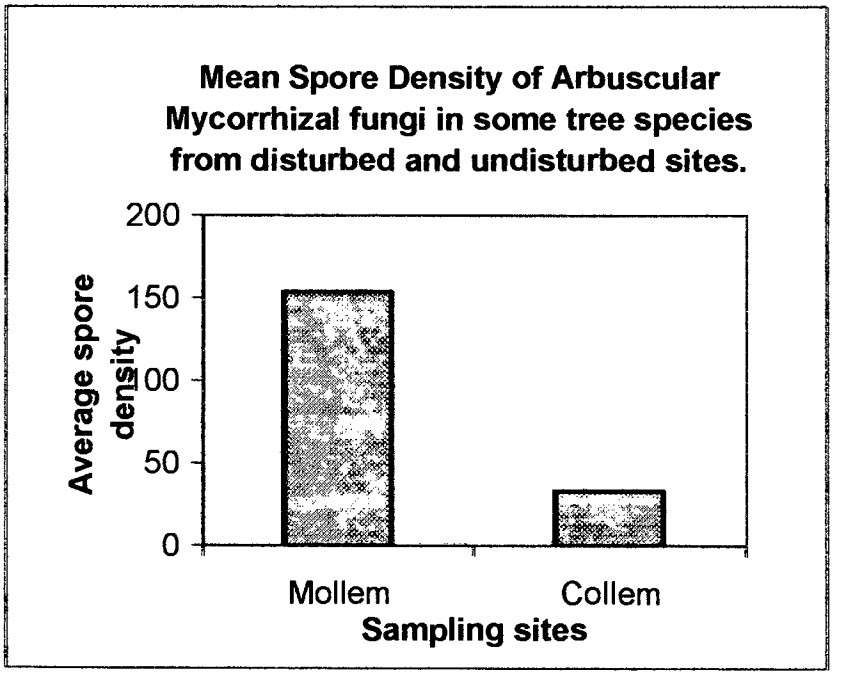

Mean Spore Density of Arbuscular Mycorrhizal fungi in some tree species from disturbed and undisturbed sites.
Average spore density
200
150
100
50
0
Mollem
Collem
Sampling sites

Fig. 9

Table 23 : Percent root colonization and mean spore density in some tree species from disturbed forest site at Collem .

Sr. No.	Tree species	Family	Root colonization▪ (%)	Spore density100g^{-1} soil▪
1	*Terminalia paniculata,* Roth	Combretaceae	42.33 ± 15.20	45.00 ± 8.56
2	*Terminalia crenulata,* Roth	Combretaceae	35.83 ± 7.78	37.00 ± 8.56
3	*Strychnos nux-vomica* L.	Loganiaceae	33.50 ± 5.54	21.83 ± 2.82
4	*Buchnania cochinchinensis,* (Lour) Almeida.	Anacardiaceae	59.50 ± 11.00	20.83 ± 3.71
5	*Careya arborea,* Roxb	Lecythidaceae	43.66 ± 5.78	46.00 ± 9.54
6	*Ervatamia heyneana,* (Wall) Cooke.	Apocynaceae	37.00 ± 11.20	39.33 ± 3.98
7	*Xylia xylocarpa,* Taub.	Mimosaceae	19.00 ± 4.51	36.66 ± 6.65
8	*Holarrhena pubescens* (Buch.-Ham.) Wallich ex Don	Apocynaceae	27.00 ± 5.47	17.00 ± 3.57
Average			**37.22 ±8.31**	**32.95 ±5.92**

▪ Values are mean of three readings. ± Indicates Standard deviation.

Table 24: Frequency of occurrence of AM species in rhizosphere soils of various tree species at Mollem and Collem forest areas.

Sr.No	Name of AM species	Frequency of occurrence of AM fungi (%)	
		Mollem	Collem
1.	*Acaulospora foveata*	50.00	25.00
2.	*A. gerdmannii*	12.50	-
3.	*A. laevis*	25.00	-
4.	*A. scrobiculata*	62.50	37.50
5.	*A. spinosa*	50.00	12.50
6.	*Gigaspora decipiens*	37.50	12.50
7.	*G. margarita*	37.50	12.50
8.	*Glomus ambisporum*	37.50	12.50
9.	*G. australe*	37.50	-
10.	*G. caledonim*	12.50	12.50
11.	*G. claroideum*	12.50	25.00
12.	*G. clarum*	12.50	12.50
13.	*G. dimorphicum*	25.00	-
14.	*G. etunicatum*	12.50	-
15.	*G. fasciculatum*	62..50	12.50
16.	*G. geosporum*	25.00	12.50
17.	*G. globiferum*	50.00	-
18.	*G. heterosporum*	12.50	12.50
19.	*G. hoi*	12.50	-
20.	*G. intraradix*	37.50	-
21.	*G. macrocarpum*	37.50	-
22.	*G. monosporum*	35.50	-
23.	*G. mosseae*	25.00	12.50
24.	*G. pansihalos*	12.50	12.50
25.	*G. pustulatum*	12.50	-
26.	*G. versiforme*	12.50	12.50
27.	*Sclerocystis rubiformis*	12.50	50.00
28.	*S. sinuosa*	50.00	12.50
29.	*S. taiwanensis*	12.50	-
30.	*Scutellospora gregaria*	-	12.50
31.	*S. pellucida*	-	12.50
32.	*S. corolloidea*	25.00	12.50

Collem, at species level *Sclerocystis rubiformis* was most abundant with a relative abundance of 14.81% **(Table 25).**

At Mollem, maximum arbuscular mycorrhizal species were recorded in *Ervatamia heyneana* (15) while minimum arbuscular mycorrhizal species (4) were recorded in *Terminalis crenulata* with the number of species given in parenthesis **(Table 26)**. At Collem, maximum arbuscular mycorrhizal fungal species were recorded in *Terminalis crenulata* (5) and *Strychnos nux-vomica* (5), while minimum arbuscular mycorrhizal fungal species (2) were recorded in *Ervatamia heyneana* with the number of species given in parenthesis **(Table 27)**.

There were distinct differences in soil properties of both the sites. When disturbed site with sparse vegetation was compared with undisturbed site with dense vegetation cover, increase in soil N, Soil P, Soil K, Available P and Organic carbon observed in the later. Similar observations have been recorded earlier by Brundrett *et al.*, (1996a) in his studies on bioassay of arbuscular mycorrhizal fungal propagules from disturbed and natural habitats in soils from Tropical Australia. It is found that the surface soil of natural forest are more acidic than the surface soil of disturbed site, this may be due to the presence of great amount of tannic and humic acids resulting from more active microbial decomposition process in the natural forest.

Table 25: Relative abundance of AM species in rhizosphere soils of various tree species at Mollem and Collem forest areas.

Sr.No	Name of AM species	Relative abundance (%)	
		Mollem	Collem
1.	*Acaulospora foveata*	5.79	7.40
2.	*A. gerdmannii*	1.44	-
3.	*A. laevis*	2.89	-
4.	*A. scrobiculata*	7.24	3.70
5.	*A. spinosa*	5.79	10.0
6.	*Gigaspora decipiens*	4.34	3.70
7.	*G. margarita*	4.34	3.70
8.	*Glomus ambisporum*	4.34	3.70
9.	*G. australe*	4.34	-
10.	*G. caledonim*	1.44	3.70
11.	*G. claroideum*	1.44	7.40
12.	*G. clarum*	1.44	3.70
13.	*G. dimorphicum*	2.89	-
14.	*G. etunicatum*	1.44	-
15.	*G. fasciculatum*	7.24	3.70
16.	*G. geosporum*	2.89	3.70
17.	*G. globiferum*	5.79	-
18.	*G. heterosporum*	1.44	3.70
19.	*G. hoi*	1.44	-
20.	*G. intraradix*	4.34	-
21.	*G. macrocarpum*	4.34	-
22.	*G. monosporum*	4.34	-
23.	*G. mosseae*	2.89	3.70
24.	*G. pansihalos*	1.44	3.70
25.	*G. pustulatum*	1.44	-
26.	*G. versiforme*	1.44	3.70
27.	*Sclerocystis rubiformis*	1.44	14.81
28.	*S. sinuosa*	5.79	3.70
29.	*S. taiwanensis*	1.44	-
30.	*Scutellospora gregaria*	-	3.70
31.	*S. pellucida*	-	3.70
32.	*S. corolloidea*	2.89	3.70

Table 26: Arbuscular mycorrhizal fungal species diversity in some tree species from Mollem forest.

Sr. No.	Name of Species	Arbuscular mycorrhizal species
1.	*Terminalia paniculata* Roth.	*Acaulospora scrobiculata, A. spinosa, Gigaspora margarita, Glomus dimorphicum, Glomus australe, G. claroideum, G. fasciculatum, G. globiferum, G. macrocarpum, G. mosseae, Sclerocystis sinuosa, Scutellopsora corolloidea.*
2.	*Terminalia crenulata* Roth	*Glomus fasciculatum, G. intraradix, Acaulspora foveata, A. spinosa.*
3.	*Strychnos nux-vomica* L.	*Acaulospora scrobiculata, A. laevis A. spinosa, Gigaspora decipiens, Glomus ambisporum, G. etunicatum, G. globiferum, G. monosporum, Sclerocystis sinuosa, S. taiwanensis.*
4.	*Buchanania cochinchinensis* (Lour.) Almeida.	*Acaulospora foveata, A. scrobiculata, Gigaspora decipiens, Glomus ambisporum, G. intraradix, G. caledonium, G. fasciculatum, G. macrocarpum.*
5.	*Careya arborea* Roxb.	*Acaulospora scrobiculata, Gigaspora decipiens, Glomus ambisporum, G. fasciculatum, G. geosporum, G. globiferum, G. monosporum, G. mosseae, G. pansihalos, Sclerocystis sinuosa.*
6.	*Ervatamia heyneana* (Wall) Cooke.	*Acaulospora. foveata, A. laevis, A. scrobiculata, A. laevis, Gigaspora margarita, Glomus ambisporum, G. clarum, G. dimorphicum, G. intraradix, G. Australe, G. fasciculatum, G. mosseae, Sclerocystis rubiformis, S. sinuosa, Scutellopsora corolloidea.*

Contd.

7.	*Xylia xylocarpa* Taub.	*Acaulospora foveata, A. spinosa, A. gerdmanni, A. scrobiculata, Gigaspora margarita, Glomus etunicatum, Glomus australe, G. fasciculatum, G. geosporum, G. globiferum, G. monosporum, G. mosseae, G. intraradix.*
8.	*Holarrhena pubescens (Buch.-Ham.) Wallich ex Don*	*Acaulospora scrobiculata, A. spinosa, Glomus etunicatum, G. hoi, G. macrocarpum, G. mosseae, G. versiforme, G. heterosporum, G. pustulatum.*

Table 27: Arbuscular mycorrhizal fungal species diversity in some tree species from Collem forest.

Sr. No.	Name of Species	Arbuscular mycorrhizal species
1.	*Terminalia paniculata* Roth.	*Glomus claroideum, G. fasciculatum, Acaulospora foveata, A. scrobiculata.*
2.	*Terminalia crenulata* Roth	*Glomus ambisporum, G. caledoneum, G. clarum, Acaulospora spinosa, Sclerocystis rubiformis.*
3.	*Strychnos nux-vomica* L.	*Glomus geosporum, Acaulospora scrobiculata, Sclerocystis rubiformis, Scutellospora coralloidea, S. pellucida.*
4.	*Buchanania cochinchinensis* (Lour.) Almeida.	*Glomus heterosporum, Gigaspora margarita, Acaulospora scrobiculata, Sclerocystis rubiformis.*
5.	*Careya arborea* Roxb.	*Glomus claroideum, G. moseae, Sclerocystis sinuosa.*
6.	*Ervatamia heyneana* (Wall) Cooke.	*Glomus pansihalos, G. mosseae.*
7.	*Xylia xylocarpa* Taub.	*Glomus geosporum, G. versiforme, Scutellospora gregaria.*
8.	*Holarrhena pubescens (Buch.-Ham.) Wallich ex Don*	*Glomus claroideum, Gigaspora decipiens, Acaulospora foeveta, Sclerocystis rubiformis.*

It is observed from the study that the plant species belonging to both disturbed and undisturbed site are mycorrhizal. Daft and Nicolson (1974) studied plants on coal wastes in Scotland and found that arbuscular mycorrhizal colonization occurs on more successful plants on mine spoils. Similar observations have been made in Pennsylvania coal spoils (Daft and Hacskaylo, 1976). Cress and Aldon (1977) have suggested that endomycorrhizae may be essential for reclamation of coal mining areas.

In the present study, the extent of mycorrhizal colonization varied among different plant species in disturbed and undisturbed areas of both the sites which confirms the earlier findings of Manjunath and Bagyaraj, (1982) who stated that the extent to which plants respond to colonization varies with the plant species. Gerdemann, (1965) has shown that the colonization pattern of arbuscular mycorrhizal fungal species can be distinctly different in various plant species. Similarly, spore density also showed variation between the plant species at each site. Thus, formation of mycorrhiza may depend on plant and fungal species as well as on the site conditions (Mason *et al.*, 1983) and soil nutrients (Hayman, 1982).

In the present study, reduced levels of colonization, spore density and diversity of arbuscular mycorrhizal fungal species was observed in the disturbed site as compared to the undisturbed site and this could be attributed to land

disturbance. It can be concluded that soil degradation by mining brings about decrease in the mycorrhizal population although differences in colonization and spore number may be observed between the sites depending upon their soil properties. It is well known that soil degradation negatively affects microbiological population particularly mycorrhizal fungi (Williams and Allen, 1984). However, in spite of unfavourable conditions, the fungi do not totally disappear which suggests their adaptation to stress that can facilitate the establishment of plants capable of surviving in these disturbed soils. Helgason *et al.*, (1998) and Johnson, (1993) found that the loss of arbuscular mycorrhizal fungal biodiversity system could therefore, decrease both plant biodiversity while increasing ecosystem instability.

Disturbance of soil has been shown to reduce the colonization of arbuscular mycorrhizal fungi (Moorman and Reeves, 1979; Diaz and Honrubia, 1993). Consequently, it has been hypothesized that if viable arbuscular mycorrhizal inoculum is reduced, then the development of the recolonizing plant community may be limited or retarded, particularly in soil that have low concentration of essential plant nutrients (Loree and Williams, 1987). Numerous studies have shown that the upper soil profiles contains the greatest number of spores of each species and the number of species decreases with increasing depth. (Menge *et al.*, 1977; Koide and Mooney, 1987). However, some species of arbuscular mycorrhizal fungi may not even produce spores (McGee, 1989).

According to Mosse, (1972 & 1975) adaptation of species of arbuscular mycorrhizal fungi appears to be associated with edaphic or other physical factors and most species have a wide host range. In the present study, many species of arbuscular mycorrhizal fungi were found to be common to both disturbed and undisturbed areas of mine sites. Thus, occurrence of some common species of arbuscular mycorrhizal fungi suggests that they exhibit little habitat specificity (Molina *et al.*, 1978).

The number of spores found in undisturbed site is more than the disturbed site. This could be due to the presence of plant species in undisturbed site which have heavy sporulating endophytes. A significantly faster decomposing rate was observed in the forest than in a mine site indicating greater activities of decomposers in the natural forest. It may also be possible that in the natural forest the germination rates of endophytic spores are more than in other sites.

According to Loree and Williams, (1984) the re-establishment of symbiont community following disturbance is clearly time dependent. Loree and Williams, (1987) in their studies on colonization of western wheat grass by arbuscular mycorrhizal fungi during revegetation of surface mine site reported that variety of spore types observed per sample increased as a function of site age, and attributed it to soil replenishment during reclamation. According to Lambert and Cole, (1980) development of mycorrhizae has been enhanced by application of topsoil. While,

investigations by Miller, *et al.*, (1985) also suggests that length of time of storage of topsoil play a major role in propagule survival. Danielson *et al.*, (1979) found that organic amendments would modify colonization rate in mine spoils.

Disturbance of natural plant communities is the first visible symptom and is often accompanied by loss of key physicochemical and biological soil properties (soil structure, plant nutrient availability, organic matter content, and/or microbial activity) (Skujins and Allen, 1986; Requena *et al.*, 2001). These properties largely determine soil quality and fertility, and thus plant establishment and productivity. Hence their degradation results in a loss of sustainability. Since soil degradation limits the potential for reestablishment of native plants (Agnew and Warren, 1996).

From the present study it is evident that the disturbance highly reduces the spore density and diversity when compared with the natural undisturbed area. Continuous and constant disturbance on the recently degraded mine which is under active mining operations might have severely affected the seedling establishment as well as colonization of arbuscular mycorrhizal fungi. Hence, it can be argued that in a stable ecosystem the increased arbuscular mycorrhizal fungal diversity influences the plant biodiversity.

CHAPTER- 5

RESPONSE OF ARBUSCULAR MYCORRHIZAL FUNGI ON GROWTH AND BIOMASS OF SELECTED TREE SPECIES.

INTRODUCTION

A mycorrhizae is a type of endophytic, biotrophic, mutualistic, symbiosis prevailed in many cultivated and natural ecosystems. The symbiotic association between plant and mycorrhizal fungi is the rule rather than the exception under natural conditions (Malloch *et al.*, 1980). They are present in the soil in the form of chlamydospores, mycelium, zygospores and azygospores. Their population varies greatly in size and composition according to the habitat, type of soil and vegetation. Phosphorus content to soil plays a very important role in distribution of arbuscular mycorrhizal fungi and establishment of arbuscular mycorrhizae.

Arbuscular mycorrhizal fungi have been reported to increase the growth of plants by improving the uptake of phosphate and other nutrients from the soil through a reduction of the distance that nutrient must diffuse to plant roots (Hatting *et al.*, 1973; Rhodes and Gerdemann, 1975) and by accelerating the rate of nutrient absorption and nutrient concentration at the absorbing surface (Cress *et al.*, 1979). They also confer increased drought tolerance, helps to control pests and fungal pathogens and contributing to the soil structure and stability (Koske *et al.*, 1975; Sutton and Sheppard, 1976; Harley and Smith, 1983; Nelson, 1987; Newsham *et al.*, 1995). Arbuscular mycorrhizal fungi increase the cytokinin concentration in both roots and leaves (Allen, *et al.*, 1980). Enhanced cytokinin levels can also promote plant growth, delay senescence and elevate photosynthetic rate (Incoll and Whitlam, 1977). Arbuscular mycorrhizae are also known to influence plant biodiversity (Van der Heijden *et al.*, 1998).

Arbuscular mycorrhizae thus have a profound influence, directly or indirectly on life on land.

Land disturbances can reduce population density of arbuscular mycorrhizal fungi in soil by destroying plant community and by mixing top soil with both subsoil and mine spoil materials (Reeves *et al.*, 1979). The reduced population of arbuscular mycorrhizal fungi in disturbed soil may limit successful establishment of the native plants. A number of studies have indicated that arbuscular mycorrhizal fungi play an important role in the revegetation of disturbed lands (Daft and Nicolson, 1974; Khan, 1978, Reeves *et al.*, 1979; Lambert and Cole, 1980; Khan, 1981). Revegetation of any site will occur naturally with time (Bradshaw, 1984) but, soils poor in plant nutrients and tend to have physical shortcomings, natural colonization can be extremely slow. The use of expensive inputs such as the use of inorganic fertilizers is not advisable as they are derived from non-renewable resources and hence, are expensive and tend to be more expensive every year. Again, their constant use is known to degrade the soil. Hence, there is an urgent need of switching on to bio-fertilizers.

A wide variation among and within different species of arbuscular mycorrhizal fungi in the ability for stimulating plant growth have been observed (Abbott and Robson, 1978; Govinda Rao *et al.*, 1983). Earlier studies on

arbuscular mycorrhizal fungi have reported the beneficial effect of inoculation on plant growth in sterile soil with low available phosphorus (Gerdemann, 1964; Mosse and Hayman, 1971). But later investigations indicated that even in unsterile soil, plants do respond to inoculation with efficient strains of arbuscular mycorrhizal fungi (Mosse and Hayman, 1971; Khan, 1972).

The beneficial effect of inoculating forest trees with arbuscular mycorrhizal fungi to improve plant growth are well known (Kormanik *et al.*, 1978, Janos, 1983; Jeltra, 1987; Bagyaraj *et al.*, 1989). Earlier studies on arbuscular mycorrhizal fungi have reported the beneficial effect of inoculation on plant growth in sterile soil with low available phosphorus (Gerdemann, 1964; Mosse and Hayman, 1971). But later investigations indicated that even in unsterile soil, plants do respond to inoculation with efficient strains of arbuscular mycorrhizal fungi (Mosse and Hayman, 1971; Khan, 1972). Reports are scanty on the effects of pre-inoculation of plants with arbuscular mycorrhizal fungi in unsterile soils (Khan, 1981; Daft and Hacskaylo, 1977; Manjunath and Bagyaraj, 1988).

All the three tree species *viz.*, *Anarcardium occidentale* L., *Artocarpus heterophyllus* Lam. and *Terminalia crenulata* Roth taken up in the present study are multipurpose tree species commonly found in Goa. They are known to grow

in nutrient deficient soils and are important sources of fruit, fuel, fodder and timber.

In the present chapter, effect of two arbuscular mycorrhizal fungal species *viz.*, *Glomus mosseae* (Nicolson & Gerdemann) Gerdemann & Trappe and *Glomus intraradix* Schenck and Smith, on growth and biomass production of three forest tree species *viz.*, *Anacardium occidentale*, *Artocarpus heterohyllus* and *Terminalia crenulata* have been studied **(Plate XI)**. The present study also determines the relative symbiotic efficiency of indigenous and introduced arbuscular mycorrhizal fungi in stimulating plant growth in forest soils and the dependence of plants on symbiotic association.

MATERIALS AND METHODS

Spores of *Glomus mosseae* (Nicolson & Gerdemann) Gerdemann & Trappe were isolated and multiplied on the roots of *Eleusine coracana* (L.) Gaertn., *Lycopersicum esculentum* Mill., *Allium cepa* L. and *Coleus sp.* as host plants. While pure culture of *Glomus intraradix* Schenck & Smith procured from Tata Energy Research Institute (TERI), New Delhi was multiplied using *Eleusine coracana* (L.) Gaertn., *Lycopersicum esculentum* Mill., *Allium cepa* L. and *Coleus sp.* as host plants. **(Plate XII)**.

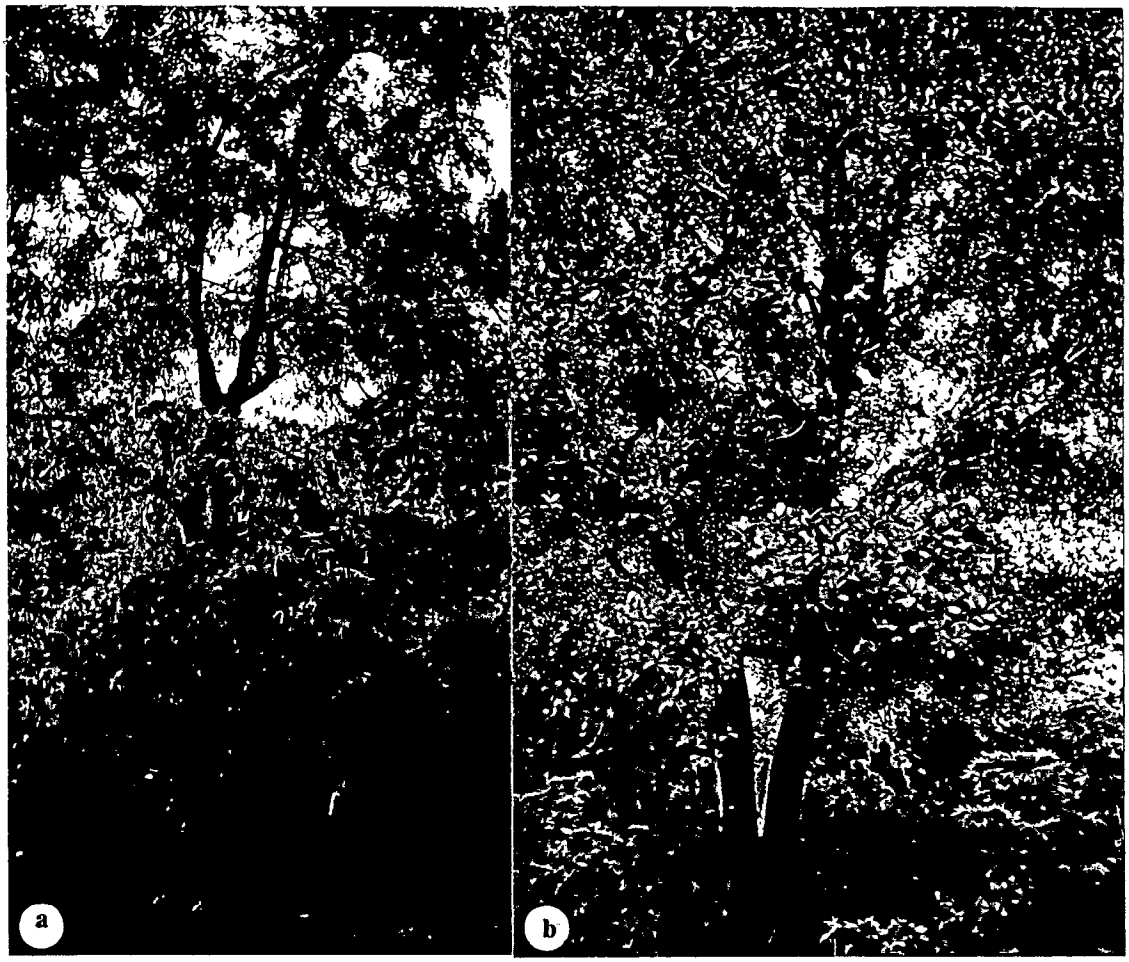
PLATE- XI

MULTIPURPOSE TREE SPECIES USED IN GROWTH EXPERIMENTS

a. *Terminalia crenulata* Roth

b. *Artocarpus heterophyllus* Lam

c. *Anarcardium occidentale* L.


a
b


c

Clayey loam forest soil (pH 5.6) of low phosphorus status was used. The soil was sterilized in an autoclave at 121° C for 2 hours daily for three continuous days to eliminate naturally occurring endophytes and other contaminants. Uniform seeds of *Anarcardium occidentale* L., *Artocarpus heterophyllus* Lam. and *Terminalia crenulata* Roth were surface sterilized for 20 minutes in 70% ethanol, soaked overnight in sterile water, then placed in moist sterile soil at room temperature until germinated.

The experiment comprised of three treatments *viz.*,

a) Unsterilized soil (Control);
b) Unsterilized soil + *Glomus intraradix* (GI) and,
c) Unsterilized soil + *Glomus mosseae* (GM).

For each treatment, polyethylene bags were filled with 4.5 Kg of unsterile forest soil and 1 germinated seed per bag was sown. For treatments b) and c), 20g of inoculum containing approximately 450 arbuscular mycorrhizal fungal spores and colonized root bits of *Glomus mosseae* (GM) and *Glomus intraradix* (GI) were applied separately as a thin layer 3 cm below the soil surface to obtain mycorrhizal plants. There were ten replicates for each treatment.

All the polyethylene bags containing plants were labeled and kept separately. Plants received sunlight, were watered to field capacity as and when

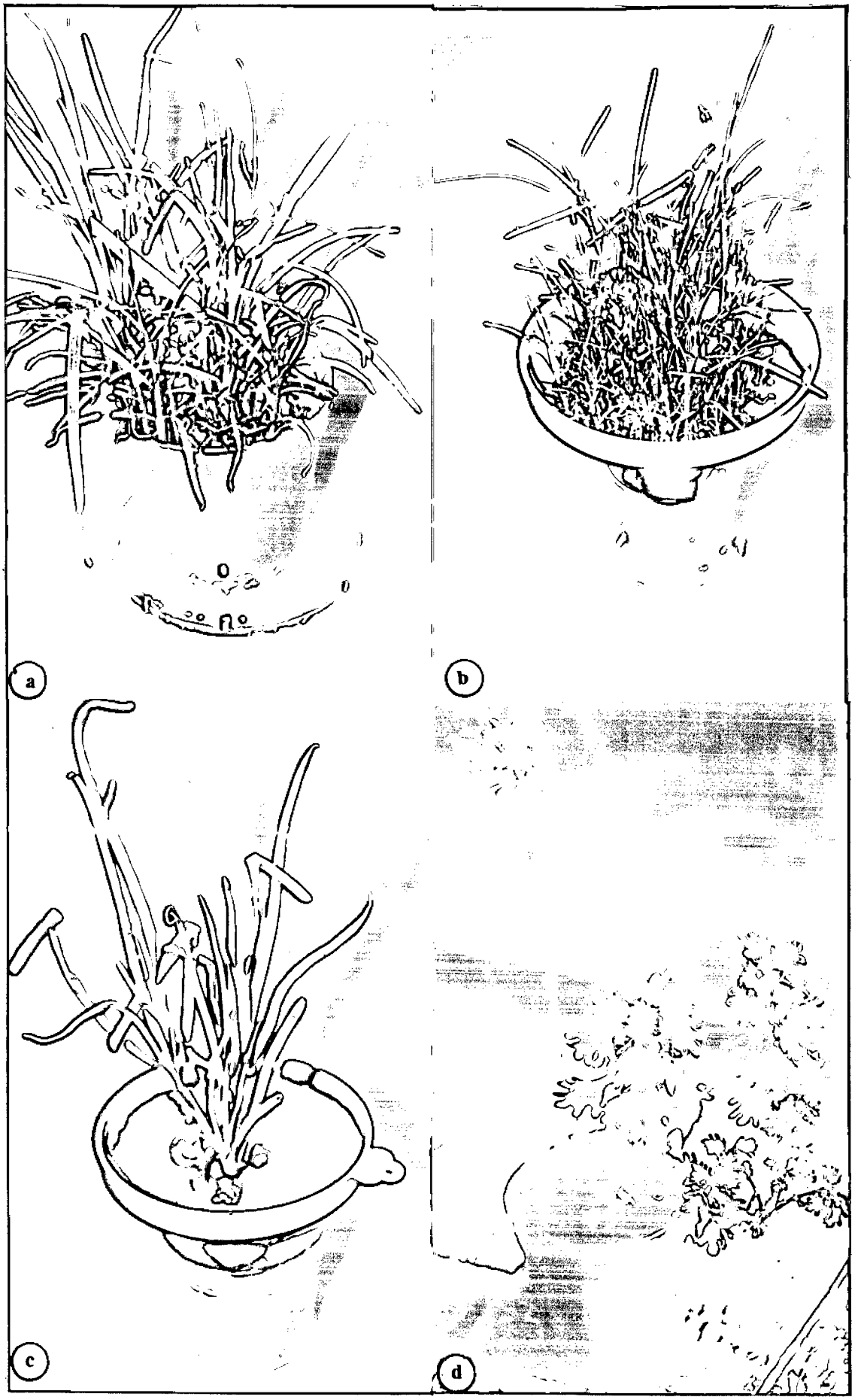
PLATE- XII

TEST PLANTS USED FOR INCOCULUM PRODUCTION

a) & b) *Eleusine coracana* L.,

c) *Allium cepa* L.

d) *Coleus sp.*


a
b
c
d

required and were fertilized with Hogland's solution lacking in phosphorus in every fifteen days.

After 45 days of inoculation with various arbuscular mycorrhizal fungal species, the roots were stained using 0.05% trypan blue in lactophenol (Phillips and Hayman, 1970) and mycorrhizal colonization was estimated using slide method (Giovannetti and Mosse, 1980). Presence of arbuscular mycorrhizal fungal structures *viz.*, hyphae, arbuscules and/or vesicles in the inoculated plants confirmed the establishment of arbuscular mycorrhizal fungi.

After 90 days of growth, response of the three plant species to different treatments were analyzed by studying various growth parameters *viz.*, shoot and root length, leaf number, leaf length (3rd leaf), stem girth and, shoot and root fresh weights. Later, the shoot and root portions of plants were dried separately at 70°C for 72 hrs and dry weights were recorded.

MYCORRHIZAL DEPENDENCY

Mycorrhizal dependency of the plants was determined using the formula proposed by Bagyaraj (1995) for Mycorrhizal Inoculation Effect (MIE).

$$\text{MIE (\%)} = \frac{\text{Dry wt. of inoculated pt.} - \text{Dry wt. of uninoculated}}{\text{Dry wt .of inoculated plant}} \times 100$$

STATISTICAL ANALYSIS

Data on plant growth was subjected to (ANOVA) analysis of variance, mean and standard deviation. Significant differences were determined at $\alpha = 0.05$.

RESULTS AND DISCUSSION

Soil analysis results revealed that the soil was acidic with pH 5.28, Electrical Conductivity (EC) 0.566 M mhos/cm, total nitrogen 0.44%, total phosphorus 0.20%, total potassium 0.17 kg/hectare, organic carbon 2.68%, available phosphorus 49.2 kg/hectare and available potassium 423.2kg/hectare **(Table 28).**

Results on growth responses to the two arbuscular mycorrhizal treatments in seedlings of three tree species *viz., Anacardium occidentale, Artocarpus heterohyllus* and *Terminalia crenulata* are depicted in **Table 29, Table 30 and Table 31 (Plate XIII)**. Mycorrhizal seedlings showed distinct variations than the non-mycorrhizal seedlings for most of the growth parameters. Inoculation with different arbuscular mycorrhizal fungal species had varied effects on the shoot and root growth, stem girth, leaf length, leaf number and, shoot and root fresh and dry weights.

PLATE- XIII

RESPONSE OF ARBUSCULAR MYCORRHIZAL FUNGI ON SOME TREE SPECIES

a) *Anarcardium occidentale* L.

b) *Artocarpus heterophyllus* Lam

c) *Terminalia crenulata* Roth

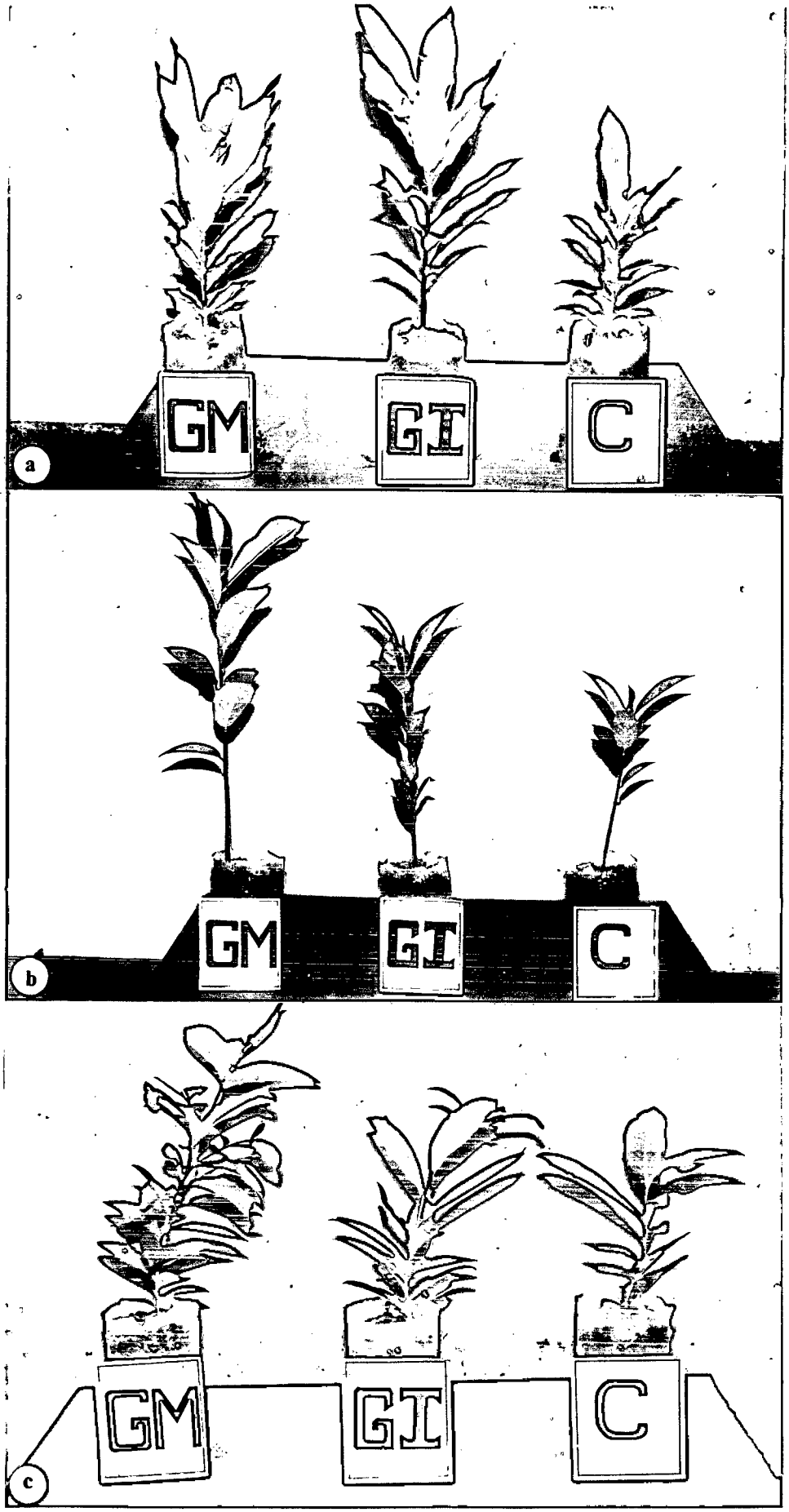

GM
GI
C
a
GM
GI
C
b
GM
GI
C
c

Table 28: Soil Characteristics of Mollem forest area.

SITE	pH	EC M mhos/cm	Total N_2 (%)	Total P_2O_5 (%)	Total K_2O Kg/hac	Available phosphorus Kg/hac	Available K Kg/hac	Organic carbon (%)
Mollem	5.28 ± 0.17	0.566 ± 0.04	0.44 ± 0.03	0.20 ± 0.02	0.17 ± 0.02	49.20 ± 1.83	423.2 ± 12.82	2.68 ± 0.04

± Indicates Standard deviation.

All values are mean of six readings.

In seedlings of *Anacardium occidentale* the leaf number, stem girth, plant height, shoot fresh and dry weights, and root fresh and dry weights varied significantly between the treatments at $P<0.05$. However, the difference in leaf length and root length was not statistically significant between the treatments **(Table 29)**. In seedlings of *Artocarpus heterophyllus* the leaf number, leaf length, stem girth, plant height, shoot fresh and dry weights, and root fresh weight varied significantly between the treatments at $P<0.05$. However, the difference in root length and root dry weight was not statistically significant between the treatments **(Table 30)**. While in seedlings of *Terminalia crenulata* only shoot fresh and dry weights varied significantly between the treatments at $P<0.05$. However, there was no significant difference in leaf number, leaf length, stem girth, plant height, root length and, root fresh and dry weights between the treatments **(Table 31)**.

Among the two arbuscular mycorrhizal treatments *viz.*, *Glomus intraradix* (GI) and *Glomus mosseae* (GM) the increase in total plant biomass was much higher in seedlings of *Artocarpus heterophyllus* and *Terminalia crenulata* inoculated with *Glomus mosseae* (GM), while the seedlings of *Anacardium occidentale* showed increased biomass when inoculated with *Glomus intraradix* (GI) **(Table 32). (Plate 13)**.

Mycorrhizal inoculation effect (MIE%) which is used to assess the extent to which introduced arbuscular mycorrhizal fungi compete with the native

Table 29: Effect of AM fungi on some growth parameters in *Anacardium occidentale* L.

Parameter	Treatment			C.D. at 0.05%
	C	G.M.	G.I.	
No. of Leaves/plant	12.00 ± 2.35	19.20 ± 1.93	19.10 ± 3.51	1.956
Leaf length (cm)	29.78 ± 51.03	13.87 ± 2.10	15.25 ± 1.98	N.S.
Stem girth (cm)	2.03 ± 0.23	2.10 ± 0.23	2.33 ± 0.20	0.176
plant height (cm)	16.17 ± 3.12	20.59 ± 2.45	25.50 ±5.23	2.937
Root length(cm)	22.60 ± 6.39	21.48 ± 5.47	21.90 ± 2.33	N.S.
Shoot fresh wt. (g)	12.04 ± 2.30	21.48 ± 3.10	25.63 ± 3.51	2.573
Shoot dry wt. (g)	3.59 ± 0.85	6.35 ± 0.90	6.97 ± 0.90	0.523
Root fresh wt. (g)	2.67 ± 0.88	4.43 ± 1.50	5.77 ± 1.34	0.953
Root dry wt. (g)	0.85 ± 0.32	1.58 ± 0.50	1.62 ± 0.45	0.204

Legend:
C = Control G.M. = *Glomus mosseae* G. I. = *Glomus intraradix.*
Values are mean of ten replicates.
± = Standard deviation.
N.S. = Non Significant.

Table 30: Effect of AM fungi on some growth parameters in *Artocarpus heterophyllus* Lam.

Parameter	Treatment			C.D. at 0.05%
	C	G. M.	G. I.	
No. of Leaves/plant	5.20±2.04	8.60±1.07	7.30±2.05	1.223
Leaf length (cm)	11.04±3.58	16.21±1.77	12.69±2.11	3.609
Stem girth (cm)	1.25 ± 0.27	2.24 ± 0.29	1.73 ± 0.24	1.124
plant height (cm)	17.04 ± 6.07	45.51 ± 6.58	33.04 ± 5.68	5.011
Root length(cm)	16.40 ± 6.22	19.60 ± 3.23	20.50 ± 3.27	N.S.
Shoot fresh wt. (g)	9.67 ± 6.35	30.40 ± 6.29	19.38 ± 6.24	1.285
Shoot dry wt. (g)	2.22 ± 1.35	8.07 ± 1.58	4.54 ± 1.77	4.215
Root fresh wt. (g)	2.36 ± 1.72	10.01 ± 2.62	7.69 ± 4.45	0.493
Root dry wt. (g)	0.43 ± 0.35	2.48 ± 0.83	1.41 ± 0.66	N.S.

Legend:

C = Control G.M. = *Glomus mosseae* G. I. = *Glomus intraradix.*

Values are mean of ten replicates.

± = Standard deviation.

N.S. = Non Significant.

Table 31: Effect of AM fungi on some growth parameters in *Terminalia crenulata* Roxb.

Parameters	Treatment			C.D. at 0.05 %
	C	G. M.	G. I.	
No. of Leaves/plant	18.50 ± 8.39	32.50± 5.75	21.1 ± 8.21	N.S.
Leaf length (cm)	13.03 ± 3.35	16.29 ± 2.18	13.66 ± 3.13	N.S.
Stem girth (cm)	1.43 ± 0.22	1.81 ± 0.16	1.57 ± 0.28	N.S.
Plant height (cm)	22.89 ± 11.19	34.27 ± 9.22	26.41 ± 14.57	N.S.
Root length(cm)	32.10 ± 7.80	32.75 ± 2.97	29.14 ± 4.47	N.S.
Shoot fresh wt. (g)	13.15 ± 8.32	24.12 ± 4.65	17.12 ± 9.87	5.563
Shoot dry wt. (g)	3.12 ± 2.10	5.60 ± 1.17	3.99 ± 1.88	1.317
Root fresh wt. (g)	8.57 ± 5.64	13.69 ± 2.28	12.06 ± 5.10	N.S.
Root dry wt. (g)	2.64 ± 1.55	3.78 ± 0.57	3.35 ± 1.62	N.S.

Legend:

C = Control G.M. = *Glomus mosseae* G. I. = *Glomus intraradix.*

Values are mean of ten replicates.

± = Standard deviation.

N.S. = Non Significant.

Table 32: Effect of selected AM fungal species on total dry weight biomass on some tree species grown in forest soils.

Plant species	Treatment	Root dry weight (g)	Shoot dry weight (g)	Total dry weight
Anacardium occidentale	C	0.85 ± 0.32	3.59 ± 0.85	4.44 ± 1.17
	G.M.	1.58 ± 0.50	6.35 ± 0.90	7.93 ± 1.4
	G.I.	1.62 ± 0.45	6.97 ± 0.90	8.59 ± 1.35
Artocarpus heterophyllus	C	0.43 ± 0.35	2.22 ± 1.35	2.65 ± 1.71
	G.M.	2.48 ± 0.83	8.07 ± 1.58	10.55 ± 2.42
	G.I.	1.41 ± 0.66	4.54 ± 1.77	5.95 ± 2.43
Terminalia crenulata	C	2.64 ± 1.55	3.12 ± 2.10	5.76 ± 3.65
	G.M.	3.78 ± 0.57	5.6 ± 1.17	9.38 ± 1.75
	G.I.	3.35 ± 1.62	3.99 ± 1.88	7.34 ± 3.51

Legend:

C = Control G.M. = *Glomus mosseae* G. I. = *Glomus intraradix.*
Values are mean of ten replicates.
± = Indicates Standard deviation.

endophyte showed variations among the two arbuscular mycorrhizal fungal treatments in the three plant species.

In *Anacardium occidentale*, maximum MIE was recorded in seedlings inoculated with *Glomus intraradix* (48.31%) followed by seedlings inoculated with *Glomus mosseae* (44.01%), while in *Artocarpus heterophyllus*, maximum MIE was recorded in seedlings inoculated with *Glomus mosseae* (75.23%) followed by seedlings inoculated with *Glomus intraradix* (55.46%). In *Terminalia crenulata*, maximum MIE was recorded in seedlings inoculated with *Glomus mosseae* (38.50%) followed by seedlings inoculated with *Glomus intraradix* (21.52 %). **(Table 33) (Fig. 10, 11 & 12).**

In the present study, arbuscular mycorrhizal inoculation with both the fungal inoculants was efficient in stimulating the growth of seedlings in all the three tree species. However, both the inoculants showed variation in their response.

The local isolate *Glomus mosseae* gave better response over *Glomus intraradix* in seedlings of two tree species *viz.*, *Artocarpus heterophyllus* and *Terminalia crenulata* while in *Anacardium occidentale* the response was better when inoculated with *Glomus intraradix*. Although there was no host specificity, a host preference for *Glomus mosseae* in *Artocarpus heterophyllus* and *Terminalia crenulata* and host preference for *Glomus intraradix* in *Anacardium occidentale*

Table 33: Mycorrhizal Inoculum Effect (MIE) in three tree species.

Tree species	Treatment	MIE %
Anacardium occidentale	G.M.	44.01
	G.I.	48.31
Artocarpus heterophyllus	G.M.	75.23
	G.I.	55.46
Terminalia crenulata	G.M.	38.50
	G.I.	21.52

Legend:

G.M. = *Glomus mosseae.*
G.I. = *Glomus intraradix.*
MIE = Mycorrhizal Inoculum Effect.

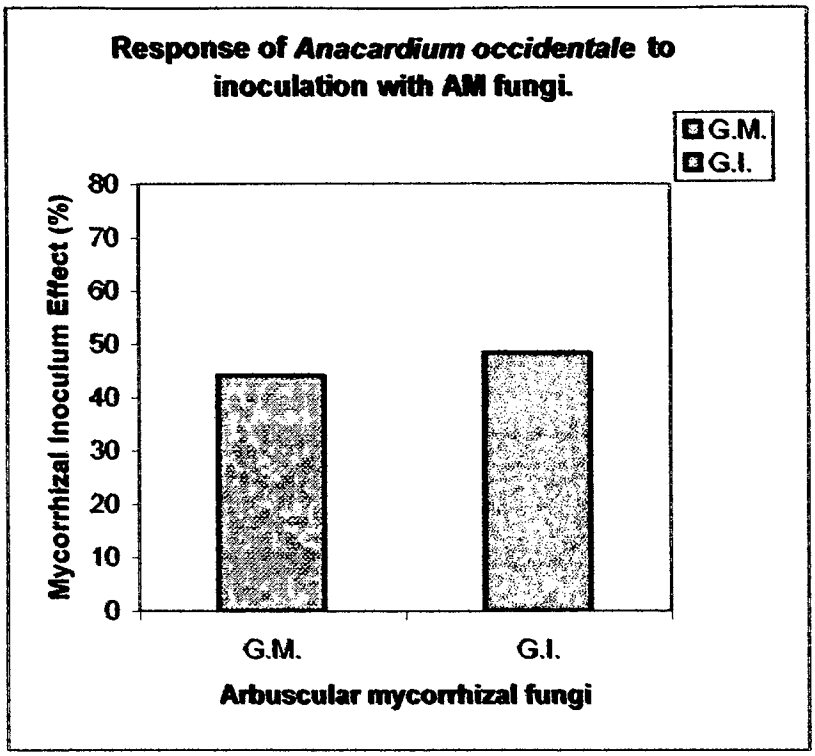

Response of *Anacardium occidentale* to inoculation with AM fungi.
G.M.
G.I.
Mycorrhizal Inoculum Effect (%)
80
70
60
50
40
30
20
10
0
G.M.
G.I.
Arbuscular mycorrhizal fungi

Fig. 10

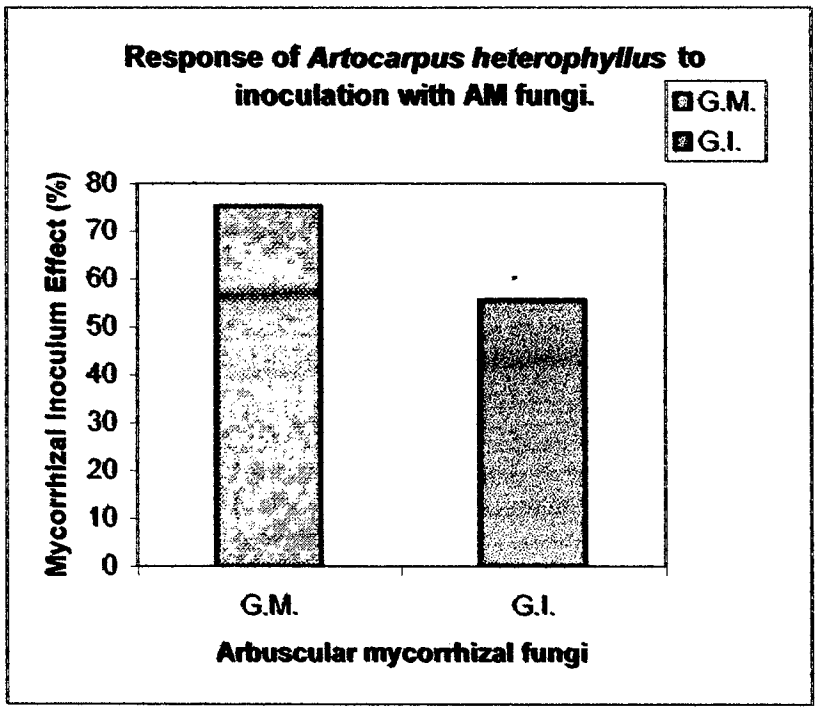

Response of *Artocarpus heterophyllus* to inoculation with AM fungi.
G.M.
G.I.
Mycorrhizal Inoculum Effect (%)
80
70
60
50
40
30
20
10
0
G.M.
G.I.
Arbuscular mycorrhizal fungi

Fig. 11

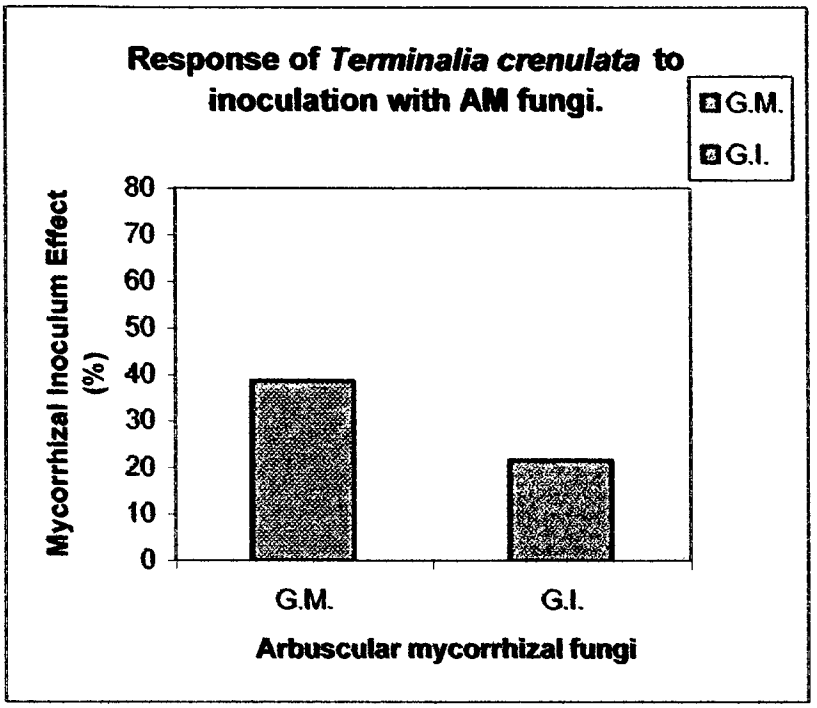

Response of *Terminalia crenulata* to inoculation with AM fungi.
G.M.
G.I.
Mycorrhizal Inoculum Effect (%)
80
70
60
50
40
30
20
10
0
G.M.
G.I.
Arbuscular mycorrhizal fungi

Fig. 12

was observed. Bagyaraj *et al.*, (1989) made similar observations wherein they screened seven arbuscular mycorrhizal fungi for symbiotic response with *Leucaena leucocephala,* of which a local isolate *Glomus mosseae* was found to be the best mycorrhizal fungus for inoculation of *Leucaena leucocephala.*

As soil erosion tends to decrease the number of mycorrhizal propagules it could be critical to re-introduce such propagules to improve the recovery rate of ecosystem. However, it is not feasible to inoculate large tracts of lands with arbuscular mycorrhizal fungi, as there are currently too many problems associated with the mass production of these fungi (Menge, 1984). It is desirable to integrate low maintenance tree species and appropriate soil micro-organisms to establish and maintain plant communities under low energy input of minimum soil fertility and cultivation. Transplantation of arbuscular mycorrhizal inoculated nursery seedlings may prove advantageous on reclamation sites, as limited nutrients in the soil would be more efficiently extracted (Jasper *et. al,* 1989, Sylvia, 1990).

The present study thus suggests the importance of isolation of local mycorrhizal fungi and including them during screening for efficiency, in order to select an efficient strain of mycorrhizal fungus best suited for a particular host plant.

SUMMARY

The investigations carried out on the ecology, taxonomy of arbuscular- mycorrhizal fungi from Mollem and Dharbandoda forests, disturbed areas of Collem forest, and the studies related to screening of efficient arbuscular- mycorrhizal fungi for some tree species are presented under five chapters and the important results are highlighted below.

The status of arbuscular mycorrhizal fungi in Mollem and Dharbandoda forest areas of Goa is reported for the first time. All the twenty eight tree species belonging to twenty two families were taken up for the study and were found to be mycorrhizal. The mycorrhizal colonization was characterized by intra-radical and extra metrical hyphae, intra-cellular coils, vesicles and/or arbuscules. Higher spore density and higher percentage root colonization was recorded from Mollem forest as compared to Dharbandoda forest area. In all a total of 31 arbuscular mycorrhizal fungal species are identified from both Mollem and Dharbandoda forest. The genus *Glomus* was found to be dominant in both Mollem and Dharbandoda forest areas.

Studies on arbuscular mycorrhizal fungi in Mollem and Dharbandoda forests, and disturbed areas of Collem forest revealed a rich diversity of arbuscular mycorrhizal fungi. A total of 33 arbuscular mycorrhizal fungal species belonging to five genera *viz.*, *Acaulospora* (6), *Gigaspora* (2), *Glomus* (19), *Sclerocystis* (3) and *Scutellospora* (3) are isolated by using trap plants and pot cultures, and described with modern terminologies. A total of 31 arbuscular mycorrhizal fungal species were recovered from Mollem site, 26

arbuscular mycorrhizal fungal species were recovered from Dharbandoda site and 22 arbuscular mycorrhizal species were recovered from Collem site.

Studies on seasonal variations of arbuscular mycorrhizal fungi with respect to root colonization, spore density and species diversity was taken up at two sites *viz,* Mollem and Dharbandoda forest areas. The study revealed that the seasonal variations have a remarkable influence on the occurrence on the spores and root colonization. It was also observed that the average spore density recorded was maximum in pre-monsoon, followed by post-monsoon and was minimum in monsoon. However, average percentage root colonization was maximum in post-monsoon, followed by monsoon and was minimum in pre-monsoon. It was also observed that the climatic and edaphic factors strongly influence the spore density and root colonization.

Two sites *viz.,* undisturbed (Mollem) and disturbed (Collem) taken up for the study indicated that the average spore density was higher in undisturbed site than disturbed site. Similarly, percentage root colonization was also higher in undisturbed site as compared to the disturbed site. Also the arbuscular mycorrhizal fungal species richness was higher in undisturbed site than in the disturbed site. The dominance of arbuscular mycorrhizal species varied at both sites.

All the tree species *viz., Anacardium occidentale, Artocarpus heterophyllus* and *Terminalia crenulata* inoculated with the two species of arbuscular mycorrhizal fungi *viz., Glomus mosseae* and *Glomus intraradix* showed better growth response compared to control. It was observed that *Anacardium occidentale* inoculated with *Glomus intraradix* showed better mycorrhizal inoculation effect (MIE =48.31%) than *Glomus mosseae* (44.01%). While, *Artocarpus heterophyllus* inoculated with *Glomus mosseae* showed better mycorrhizal inoculation effect (MIE=75.23%) when compared to inoculation with *Glomus intraradix* (MIE= 55.46%). Similarly, *Terminalia crenulata* inoculated with *Glomus mosseae* showed better mycorrhizal inoculation effect (MIE =38.50%) when compared to inoculation with *Glomus intraradix* (21.52%).

Transplantation of arbuscular mycorrhizal inoculated nursery seedlings may prove advantageous on reclamation sites as limited nutrients in the soil would be more efficiently extracted. It is essential to isolate local arbuscular mycorrhizal fungi and include them during screening for efficiency, in order to select an efficient strain of arbuscular mycorrhizal fungi best suited for a particular plant host.

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SYNOPSIS

STUDIES IN ARBUSCULAR MYCORRHIZAL (AM) FUNGI ASSOCIATED WITH SOME TREE SPECIES FROM MOLLEM AND DHARBANDODA FOREST AREAS OF GOA.

INTRODUCTION

Large number of microorganisms inhabits the rhizosphere layer of soil. These microorganisms carry out various types of activities, which are very much helpful to plants as well as to these organisms, hence they live associated with plants. The most prevalent and widespread type of association is the "Mycorrhizal Association". (Peterson and Farquhar, 1994).

More than 80% of plant species are capable of forming arbuscular mycorrhizae. They are found nearly in all ecosystems all over the world (Dhillion *et al.*, 1995) and is a determining factor for plant establishment and survival in many ecosystems (Bledose *et al.*, 1990). Arbuscular mycorrhizal fungi have shown to help plants to acquire macronutrients (Smith and Gianinazi-Pearson, 1988) and micronutrients (Azcon, 1994). Arbuscular Mycorrhizal fungi have also been found to increase water uptake and/or otherwise alter the plant physiology to reduce stress response to drought and salinity. The improved nutrient uptake and better water utilization in endomycorrhizal plants reduce the transplant shock, quick recovery after temporary wilting and survival after transplanting (Michelson and Rosendahl, 1990). An interesting arbuscular mycorrhizal effect is the increase in resistance and tolerance of arbuscular mycorrhizal plants to soil pathogens and

some of the pests. An Arbuscular Mycorrhizal fungus is a symbiotic association between the hyphae of certain fungi and the roots of most forest trees. The importance of mycorrhizae in the nutrition of most vascular plants and the health of forest ecosystem has been overwhelmingly demonstrated in recent decades (Marks and Kozlowski, 1973). Arbuscular mycorrhizal fungi enhances the uptake of nutrients from low nutrient environment, contributes to the success of plant establishment and survival.

Mollem, Dharbandoda and Collem forest areas are covered by Collem range of Sanguem Taluka and the total forest area covered by this range is about 5486.14 hectares. The climate of the tract is Tropical. The highest annual rainfall recorded is 5569 mm. The highest humidity recorded is 96% and, maximum and minimum temperatures recorded are 37.2 ^{0} C and 15.9 ^{0} C respectively. The soils are well drained gravely with silty clay loam texture associated with stone lime or gravel horizon and occur on steep to very steep sloping lands. Large areas are mapped under dense forest vegetation and kumeri cultivation.

The potential of arbuscular mycorrhizal fungi can be used to enhance plant productivity (Abbott and Robson, 1991; Read 1991). Many concepts of arbuscular mycorrhizal fungal application cannot be adequately assessed or manipulated in a rational way for field application without knowledge of their taxonomy and ecology (Walker, 1992). With this approach in mind, a detailed study related to arbuscular mycorrhizal diversity, their taxonomy and ecology is being attempted in the present research work.

AIMS AND OBJECTIVES

1. To study the root colonization and to determine spore density of arbuscular mycorrhizal fungi, occurring in Mollem and Dharbandoda forest areas.
2. To study the taxonomy of arbuscular mycorrhizal fungi in the rhizosphere soils of various plant species of Mollem and Dharbandoda forest areas.
3. To study the seasonal dynamics of arbuscular mycorrhizal fungi (root colonization and spore density) with respect to edaphic and climatic factors associated with some plant species from Mollem and Dharbandoda forest areas.
4. Comparative study of arbuscular mycorrhizal species in disturbed and undisturbed sites.
5. To study the effect of selected arbuscular mycorrhizal fungi on growth and dry weight biomass of selected forest tree species.

METHODOLOGY

1. Collection of rhizosphere soil samples and root samples from Mollem and Dharbandoda areas.
2. Examination of the roots to find out percent infection using Phillips and Hayman Method (1970).
3. Isolation of arbuscular mycorrhizal fungi from rhizosphere soil samples by wet sieving and decanting Method (Gerdmann and Nicolson, 1963).

4. Quantification of the spores and sporocarps of arbuscular mycorrhizal species in the rhizosphere soil samples (Adholeya and Gaur, 1994).
5. Identification of the spores and sporocarps isolated using the standard keys (Schenck and Perez, 1990; Morton and Benny, 1990; Wu, 1993).
6. To study the effect of selected arbuscular mycorrhizal fungal species on growth and dry weight biomass of selected forest tree species.

OBSERVATIONS

The **first chapter** deals with the general survey of arbuscular mycorrhizal fungi in Mollem and Dharbandoda forest areas. The objective of the work was to study the root colonization and to determine spore density of arbuscular mycorrhizal fungi occurring in Mollem and Dharbandoda forest areas. It was found that all the tree species taken up in the study were mycorrhizal and a high spore density in the rhizosphere soils in both the sites was recorded.

The **second chapter** deals with the taxonomy of arbuscular mycorrhizal fungi associated with some plant species from Mollem, Dharbandoda and Collem forest areas recovered during the study period. The study reports a rich diversity of arbuscular mycorrhizal fungi associated with tree species. The arbuscular mycorrhizal fungi recorded belonged to five genera viz. *Acaulospora, Glomus, Sclerocystis, Gigaspora* and

Scutellospora with *Glomus* species occurring frequently. In all, a total of 33 arbuscular mycorrhizal fungal species were recorded during the study.

The **third chapter** deals with the seasonal dynamics of arbuscular mycorrhizal fungi in Mollem and Dharbandoda forest areas. The aim of the study was to study seasonal variations in arbuscular mycorrhizal fungi (root colonization and spore density) with respect to edaphic and climatic factors associated with some plant species from Mollem and Dharbandoda forest areas. The maximum average spore density was recorded in pre-monsoon (summer) followed by post-monsoon (winter) and the minimum average spore density per/ 100g of soil was observed in monsoon (rains) collection. Root colonization was observed throughout the year.

The **fourth chapter** deals with occurrence and distribution of arbuscular mycorrhizal fungi in disturbed and undisturbed forest areas. The objective of the study was to compare the spore density, root colonization and diversity of arbuscular mycorrhizal fungi in disturbed and undisturbed forest areas. The study reveals that the spore density and species richness were more in undisturbed site than in disturbed site.

The **fifth chapter** deals with study related to the effect of selected arbuscular mycorrhizal fungi on growth of selected tree species. Two arbuscular mycorrhizal fungal species namely *G. mosseae* and *G. intraradices* have been used as inoculants on three forest tree species namely *Terminalia crenulata, Anacardium occidentale* and *Artocarpus*

heterophyllus. It was observed that innoculation with *G. mosseae* showed maximum dry weight biomass in *Artocarpus heterophyllus* and *Terminalia crenulata,* while innoculation with *G. intraradix* recorded maximum biomass in *Anacardium occidentale*.

CONCLUSION

The present study involving the two forests *viz.*, Mollem and Dharbandoda indicates that arbuscular mycorrhizal fungi are well developed feature of forest ecosystem. All the plant species studied in the present investigation were mycorrhizal. The study indicates that there is a rich diversity of arbuscular mycorrhizal fungi in forest soil. Arbuscular Mycorrhizal fungi can be used to increase the growth and productivity of forest plants. The disturbance due to human interference and mining activity has deleterious effects on arbuscular mycorrhizal fungal spore density in the soil thereby affecting the growth and productivity of the forest. Arbuscular Mycorrhizal fungi varied in their spore density and intensity of colonization with the changes in season.

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